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ABSTRACT BOOK

BOOK OF ABSTRACTS

Contents

KEYNOTES

K01

The effects of CO_2 and related water chemistry on fish: climate change v. aquaculture

Wilson, Rod W.

University of Exeter, UK

Atmospheric CO_2 is currently ~400 µatm and rising exponentially. This is causing concern for marine life, often under the banner of "ocean acidification". However, all aquatic environments are following the same trend of rising CO_2, including freshwater, so it is perhaps better to refer to "aquatic acidification", or even "aquatic carbonation" as many of the biological effects are driven by the CO_2 itself rather than the pH of the aquatic environment. Elevated CO_2 has long been known to directly affect acid-base/ion regulation, respiratory function, and aerobic performance in aquatic animals. More recently, elevated CO_2 levels projected for 2100 (e.g. ~1,000 µatm) have been shown to dramatically and negatively affect behaviours linked to sensory stimuli (smell, hearing and vision), digestive physiology and immune function in fish and invertebrates. By contrast, aquaculture has been experiencing much higher CO_2 levels (e.g. 10,000-40,000 µatm) long before "ocean acidification" was coined, with limited effects originally reported. Potential explanations for this discrepancy include the relatively benign aquaculture environment (abundant food, disease-protection, lack of predators), and inadvertent breeding/selection for CO_2-tolerance. However, effects that are directly relevant to growth, health and welfare have more recently been observed at CO_2 levels very relevant to aquaculture. It is not yet clear how high CO_2 conditions in aquaculture may play a role in the susceptibility to other stressors, including disease which causes $6 billion in losses to the industry annually. Climate change and aquaculture science can provide different but valuable insights from both marine and freshwater settings on the known and potential impacts of high CO_2 on aquatic animals. My talk will address some of the lessons emerging from these two disciplines, which are important both for predicting how wild populations will be affected in the future, and how to improve the sustainability of aquaculture. Considering other aspects of water chemistry is also key to determining the impacts of CO_2, because many variables (e.g. calcium, sodium, chloride, and alkalinity) are known to profoundly influence the tolerance of aquatic animals to CO_2. Furthermore, these aspects of water chemistry can vary enormously in both natural environments and aquaculture settings.

K02

The progressive management for improving aquaculture biosecurity: a new initiative

Reantaso, Melba B.

Food and Agriculture Organization of the United Nations, Italy

Approximately every 3-5 years, there is a new disease emerging and there is a long time lapse from the time that a disease or mortality event is first observed in the field, until the causative agent is confirmed, communicated nationally or through OIE, up to the time when a vaccine or cost-effective containment measures are put in place. While these events are happening, significant production and market losses are incurred affecting food supply, livelihoods, and export earnings. The cost would have been much less if efforts were focused on prevention compared to the costs for biosecurity measures, compensation and other alternatives.

The Progressive Management Pathway for improving aquaculture biosecurity (PMP/AB)[1], a new initiative, refers to a pathway aimed at enhancing aquaculture biosecurity by building on existing frameworks, capacity and appropriate tools using risk-based approaches and public-private partnerships. It is an extension of the Progressive Control Pathway (PCP)[2]. The PMP/AB is expected to result in sustainable:

- reduction of burden of disease
- improvement of health at farm and national levels
- minimization of global spread of diseases
- optimization of socio-economic benefits from aquaculture
- attraction of investment opportunities into aquaculture and
- achievement of One Health goals

The presentation provides information on: (i) the processes taken in the development of the PMP/AB that included an understanding and analysis of the factors, drivers and pathways to aquatic animal disease emergence; (ii) the four stages of the PMP/AB and key considerations at each stage; (iii) the benefits of PMP/AB; (iv) the entry points for countries; and (v) and the way forward for PMP/AB.

While awaiting endorsement and support from the FAO Committee on Fisheries Sub-Committee on Aquaculture 10th session, current activities include awareness raising and development of PMP/AB tools that will support implementation.

[1] The PMP/AB was developed and consensus built through two multistakeholder consultations co-organized by FAO and partners (e.g. Mississippi State University, the Norwegian Agency for Development Cooperation, the Norwegian Veterinary Institute and The World Bank (WB) hosted by the WB (Washington, DC, April 2018) and the OIE (Paris, January 2019) and a Technical Working Group meeting (FAO headquarters, Rome, March 2019).
[2] The PCP, is a step-wise approach increasingly used for the reduction, elimination and eradication of a range of major livestock and zoonotic diseases including, for example the Foot and Mouth Disease (FMD), Peste des Petits Ruminants (PPR), rabies, and African Animal Trypanosomosis (AAT). The PCPs provide systemic frameworks for planning and evaluating field interventions and enable realistic disease control objectives to be defined and achieved.

K03

The immune system of sea bass *Dicentrarchus labrax* in health and upon stimulation

Scapigliati, Giuseppe

Department for Innovative Biology, Agrofood and Forestry, University of Tuscia, Viterbo, Italy

The sea bass is maintained in aquaculture since two millennia, and at present is the main farmed species in the Mediterranean area, with a production of about 192 kt in 2016. The new milleniummillennium challenges for marine fish farming are to produce economically competitive, healthy, consumer and environment friendly sea bass in a changing aquatic environment. In this respect, the control of known and emerging sea bass pathologies through immune modulation plays a pivotal role in fish farming, with the aims of producing effective vaccines and positive stimulators of immune defences. The main pathologies affecting farmed and wild sea bass are induced by betanodavirus, by bacteria (genus: *Vibrio*, *Pasteurella*, *Flexibacter*, *Mycobacterium*), and parasites (protists, nematodes). Our group is studying the immune defences of MediterraneanEuropean sea bass (*Dicentrarchus labrax*) in the Mediterranean since 1995, and produced specific cellular markers for leukocytes, immuno-modulatory molecules, and molecular markers for expressed genes. These tools have been employed to investigate innate and acquired immune responses during changes of environmental farming conditions, antigen stimulation, vaccination, and pathologies, making the sea bass a reference marine species. This review will summarize available knowledge on these arguments.

K04

Next-generation sequencing: a revolution in the field of fish diseases

Avarre, Jean-Christophe

Institute of Evolutionary Sciences of Montpellier, DIVA (Fish Diversity and Aquaculture), France

The recent technological advances in nucleic acid sequencing, called next-generation sequencing (NGS), have revolutionized the field of genomics. Now available in almost all molecular biology platforms, NGS provides a huge amount of sequence data at relatively low cost. Research on fish pathogens has greatly benefited from these new technologies, and NGS is now increasingly used to trace aquatic viruses, to study virulence and evolution of pathogens, or to discover novel etiological agents causing fish mortalities. This presentation will first introduce the principles and characteristics of the major second-generation (Illumina) and third-generation (Oxford Nanopore, Pacific Biosciences) sequencing technologies. Then the main applications that are becoming routine tools in aquaculture will be described, such as whole genome sequencing of pathogens (both bacteria and viruses) or environmental DNA monitoring (through metabarcoding and metagenomics). A special focus will be dedicated to the challenges and limitations these applications are facing, especially regarding sample preparation and bioinformatics. Finally, concrete examples will illustrate how NGS could be used to investigate the diversity and virulence of an ecologically important fish pathogen, the *Cyprinid herpesvirus* 3.

ORAL PRESENTATIONS

Climate Changes, Ocean Acidification and Diseases

001-O

Paramoebic infections in changing environment

Nowak, Barbara (Australia)[1]; Archibald, John (Canada)[2]

1 - University of Tasmania, Australia; 2 - Dalhousie University, Canada

Introduction: Paramoebiasis are infections caused by paramoebae, which are marine free living amoebae capable of becoming parasitic. Paramoebae are characterised by the presence of obligate endosymbiont, *Perkinsela* sp., which is related to a parasitic flagellate *Ichthyobodo* sp. The most studied paramoebic infection is Amoebic Gill Disease (AGD), which was first reported from salmonids farmed in marine environment in Australia (Tasmania) and USA (Washington State) 30 years ago. Since then clinical AGD has been observed in fourteen countries across six continents and caused mortalities in seven farmed fish species. Usually, the first clinical outbreak coincided with unusually high water temperatures. The causative organism, *Neoparamoeba perurans*, has been detected in environmental samples (water and sediments).

Methodology: Literature review and small scale *in vitro* studies were undertaken.

Results: Paramoebiasis affects aquaculture and wild fisheries, including sea urchins, American lobsters and blue crabs. The effects will increase with climate change.

Conclusion: While a range of species of paramoebae have been detected in marine environment, their potential to cause infections is not fully understood. Climate change is likely to contribute to an increase in outbreaks of paramoebiasis both in farmed and wild species, as shown both by the reports of mortalities of sea urchins in Nova Scotia and AGD worldwide.

Keywords: *Neoparamoeba*, paramoebiasis, AGD, temperature

002-O*

What the history of the common cockle (*Cerastoderma edule*) can tell us about its potential future

Mahony, Kate E. (Ireland)[1]; Lynch, Sharon A. (Ireland)[1]; Culloty, Sarah C. (Ireland)[1]

1 - School of Biological, Earth and Environmental Sciences, and Aquaculture and Fisheries Development Centre, Environmental Research Institute, University College Cork, Ireland

Introduction: The common cockle (*Cerastoderma edule*) is a commercially important European bivalve species. In recent years, harvests of *C. edule* have decreased coinciding with more frequent mass mortalities. These mortalities have been attributed to multiple factors including climate and parasites. In order to protect future cockle fisheries, the history of cockles and causative factors for previous mortalities in Europe must be understood.

Methodology: "Marine Historical Ecology" techniques were used to create datasets of abundance and harvest levels of cockles at various European sites, as well as parasites, pathogens and ecological factors impacting cockles. Abundance of cockles has been shown to vary at a regional level, however this study aimed to collate all relevant literature, including grey literature, published literature and fisheries records, to examine European trends in wild and harvested cockles. These data were examined to determine if abundance and harvests of cockles were correlated with changing climate and to describe the causes of changes in cockle abundance and harvesting.

Results: Current knowledge of cockle populations is limited spatially and temporally. The results of this study highlighted the factors and drivers influencing mortalities on a regional basis. The impacts of climate varied by site and may be impacted by other factors including pollution and management regimes.

Conclusion: This analysis has provided an insight into the historical European cockle fishery but also highlighted the need for the standardisation of cockle monitoring techniques to allow for better prediction of future cockle abundance.

Keywords: *Cerastoderma edule*, common Cockle, mass mortality, marine historical ecology, climate change

Funding: COCKLES (EAPA_458/2016 COCKLES Co-Operation for Restoring CocKle SheLlfisheries and its Ecosystem Services in the Atlantic Area).

003-O*

The impacts of UV-B radiation on pacific oyster *Crassostrea gigas* health and development of pathogens *Vibrio aestuarianus* and ostreid herpesvirus-1 (OSHV-1MICROVAR)

Kett, Gary (Ireland)[1]; Jansen, Marcel (Ireland)[1]; Culloty, Sarah (Ireland)[1]; Lynch, Sharon (Ireland)[1]

1 - School of Biological, Earth & Environmental Sciences, and Aquaculture & Fisheries Development Centre, Environmental Research Institute, University College Cork, Ireland

Introduction: Since 2008 oyster cultivation sites around Europe have been experiencing increasing incidences of seasonal mass mortality events occurring over the summer months. The primary pathogens associated with oyster mortality are ostreid herpesvirus and variants (OsHV-1 mVar) and *Vibrio aestuarianus*, and a range of environmental factors can impact on the levels of infection and the health of oysters.

Medium length ultraviolet radiation (UV-B l280 - 315 nm) is highest during summer months and can fluctuate significantly between years. UV-B is known to cause damage to DNA through the formation of mutagenic lesions which can affect the performance of aquatic organisms and can increase mortality or in sub-lethal doses, induce an immune response. UV-B is also effective at inactivating free-living pathogens in water bodies. The role of UV-B radiation in the seasonal mass mortality events of *C. gigas* has not been investigated. The aim of this research is to investigate the biological impact of UV-B on the health of *C. gigas* and the development of OsHV-1 mVar and *V. aestuarianus* pathogens.

Methodology: Two laboratory trials have been carried out, exposing oyster seed and adults to UV-B radiation in both sea-water submerged and aerially exposed experiments. Field trials took place in Dungarvan Bay, Ireland, oysters were held at different intertidal heights and thus exposed to naturally different levels of solar UV-B.

Results: In the laboratory trials, it was found that exposure to UV-B radiation significantly increased the rate of mortality in oyster seed but not in adult individuals. In the short-term laboratory trial, prevalence of *V. aestuarianus* was reduced in oysters after exposure to UV-B. Preliminary results of the field trial appear to match those of the laboratory trials. Exposure to UV-B (artificial or natural) increases the rate of mortality in *C. gigas*, while also reducing the development of *V. aestuarianus* bacteria.

Keywords: Pacific oyster, *Crassostrea gigas*, *Vibrio aestuarianus*, ostreid herpesvirus -1 (OsHV-1 microVar), UV-B radiation

Funding: Research was by the European Union's Horizon 2020 project VIVALDI (No. 678589), European Regional Development Fund through the Ireland Wales Cooperation programme, BLUEFISH (No. 80991) and he Atlantic Area Interreg project COCKLES (EAPA_458/2016).

Nutrition and Health

004-O*

The guppy MODEL - developing functional feed additives against ectoparasites

Arapi, Elissavet (United Kingdom)[1]; Hunt, Rhi (United Kingdom)[1]; Nuez-Ortin, Waldo (Spain)[2]; Isern-Subich, Mercè (Spain)[2]; Coutteau, Peter (Spain)[2]; Cable, Joanne (United Kingdom)[1]

1 - Cardiff University; 2 - Nutriad - Adisseo

Introduction: Commercial aquaculture species suffer from a variety of parasites which cause major economic losses. Functional nutrition through the inclusion of health promoting feed additives is nowadays a widespread strategy for preventing and minimizing the impact of parasitic infections. The development of efficacious feed additive formulations, however, requires robust screening under an infection challenge prior to commercial application. Cohabitation studies can be used as preliminary assessment of the efficacy of functional additives against ectoparasites, however, these infection challenges are generally limited by the number of replicates and entail high inter- and intra-tank variability in infection rates.

Methods: Here, we developed and used a host-parasite model - the Trinidadian guppy *Poecilia reticulata* and its associated ectoparasite *Gyrodactylus turnbulli* – to test the potential efficacy of different functional feed additives against ectoparasites. A total of nine feeds were manufactured, each containing a different functional feed additive, and fed to infected and uninfected fish.

Results: Overall, five out of nine tested functional additives improved the host's parasite tolerance and two significantly reduced parasite burden and mortality.

Conclusion: This study highlights the suitability of the guppy model for monitoring host infection responses and corroborates the role of supplementary, functional feed additives for prevention against ectoparasites.

Keywords: nutrition, feed additive, ectoparasite

Funding: KESSII studentship (512499) and industrial partner, Nutriad (Adisseo).

005-O*

Cellular and molecular fingerprinting of feed-induced intestinal inflammation in Atlantic salmon

Park, Youngjin (Norway)[1]; Siriyappagouder, Prabhugouda (Norway)[1]; Dahle, Dalia (Norway)[1]; Kalerammana Vasanth, Ghana (Norway)[1]; Sørensen, Mette (Norway)[1]; Dias, Jorge (Portugal)[3]; Verlhac, Viviane (Switzerland)[2]; Manuel De Oliveira Fernandes, Jorge (Norway)[1]; Kiron, Viswanath (Norway)[1]

1 - Nord University; 2 - DSM Nutritional Products; 3 - Sparos, LDA

Introduction: Inclusion of more plant-derived ingredients is fast becoming the norm in the aquafeed industry. However, such diets cause inflammation among prominent farmed fish species. This non-infectious intestinal disease has to be tackled appropriately as the disease condition affects the growth and health of the fish and is a serious welfare issue. Hence, it is imperative to gather appropriate data to obtain a thorough understanding of the cells and molecules that are affected during intestinal dysbiosis.

Methodology: A five-week feeding trial was conducted on Atlantic salmon (*Salmo salar*) smolts. Two experimental groups were fed either an inflammation-inducing feed (containing full-fat soybean and soy saponin) or a control feed. Distal intestinal samples were collected to perform histological and transcriptomic studies. In addition, blood and head kidney leucocytes were examined using flow cytometry.

Results: Growth and feed performance were lower in the fish that developed diet-induced inflammation. The inflammatory condition in the intestinal folds was marked by wide *lamina propria* and infiltration of immune cells. Transcriptome analyses revealed the upregulation of 57 and downregulation of 44 genes in the inflamed intestine. Furthermore, perturbation of the MAPK signaling pathway was also evident.

Lymphocyte populations were significantly higher in the peripheral blood of fish that had inflamed intestine. Furthermore, in this fish group, phagocytic activity of macrophage-like cells from the head kidney was significantly higher compared to those on the control feed.

Conclusion: Diet-induced inflammation left significant marks at cellular and molecular levels in different tissues of Atlantic salmon. The soybean-induced enteritis in Atlantic salmon is an ideal model to investigate the inflammatory responses at the cellular and molecular level.

Keywords: soy saponin, inflammation, fish feed, Atlantic salmon, intestine

Funding: DSM Nutritional Products, Nord University.

006-O

Mycotoxin exposure poses a risk to farmed fish

Pietsch, Constanze (Switzerland)[1]

1 - Zurich University of Applied Sciences

Introduction: The continuous monitoring of mycotoxins in fish feeds is an enormous task. Therefore, a tool to estimate the risk of mycotoxin contamination in fish diets would help to judge if a particular risk for farmed fish due to the inclusion of certain feed ingredients should be assumed. For this, the levels of 10 different mycotoxins in 97 commercial fish feeds have been estimated. Since feed ingredients of differing quality may be chosen for feed production, the estimation of the mycotoxin contaminations of these fish feeds was done according to 6 different contamination scenarios.

Results: The calculations mainly focus on the mycotoxin contamination that originates from fungal growth on the field crops and not on toxins that are commonly formed during feed storage. The calculations predicted that deoxynivalenol, zearalenone, fumonisins and enniatins are the most prominent toxins in fish feeds. Whether the calculated toxin concentrations also pose an actual risk to farmed fish was estimated from Bayesian modeling, which was used to determine the critical concentrations 5% (CC5) for theoretical fish populations exposed to the different toxins. Besides fishmeal, plant-based materials such as wheat, soybean products and corn are regularly used as feed ingredients in aquaculture. These lead to the introduction of considerable amounts of mycotoxins into feeds for farm fish. The differently calculated feed contamination scenarios showed that fish are at risk to be affected by high toxin contaminations if feed ingredients of low quality are chosen for feed production.

Conclusion: Consequently, fish feed production should be based on ingredients of high quality to prevent any damage to farmed fish. In addition, more studies on mycotoxin effects on fish would be needed to yield more accurate risk assessments. And finally, more specific maximum allowable levels for several mycotoxins in fish feeds should be established in the future to address the increasing risk of mycotoxin contamination in aquaculture.

Keywords: feed quality, toxicology, fish health

109-O*

Evaluation of skin mucous cells of Atlantic salmon (*Salmo salar*) fed plant-based diets and probiotics

Nadanasabesan, Nimalan (Norway)[1]; Sørensen, Mette (Norway)[1]; Vatsos, Ioannis (Norway)[1]; Kiron, Viswanath (Norway)[1]; Fečkaninová, Adriána (Slovakia)[2]; Koščová, Jana (Slovakia)[3]; Mudroňová, Dagmar (Slovakia)[3]

1 - Faculty of Bioscience and Aquaculture, Nord University in Bodø; 2 - Department of Food Hygiene and Technology, University of Veterinary Medicine and Pharmacy in Košice; 3 - Department of Microbiology and Immunology, University of Veterinary Medicine and Pharmacy in Košice

Introduction: Commercial salmon diets used in Norwegian aquaculture is based on plant and marine ingredients. The general trend during the course of the last decades is to increase the inclusion levels of plant ingredients, while reducing those from marine origin; currently plant : marine ingredient ratio is 70:30. Plant ingredients may contain antinutritional factors that interfere with nutrient utilization and hence, they may not provide optimal levels of the necessary nutrients required for maintaining the fish health. Dietary probiotics may have a commercial value in fish farming if they can alleviate the negative effects of feed ingredients and/or strengthen the innate immune system of fish. This study investigated the histomorphology of skin of Atlantic salmon fed different diets and probiotics.

Methodology: A feeding experiment was conducted with Atlantic salmon. The fish were first fed 3 different diets and later the respective diets coated with probiotics. The ingredient composition of the diets were, Diet 1: a fish meal/fish oil based, Diet 2: a commercial-like diet with a plant/marine ingredient ratio of 70/30, and Diet 3: a fish meal/fish oil based diet in which soybean meal replaced 20% of the fish meal. Dorsal skin samples were collected from 12 fish per treatment and fixed in 4% formalin. Decalcified (10% formic acid, 5 hours) tissue sections of 4µm were prepared and stained with H&E and AB-PAS. Images (9/fish, 108/diet) were acquired and quantitative analysis was performed using ImageJ.

Results: Both the area of the mucous cells, and the area of mucous cells per epithelium of the study groups were similar. However, the number of mucous cells per epithelium area (M/E) was significantly influenced by diet and probiotics. Fish fed Diet 2 and 3 had more M/E compared to Diet 1. Addition of probiotics to Diet 1, increased the M/E and hence, we presume that this diet may have strengthened the protective barrier of the skin.

Conclusion: This study revealed that the ingredient composition of diet directly influence the number of mucous cells per epithelium. Probiotics increased the number of mucous producing cells per epithelium and may improve the barrier function of the skin.

Keywords: Atlantic salmon, skin mucous cells, plant-based diets, probiotics

Bacterial Diseases I

007-O*

The role of hypermutable strains in evolution of fish pathogenic bacteria and implications for vaccination in aquaculture

Silayeva, Oleksandra (Australia)[1]; Barnes, Andrew (Australia)[1]

1 - The University of Queensland

Introduction: Vaccine efficacy is reduced by pathogen adaptation to immune pressure achieved via serotype evolution through shifts in major antigens such as capsular polysaccharide or lipopolysaccharide. Increased evolvability is a feature of bacterial strains deficient in DNA repair genes (mutators) as a high mutation rate allows for rapid generation of adaptive variants. We investigated whether mutators promote serotype evolution using major aquatic pathogen *Streptococcus iniae* as a model organism.

Methodology: Whole genome sequences from 80 *S. iniae* strains isolated globally over 40 years were employed to infer phylogenetic relationships and identify variation in DNA repair (mutator) genes. Mutation rate phenotype and major virulence-associated traits were determined and correlation analysis was used to establish if mutations in mutator genes were associated with differences in mutation rate and virulence trait phenotypes. Oxidative stress resistance and virulence in a novel host (zebrafish) were compared between wild-type and isogenic knock-outs of *mutS* and *mutY*.

Results: Mutations in DNA repair genes correlated strongly with shifts in mutation rate and occurrence of atypical phenotypes associated with virulence. Most profound shifts in virulence determinants linked to multiple variants in mutator genes were detected in isolates from host jumps and vaccine escape outbreaks. Knockout of the *mutS* gene, that initiates DNA mismatch repair, increased resistance to oxidative stress. In the experimental cohorts of a novel host model, the wild type strain and *mutY* mutant showed extremely high and low virulence respectively. However, *mutS* knockout produced lethal infection in most of the hosts but established the chronic infection in a smaller proportion of surviving individuals.

Conclusion: Our data imply that serotype evolution, enabling epidemiologically relevant events such as vaccine escape outbreaks and host jumps, is facilitated by mutator strains. Moreover, fitness of mutators in the immune host may be increased by their intrinsic resistance to oxidative stress. Consequently, endeavours to find long term cross-protective registered vaccines against Streptococcus and other genetically variable pathogens based on conserved antigens might be difficult. We propose that greater success might be achieved for farmers via autogenous vaccines coupled with rapid strain typing and reformulation as necessary.

Keywords: *Streptococcus*, vaccines, evolution

Funding: Australian Research Council Discovery Project DP120102755.

008-O

Insights into microbial biofilm communities in an aquaculture facility

Graf, Joerg (United States of America)[1]; Testman, Todd (United States of America)[1]; Beka, Lidia (United States of America)[1]; Mcclure, Emily Ann (United States of America)[1]; Hassan, Ahmad (United States of America)[1]; Reichley, Stephen (United States of America)[2]; King, Stacey (United States of America)[2]; Wiens, Greg (United States of America)[3]; Welch, Tim (United States of America)[3]

1 - University of Connecticut; 2 - Clear Spring Foods; 3 - USDA ARS

Introduction: Microbial biofilms form rapidly on surfaces in the aquatic environment and can harbor important pathogens. In addition, bacteria in biofilms are more resistant to disinfectants and antibiotics. Some pathogens have been shown to express virulence factors in biofilms. We investigated biofilms formed in the raceway of a large commercial trout farm using a combination of techniques including 16S rRNA gene surveys, droplet digital PCR, ddPCR, and fluorescence *in situ* hybridization, FISH.

Methodology: Samples were collected using swabs at different depths and from different surfaces of the raceway and slides were suspended in the raceway for *in situ* biofilm formation. The V4 region of the 16S rRNA gene was PCR-amplified, sequenced on an Illumina MiSeq and analyzed using QIIME 2. The total amount of bacteria was determined using ddPCR. Using spectral imaging FISH, specific taxa were detected using a combination of probes specific for bacteria at various taxonomic levels.

Results: While Betaproteobacteria and Alphaproteobacteria often dominated the biofilm communities under some conditions, Gammaproteobacteria, Cytophaga, and Flavobacteriia were abundant. The analysis of the V4 region allowed the identification of some important fish pathogens to the species level, for example *Flavobacterium columnare* and *F. psychrophilum.* A ddPCR assay using universal primers was used to quantify the bacterial abundance, which allowed the comparison of absolute numbers, not just relative abundances, between samples. FISH analysis revealed multispecies biofilms with a higher degree of heterogeneity when compared to the analysis of DNA extracted from swab samples.

Conclusion: This study provides new insights into the biofilm community in active raceways and allows the determination of absolute abundances, which may serve as a better indicator for infection risk. In addition, new information regarding the spatial organization of mixed species biofilms was revealed. The combination of these technologies provides insight into the composition and structural organization of biofilms present in the raceways of active aquaculture facilities. This approach provides insight into the microbial ecology of raceway surfaces and will help to identify reservoirs of fish pathogens.

Keywords: biofilm, microbiome, *Flavobacteria*, 16S rRNA survey, FISH

009-O*

First report of pasteurellosis in meagre - genetic and biochemical identification of *Photobacterium damselae* subs. *piscicida*

Moreira, Márcio (Portugal)[1,2]; López-Sanmartín, Monserrat (Spain)[3]; López, José R. (Spain)[3]; Navas Triano, Jose Ignacio (Spain)[3]; Barata, Marisa (Portugal)[1]; Pousão-Ferreira, Pedro (Portugal)[1]; Soares, Florbela (Portugal)[1]

1 - Portuguese Institute for the Ocean and Atmosphere, Aquaculture Research Station, Av. 5 de Outubro s/n, 8700-305 Olhão, Portugal; 2 - Universidade do Algarve - Centre of Marine Sciences, University of Algarve, Campus de Gambelas, 8005-139 Faro, PORTUGAL; 3 - IFAPA Agua del Pino, Ctra. Cartaya-Punta Umbría, 21459 Cartaya, Huelva, SPAIN

Introduction: A 10% mortality was observed between December 2017 and January 2018 in 1.68 g meagre (*Argyrosomus regius*) produced in an open intensive system (9 kg/m^3) at Aquaculture Research Station, Portuguese Institute for the Ocean and Atmosphere (EPPO-IPMA).

Methodology: Microbiological analysis was performed in liver, spleen and head kidney of the affected fish. After 48 h, we obtained a pure isolate from the spleen of diseased meagre (ref. 42019IPMA). Biochemical identification of the isolate was performed, and the results were run in IDENTAX software for probabilistic identification. Identification was later confirmed by genetic analysis of the of 16S gene, that was amplified using 20F/ 1500R pair of primers and sequenced by SECUGEN (Madrid, Spain). Two specific PCRs were carried out comparing the isolate 42019IPMA with a *P. damselae* subsp. *piscicida* strain (a321), isolated from Senegalese sole (*Solea senegalensis*), and two reference strains, namely *Photobacterium damselae* subsp. *damselae* CECT 626T and *Photobacterium damselae* subsp. *piscicida* CECT 5895.

Results: The biochemical profile of the isolate 42019IPMA is similar to the ones described in bibliography for *P. damselae* subs. *piscicida*, with the negative urease, gelatinase and nitrate tests that distinguishes it from *P. damselae* subsp. *damselae*. IDENTAX software also identified the isolate as *P. damselae subs. piscicida* (96% probability). In the genetic analysis, the 16S rDNA sequence showed a high similarity value with *P. damselae* (99.6%, Eztaxon software). Moreover, molecular PCRs using specific pair of primers Ure3/ Ure5 to *P. damselae* subsp. *damselae* and CPSF / CPSR to *P. damselae* subsp. *piscicida* showed that the strain isolated has a profile identical to the *P. damselae* subsp. *piscicida*.

Conclusion: This confirms the first report of an outbreak of *Photobacterium damselae* subs. *piscicida* in a meagre inshore intensive production system.

Keywords: *Argyrosomus regius*, bacterial identification, PCR, outbreak, inshore intensive production system

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010-O*

Comparison of *Pasteurella* sp. isolated from outbreaks of pasteurellosis in lumpsucker (*Cyclopterus lumpus* L.) in Norway and Scotland

Monaghan, Sean (United Kingdom)[1]; Bartie, Kerry (United Kingdom)[1]; Bekaert, Michael (United Kingdom)[1]; Colquhoun, Duncan (Norway)[2]; Davie, Andrew (United Kingdom)[1]; Bojesen, Anders Miki (Denmark)[3]; Birkbeck, Harry (United Kingdom)[4]; Migaud, Herve (United Kingdom)[1]; Adams, Alexandra (United Kingdom)[1]

1 - Institute of Aquaculture, University of Stirling, Stirling, UK; 2 - Norwegian Veterinary Institute, Oslo, Norway; 3 - Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg C, Denmark; 4 - University of Glasgow, Institute of Infection, Immunity and Inflammation, Glasgow, UK

Introduction: Lumpsucker (*Cyclopterus lumpus* L.) have proven to be a useful cleaner fish species for delousing sea lice on Atlantic salmon (*Salmo salar* L.). Production of cultured lumpfish for deployment in sea cages is proving successful, however, as with many cultured fish species, farming intensification has lead to an increase in infectious disease in these fish. Outbreaks of pasteurellosis have notably caused high mortality rates in lumpfish (up to 100%) in Scotland and Norway, characterised typically by haemorrhaging of the fin bases and jaw, spots around the eyes and skin, and chronic visceral granulomas.

Methodology: In this study, five isolates of lumpfish *Pasteurella* sp. obtained during geographically distinct outbreaks of pasteurellosis on the west coast of Norway in 2013 were compared to five isolates from distinct outbreaks on the west coast of Scotland in 2017. The isolates were cultured on blood agar for 3-7 days. DNA was extracted from the bacterial colonies for comparative genomic analysis by repetitive PCR and whole genome sequencing (MiSeq). Bacterial pellets were also resuspended for proteomic analysis by quantitative 1D SDS PAGE and lectin Western blotting. Other members of the *Pasteurella* and *Photobacterium* genus were included in the analysis as a reference.

Results and Conclusion: The data supports previous 16S rRNA studies reporting that lumpsucker *Pasteurella* sp. is distinct from another fish *Pasteurella* sp., e.g. *P. skyensis* isolated from Atlantic salmon as well as closely related *Photobacterium damsellae* subsp. *piscicida* isolated from sea bass, at the genomic and proteomic level. However, the *Pasteurella* sp. isolates from lumpfish appear to a have a high degree of homogeneity. Intra- and inter-genomic and proteomic comparisons between isolates from the two countries will inform on the potential for development of cross-protective vaccines against pasteurellosis for the lumpsucker industry.

Keywords: *Pasteurella,* cleaner fish, lumpsucker, pasteurellosis, whole genome sequencing

Funding: Scottish Aquaculture Innovation Centre (part of Health WP4 of project: “Securing a sustainable supply and the optimal deployment of lumpsucker for sea lice control in the Scottish salmon industry”).

011-O*

A new serogroup of *Vibrio harveyi* in the Adriatic Sea – comparison of virulent vs non-virulent strains for sea bass

Pavlinec, Željko (Croatia)[1]; Zupičić, Ivana Giovanna (Croatia)[1]; Lojkić, Ivana[1]; Zrnčić, Snježana (Croatia)[1]; Fouz, Belen (Spain)[2]; Oraić, Dražen (Croatia)[1]; Mladineo, Ivona (Croatia)[3]; Jozić, Slaven (Croatia)[3]; Llorens, Amparo (Spain)[2]

1 - Croatian Veterinary Institute; 2 - University of Valencia; 3 - Institute of Oceanography and Fisheries

Introduction: *Vibrio harveyi* is a highly diverse marine pathogen, with some strains causing severe losses in aquaculture, while others being non-virulent. During recent years disease outbreaks in the Mediterranean farmed fish due to *V. harveyi* are becoming more common, posing a serious threat to the industry. The exact mechanisms and interplay of different virulence factors are still largely unclear. During the summer months of past few years, several outbreaks of vibriosis in sea bass (*Dicentrarchus labrax*) characterized by haemorrhages, corneal opacity and uncoordinated swimming were recorded in several farms along the Croatian Adriatic coast. Samples of diseased fish were submitted for laboratory examination.

Methodology: Samples of eyes, anterior kidneys, hearts and spleens were plated on both TSA supplemented with NaCl and Marine agar (MA) and cultured overnight at 25 °C. Pure colonies were obtained by streaking and restreaking on fresh MA plates. The non-virulent strain was isolated from the gut of healthy seabass. Genomic DNA was extracted from bacterial cultures using the Qiagen QIAamp DNA Mini QIAcube Kit. 16S rRNA gene was amplified using 27FYM and 1492R primers and sequenced to confirm the genus *Vibrio*, and toxR gene was amplified using toxRF1 and toxRR1 primers and sequenced to confirm the species *V. harveyi*. Slide agglutination test and ELISA were used to detect the main emerging pathogenic serogroup isolated from sea bass.

Results: Several isolates were confirmed by searching the NCBI Nucleotide database using Megablast as *V. harveyi*. Serological assays showed that strain 94/17, isolated from the eye, belongs to the virulent Serovar A previously described in Spain. However, strain 99/17, isolated from the anterior kidney, seems to belong to a new virulent serovar of the species. The results of currently ongoing WGS of described isolates and the one isolated from healthy fish will be analyzed and presented.

Conclusion: A new virulent serovar of *V. harveyi* has been isolated from diseased sea bass in the Adriatic Sea. Comparing the genome sequences of fish virulent and non-virulent strains as well as of virulent strains belonging to different serovars could shed some light on the cause of the differences in phenotype/pathogenicity.

Keywords: *Vibrio harveyi*, sea bass, serogroup, Adriatic

012-O*

Neurological signs in grey mullet *Mugil cephalus* caused by *Vibrio harveyi*

Strem, Rosa Ines (Israel)[1]; Sharon, Galit (Israel)[2]

1 - Ben Gurion University; 2 - National Center for Mariculture - IOLR

Introduction: Consumption of grey mullet (*Mugil cephalus*) has been growing worldwide. The National Center for Mariculture is one of the major producers in Israel, providing fingerlings from breading stocks held in captivity to local and foreign fish farmers. In recent years, mullet breeding stocks and fingerlings have been showing neurological signs such as uncoordinated circular swimming and a few days after, the fish die.

Methodology: To isolate and identify the pathogen from fish showing clinical signs, the methodology included pathology, bacteriology, histology, cell culture, microscopy and molecular analyses.

Results: 479 tissue samples were collected from infected fish since 2015. No ecto- or endo-parasites were found. Four cell lines were inoculated with tissue extracts and incubated at different temperatures. A cytopathic effect (CPE) was observed after several days of incubation, however, the cells recovered and CPE was no longer evident. Histological analysis indicated cavitation in brain tissue. RT-qPCR using specific primers gave negative results for viral nervous necrosis (VNN). RNA from 24 samples were processed for whole genome sequencing using a reference-based approach with MiSeQ Illumina Nextera XT. The results were negative for RNA viruses.

Bacteriological analysis showed different *Vibrio* species present in liver, spleen and kidney. *Vibrio harveyi* was the bacterium most frequently isolated from brain tissues. PCR using primers for the hemolysin and Tox R genes, specific for *Vibrio harveyi,* showed positive results in all the brain tissue samples as well as in bacteria cultures isolates from clinically sick fish. An experimental intraperitoneal re-infection with *Vibrio harveyi* isolated from a clinically diseased fish showed that 5 days later, neurological signs and oral haemorrhages could be observed in fish. A comparative analysis of the 16S gene from both the bacteria used for infection and the ones later isolated from brain of clinically diseased fish showed a 100% sequence similarity. DNA and RNA samples from both bacterial isolates gave positive results for both hemolysin and Tox R genes.

Conclusion: Results showed *Vibrio harveyi* as the cause of the neurological symptoms observed. To the best of our knowledge, this is the first report describing *Vibrio harveyi* causing neurological symptoms in grey mullets.

Keywords: *Vibrio harveyi*, *Mugil cephalus*

013-O

Pathology of mouthrot caused by *Tenacibaculum maritimum* in farmed Atlantic salmon

Frisch, Kathleen (Canada)[1]; Småge, Sverre Bang (Norway)[2]; Jakobsen Brevik, Øyvind (Norway)[2]; Duesund, Henrik (Norway)[2]; Johansen, Renate (Norway)[3]; Nylund, Are (Norway)[4]

1 - Cermaq Canada, Campbell River, Canada; 2 - Cermaq Group AS, Bergen, Norway; 3 - PHARMAQ Analytiq AS, Oslo, Norway; 4 - Fish Disease Research Group, University of Bergen, Bergen, Norway

Introduction: *Tenacibaculum maritimum* is a significant marine fish pathogen found world-wide, that is most commonly associated with tenacibaculosis, a disease characterized by frayed fins, tail rot, mouth erosion and skin lesions/ulcers. However, in the Pacific Northwest (West Coast of North America), *T. maritimum* has caused the disease mouthrot in farmed Atlantic salmon smolts since the late 80s, and continues to be a major health and welfare problem to the industry. Smolts recently transferred into saltwater are the most susceptible and affected fish have characteristic small (usually < 5 mm) yellow plaques. The fish die with little internal or external clinical signs other than these plaques and the mechanisms by which they die is unknown.

Methodology: A project was setup to increase the knowledge of mouthrot as it is seen in Atlantic salmon in the Pacific Northwest and make steps towards developing management tools that would help decrease the use of antibiotic treatments and improve fish welfare. This study included investigating the microscopic pathology (histology, scanning electron microscopy and immunohistochemistry) of bath infected smolts with Western Canadian *T. maritimum* isolates (TmarCan15-1, TmarCan16-1 and TmarCan16-5).

Results: Tissue tropism, investigated using a newly developed real-time RT-PCR assay showed that *T. maritimum* is detectable internally in infected smolts. This combined with the fact that the bacteria can be isolated from the kidney suggests that *T. maritimum* becomes systemic. The pathological investigation showed that infected smolts, both in field outbreaks and in bath challenges, have primarily mouth lesions, a disease that is similar to periodontal disease in mammals.

Conclusion: Changes in mouthrot affected fish are focal, severe, and occur very rapidly with little associated inflammation. The mechanism by which the fish die remains unknown.

Keywords: *Tenacibaculum maritimum*, pathology, histology, bacteria, lesions

Funding: The Research Council of Norway (RCN), project number 251805.

Parasitological Diseases I

015-O

A global review of parasites in finfish aquaculture

Poynton, Sarah (United States of America)[1]; Nowak, Barbara (Australia)[2]

1 - Johns Hopkins University School of Medicine; 2 - University of Tasmania

Introduction: Rising global production of finfish requires sound regional knowledge of the role and control of parasites. However, geographic coverage is uneven: (i) most geographical reports focus on host-parasite occurrence and ecology, not broader issues such as fish health infrastructure, and emerging diseases, (ii) geographic coverage is often disproportional to global, regional, or local importance of finfish aquaculture, and parasites therein, (iii) key gaps persist in literature in English, about parasites of finfish aquaculture for China, much of Asia, Russia, Africa, and some of Latin America (despite countries being significant producers).

Methodology: To address knowledge gaps, we are completing assembly of a multi-author book "Aquaculture Parasitology: Global Impacts and Management in Finfish" for publication by Wiley. The book comprises two main sections "Parasites of Economic Importance" and "Regional Reviews". The latter section has seven chapters: China, Asia (excluding China), Oceania, Europe (including Russia), Africa, North American, and Latin America and the Caribbean (following FAO (UN) geoschemes). Authors present an overview of aquaculture in their region, infrastructure for health and disease monitoring and management, current and emerging parasite diseases, current practices, and special topics. Of particular interest is current information from countries and regions typically under-represented in the English literature, including China, Iran, Russia, sub-Saharan Africa, and some countries in Latin America.

Results and Conclusion: Important contrasts include: (i) parasites of greatest economic importance range from myxozoans and digenean trematodes in North America (primarily salmonid and catfish production) to monogeneans in China (predominantly carp production), and crustaceans in Latin America (predominantly salmonid production); (ii) infrastructure ranges from substantial, as in parts of Europe and North America, and in China (with significant government investment in research and surveillance), to rather limited, as in Russia and East Africa; and (iii) prevention and treatment practices are very diverse, including rare implementation in East Africa (where there is a history of subsistence aquaculture), to inclusion of traditional herbal medicines in China. Common themes across regions included: (i) importance of non-native fish species, (ii) environmental concerns, and (iii) need for better disease control including anti-parasitics for foodfish.

Keywords: infrastructure, emerging diseases, regional reviews

016-O

Molecular analysis for the detection of significant southern bluefin tuna parasites from environmental samples

Bott, Nathan (Australia)[1]; Power, Cecilia (Australia)[1]; Nowak, Barbara (Australia)[1]

1 - RMIT University

Introduction: Biofouling naturally occurs on aquaculture infrastructure. Biofouling can be a reservoir for pests and pathogens. By studying parasite load in fouling and water samples associated with SBT pontoons it provides the opportunity to monitor pathogen load in a non-destructive manner. Significant tuna parasites have already been found and detected in the bioufouling. For example, Japanese studies showed presence of *Cardicola orientalis* and its intermediate host in the biofouling on Pacific bluefin tuna farms. Some terrebellid polychaetes that may be intermediate host of tuna blood flukes in Australia are found attached to SBT infrastructure.

Methods: Water samples, shell fragments and fouling associated with infrastructure were collected from six individual SBT lease sites. Samples were frozen and process for DNA extraction. Any polychaetes identified in samples were examined for presence of blood flukes intermediate stages. All samples will be analysed using quantitative polymerase chain reaction (qPCR) assays for the presence of *Cardicola forsteri*, *C. orientalis* and *Miamiensis avidus.*

Results: The results of qPCR surveillance for *C. forsteri*, *C. orientalis* and *M. avidus* will be discussed, and related to the overall SBT health data.

Conclusion: Monitoring environmental reservoirs may be an important non-destructive surveillance tool, further optimization of the approach may provide insights into host-pathogen interactions improving aquatic animal health management. Our approach could also be applied for the surveillance of SBT blood fluke intermediate hosts and for other potential aquaculture pathogens.

Keywords: Southern bluefin tuna, environment, *Cardicola*, *Miamiensis avidus*

017-O

The hidden genome of a marine amoeba *Neoparamoeba perurans*

Botwright, Natasha (Australia)[1]; Greenfield, Paul (Australia)[2]; Mcwilliam, Sean (Australia)[1]; Wynne, James (Australia)[3]

1 - CSIRO Agriculture and Food, St Lucia, Australia; 2 - CSIRO Energy, North Ryde, Australia; 3 - CSIRO Agriculture and Food, Hobart, Australia

Introduction: Amoebic gill disease (AGD) is a potentially fatal parasite-mediated proliferative gill condition impacting the Atlantic salmon aquaculture industry worldwide. The amphizoic marine parasite *Neoparamoeba perurans* (*N. perurans*) is the only confirmed aetiological agent of AGD. *N. perurans* is a unicellular organism which hosts both an endosymbiont and a microbial community. Currently no publicly available genome or transcriptome exists for this commercially important pathogen. In an attempt to accelerate novel treatment or prevention strategies for AGD we have sequenced and assembled a draft genome of *N. perurans*, along with its associated endosymbiont and microbial community.

Methodology: Utilising a range of sequencing technologies, including Illumina and PacBio, we sequenced the metagenome genome of *N. perurans*. Following read quality trimming a novel iterative *de novo* genome assembly was performed enriching for contigs from *N. perurans* and its endosymbiont. Several bacterial genomes were isolated independently of this process. Evidence based *ab-initio* gene prediction was completed with Maker and annotated using the Blast2GO and InterproScan pipeline. *N. perurans* contigs were validated using transcriptome sequencing. To examine genetic variation geographically, we also sequenced the genomes of *N. perurans* isolated from cultures maintained in Norway, Scotland and Ireland.

Results: A total of 22,177 contigs were assembled and represented both *N. perurans*, and its endosymbiont as well as remnants of some microbial genomes with similar GC contents. The N50 was 14,154, with the longest contig 1,166,407 bp in length. Annotation of contigs resulted in 20,885 predicted protein coding genes with diverse cellular functions. Transcriptome evidence confirms 6,890 are definitively *N. perurans* and its endosymbiont, while the remainder are either unexpressed in this dataset, or from other metagenomes.

Conclusion: The draft metagenome of *N. perurans* represents an important genomic resource for the AGD research community. This genome will enable new targeted treatment and mitigation strategies to be developed for AGD and will provide new insights into both the host-parasite interactions and also the evolutionary history of this parasite. Further we discuss the complications of assembling a metagenomic community, and the wider implications of annotation where all species have similar GC content.

Keywords: genome, AGD, endosymbiont

Funding: CSIRO Agriculture and Food.

018-O

Phenotypic characterization of *Paramoeba perurans* clones obtained from different populations of Atlantic salmon and ballan wrasse

Blindheim, Steffen H (Norway)[1]; Andersen, Linda (Norway)[1]; Trösse, Christiane (Norway)[2]; Karlsbakk, Egil (Norway)[2]; Nylund, Are (Norway)[2]

1 - The Industrial and Aquatic Laboratory; 2 - University of Bergen

Introduction: *Paramoeba perurans* is the causative agent of amoebic gill disease (AGD) in Atlantic salmon *Salmo salar* L. and a number of other marine fish world-wide. AGD is an emerging disease in Norway where it has caused major problems for the fish farming industry since 2012. Despite several decades with research and numerous studies on AGD, large knowledge gaps about *P. perurans* exist.

Methodology: Ten isolates of *P. perurans* were characterized on the basis of phenotypical traits and 18S rRNA sequences. The isolates were obtained from several counties along the Norwegian coast (Rogaland, Hordaland, Sogn & Fjordane, Møre & Romsdal and Sør-Trøndelag), and clonal strains were established for all isolates. For morphological measurements, several hanging drop preparations were made from each isolate. Growth patterns for the amoeba isolates were examined using microscopy and real-time RT-PCR under a range of different temperatures (4, 12, 15 and 23 °C) and salinities (20, 25, 30 and 34‰).

Results: Clear differences in morphology concerning the appearance of both locomotive and floating forms of the clonal amoeba isolates were seen (e.g. size, shape of pseudopodia). Different growth patterns could be seen for the clonal amoeba isolates, both when given similar conditions, and in their response to variations in temperature and salinity. A significant reduction in growth could be seen at 20 ‰ salinity. Most amoeba isolates grew well at 12 °C and 15 °C. At 4 °C amoeba grew slower and extended pseudopodia could not be seen when in their floating form. Moreover, the isolates seemed to reach the plateau phase faster at 23 °C and a higher number of rounded amoebae were observed, indicating a reduction in the number of viable amoeba in the culture.

Conclusion: The results from this study show that there are variations between clonal isolates of *P. perurans* when it comes to morphological features, growth curves and preferences for temperatures and salinities. These isolates should be further examined in experimental studies, as the observed differences could be relevant for amoebae growth potential on gills and virulence.

Keywords: phenotyping, *Paramoeba perurans*, clonal culture

Funding: Norwegian Seafood Research Fund (FHF), project no. 901053.

019-O

Novel anti-parastics in the fight against amoebic gill disease in Atlantic salmon

Botwright, Natasha (Australia)[1]; Hutt, Oliver (Australia)[2]; English, Chloe (Australia)[1]; Welch, Nicholas (Australia)[2]; Rusu, Anca (Australia)[1]; Mulet, Xavier (Australia)[2]; Muir, Ben (Australia)[2]; Wynne, James (Australia)[1]

1 - CSIRO Agriculture and Food; 2 - CSIRO Manufacturing

Introduction: Atlantic salmon aquaculture currently supplies greater than 2.2 mt p.a. of Atlantic salmon consumed worldwide. Intensively farmed Atlantic salmon are highly susceptible to Amoebic Gill Disease (AGD) and if left untreated, affected fish eventually lose respiratory capacity and succumb to the disease. The disease is initiated by attachment of the temperate marine ectoparasite *Neoparamoeba perurans* (*N. perurans*) which causes lesions on the gill surface. The current freshwater treatment is applied 8-10 times p.a., however it is unable to eliminate the disease, or remove the parasite completely from the water. Alternative bathing or in-feed treatments which eliminate or prevent attachment of the parasite are highly sought after.

Methodology: A rapid high throughput *in vitro* screening assay measuring amoeba viability was developed to assess anti-parasitic activity within *N. perurans.* A library of 44,000 small molecules was screened *in silico* to select a subset of 180 molecules based on diversity of chemical structure. These were subject to *in vitro* screening in triplicate in a seven point serial dilution series from 150 µM to assess the relative viability of *N. perurans*. The results of the preliminary screen were used to conduct a second *in silico* screen of the library for small molecules with similar structures to those which induced a reduction in *N. perurans* viability.

Results: The 187 small molecules screened showed reduced *N. perurans* viability of greater than 60% for 9 chemical structures and a further 29 reduced viability between 40 and 60%. The vast majority of small molecules, approximately 80%, screened resulted in less than a 20% reduction in *N. perurans* viability. A secondary sub-structure search of the proprietary library based on the top 14 anti-parasitic small molecules revealed a further 381 molecules for screening.

Conclusion: Our study demonstrates that rapid high throughput screening is a highly efficient method in assessing the viability of *N. perurans* against potential novel anti-parasitic compounds. Our initial preliminary trials of small molecule structures which reduce *N. perurans* viability may lead to the discovery of additional small molecules with increased anti-parasitic activity against *N. perurans*, and ultimately novel treatments for AGD in the future.

Keywords: amoebic gill diseases, Atlantic salmon, anti-parasitics, drug discovery, treatment

020-O

First report of *Tetracapsuloides bryosalmonae* and PKD in Alaskan salmonids

Gorgoglione, Bartolomeo (United States of America)[1]; Bailey, Christyn (Switzerland)[2,3]; Ferguson, Jayde A. (United States of America)[4]

1 - Aquatic Animal Health Laboratory, Dept. of Pathobiology and Diagnostic Investigation, CVM & Dept. of Fisheries and Wildlife, CANR - Michigan State University, East Lansing, MI 48824; 2 - Centre for Fish and Wildlife Health, Universität Bern, Länggassstrasse 122, 3012 Bern, Switzerland; 3 - Fish Immunology and Pathology Laboratory, Animal Health Research Center (CISA-INIA), Madrid, Spain; 4 - Alaska Department of Fish and Game, Division of Commercial Fisheries, Fish Pathology Laboratory, 333 Raspberry Rd., Anchorage, AK 99518

Introduction: Proliferative kidney disease (PKD) is an emerging infectious disease of salmonids, caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*. PKD pathogenesis results in chronic lymphoid immunopathology and strong immunosuppression, increasing host susceptibility to secondary infections. Thus far the parasite has been found in western Northern America, from California to the southern British Columbia, and widely throughout Europe, even including the southern Scandinavia and Iceland.

Methodology: Clinical PKD was diagnosed from two distinct diagnostic cases submitted to the Alaska Department of Fish and Game Fish Pathology Laboratory. Necropsies were followed by histopathology assessment and *T. bryosalmonae* confirmation using PCR, and parasite identification in histological sections using IHC.

Results: Adult chum salmon (*Oncorhynchus keta*) were sampled from Kantishna River, a tributary to the Yukon River watershed, in August/September 2011. Tumultuous histozoic extrasporogonic stages of *T. bryosalmonae* were documented in the kidney interstitium and coelozoic sporogonic stages were also observed within renal tubules. Archived samples provided molecular confirmation and local strain identification. A suspicious archived case from August 1997 was re-evaluated. Sockeye salmon (*Oncorhynchus nerka)* smolts reared in net-pens in a hydrologically isolated freshwater lake in Nanwalek, experienced elevated chronic mortality following furunculosis and concurrent infections with other parasites. *T. bryosalmonae* sporogony, and severe to resolving PKD, were confirmed also from this older case.

Conclusion: *T. bryosalmonae* is circulating, and is able to cause PKD in both wild and cultured salmonids in Alaska. Catchments of the Yukon River mainstream offer adequate conditions for its life cycle, reaching 15-20°C during the summer. The known geographic range of *T. bryosalmonae* is extended to ~170 miles south of the Arctic Circle in North America, representing one of the world's northernmost detection. Given the vast size of Alaska and its small resident population it is likely *T. bryosalmonae* remained undetected, but more recently became evident due to the clinical manifestation of PKD, possibly linked to climate change events. Undertaking a systematic and focused surveillance is necessary for generating baseline data, for detecting parasite strains, identifying local suitable environmental conditions permissive for its life-cycle, and to characterize disease dynamics in these host species.

Keywords: chum salmon, sockeye salmon, surveillance, myxozoa, parasite

Emerging and Alien Pathogen Species

021-O

Killers or bystanders? Cases of PRV-1 in Atlantic salmon and PRV-3 in rainbow and brown trout in German

Adamek, Mikolaj (Germany)[1]; Fux, Robert (Germany)[2]; Arndt, Daniela (Germany)[2]; Langenmayer, Martin C. (Germany)[2]; Hellmann, John (Germany)[3]; Flamm, Agnes (Germany)[4]; Schwaiger, Julia (Germany)[5]; Ferling, Hermann (Germany)[5]; Teitge, Felix (Germany)[1]; Fischer, Nicole (Germany)[6]; Indenbirken, Daniela (Germany)[7]; Grundhoff, Adam (Germany)[7]; Dölken, Lars (Germany)[8]; Vendramin, Niccolò (Denmark)[9]; Fey, Daniel (Germany)[3]; Rißе, Karin (Germany)[4]; Blakey, Franziska (Germany)[4]; Rimstad, Espen (Norway)[10]; Sutter, Gerd (Germany)[2]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany; 2 - Ludwig Maximilian University of Munich, Munich, Germany; 3 - North Rhine Westphalian State Agency for Nature, Kirchhunden-Albaum, Germany; 4 - Hesse State Laboratory, Giessen, Germany; 5 - Bavarian Environment Agency, Wielenbach, Germany; 6 - University Medical Center Hamburg Eppendorf, Hamburg, Germany; 7 - Leibniz Institute for Experimental Virology, Hamburg; 8 - Julius-Maximilians-Universität Würzburg, Würzburg, Germany; 9 - Technical University of Denmark, Lyngby, Denmark; 10 - Norwegian University of Life Sciences, Oslo, Norway

Introduction: Piscine orthoreoviruses (PRVs) are emerging viral pathogens causing circulatory disorders in salmonids. German aquaculture, with a significant production of rainbow trout and conservation programs for Atlantic salmon and brown trout in several river systems, is potentially vulnerable to the impact caused by these viruses. Furthermore, German brown trout populations are decimated by the proliferative darkening syndrome (PDS) a lethal disease of unknown etiology affecting this fish in the rhithral region of alpine Bavarian limestone rivers.

Methodology: Samples were collected from two German farms breeding Atlantic salmon or rainbow trout, experiencing in 2017 some health problems leading to accumulated mortalities of 10% and 20%. Furthermore, archival samples from exposure studies performed in 2008 and 2009, in which brown trout developed PDS, were used. The samples were tested for the presence of PRV RNA with PCR based methods, PDS samples from 2009 were additionally screened in a next-generation RNA sequencing pipeline for pathogen detection.

Results: Virological examination indicated a PRV-1 infection in the Atlantic salmon and a PRV-3 infection in rainbow trout. Further analyses indicated also the presence of *Aeromonas salmonicida* in internal tissues of both species. While PRV-1 was likely the causative agent of the disease in Atlantic salmon, most of the rainbow trout suffered from a systemic infection with *A. salmonicida* and not from PRV-3. Interestingly, PRV-3 RNA was also detected in several organs of the PDS-affected brown trout in 2008 but not in 2009. However, similar virus loads were measured in control fish from 2008, which were not exposed to river water presumably holding the PDS-inducing pathogen and which did not show any signs of the disease.

Conclusion: The results from Germany confirm a wide geographical distribution of both PRV-1 and PRV-3 in Atlantic salmon and rainbow trout also in continental Europe. However, a clear association with disease was hampered by the presence of an *A. salmonicida* co-infection. Results from the brown trout indicate that PRV-3 is present in brown trout populations but is not the causative agent of PDS. Nevertheless, diseases induced by PRVs should be considered when investigating mortalities in salmonids.

Keywords: PRV, Atlantic salmon, brown trout, rainbow trout

022-O

Piscine orthoreovirus-3 (PRV-3), a new pathogen for farmed rainbow trout

Vendramin, Niccolò (Denmark)[1]; Kannimuthu, Dhamotaran (Denmark)[2]; Cuenca, Argelia (Denmark)[1]; Wessel, Øystein (Norway)[2]; Dahle, Maria (Norway)[3]; Teige, Lena (Norway)[2]; Olsen, Anne Berit (Norway)[4]; Iburg, Tine (Denmark)[1]; Rimstad, Espen (Norway)[2]; Olesen, Niels Jørgen (Denmark)[1]

1 - European Union Reference Laboratory for Fish and Crustacean Diseases, National Institute of Aquatic Resources, DTU aqua. Kgs. Lyngby, Denmark; 2 - Department of Food Safety and Infection Biology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences, 0454 Oslo, Norway; 3 - Norwegian Veterinary Institute, 0454 Oslo, Norway; 4 - Norwegian Veterinary Institute, 5003 Bergen, Norway

Introduction: Piscine orthoreovirus – PRV have emerged as pathogens for salmonid aquaculture worldwide. Three different subtypes with specific host are described for this viral species: PRV-1 is the causative agent of heart and skeletal muscle inflammation (HSMI) in Atlantic salmon. PRV-2 causes erythrocytic inclusion body syndrome (EIBS) in Coho salmon. PRV-3 was discovered for the first time in 2013 in Norway in connection with disease outbreaks in farmed rainbow trout. In late 2017, the presence of PRV-3 was also reported in different countries in Europe including Scotland, Germany, France, Italy and Denmark. Interestingly, these viral isolates appear to be genetically distinct from the Norwegian isolate leading to proposition of two separate clades within PRV-3 viral type (PRV-3a and PRV-3b).

Methodology: A first series of experimental trials conducted were performed to assess its pathogenicity and pathogenesis in rainbow trout ant Atlantic salmon. A PRV-3 isolate has been purified and its genome and antigenic features analyzed. A second infection study with purified PRV-3 has been conducted to fulfill Koch's postulate. In both experiments tissue samples at selected time points have been collected and analysed for viral load by QPCR, histopathology and immune gene regulation. During field outbreak in RAS, extensive sampling and screening have been conducted to link the virus to the disease.

Results: The experiment showed that PRV-3 is highly contagious to rainbow trout whereas Atlantic salmon is less susceptible to the virus. In Rainbow trout PRV-3 replicates up to a peak and lately the virus is cleared by the host. The purified virus induces pathological heart lesions similar to HSMI, and thus fulfill Koch's postulates. PRV-3 infection upregulates IFN production, and induces specific antibody response in later phases. Field outbreak in RAS are characterized by uncoordinated swimming behavior, severe gill disease and increased mortality; necropsy findings include severe anemia and ascites. PRV-3 load increases in the target organs (heart, spleen) before clinical disease appear, whereas other pathogens are not detected in a systematic pattern.

Conclusion: PRV-3 causes heart pathology in Rainbow trout and it is a major player in the complex disease outbreak that occur in RAS in RT.

Keywords: piscine orthoreovirus, experimental challenge, pathogenesis, heart pathology, emerging disease

023-O

Fist isolation of a bafinivirus in imported goldfish in the United Kingdom - pathogenicity studies

Cano, Irene (United Kingdom)[1]; Feist, Stephen W. (United Kingdom)[1]; Savage, Jacqueline (United Kingdom)[1]; Wood, Gareth (United Kingdom)[1]; Gray, Joshua (United Kingdom)[1]; Stinton, Nick (United Kingdom)[1]; Ross, Stuart (United Kingdom)[1]; Bateman, Kelly (United Kingdom)[1]; Stone, David (United Kingdom)[1]

1 - Cefas

Introduction: A novel bafinivirus, belonging to the family Coronaviridae, has been recently isolated in the UK as part of a normal health check conducted by the Fish Health Inspectorate of a consignment of assorted goldfish on animals showing no clinical signs of disease.

Methodology and Results: The bafinivirus isolated from goldfish was characterised by TEM in EPC cells. Virus like structures were observed within the cytoplasm consisting in cylindrical tubes varying in length and measuring approximately 10 nm in diameter. In addition, spherical particles possessing distinct spike like structures on the external membrane were observed, measuring 160 nm in diameter similar in size and shape to coronavirus. This unknown virus was *de novo* sequenced using Nextera XT (Illumina). The consensus sequence shared 99% nucleotide identity overall with the Chinook salmon Bafinivirus, NC_026812.1, with two 500 nt deletions in the ORF for the Ia protein. The bafinivirus can infect and replicate in a diverse range of cyprinid, salmonid and other fish derived cells lines at a wide temperature range, from 15 to 25 °C, with faster growth at a warmer temperature. To evaluate possible risks to UK native species, Atlantic salmon and common carp were exposed to the bafinivirus by intraperitoneal (IP) injection and bath challenge. A strong inhibition of appetite, mucus production and faecal casts were observed in challenged goldfish and carp throughout the challenge. However, morbundity was observed only in 8% of the IP injected goldfish at 17 days post challenge (pc). The virus was re-isolated from IP and bath challenged carp, Atlantic salmon and goldfish at 10 days pc; and only in goldfish at 20- and 33-days pc with very low viral titres. Histopathology examinations and seroconversion are ongoing.

Conclusion: The bafinivirus isolated from gold fish is able to infect Atlantic salmon and common carp by IP and bath challenge however no associated mortalities were observed. The risk that the introduction of the bafinivirus poses to UK native species will be discussed.

Keywords: bafinivirus, imported gold fish, TEM, pathogenicity, isolation

Funding: Defra contract FB002A.

024-O

Analysis of the white sturgeon (*Acipenser transmontanus*) immune response during *Veronaea botryosa* infection

Soto, Esteban (United States of America)[1]; Fast, Mark D. (Canada)[2]; Purcell, Sara (Canada)[2]; Coleman, Denver (United States of America)[1]; Leal, Michaiah J. (United States of America)[1]; Yazdi, Zeinab (United States of America)[1]; Kenelty, Kirsten (United States of America)[1]; Yun, Susan (United States of America)[1]; Camus, Alvin (United States of America)[3]

1 - University of California, Davis; 2 - University of Prince Edward Island; 3 - University of Georgia

Introduction: Systemic phaeohyphomycosis caused by *Veronaea botryosa* is regarded as one of the most important emergent diseases in sturgeon aquaculture in North America. Animals cultured at temperatures above 13–15 °C, were recently found to be at higher risk of severe disseminated disease and higher mortalities. Despite this, little is known regarding disease pathogenesis and immune responses to infection. The main objectives of this study were to develop tools to study immunity in white sturgeon, and to investigate the response of infected fish maintained at different temperatures.

Methodology: Since there are few genomic resources for white sturgeon, recently assembled transcriptomes for Atlantic sturgeon (*Acipenser oxyrhynchus*) were used as a database source for generation of quantitative reverse-transcription real time PCR assays. White sturgeon maintained at 13 °C or 18 °C were challenged with *V. botryosa* via intra-muscular injection. At 2, 8 and 32 d post-challenge (dpc), 6 fish per treatment were bled, euthanized, and subjected to complete necropsy. Spleens were collected for investigation of interleukin pp17 (IL-17), major histocompatibility class II (MHCII), serotransferrin, haptoglobin, and serum amyloid A (SAA) expression during *V. botryosa* infection. Antibodies to acute phase proteins (APP), were used to quantify the conserved APP peptides in the serum of challenged and control fish.

Results: IL-17, serotransferrin, and haptoglobin transcripts were increased by 8 dpc only in splenic tissues of challenged fish maintained at 18 °C. By day 32, fungal exposed fish maintained at 18 °C exhibited significantly increased transcript levels when compared to other treatments and time-points ($p<0.05$). MHC II and SAA transcript abundance remained similar in splenic tissues from exposed and control fish regardless of temperature. In agreement with transcript quantification, only serotransferrin, and haptoglobin peptides were significantly increased in the challenged fish maintained at 18 °C ($p<0.05$).

Conclusion: Results demonstrate that *V. botryosa* infection induces robust APP expression correlated with IL-17 gene expression only in the spleens of sturgeon maintained at 18 °C. This response was associated with fungal dissemination and disease progression, as evidenced by increased fungal DNA detection in spleen, and supports previous histopathological studies indicating more severe lesions in fish challenged at the higher temperatures.

Keywords: caviar, cytokine, fungus, sturgeon

025-O

From lamb to lion: unleashing the beast in "virulent" *Aeromonas hydrophila*

Beck, Benjamin (United States of America)[1]; Peatman, Eric (United States of America)[2]; Shoemaker, Craig (United States of America)[1]; Mohammed, Haitham (United States of America)[2]

1 - USDA ARS Aquatic Animal Health Research Unit; 2 - Auburn University School of Fisheries, Aquaculture, and Aquatic Sciences

Introduction: An emerging pathotype of *Aeromonas hydrophila* (vAh) has been responsible for widespread farm losses in the US catfish industry over the last decade. While our genetic and biochemical understanding of vAh has been greatly enhanced in this time frame, our ability to reliably induce the disease in the laboratory remained limited.

Methodology: Utilizing established protocols for aeromonad challenges resulted in minimal mortality and inconsistent clinical symptoms. Therefore, taking cues from observed farm conditions associated with outbreaks, we perturbed iron scavenging dynamics through the addition of a xenosiderophore, deferoxamine mesylate (DFO), to the culture medium prior to challenge. We also evaluated the impact of catfish feeding status (fasted versus fed) on host susceptibility.

Results: The addition of DFO to vAh cultures prior (during the 18-20 h culture phase) to immersion challenge significantly increased virulence in several vAh isolates but not in a non-epidemic strain (43.3±17.6% survival post-DFO addition vs. 100±0.0% survival when cultured in control broth; $p<0.05$). DFO addition did not impact vAh growth dynamics or perturb iron-sensitive gene pathways, but did significantly enhance hemolysis of catfish blood at an addition rate of 0.4mM or higher. Furthermore, hours between last feeding and immersion challenge (postprandial status), was observed to be a critical determinant of catfish susceptibility. Fish with a full gastrointestinal tract had significantly lower survival than those in a fasted state (63.3% ± 7.9 vs. 89.9 ± 3.8%, $p<0.05$), and this effect was cumulative with that of DFO-enhanced vAh virulence.

Conclusion: Utilizing our more robust challenge model, we are currently examining the practical efficacy of varying protective strategies for the industry including diet modification, vaccination, genetic selection, and modulation of the pond environment. Our latest results in this vein will also be highlighted.

Keywords: catfish, aquaculture, *Aeromonas hydrophila*, motile *Aeromonas septicemia*, host-pathogen interactions

Funding: United States Department of Agriculture.

Bacterial Diseases II

026-O

***Tenacibaculosis* in the sea lice cleaner fish *Cyclopterus lumpus* L.**

Bang Småge, Sverre (Norway)[2]; Frisch, Kathleen (Canada)[1]; Knutsen, Linn Emilie (Norway)[2]; Jakobsen Brevik, Øyvind (Norway)[2]; Duesund, Henrik (Norway)[2]; Nylund, Are (Norway)[3]

1 - Cermaq Canada, Campbell River, Canada; 2 - Cermaq Group AS, Bergen, Norway; 3 - Fish Disease Research Group, University of Bergen, Bergen, Norway

Introduction: *Tenacibaculum* spp. are associated with disease in lumpfish (*Cyclopterus lumpus* L) in Norwegian salmon farming where the bacteria typically attack the spikes of the skin ("donut syndrome"). During a high mortality outbreak in lumpfish in 2015, *Tenacibaculum maritimum* was isolated and described for the first time in Norway. This isolate was found to be closely related to isolates that cause "mouthrot" in farmed Atlantic salmon smolts on the West Coast of Canada, which have been shown to be easily transmitted between fish. Recently, *T. maritimum* has been associated with gill issues in farmed Atlantic salmon in Norway.

Methodology: As part of the project "Limiting the effect of tenacibaculosis in Norwegian salmon farming (LimiT)" (FHF Project number: 901433), studies were conducted to investigate the ability of *T. maritimum* isolates collected from both Norwegian farmed Atlantic salmon and lumpfish, to cause disease in lumpfish. In challenge experiments, the transmission ability of *T. maritimum* between lumpfish and Atlantic salmon smolts was also investigated.

Results: These experiments have shown that *T. maritimum* causes tenacibaculosis ("donut syndrome") in lumpfish. Also, the bacterium transfers from infected lumpfish to naïve Atlantic salmon smolts. Results from these experiments will be presented including mortality data and pathology.

Conclusion: Norwegian *Tenacibaculum maritimum* isolates recovered from salmon and lumpfish causes tenacibaculosis ("donut syndrome") in lumpfish and are able to transfer from infected lumpfish to naïve Atlantic salmon smolts.

Keywords: lumpfish, donut syndrome, skin lesions, *Tenacibaculum maritimum*, disease

Funding: FHF Project number: 901433.

027-O

Variable *Piscirickettsia salmonis* shedding rates during infections in Pacific and Atlantic salmon

Jones, Simon (Canada)[1]; Long, Amy (Canada)[1]

1 - Fisheries and Oceans Canada

Introduction: Infection with the Gram-negative bacterium *Piscirickettsia salmonis* causes piscirickettsiosis (SRS) in farmed marine fish. In salmonids, although data from laboratory and epidemiological investigations suggest horizontal transmission of the bacterium, the timing and magnitude of transmission during the infection is not known. An isolate from British Columbia, Canada was used to compare susceptibility and bacterial shedding among species of Pacific and Atlantic salmon in controlled cohabitation trials.

Methodology: Susceptibility was assessed by cohabitation of injected and naïve salmon, held in duplicate tanks of seawater (SW). Minimum infectious doses were estimated by 60 min immersion of naïve salmon in known concentrations of bacterial suspensions prepared from colonies. Tissues from exposed fish were screened for the infection by qPCR. Individual and tank-level shedding rates were estimated from bacterial levels in SW by quantitative PCR (qPCR), adjusted to biomass and correlated to tissue burdens.

Results: Cumulative morbidity and/or mortality (MM) among injected pink and chum salmon reached 100% by approximately 30 days post-injection. Onset of MM among naïve pink salmon was 40 dpi, and reached 65% by the end of the trial at 60 dpi. In contrast, MM in naïve chum salmon was 40% by 65 dpi. In immersed pink salmon, there was no mortality over 30 days however the prevalence of hemorrhagic skin lesions increased from 9% to 94% with increasing exposure dose. Bacterial DNA was detected in SW coincident with MM in injected and naïve pink and chum salmon. At 10 dpi, data suggested negligible bacteria were shed from individual chum salmon, whereas at 15 dpi, individual shedding rates ranged from 0 to 0.6 genome equivalents ml/ g/ min.

Conclusion: We confirmed horizontal transmission of *P. salmonis* during cohabitation trials. Immersion in as few as 10^1 bacterial cells/ ml resulted in disease. Detection of bacterial DNA in SW suggested maximum shedding from injected fish occurred during the period of peak mortality. In contrast, the timing of shedding from naïve exposed salmon was variable and suggested the presence of high- and low-shedders in the affected population.

Keywords: salmon, piscirickettsiosis, susceptibility, shedding, cohabitation

Funding: Fisheries and Oceans Canada.

028-O

First pathological description of C*hlamydia* bacteria in Chilean salmonid farming

Suarez, Rudy (Chile)[1,2,3]; Godoy, Marcos (Chile)[1,2,4]; Kusch, Karina (Chile)[1,2]; Medina, Daniel (Chile)[1]

1 - Laboratorio de Biotecnología Aplicada, Facultad de Medicina Veterinaria, Universidad San Sebastián, Chile; 2 - Centro de Investigaciones Biológicas Aplicadas, Chile; 3 - Magister en Acuicultura, Universidad Católica del Norte, Coquimbo, Chile; 4 - Doctorado en Acuicultura, Programa Cooperativo Universidad de Chile, Universidad Católica del Norte, Antofagasta, Chile

Introduction: Epitheliocystis is an intracellular bacterial infection, caused by microorganism belonging to order Chlamydia. It has been associated with heavy mortality of farmed fish and reduced growth in the survivors. This kind of bacterial infection has been described in several freshwater and seawater fish species, being associated to the Proliferative Gil Disease (PGD) in Atlantic salmon (*Salmo salar*). This works describes the first description of epitheliocystis in Chilean salmonid farming.

Methodology: Fish samples were collected from 60 farming centers among 2010 to 2019. Fish tissue was removed for necropsy and organs were fixed in 10% formalin buffered for histopathological analysis. Also, gill apex was collected in 2% glutaraldehyde for ultrastructural transmission electron microscopy description (TEM) and organs samples were stored in RNA later to sequence 16S rDNA gene for bacteria taxonomic identification.

Results: No macroscopic changes or pathogenic evidences were observed in analyzed fishes. Histopathologically, intraplasmatic eosinophilic inclusion in epithelial cells were observed. TEM observation showed large cyst or branchial inclusions containing morphologically different bacterial stages. Also, 16S rDNA gene sequencing from apex gill tissue, showed the presence of bacteria belonging to order Chlamydia in fish collected from salmonid farms

Conclusion: No macroscopic clinical traits were observed in analyzed fishes. However, a high prevalence of epitheliocystis was associated to coho salmon (*O. kisutch*) in fresh and seawater farming. By contrast, a low prevalence of epitheliocystis were identified in Atlantic salmon (*S. salar*) in both fresh and seawater. Histopathological lesions and TEM study are consistent with bacterial Chlamydia infection. Also, 16S rDNA gene sequencing confirm the presence of bacteria belonging to order Chlamydia.

Keywords: Chlamydia, epitheliocystis, proliferative gill disease, bacterial disease, pathology

Funding: This work was funded by Universidad San Sebastian and by Centro de Investigaciones Biológicas Aplicadas (CIBA).

029-O*

New Zealand rickettsia-like organism: investigating the association of this bacteria with mortalities in farmed chinook salmon in New Zealand

Brosnahan, Cara (New Zealand)[1,2]; Jones, Brian (New Zealand)[3]; Davie, Peter (New Zealand)[2]; Munday, John (New Zealand)[2]

1 - Ministry for Primary Industries; 2 - Massey University; 3 - Murdoch University

Introduction: In April 2015 the intra-cellular bacteria New Zealand rickettsia-like organism (NZ-RLO) was detected for the first time in farmed chinook salmon, *Oncorhynchus tshawytscha.* These salmon were experiencing clinical signs of disease and mortalities of up to 70% during the summer months. This study presents the detection, phylogenetic analysis, distribution, and pathogenicity of NZ-RLO.

Methodology: Detection of NZ-RLO was carried out by histology, PCR and nucleotide sequencing. Evaluating the different strains of NZ-RLO present in New Zealand chinook salmon was conducted by nucleotide sequencing of the 16S rRNA gene and internal transcribed spacer region. Understanding the distribution of NZ-RLO within the farmed marine chinook salmon populations of New Zealand was carried out by qPCR. Pathogenicity of NZ-RLO in chinook salmon was assessed by subjecting salmon smolt to intraperitoneal injection of NZ-RLO with histology, qPCR, culture and in-situ hybridization used to confirm the presence of NZ-RLO in inoculated fish.

Results: Three strains of NZ-RLO were identified using the internal transcribed spacer region and 16S rRNA gene. These strains were specific to certain regions where chinook salmon are farmed. Two of these strains were associated with clinical signs of disease and found in areas where mortalities occurred. Infectivity trials were carried out and both strains were shown to cause clinical signs of disease and mortalities in chinook salmon smolt, however the two strains showed differences.

Conclusion: This study strongly suggests that certain NZ-RLO are involved in mortalities in farmed chinook salmon, however elevated temperatures are likely to have amplified the mortalities.

Keywords: Rickettsia-like, chinook salmon, pathogenicity, pathology

Parasitological Diseases II

031-O*

Duality of late glochidiosis: a gill parasitosis of salmon and key stage for freshwater mussel culture

Castrillo, Pedro Antonio (Spain)[1]; Ronza, Paolo (Spain)[1]; Varela, Catarina (Spain)[1]; Losada, Ana (Spain)[1]; Bermúdez, Roberto (Spain)[1]; Ondina, M. Paz (Spain)[1]; Quiroga, M. Isabel (Spain)[1]

1 - Universidade de Santiago de Compostela

Introduction: Most freshwater mussels play significant functions in ecosystems, nevertheless, species like *Margaritifera margaritifera* have declined dramatically, so *ex situ* conservation programmes have been implemented. Larvae of *M. margaritifera*, known as glochidia, need to parasitize the gills of salmonid fish for several months to metamorphose and detach as juvenile mussels when temperature raises. The host-parasite interaction in advanced stages of this long-term parasitism have received little attention. Particularly, no studies offered an integrated vision of the branchial morphopathological changes in relation to the development and detachment of juvenile mussels. The purpose of this study was to perform a comprehensive morphopathological evaluation of late glochidiosis.

Methodology: Atlantic salmon fry were exposed to *M. margaritifera* glochidia by bath immersion. At 202 days post-exposure (DPE) parasitized fish were submitted up to 17 ºC to synchronize the larval detachment and collection of viable juveniles around 225 DPE. Eight parasitized and control fish non-exposed to glochidia were collected and euthanized at 204, 221, 225, 232, 239 and 246 DPE. The gills were examined under the stereomicroscope and processed for light and electron microscopy.

Results: Until 225 DPE, glochidia were macroscopically observed as protruding white spots in the gill filaments, mostly located at the dorsal and ventral region of the holobranches, in clusters. Histologically a severe proliferative and inflammatory responses was seen, characterized by epithelial hyperplasia encircling the parasites and lymphohistiocytic infiltrate at the filamental core. Prior to detachment, the contact area between the gill and larvae became reduced to pedunculated structures. After 225 DPE, most lesions lacked parasites and consisted of a proliferative response with interlamellar cysts and goblet cells. A complete recovery of gill morphology generally occurred at 246 DPE. Throughout the study, the glochidial morphological development was recorded, particularly of the pedal and branchial organs, associated to the juvenile metamorphosis.

Conclusion: This study provides information about the pathogenesis of late glochidiosis, contributing to understand the gill defense and recovery mechanisms. Moreover, the evaluation of the larval morphology might help to optimize this critical stage in freshwater mussel *ex situ* conservation.

Keywords: freshwater mussel, gill histopathology, Atlantic salmon, *Margaritifera margaritifera*

Funding: Fundación Biodiversidad (MarMaCul), Xunta de Galicia and governmental FPU predoctoral contract.

032-O

Infestation and infection dynamics of tapeworms (*Eubothrium* sp.) in farmed Atlantic salmon

Hansen, Haakon (Norway)[1]; Karlsbakk, Egil (Norway)[2]; Ruud, Kristian (Norway)[2]; Sakariassen, Trine (Norway)[3]; Darrud, Mari (Norway)[1]; Sevatdal, Sigmund (Norway)[4]

1 - Norwegian Veterinary Institute, Fish Health Research Group; 2 - University of Bergen, Norway, Fish Disease Research Group; 3 - University of Tromsø, Department of Arctic and Marine Biology; 4 - VESO, Oslo

Introduction: The cestode *Eubothrium* sp. infects salmonids in seawater and is common in both wild and farmed fish in Norway. The life cycle consists of one intermediate host; a planktonic crustacean and the fish final host were the parasite reaches fertility. Infestations with tapeworms (*Eubothrium* sp.) are an increasing problem in farmed salmon in several areas, especially in the mid and southwestern part of Norway, but seems to be absent in farmed fish in the north. Infected farmed fish are treated with praziquantel (prazinoisoquinolin, dose: 10 mg/ kg biomass). There are several reports of reduced effect, which might be due to development of resistance. The use of praziquantel increased from 2010 to 2015, then the use declined. This is probably not due to fewer incidences, but medical treatment is expensive and involves a cumbersome application procedure. The parasite also seems to spread to new areas. Knowledge of the parasite biology and the impact of these cestodes on the health and growth of farmed salmon is sparse.

Methodology: To study the infestation and infection dynamics of *Eubothrium* sp. in farmed salmon, a total of 13 cohorts of Atlantic salmon of different sizes was examined; 7 cohorts stocked in sea in autumn 2017 and 6 in spring 2018. Samples of stomach and intestines from 30 fish at each sample point, together with length and weight of the fish, were taken at each sampling; the first sample from fresh water, then after 1 month at sea followed by new samples every third month. All samples were examined for the presence of all development stages of *Eubothrium* sp.

Results: The infestation and infection dynamics from selected cohorts with focus on stocking time, geographic distribution, infection period, fish size, and infection dynamics throughout the production cycle will be presented.

Keywords: cestode, tapeworm, cohort study, geographic distribution

Funding: The Norwegian Seafood Research Fund (FHF), project no 901449.

Diseases of Public Concern

033-O

Risk mapping of zoonotic parasites in Italian aquaculture

Gustinelli, Andrea (Italy)[1]; Caffara, Monica (Italy)[1]; Bisanti, Davide (Italy)[1]; Fioravanti, Maria Letizia (Italy)[1]

1 - Department of Veterinary Medical Sciences, Alma Mater Studiorum Università di Bologna, Italy

Introduction: The diffusion of new eating habits and the increase of fish products demand lead to a raising risk for consumers inherent food-borne parasitic zoonoses. In this scenario the scientific community and food security authorities are called to assess the possible risks linked to consumption of products from fishery and aquaculture and to set up strategies aimed to their management. Thus, the collection of epidemiological information on the presence of zoonotic parasites in fish products through extensive epidemiological surveys is necessary. Concerning aquaculture, according to EFSA (2010) and Regulation (EU) 1276/2011 only for the Atlantic salmon the risk of transmission of parasites to man can be considered negligible. At this purpose, in the framework of the EU funded project ParaFishControl a wide parasitological survey has been carried out on the main farmed fish species in Italy and other countries such as Spain, Greece, Denmark, Norway and Hungary. The present work reports the results from Italy.

Methodology: From 2016 to 2018 a total of 4728 farmed fish have been examined from 5 marine and 5 freshwater farms located in Italy: 1563 gilthead sea bream (GSB), 1571 European sea bass (ESB) and 1594 rainbow trout (RT) have been sampled. Besides harvest quality fish, runts were also examined. Parasitological analyses to search for anisakid nematodes, diphyllobothriid cestodes and Opisthorchioidea digeneans were performed utilizing different methodologies such as visual inspection and candling as provided by the EU regulation, implemented by UV-press method, muscular compression/artificial digestion followed by microscopic examination when required.

Results: No zoonotic parasites were found in any of the examined fish, including runts. Only one L4 specimen of the nematode *Hysterothylacium fabri* has been found on the surface of the liver in one ESB.

Conclusion: The results of this survey are very encouraging and allow to assess the risk of the presence of zoonotic parasites in farmed GSB, ESB and RT as negligible, similarly to Atlantic salmon.

Keywords: zoonotic parasites, gilthead sea bream, European sea bass, rainbow trout

Funding: ParaFishControl H2020 project (634429).

034-O

Diphyllobothriasis in Latin America, with special reference to Brazil: integrative analysis

Sampaio, Marrara (Brazil)[1]; Pavanelli, Gilberto (Brazil)[2]; Oliveira, Sueleen (Brazil)[3]

1 - Universidade Federal Fluminense; 2 - UNICESUMAR, Promoção da Saúde, ICETI, Instituto Cesumar de Ciência, Tecnologia e Inovação, Maringá, PR; 3 - Fundação Oswaldo Cruz, Rio de Janeiro, Brasil

Introduction: Diphyllobotriasis is a human parasite condition caused by cestoda of the genus *Diphyllobothrium*. In massive infections, the helminths may cause intestine obstruction, deep anemia, fatigue and mental confusion. Other symptoms may include abdominal discomfiture, nauseas, vomits, diarrhea, loss of appetite and bellyaches. It is a public health pathology with a great number of asymptomatic cases. Patients may eliminate eggs when not treated and may spread them in the water when basic sanitary conditions are lacking. The genus *Diphyllobothrium* comprises 50 species of which 12 have been detected in humans. The most prevalence species is *Diphyllobothrium latum,* with wide geographic distribution, especially in the Americas, Asia and Europe. Its transmission to humans occurs when raw or badly cooked fish, with the parasite´s infecting forms, are ingested. In several South American countries, including Brazil, there has been an increase in cases of diophyllobothriasis due to the common consumption of raw fish derived from Oriental cuisine, such as sushi, or even local titbits such as ceviche, a typical food from Peru, Chile and Argentina.

Methodology: Methodology involving integrative review of the literature in current study aims at detecting cases of diphyllobotriasis in Latin America, employing BIREME, LILACS, PubMed and SciELO databases.

Results: Two hundred and eight cases of diphyllobothriasis were detected in five Latin American countries, namely, Argentina, Brasil, Chile, Cuba and México. Low number of cases is perhaps due to non-mandatory notification, lack of information by health professional and absence of symptoms in most cases. The number of occurrences may increase due to export-import transactions among countries and the intake of raw fish by the population.

Conclusion: So that the number of cases with parasite conditions may decrease, the ingestion of cooked or fried fish is recommended or freezing up to -20 ºC for 7 days.

Keywords: diphyllobothriasis, fishes, zoonosis, Latin America

035-O*

Assessing zoonotic potential and viability of cythocotylid muscle metacercariae found in an common carp (*Cyprinus carpio*) aquaculture in Hungary

Sándor, Diána (Hungary)[1]; Gyöngy, Martina (Hungary)[2]; Nyeste, Krisztián (Hungary)[2]; Czeglédi, István (Hungary)[3]; Székely, Csaba (Hungary)[1]; Cech, Gabor (Hungary)[1]

1 - Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary; 2 - Department of Hydrobiology, University of Debrecen, Hungary; 3 - Hungarian Academy of Sciences, Centre for Ecological Research, Balaton Limnological Institute, Tihany, Hungary

Introduction: During an extensive carp monitoring for zoonotic trematodes in Hungary (ParaFishControl project) one of the aquacultures was heavily infected by muscle metacercariae. Morphological and molecular analyses were carried out for taxonomical identification. The evaluation of the food safety required to determine the viability and zoonotic ability of these muscle parasites.

Methodology: Infected fish were anaesthetised and decapitated than their musculature was digested in pepsin solution to free the metacercariae. 15 specimens were selected to investigate their morphology, furthermore 5 individuals were preserved for sequence analysis (ITS region). Zoonotic risk was estimated by infection experiments of rodents (mice and hamsters) where the animals were fed with 50 – 50 muscle metacercariae *per os* while 2 mice and 2 hamsters were used as negative control. As a positive control *Metagonimus* sp. metacercariae were used as a well-known zoonotic genus. After a week the rodents were killed by CO_2 and their intestines were under a Zeiss stereo microscope for trematode infections.

Results: A viability experiment was conducted where 10 isolated cysts were placed between two muscle pieces then treated by various temperatures (–18 °C, 20 °C, 40 °C, 60 °C) and chemical reagents (5% and 10% acetic acid, 10% NaCl solution). The “fillet sandwiches” were checked for alive or dead metacercariae in various time intervals.

Conclusion: According to our morphological and molecular results these metacercariae belong to the Cyathocotylidae trematode family but species identification was not possible. Adult flukes did not develope in mammals therefore their zoonotic potential does not seem probable, which is in agreement with the lack of history in the scientific literature. However, the *Metagonimus* sp. metacercariae used as positive control were found in the intestines of rodents in large number. All physical and chemical treatments in the viability experiment were effective in a relatively short time compared to the control. The least effective was the incubation under 20 °C which eliminated the parasites after 12 hours while 10% acetic acid as the most aggressive treatment killed instantly the flukes.

Keywords: common carp, digenea, viability, zoonosis

Funding: ParaFishControl Horizon 2020 (634429) and ÚNKP-18-3 New National Excellence Program of the Ministry of Human Capacities.

036-O

VIVALDI project

This workshop aims at crossing experiences from the VIVALDI project with research conducted overseas by members of the VIVALDI expert advisory panel. What can be done to detect the emergence of diseases as early as possible? How can we anticipate on these diseases? Combining microscopy, new sequencing tools and environmental approaches can bring new perspectives and will be discussed by the participants to the workshop.

This project has received funding from the EU's Horizon 2020 Research and innovation programme under grant agreement N° 678589

037-O

Open workshop: Bottlenecks in diagnostics of Mediterranean fish diseases

Zrnčić, Snježana (Croatia)[1]; Fioravanti, Marialetizia (Italy)[2]; Gustinelli, Andrea (Italy)[2]; Padros, Francesc (Spain)[3]; Palenzuela, Oswaldo (Spain)[4]; Brun, Edgar (Norway)[5]

1 - Croatian Veterinary Institute, Zagreb; 2 - Department of Veterinary Medical Sciences, Universita di Bologna; 3 - Fish Diseases Diagnostic Service, Universitat Autonoma de Barcelona, Catalonia; 4 - Instituto de Acuicultura Torre de la Sal, Ribera de Cabanes; 5 - Norwegian Veterinary Institute, Oslo

The goal of two EU funded projects in the frame of Horizon 2020, namely MedAID and PerformFISH, is to increase the overall competitiveness and sustainability of the Mediterranean marine fish-farming sector, throughout the whole value chain. An important aspect of both projects is managing transmissible diseases of farmed fish. Another H2020 project ParaFishControl, aiming to improve understanding of fish-parasite interaction and developing innovative solutions and tools for the prevention, control and mitigation of farmed fish parasitic diseases, interacts with these two projects in the field of the parasitic diseases. The projects are actively cooperating in all possible aspects and the workshop will cover one of the mutual interests of these three EU H2020 projects: diagnostics. PerformFISH is working on a deliverable on diagnostic methods and MedAID and ParaFishControl have prepared Diagnostic Manuals for certain diseases. Still, further efforts for the validation of diagnostic methods of infectious diseases in Mediterranean aquaculture are needed. In addition, harmonization of recommended procedures is necessary for the generation of meaningful field data at all levels, and the scope of diagnostic manuals is to standardize these procedures as much as possible.

Workshop program:

1. Presentations:
   - Diagnostic methods (PerformFISH and MedAID)
   - Diagnostic manual on viral and bacterial diseases of seabass and seabream (MedAID)
   - Diagnostic manual on parasitic diseases of European aquacultured fish. (ParaFishControl)
   - Diagnostic capacities in the Mediterranean basin (PerformFISH and MedAID)

2. Working group discussion with the following topics:
   - VNN
   - *Vibrio* and *Tenacibaculum*
   - Fastidious and intracellular bacteria, Red Rash Syndrome
   - Identification and assessment of intestinal parasites and ectoparasites

   Each group should discuss challenges in implementing standardized procedures and the bottlenecks in diagnostics of the mentioned pathogens and prepare a summary from the discussion for presentation in plenary. The discussion will be moderated by experts of MedAID, PerformFISH and ParaFishControl.

3. The final plenary session will summarize bottlenecks, discuss the way forward to overcome them and try to make conclusions. The summary of the discussions and conclusions is planned to be written in a joint publication for the EAFP Bulletin.

Keywords: Mediterranean aquaculture, diseases, diagnostics

Aquatic Animal Epidemiology

038-O

Spatiotemporal mortality patterns in Norwegian salmonid aquaculture

Bang Jensen, Britt (Norway)[1]; Toft, Nils (Denmark)[2]; Qviller, Lars (Norway)[1]

1 - Norwegian Veterinary Institute; 2 - IQinAbox

Introduction: In an ethical, sustainable production of animals, monitoring and minimizing mortality must be a top-priority, especially considering that mortality is an indicator of sub-optimal welfare of animals. In 2018, the average mortality in Norwegian sea farms cultivating Atlantic salmon was 14.7%, and 16.6% in farms cultivating rainbow trout. In total, 49 million farmed salmonids were reported to have died during production. Meanwhile, there is a lack of consensus of how these mortalities should be calculated and presented. We therefore set out to propose a logical, transparent way of calculating mortality. And to present the calculated differences between counties and years for a five-year production period.

Methodology: Since 2002, each Norwegian sea farm has been obliged to report production data to the Directorate of Fisheries every month. The data holds information about fish farm location, number and biomass of fish at the farm at any given month, and several categories of loss, including mortality. We have used the reported mortality data from 2014-2018, with the following assumptions: For the number of fish at a location, the number reported for a month constitutes the current number of fish, i.e. fish present at the end of the month. For the number of dead fish, the number of fish reported constitutes the number of fish that died during the month. The mortality rate (M_{rate}) for a month (i) is calculated as: # fish dead in month i/((# fish alive at the end of month i-1+# fish alive at the end of month i))/2. The denominator calculates the average number of fish alive during the month, by assuming that fish die, or are added/ removed uniformly throughout the month.

Results: There are relatively large differences in mortality across the different counties and between the years. The most population-dense areas are also the ones with highest mortality. The monthly mortality risk varies, but shows distinct seasonal patterns

Conclusion: Understanding the drivers for the differences in mortality is the first step towards managing mortality on a national scale.

Keywords: mortality, salmonid, aquaculture

039-O*

Development of method for detection of salmonid alphavirus (SAV) from seawater

Bernhardt, Lisa-Victoria (Norway)[1]; Myrmel, Mette (Norway)[2]; Weli, Simon (Norway)[1]; Qviller, Lars (Norway)[1]; Lillehaug, Atle (Norway)[1]

1 - Norwegian Veterinary Institute; 2 - Norwegian University of Life Sciences

Introduction: Virus-related diseases are among the major causes of the high mortality rates in the marine phase of the farmed Atlantic salmon (*Salmo salar* L.). Virus surveillance therefore constitutes an important strategy for controlling the spread of the disease agent. Pancreas Disease (PD) caused by Salmonid alphavirus (SAV), has a negative impact on fish welfare and the economy in salmonid aquaculture. Infected fish shed SAV into the water, thus seawater from the fish environment could be used for surveillance of the presence of SAV in fish populations. However, currently, in Norway, the prevalence of SAV is being mapped using intensive (monthly) sampling of organ tissue from fish.

Methodology: A six-week cohabitant challenge trial was performed using post-smolt Atlantic salmon inoculated with two different doses of SAV subtype 3 (SAV3). Seawater samples were collected together with organ tissue samples from cohabitant fish at a total of sixteen sampling time points. At each sampling, one litre of seawater was concentrated for SAV3 analysis by adsorption to an electro charged membrane filter, and elution with a buffer. Optimization of the method was done by using differently charged filters (either positive or negative) in combination with different elution buffers. Detection and quantification of the virus were performed with the use of reverse transcription quantitative PCR (RT-qPCR). Based on the above-mentioned, a similar procedure was applied in the field at a farm with Atlantic salmon diagnosed with PD and several seawater samples were collected at different water depths, both inside and outside an open seawater net pen.

Results: In the challenge trial, both seawater and cohabitant fish from low and high dose tanks were positive for SAV3, by RT-qPCR. External clinical signs, gross pathology and histopathology, associated with PD, were observed in cohabitant fish. Using these results, a relationship was established between SAV3-infected cohabitant fish and the recovery of SAV3 from seawater. Additionally, a majority of the seawater samples collected from the field were positive for SAV, by RT-qPCR.

Conclusion: Filtration of seawater has the potential to be used as a surveillance method for presence of SAV at Atlantic salmon farms.

Keywords: SAV, PD, virology, metagenomics, biosecurity

040-O*

Evolution of the piscine orthoreovirus genome linked to emergence of heart and skeletal muscle inflammation in farmed Atlantic salmon

Dhamotharan, Kannimuthu (Norway)[1]; Tengs, Torstein (Norway)[2]; Wessel, Øystein (Norway)[1]; Braaen, Stine (Norway)[1]; Nyman, Ingvild B. (Norway)[1]; Hansen, Elisabeth F. (Norway)[1]; Christiansen, Debes H. (Norway)[3]; Dahle, Maria K. (Norway)[4]; Rimstad, Espen (Norway)[1]; Markussen, Turhan (Norway)[5]

1 - Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway; 2 - Faculty of Chemistry, Norwegian University of Life Sciences, Ås, Norway; 3 - Faroese Food and Veterinary Authority, National Reference Laboratory for Fish Diseases, Tórshavn, Faroe Islands; 4 - Department of Immunology, Norwegian Veterinary Institute, Oslo, Norway; 5 - Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway

Introduction: Heart and skeletal muscle inflammation (HSMI) in farmed Atlantic salmon (*Salmo salar*) was first diagnosed in Norway in 1999. The disease is caused by *Piscine orthoreovirus*-1 (PRV-1). The virus is prevalent in farmed Atlantic salmon, but not always associated with disease.

Methodology: Phylogeny and sequence analyses of 31 PRV-1 genomes collected over a 30-year period from fish with or without HSMI, including a strain sampled in Norway in 1988, was performed.

Results: The viral sequences grouped into two main monophylogenetic clusters, one associated with HSMI and the other with low virulent PRV-1 isolates. The PRV-1 strain from Norway sampled in 1988, a decade before the emergence of HSMI, grouped with the low virulent HSMI cluster. The two distinct monophylogenetic clusters were particularly evident for segments S1 and M2. Only a limited number of amino acids were unique to the association with HSMI, and they all located to S1 and M2 encoded proteins.

Conclusion: The observed co-evolution of the S1-M2 pair coincided in time with the emergence of HSMI in Norway, and may have evolved through accumulation of mutations and/or segment reassortment. Sequences of S1-M2 suggest selection of the HSMI associated pair, and that this segment pair have remained almost unchanged in Norwegian salmon aquaculture since 1997. PRV-1 strains from the North American Pacific Coast and Faroe Islands have not undergone this evolution, and are more closely related to the PRV-1 precursor strains not associated with clinical HSMI.

Keywords: piscine orthoreovirus, PRV-1, HSMI, reassortment, virulence

Funding: The Research Council of Norway grant #237315/E40 and #235788, and the Indian Council of Agricultural Research (ICAR) through the ICAR International Fellowship for PhD.

041-O

Piscine myocarditis virus (PMCV) in Atlantic salmon: prevalence in fry and broodstock material and complications in relation to mechanical delousing

Petersen, Petra E. (Faroe Islands)[1]; Christiansen, Debes H. (Faroe Islands)[1]; Dahl, Maria M. (Faroe Islands)[1]; Dam, Kristian M. (Faroe Islands)[2]; Eysturskarð, Jonhard (Faroe Islands)[2]; Østergaard, Peter S. (Faroe Islands)[3]; Strøm, Andras (Faroe Islands)[4]; Nolsøe, Marner (Faroe Islands)[2]; Wilhelm, Marjun (Faroe Islands)[5]; Vang, Gudny (Faroe Islands)[6]; Garseth, Åse H. (Norway)[7]; Jensen, Britt B. (Norway)[7]

1 - Faroese Food and Veterinary Authority, Tórshavn, Faroe Islands; 2 - Bakkafrost, Glyvrar, Faroe Islands; 3 - Aquamed, Tórshavn, Faroe Islands; 4 - Private Veterinarian, Kvívík, Faroe Islands; 5 - Hiddenfjord, Sandavágur, Faroe Islands; 6 - Mowi, Oyndarfjørður, Faroe Islands; 7 - Norwegian Veterinary Institute, Oslo, Norway

Introduction: Cardiomyopathy syndrome (CMS), caused by the piscine myocarditis virus (PMCV), causes severe financial losses to the salmon farming industry, partially through complications related to mechanical delousing treatments. Main route of transmission is horizontal, though recent findings indicate vertical transmission. We evaluated potential vertical transmission by screening broodstock material as well as early startfeeding fry of various origin and the potential risk for PMCV development in thermal delousing treatments.

Methodology: PMCV screenings were accomplished using real-time PCR and selected results were evaluated by sequencing. Screening brood fish material and fry from all Faroese hatcheries is ongoing. PMCV development in thermal delousing was evaluated by PMCV screenings in selected net-pens at a marine farm undergoing optilicer treatment.

Results: PMCV prevalence in internal organs of mature brood fish was 36% and prevalence in ovarian fluid and milt 97% and 80%, respectively. All fertilised eggs investigated until now (N=767) have been negative for PMCV; however, in early first-feeding fry, a prevalence of 32% was detected. As Ct-values were close to the limit of detection, sequencing was used to evaluate the results. Subsequent samplings of the same batch of fry showed zero prevalence and, until now, screenings of fry from freshwater smolt stations have only revealed sporadic detections. Immediately following optilicer treatment, increased mortalities were observed in CMS units; day one mortalities were 0.24% for a CMS absence unit, 0.82% for a low CMS unit and 1.61 and 0.85 for two high CMS units. Development of infection was most distinct in the low CMS unit, where prevalence increased from 30% pre-treatment to 80% one week post-treatment. In the high CMS units, prevalence remained close to 100%, whereas prevalence in an untreated reference unit remained at zero.

Conclusion: Our results show that thermal delousing treatments pose a risk factor for the development of PMCV, thus emphasising the importance of close surveillance by fish health personnel and concordant biosecurity measures. Further, the results support recent indications of vertical transmission of PMCV and, to our knowledge, provide the first sequencing results of PMCV in early fry stages.

Keywords: PMCV, CMS, transmission, mechanical delousing

Funding: Faroese Research Council, Bakkafrost, Mowi and Hiddenfjord.

042-O

Risk factors for clinical outbreaks of cardiomyopathy syndrome (CMS) in Atlantic salmon in Norway

Bang Jensen, Britt (Norway)[1]; Mårtensson, Arthur (Norway)[1]; Kristoffersen, Anja B (Norway)[1]

1 - Norwegian Veterinary Institute

Introduction: Cardiomyopathy syndrome (CMS), is a severe cardiac disease of Atlantic salmon with a significant economic impact at both company and industry level in Norway. The disease is also found in Ireland, Scotland, and in the Faroe Islands. Estimated costs of CMS for the industry was approximately 78 mil. € in 2015. Control is based on mitigation of risk factors, since no treatment or vaccine is available. The objective of this study was to identify risk factors leading to the development of CMS.

Methodology: For this study, we collected data from one major salmon producing company. The data consisted of daily registrations on number of fish, average weight, mortality, feed, temperature, treatments, handling and movements. The mortality was divided into cause-specific categories (ie. "CMS", "wounds", "predator" etc.). The dataset included all fish groups that were stocked between spring 2012 and fall 2014, totalling 1536 fish groups in 118 farms. We constructed a model for describing the daily probability of outbreak of CMS in each fish group. The model was then run with one proposed risk factor at the time, in order to determine which factors should be included in a final multivariable model

Results: Spring smolt had significantly lower risk of developing CMS than autumn smolt. Fish grups that had previously experienced an outbreak of heart- and skeletal muscle inflammation (HSMI) or Pancreas disease (PD) had a significantly higher risk of developing CMS. The length of time at sea was also a significant risk factor. In addition, we found that fish groups from some smolt suppliers had a higher risk than from others.

Conclusion: The findings provides the producers with some options for controlling disease. For example, the link to smolt supplier suggests that the producers should be careful when selecting supplier, and perhaps start screening the smolt that is to be put into production for CMS-virus.

Keywords: CMS, risk factors, Atlantic salmon

Funding: FHF-project 901118.

043-O

No evidence of vertical transmission of ISAV-HPR0 from Atlantic salmon brood fish to offspring's in Faroese aquaculture

Christiansen, Debes H. (Faroe Islands)[1]; Petersen, Petra E. (Faroe Islands)[1]; Dahl, Maria M (Faroe Islands)[1]; Falk, Knut (Norway)[2]

1 - Faroese Food and Veterinary Authority; 2 - Norwegian Veterinary Institute

Introduction: The non-virulent subtype of infectious salmon anaemia virus, ISAV-HPR0, is the progenitor and reservoir for all virulent ISAV and thus represent a risk factor for the emergence of infectious salmon anaemia (ISA). HPR0 has been detected in all three production compartments of Atlantic salmon (*Salmo salar* L.) farming in the Faroe Islands, i.e. the land-based brood fish farm, freshwater smolt farms and marine production farms. Understanding its transmission pathways is important for proper management and mitigation strategies particularly related to large smolt production in RAS. The purpose of this study was (1) to get a better understanding of the infection routes of HPR0 in the three production compartments and (2) to elucidate if HPR0 establishes "house strains" in the RAS farms.

Methodology: The study period was from 2007 to 2016 including 1 brood fish farm, 6 freshwater smolt farms, and 24 marine production farms. Gills from Atlantic salmon were screened for the presence of ISAV by real-time RT-PCR. For phylogenetic analysis the hemagglutinin esterase (HE) gene was sequenced as outlined before Christiansen *et al*. 2017.

Results: During the 10-year study period 58506 salmon from the three compartments were screened for the presence of ISAV. Overall, 10% of the salmon were tested ISAV positive; 44% brood fish, 9% in smolt farms and 8% in marine farms. Phylogenetics of more than 250 HPR0 isolates showed that HPR0 is geographically structured. Whereas the two highly significantly different HPR0 subtypes EU-G2 and EU-NA co-circulate in the marine environment brood fish were infected with EU-G2 and smolts were infected with EU-NA. A longitudinal phylogenetic analysis of HPR0 from the 6 smolt farms demonstrated the presence of identical HPR0 strains as well as periodically new HPR0 strains.

Discussion: Our results demonstrate that HPR0 was not transmitted vertically from Atlantic salmon brood fish to their offspring's in Faroese aquaculture. Furthermore, we observed both HPR0 "house strains" and periodically introduction of new HPR0 strains into the smolt farms. Consequently, the strategy of producing large smolt could increase the risk of ISA in RAS. Production of smolt in closed-containment systems could minimize this risk.

Keywords: ISAV-HPR0, vertical transmission, HPR0 house strain

044-O

A web-based application for simulating the spread of pancreas disease after introduction in a naïve population

Qviller, Lars (Norway)[1]; Kristoffersen, Anja B (Norway)[1]; Aldrin, Magne (Norway)[2]; Bang Jensen, Britt (Norway)[1]

1 - Norwegian Veterinary Institute; 2 - Norwegian Computing Center

Introduction: Pancreas Disease (PD) is a viral disease caused by Salmonid Alphavirus (SAV). It affects 140-170 marine farms in Norway every year, and is also present in Ireland and Scotland. In Norway, PD has been notifiable since 2008. However, the disease continues to spread, and cause substantial economic losses. The aquaculture industry is growing continuously, introducing farms in new geographical areas and fish are moved between hydrogeographically divided zones for trade and slaughter. All such movements need to be approved by the competent authorities. To offer support to farmers and competent authorities when making decisions on disease management, we are building an application that can simulate the spread of PD introduced into an area where the disease is not already present. The end goal is that equip the stakeholders with a user friendly application for outbreak simulation in real-time.

Methodology: The application is based on a stochastic model for disease transmission, which utilizes data on outbreaks of PD from 2008 to 2014, and data on production from the same period. These data include geographic location of farms, and monthly data on number of fish on farm and weight. Seaway distances between each farm to every other farm in Norway has been calculated and included. The model takes into account that there are two separate epidemics of PD, caused by two separate genotypes of SAV.

Results: The model has been tested on real life data, and fitted so it describes the disease transmission well. Within the web-based application, simulations of outbreaks are demonstrated by showing the calculated risk of transmission of SAV to all farms within a 100 km radius, if SAV is introduced into a previously naïve population. This is visualized by different colors, purveying the risk of transmitting disease.

Conclusion: The Norwegian salmonid production system is exceptionally well suited for host – pathogen infection dynamics modeling because of the body of surveillance data that documents both the host population at risk of infection and pathogen/disease occurrences and distribution. The web-based application provides a transparent and intuitive tool for stakeholders and farmers to make knowledge-based decisions on disease management.

Keywords: pancreas disease, epidemiology, disease transmission, salmonid alphavirus

045-O

Longitudinal survey of *Flavobacterium* species in Icelandic fish farms

Einarsdottir, Thorbjorg (Iceland)[1]; Guttormsdottir, Gudbjorg (Iceland)[2]; Connaghan, Daniel (United Kingdom)[3]; Hjartardottir, Sigridur (Iceland)[1]

1 - Institute for Experimental Pathology, University of Iceland, Keldur, 112-Reykjavik, Iceland; 2 - Alvotech, Reykjavik, Iceland; 3 - The Ecology Consultancy, London, UK

Introduction: *Flavobacterium* species cause significant disease in salmonid farming worldwide. The etiological agent of Bacterial Cold Water Disease (BCWD), *Flavobacterium psychrophilum*, causes symptoms such as mortality in sac fry and necrosis and ulceration in fingerlings and fry. In this study, we examined *Flavobacterium* species from Icelandic aquaculture farms and a hatchery over the course of 3 years, in order to characterize the bacteria causing disease and explore transmission routes, as well as identify possible vaccine candidates.

Methodology: Bacteria were isolated from diseased Atlantic salmon (*Salmo salar*) and Arctic char (*Salvelinus alpinus*) from 5 Icelandic aquaculture farms and one hatchery. The 16S rRNA gene and multilocus sequence type genes were sequenced in order to identify the bacteria and examine variability between isolates.

Results: We found several distinct groups of flavobacteria, some of which were homogeneous and appeared to persist between years, while others were heterogeneous and transient. *F. psychrophilum* was isolated from diseased Arctic char from all fish farms, but not from Atlantic salmon, roe or water samples, where other *Flavobacterium* groups were isolated. Little variability was observed in *F. psychrophilum* between farms and over the course of time.

Conclusion: The results indicate that there may have been a limited number of introductions of *F. psychrophilum* into Icelandic fish farms. The data reveals a complex *Flavobacterium* flora in fish farms and underscores the importance of discriminating between persisting and transient *F. psychrophilum* and closely related bacterial species.

Keywords: *Flavobacterium psychrophilum*, bacterial cold water disease (BCWD)

Funding: AVS R&D Fund by the Ministry of Fisheries and Agriculture in Iceland (grant R 14 007-14).

250-O*

Fungal pathogens causing systemic mycosis in *Onchorhynchus mykiss* and *Cyprinis carpio communis*; pathogenecity characterization by SEIR modeling and histopathological approach

Magray, Aqib R (India)[1]; Ganai, Bashir A (India)[1]; Ahmad, Fayaz (India)[2]; Hafiz, Sabira (India)[1]; Mubarak, Saqib (India)[3]

1 - Centre of Research for Development, University of Kashmir, Srinagar, India 190006; 2 - Department of Zoology, University of Kashmir, J & K, India; 3 - Department of Mathematics, University of Kashmir, Srinagar, India

Introduction: Fungal infections are reeling as one on the main threats to diverse fish fauna and ecosystem health. Fishes are susceptible to most of the opportunistic fungi, when stressed or immuno-compromised because of environmental atrocities, or when concurrently infected with bacterial or viral infections. Fungal pathogens evolved as unadorned saprotrophic or biotrophic parasite of fish resulting in major losses in aquaculture economy and aquatic ecosystems. The fish fungal pathogens (*Saprolegnia*) belonging to heterokonts group, are causing saprolegniosis. Further, Fusarium and Mucor species received considerable attention among all the fungal pathogens of fish as they are known to cause systemic mycosis.

Methodology: In the present study, fungal pathogens *Saprolegnia delica*, *Fusarium aveneacium* and *Mucor hiemalis* which were previously isolated from naturally infected rainbow trout fish was used for experimental transmission in *Onchorhynchus mykiss* and *Cyprinis carpio communis*. Further, for the pathogenecity characterization Susceptible Exposed Infective Recovery (Immune)-SEIR model along with histopathological approach was employed.

Results: Our results revealed that the zoospores, cysts and mycelia were initial pathogenic components of *Saprolegnia delica*, and spores and mycelium of *Fusarium aveneacium* and *Mucor hiemalis* were developing initial infection involved in the disease progression. Histopathological approach used to analyze host organs predicted that *Saprolegnia delica*, *Fusarium aveneacium* once establish its colony on the host manage to cause systemic mycosis and takes maximum organs into its toll. Further, our data approximately matches the solution of our predicted mathematical model for fish pathogencity and survival.

Conclusion: The fungal pathogens belonging to three different diverse classes were observed to cause systemic mycosis with distinctive potential and pathogencity. The infective components were varying among the pathogens but the disease causing potential were mainly dependent upon route of infection and rate of infectivity taking multiple organs. Further, the survivorship and mortality of host predicted were satisfied by the results of our experimental model for all the distinctive pathogens.

Keywords: systemic mycosis, SEIR, histopathology, fungal pathogens, pathogencity

Funding: Council of Scientific and Industrial Research Board, India; Junior Research Fellowship (Sanction Letter No. 09/251/(0110)/2018-EMR-I).

WS: Biosecurity I

046-O

Developing a Biorisk assessment system for aquatic pathogens

Úna McCarthy[1]

1 - Marine Scotland Science, UK

There is not a unified system to classify aquatic pathogens in terms of biorisk. One of the consequences is that aquatic organisms are classified for shipping using the criteria developed for terrestrial animal pathogens. Under the Hazardous Materials Regulations[1], *Vibrio splendidus* requires shipping as a UN2900, Category A Infectious Substance – using the same stringency as for Peste des petits ruminants virus or Foot and mouth disease virus. This results in restricted and expensive shipping which may not be necessary and could hamper research activities.

The aim of this workshop is not to classify aquatic pathogens. Rather it is to agree criteria under which aquatic microorganisms can be assessed and scored, such as survival time under different environmental conditions, infectious dose, susceptible species, background levels in the aquatic environment, etc. In this way, a benchmark is established against which to test and rank existing or newly discovered aquatic microorganisms. It is anticipated that in the future, this could provide the basis for developing a biorisk classification framework which is applicable to all aquatic microorganisms.

From this workshop, suggestions for prioritising areas for research and future collaboration will be generated. In fact the information gathered for identifying such listing criteria would assist researchers and research managers in focusing their activities on aspects where a significant knowledge gap has been highlighted.

In order to facilitate the discussions, preliminary questionnaires will be distributed several weeks in advance of the workshop to assist attendees in the preparation of pertinent background information.

[1] U.S. Department of Transportation's (DOT's) Hazardous Materials Regulations (HMR; 49 CFR Parts 171-180)

Viruses and Viral Diseases I

047-O

RGNNV capsid protein amino acids 247 and 270 are involved in betanodavirus virulence to European sea bass (*Dicentrarchus labrax*)

Moreno, Patricia (Spain)[1]; Souto, Sandra (Spain)[2]; Leiva-Rebollo, Rocio (Spain)[1]; Garcia-Rosado, Esther (Spain)[1]; Borrego, Juan Jose (Spain)[1]; Bandin, Isabel (Spain)[2]; Alonso, M. Carmen (Spain)[1]

1 - Universidad de Málaga, Departamento de Microbiología, Facultad de Ciencias; 2 - Instituto de Acuicultura, Universidade de Santiago de Compostela, A Coruña

Introduction: European sea bass is severely affected by nervous necrosis disease, caused by nervous necrosis virus (NNV, *Betanodavirus* genus). The genome of this virus is composed of two single-stranded, positive-sense segments: RNA1 (viral polymerase) and RNA2 (capsid protein, CP). Two out of the four betanodavirus genotypes (RGNNV and SJNNV) have been detected in sea bass, although showing different levels of virulence to this fish species. Thus, sea bass is highly susceptible to RGNNV, whereas outbreaks caused by SJNNV have not been reported in this fish species. In the present work, we evaluate the implication of CP amino acids 247 and/or 270 in the viral replication and virulence, as well as in host immune response.

Methodology: Recombinant RGNNV viruses harbouring SJNNV-type amino acids at positions 247 and/or 270 (Mut247Dl965, Mut270Dl965, Mut247+270Dl965) were generated by reverse genetics. The effect of these modifications on viral replication was evaluated in cell culture and in infected fish. Experimental infections were also performed to analyse viral virulence and fish immune response.

Results: Differences regarding the replication of the mutated viruses on E-11 cells were reported. In particular, Mut247+270Dl965 showed the most important differences with titres significantly lower than those obtained for the wild type. *In vivo*, fish mortality caused by mutated viruses was 60% lower and viral replication in sea bass brain was reduced in comparison with the non-mutated virus. In addition, mutated viruses triggered lower induction of IFN I system- and inflammatory response-related genes. Furthermore, mutations caused changes in viral serological properties, inducing higher seroconversion and changing antigen recognition.

Conclusion: Amino acids 247 and 270 of the RGNNV CP sequence are important virulence determinants to sea bass. Differences in viral replication *in vitro* and *in vivo* suggest that the mutations considered can affect cell recognition and entry. In addition, the immunological analysis points out the importance of IFN I system and inflammatory process in response to betanodavirus infection. Finally, the double mutated virus induced the highest seroconversion, and antibodies in sera from infected animals recognized both, RGNNV and SJNNV antigens, suggesting its potential use in vaccination assays.

Keywords: Betanodavirus, reverse genetics, sea bass, virulence determinants, immune response

Funding: AGL2014-53532-C2-2-R (MINECO/FEDER); AGL2017-84644-R (MINECO/AEI/FEDER, UE).

048-O*

Betanodavirus transmission through marine invertebrates

Vázquez-Salgado, Lucía (Spain)[1]; Olveira, José G. (Spain)[1]; Dopazo, Carlos P. (Spain)[1]; Bandín, Isabel (Spain)[1]

1 - Universidade de Santiago de Compostela

Introduction: Mediterranean aquaculture is suffering great losses due to a disease named Viral Encephalopathy and Retinopathy (VER). The etiological agent is Nervous necrosis virus (NNV), a virus belonging to the Genus *Betanodavirus* that affects mainly larvae and juveniles of a wide range of fish species. Betanodaviruses are classified into four genotypes: RGNNV, SJNNV, TPNNV and BFNNV, although reassortants between RGNNV and SJNNV have been reported in Southern Europe. NNV can be transmitted horizontally through different fish species and reservoirs, as wild marine invertebrates. Therefore, the role as potential NNV reservoirs of the crustacean *Artemia salina* and the rotifer *Brachionus plicatilis*, which are commonly used as live food in marine fish hatcheries, has been investigated.

Methodology: *Artemia* and rotifer cultures were experimentally bath infected with a reassortant betanodavirus strain (RGNNV/SJNNV) for 7 days (d). Because crustacean cultures were fed with 2 types of microalgae (*Nannochloropsis gaditana* and *Isochrysis galbana*), the virus was also incubated with both microalgae water culture. In addition, NNV survival in sea water was tested. The quantification of viral RNA was performed by qRT-PCR whereas the viability of viral particles was assessed by titration in E-11 cultures.

Results: The quantification of viral load in *Artemia* and rotifer individuals showed the viral presence in both invertebrates after 7 d but with a reduction of about 2 logs from the initial inoculum. However, the incubation of the betanodavirus strain with *I. galbana* water culture showed that both viral load and titer decreased about 4 logs at d 7. Finally, no significant oscillations in viral load was observed in the sea water during the 7th d study.

Conclusion: NNV can persist into *Artemia* and rotifer individuals at least for 7 days. However, when *I. galbana* is added to cultures, as invertebrates food, viral viability is reduced, which is also demonstrated by incubating the viral strain with the microalgae water culture. That microalgae seems to release to the water environment a molecule which may alter the capsid protein and/or affect viral attachment to host cells. Studies are in progress in order to characterize this molecule and its effect on NNV.

Keywords: betanodavirus, *Isochrysis galbana*, *Artemia*, rotifer, infection

049-O*

RGNNV/SJNNV reassortant betanodavirus outbreaks in a sea bream and sea bass farm

Volpe, Enrico (Italy)[1]; Gustinelli, Andrea (Italy)[1]; Caffara, Monica (Italy)[1]; Errani, Francesca (Italy)[1]; Quaglio, Francesco (Italy)[2]; Fioravanti, Marialetizia (Italy)[1]; Ciulli, Sara (Italy)[1]

1 - Department of Veterinary Medical Sciences, University of Bologna, Italy; 2 - Department of Comparative Biomedicine and Food Science, University of Padua, Legnaro, Italy

Introduction: The Mediterranean aquaculture has suffered significant economic losses due to viral nervous necrosis mainly caused by RGNNV betanodavirus genotype primarily involving sea bass (*Dicentrarchus labrax*). Recently, a RGNNV/SJNNV reassortant betanodavirus, harbouring the RNA1 segment of RGNNV genotype and the RNA2 segment of SJNNV genotype, emerged. So far, the reassortant strain has caused a negative economic impact mainly on sea bream (*Sparus aurata*) hatcheries sparing the sea bass farming sector.

Methodology: Multiple mortality outbreaks occurred in an Italian marine farm involving both sea bass and sea bream at different life stages. Batches of sea bass and sea bream involved in the outbreaks (December 2017, May 2018 and August 2018) were investigated through a complete microbiological and molecular investigation.

Results: The cumulative mortality rates recorded during the outbreak occurred in December 2017 were 10% and 100% in larvae of sea bass and sea bream, respectively. In May 2018, sea bass survived the first outbreak (weight 4 g) showed a further outbreak with 10% of mortality. Moreover, in August 2018 a newly introduced batch of sea bream suffered a further outbreak, which led to 100% of mortality. All the batches were negative for parasites and bacteria. Betanodavirus was isolated on SSN-1 cells from all batches. Betanodavirus-typical lesions have been also found at histology. The molecular characterization of the strains isolated during all the outbreaks reported 100% nucleotide and amino acid identities, showing the involvement of the same viral strain during the different outbreaks. The phylogenetic analysis has demonstrated that the strain detected in both sea bream and sea bass involved in the multiple mortality outbreaks was a RGNNV/SJNNV reassortant betanodavirus.

Conclusion: The microbiological and molecular analyses allowed identifying a RGNNV/SJNNV reassortant betanodavirus strain as the causal agent of the outbreaks. This is the first investigation of a field mortality outbreak caused by a RGNNV/SJNNV reassortant betanodavirus involving sea bream and sea bass simultaneously. Sea bream has recorded the highest mortality rates, but sea bass seems to act as asymptomatic carriers and viral source for other susceptible species such as sea bream.

Keywords: viral nervous necrosis, betanodavirus, RGNNV/SJNNV reassortant, sea bream, sea bass

Funding: PerformFISH EU H2020 project (727610).

050-O

Viral encephalo and retinopathy (VER) in gilthead sea bream: results of experimental infections with RGNNV/SJNNV betanodavirus

Toffan, Anna (Italy)[1]; Panzarin, Valentina Maria (Italy)[1]; Biasini, Lorena (Italy)[1]; Abbadi, Miriam (Italy)[1]; Buratin, Alessandra (Italy)[1]; Pretto, Tobia (Italy)[1]; Marsella, Andrea (Italy)[1]; Pascoli, Francesco (Italy)[1]

1 - Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

Introduction: Gilthead sea bream (*Sparus aurata*) is generally believed to be resistant to VER, due to the absence of clinical signs. However, an increasing number of RGNNV/SJNNV outbreak in sea bream hatcheries has been reported in recent years, thus it was decided to carry out experimental challenges of juveniles and larvae to study the disease in sea bream, paying particular attention to fish age.

Methodology: Three experimental trials were performed. Different ages of sea bream batches were infected with the reassortant RGNNV/SJNNV. Sea bream juveniles (6-8 g) where intramuscularly infected with $10^{6,8}$ $TCID_{50}$/fish. Larvae of 75 and 21 days post hatching (dph) were challenged by immersion with $10^{5,45}$ $TCID_{50}$/ml. Fish were monitored for a period of 28 days post infection (dpi) to observe the appearance of clinical signs and confirm any mortality case. Samples were collected at different time points.

Results: Infected sea bream juveniles showed no clinical signs nor mortality. However, all brain samples collected at the end of the study tested positive for betanodavirus. Similarly, 75 dph larvae showed no clinical signs nor mortality, therefore we kept monitoring for a longer period the larvae, which tested positive in the brain for up to one year post infection. On the other hand, 21 dph larvae showed typical clinical signs 9 dpi, with affected subjects showing apathy, abnormal swimming and overinflation of swim bladder. Mortality began at 10 dpi, peaked at 11-13 dpi and then decreased but never ceased completely. Cumulative mortality was high but it was not possible to exactly quantifying it due to the impossibility to count the number of larvae at the beginning of the trial. RNA1 and RNA2 quantitative RT-PCR are currently ongoing and aim to characterize the viral replication kinetic.

Conclusion: Sea bream are susceptible to the RGNNV/SJNNV, although development of clinical signs is age dependent. This is likely related to the developmental stage of the immune system of fish. Once infected, larvae remain persistently infected for long period of time, thus becoming a potential risk as asymptomatic carriers.

Keywords: gilthead sea bream, betanodavirus, reassortant, larvae

Funding: This work was funded by Italian Ministry of Health RC IZSVE 09/15.

051-O*

Genome-wide sequencing of salmonid alphavirus suggests recent anthropogenic transmission across large distances in Norwegian aquaculture

Gallagher, Michael (United Kingdom)[1]; Karlsen, Marius (Norway)[2]; Petterson, Elin (Norway)[2]; Haugland, Øyvind (Norway)[2]; Matejusova, Iveta (United Kingdom)[3]; Macqueen, Daniel (United Kingdom)[1]

1 - The Roslin Institute, Edinburgh, UK; 2 - PHARMAQ AS, Oslo, Norway; 3 - Marine Scotland Science, Aberdeen, UK

Introduction: Salmonid alphavirus (SAV) is one of the major pathogens in salmonid aquaculture, causing pancreas disease (PD) in Atlantic salmon. While partial gene sequencing is routinely used to support SAV epidemiology and PD diagnostics, genome-wide sequencing has not yet been widely applied for this, or other fish viruses. However, genome-wide analyses have been widely applied to improve our knowledge of human pathogen dynamics and evolution. The Oxford Nanopore 'MinION' platform allows accurate viral whole genome sequencing at low per-sample cost, which we have recently successfully demonstrated for SAV.

Methodology: Using the Oxford Nanopore 'MinION' platform, whole SAV genomes were sequenced from Norwegian samples positive for SAV subtypes 2 and 3. Highly conserved PCR primers targeting long amplicons (~2 kb) were tiled across the genome to capture whole genomes. Evolutionary analysis was performed using a time-calibrated Bayesian approach and a phylogeographic model to reconstruct recent transmission routes of SAV in Norway.

Results: Whole SAV genomes were successfully generated for a number of recently sampled isolates across a wide geographic range in Norway. All recently isolated SAV3 viruses were highly similar (>99% pairwise identity) and phylogenetically closely related, contrary to what has been found previously where at least two clades of SAV3 had been identified as being co-circulating.

Conclusion: The population of SAV strains circulating in Norway seems to be dynamic with significantly differing levels of diversity being present in different years. Since previous sequencing studies, one of the two SAV3 clades appears to have declined in favour of a relatively homogeneous SAV3 Norwegian epidemic across Production Areas 2-4. This is indicative of a predominance of anthropogenic transmission routes instead of natural infection cycles including both water currents and/or wild fish movements.

Funding: This work received support from the Biotechnology and Biological Sciences 290 Research Council (grants: BB/M010996/1 and BBS/E/D/20002173) and Marine Scotland Science.

052-O*

Tuning salmonid alphavirus (SAV) virulence by modification of viral glycosylation and nuclear localization signal

Aksnes, Ida (Norway)[1]; Braaen, Stine (Norway)[1]; Markussen, Turhan (Norway)[1]; Vaaje-Kolstad, Gustav (Norway)[1]; Rimstad, Espen (Norway)[1]

1 - Norwegian University of Life Sciences

Introduction: Glycosylation is the process where sugar molecules (glycans) are attached to proteins. They are essential for proper protein folding and function of viral envelope glycoproteins. The glycan structures of mammalian alphaviruses have been characterised in several studies. In contrast, corresponding information on glycosylation in salmonid alphavirus (SAV) is limited or absent. Attachment of the glycan occurs through N-glycosylation of asparagine residues present in the Aspargine-X-Serine/Threonine consensus sequence were X can be any amino acid, with the probable exception of proline. The SAV envelope glycoproteins E1 and E2 both contain such consensus sequences, with the asparagine at positions 35 and 319, respectively. Multiple sequence alignment of the SAV E2 protein with its mammalian counterpart provides particular strong support that this protein is glycosylated also in the fish alphavirus.

Methodology: By utilizing a SAV3 infectious clone (prSAV) we successfully recovered viable SAV3 mutants harbouring an asparagine-to-glutamine or asparagine-to-alanine substitution in the E2 motif. Similarly, we introduced a mutation in the putative nuclear localization signal (NLS) in the capsid protein of SAV and recovered viable viruses. In addition, a viable virus that contained the combined E2-deglycosylation and capsid-NLS mutations were recovered.

Results: Significant differences in infectivity, development and severity of cytopathic effects were observed in cell cultures for these mutant viruses when compared with the wild type strain. An initial *in vivo* trial with the E2-deglycosylated mutant showed that it is both infectious for Atlantic salmon and transmissible to cohabitant fish. No reversion of the introduced mutation was observed during the course of the pilot experiment.

Conclusion: Tuning SAV virulence by modification of envelope glycosylation structures may lead to the development of improved prophylactic measures, and possibly yield functionally attenuated viruses optimal for improved vaccine development. Consequently, a challenge study has been initiated, and results of this will be presented.

Keywords: Atlantic salmon, salmonid alphavirus, attenuation, vaccine

Funding: Research Council of Norway: #237315/E40 (ViVaFish), #280847 (ViVaAct) and Norwegian University of Life Science.

053-O

Fast recombination in the hemagglutinin gene of salmon isavirus gives rise to new genotypes: evidence of template switching

Cortez-San Martín, Marcelo (Chile)[1]; Vasquez-Martinez, Yesseny (Chile)[1]; Montoya, Margarita (Chile)[2]; Toledo, Jorge (Chile)[3]; Alarcon, Javiera (Chile)[1]; Michelson, Sofia (Chile)[1]; Galleguillos, Claudia (Chile)[1]; Cardenas, Matias (Chile)[1]

1 - Laboratory of Molecular Virology and Pathogen Control, Faculty of Chemistry and Biology, University of Santiago, Santiago, Chile; 2 - 2Cellular Biochemistry Laboratory, Department of Biology, Faculty of Chemistry and Biology, University of Santiago, Santiago, Chile; 3 - Biotechnology and Biopharmaceuticals Laboratory, Pathophysiology Department, School of Biological Sciences, Universidad de Concepción, Chile

Introduction: *In vitro* replication capacity of new synthetic reassortant ISAV strains HPR7b, 2, 3, 10 and 14 was evaluated in ASK cells. In addition, the genetic stability analysis of Hemagglutinin gene in Segment 6 from each rISAV was performed showing highly recombinant activity in HPR region.

Methodology: Using reverse genetic system, we generate synthetic ISAV strains HPR7b, 2, 3, 10 and 14 with a virulent isavirus strain genetic backbone but the ORF of segment 6 was exchanged with each respective strain. Three blind passages using 1:10 dilution was done in ASK cells, later the sequence of each Segment 6-PCR product from the virus was determined by Sanger method. All the sequence was bioinformatical analyzed using Blast and Clustal platforms. The sequence of the putative fragment recombination was manually determined.

Results: Each virus was successfully rescued in transfected ASK cells supernatant, however the virus in the subsequent viral passage evolved into different HPR genotype in seven days range. The analysis shown novel HPR genotypes from ISAV HPR2 and 3 in passage 1, but is interesting to note that HPR7b, 2, and 14 genotypes evolved into a HPR0 in the third passage. Instead, HPR3 and HPR10 evolve into HPR7b and 2 respectively. We find a sequence movement for HPR genesis, up to 11 nucleotides; in particular, the short sequences inserted/deleted are found in isavirus segment 1, 5, 6 and 8.

Conclusion: This is the first study where clonal synthetics isavirus was rescued with different segment 6 but with the same genetic backbone. The results allowed the elaboration of a new and interesting insertion/deletion mechanism model for HPR genesis. Showing for the first time a Template Switching mechanism evidence for ISAV recombination. The analysis suggests that short sequences from genome segments of ISAV are used as a template for the fast evolution of isavirus genotype in salmon ASK cells. In contrast to the accepted hypothesis, which proposes the ISAV HPR0 as a common ancestor of each HPRΔ, in this study most of the genotypes rescued evolved into an isavirus HPR0.

Keywords: isavirus, salmon, orthomyxovirus, recombination

Funding: FONDECYT 1161006 (CONICYT); MECESUP-USACH USA1555, DICYT, DGT-VRIDEI (USACH).

Host-Parasite Interactions I

055-O*

Comparative proteomic profiling of pathogenic and non-pathogenic *Paramoeba perurans* proteins associated with AGD

Ní Dhufaigh, Kerrie (Ireland)[1]; Dillon, Eugene (Ireland)[2]; Talbot, Anita (Ireland)[1]; O'connor, Ian (Ireland)[1]; Maccarthy, Eugene (Ireland)[1]; Slattery, Orla (Ireland)[1]

1 - Galway-Mayo Institute of Technology; 2 - University College Dublin

Introduction: Three isolates of *Paramoeba perurans* of wild type and avirulent origin were cultivated in this study with the aim of elucidating virulence factors based on the reported loss of *P. perurans* virulence over time. An experimental challenge trial using Atlantic salmon smolts confirmed the loss of virulence in an *P. perurans* culture that was maintained for 3 years.

Methodology: A combination of two-dimensional gel electrophoresis (2D) in conjunction with LC MS/MS from *P. perurans* isolates (putatively virulent strains; 60 days and 1-year culture and putative avirulent stain; 3 year old culture). Gel-free MS analysis was used to detect differential protein expression in the 1-year culture and 3-year culture. 11 differential spots were selected for identification using *de novo* sequencing. Atlantic salmon smolts (n=120) were divided into 3 cohorts; controls [n=40], avirulent [n=40] and virulent [n=40]. AGD cohorts were inoculated with 2,000 cells/ L of a 3-year *P. perurans* culture (avirulent) and 1-year *P perurans* (virulent) culture. Lethal sampling occurred on 0, 7, 14- and 21-days post infection (dpi). Gill scoring, histology and qPCR of each gill was used to assess the level of infection.

Results: 2D analysis and gel free analysis confirmed differential protein expression between the avirulent and virulent *P. perurans* isolates. Over 98 differences in spot intensity were recorded in the analysis, of which 11 spots were identified. Enzymes pertaining to protecting the parasite were differentially expressed between the isolates. The challenge trial was terminated at 21 dpi, as a gill score of 2 was observed in fish inoculated with the virulent *P. perurans* isolate. *P. perurans* was not detected by qPCR at 21 dpi in fish inoculated with the avirulent isolate.

Conclusion: This study reports the loss of virulence in a 3-year *P. perurans* culture. Analysis of the *P. perurans* proteome reveals alterations to *P. perurans* protein expression over time in culture. *P. perurans* hydrophilic proteins indicate the expression of enzymes that play a role in antioxidant defence were higher in the virulent isolate when compared to the avirulent isolate.

Keywords: AGD, virulence, *Paramoeba perurans*

Funding: Department of Agriculture, Food and the Marine, Ireland.

056-O

A static *in vitro* gill model successfully reproduces *in vivo* Atlantic salmon host responses to *Neoparamoeba perurans* infection

Cano, Irene (United Kingdom)[1]; Taykor, Nick Gh (United Kingdom)[1]; Bayley, Amanda (United Kingdom)[1]; Mccullough, Robin (United Kingdom)[1]; Nowak, Barbara F (Australia)[2]; Paley, Richard (United Kingdom)[1]

1 - Cefas; 2 - IMAS

Introduction: An *in vitro* system to study host-pathogen interaction in amoebic gill disease (AGD) requires the ability to isolate and grow the parasite and the ability to maintain suitable host cells. Protocols for the isolation of the protozoan *Neoparamoeba perurans*, the causative agent of AGD, are available. *N. perurans* requires full salinity sea water and cannot be exposed to host cells in cell culture media which have lower osmolality than sea water. Transwell® culture inserts provide a permeable support on which seeded cells can attach and form confluent monolayers. By replacing apical media with either freshwater or seawater, culture conditions can be modified to establish asymmetrical systems which produce a cell culture environment that enables the establishment of effective polarised epithelia and more closely resembles the *in vivo* state.

Methodology: The rainbow trout gill derived cell line, RTgill-W1, was seeded onto permeable cell culture supports and maintained asymmetrically with apical seawater. Cells were inoculated with either a passage attenuated or a recent wild clone of *N. perurans*.

Results: Amoebae, loaded with phagocytosed fluorescent beads, were observed associated with host cells within 20 min post inoculation (pi). Due to cell monolayer disruption, the platform could not support proliferation of amoebae. Both clones induced similar host inmate immune responses, with the up-regulation of proinflammatory cytokine IL1β, complement C3 and cell receptor MHC-1. The Th2 pathway was up-regulated, with increased gene expression of the transcription factor GATA3, and Th2 cytokines IL10, IL6 and IL4/13A. PCNA and AG-2 were also up-regulated. The wild clone induced significantly higher up-regulation of IL1β, MHC-1, PCNA, lysozyme and IL10 than the attenuated clone for at least some exposure times, but AG-2 gene expression was higher in cells inoculated with the attenuated one. A principal component analysis showed that AG-2 and IL10 were key genes in the *in vitro* host response to *N. perurans*.

Conclusion: This *in vitro* model has proved to be a promising tool to study host responses to amoebae and may therefore reduce the requirement for in vivo studies when evaluating alternative therapeutants to AGD control.

Keywords: *N. perurans*, *in vitro*, salmon

Funding: EU grant ParaFishControl.

057-O*

Functional analysis with RNA interference technique of highly expressed protease genes at the invasive and parasitic stages of *Cryptocaryon irritans*

Watanabe, Yuho[1]; Itoh, Naoki (Japan)[1]; Yoshinaga, Tomoyoshi (Japan)[1]

1 - The University of Tokyo

Introduction: Cryptocaryoniasis is caused by *Cryptocaryon irritans*, an obligatorily parasitic ciliate. This parasite is a major threat to marine cage culture in tropical and subtropical waters. Many studies have been carried out aiming at the development of control methods such as therapeutic drugs and vaccines. However, these treatments are not sufficient to control cryptocaryoniasis especially in cultured food fishes. Hence, there is a need for more effective chemotherapeutic drugs or vaccines against *C. irritans*. Previous studies in our laboratory have confirmed that serine and cysteine protease genes are highly expressed in theronts (invasive stage) and trophonts (parasitic stage). In addition, the inhibitors against these proteases showed decrease in the viability and infectivity of the parasite. However, it is unknown which proteases are important in the infection process of the parasite. In this study, we conducted RNA interference (RNAi) against four protease genes which are highly expressed at invasion and parasitic stages of *C. irritans* to know that these proteases relate to the infection process of the parasite.

Methodology: Double-stranded RNAs (dsRNA) against the four protease genes were respectively synthesized *in vitro* and transfected into theronts by electroporation. The relative expression level of these target genes was examined by qPCR to confirm the effect of RNAi. Also, challenge experiments were conducted by using the RNAi treated theronts, and the number of infected parasites was counted.

Results: In the RNAi-treated parasites, the relative expression levels of the target genes were reduced as compared to the control, and suppression of these genes' expression by RNAi was confirmed. In addition, when fish were challenged with the RNAi-treated theronts, the numbers of parasites that infected and left fish after full development were reduced compared to the control.

Conclusion: It was suggested that protease genes highly expressed in the theront and trophont stages are involved in the infection process of the parasite. This information will assist the development of new chemotherapeutic drugs or vaccines against cryptocaryoniasis.

Keywords: *Cryptocaryon irritans*, RNA interference, protease, trophont, theront

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058-O

Serum metabolomics tells the story of disease degree in a fish enteritis model

Sitjà Bobadilla, Ariadna (Spain)[1]; Gil Solsona, Rubén (Spain)[2]; Estensoro, Itziar (Spain)[1]; Piazzon, M. Carla (Spain)[1]; Martos Sitcha, Juan Antonio (Spain)[3]; Picard Sánchez, Amparo (Spain)[1]; Fuentes, Juan (Portugal)[4]; Sancho, Juan Vicente (Spain)[2]; Calduch Giner, Josep (Spain)[3]; Hernández, Félix (Spain)[2]; Pérez Sánchez, Jaume (Spain)[3]

1 - Fish Pathology Group, Instituto de Acuicultura Torre de la Sal, Castellón, Spain; 2 - Research Institute for Pesticides and Water (IUPA), University Jaume I, Castellón, Spain; 3 - Nutrigenomics and Fish Endocrinology Group, Instituto de Acuicultura Torre de la Sal, Castellón, Spain; 4 - Comparative Endocrinology and Integrative Biology, CCMar, University of Algarve, Faro, Portugal

Introduction: In animal production, enteritis is responsible for serious economic losses, being intestinal parasitism a major stress factor leading to malnutrition and lowered performance and production efficiency. The intestinal myxozoan parasite *Enteromyxum leei* dwells between gut epithelial cells and causes severe desquamative enteritis in gilthead sea bream (*Sparus aurata*) that impairs nutrient absorption causing anorexia, cachexia, growth impairment, reduced marketability and increased mortality. This study aimed to outline the gut failure produced in this fish-parasite model using a multifaceted approach and to find and validate serum non-lethal markers of gut barrier dysfunction.

Methodology: Intestinal integrity was studied in parasitized and non-parasitized fish by immunohistochemistry with specific markers for cellular adhesion (E-cadherin) and tight junctions (Tjp-1 and Cldn3) and by functional studies of permeability (oral administration of FITC-dextran) and electrophysiology (Ussing chambers). Serum samples from parasitized and non-parasitized fish were analyzed using non-targeted metabolomics and some significantly altered metabolites were selected to be validated using commercial kits.

Results: The expression of the tight junction proteins Tjp-1 and Cldn3 was significantly lower in parasitized fish along all the intestine, while no differences were found in E-cadherin labeling. Some parasitized fish showed a significant increase in paracellular uptake measured by FITC-dextran detection in serum. Electrophysiology studies showed a decrease in transepithelial resistance in infected animals, which showed a diarrheic profile when compared to the normal absorptive profile of the control animals. Serum metabolomics revealed 3702 ions, from which the differential expression of 20 identified compounds significantly separated control from infected groups in multivariate analyses (PLS-DA), and even separated groups by intensity of infection. Of these compounds, inosine and creatine were identified as relevant and tested with commercial kits in serum samples.

Conclusion: This study demonstrates the loss of barrier function induced by the enteric parasite *E. leei* and underlines key markers to differentiate control and infected fish. The untargeted serum metabolomics approach did not reveal specific effects by the parasite, but more a profile typical of absorption dysfunction and anorexia, which are, of course, part of the disease signs.

Keywords: Myxozoa, *Sparus aurata*, intestinal permeability, electrophysiology, immunohistochemistry

Funding: ParaFishControl H2020 project (634429), Aquaexcel[2020] (652831, TNA AE10004-INTEBREAM), AGL2013-48560-R.

059-O

Ultrastructural studies on the early interaction between the oomycete *Saprolegnia parasitica* and fish cells

Muñoz, Pilar (Spain)[1]; Saraiva, Marcia (United Kingdom)[2]; Trusch, Franziska (United Kingdom)[2]; Milne, Gillian (United Kingdom)[3]; Holland, Jason (United Kingdom)[2]; Van West, Pieter (United Kingdom)[2]

1 - Dpto. Sanidad Animal. Campus de Excelencia Internacional Regional "Campus Mare Nostrum", Universidad de Murcia, 30100, Murcia, Spain; 2 - International Centre for Aquaculture Research and Development, University of Aberdeen; 3 - Microscopy and Histology Core Facility, University of Aberdeen

Introduction: The oomycete *Saprolegnia parasitica* causes the disease saprolegniosis in salmonids and other freshwater fish, resulting in important economic losses in aquaculture. Plant-pathogenic oomycetes employ infection structures, such as haustoria, that allow delivery of effector proteins into the host and nutrient uptake from the host. However, a detailed understanding of the cellular infection process of the animal pathogenic oomycete, *S. parasitica*, is currently unclear. Thus, the early interaction stages of *S. parasitica* with rainbow trout cells was analysed by means of transmission electron microscopy.

Methodology: *Oncorhynchus mykiss* (rainbow trout) cell lines, RTG-2 RT and Gill-W1 derived from gonadal tissue and gill explants respectively, were used. Suspensions of zoospores/cysts of *S. parasitica* isolate CBS223.65 were incubated with the cell line monolayer cultures. After 12 h of incubation the infection progression was monitored every 30 min under an inverted microscope. At different intervals (13.5, 14, 14.5, 15 and 16 h p.i.), infected cells were fixed and processed for electron microscopy.

Results: After 13.5 h of incubation, hyphae were observed in close proximity to the host cell but the integrity of plasma membranes of both the host and pathogen remained intact and the morphology of the host cells appeared normal. There was also no detectable damage to fish cell organelles at this stage. However, after 14 h, the plasma membrane of both the fish cells and the oomycete had disappeared in the contact zone and the exchange of nutrients seemed to occur. Unlike plant pathogenic oomycetes, haustorial feeding-structures connecting invaded cells with *Saprolegnia* were not observed at this stage of infection. The traffic of material became more evident at 16 h post infection, even the exchange of whole organelles was observed.

Conclusion: The present study contributes to a better understanding of *S. parasitica* pathogenesis by demonstrating the first steps of the cellular infection process of this animal pathogenic oomycete.

Keywords: oomycetes, saprolegniosis, fish, host-pathogen interaction

Funding: BBSRC, NERC and Newton Fund.

060-O

Leukocyte nucleolus and *Anisakis pegreffii*: when falling apart actually is falling in place

Buselić, Ivana (Croatia)[1]; Trumbić, Željka (Croatia)[2]; Hrabar, Jerko (Croatia)[1]; Vrbatović, Anamarija (Croatia)[1]; Petrić, Mirela (Croatia)[2]; Mladineo, Ivona (Croatia)[1]

1 - Institute of Oceanography and Fisheries; 2 - University Department for Marine Studies

Introduction: Ribosome biosynthesis (RB) is orchestrated by nucleolus whose main function is rapid production of small and large ribosome subunits. Nucleolus is also essential in the sensing of stress stimuli that target ribosome biogenesis; a condition known as nucleolar or ribosomal stress. It is exhibited through expression of several p53-dependent or p53-independent response pathways that maintain cell homeostasis. Our aim was to characterise molecular mechanisms and architectural changes in the nucleolus of the *Anisakis*-stimulated rat peripheral blood leukocytes (PBLs).

Methodology: *In vitro A. pegreffii* crude extract-stimulated Sprague-Dawley rat PBLs were harvested 1 and 12 h post-stimulation for RNA isolation. Illumina NextSeq 500 was used for paired-end sequencing of 7 pooled PBLs. Markers of ribosomal biogenesis (*Tp53*, *CDKN1*, *CCND1*, *mTOR*, *RPL5*, *RPL11*, *RPL23*, *NFE2L2*) and inflammation (*Il4*, *Il6*, *Il7*, *TNF-α*) were measured by qPCR, while nucleolar architecture was assessed by localisation of nucleophosmin, NOP58 and coilin by confocal microscopy.

Results: Differential expression (DE) analysis of rat PBLs revealed 53 (22 down and 31 up-regulated) DE transcripts 1 h post-stimulation, whose strong and significant fold changes (LogFC >> 1) were especially noted for transcripts involved in regulation of protein complex assembly, membrane polarisation, mitotic cell cycle and calcium ion binding, to name only the few. In contrast, 39 (5 down and 34 up-regulated) DE transcripts were observed 12 h post-stimulation, mainly involved in proinflammatory functions. Strong scattering of nucleophosmin from nucleolus into the nucleus was evidenced, while nucleolus granular component and Cajal bodies remained mostly unaffected.

Conclusion: Transcriptomics, targeted gene expression and confocal microscopy support the hypothesis that *A. pegreffii* CE in PBLs trigger the onset of nucleolus functional and morphological cues toward increased ribosomal biogenesis already 1 h post-stimulation, enriched in transcripts of 40S and 60S ribosomal proteins. This suggests that nucleolar rearrangements occur early and are soon balanced in order to provide an efficient proinflammatory response to the nematode.

Keywords: *Anisakis pegreffii*, nucleolus, ribosomal proteins, inflammation

Funding: Croatian Science Fundation HRZZ, project AnisCar (#8490).

030-O*

Epizootiology and genomic characterisation of a novel epitheliocystis agent in greater amberjack in Greece

Cascarano, Maria Chiara (Greece)[1,2]; Kristoffersen, Jon Bent (Greece)[1]; Tsertou, Maria Ioanna (Greece)[1]; Papandroulakis, Nikos (Greece)[1]; Katharios, Pantelis (Greece)[1]

1 - Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Heraklion, Greece; 2 - Biology Department, University of Crete, Heraklion, Greece

Introduction: Epitheliocystis has been reported to affect different *Seriola* spp. worldwide. The pathology is characterized by the presence of inclusions in the gill epithelium containing the replicating infectious agents. Epitheliocystis causes epithelial hyperplasia, respiratory distress and eventually death of the host. The obligate intracellular lifestyle of the agents makes them hard if not impossible to isolate; therefore, direct sequencing of infected material aiming to obtain insights on the phylogeny, the infection mechanism and the virulence properties of the pathogen has been used. The present study is focused on the disease dynamics and the genomic features of the epitheliocystis agent affecting cultured greater amberjack in Greece.

Methodology: The disease was monitored during one year, following the transfer of the juveniles from the hatchery to sea cages. Histology, micro-CT and TEM were used to locate, quantify and describe the epitheliocystis lesions on the gills. Molecular screening was conducted using epitheliocystis-related primers. Gill microdissection and pooling of the inclusions was used to obtain DNA for metagenomic analysis. Hybrid sequencing methods including both short and long reads sequencing technologies (Illumina Miseq and MinION), allowed building a draft genome for the novel bacterial species.

Results: The presence of the pathogen was detected with PCR one month after the fish transfer in the sea cages, with histological sections not clearly showing cysts. Abundant cysts were observed one month after the initial detection. The infection resolved spontaneously after a late state of infection dominated by granulomatous lesions. Molecular screening revealed a faint signal when using Chlamydial primers and a strong signal for *Ca*. *ichthyocystis* primers. The disease was caused by β-proteobacteria that showed 96% similarity with the recently described *Ca*. *ichthyocystis hellenicum*.

Conclusion: Epitheliocystis was confirmed to be one of the most common diseases in greater amberjack. The outbreak occurred soon after the introduction of the fish in the open sea cages. Genomic analysis showed that the bacterial agent is a member of the recently described *Ichthyocystis* genus that comprise the major epitheliocystis-related agents in the Greek aquaculture.

Keywords: epitheliocystis, genomics, NGS

Funding: European Union's Seventh Framework Programme (FP7) (KBBE-2013-07 single stage, GA 603121, DIVERSIFY).

WS: Biosecurity II

061-O

Assessment of the biosecurity in fish farms with an innovating decision-making tool

Le Bouquin, Sophie (France)[1]; Dimouchy-Pimont, Lucille (France)[1]; Broquard, Lucille (France)[1]; Pekala-Safinska, Agneszka (Poland)[3]; Moyano, Gabriel (Spain)[4]; Baron, Sandrine (France)[2]; Thomas, Rodolphe (France)[1]; Chauvin, Claire (France)[1]

1 - ANSES, Ploufragan/Plouzané/Niort Laboratory, Epidemiology, Health and Welfare Unit, Ploufragan, France; 2 - ANSES, Ploufragan/Plouzané/Niort Laboratory, Mycoplasmology Bacteriology and Antimicrobial Resistance Research Unit, Ploufragan, France; 3 - Department of Fish Diseases, National Veterinary Research Institute, Puławy, Poland; 4 - Facultad de Veterinaria, Universidad Complutense de Madrid, Spain

Introduction: Biosecurity is defined as all measures taken to prevent both the introduction and the spread of disease agents on the farm. Some risk-based assessment tools have already been developed for evaluating biosecurity in territorial livestock industry but such approaches are limited for aquaculture production system.

Methodology: Sixty rainbow trout farms located in France, Spain and Poland were investigated in 2015 through an epidemiological questionnaire consisting in 13 main thematics. Data collected were then integrated into a biosecurity grid specially design for, based on scientific literature and European expert elicitation. Two scores were obtained, for external and internal biosecurity respectively. The two scores were combined to form the final global biosecurity grade. Biosecurity was evaluated through a grade up to 100 points. The higher the grade is, the better the on-farm biosecurity is. Spidergraphs were used in order to make the outputs as clear and readable as possible.

Results: The overall score is 63/100 with significant variations between farms (min 47.9/100, max 77.3/100). The performance in terms of internal biosafety is more satisfactory than in external biosafety (60.2/100 vs 37.8/100), which is explained by an easier control. In contrast, the least satisfactory external biosecurity scores reflect the low influence of farmers on their environment. For external biosecurity, water quality (45.3/100) and people management (50/100) get the lower scores. On the other hand, eggs and fish introductions (87/100) have the highest scores. For internal biosecurity, zootechnical organization (62/100) gets the lower score but cleaning and disinfection of the installations and equipment (75.1/100) have the highest score. National tendencies are also observed, particularly concerning the size of farms and zootechnical practices.

Conclusion: This tool is a base for evaluating biosecurity in aquaculture. It has both didactic and decision making purpose, and contributes to farmer awareness about their own biosecurity performances. Users can also benchmark themselves by comparing their scores to a group reference. It could also be used for further development of biosecurity tools for smartphones or others aquaculture productions.

Keywords: biosecurity, expert elicitation, risk ranking, score, fish

Funding: EU FP7/2007-2013 n° 613754 EFFORT.

Diseases of Wild and Ornamental Fish

062-O

***Saprolegnia* infections in wild salmonids - an increasing challenge for fisheries**

Matthews, Emily (United Kingdom)[2]; James, Joanna (United Kingdom)[1]; Williams, Chris (United Kingdom)[1]; Reading, Amy (United Kingdom)[1]; Ellison, Amy (United Kingdom)[2]; Cable, Joanne (United Kingdom)[2]

1 - Environment Agency; 2 - Cardiff University

Introduction: *Saprolegnia* species are members of the oomycetes and ubiquitous in aquatic environments globally. Infections with *Saprolegnia* are typically considered as secondary and do not usually cause problems in wild salmonid populations. However, recent increases in the prevalence of *Saprolegnia* in some UK rivers has led to outbreaks of diseases and, in some cases, significant mortalities. There is therefore a need to better understand the diversity of *Saprolegnia* in UK and determine drivers for the recent increases in infection levels.

Methodology: Isolates (n = 70) of *Saprolgenia* were collected from wild Atlantic salmon and sea trout from the UK and the genetic diversity assessed by sequencing of the ITS region. Laboratory trials using naive hatchery reared juvenile salmon and trout were also conducted to investigate host specificity and pathogenicity.

Results: All but one of the isolates was identified as *S. parasitca,* but considerable within species variation was present. There were also differences in the level of pathogenicity caused by strains isolated from salmon and sea trout with some causing significantly higher levels of Saprolegniasis and death. Some isolates also appeared to be more generalist in their host range than others.

Conclusion: Whilst these appears to be little species level diversity in *Saprolegnia* in the UK numerous genetically distinct strains were detected. Differences in virulence between these strains may contribute to spatial and temporal differences in *Saprolegnia* infections. These findings are discussed in the context of the increasing problems of *Saprolegnia* infections both in wild fish and aquaculture.

Keywords: *Saprolegnia parasitica*, aquatic disease, Atlantic salmon, sea trout

Funding: Cardiff University, Environment Agency England, and KESS (Knowledge Economy Skills Scholarships).

063-O

Micro and macroparasites in pink salmon (*Oncorhynchus gorbuscha*) invading rivers in western Norway

Fjær, Marte Andrea (Norway)[1]; Moore, Lindsey J. (Norway)[1]; Madhun, Abdullah S. (Norway)[2]; Karlsbakk, Egil (Norway)[1]

1 - University of Bergen; 2 - Institute of Marine Research

Introduction: In summer-autumn 2017, the rivers in western were invaded by thousands of pink salmon, an alien species introduced to the White Sea area in Russia decades ago. Among the different concerns raised, are the spread of diseases and parasites.

Methodology: A total of 80 pink salmon caught in three rivers (2017) were deep-frozen and later examined for micro- and macroparasites. Heart, kidney and gill samples were analysed for certain viral, bacterial and protist parasite infections using qPCR, and pseudobranch and intestinal samples were analysed for *Parvicapsula* spp. and *Spironucleus* spp. infections using PCR. Microscopy was used when examining tissue samples for other spore-producing micro-parasites, and complete dissections performed for helminth and copepod parasites. Some larval helminths were identified by their 28S rDNA sequences.

Results: Viral infections (IPNV, ISAV, PMCV, SAV, IHNV) were not detected (N=40). The epitheliocyst forming bacterium *Candidatus* Branchiomonas cysticola was rare, while the microsporidian *Desmozoon lepeophtherii* and flagellate *Ichthyobodo salmonis* infections in the gills were common. The myxosporean *Parvicapsula pseudobranchicola* was detected in the pseudobranchs by PCR, but spores were absent and prevalence low (13%). *Spironucleus* spp. were not detected. A total of 14 helminth species and two copepod species were identified. Most helminths were larval or immature forms, such as the cestodes *Eubothrium* sp. aff. *crassum*, *Scolex pleuronectis* in the intestine, and *Diphyllobothrium* sp. encapsulated in the gut wall, the trematodes *Cryptocotyle lingua* and *Apatemon gracilis* occurring as metacercariae in skin and oesophagus, or the nematodes *Hysterothylacium aduncum* and *Anisakis simplex* encapsulated in the viscera. Adult parasites were represented by gastrointestinal trematodes, of which *Derogenes varicus* and *Lecithaster gibbosus* were dominant, *H. aduncum* in the gut and the copepods *Lepeophtheirus salmonis* and *Salmincola salmoneus* from skin and gills.

Conclusion: Microparasites that represent a threat to salmonid aquaculture was not detected. The use of the helminths as indicators for pink salmon feeding areas is discussed.

Keywords: pink salmon, parasites, diseases invading exotic fish

064-O

Epidemiological and genetic investigations of the cnidarian parasite of sturgeon and paddlefish eggs, *Polypodium hydriforme*

Okamura, Beth (United Kingdom)[1]; Hartigan, Ashlie (United Kingdom)[1]; Long, Paul (United Kingdom)[2]; Loi, Emily (United Kingdom)[1]; Jaimes-Bacerra, Adrian (Brazil)[3]; Smith-Easter, Katie (United States of America)[4]; Aleoshin, Vladimir (Russian Federation)[5]; Mikhailov, Kyrill (Russian Federation)[5]; Ruggeri, Paolo (United Kingdom)[1]; Marques, Antonio (Brazil)[3]; Schooley, Jason (United States of America)[6]

1 - Natural History Museum, London; 2 - King's College London; 3 - University of São Paulo; 4 - Northeastern State University, Oklahoma; 5 - Lomonosov Moscow State University; 6 - Oklahoma Department of Wildlife Conservation

Introduction: Phylogenomic and morphological evidence place *Polypodium hydriforme* as a monotypic sister clade to the diverse Myxozoa, comprising the Endocnidozoa. *Polypodium*'s one-host life cycle involves a parasitic larval stage in oocytes of acipenseriform fish (sturgeons and paddlefishes). A free-living stolon of connected tentaculate individuals emerges from spawned eggs. Subsequent fragmentation releases single individuals (up to 100) that take up benthic life. Upon maturity a specialised multicellular stage enables infection via contact with larval fish. Morphological similarity suggests a single, widely-distributed *Polypodium* species (Eurasia to North America). 78% of female Sterlet Sturgeon, *Acipenser ruthenus*, may be infected by *Polypodium* with up to 100% of oocytes carrying the parasite – a cause for concern given potential impacts on caviar production and conservation of this vulnerable species. As part of larger investigations we analysed *Polypodium* material to: a) explore whether there is genetic variation between Old and New World *Polypodium*, between New World populations, and between stolons from the same egg mass; b) characterise epidemiology of American Paddlefish infections.

Methodology: We sampled *Polypodium* from eggs of American Paddlefish (*Polyodon spathula*) in Oklahoma and Montana, USA for genetic analysis using mitochondrial and ribosomal markers, and from Russian Sturgeon (*Acipenser gueldenstaedtii*) in the Volga River delta, Russia, for genome development. Data were also gathered to analyse covariates to presence and intensity of *Polypodium* infection such as fish size, age, condition, and year of collection in Oklahoma.

Results: We obtained evidence for genetic divergence of Old and New World *Polypodium* but little between New World populations. Some 50% of fish were infected each year and infection intensity was negatively skewed. Presence of infection was positively associated with fish size/age and negatively with fish condition. Infection intensity showed no association with these variables and relationships did not vary between years. Work in progress on genetic variation amongst stolons within egg masses and further epidemiological analyses will be reported.

Conclusion: Low genetic divergence between New World populations may reflect historical connectivity within river systems. Differences in infection prevalence and intensity between Old and New World material may reflect different infection strategies or hosts.

Keywords: biogeography, genetic divergence, infection prevalence, caviar

Funding: The Leverhulme Trust.

065-O

Haplotype-specific environmental DNA detection of *Gyrodactylus salaris*

Rusch, Johannes (Norway)[1]; Vrålstad, Trude (Norway)[1]; Strand, David (Norway)[1]; Andersen, Tom (Norway)[2]; Hansen, Haakon (Norway)[1]

1 - Norwegian Veterinary Institute, Fish Health Research Group; 2 - University of Oslo, Section for Aquatic Biology and Toxicology

Introduction: Infections with *Gyrodactylus salaris* (Monogenea) result in high mortality rates of young Atlantic salmon. This in turn causes severe problems, both ecologically, economically and to animal welfare. In recent years, eDNA has emerged as a less disruptive method for determining the presence of specific organisms in aquatic systems. DNA from shed cells is used for detection, bypassing the need to capture the actual specimen. Recent research conducted at the Norwegian Veterinary Institute (NVI) confirms the possibility to detect *G. salaris* using eDNA methodology. However, several strains of *G. salaris* exist that can be characterised by sequencing of the mitochondrial cytochrome oxidase I (COI) gene and the current assays cannot distinguish between these. The detection of and differentiation between the mitochondrial haplotypes is important as some strains are pathogenic and some are non-pathogenic to its host. In addition, studies on detection limits and quantification are urgently needed.

Methodology: Droplet digital PCR (ddPCR) assays targeting three different mitochondrial haplotypes of *G. salaris* present in Norway, were designed, tested and optimised regarding specificity and sensitivity. Subsequently, water-samples from rivers with known and unknown infection status for *G. salaris* were analysed to test the efficiency and efficacy of the assays on environmental samples. Detection limits and quantification were studied in an experiment where infections with *G. salaris* on individually isolated fish (4 L of water and a defined rate of water exchange) were monitored for a period of 14 weeks. Every week, parasite numbers on the fish were counted and water samples were taken for analysis.

Results: The specificity of the assays was confirmed and the method also proved successful during field-validation. Initial data indicates a correlation between the number of parasites on a fish and the strength of the PCR signal when a certain number of parasites is reached.

Conclusion: We have developed an eDNA method that is able to detect specific haplotypes of *G. salaris*. We also show that it is possible to semi-quantify the parasite numbers – at least in a closed system – if certain precautions are taken.

Keywords: eDNA, monogenea, droplet digital PCR

Funding: NVI through the "eDNAqua-Fresh" PhD-project.

Viruses and Viral Diseases II

066-O*

Clinical and subclinical infections of coho salmon (*Oncorhynchus kisutchi*) by piscine orthoreovirus (PRV)

Godoy, Marcos (Chile)[1,2,3]; Suarez, Rudy (Chile)[1,2,4]; Medina, Daniel (Chile)[1]; Kibenge, Frederick (Canada)[5]; Wang, Yingwei (Canada)[6]; Heisinger, Alejandro (Chile)[7]; Labraña, Mauricio (Chile)[8]

1 - Laboratorio de Biotecnología Aplicada, Facultad de Medicina Veterinaria, Universidad San Sebastián, Chile; 2 - Centro de Investigaciones Biológicas Aplicadas, Chile; 3 - Doctorado en Acuicultura, Programa Cooperativo Universidad de Chile, Universidad Católica del Norte, Antofagasta, Chile; 4 - Magister en Acuicultura, Universidad Católica del Norte, Coquimbo, Chile; 5 - Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Canada; 6 - School of Mathematical and Computational Sciences, University of Prince Edward Island, Canada; 7 - Multiexportfoods, Puerto Montt, Chile; 8 - Aquachile, Puerto Montt, Chile

Introduction: Piscine orthroreovirus (PRV) belongs to the family Reoviridae, being described mainly in association with salmonid infections. The objective of this study is to describe the clinical and subclinical infections of PRV on coho salmon (*Oncorhynchus kisutch*) in Chile.

Methodology: Between years 2016 to 2019 a health surveillance program was carried out in coho salmon (*O. kisutch*) farming in Chile. For this, samples were taken from random and selected fish which were subjected to necropsy followed by histopathological analysis, specific RT-qPCR to detect PRV, and DNA sequencing to PRV genomic segments S1 and L1.

Results: We detect a predominant infection of PRV in subclinical cases, with an average cycle threshold (Ct) of 33. The clinical cases analyzed were characterized by generalized circulatory disturbances, mild to moderate myositis, myocarditis that predominantly affected the spongy layer, and the tissues had an average Ct of 25. Phylogenetic analysis showed that the PRV strains isolated belonged to genogroups Ia, Ib and IIa.

Conclusion: Piscine orthoreovirus (PRV) infections are predominantly subclinical, and occur in all stages of farming. Clinical cases were most prevalent in winter, during the ongrowing phase in estuary or sea, and were consistent with Heart and Skeletal Muscle Inflammation disease (HSMI), previously described in other salmonids species. Genetic analysis of the PRV isolates showed that all the genogroups reported in Chile are present in coho salmon farm sites in Chile, possibly as a result of the close contact with breeding centers of Atlantic salmon (*Salmo salar*) and Rainbow trout (*O. mykiss*).

Keywords: piscine orthoreovirus, viral disease, coho salmon, PRV, pathology

Funding: This work was funded by Universidad San Sebastian and by the Centro de Investigaciones Biológicas Aplicadas (CIBA).

067-O*

Piscine orthoreovirus (PRV-1) persists in erythroid progenitor cells, erythrocytes, macrophages and unknown cells in kidney of Atlantic salmon (*Salmo salar*)

Malik, Muhammad Salman (Norway)[1]; Kannimuthu, Dhamotharan (Norway)[1]; Hansen, Elisabeth Furuseth (Norway)[1]; Wessel, Øystein (Norway)[1]; Markussen, Turhan (Norway)[1]; Dahle, Maria K. (Norway)[2]; Rimstad, Espen (Norway)[1]

1 - Department of Food Safety & Infection Biology, Norwegian University of Life Sciences, Oslo, Norway; 2 - Department of Fish Health, Norwegian Veterinary Institute (NVI), Oslo, Norway

Introduction: Piscine orthoreovirus (PRV-1) is ubiquitous in farmed Atlantic salmon (*Salmo salar*) in the marine phase and cause heart and skeletal muscle inflammation (HSMI), a disease known from Norway, Chile, Scotland and Canada. PRV-1 belongs to the *Reoviridae* family, *Orthoreovirus* genus and has a double stranded RNA (dsRNA) genome of 10 segments enclosed in a double-layered protein capsid. In experimental settings, PRV-1 causes an acute infection with a peak phase lasting 1-2 weeks before the infection subsides into persistence. This study aimed to analyze viral kinetics and identify target cells during persistent phase of infection.

Methodology: Experimental Atlantic salmon were challenged with intraperitoneal injection of PRV-1 infected blood homogenate for 18 weeks. Samples were harvested every 3 weeks during the trial. PRV-1 genomic segments expression and outer capsid protein expression were measured by RT-qPCR and Western blotting respectively. Cellular localization and co-localization of PRV-1 was analyzed using singleplex and duplex *in situ* hybridization method.

Results: PRV-1 maintains a high level of transcription, but a low level of viral protein synthesis in the persistent phase. Transcription levels of the PRV-1 genomic segments tested, L1, M2, M3, S1, S2 and S3, were similar throughout the trial period. High level of PRV-1 was detected in kidney with higher level of genomic dsRNA as compared to viral transcripts (ssRNA). Plasma remained positive for PRV-1 genomic dsRNA until termination of the study, indicating that viral particles are continuously released into the circulation. *In situ* hybridization assays confirmed that PRV-1 was present in erythroid progenitor cells, erythrocytes, macrophages and unknown cells in kidney, and to a lesser degree in spleen, during persistence.

Conclusion: The results show that PRV-1 establishes a productive, persistent infection in Atlantic salmon.

Keywords: piscine orthoreovirus-1, persistent infection, Atlantic salmon

Funding: The Norwegian Seafood Research Fund (FHF) grant #901221.

068-O

Genomic characterisation of Tasmanian salmon reoviruses

Mohr, Peter (Australia)[1]; Moody, Nick (Australia)[1]; Williams, Lynette (Australia)[1]; Hoad, John (Australia)[1]; Slater, Joanne (Australia)[1]; Crane, Mark (Australia)[1]

1 - CSIRO, Australian Animal Health Laboratory

Introduction: Since 1990, Tasmanian salmon reoviruses (TSRVs) have been isolated on a regular basis during the Tasmanian Salmonid Health Surveillance Program from Atlantic salmon farmed in Tasmania, Australia. Initially, TSRV infections were rarely associated with disease, however, in recent years there is growing evidence that TSRV has been involved in disease processes for both Atlantic salmon and rainbow trout. Preliminary evaluation of TSRV isolates had identified the existence of at least two variants of the virus, termed typical and atypical. Prior to the development of a vaccine for TSRV a more comprehensive genomic study was required to assess the diversity within TSRV isolates.

Methodology: Fifteen isolates of TSRV from varying host species, age, tissues, health status, geographical location and season were selected. The isolates included 10 typical TSRV isolates from 1990 to 2013 and 5 atypical TSRV isolates from 1992 to 2014. Several different viral and nucleic acid preparation protocols were required prior to next generation sequencing with the Illumina MiSeq platform to enable assembly of all 11 double stranded RNA segments for each virus.

Results: For the first time the entire genome of typical and atypical TSRV isolates were assembled. Variation between open reading frames (ORFs) within typical or atypical TSRV isolates was <1.2%. However, significant variation of up to 18% was observed when comparing ORFs between typical and atypical TSRV isolates, confirming the existence of two TSRV variants in Tasmania. Despite the differences between the two variants, phylogenetic analysis clearly clustered all TSRV viruses into a single node with other aquareovirus A species.

Conclusion: The presence of two distinct variants of TSRV will need to be carefully considered when developing a vaccine to prevent disease associated with this virus in farmed Atlantic salmon and rainbow trout in Tasmania.

Keywords: Aquareovirus, genome, Atlantic salmon, rainbow trout

Funding: Fisheries Research and Development Corporation, the Australian Government (FRDC Project No 2011/024).

069-O

Identification of virulence determinants of viral hemorrhagic septicemia virus in rainbow trout

Vakharia, Vikram (United States of America)[1]; Yusuff, Shamila (United States of America)[1]; Li, Jie (United States of America)[1]; Mckenney, Douglas (United States of America)[2]; Tesfaye, Tarin (United States of America)[2]; Kim, Min-Sun (United States of America)[2]; Kurath, Gael (United States of America)[2]

1 - Institute of Marine & Environmental Technology, University of Maryland Baltimore County, Baltimore, USA; 2 - U.S. Geological Survey, Western Fisheries Research Center, Seattle, USA

Introduction: Viral hemorrhagic septicemia virus (VHSV) is an important fish rhabdovirus that is not naturally virulent in rainbow trout (*Oncorhynchus mykiss*), but one major genetic subgroup and several strains of VHSV have gained high virulence in rainbow trout. To identify the genetic basis of VHSV virulence in rainbow trout, we used reverse genetics approach to create chimeric recombinant VHSVs in which viral gene(s) were exchanged between a trout-virulent European VHSV strain (DK-3592B) and a trout-avirulent North American VHSV strain (MI03).

Methodology: Using infectious clones of the trout-virulent VHSV-Ia strain and a trout-avirulent VHSV-IVb strain, sixteen chimeric VHSV clones were constructed in which the coding region(s) of the nucleoprotein (N), phosphoprotein (P), N and P, matrix protein (M), glycoprotein (G), non-virion protein (NV), G and NV, or G, NV and L (large protein) genes together, were exchanged between the two clones. Recombinant VHSVs (rVHSVs) were created, including two parental rVHSVs, from the full-length plasmids. Recovered rVHSVs were characterized for viability and growth *in vitro* and used to challenge groups of juvenile rainbow trout by intraperitoneal injection.

Results: Testing of chimeric rVHSV in juvenile rainbow trout showed that exchanges of the viral G (glycoprotein), NV (non-virion protein), G and NV, G and NV and L (large protein), M (matrix protein) or P (phosphoprotein) genes had no effect on the trout-virulence phenotype of either parental clone. However, reciprocal exchanges of the viral nucleoprotein (N) gene resulted in a partial gain-of-function in the chimeric trout-avirulent virus (22% mortality), and complete loss of virulence for the chimeric trout-virulent virus (2% mortality). Reciprocal exchanges of both the N and P genes together resulted in complete gain-of-function in the chimeric avirulent virus (82% mortality) and complete loss of virulence in the chimeric trout-virulent virus (0% mortality).

Conclusion: Our results demonstrate that the N gene is an essential virulence determinant, and the P gene is a strong enhancer of the trout-virulence determinant in the N gene, but this P enhancement is not expressed without the N gene. Moreover, we have identified putative amino acids responsible for trout-virulence in the N and P proteins of VHSV.

Keywords: virulence determinant, VHSV, rainbow trout

070-O

Molecular basis for VHSV virulence in rainbow trout

Panzarin, Valentina (Italy)[1]; Cuenca, Argelia (Denmark)[2]; Toffan, Anna (Italy)[1]; Alencar, Anna Luiza Faria (Denmark)[2]; Gastaldelli, Michele (Italy)[1]; Pascoli, Francesco (Italy)[1]; Abbadi, Miriam (Italy)[1]; Blanchard, Yannick (France)[3]; Ryder, David (United Kingdom)[4]; Bremont, Michel (France)[5]; Morin, Thierry (France)[6]; Olesen, Niels Jørgen (Denmark)[2]

1 - IZSVe, Padova, Italy; 2 - DTU, Lyngby, Denmark; 3 - Anses, Ploufragan-Plouzané-Niort Laboratory, GVB Unit, Ploufragan, France; 4 - CEFAS, Weymouth, UK; 5 - INRA, Paris-Saclay University, Jouy-en-Josas, France; 6 - Anses, Ploufragan-Plouzané-Niort Laboratory, PVP Unit, Plouzané, France

Introduction: VHSV is of great concern in the EU, being the most threatening pathogen for the European trout industry. The identification of VHSV virulence markers is of utmost importance to predict the *in vivo* phenotype of viral isolates and to design molecular tests with prognostic value. As part of a European project, 6 laboratories from 5 countries have acted jointly to compile the largest dataset of *in vivo* virulence data combined with genetic information, with the aim of identifying the molecular markers responsible for VHSV virulence in rainbow trout (*Oncorhynchus mykiss*).

Methodology: Sixty-eight VHSV isolates covering all European genotypes were selected based on previous knowledge on their level of virulence in rainbow trout. Viruses were propagated in cell culture and used for infection trials by immersion in rainbow trout juveniles. For each virus, virulence degree (low, moderate, high) was assessed by estimating the survival probability. All isolates were also subject to whole genome sequencing and phylogenetic reconstruction based on protein coding regions. A genome wide association analysis (GWAS) was then performed by combining virulence and sequence data to identify molecular markers putatively involved in VHSV virulence.

Results: A high variability in terms of *in vivo* phenotype was observed, with rainbow trout survival rates ranging between 0 and 100%. Our results clearly indicate that VHSV virulence has a strong genetic basis, with almost all isolates highly virulent for rainbow trout clustering within genotype Ia. However, no clear segregation over the tree was found for viral isolates belonging to the moderate and low virulence classes. The GWAS analysis identified 33 molecular markers dispersed over the genome, which might play a role as genetic determinants for VHSV virulence in rainbow trout.

Conclusion: Our data indicate that VHSV virulence in rainbow trout is highly variable and strongly depends on viral genetic make-up, with Ia sublineage being the most pathogenic for this species. For moderately virulent viruses it was not possible to clearly identify virulence determinants, making it difficult to predict their *in vivo* phenotype. Molecular markers identified by GWAS are currently being validated with reverse genetics.

Keywords: VHSV, trout, pathogenicity, virulence markers

Funding: Anihwa ERA-Net (Novimark, G88F13000660001), UK DEFRA (C7277B).

071-O

Virulence characterization of Italian infectious haematopoietic necrosis virus strains

Abbadi, Miriam (Italy)[1]; Gastaldelli, Michele (Italy)[1]; Zamperin, Gianpiero (Italy)[1]; Pascoli, Francesco (Italy)[1]; Buratin, Alessandra (Italy)[1]; Bedendo, Giulia (Italy)[1]; Toffan, Anna (Italy)[1]; Panzarin, Valentina (Italy)[1]

1 - Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy

Introduction: Although IHNV has always been associated to a low level of mortality, in the last years its impact on Italian trout farming has progressively increased to the point of determining severe disease outbreaks and significant production losses. However, due to the confounding factors always present in the field and to the lack of record keeping on past IHN outbreaks, the increased virulence of IHNV remains just an empirical observation.

Methodology: To confirm the field evidence and to investigate the virulence of IHNV, a selection of 16 Italian isolates with different genetic features and covering a wide time span was used for challenging rainbow trout juveniles. Their virulence was described in terms of cumulative mortality as well as survival probability estimated by Kaplan-Meier curves. In addition, parametric survival models were used to explore the mortality rate profiles in order to characterize the strain-specific mortality peaks and to relate their topology to virulence and mortality. Finally, correlation between viral fitness, determined with quantitative real-time PCR, and virulence/mortality was assessed in dead and survived animals. A full-genome phylogenetic analysis was also conducted to determine the correlation between virulence phenotype and IHNV genetics.

Results: Italian IHNV presents a variety of virulence phenotypes, as demonstrated by cumulative mortality and survival probability estimates. A positive correlation between maximum mortality probability and virulence was observed for all the strains. Interestingly, our results also indicate that more virulent is the strain, the earliest and narrowest is the mortality peak. Data confirmed a progressive increase of IHNV virulence over time, with the most recent strains showing moderate to high virulence for rainbow trout. Intra-host viral quantification showed a significant correlation between viral replication and virulence. Ongoing phylogenetic analyses will be presented to clarify the relation between evolution and virulence for IHNV.

Conclusion: This study is the first systematic characterization of Italian IHNVs virulence. Results confirm field data pointing out an increased pathogenicity of recent IHNV isolates. Further studies are scheduled in order to investigate the genetic determinants for IHNV virulence in rainbow trout.

Keywords: IHNV, virulence, genetic determinants, rainbow trout

Funding: Anihwa ERA-Net Consortium (Novimark project, contract G88F13000660001).

072-O*

Development of viral reporter systems for the detection of SVCV and VHSV replication

Rice, Annabel (United Kingdom)[1,2]; Kudoh, Tetsu (United Kingdom)[2]; Stone, David (United Kingdom)[1]

1 - Cefas; 2 - University of Exeter

Introduction: Infectious disease outbreaks pose a serious risk to the aquaculture industry and can result in mass mortalities of fish. Consequently, this threatens production and has significant economic implications for fish farmers. Viral diseases, such as Spring viraemia of carp (SVCV) and Viral haemorrhagic septicaemia (VHSV) are particularly problematic due to the lack of commercially available vaccines and anti-viral treatments. Reverse genetics systems can be used to study negative-sense RNA viruses including SVCV and VHSV, allowing genetic manipulation of the viral genetic sequences to learn more about viral pathogenic mechanisms.

Methods: Viral reporter systems for the detection of SVCV and VHSV replicationwere generated, using a reverse genetics approach. Hammerhead (HH) and hepatitis delta (HD) ribozymes were used to cleave the reporter constructs, leaving precise ends of the viral 3' leader and 5' trailer sequences. Expression of green fluorescent protein (GFP) was used as a reporter upon recognition and subsequent transcription during SVCV and VHSV infection. Preliminary testing of the VHSV reporter construct was carried out in *epithelioma papulosum cyprini* (EPC) cells. Co-transfections were performed using plasmids encoding the VHSV N, P and L proteins which are required for viral replication.

Results: GFP expression was observed visually by 32-hours post-transfection of the VHSV reporter construct + N, P & L, however there was no GFP expression in cells transfected with the reporter constructs alone (without N, P and L proteins). RT-qPCR analysis showed a significant increase in the GFP transcript copy numbers at 16 hours and 40 hours post-transfection, in cells transfected with the VHSV reporter construct and N, P and L plasmids compared to cells transfected with the VHSV reporter construct alone.

Conclusion: This data suggests that the VHSV minigenome can successfully be rescued by the N, P and L viral proteins and can provide a useful tool for the visual detection of VHSV replication. Work with the SVCV minigenome is ongoing. The development of these molecular tools will improve our understanding of the pathogenesis of these important viruses, allowing us to develop more effective vaccines and treatments.

Keywords: SVCV, VHSV, reverse genetics, reporter system

Host-Parasite Interactions II

073-O

The salmon louse (*Lepeophtheirus salmonis*) salivary gland secretes immune regulatory proteins

Øvergård, Aina-Cathrine (Norway)[1]; Nilsen, Frank (Norway)[1]; Nuñez-Ortiz, Noelia (Norway)[2]; Bjerga, Gro Elin Kjerag (Norway)[3]; Hamre, Lars Are (Norway)[1]; Chettri, Jiwan Kumar (Norway)[4]; Dondrup, Michael (Norway)[5]; Dalvin, Sussie (Norway)[2]; Sandlund, Liv (Norway)[6]; Buchmann, Kurt (Norway)[4]; Grotmol, Sindre (Norway)[1]

1 - SLRC, Department of Biological Sciences, University of Bergen; 2 - SLRC, Disease and Pathogen Transmission, Institute of Marine Research; 3 - Norwegian Research Centre; 4 - Parasitology and Aquatic Pathobiology, University of Copenhagen; 5 - SLCR, Department of Informatics, University of Bergen; 6 - Pharmaq Analytic, Zoetis

Introduction: Exocrine glands of blood-feeding parasites are believed to be important in pre-digestion of food and in the host-parasite interaction. The salmon louse salivary glands are developed when lice are able to attach to its host, and probably deposit the secretions directly onto the fish. Gaining basic knowledge on the salivary gland secrete is thus of importance.

Methodology: Salivary gland genes were identified by RNA sequencing and *in situ* hybridization. Selected genes were further sequenced, expression analysis conducted, and knock down studies followed by infection studies analyzing the Atlantic salmons cutaneous immune response were done. As it is difficult to perform RNAi studies with the mobile adult lice, a recombinant protein for one gene expressed at this life stage only was successfully synthesized. Immune dampening functions of this recombinant protein were analyzed in LPS stimulated primary leucocytes.

Results: From the RNA sequencing data, ten genes were confirmed to be expressed by the salmon louse salivary gland. Bioinformatic analysis of the open reading frames revealed five proteins with no functional annotation, and five encoding conserved catalytic domains found in metallopeptidases or serine peptidases. Developmental expression analysis showed an increase in expression of most genes shortly after attachment, and these were either found to be highly expressed in the copepodid only, or also at the pre-adult and adult stages. Other salivary gland genes were mainly expressed at the later pre-adult and adult stages. RNAi studies showed dampening effect on the expression of inflammatory cytokines, IL4, MHCII, IgD and IgT. Treatment of primary leucocytes with a recombinant salivary gland protein showed decreased expression of CD8α and IgT.

Conclusion: The present study shows an immune modulatory role for some of the genes expressed in the salmon louse salivary gland. In particular, the inflammatory and humoral immune response were found to be dampened during the early lice stages, while a modulation of the T cytotoxic cell response is likely to be important at pre-adult and adult lice stages.

Keywords: immune modulators, Atlantic salmon, inflammation

Funding: This work was funded by SLCR-Sea Lice Research Centre, grant number: 203513/O30 and by EU H2020 program through ParaFishControl Project (634429).

074-O*

Identification of proteins from the secretory/ excretory products (SEPS) of the branchiuran ectoparasite *Argulus foliaceus* (Linnaeus, 1758)

Ambuali, Aisha (Oman)[1,2]; Monaghan, Sean (United Kingdom)[1]; Mclean, Kevin (United Kingdom)[3]; Inglis, Neil (United Kingdom)[3]; Bron, James (United Kingdom)[1]

1 - Institute of Aquaculture, University of Stirling, Stirling, UK; 2 - Department of Marine Science and Fisheries, College of Agricultural and Marine Sciences, Sultan Qaboos University, Oman; 3 - Moredun Proteomics Facility, Moredun Research Institute, Midlothian, UK

Introduction: *Argulus foliaceus* (Linnaeus, 1758), the freshwater louse, is a member of the branchiuran family Argulidae, which has a widespread distribution and parasitizes a broad range of freshwater fish species, including infections of pike and trout. Previous studies have reported haemorrhagic and inflammatory responses after *A. foliaceus* infection, and have suggested that the parasite secretes bioactive molecules during the feeding process to assist with the ingestion of blood and epithelial tissue. We recently described and characterised the glandular cells associated with the feeding appendages of *A. foliaceus* in depth and this study was undertaken with the objective of identifying the bioactive components secreted from these glands.

Methodology: The *A. foliaceus* secretory/ excretory products (SEPs) were collected from live healthy lice (n= 560, 5-9 lice per tube) after 24 h incubation in tubes of 1mL fresh water at 10°C. Proteins from the SEPs were separated by SDS-PAGE and subsequently identified and characterised using LC-ESI-MS/MS analysis.

Results: Of 45- confidently identified *A. foliaceus* SEPs, 44 had at least one unique peptide to confirm that the protein was present and distinct from other similar proteins. To assign functionality the proteins were searched into InterProscan, 33 proteins gave matches, covering 12 protein families. Twelve proteins gave no match which suggests that either *Argulus* spp. has unique proteins to help in modulating their host, or that these proteins contained partial sequences. SignalP 5.0 software, identified 21 proteins with a signal sequence suggestive of signal peptides. Interestingly, the functional characteristics of *A. foliaceus* proteins/ domains that were revealed have been described earlier from the salivary glands and saliva of other blood feeding arthropods such as ticks. The identified proteins have been classified as transporters, peroxidases, metalloproteases, proteases, serine protease inhibitors and secreted domains.

Conclusion: The current study represents the first proteomics-based attempt to identify and characterise the SEPs of a member of the Argulidae. Here we reveal functional roles of *A. foliaceus* SEPs in digestion and immunomodulation, which may be similar to those used by ticks and other haematophagous arthropods.

Keywords: secretions, immunomodulation, fish lice

Funding: Sultan Qaboos University, Oman.

075-O

Ceratothoa oestroides and sea bass interaction inferred by histology and 3D visualisation

Mladineo, Ivona (Croatia)[1]; Hrabar, Jerko (Croatia)[1]; Vrbatović, Anamarija (Croatia)[1]; Beraldo, Paola (Italy)[2]; Volpatti, Donatella (Italy)[2]; Keklikoglou, Kleoniki (Greece)[3]; Cascarano, Maria Chiara (Greece)[3]; Katharios, Pantelis (Greece)[3]

1 - Institute of Oceanography and Fisheries; 2 - Department of Agro-alimentary, Environmental and Animal Sciences, University of Udine; 3 - Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research

Introduction: The cymothoid isopod *Ceratothoa oestroides* is causing losses in the European sea bass (*Dicentrarchus labrax*) and meagre (*Argyrosomus regius*), and to a lesser extent gilthead sea bream (*Sparus aurata*) aquaculture. To design appropriate strategies for the treatment of *Ceratothoa* infection in aquaculture fish, it is necessary to elucidate the basic host-parasite interaction in respect to pathology and host immune response. Therefore, the aim of this study was to define histological and immunohistochemical traits between the sea bass and attached isopod, and portray it by 3D imaging micro-computational tomography (µ-CT).

Methodology: Infected and uninfected sea bass heads were fixed in Bouin's solution, decalcified, processed and embedded to obtain sagittal sections. Sections were stained with H&E, Masson, Cleveland trichrome and PAS-Alcian blue. Immunohistochemical (IHC) labelling was done to localise PCNA (proliferating cell nuclear antigen), iNOS (inducible nitric oxide syntheses), peroxidase and IgM positive cells. µ-CT was performed for 3D visualisation of the isopod feeding and attachment contact with the host tissues.

Results: The tissues reaction consists of a chronic inflammation of the oro-pharyngeal floor, characterised by a diffuse and marked epithelial hyperplasia of buccal cavity (tongue) and pharynx (gill arches and rakers). The mitotic processes observed in the epithelium, confirm the existence of compensatory tissue regeneration and growth, also evidenced by increased thickness of the epithelium at the attachment site. The inflammatory response is characterised by an abundant mixed cellular infiltrate consisting predominantly of lymphocytes, and a moderate number of eosinophilic granular cells, mostly present under basal membrane. Cell desquamation and marked hyperplasia are seen in the tongue epithelium, along focal ulcerations. Positive immunolocalisation was observed for all tested IHC markers.

Conclusion: Chronic inflammation at the feeding and attachment site of *C. oestroides* represents a defensive response triggered by traumatic action of isopod pereopods/ pleopods and feeding apparatus. The involvement of the innate and adaptive immune components is supported by positive immunolabelling of select markers. 3D visualisation greatly enhances our understanding of parasite-host interaction.

Keywords: *Ceratothoa oestroides*, histopathology, immune response, Mediterranean aquaculture, µ-CT

076-O

Piscirickettsia salmonis extracellular products would decrease *in vitro* macrophage apoptosis

F, Duarte (Chile)[1]; X, Farías (Chile)[1]; C, Maturana (Chile)[2]; J, Palomino (Chile)[1]; Am, Ramírez (Chile)[1]; Pa, Salgado (Chile)[1]; Pa, Smith (Chile)[1]

1 - Faculty of Veterinary Sciences, University of Chile, Santiago, Chile; 2 - Virbac-Centrovet, Santiago, Chile

Introduction: *Piscirickettsia salmonis* is a Gram-negative facultative intracellular bacterium. This organism causes piscirickettsiosis, an especially important disease in salmonid fish reared in sea-cages in Chile. Although *in vivo* this bacterium replicates in several type of fish cells, macrophages seem to be the main target cells for its multiplication. To improve the understanding of piscirickettsiosis pathogenesis, the *in vitro* apoptosis modulation of peritoneal macrophages of rainbow trout (*Oncorhynchus mykiss*) by the extracellular products (ECPs) of *P. salmonis* cultured in CHSE-214 cells was studied.

Methodology: A leukocyte pool obtained from peritoneal washings from ten fish (≈357 g) was used. Cell suspension aliquots were seeded in ultra-low attachment microplate wells and exposed to *P. salmonis* ECPs used in a non-diluted way and at 10^{-1} and 10^{-2} dilutions. Filtered supernatant of non-infected CHSE-214 broken cells was used as control. After exposure for 20 h at 17 °C, the suspensions of each well were tested by flow cytometry using the JC-1 cationic dye to detect apoptotic cells.

Results: Experiment outcomes showed that *P. salmonis* ECPs inhibited apoptosis in macrophage enriched populations (Kruskall-Wallis test, $p \leq 0.05$) and suggest that this effect occurs in a dose-response manner.

Conclusion: These findings are consistent with previous results obtained *in vivo* from salmonid fish and suggest that *P. salmonis* ECPs modulate the apoptosis of immunocompetent cells. It is possible that the apoptosis inhibition of macrophages facilitates the intracellular multiplication of *P. salmonis* in these phagocytic cells and therefore helps to the infectivity process of this pathogen in their fish hosts.

Keywords: *Piscirickettsia*, ECPs, apoptosis, salmonid, fish

Funding: Partially supported by the grant EQM120156 (Fondequip) of the National Commission for Science and Technology (CONICYT-Chile).

077-O*

Vibrio anguillarum expresses some virulence factors in a temperature-dependent manner

Lages, Marta A. (Spain)[1]; Balado, Miguel (Spain)[1]; Lemos, Manuel L. (Spain)[1]

1 - Universidade de Santiago de Compostela

Introduction: *Vibrio anguillarum* is the causative agent of vibriosis, an haemorrhagic septicemia that affects aquacultured warm- and cold-water fish species. As a marine pathogen, *V. anguillarum* must adapt its physiology to the variable environmental conditions of temperature and iron availability. It can grow in a wide range of temperatures (5 ºC-42 ºC) and produces siderophores for iron acquisition. An *in silico* analysis of the strain RV22 genome showed the existence of two siderophore systems, vanchrobactin and piscibactin. While vanchrobactin system is conserved in all *V. anguillarum* isolates, piscibactin is encoded by a high-pathogenicity island homolog to that found in *Photobacterium damselae* subsp. *piscicida*. The contribution of both siderophore systems to virulence showed that piscibactin contributes more to virulence for turbot than vanchrobactin. Notably, the transcriptional analysis of piscibactin genes showed that their expression is higher at 18 ºC than 25 ºC.

Methodology: We studied the adaptative response of *V. anguillarum* to temperature variations and iron deficiency by means of a transcriptomic analysis. The expression levels of the whole genome were compared under iron starvation either at 25 ºC or 15 ºC, conditions that mimic those that the pathogen faces during the infection of warm- or cold-water adapted fish species.

Results: The analysis of the transcriptome of RV22 under iron restriction showed a decrease in the expression levels of metabolic genes and an induction of several virulence factors. The expression patterns of virulence factors showed differences at 25 ºC and at 15 ºC. Genes related to chemotaxis (*che* genes), motility (*fli* and *flg* genes) and T6SSI are preferentially expressed at 25 ºC. Genes related to the hemolysin RTX toxin, T6SSII and genes associated with the synthesis of exopolysaccharides are preferentially expressed at 15 ºC. The expression of piscibactin system is favoured at 15 ºC whereas there is an up-regulation of the vanchrobactin system at 25 ºC.

Conclusion: *V. anguillarum* modulates the expression of virulence factors in response to the environmental temperature and iron availability and this work provides new insights into the mechanisms beneath the temperature-based regulation of piscibactin expression.

Keywords: *Vibrio anguillarum*, vibriosis, virulence factors, siderophores, piscibactin

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078-O

You shall not pass! 25-Hydroxycholesterol based antiviral response can block entry of CYHV-3 into common carp cells

Adamek, Mikolaj (Germany)[1]; Davies, Jonathan (United Kingdom)[2]; Beck, Alexander (Germany)[3]; Jordan, Lisa (Germany)[3]; Becker, Anna (Germany)[3]; Matras, Marek (Poland)[4]; Reichert, Michal (Poland)[4]; Zou, Jun (China)[5]; Bergmann, Sven (Germany)[6]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany; 2 - Keele University, Keele, UK; 3 - Friedrich-Alexander-University, Erlangen, Germany; 4 - National Veterinary Research Institute, Pulawy, Poland; 5 - Shanghai Ocean University, Shanghai, China; 6 - Friedrich-Loeffler-Institut, Insel Riems, Germany

Introduction: Cholesterol is essential for building and maintaining cell membranes. Furthermore, it is a main component of lipid rafts and by this modulates membrane fluidity. This makes cholesterol critical for several steps in the viral replication cycle, especially for enveloped viruses. Hence, mammalian cells respond to virus infection with the induction of the oxysterol 25-hydroxycholesterol (25HC), which cannot be sequestered by the viruses. 25HC is a soluble antiviral factor, which is produced from cholesterol by activation of cholesterol 25-hydroxylase (CH25H). This response was shown stopping the entry process of a very broad spectrum of mammalian viruses, however immune responses underlying the induction of CH25H were largely not studied in fish.

Methodology: Putative genes encoding for CH25H were identified, amplified and molecularly cloned in common carp and rainbow trout. The modulation of cholesterol 25-hydroxylase gene expression and protein levels was measured during several viral infections *in vitro* and *in vivo*. Furthermore an HPLC-MS method was established for measuring oxysterols in fish cells. Recombinant type I interferon was used to study the IFN dependence of *ch25h* induction.

Results: The *ch25h* expression is strongly modulated *in vitro* and *in vivo* during viral infections. HPLC-MS analyses showed that even fibroblastic cells are capable of producing 25HC and its metabolite 7a,25diHC. The 25HC had an antiviral activity by blocking the entry of cyprinid herpesvirus 3 (CyHV-3) but not spring viremia of carp virus (SVCV) and common carp paramyxovirus (CCPV) in KFC cells and viral haemorrhagic septicaemia virus (VHSV) and infectious pancreatic necrosis virus (IPNV) in RTG-2 cells. The stimulation of RTG-2 cells with rainbow trout recombinant type I IFN provided further evidence that despite the fact that the CH25H based antiviral response coincides with type I IFN responses; it is not type I IFN dependent.

Conclusion: Our results give substantial evidence that CH25H activation is an antiviral response to a very broad spectrum of viruses in both common carp and rainbow trout cells *in vitro*. It can functionally block the entry of CyHV-3. Interestingly, the vulnerability of CyHV-3 to 25HC is counteracted by a downregulation of *ch25h* gene expression in carp fibroblasts during CyHV-3 infection.

Keywords: CyHV-3, 25-hydroxycholesterol, cholesterol 25-hydroxylase

079-O

Applications, potential and challenges of an *in vitro* heart model: salmon cardiac primary cultures (SCPCS)

Noguera, Patricia (United Kingdom)[1]

1 - Health and Welfare, Marine Scotland Science, Aberdeen, UK

Introduction: Fish based models are well established and extensively applied in human biomedical research but, paradoxically, less frequently focused on fish issues or specifically, fish health. While mimicking the high complexity of living systems is not fully achievable *in vitro*, major advances on cellular models and a strong shift towards application of 3R principles on animal research, has made novel in vitro alternatives an attractive and necessary tool for host-pathogen interaction and pathogenesis studies also in fish research.

Methodology: Embryonated eggs with ~480 accumulated thermal units were processed to SCPCs and infected following protocols previously described.

Results: SCPCs successfully generated from both wild or farmed salmon embryos were maintained under laboratory conditions for several months with minimal support. After virus infection (salmonid alphavirus; SAV), the replication and kinetics of infection could be followed and the model was shown also suitable for electron microscopy (TEM) studies and live cell imaging.

Conclusion: An *ex vivo* heart model developed from Atlantic salmon embryos was tested as a model for host-pathogen interaction during viral infections and for genotypic differences in disease resistance. An important advantage over cell culture monolayers is the presence of cardiomyocytes, endothelial cells and fibroblast, all contributing to functional, structural, biochemical, mechanical or electrical properties as in the normal fish hearts. The removal in the model of a layer of functional complexity (acquired immunity), makes possible to focus on tissue specific, early innate immune mechanisms. A very small numbers of embryos are required to generate sufficient experimental SCPS numbers and although the small size represents a challenge, it means also less material and smaller facilitates are required, thus reducing the cost of research. The full potential of the model requires further characterizations for a deeper understanding of mechanisms of regeneration and ageing processes, metabolism or electrophysiology. The gold standard would be the comparison with results from same cohort fish under *in vivo* experiments.

Keywords: Atlantic salmon, *in vitro* heart model

Funding: Marine Scotland Science.

Diseases of Uncertain Aetiology

080-O*

Pathological characterization of emerging disease: hematomas and hepatic rupture syndrome in rainbow trout (*Oncorhynchus mykiss*)

Godoy, Marcos (Chile)[1,2,3]; Suarez, Rudy (Chile)[1,2,4]; Kusch, Karina (Chile)[1,2]; Medina, Daniel (Chile)[1]; Cardenas, Denis (Chile)[1]

1 - Laboratorio de Biotecnología Aplicada, Facultad de Medicina Veterinaria, Universidad San Sebastián, Chile; 2 - Centro de Investigaciones Biológicas Aplicadas, Chile; 3 - Doctorado en Acuicultura, Programa Cooperativo Universidad de Chile, Universidad Católica del Norte, Antofagasta, Chile; 4 - Magister en Acuicultura, Universidad Católica del Norte, Coquimbo, Chile

Introduction: Since 2012, a new pathological condition has been registered that affects mainly the cultivation of Rainbow trout (*Oncorhynchus mykiss*), known as Hepatic rupture syndrome (HRS), Anasarca anemia trout syndrome (SAAT) or officially called Syndrome idiopathic trout (SIT). The main affected farm site is located in Reloncavi Estuary and some sporadic cases have been recorded in Chiloe Island. Currently, the etiology of the disease remains unknown.

Methodology: Fish samples were collected from 17 farming sites during 2012 to 2014. A number of 103 fish were taken for necropsy to microbiological analysis, while organs from 50 fish were fixed in 10% formalin buffered for histopathological analysis. In addition, microbiological, cell culture and molecular qPCR characterization to detect relevant salmonid farming endemic and exotic pathogens were carried out.

Result: Affected fish had good nutritional status, with an average weight ranged between 0.38 to 3.12 kg. However, they presented darkening of the skin and lethargy. The external clinical signs include the presence of protruding anus, distended abdomen, exophthalmia and cutaneous edema. Internally, the main clinical signs were the presence of hydropericardium, hemopericardium, ascites, clots in the abdominal cavity and hepatic hematomas. Into the main histopathological findings, the presence of edema was the most prevalent in the tissues analyzed, also, branchial telangiectasia, splenic tissue congestion, hepatic necrosis, hepatic peliosis and gastrocalcinosis were identified. Molecular detection by qPCR shows a low pathogenic diversity, detecting mainly the presence of *Piscirickettsia salmonis* and *Nucleospora salmonis*.

Conclusion: The macroscopic and histopathological findings show that the fish with presence of hematoma syndrome and hepatic rupture present systemic circulatory disturbance. Currently, the etiology and risk factors for the presentation of the disease are unknown.

Keywords: hematomas, hepatic rupture syndrome, emerging disease, rainbow trout, pathology

Funding: This work was funded by Universidad San Sebastian and by the Centro de Investigaciones Biológicas Aplicadas (CIBA).

081-O

Cysts of unknown etiology (CUES): new insights and main factors affecting their prevalence in Mediterranean wild fish populations

Constenla, Maria (Spain)[1]; Padrós, Francesc (Spain)[1]; Moyà-Alcover, Catalina Marta (Spain)[1]; Dallarés, Sara (Spain)[1]; Pérez-I-Garcia, David (Spain)[1]; Carrassón, Maite (Spain)[1]

1 - Universitat Autònoma de Barcelona

Introduction: Cysts of unknown etiology (CUEs) are small round whitish cysts located mainly in the gills. Microscopically, they are formed by concentric acellular layers surrounding a large central core of eosinophilic material. Connective tissue and capillary vessels are usually observed in the vicinity of the cysts. The origin of these cysts remains still undetermined. Several studies associate their presence to environmental pollution but other factors may also be involved.

Methodology: An extensive survey was carried out in a wide area of north-western Mediterranean Sea in order to evaluate the potential association of biological and ecological factors with the presence of these cysts. A total of 1802 fish representing 29 different species were collected and sampled seasonally and at different depths from the coast off Barcelona, Tarragona and Balearic Islands (2007-2011). Biometric data and the trophic level of each fish, as well as data on environmental variables (T, S, O, turbidity, chlorophyll A and MO) and pollutants (heavy metals, PCBs, PAHs, HCHs and DDX) were also collected and analysed (Generalized Linear Models and Ramdon Forest) in relation to the prevalence of the CUEs detected using routine histology.

Results: None of the chondrichthyan species analyzed presented CUEs. Among the Actinopterygii, only four fish species from the platform and six from the continental slope did not presented CUEs in the gills. *Phycis blennoides* was the fish species with the highest prevalence (71%), followed by *Merluccius merluccius* and *Micromesistius poutassou* (68% and 67%, respectively). The prevalence of CUEs seems to be strongly related with phylogeny since significant differences in prevalence were observed by order, family and fish species. Fish size is also an important factor, and interacts with other factors significantly related to the presence of CUEs, such as location, season and depth. Turbidity is the only environmental variable significantly related with CUEs.

Conclusion: Contrary to common believe, pollutants do not seem to be related to the presence of these cysts. Results clearly indicate that phylogeny is the most important factor related to the presence of CUEs.

Keywords: CUEs, marine wild fish, environment

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082-O

Skin health of chinook salmon farmed in New Zealand

Nowak, Barbara (Australia)[1]; Johnston, Hazel (New Zealand)[2]; Johnston, Colin (New Zealand)[2]; Comezzi, Francesco (Australia)[1]; Walker, Seumas (New Zealand)[3]; Anderson, Kelli (Australia)[1]; Ghosh, Bikramjit (Australia)[1]; Lopez, Cesar (New Zealand)[4]; Preece, Mark (New Zealand)[4]; Symonds, Jane (New Zealand)[3]

1 - University of Tasmania; 2 - Brightwater Consulting; 3 - Cawthron Institute; 4 - New Zealand King Salmon

Introduction: Chinook salmon (*Oncorhynchus tshawytscha*), first introduced in New Zealand in 1875 from California USA by Hawkes Bay Society (McDowall, 1994), is the only salmonid species commercially farmed in New Zealand, although trout are cultured for release by Fish and Game New Zealand. There are several farming areas in the South Island of New Zealand, including Marlborough Sounds (MS), Canterbury, Otago and Stewart Island (Aquaculture New Zealand website). While the industry has not been affected by the major production diseases seen internationally, skin lesions have been recorded for many years with a significant increase in prevalence from 2012 onwards in the Marlborough Sounds associated with elevated summer mortalities on farms in 2015.

Methodology: Skin samples were collected from affected and apparently health fish and processed for histology, pathogen detection and gene expression analysis.

Results: We developed case definitions and detailed descriptions for spots, spreading spots, ulcerated spreading spots and MS ulcers, defining a positive case for three study units, including individual fish, pen/unit and farm. We have determined the relationship between those lesions and presence of New Zealand rickettsia-like organisms and *Tenacibaculum maritimum*. Risk factors such as temperature, biofouling levels on fish nets and other environmental variables have been investigated.

Conclusion: These skin conditions have most likely a multi-factorial aetiology. Our current understanding of the risk factors will focus our research into the mitigation methods to reduce the prevalence of these conditions in the farmed stocks.

Keywords: chinook, skin, RLO, *Tenacibaculum*

Funding: New Zealand's Ministry of Business, Innovation and Employment for the research programme "Aquaculture Health Strategies to Maximise Productivity and Security" (CAWX1707).

083-O

Effect of antibiotic in-feed treatment against red mark syndrome in rainbow trout

Schmidt, Jacob G. (Denmark)[1]; Olesen, Niels Jørgen (Denmark)[1]

1 - DTU Aqua, Unit for Fish and Shellfish Diseases, Kemitorvet, bld. 202, 2800 Kgs. Lyngby, Denmark

Introduction: Red mark syndrome (RMS) is a skin disease of rainbow trout, the prevalence of which has increased in Europe over the last decade or two. Hallmark symptoms are large, haemorrhagic skin lesions. It is believed that the disease is bacterial and caused by a *Midichloria*-like organism (MLO). However, the bacterium has never been isolated or cultured *in vitro*, and is only known from its 16S rDNA sequence. Thus there is no vaccine for the disease, and no other treatment for RMS symptoms has been tested under controlled experimental conditions. However, antibiotic treatment is used on some farms with apparent effect, whereas other fish farmers report that they see no apparent effect of antibiotics on RMS symptoms.

Methodology: Here we investigate for the first time the effect on RMS of in-feed treatment with three types of antibiotics: florfenicol, oxolinic acid and oxytetracycline. In short, specific pathogen free (SPF) fish were cohabited with RMS-affected seeder fish. When the SPF fish started developing RMS symptoms they were divided into 8 tanks with 20 fish in each. There were four treatment groups (3 types of antibiotics and control feed) in duplicate as well as a negative control tank with healthy SPF fish. The effect of treatment was evaluated by visual inspection of RMS lesion development as well as quantification of the putative cognate pathogen (MLO) by qPCR.

Results: All three types of antibiotics affected both of the monitored disease parameters, with less MLO detected in skin and less macroscopic skin pathology observed in treatment groups compared to controls.

Keywords: antibiotics, MLO, red mark syndrome

084-O

Epizootic skin tumors in pike-perch (*Sander lucioperca*) from Lake Constance in Austria

Lewisch, Eva (Austria)[1]; Kolodziejek, Jolanta (Austria)[2]; Dinhopl, Nora (Austria)[3]; Reifinger, Martin (Austria)[3]; Nowotny, Norbert (Austria)[2]; Schachner, Oskar (Austria)[1]; Soliman, Hatem (Austria)[1]; El-Matbouli, Mansour (Austria)[1]

1 - Clinical Division of Fish Medicine, University of Veterinary Medicine Vienna; 2 - Institute of Virology, University of Veterinary Medicine, Vienna, Austria; 3 - Institute of Pathology, University of Veterinary Medicine, Vienna, Austria

Introduction: Skin neoplasms in pike-perch (*Sander lucioperca*) with an estimated prevalence of 5% have been observed in Lake Constance by local fishers in Vorarlberg, Austria, since 2014. Gross morphology of the tumors resembled walleye dermal sarcoma (WDS) and walleye discrete epidermal hyperplasia (WDEH), both retroviral diseases not encountered in Europe so far.

Methodology: Three adult fish (one from November 2018, two from February 2019) and a tumor from another one (December 2018) were collected for examination. Macroscopic and histopathological examinations were performed. For molecular-genetic investigations 50 mg tissue samples of each tumor were stored in RNAlater at 4 °C and without any additive frozen at -80 °C until nucleic acid extraction, following routine protocols. Cell culture was performed on *epithelioma papillosum cyprini* (EPC), bluegill fry (BF-2) and chinook salmon embryo (CHSE-214) cell lines. Additionally, tumor tissue was explanted in cell culture, following a previously described protocol. For transmission electron microscopy (TEM) samples were proceeded customarily. Until now several RT-PCRs were performed on eight individual and seven pooled samples with five different primer pairs which target the sequences of the *rv-cyclin* gene (OrfA), the *gag*-gene, the *env*-gene and the LTR5' of WDSV.

Results: Upon morphological inspection, white to pale red nodules disrupting the epidermis with bleedings and a lobated surface were evident. Size varied from a few millimeters to 3 cm. Lesions were found distributed over the whole fish body, including operculum and fins. Seemingly older lesions tended to ulcerate. So far, no retrovirus-specific amplification product was detected. Also, attempts of virus cultivation and tumor cell explantation were yet unsuccessful. Histology revealed substantial differences to tumors caused by WDSV and WEHV. The tumors consisted of lymphoid/histiocytic cells invading the connective tissue of the dermis and muscle. Evaluation by TEM revealed several 90-120 nm retrovirus-like particles.

Conclusion: Gross morphology of the tumors strongly resembled an infection with WDSV or WEHV. Obvious differences in tumor histology, negative PCR results and the evidence of retrovirus-like particles provide reasons for further investigations.

Keywords: neoplasm, walleye dermal sarcoma, walleye discrete epidermal hyperplasia, retrovirus, pike-perch

Viruses and Viral Diseases III

085-O

Pike, a "discrete vector" of viral hemorrhagic septicemia virus (VHSV) and infectious hematopoietic necrosis virus (IHNV)?

Cabon, Joëlle (France)[1]; Louboutin, Lénaïg (France)[1]; Almeras, Fabrice (France)[1]; Baud, Marine (France)[1]; Pallandre, Laurane (France)[1]; Langlois, Yan (France)[2]; Morin, Thierry (France)[1]

1 - French Agency for Food, Environmental and Occupational Health & Safety (Anses), Ploufragan-Plouzané-Niort Laboratory, Unit Viral Diseases of Fish, National Reference Laboratory for listed fish diseases, Plouzané, France; 2 - DDCSPP57, France

Introduction: VHSV is a novirhabdovirus listed at the European level responsible for severe disease in several freshwater fish species, particularly rainbow trout *Oncorhynchus mykiss* (RT). Since 2011, several isolations of VHSV were reported in France, particularly in two fish farms located in an area rich in ponds containing a large diversity of white fish but also pike (*Esox lucius*), a species susceptible to VHSV (OIE 2017).

Methodology: During the most recent outbreak in 2016, VHSV from these two farms showed identical full Glycoprotein gene sequences and close relationship with previously characterized isolates, with nevertheless some significant Single Nucleotide Polymorphisms. Epidemiological and laboratory investigations were engaged to identify the putative origins for viral dissemination in this specific hydro-geographical context.

Results: Analysis of a potential carriage by wild fish allowed identifying pike infected by both VHSV and IHNV in 1 out of 9 sampled ponds located in the incriminated area. VHSV isolates belonged to Ia genotype and shared high nucleotide identities with 2014 and 2016 French isolates from RT. Pike IHNV clustered in European genotype but seemed quite different from recent isolates circulating on the French territory. Both VHSV and IHNV pike isolates were find to be highly virulent for RT through experimental contamination, with at least 85% of mortality after bath infection. No mortality was observed for one-year-old pike infected by the same route but at least 60% of pike fingerlings died after VHSV infection. The cumulative mortality reported for IHNV experimentally infected fingerlings remained very slight, despite the observation of characteristic clinical signs on dead fish. Interestingly, VHSV or IHNV were isolated from RT fingerlings put in contact with infected pike several days after the initial contamination.

Conclusion: Pike could represent a silent vector for persistence of VHSV and IHNV in particular hydro-geographic areas, contributing to recurrent infection events in salmonid farms. Complementary *in vivo* investigations should be carried out to deeply investigate its susceptibility and better characterize the level of risk associated to a potential virus release.

Keywords: viral hemorrhagic septicemia virus, infectious hematopoietic necrosis virus, pike *Esox lucius*, experimental infection, transmission

Funding: European Commission - Horizon 2020 - Research and Innovation Framework Program under Grant Agreement No. 731014 (VETBIONET; http://www.vetbionet.eu/).

086-O*

Micro-carriers for cultivation of fish cells and propagation of koi herpesvirus

Jordan, Lisa Katharina (Germany)[1]; Baldus, Stefan (Germany)[1]; Michalowski, Alexander (Germany)[1]; Schneidereit, Dominik (Germany)[2,3]; Friedrich, Oliver (Germany)[2,3]; Becker, Anna Maria (Germany)[1]

1 - Institute of Bioprocess Engineering, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany; 2 - Institute of Medical Biotechnology, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany; 3 - Erlangen Graduate School in Advanced Optical Technologies (SAOT), Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany

Introduction: *In vitro* propagation of the cyprinid herpesvirus-3 (CyHV-3, also koi herpesvirus, KHV) is needed for diagnostic and research purposes. Available cell lines for replication of this virus, e.g. common carp brain cells (CCB), are adherent. Despite all efforts to obtain reproducibly high virus titers, only rarely, titers >10^8 $TCID_{50}$/mL could be determined. This might be due to virus/cell characteristics but can also be connected to limitations regarding cultivation of adherent cell lines. In comparison, cultivation of cells in suspension is advantageous with regard to nutrient and oxygen supply as well as space. Therefore, especially when high virus titers are required, e.g. for vaccine production, cultivation of adherent cells on micro-carriers in stirred or shaken vessels is used.

Methodology: In this work, the cultivation of CCB cells on dextran based, collagen coated micro-carriers (Cytodex-3) was examined. To monitor the attachment to the carriers and cell growth, the cell density was determined by 3D fluorescence microscopy of cells stained with Hoechst 33342. Additionally, viability and glucose consumption during cultivation were assessed using photometric assays. Multiple parameters for cell growth were examined, e.g. various stirring rates, micro-carrier and cell concentrations, different vessels and culture media.

Results: A maximum cell density of >300 cells/micro-carrier (>3.5 x 106 cells/mL culture) was achieved 9 days after seeding in spinner flasks incubated at 25 °C with a stirring rate of 75 rpm and applied concentrations of 12,000 micro-carriers/mL and 30 cells/micro-carrier at seeding. Furthermore, KHV replication in CCB cultivated on micro-carriers was tested, indicating promising titers >10^9 $TCID_{50}$/mL using the experimental setup outlined above. In comparison, the maximum titers obtained with the same virus isolate and cells from cultivation in adherent cell flasks were ~10^8 $TCID_{50}$/mL.

Conclusion: The presented work provides insights into an alternative cultivation system for fish cells and its potential for virus replication. Further examination and optimization of this system might enable even higher titers, thus being very promising for further application in vaccine development and production.

Keywords: micro-carrier cell cultivation, suspension cell cultivation, *in vitro* replication, cyprinid herpesvirus-3, koi herpesvirus

Funding: German Federal Ministry of Food and Agriculture (BMEL) through the Federal Office of Agriculture and Food (BLE), grant number 2815HS010.

087-O*

Infection of European eel by anguillid herpesvirus 1: physical contacts between infected and naïve eels enhance viral transmission

Delrez, Natacha (Belgium)[1]; Zhang, Haiyan (Belgium)[1]; Denoel, Mathieu (Belgium)[2]; Mélard, Charles (Belgium)[4]; Suarez, Nicolas (United Kingdom)[3]; Davison, Andrew (United Kingdom)[3]; Boutier, Maxime (Belgium)[1]; Vanderplasschen, Alain (Belgium)[1]

1 - Immunology-Vaccinology, Department of Infectious and Parasitic Diseases, Fundamental and Applied Research for Animals & Health, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium; 2 - Laboratory of Fish and Amphibian Ethology, Behavioral Biology Unit, Department of Biology, Ecology, and Evolution, University of Liège, Liège, Belgium; 3 - MRC-University of Glasgow, United Kingdom; 4 - Aquaculture Research and Education Centre, University of Liège, Liège, Belgium

Introduction: Over the last few decades, the number of European eel (*Anguilla anguilla*) reaching Europe has declined by 99%, justifying its current classification as a critically endangered species. Among the multiple factors contributing to this decline, viral infection caused by anguillid herpesvirus 1 (AngHV-1) is thought to play a key role. Here, we aimed to investigate the pathogenesis of the AngHV-1 in his natural host using *in vivo* bioluminescent imaging system (IVIS).

Methodology: First, we produced a recombinant strain (hereafter called LucGFP strain) encoding a bicistronic reporter expression cassette inserted in the intergenic region between open reading frame (ORF) 32 and ORF33. This cassette driven by the EF1 promoter led to detectable expression of both firefly luciferase and copepod GFP in infected cell cultures. Next, AngHV-1 portal of entry was investigated in juveniles eels infected with the LucGFP strain. We used different modes of inoculation mimicking epidemiological conditions, than latter analysed fish by IVIS according to time post infection (p.i.).

Results: Ingestion of infectious material (oral contamination) led to no detectable infection. Immersion in water containing the virus (contamination by immersion) and intraperitoneal (IP) injection led to a strong bioluminescent signal by IVIS analysis from day 3 post infection. Among these groups, clear clinical signs appeared from day 6 p.i. and were characterized by lethargy, discolored skin patches, ulcers, erythema and in some cases, abnormal vertical swimming. First bioluminescent signal was detected on the skin – mainly on the head and tail-, while internal organs were still negative. Finally, naïve eels were contaminated by cohabitation with infected eels. This mode of inoculation led to rapid and efficient transmission of AngHV-1 through epidermal infection. Localization of foci of infection on the skin of contaminated eels strongly suggested the roles of physical interactions in the transmission process.

Conclusion: Together, the data obtained in the present study demonstrate that the skin is the portal of entry of AngHV-1 in eels and unravel the importance of physical interactions between infected and naïve subjects in the epidemiology of the infection.

Keywords: European eel, herpesvirus, pathogenesis

Funding: Research Fellow FNRS.

088-O

Prevention and control management of grass carp hemorrhagic disease (GCHD) in China

Wang, Qing (China)[1]; Zeng, Weiwei (China)[1]; Wang, Yingying (China)[1]; Li, Yingying (China)[1]; Yin, Jiyuan (China)[1]; Liu, Chun (China)[1]; Chang, Ouqin (China)[1]; Ren, Yan (China)[1]; Bergmann, Sven M (Germany)[2]; Shi, Cunbin (China)[1]

1 - Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences; 2 - Friedrich-Loeffler Institut

Introduction: Grass carp, *Ctenopharyngodon idellus*, is one of the main species of farmed freshwater fish in China. However, frequent outbreaks of GCHD due to grass carp reovirus (GCRV) cause approximately 20% decline of the annual production of grass carp. In present study, an inactivated vaccine against GCRV genotype II as well as an oral vaccine by combining *Bacillus subtilis* spore with GCRV vp4 protein was established and evaluated its protection in grass carp.

Methodology: The isolate HuNan1307 was produced onto the grass carp cell line PSF, and inactivated with 1% b-propiolactone for 60 h at 4 ºC. Grass carp were injected with inactivated GCRV vaccine followed by challenge. *B. subtilis* spores was used as the oral delivery system and successfully constructed the *B. subtilis* CotC-VP4 recombinant spores (CotC-VP4 spores) to evaluate its protective efficacy in grass carp.

Results: All grass carp immunized with the inactivated vaccine produced a high titer of serum antibodies and GCRV-specific neutralizing antibody. Moreover, the inactivated vaccine injection increased the expression of 6 immune-related genes in the spleen and head kidney. In addition, grass carp immunized with the inactivated vaccine showed a survival rate above 80% after the viral challenge, and the protection lasted at least for one year. Grass carp orally immunized with CotC-VP4 spores showed a survival rate of 57% and the relative percent survival (RPS) of 47% after the viral challenge. Further, the specific IgM levels in serum and the specific IgZ levels in intestinal mucus were significantly higher in the CotC-VP4 group than those in the Naive group. The immune-related genes including three innate immune-related genes, four adaptive immune-related genes, three inflammation-related genes and interferon type I (IFNI) related signaling pathway genes were significantly up-regulated in the CotC-VP4 group.

Conclusion: The inactivated HuNan1307 vaccine induced a strong humoral immune response and conferred effective protection against GCRV infection in grass carp. The CotC-VP4 spores produced protection in grass carp against GCRV infection, and triggered both innate and adaptive immunity post oral immunization. They both provided efficient vaccine candidate to control GCHD in china.

Keywords: grass carp reovirus, grass carp hemorrhagic disease, vaccination

Funding: Chinese Academy of Fishery Sciences (2016ZD0503).

251-O

First detection of infectious spleen and kidney necrosis virus (ISKNV) associated with massive mortalities in farmed tilapia in Africa

K. Paley, Richard (United Kingdom)[1]; Ramírez-Paredes, José Gustavo (United Kingdom)[2]; Hunt, William (United Kingdom)[2]; W. Feist, Stephen (United Kingdom)[1]; M. Stone, David (United Kingdom)[1]; Field, Terry (United Kingdom)[2]; Haydon, David (United Kingdom)[2]; Ziddah, Peter (United Kingdom)[3]; Duodu, Samuel (United Kingdom)[4]; Stephen Wallis, Timothy (United Kingdom)[2]; W. Verner -Jeffreys, David (United Kingdom)[1]

1 - Cefas Weymouth Laboratory, Weymouth, Dorset, England, United Kingdom; 2 - Ridgeway Biologicals Limited, Units 1-3 Old Station Business Park, Compton, Berkshire, England; 3 - Fisheries Commission, Ministry of Fisheries and Aquaculture Development, Accra, Ghana; 4 - University of Ghana, Accra, Ghana

Introduction: The tilapia industry in Ghana has expanded rapidly in the last 10 years, with more than 90% of production taking place on Lake Volta. In late 2018, unusual patterns of very high mortality (>50% production) were reported in intensive tilapia cage culture systems across Lake Volta in Ghana. Affected fish showed darkening, erratic swimming and abdominal distension and associated ascites.

Methodology: Material was taken from two farms experiencing highly elevated mortalities for histological, bacteriological, virological and molecular biological analyses.

Results: Histopathological observations revealed the presence of lesions in the tissues that were indicative of viral infection. These included haematopoietic cell nuclear and cytoplasmic pleomorphism with marginalisation of chromatin and fine granulation. Affected cells viewed using transmission electron microscopy contained conspicuous virions with typical iridovirus morphology i.e. enveloped, with icosahedral and or polyhedral geometries with a diameter 160 nm and forming arrays. PCR confirmation and sequencing identified the likely cause of the disease as infectious spleen and kidney necrosis virus (ISKNV). Samples collected from two affected farms, including samples of fry, during the mortality event, were all strongly positive for the presence of the virus by qPCR. All samples tested negative for TiLV and nodavirus by qPCR. Archived samples collected prior to the mortality event at these and other nearby farms were negative for ISKNV.

Conclusion: The results indicate that ISKNV was the cause of acute disease on the investigated farms and likely had a primary role in the mortality events. This is not the first detection of ISKNV in tilapia but is the first reported association with massive mortalities and the first detection of ISKNV in Africa.

Funding: Ridgeway Biologicals; Defra FX001 supporting the OIE Collaborating Centre for Emerging Aquatic Animal Diseases at Cefas.

Immunomodulators and Aquatic Animal Health

089-O

Significance of immunomodulators in control of aquatic animal health – over 30 years of application and actual possibility

Siwicki, Andrzej (Poland)[1]

1 - Department Microbiology and Clinical Immunology, University of Warmia and Mazury in Olsztyn, Poland

Introduction: The immune status of fish held in hatcheries, farms and aquaculture net-pens is negatively effected of many factors including poliethiological stress, environment pollution or xenobiotics and treatments with chemotherapeutics. The effects of these factors may impair the protective mechanisms of the fish and together with the accumulation of various pathogenic flora in aquatic environment, conditions become favourable to the occurrence of infectious diseases, frequently with a mixed etiology. Therefore, it is of great importance to study the early detection of immune deficiencies and the stimulation or modulation of nonspecific cellular and humoral immunity and protection against diseases.

Methodology: The collection of literature and own experience have enabled the collection of data for this review.

Results: The use of immunomodulators and vaccines in fish culture offers a wide range of attractive methods for inducing and modulating protection against infectious diseases, especially in fingerling. In general, immunostimulants and/or immunomodulators comprise a group of natural and synthetic compounds that enhance the cell-mediated and humoral-mediated immunity in human and animals. Some of them only stimulate the defence mechanisms, other are able to restore or modulate immunity after suppression induced by xenobiotics. The stimulation of the nonspecific defence mechanisms may be particularly important for fish that are raised in or released to environment where the species or serotypes of pathogens are unknown and immunization by specific vaccines may be futile. Several promising biological modifiers, adjuvants and drugs have been tested in fish. The effects of immunomodulators on the fish were controlled by special *in vitro* and *in vivo* protocol firstly presented by group of dr Douglas P. Anderson from NFHRL Leetown, USA. After we developed the methods of control the influence of immunomodulators on cellular and humoral defence mechanisms and protection against diseases by challenge test. Immunostimulants may be used in patterns similar to those of chemotherapeutics and in combination with vaccine. The fish could be prepared for a predicted event, such as seasonal exposure to pathogens or handling stress, by a treatment prior to the event.

Conclusion: Many scientists developed protocols for the use of immunostimulants in fish, including timing, dosage requirements, temperature, stability of each component, species of fish, time and length of application and influence on the environment.

Keywords: immunomodulators, immune response, prevention of diseases

090-O*

Addition of fulvic acid to fish production water increases growth performance and stimulates the immune response

Lieke, Thora (Germany)[1]; Meinelt, Thomas (Germany)[1]; Knopf, Klaus (Germany)[1]; Kloas, Werner (Germany)[1]

1 - Leibniz Institute of Freshwater Ecology and Inland Fisheries

I**ntroduction:** Intensification in aquaculture is accompanied by problems such as increased stress and infections. Negative impacts of chemical control agents and drug residues to environment and health have become more apparent. Treatment or control of diseases in aquaculture by using immune modulatory feed supplements has therefore become an increasing field of research. We evaluated the effect of addition of a fulvic acid to the production water of rainbow trout (*Oncorhynchus mykiss*).

Methodology: Juvenile rainbow trouts were exposed to 5 and 50 mg C/L fulvic acid for 4 weeks in a flow through system. Growth parameters, blood samples, gill, and head kidney were taken from 12 fish per group per sampling. Subsequently, fish were stressed by air exposure and recovery was evaluated by taking blood samples after 15 and 90 min. Two days post-sampling, remaining fish were sampled for effects of stress on immune parameters. Respiratory burst and phagocytic activity were analyzed from head kidney, furthermore, protein content, lysozyme activity and total antioxidative capacity was measured from serum and gill.

Results: Exposure to fulvic acid resulted in increased growth (length and weight) performance in the 5 mg C/L group and significant increase in the 50 mg C/L group. Furthermore, the feed conversion ratio was decreased. Immune response was improved as shown by increase of respiratory burst and phagocytoses activity in the 50 mg C/L group. Serum lysozyme and hematocrit was not affected. However, after stressor, groups exposed to fulvic acid hat significantly higher amounts of blood cells than untreated fish.

Conclusion: Exposure to fulvic acid to the production water had beneficial effects on growth performance and immune response of juvenile rainbow trouts. Our results show, that fulvic acid addition improves output of trout aquaculture production. Fish were less stressed and had better defense mechanisms, which could help to decrease infections and subsequent mortality. Although further research is need, fulvic acid can be used as supplementation and has the potential to decrease use of chemical therapeutants against diseases.

Keywords: fulvic acid, immunomodulator, water additive, rainbow trout

Funding: AiF Projekt GmbH (Federal Ministry for Economic Affairs and Energy) under project No. ZF4240502SK7.

091-O

Anti-inflammatory effects of ß-1,3/1,6 glucan supplemented feeds in farmed salmon

Midtlyg, Paul J. (Norway)[1]; Sulen Tavara, Ana Carolina (Norway)[2]; Dubey, Saurabh (Norway)[1]; Gamil, Amr (Norway)[1]; Munang'andu, Hetroney M. (Norway)[1]; Mutoloki, Stephen (Norway)[1]

1 - Faculty of Veterinary Medicine, Norwegian University of Life Sciences; 2 - Center for Molecular Medicine Norway, Faculty of Medicine, University of Oslo

Introduction: Two recently completed studies have provided evidence that orally administered ß-1,3/1,6 glucans have anti-inflammatory effects in fish.

Methodology: In both trials, groups of Atlantic salmon post-smolts were fed a commercial salmon pelleted diet without (control) or with supplementation of 0.1% of a ß-1,3/1,6 glucan (MG diet). After 4 weeks of priming, both study groups were vaccinated intraperitoneally using a multivalent, oil adjuvanted salmon vaccine. This and similar vaccine formulations are known to induce intra-abdominal inflammatory reactions in the fish during the subsequent period. Several time points after immunisation, the lymphoid organs of both feeding groups were sampled and the expression of inflammation-associated cytokine genes was assessed by qRT-PCR. The trial setup was first performed in seawater adapted smolts held at ambient temperatures of ca 8 °C, and thereafter repeated in parr reared at in a freshwater recirculation system at 14 °C in for confirmation.

Results: In head kidney and spleen there was a weak upregulation of IL-1β in both dietary groups shortly after vaccination. However, at several time points up to 60 days thereafter, a clear down-regulation of the pro-inflammatory cytokines TNF-α and IL-17a in head kidney and spleen of the MG dietary group compared to the control fish was observed. The consistency of the outcome patterns indicated that the effects were systemic. The second (freshwater) vaccination study confirmed that the expression of several inflammation-associated genes in the MG dietary group was downregulated during the post-vaccination period.

Conclusion: The systemic effects seen in fish receiving the MG-supplemented feed suggests that oral administration of beta-glucanscan reduce inflammatory damage of major relevance to salmonid farming. Examples of relevant clinical applications will be discussed.

Keywords: vaccine-induced inflammation, cytokines

Funding: Biorigin Europe NV (Belgium).

092-O

Probiotic yeast protects Atlantic salmon against *Piscirickettsia salmonis* by empowering crosstalk between microbiota and fish via micrornas modulation

Goncalves, Ana Teresa (Chile)[1,2]; Collipal-Matamala, Rayen (Chile)[1,2]; Gallardo-Escárate, Cristian (Chile)[1,2]

1 - Interdisciplinary Center for Aquaculture Research; 2 - Universidad de Concepción

Introduction: Dietary supplementation of probiotics has been studied for many years as a nutritional strategy for health improvement and disease resistance in aquaculture. Yeasts such as *Saccharomyces cerevisiae* have been shown to promote growth, immunocompetence and gut stability and health in farmed fish, including salmons, but the underpinnings of these effects are yet to be revealed. This study applied nutrigenomics to understand the protective effect of *S. cerevisiae* against a known salmon's pathogen.

Methodology: *Salmo salar* smolts (110 g) were fed control or probiotic supplemented diet (0.5%) for 30 days, after which they were injected with the bacteria *Piscirickettsia salmonis* EM90. Samples were retrieved before injection and 15 dpi when first mortalities were observed, and were processed for blood biochemical evaluation, intestinal microbiota assessment by 16S rRNA amplicon sequencing, intestinal transcriptome for mRNA and small RNA by Hiseq sequencing in Illumina platform. Growth performance and survival after 31 dpi were assessed and an integrative analysis was performed to integrate next generation sequencing data and physiological output.

Results: Salmon fed probiotic supplemented diet presented 46% less mortality by 31dpi than control fed group. Intestinal microbial diversity was changed with probiotic feeding, and with infection empowering the presence of several lactic acid bacteria but also members of *Vibrio* genus. These changes and better survival were associated with a strong miRNome modulation, namely with higher expression of miR-23b, miR-338 and miR-731 and lower expression of miR-15e, miR-27c and miR-19a. Intestinal transcriptome modulation was also observed, and higher survival was correlated with upregulation of genes participating in ECM-receptor interaction, phagosome and intestinal integrity pathways.

Conclusion: Probiotic yeast have a strong effect in intestines by modulating microbiome and host. The host responds with modulation of miRNome and transcriptome, and this interplay has physiological effects that reflect on higher resistance to an important pathogen for aquaculture. Our data suggest that there is a crosstalk between host-microbiome that can be positively modulated by a functional feed, and that some miRNAs might be the effectors of the beneficial output.

Keywords: salmon, probiotics, microbiome, intestinal transcriptome, *Piscirickettsia salmonis*

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Antimicrobial Resistance in Fish and Shellfish

093-O

The importance of using standard protocols to determine antimicrobial susceptibility

Smith, Peter (Ireland)[1]

1 - Department of Microbiology, National University of Ireland Galway

Introduction: Establishing the antimicrobial susceptibility of bacteria isolated from aquatic animals is an essential component of prudent use of these agents in aquaculture. It is also a necessary step in the performance of programmes aimed at the monitoring and surveillance of resistance associated with the use of antimicrobial agent in aquaculture that have been recommended in the Aquatic Animal Health Code of OIE. Whether undertaken for clinical or monitoring purposes it is essential that susceptibility should always be determined using internationally accepted standard testing protocols.

Methodology: A review was performed of the current state of the development of internationally accepted standard testing protocols that could be used in the susceptibility testing of 44 bacterial species that were considered to include those most frequently isolated from aquatic animals. The physiology of these 44 species were examined with respect to the conditions specified in the currently published standard testing protocols.

Results: The currently existing standard protocols were considered to be adequate for the susceptibility testing of 37 (84%) of the 44 bacterial species considered. There was, however, a lack of international harmonized, species-specific, interpretive criteria for many species.

Conclusion: The recent progress in developing international standard susceptibility testing protocols is such that there are now standard protocols for the majority of the species encountered in global aquaculture. It will be strongly argued that all future susceptibility studies should be performed using the available standard protocols. It will also be argued that there is an urgent need for studies that will generate the data required to set protocol-specific epidemiological cut-off values for the species which currently lack these interpretive criteria.

Keywords: antimicrobial agents, susceptibility, standard methods

094-O

Trans-national study: proposition of provisional epidemiological cut off value for *Vibrio anguillarum* and *Vibrio vulnificus* isolated from diseased fish

Baron, Sandrine (France)[4]; Blomkvist, Eva (Sweden)[1]; Dalsgaard, Inger (Denmark)[2]; Granier, Sophie (France)[3]; Haenen, Olga (Netherlands)[5]; Jansson, Eva (Sweden)[1]; Jouy, Eric (France)[4]; Kempf, Isabelle (France)[4]; Larvor, Emeline (France)[4]; Madsen, Lone (Denmark)[2]; Morin, Thierry (France)[6]; Mouritsen, Kári K. (Denmark)[2]; Veldman, Kees (Netherlands)[7]; Van Gelderen, Betty (Netherlands)[5]; Voorbergen-Laarman, Micha (Netherlands)[5]; Säker, Eva (Sweden)[1]; Testerink, Joop (Netherlands)[7]; Ceccarelli, Daniela (Netherlands)[7]; Smith, Peter (Ireland)[8]

1 - SVA, Department of Animal Health and Antimicrobial Strategies; 2 - DTU Aqua, Unit for Fish and Shellfish Diseases, Kongens Lyngby, Denmark; 3 - ANSES, Fougères Laboratory, National Reference Laboratory for antimicrobial resistance, Fougères, France; 4 - ANSES, Ploufragan-Plouzané-Niort Laboratory, Mycoplasmology-Bacteriology and Antimicrobial Resistance Unit; 5 - Wageningen Bioveterinary Research, Fish Disease Laboratory, Lelystad, The Netherlands; 6 - ANSES, Ploufragn-Plouzané-Niort Laboratory Viral Fish Diseases, Plouzané, France; 7 - Wageningen Bioveterinary Research, AMR laboratory, Lelystad, The Netherlands; 8 - NUI Galway, Ireland

Introduction: Antimicrobial resistance (AMR) is a major issue for both human and animal health, and requires a *One Health* approach. Aquaculture is growing fast, both in Europe and at a global scale. Antimicrobials are widely used in aquaculture. Although this usage is strictly regulated in only a few countries, there is a growing awareness of the need for good practices guidelines and other measures to support the prudent use of antimicrobials throughout the food chain. It is recognised that AMR in aquaculture may develop in fish and shellfish bacteria as a result of antimicrobial use or by contamination of the aquatic environment by human or animal waste containing antimicrobials. However, knowledge on AMR in aquaculture is poor compared to other animal species. vibriosis caused by various *Vibrio* species may have major negative economic impact in brackish and marine fish and shellfish culture, in Europe and worldwide. Good quality data on AMR in *Vibrio* spp. from aquaculture presently are scarce. The generation of these data will require the harmonization of antimicrobial susceptibility testing methods for these pathogens of cold blooded animals. The aim of this collaborative study was to harmonize methods for AMR testing of two *Vibrio* species (*Vibrio anguillarum* and *Vibrio vulnificus*) among four national veterinary public health institutes (members of CoVetLab and MedVetNet association).

Methodology: The study used an isolate collection comprised of *V. anguillarum* (n=30) and *V. vulnificus* (n=26) from three institutes that were shared among all partners. Susceptibility was determined to oxolinic acid, florfenicol, ampicillin, amoxicillin, sulfamethoxazole-thrimethoprim, chloramphenicol, tetracycline and oxytetracycline. Standard CLSI agar diffusion and/or broth microdilution protocols was performed at 28 °C.

Results: Provisional epidemiological cut-off values were determined for each species.

Conclusion: Based on these initial results, it seems possible to group data from both species to define epidemiological cut-off values.

Keywords: AMR, FISH, Ecoff value, *Vibrio* species, *V. anguillarum*, *V. vulnificus*, CLSI

Funding: CoVetLab.

095-O*

Antimicrobial resistance in the bacterial communities of the rainbow trout filet?

Helsens, Nicolas (France)[1,2]; Calvez, Ségolène (France)[2]; Bouju-Albert, Agnès (France)[1]; Rossero, Albert (France)[1]; Prévost, Hervé (France)[1]; Magras, Catherine (France)[1]

1 - SECALIM, INRA, Oniris, Université Bretagne Loire, 44307, Nantes, France; 2 - BIOEPAR, INRA, Oniris, 44307, Nantes, France

Introduction: The role of food in the routes of transmission of resistant bacteria and antimicrobial resistance genes (ARG) is yet to be explored. Farmed fish filets can carry antibiotic-resistant bacteria because of their environmental exposures (animal farms, human activities, aqueous environment…). The presence of such bacteria on foodstuff must be evaluated as it presents a risk for the consumers. To assess this risk and to extend our knowledge about the farmed fish filet resistome, ARG were sought in the bacterial communities of fresh rainbow-trout filets.

Methodology: The analyses were performed on rainbow trout filet samples obtained in two different conditions: a) filets sampled in laboratory conditions (n = 14) and b) filets sampled in an industrial facility (n = 14). For each filet, two samples were rinsed and the DNA extracted in order to perform: 1) the ARG detection and 2) the microbial community investigation. The ARG were detected and quantified using a 245 primers pairs set. The set were chosen after bibliography analyses and *in silico* verification. It was designed to detect resistances to most antimicrobial classes, including beta lactams, macrolides, cyclines, aminoglycosides, phenicols, as well as multidrug efflux pumps and biocides resistance genes. The amplification was realised thanks to the Smartchip Real-Time PCR technology (Takara). The microbial communities were analysed using 16S rDNA metagenomics, thanks to a set of primer targeting the V3-V4 region. The sequences were analysed using the FROGS pipeline.

Results: Some ARG were detected at Ct around 25 on fresh filets (tetL, tetB, sul1), as well as biocide resistance genes (qacEΔ1), suggesting that these filets carried antimicrobial-resistant bacteria. The detectable bacterial communities were mainly composed of Gammaproteobacteria such as *Pseudomonas* spp.

Conclusion: Few ARG were detected and at a low level but it could be participated in antibioresistance diffusion. It will be interesting to determine whether resistances are carried by which bacteria population, the mostly prevalent bacteria or if some minor population carry most of the ARG.

Keywords: antimicrobial resistance, rainbow trout, metagenomics

096-O

Antimicrobial susceptibility of *Aeromonas* spp. isolated from wild freshwater fish and farmed trout in France

Larvor, Emeline (France)[1]; Duprey, Héloïse (France)[1]; Perrenou, Lucas (France)[1]; Quentel, Mauryne (France)[1]; Jouy, Eric (France)[1]; Gassilloud, Benoit (France)[2]; Wilhelm, Amandine (France)[2]; Chauvin, Claire (France)[3]; Thomas, Rodolphe (France)[3]; Le Bouquin, Sophie (France)[3]; Kempf, Isabelle (France)[1]; Granier, Sophie (France)[4]; Baron, Sandrine (France)[1]

1 - ANSES, Ploufragan/Plouzané/Niort Laboratory, Mycoplasmology Bacteriology and Antimicrobial Resistance Research Unit, Ploufragan, France; 2 - ANSES, Laboratory for Hydrology, Nancy, France; 3 - ANSES, Ploufragan/Plouzané/Niort Laboratory, Epidemiology, Health and Welfare Unit, Ploufragan, France; 4 - ANSES, Fougères Laboratory, National Reference Laboratory for Antimicrobial Resistance, Fougères, France

Introduction: *Aeromonas* is an autochthonous bacteria of aquatic environment, and a major causative agent of infections in fish. Integrons, and other genetic elements are frequently detected in *Aeromonas*, in respect with these properties, *Aeromonas* spp. has been studied as an indicator of the dissemination of antimicrobial resistance in water and fish.

Methodology: *Aeromonas* isolates were collected from gills and intestinal contents of wild freshwater fish (64 fish) and from farmed trout (20 fish) by culture on GSP agar. Identification at genus level was performed by PCR and Maldi-Tof. 347 isolates were obtained, 152 from wild fish (WF) and 195 from farmed rainbow trouts (FT). Antimicrobial susceptibility testing was performed by micro broth dilution according to CLSI guidelines with homemade microplate. The agents labeled in French aquaculture flumequine, oxolinic acid, trimethoprim-sulfamethoxazole, oxytetracycline and florfenicol, and two other agents: gentamicine and ciprofloxacin were tested. To categorise *Aeromonas* isolates as either fully susceptible wild type (WT) or as non-wild-type (NWT), provisional Ecoff value determined in a previous study were used (Baron et al., 2016).

Results: Among WF isolates, 85.6% were susceptible to all antimicrobial agents tested and no isolate was multidrug resistant. Among the nine isolates displaying acquired resistance mechanisms, six isolates had lost their natural quinolone susceptibility. All isolates were susceptible to florfenicol and gentamicine. Among FT isolates, 65.6% were susceptible to the seven antimicrobials tested, and 11.2% were multidrug resistant. Acquired resistance mechanisms to quinolone was the most frequent one. Eleven isolates (5.6%) acquired resistance mechanisms to florfenicol and three to gentamicine (1.5%).

Conclusion: Due to the diversity of susceptibility profiles highlighted here, *Aeromonas* appears as relevant indicator to follow dissemination of resistance mechanisms in aquatic environment

Keywords: fish, *Aeromonas* spp, indicator, AMR dissemination

Funding: EcoAntibio.

097-O*

Antimicrobial susceptibility testing of atypical *Aeromonas salmonicida* isolates from wrasse in the United Kingdom

Buba, Elizabeth (United Kingdom)[1,2]; Desbois, Andrew (United Kingdom)[1]

1 - University of Stirling; 2 - College of Veterinary Medicine, Federal University of Agriculture Makurdi, Nigeria

Introduction: Ballan wrasse as cleaner fish could offer an effective and alternative approach for delousing farmed salmon. In the absence of commercial vaccines, disease outbreaks caused by atypical *Aeromonas salmonicida* (*aAs*) of ballan wrasse are treated with antibiotics. However, this can lead to the emergence of resistant strains. The aim of this present study was to evaluate the antibiotic susceptibility of *aAs* isolates collected from farmed wrasse in Scotland during 2013 to 2018.

Methodology: Antimicrobial susceptibility testing was performed by the disk diffusion method following the M42-A2 guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2006). The following four antibiotic disks were selected: 30 µg florfenicol (FFN), 30 µg oxytetracycline (OTC), 2 µg oxolinic acid (OXO) and 25 µg trimethoprim/sulphamethoxazole (SXT), where cut off CLSI values were available for *A. salmonicida* to categorise isolates as wild type (WT, fully susceptible) or non-wild type (NWT, reduced susceptibility). Two further agents, 10 µg ampicillin (AMP) and 5 µg enrofloxacin (ENRO) were also included with no cut off values.

Results: Overall, 89%, 88% and 82% of isolates were susceptible to FFN, OXO and SXT, respectively. Only 65% of isolates were susceptible to OTC. More recent isolates (sampled in 2017 and 2018) showed a trend of reduced susceptibility to multiple antibiotics, which could reflect increased antibiotic use. Therefore, the application of these antibiotics to treat current disease outbreaks should be adopted with caution.

Conclusion: It is important to continue to monitor antimicrobial susceptibility to inform on the most effective treatment regime for *aAs* in farmed ballan wrasse deployed as cleaner fish. Use of vaccines should be encouraged to reduce the use of antibiotics.

Keywords: antimicrobial susceptibility, atypical *Aeromonas salmonicida*, ballan wrasse

098-O

Biofilm formation by atypical *Aeromonas salmonicida* and effect of sub-lethal concentrations of antibiotics

Cook, Kira (United Kingdom)[1]; Buba, Elizabeth (United Kingdom)[1]; Desbois, Andrew (United Kingdom)[1]

1 - Institute of Aquaculture, University of Stirling

Introduction: Atypical *Aeromonas salmonicida* can infect ballan wrasse, a species of cleaner fish cultured to remove sea lice from Atlantic salmon. These bacterial infections are treated with antibiotics, particularly when diseased fish are at early life stages before vaccines provide protection. Biofilm formation is a phenotypic trait that can protect bacteria against the lethal effects of antibiotics, and biofilm formation can be induced by sub-lethal concentrations of antibiotics. Moreover, biofilm allows bacteria to persist in culture systems and it provides an environment that increases the opportunity for exchange of genetic material such as plasmids, which may encode antibiotic-resistance genes. However, little is known of biofilm formation by atypical *A. salmonicida* or the factors affecting this. Hence, this study aimed to investigate the biofilm-forming potential of atypical *A. salmonicida* and the effects of sub-lethal concentrations of antibiotics on this.

Methodology: Biofilm formation was assessed by microtitre plate assay. Twenty-three isolates of atypical *A. salmonicida* were inoculated into wells containing tryptone soy broth and cultured for 72-96 h at 22 °C. Then, plates were inverted to remove the cultures and each well was washed with distilled water. Next, 0.1% crystal violet solution was added and the plate incubated for 15 minutes at room temperature. After a second wash, 30% acetic acid was added to solubilise the remaining crystal violet. After 15 minutes, well contents were transferred to a new plate and absorbance read at 550 nm. Assays were repeated in the presence of sub-lethal concentrations of oxytetracycline and florfenicol, two antibiotics used to treat atypical *A. salmonicida* infections.

Results: Biofilm formation varied between the isolates and these could be characterised into groups with high, intermediate and low biofilm-forming potential. Sub-lethal concentrations of antibiotics increased the abundance of biofilm in multiple isolates.

Conclusion: This study shows that biofilm formation occurs in atypical *A. salmonicida* and sub-lethal concentrations of antibiotics encourage biofilm formation, which may allow the pathogen to persist in culture systems. These findings have implications for the selection and dissemination of antibiotic resistance, and further demonstrate the need for alternatives to antibiotic therapy for atypical *A. salmonicida* infections of ballan wrasse.

Keywords: ballan wrasse, atypical *Aeromonas salmonicida*, cleaner fish, antibiotic resistance, oxytetracycline

099-O

Composition of the cultivable aerobic and facultative anaerobic intestinal microbiota of dwarf gourami (*Colisa lalia*) and prevalence of antibiotic resistance

Menanteau-Ledouble, Simon (Austria)[1]; Demuth, Viktoria (Austria)[1]; Werlberger, Andreas (Austria)[1]; El-Matbouli, Mansour (Austria)[1]

1 - Klinische Abteilung für Fischmedizin, Vetmeduni Vienna

Introduction: The intestinal flora plays an important role in the digestive process as well as a first line of defence against the establishment of deleterious bacterial populations. Moreover, it is a place of intense genetic exchange between resident bacteria, including for the exchange of antibiotic resistance genes. However, little is known about the composition of the intestinal flora of the dwarf gourami (*Colisa lalia*), a popular ornamental species originating from India, Bangladesh or Pakistan.

Methodology: The intestinal track of nine dwarf gouramis were sampled, lysed and the lysates were plated on Mueller-Hinton and Triptic Soy Agars. Bacteria were sub-cultured and identified based on morphology as well as using the analytical profile index (API) 20NE and API 20. Afterwards, the isolates were tested for resistance against eight antibiotics (doxycicline, flumequine, enrofloxacine, oxolonic axid, oxytetracycline, trimetroprim-sulfadimethoxin, florfenicol and amoxicillin) using the Kirby-Meier method. Then, genomic DNAs were extracted and identification of the bacteria was confirmed using amplification and sequencing of the 16s rRNA and PCRs were performed to identify antibiotic resistance genes (*ampC*, *tetA*, *tetB*, *pKD13*, *ermA*, *ermB*, *ermC*, *aaC3I*, *aaC3III*, *acrA*, *mphA*, *mphB* and *aadA14*) on the bacterial genome.

Results: In total, 13 different bacterial species were identified with the most common being *Aeromonas* spp., *Pseudomonas* spp. and *Vibrio* spp. Results from the antibiograms showed that resistance to Amoxicillin was common among the tested bacteria while resistance to florfenicol was still rare. 41% of all the bacteria tested exhibited a multiresistant phenotype, and were resistant to more than three of the antibiotics tested. Of these multiresistant bacteria, 10 belonged to species or genus known to be associated with disease outbreaks in fish or humans.

Conclusion: Antibiotic resistances appear common within the normal intestinal flora of *Colisa lalia*. It is likely that this flora can act as a reservoir for antibiotic resistance genes and pass the associated phenotypes to pathogenic bacteria.

Keywords: gourami, *Colisa lalia*, intestinal flora

100-O*

The prevalence of plasmid-mediated quinolone resistance genes in *Chrysobacterium aquaticum* isolated from farmed salmonids in Turkey

Saticioglu, Izzet B. (Turkey)[1]; Duman, Muhammed (Turkey)[2]; Altun, Soner (Turkey)[2]

1 - Aquatic Animal Disease Department, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey; 2 - Aquatic Animal Disease Department, Faculty of Veterinary Medicine, Bursa Uludag University, Bursa, Turkey

Introduction: The aquatic environment can serve both as a natural reservoir of antibiotic resistance genes and the spreading of bacteria and antimicrobial resistance genes to environment. Plasmid-mediated quinolone resistance (PMQR) genes represent an important challenge to the effectiveness of quinolones in the treatment of human and animal infections. In this study, 70 *Chrysobacterium aquaticum* isolates recovered from farmed salmonids in Turkey were analyzed for PMQR genes.

Methodology: *C. aquaticum* strains were recovered from farmed salmonids in Turkey, 2013-2017 and identified with 16S rRNA sequence analysis. Quinolone susceptibility among the strains was determined using minimum inhibitory concentration method against oxolinic acid, and the frequency of PMQR genes (*qnr*A, *qnr*B, *qnr*S) was investigated by PCR.

Results: The isolates recovered from farmed rainbow trout exhibiting clinical signs such as darkening of skin color, exophthalmia, and caudal fin root were identified as *C. aquaticum*. The rates of reduced susceptibility in phenotypically were determined for oxolinic acid (89%). Of the 70 isolates, 28 (40%) isolates harbored *qnr*A, 23 isolates (32%) harbored *qnr*S and 17 isolates (24%) have both *qnr*A and *qnr*S. None of the isolates harbored *qnr*B.

Conclusion: This is the first report of *C. aquaticum* recovered from rainbow trout in Turkey. Its pathogenicity was not assessed previously. Further research is needed for determining the virulence mechanisms and pathogenesis of *C. aquaticum*. Our findings showed high rates of quinolone resistance (89%) and qnr genes, underlining the importance of aquatic environment as reservoirs for the dissemination of potentially possible *C. aquaticum* and horizontal gene transfer between other waterborne bacterial species. Other possible mechanisms of resistance should also be investigated for better characterization of quinolone-resistant *C. aquaticum* isolates.

Keywords: *Chrysobacterium aquaticum*, antimicrobial resistance, rainbow trout, plasmid-mediated quinolone resistance genes

Funding: The Research Fund of Erciyes University, project number TCD-2018-8586.

101-O

ParaFishControl Workshop "Mediterranean fish parasite management strategies"

While bacterial and viral diseases of cultured finfish have been extensively studied and have witnessed substantial advances in their control, parasitic diseases have received less attention and research funding. Nevertheless, disease prevention and management are essential for the sustainability of the European aquaculture industry. The diversity of species and farming practices throughout Europe involves a significant number of threats from numerous pathogens. These pathogens hamper production and require specific preventive and curative practices and tools to ensure a high level of biosecurity in aquaculture production and related seafood products.

Over the last four years, the EU-funded project **ParaFishControl** (634429) has been working to develop innovative solutions and tools for the prevention, control and mitigation of the most harmful parasitic species affecting the main European farmed fish species, namely: Atlantic salmon, rainbow trout, turbot, gilthead seabream, European sea bass and common carp.

The **"Mediterranean Fish Parasite Management Strategies" workshop** will focus on the parasites which have been most damaging to Mediterranean farmed species populations - *Amyloodinium ocellatum*, *Ceratothoa oestroides*, *Enteromyxum leei*, *Enteromyxum scophthalmi*, *Enterospora nucleophila*, *Philasterides dicentrarchi*, and *Sparicotyle chrysophrii*. During the workshop, industry representatives will provide an overview of the most prevalent issues related to parasitic diseases in Mediterranean aquaculture farms. **ParaFishControl** partners will then present the **new tools and techniques** developed within the project to **diagnose, prevent and treat** these diseases. The workshop will provide attendees with new knowledge to better manage their farms, and greatly reduce population loss in a cost-effective way.

WS: Co-infections and Multiple Stressors

102-O

Co-infections and multiple stressors in fish

Gorgoglione, Bartolomeo (United States of America)[1]; Bailey, Christyn (Spain)[2]; Bigarré, Laurent (France)[3]; Haenen, Olga (Netherlands)[4]

1 - Aquatic Animal Health Laboratory, Dept. Pathobiology and Diagnostic Investigation, CVM & Dept. Fisheries and Wildlife, CANR - Michigan State University, East Lansing MI-USA; 2 - Fish Immunology and Pathology Laboratory, Animal Health Research Centre (CISA-INIA), Madrid, Spain; 3 - ANSES, Fish Viral Pathology, Plouzané, France; 4 - Wageningen Bioveterinary Research, NRL for Fish Diseases, Lelystad, the Netherlands

Farmed and wild fish populations are typically exposed to multiple physical, chemical and biological stressors. The cumulative impact of co-infections between parasites, bacteria, viruses and (a)biotic environmental pressures may trigger complex interactions, eliciting different pathological and immunological outcomes than classically assessed in highly controlled host-pathogen interactions. New studies specifically focus on the impact and dynamics of heterogeneous co-infections affecting fish, both in salmonid and non-salmonid species. Furthermore, cross disciplinary studies attempt to measure the impact of environmental stressors in modulating the host response to pathogens. Scientific advances are needed to improve fish stock management, reduce pressure on natural populations and to design more efficient vaccination strategies and diagnostic tools. This EAFP-promoted workshop aims to raise awareness of ongoing research on the interaction between multiple infectious agents and (a)biotic environmental stressors to foster new studies and collaborations.

Keywords: co-infections, multiple stressors, workshop

103-O

Pathological synergies in co-infecting pathogens are impacted by exposure order, and host response to initial infection

Fast, Mark D. (Canada)[1]

1 - Hoplite Research group, Department of Pathology and Microbiology, Atlantic Veterinary College, UPEI, Charlottetown, PE, Canada

In wild and cultured populations of fish, exposure and response to potential pathogens is a dynamic process. Until recently our understanding of host-pathogen interactions was limited to single exposures under individual pulsed conditions. We are only now beginning to understand the importance and frequency with which co-infecting organisms impact the host. In the case of wild and cultured salmonids, the ectoparasitic copepod *Lepeophtheirus salmonis* (aka 'sea lice'), is highly prevalent and commonly causes acute and chronic immunophysiological strain on its hosts in the marine environment. When assessing and managing other diseases of salmon in open-netpens, it must be acknowledged that these diseases occur in the presence, in many cases, of a chronic sea lice infection. Our research group and others have begun to examine the immunomodulatory and immunophysiological effects sea lice infection has on the development of disease with viral, bacterial and other parasitic pathogens. In all cases, prior infection with sea lice enhanced pathology and mortality associated with subsequent infecting organisms, but this was not always linked to the intensity of sea lice infection. Furthermore, in the case of co-infection with the coldwater ulcer-associated bacteria, *Moritella viscosa*, our data suggests that the order of establishment impacted subsequent lice abundance, lesion development and ultimate survival. This work will be discussed with respect to the mechanisms underlying inefficacy of certain intervention strategies designed at single infecting organisms. It is our hope that we can utilize this more complex approach to studying pathogenesis of disease, to improve immune potentiating approaches in fish in the future.

Keywords: heterogeneous co-infections, immunophysiology, Atlantic salmon, sea lice, *Moritella*

104-O

The pathobiome in animal and plant health

Bass, David (United Kingdom)[1]; Tyler, Charles (United Kingdom)[2]; Stentiford, Grant (United Kingdom)[1]

1 - Cefas, Weymouth, UK; 2 - Exeter University

Introduction: A growing awareness of the diversity and ubiquity of microbes (eukaryotes, prokaryotes, and viruses) associated with larger 'host' organisms has led to the realization that many diseases thought to be caused by one primary agent are the result of interactions between multiple taxa and the host. Even where a primary agent can be identified, its effect is often moderated by other symbionts. Therefore the one-pathogen-one-disease paradigm is shifting towards the pathobiome concept, integrating the interaction of multiple symbionts, host, and environment in a new understanding of disease aetiology. Taxonomically, pathobiomes are highly variable across host species, ecology, tissue type, and time. Additionally, the concept of what constitutes a pathogen needs to be much more context-dependent. Therefore a more functionally-driven understanding of pathobiotic systems is necessary, based on gene expression, metabolic interactions, and ecological processes. The essence of the pathobiome concept is to provide a framework within which the combined effect of more than one symbiont on host health can be understood, taking into account environmental influences and host response/condition. This presentation explains the pathobiome concept in detail, and illustrates pathobiotic scenarios that are diverse in terms of the types and numbers of symbionts involved, and the processes by which their influence is manifested.

Keywords: pathobiome, pathobiotic, disease, microbiome, symbiont

Funding: Defra (UK government), VIVALDI (EU H2020).

105-O*

Expecting the unexpected: an analysis of multiple stressors and their physiological consequences for rainbow trout (*Oncorhynchus mykiss*)

Bailey, Christyn (Switzerland)[1]; Segner, Helmut (Switzerland)[1]

1 - Centre for Fish and Wildlife Health, Switzerland

Introduction: Freshwater fish are threatened by the cumulative impact of multiple stressors. The cumulative effect of multiple stressors may result in non-linear effects and ecological surprises leading to, antagonistic, negative, additive or even synergistic effects. The purpose of this study was to unravel the molecular and organism level reactions of rainbow trout, *Oncorhynchus mykiss*, to the combined impact of two environmental stressors occurring in the natural habitat of salmonids.

Methodology: Fish were exposed to two stressors: 1) the myxozoan parasite, *Tetracapsuloides bryosalmonae* the causative agent of proliferative kidney disease (PKD) and 2) an estrogenic endocrine disrupting compound ethinylestradiol (EE2). PKD is a slowly developing chronic immunopathological disease here we focused on a later time point (130-day post-infection; d.p.i) when parasite intensity in the fish kidney has already past the plateau phase and began decreasing. At this time point, RNA-seq was applied to the posterior kidney, the main target organ for parasite development.

Results and Conclusion: 280 (PKD), 14 (EE2) and 444 (PKD x EE2) differentially expressed genes (DEGs) were observed in the experimental groups. In fish exposed to the combination of stressors (PKD x EE2), a number of pathways were regulated that were neither observed in the single stressor groups. Parasite infection, alone and in combination with EE2, only resulted in a low intensity immune response that negatively correlated with an upregulation of genes involved in a variety of metabolic and inflammation resolution processes. This could indicate a trade-off whereby the host increases investment in recovery/resolution processes over immune responses at a later stage of disease. When PKD infection took place under simultaneous exposure to EE2 (PKD x EE2), parasite intensity decreased and pathological alterations in the posterior kidney were reduced in comparison to the PKD only condition. In these fish several T cell markers (Tbet, CD8α, foxp3-1, foxp3-2, cd3e) were downregulated, this suggests that the multiple stressor treatment (PKD x EE2) might be having a stronger immunosuppressant role than in the PKD only fish, which possibly reduced the immunopathology associated with PKD attenuating the disease impact on the fish.

Keywords: proliferative kidney disease, estrogen, anthropogenic pollution, multiple stressor, immune response

106-O*

Saprolegniosis: who is there and why

Saraiva, Marcia (United Kingdom)[1]; Van West, Pieter (United Kingdom)[1]

1 - Aberdeen Oomycete Laboratory, International Centre for Aquaculture Research and Development, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK

Introduction: Diseases are a huge threat to the aquaculture industry and for global food security. Currently, the most important disease-causing organisms both in aquaculture and in natural freshwater ecosystems are species belonging to *Saprolegnia* genus. Saprolegniosis is responsible for at least 10% mortalities in salmon hatcheries and freshwater sites affecting both fish and eggs. Polymicrobial infections are harder to treat due to increased resistance to antimicrobial therapy, as such, polymicrobial diseases can have increased mortality compared with their monomicrobial counterparts. It has been demonstrated that *Saprolegnia* is capable of biofilm formation.

Methodology: Water and fish samples from different Atlantic salmon farms across Scotland are being collected. Oomycetes in pure cultures are being obtained and ITS sequenced. Metagenomic analysis is being carried out both for bacteria and eukaryotes. Co-infections assays are being performed with the most abundant genera and *Saprolegnia parasitica*.

Results: At present we have more than 300 pure isolates obtained from fish farms and hatcheries. The second most abundant/represented genus is *Mortierella*, a soil fungus. It has been demonstrated that some *Mortierella* species can effectively transform a series of fish-toxic diterpenes and their chlorinated analogues into nontoxic metabolites. This mechanism might aid *Saprolegnia* to infect fish. Here we present our latest findings.

Conclusion: Characterising the complex interactions that occur in these mixed species communities will undoubtedly increase our understanding of host-pathogen relationships, the mechanisms that underly infection and help design/discovery novel sustainable control strategies.

Keywords: co-infections, *Saprolegnia*, polymicrobial, metagenomic

Funding: BBSRC-Link – RiFE-SOS project.

107-O

Multi-causal eel diseases in the Netherlands

Haenen, Olga (Netherlands)[1]; Voorbergen-Laarman, Michal (Netherlands)[1]; Van Gelderen, Betty (Netherlands)[1]; Davidse, Aart (Netherlands)[1]; Roozenburg, Ineke (Netherlands)[1]; Vloet, Rianka (Netherlands)[1]; Van Beurden, Steven (Netherlands)[1]; Engelsma, Marc (Netherlands)[1]

1 - Wageningen Bioveterinary Research, NRL for Fish Diseases, P.O. Box 65, 8200 AB Lelystad, the Netherlands

Introduction: In fisheries and in aquaculture, eels, *Anguilla* spp., originate from the wild. Therefore, they may be infected with various pathogens and parasites from the wild. These multi-infections appear during disease diagnosis, like we have seen in the past 30 years in our diagnostic service of eel diseases in the Netherlands. The multi-causal diseases increased since some pathogens were introduced from South East-Asia via imports of live eels in the end of last century.

Methodology: We performed diagnostics of hundreds of batches of diseased eel by standard necropsy, parasitology (through necropsy and histopathology), bacteriology (isolation and identification by biochemistry, 16S rRNA and MALDI-TOF), virology (isolation, IFAT and PCRs).

Results: In the eighties, European eels (*A. anguilla*) in West-Europe became severely infected with the swim bladder nematode, *Anguillicoloides crassus.* Secondary infections with various bacteria occurred, like with *Aeromonas hydrophila*. After anguillid herpesvirus (AngHV1) was detected for the first time in Europe in the Netherlands in 1998, and diagnostics for the three main eel viruses, AngHV1, Eel Virus European (EVE) and Eel Virus European X (EVEX) in place, combinations of two eel viruses with one or two pathogenic bacteria, like *Pseudomonas anguilliseptica*, *Vibrio vulnificus*, *Edwardsiella tarda*, *Aeromonas salmonicida*, and in exceptional cases also *Mycobacterium marinum* were diagnosed. Often, also gill trematode parasites, like introduced species of *Pseudodactylogyrus* spp. were present, and last but not least a management factor, like stress from grading the eels or a bad water quality as basis.

Conclusion: We found various multi-causal diseases in eels. Some of the pathogens were newly introduced from South East-Asia. Examples are presented.

Keywords: multiple infections, eel, casuistic diagnosis, lecture in Workshop co-infections and multiple stressors

Funding: Dutch Ministry of Agriculture, Nature Conservation and Food quality.

108-O

Flavobacterium branchiophilum co-infection can increase pathological changes during koi sleepy disease caused by carp edema virus infection in carp

Adamek, Mikolaj (Germany)[1]; Teitge, Felix (Germany)[1]; Jung-Schroers, Verena (Germany)[1]; Heling, Max (Germany)[1]; Gela, David (Czech Republic)[2]; Piackova, Veronika (Czech Republic)[2]; Kocour, Martin (Czech Republic)[2]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany; 2 - University of South Bohemia, Vodňany, Czechia

Introduction: Koi sleepy disease (KSD) affecting common carp is an often fatal condition of increasing importance for global carp aquaculture. Despite the fact that carp edema virus (CEV) is most likely the causative agent of KSD, the disease often seems to present itself as multifactorial. Therefore, we hypothesised that a CEV infection may promote infections with secondary pathogens, which subsequently increase the severity of the clinical signs presented during the disease. Therefore, in this study, we analysed a possible interaction of infections with flavobacteria and CEV for the development of clinical KSD.

Methodology: We examined 68 gill samples of carp and koi from Germany and Hungary, and performed several infection experiments including antibiotic treatment. The amounts of flavobacteria and CEV were evaluated by quantitative PCR. The typing of flavobacteria present on gills was performed by isolating the bacteria and by molecular cloning of the 16S amplicon. The development of the disease was monitored by analysis of fish behaviour and by gill histology.

Results: Screening of field samples suggested the presence of a co-infection with CEV and flavobacteria. Individuals with CEV loads over 10^5 virus copies per 250 ng DNA had also statistically higher flavobacteria numbers in gills when compared to CEV free fish. This was confirmed by five infection experiments which indicated a rapid transfer and progress of CEV and flavobacterial infections. Both infections were progressing faster in KSD susceptible koi than in the KSD resistant common carp strain called Amur wild carp (AS). *Flavobacterium branchiophilum* was identified as a possible co-pathogen during KSD by culturing and molecular methods. Antibiotic treatment prevented a *F. branchiophilum* infection and identified CEV as the primary pathogen in KSD causing an insult to the gills of carp. Results from antibiotic treatment indicated also that a *F. branchiophilum* co-infection led to elevated levels of epithelial cells hyperplasia, epithelium lifting and proliferation of the intra-lamellar cellular mass when compared with a single CEV infection.

Conclusion: Despite the fact that a *F. branchiophilum* co-infection is not required for the development of clinical KSD; it could contribute to the pathological changes recorded during the outbreaks of this disease.

Keywords: *Flavobacterium branchiophilum*, CEV, common carp

246-O

Simultaneous and sequential co-infection patterns modulating rainbow trout response to BCWD and IHNV

Gorgoglione, Bartolomeo (United States of America)[1,2]; Jones, Darbi R. (United States of America)[3,4]; Leaman, Douglas W. (United States of America)[2]; Burger, Payton S. (United States of America)[1]; Wargo, Andrew R. (United States of America)[3]

1 - Aquatic Animal Health Laboratory, Dept. Pathobiology and Diagnostic Investigation, CVM & Dept. Fisheries and Wildlife, Michigan State University, East Lansing, MI-USA; 2 - Dept. Biological Sciences, Wright State University, Dayton, OH-USA; 3 - Dept. Aquatic Health Sciences, Virginia Institute of Marine Science, William and Mary, Gloucester Point, VA-USA; 4 - Maryland Dept. Natural Resources, Cooperative Oxford Laboratory, Oxford, MD-USA

Introduction: Fish pathology and immunology studies assessing heterogeneous co-infections attract growing attention, particularly on multiple pathogen-host interactions modulating pathogen transmission, pathogenesis, and disease outcomes. *Flavobacterium psychrophilum*, causing bacterial cold-water disease (BCWD), and *Salmonid novirhabdovirus*, causing infectious hematopoietic necrosis (IHN), are major pathogens affecting rainbow trout. This is relevant for wild and farmed trout in North America although prevention tools, including vaccination and selective breeding, are typically optimized towards single infections.

Methodology: Juvenile rainbow trout (8-45 g TBW; 8-17 cm TBL) maintained at 15 °C in flow-through system were allocated to experimental groups (Mock-control; Single IHN; Sequential BCWD+IHN; Single BCWD; Simultaneous BCWD+IHN). Fish were respectively: individually IP-injected with *F.psychrophilum* suspension, or sterile TYES for control; IHNV (C-genogroup) was given in bath challenge, or sterile EMEM-10 for control. After pathogen exposure, fish were isolated into individual tanks. Clinical observations and tissue samples were retrieved from each time point, at 1, 3, 5, 7, 9 and 11 days post infection (dpi).

Results: The lab-controlled experimental infection challenge indicated heterogeneous co-infection has a stronger pathobiological impact on hosts, when compared to single infections. The simultaneous *F. psychrophilum+IHNV* co-infection induced the fastest and most exacerbated pathology, with higher splenosomatic index. Trout mortality started at 5 dpi, peaking between 6/7 dpi, with typical clinical signs of IHN (including markedly congested bulging eyes, ascites and diffuse haemorrhaging in abdominal organs). The sequential infection group showed evident pathological signs, although milder, lower, and with delayed mortality (at 8/9 dpi). Conversely, a much milder gross pathology was seen in each single infection group, with no mortality and mild disease-specific pathology. No abnormalities were detected in mock-control group.

Conclusion: This ongoing study provides clues on how co-infections alter host's ability to respond to pathogens. The sequential co-infection pattern may offer more interesting pathology and better quantifiable immune response when compared to the simultaneous co-infection, in which the combined pathogenetic effect of multiple pathogens appears too strong. Sequential infections are also likely to be more biologically relevant, while the likelihood of hosts being exposed to multiple pathogens at the same time is low. This work will provide important management insights.

Keywords: salmonid novirhabdovirus, *Flavobacterium psychrophilum*, rainbow trout, heterogeneous co-infections, pathogenesis modulation

Funding: NIH EEID grant R01GM113233, 2018 EAFP-Small Scheme Grant.

247-O

Environment and UV-irradiation affect severity and timing of multiple infections in sea-water-reared Atlantic salmon smolts in British Columbia, Canada

Jones, Simon (Canada)[1]; Long, Amy (Canada)[1]; Macwilliams, Christine (Canada)[1]; Polinski, Mark (Canada)[1]; Garver, Kyle (Canada)[1]

1 - Fisheries and Oceans Canada

Introduction: Cultured Atlantic salmon are reared in open net pens which exposes them to infectious agents. The effects of time, environmental conditions and UV irradiation on prevalence and severity of enzootic infections were assessed in salmon smolts following transfer to seawater.

Methodology: Smolts were maintained in duplicate tanks of deep-sourced raw (RSW) or UV-irradiated seawater (UVSW), or in an open net pen (NP) between 13 June 2018 and 20 February 2019. Monthly samples were collected. Gill, skeletal muscle and kidney were examined using histology or quantitative PCR for epitheliocystis, *Kudoa thyrsites*, *Desmozoon lepeophtherii* and *Piscirickettsia salmonis*.

Results: In NP, temperature, salinity and dissolved oxygen ranged from 5.9 – 21.9 °C, 19.1 – 30.9‰, and from 4.7 – 10.4 mg / L, whereas in tanks these ranged from 7.6 – 14.8 °C, 27.1 – 31.1‰, and from 7.2 – 9.8 mg / L. Epitheliocystis was observed in NP-salmon at 28, 56 and 84 d post-transfer (dpt). *Kudoa thyrsites* was first detected 84 dpt in RSW and at 140 dpt in the NP. The prevalence was significantly higher in RSW (83%, mean 0.75 mm^{-2}) compared with NP (3%, mean 0.32 mm^{-2}). In gill, *D. lepeophtherii* was first detected after 28 dpt (NP and RSW) whereas in kidney, first detection was after 56 dpt (NP) and 84 dpt (RSW). In NP, maximum prevalence (80%) occurred in gill at 56 dpt and in kidney at 140 dpt. An outbreak of piscirickettsiosis (SRS) resulted in greater mortality in the NP (34%) compared with RSW (12%) ($P<0.01$). The outbreak was controlled with oxytetracycline and UVSW-salmon were not affected. No infections were detected in UVSW.

Conclusion: We demonstrated a broad-spectrum germicidal effect of UV. In the NP, the presence of epitheliocystis and greater severity of SRS suggested either a near-surface source of infection or adverse influences of warmer SW. In contrast, there was an increased risk of exposure to *K. thyrsites* in deep SW. Infection with *D. lepeophtherii* was equally likely in NP and RSW, was acquired via gill and subsequently spread to kidney.

Keywords: salmon, *Kudoa*, *Desmozoon*, *Piscirickettsia*, UV

Funding: Fisheries and Oceans Canada.

248-O

Gill histopathology scoring vs gross morphology and transcriptome analysis in farmed Atlantic salmon (*Salmo salar*)

Noguera, Patricia (United Kingdom)[1]; Król, Ela (United Kingdom)[2]; Douglas, Alex (United Kingdom)[2]; Bickerdike, Ralph (United Kingdom)[3]; Valdenegro, Victoria (Norway)[4]; Gajardo, Karina (Norway)[4]; Martin, S.A.M. (United Kingdom)[2]

1 - Health and Welfare, Salmon and Fresh Water Fisheries, Marine Scotland Science, Aberdeen, UK; 2 - School of Biological Sciences, University of Aberdeen, Aberdeen, UK; 3 - Scottish Sea Farms, Laurel House, Laurelhill Business Park, Stirling, UK; 4 - BioMar AS, Havnegata 9, Pirsenteret, Norway

Introduction: Gill diseases in farmed Atlantic salmon are on the rise, producing welfare concerns and substantial economical loses. From the effects of well characterised agents, e.g. *Neoparamoeba perurans* (amoebic gill disease, AGD) to the combined response to AGD and other biological or non-biological factors, gill health represents a major challenge for the salmon industry. Farmers support their disease control schemes by periodically monitoring and collecting data from *in situ* scoring of gross macroscopic changes related to AGD and proliferative gill disease (PGD), aimed to support early actions on management strategies.

Methodology: Salmon gills from geographically diverse Scottish aquaculture sites were examined to explore the underlying molecular events associated with the progression of the AGD and PGD conditions. Macroscopic scoring of 200 fish was performed *in situ*, recording data from both surfaces of all 8 arches. The gill arch with the highest PGD score sampled for histopathology and RNA-seq analysis. Histological evaluation and scoring was performed on digitalised images of routine H&E stained sections. Extracted RNA from 43 fish presenting low or medium gill PGD scores were analysed by whole transcriptome analysis using RNA-seq. For each fish, 20 M reads were generated and mapped to the Atlantic salmon genome.

Results: The predominant features of gill histopathology included hyperplastic and proliferative lesions, frequently associated with a degree of lamellar fusion, inflammatory reaction, presence of amoeba and vascular lesions. The results from 43 fish representing low and medium PGD scores showed that the changes in gross morphology and histopathology were not consistent with each other, but were rather dominated by site(location) effects. Interestingly, results from the RNA seq analysis on the same individuals showed a similar trend.

Conclusion: Both histopathology and whole transcriptome data clustered together based on the sample origin (site effect), suggesting that both macro and micro scores may inform about the overall progression of gill damage but not about the underlying pathology, providing support for a complex and multifactorial aetiology of the gill condition. The minimal common responses between different sites suggests spatio-temporal location represents an additional important factor influencing fish gill response.

Keywords: Atlantic salmon, gill pathology, complex gill disease, macro /micro scoring

Funding: MSS-Biomar.

249-O*

Farmed Atlantic salmon microbiome on gills and mucous samples during an amoebic gill disease episode: towards an early prediction

Birlanga, Victor B. (Ireland)[1]; Z. Ijaz, Umer (United Kingdom)[2]; Mccormack, Grace (Ireland)[1]; Mccarthy, Eugene (Ireland)[3]; Smith, Cindy (United Kingdom)[2]; Collins, Gavin (Ireland)[1]

1 - National University of Ireland (Galway); 2 - University of Glasgow; 3 - Galway-Mayo Institute of Technology, Galway

Introduction: Nowadays, the only way to reduce Amoebic Gill Disease (AGD) impact on salmon stocks is to control *Neoparamoeba perurans* (first aetiological agent) population from an advanced stage, preventing early diagnosis. However, microbiome plays an important role in the development of many diseases. Therefore, characterizing the microbiome of salmon gills throughout different AGD stages may allow a correlation to be observed between that community and the AGD. This correlation might offer an alternative to common detection techniques or even to conventional treatments to the disease.

Methodology: Farmed Atlantic salmon gill and mucous samples were collected from a fish farm on the West of Ireland during the 2017 summer, when an AGD episode is more likely to happen. Total microbial DNA from those samples was extracted using a new method that reduces most of existing bias found in previous gill microbiome studies. The bacterial community on gills and mucous samples was characterized using universal prokaryotic primers in high-throughput sequencing. In addition, AGD development was tracked to correlate it with the prokaryotic microbiome.

Results: The richness, balance of the community and environmental pressure of the prokaryotic community in both types of samples showed a considerable increase before the AGD started, although they significantly decrease after it appeared. Moreover, some opportunistic considered bacteria (*Tenacibaculum maritimum* or *Piscirickettsia salmonis*) were present during the AGD episode, suggesting that it could have synergistic effects with other diseases. The genus *Shewanella* sp. had a maximum relative abundance 12 days before the first AGD evidences were detected. In addition, it was in the top 25 most abundant OTUs in the whole campaign. These reasons make this genus a possible target for an early AGD prediction test.

Conclusion: This study supports the idea that the Amoebic Gill Disease development has an impact on the gill and mucous prokaryotic community structure. The data also demonstrated that mucous could be a feasible non-lethal alternative for a partial characterization of the gill microbiome. Finally, based on these results, *Shewanella* sp. would be promising target for an early AGD prediction test.

Keywords: amoebic gill disease, Atlantic salmon, gill microbiome, high-throughput sequencing

Funding: Department of Agriculture, Food and the Marine (Ireland).

Fish and Shellfish Immunology I

111-O

Evolutionary characteristics I84 family of protease inhibitors suggest a lineage-specific multigene family likely functioning as immune effector mechanism in mollusks

Xue, Qinggang (China)[1]; Wu, Qianjiang (China)[1]; Lin, Zhihua (China)[1]

1 - Zhejiang Wanli University

Introduction: Protease inhibitors play an important role in host defense in plants and animals. The I84 family of protease inhibitors, originally identified from eastern oysters *Crassostrea virginica*, consists of novel proteins likely functioning in host immunity. Family members effectively inactivate subtilisin family proteases including perkinsin, a major virulence extracellular protease of *Perkinsus marinnus*, and to inhibit *in vitro* propagation of the parasite. Besides, expression levels of member gene are significantly higher in oysters with increased dermo resistance than in oysters highly susceptible to the disease. Moreover, member gene expression can be up-regulated by biological and environmental stressors. This research was to investigate the molecular evolution of the family in bivalve mollusks.

Methodology: the genome databases of the Pacific oyster *Crassostrea gigas*, the eastern oyster and the Yesso scallop *Mizuhopecten yessoensis* were tblastn-searched using amino acid sequence of known I84 family members as queries. mRNA sequences were downloaded, and the translated amino acid sequences were used for constructing phylogenetic tree using the Maximum Likelihood method after multiple alignments using Clustal Omega. Gene divergence was investigated by PCR analysis of temporal and spatial expression patterns of analogous genes.

Results: a total of 5, 17, and 26 of I84 family genes were identified in the genomes of Yesso scallop, Pacific oyster and eastern oyster correspondently. Grouped together in the phylogenetic tree were either orthologs from different species or analogs, which were identified to be present closely in the genome. The expression of representative *C. gigas* analogs in different tissues and different time post challenges is under analysis.

Conclusion: I84 family of protease inhibitors appears to expand dramatically in mollusk species via lineage-specific gene expression and rapid and active evolution, forming a multiple gene/protein family with significant difference in gene number between certain species. Give the potentials of the family in host defense, it is thus likely that I84 family of protease inhibitors constitutes a lineage specific effector mechanism in mollusk immune responses. Possibilities of the family evolution being under positive selection, and the family's mechanism of action in host defense will be further characterized in future research.

Keywords: mollusks, protease inhibitor, immune effectors, lineage specific, multigene family

Funding: NSFC project# 31672629.

112-O*

Understanding the immune response of big-belly seahorse *Hippocampus abdominalis* under bacterial infection by differential expression analysis

Lee, Sukkyoung (Republic of Korea)[1,2]; Wan, Qiang (Republic of Korea)[1,2]; Lee, Seongdo (Republic of Korea)[1,2]; Yang, Hyerim (Republic of Korea)[2]; Tharuka, M.D. Neranjan (Republic of Korea)[2]; Lee, Jehee (Republic of Korea)[1,2]

1 - Fish Vaccine Research Center, Jeju National University, Republic of Korea; 2 - Department of Marine Life Sciences, Jeju National University, Republic of Korea

Introduction: Big-belly seahorse (*Hippocampus abdominalis*) is one of the commercial and important species either as an aquarium fish or a component of oriental medicine in China, Japan, and Korea. However, some serious pathogenic infections were reported in maricultures that lead to severe economic loss. To elucidate the immune system at molecular level, analysis of differentially expressed genes (DEG) was done after exposure to lipopolysaccharide (LPS), polyinosinic:polycytidylic acid (poly I:C), and *Vibrio tubiashii*.

Methodology: *V. tubiashii* isolated from diseased seahorse was selected as a pathogenic bacterium during the pre-challenge experiment. LPS, Poly I:C, and *V. tubiashii* were diluted to final concentrations of 1.25 mg/mL, 1.5 mg/mL, and 0.1 OD, respectively, in 1X phosphate-buffered saline (PBS) at a total volume of 100 mL and injected into the seahorse abdominal cavity. Kidney tissue was sampled from five fish in each group at 0, 6, 24, and 72 h post-injection (p.i.), and immediately snap-frozen in liquid nitrogen. Total RNA was later isolated from all the extracted kidney tissues. Twelve RNA libraries were constructed, and then, sequenced by Illumina NextSeq 500 platform. DEG analysis was performed on the PBS group and each challenged group.

Results: A total of 126.73 Gbp were sequenced from 12 RNA libraries (average throughput 10.56 Gbp, 69,942,559 reads). Time-course expression patterns were first analyzed against PBS groups, and then, DEG analysis was done to compare PBS with LPS, poly I:C, or *V. tubiashii* treated groups at each time point. It was demonstrated that the up- or down-regulated genes were associated with a biological process, cellular component, or a molecular function. Especially, the differentially expressed genes were shown to be associated with immune response or immune system process.

Conclusion: We tried to elucidate the immune response of big-belly seahorse by the DEG analysis. Although the results of PBS groups were not stable enough to be compared with other challenged groups, we could validate the expression changes under immune stimulations. Based on these results, we will perform future studies on gene characterization and function.

Keywords: big-belly seahorse, DEG, immune response

Funding: This work was supported by the National Research Foundation of Korea (NRF) (NRF-2017R1C1B2008380).

114-O

Transcriptomic profiles of Atlantic salmon challenged with *Piscirickettsia salmonis* reveal a strategy to evade the adaptive immune response

Rozas-Serri, Marco (Chile)[1,2]; Peña, Andrea (Chile)[1]; Maldonado, Lucerina (Chile)[1]

1 - Laboratorio Pathovet; 2 - Newenko Group SpA

Introduction: Piscirickettsiosis is the main bacterial disease affecting the Chilean salmon farming industry and is responsible for high economic losses. The development of effective strategies to control piscirickettsiosis has been limited in part by insufficient knowledge of the host response.

Methodology: The aim of this study was to use RNA sequencing to describe the transcriptional profiles of the responses of post-smolt Atlantic salmon infected with LF-89-like or EM-90-like *Piscirickettsia salmonis* via intraperitoneal injection and cohabitation. For sequencing, we used Illumina MiSeq sequencer (Illumina Inc., San Diego, CA, USA). To validate the reliability of the RNA-seq data, quantitative real-time PCR was conducted on 12 randomly selected representatives and differentially expressed genes.

Results: Enrichment and pathway analyses of the differentially expressed genes revealed several central signatures following infection, including positive regulation of DC-SIGN and TLR5 signalling, which converged at the NF-kB level to modulate the pro-inflammatory cytokine response, particularly in the PS-EM-90-infected fish. *P. salmonis* induced an IFN-inducible response (e.g. IRF-1 and GBP-1) but inhibited the humoral and cell-mediated immune responses. *P. salmonis* induced significant cytoskeletal reorganization but decreased lysosomal protease activity and caused the degradation of proteins associated with cellular stress. Infection with these isolates also delayed protein transport, antigen processing, vesicle trafficking and autophagy. Both *P. salmonis* isolates promoted cell survival and proliferation and inhibited apoptosis. Both groups of Trojan fish used similar pathways to modulate the immune response at 5 dpi, but the transcriptomic profiles in the head kidneys of the cohabitant fish infected with PS-LF-89 and PS-MS-90 were relatively different at day 35 post-infection of the Trojan fish, probably due to the different degree of pathogenicity of each isolate.

Conclusion: Our study showed the most important biological mechanisms used by *P. salmonis*, regardless of the isolate, to evade the immune response, maintain the viability of host cells and increase intracellular replication and persistence at the infection site. These results improve the understanding of the mechanisms by which *P. salmonis* interacts with its host and may serve as a basis for the development of effective strategies for the control of piscirickettsiosis.

Keywords: RNA-seq, piscirickettsiosis, *Piscirickettsia salmonis*, LF-89, EM-90

252-O

Evidence of trained immunity for common carp macrophages

Petit, Jules (Netherlands)[1]; Embregts, Carmen W.E. (Netherlands)[1]; Forlenza, Maria (Netherlands)[1]; Wiegertjes, Geert F. (Netherlands)[1,2]

1 - Cell Biology and Immunology Group; 2 - Aquaculture and Fisheries Group, Wageningen University & Research, NL

Introduction: Trained immunity is a form of innate immune memory best described in mice and humans. Trained immunity is defined as a heightened response to a secondary infection that can be exerted toward both homologous and heterologous microorganisms. Typical criteria of trained immunity include: 1) induction upon primary infections or immunizations and subsequent protection against a secondary infection, in a T- and B-lymphocyte independent manner, 2) a response that is less specific than an adaptive immune response but that still confers increased resistance upon reinfection of the host and, 3) the involvement of innate cell types such as NK cells and macrophages involved in improved pathogen recognition and an increased inflammatory response.

Clear evidence of the evolutionary conservation of trained immunity in teleost fish is lacking. Given the evolutionary position of teleosts as early vertebrates with a fully developed immune system, we hypothesize that teleost myeloid cells show features of trained immunity common to those observed in mammalian macrophages. These would at least include the ability of fish macrophages to mount heightened responses to a secondary stimulus in a non-specific manner.

Methodology: We established an in vitro model to study trained immunity in fish by adapting a well-described culture system of head kidney-derived macrophages of common carp. A soluble NOD-specific ligand and a soluble β-glucan were used to train carp macrophages, after which cells were rested for six days prior to exposure to a secondary stimulus.

Results: Unstimulated trained macrophages displayed evidence of metabolic reprogramming, as well as heightened phagocytosis and increased expression of the inflammatory cytokines il6 and tnfα. Stimulated, trained macrophages showed heightened production of reactive oxygen and nitrogen species as compared to the corresponding stimulated but untrained cells.

Conclusion: Trained immunity is a form of innate immune memory that appears conserved in macrophages of common carp. Measurement of the production of reactive oxygen species proved particularly informative to identify ligands able to train carp macrophages.

Funding: Netherlands Organisation of Scientific Research.

Diagnostics I

115-O*

DNA-based electrochemical biosensor for the detection of ostreid herpesvirus-1 in *Crassostrea gigas*

Toldrà, Anna (Spain)[1]; Furones, M. Dolors (Spain)[1]; O'sullivan, Ciara K. (Spain)[2,3]; Campàs, Mònica (Spain)[1]

1 - IRTA, Sant Carles de la Ràpita, Spain; 2 - Departament d'Enginyeria Química, Universitat Rovira i Virgili, Tarragona, Spain; 3 - ICREA, Barcelona, Spain

Introduction: Ostreid herpesvirus-1 (OsHV-1) has been involved in massive mortality outbreaks of Pacific oyster *Crassostrea gigas* throughout the world, causing important economic losses to shellfish aquaculture. Therefore, rapid, simple, low-cost and in-situ detection tools are highly needed to provide early warnings. In this work, an electrochemical biosensor for the detection of OsHV-1 DNA based on isothermal recombinase polymerase amplification (RPA) and thin-film gold electrodes was developed.

Methodology: The method involves two steps. For the RPA step, specific primers were designed to render an amplicon with single stranded DNA tails for its subsequent detection via a sandwich hybridization assay. For the detection step, a thiolated capture probe was immobilized on gold electrodes. Following hybridization of the RPA amplicon, electrochemical detection was achieved via addition of an HRP-conjugated reporter probe. Prior to the electrochemical detection, a colorimetric assay was developed to test the feasibility of the approach and optimise the RPA conditions. Calibration curves were constructed using PCR-amplified OsHV-1 DNA. The biosensor was applied to the detection of OsHV-1 in 16 spat oysters (both control and treated) from an infectivity experiment, and OsHV-1 DNA quantifications were compared with qPCR results.

Results: A limit of detection (LOD) of ~400 target copies was achieved by the colorimetric assay using the optimised RPA conditions. When the strategy was transferred to an electrochemical platform, the biosensor provided an LOD of ~200 target copies. Oyster samples from the control aquarium were negative with both the biosensor and the qPCR assay. Regarding treated oysters, a strong degree of correlation was obtained between techniques (r = 0.982), demonstrating the reliability of the method.

Conclusion: The presented biosensor offers clear advantages compared to conventional PCR methodology used to monitor OsHV-1 disease: it uses an isothermal amplification technique, which bypasses the need for thermal cycling, and it requires simple and user-friendly detection instrumentation. Although LODs are higher than other molecular techniques, the biosensor offers great potential to be integrated into microfluidic systems to develop compact devices that could be used to perform in-field analysis.

Keywords: ostreid herpesvirus 1 (OsHV-1), *Crassostrea gigas*, electrochemical biosensor, isothermal DNA amplification

Funding: H2020 678589 (VIVALDI project).

116-O

Histological diagnostic criteria for nematocyst associated gill disease in finfish

Rodger, Hamish (Ireland)[1]; Marcos-Lopez, Mar (Ireland)[2]; Kraugerud, Marianne (Norway)[3]; Mcloughlin, Marianne (United Kingdom)[4]; Hellberg, Hege (Norway)[3]; Lie, Kai-Inge (Norway)[3]; Ostevik, Liv (Norway)[3]; Alarcon, Marta (Norway)[3]; Pearson, Marianne (United Kingdom)[4]; Herrero, Ana (United Kingdom)[4]; Scholz, Felix (Ireland)[2]; Mitchell, Susie (Ireland)[2]; Hoare, James (United Kingdom)[4]

1 - VAI Consulting; 2 - FVG Ireland; 3 - FVG Norge; 4 - FVG Ltd.

Introduction: Gill disease, is increasing in clinical and sub-clinical significance in many regions and farmed fish species. Histopathology is a vital tool to assess gill health and fish welfare and is routinely used for disease investigation purposes. Specific diagnostic histopathological criteria exist for a limited number of gill disease conditions and in many other cases the diagnostic pathology can be complex with the aetiology difficult to establish. This presentation proposes diagnostic criteria for nematocyst associated gill disease (NAGD).

Methodology: A review of experimental challenge work on finfish exposed to harmful zooplankton, hydroids and cnidarians (not including Myxozoa) was undertaken, and diagnostic cases from Fish Vet Group in Ireland and Scotland associated with exposure to these organisms compared for histopathology. Specific common histopathological changes present in all challenges and cases were identified, as well as those that were occasionally observed.

Results: Four diagnostic pathological changes were present in all acute cases (day 0 to 3 or 4 post exposure), namely a) microthrombi in lamellae; b) focal epithelial necrosis; c) focal epithelial sloughing or perforation in epithelium and d) focal haemorrhage and/or oedema in the lamellae. An additional five changes were identified in some cases comprising: e) increased thickness of the eosinophilic granular cell (EGC) layer in the filament; f) colonies of filamentous bacteria on gill or gill raker surface; g) foci of inflammatory cells in the filaments; h) presence of cnidarian or nematocyst fragments on or adjacent to gill surface; and i) focal hepatic necrosis. In chronic cases (from days 5 or 6 onwards post exposure); j) microthrombi; and k) focal lamellar hyperplasia and fusion, were consistently observed, and in some cases; l) filamentous bacterial colonisation; m) increased thickness of the EGC layer (+/- fibrin); n) focal inflammation and o) melanisation in filaments and lamellae, were evident.

Conclusion: For diagnosis of acute NAGD, all four changes a) to d) should be present, with at least one of e) to i) also evident. For chronic NAGD, there should be at a minimum of both j) and k) present with at least two more from l) to o).

Keywords: histology, diagnostic, nematocysts, gill, disease

117-O

Salmonid alphavirus subtype I isolated from clinically-diseased Atlantic salmon, *Salmo salar*, in freshwater culture

De Carvalho Soares, Silvia (United Kingdom)[1]; Elwenn, Svenja (United Kingdom)[2]; Campbell, Maria (United Kingdom)[3]; White, Patricia (United Kingdom)[3]; Still, Nichola (United Kingdom)[3]; Munro, Eann (United Kingdom)[3]

1 - Marine Scotland Science, Marine Laboratory, Aberdeen, Scotland, UK

Introduction: In May 2018, the Fish Health Inspectorate (FHI) were alerted to a mortality event in Atlantic salmon at a freshwater hatchery on the west coast of Scotland. The site was stocked with approximately 1,400,000 S0 Atlantic salmon with a mean weight ranging from 10 - 16 g. The FHI visited the site on 31 May 2018 and observed from site records that mortality levels were reported as 2.16% in week 21 and 11.67% in week 22 (the week of the visit) and then decreased to 0.55% in week 23.

Methodology: Five moribund and lethargic fish were removed for diagnostic sampling and samples were collected for analysing by the Disease Diagnostic Group.

Results: Histopathological examination revealed lesions in the pancreas, heart and skeletal muscle consistent with Pancreas Disease pathology. Pancreas disease (PD) is a viral disease of farmed salmonids and it is responsible for significant economic losses to the aquaculture industry in Ireland, Norway and Scotland due to mortality percentage, carcass and fillet quality downgrade as well as treatment and management costs. Molecular and traditional virological techniques detected the presence of salmonid alphavirus (SAV), the causative agent of PD, and the isolate was subsequently confirmed as SAV subtype I by partial sequence analysis of the E2 gene.

Conclusion: The origin of the SAV introduction at this hatchery is unknown, however, the freshwater hatchery is located less than a mile from a seawater loch, where seawater sites for farmed Atlantic salmon are located. It is possible that the virus was transmitted from a seawater source into the hatchery. This is the first published description of a confirmed clinical field case of SAV with pathology consistent with PD in freshwater Atlantic salmon and as such, is a very significant development in the epidemiology of PD.

Keywords: pancreas disease, salmonid alphavirus, salmonid freshwater production

118-O

Difference in virulence of PRV-1 strains infecting Atlantic salmon

Wessel, Øystein (Norway)[1]; Dahle, Maria K. (Norway)[2]; Hansen, Elisabeth F. (Norway)[1]; Garver, Kyle A. (Norway)[3]; Polinski, Mark P. (Norway)[3]; Timmerhaus, Gerrit (Norway)[4]; Inami, Makoto (Norway)[5]; Løvoll, Marie (Norway)[5]; Rimstad, Espen (Norway)[1]

1 - Faculty of Veterinary Medicine, Norwegian University of Life Sciences, Oslo, Norway; 2 - Norwegian Veterinary Institute, Oslo, Norway; 3 - Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, BC, Canada; 4 - Nofima AS, Norwegian Institutes of Food, Fisheries & Aquaculture Research, Ås, Norway; 5 - VESO Vikan, Vikan, Namsos, Norway

Introduction: Piscine orthoreovirus 1 (PRV-1) is a ubiquitous virus in farmed Atlantic salmon (*Salmo salar*) in Norway, and has been shown to be the etiological agent of heart and skeletal muscle inflammation (HSMI). HSMI is a prevalent disease in Norway; however, PRV-1 is also present in apparently healthy Norwegian salmon. Furthermore, in British Colombia, Canada, the virus is prevalent but HSMI is not. This indicates that the development of disease is complex, involving viral, host and environmental factors. Studies addressing the contribution of these different factors in disease development are highly warranted.

Methodology: We have developed a method to amplify and purify PRV-1 from blood, which enables more standardized studies comparing differences between virus strains. In the present study, we compared the pathogenesis of genetically different PRV-1 strains. This included two Norwegian strains; one from a severe HSMI outbreak in 2012 and a second revived from archived material dating back to 1988; approximately 10 years before HSMI appeared in farmed salmon in Norway. In addition, a Canadian PRV-1 strain not previously associated with HSMI was included. The three different strains were propagated in Atlantic salmon and used as source for virus purification. The purified virus was inspected by electron microscopy, quantified by absolute quantification RT-PCR, and Atlantic salmon were challenged by i.p. injection with an equal number of pure virus particles from the three virus strains.

Results and Conclusion: The challenge trial confirmed difference in virulence between the PRV-1 strains. The three strains replicated to similar titers in blood. However, the Norwegian 2012 strain was the only virus able to induce classical HSMI lesions. In contrast, only minor lesions were induced using the Norwegian 1988 strain and the Canadian strain. This is the first experimental confirmation of virulence differences between PRV-1 strains.

Keywords: PRV, Atlantic salmon, HSMI, virulence

119-O

Use of non-lethal sampling in pancreas disease (PD) diagnosis and surveillance

Midtlyng, Paul (Norway)[1]; Westgard, Aoife (Norway)[2]; Schlittenhardt, Sandra (Norway)[3]; Graham, David (Ireland)[4]; Savage, Paul (United Kingdom)[5]; Prendergast, Maureen (Ireland)[6]; Wilson, Camilla (United Kingdom)[7]; Mcloughlin, Marian (United Kingdom)[8]

1 - Norwegian School of Veterinary Science; 2 - Aqua Kompetanse; 3 - Mowi Ireland; 4 - Animal Health Ireland; 5 - Agri-Food & Biosciences Institute, N. Ireland.; 6 - MSD Animal Health Ireland; 7 - MSD Animal Health UK; 8 - Fish Vet Group

Introduction: Pancreas disease is an economically important disease of the European farmed salmon industry affecting all production areas including Norway, Scotland and Ireland. It is caused by an aquatic alphavirus, salmon pancreas disease virus (SPDV), also known as salmonid alphavirus (SAV). Pancreas disease is confirmed in Atlantic salmon by a combination of histopathology, PCR and serology. This presentation will highlight the pros and cons of PCR & serology diagnostic testing and highlights the advantages of use of non-lethal sampling in PD diagnosis and surveillance.

Methodology: The dynamics of SAV infection will be described in clinical and subclinical SAV infections. The results of regular PD serology monitoring and diagnostic testing in recent PD cases in Ireland and Norway will be presented to compare and contrast the usefulness of PCR and PD serology in PD diagnosis and surveillance.

Results: Both PCR and serology are shown to be sensitive and specific for the detection of SAV over a prolonged period post infection, but antibody to SAV persists until harvest and is often identified in the absence of a positive PCR signal. Proactive sampling of fish populations prior to the PD risk period and during SAV challenge can provide very useful temporal and diagnostic information to enable informed management decisions regarding the control of PD in endemic and non-endemic PD areas.

Conclusion: The use of non-lethal methods of disease diagnosis is successfully used in other farmed animal populations. The use of serology should be further developed in salmonid aquaculture as a specific, sensitive and cost effective diagnostic and surveillance tool.

Keywords: SAV, PD, serology, PCR, diagnosis

120-O

Discovery of cutthroat trout virus (CTV) in Atlantic salmon and other salmonids from the eastern Canada

Gagné, Nellie (Canada)[1]; Arseneau, Jean-René (Canada)[1]; Leblanc, Francis (Canada)[1]; Boston, Linda (Canada)[1]

1 - Fisheries and Oceans Canada, Gulf Fisheries Center

Introduction: Cutthroat trout virus (CTV), recently designated as Piscihepevirus A and a member of the Hepeviridae family, was first identified in salmonid fish in California in 1988. It is often reported from rainbow trout (*Oncorhynchus mykiss*), cutthroat trout (*Oncorhynchus clarkii*), brown trout (*Salmo trutta*), and brook trout (*Salvelinus fontinalis*) in several western states of the United States. In Atlantic Canada, through regular surveillance of salmonid health using Chinook salmon embryo cells (CHSE-214), unknown cytopathic effects were observed in 2009 and at a regular frequency afterward (up to 15 cases in 2011).

Methodology: An initial protocol was designed to semi-purify the unknown virus and perform random amplification. High throughput sequencing was performed to retrieve virus genome sequences.

Results: Sequences were recovered and allowed the identification of a virus genetically similar to CTV, with approximately ~75% identity on partial nucleotide sequences obtained. A PCR assay was designed to confirm cell culture findings. Initial screening of Atlantic salmon from various sources revealed a high prevalence on farms and in wild fish. CTV isolations in cell culture were mostly from Atlantic salmon, although brook trout, rainbow trout, and Arctic char samples were also occasionally found positive for the virus.

Conclusion: High-throughput sequencing of 7 isolates generated the full sequence of the virus. We further determined the relationship of these CTV-like virus using phylogenetic analysis, and demonstrate that two distinct genogroup of virus are present in fish from Atlantic Canada.

Keywords: cutthroat trout virus, Atlantic salmon, phylogenetic, viral culture

121-O*

Real-time PCR and droplet digital PCR: two methods for pathogen detection with key differences

Testerman, Todd (United States of America)[1]; McClure, Emily Ann (United States of America)[1]; Graf, Joerg (United States of America)[1]

1 - University of Connecticut

Introduction: The detection of rare pathogens within complex microbial communities can prove a challenging task. High background levels of DNA, inadequate taxonomic resolution, and the presence of chemical inhibitors can prevent many qualitative techniques from delivering reliable results. A major benefit of a quantitative method is that knowing the absolute number of target sequences improves the assessment of infection risks. Additionally, a fast and highly specific assay for pathogen detection can provide the guidance needed to institute an appropriate treatment plan as quickly as possible. Within an aquaculture facility, diseases can be difficult to distinguish and can spread quickly amongst fish. Real-Time PCR and Droplet Digital PCR (ddPCR) assays for various fish pathogens offer solutions for many of these considerations.

Methodology: We designed species-specific TaqMan primers and probes using the oligo designer Primer3 and checked for specificity bioinformatically on NCBI BLAST. Primers and probes were tested for functionality in Real-Time PCR and ddPCR assays with target and non-target DNA extracted from cultured cells and spiked samples.

Results: We have produced and validated 5 Real-Time PCR and ddPCR assays for economically-relevant disease agents in aquaculture; *Flavobacterium psychrophilum*, *Flavobacterium columnare*, *Flavobacterium branchiophilum*, *Yersinia ruckeri* and *Aeromonas salmonicida*. We show that these assays are specific as cross-reactivity with other related and non-related species was not observed. We were also able to show that target sequences can be detected at low concentrations and amongst complex communities of microbes. Multiplexing of up to three assays was tested for compatibility and robustness. Consistent low-level quantification of target was tested on both platforms using replicates of low copy genomic DNA samples.

Conclusion: These assays provide the specificity and sensitivity necessary to be used in clinical aquaculture settings. Real-Time PCR was found to have a broader dynamic range, be more receptive to multiplexing, and provide a cheaper cost per assay. ddPCR provided absolute quantification data without the use of a standard curve, accurate low target-level quantification, and less sensitivity to PCR inhibitors than Real-Time PCR.

Keywords: detection, qPCR, bacterial pathogen

Funding: United States Department of Agriculture (USDA).

Aquatic Animal Welfare

122-O

The animal health law - a particular challenge for EU member states with small-scale aquaculture production business

Kleingeld, Dirk Willem (Germany)[1]

1 - Lower Saxony State Office for Consumer Protection and Food Safety

Introduction: On 21 April 2021 the Directive 2006/88/EC which has been the basis for the control of aquatic animal diseases in the EU for more than 10 years will be repealed and the Regulation (EU) 2016/429 (Animal Health Law / AHL) will apply.

Methodology: In order to get an overview of the effectiveness of control measures epidemiological data from the animal disease database TSN© were evaluated. Furthermore, the AHL and its secondary legislation still to be adopted were assessed with regard to the possible impact on small-scale aquaculture.

Results: Regarding the epidemiological development a positive resume can be drawn for Germany. There is a decline of VHS, IHN and KHVD outbreaks and an increase in VHS, IHN and KHVD disease-free compartments and zones. Other listed fish diseases were not detected. Moreover the health status categorisation has proven to be successful, although more than 95% of all farms are still classified in Category-III ("not known to be infected"). The production of aquatic animals in Germany is largely done in small-scale establishments. The AHL does not envisage the maintenance of Category-III status eg regarding VHS or IHN. Three health status categories are foreseen: "disease-free", "eradication programme" and "others". However under pressure from several Member States Category-III will probably be maintained by means of delegated acts. According to the IA (EU) 2018/1882 KHVD will be only subject to surveillance from 2021 within the frame of the AHL.

Conclusion: Due to various reasons it is impossible to establish eradication programmes for many small-scale farms in Germany and elsewhere in Central Europe. Therefore it is indispensable to issue regulations for the protection of the health status of these farms within the framework of the AHL secondary legislation. On the basis of epidemiological findings it is incomprehensible that KHVD will be only subject to surveillance in future. By contrast, mollusc diseases will still be subject to obligatory/optional eradication, although the eradication of these diseases is considered to be impossible in most cases. It is questionable whether the AHL-requirements can be met by small-scale aquaculture production establisments.

Keywords: animal health law, aquatic animal disease control, EU-regulations, delegated and implementing acts, notifiable aquatic animal diseases

123-O*

An innovative two-chamber skin explant model to study skin diseases in marine fish

Vercauteren, Maaike (Belgium)[1]; Devriese, Lisa (Belgium)[2]; Decostere, Annemie (Belgium)[1]; Chiers, Koen (Belgium)[1]

1 - Ghent University, Faculty of Veterinary Medicine; 2 - Flanders Marine Institute

Introduction: Skin ulcerations are one of the most common lesions in flatfish in the Belgian part of the North Sea. Despite their high prevalence, the exact etiology is not yet pinpointed. Although we have demonstrated the involvement of bacteria, various environmental characteristics might contribute to an increased susceptibility to infection by pathogenic micro-organisms. To study these possibly influencing factors, *in vivo* experiments are often performed including a high number of laboratory animals. Using an *in vitro* or *ex vivo* model may be considered ethically superior since it implements the concept of replacement, reduction and refinement. However, to mimic the natural marine environment for the skin *in vitro*, the difference in salinity between the inner part (body fluids, ± 0.9 PSU) and outer environment (seawater, ± 31 PSU) of the fish needs to be taken into account. To do so, we have designed a "two-chamber skin explant model".

Methodology: Immediately after euthanasia, four skin samples of each side of the flatfish (>20 cm length) were collected and disinfected by immersion in antibiotic solution. Thereafter, skin samples were mounted between two 3D printed plastic plates. DMEM media supplemented with salt was added to the outer surface of the skin and DMEM to the inner part. Both media were replaced every eight hours. After 24 h, skin was fixed in formalin and the integrity of the epidermis and underlying tissues histologically evaluated. In a second study, the two chamber model was applied to study the adhesion and invasion of *Vibrio tapetis* with intact and damaged (focal removal of scales) skin, combined with changes in temperature and salinity of the medium covering the outer surface.

Results: The overall integrity of the skin was satisfactory with minor histological changes. The second study is still ongoing.

Conclusion: The two-chamber skin explant model described here may be regarded as a fairly simple, effective and feasible non-sentient alternative to the use of live fish in research. It offers the opportunity to treat the skin locally in a small treatment spot, mimicking a certain environmental impact and study a disease process.

Keywords: skin ulceration, skin explant, *in vitro* experiment, two-chamber skin explant model

Funding: Research Foundation – Flanders, Flanders Marine Institute, EMBRC-ERIC.

124-O

Recommendations for stunning and killing of common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*)

Jung-Schroers, Verena (Germany)[1]; Retter, Karina (Germany)[1]; Hellmann, John (Germany)[2]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany; 2 - State Office for Nature, Environment and Consumer Protection North Rhine-Westphalia, Germany

Introduction: In Germany stunning of animals in general is regulated in a directive and special requirements for stunning of fish are given. For all fish species these regulations prescribe stunning by percussion or electrical current. Stunning of several fish species, like common carp, is difficult by using the authorised methods.

Methodology: The whole process of stunning and slaughtering was evaluated 22 times for trout and 17 times for carp on fish farms throughout Germany. On the farms water parameters to evaluate fish welfare, like cortisol, and to evaluate the stunning procedure, like conductivity, were measured. Additionally, fish blood was analysed for different parameters, like cortisol, glucose and lactate. An evaluation score was established which includes all measured parameters about the process. With this score a gradual classification and an assessment of different techniques and methods for stunning and killing of trout and carp was possible.

Results: Most of the rainbow trout were stunned by electrical current, followed by percussion. In two farms trout were stunned by a combination of both. In contrast, in most of the documented cases carp were stunned by a combination of electrical current and percussion. Most of the rainbow trout were successfully stunned by all evaluated methods. In carp only a combination of electrical current and percussion was leading to a successfully stunning. Only around 60% of carp were successfully stunned by electrical current and around 80% of carp were successfully stunned by percussion. Water conductibility, stunning time and size and shape of the stunning tank have an important influence of the success of stunning by electrical current. Short stunning times of less than 2 minutes and water conductibility lower than 500 µS/cm or higher than 1000 µS/cm were leading to problems with stunning of especially carp. Cortisol levels in water and blood and other blood parameters were not correlated to the stunning success.

Conclusion: A combination of stunning by electrical current with adequate conductibility and adequate stunning time followed by percussion seems to be the best method for stunning of rainbow trout and carp.

Keywords: stunning, slaughtering, electrical current, percussion, water conductibility

Funding: German Federal Ministry for Food and Agriculture.

125-O*

Distress and eustress in the fish brain

Tschirren, Linda (Switzerland)[1]; Seitz, Andreas (Switzerland)[1]; Rhyner, Nicola (Switzerland)[1]; Pietsch, Constanze (Switzerland)[1]

1 - Zurich University of Applied Sciences

Introduction: While the effects of stress on the mammalian brain are under thorough investigation, it is poorly understood how distress and eustress affect the brain of teleosts. Being the centre of an elaborate nervous system, the fish brain is likely to be influenced by current and past stress, and may also alter the perception of future stressors. In a 4 year project our team seeks to better understand the complex interactions between stress and brain structures and started with investigating the gene expression levels in different parts of the brain.

Methodology: In this first step, carps were exposed to acute short-term stressors, air exposure as a distress and feeding as a eustress. The expression levels were measured using qPCR in 4 different parts of the brain, the telencephalon, the optical tectum, the hypothalamus and the cerebellum, and compared amongst the differently stressed fish and an unstressed control group. In addition, the measurements were conducted at three different time points, 10 min, 30 min and 60 min after the impact of the stressor. In order to compare the gene expression analysis with a commonly used parameter for stress, the plasma levels of several steroids, among them cortisol, were measured using a HPLC method.

Results: First results show that the stressors did alter the cortisol levels and that there are considerable differences in the expression of genes not only between the treatment groups but also amongst the brain areas. The steroid levels in the plasma show a pattern in time, with a peak 10 min after the stressors and a consecutive decrease. The corresponding gene expression data is currently analysed in the lab and results will be available by summer 2019.

Conclusion: The first preliminary results show how important the structuring of the teleost brain is when investigating the effects of stress on fish. Taking into account that different brain areas also have various functions and therefore may also be affected differently by stress is crucial to any future investigation.

Keywords: distress, eustress, brain, gene expression

Funding: IUNR, ZHAW Wädenswil.

126-O

Does flash photography cause stress in aquarium fish?

Knopf, Klaus (Germany)[1,4]; Buschmann, Katharina (Germany)[1,4]; Hansel, Martin (Germany)[2]; Radinger, Johannes (Spain)[1,3]; Kloas, Werner (Germany)[1,4]

1 - Leibniz-Institute of Freshwater Ecology and Inland Fisheries; 2 - Sea Life Berlin; 3 - University of Girona; 4 - Humboldt University of Berlin

Introduction: In public aquaria, the use of camera flashes is usually prohibited in order to avoid stress for the animals. However, the literature offers only very little information on whether photo flashes can induce stress in fish.

Methology: The Ram cichlid *Mikrogeophagus ramirezi* was used to examine whether flash photography can be a stressor for fish in aquaria. The potential stress response to camera flash-light was examined 22 min after exposure to a single flash and after repeated flashes by applying 10 flashes per minute for 8 h/ day over 2 weeks. Stress parameters were determined from whole body homogenates of individual fish. Additionally, the frequency of aggressive interactions between the fish was recorded.

Results: The stress parameters cortisol and glucose were not increased due to exposure to the flash light. In contrast, after a single flash mean cortisol values tended to be lower and mean glucose values were significantly lower than in the control group, and after repeated flashes mean cortisol and glucose values were significantly lower than in the control group. Furthermore, photo flashes caused a reduction of the frequency of aggressive interactions.

Conclusion: Neither cortisol nor glucose values indicated that photo flashes induce a physiological stress response in this fish species. Reduced values of the stress parameters in the exposed fish are explained by a possible irritation of the fish by the photo flashes, which reduces the natural aggressive interspecific behaviour and as a result the level of stress hormones.

Keywords: aquarium, flash photography, stress, behaviour

127-O

Influence of a nanofiltration - reactor on the bacterial microflora and on *Ichthyophthirius multifiliis* theronts in recirculating aquaculture systems

Jung-Schroers, Verena (Germany)[1]; Adamek, Mikolaj (Germany)[1]; Boley, Angela (Germany)[2]; Bauer, Julia (Germany)[1]; Korshun, Anna (Germany)[2]; Teitge, Felix (Germany)[1]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany; 2 - University of Stuttgart, Stuttgart, Germany

Introduction: Recirculating aquaculture systems offer the opportunity to keep high numbers of fish without the need of high amounts of fresh water due to recirculation and filtration of tank water. Problems can occur if the amount of bacteria or parasites in the water increases. As a method to reduce the amount of bacteria in the water and to keep the chemical water parameters in an optimal range, nanofiltration of the water is described.

Methodology: We tested nanofiltration reactors with integrated denitrification membranes in four different recirculation aquaculture facilities. In each facility one system was run with the reactor and identical systems without reactor were used as control. In three facilities the bacterial microflora was analysed in tank water and biofilms of tanks and in two facilities also the gills of fish were examined bacteriologically. In two of the systems cortisol measurements in water and blood samples were performed to determine the stress level of the animals in the system. In the fourth system fish were infected with the parasite *Ichthyophthirius multifiliis* and the effectivity of nanofiltration in filtering the theronts of this ciliate was determined.

Results: Overall it could be shown that by nanofiltration the total amount of bacteria in the tank water of recirculating aquaculture systems and on the gills of fish can be reduced. Additionally the diversity of bacteria was higher and cortisol levels were lower in the systems with installed reactor. A reduction of theronts of *Ichthyophthirius multifiliis* could also be detected in a system with installed reactor. One challenge was the increasing water temperature in systems with installed reactor and the operation of the reactor itself is time consuming.

Conclusion: The usage of a reactor with filtrating-membrane can have a positive influence on fish health and welfare.

Keywords: nanofiltration, *Ichthyophthirius multifiliis*, RAS, microflora

Funding: German Federal Environmental Foundation and German Federal Ministry for Food and Agriculture.

128-O*

Sea-based container culture of European lobster (*Homarus gammarus*) increases bacterial diversity of the gut and lowers susceptibility to viral infection.

Holt, Corey C. (United Kingdom)[1]; Van Der Giezen, Mark (United Kingdom)[2]; Daniels, Carly L. (United Kingdom)[3]; Stentiford, Grant D. (United Kingdom)[4]; Bass, David (United Kingdom)[4]

1 - University of Exeter/Centre for Environment, Fisheries and Aquaculture Science; 2 - University of Exeter; 3 - National Lobster Hatchery; 4 - Centre for Environment, Fisheries and Aquaculture Science

Introduction: The gut microbiome can markedly impact host fitness and survival. As such, environmental pressures on gut community composition may correlate with differential growth rates of the host and susceptibility to disease. Investigation into microbiome composition and the potential of its manipulation could, therefore, substantially benefit future aquaculture production.

Methodology: Using a large-scale mariculture project, we investigated compositional differences in the gut microbiota of the European lobster (*Homarus gammarus*), comparing sea-based animals with those retained in a land-based system, over a one-year period. Assessing the phylogenetic structuring of the gut community across the two locations, we assess the degree of determinism involved in its assembly. Furthermore, through means of a histology-lead health assessment, we correlate changes to the microbial community in relation to the incidence of a novel enteric viral infection, *Homarus gammarus* nudivirus (HgNV).

Results: High-throughput sequencing demonstrates that the gut microbiota was significantly different between land- and sea-based cultivation; with species richness and species diversity significantly increased in sea-based individuals. Furthermore, the determinism of the gut assembly in sea-based animals was greater than that of hatchery-reared individuals, indicating a higher degree of environmental filtering. The presence of viral histopathology in hepatopancreatic cells of the digestive tract correlated with a reduction of bacterial diversity in the gut and could implicate microbiome-mediated resistance to infection by the virus.

Conclusion: Our data provides the first extensive characterisation of the gut microbiome in the European lobster and indicates how better understanding of this complex community could be applied to aquaculture planning and management in order to maximise the cultivation of the host. We show that retainment of lobsters in open sea-based systems may benefit the overall health of the host by promoting the assembly of a more diverse gut bacterial community and reduced susceptibility to disease associated with the viral pathogen HgNV. Furthermore, characterisation of indigenous microbes within the gut could form the foundation of targeted probiotic development, further benefiting an ever-growing aquaculture sector.

Keywords: lobster, gut, microbiome, nudivirus, aquaculture

Funding: PhD studentship funded by Centre for Sustainable Aquaculture Futures (SAF).

Fish and Shellfish Immunology II

129-O

Early immune response in Atlantic salmon vaccinated with inactivated whole-cell bacterin from *Piscirickettsia salmonis* or infected with pathogenic isolates

Rozas-Serri, Marco (Chile)[1,2]; Peña, Andrea (Chile)[1]; Maldonado, Lucerina (Chile)[1]

1 - Laboratorio Pathovet; 2 - Newenko Group SpA

Introduction: Piscirickettsiosis (SRS) is the most challenging disease affecting the Chilean salmon industry. The control of SRS has focused mainly on chemotherapy and vaccination. Although the available vaccines based on bacterins, recombinant subunits and/or live-attenuated have not prevented SRS in Chile, they have delayed onset of the first outbreak. The aim of this study was to describe the immune response of Atlantic salmon intraperitoneally infected with the LF-89 and EM-90 isolates of *Piscirickettsia salmonis* and vaccinated with inactivated whole-cell bacterin from *P. salmonis*.

Methodology: To evaluate the expression of immune response genes, we used head-kidney samples obtained from Atlantic salmon intraperitoneally infected with LF-89 and EM-90 isolates and vaccinated with inactivated whole-cell bacterin of *P. salmonis*. Five live fish were sampled from each tank at 1 (12 Degree-Days), 3 (36 DD), 5 (60 DD), 7 (84 DD) and 14 days post-inoculation (168 DD). The genes expression was evaluated by normalized RT-qPCR.

Results: A positive correlation of the overexpression of IFNγ, IL-2, IL-10, IL-12ß, MHC-II and CD4 was seen in the PS-LF-89- and PS-EM-90-infected fish, but the proinflammatory response in the PS-EM- 90-infected fish was more exacerbated. The fish infected with PS-LF-89 showed an anti- inflammatory response, whereas this finding was not observed in the PS-EM-90-infected fish. Conversely, a positive correlation of the downregulation of IFNγ, IL-2, IL-12ß, MHC-I and CD8 was seen in the vaccinated fish. Fish infected with both *P. salmonis* isolates showed mhc1-mhc2, cd4-cd8b and igm overexpression, suggesting that *P. salmonis* promotes a CD4+ T- and CD8+ T cell response and a humoral immune response. The vaccinated-fish exhibited mhc1, mhc2 and cd4 overexpression but a significant downregulation of cd8b and igm, suggesting that the vaccine supported the CD4+ T-cell response but did not induce an immune response mediated by CD8+ T cells or a humoral response.

Conclusion: The expression patterns of genes related to the humoral and cell-mediated adaptive immune responses showed upregulation in fish infected with *P. salmonis* and downregulation in vaccinated fish. The results of this study contribute to our understanding of the immune response against *P. salmonis*and can be used in the optimization of SRS prevention and control measures.

Keywords: *Piscirickettsia salmonis*, immune response, vaccination

130-O*

Aeromonas salmonicida subsp. *salmonicida* activates rainbow trout IGM+ B cells through TLR signalling

Soleto, Irene (Spain)[1]; Martin, Diana (Spain)[1]; Tafalla, Carolina (Spain)[1]

1 - Animal Health Research Center

Introduction: *Aeromonas salmonicida* subsp. *salmonicida* is a Gram negative bacteria and the cause of furunculosis, one of the most important fish health problems in salmonid aquaculture. Although commercial vaccines are able to induce long-term protection, furunculosis outbreaks still occur. In order to improve these vaccines, it is quite relevant to investigate the effects that this bacteria produces on B cells, responsible for the production of specific antibodies. Given that B cells are singularly equipped with a B cell receptor (BCR) as well as with diverse innate receptors, B cells are able to integrate at the same time antigen-specific and innate signals.

Methodology: In this context, in the current study, we have investigated the effects of inactivated *A. salmonicida* on rainbow trout splenic IgM^+ B cells *in vitro* in the presence or absence of different inhibitors of Toll-like receptor (TLR) signalling, to establish to what degree innate signals are contributing to the activation of B cells in teleost. For this, after incubating splenocytes with inactivated *A. salmonicida* for different times, diverse parameters such as survival, proliferation, phagocytic capacities or expression of surface markers were determined through flow cytometry in the presence or absence of the inhibitors. The effect of *A. salmonicida* on the capacity of B cells to secrete IgM was also established through ELISPOT.

Results: *A. salmonicida* has a strong proliferative effect on rainbow trout IgM^+ B cells that goes along with a size increase and an augmentation of the levels of surface MHC-II on these cells. Additionally, a significant increase in the number of IgM secreting cells in the cultures was obtained after incubation with the bacteria. Interestingly, these effects were almost completely reverted in the presence of inhibitors of MyD88, an important node in TLR signal pathways.

Conclusion: Our results demonstrate that TLR signalling is essential for the activation of IgM^+ B cells and the onset of adaptive immune responses. The results derived from this study will be useful to generate novel vaccines and adjuvants against bacterial pathogens in the future.

Keywords: *A. salmonicida,* TLR, B cells, rainbow trout

Funding: ERC Consolidator Grant 725061 TEMUBLYM.

131-O

Stress reduces proliferative response of rainbow trout head kidney macrophages *in vitro*

Garcia Muñoz, Lorenzo (Spain)[1]; Romero Lopez, Maria (Spain)[1]; Revuelta Rueda, Luis (Spain)[1]; Garcia Cabrera, Jose Antonio (Spain)[1]

1 - Facultad de Veterinaria, Universidad Complutense de Madrid, Madrid, Spain

Introduction: Macrophages provides a critical first line of defence of fish against invading pathogens. Presence of an invader would lead to proliferation of head kidney macrophages due to the secretion of several growing factors (like MGF, but also respiratory burst products have been pointed as potential proliferative promoters). At this point, anything that could compromise macrophage response would, therefore, negatively affect their capacity to face and fight the harm. Aquaculture means some level of disturbance that, at some moments, can place fish in a situation beyond its normal level of tolerance. At these moments stress levels will increase, and as result fish may be more vulnerable to any aggressor.

Methodology: We studied the effect of stress (two hours crowding) on the proliferative response of head kidney macrophages. A non-stressed group was included as control. Macrophages from head kidney of both groups were obtained and placed on a 96 well plate. Then, *E. coli* was added to each well at a moi of 50:1 (50 bacteria per macrophage), and incubated at 22 ºC. Macrophages from two wells of each group were counted in duplicate at 0, 4 and 24 hours post-infection (hpi) using a Neubauer chamber.

Results: Our results show an increase in the number of macrophages in the non-stressed group from the 4 hpi count that continued up to 24 hpi. On the other hand, in the stressed group the number of macrophages suffered an initial decrease at 4 hpi, and then it increased at 24 hpi. However, in spite of the final increase, the number of macrophages at 24 hpi in the stressed group was significantly lower than that in the non-stressed group.

Conclusion: These results suggest a negative effect of stress on macrophages that would compromise the response of these cells to an infectious agent.

Keywords: stress, macrophage, rainbow trout, crowding, Neubauer chamber

132-O*

Rainbow trout preferentially respond to thymus-independent antigens upon anal administration

Martín-Martín, Alba (Spain)[1]; Díaz-Rosales, Patricia (Spain)[1]; Abós, Beatriz (Spain)[1]; Tafalla, Carolina (Spain)[1]

1 - Animal Health Research Center, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Valdeolmos, Madrid, Spain

Introduction: Thymus dependent (TD) responses are those organized in germinal centers (GCs) through cooperation of T helper cells, whereas thymus independent (TI) antigens are capable of triggering B cell responses in the absence of T cell help by B cell receptor (BCR) cross-linking or through Toll-like receptor (TLR) engagement. In mammals, while TD responses are executed by B2 cells, TI responses mainly involve other B cell subsets such as B1 cells or marginal zone (MZ) B cells, which provide an early response against pathogens. Recent evidence has pointed out many functional and phenotypic similarities between fish IgM^+ B cells and mammalian B1 cells. In this context, it is of great importance to study how fish B cells respond to different types of antigens and this is what we have undertaken in the current study in rainbow trout, administering the antigens anally.

Methodology: TNP-KLH (2,4,6-trinitrophenyl hapten conjugated to keyhole limpet hemocyanin) and TNP-LPS (2,4,6-trinitrophenyl hapten conjugated to lipopolysaccharides) were used as model TD and TI antigens respectively. Both antigens were administered to groups of rainbow trout of approximately 10 cm by anal inoculation in the absence of adjuvants. An additional group anally inoculated with saline was included as a control. The immune response elicited by the antigens was investigated after 15 and 30 days through different techniques, including ELISA, ELISPOT, real time PCR and immunofluorescence.

Results: Serum anti-TNP antibodies were mostly detected in rainbow trout stimulated with TNP-LPS. Similarly, the number of TNP-specific antibody secreting cells (ASCs) detected in spleen and kidney after anal administration of antigens, was significantly higher in rainbow trout stimulated with TNP-LPS than in trout inoculated with TNP-KLH. Furthermore, the anal administration of TNP-LPS significantly decreased the transcriptional levels of Foxp3a and Foxp3b in intestine, suggesting a breach in tolerogenic responses in response to TI stimulation.

Conclusion: Rainbow trout IgM^+ B cells preferentially respond to TI antigens when administered anally in the absence of adjuvants.

Keywords: TNP-LPS, TNP-KLH, B cells, rainbow trout

Funding: ERC Consolidator Grant 725061 TEMUBLYM.

133-O*

Immune related genes of cobia (*Rachycentron canadum*) responding to *Streptococcus dysgalactiae* infection

Maekawa, Shun (Japan)[1]; Wang, Pei-Chi (Taiwan)[1]; Chen, Shih-Chu (Taiwan)[1]

1 - Department of Veterinary Medicine, College of Veterinary Medicine, National Pingtung University of Science and Technology, Pingtung, Taiwan

Introduction: *Streptococcus dysgalactiae* is a gram-positive bacterium and a harmful aquaculture pathogen. To investigate the immune response against *S. dysgalactiae*, we performed transcriptome analysis of head kidney and spleen in cobia (*Rachycentron canadum*) using RNA-seq.

Methods: Total RNA was extracted from head kidney and spleen of cobia 1 and 2 days after treated with *S. dysgalactiae* or PBS as a control. After purification of RNA and generating cDNA library, sequencing was performed using the Illumina HiSeq™ 4000 platform. The filtering and de novo assembling transcripts were annotated using several database. To identify differentially-expressed genes (DEGs) between *S. dysgalactiae* and PBS group, the fragments per kilobase of transcripts per million fragments mapped values were calculated.

Results: After de novo assembly, a total of 106,984 transcripts was detected with an N50 of 3,020 bp. These transcripts annotated and categorized to a total of 7,608 genes based on KEGG pathway database. DEGs (2-fold difference and adjusted p value < 0.05) were calculated by comparing the gene expression levels between *S. dysgalactiae* and PBS control groups at each time point: a total of 232 from head kidney at 1 day post infection (dpi), 368 from head kidney at 2 dpi, 491 from spleen at 1 dpi, and 116 from spleen at 2 dpi, respectively. The DEGs were annotated into signal transduction and immune system categories, based on the KEGG database. The DEGs were significantly enriched in immune-related pathway functions, “Cytokine-cytokine receptor interaction”, “Complement and coagulation cascades”, and “Phagosome”.

Conclusion: In this study, immune related genes responding to *S. dysgalactiae* were detected and categorized several immune system pathways. It is suggested that the pathways enriched by DEGs will contribute the immune reaction against *S. dysgalactiae* infection. The data revealed in this study may provide offer improved strategies against *S. dysgalactiae* infection in teleost fishes.

Keywords: RNA-seq, immune response, cobia (*Rachycentron canadum*), *Streptococcus dysgalactiae*

134-O

Development of a monoclonal antibody against CD4-2 of ginbuna crucian carp, *Carassius auratus langsdorfii*

Lau, Lik-Ming (Japan)[1]; Nakanishi, Teruyuki (Japan)[2]; Sano, Motohiko (Japan)[1]; Kato, Goshi (Japan)[1]

1 - Laboratory of Fish Pathology, Tokyo University of Marine Science and Technology, Tokyo 108-8477, Japan; 2 - Department of Veterinary Medicine, Nihon University, Kanagawa 252-0880, Japan

Introduction: Teleost fish possess two divergent forms of CD4 molecules: CD4-1 resembling mammalian CD4 and CD4-2 a unique CD4 homolog in teleost fish. A monoclonal antibody (mAb) against CD4-1 of ginbuna crucian carp had been developed to study the helper function of CD4-1$^+$ cells. The CD4-1$^+$ cells facilitated a secondary Th2 humoral immunity against virus infection in ginbuna. However, there are few functional studies of CD4-2$^+$ cells in the teleost fish, so that the roles of CD4-2$^+$ cells in immune response remains unclear. In this study, we aimed to develop a mAb against ginbuna CD4-2 for further analyses of CD4-2$^+$ cells in the teleost fish.

Methodology: BALB/c mice were subcutaneously immunized with 50 µg of an expression plasmid encoding ginbuna CD4-2, then followed by 1.0 × 10^6 of BALB/3T3 cells expressing CD4-2 (Gb CD4-2$^+$-3T3) as the final boost. Expression of CD4-2 with *myc* epitope on the cell surface of BALB/3T3 cells was examined by flow cytometry (FCM) and western blotting (WB) using an anti-*myc* antibody. Hybridomas were generated by conventional techniques, and the culture supernatants were screened by FCM and immunofluorescence assay (IFA) with Gb CD4-2$^+$-3T3 cells (1st) and head-kidney leukocytes isolated from ginbuna (2nd). Further, ginbuna head-kidney leukocytes were double stained with the established mAb and anti-CD4-1 mAb, followed by FCM.

Results: All of the Gb CD4-2$^+$-3T3 cells reacted with anti-*myc* antibody in FCM and protein band corresponding to the predicted molecular weight of CD4-2 (46 kDa) was detected in WB. The first screening of hybridomas by FCM and IFA showed that five culture supernatants reacted with Gb CD4-2$^+$-3T3 cells in FCM and IFA. After the second screening, two mAbs designated as D5 and E10 which were highly reactive with ginbuna head-kidney lymphocytes, were established. Double staining with anti-CD4-1 mAb and D5/E10 mAb revealed that the lymphocytes gate in head-kidney leukocytes consists of 6.3%/8.2% of CD4-1 single positive cells, 18.7%/15.2% of CD4-2 single positive cells, and a very few CD4-1/CD4-2 double positive cells (3.2%/6.0%).

Conclusion: D5 and E10 mAb are the powerful tools to study the role of CD4-2$^+$ cells in ginbuna crucian carp, as a model fish species.

Keywords: monoclonal antibody, CD4-2, ginbuna crucian carp

135-O

Immune response in a goldfish resistant strain to cyprinid herpesvirus 2 experimental infection

Shirato, Makoto (Japan)[1]; Yoshii, Keisuke (Japan)[1]; Nakajima, Mayuri (Japan)[1]; Tanaka, Mikio (Japan)[2]; Kato, Goshi (Japan)[1]; Sakamoto, Takashi (Japan)[1]; Sano, Motohiko (Japan)[1]

1 - Tokyo University of Marine Science and Technology, Tokyo, Japan; 2 - Saitama Fisheries Research Institute, Saitama, Japan

Introduction: Herpesviral hematopoietic necrosis (HVHN) caused by cyprinid herpesvirus 2 (CyHV-2), has affected the commercial production of goldfish *Carassius auratus* and gibelio carp *C. auratus gibelio*. A goldfish strain (Azumanishiki variety) resistant to HVHN has been established by Saitama Fisheries Research Institute, and over 90% of them can survive in artificially induced HVHN. In this study, we analyzed differences in immune response between the resistant and susceptible strains to elucidate the disease resistant mechanisms.

Methodology: For investigating mortality rates, virus kinetics and immune related genes expression in fish of both strains, we conducted infection experiment using resistant and susceptible strain fish challenged by immersion infection with CyHV-2 Sat-1isolate at a concentration of $10^{1.1}$ $TCID_{50}$/mL for 1 h. The fish were reared at 25 °C with running water. Three fish were collected at 0, 1, 3, 5, 7, 10, and 14 days post infection (dpi), and the trunk kidney, spleen, gills and caudal fin were sampled for DNA and RNA extractions. Extracted DNA and RNA were subjected to qPCR for estimation of virus DNA loads and expression analysis of immune related genes including TNFα1, caspase 3, granzyme, MHC class I, CD8, interferon, Mx1 protein and STAT1, respectively.

Results: Cumulative mortality rates of susceptible and resistant strains were 100% and 10%, respectively. Virus DNA loads in resistant fish increased until 3 dpi, then decreased. Gene expression analysis showed that the expression level of TNFα1 and caspase 3 in the trunk kidney were increased in resistant fish, but decreased in susceptible fish. Expression levels of granzyme in the spleen and MHC class I in all tested organs of resistant fish were significantly higher than those in susceptible fish regardless of CyHV-2 infection. There were no significant differences in CD8, interferon, Mx1 protein and STAT1 gene expression in fish of both strains.

Conclusion: The result about TNFα1 and caspase 3 suggested induction of apoptosis of the infected cells in resistant fish, resulting in suppressing assembly of infectious virus particles in the cells and consequently decreasing virus load in fish since 4 dpi. Granzyme and MHC class I may use selective bio-markers for the resistant fish.

Keywords: goldfish, herpesviral hematopoietic necrosis, apoptosis, caspase 3, TNF α

Diagnostics II

136-O

Phylogenetic analyses and diagnostic tools improvement to optimize the control of regulated rhabdoviroses in France

Louboutin, Lénaïg (France)[1]; Cabon, Joëlle (France)[1]; Baud, Marine (France)[1]; Almeras, Fabrice (France)[1]; Chalopin, Morgane (France)[1]; Vigouroux, Estelle (France)[1]; Pallandre, Laurane (France)[1]; Bellec, Laure (France)[2]; Thiery, Richard (France)[3]; Olesen, Niels Jorgen (Denmark)[4]; Panzarin, Valentina (Italy)[5]; Pereira Dopazo, Carlos (Spain)[6]; Stone, David (United Kingdom)[7]; Ryder, David (United Kingdom)[7]; Bremont, Michel (France)[8]; Blanchard, Yannick (France)[9]; Morin, Thierry (France)[1]

1 - French Agency for Food, Environmental and Occupational Health & Safety, Ploufragan-Plouzané-Niort Laboratory, Unit Viral Diseases of Fish, National Reference Laboratory for listed fish diseases, Plouzané, France; 2 - Laboratory EPOC UMR CNRS 5805, University of Bordeaux, Pessac, France; 3 - Anses, Sophia Antipolis Laboratory, Sophia-Antipolis, France; 4 - DTU, Lyngby, Denmark; 5 - IZSVe, Padova, Italy; 6 - Instituto de Acuicultura, Universidade de Santiago de Compostela, Spain; 7 - CEFAS, Weymouth, UK; 8 - VIM, INRA, Paris-Saclay University, Jouy-en-Josas, France; 9 - Anses, Ploufragan-Plouzané-Niort Laboratory, Unit Viral Genetic and Biosecurity, Ploufragan, France

Introduction: Viral Haemorrhagic Septicaemia Virus (VHSV) and Infectious Haematopoietic Necrosis Virus (IHNV) are the causative agents of severe aquatic diseases affecting several fish species. They are regulated in European Union (EU) in order to reduce the risks of dissemination by trade. In France, a monitoring and eradication plan is currently being set up with the objective to obtain a free status for all the territory. This presentation aims to describe recent works carried out to improve the diagnostic and the epidemiological monitoring of these diseases in our territory.

Methodology: To better understand viral evolution and circulation, a Bayesian inference approach was applied to partial glycoprotein gene sequences of 88 IHNV representative strains isolated in France over the period 1987-2015. To improve diagnostic, real time RT-PCR methods (qPCR) specific to IHNV and VHSV were developed and validated in accordance with the implementing decision EU 2015/1554. They include an innovative strategy based on the use of a RNA bacteriophage as universal exogenous external control.

Results: IHNV sequences showed mean nucleotide and amino-acid identities of 97.1 and 97.8% respectively and a viral population clustered into three groups with a clear spatial differentiation suggesting a predominant role of local reservoirs in contamination. Atypical "signatures" of some isolates showed the usefulness of molecular phylogeny for tracking the spread of these viruses. qPCR methods appeared highly robust with absence of cross-reactions. A more discriminative molecular test is in development based on the identification of virulence markers following the pathotyping and full-length sequencing of 68 strains of VHSV representative of all genogroups.

Conclusion: The systematization of sequencing during VHS or IHN epidemics is helpful to identify potential sources of contamination. Deploying validated qPCR tests for official diagnosis will speed up targeted analyses. Identification of virulence markers could be used to determine the level of hazard associated with an isolate and to adapt management measures. Taking together, all these actions will contribute to the improvement of the monitoring and control of fish novirhabdovirus.

Keywords: Viral Haemorrhagic Septicaemia Virus, Infectious Haematopoietic Necrosis Virus, phylogeny, validated RTqPCR, virulence

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137-O

Nodular gill disease in rainbow trout… an emerging disease?

Bermúdez, Roberto (Spain)[1]; Losada, Ana (Spain)[1]; De Azevedo, Ana Manuela (Portugal)[1,2]; Castrillo, Pedro Antonio (Spain)[1]; Ronza, Paolo (Spain)[1]; Rubiolo, Juan Andrés (Spain)[1]; Sotelo, Miguel (Spain)[3]; Quiroga, Mª Isabel (Spain)[1]

1 - Universidade de Santiago de Compostela; 2 - Ghent University; 3 - BioMar Iberia S.A.

Introduction: Several specimens of rainbow trout (*Oncorhynchus mykiss*), with a history of respiratory stress, apathy, erratic swimming and anorexia, were sent to our laboratory.

Methodology: Necropsies were performed, and tissue samples were fixed in 10% buffered formalin and routinely processed for light microscopy.

Results: The histological examination of the gills revealed a marked hypertrophy, hyperplasia and dysplasia of the branchial lining epithelium, which led to broad lamellar fusions or even filament fusion. In most of cases, a notorious clubbing affecting the apex of the filament could be seen. Occasionally the epithelial cells showed signs of hydropic degeneration and spongiosis, with separation of the lining epithelium and accumulation of proteinaceous material and inflammatory/necrotic cells in the neoformed space. A mild to moderate mononuclear inflammatory infiltrate was observed, as well as epithelial proliferation, evidenced by the high number of mitoses. In some fish the presence of telangiectasias and thrombi in the secondary lamellae was notorious. On the surface of the hyperplastic epithelium numerous organisms compatible with amoebas, with an intense eosinophilic and vacuolated cytoplasm containing a central basophilic nucleus, were observed, as well as an elevated number of filamentous bacteria, consistent with *Flavobacterium* spp. Besides the histopathological evaluation, a PCR analysis of the gills was carried out, which detected the presence of *Cochliopodium* spp., a genus of discoid or globose amoebas, previously reported infecting salmonids in freshwater environment.

Conclusion: Based on the histological observations and molecular findings a diagnosis of nodular gill disease (NGD) was made. In recent years, several outbreaks of NGD in rainbow trout have been detected in European countries, associated with respiratory stress and high mortalities. It is a multifactorial insidious disease, where the amoebas are a constant finding. Its correct diagnosis and attention by fish health consultants are key factors to control and prevent this potential emerging disease in Europe.

Keywords: gill amoebas, freshwater amoebas, histopathology

Funding: The regional government of Galicia (Xunta de Galicia) through the project "Consolidación y estructuración de unidades de investigación competitivas (GPC2015/034)" and the Postdoctoral fellowship granted to AM de Azevedo.

138-O

Novel immunodiagnostic tools for nodavirus detection in *Dicentrarchus labrax* brain samples

Galán, Carmen (Spain)[1]; Fresco, Alba (Spain)[1]; Redondo, Elena (Spain)[1]; Rodríguez, Maria José (Spain)[1]; García, Isabel (Spain)[1]; Antonio, Sanz (Spain)[1]; Rueda, Paloma (Spain)[1]

1 - Inmunología y Genética Aplicada S.A., Hnos García Noblejas 41-4º, 28037 Madrid, Spain

Introduction: Viral nervous necrosis (VNN) is a highly infective neuropathological disease caused by piscine Betanodaviruses with significant impact in Mediterranean aquaculture. RGNNV is the most common genotype, followed by SJNNV, and reassortant strains RGNNV/SJNNV are emerging as a new threat, requiring from specific diagnostic tools and multivalent vaccines to identify and protect against those viruses. Besides RT-qPCR testing for disease surveillance, we aim to develop on-site diagnostic tests to detect and distinguish the main genotypes.

Methodology: Nodavirus virus like particles (VLPs) from RGNNV and SJNNV were obtained in baculovirus expression system and characterized by electron microscopy and thermal shift assays. VLPs were used to immunize rabbit or mice to generate polyclonal (pAbs) and monoclonal antibodies (mAbs), respectively; from which different virus-detection assays were developed. Antibodies were characterized by immunofluorescence, western blot and ELISA with infectious virus and VLPs. Polyclonal Abs were initially used to develop both ELISA-DAS and lateral flow chromatography assays that were tested with field samples.

Results: Baculovirus expression of RGNNV and SJNNV nodavirus capsid proteins lead to the formation of VLPs highly resembling the parental viruses. The yield and purity of the obtained VLPs could make them suitable vaccine candidates. Using the VLPs as immunogens, two high titer rabbit pAbs specific for RGNNV or for both genotypes with comparable sensitivity were generated. Those pAbs were used to set up ELISA-DAS and lateral flow immunoassays, reaching an assay limit of detection of 10ng/ml with the recombinant VLPs. The assays were preliminary tested with 5 field samples, using nodavirus-infected seabass brain samples of different viral load that were previously characterized by RT-qPCR and sequencing. The assay detected virus in brain samples up to Ct 15. We are currently optimizing the brain sample preparation and enlarging the number of field samples being tested. In addition, new mAbs specific for RGNNV and SJNNV were obtained and are being tested for their application in the different immunoassays.

Conclusion: Newly developed ELISA-DAS and chromatographic immunoassays could be useful for vaccine quality testing or for rapid disease verification of symptomatic individuals, respectively.

Keywords: nodavirus, VLPs, diagnostics, immunoassay, antibodies

139-O

Isolation and characterization of a bunyavirus associated with clinical infection of black bass, *Micropterus salmoides*

Soto, Esteban (United States of America)[1]; Imai, Denise M. (United States of America)[1]; Yun, Susan (United States of America)[1]; Leary, John H. (United States of America)[2]; Ng, Terry F.F. (United States of America)[2]; Rosser, Thomas G. (United States of America)[3]; Camus, Alvin (United States of America)[2]

1 - University of California, Davis; 2 - University of Georgia; 3 - Mississippi State University

Introduction: In October 2017, diseased black bass (*Micropterus salmoides*) fingerlings recently shipped from Arkansas, USA to California, USA were submitted for disease diagnostics. Approximately 132,000 fish were distributed inside an indoor greenhouse rearing facility within round fiberglass tanks receiving freshwater from a well. Upon arrival, yellow nodules were observed scattered throughout the body and fins of multiple fish, and prophylactic treatments with potassium permanganate, formalin, copper sulfate, and salt were administered. The producers reported that over the following 3 months approximately 5% of the fish developed cutaneous lesions around the mouth.

Methodology: Affected animals were subjected to complete necropsy, including histological and parasitological examinations. Samples of internal and external tissues, including buccal lesions, were pooled for virus isolation on multiple cell lines. Total nucleic acids were extracted from concentrated cell culture supernatants as well as infected cell monolayers. Samples were reverse transcribed and PCR amplified. The resulting randomly amplified DNA was pooled and subjected to metagenomic sequencing using the Illumina MiSeq platform.

Results: Gross necropsy revealed encysted trematode metacercariae within nodules suggestive of "yellow grub" infection. *Clinostomum marginatum* was confirmed following amplification and sequencing of the internal transcribed spacer region and *cox*1 gene. Histopathological evaluation of the mouth lesions revealed markedly proliferative cheilitis and anterior stomatitis characterized by epithelial hyperplasia and dysplasia, superficial ulceration, and a dense lymphohistiocytic subepithelial infiltrate accompanied by multinucleated giant cells. In some fish, the lymphocytic infiltrate was suspected to be neoplastic. One-week post-inoculation, a bluegill (*Lepomis macrochirus*) fry fibroblast-like cell line incubated at 15 °C exhibited cytopathic effects consisting of cell rounding and lifting into the supernatant. Electron microscopic analysis of the infected cells revealed 68 ± 4.6 nm viral particles within the cytoplasm of degenerate spindle cells. Next generation sequence data identified a complete viral genome representing a novel member of the genus *Bunyavirus*, family *Bunyaviridae*.

Conclusion: This study represents the first isolation and characterization of a member of the genus *Bunyavirus*, family *Bunyaviridae* infecting fish.

Keywords: bunyavirus, novel, bass

140-O*

Isolation and characterization of EVEX in glass eels (*Anguilla anguilla*)

Županičić, Ivana Giovanna (Croatia)[1]; Pavlinec, Željko (Croatia)[1]; Oriać, Dražen (Croatia)[1]; Žuvić, Luka (Croatia)[2]; Zrnčić, Snježana (Croatia)[1]

1 - Croatian Veterinary Institute; 2 - Institute for Oceanography and Fisheries

Introduction: The European eel (*Anguilla anguilla*) is a catadromous species, an inhabitant of the Croatian rivers belonging to the Adriatic watershed. The species is critically endangered according to IUCN list and is facing an extremely high risk of extinction in the wild. Besides overfishing, and trafficking, the diseases like swim bladder nematode *Anguillicoloides crassus*, pathogenic bacteria, and pathogenic viruses: eel virus European (EVE), the eel virus European X (EVEX) and the anguillid herpesvirusare threatening its presence in European rivers. Totally 252.000 of glass eels were confiscated during trafficking at the Zagreb airport and placed into the tanks of the city zoo with the intention to be used for re-population of the natural habitats in Croatia.

Methodology: Species was determinedby analyzing the nucleotide sequence of cytochrome c oxidase subunit 1. Parasitological, bacteriological and virological examinations were carried out by microscopic analysis, seeding of material from organs on blood agar;and inoculating the homogenized tissues onto BF2 and EPC cell lines. Nucleic acid extraction and purification from cell culture supernatants were performed using a commercial kit. The extracted nucleic acids were used as a template for PCR and real-time RT-PCR. Viral RNAs were tested for EVE, EVEX and AnHV. New primers were designed for amplification and sequencing of EVEX N, P, M and G genes. Phylogenetic analysis was performed with obtained sequences.

Results: The cytopathic effect (CPE) in the cell monolayer was observed under the light microscope. RT-qPCR tested positive for the rhabdovirus eel virus European X (EVEX). Bioinformatic analysis of EVEX genes showed that the isolated strain is unique and phylogenetically closest to strains previously described in France.

Conclusion: Confirmed case of EVEX poses a doubt of re-population of the glass eels into natural habitats in Croatia. However, there is no data on the health status of the European eel populations from these rivers. Future research should be undertaken to support the epidemiological status of European eel in open waters.

Keywords: EVEX, *Anguilla anguilla*, trafficking, phylogeny

141-O

Genetic identification of two variants of a sturgeon mimivirus using high-resolution melting analysis

Pallandre, Laurane (France)[1]; Lesne, Mélanie (France)[2]; De Boisséson, Claire (France)[1]; Charrier, Amélie (France)[2]; Daniel, Patrick (France)[2]; Tragnan, Arthur (France)[3]; Debeuf, Bastien (France)[4]; Chesneau, Valérie (France)[5]; Bigarré, Laurent (France)[1]

1 - Agence Nationale de Sécurité Sanitaire; 2 - Laboratory of Pyrénées and Landes; 3 - Goupement de défense sanitaire aquacole aquitain; 4 - SCEA Sturgeon; 5 - Les esturgeons de l'Adour

Introduction: Two genomic variants, var1 and var2, of the mimivirus AcIV-E infecting sturgeons in Europe, have been described. They differ at least by five single-nucleotide polymorphisms (SNP) in the Major Capsid Protein gene. A high-resolution melting (HRM) assay was set up in order to distinguish these two variants.

Methodology: A set of primers was chosen to amplify a short region of 80 bp in the MCP gene. The two distinct alleles were amplified and inserted into plasmids to evaluate the sensitivity of the assay. Virus-positive field samples from 2016 and 2017 were used to evaluate the method.

Results: The HRM assay detected as little as 100 copies of plasmids harboring cloned sequences of var1 and var2, which have melting temperatures (Tm) differing by only 1 °C. The assay was specific of AcIV-E as demonstrated by the absence of signal when testing a related, yet distinct, virus as well as DNA from an AcIV-E-negative sturgeon sample. Experiments with mixtures of two distinct plasmids revealed abnormal melting curve patterns, which showed dips just before the main melting peaks. These dips in the curves were interpreted as the dissociation of heteroduplexes fortuitously created during the PCR step. A screening of 128 AciV-E-positive field samples of Russian sturgeons from three farms revealed the presence of var2, based on the Tm. However, for 7 samples (5.4 %), the melting curves showed patterns typical of var2 as the dominant viral genome, mixed with another minor variant which proved to be var1 by Sanger sequencing.

Conclusion: In conclusion, HRM is a simple method to screen for AcIV-E var1 and var2 and can be used on a large scale in Europe in order to monitor the spread of these two variants which likely represent two genetic lineages.

Keywords: mimivirus, sturgeon, high-resolution melting, genotyping

Funding: The European Regional Development Fund (FEDER 160710), the Nouvelle Aquitaine regional council and partner fish farmers.

142-O

Simple and rapid methods for tilapia lake virus on-site detection and serological diagnostic

Weiwei, Zeng (China)[1]; Yingying, Wang (China)[1]; Qing, Wang (China)[1]; Jiyuan, Yin (China)[1]; Yingying, Li (China)[1]; Bergmann, Sven M. (Germany)[2]; Defeng, Zhang (China)[1]; Caixia, Gao (China)[1]; Chun, Liu (China)[1]; Huzi, Hu (China)[1]; Cunbin, Shi (China)[1]; Yan, Ren (China)[1]

1 - Pearl-River Fisheries Research Institute of CAFS; 2 - Friedich-Loeffler-Institute, Insel Riems

Introduction: Recently, a newly segmented RNA virus resembling orthomyxovirus named tilapia lake virus (TiLV) has been considered as a potential threat to global tilapia production. Currently, the lack of anti-viral therapeutics and no vaccines against TiLV are available. Therefore, early and rapid diagnosis is urgent need to isolate infected aqua farms and implementation of prompt control measures.

Methodology: In order to meet the practical on-site and serological diagnostic required, a RT-LAMP for detection of RNA and an indirect ELISA used for detection of antibody against TiLV were investigated. Primers according to the conserved sequence of segment 2 genes were designed and the sensitivity, specificity and the detection effect of field tissue samples were evaluated. The indirect ELISA was developed by used segment 5 encoded protein of TiLV as the coating antigen and the monoclonal antibodies against IgM of tilapia as the secondary antibody. The antigen concentration and serum dilution were optimized by using chess board titration, and in comparison with results obtained from IFA by testing of 40 serum samples to evaluate the sensitivity and specificity, and A serological survey was performed using the ELISA with tilapia field serum samples.

Results: The optimized RT-LAMP reaction was maintained the isothermal condition of 63 °C for 45 min, and the products could be verified by color change with the addition of SYBR Green I. The detection sensitivity of this method is 1.6 copies/μL, and had a diagnostic sensitivity and specificity of 100% when TiLV positive samples and non-target virus were tested. In comparison with the results IFA ,The ELISA had a diagnostic sensitivity and specificity were 90% and 96.7%, respectivel, and the seropositive rate of the 218 field serum samples was 26.8%.

Conclusion: The developed RT-LAMP and indirect ELISA is a very specific and sensitive test that will be useful for TiLV on-site detection and large-scale serological surveys.

Keywords: tilapia lake virus, detection, RT-LAMP, ELISA

Molluscs and Crustacean Diseases I

143-O

Implementing an eDNA based method for assessing the spread of a listed disease: the Irish national crayfish plague surveillance programme

O'Toole, Ciar (Ireland)[1]; Morrissey, Teresa (Ireland)[1]; Nelson, Brian (Ireland)[2]; Dore, Bill (Ireland)[1]

1 - Marine Institute; 2 - National Parks and Wildlife Service

Introduction: *Aphanomyces astaci* is the causative agent of crayfish plague, a World Organisation for Animal Health (OIE) listed disease which causes mortality in the white-clawed crayfish *Austropotamobius pallipes*. This is the only crayfish species native to Ireland, is protected under the EU Habitats Directive and is considered endangered. The first confirmed outbreak of crayfish plague in Ireland was recorded in the Erne catchment in 2015. Since then, it has been detected in six additional geographically distinct catchments, with three different strains of *A. astaci* genotyped. This genetic diversity suggests there have been at least three separate introductions of the disease into Ireland.

Methodology: An eDNA based National Surveillance Programme, funded by the Irish National Parks and Wildlife Service, was implemented in mid-2018. This programme aims to assess all Irish catchments with known *A. pallipes* populations for the presence of *A. astaci* over a two year period. The first season of sampling is complete with 15 of 30 catchments surveyed. In each catchment, six sites are sampled by filtering three replicates of five litres and retaining the filter paper for eDNA analysis. After DNA extraction, the samples are tested by qPCR for the presence of *Aphanomyces astaci.* Future screening will look for the presence of DNA from non-native crayfish species.

Results: eDNA analysis of collected water samples is on-going, with samples initially screened for the presence of *A. astaci*, and work now commencing to look for the presence of non-native crayfish eDNA. Initial results have validated the methods' ability to detect crayfish plague in Irish freshwater systems. Full results from year one of the programme will be presented here.

Conclusion: The programme aims to add to the knowledge on the extent of spread of crayfish plague in Ireland as well as understanding the possible sources of the disease and its routes of transmission. Such data is critical to developing disease control measures to protect the endangered white-clawed crayfish in Ireland.

Keywords: crayfish plague, *Aphanomyces astaci*, white-clawed crayfish, eDNA

144-O

Crayfish plague in Ireland - a complex story

Cheslett, Deborah (Ireland)[1]; Morrissey, Teresa (Ireland)[1]

1 - Marine Institute

Introduction: Crayfish plague is a highly infectious lethal disease caused by spores of *Aphanomyces astaci*, a member of the Oomycetes. This OIE listed disease affects the white-clawed crayfish *Austropotamobius pallipes*, which is the only crayfish species native to Ireland. Ecologically, the white-clawed crayfish is an important keystone species which is protected under the EU Habitats Directive and is on the IUCN Red List of Endangered species. Ireland has been regarded as a stronghold for the white-clawed crayfish in Europe, following its widespread decline due in large part to crayfish plague, but existing Irish populations could potentially be eliminated if crayfish plague becomes established nationally.

Methodology: Screening for the presence of *Aphanomyces astaci* is carried out by real time PCR according to the method previously described, which detects all 5 genotypes described to date. Linkages between mortality outbreaks in Ireland are being investigated through two genotyping approaches: microsatellite typing and a new PCR-based genotyping assay.

Results: Genotyping analysis by OIE reference laboratories in the UK and in Finland have shown there are at least three different genotypes of *Aphanomyces astaci* present in Ireland.

Conclusion: The first outbreak of crayfish plague in Ireland was confirmed by the Marine Institute, in 2015. Control measures were rapidly put in place to reduce the risk of spread and yet there have been a further 7 confirmed outbreaks across geographically distinct river catchments across the whole of Ireland. Genotyping suggests limited linkages between the outbreaks with at least three separate introductions of the disease into the country suspected. Whilst the source of the introductions has not yet been identified the risks posed by the possible introduction of non-native North American crayfish species which are established in the wild across Europe is clear. Several species are thought to be present in Ireland in private ownership as pets but have not yet been found in the wild. There is now an urgent need in Ireland for a better understanding of the possible sources of the disease, its routes of transmission, and its current spread.

Keywords: crayfish plague, Ireland, genotypying

145-O*

Identification and pathogenicity of pathogenic bacterium isolated from mud crab *Scylla serrata* larvae

Midorikawa, Yuki (Japan)[1]; Shimizu, Tomohito (Japan)[2]; Sanda, Tetsuya (Japan)[2]; Hamasaki, Katsuyuki (Japan)[1]; Dan, Shigeki (Japan)[1]; Kato, Goshi (Japan)[1]; Sano, Motohiko (Japan)[1]

1 - Tokyo University of Marine Science and Technology, Tokyo, Japan; 2 - Seikai National Fisheries Research Institute, Japan Fisheries Research and Education Agency, Okinawa, Japan

Introduction: Bacterial diseases are the major obstacles in the mud crab seed production and a pathogenic bacterium which caused mass mortality in zoea stages of mud crab *S. serrata* hatchery-reared in Ishigaki Island, Okinawa, Japan was isolated. In this study, we investigated the classification and pathogenicity of this bacterium.

Methodology: We used NY 1 strain isolated from the mud crab (5th stage zoea) in 2000. Extracted DNA from the bacterium was sequenced using Illumina MiSeq. The sequence was classified by phylogenetic analysis. We conducted infection experiments of artemia *Artemia francisca* nauplii, Japanese marsh shrimp *Caridina multidentata* 1st stage zoea, swimming crab *Portunus trituberculatus* 1st stage zoea and mud crab *S. serrata* 1st stage zoea. Larvae (n = 20-30) were immersed for one hour in the bacterial suspension at low (about 10^3 CFU/ml) and high (about 10^5 CFU/ml) challenge doses and reared in 12- or 6-well plates with sterilized seawater.

Results: Whole genome sequencing showed that the genome size of the isolate was estimated to be over 3.56 million base pairs in length with a G+C content of 32.5%. ABC transporter ATP-binding protein, endonuclease and chitinase genes had certain homology with genus *Aquimarina*. Phylogenetic analysis on 16S rRNA sequence identified the bacterium as *Aquimarina hainanensis,* which has been reported originally as a pathogen of whiteleg shrimp *Litopenaeus vannamei*. As a result of the infection experiments, mortalities of groups challenged at low and high bacterial dose were 100% and 100% in artemia larvae, 70% and 95% in Japanese marsh shrimp larvae, 20% and 90% in swimming crab larvae, and 77% and 93% in mud crab larvae, respectively.

Conclusion: The present study reveals that the bacterium caused mass mortality in mud crab seed production is *A. hainanensis* and can be pathogenic widely to crustaceans.

Keywords: mud crab, bacteria, genome sequence, pathogenicity

146-O*

Disease connectivity: using eDNA to investigate disease dynamics of the parasite *Hematodinium* sp. in shore crabs, *Carcinus maenas*

Davies, Charlotte (United Kingdom)[1]; Batista, Frederico (United Kingdom)[2]; Malkin, Sophie (United Kingdom)[1]; Thomas, Jessica (United Kingdom)[1]; Bryan, Charlotte (United Kingdom)[1]; Crocombe, Peter (United Kingdom)[1]; Coates, Christopher (United Kingdom)[1]; Rowley, Andrew (United Kingdom)[1]

1 - Department of Biosciences, College of Science, Swansea University, Swansea SA2 8PP, Wales UK; 2 - Centre for Environment, Fisheries and Aquaculture Science, Weymouth DT4 8UB, England UK

Introduction: The common shore crab, or green crab, *Carcinus maenas*, is native to all shores of Britain and Ireland but has been introduced to the USA, Sri Lanka, Red Sea, Madagascar, South Africa and Australia. This species plays host to a range of micro- and macro-parasites, including the dinoflagellate, *Hematodinium* spp., which causes bitter or pink crab disease. Due to its introduction to a wide range of areas, and sharing habitats with species of commercial importance, it is considered an important species in which to monitor diseases.

Methodology: We sampled crabs (n = 50/ location) and water (2L/ location) monthly from two distinct locations; a closed Dock and an intertidal Pier over 12 months. Molecular screening of both crab DNA and water eDNA, in addition to histological screening of crab gills and hepato-pancreas for *Hematodinium* spp. and co-infections took place.

Results: Overall, 13.6% of crabs were *Hematodinium* sp. positive from PCR analyses (14.4% Dock and 12.8% Pier location, 12.8%). Binomial logistical regression models revealed a significant seasonal pattern, with peak infections occurring during spring (March – May) both overall and in the Pier, but no apparent seasonality in the Dock location. Males were more likely to host *Hematodinium* sp. than females overall and in the Dock location, but not in the Pier. Size was a significant factor in determining the disease in the Pier location only, where crabs presenting *Hematodinium* sp. were significantly smaller. Carapace colouration, fouling (presence of epibionts), limb loss, pigment loss and location were not significant in explaining the disease. *Hematodinium* sp. was found in eDNA of just one location.

Conclusion: Due to the increasingly wide range of *C. maenas*, it is important to further understand the disease ecology of this species, including in terms of disease connectivity with other co-inhabitants of commercial significance.

Keywords: *Hematodinium*, disease connectivity, co-infections, eDNA, crabs

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147-O

Addressing the health of *Macrobrachium rosenbergii* in Bangladesh aquaculture

Hooper, Chantelle (United Kingdom)[1]; Bateman, Kelly (United Kingdom)[1]; Ross, Stuart (United Kingdom)[1]; Stentiford, Grant (United Kingdom)[1]; Rahman, Muhammad Meezanur (Bangladesh)[2]; Basak, Siddhawartha Kumar (Bangladesh)[2]; Bass, David (United Kingdom)[1]

1 - Cefas; 2 - WorldFish Bangladesh

Introduction: Bangladesh aquaculture produced in excess of 2,000,000 tonnes, valued at 1.9 billion USD in 2016 and was the world's second largest producer of freshwater prawns (*Macrobrachium rosenbergii*). *M. rosenbergii* is an attractive species for culture due to its increasing demand and value at international market. Since 2011, hatcheries have been experiencing high levels of larval mortality, depleting the number of active hatcheries and reducing the number of animals surpassing the larval stage. Poor hatchery management may contribute as a factor to the low survival of larva, however the hatcheries were still functioning well until 2010 despite large inconsistencies in water quality, biosecurity and feeding practices, suggesting that a new factor had been introduced to account for the mortalities.

Methodology: *Macrobrachium* from hatcheries and three rivers in Bangladesh were taken for molecular and histological analysis in order to identify the causative agent for the large numbers of moralities. Histological sections were stained with haematoxylin-eosin staining methods and inspected for signs of pathology. RNA was extracted and metagenomic sequence libraries were generated from adult and larval *M. rosenbergii* identified to have pathology.

Results: Histology of hatchery-reared and wild animals identified intracytoplasmic inclusion bodies in the hepatopancreas suggestive of an RNA virus. PCR screens for pathogens known to cause issues in *Macrobrachium* were negative, suggesting that the causative agent for the mortalities may be novel. Analysis of the metagenomic sequence data showed that the inclusions seen in histology could potentially be one of two ssRNA viruses to the families *Roniviridae* and *Dicistroviridae,* the same families to which the *P. monodon*-infecting viruses YHV and TSV belong respectively.

Conclusion: This study detected two new viruses that may be involved in mortalities in *M. rosenbergii* prior to the post-larval stage. Development of PCR screens for these two viruses in combination with the PCR screens also available for other pathogens of *Macrobrachium* will provide a multi-agent risk assessment toolkit to determine a possible cause for the mortalities and may lead to changes in practices to prevent spread of infectious agents both in and between hatcheries.

Keywords: *Macrobrachium*, viruses, Bangladesh, animal health

Funding: BBSRC, Newton Fund, DFID.

148-O

Handbook of pathogens and diseases in cephalopods

Gestal, Camino (Spain)[1]; Pascual, Santiago (Spain)[2]; Guerra, Angel (Spain)[2]; Fiorito, Graziano (Italy)[3]; Vieites, Juan M. (Spain)[4]

1 - Aquatic Molecular Pathobiology Group, Instituto de Investigaciones Marinas, Vigo, Spain; 2 - ECOBIOMAR, Instituto de Investigaciones Marinas, Vigo, Spain; 3 - Association of Cephalopod Research (CephRes), Naples, Italy; 4 - ANFACO-CECOPESCA, Vigo, Spain

Introduction: Cephalopods are valuable seafood for human consumption and some of them are good candidates for aquaculture and interesting models for research. The recent inclusion of cephalopods in the Directive 2010/EU regulates the use of animals for scientific purposes and obliges cephalopod researchers to promote the best health and welfare practices during aquarium maintenance or aquaculture procedures. The identification of pathogens and produced diseases are consequently of major interest to improve the cephalopod welfare and husbandry.

Methodology: Despite the increased interest in cephalopods as sea food, and the recommendations of FSA on parasite risk in fishery products, currently only fragmentary information on pathogens and diseases in cephalopods exists, and any guide to histopathological identification had been published. This open access book has been designed as a short, easy to follow "handbook" with the aim of facilitate the identification and description of the different organs as well as pathogens and diseases affecting the most representative species of cephalopods focussed on *Octopus vulgaris*, *Sepia officinalis* and *Loligo vulgaris*. These species are valuable 'morphotype' models and belong to the taxonomic groups Sepioidea, Myopsida and Octopoda, which include most of the species with a high aquaculture potential.

Results: The study is based on photographs at macroscopic and histological level in order to illustrate the role of the most important pathogens and related diseases from the view of a pathological diagnosis. The reader is able to familiarize with functional anatomy, necropsy and general histology of adults and paralarvae, as well as with the identification of different pathogens and pathologies.

Conclusion: This work is thus an invaluable guide for the diagnosis of cephalopod diseases. Besides including pathogens for non-European cephalopod species, it also provides a useful contribution encouraging marine pathologists, parasitologists, veterinarians and those involved in fishery sanitary assessments, aquarium maintenance and aquaculture practices aiming to increase their knowledge about the pathology of cephalopods.

Keywords: cephalopods, pathology, parasites, infectious diseases, aquaculture

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149-O

A new *Aggregata* species (Apicomplexa: Aggregatidae) of *Octopus bimaculatus* from Baja California, Mexico

Colunga Ramirez, Graciela Esmeralda (Mexico)[1]; Castellanos Martinez, Sheila (Mexico)[1]; Flores Lopez, Carlos Alberto (Mexico)[1]; Gestal Mateo, Camino (Spain)[2]

1 - Universidad Autónoma de Baja California; 2 - Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas

Introduction: The coccidians of the family Aggregatidae are common parasites that infect the gastrointestinal tissue of cephalopods and causatives of malabsorption syndrome. The knowledge about the diversity, ecology, and impact of this coccidian parasitic group on the cephalopod populations is under study however; taxonomy, phylogeny or effect on the host has not been investigated in Mexico.

Methodology: The cecum of 8 *O. bimaculatus* from Baja California, Mexico (29°00′ N, 113°30′ W) was collected. The infection was macroscopically observed as white spots and confirmed by histological sections of the tissue infected processed by hematoxylin & eosin (H&E) method. Morphological characterization of the parasite was performed by light (LM) and scanning electron (SEM) microscopy. Fresh sporocysts and sporozoites were measured. The molecular characterization was performed based on the amplification of the 18S rRNA gene of the parasite using primers and PCR conditions yet published. The Bayesian Interference (BI) was used to perform the phylogenetic analyses of *Aggregata* spp.

Results: The length in average of sporocysts and sporozoites of *Aggregata* spp. infecting *O. bimaculatus* was 18 µm and 21 µm, respectively. According to the analysis of fresh and histological sections of tissue infected, 11-13 sporozoites are contained on each sporocyst. The ultrastructure of sporocysts analyzed by SEM reveals the suture that allows the sporocysts to open their two valves in order to release the parasites. Additionally, surface ornamentation of mature sporocysts in *Aggregata* species shows cylindrical structures with projections in the top, herein reported for the first time. A consensus sequence of 1533 bp was obtained from the 18S rRNA gene. The phylogenetic analysis showed that *Aggregata* genus has a monophyletic origin, from which, *Aggregata* spp. from *O. bimaculatus* formed a sister group with *Aggregata octopiana*, highly supported (posterior probability (pP) = 1.0).

Conclusion: Morphological and molecular data evidence that *O. bimaculatus* is infected by a coccidia of the genus *Aggregata*, with ornamentations on the outer sporocyst surface non-previously recorded in this genus. The evidence supports that this parasite represents a new species, the first one described in a cephalopod from Mexico.

Keywords: cephalopods, *O. bimaculatus*, coccidian parasites, *Aggregata* spp.

Fish and Shellfish Immunology III

150-O*

Immersion vaccination efficacy against atypical *Aeromonas salmonicida* in juvenile farmed ballan wrasse (*Labrus bergylta*, Ascanius)

Papadopoulou, Athina (United Kingdom)[1]; Monaghan, Sean J. (United Kingdom)[1]; Bagwell, Nicola (United Kingdom)[3]; Featherstone, Paul (United Kingdom)[2]; Padrisa, Meritxell (United Kingdom)[2]; Wallis, Tim (United Kingdom)[4]; Verner Jeffreys, David (United Kingdom)[3]; Davie, Andrew (United Kingdom)[1]; Migaud, Herve (United Kingdom)[1]; Adams, Alexandra (United Kingdom)[1]

1 - Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling FK9 4LA, UK; 2 - Marine Harvest, Ltd, Stob Ban House, Glen Nevis Business Park, Forth William, PH33 6RX UK; 3 - Centre for Environment, Fisheries and Aquaculture Science, Barrack Road, The Nothe, Weymouth, Dorset, DT4 8UB UK; 4 - Ridgeway Biologicals Ltd, Units 1-3 Old Station Business Park, Compton, Nr Newbury G20 6NE UK

Introduction: Atypical *Aeromonas salmonicida*; a*As*, is an important bacterial pathogen of commercially farmed cleaner fish, ballan wrasse, *Labrus bergylta* Ascanius, 1767. The current study evaluated the efficacy of a polyvalent autogenous vaccine and the subsequent immune response induced in ballan wrasse after administration by single prime and booster immersion vaccination regimes.

Methodology: Fully weaned juvenile ballan wrasse (approx. 0.5 g – small cohort; S and 1.5 g – large cohort; L) were bath vaccinated for 4 h at 15 °C in static sea water. Mock vaccinated fish were exposed to sterile sea water (33 ppt). Whole fish samples from vaccinated and mock vaccinated fish (n= 6 per group) were preserved in RNALater pre-vaccination (0 h) and 24 h post-vaccination (pv) for analysis of immune genes expression. The L fish received a booster vaccination by bath after 495 degree days and liver, spleen and kidney (n=6 per group) were preserved in RNALater at 24 hpv. Triplicate groups of vaccinated and mock vaccinated fish were then bath challenged with a*As* subtype V at an OD 0.8 (10^8 - 10^9 cfu/mL in 5 or 8 L of static sea water for S and L fish, respectively) for 4 h at 15 °C. Control groups were exposed to sterile sea water. Fish were monitored for up to 30 days post infection. Samples were fixed in 100% ethanol for bacterial species/ subtype confirmation.

Results: The mean cumulative mortalities (CM) were not significantly different between prime vaccinated (S = 28% and L = 41%) and control groups (S = 23% and L =34%). However CM of booster vaccinated fish (L = 54%) were significantly greater than booster control groups (L = 40%) ($p < 0.05$). We recently found IgM and MHCII genes expressed in wrasse larvae, which provide ideal markers to investigate the immune responsiveness/anergy of these vaccinated juvenile wrasse.

Conclusion: The results of this study are very important with regards to vaccination in commercial ballan wrasse hatcheries with regards to time / size of vaccination and suggest that immersion vaccination should be performed at a later stage of the production cycle (e.g. size >1.5 g).

Keywords: autogenous vaccine, immersion, efficacy, immunocompetence, ballan wrasse

151-O

Adjuvant influences immune suppression and antibody avidity as well as kinetics of antibody response to a T-dependent antigen in barramundi

Zoccola, Emmanuelle (Australia)[1]; Li, Angus (Australia)[1]; Koster, Bert (Australia)[1]; Barnes, Andrew (Australia)[1]

1 - The University of Queensland

Introduction: Oil emulsion adjuvants were an enabling technology that underpinned the adoption of single injection multivalent vaccines offering lifetime protection in salmon aquaculture. In warm water aquaculture, where expansion is most rapid, vaccination faces a new set of challenges, not least the shorter farming cycle and the increased metabolic rate at high temperature. Consequently, there is a need to explore alternatives to emulsions that were developed for cold-water salmonids.

Methods: A prime-boost experiment was conducted in Asian sea bass at 28 °C e ploying a T-dependent hapten-carrier complex, DNP-KLH, to evaluate effect of adjuvants on primary and secondary antibody responses, periodically over 3 months.

Results: Adjuvants significantly slowed the development of primary immunity with serum antibody titre in fish vaccinated with antigen-only peaking before the 21-day initial sample point. Inclusion of any adjuvant in the vaccination delayed the antibody response by around 7 days with antibody peaking at or later than 28 days post immunisation. Adjuvants also substantially increased antibody avidity (by chaotrope titration ELISA) in the primary response compared to antigen alone, which was highest at 28 days post-primary immunisation and decreased over the period of the trial. In terms of secondary response, antigen boost resulted in depleted circulating antibody at 28 days, coincident with peak primary antibody levels in all adjuvant treatment groups except those vaccinated with antigen emulsified in a highly biodegradable oil, Essai 1616102. This might result from epitope masking by the primary antibody. Irrespective of the type of adjuvant used, fish mounted a strong secondary response when animals were boosted with antigen 98 days post-primary immunisation. Primary vaccination in the presence of any of the adjuvants also resulted in a higher avidity secondary response and the ability to initiate this higher affinity response was achieved much earlier post primary immunisation compared to antigen alone. Intriguingly, a water in oil emulsion formulation with Essai 1616102 resulted in significantly higher avidity antibodies than other preparations both in primary and secondary responses. Moreover, the secondary high avidity antibodies were detected when boosted much earlier post initial vaccination than when other adjuvants were used (21 days vs 72 days).

Conclusion: We conclude that using more biodegradable oils in W/O emulsions may confer significant advantages for T-dependent antigen processing in warm water fish.

Keywords: vaccine, adjuvant, antibody avidity, hapten

152-O

Development of MAB 10F8-3 and its role in characterizing CD4-1 T cells in the immune response of olive flounder (*Paralichthys olivaceus*)

Jung, Jae Wook (Republic of Korea)[1]; Lee, Jung Seok (Republic of Korea)[1]; Im, Se Pyeong (Republic of Korea)[1]; Kim, Si Won (Republic of Korea)[1]; Chun, Jin Hong (Republic of Korea)[1]; Lazarte, Jassy Mary (Republic of Korea)[1]; Kim, Young Rim (Republic of Korea)[1]; Kim, Jaesung (Republic of Korea)[1]; Jung, Tae Sung (Republic of Korea)[1]

1 - Laboratory of Aquatic Animal Diseases, College of Veterinary Medicine, Gyeongsang National University, Jinju, 660-701, South Korea

Introduction: The involvement of CD4 helper T cells in mediating the adaptive immune system has been well explicated in mammals. However, in teleost species, though they have already been identified, the analysis of CD4 positive cells at the cellular level has yet to be understood. One of the limiting factors that hinder their characterization is the lack of monoclonal antibodies. Thus, development of antibodies is imperative to have a better insight on the adaptive immune system of teleost fish.

Methodology: Recombinant CD4-1 antigen was produced through a bacterial expression system and was used to immunize mice for the production of monoclonal antibody. CD4-1 mAb (10F8-3) was check for its specificity through ELISA and Western blotting. Further characterization of CD4-1 positive T lymphocytes was performed by the use of the newly developed mAb. Additional experiments to check on the ability of the mAb 10F8-3 to properly and specifically detect the CD4-1 positive T lymphocytes were conducted through flow cytometry, immunofluorescence staining and RT-PCR. The potential role of these cells on the immune response of the olive flounder was also assessed, *in vivo*.

Results: Using the 10F8-3 mAb against CD4-1, we determined that CD4-1 positive lymphocytes expressed CD3ε, CD4-1 and TCR transcripts, while CD8, IgL and IgM transcripts were found in CD4-1 negative lymphocytes, implying that this mAb specifically detect CD4-1 lymphocytes in olive flounder. In addition, the number of CD4-1 positive lymphocytes was identified to gradually increase and lead to peak at 7 days post infection (dpi) with viral hemorrhagic septicemia virus (VHSV), and was seen to peak at 7 dpi in fish infected with viral nervous necrosis virus (VNNV), indicating that the immune response against viral infection starts at 3 to 7 dpi, depending on the species of the virus.

Conclusion: mAb 10F8-3 can detect the CD4-1 T cells in teleost, so we were able to demonstrate the basic properties of these cells and it appears that they are similar to their mammalian counterparts. In addition, the immune response related to CD4-1 in olive flounder during a virus infection is partially equivalent to the helper T cells in mammals.

Keywords: CD4-1 T cells, *Paralichthys olivaceus*, immune response, monoclonal antibody

153-O*

A novel ligand of common receptor subunit beta chain in common carp promotes development of basophils and macrophages

Nishiya, Kohei (Japan)[1]; Sawada, Mai (Japan)[1]; Miyamae, Jiro (Japan)[1]; Okano, Masaharu (Japan)[1]; Dijkstra, Johannes M. (Japan)[2]; Katakura, Fumihiko (Japan)[1]; Moritomo, Tadaaki (Japan)[1]

1 - Laboratory of Comparative Immunology, Department of Veterinary Medicine, Nihon University, JPN; 2 - Fujita Health University, JPN

Introduction: Interleukin (IL)-3, granulocyte/macrophage colony-stimulating factor (GM-CSF) and IL-5 are hematopoietic cytokines and share a common receptor subunit beta chain (βc) which form heterodimeric receptors with cytokine-specific α chain receptors in mammals. These βc ligands (βcl) are produced from activated T cells and stimulate production of various myeloid cells. Recently, βcl-related genes, tentatively designated as IL-5fam, have been predicted in genomes of cartilaginous fish, but no report in teleosts. In this study, we firstly identified a common carp βcl in teleost and characterized its function.

Methodology: A putative carp βcl was predicted by comparing genomic synteny of carp and other vertebrates, isolated with RT-PCR using carp spleen cDNA and phylogenetically analyzed. Subsequently, gene expression of carp βcl was analyzed in tissues of healthy carp and kidney leukocytes stimulated with various mitogens by quantitative RT-PCR. In addition, recombinant carp βcl (rβcl) was produced by 293T and investigated its ability to stimulate proliferation and colony formation of carp kidney hematopoietic cells.

Results: Phylogenetic analysis revealed that carp βcl and fish IL-5fam form a single evolutionary clade outside other related cytokine families such as mammalian IL-3, IL-5 and GM-CSF. In expression analysis, βcl gene expression was not detected in healthy tissues tested, whereas stimulation of kidney leukocytes with phytohemagglutinin resulted in significant enhancement of βcl expression. rβcl promoted *in vitro* proliferation of carp kidney hematopoietic cells, with the most active proliferation at 20 ng/ml. Furthermore, rβcl stimulated kidney cells to form colonies consisting of both cells with basophilic granules and macrophage-like cells. Most of these colony cells exhibited PAS staining positive and peroxidase staining negative. Furthermore, the colony cells expressed myeloid transcription factors involved in granulocyte development (*cebpα* and *gata2*) and macrophage development (*irf8*) and βc receptor.

Conclusion: Carp βcl is a novel cytokine induced by immunological stimuli and promotes cell proliferation and colony formation showing characteristics for basophils and macrophages with the βc pathway. Our findings suggest that βcl play a key role for myeloid cell development in teleosts.

Keywords: carp, hematopoiesis, IL-5, basophils

154-O

When hypnos meets thanatos to kill hygieia – physiological stress during koi sleepy disease causes suppression of T- and B-cells

Adamek, Mikolaj (Germany)[1]; Dietrich, Mariola (Poland)[2]; Teitge, Felix (Germany)[1]; Ganter, Martin (Germany)[1]; Baumann, Ilka (Germany)[1]; Jung-Schroers, Verena (Germany)[1]; Piackova, Veronika (Czech Republic)[3]; Gela, David (Czech Republic)[3]; Kocour, Martin (Czech Republic)[3]; Ciereszko, Andrzej (Poland)[2]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany; 2 - Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland; 3 - University of South Bohemia, Vodňany, Czech Republic

Introduction: Koi sleepy disease (KSD), caused by the gill infecting poxvirus carp edema virus (CEV), is a unique model for a branchial disease in carp. Affected fish are lethargic, lying at the bottom of the tank and with progressing disease, the activity of the fish decreases until almost complete stillness, followed by death. The clinical signs are related to gill dysfunction, resulting in ion dysregulation (sodium drop from >135 mmol/L in control to <90 mmol/L in infected carp blood) and ammonia accumulation but can be abolished by salt treatment.

Methodology: DIGE based proteomics was used for evaluating immune responses in gills of carp under KSD. A salt rescue model was used for further investigating immune responses by analysing mRNA expression of genes (*cd4*, *cd8*, *igm*, *casp9*, *inos*, *tcr a2*, and *mpo*) involved in different immune responses.

Results: Haematological analyses revealed that KSD affected fish experienced severe leucopenia and granulocytosis, with a 4-fold drop of leucocyte counts by 6 days post infection. DIGE based proteomic studies of gill tissue from these fish indicated that 86 proteins were significantly changed during the onset of severe KSD. Besides an up-regulation of antiviral and antimicrobial innate immune responses (Mx, LyzC and ApoAI), signs of an immunosuppression could be noticed. The down-regulation of the antimicrobial peptide NK-lysin-like could indicate lower activity of NK cells, T-cells. Down-regulation of calpain and caspase indicated a pro-apoptotic effect of the infection. Increased in inflammation was mediated by a down-regulation of the anti-inflammatory proteins GSN, ANXA1, SCIN. Increased concentrations of several heat shock proteins could indicate an elevated stress in infected gills. KSD affected koi also had significantly lower expression levels of *cd4*, *tcr a2* and *igm*. Interestingly, salt treatment abolished all these immunosuppressive effects of the CEV infection.

Conclusion: Even though poxviruses encode multiple immunomodulatory proteins, the results from the salt rescue experiment suggest that the immunosuppression observed in carp suffering from KSD was related to increased ammonia and loss of the osmotic balance. KSD related suppression of T- and B-cell responses could foster the development of secondary infections, which often accompany KSD.

Keywords: CEV, stress, immunosuppression, KSD

Diagnostics III

155-O*

Non-lethal sampling for detection and quantification of the high-fatality fish pathogen, *Nocardia seriolae*, using comparative genomics and real-time PCR

Le, Cuong (Vietnam)[1,2]; Price, Erin (Australia)[1]; Sarovich, Derek (Australia)[1]; Nguyen, Thu Thi Anh (Vietnam)[3]; Phan, Ut (Vietnam)[2]; Vu-Khac, Hung (Vietnam)[4]; Knibb, Wayne (Australia)[1]; Chen, Shih-Chu (Taiwan)[5]; Katouli, Mohammad (Australia)[1]

1 - GeneCology Research Centre, University of the Sunshine Coast, Queensland, Australia; 2 - Institute for Aquaculture, Nha Trang University, Nhatrang, Vietnam; 3 - Institute for Biotechnology and Environment, Nha Trang University, Nhatrang, Vietnam; 4 - Central Vietnam Veterinary Institute, Nha Trang, Vietnam; 5 - Department of Veterinary Medicine, College of Veterinary Medicine, National Pingtung University of Science and Technology, Taiwan

Introduction: *Nocardia seriolae*, a Gram-positive intracellular actinomycete, is the aetiological agent of nocardiosis, a disease affecting more than 50 fish species that can result in massive mortality in aquaculture settings. Current *N. seriolae* detection requires lethal sampling of fish internal organs. This diagnostic method is time-consuming, cumbersome, and prone to false-negative results due to low bacterial load in subclinical infections of chronically-infected fish and the slow growth rate of *N. seriolae*. Therefore, an alternative approach that enables the rapid, accurate, and non-lethal diagnosis and quantification of *N. seriolae* in infected fish would greatly benefit disease management strategies.

Methodology: Using gene presence/absence analysis of 126 publicly-available *Nocardia* spp. genomes and newly-sequenced genomes from 7 Vietnamese *N. seriolae* strains, we identified several candidate *N. seriolae*-specific loci. One locus, No8079000, was chosen and a Black Hole Quencher (BHQ) probe was designed targeting *N. seriolae*, to the exclusion of all other species according to Nucleotide BLAST analysis. To enhance the reliability of the newly-designed quantitative real-time PCR assay, a previously published bacterial 16S rDNA BHQ assay was incorporated into each reaction. This duplex qPCR was validated across DNA from 64 *N. seriolae* strains collected from Vietnam and Taiwan, and other bacteria species (N=30) (including *Nocardia* spp.). In addition, detectability of the assay was evaluated on fish blood, faeces and internal organ samples from experimentally and naturally-infected fish.

Results: Our *N. seriolae*-specific assay effectively amplified *N. seriolae* directly from pure cultures, from extracted DNA, and from laboratory-spiked *N. seriolae* in fish tissues. The assay was 100% sensitive, specific, and possessed limits of detection and quantification of 5 (38 fg) and 200 gene copies (0.8 pg) of purified bacterial DNA per 5 µL reaction, respectively. A similar sensitivity was observed for spiked blood, faeces, spleen and liver samples, indicating that the assay was not affected by inhibitors in fish tissue.

Conclusion: Our assay provides the first efficient, specific, and sensitive method for detecting and quantifying *N. seriolae*. We recommend that this assay replace the current diagnostic method, which will improve laboratory testing capacity and fish health monitoring without having to sacrifice them.

Keywords: *Nocardia seriolae*, WGS, real-time PCR

156-O

Construction of a qPCR-based macroarray for diagnosis, typing and evaluation of virulence of VHSV strains

López-Vázquez, Carmen (Spain)[1]; Olveira, José G. (Spain)[1]; Olesen, Niels J. (Denmark)[2]; Panzarin, Valentina (Denmark)[3]; Michelle, Gastaldelli (Italy)[3]; Blanchard, Yannick (France)[4]; Stone, David (United Kingdom)[5]; Thierry, Morin (France)[6]; Brémont, Michel (France)[7]; Bandín, Isabel (Spain)[1]; Dopazo, Carlos P. (Spain)[1]

1 - Departamento de Microbiología y Parasitología, Instituto de Acuicultura, Universidade de Santiago de Compostela; 2 - DTU, Lyngby, Denmark; 3 - IZSV, Padova, Italy; 4 - Anses, Ploufragan-Plouzané-Niort Laboratory, GVB Unit, Ploufragan, France; 5 - CEFAS, Weymouth, UK; 6 - Anses, Ploufragan-Plouzané-Niort Laboratory, PVP Unit, Plouzané, France; 7 - VIM, INRA, Paris-Saclay University, Jouy-en-Josas, France

Introduction: VHSV is of special concern in the EU, mainly for rainbow trout aquaculture. The improvement of its diagnosis has been the focus of researchers, and several RT-qPCR procedures have been reported for diagnosis and molecular typing. Aimed to the advance in the control of this disease, and as part of a European ANR-EraNet-Anihwa grant (006-02 Novimark), 6 groups from 5 countries coordinated their efforts to design a qPCR-based method to distinguish VHSV isolates with different virulence profiles. In the present communication, we show the preliminary results on the construction and evaluation of a qPCR-based macroarray for diagnosis, molecular characterization and typing of virulence of VHSV strains.

Methodology and Results: For the array construction, sets of primers/probe previously reported for universal detection and genotyping of VHSV strains were tested. TaqMan® probes were fixed in the bottom of qPCR tubes strips following a procedure previously designed by the Spanish group (to be patented). Three sets of strips were frozen at -30 °C to be tested 1 week, 3 months and 1 year after coating; a 4th set was immediately evaluated. Our results revealed the same sensitivity, specificity and typing efficiency than previously reported. Conservation of the arrays did not affect their reliability after 1 week or 3 months at -30 °C; however, its storage for 1 year reduced sensitivity ≈1 log. Simultaneously, within the framework of the NOVIMARK consortium, a series of virulence determinants (i.e. SNPs) were identified along the VHSV genome. This, together with the identification of oligosequences (18-25nc) associated with high/low virulence, allowed us to design specific probes to be used for virulence typing by qPCR-macroarray. Two approaches were adopted: a standard qPCR with TaqMan® SNP/MGB probes, and a CastPCR using TaqMan probes and strain specific MGB-capped blocking oligonucleotide primers. Since this part is still on going, these results will be discussed in the presentation.

Conclusion: The optimization and validation of a qPCR-based macroarray for diagnosis, typing and evaluation of virulence of VHSV isolates will provide an important tool for improving the control of the disease, providing lots of information

Keywords: VHSV, macroarray, diagnosis, typing, virulence

Funding: ERA NET ANIHWA 68 NOVIMARK/ INIA – AEI, Spain.

157-O

Can transport swabs maintain the viability of fish pathogens – can we stop taking agar plates into the field?

Chambers, Edel (United Kingdom)[1]; Morrell, Nadine (United Kingdom)[1]; Joseph, Andrew (United Kingdom)[1]; Gardiner, Richard (United Kingdom)[1]; Teixeira Alves, Mickael (United Kingdom)[1]; Cochrane-Dyet, Tamsin (United Kingdom)[1]; Trent, Angela (United Kingdom)[1]

1 - Centre for environment, fisheries and aquaculture science

Introduction: Commercially available transport swabs are commonly used by veterinarians and health care professionals. In the field of fish diagnostics, fish are routinely sampled on-site immediately onto solid agar plates which are transported to the laboratory for incubation. There are many reasons why this practice is not ideal. The purpose of this study was to investigate whether commercially available transport swabs could maintain the viability of several fish pathogens, also whether these pathogens could be detected in the presence of a fast-growing *A. hydrophila* isolate.

Methodology: Nine bacterial fish pathogens (*Aeromonas salmonicida salmonicida*, *Aeromonas salmonicida achromogenes*, *Aeromonas hydrophila*, *Yersinia ruckeri*, *Vibrio anguillarum*, *Flavobacterium psychrophilum*, *Streptococcus agalactiae*, *Lactococcus garviae* and *Francisella noatunensis*) were tested. Cultures were prepared to contain no more than 100 colony forming units/100µl of each isolate. Transwab® Amies Charcoal swabs (designed for the recovery of aerobes, anaerobes and fastidious organisms) were used. Duplicate swabs were inoculated and either plated immediately, stored at room temperature, or stored at 4 °C. Stored swabs were plated at either 24, 48 or 168 h post inoculation. Plates were incubated for at least 14 days and numbers of colony forming units counted. Equal volumes of *A. hydrophila* were mixed with each of the fish pathogens and tested as the individual cultures. For field trials, the Fish Health Inspectorate at Cefas took duplicate samples over a six-month period and several mortalities from an unrelated fish challenge study were also swabbed.

Results: Laboratory studies showed that swabs were capable of supporting the viability of fish pathogens. Overgrowth was minimised by keeping swabs cold between sampling and plating. Results from 184 swab/plate combinations showed that the Transwabs were at least as good as direct plating for the recovery of isolates from fish tissues. More pathogens and fewer insignificant isolates were recovered from swabs than direct plates. Plates inoculated from swabs were easier to read, there was less contamination and no issues with condensation or breakage.

Conclusion: The use of Transwab® Amies charcoal can be recommended for the isolation of non-fastidious bacteria causing clinical disease in fish.

Keywords: transwab, viability, bacterial fish pathogen

Funding: Department for Environment, Food and Rural Affairs, Defra-GOV.UK.

158-O

Non-lethal sampling for the detection of *Renibacterium salmoninarum*

Jansson, Eva (Sweden)[1]; Hjort, Maj (Sweden)[1]; Jinnerot, Tomas (Sweden)[1]; Aspán, Anna (Sweden)[1]

1 - National Veterinary Institute

Introduction: The control of Bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum*, is depending on active health monitoring, as there are no commercial vaccines or therapies available. BKD was first diagnosed in Sweden 1985 and a continuous screening program was initiated in the early 1990s, further supported by EU; additional guarantees for freedom for BKD since 2004. More than 50 new outbreaks were identified during 1985-1992. These were combated through a voluntary program with individually developed sanitary plans. Today there are no, or a few positive cases detected each year, although the methodology for detection has improved and the number of samples have increased. *Renibacterium salmoninarum* can be transmitted horizontally but also from female broodfish to the offspring. Current diagnostics is based on mandatory screening of kidney samples by an ELISA detecting the p57 protein from the bacterium. Positive results are confirmed by real-time-PCR. Sampling is lethal. A technique for diagnostics based on an *in vivo* technique has therefore been highly requested.

Methodology: Samples from Arctic char (*Salvelinus alpinus*) and rainbow trout (*Oncorhynchus mykiss*) were collected from farms classified as BKD infected and from experimentally infected rainbow trout (n = 169) for evaluation of non-lethal BKD detection. Samples were collected individually from gills and cloacae for PCR and compared with ELISA and PCR of kidney samples. Samples collected from populations classified as free from BKD were simultaneously tested (n = 88).

Results: In samples collected from populations known to harbor BKD, 34% and 40% of the kidney samples were classified as positive by ELISA and PCR, respectively. In non-lethal sampling, 57% of gills and/or cloacae were positive. There was an agreement of 95% between the PCR positive kidney samples with the non-lethal collected samples. However, more than 30% of the gill-cloacae samples from BKD-classified populations tested positive, while kidney samples were negative. All samples from BKD free populations tested negative in all tests.

Conclusion: Our results show that PCR on samples collected from gills and cloacae allows for sensitive and non-lethal detection of *R. salmoninarum* both in clinical cases as well as in latent carriers.

Keywords: BKD, bacterial kidney disease, non-lethal, sampling

Funding: Swedish Board of Agriculture.

159-O

Persistence of *Renibacterium salmoninarum* antigens in kidney samples of arctic charr, *Salvelinus alpinus*

Guðmundsdóttir, Sigríður (Iceland)[1]; Kristmundsson, Árni (Iceland)[1]; Árnason, Ívar Örn (Iceland)[1]

1 - Institute for Experimental Pathology, University of Iceland, Keldur, 112-Reykjavik, Iceland

Introduction: Studies on populations of wild salmonid species repeatedly show high percentages of fish with *R. salmoninarum* positive ELISA. Do all the ELISA positive fish harbour active bacterium or can inactive bacteria or residing bacterial antigens be responsible for some of the positive results? The question of antigen persistency was addressed by injecting bacterial antigens into disease free Arctic charr, *Salvelinus alpinus*, fingerlings.

Methodology: Icelandic isolate of *R. salmoninarum* was propagated and two different antigenic solutions made and sterile filtrated, cell surface extracts and extra cellular products. Fingerlings, of 50 g were injected i.p. with 100 mg or 400 mg of either solution (protein content). There were two control groups, saline-injected and un-injected. Fish from all groups were evenly distributed between two tanks with pathogen free borehole water, at 9 °C. The experiment was run for 43 weeks. Kidney samples were obtained before injection, 3 days later and after 4, 11, 22, 28 and 43 weeks. All samples were tested for antigen content in an ELISA using polyclonal antibodies. Additionally, kidneys were sampled for bacterial isolation (S-KDM) and for DNA analysis (snPCR) at the end of the experiment.

Results: Antigens of *R. salmoninarum* were detected in the ELISA test for both antigen-injected groups throughout the study period of 43 weeks, but the amount declined over time. Both control groups remained negative. On the final sampling day, all samples tested negative in culture and nPCR. Pathological changes were not seen and the overall survival was 100%.

Conclusion: The results underline that interpretation of ELISA result can be problematic. Positive ELISA shows that the fish has got infected but the timing is uncertain. There are speculations that in fish where ELISA is positive, but PCR and culture negative, the bacterium has either been cleared from the system of the host or that it is present in very low numbers, unevenly distributed or located outside the organs sampled. Whether or not the host can clear the infection remains an unresolved question in the biology of *R. salmoninarum* and its salmonid hosts.

Keywords: *Salvelinus alpinus*, *Renibacterium salmoninarum*, antigenic persistence

Funding: AVS R&D Fund of Ministry of Fisheries and Agriculture in Iceland.

Molluscs and Crustacean Diseases II

160-O*

Disseminated neoplasia in baltic bivalves: aetiology, characteristics and prevalence

Michnowska, Alicja (Poland)[2]; Smolarz, Katarzyna (Poland)[2]; Hallmann, Anna (Poland)[1]

1 - Department of Pharmaceutical Biochemistry, Medical University of Gdańsk; 2 - Department of Marine Ecosystems Functioning, Institute of Oceanography, University of Gdańsk

Introduction: Disseminated neoplasia (DN), cancer similar to human leukaemia, has been diagnosed in several marine taxa, with high frequency in bivalves. Its aetiology is still not defined in the case of the Gulf of Gdańsk (southern Baltic Sea), where up to 80% of local populations of bivalves were diagnosed as neoplastic. The aim of this work is to present available data concerning DN in bivalves from the Gulf of Gdańsk taking into account its characteristics, aetiology and spatial and temporal variations in its prevalence.

Methodology: DN diagnostics and characteristics were performed with various techniques: flow cytometry, cytogenetics and histology. Mitochondrial respiration was measured in order to characterise neoplastic cells, as well as cancer cell cultures were started. Determination of potential aetiological DN factor was performed by analyses of environmental concentrations of heavy metals, polychlorinated biphenyls, polycyclic aromatic hydrocarbons and various toxicity tests. Now modern molecular techniques are used in order to verify hypothesis about horizontal spread of the disease.

Results: DN affects all native Baltic bivalves: *Limecola balthica*, *Mya arenaria*, *Mytilus trossulus* and *Cerastoderma glaucum*. Frequency of DN varies between species, and our recent findings indicate that locally it still remains on high level since it was first diagnosed in 1990s. The species characterised by the highest disease frequency is *L. balthica* while the one displaying the lowest frequency is *C. glaucum* (first diagnosed as neoplastic 2017). Long – term data (1996 – 2018) highlight the occurrence of strong spatial and temporal fluctuation in DN frequency. The Gulf of Gdańsk environment encompasses various carcinogenic stimuli potentially involved in DN induction and progression such as carcinogenic pollution, hypoxia, anoxia and harmful algal blooms, although those factors were not successfully recognised as definitive aetiologial agents in DN induction and/or progression.

Conclusion: DN aetiology is still not defined in case of bivalves from Gulf of Gdańsk despite multiple research conducted on this subject. Thus, ongoing molecular analyses aim to verify infectious nature of DN, in which an infective agent is a single clonal cell horizontally transmitted on intra- and interspecies level.

Keywords: shellfish, neoplasm, Gulf of Gdańsk, leukaemia

Funding: Polish National Science Centre funding within Harmonia 9 grant.

162-O

A MALDI-TOF MS database for identification of *Vibrio* spp. that are potentially pathogenic in marine molluscs

Moussa-Pouly, Mirna (France)[1]; Travers, Marie-Agnès (France)[1]; Cauvin, Elodie (France)[2]; Thuillier, Benoit (France)[3]; Le Piouffle, Anthony (France)[3]; Treilles, Michael (France)[4]; Garcia, Céline (France)[1]

1 - Ifremer; 2 - Labéo-Manche; 3 - Labocea; 4 - Qualyse

Introduction: In mollusc aquaculture, a large number of *Vibrio* species are considered as major pathogens that can cause high losses in hatchery and field. Thus, development of effective techniques for rapid detection and accurate identification of *Vibrio* involved in mortality events appears important to build efficient mollusc diseases surveillance programs. Phenotypic, biochemical and molecular techniques based on DNA amplification and sequencing are widely used for the identification of *Vibrio* species in the environment, but are time-consuming because of the use of different markers to differentiate closely related species. To provide the correct identification of unknown bacteria and species classification of environmental isolates, a tool is increasingly used, the matrix-assisted laser desorption/ionization time of flight spectrometry (MALDI-TOF MS), a proteomic method able to generate a specific proteomic bacteria profile in few seconds. Nevertheless, existing databases do not contain spectra for *Vibrio* associated with marine molluscs, consequently, we proposed to create a MALDI-TOF VibrioBase database containing spectra of *Vibrio* species potentially responsible for molluscs diseases.

Methodology: 109 bacterial strains were analyzed in this study, belonging to 14 species: *V. aestuarianus*, *V. harveyi*, *V. jasicida*, *V. rotiferianus*, *V. tapetis* and species of *Coralliilyticus* and *Orientalis* clades. A total of 72 mass proteomic spectra per strain cultured in three different media were generated and analyzed to determine specific reference spectra for each strain in order to perform the MALDI-TOF MS database specific to marine molluscs. To increase the specificity of the reference spectra, complementary statistical methods (PCA clustering and CCI matrix) were used.

Results: The first results showed that 96% of the reference spectra created composing the VibrioBase were well-identified based on existing databases. Furthermore, we observed a good discrimination at species and subspecies levels of bacteria of the genus *Vibrio*.

Conclusion: This VibrioBase is a first step that could enable the use of MALDI-TOF MS as a routine diagnostic tool for rapid bacterial identification in marine molluscs. This new database will be extended by including others strains of marine *Vibrio* as strains of *Splendidus* clade and will be made available for all interested laboratories.

Keywords: *Vibrio*, MALDI-TOF MS, database, reference spectra, marine molluscs pathogens

Funding: Ifremer, DGAL, European Commission and Vivaldi Project.

163-O*

Francisellosis, an emerging disease causing high mortality among cultured Yesso scallops *Patinopecten yessoensis* in Japan

Kawahara, Miku (Japan)[1]; Yoshinaga, Tomoyoshi (Japan)[1]; Itoh, Naoki (Japan)[1]

1 - The University of Tokyo, Tokyo, Japan

Introduction: Recently, abscess lesions in the adductor muscle have been reported in association with high mortality and slow growth of cultured Yesso scallops *Patinopecten yessoensis* in Japan. Since this disease is considered to cause a serious economic impact on the scallop industry in Japan, the present study aimed at identifying the pathogen causing this emerging disease in Yesso scallops.

Methodology: As suggested from previous histopathological observations, the present study focused on the involvement of a bacterial pathogen in the disease. Adductor muscles with and without lesions were collected from an epidemic area and subjected to microbiome analyses. For the bacterium, suggested to be the most probable causative agent, bacterial isolation was attempted using Modified Eugon Agar supplemented with antibiotic solutions. Finally, in order to assess the pathogenicity of the obtained bacterium, experimental challenge tests against Yesso scallops were conducted.

Results: Histopathological observations revealed that the lesions were characterized by massive hemocyte infiltration, indicating intense host responses in the lesions. Additionally, neither parasites nor fungi were detected, suggesting the involvement of a bacterial pathogen. Microbiome analyses revealed that *Francisella halioticida*, a bacterium affecting Giant abalone *Haliotis gigantea*, was the most dominant bacteria species in the abscess lesions, and *F. halioticida* was successfully isolated from the lesions. In the bath challenge tests using the isolate, infection of *F. halioticida* in the challenged scallops was confirmed by both species-specific PCR and the culture-based method, and the lesions characterized with hemocyte infiltration were histologically observed in the challenged group only. Moreover, challenged scallops showed significantly higher mortality than the control groups.

Conclusion: In the present study, Koch's postulate between *F. halioticida* and the abscess lesion in Yesso scallop was fulfilled. Furthermore, the experimental challenges revealed that this bacterium causes not only abscess lesions in the adductor muscle, but also high mortality for Yesso scallops.

Keywords: *Francisella halioticida*, Yesso scallop, etiology, bacterial disease

164-O

Life cycle, host specificity, phylogeny and pathogenicity of *Merocystis kathae*, the first apicomplexan known to have two mollusc hosts

Kristmundsson, Árni (Iceland)[1]; Freeman, Mark Andrew (Saint Kitts and Nevis)[2]

1 - Institute for Experimental Pathology at Keldur, University of Iceland; 2 - Ross University School of Veterinary Medicine

Introduction: *Merocystis kathae* (Apicomplexa) was described by Dakin in 1911 from the renal tissue of the common whelk, *Buccinum undatum*. Further studies, describing its life stages in more details were subsequently published by Foulin in 1919 and Patten in 1935, showing that whelks were definite hosts for this parasite while the intermediate host was unknown. Until the previous study, this parasite had received no attention since the 1930s. The distribution of the common whelk and Iceland scallop *Chlamys islandica* is almost sympatric in the North Atlantic. In context with a presumably heteroxenous aggregatid apicomplexan species (SAP), which was responsible for a total collapse in the population of Iceland scallop, several mollusc species were examined for parasites in search for the missing link in the life cycle of SAP.

Methodology: Several mollusc species, among those Iceland scallops and common whelks, were collected from Bay Breidafjördur in Iceland, subsequently dissected, examined for apicomplexans and samples from various tissues taken for DNA and histological analysis. After initial molecular analyses, selected samples were subjected to *in situ* hybridization, using probes specific for SAP observed in diseased Iceland scallops.

Results: *M. kathae* and SAP are conspecific; whelk is the definite hosts where gamogonic and sporogonic stages develop, Iceland scallop the intermediate host, where merogony occurs. Both mollusc species acquire infections via the gastrointestinal tract, the scallops by their unselective filter feeding and the whelks by predation or scavenging of life, moribund of dead scallops.

Conclusion: This is the first dual mollusc life cycle reported for an apicomplexan. While *M. kathae* is highly pathogenic in the Iceland scallop, it does not seem to be harmful for the whelks. The sympatric distribution of the common whelk and the Iceland scallop makes transmission extremely effective. Phylogenetically, *M. kathae* sits within the Family Aggregatidae, most or all of which have heteroxenous life cycles. Scallops seem able to regulate low-level infection, as *Merocystis* exists in normal scallop populations between epizootics. Sensible fisheries of both whelk and scallop populations might minimize the occurrence of *M. kathae* epidemics and prevent damaging economic losses.

Keywords: *Merocystis kathae*, whelk, scallop, mollusc, diseases

Funding: Ministry of Industries and Innovation, Iceland.

165-O*

The effect of light on the trematode *Himasthla elongata* - from cercaria behaviour to infection success

Correia, Simão (Portugal)[1]; Magalhães, Luísa (Portugal)[1]; Freitas, Rosa (Portugal)[1]

1 - Departamento de Biologia & CESAM, Universidade de Aveiro, 3810-193 Aveiro, Portugal

Introduction: *Cerastoderma edule* is a widespread bivalve along the European and north-west African coast where displays important socio-economic role. This bivalve is the first and/ or second intermediate host of several parasite species. *Himasthla elongata* is a trematode parasite with a complex life cycle involving three host species and three transmission stages. Miracidium free-living stage, hatches from the egg and infects the *Littorina littorea* as first intermediate host. After maturation into redia, free-living cercariae is formed through asexual reproduction, emerges and swims to infect the second intermediate host, *C. edule*, where it settles as metacercariae. Metacercariae display short lifespan during which they have to ensure host-to-host transmission. The cycle is completed when the infected second intermediate host is predated by the final host, in this case seabirds, where the trematode transforms into adult stage. Knowing that light:dark cycle is one of the major drivers of life on Earth, the objective of the present study was to assess, through experimental approach, the influence of the light (no light exposure vs. light exposure, 50 mmol/ m^2/ s) on the cercariae behaviour and their subsequent efficiency to infect *C. edule*.

Methodology: *H. elongata* cercariae were released into an aquarium exposed to a light gradient and, after 10 hours lifespan, cercariae position was observed. Subsequently, cockles were individually screened to two different light condition and 25 cercariae released in each vessel. Cockle respiration rate and cercariae infection success was assessed.

Results: This trematode species presented a positive phototactic behaviour. Nevertheless, cockles under dark condition presented higher levels of infection than cockles exposed to light. Despite different infection levels observed, cockles presented similar respiration rate regardless light conditions. Therefore, the differences observed in terms of infection success can be explained by the cercariae swimming activity, which navigation showed to be guided by the light position, and the consequent distribution in the water column and not by the host presence and/ or host filtering capacity.

Conclusion: This study highlighted the influence of light on the behaviour of trematode cercariae which may be decisive on trematodes population dynamics and distributional range, particularly relevant in climate change research.

Keywords: *Cerastoderma edule*, parasitism, experimental infection, cockles

Funding: INTERREG-ATLANTIC COCKLES.

Myxozoan Diseases I

166-O

The complex evolution of Myxozoa mitochondrial genomes

Huchon, Dorothee (Israel)[1]

1 - School of Zoology, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Tel-Aviv, Israel

Introduction: Myxozoans are microscopic cnidarian parasites whose infections cause substantial damage to fish aquaculture. Unlike other cnidarians, myxozoans have been found to possess partitioned mitochondrial (mt) genomes fragmented into two to eight circular chromosomes. However, the mt genome structure has only been deciphered for members of two genera that belong to the same myxozoan lineage (the marine clade). I here present preliminary results regarding the structure of the mt genome of representative of the fresh-water clade and of the Myxozoa sister clade *Polypodium hydriforme*.

Methodology: Mitochondrial genome sequences were assembled from paired-ended reads (100 or 150 bp) obtained using Illumina technology for seven members of the fresh-water clade and *P. hydriforme*. To validate and strengthen the assembly results, long reads were also obtained for three species including *P. hydriforme* using the Oxford Nanopore technology with the MinION sequencer.

Results: The mt genome of both *P. hydriforme* and members of the fresh-water clade consists of a single circular chromosome. *Polypodium* presents an unusually large genome of 86 kbp which includes numerous repetitive non-coding elements. It only is the combination of both Illumina and Nanopore reads that allowed to resolve the mt genome structure of this species. In all Myxozoa species studied, the protein coding genes show an unusually high rate of sequence evolution and possess little similarity to their cnidarian homologs. Only five mt protein coding genes could be identified. Unlike Myxozoa, most canonical animal mt genes could be identified for *Polypodium*. Remarkably, our analyses suggest that mt tRNA genes were lost in lineage leading to Myxozoa and *Polypodium*. Surprisingly, among members of the fresh-water clade we have discovered that the species *Henneguya salminicola* has lost its mt genome, and thus the ability to perform aerobic cellular respiration.

Conclusion: These results reveal a remarkable plasticity of myxozoan mt genomes. They also show that Illumina sequencing alone is not always able to resolve mt genome structures. Evolutionary drivers for the loss of mt genome in *H. salminicola* might be related to low oxygen availability in parts of the life cycle.

Keywords: Illumina, Oxford Nanopore, MinIon, mt-tRNA, Myxosporea, gene conversion

Funding: Israel Science foundation, U.S.-Israel Binational Science Foundation.

167-O*

Functional characterisation of a myxozoan micro exon gene: utility as a biomarker and vaccine candidate

Faber, Marc Nicolas (United Kingdom)[1]; Holland, Jason W. (United Kingdom)[1]; Abós, Beatriz (Spain)[3]; Trusch, Franziska (United Kingdom)[4]; Yoon, Sohye (United Kingdom)[1]; Alnabulsi, Ayham (United Kingdom)[1]; Wiens, Gregory (United States of America)[2]; Tafalla, Carolina (Spain)[3]; Secombes, Chris (United Kingdom)[1]

1 - Scottish Fish Immunology Research Centre, University of Aberdeen, Scotland, UK; 2 - National Center for Cool and Cold Water Aquaculture Research, ARS-USDA, USA; 3 - Animal Health Research Center (CISA-INIA), Madrid, Spain; 4 - James Hutton Institute, University of Dundee, Scotland. UK

Introduction: The myxozoa are a highly diverse group of cnidarian endoparasites. Our work has focused on *Tetracapsuloides bryosalmonae*, the causative agent of PKD in farmed salmonids. The chronic pathology associated with PKD is due to abnormal proliferation of the kidney lymphoid tissues. We have characterised a promising antigen uncovered from our transcriptome and vaccine studies that represents a novel micro exon gene (MEG). MEGs were considered to be intrinsically disordered surface proteins found only in helminth parasite, which exhibit extensive antigenic variability and the potential to bind to a large repertoire of host proteins.

Methodology: The *TbMEG-1* gene was sequenced by PCR from infected bryozoan material. Using UTR primers, transcripts from different parasite populations were amplified, cloned and sequenced and mapped onto the genomic scaffold. Using *TbMEG-1* recombinant protein, ELISA assays were conducted to assess the specific IgM responses in rainbow trout from different populations. A monoclonal Ab to *TbMEG-1* was also produced for diagnostic use and sequenced to generate an antibody fragment for therapeutic intervention, alongside protein and DNA vaccination in field trials.

Results: *TbMEG-1* is encoded by 65 exons with only 5 exons > 30 bp, with the largest exons covering the N and C terminal domains. It possesses a hydrophilic repeat region that undergoes extensive alternative splicing and is expressed in different fish hosts and fish populations as numerous cDNA variants and protein isoforms. TbMEG-1 is expressed in and on the surface of the parasites and is associated with a subset of host cells within the infected kidney. Infected fish exhibit a potent anti-TbMEG-1 specific antibody response. Fish vaccinated with a DNA construct containing *TbMEG-1* show a reduction in pathology during clinical disease relative to other vaccine candidates tested. The results of an ongoing trial using recombinant antigens and a newly developed small antibody fragment targeting Tb-Meg1 will be presented and discussed.

Conclusion: Our studies provide unique insights into the host-parasite interactions driving PKD, having implications for our understanding of the evolution of antigenic variation in metazoan parasites. The specific immune response to *TbMEG-1* provides an opportunity to develop non-invasive assays to detect PKD.

Keywords: PKD, myxozan, parasite, vaccination, antigen

Funding: BBSRC (BB/S004076/1).

168-O*

The initiation of life cycle complexity in the Myxozoa: evolutionary events and potential mechanisms

Lisnerová, Martina (Czech Republic)[1,2]; Fiala, Ivan (Czech Republic)[1,2]; Cantatore, Delfina (Argentina)[3]; Irigoitia, Manuel (Argentina)[3]; Timi, Juan (Argentina)[3]; Pecková, Hana (Czech Republic)[1]; Bartošová-Sojková, Pavla (Czech Republic)[1]; Holzer, Astrid (Czech Republic)[1]

1 - Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic; 2 - Faculty of Science, University of South Bohemia in České Budějovice, České Budějovice, Czech Republic; 3 - Laboratorio de Ictioparasitología, Instituto de Investigaciones Marinas y Costeras (IIMyC), FCEYN, UNMdP-CONICET, 7600 Mar del Plata, Argentina

Introduction: Chondrichthyes as some of the first fishes play a significant role in the reconstruction of the early evolutionary history of myxozoan parasites in vertebrates. It seems that life cycle complexity in myxozoans originated by inclusion of vertebrates into simple life cycles exploiting aquatic invertebrate hosts. Nevertheless, nothing is known about the mechanisms of this event, even though it supported massive parasite diversifications. The aim of this study was to do a comprehensive search and enrich myxozoan parasite SSU rDNA sequences data from evolutionary old fishes, discover new lineages, morphotypes and reconstruct the basic branches in the phylogenetic tree of myxozoans.

Methodology: Twenty one species of sharks and rays were collected from 3 geographical areas: the Gulf of Mexico, the Atlantic off South Carolina and the Atlantic off Mar del Plata (Argentina). We analyzed myxozoan biodiversity by microscopy and molecular methods (SSU rDNA). We used phylogenetic, cophylogenetic (CoRe-PA) and character mapping (s-DIVA) methods to characterize the origins of myxozoans in Chondrichthyes, as well as host-parasite coevolution and biogeographic origins.

Results: The acquisition of 21 new unique SSU rDNA sequences allowed for new views into the evolutionary origin of the Myxozoa in sharks, skates and rays. We identified seven lineages of myxozoans, 2 of them seem to be restricted to Chondrichthyes only (*Bipteria* and basal *Chloromyxum*). With respect to a large monophyletic dataset of *Chloromyxum* spp. we demonstrate that host and parasite phylogenies are strongly correlated and that tectonic changes explain phylogeographic patterns in more recent species of skates and softnose skates. The most basal myxozoan lineages are bile parasites so we suggest trophic transmission and feed-integration as a likely mechanism establishing a complex life cycle, with entry from the gut to bile via the bile duct, following the consumption of infected invertebrates by palaeozoic predators feeding predominantly on benthic organisms (e.g. chimaeras).

Conclusion: For the first time we analyse the course, the drivers and the early evolutionary history of the oldest metazoan parasites known in their oldest vertebrate host group.

Keywords: Myxozoa, phylogeny, Chondrichthyes

Funding: Czech Science Foundation (project #16-20744S); IBERA project (CZ.02.2.69/0.0/0.0/16_028/0006247).

169-O*

Expressional profiling of minicollagens during myxozoan development

Kyslík, Jiří (Czech Republic)[1,2]; Bartošová-Sojková, Pavla (Czech Republic)[3]; Kosakyan, Anush (Czech Republic)[3]; Fiala, Ivan (Czech Republic)[2,3]

1 - Institute of Parasitology, Biology Centre CAS, České Budějovice, Czech Republic; 2 - University of South Bohemia, Faculty of Science, České Budějovice, Czech Republic; 3 - Institute of Parasitology, Biology Centre CAS, České Budějovice, Czech Republic

Introduction: Myxozoans are a group of cnidarian parasites mainly of fish hosts. The polar capsule represents a prominent nematocyst homologue, present in their spore stage, which is crucial for parasite attachment and thus for host invasion. A major constituent of the nematocyst wall is a family of cnidarian-specific structural genes including unusually short collagens, the minicollagens. Four types of minicollagens were reported in myxozoans, whereas diversity in Cnidaria may reach up to 17 types in *Hydra vulgaris*. *In situ* hybridization study in *H. vulgaris* revealed expression of minicollagens during the early stage of nematocyst morphogenesis until capsule maturation. However, the expressional patterns of minicollagens in myxozoan spore development have never been elucidated.

Methodology: Myxosporean stages in the different phases of development of *Myxidium lieberkuehni,* parasite of the Northern pike, were seasonally collected to determine the gene expression patterns of minicollagens. Newly produced de novo RNA-seq assembly of the parasite was used to mine minicollagens (Ncol-1, Ncol-3, Ncol-5) and reference (β-actin, 18S rDNA) genes in order to design specific primers for subsequent qPCR. Relative gene expression of minicollagens was calculated by ΔCT method.

Results: All examined minicollagen genes showed similar trends in expression during myxozoan development which peaked at the onset of sporogony compared to lower levels in proliferative and late sporogonic stages. The expression of minicollagens was the lowest in early plasmodia, dramatically increased in large differentiated plasmodia, some of which already contained pansporoblats and spores, and was down-regulated in late sporoblasts and spores. Interestingly, Ncol-1, the gene localized in cnidarian polar capsule wall, generally showed much higher expression in all stages compared to the overall expression of Ncol-3, Ncol-5 genes.

Conclusion: Our results emphasize the significant contribution of minicollagens, especially of Ncol-1, to myxospore development. Further experiments aimed at localization of these molecules in myxozoan spores are needed to elucidate their role in structural spore development.

Keywords: Myxozoa, minicollagens, expression, myxozoan spore development

Funding: IBERA project number (CZ.02.2.69/0.0/0.0/16_028/0006247), European Centre of IchthyoParasitology (ECIP) Centre of Excellence, Grant agency of the Czech Republic (505/12/G112).

170-O*

Description of new and known myxozoans infecting wild Indian fishes in Uttar Pradesh, India

Goswami, Urvashi (Hungary)[1]; Cech, Gábor (Hungary)[1]; Chaudhary, Anshu (India)[2]; Molnár, Kálmán (Hungary)[1]; Singh, Hridaya Shaker (India)[2]; Székely, Csaba (Hungary)[1]

1 - Institute of Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest; 2 - Chaudhary Charan Singh University, Meerut, U.P. India

Introduction: Myxozoans are highly specialized Metazoan parasites of aquatic animals with a rather strict host range. Parasites of this phylum have become increasingly important as new species are continually emerging as significant threats to the development of both farmed and natural environmental fish. In India, molecular tools were applied only recently in myxozoan studies, therefore only a few data are available in the GenBank regarding myxozoan species from India. During the present study our aim was to find myxospores of myxozoan spp. parasitizing of selected fish species at the sampling sites in India.

Methodology: Our primary task was a survey on wild fishes infected with myxozoans in the tributaries of River Ganga at the district of Hastinapur and Bijnor, Uttar Pradesh, India in 2017-2018. For better identification using of molecular techniques were planned to compare 18S rDNA sequences of the observed species.

Results: A new *Myxobolus* species, *Myxobolus ompok* n. sp. was found in the kidney tissue of *Ompok pabda* (Siluridae). Another already known species, identified as *M. cylindricus* was recorded from gill lamellae of *Channa gachua* (Channidae). Besides *Myxobolus* spp. two *Henneguya* spp. were found from gill lamellae of *Notopterus notopterus* (Notopteridae) and *Mystus vittatus* (Bagridae). Regarding the less studied myxozoans, *Myxidium* sp. were found from the kidney tissue of *Channa punctata* (Channidae) and *Monopterus cuchia* (Synbranchidae) and a *Myxobilatus* sp. was found from kidney tissue of *Anabas testudineus* (Anabantidae).

Conclusion: In India, we found *Myxobolus ompok* n. sp. from the kidney tissue of *O. pabda*, *M. cylindricus* from the gill lamellae of *C. gachua*. In addition two *Henneguya* spp. were found from gill lamellae of *N. notopterus* and *M. vittatus*. Other myxozoans, *Myxidium* sp. were found from the kidney tissue of *C. punctata* and *M. cuchia*. One *Myxobilatus* sp. was found from kidney tissue of *A. testudineus*. At present, analyses of 18S rDNA sequences are in progress for a proper identification of a new species as well as for redescription of already existing myxozoan species.

Keywords: myxozoans, Indian fishes, phylogeny

Funding: SZIU Stipendium Hungaricum for UG and the ERDF and the Government of Hungary project NOP-2.3.2-15-2016-00025.

Molluscs and Crustacean Diseases III

171-O

Ascetoporean parasites of invertebrates: an update and review

Bass, David (United Kingdom)[1]; Feist, Stephen (United Kingdom)[1]; Urrutia, Ander (United Kingdom)[1]; Ward, Georgia (United Kingdom)[2]

1 - Cefas; 2 - The Natural History Museum

Introduction: Ascetosporea are protistan parasites of invertebrates, incorporating the orders Haplosporida, Paramyxida, Mikrocytida, Paradinida, and the currently unplaced genus *Claustrosporidium*. Molluscan parasites in this class are known from the first three of these orders, including some high profile parasites of bivalves such as *Haplosporidium nelsoni*, *H. costale*, *Marteilia refringens*, *Marteilioides* and *Mikrocytos* spp. All four orders contain parasites of Crustacea, some of economic importance.

Methodology: We employed a range of complementary approaches: histopathology, molecular diagnostics and in situ hybridization, eDNA techniques, metagenomics, and phylogenetic analyses, to make a synthesis of ascetosporean diversity, evolutionary relationships, and parasitology. Group-specific PCR primers have been developed for all four orders, and subgroups within them, to improve understanding of their diversity beyond that encountered by clinical sign-led investigations of hosts.

Results: We present several new lineages associated with oyster, mussel, and crustacean hosts in the UK and Europe, including new species of *Minchinia* and *Haplosporidium*, paramyxids, and mikrocytids. We also detected a broad diversity of ascetosporean lineages in environmental and non-canonical host samples, from which inferences about the host range and preferences of these parasites, their biogeographical distributions, and lifecycles can be made. Phylogenetic and ecological insights from each order will be presented.

Conclusion: Enhanced lineage sampling informs and improves phylogenetic reconstruction of each order, and also provides a basis for understanding the evolutionary relationships between the ascetosporean orders, a goal made particularly elusive by virtue of the extreme genetic divergence of their constituent taxa. The development and use of group-specific primers for parasite radiations is a very powerful tool for many applications, from diagnostics, through ecology, and phylogenetics, particularly for groups that are poorly, or not, amplified by more broadly-targeted primers.

Keywords: Ascetosporea, Haplosporidia, mikrocytids, Paramyxida, protist

Funding: Defra (UK government), VIVALDI (EU H2020).

172-O*

Widespread distribution of Haplosporidia in the cockle *Cerastoderma edule* in Ireland

Albuixech-Marti, Sara (Ireland)[1,2]; Lynch, Sharon (Ireland)[1,2]; Culloty, Sarah (Ireland)[1,2]

1 - School of Biological, Earth and Environmental Sciences, University College Cork, Ireland.; 2 - Aquaculture and Fisheries Development Centre, Environmental Research Institute, University College Cork, Ireland.

Introduction: Species in the phylum Haplosporidia are spore-forming and endoparasitic protists of a wide range of marine invertebrates, mostly molluscs, especially of commercially important bivalves. Nevertheless, characterizing the diversity and distribution of Haploporidia parasites remains a challenge due to their patchy spatial and temporal distributions, host-restricted occurrence and poorly known life cycles. Despite that, numerous emerging haplosporidians continue to be reported, highlighting the possibility that the geographic range of Phylum Haplosporidia is significant greater than originally documented. On the other hand, parasites as Haplosporidia may reduce the tolerance threshold of host organisms to extreme environmental conditions such as oxygen depletion, extreme temperatures and salinity fluctuations. Consequently, parasite burdens that are considered harmless in favourable environmental conditions could possibly become stressful harmful agents in unfavourable environmental conditions, such as those caused by climate change. The aim of this study is to determine the distribution and seasonal impacts of emerging *Haplosporidia* spp. in the cockle *Cerastoderma edule.* In addition, the abiotic and biotic drivers or inhibitors of infection and impact on cockle population structure are being investigated.

Methodology: Cockles were collected seasonally around the coast of Ireland during 2018-2019. It was selected two different locations in Cork Harbour, one location in Youghal Bay, one location in Dungarvan Harbour and other two locations in Dundalk Bay. Cockle health status, seasonal variation and site influence on its health is being assessed. The cockles are being processed and analysed by molecular techniques (PCR, Sanger sequencing) and histopathology, focusing in the detection of *Haplosporidia* spp.

Results: Results to date displayed Haplosporidia infection in all the sampling sites throughout the year, although, in general, at low detection prevalence. Likewise, Sanger sequencing confirmed that the species detected are *Minchinia tapetis* and *Minchinia* sp. more than likely *M. mercenariae*-like.

Conclusion: Results of screening to date and pathogen diversity in the populations screened will be presented. Likewise, the widespread distribution of *Haplosporidia* spp. in the cockle *Cerastoderma edule* in Ireland will be assessed.

Keywords: cockle health, pathogens, Haplosporidia, climate change

173-O*

Development and validation of a specific real time PCR assay for the detection of the parasite *Perkinsus olseni*

Ríos, R. (Spain)[1]; Aranguren, R. (Spain)[1]; Gastaldelli, M. (Italy)[2]; Arcangeli, G. (Italy)[2]; Novoa, B. (Spain)[1]; Figueras, A. (Spain)[1]

1 - Instituto de Investigaciones Marinas, Vigo, Spain; 2 - Istituto zooprofilattico sperimentale delle Venezie, Legnaro (Pd) Italy

Introduction: *Perkinsus olseni* is a protozoan parasite that infects a wide variety of molluscs worldwide, causing economic losses in the aquaculture sector.

Methodology: In the present study, a real-time PCR (qPCR) assay was developed for the detection and quantification of *P. olseni* in the clam gill tissue and hemolymph (*Ruditapes philippinarum* and *R. decussatus*), and the results were compared with those of the standard diagnosis methods accepted by the O.I.E. (World Organization for Animal Health): the Ray's Fluid Thioglycollate culture Method (RFTM), a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay and histopathology. The efficiency, sensitivity and reproducibility of the newly described qPCR assay were also determined.

Results: The highest prevalence was detected in the qPCR assay in comparison with the classical assays, and the strongest linear correlation was obtained between the RFTM infection levels and the threshold cycle (Ct) number from the gill tissue. Although better results were obtained from the gill than from the hemolymph in the qPCR assays, especially with lower infection levels of the parasite, a significant linear correlation was observed between the Ct values from the gill and hemolymph.

Conclusion: The quantitative PCR assay that was developed in this study showed high sensitivity, specificity and reproducibility for the detection and quantification of *P. olseni*.

Keywords: *Perkinsus*, real-time PCR, *Ruditapes decussatus*, *Ruditapes phillipinarum*, thioglycollate

Funding: AGL2015-65705-R (Ministerio de Economía y Competitividad, Spain), IN607B 2016/12 (Consellería de Economía, Emprego e Industria - GAIN, Xunta de Galicia) and VIVALDI (678589) (EU H2020).

174-O*

Poor ecological fitness and mortality of manila clam caused by heavy *Marteilia granula* infection on the south coast of Korea

Lee, Hye-Mi (Republic of Korea)[1]; Kang, Hyun-Sil (Republic of Korea)[2]; Cho, Young-Ghan (Republic of Korea)[1]; Hong, Hyun-Ki (Republic of Korea)[1]; Lee, Hee-Jung (Republic of Korea)[2]; Kim, Jeong-Hwa (Republic of Korea)[1]; Shin, Jong-Seop (Republic of Korea)[1]

1 - Jeju National University; 2 - National Institute of Fisheries Science (NIFS)

Introduction: Manila clam *Ruditapes philippinarum* landings in Korea have been declined for the past decades, while may be linked to the increase in parasitic infection in Manila clam population for the past decades. In this study, we transplanted Manila clams from intertidal natural habitat to subtidal cages to enhance the growth.

Methodology: To test enhanced growth in the transplanted clams, we monitored the shell and tissue growth, reproduction, and parasite infection as a measure of the ecological fitness from October 2015 to May 2016.

Results: SL of clams in the intertidal (control) and the subtidal cage increased from 23.1 mm to 25.9 mm and 26.6 mm over 8 months, respectively. From October to February, the tissue weight increased markedly from 0.46 g to 1.51 g in the suspended cage, however, increase in the tissue weight was substantially lower (0.46 to 0.90 g) in the control. In January, we observed *Marteilia*-like organisms from clams in the suspended cage (62.1%), as well as from the control clams in the intertidal (16.7%). PCR assay using *Marteilia* species-specific markers confirmed that the pathogen was *M. granula*. In histology, *M. granula* was focalized in the digestive gland causing digestive tubule tissue necrosis. Prevalence of *M. granula* in clams in the suspended cage increased dramatically from January to February (100%) and remained 100% until May. Mortality of clams in the suspended cage increased dramatically from March (3.3%) to April (39.1%) and May (67.4%).

Conclusion: Histology revealed that *M. granula* occupied most of the digestive tubules resulting in massive destruction of digestive tubular tissues. Histology also indicated that the gonad development of clams in the suspended cage as well as in intertidal was retarded, possibly due to the heavy infection with *M. granula*. This is the first report on the clam mortality caused by Marteiliosis in the northwest Pacific region.

Keywords: *Marteilia granula*, ecological fitness, mortality, Manila clam, *Ruditapes philippinarum*, paramyxean parasite

175-O

Is natural selection enhancing resistance against marteiliosis in cockles recruited in the inner side of the Ría of Arousa?

Iglesias, David (Spain)[1]; Villalba, Antonio (Spain)[1]; Cao, Asunción (Spain)[1]; Carballal, María J. (Spain)[1]

1 - Centro de Investigacións Mariñas, Consellería do Mar da Xunta de Galicia, Vilanova de Arousa

Introduction: The common cockle *Cerastoderma edule* fishery has traditionally been the most important shellfishery in Galicia in terms of biomass. An unprecedented huge mortality of cockles, due to infection with the protistan *Marteilia cochillia*, which had never been detected previously in the region, led to cockle fishery collapse in the ria of Arousa in 2012. Since then, a survey programme is under way to monitor the evolution of marteiliosis dynamics.

Methodology: The most productive cockle bed of the Ría of Arousa, Lombos do Ulla, located in the inner side of the ria, is monthly surveyed from October 2011 to monitor the health status and the mortality of every annual cockle cohort. Additionally, in 2017 and 2018 common garden field experiments were performed to assess if natural selection is enhancing resistance against marteiliosis, by comparing cockles newly-recruited in this bed with cockles collected from a disease-free area, the Ría of Noia, and deployed in Lombos do Ulla in each summer. Marteiliosis prevalence (by histology) and mortality rate were estimated monthly.

Results: Since the first detection in 2012, new outbreaks of marteiliosis have occurred in Lombos do Ulla every year. From 2012 to 2016, the disease showed explosive dynamics; thus, once marteiliosis was detected, its prevalence rapidly increased reaching values close to 100%, causing the extinction of every newly-recruited cockle cohort. Nevertheless, in 2017 and 2018 the disease dynamics seems to have changed, because the prevalence did not reach 100% in the cockles recruited in Lombos do Ulla and their cumulative mortality was lower than previously, more markedly in 2018. On the contrary, the prevalence and the cumulative mortality quickly reached very high values, close to 100%, in the naïve transplanted cockles.

Conclusion: The impact of marteiliosis has declined in Lombos do Ulla in the two last years and the comparison between the locally recruited and the transplanted naïve cockles suggests that resistance against marteiliosis is being enhanced by natural selection.

Keywords: *Marteilia cochillia*, *Cerastoderma edule*, disease dynamics, mortality

176-O

Improving the characterisation of closely related parasites by long-range sequencing of ribosomal RNA

Hooper, Chantelle (United Kingdom)[1]; Ironside, Joe (United Kingdom)[2]; Skujina, Ilze (United Kingdom)[2]; Feist, Stephen (United Kingdom)[1]; Bass, David (United Kingdom)[1]

1 - Cefas; 2 - Aberystwyth University

Introduction: *Marteilia* spp. (Paramyxida) are known to cause large mortality events in bivalve species important to aquaculture around the globe. It is increasingly apparent that some very closely related Paramyxid lineages are parasitologically distinct and should be considered distinct biological entities. However, standard approaches to molecular diagnostics are not sufficiently powerful to resolve these differences. Here we present a new approach to discriminating between such closely related lineages using long-range PCR and sequencing of multiple regions of the rRNA gene array. We focus on two examples: *Marteilia cochillia*, associated with mass mortalities in the cockle *Cerastoderma edule* in Spain and a similar, newly detected parasite of cockles in the UK, and *M. refringens* and *M. pararefringens*, parasites with different geographical ranges and infection dynamics in oysters and mussels.

Methodology: The rRNA gene array for *Marteilia* spp. was amplified by long-range PCR using a newly designed hemi-nested PCR strategy, and then sequenced on Ilumina and PacBio platforms. The resulting sequences were analysed phyogenetically, and the nature of the differences between them determined. Regions potentially suitable for new diagnostic assays were identified.

Results: *Marteilia* rRNA amplicons of c. 4300 bp were generated using 18S forward and 28S reverse primers using long-range PCR for both Welsh and Spanish cockles and UK mussels. Sequencing showed that the ITS2 region of *Marteilia* spp. appeared to be much more variable than the 18S region; the variability in this region suggested that of *M. cochillia* from Wales and Spain, and *M. pararefringens* and *M. refringens* were genetically distinct. Phylogenetic analyses based on the long amplicons provided a much more robust evolutionary basis for lineage discrimination than the more commonly-used short diagnostic regions.

Conclusion: Short amplicons are increasingly being found to be insufficient for distinguishing closely related parasite lineages. Despite linages being closely related they are often biologically and parasitologically distinct, increasing the need for correct identification to prevent mis-characterisation. With cost decreasing and read-accuracy increasing, third-generation sequencing is becoming a more viable diagnostic tool that can access sequence data multiple genes in a single read increasing the confidence of parasite identification.

Keywords: *Marteilia*, long-range sequencing, *Cerastoderma edule*

Funding: Defra, BlueFish.

177-O

Survey of pathological conditions in two french cockle (*Cerastoderma edule*) beds

Arzul, Isabelle (France)[1]; Chollet, Bruno (France)[1]; Noyer, Mathilde (France)[1]; Serpin, Delphine (France)[1]; Lecadet, Cyrielle (France)[1]; Garcia, Céline (France)[1]

1 – IFREMER, Laboratoire de Génétique et Pathologie des Mollusques Marins

Introduction: The production of cockles *Cerastoderma edule* is important in some European regions including Galicia in Spain. Since 2012, Galician cockle beds have drastically declined due to the emergence of a new protozoan parasite *Marteilia cochillia*. Considering the dramatic impact of this parasite on cockle populations and the lack of data available on pathogens present in cockle beds in Europe, analyses are carried out in the context of the Interreg project Cockles to improve our knowledge regarding the distribution of these pathogens including *M. cochillia*.

Methodology: In 2018, a survey has been initiated in different cockle producing areas in Europe including in France. More particularly, pathological conditions have been monitored in two French cockle populations: Baie de Somme and Bassin d'Arcachon. Two cohorts (juveniles and adults) were collected in spring and autumn in the first site and seasonally in the latter one. Once collected, cockles were processed for histology following classical procedures and bacteria isolation was also done from up to 10 cockles for spring and autumn samplings.

Results: Cockles located at the surface of the sediment were only observed and collected in Arcachon. Histological examination has revealed differences between the two locations. In particular, Haplosporidia-like parasites and neoplasia were only noticed in cockles from Arcachon and not from Baie de Somme. A range of potentially pathogenic organisms were noticed in both cockle populations including Rickettsia-like organisms, trematods (metacercariae and sporocysts) and ciliates. Bacteria diversity seems more important in cockles buried in the sediment.

Conclusion: Pathological conditions of cockles are different between both studied locations and between young and adult cohorts. Complementary analyses will be carried out to characterize some of the pathogens and lesions observed by histology and bacteria sequencing is ongoing. This work will not only allow mapping the distribution of main cockle pathogens at the EU level but also establishing a reference picture for future investigation.

Keywords: cockles, *Cerastoderma edule*, pathological conditions, histology

Funding: Interreg project Cockles.

Prophylaxis and Treatment I

178-O*

Bacteriophages-bacteria dynamics: fundamental studies towards phage therapy in aquaculture and fisheries

Maestri, Alice (United Kingdom)[1]; Chevallereau, Anne (United Kingdom)[1]; Van Houte, Stineke (United Kingdom)[1]; Cano, Irene (United Kingdom)[2]; Westra, Edze (United Kingdom)[1]; Paley, Richard (United Kingdom)[2]

1 - Exeter University; 2 - Centre for Environment, Fisheries and Aquaculture Science

Introduction: *Pseudomonas aeruginosa* is an opportunistic pathogen, infecting amongst others catfish, tilapia, and mussels. Antibiotic resistance is a worldwide increasing matter and *P.aeruginosa* is among the most prevalent multidrug-resistant microorganisms. To address this issue, bacteriophage (phage) therapy is a promising solution. Phages have been already experimentally applied for the treatment of several fish and molluscs' pathogens, including *Aeromonas*, *Flavobacterium*, *Vibrio* and *Pseudomonas* spp., with encouraging outcomes, but its success can be limited by the evolution of bacterial resistance. We examine the role of the CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats - Cas associated) adaptive immune system and other defence strategies of *P. aeruginosa* in determining the success of phage therapy in fisheries and aquaculture.

Methodology: Using the system of *P. aeruginosa* SMC4386 and phage DMS3, we aim to identify the ecological conditions favouring the evolution of CRISPR-resistance upon phage infection, notably by testing whether this depends on the presence of a complex bacterial community. Reciprocally, we analyse how phages can overcome CRISPR-resistance by studying CRISPR-escape phage mutants obtained upon natural selection and/or genetic engineering (introduction of point mutations) and phages carrying anti-CRISPR genes. Strain SMC4386 naturally hosts a prophage (Pf1) that may prevent infection by other phages (superinfection exclusion, Sie). We aim to study the interactions between DMS3 and Pf1 by generating a strain cured from Pf1. Furthermore, we investigate new alternative phage-defence mechanisms by screening a transposon-mutant library to identify new genes involved in phage-resistance.

Results: Under the conditions tested, we did not observe evolution of CRISPR-resistance by strain SMC4386. Using a clone that already has CRISPR-resistance, we observed that phage escape mutants fail to arise under natural selection.

Conclusion: Given that previous work with strain PA14 of *P. aeruginosa* demonstrated phage escape mutants rapidly arise under the conditions tested, it is surprising that it is not the case in strain SMC4386. This suggests that additional mechanisms may play a role in determining the outcome of phage infection, including Sie and other host defences, which may act synergistically with CRISPR. These hypotheses are currently being explored.

Keywords: bacteriophages, phage therapy, *Pseudomonas aeruginosa*, CRISPR, coevolution

Funding: PhD scholarship from CLES (University of Exeter, UK) and CEFAS (Weymouth, UK).

179-O

The use of phages as a "smart disinfectant" for aquaculture live feeds

Katharios, Pantelis (Greece)[1]; Kokkari, Constantina (Greece)[1]; Cascarano, Maria Chiara (Greece)[1]; Triga, Adriana (Greece)[1]; Sotiriadis, Sotiris (Greece)[1,2]; Kalatzis, Panos G. (Greece)[1]

1 - Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Crete, Greece; 2 - Department of Biology, University of Crete, Greece

Introduction: Modern marine hatcheries in the Mediterranean aquaculture are areas of high biosecurity. Nevertheless, bacterial infections continue to cause losses at the early developmental stages of the fish. The entrance gate for the pathogens is the live feeds, the bacterial load of which is usually high. Vibrios are the predominant bacteria in rotifers and *Artemia* cultures and this group comprises some of the most important opportunistic pathogens. Disinfection of live feeds is applied in several occasions using disinfectants and antibiotics. This impacts the whole of the microbiota with adverse effect on the colonization of the fish gut by beneficial bacteria. Phage therapy, the use of bacterial viruses, can selectively target vibrios without affecting the beneficial bacteria of the live feeds and therefore can be used as a "smart" targeted disinfectant for live feeds. Here we describe our experience with this approach.

Methodology: Bacteriophages were isolated using standard enrichment techniques with several species of *Vibrio* as hosts. The bacterial hosts isolated from the hatchery environments using selective media were identified with molecular tools. Moreover, collection strains of known pathogenic vibrios were also used for phage isolation. Following characterization, which includes whole genome sequencing, lytic phages were selected based on their host range, lytic activity and production characteristics like the burst size. Phage cocktails were used for the selective reduction of vibrios in the live feeds under control experiments.

Results: The main opportunistic vibrios that cause problems in the hatcheries belong to the Harveyi clade and include species like *V. alginolyticus* and *V. harveyi*. Several phages were characterized exhibiting variable host range. The phages showed strong lytic activity on their hosts when tested in vitro. Genomic analysis showed that all phages selected are lytic due to the absence of temperate phages' signature genes. Combinations of these phages showed that they can significantly reduce the natural vibrio populations of live feeds when applied for 3-4 hours before feeding the fish larvae.

Conclusion: Phage therapy can be effectively applied in live feeds as a "smart" disinfectant to selectively reduce the *Vibrio* load.

Keywords: phage therapy, *Vibrio*

Funding: Greek Operational Programme for Fisheries and Sea (OPFS) 2014-2020.

180-O

Phage-resistant *Flavobacterium columnare* isolates: a challenge for phage therapy?

Kunttu, Heidi Mt (Finland)[1]; Runtuvuori, Anniina (Finland)[1]; Sundell, Krister (Finland)[2]; Wiklund, Tom (Finland)[2]; Middelboe, Mathias (Denmark)[3]; Landor, Lotta (Finland)[2]; Ashrafi, Roghaieh (Finland)[1]; Sundberg, Lotta-Riina (Finland)[1]

1 - Department of Biological and Environmental Science and Nanoscience Center, University of Jyväskylä, Jyväskylä, Finland; 2 - Laboratory of Aquatic Pathobiology, Åbo Akademi University, Turku, Finland; 3 - Department of Biology, Marine Biological Section, University of Copenhagen, Helsingør, Denmark

Introduction: Globally, the increasing problems with antibiotic resistance has directed interest towards phages as tools to treat bacterial infections in the aquaculture industry. However, phage resistance is known to evolve rapidly in bacteria, which may pose a challenge for successful phage therapy. The BONUS Flavophage project aims to develop phage-based treatment and prevention methods for *Flavobacterium columnare* infections in farmed rainbow trout (*Oncorhynchus mykiss*). We explored how phage-resistance affects phenotypic properties of *F. columnare*.

Methodology: Two phage-sensitive wild-type *F. columnare* strains, virulent FCO-F2 and less virulent FCO-F9, were exposed to phages. Exposure cultures were analysed for bacterial viability and colony morphology. Twenty-four phage-exposed isolates were further characterized for phage-resistance, antibiotic susceptibility, motility, adhesion and biofilm formation on polystyrene surface, protease (elastinase, gelatinase and caseinase) activity, and virulence on rainbow trout fry.

Results: Bacterial viability first decreased in the exposure cultures, subsequently increasing after 1-2 days. At the same time, the colony morphology of the phage-exposed isolates changed from original rhizoid to rough. Control isolates maintained the rhizoid morphology. Compared to the wild-type isolates, the rough isolates arising in phage exposure were phage-resistant, but the rhizoid isolates maintained phage-sensitivity, although it was reduced. Bacterial motility and high virulence were related to the rhizoid colony morphology and thus phage-sensitivity. Antibiotic sensitivity patterns of all the phage-resistant and phage-sensitive isolates were similar to the patterns observed in the wild-type strains. Adhesion and biofilm forming capacity were not affected by the phage-resistance in FCO-F2 isolates, but in FCO-F9, adhesion was weaker and biofilm formation stronger in phage-resistant compared to the phage-sensitive isolates. Elastinase activity was detected only in phage-sensitive FCO-F2 isolates, and caseinase activity was higher in phage-sensitive FCO-F9 isolates compared to the phage-resistant isolates. Gelatinase activity was higher in phage-sensitive than phage-resistant isolates

Conclusion: These results indicate, that phage-resistance leads to rough morphology growth form in *F. columnare* leading to a decrease in virulence and virulence-related properties. Hence, we suggest that phage-resistance is likely not a challenge for development of phage therapy for columnaris disease. However, the role of phage-exposed rhizoid and highly virulent isolates with reduced phage-sensitivity needs to be studied further.

Keywords: *Flavobacterium columnare*, columnaris disease, phage therapy, phage resistance

181-O*

Bacteriophage–coated feed to control *Flavobacterium psychrophilum* infections *in vivo* in rainbow trout fry: a prophylactic approach

Donati, Valentina Laura (Denmark)[1]; Dalsgaard, Inger (Denmark)[1]; Middelboe, Mathias (Denmark)[2]; Madsen, Lone (Denmark)[1]

1 - National Institute of Aquatic Resources, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark; 2 - Marine Biological Section, University of Copenhagen, DK-3000 Helsingør, Denmark

Introduction: Due to the rise of antibiotic resistance and the unavailability of a commercial vaccine, alternative environmentally sustainable methods able to control the spread of *Flavobacterium psychrophilum*, a worldwide-known pathogen in salmonid aquaculture, are of high ecological and economic interest. Bacteriophages, host-specific viruses of bacteria unable to replicate in eukaryotes, represent a potential alternative.

Methodology: In this study, we investigated the efficiency of a bacteriophage-based prophylactic treatment of rainbow trout (*Oncorhynchus mykiss*), where phages were orally administered through the feed. Rainbow trout fry (1-2 g) were fed with phage-coated feed for 30 days before the exposure with *F. psychrophilum* (low infection dose). Controls fed with conventional feed as well as controls not infected with the bacterium were included in the study. The effects of the prophylactic treatment on fish survival, growth and welfare were quantified and samples from several fish organs were taken over time in order to assess the spread and density of phages.

Results: Fish growth during the experiment was positive for every group and no mortality was observed prior to infection. The detection and quantification of phages in the fish organs during the experiment showed their constant presence in the intestine (10^1-10^2 CFU/mg of tissue) and a minor occurrence in the inner organs (spleen, kidney and brain) indicating that a higher concentration of bacteriophages on the feed is necessary for their diffusion in the fish inner organs. After the IP injection with *F. psychrophilum*, we observed a decrease in fish survival in all groups around 15%. Dead fish resulted positive to the presence of the bacteria and negative to bacteriophages.

Conclusion: Phage-coated feed represents a promising approach to control *F. psychrophilum* infections in rainbow trout fry. Our preliminary experiment shows that phage-coated feed does not have any negative impact on fish welfare. However, it is important to increase the concentration of phages on the feed, which can spread rapidly in the inner organs through the circulatory system and cross the blood-brain barrier. In this way, phages could create a protection against the bacterial infection.

Funding: BONUS FLAVOPHAGE project.

183-O*

The bioactive potential of fish-gut *Bacillus* to prevent aquaculture fish diseases

Santos, Rafaela A. (Portugal)[1,2,3,4]; Oliva-Teles, Aires (Portugal)[1,2]; Saavedra, Maria José (Portugal)[2,3,4,5]; Enes, Paula (Portugal)[2]; Serra, Cláudia R. (Portugal)[2,3,4]

1 - Departamento de Biologia, Faculdade de Ciências, Universidade do Porto, Rua do Campo Alegre s/n, Ed. FC4, 4169-007 Porto, Portugal; 2 - Centro Interdisciplinar de Investigação Marinha e Ambiental, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal; 3 - Centro de Investigação e Tecnologias Agroambientais e Biológicas, Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal; 4 - Centro de Ciência Animal e Veterinária, Universidade de Trás-os-Montes e Alto Douro, P.O. Box 1013, 5001-801 Vila Real, Portugal; 5 - Departamento de Ciências Veterinárias, ECAV, Universidade de Trás-os-Montes e Alto Douro, Quinta de Prados, 5000-801 Vila Real, Portugal.

Introduction: Bacterial diseases outbreaks are a major constraint in aquaculture, an industry responsible for more than 50% of global seafood consumption. Their emergence is also associated with a misuse of antibiotics, posing serious threats to public health. One promising disease-preventive strategy is the use of probiotics. *Bacillus* species are the most attractive probiotics for aquaculture due to their endosporeforming nature, important for industry, and their production of natural antimicrobial compounds (NACs) capable of antagonizing pathogens growth, biofilm formation and communication (quorum-sensing). Isolate and characterize *Bacillus* spp. from the gut of aquaculture fish, capable of producing NACs antagonistic of fish bacterial diseases.

Methodology: Heat-treated intestinal contents of *Sparus aurata*, *Diplodus sargus*, and *Dicentrarchus labrax* were used to obtain the gut sporeforming community. All isolates were screened for antimicrobial, anti-biofilm and anti-quorum-sensing activities, using established protocols. Significance of inhibition was evaluated by repeated measures ANOVA or 1-way ANOVA.

Results: A total of 176 isolates representing different colony morphologies and samples were obtained. Screening for NACs production revealed that 52% displayed antimicrobial activity against at least one pathogen tested. By characterizing the localization (intra- or extra-cellular) of the inhibitory molecules, the cell-free supernatants of three isolates (identified as *B. subtilis* by 16S rRNA sequencing), significantly ($p<0.05$) inhibited the growth and biofilm formation of several *Aeromonas*, *Vibrio*, *Photobacterium*, *Tenacibaculum*, *Edwardsiella*, *Shigella* and *Staphylococcus* species. Interestingly, the cell-free supernatant of the three strains were not capable of interfering with bacterial growth, but significantly decreased the biofilm formation of *A. salmonicida*. Moreover, the three isolates produced compounds capable of interfering with acyl-homoserine-lactone signals, used in Gram-negative bacteria quorum-sensing. These strains are being further studied to be used as future probiotics or source of bioactive molecules as tools to prevent aquaculture fish diseases.

Keywords: aquaculture, fish diseases, sporeformers, natural antimicrobial compounds

Funding: R. A. Santos is the recipient of a PhD grant (SFRH/BD/ 131069/2017) from FCT (Foundation for Science and Technology), under the POCH program.

184-O

Analysis of structural characteristics and antimicrobial activity of analogous derived from C-terminal salmonids IL-8

Santana Sepúlveda, Paula Andrea (Chile)[1]; Aróstica, Mónica (Chile)[2]; Guzmán Quimbayo, Fanny (Chile)[3]

1 - Instituto de Ciencias Químicas Aplicadas, Facultad de Ingeniería, Universidad Autónoma de Chile, El Llano Subercaseaux 2801, 8320000 Santiago, Chile; 2 - Programa de Doctorado en Biotecnología Pontificia Universidad Católica de Valparaíso/Universidad Técnica Federico Santa María, Valparaíso, Chile; 3 - Núcleo de Biotecnología Curauma, Pontificia Universidad Católica de Valparaíso (PUCV), Avenida Universidad #330, 2373223 Valparaíso, Chile

Introduction: Atlantic salmon and rainbow trout are species that develop diseases produced mainly by bacteria, which result in high economic losses. To control these diseases, has been used vaccines but only provide partial degrees of protection and antibiotics have each time less effective given its over use. Therefore, studies have focused on finding new forms of treatments like antimicrobial peptides (AMPs) that are excellent alternative. Currently, our research group has identified AMPs derived from the carboxyl-terminal end of the salmonids chemokine IL-8, which have antibacterial properties against Gram-negative bacteria, but its mechanism of action is still unknown. Thus the objective of this work was identify the key residues in antimicrobial activity of IL-8 derived peptide.

Methodology: An Alanine scanning was performed using solid phase synthesis F-moc. The native salmonids IL-8-derived peptides and its analogues were characterized by HPLC, UFLC-ESI-MS and circular dichroism spectroscopy. The determination of the antimicrobial activity of analogous against *Escherichia coli* and *Aeromonas salmonicida* were realized through a microdilution in 96-well plates.

Results: Most of the trout and salmon peptides obtained through the scan-Ala, showed a tendency to form α-helix in the solvent trifluoroethanol 30% v/v in water, with a double minimum between 210-230 nm and a maximum around 190 nm. The results of Scan-Ala for trout peptides against *E. coli* showed an activity similar to native IL-8-derived peptide, in 14 of the 16 peptides analysed (~5μM). When replacing Lysine in position 11 with alanine, the peptide loses its functionality and in position 13, its MIC increases twice. In the case of *A. salmonicida,* only 3 peptides maintained MIC close to native peptide (~5μM), 5 peptides lost their activity and the rest presented a range between 3 and 6 times MIC. For the case of salmon peptides against *E. coli*, maintained a behaviour similar to trout peptides. In the case of *A. salmonicida*, 13 of the 16 peptides analysed has antibacterial activity when the substitution was performed.

Conclusion: These bioactive molecules are a basis for the design of new drug and their used in the treatment of salmonids infectious disease.

Keywords: antimicrobial peptides, salmonids, chemoquine

Funding: FONDECYT INICIO N°11170244, REDES 180203.

Myxozoan Diseases II

185-O

***Sphaerospora molnari*: a new *in vivo* and *in vitro* model for myxozoan research**

Holzer, Astrid (Czech Republic)[1]; Pudhuvai, Baveesh (Czech Republic)[1,2]; Korytář, Tomáš (Czech Republic)[1,2]

1 - Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice,, Czech Republic; 2 - Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, University of South Bohemia, České Budějovice, Czech Republic

Introduction: Myxozoas are obligate cnidarian parasites, with some species known to cause important pathologies and losses in fish in aquaculture. Recently, myxozoan infections have been geographically expanding as a result of climate change. To develop targeted anti-myxozoan strategies, it is important to understand the molecular crosstalk between host and parasites and the mechanisms for rapid proliferation and host exploitation. A major reason for limited knowledge is the absence of available *in vivo* and *in vitro* models. Only 3-4 life cycles are continuously perpetuated in research laboratories, as their maintenance is laborious and time-consuming. An *in vitro* model is non-existent. Sphaerosporids typically proliferate in the blood of their fish hosts prior to spore formation in the target organ. Blood stages are of particular importance as they quickly multiply and evade cellular and humoral host defences. We aimed at establishing *Sphaerospora molnari* as a model for myxozoan early proliferation research in our laboratory.

Methodology: We designed and compared protocols to isolate, maintain and propagate *S. molnari* blood stages. *In vivo,* we transmit blood stages from fish to fish via intraperitoneal injection and combine this with host immunosuppressant therapy to produce large numbers of parasites. Purified parasite stages were submitted to an array of culture and cryopreservation trials and to optimize *in vitro* propagation.

Results: *S. molnari* has been IP-transmitted and propagated in our laboratory for over two years now. The lab strain has been used for genome sequencing, for infection experiments studying molecular host-parasite interaction as well as for studying immune responses of carp to *S. molnari*. *In vitro* cultures proliferate over several days and can be used for testing chemotherapeutants and inhibitors, as well as molecular interference assays.

Conclusion: Overall, the possibilities for research in our laboratory were greatly expanded by the development of laboratory culture and propagation methods for *S. molnari*, and they form a solid base for experimental approaches, with a continuous, season-independent workflow. However, despite the important advantages offered by the new myxozoan model system we also highlight remaining obstacles and restrictions for research.

Keywords: Myxozoa, laboratory model, common carp, host-parasite interaction

Funding: EC, Horizon 2020, ParaFishControl (#634429), Czech Science Foundation, AQUAPARA-OMICS (#19-28399X).

186-O*

Game of genes: tracking the major trajectories to successful parasitism of *Sphaerospora molnari* in common carp

Kosakyan, Anush (Czech Republic)[1]; Holzer, Astrid S. (Czech Republic)[1]

1 - Institute of Parasitology, Biology Center of the Czech Academy of Sciences, Ceske Budejovice, Czech Republic

Introduction: Parasitism occurs in almost all lineages across the tree of life. It was shown that parasites have independently evolved from the non-parasitic ancestors by acquiring variety of hosts and micro-habitats. Myxozoans are unique group of metazoan parasites that have undergone large scale transformations and diverged from their free-living cnidarian ancestors to obligatory microscopic endoparasites. Yet, the main genetic mechanisms and the sets of the genes driving the success and diversification of this parasite group, their ability to penetrate the host and successfully proliferate within it, remain largely unexplored. *Sphaerospora molnari* is a myxozoan parasite infecting common carp and is responsible for up to 100% moralities of some carp fingerling stocks in Czech Republic and Central Europe. We have used *Sphaerspora molnari* as a model to study the functional gene groups and the main mechanisms that allow parasite successfully invade and proliferate within the host.

Methodology: We have obtained transcriptomic data from 3 organs of infected common carp: blood, liver and gills that are related to strategic time points of parasite development within the host. We have developed specific bioinformatic pipeline for parasite assembly, differential gene expression and annotation of Illumina data mainly following Trinity package. Additionally, this pipeline includes parasite double filtration step to insure the clean parasite (host-free) assembly.

Results and Conclusion: We compare differential gene expression profiles of these developmental stages of the parasite and discuss the importance of revealed functional gene groups for better understanding the adapted parasite strategies of myxozoans in general and for potential control of parasite outbreaks in aquaculture and wild fish populations.

Keywords: Myxozoa, gene expression, transcriptomics

Funding: EC, Horizon 2020, ParaFishControl (#634429), Czech Science Foundation, AQUAPARA-OMICS (#19-28399X).

187-O

Do antibodies matter in the myxozoan infections?

Korytář, Tomáš (Czech Republic)[1]; Ganeva, Vyara (Czech Republic)[2]; Peckova, Hana (Czech Republic)[2]; Holzer, Astrid S. (Czech Republic)[2]

1 - Faculty of Fisheries and Protection of Waters, University of South Bohemia, České Budějovice, Czech Republic; 2 - Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

Introduction: Myxozoans are an ancient group of cnidarian parasites, some of which are causing massive mortalities in the aquaculture and wild fish. Despite their geographical spread and growing incidence of infections, the available therapeutic measures are still limited. Development of vaccines has thus far shown only limited success. The main reason for this delay is the lack of understanding of the antibody responses towards myxozoans and their capacity for parasite elimination.

Methodology: Using the infection model of common carp with *Sphaerospora molnari*, we investigated the development of antibody responses for a period of 63 days following infection. Additionally, we tried to identify the major *S. molnari* antigens and evaluate the presence of specific antibodies in the sera of infected fish. Consequently, we elucidated the importance of these antibodies for disease prevention in a series of *in vitro* and *in vivo* experiments.

Results: Our data provided compelling evidence for the importance of IgM in the myxozoans infection. We observed a strong increase in the expression of IgM transcripts, starting as soon as 21 dpi and increasing steadily until 42 dpi, where they reached an 11 fold increase. The prominent role of IgM was further supported by the increased number of IgM^+ B lymphocytes in the blood, which grew from 1.3 – 3 x 10^3 IgM^+ lymphocytes/ µl in the naïve individuals to 13 x 10^4 IgM^+ B cells/ µl at 56 dpi. Importantly, the infection induced specific antibodies, which recognized a 16 kDa band on the *S. molnari* lysate in western blots. Surprisingly, although the immune sera exhibit potent opsonizing capacity *in vitro*, *S. molnari* isolated from the blood of infected fish were not labelled with any carp IgM.

Conclusion: The presented findings provide novel insights into the role of IgM in the infection with *S. molnari* and suggest a presence of so far unknown strategy allowing the parasite to avoid antibody-mediated elimination *in vivo*. It remains to be determined whether this ability evolved only in the sphaerosporids, or if it is a conserved mechanism used by all myxozoans.

Keywords: Myxozoa, *Sphaerospora molnari*, IgM, Western Blot, B cells

Funding: Czech Science Foundation, Grant No. 19-25589Y.

188-O

Expressional profiling and 3D structure modeling of cysteine protease inhibitors of *Sphaerospora molnari* (Cnidaria: Myxozoa)

Bartošová-Sojková, Pavla (Czech Republic)[1]; Kosakyan, Anush (Czech Republic)[1]; Pudhuvai, Baveesh (Czech Republic)[1,2]; Eszterbauer, Edit (Hungary)[3]; Holzer, Astrid S. (Czech Republic)[1]

1 - Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Ceske Budejovice, Czech Republic; 2 - University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses; 3 - Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary

Introduction: The Myxozoa is an ancient group of cnidarian parasites. *Sphaerospora molnari* is a pathogenic myxozoan species affecting the skin and gills of common carp causing severe respiratory and osmoregulatory failure. Besides spores and plasmodia, it forms motile extrasporogonic stages in the host's blood. Cysteine protease inhibitors are a widely distributed superfamily with stefins as intracellular molecules lacking a signal peptide and extracellular cystatins with a signal peptide. They are responsible for the control of protein degradation processes and in parasites they are directly involved in host-parasite interactions. Therefore, parasite cysteine protease inhibitors have been exploited as vaccine and chemotherapeutic targets and can also be useful to control myxozoan diseases.

Methodology: 3D structures were predicted in Phyre2. Expression of stefins was assessed using the TMM normalization method from the transcriptomes of *S. molnari* proliferative (blood), feeding (liver) and sporogonic stages (gills). *In silico* gene expression was confirmed by qPCR in the same organs and parasite stages.

Results: *S. molnari* codes for an unusual stefin SF27333 that is synthesized with the signal peptide. SF27405 is a classical stefin without a signal peptide. Predicted 3D structures of both molecules are similar to the known structures of stefins of other organisms with slight differences in their terminal parts that may reflect their novel, so far unidentified functions. The transcriptomic analysis of the three parasite stages has revealed that both stefins show highest expression levels in the sporogonic stages. Stronger upregulation of gene expression in the sporogonic phase of the development compared to proliferative and feeding stages was observed for SF27333, the stefin that is likely secreted. *In-silico* results were confirmed by qPCR.

Conclusion: Due to higher demand for their expression in sporogonic stages, we assume that both stefins, especially SF27333, play important immunomodulatory roles in the inhibition of host-derived proteases aimed to recognize and eliminate the parasite during the process of gill tissue invasion and subsequent spore formation.

Keywords: Myxozoa, protease inhibitors, qPCR, protein structure prediction, gene expression

Funding: Ministry of Education, Youth and Sports (No. LTAUSA17201), Horizon 2020, RIA (ParaFishControl; No. 634429), Czech Science Foundation (AQUAPARA-OMICS; No. 19-28399X) and the National Research, Development and Innovation Office, Hungary (No. NN124220).

189-O

Novel myxozoan Kunitz type serine peptidase inhibitors and their inferred function

Hartigan, Ashlie (United Kingdom)[1]; Doonan, Liam (United Kingdom)[2]; Long, Paul (United Kingdom)[2]; Okamura, Beth (United Kingdom)[1]

1 - Natural History Museum London; 2 - King's College London

Introduction: Kunitz type peptidase inhibitors are commonly employed by parasites, functioning to modify host inflammatory and digestive responses and hence promote parasite survival. Recognised by their classic kunitz fold, these proteins inhibit serine peptidases, including trypsin and chymotrypsin. This family of inhibitors is regarded to have a single origin with expansion and diversification subsequently occurring in various pathogenic species. Kunitz type inhibitors have been found in the venom of many animals. In cnidarians Kunitz inhibitors are incorporated in venoms of cnidocysts (stinging cells) and have expanded functions, including inhibition of peptidases outside of the serine clan. Furthermore in some anthozoans, these inhibitors have a dual function involving both peptidase inhibition and potassium channel blocking activity. Here we report on the presence and diversity of Kunitz inhibitors in myxozoans and *Polypodium hydriforme.* Both groups infect fish and may be utilising Kunitz inhibitors to modulate host immune response during their invasion or development.

Methodology: Our work examines the presence and diversity of putative toxic Kunitz inhibitors in parasitic and free living cnidarians to improve our understanding of proteins involved with the host immune responses to myxozoan disease. We screened genomic, transcriptomic and proteome datasets for putative toxins including Kunitz inhibitors in a range of cnidarians to characterise sequence, structural and specific binding site changes and to present an *in silico* prediction of Kunitz activity.

Results: We identified multiple distinct kunitz homologs in our model myxozoan, *Buddenbrockia plumatellae* and other myxozoan species including a dendrotoxin like protein (identified in snake venom). We will present our current findings on the localisation of target inhibitors within the vermiform, invertebrate host-sourced stage. In addition specific serine protease activity will be assayed in worm tissue extract to demonstrate substrate and activity range of *B. plumatellae* proteins.

Conclusion: Our findings on these peptidase inhibitors have implications for mechanisms of myxozoan-induced pathogenesis as well as the evolution and adaptability of proteins in parasites.

Keywords: Myxozoa, *Polypodium*, Kunitz, inhibitor, disease

Funding: Leverhulme Trust Research Project Grant.

190-O*

Does time matter in *Enteromyxum leei* (Myxozoa) fish-to-fish experimental transmission?

Picard Sánchez, Amparo (Spain)[1]; Estensoro, Itziar (Spain)[1]; Del Pozo, Raquel (Spain)[1]; Piazzon, M. Carla (Spain)[1]; Sitjà Bobadilla, Ariadna (Spain)[1]

1 - Fish Pathology Group, Instituto de Acuicultura Torre de la Sal, Castellón, Spain

Introduction: *Enteromyxum leei* is a myxozoan histozoic parasite that infects the intestine of several teleostean fish species. In the gilthead sea bream (*Sparus aurata*) it provokes a chronic disease, with anorexia, delayed growth with weight loss, cachexia, reduced marketability and mortality. Direct fish-to-fish transmission has been demonstrated for *E. leei* via effluent, cohabitation, oral and anal routes. Effluent transmission trials in GSB are set up for long times of exposure (70-120 day) to water from tanks holding infected fish (donors, D). However, the minimum time of exposure to infect fish has not been established, nor the possible effect on the fish immune response.

Methodology: A D tank was setup to effluent water to two equal recipient (R) tanks, each with 50 naïve fish. R1 tank was kept with the same number of fish all the trial, whereas in R2 tank, some fish were left all the time (R2-13) and 10 fish were removed at 1 (R2-1), 3 (R2-3), 5 (R2-5) and 7 (R2-7) weeks post exposure (wpe) and placed in separated tanks with non-infected water. At 9 wpe, a non-lethal sampling was done to evaluate the progression of the infection and a final sampling at 13 wpe to obtain intestinal and serum samples.

Results: No effect of time of exposure was detected on prevalence of infection, as it varied between 100% (R2-1) and 80% (R2-3). Although no significant differences were found in weight, length and condition factor among R groups, the weight decrease typical of the infection was lowest in R2-1. The percentage of fish with specific antibodies against *E. leei* varied between 50 and 100%.

Conclusion: From the significant correlations found among the different variables, we can conclude that 1) the earlier the infection is achieved, the higher amount of Abs are produced, 2) the longer exposure times, the higher impact on biometrical values, 3) the higher reduction of growth, the higher extension of the infection along the intestine and the higher percentage of fish with antibodies. Further trials have to be performed under lower temperatures and exposure times shorter than one week.

Keywords: *Sparus aurata*, immune response, growth delay, exposure time

Funding: ParaFishControl H2020 (634429).

191-O

Teleost thymus is an active player in infectious episodes: the example of turbot enteromyxosis

Ronza, Paolo (Spain)[1]; Robledo, Diego (United Kingdom)[2]; Losada, Ana (Spain)[1]; Bermúdez, Roberto (Spain)[1]; Pardo, Belén (Spain)[1]; Martínez, Paulino (Spain)[1]; Quiroga, María Isabel (Spain)[1]

1 - Universidade de Santiago de Compostela; 2 - The Roslin Institute and Royal (Dick) School of Veterinary Studies

Introduction: Enteromyxosis caused by the intestinal myxozoan parasite *Enteromyxum scophthalmi* is considered a limiting factor for turbot (*Scophthalmus maximus* L.) aquaculture. The dysfunction of turbot immune response and its inefficacy in stopping the infection have been investigated, especially focusing on the spleen, kidney and local (gut) immunity. Nevertheless, the role of the thymus was never evaluated. The thymus is a primary lymphoid organ that plays a pivotal role in the adaptive immune system. Despite its importance, its characterization and role in the response against diseases have been scarcely studied in teleosts. The aims of this work were to broaden the knowledge on thymus function in turbot and to evaluate its response during enteromyxosis.

Methodology: The thymus transcriptomic profile was analyzed by RNA-seq in healthy and *E. scophthalmi*-infected turbot previously evaluated by histopathology. Differential expression, GO term and KEGG pathway enrichment were investigated. Analyses were complemented by the *in situ* evaluation of relevant gene products by immunohistochemistry. Markers of T cell, inflammation, apoptosis and cell proliferation were included.

Results: Healthy thymus reflects a high metabolic activity, with enriched functions related to energy production, protein synthesis and cell proliferation. Also, several terms were related to its immunological role in T-cell development were identified. A total of 1,888 up- and 2,225 down-regulated genes were found in infected fish compared to controls. The down-regulated genes showed evidence of declined thymic function regarding both immune-related and metabolic activities. Immunoreactivity to cell proliferation and T-cell markers was also diminished. Up-regulated genes were mainly related to inflammation and apoptosis, results also supported by the immunohistochemical observations. The general picture obtained suggests that the elevated level of pro-inflammatory mediators affects thymic function, with a negative impact on the ongoing immune response.

Conclusion: This is the first description of the thymus transcriptome in turbot, providing novel insights into its role during infection in teleosts. The significant transcriptomic changes found indicate that the thymus is affected during enteromyxosis and might contribute to the failure of turbot immune response.

Keywords: thymic function, T cells, transcriptomics, immunohistochemistry, parasitoses

Funding: The Spanish Ministry of Economy and Competitiveness and the European Regional Development Fund (ERDF) under the Projects AGL2015-67039-C3-1-R and AGL2015-67039-C3-3-R.

Molluscs and Crustacean Diseases IV

192-O

Detecting all listed pathogens of flat oysters in one step: a new PCR to diagnose *Marteilia refringens* and *Bonamia* sp.

Canier, Lydie (France)[1]; Dubreuil, Christine (France)[1]; Noyer, Mathilde (France)[1]; Serpin, Delphine (France)[1]; Couraleau, Yann (France)[1]; Chollet, Bruno (France)[1]; Garcia, Céline (France)[1]; Arzul, Isabelle (France)[1]

1 - IFREMER, EU Reference laboratory for mollusc diseases, La Tremblade, France

Introduction: *Bonamia ostreae*, *Bonamia exitiosa* and *Marteilia refringens* are protozoan parasites of the flat oysters *Ostrea edulis* responsible for the bonamiosis and the marteiliosis, two diseases which have caused significant mortalities in European populations of flat oysters. Those parasites are endemic in Europe; however some countries/ zones are still free of *Bonamia* and/or *Marteilia* parasites. Bonamiosis and marteiliosis are both listed as notifiable diseases at the European and the International levels. A wide range of PCR assays, including conventional and real-time PCRs, are currently available for the detection of either *Marteilia refringens* or *Bonamia* sp. parasites, with heterogeneous level of validation. In order to facilitate the diagnosis of these two listed diseases, the EU reference laboratory for mollusc diseases has developed and validated a new multiplex Taqman® PCR assay allowing the detection of both parasites in one step.

Methodology and Results: The new PCR assay underwent a full validation procedure and results show that it has equivalent or better diagnostic performances than currently recommended PCR assays in terms of sensitivity, specificity, repeatability and reproducibility. The new multiplex PCR is able to detect down to 10 genomes equivalent per µl for each targeted pathogens. Diagnostic sensitivity (DSe) and specificity (DSp) were assessed by testing a large panel of field samples and using a latent class analysis approach. Compared to currently recommended PCR assays, our multiplex PCR has a higher DSe and an equivalent DSp for the detection of *M. refringens*, and an equivalent DSe and DSp for the detection of *B. ostreae*. Finally, its reproducibility was evaluated in the context of an inter laboratory comparison test which involved 17 laboratories all across Europe, and showed that this new assay is easily transferable to other laboratory settings.

Conclusion: This is the first assay designed to detect both *Marteilia refringens* and *Bonamia* sp. in a single step and it should allow reducing the number of analysis to perform to demonstrate freedom from these pathogens or to monitor bonamiosis and marteiliosis in flat oyster populations in Europe.

Keywords: *Marteilia refringens*, *Bonamia*, PCR, diagnostic, flat oysters

193-O

Where can *Bonamia ostreae* and *Marteilia refringens* be found outside their bivalve host, *Ostrea edulis*?

Mérou, Nicolas (France)[1]; Pouvreau, Stéphane (France)[2]; Arzul, Isabelle (France)[1]

1 – IFREMER, Laboratoire de Génétique et Pathologie des Mollusques Marins; 2 - IFREMER-Laboratoire des Sciences de l'Environnement Marin

Introduction: *Marteilia refringens* and *Bonamia ostreae* are protozoan parasites associated with mortality of flat oysters. Since their first description in the 70-ies and 80-ies respectively, these parasites have contributed to the decline of populations of *Ostrea edulis* notably in France. Although the production of this oyster remains very low, there has been a renewed interest for this native species since these last years because of its patrimonial, economic and ecological interests. Marine mollusc disease management is limited to protective rather than curative measures and a better understanding of parasitic cycles is of particular interest for the management of wild populations such as flat oyster beds. The development of both parasites in their bivalve host is well described whereas some questions remain unresolved regarding their life cycle outside their bivalve host in particular regarding their niche and stage.

Methodology: In this context we have investigated the distribution of both parasites in the surrounding environment near a flat oyster bed located in Rade of Brest (Brittany, France), known to be endemic regarding both diseases. A seasonal based sampling has been carried out over one year and includes not only flat oysters but also water, sediment, plankton, other bivalves and benthic species associated with flat oysters. Detection of both parasites in samples is first carried out by real-time duplex PCR.

Results: Analyses are still in progress but preliminary results reveal a different seasonal dynamics of *B. ostreae* and *M. refringens* in the flat oyster bed. Interestingly, *B. ostreae* has only been detected in flat oysters, contrary to *M. refringens* which could be detected in all the tested categories of samples (sediment, water, plankton and bivalves).

Conclusion: While the presence of *B. ostreae* appears restricted to flat oysters, *M. refringens* is detected in different parts of the ecosystem including sediment. Subsequent analyses based on RNA detection and histology will also be performed to characterize more deeply the two parasites in these environmental samples.

Keywords: parasite, life cycle, flat oyster, *Bonamia ostreae*, *Marteilia refringens*

194-O*

Characterization of OSHV-1 µVAR genotypic diversity during pacific oyster mortality outbreaks in two French aquaculture areas

Delmotte, Jean (France)[1]; Galinier, Richard (France)[1]; De Lorgeril, Julien (France)[1]; Petton, Bruno (France)[2]; Gueguen, Yannick (France)[1]; Mitta, Guillaume (France)[1]; Montagnani, Caroline (France)[1]; Chaparro, Cristian (France)[1]; Escoubas, Jean-Michel (France)[1]

1 - IHPE, Université de Montpellier, CNRS, IFREMER, Université de Perpignan Via Domitia, Place E. Bataillon, 34095 Montpellier, France; 2 - LEMAR UMR 6539,UBO/CNRS/IRD/ Ifremer, 11 presqu'île du vivier, 29840 Argenton-en-Landunvez, France

Introduction: *Crassostrea gigas* juvenile oysters are subjected to frequent episodes of mortality with wide geographical distribution. These mortality named "Pacific Oyster Mortality Syndrome" (POMS) are of complex etiology. This disease requires a primary infection with Ostreid herpes virus (OsHV-1) which induces immunosuppression in the host which subsequently leads to the colonization of the oyster tissues by a consortium of opportunistic bacteria and / or pathogenic bacteria causing a secondary bacteremia inducing the mortalities. Monitoring of global mortality episodes worldwide has revealed the existence of numerous genotypic variants of OsHV-1 without being able to correlate this viral diversity with the onset and progression of the disease.

Methodology: To study the consequence of this viral diversity in POMS, we generated five genetically differentiated biparental oyster families with contrasted resistance phenotypes to POMS, that were produced from wild genitors sampled in farming and non-farming areas (from Atlantic and Mediterranean coasts). These oyster families were confronted to disease outbreaks that occurred in two farming areas (Atlantic coast and Mediterranean Sea). The dynamics of mortalities were monitor for each families during the disease outbreaks and viral diversity was analysed using RNA-Seq approach.

Results: High variability in the dynamics of mortalities and percentages of survival were observed among families within the same environment but also for the same family between the two environments. We showed that the level of susceptibility of the oyster families is directly linked to OsHV-1 load and that the transcriptional signature of viral genes was similar in all the families. Viral diversity analysis through single nucleotide polymorphisms characterization revealed that viruses present in our study are different from OsHV-1 genomes already characterized. We showed that two distinct viral populations are involved in mortality outbreaks that occurred on Atlantic and Mediterranean sites. Moreover, within each environments, the different oysters families were not infected by the same viral populations.

Conclusion: These results highlight the need to integrate viral genotypic diversity in genome-wide association studies for oyster resistance to OsHV-1 especially if they are to be used in breeding programs.

Keywords: oyster, ostreid herpesvirus, viral diversity, viral transcriptomic profiles, oyster genetic background

Funding: ANR project DECIPHER (ANR-14-CE19-0023), EU funded project VIVALDI (H2020 program, n°678589).

195-O

Transcriptomic profile and targeted genotyping on OSHV-1 resistant and susceptible *Crassostrea gigas*

Prado-Álvarez, María (Spain)[2]; Carlsson, Jeanette (Ireland)[1]; Cao, Asunción (Spain)[3]; Gestal, Camino (Spain)[2]; Culloty, Sarah C. (Ireland)[4]; Villalba, Antonio (Spain)[3]; Carlsson, Jens (Ireland)[1]

1 - School of Biology and Environmental Science, University College Dublin, Ireland.; 2 - Marine Research Institute, Spanish National Research Council, Vigo, Spain.; 3 - Centro de Investigacións Mariñas. Consellería do Mar. Xunta de Galicia. Spain.; 4 - AFDC, School of Biological, Earth and Environmental Sciences, University College Cork, Ireland

Introduction: The incidence of the OsHV-1 virus has been directly associated with significant mortalities of the Pacific oyster, *Crassostrea gigas*, the principal shellfish cultivated worldwide. Long term solutions such as selection of resistant strains have been referred to as a priority by the scientific community. In this context, novel sequencing methodologies have increased the genetic information on this specie and the development of selective breeding programs is nowadays more achievable than ever before.

Methodology: Spat survivors from a natural outbreak occurring in a producing area were collected, experimentally exposed to OsHV-1 µVar and then analyzed for gene expression by RNA-sequencing. Control animals from a virus free area were also included in the analysis. Parallel, broodstock from these two areas were used to produce pure bred families that were also exposed to OsHV-1 µVar and analyzed for gene expression.

Results: Experimental infection under controlled conditions and the natural outbreak occurring in the field caused mortalities in spat and larvae. Oysters with contrasted susceptibility also showed significant lower survival in the field. Transcriptomic analysis showed that genes related to defense were upregulated in oysters with less susceptibility to the virus. Single nucleotide polymorphisms were identified in differently expressed transcripts and thereafter analyzed in a collection of pure bred families with contrasted resistance and susceptibility to OsHV-1.

Conclusion: In this study a set of differently expressed genes related to OsHV-1 infection were identified attending to the infection status and also to the susceptibility of the stock. Genotyping analysis will help to corroborate if these genes and the nucleotide polymorphisms associated to them can be used as markers for selective breeding in the Pacific oyster.

Keywords: *Crassostrea gigas*, OsHV-1, RNAseq, genotyping, resistance

Funding: EU H2020-Marie Sklodowska Curie Actions - RESISGAL, Axudas do Programa de Consolidación e estructuración de unidades de investigación competitivas (GPC) IN6078 2018/11.

196-O

Unsuspected OSHV-1 genomic diversity at inter and intra-host level

Morga, Benjamin (France)[1]; Lamy, Jean-Baptiste (France)[1]; Faury, Nicole (France)[1]; Bietry, Antoine (France)[1]; Pepin, Jean-François (France)[1]; Renault, Tristan (France)[1]

1 - Ifremer

Intoduction: Ostreid herpesvirus 1 (OsHV-1) is a major pathogen affecting *Crassostrea gigas* production, as well as some other edible mollusk species inFrance and in the world (Australia, New Zealand, Sweden, etc.).

Methodology: The aim of this study is to characterize the simple and complex nucleotide polymorphisms of various OsHV-1viral population on infected individuals collected worldwide, in order to determine the existing phylogeny (using genome-wide dataset) between OsHV-1 specimens and to determine the inter and intra-host diversity to get better insights how disease is built during infection.

Results: First results show that OsHV-1 samples from France and New Zealand infecting *C. gigas* did not cluster together, meaning that New-Zealand OsHV1 viral population is another structural variant compare to OsHV-1 µVar. In addition, we confirm the proximity of AVNV to OsHV-1 µVar-like cluster that suggest a recent host shift from *C. gigas* to *C. farreri*.

Conclusion: The genome-wide study of simple and complex polymorphism suggests that some genomic regions are deleted in several specimens or accumulate a high level of substitution. This non random pattern of polymorphism suggests that some genomic regions are under selective process. Contrary to a common belief, we found variants within all infected individuals. The biological interpretation of these observations is discussed in detail.

Keywords: diversity, herpesvirus, oyster, polymorphism, haplotype

Funding: VIVALDI H2020.

197-O*

The potential role of invasive tunicates in the transmission of pathogens in the marine environment

Costello, Katie Ellen (Ireland)[1]; Lynch, Sharon (Ireland)[1]; O'riordan, Ruth (Ireland)[1]; Mcallen, Rob (Ireland)[1]; Culloty, Sarah (Ireland)[1]

1 - School of Biological, Earth and Environmental Sciences And Aquaculture and Fisheries Development Centre, Environmental Research Institute, University College Cork

Introduction: Many tunicate species display rapid territorial expansion when introduced beyond their native range, and as such are often considered successful invaders. To date research into the invasion pathways and impacts of tunicates is well documented, but less is known about their ability to act as carriers, reservoirs or vectors for parasites and pathogens. In recent years, culture of oyster species has been impacted by a range of pathogens that have resulted in significant mortality events and losses for the sector – the European flat oyster *Ostrea edulis* has been impacted by the haplosporidian *Bonamia ostreae* and the Pacific oyster *Crassostrea gigas* by the ostreid herpesvirus OSHV-1µVar and more recently several *Vibrio* species. This study is focusing on the potential impact of invasive tunicates on the maintenance of these pathogens and their ability to introduce new pathogens and parasites to culture sites.

Methodology: In July and November 2018, samples of the invasive tunicates *Styela clava* and *Botrylloides violaceus* were collected from Cork Harbour, at an oyster farm and marina respectively. Samples of the European flat oyster (*Ostrea edulis*) and Pacific oyster (*Crassostrea gigas*) were also collected to assess current status of the oyster stocks. A range of techniques including heart smear screening, polymerase chain reaction (PCR), quantitative polymerase chain reaction (qPCR) and Sanger sequencing were utilised to screen for *Haplosporidium* spp., *Vibrio aestuarianus* and ostreid herpesvirus in both tunicate and oyster species.

Results: Molecular screening confirmed the presence of the haplosporidian *Bonamia ostreae* in the heart and gill tissue of European flat oysters, *Ostrea edulis*. *Vibrio aestuarianus* and *Haplosporidium* sp. were detected in samples of the Pacific cupped oyster, *Crassostrea gigas*. Screening of the invasive tunicates then detected the presence of *Bonamia ostreae* in the leathery/clubbed tunicate, *Styela clava*. All samples were negative for ostreid herpesvirus.

Conclusion: Detection of *Bonamia ostreae* in *Styela clava* suggests that this invasive species could act as a potential carrier for the pathogen. Work on a series of transmission trials to determine the ability of tunicates to act as vectors will be presented.

Keywords: invasive, tunicates, oysters, parasites

245-O

Distribution of cockle disseminated neoplasia in Ireland: evaluation of their clonal structure

Díaz, Seila (Spain)[1,2]; L. Bruzos, Alicia (Spain)[1,2]; Temes, Javier (Spain)[1]; Johnson, Mark (Ireland)[3]; C. Tubío, Jose M. (Spain)[1,2]

1 - Centro Singular de Investigación en Medicina Molecular y Enfermedades Crónicas, Universidad de Santiago de Compostela, Santiago de Compostela, Spain; 2 - Estación de Ciencias Marinas de Toralla, Universidad de Vigo, Vigo, Spain; 3 - Ryan Institute, NUI Galway, Galway, Ireland

Introduction: Clonally transmissible cancers are somatic cell lineages that are transmitted between individuals via the transfer of living cancer cells. There are only three known types of naturally occurring transmissible cancers, one of which is a leukemia-like cancer found in multiple species of bivalves around the world among then cockles. Cockles *Cerastoderma edule* have been reported high prevalence of disseminated neoplasia. This disease was described in cockles from Cuskinny (Ireland) in the eighties.

Methodology: A total 615 cockles were collected in five different sandbeds around Ireland (Cork, Dublin, Tralee, Westport and Wexford). Disseminated neoplasia was diagnosed by examination of histological sections with light microscopy, discerning four stages: null, light, moderate and high intensity. Cockles have been diagnosed as high and moderate intensity, the mitochondrial genome of their circulating cells and the organ least affected by the disease, was amplified through long-range PCR and the amplicons were sequenced through Minion Oxford Nanopore Technologies to identify somatic tumor genomic variants to generate a phylogenetic tree.

Results: It has been detected that disseminated neoplasia continues to affect Irish cockles currently and the disease has been diagnosed for the first time in the west coast. The mitochondrial genetic variants studied have shown that affected tissues had different variants compared with non-affected ones in the same neoplastic cockle. A phylogeny constructed with those variants of Irish neoplastic cockles has shown a different clone compared with other cockle populations previously studied.

Conclusion: Tumor tissues cluster together far from their matched normal supporting a multiple polyphyletic origin of disseminated neoplasia meaning that multiple cancer clones originated independently have arisen along time and coexist.

Keywords: transmissible cancer, disseminated neoplasia, *Cerastoderma edule*, cockle, Ireland

Funding: EmeraldNeo AssemblePlus project and Scuba Cancers ERC project.

Prophylaxis and Treatment II

198-O

Fortior genetics, a platform to enhance disease resistance by genetic selection in aquaculture

Francois, Yoannah (France)[1,2]; Cabon, Joëlle (France)[2]; Morin, Thierry (France)[2]; Guemene, Daniel (France)[3]; Haffray, Pierrick (France)[1]

1 - Syndicat des Sélectionneurs Avicoles et Aquacoles Français, Rennes, France; 2 - French Agency for Food, Environmental and Occupational Health & Safety, Ploufragan-Plouzané-Niort Laboratory, Viral Fish Pathology Unit, Plouzané, France; 3 - Syndicat des Sélectionneurs Avicoles et Aquacoles Français, Nouzilly, France

Introduction: The *FORTIOR Genetic*s platform was created to mutualize skills (genetic and infectiology) and resources of both SYSAAF and ANSES for the benefit of the health of farmed fish and the French fish farming industry. Its aim is to propose controlled infectious challenges on siblings for several serious diseases in partnership with breeding companies. This platform contributes to a sustainable approach aiming at reducing inputs in aquaculture, reducing resistance to antibiotics and increasing sanitary fish qualities. ANSES facilities are EU approved for fish experimentations and are adapted for infectious challenges of genetic purpose on large amount of juveniles (from 1000 to 2000) of different sizes. Major parameters such as the type of water (fresh or salted), the temperature, the debit, the tanks size, can be adjusted.

Methodology: Several infectious challenges were held since the beginning of RE-SIST program on different host/pathogens couple such as sea bass/viral nervous necrosis, sea bass/vibriosis or sea bream/photobacteriosis. They are used to estimate genetic breeding values of candidates to several different traits as well as the genetic parameters (heritability, genetic correlations with growth and production traits).

Results: For example, heritabilities of resistance to nodavirosis and vibriosis were estimated at 0.11 ± 0.05 and 0.08 ± 0.03 respectively with a negative correlation of -0.45 ± 0.17 between both traits. Sea bream resistance to photobacteriosis heritability was estimated at 0.24 ± 0.07, with a strong genetic correlation with commercial weight (0.75 ± 0.10) but a low correlation with carcass yield (-0.20 ± 0.13).

Conclusion: *FORTIOR Genetics* is one of the first European tool for phenotyping farmed fish to different disease resistances in order to support domestication and genomic selection developed by breeders. Several improvement were identified to standardize infectious challenges and make them as representative as possible of the field conditions. Redefining the studied phenotypes and test new genomics tools could also help to improve the efficiency and relevance of FORTIOR Genetics results, and contribute to the generation of knowledge on fish disease resistance.

Keywords: FORTIOR genetics, infectious challenges, disease resistance, genetic selection, aquaculture

Funding: FUI-ReSist, Fishboost (FP7), FEAMP, Région Bretagne.

199-O

Pharmacokinetics and effect of antibacterial treatments of lumpfish experimentally challenged with *V. anguillarum*, atypical *A. salmonicida* and *Pasteurella* sp.

Haugland, Gyri T. (Norway)[1]; Kverme, Karen O. (Norway)[1]; Larsen, Kristina (Norway)[1]; Kallekleiv, Marielle (Norway)[1]; Hannisdal, Rita (Norway)[2]; Rønneseth, Anita (Norway)[1]; Lunestad, Bjørn Tore (Norway)[2]; Samuelsen, Ole B. (Norway)[2]; Wergeland, Heidrun I. (Norway)[1]

1 - University of Bergen; 2 - Institute of Marine Research

Introduction: Lumpfish (*Cyclopterus lumpus* L.) are increasingly used as cleaner fish for removal of sea lice on commercially farmed salmon. The production of lumpfish is successful, but there are challenges with bacterial infections and the number of prescriptions have increased the last years despite lack of established protocols for efficient treatments. This study examined uptake and elimination of the antibacterial agents florfenicol (FFC), oxolinic acid (OA) and flumequine (FLU) in lumpfish following a single oral administration of 10 mg FFC/kg fish and 25 mg OA and FLU/kg fish given in feed. Furthermore, we have tested the sensitivity of pathogenic bacteria towards FFC, OA and FLU, and investigated the effect of different antibacterials on lumpfish experimentally challenged with *Vibrio anguillarum*, atypical *Aeromonas salmonicida* and *Pasteurella* sp.

Methodology: For pharmacokinetical analyses, medical feed were given orally. Plasma and tissue samples were taken at different time points post administration, homogenized and analyzed by LC/MSMS. Minimum inhibitory concentration analysis (MIC) was performed for sensitivity-testing. For treatment experiments, fish were experimentally challenged with bacteria and treated with FFC, OA and/or FLU.

Results: The highest level of FFC and the metabolite FFC-amine was detected in head kidney 24 h post administration. The elimination half-time was 30 h. The highest level of FLU and OA was after 6-12 h and the elimination half-times were 22 h and 21 h, respectively. Sensitivity-analyses showed that all isolates of *A. salmonicida* were sensitive for FFC, but some had low sensitivity/ were resistant for OA and FLU. Treatment experiments where lumpfish were experimentally challenged with *V. anguillarum*, atypical *A. salmonicida* and *Pasteurella* sp. (all isolated from diseased lumpfish) showed that FFC had good effect to treat vibriosis (20 mg/kg/day in 10 days) and pasteurellosis (20 mg/kg/day in 15 days). None of the tested antibacterials gave satisfactory effect on atypical furunculosis.

Conclusion: Knowledge of the sensitivity of bacteria towards antibacterial agents, pharmacokinetical data and efficient treatment is essential to increase the welfare of lumpfish, to reduce the risk that lumpfish become carrier of pathogenic bacteria and to avoid development of antibiotic resistant bacteria.

Keywords: lumpfish, pharmacokinetics, antibacterial treatment, cleaner-fish

Funding: Norwegian Seafood Research Fund and Research Council of Norway.

200-O*

In vitro assessment of the antimicrobial activity of chitosan nanoparticles against major fish pathogens

Ahmed, Fatma (Austria)[1]; Saleh, Mona (Austria)[2]; El-Matbouli, Mansour (Austria)[2]

1 - Clinical Division of Fish Medicine, University of Veterinary Medicine, Vienna, Austria 2 Zoology Department, Faculty of Science, Sohag University, Sohag, Egypt; 2 - Clinical Division of Fish Medicine, University of Veterinary Medicine, Vienna, Austria

Introduction: Nanotechnology applications in aquaculture has been constantly developed over the last years for disease prevention and treatment. Chitosan nanoparticles (CSNPs) have been widely reported as an effective antimicrobial agent against bacteria as well as fungi. Additionally, they are feasible for biomedical applications due to their nontoxicity, anti-microbial activity, mucoadhesivity and haemocompatibility. These features turn CSNPs effective in prophylaxis and treatment by enhancing fish immunity and increasing their antimicrobial resistance. Our study evaluates the antimicrobial activity of CSNPs against major fish pathogens.

Methodology: CSNPs were synthesized via the ionic interaction of chitosan with sodium tripolyphosphate and characterized by transmission electron microscopy and zeta sizer. The synthesized nanoparticles were tested for their antibacterial activity against *Pseudomonas fluorescence*, *Aeromonas hydrophila*, *Aeromonas salmonicida* subsp. *salmonicida*, *Yersinia ruckeri, Edwardsiella tarda* and *Francisella noatunensis* subsp. *orientalis*. Initially, the ability of CSNPs to inhibit growth of the bacterial was evaluated. Subsequently, the minimum inhibitory concentrations (MIC) and the minimum bactericidal concentrations (MBC) were determined.

Results: CSNPs (1 mg/ml) successfully inhibited the growth of *P. fluorescence*, *A. hydrophila* and *Y. ruckeri* but couldn't inhibit the growth of other strains at this concentration. MIC values of CSNPs were 0.125 mg/ml for *P. fluorescence*, 0.5 mg/ml for *A. hydrophila*, 1mg/ml for *Y. ruckeri*, 2 mg/ml for *F. noatunensis* subsp. *orientalis* and 3 mg/ml for both *E. tarda* and *A. salmonicida* subsp. *salmonicida.* The MBC values of CSNPs were 1, 2, 3, 4 and 6 mg/ml for *P. fluorescence*, *A. hydrophila*, *Y. ruckeri*, *E. tarda* and *A. salmonicida* subsp. *salmonicida*, respectively. However, CSNPs didn't demonstrate bactericidal effects against *F. noatunensis* subsp. *orientalis*.

Conclusion: CSNPs demonstrate active and dose-dependent actions inhibiting bacterial growth. They exhibit bactericidal activity against *P. fluorescence*, *A. hydrophila*, *Y. ruckeri*, *E. tarda* and *A. salmonicida* subsp. *salmonicida* and show bacteriostatic activity against *F. noatunensis* subsp. *orientalis.* Therefore, CSNPs can be used to reduce the excessive use of antibiotics especially in cases of antibiotic resistant bacteria.

Keywords: chitosan nanoparticles, antimicrobial activity, fish pathogens, *in vitro*

202-O*

Turbot (*Scophthalmus maximus*) NK-lysin induces broad-spectrum activity against parasites, bacteria and virus

Pereiro, Patricia (Spain)[1]; Falcó, Alberto (Spain)[2]; Lama, Raquel (Spain)[1]; Medina-Gali, Regla María (Spain)[2]; Belló-Pérez, Melissa (Spain)[2]; Figueras, Antonio (Spain)[1]; Encinar, José Antonio (Spain)[2]; Novoa, Beatriz (Spain)[1]

1 - Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, Vigo, Spain; 2 - Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche, Miguel Hernández University, Elche, Spain

Introduction: Nk-lysin (Nkl) is an antimicrobial peptide (AMP) belonging to the saposin-like protein (SAPLIP) family with ability to interact with biological membranes.

Methodology: Following the recent characterization of turbot (*Scophthalmus maximus*) Nkl, a shortened synthetic peptide containing the potential core region of the turbot Nkl (Nkl_{71-100}) was designed to determine its activity against different aquaculture pathogens. Several biophysical methods, such as vesicle aggregation, leakage and fluorescence polarization, were employed to investigate the interaction of Nkl_{71-100} with different phospholipid species. Also several studies were conducted to determine the activity of Nkl_{71-100} against different pathogens.

Results: Our results showed that, at acidic pH, Nkl_{71-100} significantly interacted with phosphatidylserine (PS) and could disrupt PS membranes, allowing the content leakage from PS vesicles. Nkl_{71-100} inhibited the proliferation of two bacterial pathogens, *Escherichia coli* and *Aeromonas salmonicida*, with higher activity at higher concentrations of $Nkl_{71-100.}$ The peptide also affected the viability of *Philasterides dicentrarchi*, the scuticociliate responsible for the outbreaks in turbot farms, reducing the parasite load and increasing the survival of experimentally infected turbot. Moreover, we have demonstrated through scanning electron microscopy (SEM) a direct effect of this molecule on physical destruction of the parasite. Finally, Nkl_{71-100} showed antiviral activity using spring viraemia of carp virus (SVCV) as experimental model. The peptide inhibited not only the binding of viral particles to host cells, but also the fusion of virus and cell membranes, which requires a low pH context. Such antiviral activity seems to be due to the important role that PS plays in these steps of the replication cycle of SVCV and other families of virus.

Conclusion: All these results confirm that NK-lysin and derivates are promising broad-spectrum AMPs with potential activity against pathogens with health and veterinary relevance.

Keywords: NK-lysin, antimicrobial peptide, turbot, pathogens

Funding: EU, Horizon 2020, ParaFishControl (project reference 634429) and the Spanish project BIO2017-82851-C3-1-R.

203-O*

H_2O_2 treatment impacts immune activity in Atlantic salmon gills and causes similar mucin disruption to high grade AGD affected fish

Fernandez, Carolina (United Kingdom)[1]; Monaghan, Sean J. (United Kingdom)[1]; Mascolo, Dario (United Kingdom)[2]; Baily, Johanna L. (United Kingdom)[1]; Betancor, Monica B. (United Kingdom)[1]; Chalmers, Lynn (United Kingdom)[1]; Paladini, Giuseppe (United Kingdom)[1]; Adams, Alexandra (United Kingdom)[1]; Fridman, Sophie (United Kingdom)[1]; Bron, James E. (United Kingdom)[1]

1 - University of Stirling; 2 - Scottish Sea Farms

Introduction: Amoebic gill disease (AGD), caused by the protozoan ectoparasite *Neoparamoeba perurans,* is known to be one of the most important infectious diseases in Atlantic salmon (*Salmo salar* L.). Amoebae attach to the gills of the fish causing multifocal white raised mucoid spots which can coalesce into larger patches as the disease progresses. Although a proportion of the salmon aquaculture industry uses freshwater bathing as a treatment strategy, the availability of freshwater is often restricted. As an alternative, hydrogen peroxide (H_2O_2) is frequently used as a treatment.

Methodology: The effect of H_2O_2 treatment on the gills of Atlantic salmon was determined by gene expression (qPCR), histology (Alcian blue/PAS staining) and immunohistochemistry (IHC) looking at T-cell, B-cell and mucin markers. Unchallenged fish treated with H_2O_2 were sampled after 4 h, 24 h and 14 d post-treatment (total n = 24). Additional AGD-affected fish were used as a comparison. This latter group of fish had exhibited low (1-2) and high grade AGD (scores 3-4) after experimental challenge.

Results: Treatment with H_2O_2 resulted in upregulation of markers for T-cell activity and anti-inflammatory response in the gills 14 d post-treatment (dpt), which corroborated the findings of IHC analysis which showed an increased number of CD3+ T cells at 14 dpt. In contrast, markers for T cell and B-cell activity were significantly down-regulated as a result of high grade AGD. Additionally, the impact of the treatment on gill epithelium was evident even after 14 dpt, causing a similar disruption to mucin expression in the gills to that experienced in high grade AGD infection. This was confirmed histologically by lower mucous cell counts in H_2O_2 treated fish gills after 14 dpt. No changes were, however, observed in the low grade AGD fish except for a significantly elevated IL-10 response to the parasite.

Conclusion: These data suggest that H_2O_2 impacts immunological activity in the gills of H_2O_2-treated fish and results in similar mucin disruption to that experienced by high grade AGD affected fish.

Keywords: hydrogen peroxide, amoebic gill disease, fish immunology, mucins, gill epithelium

Funding: This study has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement no. 634429 (ParaFishControl).

204-O

The interaction of treatment temperature and hydrogen peroxide dose on amoebic gill disease affected Atlantic salmon

Taylor, Richard S. (Australia)[1]; Stratford, Chris (Australia)[2]; Slinger, Joel (Australia)[2,4]; Samsing, Francisca (Australia)[1]; Rigby, Megan (Australia)[1]; Mcculloch, Russell (Australia)[3]; Quezada Rodriguez, Petra R. (Australia)[1,4]; Wynne, James W. (Australia)[1]

1 - CSIRO, Agriculture and Food, Hobart, Tasmania; 2 - CSIRO Agriculture and Food, Bribie Island Research Centre, Queensland; 3 - CSIRO Agriculture and Food, Queensland Bioscience Precinct, Brisbane, Queensland; 4 - Institute of Marine and Antarctic Studies, University of Tasmania, Launceston, Tasmania

Introduction: Hydrogen peroxide (H_2O_2) is increasingly used as a bath treatment of amoebic gill disease (AGD) affected fish. While H_2O_2 is effective at reducing the parasitic load and alleviating associated clinical disease, it is a risky treatment above 12 °C thus its use is seasonally constrained. This trial aimed to assess treatment efficacy and host response in fish held at 16 °C that were treated at a range of H_2O_2 dose and temperatures then returned to ambient conditions.

Methodology: Fish (223 g) were inoculated with *Neoparamoeba perurans* and AGD progression monitored by gross gill score (GS). At an industry appropriate average GS (1.3) fish were bathed for 20 minutes at 0 (control), 750, 1000 and 1250 ppm and 8, 12 or 16 °C. Post treatment, 3 fish per treatment were sampled (GS, gill swab, histopathology, blood serum stress indicators) at 1H, 1D, 2D, 7D and 14D and GS recorded at 15D.

Results: There was a clear increasing H_2O_2 dose response in reducing GS and lowering amoeba load. Although there were no treatment related mortalities, significant gill damage was evident at higher H_2O_2 dose and higher treatment temperature. Only minor damage was observed at 8 °C at 1250 ppm H_2O_2. Stress indicators (cortisol, glucose, lactate) were elevated at higher dose immediately post treatment, but these resolved rapidly. There was clear evidence of increased osmotic imbalance (sodium, potassium, chlorine) at higher H_2O_2 dose which was reduced at lower treatment temperature.

Conclusion: Hydrogen peroxide doses between 750 and 1250 ppm were successful in reducing AGD, though clearance was most successful at 1250 ppm. Fish treated at temperatures below ambient (16 °C) achieved similar AGD reduction with a marked reduction in gill damage, stress response and osmotic disturbance. During periods of high ambient water temperature, H_2O_2 bathing at lower temperature offers the potential to successfully treat AGD affected fish with improved welfare outcomes.

Keywords: AGD, *Neoparamoeba perurans*, hydrogen peroxide, temperatura

Funding: CSIRO and Tassal Operations Pty Ltd.

014-O

AQUAPATHLAB: a web-based virtual pathology lab for diseases of aquatic animals

Katharios, Pantelis (Greece)[1]; Keklikoglou, Kleoniki (Greece)[1]; Cascarano, Maria Chiara (Greece)[1]; Panteri, Emmanuella (Greece)[1]; Filiopoulou, Irene (Greece)[1]; Magoula, Antonios (Greece)[1]; Arvanitidis, Christos (Greece)[1]

1 - Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Crete, Greece

Introduction: As aquaculture continues to grow, there is a parallel growth of the need for support in disease diagnostics. Recent advancements and modern tools in diagnostic methods together with the rapid progress and changes in the communication through the new web-based tools offer a unique opportunity for developing interactive e-platforms that could serve as hubs for exchanging information, education and collaboration of aquatic animal health specialists. Here we describe one such platform, the AQUAPATHLAB, a virtual fish pathology laboratory.

Methodology: Several diseases have been selected to be used as a proof-of-concept but also as demonstration for the virtual lab. For the description of the pathologies we have used the microCT technology. Several samples have been scanned using the Skyscan 1172 microtomograph (Skyscan, Bruker, Belgium) at the Hellenic Centre for Marine Research). In addition, every pathology is described through histology and electron microscopy (Scanning and Transmission). Epidemiological and phylogenetic data are also provided where available.

Results: A web portal has been established in HCMR's site (http://aquapath.hcmr.gr). The user has access to all the uploaded information regarding 6 diseases. The diseases selected include diseases caused by bacteria (epitheliocystis), parasites (*Ceratothoa oestroides*, *Sciaenocotyle pancerii*, *Henneguya aegea*) but also the non-infectious diseases, chronic ulcerative dermatopathy and systemic granulomatosis. For each disease, there is a general description and macroscopic photographs. There are also pictures from light microscopy of fresh squash preparations as well annotated histological sections. Through the use of Slice:Drop software the operator may have the ability to display and interactively manipulate the microCT datasets in 3D. Where available, molecular data have also been uploaded. Finally, the user has access to selected literature.

Conclusion: AQUAPATHLAB is a virtual fish pathology laboratory offering a collection of several imaging techniques, including microtomography, histology, SEM, TEM that can be used for disease diagnostics. The digitization of fish diseases will create searchable and retrievable datasets to be explored online.

Keywords: imaging, virtual lab, microCT

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Myxozoan Diseases III

205-O*

Transcriptome signatures in brown trout kidney during proliferative kidney disease

Sudhagar, Arun (Austria)[1]; Ertl, Reinhard (Austria)[2]; Kumar, Gokhlesh (Austria)[1]; El-Matbouli, Mansour (Austria)[1]

1 - Clinical Division of Fish Medicine, University of Veterinary Medicine, Vienna, Austria; 2 - VetCore Facility, University of Veterinary Medicine, Vienna, Austria

Introduction: *Tetracapsuloides bryosalmonae* is a myxozoan parasite that causes proliferative kidney disease in salmonids. The host molecular factors that are influenced by the proliferation of the parasite in the kidney of brown trout are poorly understood. Here, RNA-sequencing based transcriptome analysis of *T. bryosalmonae*-infected brown trout kidney was performed to explore the differentially expressed genes of the host during the disease process.

Methodology: Specific pathogen free brown trout were exposed to the spores of *T. bryosalmonae* collected from the laboratory infected *Fredericella sultana* bryozoan colonies. Brown trout kidney samples were collected from infected and control groups. The presence of numerous interstitial pre-sporogonic stages in the kidney samples was observed using immuno-histological examination. Afterwards, cDNA libraries were prepared from infected and control samples and sequenced on the Illumina HiSeq 2500. The obtained sequence reads were cleaned and mapped to a rainbow trout genome and annotated brown trout transcriptome data, and subjected to further downstream analysis.

Results: Comparison between infected and control kidney samples revealed 1169 transcripts with significant differential expression. Gene Ontology analysis showed significantly enrichment to immune responses and regulation of cellular process within biological function; and receptor activity, peptide binding, amide binding and protein binding within the molecular function. In the category of cellular components, the genes were classified as Golgi-associated vesicle, external side of plasma membrane, leaflet of membrane bilayer, plasma membrane part, cell part, and cell. Differentially expressed genes were mostly involved in inflammation mediated by chemokine and cytokine signalling pathways.

Conclusion: Most of the up-regulation of the kidney genes was involved in immune response regulation and leukocyte activation. However, down-regulation of the genes were associated with endopeptidase regular and apoptotic processes. The results obtained point towards regulation of T-cell mediated immune response in the host kidney and suggest its importance during the parasite development. This study provides new insights to understand the molecular mechanisms involved in the fish host kidney during *T. bryosalmonae* proliferation.

Keywords: *Tetracapsuloides bryosalmonae*, RNA-sequencing, differentially expressed genes, proliferative kidney disease

Funding: Austrian Science Fund (FWF) P 30981.

206-O

Is stocking in PKD positive river systems necessary to maintain the brown trout population stable? How sustainable is this measure?

Schmidt-Posthaus, Heike (Switzerland)[1]; Vonlanthen, Pascal (Switzerland)[2]; Bittner, David (Switzerland)[3]; Birrer, Christoph (Switzerland)[4]; Mehr, Christoph (Switzerland)[4]; Wahli, Thomas (Switzerland)[1]; Schneider, Ernst (Switzerland)[5]

1 - Centre for Fish and Wildlife Health, Vetsuisse Faculty, University of Bern; 2 - Aquabios Sàrl / GmbH, Cordast; 3 - Canton Aargau, Department of Construction, Transport and Environment, Office for Hunting and Fisheries, Aarau; 4 - Canton St. Gallen, Department of Economic Affairs, Office for Nature, Hunting and Fisheries, St. Gallen; 5 - Thalwil

Introduction: Proliferative Kidney Disease (PKD), caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*, was found in brown trout (*Salmo trutta*) in Swiss rivers. This disease was discussed as possible cause for brown population decline. To maintain the wild brown trout populations, stocking of brown trout fingerlings into rivers is a common practice in Switzerland. Additionally, there is a debate, whether natural selection for adapted, more disease resistant individuals is interfered by stocking. In this study we aimed to investigate how ecologically and economically worthwhile these stocking measures are, particularly in rivers with presence of PKD.

Methodology: Three river systems in different parts of Switzerland were selected. In all three river systems, PKD had regularly been documented in brown trout and all rivers had been stocked since many years. A first sampling took place when stocking was still effected (zero control), while in the following years no fish were released anymore in the rivers and samplings were continued in the same locations as before on a yearly basis. To assess the effect of PKD, in one river system, an additional PKD negative site in the upstream region was included and at the last stocking event, stocked brown trout fingerlings were marked to differentiate stocked from naturally spawned offsprings. Sampling consisted of quantitative electrofishing over a given river stretch to determine the brown trout population and in retrieving 25 young-of-the-year brown trout for the presence of *T. bryosalmonae* and the associated disease. Infection intensity was determined by qPCR and the disease status by kidney histology.

Results and Conclusion: Our observations show that the brown trout populations did not decline after discontinuation of stocking. The follow up of the marked fish in one river system indicated that hardly any of these fish survived the following two years. The low survival rate was even more pronounced under the influence of PKD. PKD prevalence, parasite abundance and pathology did not change over the years. However, given the short time of our investigation up to now, a clear effect of stocking interrupt on disease development is unlikely.

Keywords: proliferative kidney disease, brown trout, stocking, population, sustainability

207-O

Transcriptome analysis of bryozoan, *Fredericella sultana* in response to *Tetracapsuloides bryosalmonae* (Myxozoa)

Kumar, Gokhlesh (Austria)[1]; Ertl, Reinhard (Austria)[2]; El-Matbouli, Mansour (Austria)[1]

1 - Clinical Division of Fish Medicine, University of Veterinary Medicine, Vienna, Austria; 2 - VetCore Facility, University of Veterinary Medicine, Vienna, Austria

Introduction: Bryozoan *Fredericella sultana* is the most common invertebrate host of *Tetracapsuloides bryosalmonae*, the causative agent of proliferative kidney disease in salmonids. *F. sultana* grows by budding zooids that form tubular and branching colonies. Each zooid has its own independent tentacular lophophore mouth, gut, muscles, nervous and reproductive systems. The sporogenesis starts its development as single cells associated with the bryozoan body wall and then proliferates into spore-producing sacs in the body cavity. RNA-seq was applied to investigate the transcriptional response of *F. sultana* zooids during *T. bryosalmonae* development.

Methodology: *T. bryosalmonae*-infected and specific pathogen-free control *F. sultana* zooids were collected from our laboratory bryozoan culture system. RNA was extracted from both infected and control zooids and cDNA libraries were prepared with the TruSeq RNA Library Prep Kit. Libraries were sequenced on the Illumina NextSeq550 utilizing 150-bp PE reads. The sequencing data from control zooids were used for de novo transcriptome assembly and functional annotation. The generated contig sequences were subsequently used as a reference data set for RNA-seq-based gene expression analysis in parasite-infected zooid samples.

Results: For transcriptome assembly, 118 million reads were assembled in a total of 85,543 contigs, with an average length of 848 nucleotides. 23,978 contigs (28%) were annotated to known protein sequences and Gene Ontology terms. Gene expression analysis of parasite-infected zooids resulted in 1,643 differentially expressed transcripts (DETs) compared to controls. Gene ontology analysis of the DETs reveals a significant enrichment of transcripts involved in the biological processes such as immune system, response to stress and reproduction.

Conclusion: This study represents the first transcriptome assembly of *F. sultana* which provides new genetic resources for future molecular studies. Infection with *T. bryosalmonae* induces dynamic changes in the zooid transcriptome in particular the induction of genes involved in parasite defence and cellular reproduction.

Keywords: bryozoan, *Fredericella sultana*, *Tetracapsuloides bryosalmonae*, transcriptome

Funding: Austrian Science Fund (FWF) P 30981.

208-O

Omics approaches unravel structures and functions of *Ceratonova shasta* (Cnidaria: Myxozoa) nematocysts during proliferation and spore development

Americus, Ben (United States of America)[1]; Hams, Nicole (United States of America)[1]; Alama-Bermejo, Gema (Argentina)[2]; Lotan, Tamar (Israel)[3]; Bartholomew, Jerri (United States of America)[1]; Atkinson, Stephen (United States of America)[1]

1 - Oregon State University; 2 - National Scientific and Technical Research Council (CONICET); 3 - University of Haifa

Introduction: The Cnidaria are an ancient and diverse phylum of invertebrates, characterized by having nematocysts, which fire tubules to sting or entrap prey. Nematocyst structure and function varies amongst free-living cnidarian lineages Anthozoa and Medusozoa, and the parasitic Endocnidozoa, which contains the myxozoans. We hypothesize that despite their small physical and genomic sizes, myxozoans have retained nematocyst features of free-living species, *and* have adapted and optimized nematocysts for host infection.

Methods: We developed 'omics datasets for our model myxozoan *Ceratonova shasta*: a genome from infected fish; transcriptomes in both fish and annelid hosts; expression libraries in fish at 7-, 14-, and 21-days post-infection. We created a database of 31,798 venom/toxin transcripts and 7,129 proteins, from jellyfish and anemone data, and the UniProt toxin database, and compared this with the published 114 *C. shasta* nemocyst proteins using BLAST. In parallel, we used Phyre2 to predict protein tertiary structures, annotated functions based on homology to protein structures in PDB and SCP, then used phylogenetic inferences to test associations with known venoms/toxins. We mapped expression of nematocyst proteins in our time-series libraries and correlated this with nematocyst development in histology.

Results: BLAST identified 14 putative venom/toxin proteins, structural modelling identified an additional 14. These grouped in 3 categories: external attachment/interaction, immune suppression, and proliferation. Genes associated with parasite proliferation and immune system suppression were expressed at all times; genes associated with external attachment/interaction were co-expressed from day 14, which was *after* expression of a transcription factor that initiates nematocyst synthesis.

Conclusion: Structural modelling provided annotation to previously unannotated proteomic data and was more sensitive to discovering venom/toxin related proteins than BLAST. We hypothesize that many of our putative venom/toxin proteins have been re-purposed for parasitism: penetrating host tissue and suppressing host immune attack. Specifically, we suspect those external attachment/interaction "envenomation proteins" co-expressed in the time-series have been inherited from the nematocyst and venom-bearing cnidarian ancestors of myxozoans and are now playing a novel role in host invasion.

Keywords: Myxozoa, nematocyst, Cnidaria, venom, omics

Funding: Grant No. IS-5001-17C from BARD, the United States-Israel Binational Agricultural Research and Development Fund, and Grant No. 47496 from BFS, the USA-Israel Binational Science Foundation.

209-O

Evolution of *Ceratonova shasta* virulence as it encounters new hosts

Taggart-Murphy, Laura (United States of America)[1]; Alama-Bermejo, Gema (Argentina)[2]; Breyta, Rachel (United States of America)[3]; Atkinson, Stephen (United States of America)[1]; Bartholomew, Jerri (United States of America)[1]

1 - Oregon State University; 2 - National Scientific and Technical Research Council (CONICET); 3 - University of Washington

Introduction: Different genetic strains (genotypes) of *Ceratonova shasta* (Cnidaria: Myxozoa) cause a range of disease severity in their salmonid fish hosts. We now recognize that infections in *Oncorhynchus mykiss* (rainbow/steelhead trout) by genotype 0 result in a chronic infection, as opposed to the typical fatal disease from genotype II. Genotype 0-infected fish survived >2 years in the laboratory and shed myxospores while appearing healthy. We hypothesized that fish infected with genotype 0 (avirulent) show decreased expression of inflammatory cytokine genes and increased expression of immunoglobulin genes, compared to genotype II (virulent).

Methodology: Rainbow trout were exposed in the Klamath River (OR) at sites known to have genotype 0 or II present. Host gene expression post-exposure was measured in the intestine using qPCR. Tissue effects were assessed by histopathology. Genotypes were analysed using coalescent phylogenetic analysis and inferred secondary structures of their 18S-ITS-1 sequences.

Results: Expression of pro-inflammatory cytokines IL6 and IL8 was not upregulated in fish infected with genotype 0 (avirulent), compared with II (virulent). Gene transcripts for the immunoglobulins IgM and IgT were upregulated in both 0 and II infections. Histopathology showed that while fish infected with 0 did mount an inflammatory response, the parasite migrated into the intestinal lumen and developed into mature spores without compromising the intestinal epithelium. In contrast, in II infections, parasites proliferated throughout all tissue layers of the intestine and caused necrosis and sloughing of the intestinal epithelium. Phylogenetic analysis showed that genotype 0 formed a monophyletic group distinct from genotypes I and II, and that genotype 0 may be more closely related to the ancestral ceratonovid. This was consistent with structure analysis, which showed genotype 0 was distinct from genotypes I and II, with some variations shared with other closely-related ceratonovids.

Conclusion: These differences may indicate that genotype 0 in rainbow/steelhead trout is the ancestral parasite/host relationship. Our observations of avirulent, chronic natural infections, lower inflammatory responses in controlled infections, and ancestral position in evolutionary reconstruction suggest that virulence has evolved as *C. shasta* has adapted from its original *O. mykiss* host to infect more species of Pacific salmon.

Keywords: Myxozoa, virulence, genotypes, *Ceratonova*, cytokines

Funding: NSF.

210-O

Proteases and their inhibitors are parasite virulence factors of *Ceratonova shasta* (Myxozoa: Cnidaria) in salmonid fish hosts

Alama-Bermejo, Gema (Czech Republic)[1,2,3]; Atkinson, Stephen (United States of America)[2]; Bartošová-Sojková, Pavla (Czech Republic)[1]; Holzer, Astrid (Czech Republic)[1]; Bartholomew, Jerri (United States of America)[2]

1 - Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic; 2 - Department of Microbiology, Oregon State University, Corvallis, OR, USA; 3 - National Scientific and Technical Research Council (CONICET), San Antonio Oeste, Argentina

Introduction: Virulence factors are molecules responsible for the pathogenicity of an infective agent. Proteases and their inhibitors are candidate virulence factors for parasites and intervention strategies can be designed to target these molecules. *Ceratonova shasta* is a myxozoan parasite of salmonid fishes from the Pacific Northwest of North America. This cnidarian parasite represents a complex of genotypes with different degrees of virulence: from low virulence genotype 0, to moderately virulent I and IIC, to a highly virulent IIR. We investigated proteases and inhibitors genes of *C. shasta* genotypes and their relationship to virulence.

Methodology: First, we constructed a host-free reference transcriptome for candidate virulence gene mining using next generation sequencing (Illumina HiSeq 3000) of parasite genotype IIR ascites from rainbow trout, using our custom bioinformatics pipeline. Second, intestines infected with genotypes I (chinook salmon) and IIC (coho salmon) were collected to search for genetic polymorphisms between genotypes. Finally, we collected gills and intestines from rainbow trout infected with genotypes 0 and genotype IIR at 7, 15, 22 and 29 days post-infection to investigate the differential expression (qPCR) of four selected proteases and a cysteine protease inhibitor identified *in silico*: cathepsin L, Z, D, an aminopeptidase-N, and stefin.

Results: We found that genetic variation of proteases and inhibitors correlated with virulence in *C. shasta* genotypes. Three of our selected genes were more up-regulated in the virulent genotype IIR: at day 7 there was a large fold change of aspartic protease cathepsin D, presumably linked to a faster proliferation and sporogenesis in intestinal epithelium. At day 15 there was a significant fold increase of stefin, probably associated with inhibition of host cysteine proteases responding to parasite tissue invasion and sporogenesis in all intestinal layers. Late in the infection, day 22 and 29, genotype IIR showed a larger fold change of the metalloprotease aminopeptidase-N, which could be related to spore maturation.

Conclusion: These results support the hypothesis that these enzymes are important *C. shasta* virulence factors and spotlight them as promising candidates for targeted interventions against myxozoans in aquaculture.

Keywords: Myxozoa, transcriptomics, gene expression, SNPs, proteases

Funding: GACR (14-28784P), ECIP (505/12/G112), Ministry of Education, Youth and Sports (LTAUSA17201).

Vaccinology I

211-O*

***Amyloodinium ocellatum* vaccine-induced modulation of immune genes expression in European sea bass (*Dicentrarchus labrax*)**

Byadgi, Omkar (Italy)[1]; Volpatti, Donatella (Italy)[1]; Massimo, Michela (Italy)[1]; Bulfon, Chiara (Italy)[1]; Galeotti, Marco (Italy)[1]; Beraldo, Paola (Italy)[1]

1 - University of Udine

Introduction: Amyloodiniosis caused by *Amyloodinium ocellatum* (AO) has worldwide distribution and affects all marine/brackish water species. Fish surviving natural infection and/or immunized with AO develop innate and adaptive immune responses. However, few data exist regarding immune gene expression patterns in systemic and mucosal tissues, leading to innate and adaptive immune protection. Therefore, objectives were to investigate expression of innate and adaptive immunity genes, antibody titre in European sea bass (ESB) after vaccination with inactivated AO dinospores.

Methodology: Four experimental groups (N = 40) were submitted to intracoelomatic injection (200 µl/fish) as follows: VAC+ADJ group, injected with 100 µl VAC (0.5×10^6 dinospores in PBS) + 100 µl adjuvant (Montanide™ ISA763 AVG); ADJ group, injected with 100µl adjuvant + 100 µl PBS; VAC group, injected with 0.5×10^6 dinospores in 200 µl PBS; control group injected with 200 µl PBS. One week and three weeks post-vaccination (wpv), gill and anterior kidney in RNA later for the gene expression study and serum for specific antibody titre (ELISA) were collected. On 30 days post-vaccination (dpv), 15 fish/group were infected in aquaria with 10 dinospores/ml and surveyed for 15 days. Seven days post-challenge gills, head kidney and serum were collected.

Results: The VAC+ADJ fish showed an increase of specific antibody titres compared to the other groups with survival of 100%. The gills of VAC+ADJ did not show pathological lesions and the parasitic burden was significantly lower than that reported in the other groups. On 7 dpv upregulation of hepcidin was evident in gills and head kidney of VAC+ADJ with lesser proinflammatory cytokines (*il-8* and *cc1*). Further, 3 wpv genes encoding immunoglobulins (*igm* and *igt*) were not upregulated in VAC+ADJ. One week after AO challenge, VAC+ADJ fish had the higher expression of *cc1*, *hepcidin*, and *igt*, in gills and *cc1* and *igm* in head kidney, and thus, a different type of innate (*cc1*, *il-8*, *hepcidin*) and adaptive immune (*igm*, *igt*) response occurred in this group pre- and post-challenge.

Conclusion: ESB VAC+ADJ group successfully survived post AO infection developing a protective immune response. The details of experimental set up, immune genes expression, AO specific antibody titres and cumulative mortality will be presented during the meeting.

Keywords: immune response, dinoflagellates, antibody, survival, vaccination

Funding: ParaFishControl, an EU H2020-funded project (634429).

212-O*

Differences in antibody responses and C3 levels in salmon following immunization with whole sea lice antigen using different immunization protocols

Gislefoss, Elisabeth (Norway)[1,2]; Gamil, Amr Ahmed Abdelrahim (Norway)[1,2]; Evensen, Øystein (Norway)[1,2]

1 - Norwegian University of Life Sciences; 2 - Sea Lice Research Centre

Introduction: The sea louse, *Lepeophtheirus salmonis*, is a big problem facing salmon industry due to its increased resistance to the available medication. Atlantic salmon develops limited humoral and cellular immune response against the parasite. However, the involvement of these responses in protection is not well understood. Studies addressing the protective responses and vaccination can possibly help in providing knowledge that can be used to develop new control strategies.

Methodology: Three groups of Atlantic salmon were immunized with whole sea lice (WSL) antigens formulated as water in oil emulsion using different immunization protocols. Group 1 received a single intraperitoneal (IP) injection. Group 2 received an IP injection followed by an IP boost at 6 weeks post primary immunization. Group 3 received an initial IP injection in addition to an extra injection at the base of the back-fin (WSL+fin), and both injections were repeated after 6 weeks. Three control groups injected with PBS using the different immunization protocols were included. All groups were challenged with copepodids (>450 degree days post primary immunization), and serum, mucus and skin were collected 15 days post challenge. ELISA was used to measure antibody response against WSL antigens as well as complement C3 levels in serum and mucus. In addition, mRNA levels of IgM and IgT was measured in the skin using qPCR.

Results: ELISA results showed differences in antibody responses and C3 levels between the different groups. qPCR data showed also differences in the levels of IgM and IgT transcripts in the skin. The WSL given a boost had the highest levels of serum and mucus antibodies, and mRNA IgM/IgT levels in skin, but this did not correlate with the reduction in lice numbers. However, a better reduction in lice numbers was obtained in the fin-injected group(WSL+fin).

Conclusion: Our results indicate that the different immunization protocols used resulted in different levels of antibodies and C3 in serum and mucus. The data also indicate that reduction in lice numbers does not correlate with high antibody response, but seemed to be affected by the immunization protocol used.

Keywords: antibody response, sea lice

Funding: Research Council Norway, SFI-Sea Lice Research Centre, grant number 20351.

213-O*

Protection and antibody reactivity in lumpsuckers (*Cyclopterus lumpus* L.) following vaccination against *Pasteurella* sp.

Ellul, Rebecca Marie (Norway)[1]; Bulla, Jan (Norway)[1]; Brudal, Espen (Norway)[2]; Wergeland, Heidrun (Norway)[1]; Rønneseth, Anita (Norway)[1]

1 - University of Bergen; 2 - Pharmaq

Introduction: The incidence of disease caused by *Pasteurella* sp. in farmed lumpsuckers in Norway has been steadily increasing in recent years, causing significant economic losses and fish welfare issues. The disease affects lumpsuckers in hatcheries and after release into salmon cages. In previous work, exposure experiments were tested resulting in a bath challenge model being the most robust to enable vaccine development. In this work, two preliminary monovalent vaccines were developed and tested for efficacy by means of a bath challenge model.

Methodology: The vaccines were administered by intraperitoneal injection to VIE-tagged lumpsuckers weighing an average of 10 g. Control groups were vaccinated using a monovalent *V. anguillarum*-O1 vaccine and phosphate buffered saline, respectively. Blood samples were collected post-vaccination from individual fish every 100-degree days over 600-degree days, and analysed for specific antibody production using ELISA. At 500-degree days, all fish were bath challenged using *Pasteurella* sp. and progress of disease was followed. Confirmation of disease was performed by bacteriology and qPCR of head kidney samples from dead fish. *In vitro* work was also performed to study host-bacteria interactions.

Results: High levels of specific antibodies were detected following vaccination. The vaccine efficacy trial indicated that some level of protection was obtained, however high mortality was still observed. qPCR analysis showed no difference in bacteria levels between the vaccinated and non-vaccinated fish. Symptoms observed on diseased fish were similar to those encountered in previous work, and included white spots on the skin and around the eyes, frayed fins, and redness around the mouth and the base of fins.

Conclusion: Despite lumpsuckers responding to vaccination with monovalent inactivated *Pasteurella* sp. vaccines by production of specific antibodies, the protection against experimental challenge was relatively weak. This could potentially be due to specific antibodies not being enough to provide complete protection against pasteurellosis in lumpsuckers, or alternatively, that the antibodies produced were not directed towards the right epitopes. This indicates that other parts of the immune system than just the humoral part could be important for protection against pasteurellosis, highlighting the need for further work on the mechanism of infection of *Pasteurella* sp. in lumpsuckers.

Keywords: challenge, cleaner fish, infection, pasteurellosis, vaccination

214-O*

Intramuscular vaccination of Atlantic lumpfish induces inflammation and local IgM production

Erkinharju, Toni (Norway)[1,2]; Strandskog, Guro (Norway)[2]; Vågnes, Øyvind (Norway)[3]; Hordvik, Ivar (Norway)[4]; Dalmo, Roy (Norway)[2]; Seternes, Tore (Norway)[2]

1 - Norwegian Veterinary Institute, Harstad, Norway; 2 - Norwegian College of Fishery Science, UiT - The Arctic University of Norway, Tromsø, Norway; 3 - Vaxxinova Norway AS, Bergen, Norway; 4 - Department of Biology, University of Bergen, Bergen, Norway

Introduction: Atlantic lumpfish (*Cyclopterus lumpus*) is used as cleaner fish in Atlantic salmon (*Salmo salar*) sea cages, and have in short time become the second largest farmed fish species in Norwegian aquaculture. Bacterial infections are a recurring problem for lumpfish and more effective vaccines and vaccination strategies are needed. We present a study on immune responses of lumpfish after intramuscular and intraperitoneal vaccination.

Methodology: Lumpfish were vaccinated by intramuscular (IM) and intraperitoneal (IP) administration of an oil-based vaccine containing bacterial antigens from two A-layer types of atypical *Aeromonas salmonicida*. Control fish were injected with phosphate-buffered saline (PBS). Four IM injected lumpfish were sampled for skin/muscle tissue at 0, 2, 7, 21 and 42 days post immunization (dpi), and prepared for histology/immunohistochemistry (IHC). We isolated RNA from head kidney, spleen and skin/muscle samples of four IM/IP injected lumpfish at 0, 2, 7, 21 and 42 dpi, and analysed for expressions of IgM (secretory (s) and membrane-bound (m)), IgD, TCRα, CD3 and MHC class IIβ, by using qPCR.

Results: IM vaccinated fish on histological sections demonstrated vaccine-induced granulomas containing eosinophilic granular cells (EGCs), which increased in cell numbers over time, in addition to other inflammatory cells. On IHC sections, we observed immunoglobulin M positive cells (IgM^+) in small numbers at 21 and 42 dpi of IM vaccinated fish, while the diffuse intercellular form (secretory IgM) appeared at 2 dpi. Proliferating cells ($PCNA^+$) also appeared at 2 dpi, and increased in numbers over time. Head kidney and spleen samples from vaccinated fish demonstrated small changes for all genes, with the largest difference between IM vaccinated and control fish for sIgM and IP vaccinated and control fish for mIgM at 42 dpi, respectively. Skin/muscle samples from IM vaccinated fish demonstrated an increase in IgM (secretory and membrane-bound) at 21 and 42 dpi.

Conclusion: Our results show that IM vaccination of lumpfish induces local IgM production at the injection site, with no apparent proliferation of IgM^+ cells. EGCs appear early at the site of injection and persists as the inflammation progresses, which may indicate the cells have an important function in the lumpfish immune system.

Keywords: Atlantic lumpfish, immunoglobulin M, inflammation, immune response, vaccination

216-O

Immune responses induced by oil-adjuvanted inactivated vaccine against bacterial cold-water disease (BCWD) in ayu *Plecoglossus altivelis*

Isaka, Yosuke (Japan)[1]; Suzuki, Kyuma (Japan)[2]; Watanabe, Shun (Japan)[2]; Izumi, Shotaro (Japan)[3]; Endo, Makoto (Japan)[1]; Sano, Motohiko (Japan)[1]; Kato, Goshi (Japan)[1]

1 - Tokyo University of Marine Sciene and Technology; 2 - Gunma Prefectural Fisheries Experiment Station; 3 - Tokai University

Introduction: Bacterial cold-water disease (BCWD) causes serious damage to aquaculture production of ayu *Plecoglossus altivelis* in Japan. Oil-adjuvanted formalin killed cells (FKC-Adj) has been reported to be effective against BCWD in ayu. In this study, we investigated antibody titer, histopathological changes and gene expression levels of IL-8 and IL-10 in the vaccinated ayu after challenge with *Flavobacterium psychrophilum*.

Methodology: Ayu were injected with either FKC-Adj (1.3×10^7 CFU/fish), modified cytophaga broth with the adjuvant (MCY-Adj), FKC (1.3×10^7 CFU/fish) or MCY. Serum antibody titer was measured at 28 days post-vaccination by ELISA. Fish were challenged with *F. psychrophilum* (8.7×10^6 CFU/fish) at 28 days post-vaccination. The trunk kidney and spleen was collected at 0, 1 and 2 days post-infection (dpi). Paraffin embedded section of the spleen was subjected to HE staining for histopathological analysis. Furthermore, gene expression analyses for the immune related genes in the trunk kidney were also performed using real-time PCR.

Results: Cumulative mortality of FKC-Adj, MCY-Adj, FKC and MCY group was 4.4%, 61.2%, 80.0% and 84.0%, respectively. Antibody titer of FKC-Adj and FKC group was significantly higher than those of MCY-Adj and MCY group, while the highest value was observed in FKC-Adj group. Hyperplasia of the ellipsoidal tissue was observed in the spleen of FKC-Adj and MCY-Adj group at all time points. Regressive changes such as shrunken nuclei and degeneration in the ellipsoidal tissues were observed in FKC and MCY group at 1 and 2 dpi, while these changes were not observed in FKC-Adj and MCY-Adj group. IL-8 gene expression levels in FKC-Adj and MCY-Adj group were significantly higher than that in MCY group at 0 dpi. IL-10 gene expression level was significantly lower in FKC-Adj group than MCY group at all time points.

Conclusion: These data suggest that both specific antibody and activation of phagocytes are essential to clear the bacteria in ayu.

Keywords: bacterial cold-water disease, oil adjuvant, vaccine

Prophylaxis and Treatment III

217-O

Developing bioassays and determining baseline salinity tolerance of salmon lice, *Lepeophtheirus salmonis*, a problem parasite of farmed Atlantic salmon

Andrews, Melanie (Norway)[1,2]; Horsberg, Tor Einar (Norway)[1,2]

1 - Sealice Research Centre, Oslo, Norway; 2 - Norwegian University of Life Science, Oslo, Norway

Introduction: Salmon lice, *Lepeophtheirus salmonis* (Krøyer, 1837), are ectoparasitic crustacean parasites of the Atlantic salmon. If left unchecked, they have the capability to rapidly increase population size. This leads to high economic costs to the Atlantic salmon aquaculture industry in the northern hemisphere due to the loss of fish quality and the need for increased handling. Numerous chemical treatments have been extensively used to combat this parasite, in addition a number of non-chemical treatment methods such as freshwater bathing have been in use. When dealing with such an industry wide health problem, treatment resistance towards the commonly used chemical treatment methods is a known issue. To combat this, on site *in vitro* bioassays are routinely used to monitor treatment efficacy by referring to previously established baseline sensitivities. However, neither bioassay methods nor baseline sensitivity levels have been developed for freshwater bathing.

Methodology: We developed an *in vitro* bioassay protocol to determine the salinity in parts per thousand (‰) at which median survival (EC50) occurred following 24 h exposure for the copepodid and pre-adult II (PAII) stages. This study included 6 geographically distinct populations collected along the Norwegian coast. Parasites were exposed to reduced salinity seawater for 24 h. After which, a count of unaffected/affected parasites was taken.

Results: Differences were observed between populations when comparing the copepodid stage, with 4 populations having high percentage survival (+60%) following 24 h exposure to a salinity of 13‰. Such differences were not observed in the PAII stage, however all had an EC50 value below 6‰. Detailed results will be presented.

Conclusion: Differences in tolerance to extended exposure to low salinity were observed between the populations for both the copepodid and PAII stage. By identifying the baseline sensitivity levels towards freshwater bathing, in combination with our data on the possible differences between populations, we hope to provide a tool to avoid any future development of tolerance to this treatment method. In addition, we can better understand and predict how the infective copepodid stage may be able to spread between regions.

Keywords: sealice, aquaculture, Atlantic salmon, parasitic copepod

218-O

A field study assessing the effects of skirts to control salmon louse settlement and of concurrent aeration

Midtlyng, Paul J. (Norway)[1]; Aunsmo, Arnfinn (Norway)[1]; Munkeby, Martin (Norway)[3]; Storsul, Torolf (Norway)[2]; Stormoen, Marit (Norway)[1]

1 - Faculty of Veterinary Medicine, Norwegian University of Life Sciences; 2 - Midt Norsk Havbruk AS; 3 - Norse Aqua AS

Introduction: "Skirts" made of tarpaulin with a depth of up to 10 meters are being widely used in the field to shield marine farmed salmon from salmon louse (*Lepeophtheirus salmonis*) copepodids. An aeration device has been developed to increase water circulation and to improve the environmental conditions inside these skirts. The current field study attempted to provide input for quantification of both advantageous and disadvantageous effects of using skirts with the creation device.

Methodology: We used data from regular weekly louse counts to assess protection against larvae settlement, and from fixed monitoring sensors and from manual recordings to evaluate the environmental parameters. The pattern of water circulation induced by the aeration device was visualised by use of a color marker and video recording.

Results: In two marine sites having pens with or without skirts and ring, the louse settlement was very low for most part of the observation period, with little to no observed shielding effect of skirts. However, one of the sites showed a clear reduction of attached louse counts following a mid-summer period with massive influx of copepodids. No such clear effect was seen, however, in the second site that experienced a much lower copepodid challenge during the same period. Fortunately for the fish farmer but unfortunately to science, none of the enrolled sites experienced incidents of critically low oxygen conditions inside the pens, and the observed differences in environmental water parameters (oxygen saturation and temperature) were low albeit in periods slightly in favour of active aeration. A vertical water circulation pattern inside the semi-closed pen volume was observed and documented in the dye experiment.

Conclusion: In addition to the usage of skirts, other site-specific factors could explain the difference observed in one of the sites during a period of massive copepodic challenge. The study thus failed to provide clear evidence for a shielding effect of skirts with respect to louse larval attachment. With the minor environmental improvements observed, the cost-efficiency of using tarpaulin skirts with the aeration device for salmon louse control is being questioned.

Keywords: skirts, sea lice control, water environment

Funding: This study was financially supported by the Norwegian Seafood Industry's Research Fund (FHF).

219-O

Impacts of different benzolyphenylureas across various life stages of *L. salmonis*

Fast, Mark (Canada)[1]; Michaud, Dylan (Canada)[2]; Cai, Wenlong (Canada)[1]; Poley, Jordan (Canada)[3]; Macdonald, Alicia (Canada)[4]; Belliveau, Patrick (Canada)[1]; Hardardottir, Hulda (Canada)[5]; Dalvin, Sussie (Canada)[5]

1 - Atlantic Veterinary College - University of Prince Edward Island; 2 - AVC-UPEI; 3 - Center for Aquaculture Technologies Canada; 4 - Elanco Canada Ltd.; 5 - University of Bergen

Introduction: The development of drug resistance in *Lepeophtheirus salmonis* to chemical classes used in salmonid aquaculture is an important topic for the viability and longevity of the farming industry. In spite of this, the molecular modes of action for commonly applied delousing treatments for salmon lice are largely unelucidated. The benzoylphenylureas (BPUs) are chitin synthesis inhibiting compounds that are highly effective against arthropod pests and in particular the salmon louse, for which resistance has not yet been reported. Recent work with *L. salmonis* copepodids suggests lufenuron has major impacts on genes within the chitin synthesis pathway. The present work includes the first evaluations of the mode of action of lufenuron and other BPUs of interest to salmonid aquaculture, hexaflumuron and teflubenzuron, on attached parasitic life stages of *L. salmonis*.

Methodology: Copepodids were exposed *in vitro* to 50/1500 ppb lufenuron, 1500 ppb hexaflumuron or 1500 ppb teflubenzuron then evaluated for infection success on Atlantic salmon and development success to pre-adult life stages. Lice were also collected at various time points post-exposure for transcriptomic analyses.

Conclusion: Lufenuron and hexaflumeron were highly potent in inhibiting *L. salmonis* from developing into chalimus larvae at the concentrations studied, whereas teflubenzuron was not. Transcriptional profiles of multiple stages of *L. salmonis* were compared across the different compounds examined, and discussed with respect to phenotypic results. This work has major implications on the potential for development of cross-resistance, and may support informed decision making for treatment rotation within salmonid aquaculture in all regions.

Keywords: sea lice, lufenuron, moulting, teflubenzuron, life stages

Funding: NSERC- CRD, Ocean Frontiers Institute and Elanco Animal Health Canada.

220-O

Pharmacokinetic study of in-feed administered Praziquantel in greater amberjack, *Seriola dumerili*

Kogiannou, Dimitra (Greece)[1]; Nikoloudaki, Chrysanthi (Greece)[1]; Vasilaki, Antigoni (Greece)[1]; Pyrenis, Girogios (Greece)[1]; Rigos, George (Greece)[1]

1 - Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Agios Kosmas Hellinikon, 16777 Athens, Greece

Introduction: Praziquantel (PZQ) is an effective chemotherapeutic against helminths of captive fish. The distribution profile of dietary administered PZQ in cultured greater amberjack following a multiple oral dosing as a first step to optimise PZQ dosing regimens for this species was investigated.

Methodology: Medicated diet was prepared after thoroughly blending all the dietary ingredients appropriate amounts of PZQ moistening and cold pelleting with a Hobart food pellet mill. Healthy greater amberjack (84 ± 12 g) were fed the experimental diet daily for 5 consecutive days. The final dose of administered PZQ was 75 mg/kg BW. On the first day of the trial, blood samples were taken from 5 individuals from 2 to 24h after in feed administration of PZQ. On the other intervention days, blood samples were collected after at 24 h of drug administration. An HPLC-UV method was developed for PZQ measurements in plasma samples of individual fish at each time point. Separation was performed on a Luna-C18 column using an isocratic mixture of 35:65 v/v acetonitrile:water as the mobile phase with a constant flow rate of 1mL/min. Eluant was monitored at 210 nm.

Results: The recovery rates of PZQ for plasma samples were 88.5% - 101.6%. Plasma concentrations of PZQ in greater amberjack revealed values around 2.6 μg/mL and 2.3 μg/mL at 2 and 4 h post feeding, respectively. However, maximum plasma concentrations of PZQ were achieved 8 h post feeding (3.0 μg/mL) indicating that the clearance of PZQ did not follow a simple decay model. Withdrawal of PZQ occurred rapidly in greater amberjack as its concentrations diminished 24 h post-treatment in circulation (0.15 - 0.44 μg/mL).

Conclusion: Praziquantel was absorbed and distributed rapidly in greater amberjack. This kinetic profile possibly suggests a PZQ double daily dosing.

Keywords: praziquantel, phamracokinetics, greater amberjack

Funding: The project is co-funded by Greece and the European Union under the Fisheries and Maritime Operational Program 2014-2020 (75% EMFF contribution, 25% National Contribution).

221-O*

The effect of different treatment strategies on *Cardicola* spp. infection in ranched southern bluefin tuna in South Australia

Power, Cecilia (Australia)[1]; Nowak, Barbara (Australia)[1]; Bott, Nathan (Australia)[1]

1 - Centre for Environmental Sustainability and Remediation, RMIT University, Melbourne, Australia

Introduction: The aporocotylid blood flukes, *Cardicola forsteri* and *C. orientalis* are considered the most significant health concern for Southern Bluefin Tuna (SBT) ranched off Port Lincoln, South Australia. In this study we compare the effects of different treatment strategies on blood fluke infections in ranched SBT by sampling from one untreated and two praziquantel treated pontoons progressively throughout the 2018 season.

Methodology: Severity of infection was assessed through a number of different criteria including adult fluke counts from hearts, egg counts from gill filaments and the use of specific quantitative polymerase chain reaction (qPCR) for detection of *C. forsteri* and *C. orientalis* ITS-2 DNA in SBT hearts and gills.

Results: *Cardicola forsteri* was the dominant species detected in this study, and prevalence and intensity of *C. forsteri* infection in SBT was significantly higher in the untreated pontoon than the two treated pontoons from week 8 of ranching. *Cardicola orientalis* in SBT was rarely detected. No significant differences were seen in mortalities or condition of SBT between pontoons, and results suggest that location of treated pontoons may help to reduce certain stages of the *Cardicola* spp. life cycle, thereby reducing severity of blood fluke infections in untreated SBT.

Conclusion: This study provides important insights into the continued efficacy of praziquantel on blood fluke infections in ranched SBT, and highlights the need to monitor blood fluke infections as praziquantel treatment strategies continue to develop.

Keywords: blood fluke, bluefin tuna, Aporocotylidae, Scombridae, praziquantel

Myxozoan Diseases IV

223-O

The STAT3/SOCS3 axis determines the outcome of whirling disease in rainbow trout

Saleh, Mona (Austria)[1]; Srivastava, Mitaly (Austria)[1]; Friedl, Adina (Austria)[1]; Soliman, Hatem (Austria)[1]; Secombes, Christopher J. (United Kingdom)[2]; El-Matbouli, Mansour (Austria)[1]

1 - Clinical Division of Fish Medicine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Austria; 2 - Scottish Fish Immunology Research Centre, School of Biological Sciences, University of Aberdeen, Scotland, UK

Introduction: There are differences in disease susceptibility to whirling disease among strains of rainbow trout. The North American strain Tout Lodge (TL) is highly susceptible, whereas the German Hofer (HO) strain is more resistant. The reasons for the differences in susceptibility and the mechanisms of the varying levels of resistance in these two rainbow trout strains were explored. The kinetics of cytokine and innate immune response genes were investigated in the two strains to explore factors involved in host invasion and immune response mechanisms that underlie the varying levels of resistance to *Myxobolus cerebralis*.

Methods: Replicates of each rainbow trout strain (n = 60) were exposed to 1000 freshly filtered TAMs per fish. An equal number of fish was used as a negative control group. At various time points, five fish from each strain and control were euthanized and sampled. The severity of *M. cerebralis* infection at all time points was evaluated using qPCR. Caudal fin tissues were used for total RNA extraction and real-time PCR was carried out to assess the expression levels of selected genes in control and infected fish.

Results: *M. cerebralis* induced the expression of SOCS1 and IL-6-triggered SOCS3 as well as the anti-inflammatory cytokine IL-10 and the Treg associated transcription factor FOXP3 in TL at multiple time points causing a restricted STAT1 and STAT3 activity, which likely affected Th17/Treg17 balance. Conversely, in HO, the expression of SOCS1 and SOCS3 was reduced; whereas the expression of STAT1 and IL-23-mediated STAT3 was induced potentially enabling more controlled immune responses, accelerating parasite clearance and elevating resistance.

Conclusion: The expression of SOCS1 and IL-6-triggered SOCS3 is induced and perhaps constrains the activation of STAT1 and STAT3 in TL rendering the fish unable to demonstrate a protective immune response against *M. cerebralis*. Reduction of STAT1 and STAT3 activity in TL likely causes Th17/Treg17 imbalance, leaving fish unable to limit parasite burden or control inflammatory reactions promoting susceptibility to WD. On the other hand, in HO, the induced STAT1 and IL-23-mediated STAT3 expression likely promotes successful maintenance of a Th17/Treg17 balance enabling fish to limit parasite numbers and favouring resistance against whirling disease.

Keywords: *Myxobolus cerebralis*, salmonids, susceptibility, resistance

224-O

Hidden diversity of *Paramyxidium* (Myxozoa) species in European eel (*Anguilla anguilla*)

Fiala, Ivan (Czech Republic)[1,2]; Lisnerová, Martina (Czech Republic)[1,2]; Baiko, Dariya (Czech Republic)[2]; Blabolil, Petr (Czech Republic)[3]; Lövy, Alena (Czech Republic)[1]; Holzer, Astrid (Czech Republic)[1]

1 - Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic; 2 - Faculty of Sciences, University of South Bohemia, České Budějovice, Czech Republic; 3 - Institute of Hydrobiology, Biology Centre of the Czech Academy of Sciences, České Budějovice, Czech Republic

Introduction: Myxosporeans form three main phylogenetic lineages, which branch into numerous groups mostly according to site of infection in the fish host. Within the myxosporean phylogeny, however, we recognized one specific clade that contained only sequences from actinospore stages, which did not show any close relationships to known sequences from myxospore fish stages. This actinosporean clade was enlarged by many environmental OTUs what deepened the puzzle of missing myxospore stage sequences in this group. Recently, sequences of species of newly established genus *Paramyxidium* described from European eel clustered within this clade. We aimed to uncover the diversity and host specificity of myxosporeans from the *Paramyxidium* clade and assess the diversity of *Paramyxidium* species in eel, a critically endangered species for which parasites are also one of the serious factors responsible for its population decline.

Methodology: We dissected 14 European eels from five different localities in the Czech Republic. The presence of myxozoan infection was examined by light microscopy and by PCR screening. Specific PCR primers were designed to test the presence of myxosporeans from the *Paramyxidium* clade in various fish species. Phylogenetic relationships of myxozoans were inferred using maximum likelihood and Bayesian inference based on the SSU rDNA.

Results: Microscopic examination revealed 3 eels with kidney infection and 6 eels with gills infected by myxosporeans with *Myxidium*/*Paramyxidium* type of spores. All samples contained myxospores with very similar morphology and dimensions. Phylogenetic analysis of SSU rDNA revealed one *Myxidium* species infecting kidney clustering close to *Myxidium giardi* in the urinary tract clade and 7 species clustering in the clade containing *Paramyxidium* sequences. None of newly obtained sequences were identical with three *Paramyxidium* species already described from eels. Specific PCR detecting myxosporeans from the *Paramyxidium* clade were negative in all more than one hundred individuals of ten freshwater fish species suggesting that this myxosporean clade could be Anguilliformes host specific clade.

Conclusion: We identified seven new species of the genus *Paramyxidium*. Species diversity of *Paramyxidium* is extremely high in European eel and the *Paramyxidium* clade may accommodate large number of myxosporeans infecting Anguilliformes.

Keywords: phylogeny, *Paramyxidium*, *Myxidium*, European eel

Funding: Czech Science Foundation (#29-28399X and #16-20744S).

225-O

Occurrence of *Kudoa* spp. in *Scomber colias* from the Portuguese mainland and Madeira archipelago

Cavaleiro, Bárbara (Portugal)[1]; Nogueira, Sara (Portugal)[2]; Ribeiro, Liliana (Portugal)[2]; Hermida, Margarida (Portugal)[1,3]; Saraiva, Aurélia (Portugal)[2,4]

1 - Marine and Environmental Sciences Centre; 2 - University of Porto; 3 - Oceanic Observatory of Madeira; 4 - Interdisciplinary Centre of Marine and Environmental Research

Introduction: *Kudoa* Meglitsch, 1947 (Myxozoa, Myxosporea) is a widespread myxosporean parasite associated with poor flesh quality of commercially important marine fishes. It is one of the most researched genera and it is found in several fish species from Portugal mainland. Its occurrence in Madeira waters had not previously been investigated.

Methodology: A total of 62 *Scomber colias* Gmelin, 1789, 30 from Portugal mainland and 32 from Madeira archipelago, were observed for the detection of *Kudoa* spores. Three samples of muscle weighing one gram each were collected from the anterior, median and posterior region of each specimen. Samples were macerated with a scalpel and squashed with the basis of the petri dish, and observed under a Differential Interference Contrast (DIC) microscope at 400x magnification. Prevalence was determined and compared between Portugal mainland and Madeira archipelago. Prevalence and abundance were assessed for the 3 different muscle regions and compared using Cochram Q test and Friedman's Variance Analysis.

Results: Spores of *Kudoa* sp. were detected in *S. colias* from Portugal mainland with a prevalence of 60%. In the observed specimens from Madeira, not a single spore was detected. No significant differences were found in either prevalence or abundance between different body regions of infected specimens, although there was a slight decrease in prevalence from the anterior to the posterior region.

Conclusion: Infection by *Kudoa* sp. is extremely common in fishes from the coast of mainland Portugal, and can occur with high prevalence, as detected in *S. colias*. The absence of this parasite in *S. colias* from waters surrounding Madeira archipelago could be explained by its lower occurrence or difficulty in infection acquisition in the region. This could indicate the presence of two distinct Atlantic chub mackerel populations.

Keywords: *Kudoa*, myxosporean, *Scomber colias*, Madeira, Portugal

Funding: B. Cavaleiro was financially supported by ARDITI. M. Hermida was financially supported by a postdoctoral grant from ARDITI, Project M1420-09-5369-FSE-000001. This study was partially supported by FCT through the strategic project UID/MAR/04292/2019 granted to MARE and by the OOM Project (M1420-01-0145-FEDER-000001-Observatório Oceânico da Madeira-OOM).

226-O

Workshop Student Writing: Preparing for an effective poster session: content, style, and interactions

Preamble: Despite the key importance of posters as a means of communication scientific research, particularly at large conferences, many posters fail to communicate in a way that is effective, informative, and inspiring. In this workshop we will share key recommendations for content and style of the poster, and for having an enjoyable and productive dialogue with fellow investigators who visit the posters.

Workshop topics: We will consider:

(1) The poster session as a key mechanism for scientific communication, comprising the poster itself and the interactions between presenter and audience; the importance of preparing for both parts!
(2) Content: key topics to present, how to determine what to include, what to exclude
(3) Style: the reader's perspective (visual and knowledge), layout, text, images (graphic and photographs), colours, fonts
(4) Interactions: how to have an interesting and productive dialogue with your audience
(5) Recommended resources – print and electronic

Workshop format: The workshop will mix didactic elements, individual exercises, and small group work.

Workshop materials: Participants will receive lecture materials, hardcopy worksheets, and checklists for effective poster preparation and interactions.

Organizers: The workshop leaders are Dr. Barbara Nowak and Dr. Sarah Poynton. Dr. Nowak is Associate Editor of the Journal of Fish Diseases, and a member of the editorial boards for a number of other journals. Dr. Poynton is a reviewer for a diversity of aquatic animal health journals, teaches scientific and biomedical writing, and is a freelance editor.

Dr. Nowak and Dr. Poynton have taught the well-attended EAFP student workshops on scientific publishing in Las Palmas in 2015, and on titles, figures and tables in Belfast in 2017.

Workshop duration: Two hours

Vaccinology II

227-O

Field experiences with vaccination against *Yersinia ruckeri* in late sea phase

Hanche-Olsen, Ragnhild (Norway)[1]; S. Lunheim, Ane (Norway)[2]; G. Tingbø, Monica (Norway)[1]; N. Fredriksen, Børge (Norway)[1]; Guttvik, Arne (Norway)[3]

1 - PHARMAQ AS; 2 - ZOETIS; 3 - Salmar

Introduction: There has been a great increase in yersiniosis in Atlantic salmon during late sea phase in Norway the last couple of years. The disease is caused by the bacteria *Yersinia ruckeri* (*Y. ruckeri*) serotype O1b and was earlier associated with fry stage. Since 2015 it has mainly been a problem in big fish (> 1kg), causing high mortalities up to 30 % at cage level. More than 100 million salmon are vaccinated against yersiniosis in Norway each year.

Methodology: The efficacy and safety data have been obtained from field surveys and laboratory studies. The vaccine is water-based and was mainly co-injected with a multivalent emulsion vaccine. Efficacy has been assessed for both the vaccine against yersiniosis, and the co-injected multivalent vaccine, with focus on survival and disease detection as well as antibody responses measured by ELISA. Safety data evaluated were mortality during toxicity period, weight gain and side effects (local adhesions and melanin deposits). Both efficacy and safety data were obtained from the time of vaccination to slaughter.

Results: The data shows high correlation between results from laboratory studies and field surveys. The level of protection against yersiniosis is overall high, also in sites with outbreaks in cages not vaccinated against *Y. ruckeri*. There have been no registered outbreaks on fish groups vaccinated with this vaccine by injection since the start in 2016; nor has it been registered any negative effects from co-injection.

Conclusion: The water-based vaccine provides high level of protection against yersiniosis in Atlantic salmon up to slaughter (18 months post vaccination) and is safe to use in co-injection.

Keywords: vaccination, yersiniosis, Atlantic salmon

228-O

Temporal immune responses induced following primary and booster mucosal vaccination regimes against *Vibrio anguillarum* in rainbow trout (*Oncorhynchus mykiss*)

Monaghan, Sean (United Kingdom)[1]; Shahin, Kahalid (United States of America)[1,2]; Yoon, Sohyn (United Kingdom)[3]; Secombes, Chris (United Kingdom)[3]; Herath, Tharangani (United Kingdom)[4]

1 - Institute of Aquaculture, University of Stirling, Stirling; 2 - School of Veterinary Medicine, University of California, Davis, USA; 3 - Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, UK; 4 - Department of Animal Production, Welfare and Veterinary Sciences, Harper Adams University, Shropshire, UK

Introduction: Mucosal vaccines offer a number of benefits over conventional vaccination strategies, such as avoiding handling stress and the adhesions associated with intraperitoneal vaccination. Although primary vaccination through oral administration is often ineffective in salmonids, booster vaccination through this route appears to enhance protection. The objectives here were therefore to determine whether oral vaccination as a booster could enhance protective immunity systemically compared to immersion booster vaccination.

Methodology: Spleen tissues were obtained from rainbow trout vaccinated with an inactivated *Vibrio anguillarum* vaccine. The vaccination groups included fish receiving (1) an immersion prime vaccination followed by immersion boost and (2) immersion prime vaccination followed by oral boost and (3) a no vaccine control. The fish were challenged by immersion after ~300 degree days post-boost. Samples were collected at multiple time points during the course of the experiment including post-prime and boost vaccination, and post-challenge. RT-qPCR was used to assess relative immune gene expression kinetics of transcripts; IgT, IL-10, TCR, TGF, TNF-α in the spleen samples (n=5/per time points x 5 time points).

Results: The protection induced by immersion-immersion vaccination was significantly greater (75% PRS) compared to the immersion-oral vaccination regime (50% RPS). However, stronger systemic IgT and TCR expression was observed following immersion-oral vaccination compared to the immersion-immersion vaccination regime in the spleen tissue of fish. The other target transcripts (i.e. IL-10, TGF, TNF-α) were not significantly different between vaccination regimes throughout the time course. These results are in contrast to our previous observations that stronger IgT and TCR expression occurs locally in the gill of immersion-immersion vaccinated fish than that of the gut of immersion-oral vaccinated fish.

Conclusion: These findings question whether greater systemic induction of IgT and TCR expression following antigen uptake in the gut post-boost were contributing to the protection observed in these fish. Evaluation of localised tissue IgT expression using immunohistochemistry along with histopathological analysis of tissue damage and cellular responses post-vaccination and post-challenge is currently underway to give more insight into the protective immune response associated with the efficacy of these regimes.

Keywords: mucosal vaccine, oral vaccine, immersion vaccine, rainbow trout

229-O*

ST-261 Adhesins are antigenic but not effective as an injectable vaccine against *Streptococcus agalactiae* in tilapia (*Oreochromis mossambicus*)

Kawasaki, Minami (Australia)[1]; Delamare-Debouteville, Jerome (Australia)[1]; Barnes, Andrew (Australia)[1]

1 - The University of Queensland, School of Biological Sciences and Centre for Marine Science

Introduction: Group B *Streptococcus* (GBS) expresses a polysaccharide capsule (CPS) which is the major protective antigen in vaccines, but there are multiple serotypes that infect fish. Previously, we assembled the pan-genome of GBS and identified that three out of six adhesins found in the ST-261 clonal complex are conserved in all GBS isolates. In this study, we determined culture conditions that regulate ST-261 adhesins expression relative to capsule and the level of fish antibody responses to ST-261 adhesins. Also, ST-261 adhesins' efficacies as vaccines were evaluated in a tilapia challenge model.

Methodology: The genes expressions encoding the ST-261 adhesins and CpsE in different culture conditions were determined by qRT-PCR. Tilapias were injected with recombinant adhesins and whole-cell vaccines to collect serum samples. Western-blotting using the antisera were performed to identify the antigens recognised by fish in whole protein profiles under differing culture conditions. To measure the antibody response, the antisera were used in ELISA as well. The efficacies of recombinant ST-261 adhesins as injectable vaccines were evaluated by challenging tilapia with a GBS ST-261, serotype 1b strain after 4 weeks of vaccinations.

Results: The genes expressions varied inversely dependent upon the culture conditions, suggesting inverse co-regulation. However, the level of adhesins detected in whole cells by ELISA and Western blot did not seem to reflect gene expression. This may be due to post-transcriptional processing causing a disconnect between gene and protein expression. The challenge resulted in high protection in fish vaccinated with homologous challenge strain (serotype 1b) but tilapia vaccinated with 1a whole-cell or recombinant vaccines did not confer protection.

Conclusion: The lack of protection by recombinant vaccines may be due to high expression of CPS during the septicaemic phase of the infection, which is masking other antigens including the adhesins. It may also be that adhesins are not expressed during the critical stages of proliferation in the host. Future work should elucidate the role of these ST-261 adhesins in pathogenicity in fish, including where and when they are expressed on GBS during the infection.

Keywords: *Streptococcus agalactiea*, *Oreochromis mossambicus*, vaccine, adhesins

Funding: Australian Research Council Linkage Project LP130100242.

230-O

Use of a *Flavobacterium columnare* DNAk recombinant protein vaccine to guard against columnaris disease in channel catfish

Lange, Miles (United States of America)[1]; Farmer, Bradley (United States of America)[2]; Abernathy, Jason (United States of America)[2]

1 - United States Department of Agriculture, Agricultural Research Service, Aquatic Animal Health Research Unit, Auburn, AL; 2 - United States Department of Agriculture, Agricultural Research Service, Harry K. Dupree Stuttgart National Aquaculture Research Center, Stuttgart, AR

Introduction: *Flavobacterium columnare* causes substantial losses among cultured finfish species. The Gram-negative bacterium is an opportunistic pathogen that manifests as biofilms on the host's mucosal surfaces as the disease progresses. We previously established that the dominant mucosal IgM antibody response to *F. columnare* is to the DNAk protein found in the extracellular fraction.

Methodology: To establish the efficacy of using recombinant protein technology to develop a new vaccine against columnaris disease, we are reporting on two consecutive years of vaccine trials using a recombinant *F. columnare* DNAk protein (rDNAk). In year one, three groups of channel catfish (n=300) were immunized by bath immersion with a live attenuated *F. columnare* isolate, rDNAk or sham immunized. In the second year, three groups of channel catfish (n=300) were bath immunized with rDNAk alone or with rDNAk after a brief osmotic shock or sham immunized.

Results: After six weeks, an *F. columnare* laboratory challenge showed a significant increase in survival (>30%) in both the live attenuated and rDNAk vaccines when compared to the non-immunized control. A DNAk-specific ELISA revealed significant levels of mucosal IgM antibodies present in the skin of catfish immunized with rDNAk at four- and six-weeks post immunization. Year two, after six weeks a laboratory challenge with *F. columnare* was conducted and showed a significant increase in survival in the rDNAk (>25%) and in rDNAk with osmotic shock (>35%) when compared to the non-immunized control. The DNAk ELISA demonstrated significant levels of mucosal IgM antibodies in the skin of catfish groups immunized with rDNAk at six weeks post immunization. To further understand the processes which have conferred immune protection in the rDNAk group, we conducted RNA sequencing of skin explants from the non-immunized (n = 6) and DNAk treated channel catfish at one-week (n = 6) and six weeks (n = 6) post immunization. Significantly different gene expression was identified between the non-immunized and immunized skin and gene ontology analyses will be discussed.

Conclusion: Work to enhance the catfish immune response to *F. columnare* rDNAk continues; as this protein remains a promising candidate for experimental trials in a production setting.

Keywords: recombinant protein, bath immunization, antibody response, RNA sequencing

Funding: USDA Research Project # 6028-32000-007-00D.

231-O

Protection of Atlantic salmon immunized with a novel DNA vaccine against PD and infected with SAV3

Thorarinsson, Ragnar (Norway)[1]; Phillips, Lisa (Canada)[1]; Macdonald, Alicia M. (Canada)[1]; Rodriguez, Jose F. (Canada)[1]; Inami, Makoto (Norway)[2]; Sindre, Hilde (Norway)[3]; Skjerve, Eystein (Norway)[4]

1 - Elanco Animal Health; 2 - VESO Vikan; 3 - Norwegian Veterinary Institute; 4 - Norwegian University of Life Sciences

Introduction: A DNA vaccine against pancreas disease (PD) caused by salmonid alphavirus subtype 3 (SAV3) was licensed by the EU Commission in 2017. Comparative efficacy data from SAV3 cohabitation challenge coupled with viremia, plasma neutralization titers and indication of viral shedding will be presented.

Methodology: Healthy salmon parr were immunized with the DNA vaccine (Vaccine A), an oil-emulsified PD vaccine (Vaccine B) or with saline as negative controls (Saline). After an immunization period of ~1040 degree days at ~12 °C, the fish were infected with SAV3 in seawater using a cohabitation challenge model. The fish groups were sampled and evaluated just before challenge, and/or at 19, 54 and 83/84 days post-challenge (DPC). SAV3 neutralization end-titers were measured in plasma from non-challenged fish. Efficacy parameters included survival and weight gain post-challenge. Viremia was measured in the fish sampled 19 DPC. The rate of SAV3 transmission from immunized challenged fish to naïve was ascertained using real-time qPCR.

Results: Neutralization by immune plasma of SAV3 *in vitro* revealed higher end-titers in fish vaccinated with Vaccine A compared to the other groups. Challenge mortality was expectedly low, mimicking the chronic nature of the disease. Fish immunized with Vaccine A demonstrated improved survival compared to Saline but not Vaccine B. Weight gain post-challenge was greater in fish administered Vaccine A compared to the other groups. Viremia levels were lower in fish administered Vaccine A compared to the other groups. The transmission rate of SAV3 was slower in the tank of naïve fish cohabitated with fish immunized with Vaccine A.

Conclusion: The fish immunized with Vaccine A performed significantly better than the fish immunized with Vaccine B in regards to: SAV3 neutralization capacity; reduction in SAV3 viremia levels 19 DPC; weight gain post-challenge until termination at 83/84 DPC; and the rate of SAV3 transmission to naïve fish. These results are in accordance with histopathological findings also ascertained in this study (included in a companion presentation).

Keywords: pancreas disease, salmonid alphavirus, DNA vaccine

Funding: Elanco Animal Health.

232-O

Histopathological changes as efficacy indicators in Atlantic salmon immunized with a novel DNA vaccine against PD and infected with SAV3

Wolf, Jeffrey C. (United States of America)[1]; Thorarinsson, Ragnar (Norway)[2]; Phillips, Lisa (Canada)[2]; Macdonald, Alicia M. (Canada)[2]; Rodriguez, Jose F. (Canada)[2]; Inami, Makoto (Norway)[3]; Skjerve, Eystein (Norway)[4]

1 - Experimental Pathology Laboratories Inc.; 2 - Elanco Animal Health; 3 - VESO Vikan; 4 - University of Life Sciences

Introduction: A DNA vaccine against pancreas disease (PD) caused by salmonid alphavirus subtype 3 (SAV3) was licensed for Atlantic salmon by the EU Commission in 2017. The efficacy of this DNA vaccine was assessed against another commercially available vaccine and a saline control by comparing histopathological alterations following SAV3 challenge.

Methodology: Healthy salmon parr were immunized with the DNA vaccine (Vaccine A), an oil-emulsified PD vaccine (Vaccine B) or injected with saline as negative controls (Saline). After an immunization period of ~1040 degree days at ~12 °C, the fish were infected with SAV3 in seawater using a cohabitation challenge model. Heart, exocrine pancreas, and red and white muscle samples were collected at 19, 54, and 83 days post-challenge (DPC), blind-coded, and processed routinely to histologic slides. The presence and severity of various histopathological alterations were scored for each tissue type according to pre-defined morphologic criteria.

Results: The prevalence and/or severity of SAV-induced morphologic changes in heart and pancreas up to 54 DPC (interim results) were reduced in fish administered Vaccine A compared to Vaccine B and Saline. The characteristics and time-course of SAV3-induced histopathologic interim results were consistent with other published studies. Skeletal muscle and final time point (83 DPC) analyses are ongoing and will be presented.

Conclusion: Results from all sampled tissues and time points will be presented and discussed.

Keywords: pancreas disease, salmonid alphavirus, DNA vaccine

Funding: Elanco Animal Health.

233-O

DNA vaccination against VHS – does it work in all fish species?

Sepulveda, Dagoberto (Denmark)[1]; Vendramin, Niccolo (Denmark)[1]; Olesen, Niels Jørgen (Denmark)[1]; Lorenzen, Niels (Denmark)[1]

1 - Technical University of Denmark

Introduction: DNA vaccination against rhabdoviruses is traditionally based on plasmids encoding the viral glycoprotein G under the control of a strong CMV promoter. While DNA vaccination against novi-rhabdoviruses like viral haemorrhagic septicaemia virus has been demonstrated to be highly efficient in rainbow trout as well as in turbot, reports from trials in muskellunge and Pacific herring suggest a somewhat lower protective effect in those species. In a recent study we aimed at vaccination of lumpfish (*Cyclopterus lumpus*) against VHS. Disease prophylaxis in Lumpfish has received increasing interest recently due to its expanded use as cleaner fish in the Atlantic salmon farming industry. In 2015, an outbreak of VHS genotype IV among farmed lumpfish was observed in Iceland and the virus was subsequently demonstrated to be pathogenic to lumpfish under experimental conditions.

Methodology: Previously developed DNA vaccine constructs encoding the G genes of a rainbow trout virulent VHSV isolate genotype I and a rainbow trout virulent IHNV isolate respectively, were compared with new a plasmid construct encoding the G protein of the Lumpfish VHSV isolate. Lumpfish of 5-10 g and kept at 10-12 °C were given an intramuscular injection of 10 µg plasmid DNA. Approx 2 months later, the fish were challenged by ip injection of 10^6 TCID-50 of lumpfish VHSV grown on BF2 cells. Development of disease/mortality was monitored daily

Results and Conclusion: A delay in the onset of mortality was observed in the fish given with the vaccine carrying the G-gene homologous to the challenge virus but in general, DNA vaccination by im injection of plasmid constructs encoding homologous and heterologous variants of the viral glycoprotein gene G failed to induce protective immunity to VHS in lumpfish. Examination of the vaccine induced immune response is in progress, aiming at determining whether lack of induction of innate or adaptive mechanisms may explain the lack of establishment of protective immunity.

Keywords: plasmid DNA, vaccine, rhabdovirus glycoprotein, lumpfish, protective immunity

Funding: EU, Horizon 2020, ParaFishControl (project reference 634429).

234-O

Evaluation of gilthead sea bream (*Sparus aurata*) immune response after LCDV DNA vaccination

Leiva-Rebollo, Rocio (Spain)[1]; Castro, Dolores (Spain)[1]; Borrego, Juan J. (Spain)[1]; Labella, Alejandro (Spain)[1]

1 - Universidad de Málaga, Málaga, Spain

Introduction: A DNA vaccine against Lymphocystis Disease Virus (LCDV) was developed and its protective efficacy in gilthead sea bream (*Sparus aurata*) has been established. The aim of the present study is the evaluation of immune-related gene expression after vaccination to identify which genes could be relevant to control the viral infection.

Methodology: A DNA-vaccine based on the major capsid protein (MCP) of LCDV using pcDNA3.1-NT-GFP-TOPO system was used to carry out the experiment. The vaccine was administered intramuscularly to gilthead sea bream specimens (100 g weight) at 10 µg/fish dose. In addition, two control groups, injected with empty TOPO plasmid at the same dose or PBS, were established to evaluate non-specific immune response and basal response of fish, respectively. In this study 23 genes related to the immune response (*tlr5*, *tlr9*, *ifnI*, *irf1*, *irf3*, *irf9*, *pkr*, *mx1*, *mx2*, *mx3*, *isg15*, *tnfα*, *casp1*, *il1β*, *il6*, *il10*, *ck3*, *ck10*, *c3*, *nccrp1*, *mhcII*, *tcrβ*, and *ighm*) and 2 reference genes (*ef1α* and *actβ*) were analysed using real-time PCR (RT-qPCR) in samples of head kidney and intestine at 1, 3, and 8 d post-vaccination.

Results: DNA-vaccination of gilthead sea bream induced the differential expression of 9 genes in head kidney and 15 genes in intestine samples. Through the course of the experiment, 9 of those genes reached high level of up-regulation comparing to control groups. These genes were related to IFN type I pathway(*irf9* and *mx3*, in head kidney), inflammation (*il1β*, *il6*, *tnfα*, *ck10*, *c3* and *nccrp-1*, in both organs analysed), and adaptive immune response (*mhcII*, in intestine).

Conclusion: The results obtained allow us to understand which genes could be responsible for the protection against LCDV infection conferred by the DNA vaccine in gilthead sea bream. Inflammation is the biological process mainly triggered as a systemic response in vaccinated fish. Different gene expression profiles have been observed in each organ, which may indicate specialized roles relative to immune defensive mechanisms.

Keywords: lymphocystis disease virus, immune response, DNA vaccination, gilthead sea bream

Funding: P12-RNM-2261 project (Junta de Andalucía).

235-O

Development and application of vaccine to prevent tilapia lake virus in tilapia

Surachetpong, Win (Thailand)[1,2]; Tattiyapong, Puntanat (Thailand)[1]

1 - Department of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Thailand; 2 - Center for Advanced Studies for Agriculture and Food, Institute for Advanced Studies, Kasetsart University, Bangkok, Thailand

Introduction: Tilapia lake virus disease (TiLVD) is an emerging viral disease associated with high mortalities in tilapia. Since the first reported in 2014, tilapia lake virus (TiLV) was detected in diseased fish in 14 countries. Thus, there is an urgent need to develop an effective vaccine to prevent the disease and reduce the economic impact of this virus.

Methodology: TiLV strain VETKU-TV01 was grown in E-11 cells, harvested, and processed for an inactivation with 0.1% formaldehyde for 24 h. A suspension of 100 µL of vaccine mixed with Freund's complete adjuvant at a ratio of 1:1 was injected intraperitoneally into 60 red hybrid tilapia (*Oreochromis* spp.). At 28 days after vaccination, fish were challenged with a virulent TiLV using cohabitation challenge method. The cumulative mortality rate, and antibody titer were examined to demonstrate the efficacy of vaccine.

Results: In the vaccinated group, higher survival rate at 60-70% and increased antibody titer at 4 times higher than the non-vaccinated group was clearly observed. Upon re-exposure to TiLV, there was no mortality of fish either by vaccination or experimental challenge, suggesting that fish do develop a complete immunity against this virus.

Conclusion: Our results demonstrated that a potential vaccine can provide protective immunity to prevent TiLV infection. Moreover, fish do produce antibodies that control TiLV infection after exposure or in response to vaccination. Vaccine can be applied as a tool to control the spread of TiLV and reduce the economic impact of this emerging viral disease.

Keywords: Tilapia Lake Virus; TiLV; tilapia; emerging diseases; vaccine

236-O

Category E – vaccination against koi herpesvirus disease (KHVD) as a prophylactic measurement

Sven M. Bergmann (Germany)[1]; Klafack, Sandro (Germany)[1]; Schröder, Lars (Germany)[1]; Jin, Yeonwha (Germany)[1]; Hofmann, Arndt Christian (Germany)[1]; Fuchs, Walter (Germany)[1]; Avarre, Jean-Christophe (France)[2,3]; Kielpinska, Jolanta (Poland)[4]; Wang, Qing (China)[5]; Zeng, Weiwei (China)[6]; Wang, Yingying (China)[7]; Li, Yingying (China)[6]; Lusia´Stuti, Angela Maria (Indonesia)[8]

1 - Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Insel Riems, 17493 Greifswald-Insel Riems, Germany; 2 - The Institute of Evolutionary Sciences of Montpellier (ISE-M); 3 - Université de Montpellier, campus Triolet Bâtiments 21, 22 et 24; 4 - West Pomeranian University of Technology, Szczecin, Poland; 5 - Pearl-River -Fisheries Research Institute, Guangzhou, Guangdong province; 6 - PRFRI, Guangzhou, Guangdong province; 7 - PRFRI; 8 - Research Institute for Freshwater Aquaculture, Bogor, Indonesia

Introduction: Over the last 20 years, a virus threatens the koi and common carp industry. The virus known as koi herpesvirus (KHV) or *Cyprinid herpesvirus* 3 (CyHV-3) is taxonomically grouped into the *Alloherpesviridae* family under the genus *Cyprinivirus*. KHV can induce 100% morbidity and mortality depending on the virulence of the virus, the infecting dosage, the water temperature, the season as well as the genetic background of the hosts. While KHV can infect obviously at any water temperature between 4 to 29 °C, KHVD is only observed between 16 and 28 °C. However, since KHV has been detected in other fish, e.g. goldfish (*Carassius auratus*), grass carp (*Ctenopharyngodon idella*), sturgeon (*Acipenser* sp.) or rainbow trout (*Oncorhynchus mykiss*), it is likely that virus is transferred to susceptible hosts. Inside the EU, it is recently not allowed to vaccinate against KHVD. Exceptions are endemic infected areas only. This will be changed with the new Animal Health Law (AHL) when KHVD is grouped into the category E, notification only.

Methodology: One possibility is the prophylactical vaccination against KHVD. Beside the classical method of the delivery of inactivated KHV isolates, further investigations were carried out to find naturally and genetically engineered avirulent viruses. A user-friendly oral application were identified. KHV-T were replicated onto common carp brain cells (CCB) over 100 passages at 20 °C had shown to be avirulent for carp and koi from passages over 70. KHV-T was also used to delete viral genes (e.g. ORFs 25, 123, 148, 149) to obtain an avirulent vaccine virus. Different genes of KHV, single or in combinations, were deleted by homologous and the resulting viruses were tested in animal experiments after immersion for vaccination and challenge with different wild type KHV. The isolates and variants were characterized by NGS.

Results: After immunization with naturally and genetically modified viruses, no mortality and hardly morbidity were found. Thirty-five post immunization (immersion and orally) fish were challenged with a wild-type KHV and a 100% survival rate without any clinical symptoms was induced. All immunized fish developed antibodies against KHV that correlated with the protection.

Conclusion: Vaccination is a goal and a possibility.

Keywords: KHVD, vaccination, AHL

237-O*

Combating KHVD with a live attenuated vaccine by oral application and/ or immersion

Klafack, Sandro (Germany)[1]; Schröder, Lars (Germany)[1]; Jin, Yeonhwa (Germany)[1]; Hofmann, Arndt-Christian (Germany)[1]; Fuchs, Walter (Germany)[1]; Avarre, Jean-Christophe (France)[2]; Bergmann, Sven M. (Germany)[1]

1 - Friedrich-Loeffler-Institut; 2 - Institute of Evolutionary Sciences, Montpellier

Introduction: Since the late 1990th, KHV is a major threat for carp and koi industry worldwide. Because of its high fatalities and live-long persistence, eradication of the agent is hard but necessary. Unfortunately, there were many vaccines tested but none of them was approved in the EU. The only commercialized vaccine, by the Israeli company KoVax, was removed from U.S. market after one year. Hence, it is necessary to introduce a new, safe and reliable vaccine.

Methodology: Because of its high titers, attenuation experiments were based on KHV from Taiwan (KHV-T). The virus was passaged serially onto CCB cells at 20 °C for a long time (100 passages). Several viral passages were tested in vivo in carp model for attenuation. Fish were infected/immunized by emersion. In this process one of the attenuated passages could be detected as probable vaccine virus. Additional experiments were performed to improve the vaccine administration by oral delivery via alginate capsules and to improve immunity by boost vaccination. Furthermore ORF150 was deleted in a wild type background and tested in carp model.

Results: The vaccine leads to a 100% survival rate after wild-type virus challenge. It did not induce clinical signs and antibody response was quantifiable. Whole genome sequencing revealed some minor differences compared to wild-type virus. Surprisingly, a ~1400 bp deletion was found in ORF150. Database analysis revealed a putative Ubiquitin E3 Ligase, which might influence KHVs virulence. Decreased virulence was visible as well as no mortality after wild-type challenge in ORF150 deletion.

Conclusion: This vaccine is applicable either orally, by immersion or in a combination. Open reading frame 150 seems to be an important factor for virulence. Moreover, a PCR based on ORF150 can be used for differentiation of infected from vaccinated animals (DIVA).

Keywords: KHV, oral, vaccine, DIVA

Environmental and Toxicological Diseases

239-O

Microcystins effects on chinook and Atlantic salmon: investigating the etiology of net pen liver disease and sub-lethal effects

Shartau, Ryan (Canada)[1]; Snyman, Heindrich (Canada)[2]; Turcotte, Lenora (Canada)[1]; Mccarron, Pearse (Canada)[3]; Bradshaw, Julia (Canada)[1]; Johnson, Stewart (Canada)[1]

1 - Pacific Biological Station, Fisheries and Oceans, Nanaimo, Canada; 2 - Animal Health Laboratory, University of Guelph, Kemptville, Canada; 3 - National Research Council, Halifax, Canada

Introduction: Harmful Algal Blooms (HABS) and algal toxins are common problems in marine finfish aquaculture often causing large production losses due to reduced growth and/or disease. On the West Coast of North America (WCNA) there is good histological evidence that wild Pacific salmon are commonly exposed to algal toxins during marine residency. In addition to causing death, exposure to algal toxins can cause a variety of sub-lethal effects. In non-salmonids these include: reduced growth rates, osmoregulatory effects, modified behavior, reduced immune and cardiac functions, endocrine disruption leading to a reduction in the ability to tolerate other stressors, and reduced reproductive success. Very little is presently known about sub-lethal effects on salmonids. Microcystins (MCs) are cyanobacterial toxins, found in fresh and coastal waters of the WCNA, which are believed to cause toxicopathic liver disease Net Pen Liver Disease (NLD). We are currently examining the role of MCs in disease development, as well as determining their sub-lethal effects on Chinook and Atlantic Salmon.

Methodology: Post-smolt Atlantic and Chinook Salmon received a single oral exposure to either toxic *Microcystis aeruginosa* (1700, 2200 or 3200 µg MCs/kg BW), non-toxic *M. aeruginosa* or saline. Fish were sampled at 6, 12, 24, 72 h and 2 weeks post-gavage, and serum, liver, kidney, brain, and muscle were collected for histology, gene expression, and toxin analysis.

Results: A single oral exposure at these doses did not cause morbidity in either species. Preliminary histopathology indicates hepatic structural changes beginning at 72 h in both species. However, changes seen at 72 h and 2 weeks did not include the presence of hepatic megalocytosis which is a characteristic sign of NLD. We are presently examining differences between species in the nature and severity of lesions ,as well as comparing them to lesions found in wild and farmed salmon. Effects on the expression of genes associated with liver structure and function, MCs detoxification, immunity and inflammatory processes are also being examined.

Conclusion: Understanding the sub-lethal effects of MCs and other algal toxins, at the individual and population level, will inform our understanding of environmental stressors and their cumulative effects on salmon.

Keywords: toxic algae, salmon, disease, sub-lethal effects, microcystin

240-O

Emergence of a harmful algal species in Chesapeake Bay USA: aquatic animal health impacts and mechanisms of toxicity

Vogelbein, Wolfgang (United States of America)[1]; Brill, Richard (United States of America)[2]; Small, Hamish (United States of America)[1]; Hobbs, Patrice (United States of America)[1]; Jones, William (United States of America)[1]; Harris, Thomas (United States of America)[1]; Harris, Conny (United States of America)[1]; Svendsen, Morten (United States of America)[3]; Reece, Kimberly (United States of America)[1]

1 - Virginia Institute of Marine Science, College of William and Mary, Gloucester Point, VA 23062; 2 - National Marine Fisheries Service, National Oceanic and Atmospheric Administration, Atlantic Highlands, NJ 07732 USA; 3 - Marine Biological Laboratory, University of Copenhagen, Strandpromenaden 5, DK-3000, Helsingør, Denmark

Introduction: *Alexandrium monilatum* (AMON) is a toxigenic dinoflagellate that has bloomed along the US southern Atlantic coast and within the Gulf of Mexico, where it has been implicated in massive finfish and shellfish kills. In summer 2007, AMON bloomed in the York River, VA. total mortality occurred in *rapana* whelk (2007) and cownose ray (2008) flow-through systems receiving York River water. Since 2007, blooms have expanded throughout the southern Chesapeake Bay, where they were recently implicated in cultured larval and adult oyster (*Crassostrea virginica*) mortality. The primary toxin of AMON is a polyketide called goniodomin A (GDA). Toxicity in eukaryotes has been attributed to its inhibitory interactions with actin, a protein with multiple critical roles in cell physiology.

Methodology: To clarify animal health risks of GDA, we conducted 96 hr toxicity bioassays with larval and adult shellfish and finfish.

Results: Assay organisms exhibited rapid, dose-dependent mortality following exposure to both, live AMON cultures and fractions thereof, or purified GDA. Post-exposure histopathology and electron microscopy of larval finfish (*Cyprinodon variegatus*) indicated extensive pathology in only surficial tissues (gill, skin). Gill epithelia and epidermal cells exhibited rapid swelling, extensive sloughing and lysis. Given these new findings, functional pathophysiological investigations are underway. To quantify if or how GDA impacts actin-myosin function, we will measure net force contraction *in vitro*, using isolated finfish cardiac muscle strips exposed to a range of GDA concentration. To investigate impacts of GDA on cytoskeletal function, we are using a finfish macrophage cell line to quantify aspects of phagocytosis critically dependent on proper actin-myosin function and therefore, presumed susceptible to interference by GDA. Confocal laser scanning microscopy will be used to quantify macrophage motility and phagocytosis of fluorescent latex micro-beads over a gradient of GDA concentration. Finally, hemolysis assays are underway to investigate potential membrane disruption by GDA.

Conclusion: The observed pathology is the likely cause of mortality and is highly suggestive of epithelial cell membrane damage as the underlying mechanism. This suggests that either GDA has other physiological impacts beside interference with actin function, or that other bio-active compound(s) may be produced.

Keywords: HAB, *Alexandrium*, bioassay, mortality, pathology

Funding: NOAA-ECOHAB NA17NOS4780182.

241-O

Use of experimental viral infections to assess the immunotoxicity of a glyphosate chronic exposure on rainbow trout

Le Du, Jessy (France)[1]; Saliou, Florian (France)[1]; Cabon, Joelle (France)[1]; Louboutn, Lenaig (France)[1]; Dory, Daniel (France)[2]; Danion, Morgane (France)[1]; Morrin, Thierry (France)[1]

1 - French Agency for Food, Environmental and Occupational Health & Safety, Ploufragan-Plouzané-Niort Laboratory, Unit Viral Diseases of Fish, National Reference Laboratory for listed fish diseases, Bretagne Loire University, Plouzané, France; 2 - Anses, Ploufragan-Plouzané-Niort Laboratory, Unit Viral Genetic and Biosecurity, Ploufragan, France

Introduction: Glyphosate is one of the most common active substance (AS) found in herbicide products used to increase agriculture productivity. This AS is consequently highly prevalent in continental waters and oceans worldwide. Opinions published by the European Food Safety Authority suggest a limited impact of glyphosate on aquatic organisms. However, recent studies reported deleterious effects after a glyphosate or Glyphosate Based Herbicide (GBH) exposure and raise the issue of the toxicity of adjuvants formulated in these GBH.

Methodology: In this context, our objective was to assess the potential impact on the immune response to a viral infection in rainbow trout (RT), *Oncorhynchus mykiss*, previously exposed by direct or transgenerational route to simple (AS alone) or complex (GBH) sources of glyphosate. Perturbations induced on the reproductive capacities of genitors but also on the development of juveniles originated from contaminated parents were also analyzed.

Results: In 2018, 48 genitors were daily exposed to a dose of glyphosate representative of environmental concentrations (around 1 µg/l) using the AS alone or two GBH. After 8 months of contamination, genitors produced an offspring (F1-2018) exposed from embryonic stage and during two months with the same molecules then submitted to an experimental infection. Two viruses were used: infectious hematopoietic necrosis virus (IHNV) and sleeping disease alphavirus (SDV). Ten conditions were tested to integrate all combinations of herbicide and virus. Mortalities were recorded daily and organs were collected from dead fish for virological examination. Specific and non-specific immune parameters were also analyzed 96 hours and 6 weeks after infection. Preliminary results indicate a higher sensibility of chemically contaminated fish to viral infections, with variations depending of the virus species considered.

Conclusion: Our complex experimental design allowed comparing the impact of AS and GBH combining transgenerational and direct chemical exposure with experimental infections. Coupled with studies on other mechanisms like reproductive capacities or juvenile behavior, our results will generate a complete impact analysis usable to reassess risks associated to GBH.

Keywords: glyphosate, rainbow trout, direct and transgenerational exposure, viral infections, immune response

Funding: Région Bretagne, Département des Cotes d'Armor, Saint Brieuc Armor Agglomération.

242-O

Tolerance of juvenile Atlantic salmon (*Salmo salar* L.) to dissolved gas supersaturation in freshwater

Isaksen, Trond Einar (Norway)[1]; Bye-Ingebrigtsen, Einar (Norway)[1]; Pulg, Ulrich (Norway)[1]; Stranzl, Sebastian (Norway)[1]; Velle, Gaute (Norway)[1]; Andersen, Linda (Norway)[2]; Handegård, Reidar (Norway)[2]; Hjertenes, Yngve (Norway)[3]

1 - NORCE Norwegian Research Centre AS; 2 - The Industrial and Aquatic Laboratory; 3 - The Industrial and Aquatic Laboratory (former employee)

Introduction: Gas bubble disease (GBD) among aquatic animals is a major concern in river systems with high levels of dissolved gas supersaturated (DGS) caused by hydroelectric plants. Etiology and pathogenesis of the disease is well known from studies of pacific salmonids (*Oncorhynchus* spp.), but the effect of supersaturation on juvenile Atlantic salmonids (*Salmo* spp.) is poorly documented and has often been overlooked. Hence, an experimental trial was carried out to examine the tolerance of Atlantic salmon parr (*Salmo salar* L.) to different levels of dissolved gas supersaturation in freshwater.

Methodology: Experimental trial was set-up using Atlantic salmon parr in shallow tanks (30 cm) assigned to different DGS levels ranging from 100% to 130% total dissolved gas (TDG). Supersaturated water was generated by mixing atmospheric air with cooled water combined with increasing pressure inside a cone-shaped column. Fish were sampled and examined (diagnostic; gross pathology) within a period of two weeks of exposure.

Results: Minor gas bubble formation (emphysema) in fins occurred among some fish in groups exposed to supersaturation ≤ 105% TDG. Among fish exposed to 110% TDG there was no significant mortality (n = 1), but an increasing tendency of subcutaneous emphysema, hemorrhages and exophthalmia during days of exposure. Acute GBD and lethal levels occured among fish exposed to TDG levels above 110%. Fish became moribund after 24 hours exposure time in 115% TDG, after 3 hours in 120% TDG and within less than one hour in 130% TDG. Clinical signs of acute GBD included severe emphysema in fins, gas emboli in gills and hemorrhages.

Conclusion: Gas supersaturation ≥ 115% TDG was highly lethal and caused acute GBD within hours among Atlantic salmon parr. Although gas bubble formations was observed among fish exposed to low TDG levels at shallow depth, the present study suggests that depth compensated level of ≤ 105 % TDG might be sufficient to avoid severe GBD among juvenile Atlantic salmon if the exposure time is less than two weeks. Compared to similar trials, our observations indicate that juvenile Atlantic salmon may be more sensitive to gas supersaturation than juvenile Pacific salmon species.

Keywords: gas bubble disease, diagnosis, etiology, pathogenesis

Funding: Norwegian Environment Agency (M-1123/2018).

243-O*

Identification of critical factors for escalating saprolegniosis

Saraiva, Marcia (United Kingdom)[1]; Shreves, Kypher (United Kingdom)[1]; Van West, Pieter (United Kingdom)[1]

1 - University of Aberdeen

Introduction: Diseases are a huge threat for the aquaculture industry and for global food security. Currently, the most important disease-causing organisms in aquaculture and in natural freshwater ecosystems are *Saprolegnia parasitica*, *Saprolegnia diclina* and other close related oomycetes. Collectively these oomycetes are responsible for at least 10% mortalities in salmon hatcheries and freshwater sites affecting both fish and eggs. At present these oomycete pathogens rank amongst the top 3 most relevant pathogens for the rainbow trout and Atlantic salmon aquaculture industry in the UK.

Methodology: A holistic big data investigation is being carried out which involves measuring and analysing potential risk parameters across all main Scottish fish farm companies. In every fish farm there are ultimately three essential contributing elements present that determine health or disease of the fish. These are 1) the fish, 2) the pathogen and 3) the environment. Each element brings several parameters or risk factors that can, in most cases, be individually measured, which is what we are doing over a period of 3 years. The parameters are for fish: genetic and historical background, developmental stage, size of fish, anti-microbial gene expression, immune status, mucus viscosity and quality, vaccination status, treatment after vaccination, stock densities, feed and feeding practices and handling. For the pathogen: presence / absence of pathogen, spore load, species/strain, effectivity of formalin treatment, salt tolerance. For the environment: temperature, oxygen levels, CO_2 levels, toxic metals, organic content in water, salt concentration, pH of water, flow rate of water and microbial community.

Results: The project is still ongoing and at present we have not identified the main risk factor(s) for outbreaks. However, at present we have more than 300 pure oomycete isolates obtained from fish farms and hatcheries. Here we give an overview of the identified oomycetes that cause disease in the farms which are surprisingly many more than *S. parasitica* alone. Here we present our latest findings.

Keywords: *Saprolegnia*, Atlantic salmon, risk factors, holistic

Funding: BBSRC-Link grant - RiFE-SOS.

244-O

Trans-national study to define Epidemiological Cut-Off values (ECOFF) for *Vibrio* and other aquatic bacteria

Antimicrobial resistance (AMR) is a major issue for both human and animal health, and tackling it requires a *One Health* approach. Aquaculture is rapidly growing internationally. Antimicrobials are widely used in aquaculture for therapeutic or prophylactic purposes and this usage is often not strictly regulated. There is a growing concern over good practices and other measures to support the prudent use of antimicrobials throughout the food chain. Knowledge of AMR in aquaculture is poor compared to other animal species. AMR in aquaculture may develop in fish and shellfish bacteria as a result of antimicrobial therapy or by contamination of the aquatic environment by human or animal waste containing antimicrobials and antibiotic resistant bacteria and AMR genes.

Vibriosis caused by various *Vibrio* species has a major worldwide economic impact in fresh-water, brackish and marine fish and shellfish culture. *Vibrio anguillarum*, *Vibrio vulnificus* and *Vibrio parahaemolyticus* are all examples of as severe aquaculture pathogens. Data on AMR in *Vibrio* spp. from aquaculture are scarce and those are available are often not produced using internationally recocognised antimicrobial sensitivity testing guidelines and associated inter-pretative criteria. In particular there are, to date, no established epidemiological cut-off values (ECOFF or ECV) for any of these species. We have established a network of laboratories to help develop epidemiological cut off values using both disc diffussion and broth microdilu-tion-based methods for *V. anguillarum*, *V. parahaemolyticus* and *V. vulnificus* spp. The aim of this roundtable is to present results to date and to discuss what further work needs to be done to enable the generatation of more data on a larger number of Vibrio and/or other bacterial species.

The roundtable will be opened by a presentation from Peter Smith on the theorical aspects of epidemiological cut off value determination.

POSTERS

Climate Changes, Ocean Acidification and Diseases

001-P

Increasing water temperature and disease risks in aquatic systems: does climate change increase the risk of diseases?

Karvonen, Anssi (Finland)[1]; Jokela, Jukka (Switzerland)[2]; Rintamäki, Päivi (Finland)[3]

1 - Department of Biological and Environmental Science, University of Jyväskylä; 2 - EAWAG, Department of Aquatic Ecology, and ETH-Zürich, Institute of Integrative Biology (IBZ), Dübendorf; 3 - Department of Ecology and Genetics, University of Oulu

Introduction: Global warming may impose severe risks for aquatic animal health if increasing water temperature leads to increase in the incidence of parasitic diseases. Essentially, this could take place through a temperature-driven effect on the epidemiology of the disease. In our earlier work we presented one of the first long-term (1986-2006) multi-pathogen data sets on the occurrence of pathogenic bacterial and parasite infections in farmed salmonids in relation to increasing temperatures. We showed that the prevalence (i.e. proportion of fish tanks infected each year) increased with temperature. This pattern was observed in some of the diseases (*Ichthyophthirius multifiliis*, *Flavobacterium columnare*), whereas in the other diseases, the pattern was the opposite (*Ichthyobodo necator*), or absent (*Chilodonella* spp.). Here, we explore the time-series after another 12 years (1986-2018) and ask a) if the increase in water temperature has remained on the same trajectory and b) if the diseases have further increased or decreased their prevalence corresponding to the ambient environmental temperature.

Methodology: We analyse a time-series of disease dynamics in two fish farms in northern Finland in 1986-2018. The farms were producing Atlantic salmon (*Salmo salar*) and sea trout (*Salmo trutta*) smolts for stocking purposes. Each year, farms were visited 8–17 times mainly between beginning of June and end of September and samples of fish from tanks showing symptoms of a disease were examined. This included standard microscopic examination of the epidermal tissue (parasitic infections) as well as bacterial cultivation procedures. Water temperature was measured daily at the same time from the incoming water.

Results and Conclusion: We first demonstrate that the yearly mean water temperature increased significantly in both farms over the whole study period and that the increase was most pronounced in the late summer (July-September) especially in 1995–2005. These results demonstrate the effect of increasing water temperature on aquatic disease dynamics, but also emphasise the importance of biology of each disease, as well as role of local conditions, in determining the direction and magnitude of these effects.

Keywords: global warming, disease ecology, epidemiology, aquaculture, *Salmo salar*

002-P

The bacterial microbiota of octocorals living in the Sea of Marmara

Karataş, Süheyla (Turkey)[1]; Turgay, Emre (Turkey)[2]; Yardımcı, Eda (Turkey)[3]; Topçu, Eda (Turkey)[4]; Steinum, Terje (Turkey)[3]

1 - Faculty of Aquatic Sciences, Istanbul University, Turkey; 2 - Department of Molecular Biology and Genetics, Faculty of Sciences, Istanbul University, Turkey

Introduction: Corals are in serious trouble due to pollution, anthropogenic activities and climate change leading to acidification and rising seawater temperatures. Recent research imply that the microbiota of the 'holobiont' play a more important role in coral health than previously acknowledged. However, the current knowledge is limited. For this reason, the present study aimed at describing the microflora associated with some octocoral species living in less studied temperate regions like the Mediterranean basin.

Methodology: Samples from two octocoral species *Paramuricea clavata* and *Eunicella cavolini*, relatively common to the Sea of Marmara, were collected during 2018 and 2019. The microbiota of these healthy octocorals will be determined by both traditional culture-dependent methodology, that is identification by PCR and sequencing, as well as by culture-independent 16S rRNA gene based 'metagenetics'.

Results: Our preliminary culture-dependent results suggest that *P. clavata* and *E. cavolini* octocorals could have host specific bacterial genera in their microbiota and that there is a temporal difference in diversity between autumn and winter. Less bacteria were isolated during winter sampling (31 and 19 isolates, respectively) than during autumn sampling (44 and 29 isolates, respectively). Despite this fact, more bacterial genera were identified in winter than autumn samples (5 or 6 versus 3 genera, respectively).

Conclusion: Our results thus far suggest a seasonal difference in coral microbiota diversity and that some bacterial genera could be host specific. The planned 'metagenetics' part of this work will describe in much greater detail the seasonal microbiota of the said octocorals. It is our aim to learn more about the ecological functions of coral associated bacteria and their significance for 'holobiont' health. A better understanding of the normal coral microbiota will be crucial to investigations into coral disease suspected to be caused by bacteria.

Keywords: coral, microbiota, *Paramuricea clavata*, *Eunicella cavolini*

Funding: This study was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK, 117Y064).

003-P

Keystone molecules expression from *Mytilus galloprovincialis* under an acidified ocean

Dios, Sonia (Spain)[1]; Silva, Elsa (Spain)[1]; Prado-Álvarez, María (Spain)[1]; Gestal, Camino (Spain)[1]; Babarro, José M.F. (Spain)[1]

1 - Marine Research Institute, Spanish National Research Council, Vigo, Spain

Introduction: Keystone species such as *Mytilus galloprovincialis* play a special role in the structure of ecological communities and in determining biodiversity. A glycoprotein named KEYSTONEin was characterized in *M. galloprovincialis, M. edulis* and *M. californianus*. Its role is crucial since it constitutes a cue of predation for sea stars. Indeed, sea stars would not predate on mussels if KEYSTONEin is lacking. In this work we analyzed KEYSTONEin expression under a predictable ocean acidification (1200 CO_2 ppm) regarding the current situation (400 ppm) in order to determine if it could be affected by climate change.

Methodology: Mussels were acclimated to the experimental pCO_2 concentrations (400 and 1200 µatm) for a month in 9-L tanks with semi-static system (9 mL min^{-1} of flow from header tanks with a diet composed of two phytoplankton cells: T-ISO and *Rhodomonas lens*). After that, half population was exposed to crushed conspecifics of *M. galloprovincialis* for a three -week period. Four individuals were collected from each experimental group and immediately dissected. Several tissues were sampled for RNA and/or protein isolation. qPCR was carried out for KEYSTONEin and housekeeping gene 18S expression. Fold change units were calculated following ΔCt method.

Results and Conclusion: The normalized expression of KEYSTONEin exhibits a higher expression when the faux prey is introduced in the tank, for both normal and acidified conditions. This suggests that the faux predation increases KEYSTONEin synthesis independently of the environmental conditions. However, in terms of fold change values there is a tendency towards downregulation of KEYSTONEin under acidification conditions without the faux prey, but this trend was not observed with the faux prey. These results seem to suggest that ocean acidification could play a detrimental role in keystone molecules expression. This might lead to important ecological effects such us spatial distribution not only for foundation species but also for other species. Nonetheless, when the faux prey is introduced, the predation signal seems to be strong enough to mask the effect of acidification. Further research needs to be done to understand the role of KEYSTONEin under more extreme environmental conditions and/or as defense molecule in experimental infections.

Keywords: "Keystonein" gene expression, mussels, infochemicals, climate change, ocean acidification

Nutrition and Health

004-P*

Effects of fermented and unfermented duckweed as feed additive on growth and health status of common carp (*Cyprinus carpio*)

Seitz, Andreas (Switzerland)[1]; Pietsch, Constanze (Switzerland)[1]

1 - Zurich University of Applied Sciences

Introduction: Fermented and unfermented duckweed (*Spirodela polyrhiza*) powder was used as feed additive for partially substituting the protein fraction originating from animal sources in diets for common carp (*Cyprinus carpio*). The best starter culture for fermenting *S. polyrhiza* was investigated in small batch trials. Big scale fermentation was carried out with a combination of *Pediococcus pentosaceus* with a commercial mixture of microorganisms (EM 1) as starter culture. Fermented duckweed and freshly harvested duckweed were then dried and grinded for further processing. A subsequent feeding trial was conducted for assessing the performance of the experimental diets by investigating the growth performance and health status of the fish reared in three identical and independent recirculating aquaculture systems at the Zurich University of Applied Sciences. An animal protein-based diet was used as control and two other diets were formulated to contain 30 % unfermented or fermented duckweed powder respectively. Each diet was fed in triplicates to fish with an initial weight of 60 g for 84 days. Three samplings were carried out to evaluate the effect of diets on the growth performance and health status of fish by measuring their length and weight, calculating organosomatic indices and the specific growth rate. In addition, sensory tests were conducted and the filet quality and its nutrient content were investigated.

Results: Fermentation tests revealed that the fermentation of duckweed with *P. pentosaceus* and EM 1 had positive effects on the content of anti-oxidative substances by an increase, whereas the protein content was slightly reduced. The fermentation also affected the content of anti-nutritive substances. Possible advantages of feeding unfermented or fermented duckweed to common carp will be presented.

Conclusion: Fermentation remains a promising tool for improving the quality of feed additives and with minor adaptations to the fermentation process, still better results can be expected. The feeding trial yielded promising results in terms of the growth of common carp, which now requires further investigation of the health status using histological preparations of the liver and the spleen.

Keywords: duckweed, nutrition, fermentation, common carp, growth, health

005-P*

Dietary methionine on the European sea bass immune status – a proteomic approach

Machado, Marina (Portugal)[1,2,3,4]; Azeredo, Rita (Portugal)[1,3]; Fontinha, Filipa (Portugal)[1,3]; Fernandez-Boo, Sergio (Portugal)[1]; E. C. Conceição, Luis (Portugal)[5]; Dias, Jorge (Portugal)[5]; Dos Santos, Nuno (Portugal)[2,4]; Costas, Benjamín (Portugal)[1,3]

1 - Centro Interdisciplinar de Investigação Marinha e Ambiental; 2 - Instituto de Investigação e Inovação em Saúde; 3 - Instituto de Ciências Biomédicas Abel Salazar; 4 - Instituto de Biologia Molecular e Celular; 5 - Sparos Lda

Introduction: Methionine presents a pivotal role in the regulation of many cellular events with crucial impact on the immune system, as in the control of inflammation and polyamines synthesis. Accordingly, previous results showed that methionine dietary supplementation improved European sea bass (*Dicentrarchus labrax*) cellular immune status without evidence of activation of pro-inflammatory mechanisms. The present study aimed to assess the same effect of methionine supplementation through a proteomic approach searching for biomarkers of the immune improved condition previously detected.

Methodology: A feeding trial was performed were three diets were randomly distributed (three replicates per group): a control diet (CTRL) formulated to meet the established amino acid requirements for the species; and two diets supplemented with methionine at 0.5% and 1% of feed weight relative to the CTRL diet (MET 0.5 and MET 1, respectively). At 2 and 4 weeks of feeding, blood of 12 fish was withdrawn from the caudal vein and plasma collected and pooled (12 fish per tank; 3 pools per group). After protein quantification and protein digestion, the proteomic analysis of each sample was performed using liquid chromatography–mass spectrometry (LC-MS) technique and the peptide sequences aligned with proteins from European sea bass UniProt databases. From all the identified proteins, the study focused on the proteins associated with the complement pathway in fish. Data was analysed by two-way ANOVA, with time and diet as factors and followed by Tukey post-hoc test to identify differences in the experimental treatments.

Results and Conclusion: Fish fed methionine-supplemented diets showed a lack of differences at the proteomic level on the complement associated proteins found in plasma, which is in line with the lack of immune cells activation observed in our previous study. In fact only an increase in time of the C1q-b (B-chain polypeptide of serum complement subcomponent C1q, E6ZGC9_DICLA) peptides was observed. This is in accordance with the increase of plasma alternative complement pathway activity with time previously found. The proteomics analysis allows the study of proteins at a large-scale and more plasma biomarkers will be examined.

Keywords: aminoacids, proteins, complement system, immuostimulation, fish

006-P

Feed associated rainbow trout gastroenteritis (RTGE) in a recirculation aquaculture system (RAS) fish farm

Viljamaa-Dirks, Satu (Finland)[1]; Enbom, Tuulia (Finland)[1]

1 - Finnish Food Authority

Introduction: The use of recirculating aquaculture systems (RAS) is considered an important future technique as environmentally friendly production of animal protein. While the possibilities to control water parameters are better than in conventional flow through systems, each system is unique and acquires specific knowledge of the technical performance in relation to the local water parameters, and anything added to the system including fish and feed. We describe a case of elevated mortality that apparently was caused by a specific fish feed and manifested as symptoms known as rainbow trout gastroenteritis (RTGE).

Methodology: Rainbow trout 100-400 g in size were collected for laboratory examination due to elevated mortality. The fish were examined by routine pathological, bacteriological and virological methods. When suspicion of the involvement of feed was raised, the diet of two fish groups was changed. After 10 weeks one of the fish groups got again the original feed in a slightly different format, while the other group was continued with the alternative diet. Before switching the diet and after 6 weeks the two groups were studied for histopathological changes in the alimentary canal.

Results: In the first case the fish exhibited signs of digestive problems including dilatation of the stomach packed with feed or watery mucus. The intestine was unevenly filled, sometimes containing mucus. Some of the fish had hemorrhagic areas in the intestine. Microscopical examination revealed bacteria resembling RTGE associated segmented filamentous bacteria (SFB) in the gut. Histopathological examination showed necrotic areas and elevated number of lymphocytes in the intestinal epithelium. After changing the diet, most of the symptoms disappeared, but returned in a milder form in the group that was switched back to the newly formulated original diet.

Conclusion: It seems to be possible to create favorable conditions for the growth of RTGE associated SFB like bacteria in RAS based farming. The digestibility of the feed seemed here to be the main culprit.

Keywords: RTGE, rainbow trout, RAS, feed, gastroenteritis

007-P

Gut health status in farmed Atlantic salmon (*Salmo salar* L.) in different seawater production localities in Norway - Gutmatters project

Wang, Jie (Norway)[2]; Li, Yanxian (Norway)[2]; Zhou, Weiwen (Norway)[2]; Koppang, Erling Olaf (Norway)[2]; Midtlyng, Paul J. (Norway)[1,2]; Kortner, Trond M. (Norway)[2]; Løkka, Guro (Norway)[2]; Bjørgen, Hårvard (Norway)[2]; Torres, Alexander (Norway)[2]; Berge, Gerd Marit (Norway)[3]; Sæle, Øystein (Norway)[4]; Krasnov, Aleksei (Norway)[3]; Khakimov, Bekzod (Denmark)[5]; Aru, Violetta (Denmark)[5]; Engelsen, Søren B. (Denmark)[5]; Krogdahl, Åshild (Norway)[2]; Chikwati, Elvis M. (Norway)[1,2]

1 - Aquamedic AS; 2 - Norwegian University of Life Sciences; 3 - Nofima AS; 4 - Norwegian Institute of Marine Research; 5 - University of Copenhagen

Introduction: Optimal gut health is a key component in improving feed utilization, for better fish growth and health, reduced production costs and for minimizing the environmental impact of Atlantic salmon industrial production. There is a dearth of knowledge on gut health of farmed fish stock. This is being addressed in the GutMatters project in a national survey investigating prevalence of gut health disorders and their incidence during a production cycle in sea farmed Atlantic salmon.

Methodology: In the survey that ran from October 2017 to November 2018, 6 sea farming sites along the Norwegian coast were monitored starting from smolt transfer to harvest fish size (>4 kg) (sampling 3). At each of 3 samplings per site, fish weight and length; plasma; gross pathology observations of the fish, intestinal mucosa and liver; intestinal, liver, and other samples for histology, qPCR, microbiota, metabolomics, and digestive enzyme activity were analyzed from 20 fish. Additionally, site physicochemical data, fish genetics, population records, feeding regimes, growth and health history data were also collected. Results from the preliminary gut health assessment from gross pathology and intestinal histopathology will be presented.

Results: Marked infestation with cestode parasites (*Eubothrium* spp) was observed in fish from 2 of the sites on the second (May) and third (October) samplings. The chronic parasite presence appeared to induce inflammatory changes in the whole intestinal tract as observed in the histopathology of the October samples. Histopathology also revealed inflammatory changes, resembling the salmonid soybean-meal-induced distal intestinal enteritis, in some fish from all participating sites. A trend of a general increase in occurrence and severity of the enteritis over time was noted for most of the sites. Enterocyte steatosis, possibly indicating a lipid transport disorder, in the pyloric caeca and occasionally the mid intestine was another noticeable histopathology finding whose prevalence and severity appeared to increase in warmer periods of the year.

Conclusion: In conclusion, the gut health of some fish from participating farms was hampered by chronic cestode parasitism, enteritis, and enterocyte steatosis. More survey results and follow-up plans for the GutMatters project will be presented.

Keywords: Atlantic salmon, gut health, field survey, enteritis, intestinal cestodes

Funding: Norwegian Seafood Research Fund (FHF).

008-P*

Dietary histidine, threonine or taurine supplementation induced few effects on the gilthead sea bream (*Sparus aurata*) immune status.

Ramos Pinto, Lourenço (Portugal)[1,2,3]; Machado, Marina (Portugal)[1,2]; Castillo, Marta (Spain)[1]; Calduch-Giner, Josep (Spain)[4]; Pérez-Sánchez, Jaume (Spain)[4]; Conceição, Luis (Portugal)[3]; Dias, Jorge (Portugal)[3]; S. Silva, Tomé (Portugal)[3]; Costas, Benjamín (Portugal)[1,2]

1 - CIIMAR; 2 - ICBAS-UP; 3 - SPAROS Lda.; 4 - IATS-CSIC

Introduction: Histidine (HIS) plays important roles in homeostasis maintenance and detoxification of reactive species. Threonine (THR) is a major component of fish mucin which has a key role in gut integrity and function. Low dietary levels of taurine (TAU) are the cause of the green liver syndrome and reduced disease resistance, while TAU supplementation appears to display immunoregulatory properties and improve fish survival. The present study aimed to explore the effects of diet supplementation with any of these amino acids (AAs) on the gilthead sea bream (*Sparus aurata*) immune status.

Methodology: Triplicate fish groups (8.77 ± 0.13 g) were either fed a control diet with a balanced AA profile, or the CTRL supplemented with HIS, THR or TAU. After 2 and 4 weeks of feeding, samples of blood was collected for smears, plasma and mucus for humoral immune parameters, as well as head-kidney for transcriptome analysis of 29 health-related genes.

Results: After 4 weeks of feeding with AA supplemented diets, overall bactericidal and antiprotease activities increased, while IgM displayed the opposite pattern, regardless dietary treatments. Despite a transient decrease in mucus bactericidal activity after 2 weeks in fish fed TAU and HIS compared the CTRL. Mucus antiprotease activity increased in fish fed HIS regardless sampling time. Besides, fish fed HIS showed a lower content of white blood cells after 2 weeks and a decrease of lymphocytes after 4 weeks. Regarding head-kidney expression, C-type lectin was down-regulated in fish fed THR after 2 weeks compared to those fed the CTRL, while membrane IgT was up-regulated in fish fed TAU after 4 weeks compared to those fed THR. Multivariate analysis (PLS-DA) of the head-kidney expression signatures identified sampling time (2 or 4 weeks) and not dietary treatments as the most discriminant factor among groups.

Conclusion: Results suggest that dietary supplementation with these AAs did not induce a clear immunomodulatory effect at both sampling points. Further studies with other supplementation levels and focusing on disease resistance or other stressors must be planned.

Keywords: amino acids, immunology, aquaculture, functional feeds, gilthead sea bream

Funding: Projects ALISSA (ALG-01-0247-FEDER-3520) and IF/00197/2015; FEDER, COMPETE 2020 and CRESC Algarve 2020, COMPETE, Operational Human Potential Programmes, Fundação para a Ciência e a Tecnologia.

009-P

Quantitative PCR assay for rapid screening of shifts in intestinal microbiota in Atlantic salmon following nutritional challenges

Mccarthy, Una (United Kingdom)[1]; Merrifield, Daniel (United Kingdom)[2]; Rodiles, Ana (United Kingdom)[2]; Louis, Petra (United Kingdom)[4]; White, Patricia (United Kingdom)[1]; Dehler, Carola (United Kingdom)[3]; Martin, Sam (United Kingdom)[3]

1 - Marine Scotland Science; 2 - University of Plymouth; 3 - University of Aberdeen; 4 - The Rowett Institute

Introduction: A variety of plant-based diets are being developed for Atlantic salmon (*Salmo salar*) to reduce dependence on fish oil and fish meal. Although current diets are not causing high levels of gut inflammation it is known that there are changes in intestinal metabolism and the microbiota in the intestine. Microbiota diversity can be determined by next generation sequencing of 16S rDNA but this is not practical for screening large numbers of animals. To screen greater numbers of fish an assay was developed to detect indicator microbiota species to facilitate routine screening of gut health during diet development.

Methodology: Indicator species were identified in digesta from salmon smolts fed a panel of diets rich either in plant proteins or vegetable oils compared with the control diet containing marine source ingredients. Analysis of 16S sequences using Linear Discriminant Analysis Effect Size (LEfSe) identified a number of operational taxonomic units (OTUs) that were significantly different in abundance between diets. Group-specific primers were designed, validated against DNA from reference strains, and used in qPCR quantification of *Peptostreptococcus*, *Lactococcus*, *Streptococcus* and *Leuconostoc* in digesta DNA.

Results: *Peptostreptococcus, Lactococcus* and *Streptococcus* were significantly reduced in digesta of fish fed diets containing plant proteins, compared with the control diet. In contrast, *Leuconostoc* was significantly increased in fish fed plant proteins. In fish fed plant protein diets, the substitution of vegetable oil for fish oil significantly reduced the level of *Lactococcus*. No effect of oil source on other genera was detected.

Conclusion: Clear, significant differences between the diets containing marine or plant protein were detected by qPCR assay of indicator microbiota species in fish digesta. A shift in the ratio of *Leuconostoc* to *Streptococcus* may provide a diagnostic test for potentially deleterious microbiota changes during feed trials.

Keywords: salmon, diets, intestinal microbiota

Funding: BBSRC; BB/M026604/1 “Gut health and immune function: the emerging role of gut microbiota in sustainable aquaculture”.

010-P

INFLAMMAA: Unraveling the neuro-endocrine/immune modulatory roles of tryptophan during inflammation

Costas, Benjamín (Portugal)[1,2,3]; Machado, M. (Portugal)[1,2]; Azeredo, Rita (Portugal)[1]; Ramos-Pinto, L. (Portugal)[1,2]; Castro, L.F.C. (Portugal)[1,2]; Afonso, A. (Portugal)[1,2]; Engrola, S. (Portugal)[3]; Aragão, C. (Portugal)[3]

1 - CIIMAR, Universidade do Porto, Matosinhos, Portugal; 2 - ICBAS, Universidade do Porto, Porto, Portugal; 3 - CCMAR, Universidade do Algarve, Faro, Portugal

Introduction: The concept of maintaining animal health through the best possible nutrition is well-accepted in modern animal farming and functional amino acids appear to be good candidates to improve health and survival. Tryptophan in particular have known roles in the improvement of the immune response to infection and recent evidence indicates that several immune mechanisms are influenced by its availability. Still, the potential use of tryptophan supplementation for animal health management is not fully developed. The INFLAMMAA team will use an innovative multidisciplinary approach that aims to explore the links between tryptophan nutrition, immune function and endocrine-immune plasticity.

Methodology: Underlying mechanisms will be addressed by localising key elements of the opioid system, autophagy-lysosomal signalling pathway and inflammation in the head-kidney and leucocytes, and expression of genes controlling aspects of neuro-endocrine and immune systems. An innovative approach will assess tryptophan digestibility and utilisation during the onset of inflammation while high-throughput sequencing will assess the European sea bass transcriptome during acute and chronic inflammation.

Results and Conclusion: The expected impact of the INFLAMMAA project is to deepen our knowledge on the interactions of tryptophan nutrition and inflammation. Nowadays, few studies have demonstrated clear links between amino acid nutrition and endocrine/immune functioning or other key welfare aspects in fish. This is particularly important for the aquaculture industry, where few therapeutic possibilities are available against infectious episodes.

Keywords: amino acids, functional feeds, immunomodulation, stress

Funding: INFLAMMAA project (PTDC/CVT-CVT/32349/2017)

Bacterial Diseases

012-P

Mycobacteriosis in cultured koi carp *Cyprinus carpio*

Machida, Yuichiro (Japan)[1]; Yamada, Mitsuo (Japan)[1]; Sato, Shoh (Japan)[2]; Nakajima, Kazue (Japan)[2]; Matoyama, Hisato (Japan)[2]; Endo, Makoto (Japan)[1]; Sano, Motohiko (Japan)[1]; Kato, Goshi (Japan)[1]

1 - Tokyo University of Marine Science and Technology, Tokyo 108-8477, Japan; 2 - Niigata Prefectural Inland Water Fisheries Experiment Station, Niigata, 940-1137, Japan

Introduction: Mycobacteriosis has caused economic losses in fish production of koi carp in Japan. *Mycobacterium nonchromogenicum* that forms white colonies has been reported to be a causative agent of the disease. However, we have isolated other mycobacterium species that forms yellow colonies from the diseased fish. In addition, the two kind of mycobacterial colonies were sometimes isolated from a same diseased fish. In this study, we performed histopathological analyses, bacterial identification and challenge test using several isolates.

Methodology: Spontaneously diseased koi carp externally showing lethargy and emaciation with pin-head shape were obtained from culture ponds in Niigata, Japan. Paraffin-embedded sections of the visceral organs were subjected to hematoxylin and eosin staining and Ziehl-Neelsen staining. Biochemical properties such as Tween80 hydrolysis, chromogenic and growth rate were analyzed using 14 bacterial isolates. Phylogenetic analyses using ITS, 16S rRNA, RpoB, RecA and HSP65 genes were performed with Neighbor-Joining method. Further, carp were challenged with three isolates at 1.0-3.0 × 10^8 CFU/fish and the mortalities were recorded for 100 days.

Results: The diseased fish internally showed atrophy of latter half of swim bladder, accumulation of hemorrhagic ascites and adhesion of the visceral organs and their adjacent peritoneum. Hyperplasia of collagenous fibers and a marked increase of macrophages were observed in the visceral organs of the diseased fish. Ziehl-Neelsen positive bacilli were detected in the trunk kidney, while granuloma formation was not observed in the visceral organs. Bacterial isolates were classified into two groups based on the colony color: yellow colonies (Y-1 to Y-10) and white colonies (W-1 to W-4). Y-1 to Y-10 isolates showed similar biochemical characteristics to *M. gordonae*. Phylogenetic analyses also showed that Y-1 to Y-10 isolates were classified into *M. gordonae*. W-1 to W-4 were classified into rapid-growing mycobacterium (Runyon IV) based on the biochemical characteristics. However, they were not classified into known mycobacterial species by the phylogenetic analyses. The cumulative mortality of fish challenged with Y-2, W-2 and W-3 was 30%, 50% and 50% at 100 days post-challenge, respectively.

Conclusion: These data reveal that *M. nonchromogenicum*, *M. gordonae* and unknown *Mycobacterium* spp. are implicated in mycobacteriosis in cultured koi carp.

Keywords: cyprinid, mycobacteriosis, koi carp, pathogenicity

013-P

Bacteriophage therapy in aquaculture

Larsen, Ingrid Sofie (Norway)[1]; Rønneseth, Anita (Norway)[2]; Kleppen, Hans Petter (Norway)[1]

1 - ACD Pharma; 2 - Department of Biological Science

Introduction: Today, aquaculture is one of the fastest growing food-producing sectors in the world, but the industry is struggling with losses due to bacterial infections. Several species of *Vibrio* are ubiquitous in marine environments, and some of them are responsible for large disease outbreaks in fish and shellfish farms. To fight bacterial infections, farmers have turned to various antibiotics, but due to resistant bacteria, negative impacts on the natural microflora and the environment, as well as increasing consumer demand for food raised without antibiotics, there is need for novel solutions. Bacteriophages, naturally occurring viruses that specifically infect and kill bacteria, represents such a solution. ACD Pharmaceuticals AS has since 2011 developed bacteriophage products for use in aquaculture, and have recently launched the phage-based product CUSTUS™yrs, which controls infection pressure of the fish pathogen *Yersinia ruckeri*. The aim of this study is to evaluate the feasibility of using *Vibrio*-phages in a similar manner, to control different *Vibrio* species causing disease in farmed lumpfish (*Cyclopterus lumpus*).

Methodology: Together with the University of Bergen, ACD Pharma is currently isolating and characterizing potential target *Vibrio* strains and their bacteriophages from environmental samples. Bacteriophages which meet the biological and bioinformatical selection criteria for therapeutic phages, will be tested further in challenge experiments with live lumpfish larvae. This will provide documentation of the efficacy of phages in controlling *Vibrio* infection pressure, and preventing disease outbreaks.

Results and Conclusion: This is an ongoing research project in which the results are not ready to draw a conclusion from.

Keywords: bacteriophages, lump sucker, aquaculture, therapy

Funding: This project is supported by The Norwegian Research Council and Innovation Norway.

014-P

Significant mortality in farmed Atlantic salmon (*Salmo salar* L.) associated with *Pasteurella skyensis* in Scotland

Soares, Silvia (United Kingdom)[1]; Murray, Warren (United Kingdom)[1]; Garden, Alison (United Kingdom)[1]; Mcintosh, Rebecca (United Kingdom)[1]; Duguid, Sonia (United Kingdom)[1]; Munro, Eann (United Kingdom)[1]

1 - Marine Scotland Science

Introduction: Between 1995 and 2001, *Pasteurella skyensis* was reported to be responsible for several mortality events in farmed Atlantic salmon (*Salmo salar* L.) on seawater sites on the Isle of Skye, Scotland.

Methodology: In October 2017, the Marine Scotland Science, Fish Health Inspectorate (FHI) were alerted to a mortality event at a seawater site on the Isle of Lewis. The site was stocked with approximately 577,000 A. salmon with a mean weight of 3.9 kg. Mortality levels peaked at 4.9% in week 39 and reduced to 2.9% in week 40. The FHI visited the site on week 42 to conduct a disease investigation and sampled five moribund fish for diagnostic testing. In total, approximately 125,000 A. salmon (500 tonnes) were lost during the disease outbreak. In November 2017, two seawater sites, located in Loch Sunart, recorded the presence of *Pasteurella skyensis* infection. However, the mortality levels were not as significant and a site visit was not performed by the FHI. In November 2018, elevated mortalities on a seawater site located in Loch Linnhe, stocked with approximately 93,000, 3 kg and 678,000, 2 kg A. salmon, was reported. The mortality level over weeks 44 – 47 was 2.8 %. The FHI attended the site on week 48 and sampled five moribund fish for disease diagnosis.

Results and Conclusion: Histopathological examination revealed mild pathology resembling *Pasteurella skyensis*-like infections in 2017. These fish also had health issues associated with multifactorial gill disease, making the animals more vulnerable to other pathogenic agents and therefore contributing to the elevated mortalities recorded at the time. One of the sites in Loch Sunart also reported complex gill issues along with a *Pasteurella skyensis* infection. The fish sampled in 2018 demonstrated a mild systemic granulomatous inflammation consistent with *Pasteurella skyensis* infection. The bacterium was isolated by bacteriology culture from both cases and confirmed to be *Pasteurella skyensis* by 16S rRNA and *rpoB* gene sequencing. The sequencing results showed a similarity of 99% (>1100 nucleotides) to *Pasteurella skyensis* strains 98B1 and 01A1. Phylogenetic analyses will be performed along with historic *Pasteurella skyensis* isolates to determine classification.

Keywords: *Pasteurella skyensis*, Atlantic salmon, mortality

015-P*

Pathogenicity of *Pasteurella* sp. in lumpsuckers (*Cyclopterus lumpus* L.)

Ellul, Rebecca Marie (Norway)[1]; Walde, Cecilie (Norway)[2]; Haugland, Gyri Teien (Norway)[1]; Wergeland, Heidrun (Norway)[1]; Rønneseth, Anita (Norway)[1]

1 - University of Bergen; 2 - Norwegian Veterinary Institute

Introduction: Outbreaks of pasteurellosis caused by *Pasteurella* sp. in farmed lumpsuckers in Norway has been steadily increasing in recent years, causing significant economic losses and fish welfare issues. The disease affects all life stages, from eggs stages, to hatcheries, and following transfer to salmon cages. Therefore, it is important to establish robust challenge models to be used for vaccine development, which is still in its infancy due to lack of culture protocols for *Pasteurella* sp.

Methodology: In this work, we attempted to culture *Pasteurella* sp. in liquid medium. Various exposure experiments were then tested which included intramuscular and intraperitoneal injection challenge models, a co-habitation challenge model, as well as a bath challenge model, in order to identify the best route to investigate pasteurellosis in lumpsuckers. Confirmation of disease was performed by bacteriology and qPCR of head kidney samples from all the dead fish, survivors, and control fish from the cohabitation challenge experiment and from the bath challenge experiment.

Results: *Pasteurella* sp. was successfully cultured when using TSB and BHIB, both enriched with a range of foetal calf serum (FCS) concentrations. Exposure to *Pasteurella* sp. via intramuscular and intraperitoneal injection underlined the high virulence of the bacteria, while the co-habitation and bath models allowed the chronic symptoms of the disease to be studied more accurately. Skin lesions and haemorrhage at the base of fins were observed in the more acute cases of the disease. Symptoms including white spots over the skin, especially around the eyes, characterised the chronic cases. The latter were most prominent from the bath challenge model. Histopathology indicated a systemic pattern of disease, while qPCR analysis from head kidney of the challenged fish showed that bacteria may be present in survivor fish at the end of the challenges.

Conclusion: TSB supplemented with 10% FCS was confirmed as the optimal medium composition for *Pasteurella* sp. culture for the challenge experiments. In all the challenge models investigated, *Pasteurella* sp. was re-isolated from the fish, thus fulfilling Koch's postulates. These findings highlight the importance of screening of lumpsuckers prior to transfer to minimise the risks of carrying over asymptomatic carriers.

Keywords: challenge, cleaner fish, infection, pasteurellosis, pathology

016-P

Microbial activation of biofilters in recirculating aquaculture systems (RAS) for post-smolt Atlantic salmon based on lab experiments and field observations

Roalkvam, Irene (Norway)[1]; Drønen, Karine (Norway)[1]; Dahle, Håkon (Norway)[1,2]; Wergeland, Heidrun I. (Norway)[1]

1 - Department of Biological Sciences, University of Bergen; 2 - K. G. Jebsen Center for Deep Sea Research, University of Bergen

Introduction: Rearing post-smolt Atlantic salmon in recirculating aquaculture systems (RAS) has expanded greatly in Norway during the last two decades, as the RAS technology provides an environment free of the sea lice parasite. The water in RAS is purified through several operations, including biofiltration where ammonium excreted from the fish and organic matter is removed by bacteria located in biofilms on biofilter media. The ammonium is first oxidized to nitrite and then to nitrate, a process of two steps that require metabolic activity of different bacteria. The bacteria associated with the first step are easily established in the biofilter, while the bacteria associated with the second step establish much later. Hence, activation of a new RAS biofilter is time consuming, and can give periods with variable water chemistry during RAS start-up.

Methodology: The development of nitrifying bacteria in growth cultures was compared based on 16S rRNA gene sequencing and measured physiochemical water parameters. The cultures were inoculated with either commercial inoculum for RAS or transferred biofilm material from an established RAS biofilter. In addition, the activation of two new biofilters in a RAS for post-smolt Atlantic salmon was monitored during the first 4 months of operation using 16S rRNA gene sequencing.

Results: The bacterial growth experiments revealed that cultures inoculated with biofilm carriers initiated nitrite and nitrate production from ammonia much earlier than cultures with commercial inoculum, and also reached higher concentrations of the metabolic products. The two RAS biofilters were inoculated with transferred biofilm carriers, but experienced differences in amounts of fish associated to each filter. The biofilter associated with low stocking density had a higher proportion of nitrifying bacteria in the biofilm, and both steps of nitrification were activated after 4 months.

Conclusion: Lab experiments with growth cultures showed that transferred biofilm carriers from RAS are a more suitable as inoculum for marine RAS biofilter activation than commercial inoculum. The data analyses on activation of the RAS biofilters indicate that the biofilter associated with low stocking density matured more successfully.

Keywords: recirculating aquaculture systems, bacteria, biofilter, post-smolt salmon, Atlantic salmon

Funding: Fiskeri-og havbruksnæringens forskningsfinansiering (FHF) in Norway, Project number 901470 .

017-P*

Characterization of *Yersinia ruckeri* strains isolated from trout farms in northern Poland

Pajdak-Czaus, Joanna (Poland)[1]; Terech-Majewska, Elżbieta (Poland)[1]; Platt-Samoraj, Aleksandra (Poland)[1]; Szweda, Wojciech (Poland)[1]; Siwicki, Andrzej Krzysztof (Poland)[2]

1 - University of Warmia and Mazury in Olsztyn; 2 - Inland Fisheries Institute in Olsztyn

Introduction: *Yersinia ruckeri* (*Y. ruckeri*) is a causative agent of enteric redmouth diseases (ERM) in rainbow trout. It is one of the most common bacterial diseases in this species. Fish weighing 50 to 100 g are considered to be the most susceptible. There are two recognized biotypes (biotype 1 and 2) and five serotypes: O1, O2, O5, O6 and O7.

Methodology: Samples of gills, intestine and head kidney were taken for bacteriological examination from six rainbow trout farms. The first part of the sample was cultured on Columbia Blood Agar plates. Gram-negative and oxidase-negative colonies were transferred onto TSA plates to receive homogeneous bacterial cultures. Preliminary identification was conducted with the use of API 20E tests (BioMerieux). Identification was confirmed by PCR method. *Y. ruckeri* isolates were biotyped with the use of API M medium (Biomerieux) and ability to hydrolyze Tween. Microagglutination method was used in serological identification. The second part of samples was used for PCR identification directly from tissue samples, without bacteriological culture.

Results: Presence of *Y. ruckeri* was confirmed in three out of six fish farms. Different weight groups ranging from 10 to 600g were examined depending on the stock structure. In all three fish farms *Y. ruckeri* was present in older fish, and absent in younger groups. In the case of two fish farms, strains were isolated from all tissue samples. Intestine samples from fish originating from one fish farm gave a negative result in bacteriological examination but were PCR-positive. All collected strains were homogenous in API20E tests with code number 5106100, belonged to biotype 2 and serotype O1. Only in the first two fish farms, fish had clinical symptoms of the disease.

Conclusion: Although *Y. ruckeri* is considered to affect mainly fingerlings, in our study bacterial isolates were obtained from older fish. All isolates belonged to the same serotype and biotype. Biochemically examined isolates were homogenous. PCR examination of tissue material seems to be more accurate in case of intestinal samples.

Keywords: yersiniosis, enteric redmouth disease, serotype, biotype

Funding: The work was created as a result of the research project no. 2017/25/N/NZ9/00087 funded by the National Science Center, Poland.

019-P*

Monitoring of bacterial diseases in cultured salmonid and pathogenicity analysis of *Aeromonas salmonicida* causing furunculosis

Lim, Jongwon (Republic of Korea)[1]; Hong, Suhee (Republic of Korea)[1]

1 - Department of Marine Biotechnology, Gangneung-Wonju National University

Introduction: At the present, fish farms are suffering a lot of economic losses due to infectious diseases caused by various pathogens including aeromonad. In particular, *Aeromonas salmonicida* subsp. s*almonicida* is the causative agent of furunculosis in salmonid. The major virulence factors in *A. salmonicida* are known as type 3 secretion system (T3SS) and A-layer. T3SS is a protein appendage found in many negative bacteria, which plays a key role in virulence and affects the immune response of the host.

Methodology: 129 bacterial samples were collected from Atlantic salmon, coho salmon, steelhead, masou salmon and rainbow trout in 22 Farms in Gangwon-Do, and identified by 16S rDNA sequences. Aeromonad isolates were further analysed based on rpoD or gyrB gene sequences. Also, rainbow trout and coho salmon was challenged with *A. salmonicida* and *A. sobria* isolated from different farms to confirm pathogenicity. We also analyzed the presence of 12 virulence genes at gDNA level and at mRNA level in high and low pathogenic *A. salmonicida* isolates.

Results: Among 129 bacterial samples 44 isolates were identified as the genus *Aeromonas* by 16S rDNA analysis. Based on rpoD or gyrB gene sequences, *A. salmonicida* (24 isolates) in 3 farms, *A. sobria* (14 isolates) in 9 farms, *A. bestiarum* (3 isolates), *A. media* (2 isolates) and *A. popoffii* (1 isolates) were identified. At the challenge test, one of *A. salmonicida* isolates showed a high virulence (46% mortality by $5x10^3$CFU/ml), whereas *A. sobria* showed low virulence in both fish species. High and low virulent isolates possess 12 virulence genes while only ascC and ascV genes were differentially expressed in 2 isolates.

Conclusion: In this study, we found that the genus *Aeromonas* is the most abundant bacteria in Salmonid aquaculture in Gangwon Do, Korea and proved that the phylogenetic identification of *Aeromonas* species based on the sequences of housekeeping gene is more precise than the 16S rDNA sequence. We obtained a very pathogenic *A. salmonicida* isolate and found that T3SS genes are important in pathogenicity of *A. salmonicida*.

Keywords: *Aeromonas salmonicida*, furunculosis, virulence, housekeeping gene, type three secretion type (T3SS)

020-P*

Effect of phage resistance on *Flavobacterium psychrophilum* virulence

Sundell, Krister (Finland)[1]; Landor, Lotta (Finland)[1]; Dramshoj, Liv (Denmark)[2]; Jorgensen, Natasja (Denmark)[2]; Jorgensen, Johanna (Denmark)[2]; Castillo, Daniel (Denmark)[2]; Middelboe, Mathias (Denmark)[2]; Wiklund, Tom (Finland)[1]

1 - Environmental and Marine Biology, Åbo Akademi University, Finland; 2 - Marine Biological Section, University of Copenhagen, Denmark

Introduction: *Flavobacterium psychrophilum* causes severe infections and high mortality in economically important salmonid fish species in farm environments around the world. Due to the increasing problems with antibiotic resistance in aquaculture, phage therapy has been proposed as an alternative strategy for the treatment of *F. psychrophilum* infections. However, resistance to phages can develop in bacteria in response to phage exposure, potentially diminishing the usefulness of phages for disease control.

Methodology: In the present work, we assessed the effect of phage resistance on virulence-related characteristics in *F. psychrophilum*. Two highly virulent outbreak isolates of *F. psychrophilum* were exposed individually to three different bacteriophages, and 27 phage-resistant clones were subsequently isolated and subjected to comparative phenotypical analysis. In addition, virulence tests were conducted with live rainbow trout (*Onchorhynchus mykiss*) for 18 representative clones.

Results: Compared to their parental wild-types, a significant reduction in virulence was observed among the phage-resistant *F. psychrophilum* clones, most (17 of 18) of which were found to be completely avirulent. In addition, the phage-resistant clones showed a significant decrease in motility and adherence. A phage-dependent inhibition of proteolytic activity was also observed.

Conclusion: Development of phage-resistance in *F. psychrophilum* affects virulence-related phenotypic characteristics negatively, particularly by suppressing its ability to cause infection in fish. The results from this work show that phage-resistance appears indeed to be costly for *F. psychrophilum*. In the light of these results, the use of phages appear to have the potential to mitigate harmful impacts of *F. psychrophilum* in fish farm environments.

Keywords: *Flavobacterium psychrophilum*, bacteriophage, phage resistance, rainbow trout, virulence

Funding: EU, Academy of Finland (BONUS FLAVOPHAGE project).

021-P

Hematological and molecular response of *Salmo salar* challenged with two *Piscirickettsia salmonis* strains under different salinities

Mayorga, Paulina (Chile)[1]; Albornoz, Romina (Chile)[1]; Pontigo, Juan Pablo (Chile)[2]; Sánchez, Patricio (Chile)[1,3]; Rauch, María Cecilia (Chile)[1]; Yáñez, Alejandro (Chile)[3,4]

1 - Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile, Isla Teja, Valdivia, Chile; 2 - Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, Chile; 3 - Interdisciplinary Center for Aquaculture Research, Concepción, Chile; 4 - Facultad de Ciencias, Universidad Austral de Chile, Isla Teja, Valdivia, Chile

Introduction: Aquaculture is an important economic activity in Chile, and it's affected mainly by outbreaks of infectious diseases such as piscirickettsiosis (caused by the bacterium *Piscirickettsia salmonis*), which generates huge economic losses due to fish mortality and spending in control and treatment. Hematology is a helpful and relatively inexpensive diagnostic tool in fish pathology, but knowledge of hematological responses in different culture conditions and bacterial strains is still limited.

Methodology: In the present study, hematological parameters were evaluated in fish (*Salmo salar*) infected with two *P. salmonis* strains, AUS111 and AUS005, which belong to the LF and EM genogroups, respectively, and under two levels of salinity (5‰ and 20‰), using the procedures described in the Handbook of salmonid hematology. Also, gene expression at the transcript level was evaluated by qRT-PCR for several genes related to iron metabolism and erythropoiesis on head kidney tissues from the infected fish.

Results: The results show some correlations between the pathological response with susceptibility degrees, for instance, the fish challenged with the AUS005 strain under low salinity conditions display higher mortalities, which reveals the bacterium capacity for adaptation under different stimuli. In this work, the observed anemia is described as regenerative, normocytic, normochromic and hemorrhagic regardless of the salinity or bacterial strain. Additional features are the presence of absolute leukopenia, lymphopenia and neutropenia which is influenced by salinity levels on healthy fish.

Conclusion: This study provides evidence that corroborates the nutritional immune response related to a lower iron availability and involved in the erythroid response.

Keywords: *Piscirickettsia salmonis*, hematology, iron metabolism, *Salmo salar*, salinity

Funding: FONDAP-INCAR 15110027 grant.

022-P

Geographical distribution of pathogenic *Tenacibaculum* spp. strains along the Norwegian coast

Lagadec, Erwan (Norway)[1]; Småge, Sverre B. (Norway)[2]; Nylund, Are (Norway)[1]

1 - Fish Disease Research Group, University of Bergen, Bergen, Norway; 2 - Cermaq Group AS, Oslo, Norway

Introduction: Ulcerative diseases affect a wide number of marine fishes and are of major ecological significance and a heavy burden to aquaculture worldwide. In Norway, winter ulcers have been commonly associated with *Moritella viscosa*, causing skin ulcers and septicemia, and even if Norwegian farmed salmon are vaccinated against *M. viscosa*, the occurrence of ulcers is still a massive health problem. Several studies have recently highlighted the role of the widespread marine bacteria in genus *Tenacibaculum* as possible causes for the high number of outbreaks of winter ulcers since the late 1980's. While tenacibaculosis outbreaks have dramatically increased in recent years, an extensive survey has been carried to increase knowledge on several aspects of the disease in salmonids in Norway

Methodology: *Tenacibaculum* spp. have been isolated from farmed fishes during multiple tenacibaculosis outbreaks along the Norwegian coast. Bacteria have been genotyped through a MultiLocus Sequence Typing/Analysis (MLST/MLSA) scheme and the geographical occurrence of different strains and species evaluated.

Results: MLSA revealed the presence of multiple species and showed a north-south gradient of *Tenacibaculum* spp. along the Norwegian coast. Zooming in at an intra-specific level, there is a correlation between virulence and genetic variation of the recently described *Tenacibaculum finnmarkense*, with the most virulent strains being present in the north of the country.

Conclusion: MLSA is an efficient genotyping method in order to decipher the distribution structure of the *Tenacibaculum* strains along the Norwegian coast. It could also provide an accurate and fast genotyping tool for separating strains with respect to virulence, which needs to be improved in order to implement effective control measures. An emerging alternative to MLSA analysis, with respect to virulence grading, is full genome sequencing and comparison of whole genomes. Lower price of whole genome sequencing and new available software for whole genome comparison make this approach accessible and bring about new opportunities.

Keywords: Atlantic salmon, multilocus sequence analysis, Norway, tenacibaculosis

Funding: FHF 901433 LimiT project.

023-P

Gut microbiota and biochemical markers in rainbow trout, *Oncorhynchus mykiss* (Walbaum), during bacterial hemorrhagic septicemia (BHS)

Parshukov, Aleksey (Russian Federation)[1]; Fokina, Natalia (Russian Federation)[1]; Sukhovskaya, Irina (Russian Federation)[1]; Kantserova, Nadezda (Russian Federation)[1]

1 - Institute of Biology of the Karelian Research Centre of the Russian Academy of Sciences

Introduction: It is known that pathogens affect host immune status in a versatile manner including their influence on gut microflora, a nonspecific defense component of the immunity. Data of species composition and dominant groups of bacteria are the indicator of fish health status and a basis for development of innovative probiotic and prebiotic dietary substances.

Methodology: Gut microbiota were studied in 22 rainbow trout, *Oncorhynchus mykiss*, yearlings (1+) differentiated by an infectious status. The composition of intestine microbiome of farmed trout was investigated by 16S rRNA gene sequencing (NGS).

Results: Received microbiological data on gut microbiota composition and biochemical markers of oxidative stress and lipid composition of fish organs suggest that trout has been infected since the beginning of the observation period (June), with delayed disease manifestations (August). Latent period of the infection has been revealed due to specific fatty acids of bacterial origin, including pentadecanoic (15:0) and heptadecanoic (17:0) fatty acids, as well as some changes in the intestine microflora, such as lack of *Lactobacillus* and prevalence of Mycoplasmataceae with significant differences of microbiota in healthy vs. diseased fish. Also, bacterial nature of the disease was confirmed with microbiological analysis of trout internal organs resulting in isolation of microorganisms *Pseudomonas putida* and *Cytophaga psychrophila*, the causative agents of bacterial hemorrhagic septicemia. According to the metagenomic analysis of gut and stomach microbiota of the rainbow trout there were identified 2374 OTUs which have been referred to 15 phyla and 3 “phantom” groups (OD1, SR1 and TM7) of bacteria. Phyla Firmicutes (42.3%), Bacteroidetes (21.4%), Proteobacteria (18.8%), Tenericutes (8.8%), and Fusobacteria (7.8%) dominated in the rainbow trout intestinal microbiota composition.

Conclusion: These results demonstrated clear differences in gut microbiota of BHS-infected and uninfected *O. mykiss* individuals that can be used as both infection markers and targets to develop new strategies for disease control programs.

Keywords: bacterial community, NGS, rainbow trout, *Pseudomonas putida*, *Cytophaga psychrophila*, bacterial-origin odd-chain saturated fatty acids

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024-P

Tenacibaculum infections in farmed Atlantic salmon

Brevik, Øyvind Jakobsen (Norway)[1]; Frisch, Kathleen (Canada)[2]; Bang Småge, Sverre (Norway)[1]; Duesund, Henrik (Norway)[1]

1 - Cermaq Group AS, Bergen, Norway; 2 - Cermaq Canada, Campbell River, Canada

Introduction: Several bacteria are known to cause skin ulcers in farmed salmon, most of which are controlled by vaccines. This is however not the case for tenacibaculosis caused by members of the genus *Tenacibaculum.* Tenacibaculosis is characterised by the presence of frayed fins, tail rot, mouth erosion and skin lesions.

Methodology: Two species cause tenacibaculosis in farmed salmon: *Tenacibaculum finnmarkense* and *Tenacibaculum maritimum*. Through the knowledge acquired from our published work and experience from field we attempt to clarify the different clinical presentations caused by *T. finnmarkense* and *T. maritimum* in Atlantic salmon.

Results and Conclusion: Tenacibaculosis caused by *T. finnmarkense* occurs in newly sea transferred smolts. The outbreaks have an acute progression with high mortality (5 - 20%). Diseased fish display yellow tinged skin lesions in the unscaled parts of the body (head, fins and tail). Microscopy of wet mount preparations from the lesions typically reveal large amounts of thin long rod-shaped bacteria. The bacterium is usually be observed in the collagen rich dermis layer of the skin. Co-infections with *T. finnmarkense* and *Moritella viscosa* can also occur. The outbreaks have a chronic progression with low to intermediate mortality (<10%). This is typically observed at low sea temperatures <7 °C and in larger fish (1.5 - 6 kg) at first or second winter at sea. Mixed infections are identified through the presence of ulcers with yellow margins on the scale covered parts of the skin and typically on the side of the fish. Lesions in the head region can also be observed. Tenacibaculosis caused by *T. maritimum* can cause lesions in all parts of the skin, including fins. However, the clinical presentation of *T. maritimum* infections in salmon in the Pacific Northwest is different to classical tenacibaculosis and is commonly referred to as mouthrot. The disease affect smolts the first 6 months at sea. Mouthrot is diagnosed by the presence of yellow plaques associated with the mouth and primarily with the teeth causing a disease that is similar to periodontal disease in mammals.

Keywords: *maritimum*, *Tenacibaculum*, mouthrot, tenacibaculosis, *finnmarkense*

Funding: RCN#251805, RCN#241364 & Cermaq.

025-P

Serological and genetic diversity of *Flavobacterium psychrophilum* recovered from commercially raised fish in Chile: current status and perspectives

Avendaño-Herrera, Ruben (Chile)[1]; Tapia-Cammas, Diana (Chile)[1]; Irgang, Rute (Chile)[1]

1 - Universidad Andrés Bello, Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola, Facultad de Ciencias de la Vida, Viña del Mar, Chile, Centro FONDAP, Interdisciplinary Center for Aquaculture Research, Universidad Andrés Bello, Viña del Mar, Chile

Introduction: Chile is the second largest global producer of farmed salmonids, but losses due to disease outbreaks have been an industry concern since the beginning. BCWD and RTFS are caused by *Flavobacterium psychrophilum*, which is currently the most important bacteria impacting freshwater salmonid farming in Chile. This pathogen, now affects all three cultivated salmonid species: Atlantic salmon (*Salmo salar*), Coho salmon (*Oncorhynchus kisutch*), and rainbow trout (*O. mykiss*). The development of sustainable aquaculture requires a better epidemiological knowledge of circulating pathogens. In 2009, notable homogeneity was found among 20 Chilean *F. psychrophilum* isolates using diverse typing methods (Valdebenito & Avendaño-Herrera, 2009). For the present study, a larger collection of *F. psychrophilum* isolates (retrieved from Chilean farms) was subjected to antigenic and genetic analyses to (i) provide a 10-year update in knowledge, (ii) better understand pathogen origin and propagation in Chile, and (iii) propose control and management practices.

Methodology: *F. psychrophilum* isolates (n = 114) recovered between 2006 and 2018 from all three salmonid species were analyzed by RAPD, ERIC-PCR, REP-PCR, and PCR-RFLP, including 16S rRNA. Moreover, serological diversity was tested using the slide agglutination test, dot-blot assay, and immunoblotting of lipopolysaccharides, as well as the PCR-based serotyping method.

Results: Most isolates were type-2 (56.1%) or type-4 (24.6%), while some were type-1 (13.2%) or type-0 (5.3%). One isolate could not be typified, and none were type-3. Population structure analysis through PCR-based patterns showed high genetic diversity, even revealing the distribution of different genotypes within individual farms. Host-based discrimination of the isolates was unsuccessful.

Conclusion: Chilean *F. psychrophilum* isolates recovered from diseased fish over the last ten years were serologically and genetically heterogeneous, thus refuting the 2009 isolates study. These last ten years have also seen a decreased use of the ineffective commercial Flavomune vaccine, which was made with two Chilean *F. psychrophilum*isolates according to data reported by Valdebenito & Avendaño-Herrera (2009). Therefore, efforts should be made to develop alternative measures, including auto-vaccines, especially for fish farms where more than one antigenic and genetic group of *F. psychrophilum* have been detected.

Keywords: *Flavobacterium psychrophilum*, Chilean farms, serologican and genetic studies

Funding: FONDAP 15110027 and FONDECYT 1190283 from CONICYT, Chile.

026-P

New salmonid hosts for *Tenacibaculum* species: expansion of tenacibaculosis in Chilean aquaculture

Avendaño-Herrera, Ruben (Chile)[1]; Tapia-Cammas, Diana (Chile)[1]; Vargas, Reinaldo (Chile)[2]; Rojas-Carvajal, Jorge (Chile)[1]; Saldarriaga-Córdova, Mónica (Chile)[3]; Oyarzo, Eduardo (Chile)[2]; Gaete, Álvaro (Chile)[2]; Irgang, Rute (Chile)[1]

1 - Universidad Andrés Bello, Laboratorio de Patología de Organismos Acuáticos y Biotecnología Acuícola, Facultad de Ciencias de la Vida, Viña del Mar, Chile, Centro FONDAP, Interdisciplinary Center for Aquaculture Research, Universidad Andrés Bello, Viña del Mar, Chile; 2 - Servicio Nacional de Pesca y Acuicultura, Valparaíso, Chile; 3 - Centro de Investigación en Recursos Naturales y Sustentabilidad, Universidad Bernardo O'Higgins, Santiago, Chile

Introduction: After Norway, Chile is the second largest producer of salmonids worldwide. The success and sustainability of Chilean aquaculture largely depends on the control of endemic and emerging pathogens. *Tenacibaculum dicentrarchi* is classified as an emerging pathogen, despite being detected in Atlantic salmon (*Salmo salar*) since 2010 and, more recently, being described in autochthonous fish species such as the red conger eel. More recently, "*Tenacibaculum finnmarkense*" an Atlantic salmon pathogen isolated previously from Norway was demonstrated and confirmed its presence in Chile by genomic studies. However, no outbreaks of tenacibaculosis in rainbow trout (*Oncorhynchus mykiss)* and coho salmon (*Oncorhynchus kisutch*) had been reported. As part of the surveillance program overseen by the Chilean National Fisheries and Aquaculture Service (SERNAPESCA), 11 different fish farms culturing Atlantic salmon, rainbow trout, and coho salmon were sampled from November 2018 to January 2019. The collected fish presented morbidity and mortalities, evidencing severe body injuries similar to the clinical signs caused by *T. dicentrarchi*.

Methodology: A total of 43 fish were sampled from 11 fish farms, with 31 being Atlantic salmon, 9 rainbow trout, and 3 coho salmon. External lesions on different parts of the body were common among all fish species. Samples from external tissues and internal organs, such as the kidney, liver, and spleen, were collected and analyzed via classical microbiology and the PCR protocol. In addition, tissue samples were analyzed by PCR to rule out infection caused by other bacterial agents, such as *Piscirickettsia salmonis* or *Renibacterium salmoninarum*.

Results: Samples from external and internal organs rendered PCR-positive results for *T. dicentrarchi* regardless of fish species. Microbiological analyses yielded 45, 11, and 1 bacterial isolates from Atlantic salmon, rainbow trout, and Coho salmon, respectively. All were identified as members of the genus *Tenacibaculum* through polyphasic taxonomy, which included phenotypic characterization, PCR, and 16S rRNA sequencing. PCR results were negative for other bacterial pathogens.

Conclusion: This is the first documented occurrence of tenacibaculosis in farmed Chilean rainbow trout and Coho salmon, thus extending the known host distribution of this pathogen in Chile.

Keywords: tenacibaculosis, *Tenacibaculum dicentrarchi*, Chilean salmonids farms

Funding: FONDECYT 1190283 and FONDAP 15110027 from CONICYT, Chile.

027-P*

Prevalence of *Renibacterium salmoninarum* and *Mycobacterium marinum* in wild brown trout (*Salmo trutta fario*) populations in Austrian rivers

Delghandi, Seyed Mohammad Reza (Austria)[1]; Waldner, Karoline (Austria)[1]; Menanteau-Ledouble, Simon (Austria)[1]; El-Matbouli, Mansour (Austria)[1]

1 - Clinical Division of Fish Medicine, University of Veterinary Medicine, Vienna

Introduction: *Renibacterium salmoninarum* and *Mycobacterium marinum* are important Gram-positive bacterial pathogens causing chronic infections in fish: *R. salmoninarum* is the causative agent of bacterial kidney disease (BKD) and has been associated with chronic infections in all salmonid fish at low temperature while *M. marinum* causes mycobacteriosis in fish as well as humans and other mammals. The aim of this study was to evaluate the prevalence of both pathogens in wild brown trout (*Salmo trutta fario*) populations in four Austrian rivers (Kamp, Wulka, Traun and Ybbs).

Methodology: A total of 457 kidney samples were collected from wild *S. trutta fario* between 2017 (212 samples) and 2018 (245 samples). Bacterial cultivation was performed on KDM2 and Lowenstein-Jensen medium alongside histopathological examination on these samples. Moreover, genomic DNAs were extracted and PCRs were performed to determine the presence of either of these pathogens. Positive samples were tested two more times and sequencing was performed on the amplicons followed by alignment to confirm the identity of the resulting sequences.

Results: Molecular evidence in the investigated fish suggested that the average prevalence of *R. salmoninarum* across all rivers was 0.94% in 2017. Conversely, this bacterium could not be detected in 2018. *M. marinum* was only detected in the Kamp river in June 2018, but was then present at high levels of prevalence (37.03% of the fish sampled in this river) while it could not be detected in other rivers or at other sampling points.

Conclusion: This survey provided the first insight into the prevalence rate of *R. salmoninarum* and *M. marinum* in wild brown trout populations in Austrian rivers. Environmental parameters are likely to act as risk factors to facilitate outbreaks of these diseases. For example, the fact that both of these pathogens were only identified in the summer months might suggest that elevated water temperatures could act as a stressor and increase fish susceptibility and contribute to outbreaks of these diseases.

Keywords: molecular epidemiology, nested PCR, prevalence study, wild populations, *Salmo trutta fario*

028-P

Piscirickettsia salmonis outbreak in *Dicentrarchus labrax* in the Atlantic ocean

Girons, Albert (Spain)[2]; Ribeiro, Nuno (United Kingdom)[1]; Pueyo, Carles (Spain)[2]; Ruane, Neil (Ireland)[3]

1 - Aqualife Services; 2 - ICTIOVET; 3 - Marine Institute

Introduction: Infection by *Piscirickettsia salmonis* in *Dicentrarchus labrax* is a rare condition, but it has previously been described as a pathogenic agent in the Mediterranean, namely in France, Greece and Turkey, causing mortalities in both juvenile and adult Sea Bass. This is the first report of the disease in the Atlantic Ocean.

Methodology: The farm is situated on a sheltered port of the west coast of Portugal. This case took place during the transition from Autumn to Winter, and water temperature dropping from 16 to 15 ºC. The average weight was 40 g. Fish from 2 out of 16 cages started to show neurological clinical signs, with circle swimming pattern in great numbers detected by the diving teams, and later also visible from the surface. Moribund fish were necropsied and sampled on site for histopathology (H&E and Geimsa stain), bacteriology from spleen, and brain samples placed on RNA later for PCR. After PCR confirmation, the samples were sequenced.

Results: On the necropsy, fish presented signs of acute onset of disease: normal body condition, remaining gill pathology from a *Diplectanum aequans* previous outbreak plus secondary bacterial infection with *Tenacibaculum maritimum*. No feed in the digestive system and some external haemorrhagic lesions, from possible mechanical trauma during uncoordinated swimming. Bacteriology samples taken from spleen were negative. Histological analysis of brain sections presented with a severe multifocal necrotizing meningoencephalitis. A marked mixed inflammatory infiltrate and congested blood vessels were present in meninges and brain parenchyma including optic lobe. Multiple intracellular basophilic forms compatible with *Rickettsia*-like organisms (RLO) were detected in macrophages cytoplasm. Brain samples from symptomatic fish were analysed with a *Piscirickettsia salmonis* PCR and 7/8 samples were positive. *P. salmonis* strains were sequenced and found that the strain was identical to strain reported by McCarthy *et al.* (2005) from sea bass in the Mediterranean.

Conclusion: After presumptive diagnosis through histopathology, an oral florfenicol treatment was performed for 10 days. During the treatment, mortality rate didn't decrease, but neurological signs decreased significantly. Mortalities ceased 9 weeks after the outbreak started. Total mortality rates were 6.48% in one cage and 3.24% in the other affected cage.

Keywords: European sea bass, *Piscirickettsia salmonis*

029-P*

Siderophore receptors as possible antigens in the formulation of new vaccines against *Aeromonas salmonicida*

Rey, Diego (Spain)[1]; Balado, Miguel (Spain)[1]; Martínez-Matamoros, Diana (Spain)[2]; Rodríguez, Jaime (Spain)[2]; Jiménez, Carlos (Spain)[2]; Lemos, Manuel L. (Spain)[1]

1 - Universidade de Santiago de Compostela; 2 - Universidade da Coruña

Introduction: *Aeromonas salmonicida* is a Gram-negative bacterium which is the causative agent of furunculosis, a disease which severely impact salmonids and non-salmonid fish causing great economic losses in aquaculture worldwide. Therefore, the design of new prophylactic methods against furunculosis becomes a priority in this field. Many pathogenic bacteria depend directly on iron acquisition for their survival within the host; hence one of the main virulence factors is the production of high-affinity iron chelators named siderophores. *A. salmonicida* produces two different kind of catechol siderophores: acinetobactin and four amonabactin forms. In previous works the outer membrane receptor of both siderophores were identified. FstB mediates acinetobactin uptake and FstC is the amonabactins receptor. In the present work we studied the use of acinetobactin (FstB) and amonabactin (FstC) receptors as antigens to develop new vaccines against *A. salmonicida*.

Methodology: For this purpose, FstB and FstC were expressed and purified from *E. coli* membranes. Then, to evaluate immunogenicity (production of fish-specific antibodies) and protection conferred against *A. salmonicida*, two group of 50 fishes were immunized with purified FstC or FstB supplemented with Freund's adjuvant. Groups of 50 fishes immunized with a classical bacterin, PBS or adjuvant alone were used as controls. Antibody titers were determined by ELISA after 30 and after 60 days post-vaccination. Protection degree (RPS) was evaluated by an infection challenge after 60 days.

Results: Both FstB and FstC, proteins induced a significant antibody production. However, the acinetobactin receptor FstB was significantly more immunogenic than amonabactin receptor FstC. Surprisingly, while FstC conferred some protection against *A. salmonicida*, the group of fishes immunized with FstB showed mortality ratios almost identical to unvaccinated controls. Finally, the expression of both siderophore receptors was studied *in vitro* by transcriptional fusions between the siderophore promoters and a promoterless *lux* operon.

Conclusion: Results showed that acinetobactin *fstB* expression is very low compared to amonabactin receptor *fstC*. These findings suggest that acinetobactin genes would be silenced during fish infection. Overall, the results obtained suggest that FstC is the best candidate to be used as antigen in vaccine formulations against the furunculosis produced by *A. salmonicida*.

Keywords: *Aeromonas salmonicida, vaccine, furunculosis, siderophores*

Funding: project AGL2015-63740-C2-1/2-R FEDER(AEI/FEDER-EU).

030-P

Source of bacterial kidney disease (BKD) in Icelandic aquaculture

Stefánsson, Snorri Már (Iceland)[1]; Kristmundsson, Árni (Iceland)[1]; Guðmundsdóttir, Sigríður (Iceland)[1]; Bragason, Birkir Þór (Iceland)[1]; Guðbergsson, Guðni (Iceland)[2]

1 - Keldur Institute for Experimental Pathology, University of Iceland, Reykjavik, Iceland; 2 - Marine and Freshwater Institute in Iceland

Introduction: One very important prerequisite for prospering fish farming is good knowledge on infectious diseases. Since the start of fish farming in Iceland, bacterial kidney disease (BKD), caused by *Renibacterium salmoninarum* (Rs), has produced significant problems in the industry. The most effective way to prevent introduction of the bacterium to the juvenile aquaculture facilities is using pathogen free borehole water. As Rs is common and widely distributed in wild salmonids in Iceland, the use of untreated surface water opens possibilities for the pathogen to enter the aquaculture system. The objective of this study is to examine the origin of Rs infections in three land-based farms in Iceland, and furthermore to investigate the possible effect on wild salmonids in the vicinity of Rs infected Atlantic salmon farms.

Methodology: Material sampled so far: (1) Wild salmonids collected from brooks, which two farms used as a water source to some extent; (2) Fingerlings from these two farms (salmon and Arctic charr); (3) Water samples: (a) At entry into the facility; (b) inside the farms before it reaches the tanks (c) waste water disposed from the farms and into the fjord. The fish were examined for clinical signs of BKD and all samples were screened for Rs using polyclonal ELISA and qPCR.

Results: All 51 salmonids sampled from the brooks (source water) were positive for Rs antigens in ELISA while four were positive in qPCR. All fish sampled from Farm I, some of which showing clinical signs of BKD, were positive in both ELISA and qPCR, while no fish were positive from Farm II in either test. All the water samples were positive in ELISA while only one sample was positive in qPCR.

Conclusion: The results show that the Rs is present in wild salmonids and water samples from the brooks used, to some extent, as source water in the farms. That poses a risk for Rs being introduced into the farms. UV treatment, which has recently been set up in one of the farm, might solve that problem. However, using solely pathogen free borehole water is without doubt the safest option.

Keywords: bacterial kidney disease, BKD, aquaculture, Iceland, *Renibacterium salmoninarum*

032-P

Vibrio spp. isolated from pacific white shrimp (*Litopenaeus vannmei*) kept in recirculating aquaculture systems (RAS) – identification and pathogenicity

Bauer, Julia (Germany)[1]; Teitge, Felix (Germany)[1]; Neffe, Lisa (Germany)[1]; Adamek, Mikolaj (Germany)[1]; Jung, Arne (Germany)[1]; Peppler, Christina (Germany)[2]; Steinhagen, Dieter (Germany)[1]; Jung-Schroers, Verena (Germany)[1]

1 - University of Veterinary Medicine Hannover; 2 - Polyplan GmbH Bremen

Introduction: *Vibrio* spp. are ubiquitous bacteria in marine and brackish aquatic environments and these bacteria are a part of the normal flora of shrimps´ digestive tract and skin. Some species play an important role as pathogens in aquaculture of shrimps (e.g. *V. parahaemolyticus*, *V. harveyi)*, others are the cause of wound or diarrhoeal infections in humans (e.g. *V. parahaemolyticus*, *V. vulnificus*). For the detection of *Vibrio* sp. a reliable method of identification is necessary and for evaluation of the potential risk that derives from these isolates the analysis of different pathogenicity factors is possible.

Methodology: This study compared different methods of identification, which are suitable for standard diagnostics in laboratories. The *16S* rRNA gene with two different sequence lengths (V1-V5, V1-V8) and the housekeeping gene *pyrH* were sequenced. Additionally, a biochemical identification and an identification using mass spectrometry (MALDI-TOF) were conducted. Different pathogenicity factors were analysed that are known to induce an elevated pathogenicity in certain *Vibrio* spp.. These include factors that elevate the pathogenicity of *V. cholerae* (VPI, ToxR, ToxS), as well as different haemolysins (*vhh*, *vfh, tdh, trh*). The motility of the isolates was analysed and the presence of different flagelline gene loci was investigated, as these contribute to motility in bacteria and can promote the adhesion on mucous membranes.

Results: The investigations on the *Vibrio* isolates from RAS and type strains using the different identification methods show divergent results for many of the isolates. The *16S* rRNA sequencing of the variable regions V1-V8 proved to be the most reliable method. Furthermore, using MALDI-TOF mass spectrometry fast and reliable results are possible, especially for pathogenic species. Difficulties in reliably identifying some specific *Vibrio* sp. might derive from missing entries of sequences in the databases, due to low numbers of comparable isolates, and high interspecific similarities of biochemical characteristics or nucleotide sequences.

Conclusion: The identification of *Vibrio* spp. using standard diagnostic methods was difficult and for many isolates, the identification results were divergent. The investigations on isolates from different systems show that potentially pathogenic *Vibrio* spp. can be found in RAS.

Keywords: *Vibrio*, pathogenicity factors, *Litopenaeus vannamei*, identification

033-P

Silver nanoparticles treatment of bacterial diseases of common carp

Sellyei, Boglárka (Hungary)[1]; Székely, Csaba (Hungary)[1]; Shaalan, Mohamed (Egypt)[2]

1 - Institute for Veterinary Medical Research, Centre for Agricultural Research Hungarian Academy of Sciences; 2 - Department of Veterinary Pathology, Faculty of Veterinary Medicine, Cairo University, Egypt

Introduction: Fish infections caused by *Flavobacterium* species represent major threats to commercial aquaculture worldwide. Several species of the genus, including *F. johnsoniae*, *F. psychrophilum*, *F. branchiophilum*, and *F. columnare*, have been associated with clinical disease in fish. Prevention of flavobacteria epizootics is difficult due to their ubiquitous presence in waters and due to decreased susceptibility to many antimicrobial agents routinely used in aquacultural environments. The identification of multidrug resistant (MDR) strains, reported in different countries worldwide including Hungary, indicates that the control of disease outbreaks caused by *Flavobacterium* spp. remains a significant challenge. Silver nanoparticles (Ag-NPs) are known for their potent antimicrobial activity against different types of bacteria, so they are increasingly used as a novel alternative to antibiotics. Silver nanoparticles disrupt the bacterial cell membrane and direct complete cell lysis and leakage of intracellular content.

Methodology: In this study, we evaluated the antibacterial properties of silver nanoparticles (diameter = 23 nm) against *Flavobacterium johnsoniae* infection in common carp (*Cyprinus carpio*). The fish experiments included the artificial infection with *F. johnsonia*e which was followed with Ag NPs treatment (immersion or intraperitoneal injection).

Results: Assessment of antibacterial activity and the evaluation of their effect to the fish tissue were conducted. In this experiment, mortality rates reduced from 45% in infected non-treated group to 30% and 15% in intraperitoneal injection and immersion-treated groups, respectively. The clinical signs and histopathologic lesions only in gills, kidney and liver could be observed in the positive control groups , two weeks after infection.

Conclusion: The silver nanoparticles are promising as alternative to antibiotics, especially at the onset of bacterial infection including infections with antibiotic-resistant bacteria. The single dose treatment with Ag-NPs during early infection with F. johnsoniae aided in minimizing fish losses. As a therapeutic application to the fish farms, it is worth mentioning that the silver nanoparticles treatment should be conducted in the night.

Keywords: *Flavobacterium johnsoniae*, silver nanoparticles, common carp, antibacterial, fish diseases

Funding: Parafishcontrol project and the TEMPUS scholarship awarded to Mohamed Shaalan (AK-00362-002/2018).

035-P

Genotyping of Turkish *Tenacibaculum maritimum* isolates

Steinum, Terje (Turkey)[2]; Turgay, Emre (Turkey)[3]; Yardımcı, Eda (Turkey)[2]; Karataş, Süheyla (Turkey)[1]

1 - Faculty of Sciences, Department of Molecular Biology and Genetics, Istanbul University, Istanbul, Turkey; 2 - Faculty of Aquatic Sciences, Department of Fish Health, Istanbul University, Istanbul, Turkey

Introduction: Despite vaccination, tenacibaculosis still cause severe economic losses in marine aquaculture and the Turkish sea bass industry is no exception. Vaccines developed for northern Europe likely provide less protection in Turkey so characterization of endemic *T. maritimum* isolates is the first step to develop more efficient vaccines. The purpose of this work was therefore to genotype isolates from farms on the south-eastern coast of Turkey.

Methodology: 18 bacterial isolates from Turkish cultivated sea bass suffering from tenacibaculosis and their MLST profiles were compared to international isolates. The MLST scheme utilize concatenated partial sequence data from seven housekeeping genes *atpA*, *dnaK*, *gyrB*, *glyA*, *tgt*, *infB* and *rlmN* analyzed by maximum likelihood (ML) based phylogeny.

Results: It was determined that the 18 Turkish *T. maritimum* isolates could be grouped into five different sequence types (ST). However, only two of these isolates belonged to a previously defined sequence type (ST3) represented by international isolates from Spain (*Senegalese sole*) and France (European bass).

Conclusion: The remaining 16 Turkish *T. maritimum* isolates were found to constitute four novel sequence types. These new sequence types are most closely related to the defined sequence type ST2 represented by a *Senegalese sole* isolate from Portugal, to sequence type ST30 represented by a sea bass isolate from Malta and to sequence type ST22 represented by Atlantic salmon and Rainbow trout isolates from Australia and Tasmania.

Keywords: *Tenacibaculum maritimum*, multilocus sequence typing, sea bass

Funding: The Scientific Research Project Coordination Unit of Istanbul University (project no: FBA-2016-21250).

036-P

Evaluation of metabolic changes and virulence of *Piscirickettsia salmonis* strains related to biofilm formation

Haussmann, Denise (Chile)[1]; Zuñiga, Alejandro (Chile)[2]; Cartes, Carlos (Chile)[2]; Lagos, Fernando (Chile)[2]; Solis, Camila (Chile)[2]; Figueroa, Jaime (Chile)[2]

1 - Universidad Santo Tomas, Valdivia; 2 - Universidad Austral de Chile

Introduction: *Piscirickettsia salmonis* is a facultative, intracellular, bacterial pathogen and etiological agent of SRS or piscirickettsiosis, a disease that affects salmonid species and the source of great economic losses since its appearance. There are multiple bacterial species generating biofilms as an adaptive mechanism for protection against environmental challenges. The flexibility in gene expression of *P. salmonis* allows for its survival in hostile marine environments and when subjected to multiple stressful conditions. Therefore, both the persistence and proliferation of strains depend on their capacity to generate new biologically active compounds and on their resistance to antibiotic treatments applied in mass culture salmonid centers in Chile.

Methodology: The aim was to analyze genes in the toxin-antitoxin system associated with the formation of biofilms in solid and liquid culture media, and to evaluate the adaptive behavior of *P. salmonis* strains to the culture media. The expression of genes involved in the metabolism of Biofilm generation by qPCR was evaluated under different culture conditions.

Results and Conclusion: The results proved that the three strains: LF-89, IBM034, and IBM040 are strong producers of biofilms in CASO and TSA medium, showing adaptability to the environmental conditions due to an increased capacity to generate biofilms when making subcultures and new growths. The transcript levels of six genes described in other bacteria were also analyzed due to their relevance in the biofilm formation and because of their relationship with other important metabolic functions, placing the focus on evaluating the functionality of the bacteria in the biofilm. The mazE and masF genes, involved in the formation of biofilms under stress conditions, stand out from others since they are markedly up-regulated in the biofilm condition of the three strains analyzed. For its part, the expression of gene gltA, an indicator of metabolic activity and related with virulence inhibition in *S. typhimurium*, seems also to inhibit the bacterial pathogenesis mechanism in *P. salmoni*s, through inhibition of the LISO and TCF gene expression associated with virulence. Finally, the expression of glnA suggests the use of glutamine, an essential factor for the growth of *P. salmonis* biofilms.

Keywords: *Piscirickettsia salmonis*, biofilm, metabolic changes, mechanism for protection

Funding: FONDAP-INCAR-15110027.

037-P

Host-pathogen interaction in the *P. salmonis* - Atlantic salmon model: effect of salinity as a parameter of climate change

Figueroa, Jaime (Chile)[1]; Cartes, Carlos (Chile)[1]; Lagos, Fernando (Chile)[1]; Solis, Camila (Chile)[1]; Haussmann, Denise (Chile)[2]

1 - Universidad Austral de Chile; 2 - Universidad Santo Tomas, Valdivia

Introduction: Piscirickettsiosis is the most harmful diseases with a negative impact on salmon farming in Chile, producing major economic losses to the aquaculture industry. The Gram-negative, intracellular, and facultative pathogen *Piscirickettsia salmonis* is the etiological agent of this disease. Therefore, it is relevant to determine the factors that affect the pathogenicity of the various strains of this bacterium. Salinity plays a role in the survival of *P. salmonis*, and it has been found that optimal concentrations favor its growth.

Methodology: The effect of water salinity (5‰ and 20‰) on the capacity of this pathogen to infect *Salmo salar*, as a relevant parameter of climate change affecting salmon cultivation, was evaluated. The anterior kidney, an important organ in the mechanism of immune response, was employed to analyze the expression of the Toll-Like-Receptor (TLR) gene family, specifically TLR1, TLR5m, TLR9, and TLR13. The TLRs are a critical part of the innate immune response system and facilitate host recognition of pathogen-associated molecular patterns (PAMPs). Therefore, the expression of these genes seems adequate to evaluate the pathogenicity of *P. salmonis* strains and the effect of salinity. Thus, OS salmon parr (~90 g) were experimentally infected *in vivo* with strains AUS005 and AUS111 at two salinities (5‰ and 20‰). After the challenge, cumulative mortality was quantified and the levels of transcripts (qPCR) of the above-indicated TLRs at 5, 10, 15, 20, 25, 37, 44, and 48 days post infection were evaluated.

Results and Conclusion: The fish challenged with the isolate AUS005 of *P. salmonis* at a salinity of 5‰, showed higher mortality rates than fish at a salinity of 20‰ with the same bacteria. The challenge test for cohabitation with *P. salmonis* AUS0213 at 20‰ salinity showed a standard behavior, with mortalities ranging between 50-60%. The quantification analyses of TLR transcripts showed a differential response to the two salinity conditions, a feature that related with mortalities. However, TLR1 and TLR13 were up-regulated at both salinities when compared to control fish. On the other hand, TLR5m was up-regulated at the high salinity condition only and TLR9 was down-regulated with respect to the control fish.

Keywords: *Piscirickettsia salmonis*, virulence, climate change, Atlantic salmon

Funding: FONDAP-INCAR-15110027.

038-P*

Comparative genomic analysis of *Aeromonas veronii* bv *sobria* isolated from European sea bass. Virulence, antibiotic resistance and antigenic proteins

Triga, Adriana (Greece)[1,2]; Smyrli, Maria (Greece)[1,2]; Katharios, Pantelis (Greece)[1]

1 - Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Heraklion, Greece; 2 - Department of Biology, University of Crete, Greece

Introduction: *Aeromonas veronii* bv *sobria* is an emerging pathogen for European sea bass, *Dicentrarchus labrax,* farmed in the Aegean Sea, Greece. Nine strains isolated from diseased European sea bass were selected; these strains were representatives of a larger bacterial collection and were grouped according to their geographic origin, phenotype (e.g. motility, pigment production) and isolation time (2009-2015). Comparative genomic analysis was conducted targeting pathogenicity-related and antibiotic resistance genes. *In silico* analysis of the genes encoding antigenic proteins was also performed following the principles of reverse vaccinology.

Methodology: Paired-end sequencing was performed using an Illumina MiSeq platform (Illumina, Inc.). *De novo* assembly was done using the Masurca assembler. Gene identification and annotation were done using the NCBI Prokaryotic Genome Annotation Pipeline. Virulence and antibiotic resistance genes were identified using the PATRIC annotation platform. Outer membrane proteins were predicted using the PSORTb v3.0.2 tool. The Genomic Islands analysis was conducted in the online platform IslandViewer. The Geneious 9.1.8 was used for the sequences analysis and genome browsing.

Results: The nine strains generally contained the same repertoire of virulence genes and outer membrane proteins, such as secretion system (type II, III, VI), flagella, PilT, PilW, Hcp1, hemagglutinin, hemolysin D and III, aerolysin family toxin, RTX toxin, iron-binding and transport systems, the maltoporin omp48, ompA, ompW etc. The strains encoded ampicillinases, carbapenemases, quinolone, chloramphenicol resistance genes, and aminoglycoside-modifying enzymes. Sequence polymorphisms reflected mainly the geographical origin. For instance, following the comparison, the omp48 which encodes one of the most important antigenic proteins in aeromonads, has at least two antigenically distinct groups, while the type VI secretion system gene cluster is missing from the strains isolated from a specific geographic location. The loci of the virulence and antigenic genes were frequently found within Genomic Islands (GIs), indicative of horizontal gene transfer. In total, GIs constituted ten percent of each genome.

Conclusion: The isolates from European sea bass were similar in terms of pathogenicity mechanisms, antigenic proteins and antibiotic resistance mechanisms. These findings suggest the potential multidrug resistance of the pathogen and the overall genomic profile pinpoints the importance of addressing it as a future aquaculture problem.

Keywords: genomics, virulence factors

039-P*

Monitoring of bacterial infections in sturgeons reared in Italy

Pastorino, Paolo (Italy)[1]; Mugetti, Davide (Italy)[1]; Menconi, Vasco (Italy)[1]; Righetti, Marzia (Italy)[1]; Ceresa, Luisa (Italy)[1]; Foglini, Claudio (Italy)[1]; Pedron, Claudio (Italy)[2]; Prearo, Marino (Italy)[1]

1 - Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy; 2 - Medico Veterinario, Settala (MI), Italy

Introduction: Sturgeon farming has become increasingly important worldwide for the production of caviar and fish flesh, as well as for restoration programs set up to save endangered wild populations. Despite this, there is limited information about diseases that affect sturgeon farming in Italy. Accordingly, in this study we describe some cases of bacterial infection during a monitoring campaign carried out from 2014 to 2017 in Italian sturgeon farms.

Methodology: A total of 402 sturgeons from six sturgeon farms with one or more reared sturgeon species among five species (*Acipenser gueldenstaedtii*, *Acipenser baerii*, *Acipenser naccarii*, *Acipenser transmontanus* and *Huso huso*) and two hybrids (*A. baerii* x *A. gueldenstaedtii* and *Huso huso* × *Acipenser ruthenus*) were analyzed. The subjects were necropsied under aseptic conditions and evaluated for the presence of lesions such as wounds, bleeding or other pathological alterations. Collection of samples for bacteriological examination was taken from kidney and brain using first isolation media (Columbia Blood Agar). The colonies grown after 24 - 72 hours of incubation at 22 ± 2 °C were selected, cloned in selective media and identified by biochemical tests using API galleries (bioMérieux).

Results: Generally, no external or internal macroscopic lesions were present, though some specimens showed skin lesions, liver anaemia or splenomegaly, which are non-pathognomonic signs of bacterial infection. Bacteriological exam was positive in 93 individuals (23%) and negative in the remaining 309 (77%). The most isolated bacteria in these species was attributed to *Aeromonas hydrophila* and *A. sobria* (38.71% and 13.98%, respectively), *Plesiomonas shigelloides* (15.05%), *Pseudomonas* spp. (13.98%), *Yersinia ruckeri* (6.45%) and other (11.83%) environmental bacteria.

Conclusion: the majority of the isolated bacteria were opportunistic, suggesting that environmental conditions strong influenced the outbreaks of disease. This knowledge base is important to help create a bacterial profile of sturgeons reared in Italy.

Keywords: sturgeons, bacterial infections, *Aeromonas hydrophila*, *Yersinia ruckeri*

040-P

Identification of *Mycobacterium pseudoshottsii* in Italy

Mugetti, Davide (Italy)[1]; Gustinelli, Andrea (Italy)[2]; Pastorino, Paolo (Italy)[1]; Menconi, Vasco (Italy)[1]; Fioravanti, Marialetizia (Italy)[2]; Prearo, Marino (Italy)[1]

1 - Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy; 2 - Dipartimento di Scienze Veterinarie, Università degli Studi di Bologna, Alma Mater Studiorum, Ozzano dell'Emilia (BO), Italy

Introduction: *Mycobacterium marinum* is one of the most recognized species as causative agent of fish mycobacteriosis. The use of molecular biology methods allows to identify other species of mycobacteria within the *M. marinum* complex. Among them, *M. pseudoshottsii*, concurrently with *M. shottsii*, was responsible for an acute episode in striped bass (*Morone saxatilis*) in Chesapeake Bay. Subsequently, between 1999 and 2008 the same species was recognized as a causative agent of disease in white perch (*Morone americana*) in Maryland and in different marine species (*Seriola quinqueradiata*, *S. dumerili*, *S. lalandi*, *Epinephelus septemfasciatus*) bred in western Japan.

Methodology: Sixty-eight fish (46 European sea bass, 21 red drum, 1 gilthead sea bream) were analysed in the period between May and October 2018 by the Fish Diseases Laboratory of the Department of Veterinary Sciences of the Bologna University. At anatomopathological examination, splenic and renal miliary nodules were detected, which led to suspicion of an infection sustained by nontuberculous mycobacteria. All fish were subsequently sent to the Fish Diseases Laboratory of the Istituto Zooprofilattico Sperimentale of Turin for isolation and species identification of mycobacteria.

Results: In 21 fish (19 sea bass, 1 red drum, 1 sea bream) acid-fast bacilli were isolated and subjected to biomolecular analysis for species recognition. Several genes (*16S rRNA*, *hsp65*, *rpoB*) were amplified and sequenced, leading to the identification of isolates as *M. pseudoshottsii*.

Conclusion: In this work, cases of mycobacteriosis sustained by *M. pseudoshottsii* are reported for the first time in Italy, specifically in sea bass (*Dicentrarchus labrax*), red drum (*Sciaenops ocellatus*) and sea bream (*Sparus aurata*) bred in lower Adriatic and in sea bass farmed in medium Tyrrhenian. A widespread and constant health monitoring will help to understand the real danger that this mycobacterium could represent for Italian farms.

Keywords: *Mycobacterium* spp., mycobacteriosis, *Dicentrarchus labrax*, *Sciaenops ocellatus*, *Sparus aurata*

041-P

Elucidation of the role of *Tenacibaculum* spp. in atypical winter ulcer in sea-farmed Atlantic salmon in Norway

Olsen, Anne Berit (Norway)[1]; Spilsberg, Bjørn (Norway)[2]; Nilsen, Hanne Katrine (Norway)[1]; Tavornpanich, Saraya (Norway)[2]; Lagesen, Karin (Norway)[2]; Colquhoun, Duncan (Norway)[2]

1 - Norwegian Veterinary Institute, P.O.Box 1263 Sentrum, 5811 Bergen, Norway; 2 - Norwegian Veterinary Institute, P.O.Box 750 Sentrum, 0106 Oslo, Norway

Introduction: The disease "tenacibaculosis" otherwise known as "atypical winter ulcer" has become increasingly common in Norwegian Atlantic salmon farming within the last decade. This disease is primarily characterised by head and/or fin erosions. Losses may be acute and very high. The reasons for the recent emergence of "tenacibaculosis" as a common and severe disease in Norwegian salmon farming remain unknown. It is, however, well recognised that most diseases affecting farmed fish are a result of the interplay between environment, host species and pathogenic agent i.e. the presence of the "pathogen" alone may not necessarily lead to manifestation of disease. The role of *Tenacibaculum* spp. in "atypical winter ulcer" is studied.

Methodology: Affected fish farms were sampled by fish health practitioners. An epidemiological investigation, including the use of questionnaire and farm visits for data collection, was used to reveal impact of the disease on farm production and survival rate among the outbreak sites. Histopathological and microbiological investigations were performed and *Tenacibaculum* isolates were whole genome sequenced (MiSeq).

Results: 16 geographically spread outbreaks of "atypical winter ulcer" along the Norwegian coast line in 2018 were studied. Histopathological examination showed severe tissue necrosis of the head with presence of numerous bacteria, dominated by long, filamentous rods. *Tenacibaculum* was isolated in mixed or pure culture. The whole genome sequencing of 78 isolates revealed that most bacterial populations associated with the majority of outbreaks were non-clonal. A few outbreaks, however, appeared to be clonal. A large majority of the isolates were identified as "*T. finnmarkense*". *T. dicentrarchi* and as yet undescribed *Tenacibaculum* species were also isolated. Further results, including the epidemiological investigation will be presented.

Conclusion: The *Tenacibaculum* population associated with "atypical winter ulcer" in majority of outbreaks studied belongs to "*T. finnmarkense*", but is genetically heterogenous. The non-clonal population structure indicates development of localised disease outbreaks dependent on environmental conditions and fish health status.

Keywords: atypical winter ulcer, *Tenacibaculum,* epidemiology, bacteriology

Funding: FHF – Norwegian Seafood Research Fund, no. 901434.

042-P

Phylogenetic diversity among *Aliivibrio wodanis* isolates from winter ulcer outbreaks in Norwegian semi-closed containment farming of Atlantic salmon post smolt

Lotherington, Leif (Norway)[1]; Eriksen, Jonas (Norway)[2]; Fedorczyk, Kamil (Norway)[2]; Markussen, Turhan (Norway)[1]; Sørum, Henning (Norway)[1]

1 - Department of Food Safety and Infection Biology, Faculty of Veterinary Medicine, Norwegian University of Life Sciences; 2 - Department of Mechanical, Electronic and Chemical Engineering, Oslo Metropolitan University

Introduction: Classical winter ulcer outbreaks in salmonid fish are caused by the bacterium *Moritella viscosa*. However, parallel isolation of *Aliivibrio wodanis* from ulcers has been reported since the 1980s. A causative link between *A. wodanis* and winter ulcer is yet to be determined. The identification and isolation of *A. wodanis* from recent outbreaks of winter ulcer disease in semi-closed containment post smolt facilities in Norway was used to study the epidemiology of this bacterium both inside individual farming facilities and along the Norwegian coast. To investigate the diversity of *A. wodanis* during infections, isolates from three separate outbreaks of winter ulcers were selected for Multilocus Sequence Analysis (MLSA). Of particular interest was the genetic relationship between isolates and their distribution along the coast.

Methodology: Isolates were collected from winter ulcer outbreaks; i) from a land based flow-through post smolt site in Mid-Norway in December 2015, ii) from sea-based semi-closed pens in Northern Norway in June 2016, and iii) from sea-based semi-closed pens in March 2018 in close proximity to the second outbreak. Twenty-eight isolates were sub-cultured on BA 2.5% (5% bovine blood agar with 2.5% NaCl) for 48 hours before DNA extraction. Primers were designed for six housekeeping genes, and partial sequences amplified by PCR. The PCR products were Sanger sequenced and analyzed by MLSA. Multiple sequence alignments and phylogenetic analyses were preformed using AlignX (Vector NTI Advance 11 package) and MEGA-X software.

Results: Preliminary results indicate high level of similarities between the sequences analyzed. Minor sequence differences were observed for isolates originating from both within and across the three outbreaks.

Conclusion: The results so far suggest significant homogeneity between *A. wodanis* isolates originating from outbreaks separated in both time and space. This may strengthen the hypothesis that *A. wodanis* plays a causative role in the development of winter ulcer.

Keywords: winter ulcer, *Aliivibrio wodanis*, MLSA, Atlantic salmon

043-P

Short-term immune responses of meagre (*Argyrosomus regius*) juveniles against *Photobacterium damselae piscicida*

Santos, Paulo (Portugal)[1]; Machado, Marina (Portugal)[1]; Passos, Ricardo (Portugal)[2]; Pires, Pedro (Portugal)[2]; Baptista, Teresa (Portugal)[2]; Costas, Benjamin (Portugal)[1]

1 - Centro Interdisciplinar de Investigação Marinha e Ambiental; 2 - Instituto Politécnico de Leiria

Introduction: One of the challenges of fish farming industry is the occurrence of disease outbreaks which lead to important monetary losses. In this context, the study of suitable biomarkers to assess fish health status, such as haematological and immune responses during the first steps of infection could assist in the creation of measures of recognition and prevention of disease. The present study was conceived to evaluate meagre (*Argyrosomus regius*) innate immune response after infection with *Photobacterium damselae piscicida* (*Phdp*).

Methodology: A time-course study was performed at CETEMARES (Instituto Politécnico de Leiria, Peniche, Portugal) facilities with 72 animals being sampled (79.3 ± 15.1 g). Among them, 12 fish were randomly selected and sampled before infection (time 0). Thereafter, the remaining animals were randomly selected and intraperitoneally injected (i.p.) with 100 µl PBS (control group) or 100 µl of exponentially growing bacteria (10^5 CFU/mL; infected group) and distributed as a complete randomized design in 6 recirculating seawater systems (i.e. triplicates per group). Two animals per tank (n = 6) were randomly selected and sampled at 3, 6, 9, 24 and 48 h after i.p. injection. At each sampling point, fish were anaesthetized and blood samples were collected for haematological procedures such as total and differential counting of peripheral leukocytes and total circulating erythrocytes counts. The remaining blood was centrifuged and plasma was collected for innate humoral parameters determination (i.e. bactericidal, antiproteases, proteases, nitric oxide and peroxidase activities).

Results: Similarities were found among cellular and humoral parameters in challenged fish. Infected meagre presented increased peripheral white blood cells concentration compared to control. Peripheral lymphocyte numbers increased in infected meagre from 0 h to 24 h while circulating neutrophils decreased in challenged fish regardless time, most likely due to migration to the inflammatory focus. Plasma peroxidase activity increased over time in both groups and bactericidal activity increased in infected specimens after 24 h. Nitric oxide analysis resulted in increased values after 48h in infected group compared to control animals.

Conclusion: Samples of head-kidney tissue will be suited the assessment of mRNA immune-related gene expression in order to understand how *Phdp* infection influences meagre immune machinery.

Keywords: infection, leukocytes, immune response, bactericidal activity, neutrophils

044-P

Detection of virulence genes related with tissue invasion in *Tenacibaculum maritimum*

Torres, Beatriz S. (Spain)[1]; Buján, Noemí (Spain)[1]; E. Toranzo, Alicia (Spain)[1]; Magariños, Beatriz (Spain)[1]

1 - Facultade de Bioloxía-CIBUS and Instituto de Acuicultura, Dpto. de Microbioloxía e Parasitoloxía, Universidade de Santiago de Compostela, Santiago de Compostela

Introduction: *Tenacibaculum maritimum*, a Gram negative and filamentous bacterium, has been described as the etiological agent of tenacibaculosis in marine fish. It causes significant losses in a number of economically important marine fish species worldwide. This disease is characterized by frayed fins, tail rot, mouth erosion, and skin lesions that are often ulcerative. To invade and colonize the tissue before the immune response limits bacterial growth, some virulence mechanisms such us adhesion to hydrophobic surfaces, hemagglutination or extracellular products including proteolytic activity are important. *T. maritimum* has mechanisms to invade and colonize host tissues including membrane damaging enzymes potentially involved in host cells lysis like ceramidase, chondroitinase and sphingomyelinase. These enzymes are part of the mechanism responsible of the typical ulcers caused by tenacibaculosis. The objective of this work is to study the variability of the ceramidase, chondroitinase, sphingomyelinase and sialidase genes among strains of *T. maritimum.*

Methodology: To carry out this objective a group of *T. maritimum* strains isolated from different countries and years, and hosts are selected. Primers used for the amplification of ceramidase, chondroitinase and sialidase genes were designed, and for sphingomyelinase gene, we used the primers described by Pérez-Pascual et al. (2017). The activity of the enzymes chondroitinase and sialidase were tested and compared by phenotypical tests.

Results: All strains presented the four genes studied. The highest variability was found in the chondroitinase gene with 54 polymorphic sites and 16 non-synonymous changes. The chondroitinase and sialidase activities were positive in all strains with differences in the degradation.

Conclusion: The differences in the nucleotide and aminoacidic sequences of these enzymes could affect their activities *in vivo* and, consequently, the degree of virulence and penetration capacity of *T. maritimum*.

Keywords: *Tenacibaculum maritimum*, virulence factors

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045-P

Epitheliocystis in Nile tilapia (*Oreochromis niloticus*) in Costa Rica

Calderón Salazar, Sharon (Costa Rica)[1]; Rodríguez Badilla, Raquel (Costa Rica)[1]; Coto Vargas, Jazmine (Costa Rica)[1]; Blanco Peñaranda, Tania (Costa Rica)[1]; Méndez Alfaro, Kevin (Costa Rica)[1]; Chaves Madriz, Yendry (Costa Rica)[1]; Guevara Suárez, Marina (Costa Rica)[1]; Guevara Soto, Maricruz (Costa Rica)[1]; Schmidt-Posthaus, Heike (Switzerland)[2]; Hirschi, Regular (Switzerland)[2]

1 - National Technical University of Costa Rica; 2 - University of Bern

Introduction: Epitheliocystis is a skin and gill bacterial disease worldwide spread in freshwater and marine fish, a total of 90 species have been affected by the disease and has been causing high mortalities in wild fish and aquaculture farms. The disease is caused by an intracellular bacteria the most common pathogens are chlamydial or betaproteobacterium. The target organ in Nile tilapia is the gill, but usually no gross lesions are seen, the microscopy reveal the presence of multiple cysts that vary from 14 to 30 microns with a basophilic granular material into the cyst.

Methodology: In Costa Rica, epitheliocystis has been observed in several farms but not reported as a main disease of mortality in Nile tilapia, this case is found in an aquaculture facility, necropsy was performed in fingerlings of 5 cm length and no mortalities were reported ,gills and other organs were collected for histopathology examination.

Results: The microscopy reveal the presence of a multifocal cysts in several lamellas, the cyst had different sizes and consists of agranular basophilic material. The cyst were in the lamella tissue causing compression of the normal structures. Gill DNA extraction, PCR and sequencing will be performed in order to detect the bacterial agent affecting Nile tilapia in Costa Rica.

Conclusion: the results for the PCR and the sequencing we will be able to know the agent that causes this lesion in tilapia costa rican farms.

Keywords: Epitheliocystis, bacteria

Funding: *National Technical University, Costa Rica.*

046-P*

The expression analysis of hemoglobin gene in Japanese flounder infected with *Edwardsiella piscicida*

Mori, Misato (Japan)[1]; Washio, Ryota (Japan)[1]; Ito, Tasuku (Japan)[1]; Namba, Aki (Japan)[1]; Anzai, Hirosi (Japan)[1]; Nakanishi, Teruyuki (Japan)[1]; Mano, Nobuhiro (Japan)[1]

1 - College of Bioresouce Sciences, Nihon university, Kanagawa, 252-0880, Japan

Introduction: In vertebrates, hemoglobin consists of a tetramer of two α- and two β-globin chains each containing a prosthetic heme group, and is primarily involved in oxygen delivery to tissues and in redox reactions. Furthermore, the presence of α- and/or β-globin chain in tissues besides red blood cells including peripheral tissues and brain suggests that the derived peptides play additional physiological functions in each tissue. In fish, β-globin gene expression in non-erythroid cells has recently been identified in several tissues, and the β-globin derived peptides (βHd) are reported as one of antibacterial peptides. However, the knowledge regarding the effects of stress and infection on the expression is scarce. The present study investigated the expression pattern of β hemoglobin gene in each tissue of Japanese flounder *Paralichthys olivaceus* infected with *E. piscicida*.

Methodology: After acclimation for 7 days, fish were divided into two groups, and then immersed in bacterial suspension (2.0×10^7 CFU/ml, infected group) and HI broth (control group). Five fish per group were sampled at pre-infection, 3, 12 and 24 h post-infection, and epidermis, gill, spleen, kidney, liver and intestine tissues were collected for gene expression analysis by qRT-PCR.

Results: In infected group at 3 h post-injections, β hemoglobin gene expression levels significantly up-regulated in epidermis and kidney tissues, compared with control group.

Keywords: mucosa, innate immunity, Japanese flounder

Funding: This work was supported by JSPS KAKENHI Grant Number 16H04984.

Parasitological Diseases

047-P

Parasites of *Trachurus capensis* and *T. trecae* in the Benguela ecosystem

Reed, Cecile (South Africa)[1]; Nunkoo, Irfan (South Africa)[1]; Van Der Lingen, Carl (South Africa)[2]; Mackenzie, Ken (United Kingdom)[3]

1 - University of Cape Town; 2 - Department of Agriculture, Forestry and Fisheries; 3 - University of Aberdeen

Introduction: Parasites of Cape horse mackerel, *Trachurus capensis* Castelnau, 1861 were surveyed in the northern and southern Benguela ecosystems off the west coast of southern Africa to test the existence of discrete stocks of this fish species in each of these subsystems. Similarly parasites of West African horse mackerel or Cunene horse mackerel, *Trachurus trecae* Cadenat, 1950 were surveyed in the northern Benguela ecosystem where they co-exist with *T. capensis* to examine interspecifc differences in parasite communities between these two closely related fish species living in the same environment.

Methodology: Samples of *T. capensis* and *T. trecae* were collected by the Norwegian research vessel, FRS Dr Fridtjof Nansen during 2011 from the Northern Benguela, off the coasts of southern Angola and northern Namibia. While samples of *T. capensis* only were collected by the commercial fishing vessel, F.V. Dessert Diamond in March, August and September 2012 from the southern Benguela off the coast of South Africa. All fish were frozen on board and later examined for parasites using standard procedures.

Results: In total 29 different parasite species were found infecting the 175 *T. capensis* individuals examined, while 11 parasites species were found infecting the 48 *T. trecae* individuals examined. Results show that there are significant differences in parasite community structures between fish from differing size classes, between fish of differing species and between *T. capensis* from the northern and southern Benguela. This suggests that there is a host size effect for many parasite species which is consistent with much of the literature. *Trachurus capensis* and *T. trecae* from the same location, of the same size class and caught in the same season had differing parasite community compositions. No seasonality was apparent in parasite community composition of *T. capensis*.

Conclusion: These results show that parasite assemblages are useful biotags to distinguish between stocks of *T. capensis* in the northern and southern Benguela, as well as to distinguish between the closely related *T. tracurus* and *T. capensis* in the Northern Benguela.

Keywords: Africa, biological tagging, horse mackerel

Funding: UCT Research Committee Travel Grant.

048-P

Contracaecum osculatum larvae in the liver of Baltic cod and its impact on condition and mortality of the host

Podolska, Magdalena (Poland)[1]; Horbowy, Jan (Poland)[1]; Nadolna-Ałtyn, Katarzyna (Poland)[1]

1 - National Marine Fisheries Research Institute

Introduction: In the last century, parasitic infection with anisakid nematodes affected cod in the Baltic Sea only marginally. Recent studies indicated that infection level have increased markedly in 2011–2017, particularly concerning the infection of cod liver with *Contracaecum osculatum*. The objective of the study was to quantify the prevalence and intensity of the infection of cod with nematodes that occur in the liver of fish and to investigate the association between the infection and condition of cod.

Methodology: Cod *Gadus morhua* were sampled from the southern Baltic Sea between 2011 - 2017. The entire liver of each fish was inspected visually for the presence of nematodes. Then, livers were digested in artificial stomach juice, to detect parasites located in the deeper layers. Parasites were examined using a stereomicroscope and identified based on anatomo-morphological features. Generalised Linear Models (GLMs) were applied to analyse the prevalence and intensity of cod infection with *Anisakis simplex* and *C. osculatum* and to investigate the association between the Fulton's Condition Factor (FCF) and the intensity of infection.

Results: The prevalence of infection with anisakid larvae is much higher compared with previous studies undertaken over the past few decades. Remarkable increase in the infection level of cod was reported in 2011 and there was a further increasing trend in subsequent years (2012-2017), particularly concerning the infection of cod with *C. osculatum*. The presence of anisakid larvae in the liver of cod negatively affects the condition of fish and may increase mortality of large and heavily infected individuals. FCF decreased significantly with an increasing numbers of nematodes. On average, with each increase of 20 parasites, the FCF was further reduced by 1%. Condition of heavily infected fish was up to 20% lower than that of uninfected individuals.

Conclusion: The presence of anisakid larvae in the liver of cod negatively affects the condition of fish and may increase mortality of large and heavily infected individuals. Poor condition of fish and the presence of anisakids in the liver may, in combination, have adverse consequences for the Baltic Sea population of cod.

Keywords: cod, *Gadus morhu*a, condition, *Anisakis*, *Contracaecum*

049-P*

Diversity of metazoan parasites of mullid fishes from Tunisian coasts and their use as biological indicator of fish stocks

Ben Saad, Chiraz (Tunisia)[1]; Ghattassi, Souad (Tunisia)[1]; Gargouri, Lamia (Tunisia)[1]

1 - Laboratory of Diversity, Management and Conservation of Biological Systems Faculty of Sciences of Tunis, University of Tunis El Manar, 2092 Tunis, Tunisia

Introduction: Most of the research on metazoan parasites in Tunisian coastal fish is limited to a few localities, without resorting to parasitological comparisons between different sites. These comparisons can tell us about the existence of different fish stocks, hence the importance of this study.

Methodology: During our sampling, we examined 232 *Mullus barbatus* and *Mullus surmuletus* from different sites of Tunisian coasts. After the external examination, each fish was incised and the internal organs were removed (eosophagus, stomach, pyloric caeca, anterior intestine, middle intestine, posterior intestine, rectum, liver and gallbladder). Harvested parasites were identified and counted. Helminths, taken from the digestive tract of fish, are examined in vivo for determination. For a later observation, the flat worms were fixed in Bouin solution, colored with Borax Carmine, dehydrated by successive baths of alcohol, and then clarified with clove oil. For nematodes, acanthcephala, monogenean and isopods, they were fixed directly in 70% alcohol. The collected parasites were the subject of a morpho-anotomical study; a follow-up of their distribution in the digestive tract and their evolutionary dynamics of their hosts.

Results: Our investigations allowed us to identify 18 species of parasites: 1 monogenea (*Pseudempleurosoma* sp.); 11 digenea (*Proctoeces maculatus* (Looss, 1901) Odhner, 1911, *Holorchis legendrei* Dollfus, 1946, *Opecoeloides furcatus* (Bremser in Rudolphi, 1819) Odhner, 1928, *Poracanthium furcatum* Dollfus, 1948, *Prosorhynchus aculeatus* Odhner, 1905, *Stephanostomum* sp., *Proctotrema bacilliovatum* Odhner, 1911, *Timonia mediterranea* Bartoli & Prevot, 1966, *Aphallus tubarium* (Rudolphi, 1819) Poche, 1926, *Aphallus rubalo* (Bray, 1986) Bartoli & Bray, 1987, *Lecithocladium excisum* (Rudolphi, 1819) Lühe, 1901); 2 cestode larvae (1 *Tetraphyllidea* Carus, 1863 and 1 *Trypanorhyncha* Diesing, 1863), 1 nematoda (*Hysterothylacium fabri* (Rudolphi, 1819) Deardorff & Overstreet, 1980) ; 1 Acanthocephala (*Brezachanthus* sp.) and 2 isopods (*Ceratothoa oestroides* (Risso, 1816) et *Gnathia* sp.). Some parasites were signaled for the first time in mullidae of Tunisian coasts.

Conclusion: The metazoan diversity and parasitological parameters are variable depending on the sampling site in both mullid species. This variation is statistically confirmed. In addition, some metazoan present in one sampling site are absent in other. These results would make possible to use these parasites as a biological indicator of fish stocks.

Keywords: metazoan parasites, mullid fishes, biological indicator, fish stocks, Tunisian coasts

051-P*

Lousey lice: Improving knowledge and control of *Argulus* fish lice

Hunt, Rhi (United Kingdom)[1]; Cable, Joanne (United Kingdom)[1]; Williams, Chris (United Kingdom)[2]

1 - Cardiff University; 2 - The Environment Agency

Introduction: Fishing is a culturally significant hobby with livelihoods, especially in rural areas, dependent on the industry. Freshwater lice (Genus *Argulus*) are a global problem and an emerging pathogen of UK fisheries. Chemical treatments against *Argulus* are limited (and in some countries, illegal) and management strategies are ineffective. To inform and develop new control methods, we are assessing current fishery infections via a national survey and characterising parasite light attraction for use in novel traps.

Methodology: Questionnaires sent to 1000 trout fisheries assessed environmental parameters, infection levels and economic impact. To determine circadian behaviour, adult *A. foliaceus* were recorded using 24h infrared CCTV cameras under either 12 h light: 12 h dark or 24 h dark conditions. Videos were then analysed for activity (time spent swimming) at 4 h intervals over a 48 h period. Parasite light colour preference was assessed using waterproof lights randomly set to white, blue, green or red (controlling for brightness) and recording the time adult *A. foliaceus* spent at each colour.

Results: To date, 5.1% of UK trout fisheries are economically affected by *Argulus*: 78% experiencing problems > 2 years, and 31% > 10 years. *Argulus* activity peaks when transitioning from light to dark, with parasites 12% more active during dark periods. There is, however, no apparent entrained circadian rhythm as in total darkness this pattern disappears. *Argulus* also significantly preferred white and blue light over red or green, with no difference between them.

Conclusion: Due to the higher activity in darkness, trapping may be more efficient at night. As white and blue light were equally attractive, we recommend white light to develop novel traps due to easier availability. Development of a multifaceted management tool using these results aims to reduce economic loss and chemical use, facilitating industry expansion and sustainability.

Keywords: *Argulus*, fish lice, parasite, control, behavior

Funding: NERC GW4+ DTP studentship [NE/L002434/1] and CASE partner, the Environment Agency.

052-P

Infection by *Myxobolus episquamalis* at wild flathead grey mullet (*Mugil cephalus* Linnaeus, 1758) Kerchenskyi preglass of the black sea

Kazarnikova, Anna (Russian Federation)[1]; Strigakova, Tatyana (Russian Federation)[2]; Bortnikov, Yevgeniy (Russian Federation)[2]; Beraldo, Paola (Italy)[3]; Byadgi, Omkar (Italy)[3]; Galeotti, Marco (Italy)[3]

1 - Southern Scientific Center of RAS, 41, Chekhov st., Rostov-on-Don, Russia, 344006; 2 - The Azov Sea Research Institute of Fishery, 21-v, Beregovaya st., Rostov-on-Don, Russia, 344002; 3 - Università degli Studi di Udine, Dipartimento di Scienze AgroAlimentari, Ambientali e Animali, Sezione di Patologia VeterinariaVia Sondrio, 2, Udine, Italy, 33100

Introduction: Flathead grey mullet, *Mugil cephalus*, is the object of coastal fishery at Russia. Annually the harvesting of this object at the Azov and the Black Seas reached 0.1 - 0.2 thousand tons. *M. cephalus* with mass whitish cyst-like plasmodia on their scales were collected at Kerchensky preglass of the Black Sea in 2015. The prevalence of infected fish varied from 3 to 15% at spring, increased till 40% at summer and fall down to 2.5% at autumn. No fish mortality was detected at the sea.

Metodology: 15 clinically health and 15 infected fish were taken for parasitological analyses. In particular, infected scale fresh smears were set up to describe and measure of myxosporidia spores under light microscope Leica DMLB with Leica ICC50 camera and Leica LAS EZ software. Molecular analysis using universal primer have amplified consistently and yielded the 393 bp specific amplicon of 18S rRNA of Myxobolus sp. and was confirmed using sequencing.

Results: The spores were oval in frontal view, tapering anteriorly to a blunt apex. Two unequal polar capsules were pyriform and extended over the anterior half of spore. Spores were 8,2±0,03 µm (7.9 - 8.4) in length, 5.9 ± 0.23 µm (5.2 - 7.3) in width, 4.4 ± 0.17 µm (4 - 4.7) in thickness. The polar capsules were 4 ± 0.07 µm (3.3 - 4.5) in length, 1.5 ± 0.24 µm (1.1 - 1.8) in width. The investigation of nucleotide sequences of the 18S rDNA gene of the myxosporean spores from scales with universal primer A (5′-ACCTGGTTGATCCTGCCAGT-3′) and B (5′-TGATCCTTCTGCAGGTTCACCTAC - 3′) showed 100% identity with *M. episquamalis*, on 99% *Myxobolus* sp., on 99% *M. bizerti*, on 99% *M. ichkeulensis* and on 99% *M. spinacurvata* detected in mullets.

Conclusion: This report represents the first case of *M. episquamalis* infection in flathead grey mullet scales in Russian waters of the Black sea. There is a high probability of invasion for other mullet species (*Liza aurata*, *L. saliens*, *L. haematocheilus*). In connection with the foregoing, it is very important to provide the monitoring of the epizootic state of mullet populations in order to identify *M. episquamalis* in other mugilid fish in this area.

Keywords: *Myxobolus episquamalis*, infection, grey mullet, *Mugil cephalus*

Funding: MSE of RF N01201354245.

053-P*

Histopathology and inflammatory response elicited by *Masenia nkomatiensis* (Digenea) in the intestine of *Clarias gariepinus* (Clariidae)

Dumbo, Jose Chissiua (Mozambique)[1,2]; Avenant-Oldewage, Annemerie (South Africa)[1]

1 - Department of Zoology, University of Johannesburg; 2 - Biological Science Department, Eduardo Mondlane University

Introduction: *Masenia nkomatiensis* is small intestinal parasite possessing a terminal funnel-shaped oral sucker consisting of well-developed radial muscles fibers. The mouth is surrounded by two rows of 50 conical and acuminate circumoral spines, which are used to erode the tissue prior to feeding. This morphological arrangement has an important role on damage to the host tissue.

Methodology: Fish was collected, euthanized and the intestine removed for examination for digeneans. For identification, some specimens were prepared for SEM using hexamethyldisilazane, others slightly flattened and stained with acetocarmine. Infected tissue was embedded in resin and 5µm sections were cut and stained with Haematoxylin and Eosin. Immune cells were counted per section in the area of attachment, 5000 µm away and in uninfected fish.

Results: Infection was associated with degeneration of the villi epithelium and detachment of fragments of the villi. Excessive mucus secretion and catarrh occurred in the vicinity of the worm and these are indicators of inflammation. Mast cells and mucous cells were significantly more abundant at the attachment site than 5000 µm away and in uninfected fish; whereas the number of basophils and neutrophils at the attachment site did not differ significantly to 5000 µm away and in uninfected fish. In the infected intestine, destruction of the villi epithelia may impair nutrient absorptive. The inflammation commonly observed may indicate that the innate immunity plays an important role in restricting, reducing and terminating damage and no acquired immunity has been produced against infection.

Conclusion: The histological changes seen in the intestine are due to attachment and feeding of parasite, and as response, inflammation consisting on mobilization of mast cells has been activated, no acquired immunity was activated. The changes induced by *M. nkomatiensis* within the intestine of *Clarias gariepinus* is reported for first time.

Keywords: Digenea, pathology, sharptooth catfish

054-P

Perkinsus-like organism in farmed rainbow trout (_Oncorhynchus mykiss_ Walbaum) in south-western Norway

Kvinnsland, Herman Høgenes (Norway)[1]; Dalum, Alf Seljenes (Norway)[2]; Sandlund, Liv (Norway)[2]; Nylund, Are (Norway)[3]

1 - FoMAS- Fiskehlelse og Miljø AS; 2 - Pharmaq Analytiq; 3 - Universitetet i Bergen

Introduction: The *Perkinsus*-like parasite was first described in farmed rainbow trout in 2002 and in 2003 in farmed Atlantic salmon (*Salmo salar*), located in south-western Norway (Nylund et al., 2006). The parasite was analyzed using 18S rRNA and found to be part of the group Alveolata and the phylum Perkinsozoa. Phylogenetic analysis placed the parasite to belong to the genus *Perkinsus* in the phylum Perkinsozoa. The name *Pisciperkinsea* sp. has been suggested for this new genus (Nylund et al., 2006). In late fall 2018, infected rainbow trout was found in cages with increased mortality. Clinical signs were white granulomas in the liver and heart, petechiae on internal organs (gut and peritoneum) and ascites. These findings were similar to what Nylund et al. (2006) observed in 2002-03. To our knowledge, there are no previously described fish parasites in the Perkinsozoa phylum, however, several species have been described that infect and cause severe disease in molluscs and amphibians.

Methodology: Samples for histology (4% buffered formaldehyde) and RT-qPCR (RNA-later) as well as fresh heart tissue were secured from dead fish. Sampling was done during monthly visits to the site.

Results: From histopathological examination, multifocal, in many cases severe necrotic lesions with variable degree of inflammation were seen in several tissues like heart, liver, spleen and skeletal muscle signifying a systemic condition with hematogenous dissemination. Microsporidian-like structures were seen both intracellularly in necrotic cells as well as free in necrotic tissue. Both spherical, strongly basophilic structures as well as more ovoide, pale basophilic staining structures were seen, probably representing hypnospores and trophozoites respectively. Using 18S rRNA sequencing analysis the agent was found to be identical to *Pisciperkinsus* sp.

Conclusion: There is still a lot of uncertainty surrounding the parasite and so further morphological and molecular biological investigations are ongoing in order to describe this new parasite

Keywords: rainbow trout

Funding: FoMAS and Pharmaq Analytiq.

055-P

Anisakis simplex in *Crangon crangon* and *Contracaecum osculatum* in *Gammarus* sp. found *in situ* in the stomach of Baltic cod

Pawlak, Joanna (Poland)[1]; Nadolna-Ałtyn, Katarzyna (Poland)[1]; Szostakowska, Beata (Poland)[2]; Pachur, Marzenna (Poland)[1]; Podolska, Magdalena (Poland)[1]

1 - National Marine Fisheries Research Institute, Gdynia, Poland; 2 - Medical University of Gdansk, Gdynia, Poland

Introduction: Diet preferences of Baltic cod (*Gadus morhua*) are changing during the life time and are dependent on the fish capability to catch and eat the prey. Young fish occur mostly nearby the coast and feed on invertebrates. Older cod prefer fish (Clupeidae) and bigger invertebrates. Food is not only a source of nutrients for fish, but can also be a route of infection with parasites. The parasite fauna of cod is well described, but the life cycles of parasites are known only in general terms. There is still little known which species of crustacean representatives play the role of intermediate host in life cycle of *Anisakis simplex* and *Contracaecum osculatum* (Nematoda: Anisakidae) in the Baltic Sea. The aim of this study was to determine the source of infection of Baltic cod with parasites found *in situ* in invertebrates present in the cod's stomach.

Methodology: Samples containing stomachs of cod (in total 916) were collected in February 2015, November 2015 and February 2016. During analysis of cod's diet all found invertebrates were collected, identified and inspected for the presence of parasites. The found parasites were identified on the base of anatomo-morphological features and subjected to molecular identification by PCR and DNA sequencing.

Results: Stomach content analysis of cod revealed the presence of 8801 invertebrates. *C. crangon* and *Gammarus* sp. belonged to the three most frequent species in each season. *C. crangon* infected with *A. simplex* s. s. and *Gammarus* sp. infected with *C. osculatum* were found. Systematic position of parasites was confirmed by molecular analysis.

Conclusion: This is the first report of *A. simplex* and *C. osculatum* found *in situ* in invertebrates present in the stomach of cod. *C. crangon* and *Gammarus* sp. are potential sources of Baltic cod infection with these zoonotic nematodes. In the Baltic Sea, neither *C. crangon* has been previously reported as an intermediate host for Anisakidae parasites, nor *Gammarus* sp. for *C. osculatum*. Both *A. simplex* and *C. osculatum* are zoonotic species, posing a risk to the human health.

Keywords: Crustacea, *Anisakis simplex*, *Contracaecum osculatum*, *Gadus morhua*, Baltic Sea

Funding: The National Science Centre (Poland): grant number 2015/19/N/NZ9/00173.

056-P*

Impacts of temperature and salinity on the survival and invasion ability of two *Anisakis* spp. (Nematoda: Anisakidae) *in vitro*

Gomes, Tiago (Japan)[1]; Quiazon, Karl Marx (Japan)[1,2]; Yoshinaga, Tomoyoshi (Japan)[1]

1 - Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, The University of Tokyo; 2 - Freshwater Aquaculture Center and College of Fisheries, Central Luzon State University

Introduction: *Anisakis simplex* sensu stricto (s.s.) and *A. pegreffii* are among the most abundant and relevant parasitic marine nematodes. Third stage (L3) larvae parasitize the tissues of fish and cephalopods and can infect humans when fishery products are ingested raw or undercooked. Despite having low host specificity they have very distinct geographical distributions, with the former predominantly found in northern waters and the latter prevalent in more southern relative locations. The tolerance to temperature and salinity of these two sibling species were asynchronously tested *in vitro* to further understand their epidemiological status.

Methodology: The survival rates of L3 larvae of each species incubated in PBS solutions of 8 and 30 ppt salinity at 10, 20 and 25 °C for 1 and 3 weeks were assessed. This was then followed by the monitoring of their invasiveness for a 24 hour period in 1.75% agar with 0.8% nutrient broth and covered with a supernatant of 3% acetic acid in 0.85% NaCl solution.

Results: *Anisakis pegreffii* showed a higher survival rate than *A. simplex* (s.s.) in all experimental groups, particularly after 3 weeks at 20-25 °C. In the agar invasion test, *A. pegreffii* also demonstrated superior invasion ability and both species performed better at 8 ppt than at 30 ppt. Moreover, *A. pegreffii* was significantly faster than *A. simplex* (s.s.) in invading the agar.

Conclusion: These results seem to suggest that *A. pegreffii* is more adapted than *A. simplex* (s.s.) to warmer temperatures, which might help justify their geographic distribution. It also reinforces the role of *A. pegreffii* as a relevant zoonotic agent.

Keywords: *Anisakis simplex* (s.s.), *Anisakis pegreffii*, temperature, salinity

057-P*

Outbreak of nodular gill disease in farmed brook trout (*Salvelinus fontinalis*)

Perolo, Alberto (Italy)[1]; Manfrin, Amedeo (Italy)[2]; Dalla Pozza, Manuela (Italy)[2]; Pretto, Tobia (Italy)[2]; Fioravanti, Maria Letizia (Italy)[3]; Gustinelli, Andrea (Italy)[3]; Quaglio, Francesco (Italy)[1]

1 - Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro (PD), Italy; 2 - Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy; 3 - Department of Veterinary Medical Sciences, Alma Mater Studiorum University of Bologna, Ozzano Emilia (BO), Italy

Introduction: Nodular gill disease (NGD) caused by amoebic infection represents an emerging and significant pathological condition associated with extensive mortality affecting freshwater reared salmonids, mainly rainbow trout (*Oncorhynchus mykiss*). Outbreaks of NGD have been reported in arctic char (*Salvelinus alpinus*), Chinook salmon (*Oncorhynchus tshawytscha*) and brown trout (*Salmo trutta*). In December 2017 the first case of NGD in farmed brook trout (*Salvelinus fontinalis*) associated with mortalities was observed during epidemiological amoebic investigations.

Methodology: The disease occurred in a Northern Italy commercial rainbow trout culture facility where periodic NGD's episodes happened. The brook trout were imported from a Danish farm in November 2017 and allocated in a raceway supplied by river water with at 9 °C. The live weight was between 210 and 240 g. In the following month the fish showed signs of respiratory distress. The cumulative mortality, monitored from December 2017 to January 2018, reached 30%. Twenty fish from the investigated raceway were collected for necropsy, microscopical and parasitological analysis. Gills were dissected and fixed in 10% neutral buffered formalin solution for histological examination. The samples were dehydrated, embedded in paraffin, sectioned (4 µm) and stained with haematoxylin-eosin and Giemsa solution.

Results and Conclusion: The samples did not showed noticeable macroscopical lesions with the exceptions of the gills, which appeared pale and swollen with whitish nodules located especially in the distal part of the filaments. The microscopic examination of gill tissue revealed severe proliferative reaction with presence of amoebic organisms. The histology showed multi-focal epithelial hyperplasia of the gills causing lamellar fusion, cellular exfoliations, necrosis and amoebae (approximately 10×20 µm) along the surface of the affected filaments. In 9 out of 20 examined fish a massive presence of filamentous bacteria (referable to the family Flavobacteriaceae) in the interlamellar space was observed. Occurrence of a NGD in the brook trout raises several questions and the need for further investigations regarding the susceptibility of this host to the etiological agent, the role of this species in the epidemiology of the disease and the identification of the species of amoebae primarily involved as causative agents of NGD.

Keywords: nodular gill disease, brook trout, *Salvelinus fontinalis*, amoebae

058-P*

Methacarn preserves mucus integrity and improves visualisation of amoebae in gills of Atlantic salmon (*Salmo salar* L.)

Fernandez, Carolina (United Kingdom)[1]; Mascolo, Dario (United Kingdom)[2]; Monaghan, Sean J. (United Kingdom)[1]; Baily, Johanna L. (United Kingdom)[1]; Chalmers, Lynn (United Kingdom)[1]; Paladini, Giuseppe (United Kingdom)[1]; Adams, Alexandra (United Kingdom)[1]; Bron, James E. (United Kingdom)[1]; Fridman, Sophie (United Kingdom)[1]

1 - University of Stirling; 2 - Scottish Sea farms

Introduction: The technical development of mucus stabilisation through the optimisation of fixative methods would provide the means for examining and further our understanding of the complex relationship between parasite and host. The purpose of this study was a) to optimise the preservation of the mucus on the gill using alternative aqueous and non-aqueous based fixatives and b) to examine the interaction between the amoebae and mucus on the gill of the Atlantic salmon during infection with amoebic gill disease.

Methodology: Two aqueous fixation regimes (modified Davidson's solution and modified Davidson's solution with 2% (w/v) Alcian blue) were compared against two non-aqueous fixation regimes (methacarn solution and methacarn solution with 2% (w/v) Alcian blue) along with the standard buffered formalin fixation method, in an attempt to improve preservation of the mucous coat on Atlantic salmon, *Salmo salar* L., gills.

Results: Aqueous fixatives demonstrated excellent cytological preservation but failed delivering the preservation of the mucous coat when compared to the non-aqueous based fixatives; qualitative and semi-quantitative analysis revealed a greater preservation of the gill mucus using the non-aqueous methacarn solution. A combination of this fixation method and an Alcian blue/Periodic acid–Schiff staining was tested in Atlantic salmon gills infected with amoebic gill disease (AGD); lectin-labelling was also developed to confirm the mucus preservation in the methacarn fixed tissue.

Conclusion: Amoebae were observed closely associated with the mucus demonstrating that the techniques employed for preservation of the mucous coat can indeed avoid the loss of potential mucus-embedded parasites, thus providing a better understanding of the relationship between the mucus and parasite.

Keywords: fixative, fish, parasites, gill pathology, amoebic gill disease

Funding: European Union's Horizon 2020 research and innovation programme under Grant Agreement no. 634429 (ParaFishControl).

059-P

Cryopreservation of *Paramoeba perurans*

Trösse, Christiane (Norway)[1]; Kindt, Mats (Norway)[1]; Blindheim, Steffen (Norway)[1]; Andersen, Linda (Norway)[2]; Nylund, Are (Norway)[1]

1 - Department of Biological Sciences, University of Bergen; 2 - ILAB

Introduction: The marine amoeba *Paramoeba perurans* has been shown to be the causative agent of amoebic gill disease (AGD) in Atlantic salmon (*Salmo salar*). Various amoeba isolates have been previously obtained and cultured from field samples, both in Tasmania and Europe. These isolates have been in continuous culture to enable their phenotypic and genotypic characterisation and to study their pathogenicity in infection trials. Different culture techniques utilise either agar plates or cell culture flasks to which the amoebae attach. So far, no successful attempts of cryopreservation have been reported.

Methodology: In a series of freezing and thawing experiments with different amoeba isolates, utilising protocols adapted from mammalian cell culture, we determined conditions under which viable cells were recovered after thawing.

Results: After the freezing and thawing procedure, the recovered cells divided and grew normally under standard culture conditions in our lab.

Conclusion: The possibility to freeze and thaw *Paramoeba perurans* isolates is a huge improvement for the further study of this commercially important pathogen.

Keywords: *Paramoeba*, *Neoparamoeba*, *perurans*, cryopreservation

Funding: FHF project 901053.

060-P*

Is amyloodiniosis a neglected disease in aquaculture research?

Moreira, Márcio (Portugal)[1,2]; Costas, Benjamín (Portugal)[3,4]; Rodrigues, Pedro (Portugal)[2]; Marques, Cátia (Portugal)[1]; Schrama, Denise (Portugal)[2]; Soares, Florbela (Portugal)[1]

1 - Portuguese Institute for the Ocean and Atmosphere, Aquaculture Research Station, Av. 5 de Outubro s/n, 8700-305 Olhão, Portugal; 2 - Universidade do Algarve - Centre of Marine Sciences, University of Algarve, Campus de Gambelas, 8005-139 Faro, Portugal; 3 - Centro Interdisciplinar de Investigação Marinha e Ambiental, University of Porto, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208, Porto, Portugal; 4 - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

Introduction: Amyloodiniosis is a disease that is considered a major bottleneck for semi-intensive aquaculture. It is caused by one of the most common and important parasitic dinoflagellates in marine fish, *Amyloodinium ocellatum* (Brown), and considered one of the most consequential pathogens of marine fish, causing serious mortality in brackish and marine warm-water fish in different aquaculture facilities worldwide. Information regarding treatments shows that the majority of these are highly ineffective or unpractical for earthen pond semi-intensive aquaculture, the most common aquaculture production system in Southern Europe. In addition, information about the host physiological responses to this parasite is very scarce.

Methodology: In order to check gather the available data, we performed a review of all the published work in amyloodiniosis and/ or *A. ocellatum* was performed.

Results: Information regarding the life cycle of the parasite, epidemiology and available treatments for different marine fish species is available, nevertheless only fragmented information on the host physiological and immunological responses to *A. ocellatum* was found, without any available inter-species comparative studies. We also observed a lack on genetic information available for this parasite, with only 45 sequences annotated on NCBI (mainly from the small subunit ribosomal RNA gene or internal transcribed spacer (ITS) 1 and 2). Focused on Omics techniques only one work in amyloodiniosis was found.

Conclusion: This work reveals that amyloodiniosis is still an neglected disease in aquaculture in terms of investigation, with most of the work done between 1984-1992. Even if there are more people researching this subject in the last 2-3 years, mainly on the host physiological and immunological responses, there are areas that still need a strong investment in research like genetics or omics. This emphasizes the importance of further investment in research on amyloodiniosis, in order to maximize the knowledge on *A. ocellatum* and the host physiological responses to this parasite, providing important information for the development of more effective treatments and eventually the establishment of a successful vaccine.

Keywords: *Amyloodinium ocellatum*, fish, treatments, physiological responses, genetics
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061-P

First observation of the North American *Posthodiplostomum centrarchi* Hoffman, 1958 in Hungary

Cech, Gábor (Hungary)[1]; Sándor, Diána (Hungary)[1]; Molnár, Kálmán (Hungary)[1]; Papp, Melitta (Hungary)[2]; Deák-Paulus, Petra (Hungary)[2]; Bálint, Preiszner (Hungary)[3]; Vitál, Zoltán (Hungary)[3]; Varga, Ádám (Hungary)[1]; Székely, Csaba (Hungary)[1]

1 - Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences; 2 - National Food Chain Safety Office – Veterinary Diagnostic Directorate, Laboratory for Fish and Bee Diseases, Hungary; 3 - Balaton Limnological Institute, Centre for Ecological Research, Hungarian Academy of Sciences

Introduction: *Posthodiplostomum* (Digenea: Diplostomidae) species are well-known for a long time from native freshwater fishes in Hungary (Molnár, 1969). *P. cuticola* Nordmann, 1832 is an important pathogen of cyprinid fishes as the causative agent of the black spot disease. The encysted metacercariae often occur in the scales, skin, fins and skeleton muscle that can cause deformities of the vertebral column and ectopic bone formation in swim-up fries. *Posthodiplostomum* species are also native in North America; moreover the existence of *P. centrarchi* Hoffman, 1958 was reported in Bulgaria, Slovakia, Czech Republic, Portugal, Ukraine and Germany (Stoyanov et al., 2017, Kvatch et al. 2017, Ondračková et al. 2018). Our aim was the investigation of the recent distribution of native and non-native *Posthodiplostomum* species in Hungary.

Methodology: 50 pumpkinseeds *Lepomis gibbosus* L. were collected from a North Hungarian water reservoir as a routine survey. At the same time, cyprinid fishes (*Abramis brama*, *Rutilus rutilus, Scardinius erythrophthalmus*, *Blicca bjoerkna*) and pumpkinseed were sampled form the Lake Balaton, Lake Tisza and Danube. Different developmental stages of the *Posthodiplostomum* species were analysed by native microscopy and histology and sequencing the internal transcribed spacer (ITS) region and cytochrome c oxidase I (COI) were conducted to complement morphology. In addition laboratory experiments by infecting 5 chicks with 150 metacercariae/bird were carried out in order to get adult stages. Morphology of adult specimens is necessary for the exact species identification.

Results: Morphological and molecular analyses differentiated two distinct species. The first was the native *P. cuticola* found on the scales of cyprinid fishes. The second one was *P. centrarchi*, its metacercariae were found first time in Hungary on the core region of pumpkinseeds in a small water reservoir and in Sió channel, the outflow of Lake Balaton.

Conclusion: While native parasites were also present during the monitoring, there is a clear tendency that *Posthodiplostomum* parasites of the invasive fish pumpkinseed are spreading across Hungary and other countries in Europe.

Keywords: Digenea, *Posthodiplostomum*, internal transcribed spacer (ITS), cytochrome c oxidase I (COI)

Funding: GINOP 2.3.2 – 15 – 2016 – 00004 project: “Establishing the sustainable angling-aimed management of Lake Balaton”.

062-P

Differential effects of host responses in common carp and common bream against 3rd stage larvae of *Contracaecum rudolphii* type B

Molnár, K.[1]; Székely, C.[1]; Sellyei, B.[1]; Baska, F.[2]; Zuo, S.[3]; Kania, Pw.[3]; Buchmann, K.[3]

1 - Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary; 2 - University of Veterinary Medicine, Budapest, Hungary; 3 - Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Introduction: The main fish host reaction to an infection with third stage anisakid nematode larvae is a response in which host immune cells (macrophages, neutrophils, lymphocytes) in affected internal organs initially are attracted to the parasite whereafter fibroblasts may enclose the parasite. Generally the reaction is non-lethal to the parasite which may survive for years in the fish host retaining infectivity to the final host over a longer time period. This may also apply for the anisakid nematode *Contracaecum rudolphii* (having the adult stage in cormorants, using copepods as first intermediate host and zooplankton feeding fish as paratenic hosts).

Methodology: Common bream (*Abramis brama*) was obtained from the Lake Balaton, while the common carp (*Cyprinus carpio*) derived from the Héviz Lake. A total of 362 bream and 19 carp were examined by parasitological dissection. Third stage larvae of the nematode *Contracaecum rudolphii* were collected from the abdominal cavity, examined light microscopically and by molecular biological methods. Location and host effect of larvae in affected organs (gut and peritoneum) were studied in histological sections stained by haematoxillin and eosin.

Results: The present study shows that bream caught in Lake Balaton heavily infected with *Contracaecum rudolphii* larvae (enclosed by a marked host reaction in the internal organs), allowed most of the larvae to survive. In contrast, common carp in the Héviz lake, directly connected to Lake Balaton, established a host reaction with lethal effect on *C. rudolphii* larvae in the internal organs.

Conclusion: The differential survival in common carp and bream may be associated with various factors. Ecological factors, such as feeding ecology and temperature, determine exposure of fish to *C. rudolphii* infections, whereas intrinsic factors, including differential immune responses and host genetics, are possible elements which may explain the differential survival of the nematode larvae in the two cyprinids.

Keywords: susceptibility, resistance, bream, carp, nematodes

Funding: European Union's Horizon 2020 research and innovation programme, grant agreement No. 634429 (ParaFishControl).

063-P

First data on the presence of *Anguillicola crassus* in European eel from the Segura river basin (SE Iberian Peninsula)

Muñoz, Pilar (Spain)[1]; Verdiell-Cubedo, David (Spain)[2]; Romero, Diego (Spain)[3]; Barcala, Elena (Spain)[4]; Mª Dolores, Emilio (Spain)[5]; Parrondo-Celdrán, Paz (Spain)[2]

1 - Departamento de Sanidad Animal. Universidad de Murcia. Campus de Espinardo, 30100 Murcia, Spain; 2 - Asociación Columbares, C/ Adrián Viudes, 9, 30570 Murcia, Spain; 3 - Área de Toxicología, Universidad de Murcia, Campus de Espinardo, 30100 Murcia, Spain; 4 - Instituto Español de Oceanografía, C/ Varadero, s/n, 30740 San Pedro del Pinatar, Spain; 5 - Servicio de Pesca y Acuicultura, Consejería de Agua, Agricultura, Ganadería y Pesca, CARM

Introduction: *Anguillicola crassus* (Nematoda, Dracunculoidea) is a very efficient parasitic invasive species. It has expanded from its original ecosystems in Asia throughout the world and has been able to parasitize five different eel host species within the last three decades. It is one of the many threats hanging over the European eel (*Anguilla anguilla*). After its first description in Spain (Belpaire et al 1989), several studies have demonstrated that the parasite is widespread in this country. The aim of the present work was to determine whether the nematode had already invaded the Segura River Basin.

Methodology: The study area comprises a river section of the Segura River and the irrigation network of the Murcia Orchard, where the European eel is currently present in the basin. The sampling was developed between December 2017 and March 2019 by using fyke nets. Three habitat types were sampled: the Segura River channel, the irrigation channels that provide water to crop fields called *acequias* and the drainage channels that return water back to the river called *azarbes*. A total of 29 European eels were sampled. Swim bladders were dissected and pre-adult and adult *A. crassus* were removed from the swim bladder lumen of each infected eel. Swim bladders were examined under a stereomicroscope for detection of larval stages in the wall. Prevalence, mean intensity and mean abundance were calculated. Additionally, swim bladder degenerative index (SDI), which can range from 0 when no pathological signs of infection are observed to 6 in extremely damaged swim bladder, was recorded.

Results: The prevalence was 62.1% (18/29) with a mean intensity of 5.1 worms per infected eel (range 1-19) and a mean abundance of 3.17 while SDI was 1.3. The nematode was present in eels from the three habitats sampled.

Conclusion: These results represent the first recorded occurrence of *A. crassus* in the Segura River Basin. Implications for eel management are discussed.

Keywords: *Anguillicola crassus*, *Anguilla anguilla*

064-P*

Unidentified haplosporidian-like organism parasitizing gray mussel, *Crenomytilus grayanus* from Gangneung on the east sea of Korea

Cho, Young-Ghan (Republic of Korea)[1]; Kang, Hyun-Sil (Republic of Korea)[2]; Thatchaneshkanth, Subramaniam (Republic of Korea)[1]; Hong, Hyun-Ki (Republic of Korea)[1]; Lee, Hye-Mi (Republic of Korea)[1]; Kim, Jeong-Hwa (Republic of Korea)[1]; Shin, Jong-Seop (Republic of Korea)[1]; Choi, Kwang-Sik (Republic of Korea)[1]

1 - Jeju National University; 2 - National Institute of Fisheries Science (NIFS)

Introduction: The phylum Haplosporidia comprises a group of spore-forming protozoans infecting various freshwater and marine invertebrates. To identify species, characteristics of external morphology and ultrastructure along with DNA sequencing have been incorporated and applied. In the present study, we first report on the occurrence of haplosporidian-like organism (HLO) from digestive tubules of the gray mussel, *Crenomytilus grayanus* (Dunker, 1853) occurring on the East Sea of Korea.

Methodology: From September 2012 to August 2013, we collected 30 mussels monthly and prepared for histology. For the analysis, a longitudinal section was cut in the middle of the body then prepared for the histology.

Results and Conclusion: Histology revealed that the digestive tubule walls of gray mussels included the HLO. For further molecular identification, the remaining tissues of mussels with the HLO were lyophilized and homogenized to extract the DNAs. For PCR, we used the small subunit ribosomal DNA (SSU rDNA) primers specific to the genus *Haplosporidium*. We also examined external features of the HLO extracted from the paraffin blocks using SEM. Infection prevalence of the HLO in gray mussel ranged 0 to 10% over 12 months of sampling. In histology, the HLO appeared as spherical multinucleated plasmodia parasitizing the digestive tubule. These plasmodia developed into sporocysts called sporonts, forming walls around each nucleus. Immature sporonts and groups of spores were also observed in the epithelium of the digestive tubules. Mussels heavily infected with the HLO demonstrated sever hemocyte infiltration along the infected tissues. The SSU rDNA sequence (1,784 bps) similarity analysis revealed that the HLO discovered in this study was closely related to the genus *Minchinia* and *Bonamia,* with the highest sequence similarity of 87.1% to *Minchinia tapetis.* SEM revealed that the spores were ovoid and characterized with numerous parallel folds surrounding the spore. Ellipsoidal operculum-like lid was also observed at the apical end of the spore. Although the external morphology was similar to the members in the genus *Haplosporidium*, the molecular phylogeny indicated that the HLO discovered in this study is possibly a member in the genus *Minchinia* or *Bonamia*.

Keywords: haplosporidian, gray mussel, *Crenomytilus grayanus*, Korea

065-P*

Molecular characterization of *Urosporidium* sp. parasitizing metacercaria of *Parvatrema duboisi* in the Manila clam on the west coast of Korea

Cho, Young-Ghan (Republic of Korea)[1]; Le, Thanh Cuong (Vietnam)[2]; Hong, Hyun-Ki (Republic of Korea)[1]; Lee, Hye-Mi (Republic of Korea)[1]; Kim, Jeong-Hwa (Republic of Korea)[1]; Shin, Jong-Seop (Republic of Korea)[1]; Choi, Kwang-Sik (Republic of Korea)[1]; Kang, Hyun-Sil (Republic of Korea)[3]

1 - Jeju National University; 2 - Nha Trang University; 3 - National Institute of Fisheries Science

Introduction: The genus *Urosporidium* (Phylum Haplosporidia) includes hyperparasitic haplosporidians parasitizing trematodes or turbellaria infecting marine bivalves, gastropods, and crustacean. Recently, we reported an unidentified species in the genus *Urosporidium* parasitizing the metacercaria of *Parvatrema duboisi*, trematod parasite infecting the mantle tissue of Manila clams occurring on the west coast of Korea. In this study, we first isolated and characterized the small subunit (SSU) rDNA of the *Urosporidium* sp. from the metacercaria of *P. duboisi* isolated from Manila clam.

Methodology: Metacercaria of *P. duboisi* infected by *Urosporidium* sp. could be identified by the naked eyes since they appeared as block spots on the mantle surface of Manila clam. Accordingly, we harvested Manila clam mantle tissues containing *P. duboisi* metacercaria infected by the hyperparasite by dissecting the tissues, and the tissues were kept in seawater for one week at room temperature to denature the clam and the trematode tissues. A week at the seawater, both the host tissue and the metacercaria of *P. duboisi* became denatured, and intact *Urosporidium* sp. spores could be released from the host tissue. To amplify the small subunit (SSU) rDNA of *Urosporidium* spores by PCRs, two sets of primers were applied. PCR amplicons were cloned and sequenced. The DNA sequence obtained in the present study was compared using BLAST on NCBI.

Results and Conclusion: A 1,890 bps of SSU rDNA sequence obtained in this study was 92.9-93.4% similar to the sequences of *Urosporidium* spp. previously registered in NCBI. In the Neighbor-Joining cluster analysis, the sequence obtained in this study was included in a monophyletic group of *Urosporidium* spp., indicating that the hyperparasite infecting metacercaria of *P. duboisi* is truly a member in the genus *Urosporidium*. However, the sequence similarity as well as other morphological features observed by SEM suggested that the *Urosporidium* sp. discovered from the metacercaria of *P. duboisi* could be a new member in the genus.

Keywords: haplosporidian parasite, *Urosporidium*, hyperparasite, Trematoda metacercariae, Manila clam, *Ruditapes philippinarum*

066-P

Fecundity of the *Ceratothoa oestroides* (Risso, 1816) on farmed sea bass (*Dicentrarchus labrax*) in the Adriatic Sea

Čolak, Slavica (Croatia)[1]; Kolega, Matko (Croatia)[1]; Mejdandžić, Danijel (Croatia)[1]; Franov, Šime (Croatia)[1]; Barić, Renata (Croatia)[1]; Šarić, Tomislav (Croatia)[2]; Petani, Bruna (Croatia)[2]; Župan, Ivan (Croatia)[2]; Mustać, Bosiljka (Croatia)[2]

1 - Cromaris d.d., Gaženička cesta 4b, 23000 Zadar (Croatia); 2 - Department of ecology, agronomy and aquaculture, University of Zadar, Trg kneza Višeslava 9, 23000 Zadar

Introduction: *Ceratothoa oestroides* (Risso, 1816) is an isopod parasite living in fish buccal cavity. Reproduction is usually present during whole year and knowledge of the parasite reproductive biology is an important tool to apply good preventive measures in aquaculture. Fecundity is one of the factors which is crucial for recruitment of parasite in fish population. The aim of the present study was to gain accurate information about fecundity of the isopod parasite *C. oestroides*. This study analysed fecundity of *C. oestroides* in relation to biotic (size of female parasite) and abiotic factors (temperature, photoperiod).

Methodology: The study was carried out from January to December 2018. Samples of fish infected with *C. oestroides* were collected from three different fish farms in the Middle Eastern Adriatic Sea. Water temperature varied from 12.1 °C to 22.6 °C. A total of 850 fish were examined, average weight of fish were 413 ± 40 g and length 30.4 ± 3.6 cm (mean ± SD). Fecundity was determined according to the number of larvae with visible eyes in the marsupium.

Results: Our results showed that there was no significant correlation between sea temperature and fecundity. However, there was a significant difference in parasite fecundity between different months through the year. The smallest fecundity was found in November (256 ± 115 pulli) while the largest fecundity value was determined in May (470 ± 124 pulli). There was very strong positive correlation between photoperiod and fecundity ($r = 0.9$; $P < 0.05$). Also, the relationship between female size and fecundity showed a positive strong correlation ($r = 0.6$; $P < 0.05$).

Conclusion: The large-sized female parasites had larger ovaries than smaller individuals of the same species, which allowed them to produce and carry more larvae. Also, fecundity of *C. oestroides* was positively correlated with photoperiod.

Keywords: *Ceratothoa oestroides*, sea bass, fecundity

067-P

Health status of *Pinna nobilis* in the Croatian part of eastern Adriatic coast

Šarić, Tomislav (Croatia)[1]; Župan, Ivan (Croatia)[1]; Šikić, Zoran (Croatia)[1]; Peharda, Melita (Croatia)[2]; Pavlinec, Željko (Croatia)[3]; Zupičić, Ivana Giovanna (Croatia)[3]; Beck, Relja (Croatia)[3]; Oraić, Dražen (Croatia)[3]

1 - University of Zadar, Trg kneza Višeslava 9, 23000 Zadar, Croatia; 2 - Institute of Oceanography and Fisheries, Šetalište Ivana Meštrovića 6, 21000 Split, Croatia; 3 - Croatian Veterinary Institute, Savska cesta 143, 10000, Zagreb, Croatia

Introduction: The pen shell *Pinna nobilis* is largest bivalve species endemic for the Mediterranean Sea, protected under Annex IV of the Habitats Directive, Annex II of the Barcelona Convention, and national legislation in Croatia and most Mediterranean countries. In autumn 2016, *P. nobilis* populations along the Western Mediterranean coast suffered extremely high mortalities. Affected individuals showed delayed valve-closing reflex, and some of them were not even able to completely close their shells. In recently published papers, a new parasite species, *Haplosporidium pinnae*, is associated as likely cause of mass mortality events. According to available data, mass mortality events have spread from the Iberian Peninsula and the Balearic Islands to the Mediterranean part of the French coast, the Italian and Greek coasts.

Methodology: In order to monitor the health status of the *P. nobilis* in the Adriatic Sea, from summer 2017 we conducted a biannual visual inspection of the *P. nobilis* populations at 10 different locations along the Croatian Adriatic Coast. Locations were selected based on the existing information of *P. nobilis* habitats. The screening was performed aiming to identify possible symptoms of the disease as well as possible mortalities. At the beginning of 2019, tissue samples (8 samples of the mantle and 4 samples of digestive gland and gonads) of individuals from the central part along the Croatian Adriatic Coast were taken to determine the possible presence of parasites in shell tissues by PCR and histopathology analyses.

Results: Up to March 2019, symptoms of disease and mass mortality event were not noticed in the monitored populations of *P. nobilis* in the Croatian part of the Adriatic Sea. Also, in the tissues of the examined individuals by PCR assay and histological analysis, no presence of parasite *Haplosporidium pinnae* was recorded.

Conclusion: Unusual mortality of *P. nobilis* has not yet been observed in the Croatian part of the Adriatic Sea. Moreover, we did not found any evidence of the presence of parasite *H. pinnae* in examined individuals. However, tracking the health status of *P. nobilis* population is important in order to be better prepared for possible upcoming of disease.

Keywords: *Pinna nobilis*, mass mortality event, *Haplosporidium pinnae*

068-P

Early detection of *Cardicola* (Trematoda: Aporocotylidae) eggs in hatchery-reared Atlantic bluefin tuna (*Thunnus thynnus* L.)

Muñoz, Pilar (Spain)[1]; Aranda, Guillermo (Norway)[2]; Rodríguez, Carlos (Spain)[3]; Neumann, Lukas (Norway)[4]; Nowak, Barbara (Australia)[5]

1 - Dpto. Sanidad Animal, Universidad de Murcia, Campus de Excelencia Internacional Regional "Campus Mare Nostrum", 30100, Murcia, Spain; 2 - NORTUNA AS, Hoffsveien 1c, 0275 Oslo; 3 - LIPTOAQUA, C/ San Romualdo 12 – 14, 3. 28037 Madrid; 4 - Aquatic Research Center, Skretting ARC, Stavanger; 5 - Institute of Marine and Antarctic Studies, University of Tasmania, Launceston, Tasmania 7250

Introduction: In 2011, in the framework of the SELFDOTT project Atlantic Bluefin tuna (ABT), *Thunnus thynnus*, were reproduced for the first time in captivity, and juveniles were produced, which were housed in floating cages. Although, the closure of ABT life cycle in captivity comes to a hopeful solution for the development of a self-sustained aquaculture industry, ABT farming can result in an increase of outbreaks of infectious diseases. The detection of pathogens at early stages of infection is a key point for disease control in aquaculture. Thus, we aimed to investigate when infection with blood flukes from the genus *Cardicola* can be first detected in cultured ABT by checking for eggs in the gills and using a molecular approach.

Methodology: Fifty two freshly dead juvenile ABT (mean weight 119.62 ± 164.47 g, range 5.4 - 657.4 g and mean size 15.77 ± 7.69 cm, range 8.3 - 40.2) were used. Gills were observed under a stereomicroscope and the number of eggs per gram of gill tissue was estimated using gill digestion. For molecular identification of the *Cardicola* spp. present seven ABT were used.

Results: The microscopic study revealed a total blood fluke egg prevalence of 7.69%. Eggs were detected by microscopic observation in fish higher than 26.8 g. The gill digestion method revealed a total of 418.48 ± 409.58 eggs/g tissue. Oval and crescent-shaped eggs, previously ascribed to *Cardicola* sp. and *C. opisthorchis* respectively (Forte-Gil et al., 2015), were observed being simultaneously present in two specimens. In two cases, PCR detected *C. opistorchis* while it failed to detect any *Cardicola* species in the other samples.

Conclusion: The present study contributes to a better understanding of *Cardicola* spp. infection process. Implications for aquaculture of ABT in the Mediterranean are discussed.

Keywords: Atlantic bluefin tuna, *Cardicola*, blood fluke

069-P

Effects of water exchange rate on the diplomonad flagellate *Spironucleus salmonis* infection in juvenile salmonid fish

Urawa, Shigehiko (Japan)[1]; Mizuno, Shinya (Japan)[2]

1 - Hokkaido National Fisheries Research Institute, FRA; 2 - Salmon and Freshwater Fisheries Research Institute, HRO

Introduction: The diplomonad flagellate *Spironucleus salmonis* is commonly found in the intestinal tract of salmonid fishes in Europe, North America and Asia. The heavy parasite infection is associated with the growth reduction and mortality of salmonid juveniles reared at hatcheries. We assumed that the mobile trophozoites or cysts are passed into the environment with faces, facilitating the direct water-borne transmission. The present infection experiment was designed to evaluate effects of water exchange rate on the control of *S. salmonis* infection.

Methodology: Two groups of juvenile chum salmon (*Oncorhynchus keta*; mean weight 0.4 g; n = 350 each) were cohabited with *S. salmonis*-infected fish (n = 20) in 21-l tank with constant water supply at high (3.9 l/min; 11.1 turns/h) or low (1.3 l/min; 3.7 turns/h) exchange rate. In addition, two control groups (n = 350 each) were cohabited with uninfected fish (n = 20), and reared under the same conditions. Fish were fed with commercial dry pellets at 3% body weight/day for 10 weeks. The water temperature in each tank was almost constant at 11.7 ºC. Thirty fish were sampled from each groups every two weeks, and measured and weighted individually. The whole intestine was removed from each fish, and preserved in 99% ethanol for quantitative molecular analysis. Abundance of parasites in the intestine was estimated from the copy number of *S. salmonis*-specific small-subunit ribosomal RNA gene (rDNA), which was analyzed by real-time quantitative PCR.

Results: Parasite abundance increased rapidly in the infection group reared with low water supply, while it increased gradually in the infection group with high water supply. The cumulative mortality of juvenile chum salmon for 10 weeks was 10% and 16% in the infection groups reared with high and low water supply, respectively, while it was 0.6-1.6% in the uninfected control groups. There were no significant differences in the growth of juveniles among the groups.

Conclusion: The results suggest that increasing exchange rate of water supply is effective to reduce the horizontal transmission of *S. salmonis* by excluding the infectious trophozoites or cysts from the rearing environment.

Keywords: diplomonad parasite, salmonids, control

070-P*

A retrospective study on parasites of Atlantic lumpfish (*Cyclopterus lumpus* L.) used as a cleaner fish in Norwegian salmonid aquaculture

Wüstner, Stefanie C. (Norway)[1]; Erkinharju, Toni (Norway)[1]; Furnesvik, Lisa (Norway)[1]; Hansen, Miroslava (Norway)[1]; Hansen, Haakon (Norway)[1]

1 - Norwegian Veterinary Institute, Norway

Introduction: The demand for Atlantic lumpfish (*Cyclopterus lumpus* L.) as cleaner fish for salmon lice (*Lepeophtheirus salmonis* K.) infestations in Atlantic salmon (*Salmo salar* L.) has increased exponentially since 2012. The production of lumpfish has increased accordingly and lumpfish is now the second most important fish species in Norwegian aquaculture in terms of numbers of fish produced. Over time, several types of unicellular and multicellular parasites have been observed in both wild and farmed lumpfish, including ciliates, flagellates, amoeba, myxozoans, flukes, sea-lice, different types of parasitic worms, and fungal-like parasites. Most of these parasite infections do not seem to cause significant harm to the host but may under stressful conditions with high densities of hosts like in aquaculture, become serious health problems. However, our present knowledge of lumpfish parasites during farming conditions are limited and more research is needed.

Methodology: Here we present a retrospective study on diagnostic cases of Atlantic lumpfish that were analysed by the Norwegian Veterinary Institute between 2014 and 2019. Data is presented as number of farming localities with histological changes consistent with parasite infestation/infection, in gill, gastrointestinal and other organ parasites. The results were sorted according to type of farming (sea-based or land-based), time of year (winter, spring, summer or autumn) and geographic location (north or south). As most cases are based on histopathological examination, most parasites were not identified to the species-level, but were generally assigned to either genus (example *Trichodina* sp.) or type of parasite (example nematode). Histological slides were prepared using standard histological techniques.

Results: The most prevalent observations in the gills were infections with *Trichodina* sp., gill amoebas (AGD), *Ichthyobodo* sp., *Gyrodactylus* sp. and *Cryptocotyle* sp., while for gastrointestinal parasites, *Cryptobia* sp., apicomplexans, nematodes, trematodes, cestodes, *Cycloptericola* sp. and *Ichthyophonus* sp. were the most common. For the kidney, both microsporidian and myxozoans parasites were observed, while the myxozoan *Kudoa* sp. was found in skeletal muscle.

Conclusion: The data demonstrated that several gill, gastrointestinal and other organ parasites were the most prevalent in lumpfish samples submitted to the Norwegian Veterinary Institute between 2014 and 2019.

Keywords: Atlantic lumpfish, parasites, gills, gastrointestinal, retrospective study

Funding: Norwegian Veterinary Institute.

071-P

Development of alternative, ecologically safe, effective and well-tolerated control strategies against *Ichthyophthirius multifiliis*

Jung-Schroers, Verena (Germany)[1]; Kallert, Dennis M. (Germany)[2]; Teitge, Felix (Germany)[1]; Zielasko, Marcus (Germany)[3]; Loy, Christina (Germany)[2]; Schmidt, Gregor (Germany)[3]; Wedekind, Helmut (Germany)[3]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany; 2 - Kallert & Loy GbR, Adelsdorf, Germany; 3 - Bavarian State Research Center for Agriculture, Institute for Fisheries, Starnberg, Germany

Introduction: Infections of fish with *Ichthyophthirius multifiliis* can lead to severe losses. Currently, no effective drugs for the treatment of this parasite in fish for human consumption are authorized in the EU. Considering animal welfare, this is not justifiable; and additionally a disease outbreak can lead to considerable economic losses and often endangers the existence of traditional farms. The development of a non-therapeutic-based control strategy can therefore make an important contribution to promote sustainable aquaculture and preserve traditional pond management.

Methodology: Three alternative control strategies for the reduction of *Ichthyophthirius multifiliis* in ongrowing units were tested. The number of infectious parasite stages in the water and their distribution should be significantly reduced by nanofiltration and by blocking the transmission of the parasite by methods which inactivate the parasite stages in the water, prevent the host recognition or trap parasite stages. Another aspect is the development of new vaccine strategies against the parasite. Different vaccine regimes with preparations of *Ichtyophthirius multifiliis* and *Tetrahymena* spp. will be used. Evaluation of the number of parasites was done microscopically or by an established real time PCR.

Results: First results show that the number of parasites can be reduced in small water amounts by nanofiltration. Nevertheless, for larger ponds this method is not suitable. We identified solubilized and matrix-bound natural stimulants that trigger theront host finding behavior with high efficacy. Utilizing these activating compounds gave positive preliminary results for an effective transmission breach in both experimental laboratory trials and semi-field challenge setups with juvenile trout. Vaccination of fishes with preparations from *Tetrahymena* spp. did not result in a sufficient immunization and no adequate cross reaction between antigens of *I. multifiliis* and *Tetrayhmena* spp. was observed.

Conclusion: It is possible to reduce infection intensities with *Ichthyophthirius multifiliis* in fish without a therapeutic treatment. In small units, for example in hatcheries, aquaria or quarantine systems, nanofiltration can reduce the number of theronts. In larger tanks matrix-bound stimulants can prevent theront transmission. Vaccination strategies are promising as well and have to be evaluated further.

Keywords: *Ichthyophthirius multifiliis*, vaccination, transmission blocking, alternative control strategies, nanofiltration

072-P

Nodular gill disease in piedmont region (Italy): preliminary data

Varello, Katia (Italy)[1]; Prearo, Marino (Italy)[1]; Carnevale, Viola (Italy)[1]; Righetti, Marzia (Italy)[1]; Pastorino, Paolo (Italy)[1]; Giuseppina, Abbamonte (Italy)[1]; Cavazza, Giulia (Italy)[1]; Audino, Valentina (Italy)[1]; Mugetti, Davide (Italy)[1]; Bozzetta, Elena (Italy)[1]

1 - Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy

Introduction: Nodular Gill Disease (NGD) associated with amoebae is a proliferative branchitis reported in freshwater salmonids as rainbow trout (*Oncorhynchus mykiss*). This disease has been reported in North America and Europe including Italy. The aim of the work was to evaluate the spread of NGD in Piedmont region (Italy) through a systematic monitoring.

Methodology: the experimental design included sampling in 5 intensive rainbow trout farms located in different areas of the Piedmont region for a period of one year (November 2016-October 2017). Monthly, five fish from 3 tanks of each farm were sampled. Analyses of physicochemical water parameters have been carried out. All fish were subjected to anatomo-pathological examination and gills were grossly evaluated according to Taylor et al. (2009). The first left external gill arch from each trout was fixed in 10% neutral buffered formalin and processed by standard paraffin wax techniques. Samples were cut in 4±2µ sections and stained with haematoxylin-eosin and Giemsa and histologically evaluated according to Clark & Nowak (1999). In this preliminary report, we histologically analysed gills of 320 trout sampled in the four seasons (November, February, May, August) from the five farms.

Results: gross and histological lesions referable to NGD were detected and classified mainly from grade 0 to 2. Higher grades (3 - 5) were less frequently reported, especially in Autumn/Winter months. Amoebae-like organisms were found in 3 of the 5 farms, in medium-low number, always associated to typical lesions. The presence of amoebae was related to water temperatures around 10 - 13°C. Apart from a farm, where the infestation was more extensive and with high containment problems, in the others, the management by the breeders has proved effective and suitable. In a farm with high water temperatures throughout the year (spring water 16 °C), unfavourable to the development of the disease, the presence of amoebae resulted very low. Therefore, thanks to the constant application of good management practices it is possible, in conditions not particularly at risk, to manage the disease without large losses of fish.

Conclusion: these are preliminary results and the regional situation will be only defined at the end of the histological evaluations.

Keywords: NGD, trout farms, *Oncorhynchus mykiss*, Amoebae

073-P*

Recurrent infection by *Anisakis* spp. in the European hake fishery

Rodríguez, Helena (Spain)[1]; González, Ángel F. (Spain)[1]; Pascual, Santiago (Spain)[1]

1 - Instituto de Investigaciones Marinas-CSIC

Introduction: The European hake has been demonstrated to posse an emergent risk for fish raw (or lightly cooked)-eating consumers, due to the large amount of viable infective *Anisakis* larvae found in the edible part of fish. Because of this, anisakiasis is being considered within the top ten list of most important zoonotic disease in Europe. It is well known that anisakids are able to migrate from the fish gut to the flesh, likely due to different stimulus, but the burning question that remains unsolved in any fishery is the percentage rate attributable to intra-vitam or post-mortem parasite migration behaviour. In this study, we analyse the histopathological findings of the *Anisakis* infection in different European hake products available in European markets to illustrate the relative importance of acute and chronic episodes, as a prognostic probe for the existence of recurring infective chains taking place in the European hake fishery.

Methodology: Samples of belly flaps from parasitized fish of similar year-class (~1 kg; obtained from a local fish market, a major fish dealer and a supermarket; 5 each) were routinely processed for histological preparations following standard methods, and the range of histopathological responses to *Anisakis* spp. were recorded.

Results: In all hake products, the nematode infection tended to a fish response that includes granuloma formation and fibrous encapsulation. The inflammatory response included degeneration, melanization and parasite reabsorption. This marked general pattern of concurrent pathological responses to persistent heavy infections would suggest a chronic disease condition in the European hake population, although it is still unclear both the role of *Anisakis* influencing fish population dynamics and the ability of the fish to compensate physiologically for the infection.

Conclusion: Overall, the prognostic probe in each individual fish provides an important insight of the relative importance of intra-vitam migration of *Anisakis* infective larvae into the flesh in the European hake fishery. This finding makes an important improving in how a parasitized fishery must be managed under a one-health perspective.

Keywords: European hake, *Anisakis*, anisakiasis, histopathology

074-P*

Gross lesions of muscle parasites in Atlantic swordfish

Ramilo, Andrea (Spain)[1]; Abollo, Elvira (Spain)[2];Rodríguez, Helena (Spain)[1]; Pascual, Santiago (Spain)[1]

1 - Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, IIM-CSIC, Vigo, Pontevedra, Spain; 2 - Fundación Centro Tecnológico del Mar, Vigo (Spain)

Introduction: The swordfish is a pelagic and migratory fish of great commercial interest for the seafood industry, being sold as fresh and frozen pieces and slices. The presence of parasitic lesions during post-harvest processing has been noted as a major concern for the industry, due to the food quality and safety issues but mostly to the unaesthetic appearance of the macroscopic alterations in infected fish products.

Methodology: Samples of frozen slices of *Xiphias gladius* were inspected at a local seafood industry in order to identify the macroscopic artefacts of presumptive parasitic lesions. Histological examination was also carried out on muscle portions with these macroscopic artefacts and molecular identification of the parasites was performed. Traceable data for samples were used to summarise the distribution and incidence of macroscopic lesions for fishing areas. Samples and data obtained were biobanked in the PARASITE biobank (IIM-CSIC).

Results: According to the histological findings the parasitic lesions corresponded to the cestode *Molicola horridus* and to the trematode *Maccallumtrema xiphiados*, previously described in *X. gladius*. Due to the scarcity of sequences of these parasites deposited in GenBank, molecular characterization of the 18S-ITS1-5.8S-ITS2-28S region was performed. Phylogenetic analysis of combined SSUrDNA and LSUrDNA region clustered the cestode together *Molicola* sp. of *X. gladius* from Sri Lanka (100% bootstrap) and the trematode was clustered within the family Didymozoidae (100% bootstrap). Data of distribution and incidence point out the mean percentage of rejected lots in FAO areas 27, 34, 47, 51, 57 and 87 increased from 1.7% in 2016 to 3% in 2018.

Conclusion: The zoonotic and allergen risks for consumers related to the presence of these parasites in frozen swordfish slices are negligible. Nevertheless, the fact that these swordfish products are obviously contaminated with “visible parasites” (i.e. they are clearly distinguishable from the fish tissues by the naked eye) made them unfit for human consumption and accordingly with the EC Regulation 853/2004 removed from the market. The genetic characterization of *M. horridus* and *M. xiphiados* provides valuable information for the development to new molecular diagnostic assays for them.

Keywords: *Molicola horridus*, *Maccallumtrema xiphiados*, *Xiphias gladius*, genetic characterization, food quality

075-P*

Trophic transmission of *Rhadinorhynchus* spp. from mesozooplankton (Euphausiacea) to small pelagic fishes in the nw Iberian Peninsula

García-Alves, Lara (Spain)[1]; Ramilo, Andrea (Spain)[1]; Rodríguez, Helena (Spain)[1]; González, Ángel F. (Spain)[1]; Pascual, Santiago (Spain)[1]

1 - Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, Vigo, Pontevedra (Spain)

Introduction: It is well known that euphausiids may act as intermediate host of helminth and acantocephalan endoparasites, having a key role in the infection transmission to higher trophic levels. Euphausiids are considered the main prey of small pelagic fishes which present a particulate-feeding behaviour and a prey size selectivity. In this study we provide evidence on the transmission of acantocehalan endoparasites of *Rhadinorhynchus* spp between *Nyctiphanes couchii* and the European pilchard and the Atlantic mackerel.

Methodology: In the sampling framework of CALECO Project (CTM2015-69519-R) we used a multinet to collect mesozooplankton in the Ría de Vigo. A swarm of euphausiids was observed at the end of summer 2017 and these euphausiids were microscopically studied for taxonomic identification and acantocephalan detection. Adult euphausiids and acanthocephalan cystacanths were genetically analysed by PCR-sequencing of COI mitochondrial DNA and 18S rDNA to confirm their taxonomic identification. Twenty days later from the first sampling of mesozoplankton, small pelagic fishes were obtained from commercial fishing in the same study area. Stomach contents of *Sardina pilchardus* (L = 219.13 ± 16.66 mm) and *Scomber scombrus* (L = 273 ± 14.9 mm) were microscopically and genetically analysed to confirm the taxonomic identity of the acantocephalan and the Euphausiacea.

Results: A total of 414 cystacanths infecting thoracic organs of *Nyctiphanes couchii* were found in water column (0 - 85m). Prevalence of cystacanths was 0.47 ± 0.25 and mean intensity 1.03 ± 0.04. Genetic analyses confirm the presence of cystacanths (in *N. couchii*) and adults of a single species of *Rhadinorhynchus* (in *S. pilchardus* and *S. scombrus*).

Conclusion: The coupling of the life cycle of *Rhadynorhynchus* to the trophic web (Eupahausiacea-pelagic fish) in the seasonal upwelling system off Galicia is demonstrated.

Keywords: euphausiids, acantocephalan, life cycle, trophic transmission

076-P*

Unravelling the assumed relationship between a healthy and a sustainable fishery

Rodríguez, Helena (Spain)[1]; González, Miguel (Spain)[2]; Abollo, Elvira (Spain)[3]; González, Ángel F. (Spain)[1]; Pascual, Santiago (Spain)[1]

1 - Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, Vigo, Pontevedra, Spain; 2 - Servicio de Alergología, Hospital La Paz, Madrid, Spain; 3 - Fundación Centro Tecnológico del Mar, Vigo (Spain)

Introduction: Fish is an excellent protein source, but their consumption can be more harm than good if they present hazards in great amounts. In the last decade marine parasites have become an emergent biohazard in major European markets, due to two main reasons: (1) reported increasing number of allergic and gastrointestinal disorders caused by fish-borne parasitic infections, and (2) commercial impact and economic losses due to fish rejections. In this study, a single commercial fresh lot of Atlantic European hake from a certified sustainable fishery were randomly inspected for parasites to ascertain if a fishery certified as sustainable renders a healthy fish product.

Methodology: The fresh hake lot was inspected for zoonotic anisakid parasites by destructive standard procedures. Previously, each fish was inspected by visual inspection, following EC Regulation 853/2004. Demography of infection and level of exposure to parasite risk was categorized by the Fish Parasite Rating (FPR). Parasite allergens of high clinical relevance (Ani s 1, Ani s 4) were also quantified.

Results: Results revealed a significant high risk exposure associated with zoonotic and allergenic parasites in the edible part of fish. Prevalence of infection was 100%, with density values of 6.79 parasites/100 g., and a dispersed infection pattern through the fish body. Excretory-secretory protein allergens were detected in 90% (Ani s 1) and 100 % (Ani s 4) of hakes analysed, with concentrations values highest in the antero-ventral flesh sites reaching up to 86.47 µg of Ani s 1/g and 2.17 µg of Ani s 4/g. Thus, the fish lot was categorized as poor (FPR score 1.9) and visible parasites seen in 60% of examined fish.

Conclusion: Overall, the certified sustainable fish product examined is an important source of hazard exposure to anisakiasis and/or related allergy. Accordingly, as it is established in EC regulations, the inspected commercial lot should be not intended for human consumption and then managed as a fish waste. The results suggest an inconsistency between the sustainable fishery goals and healthy fish attributes. This factual evidence illustrates the importance of this problem, and the need for a one-health perspective integrated in fisheries management.

Keywords: European hake, *Anisakis*, anisakiasis, sustainable fishery

077-P

Source, transmission and development of *Ichthyophonus hoferi* infection in the Icelandic summer-spawning herring

Pétursson, Hrólfur S. (Iceland)[1]; Kristmundsson, Árni (Iceland)[1]; Bragason, Birkir T. (Iceland)[1]; Óskarsson, Guðmundur J. (Iceland)[2]

1 - Institute For Experimental Pathology, University of Iceland, Keldur, Keldnavegur 3, IS-112, Reykjavík, Iceland; 2 - Marine and Freshwater Research Institute in Iceland, Skúlagata 4, IS-101, Reykjavík, Iceland

Introduction: In the mid-2000s, the Icelandic herring (*Clupea harengus*) population had been relatively strong and stable since its collapse in the 1960s, which was likely due to overfishing. However, in 2008, severe and highly prevalent (up to 70 - 80%) *Ichthyophonus hoferi* infections were experienced in older cohorts of herring, followed by a significant decline in the stock. The reason for this sudden increase of infections caused by endemic pathogen is unclear and a decade later, the epidemic is still ongoing. The aim is to investigate the source, transmission and development of *Ichthyophonus* infections to shed light on this prolonged epidemic in the Icelandic population of summer spawning herring.

Methodology: Different age groups of herring and various species of pelagic crustaceans, as well as haddock, cod and plaice are examined for infections using PCR, qPCR, conventional histology and *in situ* hybridization.

Results: Results indicate that various pelagic crustacean species carry *Ichthyophonus* infections and are therefore a reservoir for the parasite. Furthermore, infections do not seem to be restricted to any specific age-groups, as asymptomatic juvenile herrings, previously thought to be free of infections, have subclinical infections suggesting that the apparent age-related presence of clinical signs cannot be explained by a difference in diet.

Conclusion: Factors such as consumption of infected crustaceans over extended periods or/and the stressful process of maturation may lead to intensified infections and development of clinical disease. Environmental changes cannot be disregarded but data is lacking for further support. An *in situ* hybridization technique, intended to reveal the transmission route, has been successfully optimized and applied to samples highly positive for *I. hoferi*. The route of transmission and the development of the parasite in fish and crustacean hosts, remain unclear. Hints of free-living cell stages in crustaceans from PCR positive samples were observed with calcofluor-white chitin staining, further supporting infection via crustacean consumption.

Keywords: *Ichthyophonus*, herring, epidemic, Iceland, PCR

078-P*

Biochemical and proteomic characterisation of secretory proteins from the protozoan parasite *Paramoeba perurans* revealed by an in vitro model

Ní Dhufaigh, Kerrie (Ireland)[1]; Maccarthy, Eugene (Ireland)[1]; Slattery, Orla (Ireland)[1]

1 - Galway-Mayo Institute of Technology

Introduction: The parasite *Paramoeba perurans* is suspected to have a set of extracellular virulent proteins (Butler and Nowak, 2004; Bridle et al, 2015) that result in the clinical manifestation of AGD in susceptible farmed fish. Bridle et al (2015) demonstrated that extracellular secretions from a virulent "wild type" *P. perurans* isolate produced a greater cytotoxic response in a Chinook salmon embryo (CHSE -214) cell line compared with secretions from a long-term cultured *P. perurans* isolate. However, the causative proteins responsible for the host cytotoxic response were not determined. This study aims to characterise the extracellular proteome of *P. perurans*.

Methodology: Media was collected and pooled from *P. perurans* cultures and filtered through a 0.22 µm polyethersulfone filter to obtain cell-free supernatants. The supernatants were concentrated 10-fold using centrifugal concentrators and protein quantification measured with the BCA assay. One dimensional (1D) gels coupled with LC- MS/MS was used in separating out the soluble extracellular fraction of a virulent and non-virulent isolate of *P. perurans*. The extracellular proteins were also subjected to enzymatic activity using a protease assay and extracellular degradation capabilities of the parasite was also determined using gel zymography. An epithelial cell line was used to assess the host cytotoxic effects of the extracellular proteins from *P. perurans*. The epithelial gill cell line viability was assessed using a trio of assays.

Results: Cytotoxicity was detected when the epithelial cell line was incubated with *P. perurans* extracellular proteins. Confirmation of protease activity via the protease assay and gel zymography was also noted for both the virulent and avirulent strain. The proteins responsible for the cytotoxicity are currently being identified via LC MS/MS.

Conclusion: A trio of assays was used to validate cytotoxictiy of the virulent and avirulent *P. perurans* strain on an epithelial cell line. The causative cytotoxic proteins are currently being analysed and identified using LC MS/MS.

Funding: Department of Agriculture, Food and the Marine, Ireland.

079-P

Emerging enteric parasitic diseases in farmed gilthead sea bream (*Sparus aurata*)

Mazzone, Angelica (Italy)[1]; Caffara, Monica (Italy)[2]; Gustinelli, Andrea (Italy)[2]; Scaturro, Giuseppe (Italy)[3]; Colak, Slavica (Croatia)[4]; Fioravanti, Maria Letizia (Italy)[2]

1 - Department of Veterinary Medical Sciences, Alma Mater Studiorum Università di Bologna; 2 - Department of Veterinary Medical Sciences, Alma Mater Studiorum Università di Bologna, Italy; 3 - Gruppo Del Pesce, Italia; 4 - Cromaris, Croatia

Introduction: Enteric parasites affecting farmed gilthead sea bream (GSB) have become a serious threat for Mediterranean aquaculture in the last few years; among these parasites *Enteromyxum leei, Enterospora nucleophila* and *Cryptosporidium molnari* are undoubtedly the most concerning ones. While the enteric myxozoan *E. leei* has been broadly studied, the available data on the occurrence of *E. nucleophila* and *C. molnari* infections are still scarce. Aim of this work was to improve the knowledge about these latter two parasites in Mediterranean aquaculture by carrying out an epidemiological survey in GSB farmed in Italy and Croatia.

Methodology: 308 GSB of which 40 from an Italian hatchery, 174 from three cage farms located in Italy and 94 GSB from one cage farm located in Croatia were tested with qPCR and PCR to assess the presence of *E. nucleophila* and *C. molnari*. Histology was also performed on infected GSB.

Results: All the examined farms tested positive for both parasites: 60% of the fish examined from hatchery were positive for *E. nucleophila*, while 22.5% resulted positive for *C. molnari*. Concerning caged fish, *E. nucleophila* was found in 63.2% of the GSB coming from Italian farms and in 45.7% of fish from Croatia. *C. molnari* was detected in 3.4% of Italian GSB and in 2.1% of the Croatian ones. Histological lesions were consistent with those already reported in literature for these enteric parasitic infections.

Conclusion: This study showed a diffuse presence and a high prevalence of *E. nucleophila* in Italian and Croatian farmed GSB. Although at lower prevalence, also *C. molnari* showed to occur in farmed GSB, especially in hatchery and juveniles. Thus, further investigations are required to establish their epidemiology, transmission routes and pathogenic role in farmed GSB along its production cycle in order to assess and manage the risks arousing from these emerging enteric parasites.

Keywords: *Enterospora nucleophila*, *Cryptosporidium molnari*, gilthead sea bream, *Sparus aurata*

Funding: PerformFISH H2020 project (727610).

080-P

Infections of *Glugea hertwigi* in juvenile smelt *Osmerus eperlanus* – does warmer and slower reduce survival of the fattest?

Williams, Chris (United Kingdom)[1]; Fauconier, Georgina (United Kingdom)[2]; Cousins, Tom (United Kingdom)[1]; Reading, Amy (United Kingdom)[1]; Bradley, Hannah (United Kingdom)[1]; James, Joanna (United Kingdom)[1]; Cox, Thea (United Kingdom)[3]; Baynes, Alice (United Kingdom)[2]

1 - Environment Agency; 2 - Brunel University; 3 - Zoological Society of London

Introduction: The European smelt, *Osmerus eperlanus*, is a commercially important anadromous fish and important indicator species of freshwater environments. Once common, populations of *O. eperlanus* have declined throughout much of the UK, highlighting a need for better understanding of the environmental and anthropogenic pressures acting on extant populations.

Methodology: During 2018, routine surveys of juvenile smelt conducted by the Environment Agency throughout the River Thames catchment, England, revealed a high prevalence of fish exhibiting pronounced abdominal swellings. Fish samples were sent to the National Fisheries Laboratory for diagnostic examination.

Results: Parasitological and histopathological examinations confirmed these infections to be caused by a microsporidian parasite, consistent with the species *Glugea hertwigi*. Over 80% of juvenile smelt were infected with most exhibiting lateral and ventral swellings as a consequence of xenomas throughout the body. In some cases, xenomas constituted up to 40% of the total body mass. Histopathological changes were characterised by organ displacement, disruption of the intestine, connective tissue proliferation and inflammatory changes. Lesions were predominantly observed in the anterior body region, although diffuse infections involving the posterior gut, caudal musculature and even the head were observed.

Conclusion: Heavily parasitised *O. eperlanus* were considered unlikely to survive these infections, with potential loss of a large proportion of the 2018 year class. The role of environmental variables, notably elevated water temperature and low river flows are discussed as possible drivers for the emergence of disease. Future developments for the detection of this parasite in wild fish populations are discussed.

Keywords: microsproidian; smelt; *Glugea*

Funding: Environment Agency.

081-P

Transfer of *Sparicotyle chrysophrii* and *Ceratothoa oestroides* between wild and aquaculture fish inferred by ddRAD sequencing

Mladineo, Ivona (Croatia)[1]; Trumbic, Zeljka (Croatia)[2]; Tsakogiannis, Alexandros (Greece)[3]; Manousaki, Tereza (Greece)[3]; Vrbatovic, Anamarija (Croatia)[1]; Taggart, John (United Kingdom)[4]; Rigos, George (Greece)[3]; Sitjà-Bobadilla, Ariadna (Spain)[5]; Trumbic, Paola (Italy)[6]; Tsigenopoulos, Costas (Greece)[3]

1 - Institute of Oceanography and Fisheries; 2 - University Department of Marine Studies; 3 - Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research; 4 - Institute of Aquaculture, University of Stirling; 5 - Fish Pathology Group, Institute of Aquaculture Torre de la Sal; 6 - Department of Agro-alimentary, Environmental and Animal Sciences, University of Udine

Introduction: The monogenean *Sparicotyle chrysophrii* and the cymothoid isopod *Ceratothoa oestroides* are among the most devastating ectoparasites in Mediterranean aquaculture, causing losses in gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) production. Their life cycles and modes of transmission have not been fully elucidated and molecular resources for their investigation are extremely scarce. Being attracted by signals from conspecifics in aquaculture installations, the food and shelter availability in the farms, wild fish are generally recognised as reservoirs for parasite transmission to farmed fish. Vice versa, cultured stocks may also act as reservoirs of infectious stages, as they are present in concentrated numbers and density, and potentially more susceptible to disease than wild fish.

Methodology: The potential extent of transmission of these two ectoparasites between wild and farmed fish populations has been evaluated by genotyping-by-sequencing approach, double digest Restriction-site Associated DNA sequencing (ddRAD-Seq), to generate a genome-wide SNP marker dataset. Parasites sampling was done across four large Mediterranean farming areas in Spain, Italy, Croatia and Greece, representing the largest collection of genotypes obtained from aquaculture and wild fish populations (n = 600).

Results: Expectedly, a considerable variability in number of reads, polymorphic loci and SNPs between individuals for both species was observed, while transfer between wild and reared parasite population was more enhanced in case of the isopod.

Conclusion: The results provide a baseline for the development of new ecological and epidemiological measures for mitigation of two parasites, as the observed transfer seems to be highly conditioned by parasite and environment traits.

Keywords: *Ceratothoa oestroides*, ddRAD, Mediterranean aquaculture, *Sparicotyle chrysophrii*, transfer

Diseases of Public Concern

082-P

Emergence of the zoonotic biliary trematodes (Opisthorchiidae) in fish of North-Western Russia

Voronin, Vladimir (Russian Federation)[2]; Kudriavtseva, Tatjana (Russian Federation)[2]; Viljamaa-Dirks, Satu (Finland)[1]

1 - Finnish Food Authority; 2 - St. Petersburg State Academy of Veterinary Medicine

Introduction: Opisthorchiasis is a focal parasitic zoonotic disease caused by the trematodes of family Opisthorchiidae. The complex life cycle of these parasites includes a broad range of fish-eating mammals as definitive hosts and two intermediate hosts: freshwater gastropods and cyprinid fish. In Europe, the most dangerous fish-borne trematodes for human health are *Opisthorchis felineus*, *Metorchis bilis* and *Pseudamphistomum truncatum*. *P. truncatum* is known to occur in seals in the Baltic Sea. Until now, among Baltic Sea countries these parasites were reported in fish from Germany and Denmark, and most recently from the sea waters of Russia and Finland.

Methodology: Between 2015 and 2018, as many as 982 specimens of different species of cyprinid fish from the Russian part of the Finnish Bay and the lakes Ladoga, Pskov and Ilmen were subjected to morphological parasitological investigation to detect trematode metacercaria.

Results: Six species of cyprinid fish (roach, ide, rudd, vimba, bream and bleak) were found infected with metacercaria of *P. truncatum* in the Finnish Bay and Lake Ilmen. The total level of the infection in the roach was 75.6% in the Finnish Bay and 54.3% in Lake Ilmen, respectively. Unexpectedly, the bream, which is the main cyprinid fish for human consumption in Russia, had a minimal level of infection in contrast to the other fish species. No metacercaria of *P. truncatum* were found in the other two lakes.

Conclusion: The new data about the wide distribution of *P. truncatum* in the Baltic Sea area is of large epidemiological significance. The results of the first limited parasitological investigation of the roach from the Finnish Bay in Finland concerning *P. truncatum* were reported being very similar to this study. The prevalence of this parasite in the Finnish lake area is so far largely unknown. Future research should be focused on understanding the life cycle of the parasite under the northern conditions and the overall prevalence, especially because of the zoonotic potential through the human consumption of fish.

Keywords: trematodes, cyprinids, Baltic Sea, *Pseudamphistomum truncatum*, zoonosis

083-P*

Identification of the new *Pseudomonas* species for aquaculture and important biochemical characteristics

Duman, Muhammed (Turkey)[1]; Saticioglu, Izzet Burcin (Turkey)[2]; Altun, Soner (Turkey)[1]

1 - Aquatic Animal Disease Department, Faculty of Veterinary Medicine, Bursa Uludag University, Bursa, Turkey; 2 - Aquatic Animal Disease Department, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey

Introduction: The genus *Pseudomonas* is one of the most complex Gram-negative bacterial genera as it currently is the genus with the largest number of species. While *Pseudomonas* cause severe disease for humans, the incidence or transmission route not known very well from aquaculture to humans. Recently, there are many possible novel *Pseudomonas* species identified, but a few of them identified from fish. In the present study, the identification of *Pseudomonas* species recovered from farmed salmonid species and the determination of the most important biochemical characteristics were aimed.

Methodology: *Pseudomonas* species were isolated from farmed salmonids in Turkey, 2013 - 2018. The strains were identified by 16S rRNA gene sequence analysis with the similarity index on NCBI database. Growth condition of *Pseudomonas* spp. on different salinity, incubation temperature, distilled and tap water were showed by using Tryptic soy broth media. In addition, the fluorescent pigment and biofilm production were determined by using *Pseudomonas* selective agar and broth media.

Results: The *Pseudomonas* species isolated from rainbow trout (*Onchorhynchus mykiss*), local trout species (*Salvelinus fontinalis*, *Salmo trutta magrostigma*, *Salmo trutta labrax*), Carp (*Cyrpinus carpio*) and also farm water samples. More than ten different *Pseudomonas* species were identified with the similarity of NCBI database such as *P. jessenii*, *P. madelii*, *P. gessardii* and a number of them are possible for novel species. The most of our isolates could growth on 0 - 6% NaCl-TSB medium, distilled and tap water and also 4 - 37 °C incubation temperature.

Conclusion: This is the first comprehensive research about *Pseudomonas* species determination on aquaculture in Turkey. The identified species were determined in the first time in salmonids and also some of them are possible novel *Pseudomonas* species isolated from aquaculture. Also our findings showed that the *Pseudomonas* can growth on distilled and tap water in addition broad range of salinity and incubation temperature. Thus, the transmission of this agent from aquaculture to human/animal/environment via water pose a big risk factor.

Keywords: *Pseudomonas,* new species, biochemical characteristics

Funding: Scientific and Technological Research Council (TUBITAK) of Turkey, project number: 118O420.

084-P

Functional interpretation of *Anisakis pegreffii* infective third stage larvae transcriptomes in accidental and paratenic hosts

Trumbic, Zeljka (Croatia)[1]; Buselic, Ivana (Croatia)[2]; Hrabar, Jerko (Croatia)[2]; Vrbatovic, Anamarija (Croatia)[2]; Petric, Mirela (Croatia)[1]; Mladineo, Ivona (Croatia)[2]

1 - University Department of Marine Studies, University of Split; 2 - Institute of Oceanography and Fisheries, Split

Introduction: *Anisakis* spp. are marine nematodes which can cause zoonotic infection in humans if accidentally ingested alive in raw/undercooked fish or cephalopod meat. The aim of this study was to explore the transcriptomes of *Anisakis pegreffii* L3 larvae in two remarkably different infection systems, an evolutionary familiar and unfamiliar host.

Methodology: Experimental infection was performed on European sea bass (*Dicentrarchus labrax*) (N = 24, sampling 4, 6, 10, and 12 h post-infection), representing a typical paratenic host, and Sprague-Dawley rats (N = 35, sampling 6, 10, 18, 24, and 32 h post-infection), representing a mammalian "novel-host" model, simulating accidental human infection. Larvae were collected while penetrating various host tissues in active migration or in passive transport through gastrointestinal tract. Samples of extracted RNA from larvae collected from both hosts were paired-end sequenced using Illumina NextSeq 500.

Results: In total, there were 132 (69 up and 63 down) differentially expressed (DE) transcripts in migrating compared to non-migrating *A. pegreffii* larvae in sea bass, out of which 110 had logFC > |1|. Biologically significant DE transcripts were included in carbohydrate transport and metabolism, translation, ribosomal structure and biogenesis, cell cycle control, cell division energy production and conversion, to name a few. In rats, there were 2799 (1606 up and 1193 down) DE transcripts in migrating compared to non-migrating *A. pegreffii* larvae, out of which 814 had logFC > |1|. In addition to cell cycle control, cell division, energy production and conversion, which were mentioned in larvae from sea bass, biologically significant DE transcripts in larvae from rats were also included in intracellular trafficking, secretion, and vesicular transport, secondary metabolites biosynthesis, transport and catabolism.

Conclusion: Studies of transcriptomes of parasites give insights into aspects of gene expression, regulation and function and they represent a significant step in understanding parasites' biology and interactions with their hosts and disease. Use of transcriptomics to reveal mechanisms of interactions of *Anisakis* and its paratenic and accidental host is applied for the first time in this study.

Keywords: transcriptomics, RNA-Seq, anisakiasis, experimental infection

Funding: Croatian Science Foundation, HRZZ (Angel, project no. 5576)

085-P

Anisakis spp. L3 larva revealed by transmission electron microscopy (TEM)

Hrabar, Jerko (Croatia)[1]; Vrbatovic, Anamarija (Croatia)[1]; Mladineo, Ivona (Croatia)[1]

1 - Institute of Oceanography and Fisheries

Introduction: Members of the genus *Anisakis* Dujardin, 1845 are marine parasitic nematodes with an indirect life cycle, that utilize marine mammals, primarily cetaceans, as definitive hosts and fish and cephalopods as paratenic hosts. Several members of this genus are the causative agent of human zoonotic disease anisakiasis, which can be contracted by consumption of raw or thermally lightly processed sea food infected with live third stage larvae (L3). Despite their cosmopolitan distribution and high abundance, much remains unknown about their biology, especially their morphology. This study presents the comprehensive ultrastructure of *Anisakis* spp. L3 larvae throughout the nematode body.

Methodology: Live L3 were cut with biopsy punches into 1 mm pieces, representing different body regions. The samples were immersed in 20% (w/v) BSA, fixed by high pressure freezing (HPF) and freeze substituted (FS) in dry acetone containing 2% OsO_4 over a 7-days period. Fixed samples were then resin embedded, sectioned at 70 µm, double contrasted and inspected under 80 kV Jeol microscope.

Results: Muscle layer, located below the multi-layered cuticle, is formed by elongated cells with coarsely granular cytoplasm indicative of glycogen storage and rich with crista-type mitochondria and prominent T-tubules. Spindle-shaped epithelial cells of alimentary tract, except for oesophagus, are lined with microvilli, with numerous electron dense and electron lucent vesicles and multivesicular bodies. Single-celled excretory gland is composed of tightly packed vesicles of different size, surrounding large nucleus with numerous prominent nucleoli. The gland is drained by one major and several minor collecting ducts. In the hind part of the worm, several exosomal vesicles were found, containing amorphous matter that was found lining the cuticle from the outside.

Conclusion: Presence of different vesicles and their localisation in intestinal cells and excretory gland, abundant rough endoplasmic reticulum and euchromatin suggests active synthesis of secreted products, aiding the infective larvae in invading host tissue.

Keywords: *Anisakis*, morphology, ultrastructure, TEM

Funding: EAFP Small Grant Scheme (to Hrabar, Jerko), and Croatian Science Foundation, HRZZ (AnisCar, #8490).

Aquatic Animal Epidemiology

087-P*

Fish welfare – big brother is watching you

Hofmann, Arndt Christian (Germany)[1]; Bergmann, Sven M. (Germany)[1]

1 - Friedrich-Loeffler-Institut Insel Riems, Südufer 10, 17493 Greifswald-Insel Riems, Deutschland

Introduction: Koi, which ornamental importance for recreational fish keeping is persistent growing, and Common carp, which can undertake the task to provide affordable high quality protein for human consumption, form together a big market with high economic value and potential growth. For various reasons the health and well-being of their fish is a primary target for private and commercial keepers, breeders and the aquaculture-industry. Hence we want to establish a system that can warn the responsible person soon enough about potential risks and uprising health threats.

Methodology: In an ongoing 18 months lasting animal experiment 37 carp, respectively koi per every four basins with different temperature regime, are continuously observed by different camera-prototypes. At the same time water-parameters like temperature, pH value, oxygen, ammonium, water turbidity and pump pressure are recorded and the social- and feeding-behaviour is interpreted and evaluated. Over the course of the project, this whole process is more and more automated and digitalised. Furthermore, every month samples of gills and skin for histology and serum for leucogram are taken.

Results: Since this long-term experiment is still ongoing, finalised statements of the outturn can't be given at the moment but we will be presenting out most recent data and give an insight into the current state of the project. This includes findings about basic behaviour, exceptional complex occurrences, the progress of digitalisation and data processing.

Conclusion: Conclusions will be possible at the end of the experiment.

Keywords: aquatic animal welfare, surveillance, carp, koi

Funding: BMWI ZIM Vita-Check.

088-P

Acquired immunity in freshwater aquaculture systems can mitigate pathogen risks to wild fish

Teixeira Alves, Mickael (United Kingdom)[1,2]; Taylor, Nick (United Kingdom)[1,2]

1 - Cefas; 2 - International Centre of Excellence for Aquatic Animal Health

Introduction: Experimental and field study infection data were used to describe dynamics of *Ichthyophthirius multifiliis* in farmed rainbow trout and wild freshwater finfish populations. Interactions between farmed and wild stocks were then investigated based on flow-through farming systems that facilitate pathogen transfer between farmed and wild fish populations.

Methodology: The parasite life-cycle and mechanisms involved in disease transfer were described using simple deterministic models. The macroparasite model considered parasite aggregation, induced mortality and host resistance via acquired immunity. Parameters were based on field data and on an epidemic observed in a study on juvenile (circa 5 g) farmed rainbow trout, assuming an optimal temperature of 19 °C for the parasite.

Results: In farmed fish, around 28% of hosts died due to the parasite and most of the surviving population became resistant (99.98%), which subsequently prevented further persistence of the parasite. The wild fish population oscillated and provided a natural reservoir for the parasite, which maintained it and allowed it to initiate farm infections as naïve stocks were introduced. The parasite caused no mortality but induced resistance in 3.45% of the wild fish population. Transfer of the parasite from a single age-class farm system increased mortality in wild fish by 4.67% and led to a substantial increase in the proportion of resistant wild fish (99.79%). The multi age-class farming system however caused the prevalence of resistance to increase with age-class in farmed fish. This reduced the transfer of the parasite to the wild fish population, and thus did not influence the mortality in wild fish but induced an increase of the immune response of the latter (99.93%).

Conclusion: The trends described in this study indicate that farmed fish that acquire immunity can act as a biological filter for the parasite by increasing the number of dead-end contacts for the parasite, thus affecting the overall infection dynamics, which in turn could be used to mitigate parasite risks to wild fish. It is also reassuring to note that in this case study, aquaculture is unlikely to induce detrimental effects on wild fish survival.

Keywords: *Ichthyophthirius multifiliis*, host immunity, freshwater aquaculture

Funding: Horizon 2020 (No. 634429 ParaFishControl).

089-P

Long term data on *R. salmoninarum* screening of wild Atlantic salmon broodfish in Icelandic rivers

Hafstað Ármannsdóttir, Edda Björk (Iceland)[1]; Guðmundsdóttir, Sigríður (Iceland)[1]; Kristmundsson, Árni (Iceland)[1]

1 - Institute for Experimental Pathology, University of Iceland, Keldur, 112-Reykjavik, Iceland

Introduction: Enhancement programs of wild salmon populations have been ongoing in Iceland for decades. Wild salmon broodfish are collected, stripped, their offspring cultured in aquaculture facilities and smolts subsequently released into their river of origin. *Renibacterium salmoninarum* (Rs), the causative agent of bacterial kidney disease (BKD), is common and widespread in wild Icelandic salmonids. As Rs can be transmitted both horizontally and vertically, all wild female broodfish used for this purpose are screened for the presence of Rs. Subsequently, eggs from all Rs positive fish are discarded, in order to minimize BKD episodes during the productions of smolts and to avoid the release of infected fish into the rivers. This practice has been mandatory in Iceland for decades.

Methodology: For 28 consecutive years (1991 - 2018), around 18,000 female broodfish, originating from numerous Icelandic rivers, were screened for the presence of Rs using polyclonal ELISA.

Results: The prevalence of Rs-positive fish was low in the first 15 years, never exceeding 3%. In 2006, a significant prevalence increase was observed when 12% of the broodfish tested positive for Rs-antigens. The subsequent years, a further increase was observed, reaching a peak of 25 - 28% in 2008 - 2009. Associated with this increase, a considerable portion of fish from several rivers showed clinical signs of BKD in captivity. The following two years the prevalence dropped to around 10% and since 2012, it has been relatively low; between 2 - 6%, i.e. similar to the situation before 2006.

Conclusion: The intensive increase in Rs-prevalence during 2006 - 2009 is not fully understood. However, bad husbandry practices in these years were without doubt of significance, where fish were stored in small containers in the rivers for weeks prior to stripping. Research made on Rs-status of emigrating smolts and returning salmon of the same cohort showed that adult salmons were almost Rs-free when entering the freshwater. The prevalence of Rs-positive fish gradually increased the longer the fish stayed in the freshwater system. In light of these results, most broodfish used for enhancing natural populations are now caught relatively soon after their freshwater entry and transferred to facilities with Rs-free water supply.

Keywords: Iceland, Atlantic salmon, *Renibacterium salmoninarum*, wild broodfish, enhancement programs

Funding: Keldur.

090-P

Epidemiological study on the occurrence and the pathogenicity of the carp edema virus (CEV) in fish in Germany

Jung-Schroers, Verena (Germany)[1]; Heling, Max (Germany)[1]; Adamek, Mikolaj (Germany)[1]; Teitge, Felix (Germany)[1]; Bauer, Julia (Germany)[1]; Kleingeld, Dirk Willem (Germany)[2]; Welzel, Alice (Germany)[2]; Bergmann, Sven M. (Germany)[3]; Sauter-Louis, Carola (Germany)[3]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany; 2 - Lower Saxony State Office for Consumer Protection and Food Safety, Hannover, Germany; 3 - Friedrich-Loeffler-Institut, Insel Riems, Germany

Introduction: Infections with the carp edema virus (CEV) can cause Koi sleepy disease (KSD) that seems to pose a potential risk to carp aquaculture and koi trade.

Methodology: During the years 2015 and 2016 an epidemiological study on the occurrence of CEV in fish in Germany was performed and in total 651 gill samples were analyzed.

Results: CEV genome fragments were detected in 248 samples and most detections were made in samples from koi carp (n = 179) and common carp (n = 61). The amounts of viral DNA were also highest in samples from koi carp (1.00E+00 – 4.82E+06) and common carp (1.00E+00 – 4.03E+06). In 1 - 2 samples each of *Ctenopharyngodon idella*, *Esox lucius*, *Gymnocephalus cernua*, *Perca fluviatilis*, *Sander lucioperca* genome fragments of CEV were found in low amounts (1.10E+00 – 1.19E+03). All DNA fragments were sequenced and isolates from common carp belonged mainly to genogroup 1 whereas isolates from koi carp belonged mainly to genogroup IIa. In koi carp disease outbreaks due to CEV were mostly seen when the water temperature was between 17 - 18 °C, whereas in common carp at water temperatures between 9 - 13 °C CEV was detected most frequently. Characteristic symptoms for an infection with CEV were enophthalmus, anorexia, gill necrosis, gill swelling and lethargic behavior. In 46.66% of samples taken from clinically healthy koi or carp from retailers, CEV was detected. Taken all samples from clinically healthy koi and carp, CEV could be detected only in 26.32% of all examined fish. Purchasing fish from retailers might be one risk factor for the introduction of CEV in a pond. In common carp more frequently diseases signs and mortalities were recorded compared to koi carp.

Conclusion: Fish health services should be aware of the presence of CEV and testing of koi and carp for CEV should become part of fish disease surveillance programs of national and regional fish disease laboratories.

Keywords: CEV, koi sleepy disease, KSD, epidemiology

091-P

A generic model for assessing risk of introduction and spread of viral diseases in mediterranean sea bass and sea bream farms

Tavornpanich, Saraya (Norway)[1]; Brun, Edgar (Norway)[1]

1 - Norwegian Veterinary Institute

Introduction: The study is part of the project entitled Mediterranean Aquaculture Integrated Development (MedAID) funded by the European Commission, Horizon 2020. The study focuses on diseases and health management of two main species produced in Mediterranean marine aquaculture, i.e., seabass (European sea bass: *Dicentrarchus labrax*) and sea bream (gilthead sea bream: *Sparus aurata*). Viral nervous necrosis (VNN) is used as considered the most serious viral infection of sea bass and sea bream. The objectives of this study are to quantitatively assess the risk of introduction and spread of the virus infection within a farm, and identify probable prevention and control measures to minimize the risk and losses in production and performance.

Methodology: The approach used in the study is multidisciplinary combing a quantitative analysis of risk profiling for a farm, and a simulation modeling of infectious diseases within a farm. Data are collected from various sources including on-farm databases, literature review, scientific reports, reference and national diagnostic labs, and expert opinion.

Results: Results indicate important pathways for acquiring and spreading viral diseases within a farm and a range of factors and their values contributing to each pathway. A simulated spread of VNN within a farm provides a visualization of epidemic course over time, and can be used for comparing the effectiveness of different control measures, e.g., vaccine, early detection, improved hygiene and biosecurity, an effect of rearing density, etc.

Conclusion: A platform for quantitative risk estimation of viral disease introduction and spread in Mediterranean marine sea bass/sea bream is developed and can be used to facilitate the decision-making process of the industry, veterinary authorities, and other stakeholders.

Keywords: Mediterranean aquaculture, risk assessment, viral nervous necrosis (VNN), European sea bass, gilthead sea bream

093-P

Sequence analysis of the HPR and F-gene of HPR0 isolates of ISAV in Iceland

Bjornsdottir, Thorunn S. (Iceland)[1]; Gunnarsdottir, Harpa M. (Iceland)[1]; Natale, Sabrina (Iceland)[1]; Einarsdottir, Þorbjorg (Iceland)[1]; Sigurdardottir, Heida (Iceland)[1]; Bragason, Birkir T. (Iceland)[1]; Gudmundsdottir, Sigridur (Iceland)[1]

1 - Institute for Experimental Pathology, University of Iceland, Keldur, 112-Reykjavik, Iceland

Introduction: Infectious salmon anemia (ISA) is a serious viral disease of Atlantic salmon (*Salmo salar* L.) caused by the ISA virus (ISAV) and is notifiable to the World Organization for Animal Health (OIE). Virulent strains ISAV-HPRdel have deletions in a highly polymorphic region (HPR) of the hemagglutinin-esterase (HE) gene on segment 6 whereas avirulent strains ISAV-HPR0 have none. In addition, a Q266L substitution or insertion adjacent to the putative proteolytic cleavage site of the fusion protein (F) encoded by segment 5 has been suggested as a virulence marker. Outbreaks of ISAV-HPRdel have occurred in farmed Atlantic salmon in countries geographically close to Iceland, but only ISAV-HPR0 has been detected in Iceland.

Methodology: Group I 25037 samples from Atlantic salmon farmed in land-based facilities 2011-2018. Group II 984 Atlantic salmon samples from a) wild parr from salmon rivers b) parr of wild origin used for restocking and returning brood-fish c) parr and returning brood-fish in sea-ranching d) smolts released into net pens and ongrowing fish. Total RNA was extracted from pooled organ samples for each individual fish. ISAV was confirmed by One-step RT-qPCR of segments 7 and 8. Amplification of the HPR on segment 6 and the F-gene on segment 5 was done by One-step RT-PCR. PCR products were examined on an Agilent 2100 Bioanalyzer and sequenced.

Results: In group I 278 (1.1%) samples were ISAV positive. ISAV-HPRdel was not detected. Sequencing of 111 ISAV-HPR0 isolates showed no variation in the HPR. The Icelandic ISAV-HPR0 isolates were most similar to Norwegian and Faroese isolates. Preliminary sequencing results of the F-gene on segment 5 also suggest little variation. ISAV was not detected in group II.

Conclusion: This study shows that ISAV-HPR0 positives are in low abundance in farmed Atlantic salmon in Iceland. The Icelandic ISAV-HPR0 isolates show high homogeneity. The sequences of the Icelandic isolates are most similar to published sequences from Norway and the Faroe Islands. The information gathered for the F-gene further supports these observations. Knowledge of the genotypes of the Icelandic ISAV-HPR0 isolates will be useful for further research, analyses and risk assessment for ISAV in Iceland.

Keywords: Atlantic salmon, ISAV, Iceland

Emerging and Alien Pathogen Species

094-P

Thermoadaptation of *Aeromonas salmonicida*, widening of host species window

Schmidt-Posthaus, Heike (Switzerland)[1]; Rupp, Melanie (Switzerland)[1]; Pilo, Paola (Switzerland)[2]; Knuesel, Ralph (Switzerland)[3]; Von Siebenthal, Beat (Switzerland)[4]; Sindilariu, Paul-Daniel (Switzerland)[5]; Frey, Joachim (Switzerland)[2]

1 - Centre for Fish and Wildlife Health, Vetsuisse Faculty, University Bern; 2 - Institute of Veterinary Bacteriology, Vetsuisse Faculty, University of Bern; 3 - fishdoc GmbH; 4 - Valperca SA, Succursale Percitech; 5 - Tropenhaus Frutigen

Introduction: *Aeromonas salmonicida* is known to cause focal ulcerative dermatitis in non-salmonid fish species, called atypical furunculosis. However, systemic infections are usually rare in these fish species. *A. salmonicida* strains can harbor a type three secretion system (TTSS) which is one of the main virulence factors of pathogenic *A. salmonicida*. TTSS are nanosyringes that allow the transfer of effector proteins from the cytosol of bacteria to host cells. When *A. salmonicida* is grown at temperatures above 20 °C, plasmids harboring these genes can be cured or partial deletions in genes encoding for the TTSS can occur.

Methodology and Results: In a Swiss warm water recirculation aquaculture system, a systemic A. salmonicida infection got economically important in European perch (*Perca fluviatilis*) due to high mortality and degenerative muscle changes leading to discarding market size fish fillet. The causative agent revealed to be a TTSS positive *A. salmonicida*. The function of the TTSS was confirmed in a cytotoxicity test using bluegill fry and *epithelioma papulosum cyprini* cells. The genes encoding the TTSS in this strain could be deleted during cultivation at temperatures of at least 28 °C.

Conclusion: This increased thermotolerance is in contrast to *A. salmonicida* strains from cold water salmonid fish such as Atlantic salmon or arctic char where TTSS genes are lost at 20 °C. TTSS positive *A. salmonicida* with higher thermotolerance opens a new range of warm water fish as hosts for this bacterial species, possibly leading to increased losses in the European perch aquaculture.

Keywords: *Aeromonas salmonicida*, perch, temperature, type three secretion system, systemic disease

095-P

Histopathology caused by the alien fish parasite *Neoergasilus japonicus* on the skin of its hosts in south Africa

Avenant-Oldewage, Annemarie (South Africa)[1]

1 - University of Johannesburg

Introduction: *Neoergasilus japonicus* is a recent fish parasitic introduction in South Africa. It was noticed for the first time in 2009 on cyprinids in the Vaal Dam in the Vaal River and has since been reported on hosts in both the Vaal and Olifants River systems. Recently, it was also noticed on a variety of host in the Crocodile River. Mature egg bearing females attach to the skin and in particular the fins of their hosts.

Methodology: Fishes were collected, euthanized and examined with a dissection microscope for parasites. Some specimens were removed and fixed in 70% ethanol and prepared for scanning electron microscopy (SEM) using hexamethyldisilazane for dehydration and mounted on carbon tape and coated with gold. Some ethanol fixed parasites were cleared in 90% lactic acid with cotton blue and the mouth parts were dissected out and studied with light microscopy. After removal of the affected fin from dead fish, the remainder of specimens were fixed *in situ* on the host in 10% buffered formaldehyde for histological section. These parasites and host tissue were dehydrated in acetone and imbedded in resin, thereafter sectioned with glass at 5µm and subsequently stained with haematoxylin and counterstained with eosin.

Results: A large variety of freshwater fish species are infected with *N. japonicus*. Mature female parasites attach to the host by means of highly modified antennae that penetrated superficially in the skin of the host. The attachment site lacked external signs of inflammation. The mandible, maxillule, maxilla of the parasite parasites bears lancet-like brush-like terminal segments enabling cutting and brushing of superficial tissue into the buccal cavity of the parasite. Sections of the intestine exposed the content and confirmed the parasitic nature of the association.

Conclusion: Gross pathological observation indicated the absence of inflammation at the point of attachment of the parasite on its hosts. The content of the intestine however confirmed a parasitic relationship.

Keywords: Copepoda; cyprinid fish; mouthparts

Funding: National Research Foundation and Uiversity of Johannesburg.

096-P

Whole-genome analysis of *Lactococcus petauri* isolated from lactococcosis case in rainbow trout: first case reported in Greece

Bitchava, Konstantina (Greece)[1]; Kotzamanidis, Charalampos (Greece)[2]; Malousi, Andigoni (Greece)[3]; Vafeas, George (Greece)[2]; Chatzidimitriou, Dimitris (Greece)[4]; Papadimitriou, Evaggelia (Greece)[5,6]; Chatzopoulou, Fani (Greece)[7]; Skoura, Lemonia (Greece)[7]; Chatzidimitriou, Maria (Greece)[8]; Zdragas, Antonios (Greece)[2]

1 - Hellenic Agricultural Organisation-DEMETER, Veterinary Research Institute of Thessaloniki, Campus of Thermi, Thermi, 570 01; 2 - Hellenic Agricultural Organisation-DEMETER, Veterinary Research Institute of Thessaloniki, Campus of Thermi, Thermi, 570 01, Greece; 3 - Lab of Biological Chemistry, Medical School, Aristotle University of Thessaloniki, Greece; 4 - Laboratory of Microbiology, School of Medicine, Aristotle University of Thessaloniki, Panepistimiouli, Thessaloniki, Greece; 5 - Laboratory of Microbiology, School of Medicine Aristotle University of Thessaloniki, Thessaloniki, Greece; 6 - National AIDS Reference Centre of Northern Greece, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece; 7 - Department of Microbiology, AHEPA University Hospital, S. Kiriakidi Str. 1, 54636, Thessaloniki, Greece; 8 - Medical Laboratories, Alexandrion Educational Technological Institute of Thessaloniki, Greece

Introduction: In this study, we report for the first time the whole genome sequence of *Lactococcus petauri* isolated from rainbow trout suffering from lactococcosis. Lactococcosis is a disease encountered in a wide variety of fish species all over the world that can result in a loss of 50% of total production. *Lactococcus garvieae*, a highly diverse species, is considered to be the causative agent of the disease. The first isolation of the bacterium in Greece was reported in 2003 from an epizooty in rainbow trout.

Methodology: Since whole genome sequence analysis provides a better understanding of the bacterium pathogenicity, we used a strain of the pathogen isolated from a lactococcosis outbreak in Greece. The LG_SAV_20 strain that was sequenced on an Illumina MiniSeq platform in paired-end mode with a 2×151 bp read length, had been initially identified as a representative of *L. garvieae*, based on biochemical profiling and confirmed by a PCR assay which specifically identifies *L. garvieae*.

Results: Based on the results of whole genome shotgun sequencing the genome sequence of the isolate was 98.393% identical by average nucleotide identity (ANI) to the type genome of *L. petauri*, with 89.5% coverage of the genome. The genome of the *L. petauri* isolate includes 2,079,009 bases with an average G+C content of 38.05% and it contains 1,950 coding sequences (CDs) and 51 tRNAs. Functional annotation of the predicted coding genes using mappings to clusters of orthologous groups (COGs) revealed 22 functional COG groups and 6.7%, 5.8%, and 3.8% of the sequences included genes related to translation/ribosomal structure/biogenesis, carbohydrate metabolism/transport, and amino acid metabolism/transport, respectively. In addition, we identified on the assembled genome putative virulence factors from phylogenetically related bacteria as well as the Mdt(A) gene which confers multiple antibiotic resistance.

Conclusion: Based on the data presented in this study, the availability of the genome sequence of *L. petauri* LG_SAV_20, deposited on GenBank under the NZ_SIVY00000000.1 accession number, will allow us to better understand the role of this novel species as an etiological agent of lactococcosis.

Keywords: lactococcosis, rainbow trout, *Lactococcus petauri*

097-P

Presence and genetic variability of piscine Orthoreovirus genotype 3 (PRV-3) in Denmark

Cuenca, Argelia (Denmark)[1]; Soerensen, Juliane (Denmark)[1]; Iburg, Tine (Denmark)[1]; Vendramin, Niccoló (Denmark)[1]; Olesen, Niels (Denmark)[1]

1 - European Union Reference Laboratory for Fish and Crustacean Diseases, DTUaqua

Introduction: Piscine orthoreovirus (PRV) belongs to the subfamiliy Spinareovirinae in the Reoviridae family. PRV is characterized by an 80 nm large particle with icosahedral symmetry with two concentric layers of capsid proteins surrounding ten linear double strand RNA genomic segments. In 2015, a new genotype of PRV (PRV-3) was discovered in Norway in a HSMI-like outbreak in rainbow trout. In 2017, PRV-3 was detected for first time in Denmark in association with complex disease cases in rainbow trout reared in recirculating aquaculture systems (RAS).

Methodology and Results: In order to better understand the epidemiology of PRV-3 in Denmark, a prevalence study was carried out in the country in 2018. After validation of sampling procedures and diagnostic methods, 56 farms, including both flow through and RAS, were screened for PRV-3. The sampling sites were characterized according to species produced and farm type (broodstock, grow-out and re-stocking). PRV-3 was detected in at least 36 out of 56 farm examined, with the highest prevalence in grow-out farms. Notably disease outbreaks were observed only in RAS. For each farm, one sample was selected for sequencing of the S1 segment, encoding for the S3 (capsid) protein, and the M2 segment. Preliminary results do not support a single introduction of PRV-3 into the country, as well as it was not possible to find a clear association between clades/subtypes and isolates associated to disease outbreaks. Preliminary data may show re-assortment of viral segments.

Conclusion: PRV-3 is an emergent pathogen for reared rainbow trout in Denmark. Within a relatively short time the virus is widely spread within the country, infecting different kind of farms and production systems. All re-stocking farms tested positive for the virus, but no disease outbreak were recorded, supporting that brown trout is a susceptible host to PRV-3 and potentially act as reservoir. In Rainbow trout, PRV-3 has been shown to cause heart pathology under experimental condition; future investigation will address which environmental factor trigger the clinical manifestation seen in RAS.

Keywords: PRV-3, rainbow trout, RAS, prevalence study

Funding: The Henrik Henriksens Fond (Denmark) and the European Union Reference Laboratory of Fish and Crustacean Diseases.

098-P

From detection to regulation – 20 years of new & emerging diseases in freshwater fisheries

Williams, Chris (United Kingdom)[1]; Cable, Joanne (United Kingdom)[2]; James, Joanna (United Kingdom)[1]; Britton, Robert (United Kingdom)[3]

1 - Environment Agency, National Fisheries Laboratory, Brampton, UK; 2 - School of Biosciences, Cardiff University, Cardiff, CF10 3AX, UK; 3 - Bournemouth University, Poole, BH12 5BB, UK

Introduction: Freshwater fisheries in England and Wales contribute over £ 2 billion to the economy annually and have important societal and environmental benefits. New and emerging diseases pose a significant threat to the value, performance and ecology of these waters.

Methodology: In the last 20 years, a range of novel pathogens and emerging disease conditions have been detected in freshwater fisheries, affecting a variety of host species in both still water and riverine environments. Most of these have been detected, monitored or managed nationally by the Environment Agency - the primary government authority for environmental protection and development of freshwater fisheries in England.

Results: Notable examples of these diseases include Spring Carp Mortality Syndrome (SCMS), Tench Rhabdovirus (TRv), Koi Herpesvirus (KHV), Red Vent Syndrome (RVS), Anguillid herpesvirus-1 (Ang-HV1), Puffy Skin Disease (PSD), the Rosette Agent *Sphaerothecum destruens*, *Gyrodactylus sprostonae* and most recently, Carp Edema Virus (CEV). These diseases are reviewed chronologically with detail of their detection and current distribution. The clinical characteristics, pathology and impacts of these diseases are described, along with the benefit and limitations of regulatory controls to limit spread and protect wild fish populations.

Conclusion: New and emerging diseases pose a growing threat to the ecology and economic value of freshwater fisheries globally. The recent emergence of fish diseases in England highlights the importance of broad scale surveillance, rapid risk assessment frameworks, development of novel approaches for pathogen monitoring and prompt fish movement regulations making best use of both international and domestic controls to protect native aquatic resources.

Keywords: emerging disease, fisheries, new, emerging

Funding: Environment Agency.

099-P

Carp Edema Virus (CEV) in Polish carp aquaculture

Stachnik, Magdalena (Poland)[1]; Matras, Marek (Poland)[1]; Borzym, Ewa (Poland)[1]; Maj-Paluch, Joanna (Poland)[1]; Reichert, Michał (Poland)[1]

1 - National Veterinary Research Institute, Puławy, Poland

Introduction: Carp edema virus - (CEV) is an etiological factor of koi sleepy disease (KSD). An infection with this poxvirus causes lethargic and sleepy behaviour in the fish. In up to 80 per cent of the cases, the infection is fatal. Infected fish typically lie at the bottom of the ponds exhibiting extreme apathy and typical symptoms include sunken eyes, dermatological changes and swollen gills. KSD was first reported in Japan in the 1970s. Recently, it has been detected in many European countries. The aim of this study was to determine the spread of the pathogen in carp farms in Poland in samples collected in the NVRI laboratory in years 2013 - 2018.

Methodology: Fish samples initially collected by veterinarians on the common and koi carp farms in Poland between 2013 and 2018 as part of a KHV surveillance programme, were also tested for CEV by qPCR. Total DNA was isolated from gill and kidney and stored at -80 °C. The DNA determined as positive in real-time PCR assay was further processed by nested PCR to obtain sequencing material.

Results: Analysis of samples collected in 2013 - 2018 showed that the virus was present in 20 - 65% of investigated farms (average for 2013 - 2018). All samples from koi carp farms were positive, which confirms the hypothesis that the KSD could have been introduced with ornamental form of carp as KHV disease. Sequence alignments and phylogenetic analysis assigned the Polish CEV sequences into three distinct genogroups.

Conclusion: Koi sleepy disease (KSD), as well as koi herpes virus infection (KHV), is currently the subject of serious concern for cyprinid fish farmers in Europe. Our analysis have shown, that infection could be widespread among koi and carp stock in Polish aquaculture at least for several years. Especially in the spring, mortality outbreaks are caused by environmental factors such as water temperature and stress conditions combined with carp edema virus infection. Koi sleepy disease can be a serious threat to carp farming in Poland - if it repeats the course of events that occurred with the spread of the KHV virus.

Keywords: carp edema virus, koi sleepy disease, carp

100-P

Identification and molecular characterisation of iridoviruses of sturgeon in Poland

Stachnik, Magdalena (Poland)[1]; Matras, Marek (Poland)[1]; Borzym, Ewa (Poland)[1]; Maj-Paluch, Joanna (Poland)[1]; Reichert, Michał (Poland)[1]

1 - National Veterinary Research Institute, Puławy, Poland

Introduction: Sturgeon farming is an extremely important and rapidly growing aquaculture sector, and almost all sturgeon species in natural waters are threatened with extinction, of which more than half are classified as critically endangered species. Recently, Poland has become the third largest producer of sturgeon and caviar in Europe. As in other fish species, diseases are the main factor limiting sturgeon production. Among them, viral infections cause the most severe economic losses in breeding fish. Lately, as the restoration of the Atlantic sturgeon (*Acipenser oxirynchus*) population has been started in Poland, research was undertaken to analyse the occurrence of viral pathogens in the species most often kept on fish farms, in particular the Siberian sturgeon (*Acipenser baerii*).

Methodology: Samples were collected at fish farms in 2016 - 2018 from various sturgeon species: Russian, Siberian and bester. Total DNA, isolated from gill, skin and kidney, was analysed for the presence of iridoviruses using the qPCR method. DNA samples determined as positive during the real-time PCR assay were further processed to obtain sequencing material. Nucleotide sequences encoding the MCP fragment from the Polish, European and other sturgeon isolates were analysed using Genious software.

Results: 24 of the 158 collected samples were verified as positive by Real Time PCR. Half of them after PCR amplification were sequenced and confirmed to belong to the *Acipenser* iridovirus species. Polish isolates show high homology with each other and significant resemblance to European isolates, creating with them one genogroup - AIV-E (European) sequence. Isolates from the European and American genogroups show sequence variation in only 15%.

Conclusion: The low variability of European and American sequences indicates that origin of this sturgeon virus isolates, which could be brought to Europe along with stocking material from America and thus spread to European sturgeon fish farms. Carriers of pathogens, belonging to the most frequently bred species in Poland, Siberian and Russian sturgeon, may pose a threat to other more sensitive sturgeons, including the Atlantic sturgeon reintroduced recently in Poland, as the closest genetically to the earlier inhabiting sturgeon species.

Keywords: sturgeon, iridovirus, major capsid protein

Bio-security in Aquaculture and Veterinary Labs

101-P

A systematic risk profiling for Mediterranean Sea bass and sea bream farms

Leandro, Margarida (Portugal)[1]; Tavornpanich, Saraya (Norway)[2]

1 - Faculdade de Medicina Veterinária, Universidade de Lisboa; 2 - Norwegian Veterinary Institute

Introduction: The study focuses on diseases and health management of two main species produced in Mediterranean marine aquaculture i.e. sea bass (European sea bass, *Dicentrarchus labrax*) and sea bream (gilthead sea bream, *Sparus aurata*). With the increasing growth, biosecurity has become a critical part of aquaculture production for minimizing disease introduction and spread.

Methodology: The study uses a systematic scoring system to quantify the level of biosecurity practices and develops a risk profiling for Mediterranean sea bass and sea bream farms. About 78 farms both land-based and sea-cages located in Croatia, France, Greece, Italy, Spain, Tunisia, and Turkey are participated in the survey. The questionnaire survey consists of questions on farm characteristics and production statistics, potential pathways of disease introduction and spread (introduction of live fish, water exposure, mechanical transmission within close proximity, distance independent mechanical transmission), biosecurity and management practices, vaccination, and diagnostic competence and capacity. Each question in the questionnaire is given a weight by the subject experts, for a general risk, and pathogen-specific risk.

Results: Answers of the questionnaire are reformatted, and internal and external biosecurity scores are estimated according to Biocheck.ugent®. The approach proven to be well applicable for development of risk-based, weighted scoring system for aquaculture. In addition, the weight-adjusted sum of the total score of a farm is divided by the best score that the farm in this category could have achieved, then are used for profiling the risk of each individual farm. Detailed results will be further explained and presented.

Conclusion: The quantitative scores and ranking profiles received from the study would help farmers to ascertain which biosecurity measures they should apply to improve their overall biosecurity, and help providing farmers with the overall population risk and their individual personalized risk for disease introduction and spread.

Keywords: biosecuirty, sea bass, sea bream, Mediterranean, scoring system

Funding: EU Horizon 2020 MedAID, Norwegian Veterinary Institute.

Viruses and Viral Diseases

103-P

Presence of carp edema virus in aquaculture of the Czech Republic in 2017 – 2018

Matejickova, Katerina (Czech Republic)[1]; Pokorova, Dagmar (Czech Republic)[1]; Pojezdal, Lubomir (Czech Republic)[1]; Reschova, Stanislava (Czech Republic)[1]; Syrova, Eva (Czech Republic)[1,2]; Piackova, Veronika (Czech Republic)[3]; Palikova, Miroslava (Czech Republic)[2]; Vesely, Tomas (Czech Republic)[1]

1 - Veterinary Research Institute, Brno, Czech Republic; 2 - University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic; 3 - University of South Bohemia in Ceske Budejovice, Vodnany, Czech Republic

Introduction: Carp edema virus (CEV) causes disease, known as koi sleepy disease (KSD), which is characterized by extreme lethargic behaviour, gill damage, overproduction of mucus on the skin and gills, enophthalmia and generalized edema. The first reports about KSD came from Japan in the 1970s. In Europe, KSD was reported for the first time in 2009 and then 2011 in the UK in imported koi carp and in 2012 also in the UK in common carp. In the Czech Republic, CEV was first confirmed in 2014 in koi carp and additionally in 2013 in common carp. In this study, we report the data of the presence of CEV in common carp and koi in the Czech Republic in 2017 and 2018.

Methodology: Twenty-one localities (17 common carp localities and 4 koi localities) in 2017 and twenty-eight localities (26 common carp localities and 2 koi localities) in 2018 were examined for the presence of CEV infection based on the clinical signs, lethargic behaviour, increased mortality and CEV history. Samples were examined by PCR.

Results: In 14 of 21 localities (10 common carp localities and 4 koi localities) in 2017 and in 4 of 28 localities (2 common carp localities and 2 koi localities) in 2018, PCR products were amplified by nested PCR for the detection of CEV. The presence of CEV in all positive samples was confirmed by sequence analysis of the PCR products. Phylogenetic analysis revealed that all CEV isolates from common carp belonged to genogroup I, which includes CEV isolates previously detected in common carp cultured in Europe. Furthermore, all CEV isolates from koi were found to be virus variants from genogroup IIa, which often occurs in koi.

Conclusion: This study provides new data about the presence, distribution and genetics of CEV in the Czech Republic.

Keywords: common carp, koi carp, carp edema virus, PCR, phylogenetic analysis

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104-P*

Presence of piscine orthoreovirus 3 confirmed in wild brown trout (*Salmo trutta fario*) in the Czech Republic

Pojezdal, Lubomir (Czech Republic)[1]; Adamek, Mikolaj (Germany)[2]; Steinhagen, Dieter (Germany)[2]; Reschova, Stanislava (Czech Republic)[1]; Palikova, Miroslava (Czech Republic)[3,4]; Vesely, Tomas (Czech Republic)[1]

1 - Veterinary Research Institute, Brno, Czech Republic; 2 - University of Veterinary Medicine, Hannover, Germany; 3 - Mendel University in Brno, Czech Republic; 4 - University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

Introduction: Piscine orthoreoviruses are emerging pathogens of salmonids associated with the heart and skeletal muscle inflammation in farmed Atlantic salmon (*Salmo salar*). In the last few years the role of the piscine orthoreovirus subtype 3 (PRV-3) as a pathogen of freshwater brown trout (*Salmo trutta fario*) is a matter of scientific discussion. Due to an increasing number of reports on the presence of PRV-3 in freshwater salmonids of continental Europe, archived samples from wild salmonids from the Czech Republic were examined using conventional RT-PCR.

Methodology: The fish were collected in 2015 - 2017 from 8 locations. Spleen, heart and cranial kidney tissue from up to ten individuals were pooled, mechanically lysed and suspended in cell culture medium, supernatant of which was stored at -80 °C and subsequently submitted for RT-PCR analysis using an assay targeting the S1 segment of PRV-3-specific RNA.

Results: RT-PCR confirmed the presence of the PRV-3 virus in brown trout originating from the rivers Jihlava and Oslava collected in 2015. From these two locations, 4 out of 8 samples contained the virus. Additionally, quantitative PCR was performed to assess viral loads utilizing a plasmid-based quantification normalizing the PRV-3 S1 segment copies against 10000 copies of the trout elongation factor 1 gene. The calculated viral load ranged from 1.27 x 10^3 to 2.73 x 10^5, which indicates a comparatively high virus content for the culture medium supernatant. None of the samples tested positive for the presence of viral haemorrhagic septicaemia virus or infectious hematopoietic virus by qRT-PCR and for infectious pancreatic virus or salmonid alphavirus 2 by RT-PCR. The amplified PRV-3 cDNA was analyzed by Sanger sequencing with subsequent phylogenetic analysis of the 371bp segment of viral nucleic acid identifying the virus as subtype PRV-3b, with high levels of similarity with isolates from continental Europe and Chile.

Conclusion: Despite the detection of PRV-3 in brown trout in the Czech Republic, an association of this pathogen with any clinical disease leading to diminishing populations of this fish remains to be evaluated.

Keywords: PRV, phylogenetic, PCR, freshwater

Funding: Ministry of Agriculture of the Czech Republic MZE-RO0518 and Project PROFISH CZ.02.1.01/0.0/0.0/16_019/0000869 financed by ERDF in the operational programme VVV MŠMT.

105-P

First detection of a sturgeon mimivirus in Ukraine

Rud, Yuriy (Ukraine)[2]; Bigarré, Laurent (France)[1]; Buchatsky, Leonid (Ukraine)[3]

1 - Agence Nationale de Sécurité Sanitaire des Aliments; 2 - Institute of Fisheries; 3 - Institute of Biology and Medicine

Introduction: Specific sturgeon nucleocytoplasmic large DNA viruses (NCLDV) infect several species from the *Acipenseridae* family. Recently, it was shown that these viruses shared striking homologies with the *Mimiviridae* family (Clouthier *et al* 2018). One of these viruses, Acipenser iridovirus-European (AcIV-E), is present in farms across Europe where it has occasionally caused mild to severe losses to sturgeons. We describe the first detection of AcIV-E on Siberian sturgeon (*Acipenser baerii*) in a farm in Ukraine.

Methodology: In summer 2018, a pool of organs of dead Siberian sturgeons was sampled in a farm near Kyiv. Total DNA was extracted and submitted to a generic PCR targeting the A portion of the MCP gene of sturgeons NCLDVs, followed by direct Sanger sequencing.

Results: Mortalities affected 1-2 years-old Siberian sturgeons. Some fish showed uncoordinated spiral swimming; other fish showed hypermelanosis and appeared weak and lethargic. Preliminary examination of moribund fish revealed a range of lesions in gill tissues, which appeared pale and necrosis. When testing the presence of sturgeon NCLDV by PCR, a product of the expected size was obtained (730 bp). Its sequence exhibited 99% of identity with AcIV-E.

Conclusion: This is the first report of a NCLDV on sturgeon in Ukraine. AcIV-E was detected on sick Siberian sturgeons showing symptoms similar to those induced by this virus elsewhere in Europe. Therefore, AcIV-E probably played a role in the pathology, even if the presence of other opportunistic pathogens should be studied. The presence of NCLDVs in other farms along the Dnipro river is also to investigate. Since sturgeon farms near Kyiv are located along the river, there is a high risk of virus spread to the wild fish and to the farms downstream.

Keywords: sturgeon, mimivirus, NCLDV

106-P

Development of genetic markers associated with resistance to herpesviral hematopoietic necrosis in goldfish

Nakajima, Mayuri (Japan)[1]; Shirato, Makoto (Japan)[1]; Tanaka, Mikio (Japan)[2]; Dairiki, Keitaro (Japan)[2]; Uchino, Tsubasa (Japan)[1]; Kato, Goshi (Japan)[1]; Sakamoto, Takashi (Japan)[1]; Sano, Motohiko (Japan)[1]

1 - Tokyo University of Marine Science and Technology; 2 - Saitama Fisheries Research Institute

Introduction: Herpesviral hematopoietic necrosis (HVHN) caused by cyprinid herpesvirus 2 (CyHV-2) has damaged aquacultures of goldfish *Carassius auratus* and gibelio carp *C. auratus gibelio*. A goldfish strain (Azumanishiki vareity) resistant to HVHN has been established by Saitama Fisheries Research Institute. So far, the selection of broodstock has been conducted by virus challenge, but it may cause the surviving fish to become virus carrier. Therefore, in this study, we investigated genetic markers as an alternative selection method.

Methodology: F_1 fish between the resistant and susceptible (Kurodemekin: KD) strains that showed resistant to HVHN, were backcrossed with the KD strain, and the progenies (BC fish) were produced. We conducted infection experiment using the fish of 4 BC crosses by cohabitation infection with the goldfish infected with CyHV-2. The cumulative mortality rates in ♂F_1×♀KD No.1 or 2 and ♀F_1 No.1 or 2×♂KD were 49.5%, 45.1%, 34.5% and 29.1%, respectively. The dead and surviving fish of a cross group (♂F1×♀KD No.1) and their parents were processed for genomic DNA extraction. DNA libraries of the samples by genotyping-by-sequencing method were subjected to Illumina HiSeq 4000 sequencing. After processing the data obtained, we constructed the genetic linkage map and searched SNPs associated with the resistance.

Results: We mapped 208 SNPs in the 45 genetic linkage groups. The linkage analysis of the SNPs with surviving or dead fish showed two SNPs significantly linked with the phenotype with a LOD score over 15. It suggests that these SNPs are linked with the resistance. The SNP (297316) was further tested using fish of the 4 crosses. The results showed that 95.5% of surviving fish on average had the SNP and 97.6% of dead fish on average did not have it in groups of ♂F_1×♀KD. In groups of ♀F_1×♂KD, 67.5% of survivors on average had the SNP and 73.3% of dead fish on average did not have it. The results suggest that the recombination rate between the SNP and the disease resistant locus is different in female and male.

Conclusion: The SNP obtained in this study can be used for marker-assisted selection of male broodstock of the resistant strain.

Keywords: genetic marker, resistant fish, goldfish, herpesviral hematopoietic necrosis, CyHV-2

107-P

Effect of FBS concentration variation on fish cell lines inoculated with CYHV-3

Kim, Hyoung Jun (Republic of Korea)[1]; Kwon, Se Ryun (Republic of Korea)[2]; Yuasa, Kei (Japan)[3]

1 - National Fishery Products Quality Management Services; 2 - Sunmoon University; 3 - National Research Institute of Aquaculture Fisheries Research Agency

Introduction: This study was to confirm the effect of different concentrations of fetal bovine serum (FBS) on CPE development and replication of CyHV-3 on CCB and KF-1 cell lines.

Methodology: NRIA0301 was isolated in 2003 from common carp naturally infected with CyHV-3 on a cultured fish farm in Japan. The viral titer was $10^{5.8}$ $TCID_{50}$/mL from the viral stock solution using CCB cell line. CCB and KF-1 cells were inoculated with 50 uL of viral stock solution at 20 °C in MEM10 (Gibco, Invitrogen Co.) supplemented with 0, 2, 5, and 10 % FBS and 1 × Antibiotic-Antimycotic liquid (Gibco, Invitrogen Co.) in a 25 Cm^2 flask (Falcon). CPE development was monitored daily for two weeks, and 180 uL of supernatant from each of the inoculated flasks were sampled daily to check for variation in viral copy numbers using the real-time qPCR machine.

Results: At seven days post infection (dpi), clear vacuolations were observed in both cell lines treated with 0 and 2% FBS, but not with 5 and 10%. At 14 dpi, CPE and vaculations was clearly observed on both cell lines at all FBS concentrations, except in CCB cells with no FBS supplementation where the cells were affected by low nutrition. Quantitative real-time polymerase chain reaction results at 14 dpi showed no difference in CyHV-3 genomic copy numbers for both cell lines at all FBS concentrations. Gene copy numbers in CCB and KF-1 cell lines at seven dpi were the highest at 2% FBS (3.15×10^6 copy numbers) and 0 % FBS (2.49×10^5 copy numbers), respectively.

Conclusion: This study demonstrated that FBS concentration was related to CPE development and CyHV-3 viral replication. For use as a diagnostic method, we recommend 0 and 2% FBS concentrations for KF-1 cells and a 2% FBS concentration for CCB cells. In cases of vaccine production or mass virus culturing, we recommend a 10% FBS concentration for CCB cell line.

Keywords: Koi herpesvirus, CyHV-3, FBS concentration, CPE

108-P

Amino acid substitutions in the polymerase N-terminal region of a reassortant betanodavirus strain affect its replication capacity at high temperatures

Souto, Sandra (Spain)[1]; Vázquez-Salgado, Lucía (Spain)[1]; Olveira, José G. (Spain)[1]; Bandín, Isabel (Spain)[1]

1 - Instituto de Acuicultura-Departamento de Microbiología. Universidade de Santiago de Compostela

Introduction: Nervous necrosis virus (NNV), a member of G. *Betanodavirus,* is the causative agent of viral encephalopathy and retinopathy (VER), a neuropathological disease that causes fish mortalities worldwide. NNV genome is composed of two molecules, RNA1 and RNA2, encoding the RNA dependent RNA polymerase (RdRp) and the coat protein, respectively. Betanodaviruses are classified into four genotypes RGNNV, SJNNV, BFNNV and TPNNV. In Southern Europe the presence of RGNNV, SJNNV and their natural reassortants has been reported. Pathology caused by these genotypes is closely linked to water temperature and the RNA1 segment encoding amino acids 1-445 has been postulated to regulate viral sensitivity to temperature. Reassortants RGNNV/SJNNV isolated from Senegalese sole show 6 substitutions in this region when compared with the RGNNV genotype (positions 41, 48, 218, 223, 238 and 289).

Methodology: Five strains were used in this study: SGWak97 (RGNNV), SJNag93 (SJNNV), the natural reassortant SpSsIAusc160.03 (herafter wt160) showing an RGNNV RNA1 and an SJNNV RNA2, r160 (a recombinant virus with a genomic sequence identical to wt160) and r1_445 (a recombinant harbouring 6 point mutations which removed the differences observed between wt160 and SGwak97 in the N-terminal region of the RdRp). Viral replication was tested in cell culture at 15, 20, 25 and 30ºC and experimental infections in juvenile sole were performed at 15, 20 and 25ºC. Viral quantification was performed by $TCID_{50}$ and RT-qPCR.

Results: The *in vitro* replication of r1_445 was significantly lower than that of wt160 and r160 at 25 ºC and 30 ºC and delayed in time at 30 ºC. The analysis of the viral load showed similar results. The experimental infections confirmed the impact of the mutations on viral replication because at 25 ºC the viral load and the mortality rate were significantly lower in fish infected with the mutant than in those challenged with the non-mutated virus. Unfortunately, it was not possible to challenge fish at 30 ºC because of the scarce tolerance of sole to this temperature.

Conclusion: Our findings indicate that r1_445 shows a temperature sensitivity phenotype and confirm that the 1-445 region of RNA polymerase is involved in the temperature adaptation of NNV.

Keywords: betanodavirus, polymerase, temperature

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109-P*

Influence of infectious pancreatic necrosis virus on humoral immune response in rainbow trout

Pajdak-Czaus, Joanna (Poland)[1]; Terech-Majewska, Elżbieta (Poland)[1]; Platt-Samoraj, Aleksandra (Poland)[1]; Szweda, Wojciech (Poland)[1]; Siwicki, Andrzej Krzysztof (Poland)[2]

1 - University of Warmia and Mazury in Olsztyn; 2 - Inland Fisheries Institute in Olsztyn

Introduction: Infectious pancreatic necrosis virus (IPNV) belongs to family *Birnaviridae* and is the aetiological agent of infectious pancreatic necrosis (IPN) of salmonids. It may cause important economic loses due to mortalities and weakening of surviving fish to other fish pathogens. Increased susceptibility of survivors may be caused by the immunosuppressive influence of the virus. The aim of the study was to determine the influence of IPNV on humoral immune response in rainbow trout (*Oncorhynchus mykiss*).

Methodology: Experimental infection of rainbow trout (n = 60, weight ~80g) was conducted by intraperitoneal infection. Samples were taken from fish (n = 5) before infection, after 24 h, 4 8h, 7 d, 14 d, 21 d, 45 d. The activity of lysozyme (Lys) in serum was measured with the use of the turbidimetric method using the bacteria Micrococcus lysodeikticus (Sigma). Total protein content (TP) was determined spectrophotometrically with the biuret method using the Diagnostic Kits – Protein Total Reagents according to the manufacturer's recommendations (Sigma). The level of immunoglobulins (Ig) was determined spectrophotometrically using the biuret method (Diagnostic Kits – Protein Total Reagents - Sigma) and polyethylene glycol 10,000 kDa (Sigma). The level of ceruloplasmin (Cer) was determined by spectrophotometry. The presence of IPNV in infected fish was confirmed with the use of cell culture and ELISA test.

Results: Level of lysozyme statistically decreased after 48 h post-infection and remained statistically lower until the end of the experiment. Total protein content and ceruloplasmin level were statistically higher only 24 h after infection and returned to levels not statistically different from control group. Immunoglobulins level was increased 48 h post-infection. No mortalities were observed during the experiment.

Conclusion: IPNV infection had the biggest suppressive impact on lysozyme level. The Lys activity is one of the indicators of nonspecific resistance. Lowered Lys activity may lead to increased susceptibility towards bacterial infections.

Keywords: immunosuppression, lysozyme, ceruloplasmin, total protein

Funding: research project no. 2017/25/N/NZ9/00087 funded by the National Science Center, Poland.

110-P*

Development of a model system for studying cell-mediated immune responses to salmonid Alphavirus

Veenstra, Kimberly (Germany)[1]; Belmonte, Rodrigo (Norway)[2]; Hodneland, Kjartan (Norway)[2]; Quillet, Edwige (France)[4]; Kawanobe, Motokazu (Japan)[3]; Furihata, Mitsuru (Japan)[3]; Fischer, Uwe (Germany)[1]

1 - Friedrich-Loeffler-Institut; 2 - MSD Animal Health Innovation; 3 - Nagano Prefectural Fisheries Experimental Station; 4 - Institut National de la Recherche Agronomique

Introduction: Disease outbreaks remain a significant problem in terms of production loss and animal welfare for salmonid aquaculture. Salmon Pancreas Disease Virus (SPDV) (family *Togaviridae*, genus *Alphavirus)*, frequently referred to as Salmonid Alphavirus (SAV), is an enveloped, single-stranded, positive-sense RNA virus known to be a serious pathogen for Atlantic salmon (*Salmo salar*) (pancreas disease) and rainbow trout (*Oncorhynchus mykiss*) (sleeping disease) in Europe. Currently, in-depth knowledge of the interplay between virus and host immunity remains elusive. Therefore, we aimed to develop a model system in rainbow trout for examining these aspects of the disease with a focus on cell-mediated cytoxicity (CMC).

Methodology: Infectibility of different target cells and inducibility of CMC was studied *in vivo* and *in vitro* with SPDV using MHC class I matched effector and target cells.

Results: Insights were gained on the virus-host interactions and the pathogenesis of SPDV infections, as well as on the immune response against this virus.

Conclusion: The establishment of an *in vitro* model for studying CMC against SPDV-infected cells will lead to greater understanding of fish defence mechanisms against viruses.

Keywords: cell mediated cytotoxicity, rainbow trout, model system, salmon pancreas disease virus

Funding: MSD Animal Health Innovation.

112-P

Transcriptomic analysis of rhabdovirus infected rainbow trout and flounder cell lines

Cho, Miyoung (Republic of Korea)[1]; Kim, Kwang Il (Republic of Korea)[1]; Min, Eunyoung (Republic of Korea)[1]; Kim, Yi Kyung (Republic of Korea)[2]; Kim, Jinwoo (Republic of Korea)[2]; Jung, Sung-Hee (Republic of Korea)[1]; Park, Jung Woo (Republic of Korea)[3]; Hong, Suhee (Republic of Korea)[2]

1 - Pathology Research Division, National Institute of Fisheries Science; 2 - Department of Marine Biotechnology, Gangneung-Wonju National University; 3 - School of Biological Sciences, Ulsan University

Introduction: Infectious hematopoietic necrosis virus (IHNV) and Hirame rhabdovirus (HIRRV) are important pathogens causing clinical disease and mortalities in cultured rainbow trout and olive flounder in Korea, respectively. In recent years, RNA seq-based transcriptome analysis has been employed to understand pathogenic process and the defense strategies during viral infection in fish. In this study, we have investigated the infection mechanisms of two genotypes of IHNV (i.e., IHNV-Shizuoka and IHNV-Nagano) in rainbow trout-derived RTG-2 cell line and HIRRV in flounder-derived HINAE cell line by RNA sequencing.

Methodology: RTG-2 and HINAE cell lines were infected with above rhabdoviruses at 20 °C for 12 hrs or 24 h to analyze transcriptome at early or late stage of infection. After mapping the RNA sequences to each genome, DEG analysis and KEGG pathway analysis were performed.

Results: We found that N, M, NV and L gene expressions are important in pathogenicity and intracellular growth of IHNV. Indeed, in host cells, both genotypes of IHNV seemed to modulate the expressions of genes involved in regulation of ribosome, protein export, oxidative phosphorylation and IFN signaling pathway, indicating that they promote viral growth by inhibiting IFN signaling and induction of SOCS. And both genotypes control the expression of different IFN target genes. In addition, IHNV-Nagano genotype increases the MAPK signal while IHNV-Shizuoka increases the insulin signal in the cell.

Conclusion: Therefore, it is thought that differences in the intracellular growth and pathogenicity of IHNV-Shizuoka and IHNV-Nagano are caused by common and differential gene expression regulatory mechanisms in infecting rainbow trout-derived cell lines of both genotypes of IHNV. HIRRV is thought to be infected through various pathways such as ribosome regulation, phagosome, TGF-b signaling, glycoprotein synthesis, Toll/IFN signaling.

Keywords: transcriptome, infectious hematopoietic necrosis virus (IHNV), Hirame rhabdovirus (HIRRV), rainbow trout, flounder

113-P*

Study on tissue tropism of VHSV in juvenile olive flounder using *in situ* hybridization (RNA-ISH)

Qadiri, Syed Shariq Nazir (Republic of Korea)[1]; Kim, Soo-Jin (Republic of Korea)[1]; Krishnan, Rahul (Republic of Korea)[1]; Kim, Jae-Ok (Republic of Korea)[1]; Kole, Sajal (Republic of Korea)[1]; Kim, Wi-Sik (Republic of Korea)[1]; Oh, Myung-Joo (Republic of Korea)[1]

1 - Department of Aqualife Medicine, Chonnam National University

Introduction: Previously, we developed an *in-situ* hybridization (RNA-ISH) assay to detect viral hemorrhagic septicemia virus (VHSV) in an *in vitro* model. Here, we utilized its potential and applicability *in vivo* to further our understanding about the localization and tissue tropism of VHSV in experimentally infected juvenile olive flounder (*Paralichthys olivaceus*), an economically important flatfish in Asian aquaculture.

Methodology: DIG-labeled antisense RNA probes complementary to positive-stranded viral mRNAs were generated from PCR products targeting the nucleoprotein (N) and glycoprotein (G) genes of VHSV. Labeled RNA probes were then employed to localize VHSV in five infected flounder tissues viz., kidney, spleen, heart, liver and brain. The tissue sections were fixed and processed for RNA-ISH assay which included probe hybridization, probe detection and final coloration step. The scoring was done to measure levels of ISH in tissues and cohen's kappa (k) was used to compare the virus detection and rule out any chance agreement.

Results: VHSV localization was observed in the glomerulus, lumen of tubules, necrotic debris and tubular epithelial cells of the kidney. In spleen, prominent signals were observed in the white pulp and congested ellipsoids. In heart, the signal was strong and localized to the muscle fibers of myocardium. In liver, the signals were concentrated around the hepatic portal vessels and hepatocytes. Brain showed very less signal compared to other organs and viral genome was sporadically localized within the neural tissue. Viral mRNAs were localized within same cells of all the tested tissues using both the riboprobes, thereby, highlighting the *per-se* specificity of each DIG-labeled riboprobe to complement each other in viral localization.

Conclusion: Our study for the first time employed RNA-ISH assay to study the VHSV tissue tropism in olive flounder using two separate DIG-labeled riboprobes. Viral localization was observed in different cells and areas of tested tissues. The signal strength was highest in heart followed by spleen and kidney, and least in brain. The present study sheds light on VHSV tissue tropism which can help to better elucidate VHSV pathogenesis in olive flounder.

Keywords: tissue tropism, localization, VHSV, RNA-ISH

114-P*

Histopathological characterization and immuno-histochemical detection of VHSV in tissues of juvenile olive flounder

Qadiri, Syed Shariq Nazir (Republic of Korea)[1]; Kim, Soo-Jin (Republic of Korea)[1]; Krishnan, Rahul (Republic of Korea)[1]; Kim, Jae-Ok (Republic of Korea)[1]; Kole, Sajal (Republic of Korea)[1]; Kim, Wi-Sik (Republic of Korea)[1]; Oh, Myung-Joo (Republic of Korea)[1]

1 - Department of Aqualife Medicine, Chonnam National University

Introduction: Immunohistochemistry (IHC) assay is useful in diagnosing viral infection with regard to both sensitivity and specificity. Also, a histological approach to assess the tissue damage provides information on pathology associated with any infection. The present study used a combination of IHC and histopathology to correlate as well as elucidate the localization of VHSV in olive flounder.

Methodology: Moribund VHSV-infected and sterile MEM-exposed flounder tissues (kidney, spleen, heart, liver and brain) were sampled for histopathology using conventional hematoxylin and eosin (HE) staining. Dissected tissues were fixed, dehydrated, embedded in paraffin, sectioned, stained and examined under the light microscope. Whereas, immunohistochemical detection was carried out using primary antibody against nucleoprotein (N) of VHSV. IHC assay was done using standard protocol by blocking the endogenous peroxidase activity followed by antigen revival, non specific protein binding and final coloration. The scoring was done to measure levels of IHC staining in tissues and cohen's kappa (k) was used to compare the virus detection and rule out any chance agreement.

Results: In kidney, a positive signal was evident in glomerulus, lumen of tubules, necrotic debris, renal tubular epithelial cells, and hematopoietic tissue. A clear signal was observed in the white pulp of the infected spleen as well as in the melanomacrophage centers (MMCs). IHC results showed prominent positive signals within the cardiac muscle fibers of the ventricular myocardium in the infected heart tissue. Further, the liver showed a prominent positive signal around the hepatic blood vessels and hepatocytes. In brain, scattered signal was observed within the neuronal tissues.

Conclusion: Protein based detection (IHC) and histopathological alterations confirmed the viral localization in tested tissues. Among the target tissues, heart showed strong positive reaction followed by spleen and kidney suggesting the importance of these organs in flounder VHSV pathogenesis. The present findings reveal that VHSV shows a strong tropism for endothelial cells as virus was frequently localized in the areas having direct connection with blood cells.

Keywords: localization, tissue tropism, VHSV, IHC

115-P*

Susceptibility of finnish rainbow trout to three different genogroups of IPNV: an infection trial

Eriksson-Kallio, Anna Maria (Finland)[1]; Holopainen, Riikka (Finland)[1]; Koski, Perttu (Finland)[1]; Nousiainen, Antti (Finland)[2]; Koskinen, Heikki (Finland)[2]; Kause, Antti (Finland)[2]; Gadd, Tuija (Finland)[1]

1 - Finnish Food Authority; 2 - Natural Resources Institute Finland

Introduction: IPNV (infectious pancreatic necrosis virus) is a highly prevalent virus in Finnish fish farms. Several genotypes exist in Finland and nearby water, but their clinical symptoms and pathogenicity are inadequately known, hampering their management. Three genogroups of IPN-virus, 2, 5 and 6, have been isolated in Finland, of which genogroups 2 and 5 are encountered annually. Genogroup 2 is the most widely spread geographically and has been the only genogroup associated with clinical disease in field observations. According to previous studies, all three genogroups demonstrate amino acid patterns previously associated with avirulence in genogroup 5 viruses.

Methodology: To find out more about the pathogenicity of the different IPNV genogroups on Finnish rainbow trout (*Oncorhyncus mykiss*) strain, an infection trial was performed at VESO Vikan, Norway. Rainbow trout fry originating from IPNV test-negative Finnish parental fish were challenged by a bath model at start feeding. Three Finnish IPNV strains, one Norwegian strain (positive control, genogroup 5) and a negative control (cell culture medium) were used in triplicate tanks, except for genogroup 2 where fish in 9 tanks were challenged. Mortalities were recorded daily for eight weeks.

Results: Highest cumulative mortalities were noted for the Finnish genogroup 5 (38.2 - 10.3 %). For Finnish genogroup 2, variation in mortalities between different groups was high (28.3 - 3.5%). Finnish genogroup 6 caused only low mortalities (8-2.6%), whereas negative control treatment (3.4 - 1.7%) and positive control genotype 5 treatment (3.7-1.7 %) showed only minimal/neglectable mortalities. Histopathological findings showing lesions associated with IPN-infection and IPN-positive immunohistochemistry were noted for Finnish genogroups 2 and 5.

Conclusion: Based on mortality recordings, histopathological lesions and viral RNA measurements, the Finnish rainbow trout was more susceptible to the Finnish genogroup 2 and 5 IPNV in comparison to genogroup 6 IPNV.

Keywords: IPNV, Infection trial, histopathology, RNA-analysis, pathogenicity

Funding: Finnish operational program of the European Maritime and Fisheries Fund.

116-P

Sequencing of fish viruses: quality data assurance for NGS bioinformatics

Zamperin, Gianpiero (Italy)[1]; Lucas, Pierrick (France)[2,3]; Cano Cejas, Irene (United Kingdom)[4]; Ryder, David (United Kingdom)[4]; Abbadi, Miriam (Italy)[1]; Cuenca, Argelia (Denmark)[5]; Vigouroux, Estelle (France)[6]; Stone, David (United Kingdom)[4]; Blanchard, Yannick (France)[2]; Panzarin, Valentina (Italy)[1]

1 - IZSVe, Padova, Italy; 2 - Anses, Ploufragan-Plouzané-Niort Laboratory GVB Unit, Ploufragan, France; 3 - Bretagne Loire University, France; 4 - CEFAS, Weymouth, UK; 5 - DTU, Lyngby, Denmark; 6 - Anses, Ploufragan-Plouzané-Niort Laboratory, PVP Unit, Plouzané, France

Introduction: Next generation sequencing (NGS) is becoming widely used among diagnostics and research laboratories, and nowadays it is applied to a variety of disciplines including veterinary virology. NGS workflow comprises several steps, i.e. sample processing, library preparation, sequencing and primary/secondary/tertiary bioinformatics (BI) analyses. This latter step is an extremely complex process which is difficult to standardize, due to the variety of tools available and metrics. Thus, it is of utmost importance to assess the comparability of the BI pipelines, in order to ensure the consistency of sequence data obtained through different methods and in different laboratories.

Methodology: We have organized a proficiency testing (PT) exercise focused on the BI components and aimed at generating complete genome sequences of salmonid rhabdoviruses (i.e. IHNV and VHSV). Three partners performed NGS using different commercial library preparation kits and sequencing platforms and shared a unique set of 75 raw data, which was analyzed separately by the participants according to their own BI pipeline to produce a consensus sequence. A calibrator sample constituted by a recombinant virus was also analyzed. Consensus sequences were compared to highlight discrepancies (SNPs and indels), and a subset of inconsistencies was investigated more in detail to assess the origin of the discrepancies.

Results: Our analysis revealed 526 discrepancies, with an average of 7 discrepancies per sample. Overall, 39.5% of discrepancies were located at genome termini, 14.1% within intergenic regions and 46.4% affected coding regions. Ten SNPs and 99 indels caused changes in the protein products. According to the subset of inconsistencies investigated more in-depth, manual curation appeared the most critical step affecting sequence comparability, suggesting that this phase requires greater efforts to obtain harmonized results. The analysis of the calibrator allowed assessing BI accuracy and repeatability, which were 99.98% and 99.94%, respectively.

Conclusion: We successfully performed a PT test for NGS BI analysis applied to fish viruses, demonstrating its feasibility in this context. We suggest a wider implementation of PTs for NGS to guarantee sequence data comparability among different laboratories.

Keywords: proficiency testing, NGS, fish viruses

Funding: Anihwa ERA-Net Consortium (Novimark project, contract G88F13000660001); UK Department for Environment, Food and rural Affairs (Defra) (contract C7277B).

117-P*

Using nanopore sequencing for whole genome sequencing of viruses from aquaculture

Tighe, Andrew (Ireland)[1,2]; Gallagher, Michael (United Kingdom)[3]; Carlsson, Jens (Ireland)[2]; Matejusova, Iveta (United Kingdom)[4]; Swords, Fiona (Ireland)[1]; Macqueen, Daniel (United Kingdom)[3]; Ruane, Neil (Ireland)[1]

1 - Marine Institute, Oranmore, Ireland; 2 - University College Dublin, Dublin, Ireland; 3 - University of Edinburgh, Midlothian, United Kingdom; 4 - Marine Scotland Science, Aberdeen, United Kingdom

Introduction: The long read capabilities of nanopore sequencing platforms allow for a simpler and faster way to sequence viral genomes, using a minimal number (2 - 4) of overlapping amplicons. Combined with the low cost of purchasing a MinION sequencer with consumables, and incorporating sample specific genetic barcodes, the price per genome sequenced is vastly reduced from previous methods.

Methodology: Using this approach, we recently sequenced the genome of a strain of salmonid alphavirus (SAV) found in Ireland in wrasse. Four overlapping amplicons (2,000 - 4,000 bp) were amplified using long range PCR, sequenced via MinION, and resulting reads were then mapped to an existing SAV genome to construct the genome. This approach is now being applied to piscine myocarditis virus (PMCV), which is the causative agent for cardiomyopathy syndrome (CMS) in Atlantic salmon, in samples from Ireland. The PMCV genome was amplified in two over lapping 4,000 bp amplicons and mapped to an existing genome.

Results: The whole genome phylogeny showed the strain of SAV in this study to be distinct from other SAV subtypes. The first genome generated for PMCV to date shows close relatedness to the only other available genome, which was sequenced in Norway in 2010.

Conclusion: The phylogeny for SAV strongly suggested that this was a novel SAV subtype, SAV7. In addition we have suggested that the species be renamed piscine alphavirus to incorporate the growing number of fish this viral species infects. As more PMCV genomes are sequenced it will be possible to build a clear picture of the genetic diversity of PMCV in Ireland. Whole genome data will also make it possible to examine the whole genome in order to link any possible mutations to pathogenicity, as many healthy fish test positive for PMCV with no clinical signs of CMS. In addition, single molecule sequencing allows viral diversity from within a sample to be examined, with previous studies suggesting that a higher diversity of viral quasispecies within a host could be linked to a higher risk of developing disease.

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118-P*

Steric exclusion chromatography as a method for purification of koi herpesvirus

Jordan, Lisa Katharina (Germany)[1]; Eilts, Friederike (Germany)[2]; Wolff, Michael W. (Germany)[2]; Becker, Anna Maria (Germany)[1]

1 - Institute of Bioprocess Engineering, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany; 2 - Institute of Bioprocess Engineering and Pharmaceutical Technology, University of Applied Sciences Mittelhessen, Giessen, Germany

Introduction: Steric exclusion chromatography (SXC) is a technique that is frequently used to purify viral particles. A crude sample containing virus particles is mixed with a non-ionic polymer, i.e. polyethylene glycol (PEG), and loaded onto a hydrophilic stationary phase (cellulose membranes). PEG is sterically excluded from the stationary phase creating a PEG-deficient zone with lower PEG concentrations than in the bulk solvent. At higher PEG molecular weight and concentrations, virus particles are excluded from the bulk solvent and thus are associated with the hydrophilic membranes, reducing the surface area between the PEG-deficient zones and the PEG-rich bulk solvent. Low molecular weight impurities are not part of these constructs and are washed away. Finally, the virus particles are recovered by decreasing the PEG concentration in the mobile phase. To evaluate the applicability of SXC for purification of *Cyprinid herpesvirus*-3 (CyHV-3, also *koi herpesvirus*, KHV) various analytical methods, e.g. real time polymerase chain reaction (qPCR) for evaluation of viral DNA content, endpoint dilution assay for determination of infective viral particles, are needed. However, prior to purification experiments with SXC, it had to be investigated if the chemicals PEG and NaCl (applied for virus elution) influence these analytical methods, leading in consequence to biased data.

Methodology: qPCR procedures were conducted using various dilutions of virus stock, mixed with different concentrations of PEG (0.1 to 10%) or 2.0 M NaCl. Furthermore, cell toxicity of PEG (5 and 10%) and NaCl (0.21 to 2.1 M) were tested to assess the maximum concentrations applicable in the cell culture-based endpoint dilution assay.

Results: Regarding qPCR procedures, no clear influence of PEG and NaCl at tested concentrations could be determined. However, cell viability was affected by NaCl concentrations above 0.21 M and PEG concentration of 10%. Based on these results, samples subjected to qPCR or endpoint dilution assay will be prediluted to ensure concentrations ≤ 1% of PEG and ≤ 0.21 M of NaCl.

Conclusion: Since analytical assays are now established, the suitability of SXC procedure for KHV purification can be evaluated.

Keywords: steric exclusion chromatography, koi herpesvirus, cyprinid herpesvirus-3, virus purification

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119-P*

A newly developed carp cell line for *in vitro* replication of cyprinid herpesvirus-3

Jordan, Lisa Katharina (Germany)[1]; Kim, Kwangmin M. (Germany)[1]; Wicklein, Moritz (Germany)[1]; Christian, Jürgen (Germany)[2]; Rakers, Sebastian (Germany)[3]; Becker, Anna Maria (Germany)[1]

1 - Institute of Bioprocess Engineering, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany; 2 - Institute for Animal Health II, Bavarian Health and Food Safety Authority, Erlangen, Germany; 3 - Aquatic Cell Technology & Aquaculture, Fraunhofer Research Institution for Marine Biotechnology and Cell Technology (EMB), Luebeck, Germany

Introduction: To enable research on *Cyprinid herpesvirus*-3 (CyHV-3, also *koi herpesvirus*, KHV), a reliable and stable *in vitro* propagation of the virus with high titres is necessary. Therefore, in this work, the *in vitro* replication of KHV in newly established *Cyprinus carpio* pin cells (CCApin) was characterised. Additionally, it was investigated if CCApin might be better suited for replication of this virus than the widely used common carp brain cells (CCB) and thus, could be an alternative for future diagnostic procedures and vaccine development.

Methodology: To characterise KHV replication in CCApin, parameters such as: medium, content of fetal bovine serum, time of infection (TOI, cell density at virus inoculation), multiplicity of infection (MOI, ratio of applied virus particles per cell) and time of harvest (TOH) were varied. Virus replication was monitored using the 50% tissue culture infective dose assay ($TCID_{50}$) to determine the number of infective particles. Viral DNA content was assessed using real time polymerase chain reaction (qPCR).

Results: The highest titres of 10^7 $TCID_{50}$/mL were reproducibly achieved with TOI ranging from 20,000 to 60,000 cells/cm^2, independently of the applied MOI (from 0.005 to 3). Furthermore, to achieve the highest virus yields of 10^7 $TCID_{50}$/mL, low MOI (between 0.001 and 0.01) in combination with an intermediate TOI of ~30,000 cells/cm^2 proved beneficial. In comparison, using lower TOI ranging from 5,000 cells/cm^2 to 20,000 cells/cm^2 resulted reproducibly in maximum titres of only 10^6 $TCID_{50}$/mL, again with no correlation to used virus load. It was therefore concluded that virus replication in CCApin is more efficient when using higher TOI (>20,000 cells/cm^2). Comparison of KHV replication in CCApin and CCB cells showed that similar titres were generated, indicating that both cell lines are equally suited for KHV replication.

Conclusion: To enable higher KHV titres in the future, replication in other, newly developed cell lines might prove successful. Additionally, the use of suspension cell culture might be beneficial for the generation of high titres.

Keywords: cyprinid herpesvirus-3, koi herpesvirus, *in vitro* replication, *Cyprinus carpio* pin cells

Funding: This work was financially supported by the German Federal Ministry of Food and Agriculture (BMEL) through the Federal Office of Agriculture and Food (BLE), grant number 2815HS010.

120-P

Origanum vulgare and *Cinnamomum zeylanicum* essential oils as health promoters in gilthead sea bream experimentally infected with lymphocystis disease virus

Dedeloudis, Dimitrios (Greece)[1]; Billinis, Charalampos (Greece)[2]; Panagiotaki, Panagiota (Greece)[1]; Golomazou, Eleni (Greece)[1]

1 - Department of Ichthyology and Aquatic Environment and Aquaculture Laboratory, School of Agricultural Sciences, University of Thessaly, Volos, Greece; 2 - Faculty of Veterinary Medicine - Laboratory of Microbiology and Parasitology, University of Thessaly, Karditsa, Greece

Introduction: Lymphocystis disease (LCD) is a common viral self-limiting disease affecting fish species. Low growth rate of infected fish retards the expected breeding time, causing significant economic losses. LCD control is based on preventive husbandry practices, as no effective treatments or commercially available vaccines currently exist. Also, better nutrition supplemented with immunostimulants, like herbal extracts and probiotics may reduce the incidence of LCD. Presently, the impact of two essential oils added on fish feeds, was assessed in case of fish infected with Lymphocystis Disease Virus (LCDV).

Methodology: *Origanum vulgare* and *Cinnamomum zeylanicum* essential oils were used as nutritional supplements to the diet of the experimental gilthead sea bream. Essential oils were applied on commercial dry pellets. Four experimental fish groups – treatments were fed with feed supplemented with essential oils, CIN1: 1% *C. zeylanicum*, CIN2: 2% *C. zeylanicum*, OR1: 1% *O. vulgare* and OR2: 2% *O. vulgare*, while a control group (C) was fed with commercial fish feed. 90 days post-treatment experimental infection was applied, by cohabitation with fish infected with LCDV. Prevalence and mortality rate were recorded 5, 10, 15, 20, 30, 45, 60, 75 and 90 days post-exposure.

Results: Nodular lesions were firstly observed in case of the control group at prevalence 6.67% (day 5). During 15 days, LCDV was transmitted to all experimental groups. In case of CIN1 and CIN2 groups, the highest prevalence (16.67% and 20%) was mentioned 20 and 15 days post-exposure, respectively. Bothcinnamon groups were self-treated until day 30. In case of OR1 and OR2 groups, the highest prevalence (30% and 33.33%) was mentioned 30 days post-exposure, while nodular lesions were not observed after 45 days. The prevalence of the control group was 76.67%, 20 days post-exposure and it was gradually minimized during 90 days. Also, the highest cumulative mortality was calculated in case of the control group, while in case of CIN1 and OR1 groups no mortalities were observed.

Conclusion: Both *Origanum vulgare* and *Cinnamomum zeylanicum* essential oils used as nutritional supplements to the diet of the experimental gilthead sea bream proved to be effective against LCDV, reducing the time of self-treatment and minimizing mortalities.

Keywords: essential oils, gilthead sea bream, lymphocystis disease virus

121-P

Viability of Infectious Haematopoietic Necrosis Virus (IHNV) and Viral Haemorrhagic Septicaemia Virus (VHSV) adsorbed to sediment and soil

Joiner, Claire (United Kingdom)[1]; Paley, Richard (United Kingdom)[1]

1 - Cefas, Weymouth, Dorset, UK

Introduction: Adsorption of virus particles to sediment and soil is considered important for disease control and risk analysis. Few studies have been published on the survival of VHSV and IHNV in sediment (Oidtmann et al., 2017). They indicate adsorption occurs, but most do not separate the liquid from the sediment. This study investigated further whether the decrease in detectable viral titres observed was due to inactivation or adsorption to river sediment and 7 typical soil types.

Methodology: The survival of IHNV isolates HV-90 and 32:87 and VHSV J167 were investigated in a mix of unsterile and sterile sediment/a variety of soils and river water (1:1). The liquid portion containing the initial inoculum was removed and titrated after 1h. The solid portion was then tested directly and indirectly on fish cell lines. Several elution methods were assessed for their ability to release viable virus from the sediment.

Results: Cell culture titration ($TCID_{50}$) shows rapid decrease in virus titres in the liquid portion of the sediment mixture at higher temperatures (1 h - 1 d at 25 °C) with longer survival at lower temperatures (up to 49 d at 4 °C). Soil particles blocked the visibility of the cells at dilutions up to 10^{-3} and were too heavy to be washed from the cells by multiple pipetting. At higher dilutions however, cells were visible and cytopathic effect was evident, proving virus remains associated with the sediment and is potentially infectious to its host. Methods allowing indirect contact of the sediment and soil had varying results. Viable virus adsorbed to all the soil types tested, with highest adsorption to clay-based soils. Organic content decreased the amount of viable virus present. The adsorbed virus was eluted by subsequent washing and increased titres were observed with addition of 10% FBS. Preliminary results indicate greater adsorption for IHNV compared to VHSV but testing of additional isolates are required.

Conclusion: Aquatic viruses could remain adsorbed, and infectious, to the sediment after infected fish have been removed and the virus is undetectable in the surrounding water. Infectious virus remaining associated with sediment should be considered when undertaking disease control risk assessments.

Keywords: adsorption, IHNV, VHSV, survival

Funding: Defra.

122-P

The selective breeding of rainbow trout for resistance to Viral Haemorrhagic Septicaemia (VHS) using few parental fish

Ito, Takafumi (Japan)[1]; Olesen, Niels Jørgen (Denmark)[2]

1 - Diagnosis and Training Center for Fish Diseases, National Research Institute of Aquaculture, Fisheries Research Agency; 2 - National Institute of Aquatic Resources, Technical University of Denmark

Introduction: Viral haemorrhagic septicaemia virus (VHSV) is known as the causative agent of severe disease outbreaks with high mortalities in farmed rainbow trout. Breeding for resistance to VHS in rainbow trout likely a suitable strategy to reduce the impact of the disease since a commercial vaccine for the disease has not yet been developed. Selective breeding for resistance to fish diseases should be practically possible for fish farmers. In this study, we evaluate the possibility of conducting selective breeding for resistance to VHSV using progeny from crosses of parental fish showing various susceptibility to VHS. Experimental infections were conducted on 6 rainbow trout strains which were bred from crossings of 4 females and 3 males and using the high virulent VHSV DK-3592B isolate for the infection.

Methodology: Two experiments were conducted, in the first (#1), 20 progeny of two rainbow trout strains were used. Ten fish of each group were IP injected $10^{6.0}$ $TCID_{50}$ VHSV and 10 fish were mock infected. In the second experiment #2, 40 progeny from each of six breedings were used. The rainbow trout were infected by immersion in $10^{5.0}$ $TCID_{50}$ VHSV/mL at 13 °C for 2 h. Control fish were exposed to medium without virus. Water temperature throughout the studies were approximately 13 °C, and conducted for 1 month. All dead and surviving fish were collected and all samples were tested on cell cultures.

Results: In experiment #1, the VHS IP infected Samegai family and the Tamaki family had cumulative mortalities of 10 and 90%, respectively. In experiment #2, the mortalities in the 6 families varied from 40% to 95%, and the differences among several rainbow trout families were significant. VHSV was re-isolated from most of the kidney and brain samples collected from rainbow trout that died due to infection.

Conclusion: It was earlier shown that heritability for VHS resistance is relatively high and our results confirmed that differences in VHS susceptibility varied significantly between rainbow trout families originating from parental fish with different susceptibility to VHS. The method could be a practical way for fish farmers to select for more VHS resistant rainbow trout families.

Keywords: VHSV, rainbow trout, breeding, resistance

123-P

Betanodavirus infection in gilthead sea bream larvae: an immunohistochemical study

Pretto, Tobia (Italy)[1]; Marsella, Andrea (Italy)[1]; Toffan, Anna (Italy)[1]; Pascoli, Francesco (Italy)[1]

1 - Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

Introduction: An increasing number of viral encephalo-retinopathy (VER) outbreaks in gilthead sea bream (*Sparus aurata*) hatcheries with high mortality rate has been reported. Isolated betanodaviruses causing the disease were characterized as RGNNV/SJNNV. Limited knowledge is available on reassortant VER infections in sea bream larvae, therefore this study aimed to investigate its pathogenesis by histology and immunohistochemistry (IHC).

Methodology: A batch of 10,000 twenty-one days post hatching larvae was distributed in two 300 l tanks: one control and one challenged-group tank both maintained at 19.5 °C and 37 ‰ salinity. The challenge was performed by bath with $10^{5.45}TCID_{50}$/ml of RGNNV/SJNNV betanodavirus. Ten larvae were fixed in 10% formalin at different time points: 24 h, 48 h, 72 h, 96 h and 5, 6, 7, 12, 13, 19, 21, 24, 30 days post infection (dpi). Samples were processed for histology, stained with hematoxylin/eosin and IHC was performed with an in-house serum against SJNNV.

Results: Clinical signs appeared after 9 dpi, with affected specimens showing apathy, abnormal swimming behavior and overinflation of the swim bladder. Mortality began at 10 dpi, peaked between 11 - 13 dpi and then decreased but never ceased completely. IHC analyses showed the first immunoprecipitates in specimens sampled at 72 hours post infection (hpi) in the brain and spinal cord. Immunoprecipitates started to appear in the eye from 96 hpi onwards. The brain was the first organ to become extensively positive at 96 hpi, followed at 6 dpi by the eye. IHC positivity in the spinal cord was persistent until 24 dpi but less severe than in the brain and eye. Notably, the first vacuoles appeared at 12 dpi, significantly later than clinical signs and IHC signal. Immunoprecipitates started to decrease first in the brain and then in the eye, persisting only in scattered foci at 30 dpi.

Conclusion: Progression of reassortant betanodavirus infection in sea bream larvae was characterized through IHC for the first time. Interestingly, clinical signs appear not to be directly correlated to an anatomical lesion of nervous tissues (vacuolation) but instead to a massive virus replication.

Keywords: betanodavirus, gilthead sea bream, immunohistochemistry, reassortant, larvae

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124-P

Lumpfish (*Cyclopterus lumpus*) is susceptible to viral nervous necrosis: result of an experimental infection with different genotypes of betanodavirus

Toffan, Anna (Italy)[1]; De Salvador, Maria (Italy)[2]; Scholz, Felix (Ireland)[3]; Pretto, Tobia (Italy)[1]; Buratin, Alessandra (Italy)[1]; Cuenca, Argelia (Denmark)[4]; Vendramin, Niccolò (Denmark)[4]

1 - Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy; 2 - University of Udine, Italy; 3 - FishVet Group Ireland, Oranmore, Co. Galway, Ireland; 4 - National Institute of Aquatic Resources, EURL for fish disease Technical University of Denmark, Kongens Lyngby, Denmark

Introduction: Cleaner fish are extensively used for sea lice control in Atlantic salmon farming in Europe. Infection with Nervous Necrosis Virus (NNV) was reported in wild wrasses. Lumpfish and wrasses share the same environment and use, but at present there is no data about lumpfish susceptibility to NNV. The aim of this study was to determine the susceptibility of lumpfish (*Cyclopterus lumpus*) to different betanodaviruses.

Methodology: Three different NNVs were used: 459.18/I12 (RGNNV), Ah95NorA (BFNNV) and SK-07 1324 (BFNNV). Ninety lumpfish (divided in 3 tanks) were injected IM with 10^5 $TCID_{50}$/ml. 60 fish were mock infected. Temperature was 12 °C. Clinical disease and survival was monitored for 28 days and all diseased fish were euthanized and sampled. One healthy fish per tank was sampled once a week as were all survivors. Brain samples were subjected to molecular testing and virus isolation or to histological and immunohistochemical (IHC) analyses.

Results: Survival ranged from 80.52 - 81.02 % with no statistically significant variations between groups. Reduced survival in the first two weeks was attributed to tail biting, a well-known behavioural problem in lumpfish. No nervous signs were observed in infected fish despite all tested positive by real time RT-PCR. Virus isolation revealed a higher viral titer in Ah95NorA and SK-07 1324 compared to the 459.18/I12 infected group. Viral titer also appeared to increase over time, supporting viral replication in this host. Histology and IHC evidenced typical vacuoles surrounded with immunoprecipitates in brains and eyes of Ah95NorA and SK-07 1324 survivor fish. Mild or no IHC signal was observed in lumpfish infected with 459.18/I12.

Conclusion: Reduced survival early in the trial was not attributable to NNV. However, lumpfish are susceptible to NNV as proven by the high viral titre and severe lesions detected in the brain. Future long-term studies are needed to determine if NNV could cause clinical disease in lumpfish in different environmental conditions. Differences in viral titre amongst infected groups are due to different optimal replication temperatures of the NNVs used.

Keywords: lumpfish, cleaner fish, betanodavirus, RGNNV, BFNNV

Funding: This work was funded by H2020 Parafishcontrol and H2020 Aquaexcel 2020.

125-P

Study of the occurrence and genetic diversity of CEV strains circulating in France through the use of optimized diagnostic tools

Fabrice, Almeras (France)[1]; Laurane, Pallandre (France)[1]; Loeiz, Maillet (France)[1]; Thierry, Morin (France)[1]; Lénaïg, Louboutin (France)[1]; Marine, Baud (France)[1]

1 - French Agency for Food, Environmental and Occupational Health & Safety (Anses), Ploufragan-Plouzané-Niort Laboratory, Unit Viral Diseases of Fish, National Reference Laboratory for listed fish diseases, Plouzané, France

Introduction: Carp edema virus (CEV) disease, also known as koi sleeping disease, induces characteristic pronounced lethargy and unresponsiveness in infected koi and common carp *Cyprinus carpio,* which frequently result in significant mortalities. Molecular epidemiology analyses have suggested the existence of distinct viral populations infecting preferentially koi or common carp. Originally described in Japan in 1974, first cases were reported in Europe in the late 2000s. The establishment of a surveillance system in our territory requires the availability of powerful and validated diagnostic tools allowing detection and characterization of circulating strains.

Methodology and Results: In this work, we report the partial validation of a real time PCR test developed by Cefas (Way et al., 2017) according to the NF standards U47-600-1 and -2 (2015) as well as the genetic description of CEV isolates detected by our National Reference Laboratory since the first detection of the disease in France in 2010. An inclusivity of 100% was obtained by testing a panel of CEV representative strains but no detection was observed for other virus species (including cyprinid herpesvirus type 1, 2 or 3 and sturgeon iridovirus). The analytical and diagnostic sensitivities were estimated at 12.5 copies of plasmid DNA/reaction and 100 copies of plasmid DNA/reaction respectively. The 47 isolates detected in France since 2010 were partially sequenced on the P4a gene using a new strategy developed by our laboratory enabling the generation of a longer (894 nucleotides) and more informative sequence than the one conventionally used. Phylogenetic tree showed at least two different genetic lineages in France, one specific to common carp and the other one to koi. Nucleotide identities within viruses constituted koi carp and common carp groups are respectively between 95.9 and 99.8% and 98.5 and 99.8%.

Conclusion: The use of this validated qPCR on field samples coupled with the characterization of the isolates detected will enable us to determine more precisely the prevalence of CEV in our territory and to better understand strains diversity and circulation.

Keywords: carp edema virus, *Cyprinus carpio*, real time PCR, molecular epidemiology

126-P

Susceptibility of rainbow trout (*Oncorhynchus mykiss*) fed with fishmeal substitutes (*Hermetia illucens*, *Arthrospira platensis*) to the viral haemorrhagic septicaemia virus

Bauer, Julia (Germany)[1]; Adamek, Mikolaj (Germany)[1]; Miebach, Anne-Carina (Germany)[1]; Gährken, Jakob (Germany)[2]; Wessels, Stephan (Germany)[2]; Matras, Marek (Poland)[3]; Stachnik, Magdalena (Poland)[3]; Reichert, Michal (Poland)[3]; Jung-Schroers, Verena (Germany)[1]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover; 2 - Georg-August-Universität Göttingen; 3 - National Veterinary Research Institute, Puławy

Introduction: In the evolution of fish populations, genetic adaptions to new environmental conditions are crucial. Sudden changes in nutrition can occur, especially in aquaculture facilities due to changes in feed composition. The use of fishmeal in the feed of cultured fish is controversial due to overfishing of the oceans. Therefore, the replacement of fishmeal with alternative protein sources is a main and timely scientific focus. Supplementations on the other hand might lead to slower growth or affects fish health or animal welfare. The cooperative project "Sustainable Trout Aquaculture Intensification - SusTAIn" explores the genetic variability of trout to adapt to novel resources in order to use these for a sustainable aquaculture. The project deals with the impact of feeding substitute protein sources on the health of fish by evaluating chronic stress and analysing gene expression in order to assess immunological responses and the susceptibility to trout specific pathogens.

Methodology: Several local strains of rainbow trout were compared to a commercially available strain in growth performance and susceptibility to infection with viral haemorrhagic septicaemia virus (VSHV). The susceptibility of trout to VHSV was evaluated by *in vitro* studies using fin tissue and primary cell cultures from scales from different genetic trout strains, which were fed with the differently supplemented feeds. Grade of replication of VHSV in the *in vitro* infections was evaluated using titration on RTG-2 cell cultures with determination of the 50 % tissue culture infective dose ($TCID_{50}$).

Results: Analysis of *in vitro* infection susceptibility of fin tissue of parents of different trout strains under control feed did not show any significant differences in virus replication. Results of *in vivo* experiments indicated that the some trout strains showed significant differences in mortality rates with over 75 percent difference between most susceptible and resistant strain. The mortality was independent from growth performance of given strains of fish, also feed supplements had no impact on mortality under VHSV-infection.

Conclusion: Preliminary results indicate that under severe disease pressure the survival of rainbow trout from VHSV-related disease is predominantly influenced by the genetic background independently from feeding of fishmeal substituted feed and growth performance.

Keywords: alternative feed, susceptibility, viral haemorrhagic septicaemia virus, rainbow trout

127-P

Confirmation of spring viremia of carp virus in wild common carp (*Cyprinus carpio* L.) in Mexico

Ortega, Cesar (Mexico)[1]; Cañas-Lopez, Leticia (Mexico)[2]; Irgang, Rute (Chile)[3]; Fajardo, Raúl (Mexico)[1]; Poblete-Morales, Matías (Chile)[3]; Valladares-Carranza, Benjamin (Chile)[1]; Tapia-Cammas, Diana (Chile)[3]; Avendaño-Herrera, Ruben (Chile)[3]

1 - Universidad Autónoma del Estado de México; 2 - Universidad Autónoma del Estado de México; 3 - Universidad Andrés Bello

Introduction: This study confirms the presence of spring viremia of carp virus (SVCV) in wild common carp (*Cyprinus carpio* L) in central Mexico. Fish exhibited lesions suggestive of SVC, and samples were analyzed by cell culture, molecular techniques, gene sequencing, and electronic microscopy, resulting in the isolation and identification of SVCV.

Methodology: Ten specimens of common carp were collected from a natural freshwater lagoon where no disease outbreaks and/or disease-related mortalities have been reported. Internal samples were processed to describe histopathological alterations; fragments of kidney were collected and processed for virus isolation in cell monolayers of *epithelioma papulosum cyprini* (EPC), according to the OIE Manual and for electron microscopy. Total RNA was extracted from the supernatant of cell cultures and the molecular diagnosis of SVCV was performed according to OIE Manual; samples were considered positive if the expected sizes of the primary and secondary PCR products were 714 and 606 bp, respectively. The secondary amplification products were sequenced, and alignments were performed with the ClustalW algorithm and the two-nucleotide sequences were deposited in GenBank.

Results: Five fish presented signs of septicemic disease, with sero-hemorrhagic ascites and adhesions between abdominal organs, and diffuse hemorrhages in the coelomic cavity; histologically, the internal organs presented systemic damages associated with SVCV. The kidney homogenates showed a CPE between 24 and 48 h post-inoculation in EPC cell cultures; the electron microscopy revealed the characteristic bullet-shaped viral particles with structural traits typical of rhabdovirus (110 - 123 nm long, 75.5 - 78.1 nm wide) and also the expected specific amplification product for SVCV was observed (716 bp for the first reaction and of 606 bp for the second round). The phylogenetic analyses of partial SVCV glycoprotein gene sequences of Mexican SVCV isolates were classified into the Ia genogroup.

Conclusion: The analyses confirm the presence of SVCV in common carp in Mexico. The phylogenetic analyses classified the isolates into the Ia genogroup. However, it is difficult to estimate the risk of SVCV for other wild/feral cohabitating cyprinid species in the lagoon. The status of this virus in other water sources within this region and in the country is also unknown

Keywords: SVCV, carp, Mexico, viremia

128-P*

Unravelling the pathogenesis of salmon gill poxvirus in freshwater Atlantic salmon

Allshire, Rosa (United Kingdom)[1]; Weidmann, Manfred (United Kingdom)[1]; Baily, Johanna (United Kingdom)[1]; Wands, Ola (United Kingdom)[2]; Robertson, Rhona (United Kingdom)[2]

1 - Institute of Aquaculture, University of Stirling; 2 - Cooke Aquaculture, Scotland

Introduction: Salmon gill poxvirus is an emerging viral disease in the Scottish salmon farming industry and has been associated with high mortalities throughout the production cycle. Although severe gill pathology is generally associated with disease outbreak, little else is known about the pathogenesis of the disease. In order to better understand the transmission routes and potential outbreak triggers, two comparable cohorts of farmed salmon were studied over a 6 month period and through a clinical SGPVD outbreak.

Methodology: Two cohorts of Atlantic salmon fry in a hatchery were studied concurrently in separate recirculation systems with varying histories of SGPV. Both systems share similar husbandry techniques, water source and fry intake, but only one has had SGPV in recent years. During the study period, one cohort became infected with the virus while the other remained naïve. The progression and effects of the disease were studied and compared to the naïve group in order to better understand the prevalence of the virus and it's correlation to disease outbreak. Blood, serum, histology and gill samples were taken from 14 randomly sampled fish from each cohort, at two week intervals, over a six month period. Once SGPV was detected, sampling was increased to twice weekly for two weeks in order to document the disease outbreak. As a result, the virus prevalence, spread and effects in various organs were documented. Gill tissue was tested for viral load using qPCR, histopathology samples were screened for any pathology caused by the disease, blood samples were analysed for red blood cell count, total and differential white blood cell counts and packed cell volume and serum was taken for serology and potential biomarker identification.

Results: Results of the comparative study of naïve and infected cohorts will be presented in the final poster as analysis is ongoing.

Keywords: salmon gill virus, epidemiology, freshwater

Funding: Cooke Aquaculture and University of Stirling.

129-P

Haemathology of common carp (*Cyprinus carpio* L.) in field cases of spring mortality

Piackova, Veronika (Czech Republic)[1]; Kocour Kroupova, Hana (Czech Republic)[1]; Zuskova, Eliska (Czech Republic)[1]; Syrova, Eva (Czech Republic)[2]; Papezikova, Ivana (Czech Republic)[2]; Vesely, Tomas (Czech Republic)[3]; Palikova, Miroslava (Czech Republic)[2]

1 - University of South Bohemia in Ceske Budejovice; 2 - University of Veterinary and Pharmaceutical Sciences Brno; 3 - Veterinary Research Institute

Introduction: In the last few years, increasing of spring mortality of common carp in European ponds was recorded. In 2011, first detection of a virus from the case of spring mortality of carp was noticed in CEFAS. It was very similar to Carp Edema Virus (CEV; family Poxviridae) isolated from koi suffering from Koi Sleepy Disease in Japan in the late 1970s. Clinical and pathological symptoms of the „CEV disease“ in carp noticeably remind koi herpesvirus disease (KHVD), but they usually evolve in lower temperature (8 - 20 °C). In the Czech Republic, mortality caused by CEV was first confirmed by PCR in 2015 in archived samples from 2013 and 2014.

Methodology: Fish from twenty localities (four koi and sixteen carp ponds) were investigated in 2017. Clinical and pathological signs were as lethargy, asphyxia, gathering of fish at the surface and near the shore or inflow, irregular mucus layer on the skin, sunken eyes, necrotic gill etc. In all localities, increasing mortality was reported. The temperature of water ranged from 5 to 13 °C. Testing for CEV presence employed a two-round PCR developed at CEFAS. Haematological and immunological examinations were performed in some localities.

Results: Nine localities were CEV DNA positive (3 koi, 6 carp) but not all fish from these localities were positive although they showed similar signs. In CEV-positive koi, significant decrease of haematocrit and erythrocyte count was noticed. In common carp, the differences were in accordance rather to the level of damage of the fish organisms by than the presence of CEV DNA.

Conclusion: According to the results, spring mortality of carp can be caused by CEV but it is not only one causal agent in these cases. Results of haematological examination of carp in field cases were in accordance rather to the level of damage of the fish organisms by than the presence of CEV DNA.

Keywords: common carp, CEV, haematology

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130-P

Molecular detection of a novel cyprinid herpesvirus in roach (*Rutilus rutilus*) and asp (*Leuciscus aspius*)

Sellyei, Boglarka (Hungary)[1]; Varga, Adam (Hungary)[1]; Szekely, Csaba (Hungary)[1]; Doszpoly, Andor (Hungary)[1]

1 - Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences

Introduction: The family *Alloherpesviridae* includes the herpesviruses detected or isolated from amphibian and fish species. The family contains four genera, one of them the genus *Cyprinivirus* comprises the herpesviruses of cyprinids and that of the European eel (*Anguilla anguilla*). The genus contains four species accepted by the International Committee on Taxonomy of Viruses: *Cyprinid herpesvirus 1*, *Cyprinid herpesvirus 2*, *Cyprinid herpesvirus 3* and *Anguillid herpesvirus 1*. A fifth, not yet accepted virus species, the Cyprinid herpesvirus 4 was described from Sichel (*Pelecus cultratus*) few years ago from Lake Balaton, Hungary.

Methodology: In the early spring of 2018, also in Lake Balaton, Hungary, a roach (*Rutilus rutilus*) and an asp (*Leuciscus aspius*) were found in a fish trap showing typical signs of the so called carp pox disease caused by the *Cyprinid herpesvirus 1*. Tissue samples were homogenized and DNA extracion was carried out. Subsequently, PCRs targeting conserved genes of alloherpesviruses were conducted.

Results: The routine molecular investigations showed the presence of the DNA of an unknown alloherpesvirus. Three genes (DNA polymerase, major capsid protein and terminase) were amplified and sequenced partially from the presumed alloherpesviral genome. The sequences obtained from the two different species shared 99.9% nucleotide identity.

Conclusion: Phylogenetic tree reconstructions, based on the concatenated sequence of the genes, implied that the virus undoubtedly belongs to the genus *Cyprinivirus* within the family *Alloherpesviridae*. The sequences of the novel alloherpesvirus diverge from those of the five previously described cyprinivirus species, putatively representing the sixth virus species in the genus. In the present year, screening for the novel virus in the Lake Balaton is being conducted. Up to now, we found the novel virus only in roach showing the same symptoms.

Funding: The Hungarian Scientific Research Fund (OTKA K127916) and GINOP-2.3.2-15 -2016-00004.

131-P

It is everywhere! Detection of carp edema virus in Hungary, Croatia, Serbia and Lithuania confirm wide distribution in Europe

Adamek, Mikolaj (Germany)[1]; Baska, Ferenc (Hungary)[2]; Nienius, Darius (Lithuania)[3]; Radosavljevic, Vladimir (Serbia)[4]; Zrnčić, Snježana (Croatia)[5]; Brnić, Dragan (Croatia)[5]; Oraić, Dražen (Croatia)[5]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany; 2 - University of Veterinary Medicine, Budapest, Hungary; 3 - National Food and Veterinary Risk Assessment Institute, Vilnius, Lithuania; 4 - Scientific Institute of Veterinary Medicine of Serbia Belgrade, Serbia; 5 - Croatian Veterinary Institute, Zagreb, Croatia

Introduction: Koi sleepy disease (KSD) caused by carp edema virus (CEV) infection is considered an emerging disease in certain regions of the world. Especially in Europe the virus was detected only in recent years. However, after its initial detection in UK it came more into focus of diagnostic laboratories, which was followed by multiple detections. Based on this we hypothesise that both CEV and KSD exist in the European common carp aquaculture since long time and only due to a particular set of circumstances like: Less dramatic clinical presentation, the season of occurrence, similarity of clinical signs to koi herpesvirus disease (KHVD) or intoxication with ammonia and no ability to detect the virus by cell culturing, it was not detected.

Methodology: A result which could support this hypothesis would be a wide geographical distribution of the virus in main carp producing countries and in countries with a limited carp production. Therefore, samples were collected in Hungary, Serbia, Croatia and Lithuania in 2015-2018 and screened for the presence of CEV DNA with quantitative PCR.

Results: Prevalence of CEV in Hungary, one of the largest European carp producer, was the highest with 76% (13 CEV positive out of 17 locations screened). In contrast, the prevalence in countries with smaller crap production was lower: 14% prevalence was recoded in Croatia where 6 out of 44 locations were CEV positive, 30% prevalence was recorded in Lithuania (6 CEV positive locations out of 20 checked). In Serbia fish with clear signs of clinical KSD from only two farms were sampled and both locations were confirmed to be CEV positive. Phylogenetic analyses indicated that in some cases the detected virus isolates are distributed in geographically related locations. For instance, the same virus was found in neighbouring regions of Hungary and Serbia.

Conclusion: Our findings indicate that CEV (especially from genogroup I) is widely spread in the European carp aquaculture. This could indicate that rather than being an emerging disease this virus was previously overlooked/misdiagnosed. Therefore, KSD should be considered in whole Europe when investigating disease outbreaks in common carp at temperatures below the optimum for KHVD.

Keywords: CEV, common carp

132-P

Phylogeny of Viral haemorrhagic septicaemia virus (VHSV) based on full genome sequences

Cuenca, Argelia (Denmark)[1]; Blanchard, Yannick (France)[2]; Ryder, David (United Kingdom)[3]; Panzarin, Valentina (Italy)[4]; Abbadi, Miriam (Italy)[4]; Zamperin, Gianpiero (Italy)[4]; Morin, Thierry (France)[5]; Olesen, Niels (Denmark)[1]

1 - European Union Reference Laboratory for Fish and Crustacean Diseases, DTU aqua, Kgs. Lyngby, Denmark; 2 - Anses, Ploufragan-Plouzané-Niort Laboratory, GVB Unit, Ploufragan, France; 3 - CEFAS, Weymouth, UK; 4 - IZSVe, Padova, Italy; 5 - Anses, Ploufragan-Plouzané-Niort Laboratory, PVP Unit, Plouzané, France

Introduction: VHSV is a rhabdovirus with a negative sense, single strand RNA genome of about 11 kb. One of the most intriguing characteristics of VHSV is its ability to cross species boundaries, not only to cause sporadic infection, but also to create stable intra-species transmission in novel fish species. Indeed, since its first isolation from cultured rainbow trout in 1963, VHSV has been found in more than 90 different fish species in freshwater and marine environments. Molecular characterization of VHSV are mostly based on the sequence of the glycoprotein (G-gene). Although more than 800 full G-gene sequences are publicly available to date, it remains difficult to establish the phylogenetic relationships among the different sublineages within the largest genotype I.

Methodology: A dataset comprising more than 100 VHSV isolates were sequenced by NGS to obtain their full genome. Special emphasis was put in sequencing a large number of marine isolates, as these have been largely underrepresented in previous studies. To resolve the phylogenetic relationships among genotypes, analyzes were done both based on complete genome sequences and on individual genes.

Results: Congruency of trees topology based on different parts of the genome was evaluated, finding few strongly incongruent regions caused by chimeric consensus sequences resulting from mixed samples. After cleaning the dataset from ambiguous sequences, overall topology coincides with results obtained using the glycoprotein gene alone. However, some discrepancies among single gene analyses were confirmed, especially in the placement of genotypes Ia, Ib, Ic, and Iu. The phylogenetic tree based on full genome sequences provides a larger resolution in some areas of the tree. This is particularly true for some previously underrepresented marine isolates.

Conclusion: To our knowledge, this is the first phylogeny of VHSV based on full genome sequences, and including more than 100 isolates. This dataset could be used as a backbone to further test hypothesis of the tempo and mode of the genome evolution of VHSV.

Keywords: VHSV, phylogeny, virulence

Funding: Novimark Project (G88F13000660001) funded by the Anihwa ERA-Net Consortium, the UK Department for Environment, Food and rural Affairs (contract C7577B), and the European Reference Laboratory of Fish and Crustacean Diseases.

133-P

Abandon all hope? Is *in vitro* culturing of carp edema virus a lost cause?

Adamek, Mikolaj (Germany)[1]; Abd El-Rahman, Sahar (Egypt)[2]; Felten, Martin (Germany)[1]; Teitge, Felix (Germany)[1]; Jung-Schroers, Verena (Germany)[1]; Paley, Richard (United Kingdom)[3]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany; 2 - Mansoura University, Mansoura, Egypt; 3 - Centre for Environment, Fisheries and Aquaculture Science, Weymouth, UK

Introduction: Carp edema virus (CEV) is an epitheliotropic virus infecting predominantly the branchial tissue of common carp. Multiple attempts to re-isolate and cultivate CEV were performed without success, this significantly hampers research on this virus. We present further efforts to develop a replication system for CEV based on gill derived cells. Despite the significant limitations experienced we evaluated cases where an *in vitro* culturing of CEV could be used.

Methodology: Several approaches were taken to find a suitable *in vitro* CEV replication system: explant cultures of gills, precision cut gill slices (PCGS), primary and permanent gill cell cultures. The presence of virus particles or viral mRNA expression and changes in virus abundance or the mRNA-expression level were used as indicators of virus replication. A successful culture system was used for studying the temperature permissivity of the virus in *in vitro* and compare this with *in vivo* data.

Results: The CEV remains unculturable *in vitro* in the classical sense. The virus cannot be replicated in primary or permanent cultures of gill cells. Explants and PCGS can be infected but with very limited success and they do not produce noticeable amounts of new virus particles. The only valid approach for working *in vitro* with CEV is the use of explants/cells collected from infected fish. We successfully used such explants to show that there were significant differences between CEV replication at18 °C and 29 °C, where it was significantly limited. *In vivo* infection experiments confirmed that virus replication was severely impaired at 29 °C and fish were able to clear the infection when compared to lower temperatures. Furthermore, the separation of the cells from the gills of infected fish in a Percoll gradient provided insights into the cell type which is most susceptible to a CEV infection and can provide virus replicating cells for short culturing period.

Conclusion: Currently, the only valid approach to *in vitro* work with CEV is the use of gill material collected from infected fish. This can provide supportive information of results obtained during in *in vivo* studies and could lead to investigations into the gill cell population that replicates the virus.

Keywords: CEV, *in vitro* culturing

134-P

The influence of virus infection on cell line characteristics: CYHV-3, CCPV and CEV VS new cell lines from common carp

Adamek, Mikolaj (Germany)[1]; Felten, Martin (Germany)[1]; Rakers, Sebastian (Germany)[2]; Gebert, Marina (Germany)[2]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany; 2 - Fraunhofer Research Institution for Marine Biotechnology and Cell Technology, Luebeck, Germany

Introduction: Several factors influence the susceptibility of cell lines to infection by different viruses. They can be related to tissue specificity of the viruses, physiological status of the cells, their differentiation level and capacity to mount immune responses to combat virus infection. To study interactions of carp infecting viruses with cells, several new cell lines where raised from common carp and exposed to infection with *cyprinid herpesvirus 3* (CyHV-3), carp edema virus (CEV) and a yet not fully characterized common carp paramyxovirus (CCPV).

Methodology: Newly developed cells from common carp gills (CCAgill), brain (CCAbre), fins (CCApin) and heart (CCAcar) were compared to common carp brain (CCB) cells in virus susceptibility, expression of cellular markers, and type I IFN responses. Susceptibility to virus infection was measured by CPE formation, estimation of viral particles produced by the cells, and presence of viral mRNA in the cells. Virus susceptibility was compared with the level of type I IFN responses. Changes in cell characteristics were measured by mRNA expression of three epithelial (cadherin 1, occludin and cytokeratin 15) and one mesenchymal cell marker (vimentin).

Results: All cell lines were susceptible to CyHV-3 and CCPV, but not to CEV infection. The cell lines had different levels of type I IFN responses towards the viruses. Typically, CyHV-3 did not induce high type I IFN responses while the paramyxovirus induced high responses in CCAbre, CCAcar, CCApin cells but no response in CCAgill cells. Consequently, the type I IFN response modulated cell susceptibility to CCPV but not to CyHV-3. Interestingly, when three different passage levels of CCB cells were examined, the susceptibility of one passage was significantly lower for CyHV-3 and higher for CCPV infection. This coincided with a loss of epithelial markers and lower type I IFN responses.

Conclusion: The susceptibility of cells for virus infection was different for each of the viruses. Depending on the vulnerability of the virus to type I IFN responses, cells with a lower IFN-response can be superior for replication of some viruses. Batches of CCB cells can differentiate and by this have significantly different susceptibility to certain viruses.

Keywords: CyHV-3, CEV, common carp, common carp paramyxovirus

135-P

Carp Oedema Virus: a new threat for the cyprinids inhabiting open water

Zrncic, Snjezana (Croatia)[1]; Pavlinec, Zeljko (Croatia)[1]; Zupicic, Ivana Giovanna (Croatia)[1]; Adamek, Mikolaj (Germany)[2]

1 - Croatian Veterinary Institute, Zagreb, Croatia; 2 - Fish Disease Research Unit, Institute for Parasitology, University of Veterinary Medicine, Hannover, Germany

Introduction: Carp oedema virus (CEV), the cause of koi sleepy disease, is a DNA virus from the family *Poxviridae*. The disease was first reported as mass mortalities of koi carps in Japan, but during the last decade, it has been described throughout Europe. The disease is characterized by lethargy, body swelling, ulceration around the mouth and fin basis, gill necrosis and concomitant mortalities rise up to 80%. Due to losses in koi carp and common carp industry, CEV is considered as an emerging disease in European carp aquaculture. The highest losses in Japan where reported to occur in spring and summer with a water temperature of 15 to 35 °C while in the UK the losses were noticed during the winter months, at 6 to 9 °C. In Croatia, the surveillance for the presence of the virus was performed using the samples collected for detection of KHV during the warmer part of the year. A low prevalence of virus, without notification of mortalities or clinical signs, was detected. However, high mortality of carp from the open water occurred during the end of April 2018. Carps weighing 3 to 5 kilograms were mostly affected. The water temperature was up to 25 °C. Samples were submitted to the laboratory for a diagnostic procedure.

Methodology: Parasitological, bacteriological and virological examinations were carried out by microscopic analysis, streaking of material from organs onto blood agar and by inoculating the homogenized tissues onto BF2 and EPC cell lines. Nucleic acid extraction and purification from gills and kidney tissues were performed using a commercial kit. Nested PCR was performed using the method described by Matras et al. 2016. Phylogenetic analysis was performed with obtained sequences.

Results: Parasitological and bacteriological examinations were negative. All samples tested negative for Koi herpes virus (KHV) using real time PCR. However, samples were positive for CEV.

Conclusion: Although there was no proved connection between the introduction of carps for repopulation of open waters from CEV positive carp farms and the mortality event special attention should be employed in such cases. Phylogenetic analysis will elucidate possible pathways of the virus introduction.

Keywords: CEV, open waters, common carp

136-P

Virulence factors in VHSV: which replication step is responsible for VHSV strains virulence?

López-Vázquez, Carmen (Spain)[1]; Panzarin, Valentina (Italy)[2]; Toffan, Anna (Italy)[2]; Blanchard, Yannick (France)[3]; Brémont, Michel (France)[4]; Olesen, Niels J. (Denmark)[5]; Stone, David (United Kingdom)[6]; Morin, Thierry (France)[7]; Bandín, Isabel (Spain)[1]; Dopazo, Carlos P. (Spain)[1]

1 - Departamento de Microbiología y Parasitología, Instituto de Acuicultura. Universidade de Santiago de Compostela, Spain; 2 - IZSV, Padova, Italy; 3 - Anses, Ploufragan-Plouzané-Niort Laboratory, GVB Unit, Ploufragan, France; 4 - VIM, INRA, Paris-Saclay University, Jouy-en-Josas, France; 5 - DTU, Lyngby, Denmark; 6 - CEFAS, Weymouth, UK; 7 - Anses, Ploufragan-Plouzané-Niort Laboratory, PVP Unit, Plouzané, France

Introduction: The risk of VHSV for aquaculture is of serious concern in the EU. Recently, some RT-qPCR procedures have been reported for diagnosis and typing. Aimed to the advance in the control of this disease, and as part of a European ANR-EraNet-Anihwa grant (0006-02 NOVIMARK), 6 groups from 5 countries have coordinated efforts to design a qPCR-based method for typing the virulence of VHSV to rainbow trout. In this communication, the results of the second objective (Is a failure in replication responsible for the low virulence of certain VHSV strains?) are presented and discussed.

Methodology and Results: As part of the first objective, around 150 strains were subjected to complete genome sequence, and for around 70 their level of virulence (low, L; moderate, M; high, H) was tested in challenges with rainbow trout. In parallel, a set of recombinant viruses was constructed with mutations in specific locations of the genome, and their virulence also tested. To approach the second objective, among all, 13 strains (6 H, 3 M, 4 L) where selected to study their replication cycle to determine the steps involved in virulence. Viral titration and RT-qPCR vRNA quantification were applied in time course studies, with special emphasis in the adsorption period. No significant differences between L and H strains were observed in intracellular RNA synthesis. Total viral production, expected to be higher in H strains, only followed our hypothesis with Danish strains, and among the remaining the highest titer in RTG-2 was shown by L strain; in these cases, no differences in extracellular RNA copies were observed, suggesting that a possible failure in morphogenesis could produce non-infective particles. Most interesting, the efficiency of adsorption was markedly affected, in all cases, in the L strains.

Conclusion: These results clearly demonstrate that, apart from possible failures in the synthesis and/or morphogenesis steps, the main reason for the low virulence in VHSV is in the adsorption, which might reduce the efficiency of entrance and spreading of the virus in the host. This is the focus of the ongoing experiments.

Keywords: VHSV, virulence, replication

Funding: ERA NET ANIHWA 68 NOVIMARK / INIA – AEI, Spain.

137-P

Atlantic salmon pseudobranch tissue: a potential link in the pathogenesis of salmonid alpha virus

Weli, Simon (Norway)[1]; Tartor, Haitham (Norway)[1]; Bernhardt, Lisa-Victoria (Norway)[1]; Mohammad, Saima Nasrin (Norway)[2]; Myrmel, Mette (Norway)[3]; Lillehaug, Atle (Norway)[1]

1 - Norwegian Veterinary Institute, PO Box 750 Sentrum, 0106 Oslo, Norway; 2 - Norwegian Veterinary Institute, PO Box 750 Sentrum, 0106 Oslo, Norway; 3 - Norwegian University of Life Sciences, Ullevålsveien 72, 0454 Oslo, Norway

Introduction: Alphaviruses (family Togaviridae) are a diverse group of small, spherical, enveloped viruses with single-stranded, positive-sense RNA genomes. Pancreas disease (PD), caused by salmonid alphavirus (SAV) is among the most common viral diseases in Norwegian aquaculture, with 163 cases reported in 2018. SAV infection is characterized by necrosis and loss of exocrine pancreatic tissue, as well as degeneration and inflammation of the heart and skeletal muscle. Fish with PD appear lethargic, and unresponsive to visual challenge that may suggest blindness. In severe cases of PD, swollen or papillate pseudobranch has been observed in Atlantic salmon, that may underline a link with SAV. The Atlantic salmon pseudobranch, thought to originate from the efferent arterial gill has been found to be involved in functions that include vision, osmoregulation and secretion. However, the role of Atlantic salmon pseudobranch in SAV pathogenesis has yet to be elucidated. This work investigated the role of pseudobranch in the pathogenesis of SAV in the teleost fish species Atlantic salmon (*Salmo salar* L.).

Methodology: Cohabitant challenge trial with high and low dose SAV subtype 3 (SAV3) was performed for six weeks using post-smolt Atlantic salmon (weighing 110 g). At 16 different time point pseudobranch tissue was collected from fish in control, low and high dosage tanks and analysed by histology and immunohistochemistry. SAV detection and immune genes expression were analyzed by reverse transcription quantitative PCR (RT-qPCR).

Results: Analysis of pseudobranch tissue revealed gross pathology and histopathology, associated with PD in the SAV challenged group. Tissues from SAV challenged fish were positive for SAV at days 16-29 post-challenge. Innate and adaptive immune genes such as viperin, MX, MHC-I, sIgM and sIgT-B, were significantly upregulated in tissue from SAV- challenged fish compared with the control group at days 16-29 post challenge. The pattern of gene expression observed in both high and low viral-dose groups correlates with result obtained for SAV.

Conclusion: Our results demonstrate a clear immune response in pseudobranch tissue due to SAV infection, which suggests that the pseudobranch may have a function in the pathogenesis of SAV.

Keywords: Atlantic salmon, pseudobranch, SAV, immune response, disease

138-P

Studies into an *in vitro* system for Tilapia Lake Virus replication

Adamek, Mikolaj (Germany)[1]; Surachetpong, Win (Thailand)[2]; Abd El-Rahman, Sahar (Thailand)[3]; Paley, Richard (United Kingdom)[4]; Zou, Jun (China)[5]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany; 2 - Kasetsart University, Bangkok, Thailand; 3 - Mansoura University, Mansoura, Egypt; 4 - Centre for Environment Fisheries and Aquaculture Science, Weymouth, UK; 5 - Shanghai Ocean University, Shanghai, China

Introduction: Infections of Nile tilapia with tilapia lake virus (TiLV) cause an emerging disease threatening the production of this fish and food security in several large developing countries. The virus is already present on three continents (Asia, African and South America), thus coordinated effort has to be taken to limit its impact and spreading. We identified several challenges in working with TiLV: 1) the lack of widely available tilapia cell lines, 2) scarcity of molecular tools for currently used E-11 cells for routine TiLV work, originating from snakehead. Therefore, in a first line of studies we explored possibilities of repurposing some cyprinid or salmonid cell lines, and the development of a tilapia based *in vitro* culture system for TiLV studies.

Methodology: Precision cut slices cultures (PCSC) from tilapia brain, gills and liver were used *in vitro*. Furthermore, attempts to raise the cell line form tilapia scales or fins were made. The susceptibility of several cell lines to TiLV (Thai isolate) was measured using: RTG-2, RTgill-W1 (rainbow trout), KFC (common carp) and ZF4 (zebrafish) and compared to the E-11 reference. Susceptibility was measured by observation of CPE, estimation of viral particles produced by the cells and presence of viral RNA in the cells with $TCID_{50}$ and RT-qPCR.

Results: Over eight days culturing periods gill and brain PCSCs were replicating the virus better that liver cultures, they produced more viral particles released to the culture medium, had also higher virus RNA level in the cells. Interestingly, both rainbow trout cell lines were replicating the virus at similar levels as E-11 cells despite their cultivation at suboptimal temperature of 25°C. Also zebrafish cells were able to replicate the virus although at much lower level, while common carp cells were nonpermissive for TiLV.

Conclusion: The use of PCSC will allow monitoring type I IFN responses in tissues susceptible to TiLV, while the use of rainbow trout cells will allow to measure the vulnerability of the virus to type I IFN responses by using recombinant IFN and poly I:C stimulation. Furthermore, lipid raft dependence of viral entry and its blockage by 25-hydroxycholesterol will be studied.

Keywords: TiLV

139-P

Phylogenetic analysis and genetic diversity of infectious Hematopoietic Necrosis Virus (IHNV) isolates in Japan over the past 10 years

Namba, Aki (Japan)[1]; Minakami, Kai (Japan)[1]; Takee, Taro (Japan)[1]; Mitsui, Masako (Japan)[1]; Yasuda, Syuuta (Japan)[1]; Mano, Nobuhiro (Japan)[1]

1 - Nihon University

Introduction: Infectious hematopoietic necrosis virus (IHNV) is one of the most serious viral pathogens of salmonid fish. IHNV has been subdivided into five major genotypes: upper (U), middle (M), and lower (L) genotypes representing North American isolates, a fourth genotype representing European isolates sharing a common source with genotype M, and a fifth genotype representing Asian isolates sharing a common source with genotype U (Nishizawa *et al.,* 2006). Furthermore, IHNV isolates in Japan were classified into U and Asian isolates including two lineages, JRt-Shizuoka (S) and JRt-Nagano (N) (Mochizuki *et al*., 2009). However, knowledge regarding situation of IHNV in Japan over the past 10 years is limited. In this study, we report the phylogenetic analysis and genetic diversity of IHNV isolates obtained from diseased salmonid fish in Japan over the past 10 years.

Methodology: We collected IHNV isolates which had been isolated from diseased salmonid fish in 16 prefectures of Japan after 2008, and analyzed the G-protein full-length nucleotide sequences of each isolate.

Results: Phylogenetic analysis classified all IHNV isolates into N, S and new lineages, and most isolates belonged to the N lineage. The range of nucleotide sequence identity within N lineage was 95 - 100%.

Conclusion: The Japanese isolates obtained from 2008 were classified into 3 lineages, and the maximum nucleotide diversity among the N lineage was 5%.

Keywords: IHNV, rainbow trout, phylogenetic analysis

Funding: This study was supported in part by the Japanese Fisheries Resource Conservation Association and the Ministry of Agriculture, Forestry and Fisheries in Japan.

140-P

Susceptibility of European rainbow trout to a ranavirus isolates

Borzym, Ewa (Poland)[1]; Stachnik, Magdalena (Poland)[1]; Maj-Paluch, Joanna (Poland)[1]; Matras, Marek (Poland)[1]; Reichert, Michał (Poland)[1]

1 - Department of Fish Disease, National Veterinary Research Institute, Pulawy, Poland

Introduction: Rainbow trout (*Oncorhynchus mykiss*) fry was obtained from polish fish farms based on their current aquaculture status, besides in this farms was no history viruses infections before. Before examination fish were housed in glass aquaria. Fish were divided and maintained each species at two temperatures 13±1°C and 21±1°C. Under the same conditions were maintained control fish groups. The European sheatfish iridovirus (ESV) isolated from clinically affected adult sheatfish (*Silurus glanis*) in Poland in 2014 year and epizootic haematopoietic necrosis virus (EHNV) isolate 86/8774, classified as reference material after proficiency test performed by EURL were used for experimental trials.

Methodology: ESV and EHNV isolates were propagated in *epithelioma papulosum cyprini* (EPC) cells. During infection experiments, experimental fish were kept in 180 L aquaria. Each groups of 80 rainbow trout were exposed by bath infection to 2 x 10^7 $TCID_{50}$ mL^{-1}. Control fish were exposed to water containing cell culture medium without virus. during experiment (35 dpi) fish were observed and tissue were collected inoculated onto EPC cell for virus isolation and next confirmation by PCR.

Results: Infected rainbow trout showed no clinical changes and mortality during the infected at ESV at both temperatures. Clinical signs were observed in EHNV infected fish in 7 to 30 dpi in two temperatures, fish had dark skin, weakness, lack of appetite and symptoms of nervous system. After the section was observed anemia of gills and internal organs, splenic swelling and swollen kidneys, mortality rate was 14% at 13 °C and 28% at 21 °C. Cytopathic effect on EPC cell inoculated with tissue homogenates from all samples (tissue from infected fish), was observed. Although the fish do not always demonstrated clinical signs and mortality but ESV was reisolated.

Conclusion: The results proves the rainbow trout in Europe were susceptibility on ranaviruses and can be a vector of this viruses.

Keywords: rainbow trout, susceptibility, experimental infection, ESV, EHNV

Funding: KNOW (Leading National Research Centre) Scientific Consortium „Healthy Animal-Safe Food", decision of Ministry of Science and Higher Education, No. 05-1/KNOW2/2015.

141-P

Whole genome sequencing of the piscine myocarditis virus (PMCV) directly from field samples

Christiansen, Debes H. (Faroe Islands)[1]; Dahl, Maria M. (Faroe Islands)[1]; Petersen, Petra E. (Faroe Islands)[1]

1 - Starvstovan

Introduction: Cardiomyopathy syndrome (CMS) has proved to be of increasing concern to the farming industry. CMS is characterized by severe inflammation and degeneration of the heart, which in acute cases causes it to rupture. The causal virus, Piscine Myocarditis Virus (PMCV), is a dsRNA virus and has as of yet not been successfully cultivated, which has limited the full characterization of it. The main aim of this study was to develop a method for whole genome sequencing of PMCV directly from field samples.

Methodology: A multiplex PCR with 18 overlapping fragments was designed with fragment sizes varying from 452 bp to 536 bp. All primer pairs were designed with the online primer design tool, Primal Scheme (Quick et al., 2017) with the 6688 bp sequence from Haugland et al. (2011) as template, which to date is the only published whole genome sequence of PMCV. By pooling primers of overlapping fragments in two separate pools, the whole genome of PMCV could be amplified in only two multiplex PCR reactions and was subsequently sequenced on an Illumina MiSeq system (v3).

Results: MiSeq sequencing yielded a 6682 bp sequence with 100% coverage. Depth of coverage varied between fragments from a minimum of 2.600 to a maximum of 31.800 reads. The Faroese PMCV strain differed slightly from the template and primers will be slightly modified to prevent any primer bias. The sequencing results further detected more than one variant of the virus present in the sample, which is not uncommon for RNA viruses and can be important in the evolution of virulence.

Conclusion: The multiplex PCR amplification directly from field samples followed by a MiSeq sequencing allowed for a whole genome characterization of PMCV. This method is also suitable for samples with a low amount of virus and can therefore sequence possible variations in pre-clinical samples. The identification of these genetic divergence is crucial in determining transmission pathways and epidemiologically significant events.

Keywords: PMCV, multiplex PCR

Funding: Faroese Research Council, Bakkafrost, Mowi and Hiddenfjord.

142-P

Lymphocystis disease in flounder from the south Baltic sea

Stachnik, Magdalena (Poland)[1]; Nadolna-Ałtyn, Katarzyna (Poland)[2]

1 - National Veterinary Research Institute, Puławy, Poland; 2 - National Marine Fisheries Research Institute, Gdynia, Poland

Introduction: The LCDV virus belongs to the iridoviruses found in many fish species. Genus of lymphocystis virus in marine fish has been genetically classified into three species: LCDV-1, which occurs in flounder and plaice, LCDV-2 usually found in common dab and LCDV-3 in gilthead sea bream. Lymphocystis can cause high mortality and economic losses in farmed, ornamental and wild fish, as well as occur in a chronic form manifested by visible changes on the skin of fish. The aim of this study was to determine the distribution of LCDV virus in the species *Platichthys flesus* - a flounder from the Baltic Sea and the genetic classification of obtained isolates.

Methodology: The samples were collected in 2016 from various fishing areas of the Baltic Sea: Gulf of Gdańsk, Słupsk Furrow, Middle Pomerania Coast and South Bornholm Coast. DNA was isolated from internal organs stored in ethanol during the cruise until analysis. The PCR products were sequenced and then analysed using the Genious software.

Results: Investigations of collected samples based on the PCR method revealed the presence of LCDV virus in a total of 21 samples of pooled organs on 143 tested fish. The highest percentage of infected fish was recorded in the fishing area of Bornholm Coast (60%). The actual percentage of fish infected with lymphocystis virus is difficult to estimate due to the fishing technique error of the cathing method. Analysis of the obtained sequences showed that all isolates from the flounder belong to the LCDV-1 genogroup. Their internal diversity reaches even 20%, what may be related to the of various genetic stocks of flounder in the Baltic Sea and the diversification of breeding period.

Conclusion: Our results confirmed the presence of the lymphocystis disease virus in *Platichthys flesus* from the Southern Baltic and the genetic variation of isolates depending on the fishing zone. The diversity of breeding period in fish stocks and the higher percentage of infected fish found in some areas of the Baltic Sea may be the reason for the reduction of the flounder stocks, which is confirmed by the annual reports of ICES.

Keywords: lymphocystis disease virus, LCDV, flounder

143-P

Infection dynamics of PMCV infection in Atlantic salmon

Evensen, Oystein (Norway)[1]; Mikalsen, Aase (Norway)[1]; Fredlund, Snöa (Norway)[1]; Xu, Cheng (Norway)[1]

1 - Norwegian University of Life Sciences

Introduction: Piscine myocarditis virus (PMCV) is the causative agent of CMS (cardiomyopathy syndrome) in Atlantic salmon. It is one of the most important viral infection of salmon in Norway, associated with acute mortalities in slaughter-size salmon, typically seen during handling or transport. 200 sites were diagnosed with the disease in 2018. The virus cannot be cultivated in cell culture and studies of pathogenesis/pathogenicity have been difficult. Previous studies show that the virus has a relatively homogeneous genome across outbreaks, but with variations in ORF1 (capsid) and ORF3 (unknown viral protein).

Methodology: We sequenced a number of isolates from CMS outbreaks and performed *in vitro* assessment of biological effects of the ORF3 protein expressed *in vitro* in cell culture.

Results: *In vitro* expression of the ORF3 protein post transfection in EPC cells shows distinct cytotoxicity to the cells. Contrary to previous findings, we see high variation in the ORF3 sequences and can link differences in cytotoxicity post transfection to variants. We find that a consensus sequence is always present, the original ALV708 sequence. Mutants of ORF3 ALV708 is found in samples from different sites and within sites, or even within fish, typified by expansion of clones and mutant variants. There is variation between sites as to the "size" of the sequence space explored, *i.e.* to what extent the virus explores the mutant spectrum.

Conclusion: Our hypothesis is that the infective stage of the virus is an ALV708-like strain that it is diversified in the course of the infection. Further, there is a possibility that infection constitutes a bottle-neck event and over the course of infection diversification (mutations) occur and results in development of intra-host quasi-species. Another hypothesis can be that a successful infection occurs only when ALV708 occurs as quasi-species and it is the viral cloud that enables infection in the target organ (heart). Sequential analysis of virus variants in various organs in the course of an infection may be an approach to clarifying these questions/hypothesis.

Keywords: PMCV, cardiomyopathy syndrome, Atlantic salmon, infection mechanisms

Funding: FHF - Norwegian Seafood industry research fund, project no. 901179.

Host-Parasite Interactions

145-P*

Myxosporidian infection of grey mullet, *Mugil cephalus*, in Russian waters of the Black Sea

Kazarnikova, Anna (Russian Federation)[1]; Strigakova, Tatyana (Russian Federation)[2]; Boyko, Natalya (Russian Federation)[2]; Bortnikov, Yevgeniy (Russian Federation)[2]; Ryszhiskaya, Larisa (Russian Federation)[2]

1 - Souther Scieific Center of Russian Academy of Scieces, 41, Chekov st., Rostov-on-Don, Russia, 344006; 2 - The Azov Sea Research Institute of Fishery, 21-v, Beregovaya st., Rostov-on-Don, Russia, 344002

Introduction: The first case of *Myxobolus episquamalis* infection in flathead grey mullet, *Mugil cephalus*, scales in Russian waters of the Black Sea is presented.

Methodology: Wild, adult flathead grey mullet were collected by trawl fishing in May, June and October, 2015. 170 fish were captured, 24 fish were taken for parasitological and physiological analysis.

Results: Sick fish were characterized by extensive scale lesions. Most often, flat, milky-white cyst-like plasmodia were found on ventral, dorsal and lateral surfaces of the body, on the caudal fin, and less often on the head and caudal fin of fish. Inflammation of underlying tissues has often been noted. The lesion area ranged from 30 to 90% of the body surface. The parasite was not found on the gills, in internal organs and in the muscles of fish. The most acute phase of the disease was noted in the prespawning period. The changes in organ indexes (including the gonad index), a decrease in the protein content, fat content and the amount of dry matter in the tissues, including reproductive organs, was noted. Part of the infected fish (30%) was unable to spawn due to impaired metabolic processes in the body and lack of resources to complete the formation of sexual products.

Conclusion: Activation of the humoral immunity was detected in *M. cephalus* females during the spawning period. The content of protein and fat in the gonads was within the normal range. That indicated the preservation of reproductive function in some infected fish. In the autumn, the morphofunctional and immunological indexes of all fish examined indicated the stabilization of metabolic processes in the body of flathead grey mullet.

Keywords: *Myxobolus episquamalis*, infection, grey mullet, *Mugil cephalus*

Funding: Ministry of science RF No. 01201354245.

146-P

Skin and mucosal responses of rainbow trout and Atlantic salmon to sea lice (*Lepeophtheirus salmonis*) infection

Heavyside, Rebecca (United Kingdom)[1]; Mullins, Julia (Norway)[1]; Mcgurk, Charles (Norway)[1]; Secombes, Chris (United Kingdom)[2]; Wang, Tiehui (United Kingdom)[2]

1 - Skretting ARC; 2 - School of Biological Sciences, University of Aberdeen

Introduction: Sea lice continue to be one of the greatest challenges to salmonid farming. Although not on the same commercial scale as Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) marine farms are also affected by sea lice, yet receive much less attention in research. The aim of this study was to characterise and compare the responses of rainbow trout and Atlantic salmon to sea lice infection.

Methodology: Rainbow trout and Atlantic salmon were infected with copepodid-stage *L. salmonis* in full-strength seawater at 12 °C. At 1 day pre-infection and 10 days post-infection, skin and mucus samples were taken. Skin samples, taken from sea louse attachment sites and non-attachment sites, were analysed for the expression of genes related to inflammatory, wound healing, and antimicrobial parameters. Mucus samples were analysed for viscosity, total protein, polysaccharide, and lysozyme content.

Results: The viscosity of rainbow trout mucus (0.21 centipoise) was less than that of Atlantic salmon (0.42 cP, $p < 0.01$) and was not altered by sea lice infection. Total protein content (0.07 mg/mL v 0.22 mg/mL, $p < 0.0001$) and lysozyme activity (36.4 U/mL v 155.6 U/mL, $p<0.0001$), however, increased more than 3-fold post-infection. Gene expression analyses showed a broad suppression of T-cell responses in rainbow trout post-sea lice infection compared to a broad up-regulation in Atlantic salmon. Markers of wound healing and antimicrobial peptides were indicative of local sea lice infection in both species. Differences in the bodily distribution (skin : fins) of sea lice between the species was also noted.

Conclusion: This work presents the first known attempts to quantify the viscosity and biochemistry of skin mucus as well as the modulation of gene expression in rainbow trout. It was found that rainbow trout elicited a stronger mucosal response, but a lesser T-cell response than susceptible Atlantic salmon. These factors may therefore be relevant in host resistance mechanisms.

Keywords: sea lice, Atlantic salmon, rainbow trout, immune response, mucus

Funding: Skretting ARC and the Norwegian Research Council.

148-P

A secreted nuclease of *Saprolegnia parasitica* translocates into fish cells via a GP96-like receptor

Trusch, Franziska (United Kingdom)[1]; Loebach, Lars (United Kingdom)[1]; Wawra, Stephan (United Kingdom)[1]; Durward, Elaine (United Kingdom)[1]; Iberahim, Nurul Aqilah (United Kingdom)[1]; De Bruijn, Irene (United Kingdom)[1]; Mckenzie, Kevin (United Kingdom)[1]; Willems, Ariane (United Kingdom)[1]; Toloczko, Alexandra (United Kingdom)[1]; Dieguez-Uribeondo, Javier (Spain)[2]; Rasmussen, Tim (United Kingdom)[1]; Schrader, Thomas (Germany)[3]; Bayer, Peter (Germany)[3]; Secombes, Chris (United Kingdom)[1]; Van West, Pieter (United Kingdom)[4]

1 - International Centre for Aquaculture Research and Development, University of Aberdeen, Aberdeen, Scotland; 2 - Real Jardín Botánico CSIC, Madrid, Spain; 3 - University of Essen, Duisburg, Germany

Introduction: Oomycetes, or water moulds are eukaryotic microbes that are among the most devastating pathogens of animals and plants with a huge economic and environmental impact in cultured as well as natural ecosystems. The animal-pathogenic oomycete *Saprolegnia parasitica* causes serious losses in aquaculture by infecting and killing freshwater fish. Like plant-pathogenic oomycetes, *S. parasitica*employs similar infection structures and secretes effector proteins that translocate into host cells to manipulate the host.

Methodology: Numerous.

Results: Here, we show that the host-targeting protein SpHtp3 enters fish cells in a pathogen-independent manner. This uptake process is guided by a gp96-like receptor and can be inhibited by supramolecular tweezers. The C-terminus of SpHtp3 is responsible for the uptake into host cells. Following translocation, SpHtp3 is released from vesicles into the cytoplasm by another host-targeting protein where it degrades nucleic acids of the host.

Conclusion: Our research has given novel insights into how a fish- pathogenic oomycete establishes an infection. A detailed understanding of the infection processes at the molecular level is very important for the development of new control strategies against oomycetes that will address global challenges in sustainable food security.

Keywords: oomycete, effector, receptor, *Saprolegnia*

Funding: BBSRC, NERC, Newton Fund and EU (ITN SAPRO).

149-P*

Characterising association of *Neoparamoeba perurans* to RTGILL-W1 cells in an *in vitro* model for amoebic gill disease (AGD)

Hooper, Patrick (United Kingdom)[1,2]; Bateman, Kelly (United Kingdom)[2]; Santos, Eduarda (United Kingdom)[1]; Paley, Richard (United Kingdom)[2]; Cano, Irene (United Kingdom)[2]

1 - Biosciences, College of Life & Environmental Sciences, Geoffrey Pope Building, University of Exeter, Exeter, EX4 4QD, United Kingdom; 2 - International Centre of Excellence for Aquatic Animal Health, Cefas Weymouth Laboratory, Barrack Road, Weymouth, Dorset, DT4 8UB, United Kingdom

Introduction: Amoebic gill disease (AGD) is a severe disease caused by the amphizoic agent, *Neoparamoeba perurans.* AGD outbreaks in salmon farms can cause significant mortalities and subsequent economic losses. Current treatment of AGD relies on continued freshwater or hydrogen peroxide bathing. Both of which practices are technically difficult and add significantly to production costs, with infrastructure for freshwater treatment estimated to account for 20% of expenditure in Tasmanian salmon farms. There is therefore significant interest in the development of alternative chemotherapeutic treatments for AGD. However, there are currently two main limitations to the evaluation of potential chemotherapeutants. Firstly, a lack of understanding about the infection process of the causative agent, *N. perurans,* and secondly, the cost and time expenditure required for *in vivo* treatment challenge trials. Recently, a static *in vitro* system has been shown to successfully reproduce *in vivo* host immune responses to *Neoparamoeba perurans* infection[1]. However, it is currently unknown if this model sufficiently replicates pathogen attachment.

Methodology: The morphology and ultrastructure of *N. perurans* within the *in vitro* model will be characterised using scanning electron microscopy (SEM).

Conclusion: Previously, amoebae have been shown to embed within host gill epithelium *in vivo* and possible impact of pathogen extracellular products on the host tissue. Characterisation of the attachment process of *N. perurans* in the *in vitro* system by SEM will significantly contribute to overcoming these previously-described limitations. Both in identifying physical host-pathogen interactions in the *in vitro* system and furthermore confirm if these faithfully recreate *in vivo* interactions. Understanding these interactions is therefore essential for utilising this *in vitro* model in future evaluation of alternative chemotherapeutic treatments for AGD.

Keywords: amoebic gill disease, AGD, SEM, *Neoparamoeba perurans*

150-P*

Development a dynamic *in vitro* model of host-pathogen interactions for amoebic gill disease (AGD)

Hooper, Patrick (United Kingdom)[1,2]; Cano, Irene (United Kingdom)[1]; Santos, Eduarda (United Kingdom)[2]; Paley, Richard (United Kingdom)[1]

1 - International Centre of Excellence for Aquatic Animal Health, Cefas Weymouth Laboratory, Barrack Road, Weymouth, Dorset, DT4 8UB, United Kingdom; 2 - Biosciences, College of Life & Environmental Sciences, Geoffrey Pope Building, University of Exeter, Exeter, EX4 4QD, United Kingdom

Introduction: Amoebic gill disease (AGD) is a severe disease reported in every salmonid farming region globally. Recent outbreaks have had significant economic impacts for European salmon farms, with US$81 million mortality-associated losses in Scotland in 2011. Despite these significant impacts, relatively little is known about the causative agent, *Neoparamoeba perurans*. *N. perurans* is one of twelve free-living marine amoebae reported to colonise the gills of AGD fish. However, only colonisation by *N. perurans* induces hyperplasia and lamellar fusion of host epithelia, the symptoms of AGD. Therefore, characterising early-stage attachment interactions is key to understanding AGD. Current research into AGD is largely reliant on *in vivo* cohabitation challenges. In keeping with the 3Rs of ethical animal research, there is increasing interest in developing *in vitro* platforms to model host-pathogen interactions. This study develops on a recently-published static *in vitro* model for AGD[1], by adding a flow element to better model *in vivo* attachment in the host gill environment.

Methodology: A rainbow trout gill-derived cell line (RTgill-W1) was seeded onto a permeable Transwell™ support. A seawater suspension of *N. perurans* trophozoites, loaded with phagocytosed fluorescent beads, was then flowed across the apical surface, using a commercially-available cell culture flow system.

Results: The phagocytosis assay allowed clear visualisation of the amoebae when inoculated on the RTgill-W1 monolayer under fluorescence. Amoebae were observed in the dynamic Transwell™ inserts containing RTgill-W1 cells by fluorescence microscopy after 1 hour.

Conclusion: Future studies will compare attachment in virulence and passage-attenuated *N. perurans* isolates to ascertain the significance of colonization in virulence. The dynamic *in vitro* model therefore has significant potential in reducing reliance on *in vivo* studies.

Keywords: *Neoparamoeba perurans*, amoebic gill disease, RTgill-W1, *in vitro* models, QuasiVivo® system

151-P

A multi-modal approach for investigating host-parasite interactions associated with the infection of European sea bass by *Amyloodinium ocellatum*

Massimo, Michela (Italy)[1]; Volpatti, Donatella (Italy)[1]; Calligaro, Carla (Italy)[1]; Byadgi, Omkar (Italy)[1]; Galeotti, Marco (Italy)[1]; Ireland, Jacquie (United Kingdom)[2]; Bron, James (United Kingdom)[2]; Beraldo, Paola (Italy)[1]

1 - Dipartimento di Scienze AgroAlimentari, Ambientali e Animali, University of Udine; 2 - Institute of Aquaculture, University of Stirling

Introduction: To date the absence of effective licensed therapeutants against the ectoparasite *Amyloodinium ocellatum* (AO) makes the control of its infection very hard. To formulate better targeted prophylaxis/therapies against AO, a multi-modal study was performed to deepen the knowledge on AO biology by exploring the host-parasite relationship.

Methodology: European sea bass (ESB) gill samples collected during AO natural and experimental infections and from uninfected ESB were immediately fixed in Bouin's solution, formalin or 4% paraformaldehyde according to the specific protocol for different laboratory analyses. With conventional histology the biological samples were stained with haematoxylin and eosin (H-E), PAS-Alcian blue, Masson and Azan trichrome, Cleveland trichrome, Giemsa and Twort Gram. The immunohistochemical (IHC) labelling was performed using selected mono-or polyclonal antibodies (CD16, CYP1A, cytokeratine, ESB IgM, GM-CSFRα, iNOS, PCNA, TLR2, TLR4 and TNF-α). The riboprobe specific for the transcript Chemokine CC1 was for the first time applied in the fluorescent mRNA *in situ* hybridization (FISH) protocol developed at the Institute of Aquaculture in Stirling, where confocal laser scanning microscopy (CLSM) investigations were executed too. By CLSM a panel of fluorescent stainings was examined (H-E, DAPI, TRITC phalloidin, fluorescent lectins and Calcofluor white+Propidium iodide double staining).

Results: Histology was applied for the diagnostic confirmation of the infection and for a better characterization of the tissutal alterations. Some of the morphological and structural features of the parasite were better detailed in the gills of infected ESB by CLSM. Furthermore, the labelling and localisation of both host cell populations, recruited to combat the parasite, and molecules synthesised as part of the host immune response to AO were characterised by IHC and FISH.

Conclusion: The approaches applied in this study allowed to better comprehend how AO interacts with its host and how the host responds to AO. This understanding improvement is indispensable to formulate better targeted prophylaxis/therapies against AO.

Keywords: histology, immunohistochemistry, *in situ* hybridization, confocal laser scanning microscopy

Funding: EU Horizon 2020 ParaFishControl, EU Horizon 2020 AquaExcel.

152-P*

Influence of host vertical distribution on parasitism: patterns of trematode infection in the cockle *Cerastoderma edule*

Correia, Simão (Portugal)[1]; Magalhães, Luísa (Portugal)[1]; Picado, Ana (Portugal)[2]; M. Dias, João (Portugal)[2]; De Montaudouin, Xavier (France)[3]; Freitas, Rosa (Portugal)[1]

1 - Departamento de Biologia & CESAM, Universidade de Aveiro, 3810-193 Aveiro, Portugal; 2 - Departamento de Física & CESAM, Universidade de Aveiro, 3810-193 Aveiro, Portugal; 3 - Université de Bordeaux, EPOC, UMR 5805 CNRS, 2, Rue du Pr Jolyet, F-33120 Arcachon, France

Introduction: The cockle, *Cerastoderma edule*, is a dominant and widely distributed bivalve. It is usually associated to intertidal areas but can also occur subtidally. Cockles have an important socio-economic and ecological role. They are the first and/or second intermediate host of several trematode species, the most prevalent macroparasite clade in coastal waters. Trematodes are able to modulate cockles population dynamics however, the patterns by which they govern are poorly understood. The objective of this study was to assess the effect of host vertical distribution (intertidal vs. subtidal) on trematode mean infected rate comparing upstream and downstream areas of the Ria de Aveiro, a Portuguese coastal lagoon. The questions were 1) does cockles tidal position influence the level of individual infection? and 2) small scale habitat variations overlap the effect of tidal position?

Methodology: Cockles were collected in 4 intertidal and subtidal sites where their density, trematode infection, macrofauna community diversity and sediment and water physico-chemical features were assessed. To characterize lagoon hydrodynamics, simulations with the numerical model Mohid were performed.

Results: Intertidal cockles displayed significantly higher individual infection than those from subtidal sites, highlighting that host vertical distribution represents an important driver of trematode infection success, possibly due to shorter distance from first intermediate host or to lower capacity of cercariae to find their suitable host in a subtidal environment and then to complete the life cycle. Overall, the individual infection was significantly different comparing cockles from the two sampled areas, suggesting an important influence of other environmental drivers. Multiple linear regression analysis demonstrated cockle density and dissolved oxygen were positively correlated to trematodes mean prevalence and abundance. In contrast, sediment contamination and opportunistic macrofauna diversity were negatively correlated to cockles infection rate. Additionally, the good ecological status of the studied sites showed a positive effect on cockles individual infection and trematodes diversity.

Conclusion: The present study demonstrated the importance of host tidal position on trematode infection success, highlighting the complexity of trematode/cockle system dynamics, driven by the interaction of several biotic and abiotic factors, and showed the potential of trematodes as indicators of ecosystem ecological status.

Keywords: Ria de Aveiro, coastal lagoon, intertidal, subtidal, hydrodynamics

Funding: INTERREG-ATLANTIC COCKLES.

153-P*

Microbial communities of the external integuments and the gastrointestinal tract of fish at ecto- and endoparasites

Kashinskaya, Elena (Russian Federation)[1]; Simonov, Evgeniy (Russian Federation)[1]; Andree, Karl (Spain)[2]; Solovyev, Mikhail (Russian Federation)[1]

1 - Institute of Systematics and Ecology of Animals of Siberian Branch of Russian Academy of Sciences, Novosibirsk, Russia; 2 - IRTA-SCR, San Carlos de la Rapita, Tarragona, Spain

Introduction: This study presents analysis of the structure of bacterial communities associated with the external epithelium and the gastrointestinal tract of fish harboring ecto- and endo-parasites, while in parallel studying the microbiota of the parasites, water and sediments as potential sources of pathogenic microorganisms.

Methodology: Microbial communities associated with the external epithelium of gibel carp *Carassius gibelio* and the gastrointestinal tract of perch *Perca fluviatilis* harboring ecto- and endo-parasites, respectively were studied using next-generation high-throughput sequencing of the 16S ribosomal RNA genes.

Results: In the microbial community associated with the parasitic crustaceans *Argulus* sp. and *Lernaea* sp., along with representatives of the normal microbiota, there were identified microorganisms that could be potential agents of infectious diseases in fish (*Flavobacterium* sp., *Aeromonadaceae* sp., *Corynebacterium* sp. and *Streptococcus* sp.). Each parasite is characterized by a specific structure of its associated microbiota, which, apparently, may indicate their role as vectors of different infectious disease. The study of the taxonomic diversity of bacteria from endo-parasites in the perch *Perca fluviatilis* also revealed some features in the structure of the bacterial communities. Bacteria from the genera *Mycoplasma*, *Serratia* and *Pseudomonas* were the dominant taxa in the microbiota of intestinal mucosa and intestinal contents of perch. The microbiota of stomach mucosa and content and pyloric caeca were varied among individuals, with common bacterial taxa represented by the genera *Serratia*, *Pseudomonas*, *Enterobacteriaceae* and *Mycoplasma*. In cestodes of the genus *Proteocephalus*, a rich and diverse microbiota was found. Along with the typical representatives of the normal microbiota of fish, bacteria of the genus *Mycoplasma* were detected for the first time on the surface of the parasite's tegument, the high abundance of which is also recorded in the intestinal mucosa and content of fish intestines.

Conclusion: The results of the conducted studies may be useful in the diagnosis of mixed infections in fish. Noteworthy among results are the first reports of data on the composition of the microbiota associated to the parasitic crustaceans and cestodes from genus *Proteocephalus*.

Keywords: fish, microbiota, parasitic crustaceans, cestodes

Funding: Russian Science Foundation, project no.17-74-10054.

154-P*

Immune response of sea bass peripheral blood leukocytes to *Anisakis pegreffii* crude extract: what does RNA-seq reveal?

Buselic, Ivana (Croatia)[1]; Trumbic, Zeljka (Croatia)[2]; Hrabar, Jerko (Croatia)[1]; Vrbatovic, Anamarija (Croatia)[1]; Petric, Mirela (Croatia)[2]; Mladineo, Ivona (Croatia)[1]

1 - Institute of Oceanography and Fisheries, Split; 2 - University Department of Marine Studies, University of Split

Introduction: Nematodes of the genus *Anisakis* Dujardin, 1845 have an indirect life cycle and their life-stages are propagated through trophic webs of their marine hosts. About 200 marine fish species and 25 cephalopod species are confirmed as *Anisakis* spp. paratenic or secondary intermediate hosts. Previous research suggested that excretory/secretory *Anisakis simplex* products could have immunomodulatory role in fish hosts. The aim of this study was to assess the immunogenic potential of *Anisakis pegreffii* crude extract (CE) in European sea bass (*Dicentrarchus labrax*) peripheral blood leukocytes (PBLs).

Methodology: The experiment was performed on *in vitro* stimulated PBLs of sea bass, prepared using 51% iso-osmotic Percoll solution, seeded in 6-well plates with L-15 medium, supplemented with 15% FCS and 1% penicillin/streptomycin. Two hours (h) after settlement of PBLs (final concentration of 10^7 cells/ml), 5 µg/ml of previously prepared *A. pegreffii* CE was added to test wells. In total, 18 replicates were collected 1 and 12 h post-stimulation and preserved for RNA isolation, including control wells. Illumina NextSeq 500 was used for paired-end sequencing of total of 7 pooled PBLs samples prepared from test and control treatments.

Results: After quality control, filtering and mapping, differential expression (DE) analysis of sea bass PBLs revealed 195 (60 down and 135 up-regulated) DE transcripts 1 h post-stimulation and 968 (206 down and 762 up-regulated) DE transcripts 12 h post-stimulation with *A. pegreffii* CE. Strong and significant fold changes (LogFC >> 1), with steady up-regulation as the experiment progressed, was especially noted for transcripts of pro-inflammatory cytokines (interleukin-1 beta and tumor necrosis factor alpha), as well as interleukin-27 subunit beta, known for its diverse roles in innate immunity.

Conclusion: Overall, the most frequent gene ontology terms associated with DE transcripts were positive regulation of cell proliferation, response to stimulus, immune response, and chemokine activity. These results help explain the nature of interaction between sea bass PBLs and *A. pegreffii* CE.

Keywords: European sea bass, *in vitro*, PBLs, total protein, nematode

Funding: Croatian Science Foundation (project no. 5576).

155-P

Attempts to culture Carp Edema Virus on *in vitro* gill primary cell epithelia in asymmetrical conditions

Ivory, Dylan (United Kingdom)[2]; Cano, Irene (United Kingdom)[1]; Joiner, Claire (United Kingdom)[1]; Paley, Richard (United Kingdom)

1 - Centre for Environment, Fisheries and Aquaculture Science (Cefas), Weymouth, UK; 2 - University of Exeter, UK

Introduction: Carp Edema Virus (CEV) is a poxvirus which infects carp (*Cyprinus carpio*). First described in Japan, it has since been identified across Europe, Asia, North and South America. Outbreaks can lead to mortalities of 75-100% in juvenile koi, while lower mortalities are typically observed in common carp. In the UK CEV has been linked with Spring Carp Mortality Syndrome[1]. Despite attempts in over 30 different cell lines CEV is non-culturable - the lack of virus culture inhibits full characterisation and further research on this disease.

Methodology: We developed an *in vitro* model of the carp gill epithelium on Transwell® permeable membrane inserts using a double seeding technique[2] with purified carp gill primary cell fractions to attempt to culture CEV. Gills were perfused *in situ* with PBS, excised, rinsed then cells removed by trypsin digestion. Harvested cells were seeded onto insets and incubated overnight. A second seed was performed using Percoll® density gradient separated cells enriched for chloride and goblet cells. The apical media was replaced with freshwater after 14 days culture. Change in transepithelial electrical resistance (TEER) was measured using an EVOM2 epithelial voltohmeter (World Precision Instruments). Epithelia were inoculated with clarified supernatants of infected gill tissue and incubated at 20°C. Viral replication was monitored by microscopic observation for cytopathic effect and qPCR analysis.

Results: A method was established to produce confluent, heterogeneous carp gill cell monolayers which survive for 2-4 weeks and withstand exposure to asymmetrical conditions. Transepithelial resistance markedly increased on exposure to apical freshwater indicating tight junction formation and a functioning epithelium. Inoculation with gill homogenates from CEV positive carp resulted in disruption of the monolayers with some but limited replication of CEV in pavement cells lodged on the “epithelium”.

Conclusion: The permeable membrane allows for the simulation of a more physiological environment through asymmetrical culture conditions – In this way, the cell monolayer behaves as a model gill. With further refinement the *in vitro* gill system may represent a potential route to culture CEV.

Keywords: Carp Edema Virus, CEV, *in vito*, gill

Funding: Defra grants FB002, F1172 and FC1214.

156-P

Comparative transcriptomics analysis reveals immune evasion associated with higher virulence of viral haemorrhagic septicaemia virus *in vitro.*

Cano, Irene (United Kingdom)[1]; Paley, Richard (United Kingdom)[1]; Santos, Eduarda (United Kingdom)[3]; Van Aerle, Ronny (United Kingdom)[2]

1 - International Centre of Excellence for Aquatic Animal Health, Cefas Weymouth Laboratory, Weymouth, UK; 2 - 1. International Centre of Excellence for Aquatic Animal Health, Cefas Weymouth Laboratory, Weymouth, UK, 2. Centre for Sustainable Aquaculture Futures, University of Exeter, Exeter, UK; 3 - Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, UK.

Introduction: Freshwater viral haemorrhagic septicaemia virus (VHSV) likely emerged from an ancestral marine virus. Expansion of those new freshwater genotypes within rainbow trout aquaculture originated lethal infectious disease. Despite the extensive economic losses associated to freshwater VHSV isolates, the pathogenic mechanisms of virulent VHSV remain largely unknown.

Methodology: The transcriptomes of RTG-2 cells inoculated with two pathogenic (J167 and DK-5131) and two non-pathogenic (96 - 43/8 and 1 p49) isolates were analysed at 3, 6, and 12 hours and compared to control samples using RNA-seq.

Results: Although VHSV isolates showed the same pattern of viral replication, the transcriptomic profiles in RTG-2 cells were dramatically different between pathogenic and non-pathogenic isolates, revealing a lack of sensing of the viral replication in cells inoculated with both pathogenic VHSVs at early stages of infection. Functional annotation analysis of differentially-expressed genes between non-pathogenic VHSV and controls revealed an enrichment of pathways involved in the defense to biotic stimulus and metabolic processes (strong up-regulation of genes), and lipid metabolism and cell cycle (down-regulation of genes). In contrast, cholesterol and cytoskeleton mobility pathways were enriched (up-regulation of genes) by both pathogenic VHSV. Furthermore, an increasingly higher number of GRP78/BiP transcripts in cells inoculated with the pathogenic VHSV suggests a role of the unfolded protein response in the VHSV immune evasion.

Conclusion: Our transcriptomic analyses strongly suggest that freshwater pathogenic VHSV uses immune evasion mechanisms to enhance viral fitness.

Keywords: transcriptomics, immune evasion, host-pathogen interactions

Funding: UK Department for Environment, Food & Rural Affairs (Defra) Projects C6561 and C7277B.

157-P

Characteristics of tapeworm-produced proteinase inhibitors and their possible role in fish-cestode interactions

Solovyev, Mikhail (Russian Federation)[1]; Frolova, Tatyana (Russian Federation)[2]; Rogozhin, Eugene (Russian Federation)[3]; Izvekov, Evgeny (Russian Federation)[2]; Izvekova, Galina (Russian Federation)[2]

1 - Institute of Systematics and Ecology of Animals SB RAS; 2 - Papanin Institute for Biology of Inland Waters, RAS; 3 - Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, RAS

Introduction: Cestodes are known to inhibit the activity of host digestive enzymes, but little is known about this ability in fish tapeworms. We aimed to estimate the capacity of these tapeworms to inhibit the proteolytic activity of host intestine and commercial trypsin and identify the tapeworm-produced inhibitors. Three cestode species *Eubothrium rugosum*, *Caryophyllaeus laticeps* and *Triaenophorus nodulosus* dwelling in the intestines of burbot *Lota lota*, bream *Abramis brama* and pike *Esox lucius*, respectively, were studied.

Methodology: The ability of these worms (whole tapeworm, tegument and incubation medium) to inhibit the fish intestinal proteases (taken from host and non-host species) as well as commercial trypsin was estimated using spectrophotometric and SDS-PAGE approaches. To isolate and identify the components of the worm extract responsible for trypsin inactivation, a two-step separation including SPE and RP-HPLC techniques, and N-terminal amino acid sequencing were applied.

Results: The treatment of *E. rugosum* worms by Triton x-100 followed by centrifugation has shown their ability to inhibit proteolytic enzymes by the brush border of cestodal tegument exclusively, while the tapeworm lacking the tegument membrane loses such ability. In addition, the species-specificity of the protease inhibitor produced by *E.rugosum* was revealed by comparing its suppressive effects upon the intestinal proteinases of the host (burbot) and six non-host freshwater fishes. In *C. laticeps*, SDS-PAGE analysis revealed three common protein bands on the phoregrams of the incubation medium and the extract of cestodes, with estimated molecular weights from 19 to 47 kDa. According to casein-zymography of the bream's intestinal mucosa, the target host proteinases for a putative cestode inhibitor have an approximate molecular weight of 28–53 kDa. The components extracted from *T. nodulosus* which are responsible for trypsin inactivation were also tested. They contained four fractions represented by both polypeptides (1–45 kDa) and low-molecular hydrophobic compounds (below 1 kDa). The research revealed two new Kunitz-type proteins (Tn-KTTI-1 and Tn-KTTI-2) potentially responsible for the inhibitory capacity of these tapeworms against trypsin.

Conclusion: Studied tapeworms are able to produce the inhibitors of host alkaline proteases localized in the tapeworm's tegument and characterized by a certain degree of host-parasite species-specificity.

Keywords: *Eubothrium rugosum*, *Caryophyllaeus laticeps*, *Triaenophorus nodulosus*, trypsin, Kunitz-type proteins

Diseases of Wild and Ornamental Fish

158-P

First report of horizontal transmission and infection of *Piscirickettsia salmonis* between *Eleginops maclovinus* and *Oncorhynchus mykiss* in experimental conditions

Quintanilla, Juan Carlos (Chile)[1]; Gonzalez, Margarita (Chile)[1]; García, Juan Pablo (Chile)[1]; Olmos, Paola (Chile)[1]; Rivas, Mario (Chile)[1]

1 - Instituto de Fomento Pesquero

Introduction: *Piscirickettsia salmonis* is the aetiological agent of piscirickettsiosis, a bacterial disease that affects farmed salmonids, causing high mortalities and significant economic losses. Considering the importance of the species *Eleginops maclovinus* (Patagonian blennie), which live close or associated with salmon farms, it is relevant to clarify the epidemiological role that this species could play in the transmission and dissemination of this bacteria. The aim of this study was to determine the bidirectional transmission of the pathogen *P. salmonis* between the native species of fish Patagonian blennie (*E. maclovinus*), and Rainbow trout (*Oncorhynchus mykiss*), through a model of cohabitation challenge.

Methodology: Fishes were randomly assigned in fiberglass tanks of 1 m^3 at a density of 20 kg m^{-3}. An isolate of *P. salmonis* was used to inoculate the trojan fishes. After inoculation, 3 experimental groups were formed: group 1 ($Pb_{trojan}/Rt_{cohabitant}$), group 2 ($Rt_{trojan}/Pb_{cohabitant}$), and control group ($Rt_{trojan}/Rt_{cohabitant}$). The mortality was removed daily during the course of the challenge, and morphometric analysis and necropsy were performed. Organ samples (kidney-spleen) were taken, and then analyzed by PCR-*P. salmonis*.

Results: The results of this study show the transmission of the bacteria from the Patagonian blennie species to rainbow trout. The mortality began at day 13 post inoculation (pi), increasing from day 45 pi, and reaching 46% of specific mortality at the end of study. Necropsy of the specimens revealed clinical signology of the disease, and positive PCR result for *P. salmonis* in the analysis of organ samples, with reisolation of the bacteria from tissues. No mortality of Patagonian blennie specimens were recorded in the challenged experimental groups.

Conclusion: The present study corresponds to the first report showing the horizontal transmission of *P. salmonis*, from a non-salmonid native species such as Patagonian blennie (*E. maclovinus*) to a salmonid species rainbow trout (*O. mykiss*), inducing infection and mortality in rainbow trout through a challenge of cohabitation under controlled conditions.

Keywords: *Piscirickettsia salmonis*, *Eleginops maclovinus*, transmission, cohabitation challenge, native fish

159-P*

Pathological effects of *Cichlidogyrus philander* Douëllou, 1993 (Monogenea, Ancyrocephalidae) on the gills of *Pseudocrenilabrus philander* (Weber, 1897) (Cichlidae)

Avenant-Oldewage, Annemarie (South Africa)[1]; Igeh, Patience Chwe (Nigeria)[2]

1 - Department of Zoology, University of Johannesburg, PO Box 524, Auckland Park Johannesburg, 2006. South Africa; 2 - Department of Microbiology, Plateau State University, PO Box 2012, Jos, Nigeria

Introduction: Cichlid fish is an important source of protein and this has led to an increase in tilapia production globally. Despite this, the role of parasites on fish health is frequently overlooked. It becomes a concern when they affect a fish species of interest, causing adverse effect on the economy, recreational activity or commercial fishery. The histopathological changes caused by *Cichlidogyrus philander* Douëllou, 1993 on the gills of *Pseudocrenilabrus philander* (Weber, 1897) were studied using light and scanning electron microscopy.

Methodology: Collected fish were euthanized, dissected, gills removed and parasites identified using standard procedures. Infected gill arches and sampled parasites were preserved in 70% ethanol for scanning electron microscopy, while others were preserved in 10% neutral buffered formalin for histopathological investigation. Using standard SEM and histopathological procedures prepared specimens were examined with a TESCAN Vega 3 LMH SEM and a Zeiss Axioplan 2 imaging light microscope images taken.

Results: The parasite attach to its host with the prohaptor (temporary attachment or feeding) or haptor (firm and secured attachment). The sharp terminal ends of the anchors are inserted basally into the gill filament, between two adjacent secondary gill lamellae and the marginal hooklets assist by superficially penetrating, holding and lifting epithelial tissue in the proximal region of the secondary gill lamella. These attachment resulted in compression, tearing of the interlamellar epithelium, change in the organization of epithelial cells in both primary and secondary gill lamella, displacement of the extracellular cartilaginous matrix, occasional rupturing of blood vessels and some cells becoming ill defined. At the site of attachment, the host response comprises of hyperplasia, occasional occurrence of neutrophils and mild secretion of mucus.

Conclusion: The histopathological effect of *C. philander* on the gills of *P. philander* was focal and related to attachment as well as feeding which is also mild in natural infections.

Keywords: Monogenea, prohaptor, haptor, anchors, hooklets

Funding: University of Johannesburg and TETFUND.

160-P*

Granulomatous aerocystitis by *Phoma herbarum* in a wild greater amberjack (*Seriola dumerili* Risso)

De Sales-Ribeiro, Carolina (Spain)[1,3]; Sanchez-Henao, Andrés (Spain)[2]; García-Álvarez, Natalia (Spain)[2]; Real, Fernando (Spain)[2]; A. Rivero, Miguel (Spain)[3]; Fernández, Antonio (Spain)[3]; Caballero Cansino, Maria José (Spain)[1,3]

1 - Fish Pathology Unit, Institute for Animal Health and Food Safety (IUSA), Veterinary School, University of Las Palmas de Gran Canaria, Arucas, Canary Islands, Spain; 2 - Division of Infectious Diseases and Ichthiopathology, Institute for Animal Health and Food Safety (IUSA), Veterinary School, University of Las Palmas de Gran Canaria, Arucas, Canary Islands, Spain; 3 - Division of Histology and Animal Pathology, Institute for Animal Health and Food Safety (IUSA), Veterinary School, University of Las Palmas de Gran Canaria, Arucas, Canary Islands, Spain

Introduction: *Phoma herbarum* is a filamentous fungus with a ubiquitous distribution. In fish, *P. herbarum* has been reported to act as a facultative pathogen, causing a chronic and lethal visceral mycosis, being the swim bladder the most affected organ. Previous reports of spontaneous infections by *P. herbarum* in fish have been limited to hatchery-reared fish in freshwater conditions. Postulated portals of entry suggested that conidia could pass through the pneumatic duct from the digestive tract to the swim bladder. Yet, this theory is apparently only valid for physostomous species.

Methodology: Samples from the swim bladder, kidney, liver, stomach, intestine, heart, gills and gonads were fixed in 10% phosphate-buffered formalin solution, embedded in paraffin was, sectioned and stained with hematoxylin and eosin, Gram, Ziehl-Neelsen, Gomori's Methenamine Silver and Periodic Acid Schiff. Formalin fixed-paraffin embedded samples from the swim bladder were submitted to Instituto Valenciano de Microbiología for fungal identification by PCR.

Results: In the present work, we describe a severe granulomatous aerocystitis by *P. herbarum* in greater amberjack. Internal examination showed complete loss of morphology of the swim bladder, with fluid filled cystic lesions admixed with yellow to brownish nodules replacing the normal structure of the organ. Histopathology revealed multiple granulomas with filamentous branching septate hyphae and numerous cysts. *P. herbarum* was identified by PCR.

Conclusion: To our knowledge this is the first report of a spontaneous granulomatous aerocystitis by *P. herbarum* in greater amberjack, a wild marine physoclist with great potential for diversification in aquaculture.

Keywords: *Phoma herbarum*, aerocystitis, greater amberjack, *Seriola dumerili*, fish pathology

Funding: Fish Pathology Unit, Institute for Animal Health and Food Safety (IUSA), Veterinary School, University of Las Palmas de Gran Canaria.

161-P*

Diversity of microscopic cnidarians parasites of ornamental fish from Amazon basin

Mathews Delgado, Patrick (Brazil)[1]; Silva Gomes, Ana Lucia (Brazil)[2]; De Sousa, Fabrício B. (Brazil)[2]; Mertins, Omar (Brazil)[3]; Madrid Martinez, Rafael Ricardo (Brazil)[3]; Carrara Morandini, Andre (Brazil)[1]

1 - Department of Zoology, Institute of Biosciences, University of São Paulo, São Paulo, Brazil; 2 - Institute of Biological Sciences, Federal University of Amazonas, Manaus, Brazil; 3 - Department of Biophysics, Paulista Scholl of Medicine, Federal University of São Paulo, São Paulo, Brazil

Introduction: The Amazon basin is one of the most important sources of wild-caught freshwater fishes in the global aquarium trade. There are, however, few published surveys of myxosporeans in Amazonian ornamental fish. Thus the diversity and pathogenecity of myxosporeans parasites was evaluated in several species of freshwater ornamental fish from Amazon region of Brazil and Peru.

Methodology: Samples of fish were collected alive from natural environment and immediately placed in plastic bags containing one-fourth water and three-fourths oxygen and than transported to the field laboratory. A total of seven species of ornamental fish were analyzed: *Corydoras schwartzi*, *Apistogramma cacatuoides*, *Symphysodon discus*, *Hemigrammus* sp., *Osteoglossum bicirrhosum*, *Pristobrycon striolatus* and *Apistogramma agassizii.* Organ/tissue infected with myxosporid cyst were removed and examined using a stereo microscope and differential interference contrast microscopy. The species of myxosporean were characterized based on morphological, ultrastructural, histological and molecular features using light and transmission electron microscopy and with nucleotide sequence of the 18S small subunit ribosomal RNA (SSU rRNA) gene.

Results: A total of seven new species of myxosporean were found in the analyzed ornamental fish. The myxosporeans belonging to the genus *Myxidium* (one species) were parasitizing *A. cacatuoides*; *Ceratomyxa* (one species) in *S. discus*; *Myxobolus* (three species) in *P. striolatus*, *C. schwartzi* and *O. bicirrhosum*; *Ellipsomyxa* (one species) in *A. agassizii*; and *Henneguya* (one species) in *Hemigrammus* sp. In our study, no clinical signs were observed in the infected specimens.

Conclusion: In our study, a great diversity and high occurrence of myxosporeans was observed in many species of wild ornamental fish from Amazon basin.

Keywords: Myxozoa, ornamental fish, Amazon basin

Funding: São Paulo Research Foundation (FAPESP) and FAPEAM.

162-P

Microbiological and pathological findings in koi carp (*Cyprinus carpio*) affected by swim bladder flooding

Sirri, Rubina (Italy)[1]; Zamparo, Samuele (Italy)[1]; Barbè, Tim (Belgium)[2]; Ciulli, Sara (Italy)[1]; Volpe, Enrico (Italy)[1]; Tura, Giorgia (Italy)[1]; Errani, Francesca (Italy)[1]; Mandrioli, Luciana (Italy)[1]

1 - University of Bologna; 2 - Veterinary Practitioner

Introduction: The swim bladder (SB) disorders include fluid accumulation, collapse, over inflation and herniation. Common causes are infections, genetic-based anomalies and neoplasms. Clinical signs include abdominal swelling, abnormal swimming behaviour and loss of neutral buoyancy. The aim of this work was to investigate koi carp with clinical signs suggesting SB disorders.

Methodology: Eight koi carps including showa, utsuri and chagoi varieties, examined for abnormal swimming behaviour between 2016 and 2019, were studied. They were characterized by the presence of SB fluid detected and sampled with ultrasound-guided fine needle aspiration (FNA). Four of these cases were cytologically and bacteriologically investigated. The SB fluid was cultured on Columbia Blood Agar. Pure colonies (n = 4) were genetically characterized through 16S rRNA gene amplification and sequencing. Antimicrobial susceptibility pattern was tested with Kirby-Bauer method. The smears of SB fluid were Gram stained. The SBs of dead or euthanized fishes (n = 6) were *in toto* formalin-fixed for histology.

Results: Bacteria isolated from SB fluid were identified as *Shewanella xiamenensis* (99.7% nucleotide identity with ATCC116732). The antimicrobial susceptibility pattern revealed a diffuse sensitivity to penicillins, phenicols, aminoglycosides and enhanced sulphonamides; only one strain showed resistance to tetracycline and ciprofloxacin. Subgrossly, the thickness of the SB walls ranged from 0.7 to 7.7 millimeters. Histologically, five SBs showed a chronic aerocystitis; microrganisms were not detected by hystology. In three out of five cases, a macrophagic infiltrate associated with granulation tissue was present, in the other two cases the main finding was a severe fibrosis of the wall. In one case, a mycotic aerocystitis was recognized. In three cases, a mucous or squamous metaplasia of the epithelium was present.

Conclusion: Swim bladder flooding represents an underdiagnosed pathology in koi carp characterized by the presence of fluid within the SBs of the affected animals. Histological investigations showed a chronic inflammation and adaptive responses. Bacteriology pointed out pure cultures of *Shewanella xiamenensis*. *Shewanella* spp. are usually referred as opportunistic pathogens, but in this case it is possible to suppose its presence as the main actor in the manifestation of the described SB disorder.

Keywords: koi carp, swim bladder flooding, *Shewanella* spp., aerocystitis, antimicrobial, susceptibility pattern

163-P*

Endohelminth parasites of Albacore, *Thunnus alalunga*, from Madeira archipelago, eastern Atlantic

Hermida, Margarida (Portugal)[1,2]; Cavaleiro, Bárbara (Portugal)[1]; Saraiva, Aurélia (Portugal)[3,4]

1 - MARE - Marine and Environmental Sciences Centre; 2 - Oceanic Observatory of Madeira; 3 - University of Porto; 4 - Interdisciplinary Centre of Marine and Environmental Research

Introduction: Albacore, *Thunnus alalunga* (Bonnaterre, 1788), is a commercially important tuna species occurring in temperate and tropical waters worldwide. There are very few studies on its parasites. Jones (1991) used parasites as biological tags to study the migrations of albacore tuna in the Pacific and, recently, Mele et al. (2010) conducted a study on the gill parasites of albacore from the Mediterranean; however there are no studies on the albacore endohelminth parasite assemblages in the Atlantic Ocean.

Methodology: 30 specimens of *T. alalunga* captured in Madeira EEZ were obtained from commercial fisheries and subject to a parasitological analysis. The body cavity and internal organs were observed and all metazoan parasites were identified to the lowest possible taxon.

Results: A total of 14 helminth taxa were detected in the body cavity and internal organs of albacore tuna observed, including 7 trematode species, most of them didymozoids, 3 nematodes, 2 cestodes and 2 acanthocephalans. The acanthocephalan *Bolbosoma vasculosum* was the most prevalent species, occurring in 100% of hosts, while the didymozoid trematode *Koellikerioides internogastricus* was the most abundant species (MA=144.1 ± 182.8).

Discussion and Conclusion: Albacore tuna from the Eastern Atlantic presented high levels of infection by endoparasitic helminths. Prevalence of the nematodes *Anisakis* spp. and *Oncophora melanocephala* was higher in this study than in Pacific albacore of comparable size (Jones 1991), whereas *Rhadinorhynchus* sp. prevalence was lower. Although *Anisakis* sp. was found surrounding the viscera and not in the muscle, due to its high prevalence we recommend that albacore tuna should be thoroughly cooked or, alternatively, frozen prior to consumption, in order to inactivate any anisakid parasites which might be present.

Keywords: albacore; *Thunnus alalunga*; parasites; Atlantic; Madeira

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164-P

Detection of infectious spleen and kidney necrosis virus (ISKNV) in ornamental fish in Germany

Jung-Schroers, Verena (Germany)[1]; Adamek, Mikolaj (Germany)[1]; Bauer, Julia (Germany)[1]; Teitge, Felix (Germany)[1]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany

Introduction: Infectious Spleen and Kidney Necrosis virus (ISKNV) belongs to a subgroup of megalocytiviruses, a group in the family *Iridoviridae*. It causes systemic infections in a wide range of marine and freshwater fishes and can induce heavy losses. In Germany the first reported case of an ISKNV infection of ornamental fish, namely platy and angelfish, was described in 2014 by the authors of this study. The infected fish showed anorexia, lethargy, gill swelling and skin alterations and mortality was up to 100%. Histological examination of tissue of infected fish resulted in profound alterations in almost all internal organs. Especially necrosis in spleen, kidney and liver and a high number of hypertrophic, intensively pink stained cells which were distributed in liver, spleen and kidney, could be detected. Since March 2016 fish retailers have to examine fish for ISKNV, if the fish should be exported to Australia. Therefore ISKNV examination was included in the routine diagnostic at the Fish Disease Research Unit at the University of Veterinary Medicine in Hannover.

Methodology: Between 2016 and 2018 in total 456 examinations for ISKNV were evaluated by PCR. In total 32 different fish species from different families from freshwater as well as seawater were examined. All examined seawater species originated from tropic areas. The freshwater species originated from North, Middle, and South America, and from South Asia, South-East Asia and East Africa.

Results: In eight examined fish ISKNV genome fragments could be detected. One *Apistogramma nijesseni*, one *Symphysodon* sp., two *Betta splendens*, two *Colisa lalia*, one *Xiphophorus hellerii* and one *Xiphophorus maculatus* were tested positive for ISKNV. In none of the examined marine fish species and in none of the 412 tested cichlids from East Africa ISKNV could be detected.

Conclusion: Until today there are no reports on infections of East African cichlids with ISKNV and it remains unclear if these fish are susceptible to the virus. Because of the severe progressive form of this infection and the high mortalities that can occur in infected fish, it should be considered to keep ornamental fish separately in quarantine and to examine them before selling.

Keywords: ISKNV, megalocytivirus

165-P

Intraepithelial hyaline globules (thanatosomes) occurring in the gut of a *Rhyna ancylostoma*

Magi, Gian Enrico (Italy)[1]; Rossi, Giacomo (Italy)[1]; Galosi, Livio (Italy)[1]; Pussini, Nicola (Italy)[2]; Sommer, Matteo (Italy)[2]; Gili, Claudia (Italy)[2]

1 - School of Biosciences and Veterinary Medicine, University of Camerino, Italy; 2 - Costa Edutainment SpA c/o Acquario di Genova, Genoa, Italy

Introduction: In human medicine hyaline globules (thanatosomes) have been described in various cell types and tissues and associated with normal, nonneoplastic, and neoplastic disorders, representing a well-defined morphological and functional entities related to degeneration and apoptosis.

Methodology: Tissue samples from various organs of an adult *Rhyna ancylostoma* suffering of systemic bacterial infection died from sepsis were collected for histological analysis. 3-µm-thick sections were stained with haematoxylin-eosin (HE) and periodic acid–Schiff (PAS). In addition, sections of intestine were selected for immunohistochemical analysis by an avidin–biotin–peroxidase-complex (ABC) technique for cytokeratins AE1/AE3.

Results: Histopathological analysis revealed the presence of numerous hyaline globules within the cytoplasm of intestinal epithelium as discrete or prominent, spherical or ovoid, eosinophilic amorphous globular bodies, filling the cytoplasm. Thanatosomes stained positively for cytokeratins AE1/AE3 and were weakly PAS-positive.

Conclusion: There have been no published reports to date of hyaline globules in the gastro-intestinal epithelium of a fish species nor hyaline globules are reported as common background findings of gastro-intestinal epithelium in fish histopathology studies. Thanatosomes have been reported in the gastrointestinal epithelium of humans with various disorders. In veterinary medicine intracytoplasmic hyaline globules of neurosecretory origin have been reported sporadically in the adrenal medulla of laboratory animals dying of polychlorinated biphenyl or dioxin toxicosis, severe bacterial infections, or unknown causes and as ganglionic inclusions in captive coatis. In this case the hyaline globules were present only in the intestinal epithelium. Although thanatosomes are a nonspecific microscopic phenomenon, they represent a relatively constant and useful histologic marker of enhanced cell turnover with ischemic injury and apoptotic insult. The occurrence of hyaline globules in this case report could suggest that also in fish thanatosomes could be considered of some diagnostic and differential diagnostic importance in order to confirm these pathophysiologic conditions.

Keywords: hyaline globules, gut, *Rhyna ancylostoma*, intestinal disorders, thanatosomes

166-P

Description of a hamartoma-type odontoma in angelfish (*Pterophyllum scalare*)

Ortega, Cesar (Mexico)[1]; Fajardo, Raúl (Mexico)[2]; Valladares-Carranza, Benjamín (Mexico)[2]; Martínez-Castañeda, Simón (Mexico)[2]; Guzmán-Granados, Luis Ernesto (Mexico)[3]

1 - Centro de Investigación y Estudios Avanzados en Salud Animal, FMVZ, Universidad Autónoma del Estado de México, Toluca, México, CP 50200; 2 - Centro de Investigación y Estudios Avanzados en Salud Animal, FMVZ, Universidad Autónoma del Estado de México, Toluca, México, CP 50200; 3 - Comité estatal de sanidad acuícola del Estado de Morelos A.C.

Introduction: Twenty angelfish (*Pterophyllum scalare*) were remitted from an ornamental fish farm to be examined for single or multi-lobed masses with a diameter of 0.2 - 0.4 cm in the frontal region of the mouth, in both the maxillary and mandibular lips. The fishes did not show any other sign and no other lesions were observed.

Methodology: Mass sections were prepared for electron microscopy to demonstrate the presence of viral particles, and were also added to Leibovitz's L-15 medium (Gibco BRL) for virus isolation in monolayers of *epithelioma papulosum cyprini* (EPC) cells and bluegill fry (*Lepomis macrochirus*, BF-2) cells. The mass and other sections of internal organs were fixed in 10% buffered formalin and stained with hematoxylin and eosin for histologic examination. Samples from the presumed tumor area were collected for bacteriology and imprint smears, these last were stained with Gram´s method and acid-fast stain.

Results: The tumors were spherical or semi-spherical, between 0.2 to 0.4 cm in diameter, with edematous appearance and a whitish, pink or reddish coloration. Histologically, the mass was surrounded by a hyperplastic stratified squamous epithelium, with numerous well-differentiated dental structures (denticles) that show variable differentiation stages (organization), which make up a compound odontoma. There were no external projections of the dental forms. Cell culture and electron microscopy did not show virus forms. There was also no growth of bacteria, and the imprints were negative.

Conclusion: In angelfish, tumors of similar macroscopic appearance to these have been described as lip fibroma or as ameloblastoma; however, this is the first case of a hamartoma-type odontoma in angelfish. Lesions were present in approximately 15% of a 300 fish population. Similar macroscopic and microscopic lesions in species other than *P. scalare*, have only been reported in two individual cases in long-finned clownfish (*Amphiprion ocellaris*). The analysis made suggests that this odontoma has its origin at cellular level, on a hereditary basis.

Keywords: odontoma, tumor, odontogenic, angelfish

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167-P

Swim bladder mycosis in *Polyprion americanus*

Losada, Ana (Spain)[1]; Ronza, Paolo (Spain)[1]; De Azevedo, Ana Manuela (Portugal)[1,2]; Castrillo, Pedro Antonio (Spain)[1]; García, Cristina (Spain)[3]; Bravo, David (Spain)[3]; Robles, Alvaro (Spain)[3]; Bermúdez, Roberto (Spain)[1]; Quiroga, Mª Isabel (Spain)

1 - Universidade de Santiago de Compostela; 2 - Ghent University; 3 - Aquaculture Division, Isidro 1952 S.L.

Introduction: A 20 year-old male wreckfish, *Polyprion americanus*, coming from a farm in NW Spain was remitted to our Laboratory of Anatomic Pathology with a history of apathy, erratic swimming and abdominal distension.

Methodology: For assesing the cause of death, necropsy and sampling procedures for histopathology were performed.

Results: In the external inspection it was observed some hyperaemic eroded areas on the skin surface of the head, pectoral and ventral fins; and anaemic gills. Internally, the peritoneum was splashed with blackish miliary dotty lesions with uncountable fibrous adherences between the viscera. The swim bladder was swollen and showed a granular fibrous thickened wall filled with a firm green-blackish mass. The gut was congestive with yellow-greenish catarrhal content. Histopathology revealed an extensive granulomatous inflammatory response with brown to black fungal hyphae in the serous surface of abdominal viscera and in the lumen and wall of the swim bladder.

Conclusion: These pigmented fungi are known as deuteromycetes and include a few pathogenic species and many opportunistic pathogens that are ubiquitous in the aquatic environment. This kind of fungal infections in marine fish are rarely reported. The source of infection could not be established but might be related with a sub-clinical skin infection stimulated by the stressors of capture. To our knowledge, this is the first description of a swim bladder mycosis in *Polyprion americanus*.

Keywords: histopathology, deuteromycosis, wreckfish

169-P

Mysterious syndrome causing high mortality in wild brown trout in eastern Switzerland, similar to proliferative darkening syndrome

Schmidt-Posthaus, Heike (Switzerland)[1]; Birrer, Christoph (Switzerland)[2]; Mehr, Christoph (Switzerland)[2]; Wahli, Thomas (Switzerland)[1]; Leib, Vera (Switzerland)[3]; Kugler, Michael (Switzerland)[2]

1 - Centre for Fish and Wildlife Health, Vetsuisse Faculty, University of Bern; 2 - Canton St. Gallen, Department of Economic Affairs, Office for Nature, Hunting and Fisheries, St. Gallen; 3 - Canton St. Gallen, Department of Construction, Office for Water and Energy, St. Gallen

Introduction: In the Thur, a river situated in the Eastern part of Switzerland, massive mortalities regularly occurred in brown trout since 2016. Dead fish were recorded in a period of few weeks in July / August. The majority of the affected animals were one year old. In the course of the river, affected brown trout were seen with a sharp demarcation between affected and unaffected river stretches. Macroscopically, clinically sick fish showed dark coloration and apathy. In histology, main findings were extensive liver necrosis and hemorrhage and severe lymphohistiocytic myocarditis.

Methodology: From June to November 2018, one year old brown and rainbow trout were exposed in Thur water. From each species 5 fish were sampled every two weeks in the first month, afterwards every week until the end of the experiment. On sampled fish, parasitology, bacteriology, virology and histology were performed. Water temperature was measured regularly. Water samples were taken every ten minutes and pooled for daily samples for a period of six months. Non-target screening, target screening and suspect screening were performed. As the alterations were in agreement with those recently described for brown trout suffering from Proliferative Darkening Syndrome (PDS) in Germany, severely affected specimens were analysed for Piscine Reovirus (PRV) 1 and , the agents proposed to be involved in PDS.

Results: No consistent pathogenic agents were isolated. Organ alterations were exclusively detected in brown trout. First liver and heart lesions were found in mid-August, while the highest degree of alterations occurred from end of August to October. Full recovery did not occur as also in the last sampling end of November, lesions were still present. None of the examined samples revealed to be positive for PRV 1 and 3. Results from water analyses are still outstanding.

Conclusion: Up to now, the cause of the described syndrome remained unclear. Further examinations are ongoing.

Keywords: Brown trout, mortality, Switzerland, proliferative darkening syndrome, pathology

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170-P*

Invagination caused by adenocarcinoma in rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792)

Hoitsy, Márton (Hungary)[1]; Baska, Ferenc (Hungary)[3]; Hoitsy, György (Hungary)[2]

1 - Kaposvár University, Animal Science Doctoral School; 2 - Lillafüred Trout Breeding Farm; 3 - University of Veterinary Medicine Budapest

Introduction: Rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) is one of the most commonly bred fish species in Europe. The brood stock has a significant value at the farms, therefore the prevention of illnesses is crucial. During our research we investigated an idiopathic intestinal tumor in rainbow trout. All the examined individuals were 3-6 years old, as tumors need a longer period to develop and therefore cannot be found in fingerlings, summerlings and consumable fish. The healthcare of the valuable broodfish is of great importance in fish farms. Nowadays, the investigation of tumors growing in trouts is considered a pioneering field of research.

Methodology: During the necropsy, tumors found in the intestines, in the liver and on the gills of broodfish were identified with the help of histopathological tests. The tissues were fixed in 10% buffered formaline and immunohistochemical tests (pan-cytokeratin, E-cadherin and claudin-5).

Results: The primary adenocarcinoma developed in the small intestine. The malignant tumor tissue with a high mitotic index infiltrated and broke through the basal membrane and the propria, and spread asymmetrical to the cross-section of the intestine narrowing the lumen of the intestine causing the passage disorder of the intestinal contents and emaciation among the fish. The primary tumor formed metastases in the vessels of the gill in all cases and many times in the liver too. In some cases the tumor led to invagination in the lumen of the intestine and consequential ileus. The intestinal carcinoma of the trout gave positive results to pan-cytokeratin and E-cadherin in immunohistochemical tests, the peritumoral endothel cells also showed claudin-5- positivity. The other tests with anti-vimentin, anti-alfa-smooth muscle actin, anti-S-100 protein, anti-NSE antibody led to negative results.

Conclusion: During the research 2% of the broodstock were concerned with intestinal adenocarcinoma. Most of the rainbow trouts have died due to an invagination in the small intestines.

Keywords: invagination, rainbow trout, adenocarcinoma, intestine

171-P

European sea bass in a recirculating aquaculture system presenting petechial rash-like skin lesions

Girons, Albert (Spain)[1]

1 - ICTIOVET

Introduction: Sea bass presenting skin lesions were submitted for histopathological and bacteriological analysis. Average weight of affected fish was 330 g. A failure of water heating system caused a sharp water temperature decrease in the holding facilities from 21 ºC to 17.8 ºC. No associated mortalities were recorded. Lesions resolved spontaneously after 1.5 month from onset of condition without any treatment.

Methodology: Routine histopathological analysis was performed using paraffin-embedded tissues plus H&E, Gram and Giemsa stained sections. Routine bacteriology work included blood agar and TCBS cultures from spleen and head kidney

Results: affected fish presented with raised reddened skin lesions on flanks. Histopathological signs included a marked multifocal lichenoid-type inflammatory infiltrate distributed in superficial dermis and epidermis, proliferation of congested blood vessels in superficial dermis, marked associated lymphocytic infiltrate surrounding blood vessels and scale pockets plus occasional reabsorption of scales. Epidermis presented with moderate hyperplasia, diffused spongiosis and marked lymphocytic infiltrate distributed along basal membrane. Deep dermis (*stratum compactum*), subdermal space and underlying skeletal muscle presented with no abnormalities. No bacteria were isolated from bacteriological analysis.

Discussion: clinical presentation and histological lesions of this non-ulcerative lymphohistiocytic dermatitis is equivalent to those described in other dermatitis reported in rainbow trout (Red Mark Syndrome/ Cold Water Strawberry Disease) and gilthead sea bream (Petechial Rash). No specific aetiological agent was associated with this lesions and further research is required.

Keywords: sea bass, petechial rash, Red Mark Syndrome, strawberry disease

172-P

Immunoreactivity of red mark syndrome (RMS) trout skin to TLR5

Magi, Gian Enrico (Italy)[1]; Rossi, Giacomo (Italy)[1]; Galosi, Livio (Italy)[1]; Volpatti, Donatella (Italy)[2]; Galeotti, Marco (Italy)[2]

1 - School of Biosciences and Veterinary Medicine, University of Camerino, Italy; 2 - Department of Agricultural, Food, Environmental and Animal Science, Veterinary Pathology Section, University of Udine, Italy

Introduction: RMS is an inflammatory skin disorder of farmed rainbow trout (*Oncorhynchus mykiss*) occurring in several European countries and which aetiology has been widely discussed. Recently specific investigations identified a *Rickettsia*-like-organisms (RLO)-related DNA or a *Midichloria*-like organisms (MLO)-related DNA in tissues and detected intracytoplasmic microorganisms morphologically consistent with bacteria belonging to *Rickettsiales* order. The aim of this study was to evaluate the tissue immunoreactivity of Toll-like receptor 5 (TLR5), a receptor for bacterial flagellin that plays a critical role in early innate immunity.

Methodology: Skin tissues from 17 trout with RMS and 5 unaffected trout were analysed by immunohistochemistry to determine the labelling expression of TLR5 using a rabbit polyclonal anti-TLR5 antibody (Sigma-Aldrich) with an avidin–biotin–peroxidase-complex (ABC) technique. A blocking peptide-based protocol for TLR5 was used as negative control. Labelling was evaluated by one author semi-quantitatively based on the percentage of immunopositive cells and staining intensity to obtain an immunoreactivity score. A total of 10 HPF fields were assessed for each sample. Scores from 1 to 4 related to a low expression, scores from 5 to 8 as intermediate whereas scores greater than 8 represented high expression.

Results: Trout with RMS showed a diffuse and intense immunoreactivity of TLR5 both in the epidermis and in the layers involved by inflammatory infiltrates. Especially macrophages were strongly immunolabeled. A significant difference in TLR5 expression between RMS and normal skin was observed since the expression score in trout with RMS had a mean value of 9.9 whereas in normal skin it was 3.6.

Conclusion: In the veterinary literature several biomolecular studies on fish response to bacterial diseases demonstrated an overexpression of TLR5. Also our research confirms an up-regulation of TLR5 in the affected tissues compared to the controls and suggests that TLR5 could have an important role for the trout innate immune response *versus* the RMS etiological agent. A recent study demonstrated the presence of a flagellar apparatus in '*Candidatus* Midichloria mitochondrii', a member of the order *Rickettsiales*, thus the evidence in trout with RMS of bacteria belonging to *Midichloraceae* might be associated to TLR5 activation.

Keywords: trout, Toll-like receptors, skin disorders, RMS, TLR5

173-P

Rainbow trout (*Oncorhynchus mykiss*) red mark syndrome - a standardised approach to histopathological scoring

Orioles, Massimo (Italy)[1]; Günther Schmidt, Jacob (Denmark)[2]; Moesgaard Iburg, Tine (Denmark)[2]; Galeotti, Marco (Italy)[1]

1 - Università degli studi di Udine; 2 - Technical university of Denmark

Introduction: Red mark syndrome (RMS) is an infectious disease affecting farmed rainbow trout in Europe, the Middle East, and the Americas. No aetiological agent has been unequivocally identified for RMS, which is thus presently defined by histopathological features.

Methodology: Histolopathological features have previously been reported from RMS-affected fish from farms, but here we propose a semi-quantitative scoring system of RMS lesions using samples from a controlled direct cohabitation infection model established at DTU Aqua (Denmark). RMS was followed from early lesion development until late healing stages, and samples were taken at several time-points. H&E stained sections of a total of 94 full-thickness skin samples were shipped from DTU to the Department of Veterinary Pathology to Udine University, where they were analysed blindly. At the macroscopic level, lesions were assessed on several time-points before sampling and classified depending on factors such as colour, swelling, and scale resorption. At the microscopic level, epidermis, scale pockets, dermis spongiosum, dermis compactum, hypodermis and muscular tissue were analysed. The samples were classified using a semi-quantitative scoring system (negative to mild, mild, moderate, severe), considering intensity of inflammatory infiltrate and several features as integrity of each layer, presence of necrosis, oedema, congestion, haemorrhages, absence of scale pockets, scale pockets resorption. The samples were further classified based on the intensity of scale regeneration (no regeneration, mild regeneration, moderate regeneration, severe regeneration).

Results: 87 samples were considered of good quality and submitted for analysis. Cases considered mild were predominant (48), followed by negative to mild cases (23), moderate (8) and severe cases (8). Regeneration was observed in 50 cases, with mild signs of regeneration found in 34 cases. Severe regeneration (6) was only observed in mild cases. Evident correspondences were found between microscopic and macroscopic classifications of the samples especially when moderate and severe cases and regenerative classification were considered.

Conclusion: The semi-quantitative histopathological scoring system proposed in this study can provide a valuable standardized approach and guide the pathologist in the analysis of suspected RMS lesions. The correspondence found with macroscopic classification can further explain timing and specific features of RMS lesion development.

Keywords: RMS, rainbow trout, histopathology

Immunomodulators and Aquatic Animal Health

174-P

Effect of the HMB on nonspecific defence mechanisms and protection against *Shewanella putrefaciens* infections in carp (*Cyprinus carpio*)

Siwicki, Andrzej (Poland)[1]; Kazuń, Barbara (Poland)[1]; Kazuń, Krzysztof (Poland)[1]

1 - Department of Fish Pathology and Immunology, Inland Fisheries Institute in Olsztyn, Poland

Introduction: b-hydroxy-b-methylbutyrate (HMB) is a metabolite of the amino acid leucine and applicated in pellets for feeding significantly increased the cell-mediated and humoral-mediated immunity in different species of fish. In Poland, for over 10 years were noted mainly in spring the bacterial infections induced by *Shewanella putrafaciens* in intensive carp culture. The range of mortality is about 40% and clinical signs, prevalently lethargy, skin lesions with ulceration have been observed. The anatomopathological examination haemorrhage in the spleen and kidney was notified. The aim of the study was to examine the influence of feeding with leucine metabolite HMB on the innate immunity and on resistance against shewanelloses in fingerling of carp (*Cyprinus carpio*) grown in a intensive system of culture.

Methodology: The juvenile carp were reared in circular tanks, 200 L each, with water temperature maintained at about 22 °C. The fish of approximately 50 g were fed with commercial carp feed using automatic band feeders. The carp were fed with 100 mg HMB per kg body weight per day for 4 weeks. The control group was fed pellets without HMB. The disease challenge test using *Shewanella putrefaciens* were conducted after 4 weeks of feeding. Briefly, 100 fish from each control end experimental group were given a single intraperitoneal injection of 48 -72 h growth of *Shewanella putrefaciens* (0.2 ml). Mortalities were tabulated and the presence of pathogens was confirmed by isolation from the kidney.

Results: The results showed that HMB stimulated the macrophage and lymphocyte activity. Also HMB increased the lysozyme activity and total immunoglobulin (Ig) levels in serum. The challenge test showed that dietary supplementation of HMB decreases the mortality after experimental infection. Feeding with HMB resulted in a lower cumulative mortality (5%), compared to the control group (35% cumulative mortality).

Conclusion: The dietary supplementation of HMB decreases the mortality of carp after *Shewanella putrefaciens* experimental infection.

Keywords: *Shewanella putrefaciens*, immunity, HMB, immunostimulation

175-P

Proteomic comparison of *Ostrea edulis* granulocytes and hyalinocytes after *in vitro* stimulation with immune response inducers

De La Ballina, Nuria R. (Spain)[1]; Villalba, Antonio (Spain)[1]; Cao, Asunción (Spain)[1]

1 - Centro de Investigacións Mariñas

Introduction: Most European flat oyster *Ostrea edulis* populations are exhausted due to overfishing and diseases. Recovery and maintenance of wild populations is a priority for marine ecosystem health. Bonamiosis is one important constraint for oyster bed restoration and oyster aquaculture. Understanding oyster immune system would contribute to design effective strategies to fight oyster diseases like bonamiosis. Haemocytes play a crucial role in oyster immune response. There are two basic cell types of bivalve haemocytes, hyalinocytes and granulocytes, which are morphologically distinguishable, although their functional differences and specific abilities are poorly understood. While granulocytes are believed to be more efficient in killing microorganisms, hyalinocytes are thought to be more specialized in clotting and wound healing.

Methodology: This study aims to enlighten the differences in immune capacity among the haemocytic types of *O. edulis*, analysing the proteomes of granulocytes and hyalinocytes after in vitro stimulation with various inducers of immune response, namely lipopolysaccharide (LPS), Poly I:C and Zymosan A, to simulate the confrontation with bacteria, viruses and fungi, respectively. Both oyster haemocyte types were separated by Percoll density gradient centrifugation of oyster haemolymph. Granulocytes and hyalinocytes were separately stimulated with LPS, Poly I:C and Zymosan A for various time-lengths. Then, cell proteins were separated by 2D-PAGE and the protein profiles of granulocytes and hyalinocytes were analysed and compared with PD Quest software. The protein spots exclusive for each haemocyte type and treatment were excised from gels and analysed by MALDI-TOF/TOF with a combination of mass spectrometry (MS) and MS/MS for sequencing and protein identification.

Results: A total of 12 proteins were identified, 6 exclusive of granulocytes stimulated with LPS and Poly I:C and 6 exclusive of hyalinocytes stimulated with Poly I:C and Zymosan A. The identified proteins are involved in important biological processes for the preservation of organism integrity, such as signal transduction, defence, functions related with cytoskeleton, protein biosynthesis.

Conclusion: Our results contribute to identify differential roles of each haemocyte type and to better understand the oyster immune mechanisms.

176-P

Influence of bacteriophages cocktail on European eel (*Anguilla anguilla*) immunity

Schulz, Patrycja (Poland)[1]; Robak, Stanisław (Poland)[2]; Dastych, Jarosław (Poland)[3]; Siwicki, Andrzej Krzysztof (Poland)[1]

1 - Department of Microbiology and Clinical Immunology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland; 2 - Inland Fisheries Institute in Olsztyn, Poland; 3 - Proteon Pharmaceuticals S.A., Łódź, Polska

Introduction: Due to intensive fish farming practices, infectious disease pose a major problem in aquaculture industry globally, especially causing heavy loss to farmers. Finding a preparation that could be used both prophylactically to increase the resistance of fish against infection with pathogenic bacteria and therapeutically, without side effects for fish and aquatic environment could be a great solution to this problem. In this study, we evaluated the response of European eel to contact with novel bacteriophage-based preparation BAFADOR®, through the monitoring of different immunological parameters.

Methodology: The experimental material comprised of 90 European eels (*Anguilla anguilla*). After 14 days of acclimation, the animals for immunological tests were randomly divided into three equal groups- control, immersion, and fodder group. Six fish of each group were sampled on day 1, 7, 14 and 21 of the experiment. Blood samples were collected from a caudal vein for blood serum and stored in -80 °C until analysis. The following parameters were determined: total protein and total-Ig- contents, lysozyme and ceruloplasmin activities in blood serum.

Results: Application of BAFADOR® increased total protein level, immunoglobulin level, lysozyme activity and ceruloplasmin level in European eel serum. Our results showed that preparation BAFADOR® containing bacteriophages against *Aeromonas hydrophila* and *Pseudomonas fluorescens* administered in feed and immersion is well tolerated by the fish organism causing immunity stimulation.

Conclusion: BAFADOR®, the new bacteriophage-based preparation dedicated to fight fish bacterial pathogens, has the potential to stimulate the nonspecific immune response in fish which can improve resistance to bacterial infection.

Keywords: innate immunity, *Aeromonas hydrophila*, *Pseudomonas fluorescens*, immune response

Funding: POIG.01.04.00-10-098/12.

177-P

Influence of bacteriophages cocktail on European eel (*Anguilla anguilla*) survival after experimental challenge

Schulz, Patrycja (Poland)[1]; Robak, Stanisław (Poland)[2]; Dastych, Jarosław (Poland)[3]; Siwicki, Andrzej Krzysztof (Poland)[2]

1 - Department of Microbiology and Clinical Immunology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland; 2 - Inland Fisheries Institute in Olsztyn, Poland; 3 - Proteon Pharmaceuticals S.A., Łódź, Polska

Introduction: *A. hydrophila* and *P. fluorescens* are two most frequent bacterial pathogens in Poland, of both eel and other fish species. They are known to cause a variety of diseases in fish such as hemorrhagic septicemia, infectious dropsy and fin rot leading to heavy mortality in aquaculture farms in different fish species. Antibiotic resistance occurring in pathogens creates a global health problem. In this study we examined prophylactic and therapeutic effectiveness of bacteriophage-based preparation BAFADOR®.

Methodology: 175 European eels was used to determine the prophylactic and therapeutic effects of the preparation. After 14 days of acclimation, the fish intended to test the therapeutic effect of BAFADOR® were divided into 5 groups and fish intended for prophylactic testing of BAFADOR® into 3 groups. Fish from infected groups were given a single intraperitoneal injection of *A. hydrophila* and *P. fluorescens*. The fish from the uninfected group received intraperitoneal injection of 0.2 mL PBS. The fish were observed for 14 days for mortality and post-challenge survival percentage was calculated.

Results: In the therapeutic application study, the highest cumulative percentage of survival was registered in group where BAFADOR® was given in the shortest time from experimental infection, that is 24 hours after infection. It was 40% higher compared to the infected control and reached 80%. The delay in therapy resulted in a decrease in survival to 65% and 55% for every 24 h. In prophylactic study In addition, we received survival rate not differing from the negative control (98%) despite the one-time use of the preparation.

Conclusion: BAFADOR®, the new bacteriophage-based preparation fulfils its role as a therapeutic preparation limiting the European eel's death with a mixed infection of *A. hydrophila* and *P. fluorescens* so can be treated as a new strategy for better health management.

Keywords: *Aeromonas hydrophila*, *Pseudomonas fluorescens*, infection

Funding: Project POIG.01.04.00-10-098/12.

178-P

Stimulation of innate immunity in huchen (*Hucho hucho*) growing in an intensive culture system

Siwicki, Andrzej Krzysztof (Poland)[1]; Kowalewski, Mieczysław (Poland)[3]; Kazuń, Barbara (Poland)[1]; Kazuń, Krzysztof (Poland)[1]; Schulz, Patrycja (Poland)[2]

1 - Department Fish Pathology and Immunology, Inland Fisheries Institute, Olsztyn, Poland; 2 - Department Microbiology and Clinical Immunology, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland; 3 - Polish Anglers Association, Trout Farm, Łopuszna, Poland

Introduction: Innate immunity is a very important part of immunological mechanisms and performs a key role in the protection against diseases in fish. Huchen is a very stressful fish and high mortality of fingerling in the intensive system of culture was noticed. The aim of this study was to examine the influence of Levamisole (Biovet Drwalew, Poland) and 1.3-1.6 β-glucan (Biolex, Leiber GmbH Germany) on the cell-mediated and humoral-mediated defense mechanisms of huchen (*Hucho hucho*) grown in an intensive culture system.

Methodology: For this study 300 healthy huchen with average weight of 50 g were used. The fish were fed commercial pellets containing Levamisole at dose 500 mg per kg of feed (100 fish) and Biolex at dose 500 mg per kg of feed (100 fish). The control group (100 fish) was fed commercial pellets. Two and four weeks after, blood, spleen and headkidney samples (from 20 fish of each group) were taken for immunological study. The metabolic activity and potential killing activity of spleen phagocytes were examined. The proliferative response of headkidney lymphocytes stimulated by mitogens ConA or LPS were determined. Also, the lysozyme and ceruloplasmin activities in plasma and total immunoglobulin levels in serum were examined. Challenge test with *Aeromonas salmonicida* was performed.

Results: The results showed that Levamisole and Biolex increased the innate immunity parameters in huchen. The higher cell-mediated and humoral-mediated immunity was observed in huchen fed two weeks with Biolex and Levamisole, as compared to the four weeks fed. The lover mortality after challenge test with *Aeromonas salmonicida* was observed in huchen fed two weeks with Biolex and Levamisole (30% and 20%), compared to the control group (80%).

Conclusion: Based on the results of our study, the use of natural or synthetic immunomodulators for the activation of nonspecific defence mechanisms and protection of bacterial diseases in intensive culture of huchen seems to hold great promise.

Keywords: *Hucho hucho*, immunomodulators, immune system

179-P

Influence of effective microorganisms on the defence mechanisms of pikeperch

Terech-Majewska, Elżbieta (Poland)[1]; Pajdak-Czaus, Joanna (Poland)[1]; Naumowicz, Karolina (Poland)[1]; Kaczorek-Łukowska, Edyta (Poland)[1]; Rożyński, Maciej (Poland)[2]; Kowalska, Agata (Poland)[2]; Zakęś, Zdzisław (Poland)[2]; Siwicki, Andrzej Krzysztof (Poland)[2]

1 - University of Warmia and Mazury in Olsztyn; 2 - Inland Fisheries Institute in Olsztyn

Introduction: Effective Microorganisms (EM) uses microorganisms to work in the surrounding environment. Complex microbiological preparations show synergistic effects, combining the effects of probiotics, prebiotics and synbiotics. Fish farmers reach for the preparations in search of effective growth, conditioning and immunity enhancer. The aim of the study was to evaluate the effect of original EM-Probiotic (Greenland, Poland) on the development of cellular and humoral non-specific resistance in pikeperch (*Sander lucioperca*), in the initial stage of rearing, in closed water circulation RAS.

Methodology: The experimental diet was administered for 28 days with 0% EM (control group), 2% and 4% of feed. RBA, PKA and MTT tests were determined on cells isolated from the kidney of the head and spleen. In the blood serum, Lyz, TP, Cer, Ig were determined. After 84 days of experiment, fish were contaminated experimentally with a virulent strain of *A. hydrophila* and *A. salmonicida* (0.2 ml of bacterial suspension 1x 10^5).

Results: The results of the study showed that EM at the initial stage of pikeperch development suppresses cellular defense mechanisms. The inhibitory effect was demonstrated in all parameters in both experimental groups at a statistically significant level. The results from the humoral parameters showed differentiated EM activity at a statistically insignificant level only in 4% group. The results of the study indicate that EM enhances non-specific immune responses and reduces mortality after experimental infection.

Conclusion: Bearing in mind that fish in RAS systems are also susceptible to potentially pathogenic agents, immunosuppression of these mechanisms may aggravate the disease.

Keywords: effective microorganisms, sander, immunity

180-P

Effect of dietary β-glucan on oxidative stress biomarkers in the muscle tissue of rainbow trout (*Oncorhynchus mykiss*, Walbaum)

Tkachenko, Halyna (Poland)[1]; Grudniewska, Joanna (Poland)[2]; Terech-Majewska, Elżbieta (Poland)[3,4]; Siwicki, Andrzej Krzystzof (Poland)[2]

1 - Pomeranian University in Słupsk; 2 - Inland Fisheries Institute in Olsztyn; 3 - Universit; 4 - University of Warmia and Mazury in Olsztyn

Introduction: In recent years, the effective immunomodulatory properties of β-glucans have been extensively proved, not only in mammals but also in fish. β-glucan naturally form polysaccharides with glucose linked by β-glycosidic bonds and can stimulate macrophages to actively fight against fish pathogens. Little is known about the biochemical changes in various tissues of rainbow trout after oral administration of β-glucan. Therefore, the aim of the present study was to evaluate the effects of dietary β-glucan on oxidative stress biomarkers by detecting relevant lipid peroxidation (2-thiobarbituric acid reactive substances, TBARS) and protein oxidation biomarkers [aldehydic and ketonic derivatives of oxidatively modified proteins (OMP)] in the muscle tissue of rainbow trout.

Methodology: This trial was conducted at Department of Salmonid Research, Inland Fisheries Institute (Poland). Briefly, 150 farmed individuals were randomly placed and fed with commercial dry pellets without β-glucan inclusion prior to the start of the trial. Fish were fed with the corresponding pellets at a feeding rate of 1.5% body weight with a feeding frequency of four times daily for 14 days. The basal diet (commercial diet) was used as the control diet. For the experimental diets, the basal diet was supplemented with β-1,3-glucan obtained by chemically synthesis and naturally extracted from *Saccharomyces cerevisiae* in dose 1 kg per 100 kg of basal diet. The muscle tissue was excised, weighted and washed in 100 mM Tris-HCl ice-cold buffer. Biomarkers of oxidative stress were assessed in the homogenates. The significance of differences (significance level, $p<0.05$) was examined using the Mann-Whitney *U* test. All statistical calculation was performed with Statistica 8.0 software (StatSoft, Poland).

Results: Our results showed that feeding with low doses of β-glucans induced the decrease of TBARS level (by 27%, $p = 0.019$), aldehydic and ketonic derivatives of oxidatively modified proteins (by 43.3% and 45%, $p = 0.000$, respectively).

Conclusion: This study confirms that dietary β-glucan is beneficial for promoting growth and enhancing antioxidant capacity against oxidative stress in rainbow trout. Indeed, we cautiously hypothesized that feeding low β-glucans doses may help to boost antioxidant function, especially by the decrease of oxidative stress-induced biomarkers level in the muscle tissue of rainbow trout.

Keywords: *Saccharomyces cerevisiae*, TBARS, OMP

182-P

Recombinant flagellin and its ND1 domain from *Vibrio anguillarum* promote *in vivo* over-expression of IL-1BETA and IL-8 in *Salmo salar*

González-Stegmaier, Roxana (Chile)[1,2]; Aguirre-Gil, Isabel (Chile)[3]; Villarroel-Espíndola, Franz (Chile)[4]; Enríquez, Ricardo (Chile)[3]; Figueroa, Jaime (Chile)[1,2]

1 - Laboratorio de Biología Molecular de peces. Instituto de Bioquímica y Microbiología, Universidad Austral de Chile, Valdivia, Chile; 2 - Centro FONDAP: Interdisciplinary Center for Aquaculture Research, Chile.; 3 - Laboratorio de Biotecnología y Patología Acuática. Instituto de Patología Animal, Universidad Austral de Chile, Valdivia, Chile; 4 - Laboratorio Medicina Traslacional, Instituto Clínico Oncológico, Fundación Arturo López Pérez, Santiago, Chile

Introduction: Flagellin is the major component of flagellum in bacteria, it binds and activates the Toll-like receptor 5 promoting the expression of proinflammatory cytokines and chemokines. As reported, two recombinant molecules of *Vibrio anguillarum*, flagellin (rFLA) and the amino-terminus of the D1 domain (rND1) from the same molecule induce an *in vitro* upregulation of proinflammatory genes in gilthead sea bream and rainbow trout. In this work, we studied *in vitro* and *in vivo* biological properties of this molecules in *Salmo salar*.

Methodology: SHK-1 cells and isolated head kidney leucocytes (HKL) were exposed for 3h to 0,1 mg/mL rFLA, or 1 mg/mL rND1 and proinflammatory cytokines were measured by RT-qPCR. We compared *in vivo* effectivity after intraperitoneal injection with 5 µg rFLA or 15 µg of rND1 alone or in combination with a commercial vaccine (CV). IL-1β and IL-8 induction was measured in head kidney at 4, 24, and 72 hours.

Results: The results for *in vitro* assays were comparable and overall showed that IL-8 transcript increased 6 - 10-fold using rFLA and 2 - 6-fold using rND1, IL-1β transcript increased 3-4-fold with rFLA and 1.1-1.8-fold using rND1. The results for *in vivo* assays showed that rFLA and rND1 induced a time-dependent acute pro-inflammatory response. IL-1β upregulation reached 25-fold above the PBS-control after 4 hours and it decreased progressively until 3 to 6-fold. IL-8 showed an acute response, reaching a 13-fold change above basal levels using rFLA or rND1 at 4 hours post injection. After 24 hours IL-8 was almost undetectable. The combined challenge (CV plus one single recombinant) showed differential responses based on IL-8 and IL-1β overexpression. For both combinations, an acute IL-8 upregulation of 3-fold change in head kidney after 4 hours was observed. However, the rFLA effect on IL-8 had a shorter duration than rND1 which response was stable until 144 hours after challenge. IL-1β was shortly upregulated, 2-fold by rFLA but not by rND1, this induction was sustained in time.

Conclusion: We suggest that rFLA and rND1 can drive non-redundant cytokines upregulation and both recombinants are valid candidates to be used as an immuno-stimulant or adjuvant in farmed salmon.

Keywords: flagellin, cytokines proinflammatory, immune response, adjuvant, immuno-stimulant

Funding: Fondecyt_Postdoctoral_3170356, Fondap_15110027, VIDCA_UACH.

183-P

Molecular insights and functional analysis of copper-zinc-superoxide dismutase in redlip mullet, *Liza haematocheilia*

Sirisena, D.M.K.P. (Republic of Korea)[1]; Janson, N.D. (Republic of Korea)[1]; Lee, Jehee (Republic of Korea)[1]

1 - Department of Marine Life Sciences and Fish Vaccine Research Center, Jeju National University, Jeju Self Governing Province 63243, Republic of Korea

Introduction: Copper-zinc-superoxide dismutase (CuZnSOD) is a nuclear-encoded antioxidant metalloenzyme. Its main function is dismutation of the hazardous superoxide anion (O^{2-}) into less harmful hydrogen peroxide (H_2O_2) and oxygen (O_2).

Methodology: Structural analysis of mullet CuZnSOD (MuCuZnSOD) was performed using different bioinformatics tools. The relative expression levels of *MuCuZnSOD* were explored in healthy mullet tissues and immune challenged tissues using qPCR. Antioxidant activity of recombinant MuCuZnSOD (rMuCuZnSOD) was determined by xanthine oxidase (XOD) assay.

Results: *Oplegnathus fasciatus* CuZnSOD indicated 94.2% sequence identity with mullet CuZnSOD. Multiple sequence alignment showed that the CuZnSOD domain contained SOD_CU_ZN_1 signature and SOD_CU_ZN_2 signature. The qPCR analysis revealed the highest *MuCuZnSOD* mRNA expression in blood. *Lactococcus garvieae* infection led to highest MuCuZnSOD expression at 24 hours post-infection in blood. In XOD assay, the optimum temperature and the pH for XOD activity were 25 °C and 9, respectively. Relative XOD activity was significantly increased following treatment with rMuCuZnSOD. The activity of rMuCuZnSOD was significantly inhibited by potassium cyanide (KCN).

Conclusion: Reactive oxygen species (ROS) generation was higher in the blood compared to the other organs. Therefore, the mullet antioxidant defense system may activate and produce more MuCuZnSOD transcripts and lead to its increased expression level. When pathogens attacked immune tissues, ROS generation was increased due to the respiratory burst. Hence, antioxidant defense mechanism of mullet may induce upregulation of expression of *MuCuZnSOD*. Xanthine oxidase assay (XOD assay) revealed the ROS-scavenging ability of purified recombinant protein (rMuCuZnSOD). Taken together, the results of the present study revealed that MuCuZnSOD acts as an antioxidant enzyme and is associated with mullet immunity.

Keywords: antioxidant, CuZnSOD, oxidative stress, XOD assay

184-P

Molecular characterization of C-FOS homolog in red lip mullet (*Liza haematocheila*) and its potential immune role in fish immunity

Janson, N.D. (Republic of Korea)[1,2]; Kim, Gabin (Republic of Korea)[2]; Lee, Jehee (Republic of Korea)[1,2]

1 - Marine Science Institute, Jeju National University, Jeju Self-Governing Province 63333, Republic of Korea; 2 - 1Department of Marine Life Sciences & Fish Vaccine Research Center, Jeju National University, Jeju Self-Governing Province 63243, Republic of Korea

Introduction: c-Fos is one of the key components of the transcription factor activator protein-1 (AP-1). It is a highly conserved nuclear protein that regulates cell proliferation, survival, differentiation, cellular migration, immune responses, apoptosis, and inflammation. AP-1 is a dimeric protein consisting of region-leucine zipper (bZIP) domains which belong to Jun, Fos, Maf, and ATF subfamilies.

Methodology: The *c-Fos* homolog was identified from a transcriptomic database of *Liza haematocheila* and designated as *Lhc-Fos*. The mRNA expression profile of *Lhc-Fos* in gill, blood, liver, and spleen tissues was determined using qRT-PCR following immune challenges (LPS, poly I:C, and *Lactococcus garvieae*). The nuclear localization of *Lhc*-Fos was attested using mullet kidney cells. The potential AP-1 activity in *Lhc*-Fos was assayed in HEK293T cells using AP-1 luciferase assay.

Results: The immune challenge experiment revealed a significant upregulation of *Lhc-Fos* against all three immune stimuli. Further analysis showed an exclusive nuclear localization of *Lhc*-Fos in mullet kidney cells. AP-1 luciferase reporter assay gave a clear indication of AP-1 promoter activity in *Lhc-Fos*-overexpressing HEK293T cells.

Conclusion: The transcriptional expression pattern upon immune stimuli, exclusive nuclear localization, and transcriptional regulation of AP-1 promoter strongly indicated the potentially critical role of *Lhc-Fos* in immune defense against viral and bacterial infections.

Keywords: AP-1, c-Fos, nuclear localization, qRT-PCR, immune challenge

Antimicrobial Resistance in Fish and Shellfish

185-P

The underestimated danger: antibiotic resistance in aquaculture and pet fish

Schmidt-Posthaus, Heike (Switzerland)[1]; Delalay, Gary (Switzerland)[1]; Diserens, Nicolas (Switzerland)[2]; Berezowski, John (Switzerland)[3]

1 - Centre for Fish and Wildlife Health, Vetsuisse Faculty, Unversity of Bern; 2 - Koipraxis GmbH, Ulmiz; 3 - Veterinary Public Health Institute, University of Bern

Introduction: The demand for fish worldwide as well as in Switzerland continues to grow because of the increase in fish consumption per person. As a consequence, aquaculture has become one of the fastest growing food industries. Infectious diseases, especially those caused by bacterial pathogens, are the leading cause of mortality in aquaculture and are important because of their impact on economics and welfare. Antibiotic therapy can lead to resistance, especially when treatment is inappropriate. The purpose of this study was to assess the resistance status of bacteria cultivated at the Centre for Fish and Wildlife Health (CFWH), University of Bern, Switzerland during the period from 2000 to 2017 and to identify potential risk factors for antibacterial resistance that could be tested in future studies.

Methodology: 1448 resistance tests of bacterial isolates collected from 1134 different submissions of clinically ill food and pet fish were included. Resistance profiles were analysed and compared with data from fish farms and ponds.

Results: Resistance to all antibiotics was found more often in ornamental pond fish compared to farm fish or fish kept in private aquaria. In our study, bacterial isolates from aquaculture fish originating from farms using recirculation systems were observed to develop more resistance to antibiotics than isolates from fish originating from farms with other systems. Flavobacteriaceae isolates had much less resistance to the antibiotics tested compared to other bacteria classes.

Conclusion: The majority of Swiss fish farms discharge their wastewater directly into surface water. This has the potential to allow sensitive bacteria from the river to develop resistance when in contact with antibiotic treated effluent. Additionally, resistant bacteria from the farms are released into the river which may be a risk factor for transmission of multiresistant pathogens to wild fish and possibly other species. There is also a risk of exchange of the resistance capability from aquaculture bacteria to environmental bacteria, which is an additional risk factor for terrestrial species.

Keywords: antimicrobial resistance, aquaculture, pet fish, Switzerland, retrospective data

186-P

Antimicrobial activity of the sea urchin *Paracentrotus lividus* coelomic fluid against pathogenic bacterial strains from fish and shellfish

García, Ana (Portugal)[1]; Santos, Rafaela (Portugal)[1,2]; Azevedo, Joana (Portugal)[1,2]; Leão, Pedro Nuno (Portugal)[1]; Costas, Benjamin (Portugal)[1,3]; Serra, Claudia (Portugal)[1]; Fernández-Boo, Sergio (Portugal)[1]

1 - Centro Interdisciplinar de Investigação Marinha e Ambiental, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal; 2 - Universidade do Porto, Faculdade de Ciências, Departamento de Biologia, Rua do Campo Alegre, s/n, Edifício FC4, 4169-007 Porto, Portugal; 3 - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

Introduction: Echinoderms are exposed to challenging environments and, unlike vertebrates, they lack of an adaptive immune system. In these organisms, as in most of the invertebrates, antimicrobial peptides play a major role in host defense response against pathogens. Peptides with antimicrobial proprieties were already identified in coelomic fluid of some species of sea urchins. However, despite of the commercial value in the market, studies elucidating the interaction of these species with pathogens are still scarce. The aim of this study was to identify antimicrobial peptides from the sea urchin *Paracentrotus lividus*.

Methodology: Coelomic fluid, composed of coelomocytes and perivisceral fluid (PF) was collected from 100 specimens in the coast of Vila Chã, Porto. After centrifugation, coelomocytes and PF were separated and both fractions were lyophilized. Coelomocytes were subjected to a liquid-liquid extraction and solid phase extraction (SPE) on a C_{18} cartridge. Six different fractions were obtained: an ACN rich phase and 5 water fractions after elution of the column with mQH_2O and 10%, 40% 80% and 100% ACN. All fractions were dried under a rotary evaporation and further resuspended in mQH_2O to a final concentration of 10 mg/ml. The different fractions were tested for antimicrobial activity by microplate growth inhibition assay. Tests were performed against bacterial strains known as etiological agents responsible for diseases in aquaculture, such as: *Aeromonas hydrophila*, *Vibrio anguillarum*, *Vibrio parahaemolyticus*, *Photobacterium damselae* subsp. *piscicida* and *Tenacibaculum maritimum*.

Results: Preliminary results indicate that some coelomic fluid fractions seem to have an inhibitory effect against the growth of *Aeromonas hydrophila*, *Vibrio anguillarum* and *Vibrio parahemolyticus*. The most pronounced effects were observed for ANC-rich fraction and fractions resulting from the 10 and 40% ACN elution of the SPE column. No clear effects were observed against *Photobacterium damselae* subsp. *piscicida* and *Tenacibaculum maritimum.*

Conclusion: Coelomocyte extracts of *Paracentrotus lividus* showed growth inhibitory effect against some bacterial strains, specially, *Vibrio* and *Aeromonas* spp. Additional tests will be performed to confirm those results and, also the effect against more bacterial strains will be further evaluated.

Keywords: antimicrobial peptides, *Paracentrotus lividus*, microbicidal activity, pathogen bacteria

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187-P

Shellfish as an exemplar for assessing the burden of antimicrobial resistance in the environment

Chambers, Edel (United Kingdom)[1]; Card, Roderick (United Kingdom)[2]; Cawthraw, Shaun (United Kingdom)[2]; Marsella, Laura (United Kingdom)[2]; Stone, Michelle (United Kingdom)[1]; Baker-Austin, Craig (United Kingdom)[1]; Verner-Jeffreys, David (United Kingdom)[1]

1 - Centre for Environment, Fisheries and Aquaculture Science; 2 - Animal and Plant Health Agency

Introduction: There is growing concern that aquatic environments represent hotspots for the evolution, retention and dissemination of antimicrobial resistant (AMR) bacteria. However, prevalence data for AMR in aquatic environments is limited. Filter-feeding shellfish present a good model to help address this gap as they can concentrate bacterial contamination from the environment and are already examined in Europe for faecal contamination as part of EU Food Hygiene Regulations.

Methodology: The aim of this study was to look at the incidence of AMR in *Escherichia coli* from shellfish, utilising isolates collected between Nov 2017 and Nov 2018. Samples were collected under the Food Safety Scotland monitoring programme and UK research projects. The minimum inhibitory concentration was determined for 106 *E. coli* isolates against 14 antimicrobials using broth microdilution. EUCAST epidemiological cut-off values were used to categorise results. All isolates were whole genome sequenced using the Illumina MiSeq platform. Sequences were assembled, annotated and analysed for the presence of AMR and virulence genes and phylogenetic relatedness, based on single nucleotide polymorphisms, using standardised bioinformatic pipelines.

Results: A total of 83 isolates showed no resistance to any of the antibiotics tested. The most common microbiological resistance phenotypes observed were to tetracycline and ampicillin. Two isolates had an extended-spectrum beta-lactamase (ESBL) resistance; one harboured *bla*CTX-M15 and the other *bla*CTX-M27. Examination of the core genome SNP-based phylogenetic tree indicated considerable diversity.

Conclusion: This preliminary work suggests there may be a low burden of AMR bacteria in UK shellfish, with a concomitant low threat to the human food chain. However, the presence of ESBL-producing *E. coli* indicates that ongoing surveillance is warranted. Shellfish provide an informative and useful surveillance target to monitor AMR in the environment and its potential to enter the human food chain.

Keywords: AMR, shellfish, environment

Funding: UK Gov, Department for Environment, Food and Rural Affairs through the FAO AMR Reference Centre.

188-P

Multiresistant *Aeromonas salmonicida* subsp. *salmonicida*: presence and transfer of antibiotic resistance determinants

Wiklund, Tom (Finland)[1]; Pelkola, Kirsti (Finland)[2]; Landor, Lotta (Finland)[1]; Silvennoinen, Amanda (Finland)[1]

1 - Laboratory of Aquatic Pathobiology, Åbo Akademi Univeristy, Turku, Finland; 2 - Finnish Food Authority, Helsinki, Finland

Introduction: The extensive use of antibiotics is regarded as a contributing factor in the development and spread of antibiotic resistance in the environment. Also in the aquaculture industry, the presence of antibiotic resistant bacteria is considered an increasing problem.

Methodology: Multi-resistant *Aeromonas salmonicida* subsp. *salmonicida* (ASS) were isolated from farmed sea trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*) in Finland during 2015 – 2018. Sensitivity to different antibiotics were evaluated using disc diffusion technique. The presence of selected antibiotic resistance genes (ARG) were examined using PCR. Transfer of selected ARGs under different environmental conditions was evaluated, using co-culture of resistant (donor) and sensitive (recipients, *Aeromonas hydrophila* and *Vibrio* sp.) isolates.

Results: The ASS were found to be resistant to at least five antibiotics, chloramphenicol, florfenicol, streptomycin, tetracycline and trimethoprim/sulfamethoxasol, and to mercury. The resistances, including mercury resistance, were shown to be transferrable to the recipients. Resistances were transferred at all examined temperatures (5 – 28 °C), and at low nutrient (1% fish feed) conditions, but not at nutrient free (pure lake water) conditions. Resistance genes for florfenicol and mercury were successfully detected among the donors and recipient using PCR. However, the mobile genetic element of the bacteria could not be identified in this study.

Conclusion: The results show that ASS, resistant against all antibiotics presently in use, are circulating in Finnish fish farm. This is of potential concern for the aquaculture industry in the area. The results suggest that the examined resistance determinants can be transferred from ASS to other bacterial species. This can lead to the spread of multiple resistances to environmental bacteria, from where they potentially can be further transferred under antibiotic pressure to other fish pathogenic bacteria. Environmental mercury pollution could also allow for the persistence of these genetic elements, even in the absence of antibiotic exposure.

Keywords: *Aeromonas salmonicida*, multi-resistance

189-P

Development of antibiotic resistances in bacteria isolated between 2005 and 2018 from fish for food production and ornamental fish

Jung-Schroers, Verena (Germany)[1]; Jung-Schroers, Arne (Germany)[1]; Bauer, Julia (Germany)[1]; Teitge, Felix (Germany)[1]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany

Introduction: Antibiotic resistance is one of the biggest threats to human and animal health. It can occur naturally, but misuse of antibiotics is accelerating the process.

Methodology: All bacteria isolated from diagnostic samples submitted to the Fish Disease Research Unit between 2005 and 2018 were analysed for resistance against antibiotic substances. In total 19 substances were tested, whereas some substances were tested during the whole period of 14 years and others were tested only for two to 13 years. The antimicrobial susceptibilities of bacterial isolates were determined by the use of the disk diffusion method. Bacterial isolates were inoculated on blood agar plates. Antibiotic disks containing amoxicillin (10 µg), ampicillin (10 µg) chloramphenicol (30 µg), chlortetracycline (30 µg), colistin (50 µg), doxycyclin (30 µg), enrofloxacin (5 µg), erythromycin (15 µg), florfenicol (30 µg), flumequine (30 µg), furazolidone (100 µg), gentamicin (10 µg), kanamycin (30 µg), neomycin (10 µg), oxolinic acid (10 µg), oxytetracycline (30 µg), trimethoprim/sulfonamide (25 µg) tulathromycin (30 µg), or tylosin (30 µg) were used according to the manufacturer's instructions. Inhibition zone diameters were measured and evaluated inspired by CSLI if possible. According to the diameter of the inhibition zone, the results were given in resistant (R), intermediate (I) and sensitive (S).

Results: The detected bacteria showed mainly resistances against amoxicillin, ampicillin, neomycin, oxolinic acid and tylosin. Yet, over the last 14 years for most tested substances the resistance situation improved. For single substances, like trimethoprim/sulphonamide, the number of resistant bacteria increased. Differences were seen in the resistant patterns of bacteria isolated from fish from different keeping units. Especially in bacteria isolated from ornamental fish at wholesaler facilities more resistances were detected, whereas in bacteria isolated from fish for human consumption fewer resistances were found. Also differences were detected in the resistances of specific bacterial species and especially Flavobacteria, some species of motile Aeromonads as well as Pseudomonads showed frequently resistances against a number of antibiotic substances.

Conclusion: Over the last 14 years, resistances of bacteria against antibiotic agents were decreasing in total.

Keywords: antibiotic resistance, diagnostic samples

190-P

The CEFAS aquatic AMR centre of excellence

Verner-Jeffreys, David (United Kingdom)[1]; Chambers, Edel (United Kingdom)[1]; Stone, Michelle (United Kingdom)[1]; Joseph, Andrew (United Kingdom)[1]; Hughes, Alexandra (United Kingdom)[1]; Baker-Austin, Craig (United Kingdom)[1]

1 - Cefas

Introduction: Antimicrobial resistance, AMR, is one of the biggest threats facing humankind. The aquatic environment, both freshwater and marine, acts as a sink into which antimicrobials and microorganisms come together from industry, health care facilities, agriculture, aquaculture, and human activities. There is opportunity within this environment for resistance to both emerge and transfer between microorganisms. These resistant microorganisms can affect the sustainability of aquaculture production and potentially spread back into the human and terrestrial animal populations.

Methodology: Cefas, with funding from the Fleming Fund and the UKs Department for Environment Food and Rural Affairs, has set up the Aquatic AMR Centre of Excellence, which forms part of the new UK International AMR Reference Centre, in collaboration with our colleagues from the UK's APHA (Animal and Plant Health Agency) and VMD (Veterinary Medicines Directorate). A particular focus of the International AMR Reference Centre is on helping Lower- and Middle-Income Countries develop AMR action plans, through the development and implementation of surveillance programmes for AMU and AMR, via a One Health perspective.

Results: This presentation will outline the vision of this new Centre and the initial work we are already involved in. This includes; results of recent visits to Bangladesh to help deliver workshops on AMR and AMU in aquaculture and follow on AMR testing projects there with WorldFish and other partners; surveillance of environmental *E. coli* samples for AMR within several of the Gulf States; testing of shellfish samples collected from the UK for AMR; and contributing to the establishment of standard methods and interpretative criteria leading to the establishment of epidemiological cutoff points for the human and aquaculture pathogens, *Vibrio parahaemolyticus* and *Vibrio vulnificus*.

Conclusion: The Cefas Aquatic AMR Centre of Excellence is an example of an initiative that feeds into the pressing requirement for truly cross-sectoral "One Health" international programmes to tackle the threat of AMR. We aim to play our full part in the development by FAO, OIE and WHO of networks of laboratories to provide critical support for this vital challenge.

Keywords: AMR, reference centre

191-P

A reproducible plate-based method to assess disruption of *Yersinia ruckeri* biofilm by phytobiotic extracts

Parks, Ellie (United Kingdom)[1]; Austin, Dawn (United Kingdom)[2]; Herath, Tharangani (United Kingdom)[1]

1 - Department of Animal Production, Welfare and Veterinary Sciences, Harper Adams University, Newport, Shropshire, England, UK; 2 - Institute of Life and Earth Science, Harriot Watt University, UK

Introduction: Plant derived products with antimicrobial properties are increasingly popular as feed inclusions to improve disease resistance in farmed fish and shellfish. Although antimicrobial properties and capacities of these products are relatively well known, their effect on bacterial biofilms are not widely assessed. This research was therefore attempted to develop a rapid and reproducible plate-based method to assesses anti-biofilm properties of phytobiotic products using *Yersinia ruckeri* as a model pathogen.

Methodology: A dye-based microliter plate assay was developed to quantify *Y. ruckeri* biofilm, and to assess where there is any effect by phytobiotic on their establishment. A time course experiment was performed with different starting number of bacteria (CFU 1 x 10^9 – 1 x 10^2) to estimate time required to form a fully-grown biofilm. To estimate the ability of phytobiotic extract to inhibit biofilm, bacteria was grown in the presence of different concentrations of phytobiotics including, ginger, aloe vera and cinnamon. Biofilm disruption were assessed using crystal violet staining at 72 h and 96 h post bacterial inoculation.

Results: Crystal violet staining showed all bacterial dilutions tested were able to produce biofilms. From the time point assessed, 48 and 72 h post inoculation time points and CFU = 1 x 10^6 were selected for testing anti-biofilm properties of the phytobiotics. The ginger extract able to produce strongest antibiofilm properties and it was significantly different compared to effect caused by cinnamon and aloe vera.

Conclusion: The crystal violet-based assay optimised for *Y. ruckeri* in this study able to provide rapid, reproducible, quantitative method to enumerate biofilms. This method can easily be adopted for testing biofilms formed by other pathogenic bacterial species of fish. All plant extract tested in this study able to inhibit formation of bacterial biofilms, but with different degrees. Further studies are currently on going to test effect of ginger extract on different isolates of *Y. ruckeri* with high and low virulence.

Keywords: biofilm, *Yersinia ruckeri*, phytobiotics

Co-infections and Multiple Stressors

192-P*

Multiple co-infections and environmental stressors as causes of chronic mortalities in juvenile sturgeons (*Huso huso*)

Ciulli, Sara (Italy)[1]; Volpe, Enrico (Italy)[1]; Tura, Giorgia (Italy)[1]; Zavatta, Emanuele (Italy)[1]; Renzi, Andrea (Italy)[1]; Toffan, Anna (Italy)[2]; Pretto, Tobia (Italy)[2]; Mordenti, Oliviero (Italy)[1]; Errani, Francesca (Italy)[1]; Sirri, Rubina (Italy)[1]; Sarli, Giuseppe (Italy)[1]; Serratore, Patrizia (Italy)[1]; Mandrioli, Luciana (Italy)[1]

1 - Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy; 2 - Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padova, Italy

Introduction: Although several species of Acipenseridae are reared, information about diseases is limited. Recently, mortality outbreaks were described in association to NCLDVs (AcIV-E, NV-like virus), *Veronaea botryosa* phaeohyphomycosis and bacteria. In *Huso huso*, reports are limited to bacterial infections (*Vibrio vulnificus*, *Aeromonas hydrophila* and *Yersinia ruckeri*). The aim of this study was to describe an episode of chronic mortality of juvenile *Huso huso*.

Methodology: During 2018, 46 fingerlings were kept in a recirculating aquaculture system for restocking purposes. Water temperature ranged from 15 to 19 °C. After the starting of a chronic "dripping" mortality the moribund or recently dead juveniles (37 - 50 cm) were examined. Samples of brain, kidney, spleen, gill, skin were taken for bacteriological analysis, virological investigation in WSSK cell line and molecular investigations for Betanodavirus and NCLDVs (AcIV-E, NV-like virus). Cytology of celomatic effusions was performed. Main organs were sampled for histology.

Results: Animals showed neurologic signs such as abnormal swimming (inverted or circular), sudden movements, hyperactivity to stimuli alternated to prolonged resting on the bottom or laying on side. Two cases presented U-shaped body, one case showed epaxial muscles softening, and three cases had multifocal ulcerative dermatitis. Two cases had a sero-hemorrhagic celomatic effusion, septic and mycotic-septic respectively. Bacteriology showed septicaemia in four fish due to *Aeromonas veronii*, *Shewanella* spp. and *Citrobacter freundii*. No viral growth was obtained on WSSK cell line nor investigated viruses were detected by PCR/RT-PCR. Histology showed rarefaction of hematopoietic lymphoid tissue (renal, meningeal spinal, splenic and pericardial), splenic vessels hyalinosis, degeneration and atrophy of gangliar neurons in the animals presenting abnormal body shape and myofiber atrophy. One animal showed a systemic mycosis. The renal parenchyma showed glomerular regeneration and sporadic intratubular mineral deposits.

Conclusion: Although the histological findings were variable, the microbiological analyses highlighted systemic bacterial infections due to opportunistic species, thus suggesting the presence of other primary *noxae*. The depletion of hematopoietic tissue and ganglion cell degeneration could suggest a viral aetiology, although not yet been confirmed by investigations conducted so far. Finally, nephrocalcinosis and vessel hyalinosis suggested the presence of predisposing environmental stressors that may have facilitated the infectious onset.

Keywords: bacterial co-infections, environmental stressors, *Huso huso,* sturgeon, histopathology

193-P

Component causes of severe gill damage in rainbow trout farmed under conditions of RAS

Palikova, Miroslava (Czech Republic)[1,2]; Dykova, Iva (Czech Republic)[1]; Papezikova, Ivana (Czech Republic)[1,2]; Mares, Jan (Czech Republic)[1]

1 - Mendel University, Brno; 2 - University of Veterinary and Pharmaceutical Sciences, Brno

Introduction: In the fall of 2018, a rainbow trout farm using an indoor RAS reported disease problems and deaths among stock of marketing weight.

Methodology: In order to assess the health condition of the entire system, we combined detailed examination of diseased fish with a series of tests of the water quality and water treatment effects.

Results: Enormous gill swelling was present in all individuals. This finding indicated a histopathological examination and attempts at an isolation of bacterial fish pathogens and amoebae. In five repeated samplings, these isolation attempts were negative, while histopathological lesions were present constantly. They included lamellar hypertrophy (similar to that described after an exposure to high ammonia levels), lamellar fusion, and enormous hyperplasia of the epithelium in the distal part of filaments (known as "clubbing"). Lesions resembling those described in BGD were found on one sampling date, however, on the same date and on three following dates, a dense accumulation of eosinophilic granular cells along the vascular axis of gill filaments was an evidence of chronic branchitis. Microscopically, the presence of *Ichthyophthirius multifiliis* (0 – 20 individuals in the field of view at a × 50 magnification) on gills was found in the first sampling only. During microbiological examination *Pseudomonas koreensis, Aeromonas hydrophila*, *A. eucrenophila* and *A. bestiarum* were isolated. The presence of bacterial clusters was confirmed by histological examination. Using hydrochemical analysis, hypersaturation with oxygen (up to 200%) was found, corresponding with the presence of gas bubbles on fins in one sampling. Therapeutic measures taken by the breeder included water ozonation, daily application of peracetic acid, or addition of salt and formaldehyde in a long-term bath.

Conclusion: Based on the above listed results, the exact cause of the gill damage could not be identified; it is only assumed that the primary cause was infective agents (bacteria or Ich). Long-term exposure to multiple chemicals used for treatment in combination with gas hypersaturation could lead to the development of severe gill damage which lasted long after the disappearance of infectious agents.

Keywords: gill damage, RAS, hyperplasia, chronic branchitis

Funding: Institutional Funds of VFU Brno and by the project PROFISH CZ 02.1.01/0.0/0.0/16_019/0000869.

194-P

Pathogen interactions during experimental co-infection with *Piscirickettsia salmonis* and Piscine Orthoreovirus in *Salmo salar*

Aguirre-Gil, Isabel (Chile)[1]; Mancilla-Nova, Alejandra (Chile)[1]; Navarrete-Talloni, María José (Chile)[2]; Peredes-Herbach, Enrique (Chile)[2]; González-Stegmaier, Roxana (Chile)[1]; Enríquez-Sais, Ricardo (Chile)[1]

1 - Laboratorio de Biotecnología y Patología Acuática, Universidad Austral de Chile, Valdivia, Chile; 2 - Laboratorio de Patología Animal, Universidad Austral de Chile, Valdivia, Chile

Introduction: Piscine Orthoreovirus (PRV) infections are widespreadly distributed in Chilean salmon farms. And it is estimated that over 60% of freshwater Atlantic salmon is infected predominantly with PRV-1. In this scenario, mixed infections with other viruses or bacteria are likely to occur, and typical clinical signs could be misdiagnosed due to different responses triggered during a simultaneous infection with two or more pathogens. As an example, co-infections with *Piscirickettsia salmonis* (*P. salmonis*) could occur, which is the most important bacterial pathogen for the Chilean salmon industry. Therefore, the objective of our pilot study was to investigate viral and bacterial presence, some aspects of innate immune responses and histopathological features during an experimental challenge with *P. salmonis* in a population of Atlantic salmon smolt infected with PRV-1.

Methodology: From a population of 240, PRV-1 positive smolt (100 g), 84 shedder fish were intraperitoneally infected with *P. salmonis* and then located with 156 co-habitant smolts. Sampling was carried out at 14, 21, and 30 days post-challenge (dpc). Blood, head kidney and spleen samples were directed to molecular analysis, and head kidney, spleen, liver, heart, and gills were obtained for histological examination.

Results: Our results showed that viral loads and Interferon I (IFN-I) transcript overexpression diminished significantly from 14 dpc to 21 dpc and 30 dpc, but they did not disappear. Meanwhile, the percentage of *P. salmonis* positive fish fluctuated from 30% at 14 dpc to 51% at 21 dpc decreasing to 22% at 30 dpc. This last is consistent with the histological and innate immune response findings. Since no lesions or only mild changes were found in tissues and interleukin-8 (IL-8), and IL-1beta transcripts from 14, and 30 dpc and only moderate, initial HSMI or SRS compatible lesions were observed at 21 dpc.

Conclusion: These results suggest that PRV-1 infection does not contribute to a more severe SRS presentation, but it could exert an apparent masking effect on the host against the bacterial infection diminishing the severity of SRS clinical and histopathological signs.

Keywords: co-infection, Piscine Orthoreovirus, *Piscirickettsia salmonis*, HSMI, SRS

Funding: FIE-Sernapesca 2015-V014, VIDCA UACH.

Fish and Shellfish Immunology

195-P*

Bacterial outer membrane vesicles of *Aeromonas salmonicida* induce a proinflammatory immune response *in vitro* and *in vivo*

Ostermann, Sven (Germany)[1]; Kroniger, Tobias (Germany)[2]; Köllner, Bernd (Germany)[1]

1 - Friedrich-Löffler Institute, Institute of Immunology; 2 - Institute of Microbiology, University of Greifswald

Introduction: High mortality rates after bacterial infections cause huge annual losses for the aquaculture industry. As treatment with antibiotics is not an alternative, bacterial vaccines for intramuscular or intraperitoneal injection were developed resulting in protection but also in inflammatory granulomas and stress. Here we propose the design of a modular vaccine based on outer membrane vesicles (OMV´s) of the bacterial fish pathogen *Aeromonas salmonicida* (*A. salmonicida*). The simple preparation, the safety due to their non-replicative nature as well as the composition of natural surface exposed membrane antigens in their native confirmation are the advantages of such a vaccine design.

Methodology: In the present project, the innate immune response to OMV´s in comparison to bacterial stimulation was characterized using a peritoneal model for rainbow trout (*Oncorhynchus mykiss*). The distribution, recruitment and kinetics of leukocyte populations in peritoneum, blood, spleen and head kidney were compared using lineage marker specific monoclonal antibodies.

Results: *In vivo* trials indicate that a first immune response against OMV´s is based rather on myeloid cells than lymphocytes as it has been described for stimulation with inactivated *A. salmonicida*. For further investigation of those differences in the innate immune response, the monocyte/macrophage cell line RTS-11 was used to characterize the mRNA profile response of phagocytes to OMV´s and *A. salmonicida* bacterial particles. Next steps will include engineering of recombinant *A. salmonicida*, which produce OMV´s, presenting the immunogenic G-proteins of viral hemorrhagic septicemia virus (VHSV), infectious hematopoietic necrosis virus (IHNV) and spring viraemia of carp virus (SVCV). Those OMV´s will be used to analyze the innate immune response against bacterial and viral pathogens concerning induction of protective immune memory.

Keywords: *Aeromonas salmonicida*, outer membrane vesicles, *Oncorhynchus mykiss*, innate immune response, vaccine

196-P*

Mechanism of interleukin 12 production against intracellular bacterial infection in amberjack *Seriola dumerili*

Matsumoto, Megumi (Japan)[1]; Fujita, Sinsuke (Japan)[2]; Kubota, Taisei (Japan)[2]; Shiozaki, Kazuhiro (Japan)[2]; Kishida, Shosei (Japan)[3]; Yamamoto, Atsushi (Japan)[2]

1 - Tokyo University of Marine Science and Technology; 2 - Kagoshima University, Faculty of Fisheries; 3 - Kagoshima University, Faculty of Medicine

Introduction: Mycobacteriosis and nocardiosis are intracellular bacterial infections that causes huge economic losses in the aquaculture industry. The induction of cell-mediated immunity (CMI) is important for protection against these bacterial infections. Our previous study results suggested the importance of endogenous interleukin-12 (IL-12) production for the induction of CMI in amberjack. In the present study, we clarified the mechanism of IL-12 production in amberjack, which may be useful for the development of novel CMI-inducing vaccines.

Methodology: First, candidates IL-12 transcription factors were examined by promoter assay. Subsequently, alterations in the expression of these transcription factors in response to *Nocardia seriolae* living cell (LC) were investigated in amberjack leukocytes. Second, the effect of phagocytosis on IL-12 production was examined. Finally, the IL-12 production induced by intracellular parasitism of LC was investigated by comparing the response to LC with that to exported-repetitive protein (erp)-like gene deleted *N. seriolae* (Δ*erp-L*) which cannot establish intracellular parasitism due to changes in cell wall components, i.e, glycolipids.

Results: Result of the promoter assay and transcription factor gene expression analysis showed that the *IRF-1* and *AP-1* expression levels corresponded to the IL-12 production pattern. In addition, neutrophil phagocytosis induced IL-12 production accompanied by *IRF-1* and *AP-1* gene expression. Furthermore, Δ*erp-L* could not induce IL-12 production, nor the expression of *IRF-1* or *AP-1*, even though Δ*erp-L* was phagocytosed by neutrophils.

Discussion: In this study, we clarified several important factors for IL-12 production in amberjack: i) regulation by *AP-1* and *IRF-1*; ii) phagocytosis of LC by neutrophils; and iii) intracellular parasitism of LC. In particular, cell wall glycolipids could induce IL-12 production. In our previous study, we showed the adjuvant effect of cell wall glycolipids in amberjack in a challenge study. Taken together, glycolipids on the surface of intracellular bacteria induce host IL-12 production, and these glycolipids may thus be useful as CMI-inducing vaccine adjuvants in fish.

Keywords: intracelluar bacterium, interleukin 12, cell-mediated immunity, *Seriola* species, cell-wall glycolipids

197-P

Interaction between the soluble- and membrane-forms of tlr5 induces expression of IL-1B gene in Japanese flounder, *Paralichthys olivaceus*

Sakai, Masahiro (Japan)[1]; Morimoto, Natsuki (Japan)[2]; Kono, Tomoya (Japan)[1]; Hikima, Jun-Ichi (Japan)[1]

1 - Faculty of Agriculture, University of Miyazaki; 2 - Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki

Introduction: Bacterial flagellum is essential for their motility and consists of several different proteins whose flagella fiber possesses D1, D2 and D3 domains. In mammals, the D1 domain of flagellin is recognized by toll-like receptor 5 (TLR5) to activate innate immune responses. In fish, there are two forms of TLR5, membrane (-M) and soluble (S) forms. The TLR5S is observed only in fish and still unknown the mechanism of recognition pathway with flagellin. In this study, we attempted to understand the function of TLR5S and -M with flagellin to induce immune response in Japanese flounder (*Paralichthys olivaceus*).

Methodology and Results: Over-expressing vector DNAs encoding *Edwardsiella tarda* flagellin, TLR5S and TLR5M genes (i.e. pEtFliC, pTLR5S, pTLR5M) were transfected into the flounder embryonic (HINAE) cells. The expression of interleukin (IL)-1β gene was more strongly induced in the cell transfected with pEtFliC + pTLR5S or pEtFliC + pTLR5M compared to transfected with the empty vector or pEtFliC only. The expression levels of IL-1β gene in the cells transfected with both of pTLR5S and -M were much higher than those of the cells transfected with one of them. Furthermore, mutation of two cysteine residues, C593 or/and C620 in TLR5S indicated that C620 could be involved in dimerization of homo- or hetero-TLR5s to induce the expression of IL-1β gene in HINAE cells.

Conclusion: These results suggest that TLR5S interacts with TLR5M to activate immune response in Japanese flounder.

Keywords: TLR5, Japanese flounder, IL-1beta

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198-P*

Evidence of IGD-secreting plasmablasts and specific molecular signatures in rainbow trout gills and gut

Benedicenti, Ottavia (Italy)[1]; Periguero, Pedro (Spain)[1]; Martin-Martin, Alba (Spain)[1]; Morel, Esther (Spain)[1]; Castro, Rosario (Spain)[2]; Muñoz-Atienza, Estefania (Spain)[1]; Soleto, Irene (Spain)[1]; Diaz-Rosales, Patricia (Spain)[1]; Cerutti, Andrea (Italy)[3,4]; Tafalla, Carolina (Italy)[1]

1 - Animal Health Research Center (CISA), Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Valdeolmos, Madrid, Spain; 2 - Virologie et Immunologie Moléculaires, Institut National de la Recherche Agronomique, Université Paris-Saclay, Jouy-en-Josas, France; 3 - Department of Medicine, Immunology Institute, Icahn School of Medicine at Mount Sinai, New York, USA; 4 - Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Catalan Institute for Research and Advanced Studies (ICREA), Barcelona, Spain

Introduction: IgD is an ancient immunoglobulin for which many aspects of its regulation and function remain unclear. In teleost fish, a subset of $IgD^{+}IgM^{-}$ B cells was previously reported in rainbow trout gills and in catfish blood. However, the immune role of these cells and of secreted IgD is still not completely understood in teleosts.

Methodology: The presence of $IgD^{+}IgM^{-}$ B cells was investigated in gills, gut and spleen through flow cytometry and immunofluorescence techniques. A complete repertoire analysis of IgD in comparison to IgM and IgT was also performed in these three tissues through high throughput sequencing. Furthermore, the effects of purified IgD were determined on kidney leukocytes, characterizing through flow cytometry the cell population which was binding and responding to secreted IgD within this organ.

Results: Our results confirmed the presence of $IgD^{+}IgM^{-}$ B cells in gills and established that this cell population accounts for 83% of IgT^{-} B cells in the gut. A complete repertoire analysis of IgD confirmed the clonal expansion of IgD in these two mucosal sites but not in spleen. Remarkably IgD sequences in gills and gut share common molecular features, different from those found in spleen. Secreted IgD induced an upregulated transcription of many immune genes in the kidney, including pro- and anti-inflammatory cytokines and complement factors. These effects seem to be exerted through the binding of secreted IgD to a small subset of kidney leukocytes.

Conclusion: IgD-secreting plasmablasts represent a major B cell subset in rainbow trout gills and gut. Moreover, the IgD sequences derived from the clonal expansion of B cells found in these two tissues differ greatly from the IgD found in spleen on the cell surface in association with IgM. Secreted IgD seems to have an immunomodulatory role regardless of its antigen specificity.

Keywords: *rainbow trout, IgD, repertoire, immunomodulation*

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199-P

Identification of the immune genes of *Paracentrotus lividus* involved in response to *Vibrio anguillarum* bacterial challenge.

Fernández-Boo, Sergio (Portugal)[1]; Machado, André (Portugal)[1]; Azeredo, Rita (Portugal)[1]; Castro, Luis Filipe (Portugal)[1,2]; Costas, Benjamin (Portugal)[1,3]

1 - Centro Interdisciplinar de Investigação Marinha e Ambiental, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal; 2 - FCUP-Faculty of Sciences, Department of Biology, University of Porto, 4169-007 Porto, Portugal; 3 - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

Introduction: The edible sea urchin *Paracentrotus lividus* is the main species of sea urchin in Portuguese coasts. This species is affected for a bacterial disease caused by *Vibrio anguillarum* which provokes loss of spines, cuticula and finally death of the animal. The mechanisms underlying the immune response of sea urchins to bacterial infection are poorly understood. Some lectins, antimicrobial peptides and proteases, among other proteins such as heat shock proteins or proteins related with oxidative stress, were reported in previous studies, but the genes involved in pathogen recognition and the early response of the species are still unknown.

Methodology: In order to get deeper insights on the sea urchin immune response, a bacterial challenge was done. A total of 60 commercial size sea urchins (6.83 ± 0.49 cm) were collected from Vila Chã (41.295160ºN, 08.737073ºW), acclimated for one week and equally distributed in 6 tanks of 50 liters, 3 of them were used as control while another 3 tanks were subjected to *V. anguillarum* infection. The bacterium was cultured and bacteria was diluted to a final concentration of $3.07 \cdot 10^{12}$ bacteria/liter in the tank. 4 following challenge, sea urchins were sampled and the coelomic fluid (CF) was extracted from the internal cavity with a syringe and a 23G needle inserted through the peristomial membrane. Coelomocytes were separated from the CF by centrifugation and the main cellular and humoral immune parameters were measured. Also, coelomocytes from 3 sea urchins per tank were collected for gene expression. RNA was extracted individually and a pool of RNA from 3 sea urchins/tank was done and subjected for RNAseq analysis.

Results: Differences in nitric oxide concentration, bactericidal activity and RNA expression was seen between challenge and non-infected individuals.

Conclusion: Bacterial challenge of *Vibrio anguillarum* provokes a clear response of sea urchin immune system based on coelomocytes activities also in this presentation we will unveil, for the first time, the genes involved in pathogen recognition as well as in the defence of sea urchins to infections.

Keywords: *Paracentrotus lividus*, immune response, *Vibrio anguillarum*, transcriptome, bacterial challenge

Funding: CRAGIAMP project – FCT 02/SAICT/2017-030232 supported by COMPETE 2020, PORTUGAL 2020, FEDER and FCT.

200-P

Rockfish (*Sebastes schlegelii*) MYD88 molecular identification and functional analysis

Shanaka, K.A.S.N (Republic of Korea)[1]; Priyathilaka, Thanthrige Thiunuwan (Republic of Korea)[1]; Lee, Jehee (Republic of Korea)[1,2]

1 - Marine Molecular genetics LAB, 5373, Department of Marine Life Sciences, School of Marine Biomedical Sciences, Collage of Ocean Science 4, Jeju National University, 102 Jejudaehakro, Ara-Dong, Jeju Special Self-Governing Province 63243, Republic of Korea; 2 - Marine Molecular genetics LAB, 5373, Department of Marine Life Sciences, School of Marine Biomedical Sciences, Collage of Ocean Science 4, Jeju National University, 102 Jejudaehakro, Ara-Dong, Jeju Special Self-Governing Province 63243, Republic of Korea

Introduction: Studies on innate immune signal transducers hold paramount importance in fish vaccine development, wherein in this study, signal transducing adaptor protein, myeloid differentiation primary response 88 (Myd88) in economically important rockfish was characterized in *in silico*, *in vivo*, and *in vitro*.

Methodology: Coding sequence of rockfish *MyD88* (*SsMyD88*) was identified from a previously constructed rockfish transcriptome database. To evaluate the expression under no immune stimulant, tissue samples were obtained from healthy rockfish. To evaluate the effects of immune stimulants, rockfish were injected with immune stimulants, including polyinosinic-polycytidylic acid (poly I:C), lipopolysaccharide (LPS), and *Streptococcus iniae* (*S. iniae*), and then, the post-injection spleen tissue samples were obtained. RNA extraction, followed by cDNA synthesis, was carried out and the relative gene expression was determined by qPCR. *SsMyD88* bearing vector was transiently transfected into the HEK-293 cells along with vector bearing NF-κB luciferase construct for the luciferase reporter assay

Results: SsMyD88 is a 288 amino-acids long protein; its pI and molecular weights are 5.1 and 33.1 KDa, respectively. SsMyD88 contains two main domains, N-terminal death domain (DD) and C-terminal Toll/interleukin-1 receptor (TIR) domain. Immune-unchallenged rockfish exhibited highest expression of *SsMyD88* in liver, whereas other important immune organs, such as spleen and gills, exhibited relatively high expression. Immune challenge experiment revealed upregulation of *MyD88* transcription after exposure of poly I:C, LPS, and *S. iniae* in spleen. Luciferase assay revealed that rockfish MyD88 induced significant and enhanced activation of NF-κB compared to the control.

Conclusion: High expression of *SsMyD88* was found in important immune organs compared to non-immune organs, revealing its importance in immune pathways. During immune challenge experiment, *SsMyD88* expression was induced by all the distinct immune stimulants used, and therefore, SsMyD88 may be involved in diverse range of immune pathways. Luciferase assay results further confirmed the capability of SsMyD88 to activate the NF-κB. All these findings confirm the diverse and important roles of SsMyD88 during the innate immune defense.

Keywords: *Sebastes schlegelii*, immune challenge, mRNA expression, innate immunity, luciferase assay

201-P

Effect of a commercial immunostimulant on the immune performance and immune-related gene expression of meagre (*Argyrosomus regius*) juveniles

Pires, Pedro (Portugal)[2]; Passos, Ricardo (Portugal)[2]; Simões, Marco (Portugal)[2]; Ferreira, Inês (Portugal)[2]; Marques, Cátia (Portugal)[1]; Soares, Florbela (Portugal)[1]; Barata, Marisa (Portugal)[1]; Pousão-Ferreira, Pedro (Portugal)[1]; Baptista, Teresa (Portugal)[2]

1 - Portuguese Institute of the Ocean and Atmosphere, Aquaculture Research Station, Av. 5 de Outubro s/n, 8700-305, Olhão, Portugal; 2 - Marine and Environmental Sciences Centre, ESTM, Instituto Politécnico de Leiria, Peniche, Portugal

Introduction: Intensification of aquaculture has become an important practice in recent years, leading to an increase in stress factors for produced fish. It is well known that nutrition may affect fish health and immune response, therefore, the purpose of this work is to evaluate the performance of meagre juveniles fed with a diet enriched with a commercial immunostimulant (a yeast-based product, rich in bioactive ingredients) and subjected to a stress challenge.

Methodology: Meagre juveniles were obtained from Aquaculture Research Station-EPPO. Afterwards 240 fish (77.1 ± 11.9 g) were randomly distributed in six circular 500L tanks organized in two closed recirculation systems of triplicate tanks: Treatment A – Commercial diet supplemented with the immunostimulant; Treatment B – Commercial diet (control). Water temperature kept at 17.4 ± 1.2 °C, salinity at 35 ± 3 with a fish density of 6 kg/m^3. At the 8th week, eight fish per tank were blood sampled and three were tissue sampled for gene expression. The remaining fish were subjected to a stress challenge (air exposure during 3 minutes). Samplings were repeated at 6 hours and 2 weeks after the challenge. Plasma lysozyme, peroxidase and bactericidal activity were evaluated in the two groups. The expression of immunity related genes was also assessed.

Results: Two weeks after the stress, the immunostimulant fed fish displayed a higher lysozyme concentration, suggesting an inflammatory response, and a lower peroxidase activity, indicating an increase of oxidative stress and a reduction of immunity. Six hours post-stress the supplement fed fish had higher bactericidal activity, which could indicate an acute stress response.

Conclusion: Although the results for lysozyme and peroxidase activities were somehow contradictory, suggesting that the fish may have been under a constant stress, the results for lysozyme and bactericidal activity are promising in regard to the immunostimulant effect of the diet.

Keywords: immunostimulant, gene expression, meagre

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202-P*

Hallmark pro-inflammatory cytokines of lumpfish (*Cyclopterus lumpus*)

Eggestøl, Håvard Øritsland (Norway)[1]; Lunde, Harald Sæbø (Norway)[1]; Haugland, Gyri Teien (Norway)[1]

1 - University of Bergen, Department of Biological Sciences

Introduction: Lumpfish is a novel and highly popular fish in Norwegian aquaculture, however, little is known about its health and biology and many inflammatory diseases are prevalent. Besides its economic importance, the lumpfish represent a poorly described clade of teleosts in terms of fish immunology and is therefore of great comparative interest. The hallmark pro-inflammatory cytokines, IL-1β, IL-6, IL-18 and TNF-α, are described in teleosts as well as in mammals. In addition, two teleost specific IL1 family members, nIL-1F1 and IL-1Fm2, have been described. Fish displays a high degree of genetic variations due to several whole genome duplication events, causing many cytokines to be duplicated or triplicated. Furthermore, much of the fish cytokine functions is unknown.

Methodology: We identified and molecular characterized the four teleost members of the IL-1 family, IL-6 and TNF-α in lumpfish, studied their current phylogeny in teleosts, studied their tissue dependent gene expression and their stimulation potential by PAMPs *in vitro*.

Results: The phylogenetic analysis grouped lumpfish cytokines with other neoteleostei orthologs, although other clades showed previously undescribed evolutionary relationships. Sequence characterization identified expected motifs of other teleost sequences, with the exception of IL-6. Lumpfish IL-6 had a 5' insert and transcription of the genomic area corresponding to the first intron of other teleost IL-6 sequences occurred. Moreover, IL6 displayed a unique tissue distribution being highly expressed in immune privileged organs: brain, eye and gonad. The expression levels of IL6 and TNF-α were paralleled in all non-immune-privileged organs, at its lowest in liver and highest in immune related organs, head kidney, spleen and thymus. IL-6, TNF-α, IL-1β and nIL-1F1 showed a similar expression pattern in the *in vitro* PAMP-induction experiment, being highly induced of flagellin and moderately induced of poly(I:C) and CpG.

Conclusion: Lumpfish have and express the hallmark pro-inflammatory cytokines. They are highly regulated by flagellin and moderately induced by poly (I:C) and CpG. Lumpfish IL-6 displays a unique genomic organization.

Keywords: cytokine, inflammation, PAMP-induction, lumpsucker, phylogeny

Funding: The Research Council of Norway and University of Bergen.

203-P*

Characterization and immune responses of liver-expressed antimicrobial peptide 2A (LEAP 2A) from redlip mullet (*Liza haematocheila*)

Liyanage, Dileepa Sripal (Republic of Korea)[1]; Omeka, Welivitiye Kankanamge Malithi (Republic of Korea)[1]; Yang, Hyerim (Republic of Korea)[1]; Lee, Jehee (Republic of Korea)[1]

1 - Jeju National University

Introduction: The redlip mullet has a widely distributed in the sea and significantly used as a food source in different parts of the world. According to the statistics published by the Korean ministry of maritime affairs and fisheries, 2012, the mullets have accounted for 8% of the total food consumption and cultivation in Korea. They are susceptible to infection due to different kinds of microorganisms, such as *Lactococcus garvieae*, during the cultivation.

Methodology: In this study, liver-expressed antimicrobial peptide 2A from mullet was characterized using *in silico* analysis and temporal and spatial expression analysis.

Results: Evolutionary analysis revealed that LEAP2A belongs to LEAP2 superfamily, which had A, B, and C subclasses in fish. LEAP2A consisted of 103 amino-acids with molecular weight 11.4kDa. Pairwise alignment of LEAP2A with orthologs showed that highest sequence similarity (85.8%) and identity (82.1%) with that of Lates calcarifer. LEAP2 peptide is composed of N-terminal signal peptide, prodomain, and C-terminal mature peptide. A conserved motif (RXXR) is located before the cleavage site of the sequence separating the prodomain and the mature peptide. In addition, the C-terminal mature peptide harbored a conserved motif (MTPLWR) and minor variation was found within the motif. Quantitative real-time PCR results revealed highest tissue-specific immune expression in the liver and blood; a small level of expression was observed among twelve different tissues from healthy mullet fish. As the liver and blood tissues actively participate in the microbe invading process, high expression was observed. Mullets were subjected to immune stimulation with lipopolysaccharides, polyinosinic:polycytidylic acid, and *Lactococcus garvieae* to observe the change in transcriptional pattern in LEAP2. Blood tissue-specific immune expression showed upregulation during 6-24 hours post-infection, after which downregulation occurred.

Conclusion: In response to the immune stimulation, liver exhibited high expression of LEAP2 followed by cleavage of the mature peptides by endoprotease; it activated the immune response and alternative splicing occurred in LEAP2 gene by cleavage. Mature peptide showed selective antimicrobial activities by four conserved cysteine di-sulfide bonds and play a major role in immunity against harmful microorganisms. Altogether, the LEAP2 gene can be identified as an immunologically important gene associated with innate immune system.

Keywords: LEAP2, antimicrobial peptide

206-P

A key regulator of the mitochondrial apoptotic pathway, bax from redlip mullet (*Liza haematochelia*); molecular characterization and expression analysis

Sandamalika, Gayashani (Republic of Korea)[1]; Yang, Hyerim (Republic of Korea)[1]; Lee, Jehee (Republic of Korea)[1]

1 - Jeju National University

Introduction: Apoptosis, also referred to as programmed cell death, plays a significant role in maintaining homeostasis, immune defense, and development, which is responsible for the ecological success of an organism. Bcl-2 family proteins regulate the mitochondrial apoptotic pathway.

Methodology: A pro-apoptotic member of the Bcl-2 family was identified from redlip mullet database and referred to as *LhBAX*. The corresponding amino-acid sequence was determined by ORF finder, an online server. Molecular and structural characteristics, homology, and phylogenetic analysis were performed using various bioinformatics tools. In order to perform the tissue-specific expression analysis of *LhBAX*, quantitative real-time PCR was carried out with different tissues of mullet. Furthermore, fish were challenged with different immune stimulants (poly I:C, LPS, and *Lactococcus garvieae*) and the transcriptional responses were determined in a time-course manner.

Results: LhBAX consisted of 203 amino-acids with 22.9 kDa molecular weight. Based on sequence analysis, LhBAX contained Bcl-2-like apoptosis inhibitor domain and showed high similarity with *Monopterus albus* (77.9%) BAX. In the phylogenetic tree, LhBAX clustered together with other fish BAX, showing its closest relatedness with *Amphiprion ocellaris* and *Monopterus albus* BAX. The tissue distribution analysis showed its highest expression in spleen (10.9-fold vs. control). After induction with poly I:C, LPS, and *Lactococcus garvieae*, *LhBAX* expression was significantly upregulated within 24 h.

Conclusion: This study provides an experimental insight into the molecular and transcriptional characteristics of a key regulator of the mitochondrial apoptotic pathway, BAX, from red lip mullet, *Liza haematocheila*. The structural characteristics and phylogenetic analysis of LhBAX confirmed it to be a Bcl-2 family member. Their expression patterns among various tissues were determined, and furthermore, the significant changes in their expression after immune challenge allowed us to speculate possible functions of *LhBAX* in the innate immune system of red lip mullets.

Keywords: apoptosis, redlip mullet, BAX, immune responses

207-P

Molecular and transcriptional analysis of peroxiredoxin 3 (HAPRX3), and its innate immune responses in big belly seahorse (*Hippocampus abdominalis*)

Samaraweera, Anushka Vidurangi (Republic of Korea)[1]; Priyathilaka, Thanthrige Thiunuwan (Republic of Korea)[1]; Lee, Sukkyoung (Republic of Korea)[1]; Lee, Jehee (Republic of Korea)[1]

1 - Jeju National University

Introduction: Peroxiredoxins are group of antioxidant proteins that help to survive under H_2O_2-mediated oxidative stress conditions through their peroxidase activity. Peroxiredoxin 3 (Prx3) belongs to the typical 2-Cys Prx class, based on their catalytic mechanism and position of Cys residues. Prx3 is mitochondrial protein which belongs to the thioredoxin-like superfamily. During the catalytic mechanism, peroxidatic cysteine in one subunit reacts with resolving cysteine from other subunit of homodimer to form intersubunit disulfide bond and disulfide is reduced using NADPH under the actions of thioredoxin and thioredoxin reductase.

Methodology: The *HaPrx3* cDNA sequence was identified from the big belly seahorse transcriptome library and bioinformatics analysis was done. The tissue distribution of *HaPrx3* was analyzed in healthy seahorse using qPCR. The immune challenge experiment was done using lipopolysaccharide (LPS), polyinosinic:polycytidylic acid, *Edwardsiella tarda*, and *Streptococcus iniae*. The mRNA expression level of *HaPrx3* in blood was explored by qPCR. The recombinant protein (rHaPrx3) was expressed and purified using the pMAL Protein Fusion and Purification System.

Results: The open reading frame of *HaPrx3* is 726 bp which encodes for 241 amino-acids long protein with 26.20 kDa molecular weight. The calculated pI is 7.04. Pairwise sequence alignment showed the highest similarity and identity with the *Oplegnathus fasciatus* (striped beakfish) and multiple sequence alignment contained conserved 2-cys Prx motif 1(FYPLDFTFVCPTEI) and motif 2 (GEVCPA), including peroxidatic and resolving cysteines. Highest expression for the *HaPrx3* was observed in ovaries among fourteen tissues. The mRNA level of *HaPrx3* was significantly upregulated after the immune challenge experiment in the seahorse blood cells.

Conclusion: Taken together, we characterized the molecular and functional levels of *HaPrx3* of big belly seahorse. Transcription levels of *Haprx3* were studied in wide range of tissues and prominently in ovaries. Moreover, immune challenge experiment revealed that HaPrx3 plays an important role in the innate immunity of seahorse against bacterial and viral pathogenic attack.

Keywords: innate immunity, peroxiredoxin, cDNA, antioxidant

208-P

Molecular characterization, and expression analysis of calreticulin from big belly seahorse *Hippocampus abdominalis*

Sellaththurai, Sarithaa (Republic of Korea)[1]; Priyathilaka, Thanthrige Thiunuwan (Republic of Korea)[1]; Lee, Jehee (Republic of Korea)[1]

1 - Jeju National University

Introduction: Calreticulin (CRT) is a multifunctional protein present in a wide range of eukaryotic cells. CRT was initially isolated from endoplasmic reticulum, and later, it was isolated from other cellular structures, such as cytoplasm, cell membrane, and extracellular matrix. CRT is involved in the extracellular Ca++ homeostasis and facilitates the correct folding of other proteins, including major histocompatibility complex (MHC) class 1 molecules and their assembly factor, tapasin. Thus, it plays a several roles in immune response.

Methodology: In this study, we molecularly characterized the homology of Seahorse (*Hippocampus abdominalis*) calreticulin (designated HaCRT) using *in silico* analysis and analyzed the response after immune challenge experiment followed by qPCR.

Results: *HaCRT* ORF consisted of 1269 bp, encoding a 423 amino-acids long protein with the predicted molecular weight of 49.04 kDa, and an estimated isoelectric point of 4.37. NCBI conserved domain search revealed CRT super family from HaCRT (F24-I333). The predicted amino-acid sequence of HaCRT showed 86.2%, 86.0%, 72.4%, 72.4%, and 60.9% identity with the CRT sequence of *Oreochromis niloticus*, *Maylandia zebra*, *Homo sapiens*, *Lonchura striata domestica*, and *Drosophila melanogaster*, respectively. In the phylogenetic analysis, HaCRT was subclustered with CRT of *Maylandia zebra* and *Oreochromis niloticus*. The *HaCRT* mRNA transcript was detected in all extracted tissues using real-time PCR (blood, brain, gill, heart, intestine, liver, kidney, muscle, ovary, pouch, skin, spleen, stomach, and testis). Brain and ovaries showed highest *HaCRT* mRNA expression compared to other tissues. The variation in *HaCRT* mRNA levels after immune challenge was quantified by real-time PCR using blood and liver tissue samples. Significant upregulation of *HaCRT* were observed following treatment with all four stimulants (*Edwardsiella tarda*, *Streptococcus iniae*, polyinosinic:polycytidylic, and lipopolysaccharide) in both tissues within the experimental period (3 h, 6 h, 12 h, 24 h, 48 h, and 72 h).

Conclusion: According to the *in silico* study, HaCRT contained the conserved domain of CRT super family found in previously identified calreticulins. In addition, the pairwise alignment and phylogenetic analysis reinforced the homology of HaCRT with CRT of other teleost species. The observed significant upregulation post-immune challenge suggested that HaCRT might have a potential role in host immunity.

Keywords: *Hippocampus abdominalis*, calreticulin, immune challenge, qPCR

209-P

Antiviral activity against VHSV infection, transcriptional regulation in response to immune stimulants of IRF6 And IRF8 in *Hippocampus abdominalis*

Tharuka, M.D. Neranjan (Republic of Korea)[1]; Sellaththurai, Sarithaa (Republic of Korea)[1]; Lee, Jehee (Republic of Korea)[1]

1 - Department of Marine Life Sciences & Fish Vaccine Research Center, Jeju National University

Introduction: Interferon regulatory factors (IRFs) act as one of the most important transcription mediators with multiple biological functions, such as antiviral and antimicrobial defense, cell differentiation, immune modulation, and apoptosis.

Methodology: Three IRF family members (HaIRF6, HaIRF8) of big belly seahorse (*Hippocampus abdominalis*) were molecularly and functionally analyzed for their sequence, expression level, and antiviral capacities. Expression levels were detected under immune challenged (LPS, poly I:C, *Streptococcus iniae*) and unchallenged conditions. The challenges were administrated intraperitoneally and tissues collected at different time points. GFP-tagged HaIRFs were transfected and visualized after staining under the fluorescence microscope.

Results: Gene expression of HaIRFs was detected *in vivo* in transfected FHM cells against VHSV infection. IRFs significantly reduced viral gene expression at 24 h and 48 h post-infection. Cellular localization of HaIRFs was observed using GFP-tagged expression vectors in FHM. IRFs were ubiquitously expressed in all analyzed tissues. The mRNA expressions of IRF6 and IRF8 increased significantly at early post-injection time point in blood and gill following LPS, poly I:C, and *Streptococcus iniae* challenges.

Conclusion: These findings revealed the involvement of seahorse IRFs in host defense mechanism against immune stresses and their effective antiviral potentials against VHSV infections.

Keywords: *Hippocampus abdominalis*, antiviral activity, immune stimulants

210-P*

In silico study of the genes involved in alternative complement system in big-belly seahorse *Hippocampus abdominalis*

Jeong, Tae Hyug (Republic of Korea)[1,2]; Lee, Sukkyoung (Republic of Korea)[1,2]; Kwon, Hyukjae (Republic of Korea)[2]; Liyanage, D.S. (Republic of Korea)[2]; Omeka, W.K.M. (Republic of Korea)[2]; Lee, Jehee (Republic of Korea)[1,2]

1 - Fish Vaccine Research Center, Jeju National University, Republic of Korea,; 2 - Department of Marine Life Sciences, Jeju National University, Republic of Korea

Introduction: The complement system plays an important role in host immunity. There are three types of complement pathways, namely the classical, the lectin, and the alternative pathways. Complement component 3 (C3) is the main molecule that triggers the alternative pathway via interactions with complement factor B (CFB) and D (CFD). When this pathway activates, a membrane attack complex is formed by complement component 5 (C5), 6 (C6), 7(C), 8 (C8), and 9 (C9) to induce cell lysis of pathogens. In this study, the genes involved in alternative complement system of big-belly seahorse *Hippocampus abdominalis* were characterized.

Methodology: The complement-related genes were identified from the big-belly seahorse transcriptomic database that was developed using the 454 GS FLS™ and PacBio sequencing data. These cDNA sequences of complement components were analyzed using *in silico* tools, such as CLC main workbench 8 software, NCBI blastx and blastp, MEGA 7, NCBI Conserved Domain Database (CDD), ExPASy PROSITE, SMART, SignalP 4.1 online server, and NetNGlyc 1.0 server. The spatial and temporal transcriptional profiling was done in immune challenged fish using quantitative real-time PCR (qPCR).

Results: The partial or complete ORF sequences were confirmed to be associated with the alternative complement system and designated as HaC3, HaC5, HaC6, HaC7, HaC8a×b×g, HaC9, HaCFB, and HaCFD. The size range of complete ORF sequences was 813 bp to 5076 bp. The proteins encoded by these genes were mainly composed of thrombospondin type 1 repeats (TSP1), low-density lipoprotein receptor class A domain (LDLa), MAC/Perforin domain (MACPF), complement control protein (CCP), and factor I membrane attack complex (FIMAC) domains.

Conclusion: According to the *in silico* analysis, spatial and temporal expression results of the selected genes shows association with alternative complement system. Therefore, we can conclude that these genes are important in immune and host defense mechanisms in *Hippocampus abdominalis*.

Keywords: big-belly seahorse, complement component, alternative complement system, immune response

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211-P*

Immune response of koi carp and amur wild carp infected with KHV

Machat, Radek (Czech Republic)[1]; Vesely, Tomas (Czech Republic)[1]; Hodkovicova, Nikola (Czech Republic)[1,2]; Minarova, Hana (Czech Republic)[1,2]; Ondrackova, Petra (Czech Republic)[1]; Matiasovic, Jan (Czech Republic)[1]; Faldyna, Martin (Czech Republic)[1]

1 - Veterinary Research Institute, Brno, Czech Republic; 2 - University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

Introduction: Koi herpesvirus (KHV) causes serious disease (KHVD) affecting common carp (*Cyprinus carpio L.*) and some of its hybrids with other members of *Cyprinidae* family. KHV mortality rates are influenced, besides environmental factors, age and health conditions, by carp strain, and may reach 100%, but also a couple of percents. Influence of KHV infection on expression levels of cytokines and other genes affecting immune system of carp was tested for revealing its possible influence on KHV mortality.

Methodology: Two subspecies of common carp were chosen for KHV infection: koi carp (*C. c.* "koi"), which is very susceptible to the disease and Amur wild carp (*C. c. haematopterus*), strain resistant to diseases including KHVD. Carps of both subspecies were divided into two groups: control and infected. The virus was administered intraperitoneally with dose corresponding 10^4 $TCID_{50}$/ml. Samples of blood, skin, gills, head kidney, spleen, hepatopancreas and gut were collected at two time points: 3 dpi and 7 dpi. Time points were chosen to record early immune response, between first clinical signs and early mortality. Expression of several immune reaction related genes were detected using qPCR method. The study was focused on pro-inflammatory cytokine IL1β, anti-inflammatory cytokine IL10 and some members of antiviral signaling pathway of class I IFN.

Results: Symptoms of KHV were not detected in infected Amur wild carps. On the contrary in infected koi carps was observed increased excretion of mucus at time point 7dpi, but no more symptoms of the disease. According to present data, the induction of expression of *Il10* was detected in skin of infected Amur wild carps in contrast to its suppression in infected koi carp. Significantly higher expression of *il1β* was detected within gills and skin of koi. Meanwhile there were not differences between levels of expressions of chosen members of IFN I signaling pathway.

Conclusion: Obtained data shown difference between intensity of immune reaction of koi carp and Amur wild carp on KHV infection, but so far there was no observed reason of lesser susceptibility of Amur wild carp to KHV.

Keywords: KHV, koi carp, amur wild carp, qPCR, cytokines

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212-P

Molecular cloning and functional analysis of B cell activating factor in rockfish (*Sebastes schlegelii*)

Madhuranga, W.S.P. (Republic of Korea)[1]; Lee, Jehee (Republic of Korea)[1]

1 - Department of Marine Life Sciences and Fish Vaccine Research Center, Jeju National University, Jeju Self Governing Province 63243, Republic of Korea

Introduction: B cell activating factor (BAFF) is a type II membrane binding protein that belongs to the tumor necrosis factor ligand superfamily member 13B (TNFSF13B). The important roles of this protein include B-cell proliferation, maturation, and survival.

Methodology: Rockfish (*Sebastes schlegelii*) B cell activating factor (*SsBAFF*) homolog was identified and its biological functions were characterized. Complete cDNA of rockfish BAFF was obtained from rockfish cDNA transcriptomic database. Structural and functional features of protein were identified using several bioinformatics tools, NCBI BLAST program, ExPASy PROSITE, and Signal P. *BAFF* mRNA expression level of rockfish was analyzed by quantitative real time PCR technique. The conserved domain parts were identified using multiple sequence alignment tool Clustal W. The phylogenic evolutionary analysis was done according to the Neighbor-Joining (NJ) method using the MEGA 5.0 software. The putative *SsBAFF* protein size was determined by SDS-PAGE analysis. Putative protein 3D structure was designed by the SWISS-MODEL online protein modeling tool and PyMOLv1.5 viewer software. Cell proliferation and survival rate following introduction of the SsBAFF-MBP protein was determined by the flow-cytometry assay.

Results: The transmembrane domain, TNF family domain, D-E loop, and N- glycosylation sites were identified using the online software tools. The *SsBAFF* mRNA expression was higher in rockfish spleen tissue compared to other tissues. *Epinephelus awoara BAFF* gene sequence aligned with *SsBAFF* sequence with more than 85% identity in the pairwise homology analysis. Phytogenic evolutionary analysis showed that the *Epinephelus awoara BAFF* and *SsBAFF* have close evolutionary relationship with each other. *SsBAFF* protein molecular weight was determined to be 29.5 kDa using SDS-PAGE. Flow-cytometry analysis revealed enhanced B cell proliferation and viability after addition of recombinant *SsBAFF* protein.

Conclusion: We examined the structural and functional aspects of recombinant *SsBAFF*. We identified the conserved domains of *SsBAFF* and found its evolutionary stage. We quantified the *SsBAFF* mRNA levels in various tissues and found that it was significantly induced with certain immune stimulants and pathogens. Flow-cytometry analysis revealed that cell proliferation and survival rate was significantly enhanced after treatment with *SsBAFF*.

Keywords: BAFF, flow-cytometry, proliferation

213-P

Diverse rainbow trout lineage susceptibilities to PKD and furunculosis

Palikova, Miroslava (Czech Republic)[1,2]; Mendel, Jan (Czech Republic)[3]; Syrova, Eva (Czech Republic)[2]; Papezikova, Ivana (Czech Republic)[1,2]; Minarova, Hana (Czech Republic)[4]; Pojezdal, Lubomir (Czech Republic)[4]; Vesely, Tomas (Czech Republic)[4]; Navratil, Stanislav (Czech Republic)[2]; Mares, Jan (Czech Republic)[1]

1 - Mendel University, Brno; 2 - University of Veterinary and Pharmaceutical Sciences, Brno; 3 - Institute of Vertebrate Biology of Czech Academy of Sciences, Brno; 4 - Veterinary Research Institute, Brno

Introduction: The aim of this study was to assess the differences in susceptibility to *Tetracapsuloides bryosalmonae* and *Aeromonas salmonicida* infections between two breeding lineages of rainbow trout originating from Northern Ireland and from Italy.

Methodology: Dissimilarity of the rainbow trout lineages was determined using a genotyping method with polymorphic microsatellite loci. Cumulative mortality associated with PKD was recorded on the farm. A total of 40 samples of renal tissue from randomly selected fish were taken. The numbers of parasites in the renal tissue were detected by immunohistochemistry. The extracted kidney DNA was used for qualitative and quantitative parasite examination of *T. bryosalmonae* by PCR and real-time PCR (qRT-PCR). Experimental challenge with *A. salmonicida* was performed in both lineages of rainbow trout under laboratory conditions. Titers of specific antibodies were detected using the ELISA method.

Results: The microsatellite analysis unambiguously differentiated between the two rainbow trout lineages and pointed to differences in their origin as well as genetic basis. This differentiation was supported by the high number of private alleles (10) within populations and also the values of the fixation index (F_{ST} = 0.082) and Nei's genetic distance (0.225). Cumulative mortalities associated with PKD were up to 35% and 12% in rainbow trout originating from Italy and from Northern Ireland, respectively. Similarly, pathological changes in the kidneys and numbers of *T. bryosalmonae* were higher in the rainbow trout originating from Italy. Statistically significant differences between both lines were found in titers of specific antibodies measured 4 and 8 weeks after the experimental challenge with *A. salmonicida*. The breeding lineage that originated from Italy showed almost a double amount of antibody titers.

Conclusion: In the two genetically different lineages under study, significant differences were found in susceptibilities to both PKD and furunculosis. The lineage of rainbow trout more resistant to PKD showed higher susceptibility to furunculosis.

Keywords: lineage resistance, microsatellite loci, challenge, antibodies

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214-P*

Rainbow trout express several PRDM1/BLIMP-1 genes

Gómez-Esparza, Maria Cristina (Spain)[1]; Perdiguero, Pedro (Spain)[1]; Tafalla, Carolina (Spain)[1]; Diaz-Rosales, Patricia (Spain)[1]

1 - Animal Health Research Center (CISA-INIA)

Introduction: B lymphocyte-induced maturation protein 1 (Blimp-1), also known as PR domain containing 1 (PRDM1), encoded by *Prdm1*, is a key transcriptional regulator of B cells linked to plasma differentiation. Upon antigen encounter, B cells differentiate into antibody secreting cells (ASCs), process controlled by a complex network of transcription factors. Within this network, in mammals, the expression of the transcription factor Blimp-1 promotes the initiation of a gene expression program that drives plasma cell differentiation. Despite the importance of this process in acquired immunity, how it is regulated in teleosts has been largely unexplored. Until now, only one *Prdm1* had been reported in teleosts. In this study, we describe a complete repertoire of *Prdm1* genes in rainbow trout (*Oncorhynchus mykiss*), revealing a protein family likely forged through ancient duplication events.

Methodology: The rainbow trout genome was used to search for *Prdm1* homologs. Constitutive and tissue-specific expression of these different genes was analyzed through real-time PCR. To further explore the regulation of these Blimp-1, *in vivo* experiments were conducted. Thus, bath and intraperitoneal injection challenges with viral hemorrhagic septicemia virus (VHSV) were performed and gills and spleen collected to analyze the *Prdm1* gene expression. Finally, cells that phenotypically resemble plasmatic cells were isolated by FACS from the peritoneal cavity along with naïve B cells, and used to determine the levels of *Prdm1* expression.

Results: Eight different *Prdm1* genes have been identified in the rainbow trout genome. All of these genes are expressed constitutively in different tissues. In addition, three of these genes expressed isoforms generated by alternative splicing. We further demonstrated that many of these Blimp-1 forms were regulated during the course of a VHSV infection. Finally, cells that phenotypically resemble plasmatic cells contained significantly higher quantities of many of these Blimp-1 transcripts than those obtained in naïve B cells.

Conclusion: Rainbow trout possess many functional *Prdm1* genes, many of which seem associated with differentiation of B cells. The present results provide the basis for multiple future research studies aimed at better understanding the process of plasma cell differentiation in teleosts.

Keywords: rainbow trout, Blimp-1, B cells

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215-P

Characterization of a specific monoclonal antibody against CD8A in koi carp (*Cyprinus carpio*)

Chun, Jin Hong (Republic of Korea)[1]; Jung, Jae Wook (Republic of Korea)[1]; Lee, Jung Seok (Republic of Korea)[1]; Im, Se Pyeong (Republic of Korea)[1]; Kim, Si Won (Republic of Korea)[1]; Lazarte, Jassy Mary (Republic of Korea)[1]; Lee, Ae Rin (Republic of Korea)[1]; Kim, Young Rim (Republic of Korea)[1]; Kim, Jae Sung (Republic of Korea)[1]; Jung, Tae Sung (Republic of Korea)[1]

1 - Gyeongsang National University

Introduction: The koi carp (*Cyprinus carpio*) is one of the important subspecies of the aquaculture industry and one of the most popular ornamental fish in worldwide. Therefore, studies about their immunity is essential in the improvement of their productivity. Although many studies about CD8α-related immunity have been established in teleost, it is mainly at the genetic level, and little is known about its function and role in the immune response at the cellular level. In this study, we produced monoclonal antibody (mAb) 1c9 which can detect koi CD8α protein.

Methodology: Koi CD8α-2,3(CPDEAKYKVNKIGKV, CGEPDPATSPPKIAP) peptide was synthesized as an antigen, and were used for the production of mAb 1c9. Western Blotting was performed to confirm the specificity of mAb 1c9. Leukocytes recognized by 1c9 was examined under fluorescence and confocal microscope. Flow cytometry analysis was performed to determine the distribution of CD8α-positive cells among vital organs.

Results: Western blotting of leukocytes extracted from tissues of koi showed 16 kDa size band corresponding to CD8α protein. In CD8α-positive cells, many lymphocytes were positively stained with FITC. This indicates that mAb 1c9 binds specifically to the surface of the CD8α lymphocytes. To examine the distribution of CD8α-positive cells, flow cytometry analysis is still on-going.

Conclusion: The monoclonal antibody mAb 1c9 we developed specifically detects the CD8α-positive lymphocytes of koi carp. Through the use of this mAb, we were able determine the distribution of these lymphocytes in the organs of koi carp. This information will give a better understanding of the role of CD8α-expressing cells in the adaptive immunity of koi carp.

Keywords: CD8 alpha, monoclonal antibody

216-P*

Molecular characterization and structural analysis of CD4 homologues in brown trout (*Salmo trutta*)

Ashfaq, Hassan (Austria)[1]; Soliman, Hatem (Austria)[1]; El-Matbouli, Mansour (Austria)[1]

1 - Clinical Division of Fish Medicine, University of Veterinary Medicine, Vienna

Introduction: Brown Trout is a geographically diverse fish, habituating more towards European conditions. Fish possess both innate as well as adaptive immunity. The adaptive immune system recognizes pathogens by means of two cellular receptors, the B-cell and the T-cell receptors (TCR). A cluster of differentiation 4 (CD4), a co-receptor of TCR, is a transmembrane glycoprotein that is acting as a T helper cells (T_h) marker. T_h lymphocytes govern immune responses through specific antigen recognition and subsequent secretion of effector and regulatory cytokines. This study aims at identifying and characterizing of the CD4 homologues of the brown trout.

Methodology: Head kidney was sampled from the brown trout (n = 5, weight 100 - 120 g) and preserved in RNA*later* for RNA extraction. Different primers, targeting CD4 genes, were designed based on the CD4 sequences of other fish species and used to amplify the brown trout CD4 fragments. Rapid amplification of the cDNA ends (RACE) was used to get the complete CD4 genes. Amplified products were then subjected to bioinformatics analysis for identification and characterizations.

Results: Three CD4 genes (CD4-1, CD4-2a and CD4-2b) were identified and their genomic structural organization analyzed in brown trout. The cDNA of CD4-1 was 1796 bps in length with 490 amino acids, while CD4-2a and CD4-2b´s cDNAs contain 1798 and 1592 nucleotides with 314 and 332 amino acids residues respectively. All the three genes were structurally similar to other vertebrates CD4 in term of genomic organization; Ig-like domains, transmembrane region and a cytoplasmic domain with CXC (LCK binding) motif. The occurrence of these configurations proposed the same phenomenon for T- cell activation in all vertebrates i.e. disulphide bond, p56LCK.

Conclusion: Three CD4 homologues identified in brown trout and their genomic structure was analyzed. Brown trout CD4-1 gene lacks cysteine residue in domain (D1) which is essential for formation of disulphide bond for structural stability. Identification of these CD4 genes will pioneer to investigate the response of T-cells to different invading pathogens in combination with already published MHC II proteins in brown trout and the further assessment of Th cell subsets.

217-P*

In vitro effect of temperature on phagocytic activity in rainbow trout (*Onchorhynchus mykiss*)

Minarova, Hana (Czech Republic)[1,2]; Palikova, Miroslava (Czech Republic)[2]; Mares, Jan (Czech Republic)[3]; Faldyna, Martin (Czech Republic)[1]; Ondrackova, Petra (Czech Republic)[1]

1 - Veterinary Research Institute, Brno, Czech Republic; 2 - University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic; 3 - Mendel University in Brno, Czech Republic

Introduction: Phagocytosis assay represents a useful method for assessing the fish immune system and its ability to react to stimulants. During this assay, peripheral blood cells are incubated with various particles (e.g. zymosan, latex beads) in conditions specific for each fish species. Leukocytes are subsequently isolated by haemolysis in a hypotonic environment or gradient centrifugation. Measurement by flow cytometry is very effective and allows us to differentiate individual cell populations and easily evaluate the intensity of phagocytic activity. In fish, phagocytes comprise neutrophils, monocytes and also a small proportion of lymphocytes. Methodology varies between different authors, and there is a high natural variability between individual fish that needs to be considered. In this study, the optimal incubation temperature for rainbow trout phagocytes was investigated.

Methodology: Peripheral blood was collected from the caudal vein of rainbow trout (*Oncorhynchus mykiss*) into a heparinized syringe. Sample manipulation and incubation were performed at three different temperatures -4 °C, 15 °C and 22 °C (37 °C). Leukocytes were incubated with various particles (e.g. zymosan Texas Red) and isolated by haemolysis in a hypotonic environment. Isolated cells were centrifuged and fixed with EDTA. Differences between phagocyte numbers and the intensity of phagocytic activity at different temperatures were evaluated by flow cytometry.

Results: The highest viability of phagocytic cells was obtained with the incubation at 4°C. However, phagocytic activity was suppressed at this temperature. Cell viability was further decreasing with rising temperature. The highest levels of phagocytosis were recorded with the room temperature incubation (i.e. higher than the optimum temperature for rainbow trout). At 37°C, cell viability as well as phagocytic activity were decreased.

Conclusion: Room temperature provided optimal conditions for *in vitro* phagocytosis of rainbow trout peripheral blood cells. However, the phagocytes viability was reduced compared to the incubation at lower temperatures; the assay therefore requires fast processing.

Keywords: zymosan, particle, ingestion, haemolysis, flow cytometry

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218-P

TLRS and TLR signaling in lumpfish (*Cyclopterus lumpus* L.)

Eggestøl, Håvard Ø. (Norway)[1]; Lunde, Harald S. (Norway)[1]; Haugland, Gyri T. (Norway)[1]

1 - University of Bergen

Introduction: Recognition and degradation of potential pathogens are essential for clearance of microbes and to ensure the elicited immune response is tailored to the invading pathogen. The immune response is triggered by recognition of pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs). The main families of PRRs are Toll-like receptors, Nucleotide binding and oligomerization domain (NOD)-like receptors (NLRs), retinoic acid inducible gene I (RIG-I)-like receptors (RLRs), C-type lectin receptors (CLRs) and absent in melanoma 2 (AIM2)-like receptors (ARLs). The PRRs are germ-line encoded receptors conserved during evolution, but the PRRs repertoire in teleosts is known to be expanded.

Methodology: Head kidney leukocytes were infected with V.anguillarum (MOI1:10) and incubated for 6 and 24 hours. RNA sequencing, transcriptome assembly and differential gene expression (DEG) was performed as described earlier. Domain search were performed with Interproscan and phylogenetic trees was constructed from multiple sequence alignment by PhyML maximum likelihood.

Results: We have characterized TLRs and components of the TLR signaling pathway in the teleost lumpfish. We identified TLR1, 2, 3, 5M, 5S, 7, 8, 9, 13, 14, 21, 22 and 28 and the adaptor proteins; MyD88, TICAM1(TRIF), TIRAP (MAL) and SARM. TICAM2 (TRAM) was not identified in lumpfish, similarly to other teleosts. Here, we give an overview and molecular characterization of the TLRs, adaptor proteins and signaling components such as IRAKs and TRAFs. Transcriptome-wide analysis of the TLR signaling pathway upon bacterial exposure showed that the soluble version of TLR5 (TLR5S) was highly upregulated, while interestingly TLR13 was downregulated. The DEG analyses showed that it was primarily components of the Nfκβ signaling pathway that were upregulated, such as Nfκβ and inhibitors of its kinases. Transcripts involved in the MAPK signaling pathway, showed little change in transcript-level.

Conclusion: Our data is interesting for comparative studies and make a basis for further functional analyses of immune and pathogenicity mechanisms. In addition, it may benefit design of immunoprophylactic measures in lumpfish, which has become an economical important species as it is used as a cleaner fish for removal of sea lice from farmed Atlantic salmon.

Keywords: lumpfish, TLR, cell signaling, transcriptome

Funding: RCN and UiB.

219-P

Comparative analysis of immune related genes in teleost responding to bacterial infection from transcriptomic data

Chen, Shih-Chu (Taiwan)[1]; Maekawa, Shun (Taiwan)[2]; Wang, Pei-Chi (Taiwan)[2]

1 - National Pingtung University of Science and Technology; 2 - National Pingtung University of Science and Technology, Pingtung, Taiwan

Introduction: Transcriptome analysis using RNA-sequencing is a powerful tool for a deep understanding of complicated physiological pathways, including immune responses. There are many reports that transcriptome analysis demonstrated the immune-related pathway responding to pathogen infection in teleost. While, there has been little information regarding the diversity and universality of immune responses to pathogen infection in teleost. The aim of this study is comparative analysis of transcriptome data in bacterial infected teleost.

Methodology: To highlight comparative studies of immune responses against bacteria, we used our previous findings in largemouth bass (*Micropterus salmoides*) against *Nocardia seriolae*, grey mullet (*Mugil cephalus*) against *Lactococcus garvieae*, orange-spotted grouper (*Epinephelus coioides*) against *Vibrio harveyi*, and koi carp (*Cyprinus carpio*) against *Aeromonas sobria*, using RNA-seq techniques. We used differential expressed genes (DEGs; transcripts from spleen at 1 dpi with log2 > 1 or < -1 between infected and control group) from each data sets, and identified overlapping and specific genes that were up- or down- regulated in each species.

Results: We demonstrated that only 39 DEGs were present in all species. The number of specific DEGs in each species was relatively higher than that of common DEGs; 493 DEGs in largemouth bass against *N. seriolae*, 819 DEGs in mullets against *L. garvieae*, 909 in groupers against *V. harveyi*, and 1471 in carps against *A. sobria*. We also found that DEGs in different fish species were also representative of specific immune-related pathways.

Conclusion: The results of this study will enhance our understanding of the immune responses of fish, and will contribute to fish immunotherapy for the prevention and treatment of bacterial infections through the design of more specific and effective immune stimulants, adjuvants and vaccines.

Keywords: transcriptome, RNA-seq, immunity-related genes, bacteria

220-P

Evaluation of zebrafish (*Danio rerio*) susceptibility to new viral infections and its utilisation in C-type lectin receptor studies

Adamek, Mikolaj (Germany)[5]; Rakus, Krzysztof (Poland)[1]; Breitkopf, Veronika (Germany)[5]; Monteiro, Joao (Germany)[5]; Podlasz, Piotr (Poland)[2]; Mojżesz, Miriam (Poland)[1]; Pooranachandran, Niedharsan (Poland)[1]; Widziołek-Pooranachandran, Magdalena (Poland)[1]; Rakers, Sebastian (Germany)[3]; Way, Keith (United Kingdom)[4]; Chadzinska, Magdalena (Poland)[1]; Lepenies, Bernd (Germany)[5]; Steinhagen, Dieter (Germany)[5]

1 - Jagiellonian University, Krakow, Poland; 2 - University of Warmia and Mazury, Olsztyn, Poland; 3 - Fraunhofer Research Institution for Marine Biotechnology and Cell Technology, Luebeck, Germany; 4 - Centre for Environment Fisheries and Aquaculture Science, Weymouth, UK; 5 - University of Veterinary Medicine, Hannover, Germany

Introduction: Zebrafish (*Danio rerio*) with the significant biotechnological progress in genetics is becoming an interesting model for studying immune responses to viral pathogens which cause diseases in farmed fish. Here *in vitro* and *in vivo* studies were performed in the search for new viral models which would help explore immune function of novel C-type lectin receptors (CTLR) in zebrafish.

Methodology: Initially zebrafish derived ZF4 and SJD.1 cells were tested for susceptibility to several fish pathogenic viruses including cyprinid herpesvirus 1 and 3 (CyHV-1 and CyHV-3), *chum salmon reovirus* (CSV), common carp paramyxovirus (CCPV), common carp orthomyxovirus (CCOV), and common carp birnavirus (CCBV). As positive control spring viremia of carp virus (SVCV) was used. Antiviral responses based on *vig-1* and *mxa* gene expression were measured by RT-qPCR. Based on the *in vitro* results, CyHV-3, CSV and SVCV were selected for testing *in vivo*. The infections were performed by intraperitoneal injection of the virus into adult zebrafish and by immersion of larvae in virus suspensions. Furthermore, several putative CTLR genes were located in the zebrafish genome, molecularly cloned and analysed for structural transmembrane classification.

Results: *In vitro* studies demonstrated that apart from SVCV, CSV, CyHV-1 and CyHV-3 but not CCPV, CCOV and CCBV were able to replicate in the zebrafish cell lines ZF4 and SJD.1. *In vivo* studies showed that both CSV and CyHV-3 induce an up-regulation of *vig-1* and *mxa* expression in kidney and spleen of adult zebrafish after i.p. injection but not in larvae after infection by immersion. SVCV infection was the strongest inducer of an antiviral response. When mRNA expression of the two putative CTLR encoding genes *up463* and *up690* (likely homologues of mammalian MINCLE and SIGNR3) was measured, a strong down-regulation was noticed in adult fish under infection.

Conclusion: The presented results give a good basis for further functional studies of CTLR. These will include *in vitro* studies on the binding of bacterial and viral pathogens to recombinant proteins and further *in vivo* studies in which up463 and up690 CRISPR-Cas knockout zebrafish embryos will be infected with various viral or bacterial pathogens.

Keywords: *Danio rerio*, C-type lectin receptors, viral infection

221-P*

Influence of triploydy in lysc and lysg expression in response to *Aeromonas jandaei* infection in the Brazilian fish *Astyanax altiparanae*

Carriero, Mateus (Brazil)[1]; Meira, Caroline (Brazil)[1]; Gato, Igor (Brazil)[1]; Levy-Pereira, Nycolas (Brazil)[1,2]; Yasui, George (Brazil)[2]; Maia, Antonio (Brazil)[1]

1 - Department of Veterinary Medicine, Faculty of Animal Science and Food Engineering, University of São Paulo; 2 - National Center for Research and Conservation of Continental Fish, Chico Mendes Institute of Biodiversity Conservation

Introduction: Triploid fish are gaining increasing interest in commercial aquaculture due to benefits provided by their sterility such as improved meat quality and better growth rates resulted by the lack of sexual maturation. However, triploid fish are generally perceived as more susceptible to diseases and less able to deal with sub-optimal environmental conditions. The present study aimed to characterize the full-length LysC and LysG cDNA sequences from lambaris (*Astyanax altiparanae*) and evaluate their expression in diploid and triploid *Aeromonas*-infected fish.

Methodology: The full-length LysC and LysG cDNA sequences were cloned and characterized. Diploid and triploid fish were intraperitoneally injected with a pathogenic strain of *A. jandaei* and LysC and LysG expression in the spleen was compared with PBS-injected control groups after 12h by qPCR.

Results: The LysC cDNA sequence was composed by 726 nt with a 432-nt open reading frame (ORF) that encoded a deduced 144-amino acid sequence and the LysG cDNA contained 1029 nt with a 185-nt ORF encoding 185-amino acid residues. LysC revealed the presence of a signal peptide and the LYZ1 domain, while the LysG showed a transglycosylase SLT domain, with no signal peptide. Physiological baseline levels of both LysC and LysG transcripts were significantly higher (around 2-fold) in diploid fish when compared to triploids. In *Aeromonas*-infected fish, LysC showed a significant 43.6-fold up-regulation, whereas LysG showed a 2.2-fold up-regulation in diploids. No significant variation was observed in LysC and LysG transcript amounts in triploid fish after the bacterial challenge.

Conclusion: The full-length cDNA sequences of LysC and LysG were cloned and characterized for the first time for a fish from the Characiformes order. Basal expression of LysC and LysG is significantly lower in triploid fish when compared to normal diploids. Moreover, LysC and LysG are involved in the response against bacterial infections in diploid *A. altiparanae*, with LysC showing a significant higher involvement than LysG. The expression of lysozymes in triploid fish was not altered by bacterial infection suggesting that these fish have a different expression pattern not only under physiological conditions, but also under infections.

Keywords: triploids, molecular immunology, lambari, lysozyme, qPCR

Funding: FAPESP (Grants 2017/18562-6 and 2017/26996-6) and CNPq (Grant 408281/2018-9).

222-P

Zebrafish c-reactive protein-like molecules inhibit interrelated SVCV replication and autophagy pathways

Belló-Pérez, Melissa (Spain)[1]; Pereiro, Patricia (Spain)[2]; Coll, Julio (Spain)[3]; Novoa, Beatriz (Spain)[4]; Pérez, Luis (Spain)[1]; Falcó, Alberto (Spain)[3]

1 - Instituto de Investigación, Desarrollo e Innovación en Biotecnología Sanitaria de Elche, Miguel Hernández University, 03202 Elche, Spain; 2 - Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, 36208 Vigo, Spain; 3 - Instituto Nacional de Investigaciones y Tecnologías Agrarias y Alimentarias (INIA), Dpto. Biotecnología, 28040 Madrid, Spain; 4 - Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, 36208 Vigo, Spain

Introduction: C-reactive proteins (CRP) are considered circulating pattern-recognition molecules that are involved in the first-line of host's innate defense by mostly mediating the clearance of bacterial pathogens and damaged cells. Recent comparative studies of their ancestral orthologs are revealing unknown activities, such as antiviral ones. However, little was known about the mechanisms involved.

Methodology: There were employed several molecular techniques such as SVCV-focus forming assay, RT-qPCR, LC3B fluorescence immune-labeling and *in vivo* assays with GFP-LC3 transgenic zebrafish. Additionally, it was studied the effect of several autophagy modulators on zebrafish CRP-like molecule (zfCRPs) antiviral activity as well as on viral replication.

Results: In the present work, it is demonstrated the antiviral activity against SVCV for each zfCRP isoform. The exploration of the potential antiviral mechanisms involved suggests that such effect is mostly indirect (i.e. by inducing an antiviral state in the host) and excludes the direct blocking of the early steps of SVCV replication cycle, as well as the stimulation of the interferon system. The results obtained suggest that the antiviral protection conferred by zfCRPs is mainly due to the inhibition of the endocytic pathway at the viral entry stage, which appears to share common factors or to converge with the autophagic process.

Conclusion: This knowledge on primitive vertebrate CRP-like molecules sheds light on the evolution of this arm of the innate immune system and opens a new research field to explore with potential therapeutic applications.

Keywords: C-reactive proteins, zebrafish, SVCV, autophagy

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223-P*

Interaction between *Vibrio splendidus* and the immune system of *Mytilus galloprovincialis*

Rey-Campos, Magalí (Spain)[1]; Saco, Amaro (Spain)[1]; Novoa, Beatriz (Spain)[1]; Figueras, Antonio (Spain)[1]

1 - Instituto de Investigaciones Marinas, Consejo Superior de Investigaciones Científicas, 36208 Vigo, Spain

Introduction: Bivalves are susceptible to numerous diseases that compromise their culture, producing economic losses all over the world. Mussels that cohabitate in the same areas as oysters and clams have experienced massive mortalities due to viral and bacterial infections. As an example, the ostreid herpesvirus 1 (OsHV-1) has caused massive mortalities in oysters (*Crassostrea gigas*) in different parts of the world. However, until now, high mortalities have never been reported in the field for Mediterranean mussel (*Mytilus galloprovincialis*). This resistance could be due to the high expression of antimicrobial peptides that has been observed in mussel.

Methodology: In this work we have compared the interaction of *Vibrio splendidus* with the immune system of *M. galloprovincialis* after *in vivo* infections by bath or by injection, and we also analyzed the bactericidal activity after an *in vitro* infection of mussel hemolymph.

Results: Our results showed that Mediterranean mussels have a potent capacity to eliminate the bacteria load after bath infection, pointing out that mucus and/or gills could be effective barriers to fight against the bacteria. However, the expression of genes putatively involved in the resistance against the infection is different depending on the infection route.

Keywords: Mediterranean mussel, *Vibrio splendidus*, antimicrobial peptides, bacterial clearance

Funding: EU, Horizon 2020, VIVALDI (678589) and the Spanish project AGL2015-65705-R.

224-P*

Evaluation of toxin A/B-specific recombinant antibodies and the effect on the virulence of *Vibrio parahaemolyticus*

Lazarte, Jassy Mary (Republic of Korea)[1]; Kim, Jaesung (Republic of Korea)[1]; Lee, Jung Seok (Republic of Korea)[1]; Im, Se Pyeong (Republic of Korea)[1]; Jung, Jae Wook (Republic of Korea)[1]; Chun, Jin Hong (Republic of Korea)[1]; Kim, Si Won (Republic of Korea)[1]; Kim, Young Rim (Republic of Korea)[1]; Jung, Tae Sung (Republic of Korea)[1]

1 - Laboratory of Aquatic Animal Diseases, College of Veterinary Medicine, Gyeongsang National University, Jinju, 660-701, South Korea

Introduction: The causative agent of acute hepatopancreatic necrosis disease (AHPND) is the bacterium, *Vibrio parahaemolyticus*, which secretes a toxin in the gastrointestinal tract of the host. Toxins A and B are implicated in the pathogenesis of this disease thus, they are the subject of studies involving diagnosis and treatment. Recently, the use of recombinant antibody based on hagfish immunoglobulin gene is being explored and it has shown promising results in the neutralization of some viruses.

Methodology: Recombinant Toxin A and B were produced using a bacterial expression system. These were then used as antigens in the screening of hagfish immunoglobulin cDNA library to obtain the toxin A/B-specific antibodies. Once specificity was confirmed through Western blotting and ELISA, they were tested for their protective effect on cells. *Vibrio parahaemolyticus* AHPND-causing strain was cultured in brain heart infusion (BHI) broth with 3% NaCl at 30 °C. When the bacteria reached its growing phase (O.D. 0.5), toxin A/B-specific antibodies were introduced into the culture. Different concentrations of these antibodies (1/2, 1/10 and 1/50 times diluted) were used and at three different time points (30 min, 1 and 3 h post incubation) the supernatants were collected. The supernatants from the culture growth were tested for the possibility of toxicity to shrimp, by immersion challenge.

Results: The recombinant Toxin A and B antibodies specifically detect the Toxin A and B protein in both Western blot and ELISA results. Furthermore, mortality rate is lower in the shrimp infected with the highest concentration of Toxin A/B antibody suggesting the capacity of these antibodies to protect shrimp against AHPND.

Conclusion: Toxin A/B-specific recombinant antibodies can significantly inhibit the virulence of *Vibrio parahaemolyticus* by diminishing the effect of the bacterial toxins A and B. These findings demonstrate the use of this recombinant antibody as an alternative for immunoglobulin-based antibody which can be utilized for the proper management of AHPND especially, in shrimp farms.

Keywords: acute hepatopancreatic necrosis disease, *Vibrio parahaemolyticus*, recombinant antibody, hagfish immunoglobulin, toxin A/B

225-P

Potential application of recombinant antibodies from hagfish variable lymphocyte receptor (VLR) gene: a novel alternative antibody

Kim, Jaesung (Republic of Korea)[1]; Lee, Jung Seok (Republic of Korea)[1]; Im, Se Pyeong (Republic of Korea)[1]; Lazarte, Jassy Mary (Republic of Korea)[1]; Kim, Si Won (Republic of Korea)[1]; Chun, Jin Hong (Republic of Korea)[1]; Jung, Jae Wook (Republic of Korea)[1]; Kim, Young Rim (Republic of Korea)[1]; Jung, Tae Sung (Republic of Korea)[1]

1 - Laboratory of Aquatic Animal Diseases, College of Veterinary Medicine, Gyeongsang National University, Jinju, 660-701, South Korea

Introduction: Hagfish and lamprey are extant animals characterized as jawless animals. Unlike lampreys which exist in both freshwater and marine waters, hagfish, on the other hand, thrives only in sea water. Concerning the immune system of the cyclostomes, it had been known that jawed vertebrates had only adaptive immune system until variable lymphocyte receptors (VLRs) were discovered from lamprey. These VLRs are included in the leucine-rich repeat (LRR) family and were shown to mediate adaptive immune responses in the jawless vertebrates. The VLRs have several different characteristics compared with vertebrates' immunoglobulins (Igs) in terms of shape, mechanism of combination and possible antigen recognition.

Methodology: Globular-shaped VLRs were explored by purifying them from hagfish sera and were examined through Electron Microscopy (EM) and Immuno-EM examination. Recombinant antibodies after cloning VLR genes from hagfish were produced and applied for establishing Sandwich ELISA (S-ELISA) as coating antibodies. These VLR-based antibodies were used to measure the extracellular vesicles from *Staphylococcus aureus*.

Results: EM and single-particle analysis showed that the multimerized VLRBs form globular-shaped clusters with an average diameter of 28.7 ± 2.2 nm. The presence of VLRBs in the complex was confirmed by immune-EM analysis using an anti-VLRB antibody. The recombinant antibodies showed high capacity as coating antibodies and were capable of capturing the extracellular vesicles from *S. aureus* specifically.

Conclusion: In this study, VLRs from hagfish were evaluated to be globular-shaped, which were completely different from lamprey VLRs *in vivo* and it was suggested to be a primordial antibody with respect to its evolution. Furthermore, we were able to modify the globular-shaped VLRs by applying molecular engineering and used them in the development of recombinant antibodies. These modified VLRs showed high binding avidity for several different viral antigens and were able to capture some of bacterial vesicles in S-ELISA.

Keywords: variable lymphocyte receptor, hagfish, recombinant antibody, adaptive immune system, *Staphylococcus aureus*

226-P

RNA-seq gives insights into mechanism behind different levels of koi herpesvirus disease resistance in common carp strains

Adamek, Mikolaj (Germany)[1]; Metzger, Julia (Germany)[1]; Jung, Klaus (Germany)[1]; Kosch, Robin (Germany)[1]; Matras, Marek (Poland)[2]; Dawson, Andy (United Kingdom)[3]; Piackova, Veronika (Czech Republic)[4]; Gela, David (Czech Republic)[4]; Kocour, Martin (Czech Republic)[4]; Adamek, Jerzy (Poland)[5]; Kaminski, Rafal (Poland)[5]; Reichert, Michal (Poland)[2]; Distl, Ottmar (Germany)[1]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany; 2 - National Veterinary Research Institute, Pulawy, Poland; 3 - Keele University, Keele, UK; 4 - University of South Bohemia, Vodňany, Czechia; 5 - The Stanislaw Sakowicz Inland Fisheries Institute, Olsztyn, Poland

Introduction: Koi herpesvirus disease (KHVD) caused by cyprinid herpesvirus 3 (CyHV-3) is one of the deadliest diseases in carp global aquaculture. However, carp strains with a different genetic background are presenting diverse susceptibility to this viral pathogen. This gives opportunity to mitigate the impact of the disease on aquaculture, on the other hand provides a potential model for studying the immunological basis of resistance to alloherpesviruses in fish.

Methodology: Four strains of common carp (AS, Rop, PS and koi) were infected with CyHV-3 by bath. The mortality was observed for 26 days. The development of clinical signs was linked with an evaluation of CyHV-3 spreading through selected tissues at several time points. The RNAseq analyses was performed from selected tissue/time points from the most susceptible/resistant carp strains on Illumina NextSeq, two versions of common carp genome and the zebrafish genome were used in bioinformatic analyses.

Results: An infection experiment confirmed significant differences in mortality and virus load during CyHV-3 infection with Rop being most resistant to the disease (22% mortality) and koi most susceptible (90% mortality). Based on the evaluation of the infection process the head kidney at 96 h post infection was selected for RNA-seq analyses. Preliminary evaluation using different reference genomes gave divergent results. The use of an early version of the common carp genome indicated that differences in resistance to CyHV-3 could be related to the expression 18 genes, with 16 genes playing a role in the koi strain and two genes in Rop. In particular the down-regulation of genes involved in the activity of natural killer cells as well as the activity of the nuclear transcription factor NFκB in CyHV-3 infected koi could indicate an impairment of innate immune responses in these carp under infection. In addition, the difference in expression levels of spire-1 in non-infected koi and non-infected Rop might pre-dispose koi for infection with the virus.

Conclusion: The resistance to the KHVD can be influenced by interplay of the magnitude of innate immune responses towards the virus and by predisposition of certain strains to develop a lethal phase of the disease.

Keywords: RNA-seq, CyHV-3, KHVD

228-P

Transcriptome analysis shows that concurrent IFN-I treatment and SAV3 infection enriches the MHC-I antigen processing and presentation pathways in to-cells

Xu, Cheng (Norway)[1]; Evensen, Øystein (Norway)[1]; Munang'andu, Hetron (Norway)[1]

1 - Norwegian University of Life Sciences, Faculty of Veterinary Medicine, Department of Basic Sciences and Aquatic Medicine, P.O. Box 369, NO-0102, Oslo, Norway

Introduction: Type I interferons (IFNs) play a pivotal role in modulating adaptive immune responses in apart from to their antiviral properties in immune cells. Our aim was to profile the type of IFN-I-induced pathway genes involved in antigen-presenting pathways in an Atlantic salmon-derived (*Salmo salar* L.) macrophage cell line (TO-cells).

Methodology: We carried out a *de novo* transcriptome comparative analysis of cells treated with IFN-I and concurrently infected with salmonid alphavirus subtype 3 (SAV3) with IFN-I untreated cells infected with SAV3.

Results: Our findings show that concurrent treatment of TO-cells with IFN-I and SAV3 infection (SAV3/IFN$^+$) significantly enriched the MHC-I pathway unlike the non-IFN-I treated TO-cells (SAV3/IFN$^-$) that had lower expression levels of MHC-I pathway related genes. Differentially expressed genes (DEGs) in the SAV3/IFN$^+$ cells and not in the SAV3/IFN$^-$ cells included the proteosomal activator (PA28) and β-2 microglobulin (β2M). Our findings show that MHC-I pathway genes like heat shock protein 90 (Hsp90), transporter of antigen associated proteins (TAPs) and tapasin had higher expression levels in the SAV3/IFN$^+$ cells than in the SAV3/IFN$^-$ cells. No MHC-II pathway related genes were upregulated in SAV3/IFN$^+$ treated cells while cathepsin S linked to degradation of endosomal antigens in the MHC-II pathway were downregulated in the SAV3/IFN$^-$ cells. In summary, our findings show that concurrent IFN-I treatment of TO-cells and SAV3 infection enriched gene expression linked to the MHC-I antigen presentation pathway.

Conclusion: Data presented indicate a role of type I IFNs in strengthening antigen processing and presentation that may facilitate activation of CD8+ T-cell responses after exposure to SAV3 infection, of which SAV3 infection alone downplay MHC-II pathways.

Keywords: MHC-I, antigen, SAV3, interferon

Funding: Research Council of Norway Grant No. 226275 and TargetFish grant agreement 311993.

Diagnostics

230-P

Development of new primers for detection of S*hewanella* spp.

Paździor, Ewa (Poland)[1]; Pękala-Safińska, Agnieszka (Poland)[1]; Pruss, Łukasz (Poland)[2]; Bomba, Arkadiusz (Poland)[2]; Zając, Magdalena (Poland)[3]; Wasyl, Dariusz (Poland)[4]

1 - National Veterinary Research Institute, Department of Fish Diseases; 2 - National Veterinary Research Institute, Department of Omics Analyses; 3 - National Veterinary Research Institute, Department of Microbiology; 4 - National Veterinary Research Institute, Department of Microbiology and Department of Omics Analyses

Introduction: The different species of *Shewanella* are a ubiquitous group of microorganisms causing health disorders in fish. Interestingly, some of them may be previously undescribed species. The diagnostic of these group of bacteria is carried out based on biochemical properties and analyses of 16S rRNA gene. However, in many cases available techniques are not sufficient to their identification.

Methodology: Isolates of *S. baltica* (38), *S. oneidensis* (11), *S. xiamenensis* (8), *Shewanella* probably undescribed species (29) collected from marine and freshwater fish as well as reference strain ATCC8071 were used in our study. The whole genome sequencing of bacteria was conducted by Illumina Miseq. The primers were designed based on whole genome sequences as well as data from GenBank using the MegaX and SnapGeneViewer softwares.

Results: Of 1255 genes identified all tested *Shewanella* strains, 13 were found suitable targets: *sec*E, *hpf*, *pri*B, *rps*T, *yqe*Y, *sdh*C, *sdh*D, *rpm*I, *ybf*E, *efp*, *ihf*A, *fis* and *rnp*A. Fourteen primer pairs were developed offering melting temperature about 59 °C and amplification size ranging from 74 to 687 bp. *In silico* tools for primers evaluation showed that all designed primers might be specific and sensitive for *Shewanella* species confirmation.

Conclusion: Diversity of *Shewanella* species, with often unknown spectrum of biochemical features requires unconventional analytical approach based on currently available bioinformatic analysis of WGS data. Further laboratory tests are needed to verify usefulness of *in silico* developed primers for identification of *Shewanella*.

Keywords: *Shewanella*, primers, fish

Funding: This work was supported by the National Science Centre, Poland, project „Studies on genotypic characterisation of *Shewanella putrefaciens* group isolates from freshwater fish in Poland" (Grant No 2015/19/N/NZ7/01687) and donation Nr 447/E-180/S/2018-1 from the Ministry of Science and Higher Education.

231-P

New PCR tools for the detection and genetic characterization of percid perhabdoviruses

Pallandre, Laurane (France)[1]; Feuvrier, Claudette (France)[2]; Pozet, Françoise (France)[2]; Bigarré, Laurent (France)[1]

1 - Agence Nationale de Sécurité Sanitaire; 2 - Laboratory of analysis of Jura

Introduction: Perhabdoviruses PRV (*Perch rhabdovirus*) and STRV (*Sea trout rhabdovirus*) are threats for percid farming (*Perca fluviatilis* and *Sander lucioperca*) in Europe. Unfortunately, molecular diagnostics is still poorly developed and the genetic studies are limited. Focusing on the complete nucleoprotein gene (*n*), we have characterized a collection of viruses isolated in France since 1999. The full-length *n* genes of 13 isolates were obtained and compared one to another and to sequences available in GenBank. Specific cPCRs were tentatively designed for diagnostics.

Methodology: From viruses isolated in cell culture, total nucleic acids were extracted and submitted to PCRs targeting the *n* gene: either generic PCR for sequencing or specific PCRs for diagnostics. Sanger sequencing was performed on cloned PCR products. A ML method was used for the phylogeny (Seaview).

Results: Globally, the levels of nucleic acid identities varied between 68.3 and 100%. Three new isolates exhibited clear differences with PRV and STRV: isolates 18 - 203 and 18 - 206, almost identical one to another, exhibited maximal identities of 82.7 % with a variant of STRV, while isolate 18 - 193 was 88% similar with a previously described isolate (N4925) and only 75% and 70% with PRV and STRV. Therefore, four genetic clusters were distinguished in a phylogenetic tree: STRV, PRV, cluster N4925/18 - 193 and a fourth cluster with 18 - 203 and 18 - 206. Isolates highly related to PRV were detected using a new real-time PCR which showed a high specificity. Four cluster-specific cPCR were developed to be run in a single assay. Each PCR produced the expected signal for its cognate cluster (330, 384, 430 and 507 bp), and no (or very low) signals for other clusters.

Conclusion: A high genetic diversity is observed among percids perhabdoviruses, with four distinct genogroups, that might be considered as four species. This diversity is a challenge for the molecular diagnostics. By scrutinizing differences and similarities among all the sequences, one PRV-specific real-time PCR and four cluster-distincts specific cPCR were designed. These new tools will be useful for testing the presence of perhabdoviruses in percids affected by mortalities in Europe.

Keywords: perhabdovirus, percid, PCR

Funding: European maritime and fisheries fund (project PERCI-HATCH).

232-P

Koi herpesvirus (KHV) outbreak causing mass mortality of carp in Iraq

Toffan, Anna (Italy)[1]; Marsella, Andrea (Italy)[1]; Abass, Salah (Iraq)[2]; Al-Adhadh, Basem (Iraq)[2]; Abbadi, Miriam (Italy)[1]; Stone, David (United Kingdom)[3]

1 - Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy; 2 - Veterinary Directorate, Wazariyah, Baghdad, Iraq; 3 - Centre for Environment, Fisheries and Aquaculture Science, Weymouth, UK

Introduction: At the end of October 2018 a mass mortality event occurred in Iraq, involving exclusively cultured and wild common carps (*Cyprinus carpio*) along Euphrates and Tigris rivers. Mortality was 99.42% and it appeared suddenly and rose sharply, lasting 7 - 10 days. Fish were found dead or moribund, swimming erratically at the surface. Affected subjects showed dark skin coloration, excessive mucus production, flared gills, lethargy and dyspnea. Oxygen depletion and/or toxic contamination and/or infectious disease were considered as differential diagnosis.

Methodology: Affected fish were subjected to necropsy and sent for bacteriological and mycological investigations at Iraqi Central Veterinary Laboratory. Samples collected in different provinces were shipped to IZSVe and were tested for Cyprinid Herpesvirus type 3 (CyHV-3) and Carp Edema Virus (CEV) through real-time quantitative PCR. Pool of organs were also tested for Spring Viraemia of Carp by reverse transcription quantitative PCR and by virus isolation. Positive results were confirmed by conventional PCR and sequence analysis. Gills samples were sent to Cefas as OIE reference laboratory for CyHV-3 for disease confirmation.

Results: Water temperature was reported to be 20 - 25 °C. Fish subjected to necropsy displayed patches of gill necrosis with excessive mucus production, dark skin coloration and enophthalmos. Bacteriological and mycological examinations resulted in isolation of *Aeromonas hydrophila, Aspergillus flavus*, *Branchiomyces* spp. and *Saprolegnia* spp. All virological test were positive for CyHV-3, except for three which tested positive for CEV. Partial polymerase and thymidine kinase genes sequences of CyHV-3 isolates showed 100% nucleotide similarity with CyHV-3 strain TUMST1 and CEV partial 4a gene sequences showed 99% similarity with the genogroup Ia CEV isolate 436-2014. All positive results were confirmed by Cefas.

Conclusion: During the outbreak, 2,391,000 carps were affected, jeopardizing Iraqi aquaculture production. Results were consistent with CyHV-3 as etiological agent of the mortality. The positivity of CEV is another interesting finding, since clinical signs and macroscopic lesions were similar to CyHV-3 infection. Other stressors such as poor water quality, concurrent infection/infestation may have acted as facilitating factors increasing the magnitude of the event. This is the first report of both diseases in common carps in Iraq.

Keywords: common carp, Iraq, KHV, CEV, outbreak

233-P

OIE laboratory twinning project: improving the IRVT diagnostic capacity for viral encephalopathy and retinopathy of marine fish

Toffan, Anna (Italy)[1]; Panzarin, Valentina Maria (Italy)[1]; Dalla Pozza, Manuela (Italy)[1]; Manfrin, Amedeo (Italy)[1]; De Battisti, Christian (Italy)[1]; Abbadi, Miriam (Italy)[1]; Hammami Damak, Mounira (Tunisia)[2]; Tlatli Attia, Aida (Tunisia)[2]; Omri Ayari, Yosra (Tunisia)[2]; Megdiche Krichen, Aida (Tunisia)[2]; Haddouchi, Selma (Tunisia)[2]; Tliba, Ines (Tunisia)[2]; Sghaier, Sofiane (Tunisia)[2]; Ayari Fakhfakh, Emna (Tunisia)[2]

1 - Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padua, Italy; 2 - Institut de la Recherche Vétérinaire de Tunisie, Tunis

Introduction: The Twinning project involved the IZSVe as parent institute and the IRVT as candidate institute. The main objective of the project was to contribute to the control of viral pathologies in Tunisian aquaculture, with particular reference to viral encephalopathy and retinopathy (VER) caused by betanodavirus, the most economically relevant pathogen for marine farmed species. In order to improve VER control in sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) farms, the immediate objective of the project was to implement disease management on site, by setting up at the IRVT accurate and sensitive laboratory tests for reliable diagnosis of VER.

Methodology: The project was structured in theoretical and practical training exercises covering the following topics: fish pathology, virological and molecular diagnosis, quality assurance of laboratory data and epidemiology applied to fish diseases. Other activities included pre-project and post-training laboratory assessments, and the organization of a proficiency test (PT) for VER to evaluate the acquired skills. A closure meeting was organized to review the outputs and to disclose the project's achievements to the representatives of northern Africa.

Results: The project started in February 2018 and was concluded in July 2019. The technical and scientific skills provided and the equipment available at the IRVT will make it possible to set up a reliable and efficient service for VER diagnosis. The PT organization, as well as the post-training assessment, demonstrated the successful technology transfer and certified the competences acquired by the IRVT staff members. An additional result was the implementation of a real time RT-PCR protocol for the detection of Tilapia Lake Virus, another emerging pathogen affecting African aquaculture.

Conclusion: OIE Twinning projects are of paramount importance to facilitate capacity building of applicant laboratories, encourage technology transfer and create networks among diagnostic laboratories. Divulgation of the activities will hopefully promote the newly acquired diagnostic offer for VER of IRVT and, more in general, sensitize authorities, professionals, experts and scientists of the aquaculture sector to a better sanitary management of fish farms.

Keywords: twinning, viral encephalopathy and retinopathy, diagnostic capacity, Mediterranean Sea

Funding: The OIE Laboratory Twinning.

234-P

Importance of the 3'-terminal nucleotide of the forward primer for conventional RT-PCR for VHSV gene detection

Kim, Hyoung Jun (Republic of Korea)[1]; Kwon, Se Ryun (Republic of Korea)[2]

1 - National Fishery Products Quality Management Services; 2 - Sunmoon University

Introduction: Viral haemorrhagic septicaemia virus (VHSV), causing severe diseases in farmed fish, is detected and genotyped using conventional RT-PCR (cRT-PCR) targeting the N gene with corresponding VN F and VN R primers. However, these primers have low sensitivity to VHSV subtype IVa; We investigated the cause for the poor cRT-PCR performance using various primer combinations.

Methodology: The VN F and VN R primers were designed for cRT-PCR as described in the OIE manual of diagnostic tests for VHS. The modified VN IVa F and VN IVa R primers were derived from the VN primer sequences to improve the detection of subtype IVa. The Trial 1F and 2F primers were modified versions of the VN primers to investigate the sensitivity issue of the cRT-PCR assay using the primers to detect subtype IVa; they were designed using the VHSV KJ2008 N gene sequence and the alignment of VHSV N genes. Specifically, the 24-nucleotide VN F primer was changed at position 9 (A to G) to derive the Trial 1F primer and at position 24 (G to T) to obtain the Trial 2F primer.

Results: The VN primers were detected the target gene in the 10^{-3} dilution of IVa stock solution. However, primers fully matching subtype IVa sequences exhibited a 10,000-fold higher sensitivity, detecting the target in the 10^{-7} dilution. Furthermore, cRT-PCR results indicated that VN F impaired the sensitivity to subtype IVa due to nucleotide mismatches, whereas VN R did not affect the assay. Using subtype IVa sequences to design forward test primers Trial 1F and Trial 2F, the 24-nucleotide VN F primer was changed at the mismatched position 9 (A to G) and the mismatched 3'-end position (G to T), respectively. The cRT-PCR assay detected subtype IVa in the 10^{-4} dilution using Trial 1F/VN R and in the 10^{-6} dilution using Trial 2F/VN R.

Conclusion: The 3'-end mismatch in the VN F primer caused the reduced sensitivity to subtype IVa, whereas other mismatched internal nucleotides did not affect the assay. Hence, the 3'-terminal nucleotide of VN F is critical for detecting VHSV by cRT-PCR.

Keywords: VHSV, low sensitivity, conventional RT-PCR, 3'-terminal region of forward primer

235-P

Development of real-time PCR with a PNA-probe-based melting curve analysis for the detection of WSSV

Kim, Youngjin (Republic of Korea)[1]; Kim, Sangjo (Republic of Korea)[1]

1 - National Fishery Products Quality management Servi

Introduction: Newly developed analysis method in this study is designed to detect white spot syndrome virus, which OIE appoints. We compared existing analysis method recommended by OIE and our newly designed method.

Methodology: Experiment sample consisted of total of 100 frozen imported and distributed shrimps of which designated to test for WSSV infection when it is imported to Korea. DNA was extracted using Qiagen, Dneasy blood & tissue kit in the market. For the analysis method in this study, the same primer of Taqman real time PCR method as recommended by OIE was utilized, and PNA (Peptide nucleic acid) probe for WSSV detection was newly designed. Extracted DNA was analyzed with IQ-2000 WSSV detection and prevention systems (GeneReach Biotechnology Corp) listed in OIE, and the result got compared with the newly designed method in this study. Also, for the samples that resulted in a positive in the test, additional genetic analysis was conducted.

Results: All samples were negative with IQ-2000 test; however, 100 out of 3 resulted in a positive when analyzing with the newly designed analysis method. Also, additional genetic analysis was utilized to detect WSSV.

Conclusion: Analysis method with PNA probe designed in this study could detect WSSV with higher sensitivity compared to IQ-2000, and results in shorter analysis time. However, not only IQ-2000 but also other methods listed up in OIE should be compared with this new method. Also, it requires additional experiments constantly in order to settle as quarantine analysis method for domestic fishery product.

Keywords: WSSV, melting curve analysis, real-time PCR, PCR

236-P

Identification of the zoonotic clonal complex of *Vibrio vulnificus* pathovar *piscis* by MALDI-TOF

Boonstra, Mirjam (Netherlands)[2]; Fouz, Belén (Spain)[1]; Van Gelderen, Betty (Netherlands)[2]; Jansson, Eva (Sweden)[3]; Madsen, Lone (Denmark)[4]; Dalsgaard, Inger (Denmark)[4]; Amaro, Carmen (Spain)[1]; Haenen, Olga (Netherlands)[2]

1 - Department of Microbiology and Ecology and ERI Biotecmed, University of Valencia; 2 - Fish, Crustacean and Shellfish Disease Laboratory, Wageningen Bioveterinary Research; 3 - National Veterinary Institute Sweden; 4 - Unit for Fish and Shellfish Diseases, National Institute of Aquatic Resources, Technical University of Denmark

Introduction: *Vibrio vulnificus* (*Vv*) pathovar *piscis* (formerly Biotype 2) is a polyphyletic group within *Vv* that comprises the strains pathogenic for fish grouped in three serovar-related sublineages. The zoonotic strains within pv. *piscis* constitute a serologically homogeneous clonal complex known as SerE that is worldwide distributed. The current method for identifying SerE is through PCR using SerE specific primers. Several pathogenic species can be identified by MALDI-TOF, which can be also used to differentiate subgroups within a species. Here we test the suitability of MALDI-TOF for distinguishing the different sublineages of *Vv* pv. *piscis*.

Methodology: Strains of the three *V. vulnificus* pv. *piscis* sublineages characterised by serology and PCR (SerE) as SerE, SerA or non SerAE where grown on HIS-Blood agar for 24 h at 22 °C. Colonies were harvested and proteins where extracted using the Bruker Daltonics extended Formic acid extraction method. Matrix used was HCCA and spectra were obtained for proteins between 1KDa-20KDa on a Bruker MALDI-TOF Microflex. Spectra were screened for identifying peaks and Main Spectra (MSPs) were created using Bruker Daltonics Flexanalysis and Biotyper 3 software. Peak weighing was adjusted for subtyping.

Results: We found a peak–pattern specific for each sublineage that allowed us to distinguish the strains belonging to each sublineage or serovar. Thus, SerE and SerA strains presented two distinct peaks while non SerAE strains presented one in common with SerA strains and another in common with SerE strains. Testing of several strains from different sublineages against the subtyping MSPs resulted in the successful identification of all of them. The identification was serologically confirmed, and in the case of SerE, was also confirmed by PCR.

Conclusion: MALDI-TOF can distinguish the three different serovar-related sublineages within *Vv* pv. *piscis*. MALDI-TOF can be implemented as a fast and accurate alternative method to PCR or serological tests to identify the zoonotic SerE *of V. vulnificus* pv. *piscis*.

Keywords: zoonosis, MALDI-TOF, *Vibrio vulnificus*

237-P

Novel histological evidence of atrio-ventricular (AV) node in Atlantic salmon (*Salmo salar* L.)

Yousaf, Muhammad Naveed (Norway)[1]; Amin, Anil B. (Norway)[2]

1 - Skretting Aquaculture Research Center, Stavanger, Norway; 2 - Patologi consult AS

Introduction: The mammalian cardiac conduction system consists of three main parts such as (1) the sino-atrial node (pacemaker), (2) the atrio-ventricular node (AV node) and (3) the His-Purkinje system. The heartbeat starts in the autonomous pacemaker cells (SA node), slows at AV node and propagates to rest of the heart by the His-Purkinje system. So far, the pacemaker tissue has been identified in teleost (Atlantic salmon) histologically.

Methodology and Results: Serial histological sections of buffered formalin-fixed complete hearts were stained with haematoxylin and eosin (HE) to study the cardiomyocytes and the presence of neural tissue. Here we present the novel histological evidence of the atrio-ventricular node (AV node) in Atlantic salmon (*Salmo salar* L.). Several fish hearts showed few to several ganglion cells at atrio-ventricular border. It lies endocardially in AV valve with focal melanisation of few ganglion cells. Special and immunohistochemial staining were performed to confirm the presence of nodal tissue.

Conclusion: This finding suggests that teleost (Atlantic salmon) harbour similar cardiac conduction system components such as pacemaker and AV node (present study) as of mammals.

Keywords: heart, atrioventricular node, ganglion, Atlantic salmon

238-P

Multiple liposarcomas in farmed Russian sturgeon (*Acipenser gueldenstaedtii* Brandt & Ratzeburg) in France: first gross and histopathological description.

Labrut, Sophie (France)[1]; Armand, Lautraite (France)[2]

1 - LABOCEA; 2 - Grisolles, France

Introduction: Two years-old Russian sturgeon (*Acipenser gueldenstaedtii*) showed noticeable swelling of the musculature mostly situated in the dorso-anterior part of the body. Only a few individuals (less than 1%) were concerned by the condition without any associated mortality.

Methodology: Necropsy of euthanized affected individuals revealed intramuscular, well delineated, yellowish and rather firm masses, underneath the swelling region but also several (around ten) other unnoticed masses, smaller in size, situated along the fillet. Some of these masses were sampled for further examination and formalin-fixed.

Results: Microscopical examinations of fresh mount or stained imprints (RAL 555) of tissue did not provide any parasitic nor bacterial signs of infection. Histopathological examination revealed well delineated within skeletal muscles but infiltrating tumors. Tumoral cells were densely arranged in sheets and some whorls in a fine collagenic stroma. These cells were round to multifocally spindle-shaped, moderately large (20-25 micrometers in diameter) and well demarcated. In some tumors, a large vacuole in the cytoplasm and a small peripheral hyperchromatic nucleus characterized most of the tumoral cells (consistent with adipocytes). In others, none or some microvacuoles in the cytoplasm and an oval euchromatic, rarely nucleolated, nucleus were characteristic of most of the tumoral cells (consistent with lipoblasts). Cytonuclear atypia were mild to moderate and the mitotic index low to multifocally moderate. These tumors were consistent with intramuscular, multiple, well differentiated liposarcomas or atypical infiltrative lipomatosis.

Conclusion: Liposarcomas have been rarely described in fish, never in sturgeons. In this case, their multicentric aspect could be a sign of an on-going metastatic dissemination. Tumors were only noticed within skeletal muscles. Their etiology remains mysterious, to date.

Keywords: liposarcomas, sturgeon, histopathology

Funding: Labocea, France.

239-P*

Evaluation of a gross "total" gill score against a standardized histologic score (and qPCR) in farmed Atlantic salmon (*Salmo salar*)

Østevik, Liv (Norway)[1]; Alarcon, Marta (Norway)[1]; Lie, Kai-Inge (Norway)[1]; Nødtvedt, Ane (Norway)[2]; Kraugerud, Marianne (Norway)[1]; Hellberg, Hege (Norway)[1]; Stormoen, Marit (Norway)[2]; Simensen, Benedikt (Norway)[4]; Manji, Farah (Norway)[4]; Rodger, Hamish (Ireland)[3]

1 - Fish Vet Group; 2 - Norwegian University of Life Sciences; 3 - VAI Consulting; 4 - Mowi AS

Introduction: Compromised gill health has emerged as a major factor in the farming of Atlantic salmon in Norway, leading to economic losses and reduced animal welfare in both the marine and freshwater phase of production. There are non-infectious and infectious elements involved in the development and impact of gill disease which can present as a simple or complex set of conditions. Identification of useful indicators of gill health are necessary to identify management strategies that can prevent and reduce the impact of gill disease. Histopathological evaluation is an excellent tool for investigating gill damage and remains the gold standard for many disease conditions, while qPCR is an efficient and highly sensitive tool to detect pathogens. However; both histology and qPCR analysis performed on tissue samples require euthanasia of the fish and shipment to a specialized laboratory for processing. Gross gill scoring is widely used for monitoring of amoebic gill disease, however a gill score system encompassing all gill lesions likely associated with reduced gill health may provide useful information when making decisions with regards to treatment and other management operations. This presentation describes newly developed gross and histologic gill scoring systems and preliminary results on test agreement between different scoring methods.

Methodology: Gross and histologic scoring systems based on previously reported and observed gill lesions were developed. The score systems will be used on 8 sea farms, with repeated parallel gross scoring and sampling for histology and qPCR of 30 fish from 2 pens (total of 60 fish) every 6th week for a 12-month period. Agreement between standardized histological scoring results and scoring results for the same gill arch will be assessed. Similarly, possible associations between gross and histology gill scores and water plankton levels and qPCR results for the gill pathogens *Neoparamoeba perurans*, *Desmozoon lepeophtherii*, *Candidatus Branchiomonas cysticola* and salmon gill poxvirus will be evaluated.

Results: Preliminary results will be presented at the conference.

Keywords: gross gill score, Atlantic salmon, gill disease, histology, qPCR

240-P

MHC gene as genetic marker of resistance to bacterial and viral disease: environmental pressure as enhancer of haplotype frequency shifts

Colussi, Silvia (Italy)[1]; Fariano, Lucio (Italy)[2]; Campia, Valentina (Italy)[1]; Riina, Maria Vittoria (Italy)[1]; Pastorino, Paolo (Italy)[1]; Acutis, Pier Luigi (Italy)[1]; Prearo, Marino (Italy)[1]

1 - Istituto Zooprofilattico Sperimentale of Piemonte, Liguria and Valle d'Aosta, Turin, Italy; 2 - Azienda Agricola Canali Cavour, Centallo (CN), Italy

Introduction: one of the main concerns affecting aquaculture production are infectious diseases. Many studies have been published reporting results about the association among genetic/genomic markers and the resistance to diseases. In particular MHC genes of class I and II have been reported to be associated with resistance to different diseases in different species. The exploitation of genetic features of the host through the implementation of marker assisted selection could represent a reliable method to provide long-term control over diseases. An MHC class II genetic screening on trout broodstocks and their progeny has been carried out in a farm with the aim to detect MHC polymorphisms related to resistance to lactococcosis and to infectious haematopoietic necrosis virus. During these screening a high and anomalous frequency of a a particular MHC haplotype (called 8) was found. We assumed that this was due to the adaptation of MHC to the environment favouring beneficial haplotypes.

Methodology: for MHC genetic screening buccal swabs were collected from 100 broodstock and 250 trout belonging to their progeny. For the evaluation of environmental pressure on MHC gene, buccal swabs were collected from 200 genetically related trout (siblings); 100 reared in contained environment not exposed to pathogens and 100 naturally exposed to pathogens. DNA was extracted from buccal swabs and direct sequencing was carried out. Haplotypes definition was carried out through the software PHASE 2.1.

Results: high frequency of haplotype 8 and high number of homozigous trout was found in broodstocks and their progeny not explicable by the population genetics rules. Results obtained from siblings analysis are not still completed but partial results showed a higher number of homozygous trout in the exposed population: results from haplotypes analysis (permutation test) was significant ($p = 0.05$), indicating differences between the two populations.

Conclusion: If our assumption will be confirmed by the final results, this will be the first report of a natural selection of the fittest MHC haplotype operated by the interaction between the host trout and different pathogens naturally present in the aquatic environment.

Keywords: MHC gene, bacterial diseases, viral diseases, haplotype frequency

241-P*

Low-cost, simple and reliable SNP genotyping method for discriminating subspecies and subpopulations of fish pathogen, *Nocardia seriolae*

Le, Cuong (Australia)[1,2]; Price, Erin (Australia)[1]; Sarovich, Derek (Australia)[1]; Nguyen, Thu Thi Anh (Vietnam)[3]; Knibb, Wayne (Australia)[1]; Chen, Shih-Chu (Taiwan)[4]; Katouli, Mohammad (Australia)[1]

1 - GeneCology Research Centre, University of the Sunshine Coast, Queensland, Australia; 2 - Institute for Aquaculture, Nha Trang University, Nhatrang, Vietnam; 3 - Institute for Biotechnology and Environment, Nha Trang University, Nhatrang, Vietnam; 4 - Department of Veterinary Medicine, College of Veterinary Medicine, National Pingtung University of Science and Technology, Taiwan

Introduction: The fish pathogen, *Nocardia seriolae*, has caused significant losses in Asian aquaculture. Whole-genome sequencing (WGS) has been used extensively to trace infectious diseases, and offers the highest level of resolution to discriminate closely-related strains. However, WGS is time-consuming and costly, and thus not practical for rapid epidemiological investigations. To overcome this issue, we used comparative WGS to identify informative single nucleotide polymorphisms (SNPs) within the *N. seriolae* core genome that enable highly accurate clade-level characterisation of this fish pathogen. We then targeted two of these SNPs using a low-cost and simple allele-specific real-time PCR assay to identify the putative origin and diversity of *N. seriolae* strains in Vietnam, where *N. seriolae* has emerged in recent years and has caused devastating losses to the Vietnamese aquaculture industry.

Methodology: Seven *N. seriolae* strains isolated from infected pompano in four Vietnamese provinces were subjected to WGS on the Illumina NextSeq 500 platform. SPANDx v3.1 was used to identify core-genome, biallelic SNPs among Vietnamese strains and seven published strains from other Asian countries. Based on phylogenomic reconstruction using 3,231 high-confidence SNPs, we designed SYBR Green-based mismatch-amplification-mutation (SYBR-MAMA) real-time PCR assays to: 1) differentiate Vietnamese strains from those of other countries (SNP1), and 2) distinguish between the two Vietnamese clades (SNP2). To ensure SYBR MAMA accuracy, sequenced strains were included as DNA controls for both SYBR-MAMA PCRs. Subsequently, 48 other Vietnamese *N. seriolae* strains and 9 strains from Taiwan were screened.

Results: Our SYBR-MAMA assays yielded results identical to those obtained by WGS. The difference in cycles-to-threshold value, $|\Delta C_T|$, between the Vietnamese and non-Vietnamese strains (SNP1 assay) was ≤ 3.74 and ≥ 7.11 for Vietnamese and non-Vietnamese strains, respectively, while these values for the SNP2 assay was ≥ 11.36 for Vietnamese Clade 1 and ≤ 3.99 for Vietnamese Clade 2.

Conclusion: Our SYBR-MAMA PCRs provide a simple, cost-effective, and rapid method for discriminating Vietnamese *N. seriolae* strains from those found in other Asian countries. We demonstrated that all tested Vietnamese strains belong to one of only two very closely-related clades, confirming recent importation of this fish pathogen into Vietnam.

Keywords: SNP genotyping, *Nocardia seriolae*, whole-genome-sequencing

Funding: USC funding.

242-P

Detection of the microsporidian *Nucleospora cyclopteri* in tissue samples from lumpfish (*Cyclopterus lumpus*) using *in situ* hybridization (ISH)

Hansen, Haakon (Norway)[1]; Markussen, Turhan (Norway)[4]; Karlsbakk, Egil (Norway)[3]; Weli, Simon (Norway)[2]; Faller, Randi (Norway)[1]; Thoen, Even (Norway)[2]

1 - Norwegian Veterinary Institute, Fish Health Research Group; 2 - Norwegian Veterinary Institute, Research Group for Biosecurity and Emerging Diseases; 3 - University of Bergen, Fish Disease Research Group; 4 - Norwegian University of Life Sciences, Department of Food Safety and Infection Biology

Introduction: Lumpfish (*Cyclopterus lumpus*) is increasingly used as a cleaner fish to control infections of salmon lice in the Norwegian aquaculture industry. However, lumpfish is itself infected by a number of pathogens with mostly unknown impact on the fish health. One of these is the microsporidian parasite *Nucleospora cyclopteri* that contributes to mortality in both wild and farmed lumpfish. This parasite develops within the nuclei of leucocytes and induces a systemic infection with swollen kidneys (renomegaly) as the most prominent clinical observation. To enable detailed studies of the distribution of pathogens, and their localization and impact in various tissue types, methods that are both highly sensitive and specific for the particular pathogen are needed. Traditional methods of staining such as hematoxylin-eosin (HE), gram-twort and calco-fluor-white (CFW) are able to stain microsporidian spores. However, pre-sporogonial stages that dominate in many samples are not stained by these methods. In addition, most traditional staining methods are non-specific and stain bacteria and fungi as well as microsporidia.

Methodology: Locked nucleic acid (LNA) modified oligonucleotide probes labelled with either digoxigenin (DIG) or the fluorescent dye TYE665 were designed to specifically target *Nucleospora cyclopteri* 18S rDNA/rRNA for *in situ* hybridization (ISH) and fluorescence *in situ* hybridization (FISH), respectively. Both probes were tested on paraffin-embedded tissue sections from parasite-positive lumpfish as determined by real-time PCR. Several parameters were varied, tested and optimized ISH/FISH protocols were developed. The infections were confirmed by light microscopy and compared with neighbouring sections stained with traditional staining methods.

Results: Both the ISH and FISH methods successfully stained *N. cyclopteri* in the tissue. Mature spores were generally not stained by ISH but appeared to be stained when using FISH.

Conclusion: Specific and sensitive *in situ* hybridization methods targeting 18S rRNA/rDNA of *N. cyclopteri* were developed. The two methods complement each other as well as other traditional methods of staining, they stain the pre-sporogonial stages and provide details on the infection that contribute to understanding the impact this parasite has for the health of lumpfish.

Keywords: Microsporidia, *in situ* hybridization, fish parasites

Funding: The Norwegian Seafood Research Fund (FHF), project number 901320.

243-P*

Evaluation of histological postmortem changes in Norwegian farmed Atlantic salmon (*Salmo salar* L.) at varied time intervals and storage temperatures

Erkinharju, Toni (Norway)[1,2]; Naveed Yousaf, Muhammad (Norway)[1]; Furnesvik, Lisa (Norway)[1]; Seternes, Tore (Norway)[2]

1 - Norwegian Veterinary Institute, Harstad, Norway; 2 - Faculty of Biosciences, Fisheries and Economics, Norwegian College of Fishery Science, UiT- The Arctic University of Norway, Tromsø, Norway

Introduction: In many cases, stained histological sections are suggested as the gold standard for disease investigations in fish. Thus, the histological evaluation of tissue is crucial to diagnostics. Immediate fixation is required for best preservation for histology. There are scarce data about preserving fish carcasses to minimize postmortem autolytic changes when immediate necropsy cannot be performed in colder northern Norway environment.

Methodology: The aim of this study was to identify and score histologic postmortem autolytic changes in Atlantic salmon (cca 70 g). A total of 39 fish were stored either at room temperature, refrigerated at 4 ºC or frozen at -20 ºC and necropsy was performed at 0, 1, 4, 24 and 48 h post storage. Haematoxylin and eosin stained slides were evaluated by light microscopy and scored by using semi-quantitative scoring system as follows: minimal (< 5%), mild (5 - 10%), moderate (10 - 50%) and severe (> 50 %) autolysis.

Results: Preliminary work has shown promising results in the form of postmortem changes in room temperature, refrigerated and frozen fish groups. Detailed overview of postmortem changes and future recommendations will be presented.

Conclusion: Atlantic salmon kept at different storing temperatures will over time lead to postmortem changes in sampled tissues. This can lead to complications for histological evaluation of the tissue, and sampling should be performed to minimize these changes.

Keywords: Atlantic salmon, postmortem changes, histology, storage temperature, time

Funding: Norwegian College of Fishery Science – UiT The Arctic University of Norway, Norwegian Veterinary Institute.

244-P

Toward the design of nanomaterials-based sensing e-bioplatform for nodavirus detection to assess fish health status in fish farms

Chérif, Nadia (Tunisia)[1]; Amdouni, Fatma (Tunisia)[1]; Meftah, Marwa (Tunisia)[3]; Kssouri, Ayoub (Tunisia)[2]; Bouhaouala-Zaharc, Balkiss (Tunisia)[2]; Raouefi, Noureddine (Tunisia)[3]

1 - National Institute of Sea Sciences and Technologies; 2 - Pasteur Institute; 3 - Science Faculty of Tunis

Introduction: To further facilitate betanodavirus early detection with a promising fast, reliable and cost-effective technique, the aim of this study was to develop and optimize a simple and ultrasensitive electroanalytical methodology to detect different nodavirus genotypes that can be introduced to fish farming facilities.

Methodology: To achieve this goal, we studied and selected a structural conserved region within the viral RNA coding sequence for the capsid protein to design the recognition DNA probe that will be tethered onto the surface of nanostructured disposable electrodes, in competitive assays. In a proof-of-principle test, viral samples were prepared by extracting RNA from healthy and infected fish samples and subjected to a direct hybridization with a specific thiolated DNA probe self-assembled together with mercaptohexanol (MCH) onto gold nanoparticles (AuNP)-modified screen-printed electrodes (SH-DNA/MCH/AuNPs/SPCEs). The stepwise modification of the electrodes was accessed using cyclic voltammetry and electrochemical impedance spectroscopy.

Results: The competition between the target RNA and the biotinylated target RNA toward the probe was assessed using HRP-streptavidin conjugate, which was used to reduce hydrogen peroxide in presence of hydroquinone as an electron donor. The amount of target RNA is proportional to the reduction current of quinone formed in the catalytic reaction.

Conclusion: The preliminary results are promising and in progress toward better knowledge of the fish health status. The valuable tool will allow more effective diagnosis and risk assessment of viral diseases in aquaculture.

Keywords: biosensing, nodavirus, aquaculture, screening

245-P

Diagnostic methods for identification of *Aeromonas* spp. and examination of pathogenicity factors, cytotoxicity and adherence to fish mucus

Jung-Schroers, Verena (Germany)[1]; Arne, Jung (Germany)[1]; Ryll, Martin (Germany)[1]; Bauer, Julia (Germany)[1]; Teitge, Felix (Germany)[1]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany

Introduction: The genus *Aeromonas*, belonging to the class *Gammaproteobacteria* and the family *Aeromonadaceae*, contains Gram-negative, non-spore forming, rod-shaped, facultative anaerobic bacteria. Aeromonads are ubiquitous in the environment, especially in aquatic habitats and act as obligatory or facultative pathogens of aquatic animals and man. *Aeromonas salmonicida*, the only non-motile species within the genus *Aeromonas* and the causative agent for furunculosis in salmonids, is an obligate fish pathogen whereas many motile aeromonads are known as opportunistic pathogens of fish, amphibians and other aquatic animals and also in human disease outbreaks are described.

Methodology: In this study 44 already characterized isolates of *Aeromonas* spp. were analysed. For species identification biochemical techniques, 16S rRNA sequencing, sequencing of the *gyrB* gene that encodes the b-subunit of DNA gyrase, MALDI-TOF MS and the Sherlock Microbial Identification System (MIS) based on the composition of fatty acid ethyl esters were compared. The phylogenetic relationship, cytotoxicity in vitro, adherence to mucus in vitro and resistance against antibiotics were tested.

Results: The most reliable method for species identification was MALDI-TOF MS and *gyrB* sequencing. Most virulence factors were found in isolates of *A. dharkensis*, *A. hydrophila*, and *A. salmonicida* and especially isolates of *A. dharkensis* and *A. hydrophila* showed a high cytotoxic activity. Nevertheless, the virulence of aeromonads is probably not only depending on the species but on the isolate itself. Many isolates of *Aeromonas* spp. were showing multi-resistances against antibiotic substances. This result has to be regarded as critical, because of the ubiquitous nature of *Aeromonas* sp. and the widely distributed virulence mechanisms.

Conclusion: Testing the susceptibility of antibiotic substances before treating diseased fish should be a standard procedure, to reduce the development of resistant isolates and to ensure that an adequate substance is used for treatment.

Keywords: *Aeromonas* spp., MALDI-TOF, gyrase-B sequencing, pathogenicity

246-P

Nothing is as simple as it seems - quantitative diagnostics of gill diseases in koi reveals co-infection with multiple pathogens.

Adamek, Mikolaj (Germany)[1]; Teitge, Felix (Germany)[1]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany

Introduction: During a disease outbreak, affected fish exhibit particular clinical signs and the task in veterinary diagnostics is to identify the causing agent(s) as a prerequisite for appropriate treatment measures. This task is more challenging when several potentially deadly pathogens are present in the affected organ and could be responsible for the observed clinical signs. In a case of gill disease in koi, we performed individualized quantitative diagnostic to evaluate whether the observed mortality was caused by the same pathogen in all affected individuals during a co-infection scenario.

Methodology: Tissue samples from gills, brain, fins, kidney, head kidney, liver, spleen gut and heart were collected from dead fish during an outbreak of a multifactorial gill disease in a small cohort of ornamental koi with gill necrosis as the main exterior clinical sign. RNA and DNA were extracted from the samples and involved pathogens were identification and their abundance was determined by end-point or quantitative PCR in various tissues of affected individuals. Furthermore material collected from gills was inoculated into CCB cell monolayers.

Results: The results indicated presence of several infectious agents in gills and other tissues. Interestingly, the mortality of individual fish was most likely caused by different agents, despite the fact that it occurred in the same cohort of fish during a period of less than a week. Three out of five diseased individuals suffered from koi herpesvirus disease associated with a systemic infection with cyprinid herpesvirus 3, one fish succumbed to koi sleepy disease caused by a high carp edema virus load in the gills and with CyHV-3 and flavobacteria as co-infecting agents, while the last fish had low loads of both viruses but high flavobacteria and *Ichthyobodo* burdens and most likely died from an interaction of these bacterial and parasitic agents.

Conclusion: The results indicated that a correct identification of the infective agent responsible for observed clinical signs or mortality would require quantitative determination of the abundance of the pathogens in individual fish as well as a detailed knowledge of the infection biology of the pathogens involved.

Keywords: KHV, CyHV-3, CEV, koi

247-P

Methods for identification and differentiation of *Shewanella* spp. isolates from diagnostic samples

Jung-Schroers, Verena (Germany)[1]; Jung-Schroers, Arne (Germany)[1]; Ryll, Martin (Germany)[1]; Bauer, Julia (Germany)[1]; Teitge, Felix (Germany)[1]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany

Introduction: *Shewanella* spp. are Gram-negative, rod-shaped, motile bacteria that are widely distributed in marine and freshwater environments. They are present in the physiological microflora of fish from temperate waters and are known as fish-spoilage species. From clinically healthy fish as well as from fish with skin ulcerations *Shewanella* spp. are regularly isolated, indicating a possible role as fish pathogen.

Methodology: In this study 74 isolates of *Shewanella* spp. were analysed. For species identification biochemical techniques, 16S rRNA sequencing, MALDI-TOF MS and the Sherlock Microbial Identification System (MIS) based on the composition of fatty acid ethyl esters were compared. The phylogenetic relationship, cytotoxicity in vitro and resistance against antibiotics were tested.

Results: The most reliable method for species identification was the sequencing of the 16S rRNA gene. Different *Shewanella* species were isolated from diseased fish, clinically healthy fish and the aquatic environment. This indicates that *Shewanella* spp. are widespread in the aquatic milieu and act as a secondary pathogen. The virulence of *Shewanella* spp. is probably not depending on the species but on the isolate itself. Many isolates of *Shewanella* spp. were showing multi-resistances against antibiotic substances, especially in samples derived from retailers.

Conclusion: Different *Shewanella* species were isolated from diseased fish, clinically healthy fish and the aquatic environment. This indicates that *Shewanella* spp. are widespread in the aquatic milieu and act as a secondary pathogen. In routine diagnostics all *Shewanella* spp. should be tested for resistances against antibiotic agents.

Keywords: *Shewanella* spp., 16S rRNA sequencing, MALDI-TOF

248-P

Molecular evidences that the cell line CHSE-214 is derived from the salmon *Oncorhynchus tshawytscha* rather than the bluegill *Lepomis macrochirus*

Menanteau-Ledouble, Simon (Austria)[1]; Oskar, Schachner (Austria)[1]; El-Matbouli, Mansour (Austria)[1]

1 - Klinische Abteilung für Fischmedizin, Vetmeduni Vienna

Introduction: The Chinook Salmon Embryo-214 (CHSE-214) cell line was established in the 1960-ies and its morphology is described as epitheloid. This cell line is still used for virus propagation and it has been reported that CHSE-214 cells had been confused with cells originating from the bluegill *Lepomis macrochirus*, rather than *Oncorhynchus tshawytscha*. However, the fibroblastoid morphology and biology of the most common cell line derived from bluegill *L. macrochirus* (BF-2) clearly differs from CHSE-214 cells making confusion implausible. Because of this uncertainty, it was decided to confirm the origin of CHSE-214.

Methodology: Five primer pairs were designed targeting randomly selected genes from the genome of *O. tshawytscha* and confirmed not to match any significant segment of the genome of *L. macrochirus* using Basic Local Alignment Sequence Tool (BLAST). Conversely, five additional primer pairs were designed from *L. macrochirus* and BLAST was used to confirm that they did not match any salmonid genomes' sequences in the Entrez repository. Afterwards, PCRs were performed using these primers on CHSE-214 genomic DNA. Resulting products were purified and sequenced to confirm their identity.

Results: All of the *O. tshawytscha* primers produced amplicons of the expected size and matching the *O. tshawytscha* genome while the *L. macrochirus* primers produced no amplicons.

Conclusion: The primers designed for *O. tshawytscha* matched sequences on the CHSE genome while primers designed for *L. macrochirus* did not. Therefore, it appears that at least some of the CHSE cell lineages currently used most likely derive from *O. tshawytscha*.

Keywords: cell line, characterisation

249-P*

Are early inflammatory lesions in *Sparus aurata* indicative of the hatchery phase quality? Invisible injuries as essential histological indicators

Pacorig, Valentina (Italy)[1]; Beraldo, Paola (Italy)[1]; Volpatti, Donatella (Italy)[1]; Galeotti, Marco (Italy)[1]

1 - University of Udine

Introduction: Gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) are two of the most farmed fish species in EU. In recent years, the rapid emergence of non-EU competitor farmers has been leading to an economic stagnation. The H2020 PerformFISH project mission is to promote the sustainability/competitiveness of Mediterranean aquaculture. In this light, the marine aquaculture weakness is still represented by the hatchery phase, adversely affecting the final product quality and economic profitability. Thus, it is essential to set up prognostic indicators of larval and juvenile quality for gilthead sea bream (GSB) and European sea bass. The aim of this study is to investigate if some early inflammatory lesions in GSB larvae can be used as quality indicators.

Methodology: Larvae/juveniles GSB batches coming from 4 different European hatcheries were sampled at different ages (5, 25, 35, 50, 85-100 days post hatching DPH), fixed in Bouin's solution (n=20 for each batch and age), then histologically processed to obtain serial sections stained with HE and other histochemical and immunohistochemical protocols according to the study purposes. The specimens were observed under DMRB light microscope (Leica) equipped with digital camera (Nikon) and imaging software (NIS-Elements Br, Nikon). A multiparametric semiquantitative scoring was specifically set up for histological evaluation.

Results: Currently only in early juveniles (85-95 DPH) pancreatitis and steatitis were observed, characterised by diffuse cellular infiltrate mainly composed by eosinophilic granulocytes but also by lymphocytes and eosinophilic granular cells (mastocytes), associated to focal necrosis and slight pancreatic atrophy. Further results will be discussed.

Conclusion: Since *"what is essential is invisible to the eye"*, the histology seems to be an adequate tool to promptly recognise health problems in a fish population, helping farmers to resolve critical issues in the hatchery phase.

Keywords: gilthead sea bream, hatchery phase, histology, scoring, cellular infiltrate

Funding: PerformFish an EU H2020-funded project (727610).

250-P

Histology and anatomy of clinically healthy Atlantic lumpfish, *Cyclopterus lumpus*

Klingenberg, Oda (Norway)[3,4]; Hansen, Haakon (Norway)[1]; Karlsbakk, Egil (Norway)[2]; Seternes, Tore (Norway)[3]; Thoen, Even (Norway)[1]

1 - Norwegian Veterinary Institute, Fish Health Research Group; 2 - University of Bergen, Norway, Fish Disease Research Group; 3 - University of Tromsø, The Norwegian College of Fishery Science, Aquaculture and Environment; 4 - University of Tromsø, The Norwegian College of Fishery Science

Introduction: Lumpfish, *Cyclopterus lumpus*, has become an important species in the Norwegian aquaculture industry as it is used as a biological control measure for controlling infections with salmon lice. The lumpfish is itself exposed to a number of infections, both parasitic, bacterial and virological, and the knowledge of these infections and their importance is deficient. In order to assess the importance of infections on the host and in different types of tissues, a detailed knowledge of the normal histology is crucial for tissue changes to be detected and compared with the normal state.

Methodology: Relevant diagnostic tissues from lumpfish that were clinically healthy and had been screened for known pathogens were analysed. The fish were from 5 to 130 grams and and from each fish, tissue samples from gills, heart, skin, muscle, liver, spleen, pancreas and several samples from the kidney were taken. Tissue samples were fixed in formalin and embedded in paraffin wax. Histological sections (5 µm) were prepared and stained with haematoxylin and eosin (HE) and examined under the microscope. In addition, a selection of sections were stained with Alcian blue/periodic acid–Schiff (AB-PAS), Van Gieson and May-Grünewald/Giemsa. Images were taken at 5x, 10x, 20x, 40x and 100X magnification and the characteristics of the tissues were described.

Results: We present here a selection of the results from this study. Images of the fish tissues are presented and compared with tissue sections from salmon along with selected, relevant pathological changes associated with disease states in the lumpfish. The macroscopic and microscopic images of the normal anatomy and histology accompanied with descriptions of the tissues will at the end of the project be made publicly available through an online image database.

Conclusion: The detailed knowledge obtained from the current project and the images and descriptions of the normal histology of the lumpfish will be very valuable for diagnosticians and researchers working on histopathology and biology of this fish species.

Keywords: histology, cleaner fish, histopathology

Funding: The Norwegian Seafood Research Fund (FHF), project number 901320.

251-P

Development of a point-of-care test for amoebic gill disease (AGD)

Cano, Irene (United Kingdom)[1]; Mccullough, Robin (United Kingdom)[1]; Mulhearn, Brian (United Kingdom)[1]; Paley, Richard (United Kingdom)[1]

1 - Cefas

Introduction: Amoebic gill disease (AGD) is a serious disease affecting Atlantic salmon farmed in the marine environment. AGD is characterised by multifocal white patches on the gill surface. The causative agent is the protozoan *Neoparamoeba perurans*. Control of AGD includes general measures that prevent parasite introduction into the production system. Loop-mediated isothermal amplification (LAMP) amplifies nucleic acids with high specificity, sensitivity and rapidity under isothermal conditions.

Methodology: A LAMP assay was designed targeting the *N. perurans* 18S rRNA gene. The assay showed high specificity to *N. perurans*, with no amplification of the close relatives *N. pemaquidensis* and *N. branchiphila* or host tissue. Three commercially available isothermal master mixes were tested. Of these, The ISO004 (Optigene) showed the fastest amplification, with the detection of 10^6 copies of the recombinant plasmid under 11 min and 10^2 copies under 30 min. Five different "dirty and fast" DNA extraction protocols were evaluated for its use in the field and compared with the laboratory reference extraction method (EZ1 BioRobot). Those methods were further validated using a published Taqman qPCR test. The QuickExtract buffer was selected as the easier and faster DNA extraction method, which allows for qPCR validation. An additional LAMP assay to amplify the Atlantic salmon EF1a was designed and used as internal control for quality control of the extracted DNA. Non-lethal Isohelix gill swabs were taken from 76 Atlantic salmon showing different degrees of AGD (gill score between 4 to 0.5) and 5 control fish. The LAMP assay detected the presence of the pathogen from the gill swabs in under 23 min (average 18 min) for fish showing clinical sings (gill score 2-4); and positive results were obtained under 39 min (average 29 min) for fish showing mild AGD signs (gill score 0.5-2). Invalid test results (no amplification of the internal control) were obtained for 2% of the samples analysed.

Conclusion: The LAMP assay successfully detects *N. perurans* in non-lethal gill swabs taken from clinically infected Atlantic salmon allowing for its use as point-of-care test.

Keywords: AGD, LAMP, *N. perurans*, salmon

Funding: This project has received funding from the EU grant ParaFishControl.

252-P

Haematology and biochemistry in the assessment of turbot intestinal health

Losada, Ana (Spain)[1]; Ronza, Paolo (Spain)[1]; Bermúdez, Roberto (Spain)[1]; Quiroga, Mª Isabel (Spain)[1]

1 - Universidade de Santiago de Compostela

Introduction: Haematology and biochemistry are effective tools to monitor animal health. These economical and non-lethal techniques may complement and integrate with other diagnostic methods. These studies applied to fish are scarce, and data available on various species show wide variations. Determining the reference values in haematology and biochemistry of turbot, aquatic production of great economical impact in NW Spain, would serve as health and welfare indicators, as well as prognostic values in field cases. The aim of this work was to determine the ranges of normal values in haematological and biochemical tests in healthy turbot; and establish the variations in these values in cases of disease, using the turbot enteromyxosis as a model.

Methodology: For this purpose, a group of turbot was orally inoculated with *Enteromyxum scophthalmi* and other healthy group was maintained as control. Samples of blood and internal organs were collected and analysed. Blood cells were counted, and leukocyte formula was calculated. Biochemistry was performed in citrate plasma samples. Tissues were routinely processed for histopathology.

Results: Light microscopy of tissue samples showed mild to severe enteritis associated to stages of *E. scophthalmi*, with epithelial desquamation and mixed inflammatory infiltrates in the lamina propria-submucosa. Comparing to the normal values established in the control group, infected turbot had diminished haematocrit, red blood cell count and mean corpuscular value; increased white blood cell count and changes in the leukocyte formula (granulocytosis and lymphopenia). Biochemical alterations during infection consisted in raise of potassium, urea, transaminases and drop of total protein and glucose.

Conclusion: The preliminary results reflect the cellular response, anaemia, hypoproteinaemia, hypoglycaemia and hepatic and renal function disturbances during *E. scophthalmi* infection in turbot.

Keywords: blood tests, fish welfare, prognosis

Funding: The Spanish Ministry of Economy and Competitiveness and the European Regional Development Fund (ERDF) under the Project AGL2015-67039-C3-1-R.

253-P*

Establishment and application of two brain cell lines from tilapia and hybrid snakehead for detection of tilapia lake virus

Wang, Yingying (China)[1]; Wang, Qing (China)[1]; Zeng, Weiwei (China)[1]; Li, Yingying (China)[1]; Yin, Jiyuan (China)[1]; Liu, Chun (China)[1]; Bergmann, Sven M. (Germany)[2]

1 - Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences; 2 - Friedrich Loeffler Institut

Introduction: Tilapia lake virus (TiLV) is associated with highly contagious disease outbreaks in cultured tilapia worldwide. Cell culture isolation is considered to be the golden standard for virus identification. In present study two brain cell lines from tilapia (TiB) and hybrid snakehead (CAMB) were established and applicated for detection of tilapia lake virus .

Methodology: Primary culture of TiB and CAMB cells were initiated by trypsin digestion methods, and the cells were successfully subcultured after optimization of the culture medium and culture conditions. The 60th generation of TiB and CAMB were treated with the colchicine to get the chromosomes. The susceptibility of two cells to TiLV was determined using TiLV-2017A isolate. The viral replication in the cells were confirmed by cytopathic effect, transmission electron microscopy, immunofluorescence assays and virus titers, indicating the susceptibility of TiB and CAMB cells to TiLV-2017A.

Results: The TiB cell line was optimally maintained at 27 °C using medium 199 supplemented with 10% fetal bovine serum (FBS). And the most favorable condition for CAMB is in Leibovitz's L-15 medium containing 10% FBS at 27 °C. The TiB and CAMB cells were epithelioid cell shape and grew actively under the optimal medium and culture conditions. Chromosome analysis of the cell cultures revealed that the TiB and CAMB cells maintained the abnormal triploid chromosome number 2n = 50 and 2n = 64 whereas the number of chromosomes in tilapia and hybrid snakehead were 44 and 45. The two cells were susceptible to TiLV-2017A and the viral replication were confirmed by electron microscope observations, immunofluorescence assays and virus titers which suggested the two cell lines' application potential in studies of TiLV. The titration of viral infectivity in three cell lines (E-11, TiB, CAMB) were showed that TiB cells were most susceptible to TiLV, and then was the CAMB cells followed by E-11cells.

Conclusion: In summary, two continuous fish cell lines from brain in tilapia and hybrids snakehead have been established successfully. The developed two brain cell lines would pave way for researching TiLV detection methods, vaccine preparation, diseases control and prevention.

Keywords: cell lines, freshwater fish, Tilapia Lake Virus

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Aquatic Animal Welfare

254-P*

The effect of sudden temperature decrease on common carp under experimental conditions

Hodkovicova, Nikola (Czech Republic)[1,2]; Mares, Jan (Czech Republic)[3]; Sehonova, Pavla (Czech Republic)[1]; Blahova, Jana (Czech Republic)[1]; Modra, Helena (Czech Republic)[3]; Faldyna, Martin (Czech Republic)[2]; Vrankova, Andrea (Czech Republic)[1]; Svobodova, Zdenka (Czech Republic)[1]

1 - Department of Animal Protection, Welfare and Behaviour, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic; 2 - Department of Immunology, Veterinary Research Institute, Brno, Czech Republic; 3 - Department of Zoology, Fisheries, Hydrobiology and Apiculture, Faculty of AgriSciences, Mendel University Brno, Czech Republic

Introduction: One of the most important stress in fisheries is thermal stress caused by sudden temperature change leading to inability of keeping the homeostasis. The objective of the present study was assessment of stress caused by temperature decrease on common carp under experimental conditions.

Methodology: Total of the 56 tested individuals of *Cyprinus carpio* (94.54 ± 27.99 g) were exposed to temperature 27.8 °C (T_1). After 14 days of acclimatization, 28 individuals were moved to the 16.8 °C (T_2); the rest was kept in the original water (T_1). The water analysis together with the gaining of samples (n = 7) for haematological and biochemical analysis, biometrical data and analysis of digestive tract activity was performed after 6, 12, 24 and 48 hours for both tested groups.

Results: Significance was found for weight of digestive tract and weight intestinal content between both temperature groups. For almost all fish in colder T_2 water, the digestion was slowed and finished after 48 hours; however, in warmer T_1 water, the intestine was empty already after 24 hours. Haematological findings showed the similar trend among T_2 groups presented as decreased values for haematocrit, haemoglobin, erythrocytes and leucocytes count when compared to T_1 water. The neutrophils to lymphocytes ratio was found to be higher among all samplings made in T_2 groups. Observed biochemical parameters (ammonia, triglycerides, total protein and albumin) were decreased when fish were exposed to the colder T_2 water; in opposite, lactate was increased in T_2.

Conclusion: The digestion process was slowed when fish were exposed to colder T_2 water and the liquid constitution of digested feed in intestine was observed along with the visibly inflated intestine. Examined haematological and biochemical parameters were found to be decreased in T_2. The lower ability of detoxification and excretion of endogenous ammonia was reported for T_2 at 6 and 12 hours in conclusion to water analysis. However, the concentration of ammonia in fish plasma was at comparable levels in the same time interval for both tested groups.

Keywords: cyprinidae, stress, ammonia intoxication, digestion, immunity

Funding: Resources of institutional research FVHE and the ERDF/ESF “PROFISH” [No. CZ. 02.1.01/0.0/0.0/16_019/0000869].

256-P

Illegal fishing with electro-fishing devices in the Po river basin, Emilia Romagna, Italy

Rubini, Silva (Italy)[2]; Perolo, Alberto (Italy)[1]; Centelleghe, Cinzia (Italy)[1]; Corazzola, Giorgia (Italy)[1]; Gustinelli, Andrea (Italy)[3]; Fioravanti, Maria Letizia (Italy)[3]; Garbarino, Chiara Anna (Italy)[2]; Fontana, Maria Cristina (Italy)[2]; Gaudio, Rosa Maria (Italy)[4]; Frisoni, Paolo (Italy)[4]; Quaglio, Francesco (Italy)[1]; Mazzariol, Sandro (Italy)[1]; Fiore, Rosa Maria (Italy)[5]

1 - University of Padua - Department of Comparative Biomedicine and Food Science; 2 - Experimental Zooprophylactic Institute of Lombardy and Emilia Romagna; 3 - Department of Veterinary Medical Sciences, Alma Mater Studiorum University of Bologna, Bologna, Italy; 4 - Department of Medical Sciences, University of Ferrara, Ferrara, Italy; 5 - Freelance biologist

Introduction: Electric fishing is an illegal hunting method, forbidden in normal fishing circumstances, but unfortunately this is widely used by poachers. In Italy it is authorized only for scientific and/or conservative purposes. The principle of electrofishing consists in the application of an electric field into a water basin to paralyze fish; this allows to catch many animals in less time respect other traditional fishing technics. The characteristic behavior and immobilization of the fish are supposed to be the results of the electrical field that stimulates a muscular reaction, either involving the central and/or autonomic nervous system or not.

Methodology: Between 2014 and 2018 the Ferrara laboratory of the *Experimental Zooprophylactic Institute* received 13 cases (more than a ton of fish) of potentially illegal electric fishing including different species such as freshwater bream (*Abramis brama*), black bullhead catfish (*Ameiurus melas*), asp (*Aspius aspius*), European crucian carp (*Carassius carassius*), common carp (*Cyprinus carpio*), silver carp (*Hypophthalmichthys molitrix*), largemouth black bass (*Micropterus salmoides*), flathead grey mullet (*Mugil cephalus*), pike-perch (*Sander lucioperca*), wels catfish (*Silurus glanis*). Necropsies were performed following standard protocols and samples of different tissues were collected and examined using histochemical and immunohistochemical (IHC) techniques.

Results: The gross lesions most frequently observed were moderate to severe multifocal cutaneous hemorrhages, gills congestion, cerebral hyperemia and hemopericardium. In particular, hemopericardium was a constant finding between all the animals examined. Exophthalmos was detected in same fish. At the histological examination all the animals showed diffuse congestive/haemorrhagic phenomena and focal Zenker's necrosis in the skeletal muscle, that possibly could be attributed injuries from electric current, as already reported in literature. Immunohistochemical investigations confirmed the degenerative and necrotic picture with myoglobin depletion in the sarcoplasm that corresponded to an accumulation of fibrinogen. Myoglobin globules were also detected in the renal parenchyma, accurately in the cytoplasm of the epithelial tubular cells, suggesting myoglobinuria following muscle damage.

Conclusion: The results of this study allowed to correlate electric fishing to gross, histologic and IHC lesions, which together constitute a pathognomonic picture to be considered as a reference standard in this type of illegal controversy.

Keywords: electro-fishing, illegal fishing, Po river Italy

257-P*

Regulation of appetite gene expression in response to stress exposure in common carp (*Cyprinus carpio*)

Rhyner, Nicola (Switzerland)[1]; Tschirren, Linda (Switzerland)[1]; Seitz, Andreas (Switzerland)[1]; Pietsch, Constanze (Switzerland)[1]

1 - Zurich University of Applied Sciences

Introduction: To investigate effects of different stress exposures on appetite gene regulation in the common carp (*Cyprinus carpio*) brain, we use real-time quantitative polymerase chain reaction (qPCR) for relative quantification of genes involved in appetite adaptation and feed intake.

Methodology: All fish were acclimatized in an experimental tank before stress trials took place. Two groups of fish were exposed to either eustress using a feed reward approach or to distress by controlled air exposure. The samplings were performed after 10, 30 and 60 min past exposure. A control group was included, which experienced only minor stress at the beginning of the time series (due to opening and closing of the curtains surrounding the experimental tanks). Additionally, one control group was sampled before trials started (time 0) to serve as a reference state. Sampled brain tissue was stored in RNA later immediately and individual brains were subdivided into telencephalon, optic tectum, cerebellum and hypothalamus prior to performing RNA extraction and reverse transcription for subsequent gene expression analyses. During the samplings, blood was taken from each sampled fish for cortisol quantification which was analyzed using high-performance liquid chromatography. Relative quantification of appetite gene expression was achieved using a set of reference genes. All trials were conducted according to the regulative permissions for animal experiments.

Results: The highest blood cortisol levels among both treatments and the control were at the 10 min sampling, before concentrations decreased over time. Thus, we could show that the conducted trials resulted in expected patterns of stress responses when looking at cortisol measurements as it is commonly done when addressing stress quantification in animals. Different regulation of appetite gene expression was observed not only between the treatment groups but also amongst the four distinct brain areas. Analysis will be completed in summer 2019 and data will be discussed for the different stress scenarios conducted in this trial.

Keywords: stress, gene expression, appetite, qPCR, *Cyprinus carpio*

258-P

Can decapods successfully be stunned by electricity?

Wahli, Thomas (Switzerland)[1]; Von Siebenthal, Beat (Switzerland)[2]

1 - Centre for Fish and Wildlife Health, University of Bern; 2 - Valperca SA, Chavornay

Introduction: Decapods are caught for human consumption (e.g. lobster, edible crab) but also in the frame of management measures to eliminate invasive species (e.g. American crayfish) from aquatic habitats. Decapods are considered to be pain-sensitive. Therefore the provisions for handling these animals have been tightened in a recent amendment of the Swiss legislation. Thus decapods have always to be stunned before killing. Thus an efficient and reliable stunning method must be available. Electro-stunning is indicated by scientific and welfare organisations as a method rendering decapods senseless immediately. Effective electro-stunning devices are thus highly needed. The aim of the project presented here was to test the suitability of a new device for stunning decapods.

Methodology: Three decapod species (American lobster, edible crab and European crayfish) were tested with different settings for consciousness at 10 and 60 minutes after stop of exposure to the current, using a score considering movements of appendices and reactivity to sensory stimuli.

Results: Only one of different available settings allowed stunning animals of all three species successfully for a duration of at least 10 minutes. The effect of the electricity was immediate. No flight reaction was seen in animals of any of the three species nor was there any loss of appendices. Very slow movements of appendices immediately after exposure and ten minutes after stop of exposure were recorded. But neither touching nor taking out of the water provoked any targeted reaction. Despite an increase of movements between 10 and 60 minutes after stop of electricity exposure, none of the animals recovered. On the other hand, with the exception of the edible crabs, euthanasia was not achieved. Histological evaluation of the muscle tissue did not reveal any electricity related tissue alterations.

Conclusion: The results showed that the tested equipment can be used to stun decapods for at least 10 minutes. However, it is not suitable to kill the animals. Therefore, after stunning another method has to be used to kill the animals, whereby this has to be done within 10 minutes at latest after stunning.

Keywords: crayfish, electricity, stunning

259-P

Effect of tank color in the pigment adaptation, cooked color, growth performance and immune response of the shrimp *Litopenaeus vannamei*

Costa, Bruno (Portugal)[1,2]; Fernández-Boo, Sergio (Portugal)[3]; Serradeiro, Renata (Portugal)[1]; Costas, Benjamin (Portugal)[3,4]; Pacheco, Mário (Portugal)[2]

1 - Riasearch Lda. Cais da Ribeira de Pardelhas 21, 3870-168 Murtosa, Portugal; 2 - Departamento de Biologia, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal; 3 - Centro Interdisciplinar de Investigação Marinha e Ambiental, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208 Matosinhos, Portugal; 4 - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal

Introduction: The culture of the whiteleg shrimp *Litopaneaus vannamei* in controlled environments is a growing industry all over the world, getting increased attention in Portugal. Still, several parameters should be improved before starting its production not only in terms of growth but also in animal welfare and thus the appearance to consumers. In this way, the impact of tank color in the physiological and immune response of the whiteleg shrimp as a mean to improve production and rearing conditions was studied.

Methodology: Shrimps with an initial weight of 12.96 ± 1.22 g (mean ± SD) were reared in four different tank colors, *viz.* white, yellow, red, and black. Tank shape and water conditions were identical among treatments. Growth parameters, pigment adaptation, and cooked color, as well as immune parameters such as, hemocyanin and protein content, reactive oxygen species, protease and pro phenol-oxidase activity in hemolymph, were assessed after a 40-days trial.

Results: No significant differences in growth performance and immune response were observed for the four tested colors. However, significant differences in uncooked and cooked color of the shrimp were found. Color analysis, using a CR-400 colorimeter (Konica Minolta Sensing, Tokyo, Japan), indicated that shrimps reared in darker colored tanks (red and black) were significantly darker ($L < 39.83$) than those reared in lighter tanks (white and yellow) ($L > 44.72$). Uncooked shrimps from the black and yellow groups showed significant differences in the b color scale, having bluer and yellower colorations, respectively, when compared to the other treatments. After cooking, all shrimps shifted their coloration to a red/orange color with a substantial rise in color lightness (*L* scale).

Conclusion: Shrimps growth in darker tanks revealed to positively influence the presence of yellower ($b > 28.26$) and redder ($a > 11.97$) pigments in cooked shrimps, favoring the achievement of a final product with greater consumer acceptance. Any tank colour have induced growth reduction or immune depression.

Keywords: color effect, *Litopenaeus vanamei*, immune status, pigment

260-P

Kidney pathology in form of nephrocalcinosis and inflamation in Atlantic salmon (*Salmo salar*) produced in hatcheries in Norway

Kolstø, Stine (Norway)[1]; Bjerke, Hanna (Norway)[1]

1 - FoMAS- Fish Health Service

Introduction: Since 2014 a range of recirculation aquaculture systems (RAS) have been built on the west coast of Norway. The production of smolt in RAS is well established and the size of sea-transferred salmon has increased up to 1 kilogram. Over the last couple of years, FoMAS - Fish Health Service has observed an increased frequency of kidney changes in Atlantic salmon produced in RAS.

Methodology: FoMAS Fish Health Service studied smolt produced from two RAS sites and one flow through smolt producing site over time. The kidney changes were scored microscopically and examined histological on dead, weak and healthy fish. In addition water quality, fish size and production sites were analyzed to identify risk factors for developing changes in kidneys of Atlantic salmon before and after sea transfer.

Results: Macroscopic observations in field are swollen kidneys, extended ureters, precipitation of nephrocalcinosis and/or extended inflammation in the kidney tissue. The inflammation is found as seepage of yellow to white exudates in lesser or larger parts of the kidney tissue. The study showed that these kidney changes are found in both RAS and flow through smolt producing sites. Fish with more severe macroscopic changes (score 3 and 4) are only found on dead or weak fish.

Conclusion: Fish with these severe changes are not likely to regenerate their kidney tissue. These fish will most likely die after a short time. The study showed that fish size is an important factor for development of these severe macroscopic changes. We also observed that severe changes occurred more frequently when the fish were exposed to brackish-water and/or sea-water. During the next couple of months we will do more field observations to support current observations.

Keywords: kidney, changes, Atlantic salmon

261-P

Stress affects rainbow trout macrophage capacity to reduce bacterial load after *in vitro* infection

Garcia Muñoz, Lorenzo (Spain)[1]; Romero Lopez, Maria (Spain)[2]; Revuelta Rueda, Luis (Spain)[2]; Garcia Cabrera, Jose Antonio (Spain)[1]

1 - UCM, EAFP; 2 - UCM

Introduction: Fish farming systems inevitably introduce a number of stressors to the fish, most of which are difficult to avoid, like crowding and pre-slaughter feed withdrawal. During harvest fish are usually crowded, and depending of the systems, this may last from a few minutes to several hours. It may be reasonable that the longer trout are crowded, the greater is the stress. Stress is widely known to affect the immune response, and therefore, stressed organisms are more susceptible to infections. In fish, macrophages are the first line of defence against health threats, so any factor affecting these cells could give an advantage to infectious agents.

Methodology: We have studied the *in vitro* response of macrophages from stressed and non-stressed rainbow trout to a bacterial agent. Macrophage response was evaluated by the bacterial survival rate 24h after infection, and by the respiratory burst (NBT reduction test). Influence of water temperature at sampling on the macrophage response is also considered.

Results: At low temperatures (below 10 ºC) both groups were able to reduce bacterial survival similarly, although respiratory burst of non-stressed trouts was higher than in stressed. Between 10 - 15 ºC, bacterial survival increased in both groups compared to results below 10 ºC. But in this case, clear differences between both groups were seen; with a significant higher reduction in non-stressed trouts, although respiratory burst was similar in both groups. Finally, at temperatures between 15 - 20 ºC, bacterial survival increased over results observed at lower temperatures in both groups, while respiratory burst decreased below results at lower temperatures.

Conclusion: Our results show a lower efficiency of macrophages to coup with the bacterial threat as temperature increases in both groups, but up to 15 ºC macrophage response from stressed trouts seems to be compromised more rapidly.

Keywords: stress, macrophages, rainbow trout, bacterial survival, water temperature

262-P

Effect of UV treatment on microbial community structure in inlet water sampled from two different land based aquaculture systems

Drønen, Karine (Norway)[1]; Roalkvam, Irene (Norway)[1]; Dahle, Håkon (Norway)[2]; Nilsen, Hanne (Norway)[3]; Olsen, Anne Berit (Norway)[3]

1 - Department of Biological Science, University of Bergen; 2 - K.G. Jebsen Centre of Deep Sea Research, University of Bergen; 3 - Norwegian Veterinary Institute

Introduction: Food production in land based aquaculture systems expands worldwide. The improvement of water recirculation aquaculture systems (RAS) technology is important in this context, as it reduces the amount of high quality rearing water needed for production. In Norway, more and more Atlantic salmon spend long periods in RAS during rearing, while fish farms with direct flow through systems (FTS) have lately been established numerous for rearing lumpfish. The latter species has become an important tool for biological mitigation of salmon sea lice. Both RAS and FTS have particulate removal and UV treatment of the inlet water, which aims to remove debris and fish pathogenic bacteria, respectively. These UV systems guarantee 99 removal of bacteria. However, there has been a long, ongoing debate whether UV filtration kills selectively or follows the "cheese cutting" principle, meaning that cutting a piece of a homogenous cheese will produce a smaller, yet identical cheese. Here we report the effect of UV treatment of the inlet water on bacterial content of the water in a RAS unit for post smolt salmon and FTS unit for lumpfish.

Methodology: DNA was extracted from 240 ml water sampled prior the UV lamps and 1800 ml water sampled directly after, followed by 16S rRNA gene sequencing. The number of bacterial taxa and their relative abundances were compared.

Results: The analysis showed clearly that the majority of the taxa were present also after the UV treatment in both rearing units, and that their relative abundances remained the same.

Conclusion: Thus, the inlet water UV treatment systems followed the "cheese cutting" principle. This implies that the abundance of pathogens prior to filtration determines whether an infective dose are still present after filtration, and that competition and selection pressure inside the farm decides the bacteria to fill the outgrowth potential given by the UV filtration.

Keywords: UV filtration, bacteria, relative abundance

Funding: The Norwegian research council.

264-P

Development of essential prerequisites to monitor fish welfare in the framework of a national animal welfare monitoring in Germany

Steinhagen, Dieter (Germany)[1]; Adamek, Mikolaj (Germany)[1]; Jung-Schroers, Verena (Germany)[1]; Lugert, Vincent (Germany)[2]; Reiser, Stefan (Germany)[2]

1 - University of Veterinary Medicine, Hannover, Germany; 2 - Thünen Institute of Fisheries Ecology, Bremerhaven, Germany

Introduction: The discussion to which extent animal welfare is safeguarded in livestock farming is extremely controversial, and the available information on the state of animal welfare is generally low. Data on animal-related indicators for all relevant health areas, resource-related indicators and management-related indicators are scarce and not systematically collected. This difficulty is experienced not only in terrestrial farm animals but even to a larger extent in aquatic animals. Thus, in a collaborative research project, prerequisites for a national animal welfare monitoring in rainbow trout and carp farms will be developed.

Methodology: Indicators that are suitable for the assessment of animal welfare in German rainbow trout and carp farms will be identified. A first list of suitable and valid indicators will be evaluated in workshops in consultation with fish farmers and other professionals from the aquaculture sector, like veterinarians, government representatives, NGOs, and fisheries associations. These indicators will then be tested on a small number of farms and the most suitability and practical indicators will be identified. Subsequently, the most suitable indicators will be defined and tested by visiting a larger number of farms throughout Germany and fish welfare will be evaluated according to the defined indicators.

Results: The first results on the chosen animal-related, resource-related and management-related indicators that will be tested on aquaculture farms for rainbow trout and carp will be presented.

Conclusion: Animal welfare is gaining serious attention in public and the media. Politicians see scientifically calibrated indicators as a key priority. However, such indicators also need to be widely accepted by the means of society, operators and farmers. Further, they need to be practicable, and scientifically valid. In this project a set of measurable indicators, suitable and meaningful for the assessment of animal welfare in highly fragmented rainbow trout and carp aquaculture sector in Germany will be compiled and evaluated. The indicators can subsequently be used for a national animal welfare program which will give important information on the welfare current status of fish in German aquaculture.

Keywords: fish welfare, national animal welfare program

265-P

Spinal damages in eels after a possible passage through a hydroelectric power plant

Jung-Schroers, Verena (Germany)[1]; Teitge, Felix (Germany)[1]; Bauer, Julia (Germany)[1]; Guddorf, Vanessa (Germany)[1]; Steinhagen, Dieter (Germany)[1]

1 - University of Veterinary Medicine, Hannover, Germany

Introduction: Eels are katadrome migratory fish that are migrating downstream the rivers to spawning areas in the sea. During their way, they are exposed to numerous risks, like passages through hydroelectric power plants. Because of their body shape and length as well as their swimming behavior and their preference to swim with the mainstream, eels are more prone to be injured by a turbine passage than other fish species. Injuries might result in higher mortalities or in reduced swimming abilities. For the preservation of the European eel, it is existential to migrate into the sea for reproduction, since artificial propagation of the species is still not possible. Therefore, examinations of the impact of hydroelectric power plants as a migratory obstacle are important. The damages have to be evaluated and the used turbines should be adapted if necessary.

Methodology: 77 eels were caught downstream of a hydroelectric power plant. The animals were euthanized and examined for external and internal injuries. Additionally, all eels were x-rayed and the spinal damages were evaluated.

Results: Mainly mild external damages were seen in the fish. In 61 examined eels skin abrasions were detected. 39 eels showed bleedings in the skin and in 32 fish tearing of the skin occurred. In 27 eels internal bleeding could be detected. By x-raying, changes of the spinal column could be observed in 36 eels. The detected injuries of the spinal columns were mostly severe and the defects ranged from compression and fractures of the vertebral bodies, displacements of the vertebral bodies against each other and tears of the spinous processes. These defects occurred mainly in the second third of the body and more often in larger animals. By an external examination the existence of internal damages and especially of damages of the spinal column could not be evaluated, as about half of the externally intact animals and the animals showing only very mild external alterations had damages of the spinal column.

Conclusion: For examination of eels for damages due to a passage through a hydroelectric power plant taking x-rays is absolutely necessary to detect possible damages.

Keywords: European eel, hydroelectric power plant, spinal damages, x-ray

266-P

A sanitary control scheme for *Garra rufa* welfare in Italian beauty centers

Pastorino, Paolo (Italy)[1]; Pennazio, Roberta (Italy)[2]; Righetti, Marzia (Italy)[1]; Arsieni, Paola (Italy)[1]; Briata, Maria Paola (Italy)[2]; Prearo, Marino (Italy)[1]

1 - Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta,Torino, Italy; 2 - ASL 2 Sistema Sanitario Regione Liguria, Savona, Italy

Introduction and Methodology: *Garra rufa* also known as "doctor fish" is a native fish of the Northern and Central Middle East basins and it is famous for its ability to eat dead or diseased human skin while leaving healthy skin untouched. The use of *G. rufa* is long established in Turkey and India, but it is also becoming popular in some European countries. Nowadays research about the management, welfare and diseases of this species in beauty shops are very limited also due to the lack of specific legislation. The aim of this work was to set up a sanitary control scheme for Italian beauty centres owners in order to determine the health status and to guarantee the welfare of fish used in ichthyotherapy.

Results: This scheme will allow to shop owners to perform a daily set of self-check tests on fish batch and periodically some random tests on fish from each tank. Furthermore, it will allow assurance to patients in using this treatment. All biosecurity measures are given both for fish recruitment (e.g. from farmers and not from wild) and for ichthyotherapy activities (as disinfections measures, etc.).

Conclusion: the shop owners should follow all the suggested operations on fish and on tanks as well as the duration of a single treatment to guarantee fish welfare and public health. The proper implementation of the scheme will allow to avoid the onset of diseases too. It will also be necessary to carry out regular official controls by competent authorities with the support of specialist laboratories to check the health status of fish.

Keywords: sanitary monitoring, public health, *Garra rufa,* doctor fish

268-P*

Let's talk about stress: how to quantify the chronic stress level of the fish and its impact

Vercauteren, Maaike (Belgium)[1]; De Swaef, Evelien (Belgium)[1]; Devriese, Lisa (Belgium)[2]; Decostere, Annemie (Belgium)[1]; Chiers, Koen (Belgium)[1]; Aerts, Johan (Belgium)[3,4]

1 - Ghent University, Faculty of Veterinary Medicine; 2 - Flanders Marine Institute; 3 - Stress Physiology Research Group, Flanders Research Institute for Agriculture, Fisheries and Food; 4 - Stress Physiology Research Group, Faculty of Science, Ghent University

Introduction: Marine fish reside in an ever changing and potentially stressful environment. Offshore activities (e.g. in relation to wind energy, fishing activities, shipping, dredging) but also rising seawater temperature, changing salinity and other factors can cause a disruption in the environment hereby increasing the stress level of the fish. Stressful stimuli evoke a stress response mediated by the hypothalamus - pituitary gland - interrenal (HPI) axis which results in the release of glucocorticoids, in particular cortisol or corticosterone (depending on the species), in the plasma. Short term elevations of cortisol (acute stress) are considered adaptive as they help the fish to cope with the stressful stimuli. In contrast, long-term elevations (chronic stress) cause harmful effects on the health, growth and reproduction of fish. Despite its importance, only recently a method was pinpointed to quantify the chronic stress level of fish by evaluation of the glucocorticoid profile in the scales, which provides a retrospective view on HPI axis (re) activity. However, until now, this method was only tested in aquaculture species and never in wild caught fish. Hence, the main aim of the study was to investigate the scale cortisol levels in wild caught common dab (*Limanda limanda*).

Methodology: In total 111 fish were caught during multiple sea trips in different seasons. While in 75 fish, scale cortisol of the ontogenetic scales was analyzed shortly after catch, the remaining 36 fish were kept in RAS systems for 4 (18 fish) or 6 (18 fish) months after which the scale cortisol was analyzed.

Results: Scales of fish that were analyzed shortly after catch contained on average 0.004 ± 0.006 µg cortisol per kg scales. Scales of fish that were kept under artificial circumstances contained 0.066 ± 0.066 µg cortisol per kg scales.

Conclusion: These results demonstrate that common dab, as other teleost fish, are able to store cortisol in their scales, hereby further confirming its potential in assessing the impact of anthropogenic activities on wild fish in monitoring studies.

Keywords: chronic stress, cortisol, common dab, scales

Funding: Flanders Research Fund (FWO).

Molluscs and Crustacean Diseases

269-P

First histopathological survey of the chilean oyster *Ostrea chilensis* (Ostreae) in Pullinque Ostrich reserve in southern Chile

Ovalle, Loreto (Chile)[1]; Gonzalez, Margarita (Chile)[1]; Olmos, Paola (Chile)[1]; Rivas, Mario (Chile)[1]; Subiabre, Katerine (Chile)[1]

1 - Departamento de Salud Hidrobiológica, División de Investigación en Acuicultura, Instituto de Fomento Pesquero, Puerto Montt, Chile

Introduction: Molluscs culture is a relevant social and economic activity in Chile, being the main reason to know the sanitary condition of natural banks which are considered as genetic reserves of great commercial importance mollusc species.

Methodology: A total of 350 specimens of *Ostrea chilensis* were collected during 2018 from the natural bank of the Pullinque Ostrich Reserve and analyzed by histological techniques.

Results: Most of the specimens were free of parasites and findings (74%). However, different degrees of hemocytic neoplasia in organs such as gills, connective tissue and mantle were observed in a frequency of 1.15%, and also different degrees of hemocytic infiltration in organs (24.57%). No associations among neoplasia and infiltration with pathogens and / or parasites were detected. In addition, the presence of a turbellarian ciliate organism similar to *Paravortex* was observed in the lumen of the digestive system of an adult specimen of oyster, apparently without causing a reaction in the host.

Conclusion: The studied ostrea specimens were free of pathogens that are describe by the OIE as the cause of high risk diseases by histological techniques. This study corresponds to the first histological approach of the sanitary status of the Chilean oyster *Ostrea chilensis* in the Pullinque Ostrich Reserve in southern Chile. Carry-on this study, will provide the necessary information that allows the establishment of a Surveillance Program for this shellfish resource in this protected area.

Keywords: histopathology, chilean oyster, natural bank, sanitary status, shellfish

270-P

Genetic variability and *Perkinsus* infection level analysis to support founder population selection for a breeding program of *Ruditapes decussatus*

Cruz, Andreia (Portugal)[1]; Da Costa, Fiz (Portugal)[1]; Fernández-Pérez, Jenyfer (Spain)[2]; Nantón, Ana (Spain)[2]; Fernández-Boo, Sergio (Portugal)[3]; Insua, Ana (Spain)[2]; Méndez, Josefina (Spain)[2]

1 - Oceano Fresco S.A., Edifício Mira Center, Rua do Matadouro, piso 2, Lab. B2, Valeirinha, 3070-436 Mira, Portugal; 2 - Grupo Xenomar, Departamento de Bioloxía, Facultade de Ciencias and CICA, Universidade da Coruña, Campus de A Zapateira, A Coruña, Spain; 3 - Centro Interdisciplinar de Investigação Marinha e Ambiental, Terminal de Cruzeiros do Porto de Leixões, Matosinhos, Portugal

Introduction: Clam farmers worldwide face several challenges, including irregular seed supply and mortalities due to pathogenic organisms such as *Perkinsus* sp. In Europe, there is a high unmet consumer demand of native clam species such as *Ruditapes decussatus*. The higher market price of *R. decussatus* makes the culture of this species potentially more attractive than the culture of the alien species *Ruditapes philippinarum*. Thus, there is a market opportunity in breeding and producing *R. decussatus* at industrial scale. A selective breeding program to improve *R. decussatus* performance will be carried out in Portugal; and the first critical step to develop such a breeding program is the establishment of a founder population.

Methodology: In this study, intra- and interpopulation genetic diversity was assessed using 13 microsatellite markers in eight natural beds located in Portugal, Spain and Italy. Also, allele and genotypic frequencies of each microsatellite were assessed discriminating between *Perkinsus olseni* infected and non-infected clams.

Results: All locations showed similar values for several genetic diversity parameters. Analyses of population differentiation (*F*st, Bayesian clustering and AMOVAs) revealed five genetically differentiated regions: Rías Altas and Rías Baixas (NW Spain), North/Central Coast of Portugal, Gulf of Cadiz and Adriatic Sea. The results showed significant differences in the allele and genotypic frequency distribution between infected clams and non-infected ones at some microsatellite loci.

Conclusion: Integrating results of genetic diversity within and between populations and *Perkinsus* infection levels, a founder population for an *R. decussatus* breeding program composed by individuals from Barallobre (Rías Altas), Pontevedra or Cangas (Rías Baixas), Óbidos (North/Central Coast of Portugal), Algarve (Gulf of Cadiz) and Venice (Adriatic Sea) is proposed.

Keywords: *Ruditapes decussatus*, *Perkinsus olseni*, genetic variability, breeding, founder population

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Looking for hosts of *Marteilia cochillia* in the zooplankton

Carballal, María J. (Spain)[1]; Iglesias, David (Spain)[1]; Abollo, Elvira (Spain)[3]; Cao, Asunción (Spain)[1]; García, Lara (Spain)[2]; Ramilo, Andrea (Spain)[2]; González, Ana I. (Spain)[1]; González, Ángel F. (Spain)[2]; Pascual, Santiago (Spain)[2]; Villalba, Antonio (Spain)[1]

1 - Centro de Investigacións Mariñas, Consellería do Mar da Xunta de Galicia, Vilanova de Arousa; 2 - Instituto de Investigaciones Marinas - CSIC, Vigo; 3 - Centro Tecnológico del Mar, Fundación, Vigo

Introduction: The protozoan *Marteilia cochillia* infects cockles *Cerastoderma edule* in the rias of Arousa, Pontevedra and Vigo (Galicia, NW Spain). Outbreaks of this parasite are detected every year since 2012, affecting newly recruited cockles and causing high mortality. Direct transmission of the parasite from infected to healthy cockles has been discarded through cohabitation experiments, while the involvement of zooplankton species as intermediate hosts of other *Marteilia* spp. has been proposed. The aim of this study was to look for zooplankton species candidates to act as intermediate hosts of *M. cochillia*.

Methodology: Zooplankton was sampled periodically in three shellfish beds of the ria of Arousa: Lombos do Ulla, Corón and O Sarrido, from November 2015 to March 2017. One part of each sample was fixed in 95% ethanol and used for DNA extraction and the other part was fixed in formalin to count and identify zooplankton species. PCR assays were used to detect *M. cochillia* in the zooplankton samples. Monthly samples of cockles from those beds were processed by histology to diagnose *M. cochillia*.

Results: *M. cochillia* DNA was mainly detected in copepods and appendicularians and less frequently in larvae of crustaceans and equinoderms. Five PCR positives were detected in the copepod *Paracartia grani* and 2 positives in the copepods *Oithona plumífera*, *Temora longicornis*, *Acartia discuadata* and appendicularians. *Paracartia grani* was more abundant and showed more PCR positive cases in Lombos do Ulla, where infection was more prevalent and intense. Remarkably, *P. grani* has been proposed as intermediate host of other *Marteilia* spp. PCR positives in zooplankton were recorded in May 2016 in Lombos do Ulla and O Sarrido, when sporulation stages of *M. cochillia* occurred in cockles, which suggested transmission of spores from cockles to zooplankton. Most PCR positives in zooplankton were detected in autumn-early winter in both beds, when the infection prevalence in cockles was low or null and most infected cockles showed pre-sporulation stages.

Conclusion: DNA of *M. cochillia* was detected in zooplankton, mainly in copepods. *P. grani* is the most probable candidate to act as intermediate host of *M. cochillia*, which has to be confirmed.

Keywords: cockles, *Cerastoderma edule*, intermidate host, disease transmission, life cycle

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Sequence analysis of virulence genes, OMPU and VSM, and bath challenge using *Vibrio* isolates from Scottish blue mussels

McCarthy, Úna (United Kingdom)[1]; Chapuis, Ambre (United Kingdom)[1]; Anderson, Nicole (United Kingdom)[1]; Graham, Jennifer (United Kingdom)[1]; Bradley, Yee Mai (United Kingdom)[1]; Matejusova, Iveta (United Kingdom)[1]

1 - Marine Scotland Science

Introduction: Vibrios isolated following unexplained mortality events in Scottish blue mussels, were subjected to phylogenetic analysis of virulence gene sequences to assess genetic relationships with the oyster pathogen *V. tasmaniensis* LGP32 (LGP32) and other members of the *Splendidus* clade. The virulence of two Scottish isolates, M1 and M40, was tested by bath challenge in pre-adult mussels.

Methodology: Partial gene sequences of the outer membrane porin *ompU*, and the metalloprotease *vsm,* were used to examine phylogenetic relationships between Scottish isolates, LGP32, *V. splendidus* and other vibrio type strains. Additional primers were designed to obtain longer *ompU* sequence from isolates M1, M31, M36 and M40 and an azocasein digestion assay was used to test for the presence of metalloprotease in bacterial extracellular products. Pre-adult mussels were challenged by immersion for 2 h in M1, M40 and LGP32, and held for 144 h (6 d) in static seawater with aeration. Samples were collected at 0, 3, 24, 48 and 144 h. Expression of immune genes in gill tissue was measured by real-time RT-PCR using primers against Toll-like receptor (TLR-i), myeloid differentiation factor (Myd)88, IkB1, LPS-binding protein (LBP) and interleukin (IL)-17, with elongation factor 1-α as the reference gene. Moribund mussels were enumerated on termination of the challenge.

Results: Scottish isolates clustered within the *Splendidus* clade, although the phylogenetic trees confirmed previous findings of incongruence between the *ompU* and *vsm* genes. The isolates M31, M36 and M40 shared almost full identity in the *ompU* gene with each other and with LGP32, but no *vsm* gene was detected in these isolates, nor in M1. The outcome of the challenge experiment suggests that although M40 had no detectable effect on mussel immune responses, this strain was capable of causing limited mortality in pre-adult mussels, even in the absence of the *vsm* gene.

Conclusion: The distribution of similar sequences in mussel vibrio isolates from different locations raises questions about the geographic and temporal spread of virulence genes, and suggests a possible mechanism whereby assemblies of vibrio strains could provide the combination of virulence factors required to cause disease in mussels.

Keywords: *Mytilus*, *Vibrio*, virulence genes, challenge

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273-P

Mass mortality events in marine protected areas: the case of *Pinna nobilis* (Mollusca, Bivalvia)

Tedesco, Perla (Italy)[1]; Gustinelli, Andrea (Italy)[1]; Quaglio, Francesco (Italy)[2]; Caffara, Monica (Italy)[1]; Fioravanti, Maria Letizia (Italy)[1]; Fai, Sergio (Italy)[3]; Patarnello, Pierpaolo (Italy)[4]; Terlizzi, Antonio (Italy)[5,6]

1 - Dipartimento di Scienze Mediche Veterinarie, Alma Mater Studiorum Università di Bologna; 2 - Dipartimento di Biomedicina Comparata e Alimentazione, Università degli Studi di Padova; 3 - Area Marina Protetta, Porto Cesareo; 4 - Fish Pathologist, Private Expert, SIAV B, ASL Lecce; 5 - Dipartimento di Scienze della Vita, Università di Trieste; 6 - Stazione Zoologica Anton Dohrn, Napoli

Introduction: Marine Protected Areas (MPAs) represent a privileged observatory to monitor disease outbreaks in the marine environment. Recently, several episodes of mass mortality events (MME) of the noble pen shell *Pinna nobilis* occurred in different parts of the Mediterranean Sea. In the present work we report a MME occurred in 2018, involving a protected population of *P. nobilis* within the MPA of Porto Cesareo (Southern Italy, Ionian Sea) which had been previously characterized and monitored within the framework of the "Marine Strategy" Directive (2008/56/EC).

Methodology: Data on abundance and size structure of the *P. nobilis* population had been collected since September 2017 through scuba diving visual census. Following a MME occurred in June 2018, one moribund specimen was sampled by professional divers of Porto Cesareo MPA within the reserve boundaries. The specimen was subjected to a complete diagnostic exam, including parasitological, histopathological and molecular analyses.

Results: The mortality affected 100% of the protected population. The diagnostic exams allowed to detect the presence of *Haplosporidium pinnae*, identified through amplification and sequencing of partial 18S rDNA region, in the intestine and digestive gland. Histopathological analyses revealed the presence of haplosporidian-like protozoa in different life cycle stages within the digestive gland. The epithelium of digestive tubuli showed a diffused degeneration with extended infiltrate of brownish pigment referable to brown cells, which replaced almost completely the decaying glandular tissue.

Conclusion: *Haplosporidium pinnae* has been already indicated as responsible for other MME of *P. nobilis* occurred in Western and Central Mediterranean Sea. The implementation of routine monitoring programs to assess the presence and distribution of transmissible agents in wild marine environment is an essential step to protect biodiversity. Such procedures are especially relevant within the context of a MPA, and should focus on the identification of sanitary risks that could impair conservation efforts. With respect to *H. pinnae*, further studies aimed at elucidating the life-cycle of the parasite and its causative role in recent MME of noble pen shell are of primary importance for a correct management and successful conservation of *P. nobilis* populations.

Keywords: mass mortality, marine protected areas, *Pinna nobilis*, *Haplosporidium pinnae*

274-P

Observation of *Bonamia exitosa* (Haplosporidia) in European flat oyster (*Ostrea edulis*) at the Croatian Adriatic Sea

Oraić, Dražen (Croatia)[1]; Beck, Relja (Croatia)[1]; Pavlinec, Željko (Croatia)[1]; Zupičić, Ivana Giovanna (Croatia)[1]; Zrnčić, Snježana (Croatia)[1]

1 - Croatian veterinary institute

Introduction: The production of the European flat oyster (*Ostrea edulis*) has a long tradition in Croatia and the total annual production is about 60 tons. There are several cultivation areas but the majority of production is located in the southern part of the Adriatic Sea, Mali Ston Bay. The molluscs diseases surveillance program is in force and several sampling points are defined as well as timing and number of samples. Flat oysters are submitted for listed diseases analysis, bonamiosis and marteiliosis, twice per year, in springtime and autumn. The positive *Bonamia exitiosa* samples were observed during the period from 2016 until now.

Methodology: Totally 60 samples (n=30) of oysters were analysed for the presence of *Bonamia* spp. The diagnostic tests employed for the detection of *Bonamia* spp. were a cytological examination of stained heart tissue impression smears, polymerase chain reaction (PCR) and histology. PCR positive samples were sequenced for small subunit ribosomal DNA gene. All sequences were subjected to a homology search through BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). Samples of Pacific oyster (*Crassostrea gigas*) as putative vector species were analysed using the same protocol.

Results: *B. exitiosa* was detected at two locations namely the Bay of Medulin where 2/30 oysters were infected and on 5 locations in the Bay of Mali Ston where 17/150 oysters were infected. The finding of *B. exitiosa* was repeated in the Bay of Medulin in November 2016 where 1/30 examined oysters, and in July 2017 where 3/30 oysters, and finally, in November 2017 where 1/30 oysters were positive. In 2018, *B. exitiosa* was detected in June at another two locations; 3/30 in Savudrija and 2/30 Limski Bay while location tested positive in previous years was negative. The parasites were not visualized in smears or histology, but the PCR confirmed its' presence. Phylogenetic analysis revealed 100% homology with various isolates of *B. exitiosa*.

Conclusion: The prevalence of *B. exitiosa* was very low and findings were not accompanied by mortality events. The further work should be focused on epidemiology research which should clarify the onset of the parasite.

Keywords: *Ostrea edulis*, *Bonamia exitiosa*, Croatian Adriatic Sea

275-P

Understanding the mortality event of *Polititapes rhomboides* in 2010 in Galicia (NW Spain)

Darriba, Susana (Spain)[1]; Villacieros-Robineau, Nicolás (Spain)[2]; Febrero, Fernando (Spain)[3]; Rodríguez, Luis (Spain)[4]; Gil-Coto, Miguel (Spain)[2]; Montero, Pedro (Spain)[1]; López, Carmen (Spain)[3]

1 - Instituto Tecnolóxico para o Control do Medio Mariño de Galicia, 36611, Vilagarcía de Arousa. Pontevedra, Spain; 2 - Departamento de Oceanografía, Instituto de Investigacións Mariñas, Vigo, Pontevedra, Spain; 3 - Centro de Investigacións Mariñas, 36620, Vilanova de Arousa, Pontevedra, Spain; 4 - Xefatura Territorial de Vigo, Consellería do Mar 36201, Vigo, Pontevedra, Spain

Introduction: Banded carpet shell (*Polititapes rhomboides*) populations in Ria of Vigo (Galicia) dropped dramatically since the mortality event occurred in 2010. A severe infection caused by prokaryotic intracellular colonies (Rickettsiales-like), disrupting clam gills, was associated with this mortality. A multidisciplinary study was performed to assess the post-event populations' status and to look for environmental factors implicated in the bloom of the prokaryotic colonies.

Methodology: From 2010 to 2016 an integral study of *P. rhomboides* from Ria of Vigo was performed including population structure, histopathology and molecular techniques. Population structure was evaluated based on sizes distribution and relative abundances. A quantitative scale was applied on slides, stained with haematoxylin and eosin, to follow clams infection with prokaryotic colonies associated with mortality. DNA from infected clams was extracted and molecular studies were performed. In addition, from October 2009 until December 2010, meteorological and oceanographic conditions of this Ria were studied.

Results: The prokaryotic colonies infection achieved the highest intensity levels during the mortality event of 2010, which remained at high levels along 2011, started to decrease in December 2011 and achieved low levels since April 2015. Through molecular studies, the prokaryotic colonies were placed in the Endozoicomonaceae family. On the other hand, sizes population structure achieved the equilibrium 5 years after the mortality event. Consequently, average size and relative abundances rose since 2015. Regarding the oceanographic conditions prior to the mortality event, a long downwelling season (October 2009 - April 2010), characterized by strong south-westerly storms, produced intense significant wave heights inside the ria ($H_s > 0.5$ m) and exerted intense pulses of shear stress on the sea bottom. In addition, this period marked by historical low values of the NAO (North Atlantic Oscillation) index, highlighted by low salinity episodes and warmer waters (0.5 to 1 ºC above climatic means).

Conclusion: This multidisciplinary study allows us to establish a hypothesis to understand the mortality event. Adverse meteorological conditions and seawater warming registered prior to the event caused population stress resulting in clam weakness and favorable conditions for prokaryotic proliferation. Years later, prokaryotic colonies restore low levels and the size population structure got back to an equilibrium.

Keywords: clams, mortality, prokaryotic, physical oceanography

276-P

Mitochondrial respiration in clams with haemocyte neoplasia, a case study with Baltic *Limecola balthica*

Hallmann, Anna (Poland)[1]; Smolarz, Katarzyna (Poland)[2]; Krajewska, Magdalena (Poland)[1]; Michnowska, Alicja (Poland)[2]; Chomiczewska, Agnieszka (Poland)[1]

1 - Department of Pharmaceutical Biochemistry, Medical University of Gdańsk, Poland; 2 - Department of Marine Ecosystem Functioning, University of Gdańsk, Poland

Introduction: Neoplasia is a proliferative disorder with polymorphic cells presenting a high nucleus-to-cytoplasm ratio and a high mitotic activity. Neoplastic disorder is characterized by the presence of hypertrophic and anaplastic cells with a high level of aneuploidy, lower or even absent phagocytic ability, relocation of the p53 tumour suppressor gene and new surface antigens. These cells also have many ultrastructural features in common with malignant vertebrate cells, including swollen mitochondria and altered Golgi complexes. Recent findings highlighted that vertebrate neoplastic cells lose their capacity for mitochondrial respiration at the level of complex I (NADH-coenzyme Q reductase) and complex II (succinate-coenzyme Q reductase), the major sites for entry of electrons into the respiratory chain. Our study is based on a Baltic clam *Limecola balthica* developing haemic neoplasia (leukaemia like cancer) in the natural environment. Here, we present a comparative study highlighting differences in the mitochondrial respiration in healthy and neoplastic model clams.

Methodology: The clams were collected from the area of the Gulf of Gdansk (Baltic Sea, Poland) historically characterised by high frequency of neoplasia (up to 90% at occasions). After collection, haemolymph sample was collected from the pericardium area and the remaining soft tissue was stored in Davidson fixative (and later in formol solution). Disease diagnosis was performed based on haemolymph subsample stained with methylene blue and confirmed using basic histology. Mitochondria were isolated from cancer cells as well as foot muscle. Mitochondrial oxygen consumption rate (OCR) was measured using Seahorse XFp Analyzer by examination of electron transport chain (ETC) and the oxidative phosphorylation machinery (OXPHOS).

Results: The obtained results indicate that the mussels with neoplasia have dysfunctional mitochondria: low oxidation of energy substrates was detected and the OCR level was significantly reduced in complex I of the respiratory chain.

Keywords: Baltic clams, laeukemia-like cancer, mitochondrial respiration

277-P

Preliminary microbiological and histopathological data on *Tritia mutabilis*

Ciulli, Sara (Italy)[1]; Zamparo, Samuele (Italy)[1]; Errani, Francesca (Italy)[1]; Lorito, Luna (Italy)[1]; Volpe, Enrico (Italy)[1]; Tura, Giorgia (Italy)[1]; Zavatta, Emanuele (Italy)[1]; Mandrioli, Luciana (Italy)[1]; Serratore, Patrizia (Italy)[1]

1 - University of Bologna

Introduction: Marine gastropods have been used as food from the earliest times. Recently, their consumption has increased throughout European countries. Actually, the scientific knowledge about the European edible species is poor; our previous preliminary survey in gastropod edible species showed high *Vibrio* spp. load and the presence of betanodavirus in 43% of samples (*Tritia mutabilis*, *Bolinus brandaris*, *Rapana Venosa*). Therefore, there is a need to develop detailed studies on these animals, particularly on the possibility to harbor bacteria and viruses of human and animal health concern. The aim of this study was to provide preliminary data on *T. mutabilis*, formerly *Nassarius mutabilis*, the catch of which is an important activity carried out by artisanal fisheries in the central and northern Adriatic Sea, Italy.

Methodology: From January to March 2019 samplings of T. *mutabilis* were performed. Total *Vibrio* spp. count and the presence of vibrio species pathogenic for humans (*Vibrio cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*) were determined through phenotypical and molecular methods. Virological analyses, targeting human pathogens such as Norovirus and HAV and fish pathogen such as betanodavirus were performed through molecular methods. Histomorphological analysis was focused on foot, mantle, digestive apparatus, gonads, blood gland and nervous system.

Results: The mean value of *Vibrio* spp. resulted 5.11 $\log_{10}$ CFU/g ± 1.92 (SD). Pathogenic vibrios were not detected except *V. alginolyticus*. Viral human pathogens were not detected. Regarding betanodavirus, it was detected in one out of 7 samples (14%). Microscopically, most of the animals were normal. Only few lesions were found in some specimens (granulocytomas). In one case a marked vacuolization of the mantle nerves was detected in association with the presence of betanodavirus.

Conclusion: Microbiological investigation showed high *Vibrio* spp. load in the whole body, confirming the findings of a previous survey, and positivity for *V. alginolyticus* in 1 out of 15 samples (6.7%). The finding of the fish pathogen betanodavirus pointed out a possible vector role of these invertebrates. Furthermore, the association of betanodavirus presence with a specific histopathological finding needs further investigations to define the pathological role of this virus in this host species.

Keywords: gastropod, *Vibrio* spp., human pathogens, betanodavirus, histopathology

278-P

Detection of OSHV-1 on the cephalopod *Octopus vulgaris*

Prado-Álvarez, María (Spain)[1]; García-Fernández, Pablo (Spain)[1]; Faury, Nicole (France)[2]; Morga, Benjamin (France)[2]; Azevedo, Carlos (Portugal)[3,4]; Gestal, Camino (Spain)[1]

1 - Marine Research Institute, Spanish National Research Council (CSIC), Vigo, Spain; 2 - Institut Français de Recherche pour l´Exploitation de la Mer, La Tremblade, France; 3 - Interdisciplinary Center of Marine and Environmental Research, University of Porto, Portugal; 4 -Institute of Biomedical Sciences Abel Salazar, University of Porto, Portugal.

Introduction: The ostreid herpes virus (OsHV-1) has globally impacted in the oyster *Crassostrea gigas* production. In the present study we described for first time the occurrence of the herpes virus in different life stages of the common octopus *Octopus vulgaris*. This species has acquired high interest for aquaculture operations due to the need for diversification to satisfy the increasing global demand.

Methodology: Archival octopus samples from different life stages were analyzed by standard PCR and sequencing. In situ hybridization and transmission electron microscopy (TEM) was carried out in positive animals. In order to ascertain a putative transmission between species, experimental trials were carried out on octopus paralarvae by cohabitation with OsHV-1 injected oysters and by direct immersion in purified OsHV-1 suspension.

Results: Prevalence of detection reached mean values of 87.5% in adult octopus and 65% and 62% in embryo and paralarvae samples, respectively. Analysis of positive amplicons reveals 100% identity to OsHV-1 µVar genotype. A significant increase in mortality was observed in octopus paralarvae after 48 h of cohabitation with OsHV-1 injected oysters associated to viral detection by standard PCR. Gene expression analysis in octopus tissues revealed no expression of viral genes and an increase in the expression of genes previously described to be related to OsHV-1 infection.

Conclusion: The genotype OsHV-1 µVar was detected in octopus tissues and mortality occurred after contact with the virus. Microscopy examinations by TEM did not revealed the existence of viral capsids. Moreover, no viral replication was detected in the samples examined. However, our results suggested that a defensive response against the virus might be occurring as immune related genes are activated after contact. The effect of OsHV-1 on *O. vulgaris* deserves to be clarified if the aquaculture procedures succeed on this species.

Keywords: *Octopus vulgaris*, OsHV-1, PCR, sequencing, gene expression

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279-P

DNA methylation profiling on *Crassostrea gigas* families with different susceptibility to OSHV-1

Prado-Álvarez, María (Spain)[1]; García-Fernández, Pablo (Spain)[1]; Cao, Asunción (Spain)[2]; Villalba, Antonio (Spain)[2]; Gestal, Camino (Spain)[1]

1 - Marine Research Institute, Spanish National Research Council, Vigo, Spain; 2 - Centro de Investigacións Mariñas, Consellería do Mar; Xunta de Galicia

Introduction: Epigenetic mechanisms are important gene expression regulators with heritable modifications. Among them, DNA methylation, a process that consists of adding a methyl group to cytosines was previously described in *Crassostrea gigas* associated to developmental processes. In this study we investigated the effect of OsHV-1, a virus causing massive mortalities, on the DNA methylation pattern.

Methodology: Broodstock showing less susceptibility to OsHV-1 were crossed to produce pure bred families that were naturally exposed to OsHV-1 in the field. Susceptible stocks of oysters from a naïve site were also crossed and the produced families exposed to the same conditions. Tissue samples were collected for DNA extraction before and after a mortality outbreak for the two groups of families. Genomic DNA was analyzed by methylation sensitive amplified polymorphism (MSAP) for global DNA methylation determination.

Results: A total of 237 polymorphic loci were identified considering the total number of samples. Similar number of methylated susceptible loci (MSL) and percentage of polymorphic loci (PL) were observed on less susceptible oysters (176 MSL, 75% PL) and naïve oysters (175 MSL, 78% PL). Principal Coordinates Analysis showed that C1 accounts for the 22.5% and the 17.4% of variation between samples collected before and after the natural outbreak in less susceptible and naïve families, respectively.

Conclusion: The global DNA methylation level varied after a natural infection with OsHV-1 in the two stocks of oysters analyzed, naïve and less susceptible oysters. Whether variation in methylation patterns could be related to resistance or susceptibility to OsHV-1 remains unknown. However, it deserves to be further investigated to decipher the role of the epigenetic mechanisms as gene modulators during infection.

Keywords: *Crassostrea gigas*, OsHV-1, resistance, DNA methylation, MSAP

Funding: EU H2020-Marie Sklodowska Curie Actions - RESISGAL, and Axudas do Programa de Consolidación e estructuración de unidades de investigación competitivas (GPC) IN6078 2018/11.

280-P

Bacteria-driven infections in an invasive *Rangia cuneata* from the Vistula lagoon, Poland

Smolarz, Katarzyna (Poland)[1]; Świątalska, Agnieszka (Poland)[2]; Świeżak, Justyna (Poland)[1]; Michnowska, Alicja (Poland)[1]; Kornijów, Ryszard (Poland)[3]

1 - Department of Marine Ecosystem Functioning, University of Gdańsk; 2 - Department of Veterinary Hygiene, Voivodoship Veterinary Inspectorate, Gdańsk; 3 - Department of Fisheries Oceanography and Marine Ecology, Marine Fisheries Institute, Gdynia

Introduction: One of the recent alien species which invaded the Baltic Sea is a clam *Rangia cuneata* originating from the Gulf of Mexico. In the Polish coastal waters *R. cuneata* successfully colonized the Vistula Delta and the Vistula Lagoon. Despite being under strong biotic and abiotic pressure, the clam population in the Vistula Delta is stable while the one in the Vistula Lagoon shows strong fluctuation in population abundance and density. Here, we examine the role of microorganisms in ecosystem resistance (i.e. the ability of native ecosystem to limit introduced species).

Methodology: 200 clams from the Vistula Lagoon were collected in July 2018 and November 2018. After dissection, from approx. 100 clams a sample was taken from the gonads, gills and mantle for a bacteria culture test. Next, the soft tissue was fixed in Davidson fixative for 24 hours, and routine histology was performed. Biochemical analyses were performed on remaining 100 individuals.

Results and Conclusion: All clams were characterized by massive infiltration of haemocytes in gills, mantle and digestive gland. Observed inflammatory response was morphologically classified as mild to severe infiltrative hemocytosis and appeared less significant in bivalves sampled in colder months. In various cases, histological appearance of affected clams was similar to infiltrative hemocytosis seen in severe systemic infections. Biochemical examination revealed the lack of glycogen and low lipid content (not exceeding 7%). All clams were affected by a massive growth of bacteria from genus *Aeromonas* (*A. sobria*, *A. hydrophila*). Additionally, microbiological examination revealed the presence of *Serratia fonticola* and *Flavobacterium* (July) and *Kocuria luteus*, *Pseudomonas fluorescens*, *Burkholderia cepacia* and *Shewanella putrefaciens* (November). All microorganisms (apart from pathogenic *S. putrefaciens*) belong to opportunistic pathogens causing various diseases in fish and shellfish and are normally present in the water. Performed gram staining revealed the presence of G(+) bodies in hepatopancreas with pathogens becoming intracellular within hemocytes, while G(-) bodies were often observed in the epithelial tissue of gills and mantle. Moreover, sexually mature males and underdeveloped females were found what possibly affected reproduction success of *R. cuneata*.

Keywords: invasive species, inflammatory response, microbiology, South Baltic

281-P

Neoplastic disorder in bivalves from the gulf of Gdańsk - genetic and immunological perspectives

Smolarz, Katarzyna (Poland)[1]; Hallmann, Anna (Poland)[2]; Chomiczewska, Agnieszka (Poland)[2]; Michnowska, Alicja (Poland)[1]

1 - Department of Marine Ecosystem Functioning, University of Gdańsk; 2 - Department of Pharmaceutical Biochemistry, Medical University of Gdańsk

Introduction: Haemocyte neoplasia (HM) is a cancer that occurs at high prevalence in Baltic clam *Limecola balthica*, but has also been detected in other bivalves species from the Gulf of Gdańsk (Poland). A considerable number of results suggest that the prevalence and rate of progression of neoplasia in bivalves are elevated in seriously polluted environments under increasing anthropogenic pressure. Despite the accumulation of evidence on the role of pollution in this disease, recent studies suggest that the transmission of independent cancer lineages between individuals of the same species and within different species also occur in marine environments and may be more widespread than previously thought. Here, we present preliminary results linked to i) screening for neoplastic individuals in geographical areas of historically high neoplasia frequency, ii) examination of neoplastic and non-neoplastic DNA using COI and EF1α sequences, iii) characteristic of neoplastic and non-neoplastic cells, and iv) determination of the presence of *Steamer*-like elements (SLEs) in cohabiting invertebrate species.

Methodology: 250 clams were collected from the area of the Gulf of Gdańsk (Poland) characterized by high frequency of neoplasia. The diagnosis was performed based on general histology and haemolymph smears stained with methylene blue. Haemolymph and solid tissue was further collected and frozen in -80 for molecular analyses including the presence of SLEs. Determination of the presence of SLEs was based on DHKPL and PXRPW primers and qPCR analyses performed on *Mya arenaria* and *L. balthica* genomic samples.

Results and Conclusion: Out of 250 analyzed clams, 30 were characterized by typical for leukemia-like cancer features and thus diagnosed as neoplastic. Indeed, in leukemic clams the genotype of neoplastic cells appears distinct from normal tissue of the host. Performed comparative studies using neoplastic and non-neoplastic haemocytes revealed differences in their morphology, concentration, viability, intracellular oxidative activity, lysosomal content and phagocytosis capacity. Comparative studies of SLEs present in *M. arenaria* and *L. balthica* genomes highlighted 99% similarity between original SLEs sequence and sequenced areas of the genomes.

Keywords: leukaemia-like cancer, haemocytes, steamer-like element

Funding: The Polish Ministry of Science and Higher Education no. UMO 2017/26/M/N28/00478.

282-P

Screening for *Bonamia ostreae* in European flat oysters and Pacific oysters from the same areas of Limfjorden in Denmark

Madsen, Lone (Denmark)[1]

1 - Technical University of Denmark, National Institute of Aquatic Resources

Introduction: Limfjorden in Denmark is recognized as a unique production area for the European flat oyster, *Ostrea edulis*. In later years, the Pacific oyster (*Crassostrea gigas*), an invasive species for the area, has been found to reproduce itself in Limfjorden, too. *Bonamia ostreae*, a parasite known to be the cause of devastating disease in flat oyster species, was found in native flat oysters from Limfjorden for the first time in samples taken in 2014, with no observed elevated mortalities. The following years, the parasite has been found in flat oysters from several different areas of Limfjorden. Especially in one area of Limfjorden, the prevalence seen for *Bonamia* was high (50%), whereas the prevalences in other areas have been very low (3%).

Methodology: Screening of the two oyster species originating from the same area, both the area found to have high prevalence of *Bonamia* as well as an area found to have low prevalence of *Bonamia*, have been and are currently done and will be presented. Methods used for the screening are primarily molecular techniques, like PCR (Real-Time PCR as well as a conventional PCR), whereas histology and heart imprints are used as confirmatory methods.

Results and Conclusion: To date *Bonamia* has not been found in Pacific oysters originating from areas, where the parasite has been found in flat oysters, but the screening is still ongoing. The potential effect of the invasive species, the Pacific oyster, on the occurrence of *Bonamia* in the native flat oyster in Limfjorden, will be discussed.

Keywords: *Bonamia ostreae*, *Ostrea edulis*, *Crassostrea gigas*, PCR

Funding: European Maritime and Fisheries Fund and Danish Fisheries Agency project GIGAS.

283-P

High levels of a novel *Endozoicomonas* bacteriophage are linked to lower severity of the bacterial infection in king scallop

Cano, Irene (United Kingdom)[1]; Hooper, Patrick (United Kingdom)[1]; Van Aerle, Ronny (United Kingdom)[1]; Feist, Stephen W. (United Kingdom)[1]

1 - Cefas

Introduction: The Lyme Bay Marine Protected Area (MPA) host a valuable population of king scallop *Pecten maximus* L. An *Endozoicomonas*-like organism (ELO) causing a persistent infection in gill tissues have been identified in the lesions, sporadically associated to mass mortality. Histologically, two types of intracellular microcolonies (IMC) type I and type II have previously been identified. IMC type I were associated to gill disruption and haemocyte infiltration, while type II were associated with smaller IMC size and reduced tissue damage.

Methodology and Results: In the present study, a bacteriophage infecting ELO in king scallop was characterized. Ultrastructural examinations of thin sections of gill scallop infected with ELO have shown phage like particles with icosahedral-isometric head and long flexible tail. Metagenomic analysis identified a novel double stranded DNA phage genome with some similarity to protein sequences of *Siphoviridae* family. Using the metagenomic constructed genome of the phage, specific probes were designed for *in situ* hybridisation. The ELO-phage probe revealed that the IMC type II were highly infected by the phage. Environmental samples were examined by histology to determinate the prevalence of IMC types I and II and the intensity and severity of the gill lesions. In parallel, the number of copies of the phage terminase gene and the ELO 16S rRNA gene were then quantified by Taqman qPCR and compared with the histopathology. Histological sections of animals sampled in 2014 (associated to scallop mass mortality event) possessed a higher gill lesion severity score, lower ratio of IMC type II and lower number of the phage terminase gene copies; whilst animals obtained from outside of the mass mortality event, sampled from 2015 to 2017, showed a significantly ($p<0.05$) higher number or IMC type II, higher ratio of the phage/ELO number of copies and lower gill severity score.

Conclusion: The lower number of phage copies are inversely related with higher severity of the ELO lesions. Those preliminary results suggest that the hyperparasite is acting as a limiting factor influencing severity of ELO infection.

Keywords: king scallop, *Endozoicomonas*, phage, hyperparasite, MPA

Funding: This project has received funding Defra contract FB002A and VIVALDI (H2020 program, no. 678589).

Myxozoan Diseases

284-P

De novo genome sequencing project of the fish-parasitic *Myxobolus pseudodispar* (Myxozoa): preliminary results

Eszterbauer, Edit (Hungary)[1]; Sipos, Dóra (Hungary)[1]; Orbán, László (Hungary)[2]; Singh, Siddharth (United States of America)[3]

1 - Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary; 2 - University of Pannonia, Keszthely, Hungary; 3 - Pacific Biosciences

Introduction: The majority of microscopic myxozoan species (Cnidaria: Myxozoa) infect vertebrate hosts without clinical signs, however, some species cause typical symptoms and significant mortality among fish. Despite their economic and ecological damage, an effective control measure or therapy is not available yet. This may be due - among other things - to the fact that laboratory maintenance of myxozoans is extremely difficult, and that their *in vitro* culturing is not solved yet. For the identification of parasite genes having therapeutic potential, the whole genome and transcriptome sequencing may supply solution. Apart from the free-living cnidarians, of which the genome of several species has already been identified, partial genome data are available for some highly-virulent myxozoan species mainly from marine environment or freshwater ones from the far East.

Methodology: One of the main goal of our research was therefore to determine the genome of *Myxobolus pseudodispar*, a freshwater myxozoan with moderate pathogenicity and common in Europe. This makes possible the group-level genome comparison and the identification of therapeutic target genes. DNA sequencing was performed with single-molecule real-time (SMRT) technology on PacBio Sequel new generation sequencing system.

Results: Sequencing provided data over 7.5 Gb (gigabase). The average length of 1.6 million reads was 9250 bp. After filtering the poor quality and contaminating sequences, the assembly was done using FALCON, HGAP4 and Canu 1.6 software. The genome assembly predicted a genome size of about 150 Mb, and revealed an extremely high AT content (~70%). Genome assembly is further polished using Illumina sequencing data.

Keywords: myxozoans, cyprinids, freshwater, whole genome sequencing, PacBio

Funding: The National Research, Development and Innovation Office, Hungary (Grant No. NN124220).

285-P

Genetic diversity of serine protease inhibitors in fish-parasitic Myxozoa (Cnidaria)

Eszterbauer, Edit (Hungary)[1]; Sipos, Dóra (Hungary)[1]; Herczeg, Dávid (Hungary)[1]; Holzer, Astrid (Czech Republic)[2]; Bartošová-Sojková, Pavla (Czech Republic)[2]

1 - Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary; 2 - Institute of Parasitology, Czech Academy of Sciences, Ceske Budejovice, Czech Republic

Introduction: Myxozoans are obligate cnidarian parasites with a complex two-host life cycle, infecting both vertebrates (mostly fish) and invertebrates (mostly worms) in freshwater and marine environments worldwide. Myxozoan parasites affect the health of both farmed and wild fish populations, causing diseases and mortalities. Despite their global impacts, no effective protection of fish against these parasites exists. Serine protease inhibitors (serpins) are a large and broadly distributed superfamily of protease inhibitors. Serpins were reported as important factors for invasion and immune evasion of parasites, and due to the disparate nature of these protease inhibitors compared to the host orthologous proteins, parasite serpins are promising targets for the development of antiparasitic therapy.

Methodology: In the present study, we intended to identify and compare serpins in the genome and transcriptome of various freshwater and marine myxozoan species. Serpin sequences were mined in the genome/transcriptome of myxozoan species available in public databases (i.e. partial genome and transcriptome of *Myxobolus cerebralis*, *Thelohanellus kitauei*, and *Kudoa iwatai*, partial genome of *Enteromyxum leei*, transcriptome of *Myxobolus pendula* and *Myxidium lieberkuehni*). Furthermore, the blast search was extended to - yet unpublished - genomic and transcriptomic databases of myxozoan species (e.g. *Myxobolus pseudodispar* and *Sphaerospora molnari*). Besides detecting genetic variability among serpins, their phylogenetic positions were also reconstructed using maximum likelihood and Bayesian inference methods.

Results: High intraspecific and interspecific genetic variability was detected among the identified serpins. Whereas some of the serpins showed over 90% similarity in the amino acid-based alignment, only 20% similarity was detected for others. Genetic variability was in loose correlation to myxozoan species. In comparison to free-living and other parasitic cnidarians, unique serpin signatures and functional subgroups were detected. Furthermore, phylogenetic analyses of the obtained sequences enabled us to gain essential data on the distribution of serpins among freshwater and marine myxozoans.

Keywords: serpin, myxozoans, phylogeny, genomics

Funding: The National Research, Development and Innovation Office, Hungary (Grant No. NN124220) and the Ministry of Education, Youth and Sports, Czech Republic (Grant No. LTAUSA17201).

287-P*

Novel *Henneguya* species (Cnidaria, Myxozoa) parasitizing *Plagioscion squamosissimus* in the Amazon basin, Brazil

Zatti, Suellen (Brazil)[1]; Milanin, Tiago (Brazil)[1]; Adriano, Edson (Brazil)[2]; Maia, Antônio (Brazil)[1]

1 - University of São Paulo - FZEA/USP; 2 - Federal University of São Paulo, UNIFESP

Introduction: Myxozoa is a group of highly specialized endoparasites, characterized by morphologically reduced spores, and complex life cycles that involve both vertebrate (predominantly fish) and invertebrate (annelids or bryozoans) hosts. They represent about one-fifty of cnidarian diversity, with over 2.400 species. Some species are highly pathogenic and give rise to serious ecological and economic impacts on their host fish populations. Here, we describe a novel myxosporean species from *Plagioscion squamosissimus* (Perciformes, Scianidae), an economically important and endemic fish from the Amazon basin.

Methodology: Forty-three fish were collected by net from the Tapajós River, Pará State, Brazil, in May 2018 (SISBIO n° 44268-4). Myxospores morphometry, morphology, and ssrDNA sequences were used in the taxonomic analyses. Histopathological study was also performed. Phylogenetic analyses were performed to assess the position of the novel species among its closest relatives.

Results: *Henneguya* n. sp. was found infecting the kidney (1/43), the gill filaments (6/43), and the fins (9/43) of *P. squamosissimus* from the Tapajós River. The average prevalence was 37% (16/43). Spores of *Henneguya* n. sp. from the different organs had identical morphometrics and ssrDNA sequences. No inflammatory reaction was observed, although the plasmodia caused changes in tissue structure. In comparison with *Henneguya* spp. from South America and from different geographic regions, the species here described was morphometrically and molecularly distinct to any other. ML and BI analyses showed the novel species clustering as sister to a clade of *Henneguya* parasites of cichlids, all from the Amazon basin. Our results revealed that the strongest evolutionary signal for *Myxobolus*/ *Henneguya* was the phylogenetic affinity of the fish hosts, with clusters occurring mainly based on the host order and/or family.

Conclusion: Morphological and molecular data supported description of a novel *Henneguya* species. This description forms an important part of documenting myxozoan diversity in the Amazon basin, Brazil.

Keywords: Myxobolidae, Cnidaria, freshwater, South America, phylogeny

Funding: FAPESP - São Paulo Research Foundation (Proc. n° 2018/19285-9).

288-P

Putting together the puzzle: long term monitoring data give insight in the development of proliferative kidney disease

Wahli, Thomas (Switzerland)[2]; Berger, Robin (Switzerland)[1]; Schmidt-Posthaus, Heike (Switzerland)[2]

1 - La Maison de la Rivière, Tolochenaz, Switzerland; 2 - Centre for fish and wildlife health, University of Bern, Bern, Switzerland

Introduction: Since the 1980-ies catches of brown trout by anglers are dramatically decreasing in Switzerland. Nationwide projects have indicated different potential causes for this decrease, among them increased mortality due to proliferative kidney disease (PKD) caused by the myxozoan parasite *Tetracapsuloides bryosalmonae*.

Methodology: In order to assess the potential impact of this infection on brown trout populations, the presence of the disease in Swiss rivers has been investigated in the frame of both, routine diagnostic work and various projects, respectively. For most samplings, a defined number of young of the year trout have been included and tested by means of routine histological methods for the presence of the parasite in the kidney, for the degree of infection as well as for the degree of kidney alterations.

Results: Based on these investigations a unique database comprising river sites all over Switzerland for a period of 30 years could be built up. Although sampling sites have not been selected randomly, the database allows to analyse the data for a range of different criteria, among them: i) Distribution of sites with PKD – positive fish within the country: The vast majority of sites with infected fish is located in the Swiss midlands; ii) Spread of the disease over time: A slight trend in upriver migration of the disease was seen; iii) Change of prevalence and infection intensity over time within selected rivers: Neither in prevalence nor in infection intensity a clear trend was seen; iv) Change of distribution over time in relation to changing water temperature using altitude as a proxy for the temperature: Most sites with infected fish were below an altitude below 800 meters above sea Level, but below this Level, no clear distribution was seen.

Conclusion: The results of these evaluations are an important base to create models for predicting the future development of brown trout populations in consideration of the impact of PKD.

Keywords: *Tetracapsuloides*, PKD, brown trout, longterm data, Switzerland

290-P*

Prospecting for malacosporeans infecting freshwater fish from the Amazon basin

Naldoni, Juliana (Brazil)[1]; Okamura, Beth (United Kingdom)[2]; Adriano, Edson Aparecido (Brazil)[1]

1 - Departamento de Ciências Biológicas, Universidade Federal de São Paulo, Diadema, SP, Brazil; 2 - Department of Life Sciences, Natural History Museum, London, UK

Introduction: Myxozoans are widespread and common endoparasites of fish with complex life cycles, infecting vertebrate and invertebrate hosts. There are two major groups: Myxosporea and Malacosporea. To date some 2,500 myxosporean species have been described. By comparison, there are only five described malacosporean species although molecular and ultrastructural assays provide evidence of a hidden diversity of malacosporean species infecting a variety of fish and bryozoan hosts. In the last decade investigations of myxosporeans have been intensified in South America and some 140 species have now been described in freshwater fish in this continent. The malacosporean *Buddenbrockia* infecting bryozoans was reported in southeast Brazil, although at the time this was not recognised to be a myxozoan. However, malacosporeans have never been reported in South American fish. The aim of this study was to investigate the presence and potential diversity of malacosporeans infecting a range of freshwater fish from the Amazon basin.

Methodology: The material examined so far was collected from the municipalities of Santarém and Manaus in Brazil and includes 146 fish belonging variously to the Sciaenidae, Pimelodidae, Cichlidae, Curimatidae, Cynodontidae, Anostomidae, Potamotrygonidae, Prochilodontidae, Achiridae, Triportheidae, Serrasalmidae and Loricariidae. Kidney material from each fish was fixed appropriately for DNA sequencing and ultrastructural study. Malacosporean diversity is currently being assessed by DNA sequence data and development by ultrastructural examination of kidneys positive for infection.

Results: Preliminary evidence reveals infections of *Buddenbrockia* and *Tetracapsuloides* species at varying prevalences in ten fish species.

Conclusion: These results expand our understanding about the diversity, distribution and range of fish hosts exploited by malacosporeans.

Keywords: Myxozoa, fish kidney parasites, Amazonian fish hosts, *Tetracapsuloides*, *Buddenbrockia*

Funding: São Paulo Research Foundation - FAPESP #2016/22047-7 and FAPESP #2014/22700-7.

Prophylaxis and Treatment

292-P

Developing bioassays to determine effect of warm water bathing on salmon lice, *Lepeophtheirus salmonis*, a parasite of farmed Atlantic salmon

Andrews, Melanie (Norway)[1,2]; Horsberg, Tor Einar (Norway)[1,2]

1 - Norwegian University of Life Science, Oslo, Norway; 2 - Sealice Research Centre, Oslo, Norway

Introduction: Salmon lice, *Lepeophtheirus salmonis* (Krøyer, 1837), are ectoparasitic crustacean parasites of the Atlantic salmon. If left unchecked, they have the capability to rapidly increase population size. This leads to high economic costs to the Atlantic salmon aquaculture industry in the northern hemisphere due to the loss of fish quality and the need for increased handling. Numerous chemical treatments have been extensively used to combat this parasite. However, there has been a shift towards the use of and non-chemical treatment methods such as warm water bathing. When dealing with such an industry wide health problem, treatment resistance towards the commonly used chemical treatment methods can become an issue. To combat this, on site *in vitro* bioassays are routinely used to monitor treatment efficacy by referring to previously established baseline sensitivities. However, in the case of warm water bathing, neither bioassay methods nor baseline sensitivity levels have been identified.

Methodology: We have developed *in vitro* bioassay methods to identify the water temperature at which median survival (EC50) was obtained for both the copepodid and pre-adult II (PAII) stages following short term exposure. This study included 6 geographically distinct salmon lice populations collected along the Norwegian coast. Parasites were exposed for 2 min to a range of water temperatures (12 ºC, 30 - 40 ºC). Immediately following exposure, a count of unaffected/affected parasites was taken, they were then left undisturbed for 24 h after which another count of unaffected/affected parasites was taken.

Results: Copepodid and PAIIs were affected (no movement/attachment) immediately following exposure. At the 24 h count, we observed that the salmon lice recovered from the treatment. Population differences were observed for both stages of salmon lice at the 2 min and 24 h count. Detailed results will be presented.

Conclusion: We found that warm water bathing has an immediate immobilising effect on the salmon lice, however, rapid recovery was observed with high survival counts 24 h following treatment. We identified population differences in sensitivity to warm water bathing. By identifying the baseline sensitivity levels towards warm water bathing we hope to provide a tool to avoid any future development of tolerance to this treatment method.

Keywords: aquaculture, Atlantic salmon, sealice, parasitic copepod

293-P*

Proteolytic effect of commercial protease, NEUTRASE®, on viral membrane of fish viruses

Jin, Yeonhwa (Germany)[1]; Klafack, Sandro (Germany)[1]; Bergmann, Sven (Germany)[1]

1 - Friedich-Loeffler-Institut, Insel Riems, Germany

Introduction: Since the late 1990th, KHV is a major threat for carp and koi industry worldwide. Because of its high fatalities and live-long persistence, eradication of the agent is hard but necessary. Unfortunately, there were many vaccines tested but none of them was approved in the EU. The only commercialized vaccine, by the Israeli company KoVax, was removed from US market after one year. Hence, it is necessary to introduce a new, safe and reliable vaccine.

Methodology: Because of its high titers, attenuation experiments were based on KHV from Taiwan (KHV-T). The virus was passaged serially onto CCB cells at 20 °C for a long time (100 passages). Several viral passages were tested in vivo in carp model for attenuation. Fish were infected/immunized by emersion. In this process one of the attenuated passages could be detected as probable vaccine virus. Additional experiments were performed to improve the vaccine administration by oral delivery via alginate capsules and to improve immunity by boost vaccination. Furthermore ORF150 was deleted in a wild type background and tested in carp model.

Results: The vaccine leads to a 100% survival rate after wild-type virus challenge. It did not induce clinical signs and antibody response was quantifiable. Whole genome sequencing revealed some minor differences compared to wild-type virus. Surprisingly, a ~1400 bp deletion was found in ORF 150. Database analysis revealed a putative Ubiquitin E3 Ligase, which might influence KHVs virulence. Decreased virulence was visible as well as no mortality after wild-type challenge in ORF 150 deletion.

Conclusion: This vaccine is applicable either orally, by immersion or in a combination. Open reading frame 150 seems to be an important factor for virulence. Moreover, a PCR based on ORF 150 can be used for differentiation of infected from vaccinated animals (DIVA).

Keywords: protease, KHV, VHSV, IHNV, IPNV

294-P

Praziquantel treatment of grass carp (*Ctenopharyngodon idella*) infecting with eye fluke (*Diplostomum* sp.) in field conditions

Zusková, Eliška (Czech Republic)[1]; Máchová, Jana (Czech Republic)[1]; Piačková, Veronika (Czech Republic)[1]

1 - University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Research Institute of Fish Culture and Hydrobiology, Zátiší 728/II, 389 25 Vodňany, Czech Republic

Introduction: Trematodes of the genus *Diplostomum* (von Nordmann, 1832) are some of the most common parasite infections in pond stocked fish, where causes considerable losses particularly in younger fish species of littoral habitats (offshore zone with plants). The life cycle of *Diplostomum* sp. including passage through birds, snails and fish. Free-living stages (cercariae) are released from snails in high numbers to infect the lens of the fish eye, where are transformed to other larval stages named metacercariae. In one fish, tens to hundreds of metacercariae can parasitize in the eyes. Fish have impaired vision associated with behavioral changes. Recently, the biggest problems with diplostomosis have been reported in grass carp (*Ctenopharyngodon idella*). The aim of the study was to verify the efficacy of the antiparasitic praziquantel, administered in the feed, on the metacercariae of the eye fluke in grass carp.

Methodology: During the field pilot trial, two feeding tests were carried out - the first one in the storage tanks, the other in the model ponds, where the occurrence of intermediate hosts can be expected. Fish were fed in various intervals with praziquantel medicated feed prepared in two concentrations (1.25 and 2.5 g/ kg feed). The prevalence and intensity of infection was determined on the beginning and the end of the 7 weeks lasting tests using eye dissection.

Results: The complete disappearance of metacerkariae *Diplostomum* sp. was observed in the two groups tested, when the most economically effective treatment was six times repeated administration of medicated feed (the feed containing PQ in the concentration 1.25 g/kg of feed) (every other day) with a 14-day break, when only no-medicated feed was served.

Conclusion: Praziquntel seems to be usable and effective treatment of diplostomosis infection of pond fish in case that all requirements and approvals of its using are followed.

Keywords: anthelmintic, medicated feed, fish treatment

Funding: CENAKVA (LM2018099) and PROFISH (CZ.02.1.01/0.0/0.0/16_019/0000869).

295-P

Proteases as alternative disinfection agents against fish viruses?

Becker, Anna M. (Germany)[1]; Oberle, Martin (Germany)[2]; Christian, Jürgen (Germany)[3]; Bergmann, Sven M. (Germany)[4]

1 - Friedrich-Alexander University Erlangen-Nürnberg; 2 - Bavarian State Research Center for Agriculture, Institute for Fisheries, Department for Carp Farming, Höchstadt an der Aisch; 3 - Bavarian Health and Food Safety Authority, Institute for Animal Health II, Erlangen; 4 - Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Infectology, Greifswald - Insel Riems

Introduction: In the search for novel and environmentally friendly disinfection agents against fish viruses, such as koi herpesvirus (KHV), viral hemorrhagic septicemia (VHSV) or infectious hematopoietic necrosis virus (IHNV), proteolytic enzymes were investigated. For this purpose, two biotechnologically produced protease solutions - Neutrase® and Alcalase® (Novozymes) - as well as papain extracted from *Carica papaya* (Roche) were examined in this work.

Methodology: Toxic effects of the enzymes on the cell lines used for *in vitro* propagation of these viruses were investigated and half-maximal effective concentrations (EC_{50}) (48 h) calculated. Inactivation experiments with selected viruses and proteases at 8 and 25 °C were performed, the titer before and after the treatment determined, and a reduction of the virus titer ($T_R = \log_{10}Titer_{Start} - \log_{10}Titer_{End}$) calculated. Moreover, the proteolytic activities of Neutrase® and Alcalase® in various aqueous samples and at various temperatures were examined and virus inactivation due to the short incubation (up to 4 h at 8 and 25 °C) with these two enzyme formulations were tested.

Results: All proteases showed cell toxicity, however with different EC_{50}-values. While the EC_{50} for Alcalase® lay in the range of $\mu g_{enzyme\ solution}$/mL, the application of Neutrase® and Papain yielded a value-range of $mg_{enzyme\ solution}$/mL for all three investigated cell lines. All tested enzyme solutions were able to inactivate the selected viruses, showing the best results and the highest titer reductions at the highest investigated concentrations and 25 °C (up to $T_R = 6.4$). Higher titer reductions at 25 °C correspond well with increased proteolytic activity of Neutrase® and Alcalase® at this temperature in comparison to 8°C. Furthermore, the disinfection of KHV was reached already after 0.5 h incubation with Neutrase®.

Conclusion: In summary, our study showed that proteases are able to rapidly inactivate the investigated viruses and thus have a great potential as alternative to classic disinfection agents. Nevertheless, further studies in regard to their efficiency at various environmental conditions are needed.

Keywords: KHV, VHSV, IHNV, disinfection, proteases

Funding: The German Federal Ministry of Food and Agriculture (BMEL) through the Federal Office of Agriculture and Food (BLE), grant number 2815NA062.

296-P*

The 'BEST' method for characterising the lumpfish (*Cyclopterus lumpus*) microbiome

Christie, Lyndsay (United Kingdom)[1]; Verner-Jeffreys, David (United Kingdom)[3]; Feil, Ed (United Kingdom)[2]

1 - Cefas & University of Bath; 2 - University of Bath; 3 - Cefas

Introduction: The salmon farming industry has been plagued by the parasitic salmon louse (*Lepeophtheirus salmonis*) in recent years. Biocontrol is being increasingly used to mitigate this problem by introducing Lumpfish (*Cyclopterus lumpus*) to salmon pens to feed on the lice. Hatchery production of lumpfish is a growing industry; however research is needed to improve husbandry and disease management techniques. The fish gut microbiome is known to be important for health, disease resistance and immune development but the lumpfish microbiome is completely uncharacterised. This study aimed to validate a method for charactering the lumpfish gut microbiome and understand how it influences host health.

Methodology: Hatchery reared juvenile lumpfish at 1 g and 25 g were sampled by dissecting out gut sections and extracting the DNA. The microbiome was characterised using 16s amplicon sequencing and analysed using qiime2, allowing high throughput analysis of multiple samples. Selected samples were also sequenced using shotgun metagenomics.

Results: The 16s sequencing showed the microbiome composition was significantly different between the 1 g and 25 g lumpfish, with increased alpha diversity in the 1g lumpfish. The guts of half the 25 g fish were dominated by *Mycoplasma* spp., accounting for up to 90% of the total bacteria, the 1g lumpfish had no *Mycoplasma* in the gut. In individuals with lower levels of *Mycoplasma*, the guts were dominated by *Vibrio* spp., accounting for 20 - 70% of the total bacteria. The shotgun metagenomics results disagreed with the 16s results and instead suggested *Pseudomonas* spp. were dominating the gut.

Conclusion: The lumpfish gut microbiome is dynamic and changes significantly during development, coinciding with key life events (e.g. changes in diet), which is likely to be important for the development of the immune response. The high abundance of opportunistic pathogenic species, *Mycoplasma* and *Pseudomonas*, could have adverse effects on the health status of lumpfish. The low diversity observed in the microbiome may increase susceptibility to disease in lumpfish, especially post-deployment when they are introduced to a novel environment. Long read nanopore sequencing will be used to overcome the potential bias of the other two sequencing platforms to identify the true composition of the lumpfish microbiome.

Keywords: microbiome, cleaner fish, Mycoplasma, 16s

297-P*

The efficacy of commonly used disinfectants on lumpfish (*Cyclopterus lumpus*) embryos

Christie, Lyndsay (United Kingdom)[1]; Verner-Jeffreys, David (United Kingdom)[2]; Feil, Ed (United Kingdom)[3]

1 - Cefas and University of Bath; 2 - Cefas; 3 - University of Bath

Introduction: Demand for lumpfish (*Cyclopterus lumpus*) is increasing as they are used as cleaner fish in the salmon farming industry. Hatcheries have been set up in the UK to meet this demand, producing approximately four million lumpfish per year. In hatcheries embryo disinfection is common practice to help manage disease risk by removing pathogens. Many 'off the shelf' disinfectants are available, with varying modes of action and efficacy. The aim of this study was to compare the effects of different disinfectants for use on lumpfish embryos.

Methodology: Lumpfish embryos were collected from a UK lumpfish hatchery and separated into individual wells of a multi-well plate, filled with seawater. Three disinfectants were trialled, Buffodine (iodine based), Formalin (formaldehyde based) and Pyceze (bronopol based). The embryos were disinfected daily following manufacturer's instructions for two weeks. The effect of the disinfectants on embryo survival, hatch rate and bacterial growth were measured and recorded throughout the study.

Results: Bacterial growth was detected on the surface of embryos prior to disinfection. All three disinfectants reduced bacterial growth on the embryo surface and in the water. At the end of the study, Pyceze was found to cause the most significant reduction in bacterial growth. Pyceze and Formalin significantly increased the hatch rate of embryos compared to the controls by 130% and 180% respectively. The embryos treated with Buffodine saw the least reduction in bacterial growth and a 200% increase in embryo mortality. *Vibrio* spp. were most commonly isolated from the embryo surface.

Conclusion: Pyceze was the most effective disinfectant at reducing bacterial growth however it caused an increase in hatch rate, which may have adverse consequences for larvae if the hatching was premature. Buffodine had the least disinfectant activity and caused an increase in embryo mortality, so may not be suitable for use in lumpfish. Both Pyceze and Formalin were able to reduce bacterial growth, however, they may be removing the protective natural flora of the embryo. The species of bacteria isolated from the embryos will be identified to determine whether disinfecting fish embryos is truly good husbandry practice.

Keywords: cleaner fish, disinfectant, bacteria, embryo

298-P*

Effect of two acidic antiseptics on hatching of common carp (*Cyprinus carpio*)

Naumowicz, Karolina (Poland)[1]; Kujawa, Roman (Poland)[1]; Zalas, Bartłomiej (Poland)[1]; Terech-Majewska, Elżbieta (Poland)[1]; Wąsowicz, Krzysztof (Poland)[1]

1 - University of Warmia and Mazury in Olsztyn

Introduction: Due to the high temperature of incubation, eggs of common carp (*Cyprinus carpio*) are very susceptible to water mold infections, which are mainly caused by algae-like fungi from *Saprolegniaceae* family. Infection commonly begins in unfertilized eggs and can spread to healthy eggs, resulting in high loss of the brood. The main symptom of infection is cottony, proliferative growth on opaque white eggs. A crucial element of prophylactic treatment during artificial egg incubation is antisepsis. There is a need to test new antiseptics, which should be safe for both eggs and the environment.

Methodology: In 16 mini Weiss jar incubators (0.7 L volume), 0.1 L of fertilised eggs from one female of common carp were incubated in 24 ºC temperature. We used two commercial stabilized acidic antiseptics solutions containing peracetic acid (PAA), acetic acid, hydrogen peroxide, and water. The experimental design contains control group, Solution-1 in the concentration of 400, 500, and 600 ppm and Solution-2 in the concentration of 300, 400, 500, and 600 ppm (all groups in two repetitions). Eggs were treated with prophylactic antiseptics per 30 minutes daily till hatching. The prevalence of living hatched fry was counted.

Results: In the control group, we observed a very strong water mold infection after 20 hours of incubation. Both acetic antiseptics inhibited the growth of algae-like fungi in all examined concentrations and caused 4 hours faster hatching comparing to control group. The lowest prevalence of living hatched fry was in the control group as well as in the 600 ppm Solution-2 groups.

Conclusion: Antisepsis is a very important strategy of pathogen inactivation. Examined antiseptics can be helpful in decreasing water mold infections. On the other hand, peracetic acid, acetic acid and hydrogen peroxide are strong oxidants and can influence chorion structure. They quicken hatching by weakening the chorion. Moreover, too high concentrations of solution can irreversible damage not only chorion but also embryo, leading to high mortality. That is why choosing the proper dosage is very important. Checking concentrations lower than 300 ppm and finding the lowest concentration with antifungal effect should be taken into consideration in future research.

Keywords: water molds, saprolegniosis, algae-like fungi, artificial incubation in common carp

299-P

In vitro screening of 35 compounds against *Saprolegnia* spp.

Tedesco, Perla (Italy)[1]; Fioravanti, Maria Letizia (Italy)[1]; Dirks, Ron (Netherlands)[2]; Galuppi, Roberta (Italy)[1]

1 - Alma Mater Studiorum, University of Bologna. Department of Veterinary Medical Sciences; 2 - Future Genomics Technologies BV, Leiden, The Netherlands

Introduction: Oomycetes belonging to the genus *Saprolegnia* cause high mortality rates in freshwater fish culture. The lack of alternative treatment with effectiveness comparable to the banned malachite green (MG), urge the identification of new molecules active against this pathogen. This work focused on an *in vitro* screening aimed at assessing the effectiveness of 35 compounds against *Saprolegnia* spp. in order to select molecules that could be of major interest for further *in vivo* investigations and applications in aquaculture.

Methodology: *In vitro* trials were carried out on two *Saprolegnia parasitica* strains and one *S. delica* strain. Tests were performed according to existing protocols to determine the Minimum Inhibitory Concentration (MIC) in agar and the Minimum Lethal Concentration (MLC) after one hour of contact in water. Compounds were provided diluted in DMSO at a concentration of 10 mM (with the exception of Dequalinium Chloride, diluted at 2 mM) that were further diluted to working concentrations of 0.00001; 0.0001; 0.001; 0.01; 0.1 and 0.25 mM. Pure DMSO was also screened at the same amount present in different concentrations, until 1mM, in order to check its own inhibitory or lethal effects. Each strain was tested in triplicate.

Results: Triplicates were consistent among each other. DMSO showed no inhibitory effects against *Saprolegnia* at concentrations from 0.00001 to 0.25 mM, but was able to inhibit the oomycete at higher concentrations also when used alone. MICs were determined for 15 out of 35 compounds examined. The lowest MIC was defined for zinc pyrithione (0.01 mM). Seven compounds had a MIC of 0.1 mM, seven of 0.25 mM, while the other 20 were ineffective at the tested concentrations.

Conclusion: Although it was not possible to define MICs for several compounds at the tested concentrations, most of the molecules showed the ability to slow down the radial growth and/or to inhibit the aerial mycelium. These compounds, although not showing a high efficacy against *Saprolegnia* in *in vitro* trials, could be effective in decreasing the invasive capacity of the oomycete in the host, therefore representing good candidates for further *in vivo* studies.

Keywords: *in vitro* test, MIC, MLC, *Saprolegnia*

Funding: ParaFishControl H2020 project (634429).

300-P*

Effective treatment of fish endoparasites with oral administration of drug delivery nanoparticles

Mathews Delgado, Patrick (Brazil)[1]; Madrid Martinez, Rafael Ricardo (Brazil)[2]; Fernandes-Pattta, Ana Carolina Monge (Brazil)[2]; Rigoni Silva, Vera Lucia (Brazil)[2]; Ramirez Baquero, Carlos Alberto (Brazil)[2]; Mertins, Omar (Brazil)[2]

1 - Department of Zoology, Institute of Biosciences, University of São Paulo, São Paulo, Brazil; 2 - Department of Biophysics, Paulista Scholl of Medicine, Federal University of São Paulo, São Paulo, Brazil

Introduction: Parasites in fish cause severe economic impact worldwide. Currently, the nanotechnology provides means to treat the infections via drug delivery materials. We developed an efficient composite nanoparticle of easy oral administration, which carries and delivers anti-parasite drugs to the intestines of ornamental fish, providing effective elimination of nematodes and digenetic trematodes.

Methodology: The nanoparticles were produced with biocompatible chitosan-arginine and alginate in profit of the electrostatic interaction between the polysaccharides. The hydrophobic anti-helminthic drug praziquantel was encapsulated in optimized conditions and the powder form of the drug carrying particles was orally administrated to *Corydoras schwartzi* fish, highly infected with intestinal parasites.

Results: Fish eat the powder form of the drug delivery nanoparticles after dispersion in the aquarium water, evidencing high acceptance. The mucoadhesive and pH responsive nanoparticles were found interacting on the intestine tissues of the fish and on external and internal membranes of the intestinal parasites. The parasites infection treatment was effective following a three days administration of particles containing 0.2 mg/kg of fish body weight. Highly infected fish were completely clean after 30 days of the oral administration of the nanoparticles.

Conclusion: The mucoadhesive property of the bio-nanoparticles in intestines of teleost fish is effective and provides a prolonged retention of the structures on the mucosal tissues, thus optimizing the drug delivery and efficacious treatment of fish endoparasites is achieved. The new biocompatible nanoparticle is of strong commercial appeal.

Keywords: nanoparticles, fish parasites, treatment

Funding: São Paulo Research Foundation (FAPESP).

301-P

Optimization of *Rhus verniciflua* stokes extract against edwardsiellosis and its pharmacokinetics in olive flounder

Lim, Jae-Woong (Republic of Korea)[1]; Jung, Sung-Ju (Republic of Korea)[1]; Kang, So Young (Republic of Korea)[1]

1 - Chonnam National University

Introduction: This study aimed to optimize extraction conditions of *Rhus verniciflua* Stokes lignum (RVSL) for antibacterial activity using Box-Behnken design, to identify antibacterial compounds from the optimized extract (OE) and to evaluate pharmacokinetics of active compounds in olive flounder *Paralichthys olivaceus*.

Methodology: To get an OE from RVSL, extracting parameters were optimized by using Box-Behnken Design based on the single-factor experiments. OE was orally administered at doses of 30, 100 and 300 mg/kg b.w./day for 2 and 10 weeks in olive flounder for efficacy studies. By antibacterial activity-guided isolations over OE, seven compounds were isolated and identified through spectroscopic analyses including NMR (nuclear magnetic resonance) and MS (mass spectrometry). Among them, the most active compound, ethyl gallate (EG) was studied for pharmacokinetics using LC-MS/MS after intramuscular injection at 20 mg/kg b.w. in olive flounder.

Results and Conclusion: The optimum extraction conditions were ethanol concentration 60%, extracting temperature 85 ºC and the ratio of solvent to raw material (v/w) 30. Fish administered with OE showed significant efficacies against *Edwardsiella tarda* infection, with relative percent of survival of 14.3 to 70.0% ($P < 0.01$). Seven compounds isolated from OE were identified to be gallic acid (1), ethyl gallate (2), fustin (3), fisetin (4), butin (5), garbanzol (6) and sulfuretin (7). Among them, 2 showed the highest antibacterial activity against *E. tarda* showing MIC of 31.25 µg/mL. Compounds 1, 3, 4, 5, 6 and 7 were also found to be active, with MICs of 250 - 1000 µg/mL. Pharmacokinetic analysis was performed for the most active EG. Peak plasma concentration following a single intramuscular injection of 20 mg/kg b.w. was 6.47 (T_{max} 1.42 h) µg/mL. The elimination half-life of EG was 3.31 h. These pharmacokinetic properties of EG seems to be due to its rapid distribution and elimination. Method validation results showed good linear regression in the calibration curve ($r^2 > 0.99$) and extraction recoveries of analytes of 90.7 to 100.1 %. This is the first report on a pharmacokinetic study for EG in olive flounder. Taken together, these results suggested that OE from RVSL may be used as effective antibacterial alternatives in aquaculture.

Keywords: optimization, *Rhus verniciflua*, edwardsiellosis, olive flounder, pharmacokinetics

302-P

Screening of marine natural products (MNPS) as new antibacterial substances against fish pathogens affecting *Dicentrarchus labrax* and *Sparus aurata*

Bulfon, Chiara (Italy)[1]; Pacorig, Valentina (Italy)[1]; Zamperoni, Daniela (Italy)[1]; D'ippolito, Giuliana (Italy)[2]; Nuzzo, Genoveffa (Italy)[2]; Volpatti, Donatella (Italy)[1]; Pretto, Tobia (Italy)[3]; Manfrin, Amedeo (Italy)[3]; Fontana, Angelo (Italy)[2]; Galeotti, Marco (Italy)[1]

1 - University of Udine; 2 - CNR; 3 - IZS Venezie

Introduction: The development of antibiotic resistance due to the indiscriminate use of commercial drugs for the treatment of fish bacterial diseases is a serious problem. Therefore, intense research is in progress towards the search for natural remedies. The present study aimed at investigating the *in vitro* antibacterial activity of some marine natural products (MNPs) against relevant fish pathogens affecting European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*).

Methodology: The bacterial strains (*Vibrio anguillarum* serotype O1, *Photobacterium damselae* subsp. *piscicida*, and *Photobacterium damselae* subsp. *damselae*) were isolated during spontaneous outbreaks (Udine University and IZSVe biobanks). MNPs were provided by the CNR as both raw extracts and enriched fractions obtained by a validated solid-phase extraction (SPE) method, that allows high recovery and separation of primary and secondary metabolites from peptides to oil. The antimicrobial potential of MNPs was assessed by determining the minimal inhibitory and bactericidal concentrations (MIC and MBC) by the broth micro-dilution method in microtiter plate.

Results: Evident differences in the antibacterial activity of the compounds were found, depending on the marine organism used for MNPs extraction and on the target bacterial species. The extracts/fractions purified from the invertebrates *Gastropteron meckeli* and *Crambe crambe* exhibited a broad spectrum of inhibitory effects against the tested strains, displaying MICs ranging from 2.1 to 8.3 µg/ml, MBCs ranging from 4.2 to 16.7 µg/ml, and MICs ranging from 4.2 to 33.4 µg/ml, MBCs ranging from 8.3 to 66.7 µg/ml, respectively. The extract/fractions purified from other marine organisms (*Cyclotella cryptica*, *Thalassiosira weissflogii*, *Amphidinium carterae*, *Amphidinium massarti*, *Alexandrium minutum*, *Chondrilla nucula*, *Skeletonema marinoi*) showed negligible antibacterial effects.

Conclusion: To the best of our knowledge, this is the first report on the antibacterial properties of these MNPs against fish pathogens. Further investigations will be conducted in order to identify other MNPs demonstrating a stronger antibacterial activity and the results will be discussed. The present study seems to be promising for the identification of novel natural antimicrobials that might be used in fish farming for controlling bacterial infections.

Keywords: marine natural products, antibacterial activity, *Vibrio anguillarum, Photobacterium damselae* subsp. *piscicida, Photobacterium damselae* subsp. *damselae*

Funding: AdriAquaNet project (application ID 10045161), 2014-2020 Interreg V-A Italy-Croatia CBC Programme, Priority Axis: Blue innovation.

303-P

In vitro testing of disinfectants against nervous necrosis virus and *Tenacibaculum* spp.

Carnicero, Beatriz (Spain)[1]; Magariños, Beatriz (Spain)[1]; Dopazo, Carlos P. (Spain)[1]

1 - Departamento de Microbiología y Parasitología, Instituto de Acuicultura, Universidade de Santiago de Compostela, Spain

Introduction: As part of a research project for the diversification of aquaculture in Spain by the optimization of the culture of greater amberjack (*Seriola dumerili*), we have tested a number of disinfectants commonly cited in the literature, against 2 species of the bacterial genus Tenacibaculum and to the nervous necrosis virus (NNV), both found during the study.

Methodology and Results: Inibol, benzyl alcohol, glutaraldehyde, H_2O_2, peracetic acid, cloramine T and proxitane were first tested in BF-2 and E-11 cells to assess their toxicity in cells at different concentrations and times. The antiviral activity was tested by inoculation of the virus in a solution with the disinfectant at different concentrations; after different times of incubation the virus was concentrated by ultracentrifugation and washed twice before titrating the remaining infective virus; as reference, untreated virus was subjected to the same process. For testing antibacterial activity, H_2O_2, glutaraldehyde, cloramine T or proxitane were added at different concentrations in medium FMM with *T. maritimum* or *T. soleae*, and after 15 min, 30 min or 24 h, samples were removed and seeded into FMM plates to quantify the reduction of bacterial concentration. Only inibol showed no toxicity to cells but also low activity (1 log reduction); glutaraldehyde (2%, 5 min), peracetic acid (0.8 - 0.2%, ≥ 15 min), cloramine T (1.5%, ≥ 30 min), and proxitane (≥ 1:80, 5 min) totally inhibited the virus, but also showed toxicity to monolayers. In the case of bacteria, only proxitane showed activity to both species with just 15 min of treatment; for treatments of ≥ 30 min, 2% glutaraldehyde and H_2O_2 (240 ppm) were also effective.

Conclusion: Toxicity and disinfectant activity have been in most cases associated. Only glutaraldehyde and proxitane were effective for both, the bacteria and the virus. *In vivo* assays must be performed to assess their effectivity and toxicity in the fish.

Keywords: *Tenacibaculum*, NNV, disinfectants

Funding: Proyect SERIOLA-JACUMAR/Ministerio de Agricultura y pesca, Alimentación y Medio Ambiente.

304-P

Efficacy and mechanisms of action of extract optimized from *Sanguisorba officinalis* L. roots against viral hemorrhagic septicemia in olive flounder

Kang, So Young (Republic of Korea)[1]; Lim, Jae-Woong (Republic of Korea)[1]; Seo, Joong-Kyeong (Republic of Korea)[1]; Jung, Sung-Ju (Republic of Korea)[1]

1 - Chonnam National University

Introduction: Viral hemorrhagic septicemia (VHS) causes significant economic losses for olive flounder aquaculture in Korea. In our on-going research to discover natural products to be used as antiviral agents for VHS, we derived the extraction condition showing optimal antiviral activity and extraction yield of *Sanguisorba officinalis* L. roots via response surface methodology (Box-Behnken design) and studied the efficacy of optimized extract (OE) in olive flounder.

Methodology: Extracting parameters were optimized by Box-Behnken design based on the single-factor experiments for both *in vitro* antiviral activities (CPE reduction assay using FHM cells) and extraction yield. To evaluate preventive effects of OE against VHS, olive flounders were orally pre-administered with OE at doses of 25 and 50 mg/kg b.w/day for 2 weeks. And its therapeutic effects were also tested at the same doses. Ribavirin was used as a reference control. The chemical profile of OE was analyzed and ziyuglycoside I was quantitated as a marker by LC-MS/MS (QTOF and triple qaudrupole, respectively). For OE-administered olive flounders (25 mg/kg b.w/day for 2 weeks), serum lysozyme and bactericidal activities were determined and immune gene expressions analyzed by real time qPCR.

Results and Conclusion: The optimum conditions were ethanol concentration 55%, extracting temperature 50 ºC and extracting time 5 h. OE prepared under these conditions showed a significantly potent antiviral activity with EC_{50} value of 0.28 μg/ml. In the efficacy studies, the OE groups showed significant preventive or therapeutic effects with relative percent survival of 30 to 60 % ($P < 0.05$). The therapeutic effect of OE against VHS was significantly higher than ribavirin. Oral administration of OE significantly enhanced serum lysozyme and bactericidal activities in the olive flounders. Furthermore, it significantly induced inflammatory cytokine responses (IL-1β, NF-kB, and TNF-a) at 1 and 2 days post-oral administration (dpa). Additionally, ISG15 (1, 2 and 7 dpa) and Mx (10 dpa) were significantly activated in the olive flounder. These results suggest that OE from *S. officinalis* can be proposed as an antiviral agent against VHS in olive flounder aquaculture.

Keywords: *Sanguisorba officinalis*, viral hemorrhagic septicemia, olive flounder, response surface methodology, antiviral

Funding: NRF-2015R1C1A2A01053487.

305-P*

Mitochondrial and nuclear single nucleotide markers for detection of deltamethrin resistance in salmon lice

Tschesche, Claudia (United Kingdom)[1]; Bekaert, Michael (United Kingdom)[1]; Bron, James (United Kingdom)[1]; Sturm, Armin (United Kingdom)[1]

1 - Institute of Aquaculture, University of Stirling, Stirling, United Kingdom

Introduction: During salmon production, the pyrethroid deltamethrin (DTM, AMX®, PHARMAQ) is used to treat infections by parasitic salmon lice (*Lepeophtheirus salmonis* (Krøyer, 1837)). DTM toxicity is believed to be based on blockage of voltage gated sodium channels (Na_v1). However, the use of DTM for salmon delousing is threatened by resistance development. DTM resistance in terrestrial arthropods typically involves target-site mutations rendering Na_v1 insensitive (knock-down-resistance, *kdr*), or increased metabolic detoxification. In *L. salmonis,* DTM resistance has been shown to be mainly inherited maternally and to be associated with mitochondrial mutations. In addition, a putative *kdr*-type mutation has been identified in one of three Na_V1 homologues. This study aims to obtain insights into the mechanisms of DTM resistance in *L. salmonis* by combining pharmacological and genotyping approaches.

Methodology: *L. salmonis* originated from DTM resistant and susceptible laboratory strains, farm bioassays, and a cross between a DTM resistant male and a DTM susceptible female. DTM susceptible and resistant lice were genotyped by PCR based genotyping assays to detect the mitochondrial single nucleotide polymorphisms (SNPs) t3338c, t8600c, a8134g, and a5889g, and the Na_v1 SNP a3041g, which were associated with DTM resistance in previous studies. In addition, *L. salmonis* bioassays were performed with the pseudo-pyrethroid etofenprox, which cannot be metabolised by esterases.

Results and Conclusion: The allele frequencies of the putative *kdr* mutation a3041g in Na_v1 did not differ ($P > 0.05$) between DTM resistant and susceptible lice from farm sites and did not correlate with DTM resistance of F2 progenies from a cross between a DTM resistant male and a DTM susceptible female. In insects, *kdr* mutations cause cross-resistance to etofenprox. In contrast, DTM resistant *L. salmonis* were not cross-resistant to etofenprox. Thus, DTM resistance in *L. salmonis* seems to be unrelated to target-site mutations in Na_v1. The allele frequencies of the mitochondrial SNP alleles g3338a, t5889c, g8134a, and t8600c were higher in DTM resistant lice than in susceptible lice ($P < 0.001$), indicating an association with DTM resistance. However, while the SNP t8600c, leading to the missense mutation Leu107Ser in COX1, was present in all DTM resistant lice, it was also found in some susceptible lice.

Keywords: sea lice, deltamethrin, resistance, mtDNA, sodium channel

306-P*

Deltamethrin resistance in salmon lice: the carboxylesterase family

Tschesche, Claudia (United Kingdom)[1]; Bekaert, Michaël (United Kingdom)[1]; Bron, James (United Kingdom)[1]; Sturm, Armin (United Kingdom)[1]

1 - Institute of Aquaculture, University of Stirling, Stirling, United Kingdom

Introduction: During salmon production, the pyrethroid deltamethrin (DTM, AMX®, PHARMAQ) is used to treat infections by parasitic salmon lice (*Lepeophtheirus salmonis*). However, the use of DTM for salmon delousing is threatened by resistance development. In insects, pyrethroid resistance typically involves mutations of the compound's molecular target site or increased detoxification by enhanced expression of biotransformation enzymes, such as carboxylesterases (CaEs). CaEs possessing a catalytic triad can hydrolyse the central ester bond of pyrethroids and thus, promote its degradation. The aim of this study is to obtain insights into *L. salmonis* genes potentially involved in DTM hydrolysis. CaEs are identified in sequence databases and phylogenetic relationships are established.

Methodology: *L. salmonis* CaEs homologues were identified by homology searches in a *L. salmonis* transcriptome assembly (EBI ENA reference ERS237607) and genome assembly LSalAtl2s (metazoan.ensembl.org), using the entire complement of *Drosophila melanogaster* CaEs as queries. Phylogenetic analyses of *L. salmonis* CaEs sequences retrieved by this strategy took into account CaEs of *D. melanogaster* and *Apis mellifera*. Protein sequences were aligned using the programme Clustal Omega and subjected to phylogenetic analysis using the RAxML package.

Results and Conclusion: The *D. melanogaster* and *A. mellifera* genomes contain 35 and 24 CaEs, organised in 11 and 9 clades, respectively, which assign to three major classes. Most CaEs associated with metabolic resistance to pesticide belong to the first class. In *L. salmonis*, 27 CaEs were identified, of which 17 CaEs occur in five different clades in two classes. No sequences could be assigned to the first class, containing intracellular enzymes with detoxification functions. *L. salmonis* has 16 members of the third neuro/ developmental class, including two putative achetylcholinesterases, 12 putative neuroligins, and one putative gliotactin and neurotactin. Thus, *L. salmonis* has twice as many neuroligins as *D. melanogaster* and *A. mellifera*. Neuroligins are postsynaptic cell adhesion proteins involved in formation and specification of synapses. *L. salmonis* has only one member of the second secreted catalytic class in a small clade, which contains integument esterases. Ten identified *L. salmonis* CaEs could not be assigned to clades, which are present in insects. Their function is yet to be clarified.

Keywords: sea lice, deltamethrin, esterase, metabolic resistance

Vaccinology

307-P*

Immune response of tilapia (*Oreochromis niloticus*), injected with autovaccine and challenged with *Streptococcus agalactiae*, regulation of immune-related genes expression

Ben Hamed, Said (Brazil)[1]; Tapia-Paniagua, Silvana (Spain)[2]; Ángel Moriñigo, Miguel (Spain)[2]; Tachibana, Leonardo (Brazil)[1]; Massatoshi Ishikawa, Carlos (Brazil)[1]; Carla Dias, Danielle (Brazil)[1]; Ranzani-Paiva, Maria José (Brazil)[1]

1 - Fisheries Institute; 2 - Málaga University

Introduction: Streptococcosis, caused by *Streptococcus agalactiae*, is one of the most important bacterial diseases affecting freshwater fish species in aquaculture around the world. Resolving this emergent disease problem with antibiotic led to environmental contamination and disturbance affecting the consumer‘s health and the outbreak of pathogen resistant to antibiotic. In this study, we have evaluated the protective efficacies of inactivated vaccine and analyzed the regulation of genes expression related to immune response in tilapia.

Methodology: Three groups of tilapia (n = 50) healthy and weighed 30 ± 3.0 g averagely were reared in a controlled system. The fish were vaccinated with prime-injection and after 15 days a booster-injection were performed both with 0.1 mL of inactivated *S. agalactiae* (10^9 UFC/ mL). Relative percentage of survival (RPS), during two weeks post vaccination (dpv), was evaluated after challenge with virulent *S. agalactiae* (10^6 UFC/ mL). Spleen of control and vaccinated tilapia was sampled one, two and three weeks post-challenge. Gene expression feature of GADPH, EF1α, Ilβ, TNFα, C8β and HSP70 was analyzed.

Results: It was showed that the RPS of vaccinated tilapia, three weeks post challenge, were significantly high and reach 96.6%. Control fish (unvaccinated and challenged) presented several clinical signs of streptococcosis disease essentially loss of appetite, spine displacement, hemorrhages in the eye, corneal opacity, hemorrhages at the base of the fins and in the opercula, uni or bi-lateral exophthalmia and erratic swimming. For gene expression in control group, all the studied genes were greatly up-regulated upon challenge test compared to pre challenge status. Likewise, all the genes analyzed (except HSP70) were similarly modified upon infection in vaccinated group. Interestingly the up-regulation noted was progressively increasing a long-time. For the genes HSP70, we noted a down-regulation that start two weeks after challenge.

Conclusion: Comparison of RPS and analysis of modulation of immune-related genes for control and vaccinated fish demonstrated that the developed vaccine improves the immunity and survival of Nile-tilapia upon infection with *Streptococcus agalactiae.*

Keywords: inactivated vaccine, streptococcosis, immunity, bacterial disease

Funding: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), São Paulo, Brasil, Processes number: 2016/19816-9, 2017/05183-7 and 2018/06012-4.

308-P

Biomarkers of mucosal vaccination and challenge with *Flavobacterium psychrophilum* by proteomic profiling of skin mucus from rainbow trout (*Onchorynchus mykiss*)

Hoare, Rowena (United Kingdom)[1]; Shahin, Khalid (United States of America)[2]; Mclean, Kevin (United Kingdom)[3]; Thompson, Kimberley (United Kingdom)[3]; Adams, Alexandra (United Kingdom)[1]

1 - University of Stirling; 2 - University of California Davis; 3 - Moredun Institute

Introduction: *Flavobacterium psychrophilum* is one of the most important pathogens affecting cultured rainbow trout and affects fry when they are too small to inject. We developed a mucosal vaccine for *F. psychrophilum*, which provided a high level of protection to trout fry against challenge by immersion (RPS 84%).

Methodology: As mucus is the first barrier of the fish, playing a vital role in protection against pathogens, we examined the protein profiles of skin mucus of rainbow trout following immersion vaccination and immersion challenge with *F. psychrophilum*. Skin mucus was subjected to 2D-SDS-PAGE and spots differentially expressed between vaccinated and control fish analysed by mass spectrophotometry (MALDI-TOF). The influence of route of infection on mucus proteins was also investigated by comparing skin mucus collected following intra-muscular challenge with *F. psychrophilum* with mucus collected following immersion challenge with *F. psychrophilum*.

Results: Immersion vaccination resulted in an increased expression of annexin-like proteins (Annexin A1 and A5) in the skin mucus of trout. Annexin A1 belongs to the annexin family of Ca2+-dependent phospholipid-binding proteins with phospholipase A2 inhibitory activity in mammals. The main mechanism of glucocorticoids' anti-inflammatory effects is to increase the synthesis and function of annexin A1. Annexin A5 has been proposed to play a role in the inhibition of blood coagulation by competing for phosphatidylserine binding sites with prothrombin and also to inhibit the activity of phospholipase A1. The results also show that route of challenge has a significant impact on the protein profiles of skin mucus with beta-actin occurring only in fish challenged by immersion. Beta-actin is one of the two non-muscle cytoskeletal actins. Actins are highly conserved proteins that have roles in cell motility, cytoplasmic streaming, phagocytosis and cytokinesis. Levels of actin in skin mucus have also been associated with sea lice infection. The occurrence of intracellular proteins in the skin mucus may result from either direct expression by epidermal cells, leakage of plasma or via a secondary circulation system.

Conclusion: These results contribute to our knowledge of mucosal immune reactions in rainbow trout following infection and vaccination and provide potential biomarkers for successful mucosal vaccination.

Keywords: mucosal vaccination, *Flavobacterium psychrophilum*, proteomics, mucus

Funding: EU Fp7 TargetFish.

309-P

Evaluation of outer membrane vesicles as antigen delivery-platforms for fish

Coutinho, Filipe (Portugal)[1]; Lima, Steeve (Portugal)[3,4,5]; Oliva-Teles, Aires (Portugal)[1,2]; Oliveira, Paulo (Portugal)[3,4]; Serra, Claudia (Portugal)[1]

1 - Centro Interdisciplinar de Investigação Marinha e Ambiental; 2 - Departamento de Biologia, Faculdade de Ciências, Universidade do Porto; 3 - i3S – Instituto de Investigação e Inovação em Saúde; 4 - Instituto de Biologia Molecular e Celular; 5 - Instituto de Ciências Biomédicas Abel Salazar

Introduction: *Mycobacterium marinum* is the causative agent of mycobacteriosis, a fish bacterial disease characterized by granulomatous inflammation in multiple organs, with a mortality rate that can range between 30 - 100%. Mycobacteriosis outbreaks have important effects on commercial fish production, in particular that of European sea bass (*Dicentrarchus labrax*), a fish species of high economic interest. Mycobacteriosis is also an important infectious disease in zebrafish (*Danio rerio*), associated with severe losses in research facilities. Because no satisfactory vaccine or treatment is available, once a population of fish is infected, the most likely scenario is euthanasia of the entire group. Thus, it is imperative to develop an efficient strategy to prevent mycobacteriosis infections. One possibility is the use of bacterial outer membrane vesicles (OMVs), which are spherical bilayered structures naturally liberated from the outer membrane of Gram-negative bacteria that have been progressively used as carriers of immunogenic antigens. An example is the commercially-available OMV-based vaccine Bexsero® against *Neisseria meningitidis* serogroup-B15 in humans.

Methodology: Here, we explore tolerance to various types of OMVs derived from non-pathogenic and environmentally friendly bacteria (cyanobacteria) in zebrafish embryos (3 dpf), by monitoring embryos viability for 5 days. OMV ranged between 50 and 500 µg LPS/ml. Commercial *Pseudomonas aeruginosa* LPS (Sigma) was used in the same concentrations, as positive control, while PBS (solvent of OMVs and LPS) was used as negative control. At days 1 and 5, total RNA was extracted from pools of 5 embryos of each replicate (n=3) of all experimental conditions, for analysis of transcript levels of *tnf1α*, *il1β*, *il6,defbl1* and *defbl2* by qPCR, using established oligonucleotides.

Results: There was no significant differences on zebrafish embryos survival or LPS-induced inflammatory responses (inferred by gene-expression analysis of tumor necrosis factor *tnf1α*, interleukins *il1β*, *il6* and β-defensins *defbl1* and *defbl2*) when exposed to all OMVs tested and at the doses tested, when compared to the negative control.

Conclusion: Cyanobacterial OMVs are good candidates to be used as carriers of immunogenic antigens in zebrafish. Future work will engineer OMVs with heterologous *M. marinum* antigens, and evaluated these as delivery vehicles to immunize zebrafish and European sea bass against *M. marinum*.

Keywords: *Mycobacterium marinum*, outer membrane vesicles, antigen-delivery

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310-P*

Transcriptomic analysis of the headkidney provides insights into the immune response to scuticociliate vaccination in *Paralichthys olivaceus*

Yang, Hyerim (Republic of Korea)[1,2]; Kwon, Hyukjae (Republic of Korea)[1,2]; Kim, Gaeun (Republic of Korea)[1,2]; Lee, Seungdo (Republic of Korea)[1,2]; Wan, Qiang (Republic of Korea)[1,2]; Lee, Jehee (Republic of Korea)[1,2]

1 - Fish Vaccine Research Center, Jeju National University, Republic of Korea; 2 - Department of Marine Life Sciences, Jeju National University, Republic of Korea

Introduction: Olive flounder (*Paralichthys olivaceus*) is one of the most economically important aquatic species. However, during its production, this organism encounters various diseases. *M. avidus* is one of the scuticociliate which cause crucial a disease of farmed flounder. To develop effective scuticociliate vaccines, it is important to understand the molecular mechanisms underlying the host's protective response after vaccination. The purpose of this study is to investigate which genes are involved in the immune response of olive flounder after scuticociliate vaccination.

Methodology: We injected olive flounder with formalin-killed *M. avidus* vaccine. To evaluate the vaccination-induced changes in the expression profiles of genes, the head kidneys were collected from control and vaccinated fish at 9, 48, and 168 hours after vaccination and gene expression profiling was performed by RNA sequencing.

Results: In comparison with the control, vaccine group showed more than 2,000 DEGs at 9 and 48 hours post-injection (hpi). Among them, several genes were clustered into immune-related pathways, including immunoglobulin production, complement, and coagulation cascades pathways.

Conclusion: In this study, we acquired transcriptome profile data for immune response after scuticociliate vaccination. These data insights into molecular mechanisms in olive flounder after scuticociliate vaccination might facilitate development of highly immunogenic vaccines.

Keywords: transcriptomic analysis, scuticociliate vaccination, *M. avidus*, olive flounder

311-P

Development of yersiniosis vaccines for Atlantic salmon

Gausdal Tingbø, Monica (Norway)[1]; Sandtrø, Ane (Norway)[2]; Nilsen Fredriksen, Børge (Norway)[1]; Hanche-Olsen, Ragnhild (Norway)[1]

1 - Pharmaq; 2 - Pharmaq/ Zoetis

Introduction: Yersiniosis was traditionally a bacterial disease affecting salmon fry in fresh water phase. However, during recent years, the Norwegian aquaculture industry has experienced increasing numbers of yersinosis outbreaks in farmed Atlantic salmon after sea transfer and even into the second year in sea. Massive and acute mortalities have been registered in certain cages. The disease occurring at such a late stage in the production cycle causes huge economic losses in addition to the obvious animal welfare challenges.

Methodology: Bacterial samples were analysed using Western blot and slide agglutination. Clinical studies were performed using Atlantic salmon of different sizes and different administration methods.

Results and Conclusion: Our data show that Salmon isolates previously serotyped to O1, more specifically belong to subgroup O1b and that there are obvious differences in O-antigen structures between O1a and O1b sub-serotypes. Results from clinical studies, demonstrating the effect fish size on vaccine efficacy during dip administration and dose-response results for water-based as well as oil-adjuvanted vaccine systems for injection will be presented. We conclude that there are differences in the immune response to yersiniosis vaccination between salmon and trout. There are also structural differences between the sub-serotypes that cause ERM/ yersiniosis in these species.

Keywords: yersiniosis, salmon, vaccines

312-P

Clinical documentation of a live attenuated vaccine against *Piscirickettsia salmonis*

Olsen, Rolf Hetlelid (Norway)[1]; Finne-Fridell, Frode (Norway)[1]; Bordevik, Marianne (Norway)[1]; Norderhus, Edel Anne (Norway)[1]

1 - PHARMAQ part of Zoetis

Introduction: The majority of commercially available vaccines for the aquaculture industry are adjuvanted, oil based formulations containing inactivated antigens. Introduction of effective vaccines have in general resulted in significant improvements of fish health and welfare the last 30 years, resulting in a tremendous reduction in the use of antibiotics. Salmonid Rickettsial Septicemia (SRS) caused by the intracellular bacterium *Piscirickettsia salmonis* historically resulted in high mortalities and in high use of antibiotics in Chile. In an attempt to improve this situation, PHARMAQ has developed a live attenuated vaccine against the disease. Several vaccine candidates were examined in controlled laboratory studies, before extensive documentation studies were performed on the chosen candidate based upon European and Chilean regulatory requirements.

Methodology: Clinical documentation of safety was performed in series of clinical trials including tissue-tropism studies, reversion to virulence studies, investigation of potential horizontal transmission of the live vaccine strain to target animal species and to marine species and sea lice, in addition to examination of local reaction and growth. Clinical documentation of efficacy was performed with validated challenge models at different time-points after vaccination.

Results: A summary of the clinical documentation of this live attenuated vaccine will be presented with emphasis on differences related to classical inactivated vaccines. The vaccine has now been approved for commercial use in Chile under the product name ALPHA JECT LiVac SRS.

Conclusion: A live attenuated vaccine against *Piscirickettsia salmoni*s developed by PHARMAQ AS is proven to be both safe and effective. The effect is proven to last up to 13 months in laboratory studies.

Keywords: salmon, *Piscirickettsia salmonis*, live vaccine

Funding: PHARMAQ AS.

313-P*

Protection induced by vaccines against vibriosis is affected by the phylogenetic diversity of *Vibrio anguillarum* serotype O2A

Sepúlveda, Dagoberto (Denmark)[1]; Hansen, Mie Johanne (Denmark)[1]; Dalsgaard, Inger (Denmark)[1]; Skov, Jakob (Denmark)[1]; Lorenzen, Niels (Denmark)[1]

1 - National Institute of Aquatic Resources, Technical University of Denmark

Introduction: Vibriosis outbreaks in rainbow trout in Danish aquaculture are caused mainly by *V. anguillarum* serotypes O1 and O2A. Therefore, isolates from these serotypes are part of the formulation of the commercial vaccine used in Denmark. However, it is important to consider that, on the contrary to the serotype O1, there is a high phylogenetic diversity among strains belonging to serotype O2A, which could potentially reduce the protection induced by a vaccine depending on the infecting isolate. This work aimed to study the variability of the protection induced by both a commercial vaccine and an experimental vaccine against vibriosis caused by different *V. anguillarum* O2A isolates.

Methodology: Specific reactivity of sera samples of fish vaccinated with either the commercial vaccine or the experimental vaccine were analyzed by ELISA against nine isolates of *V. anguillarum* serotype O2A. Phylogenetic analysis was performed with the same isolates to determine any correlation between the genetic variability of *V. anguillarum O2A* and the specific reactivity test.

Results: The study showed that four isolates were only recognized by the sera of fish vaccinated with the experimental vaccine (group A), and five isolates were only recognized by the sera of fish vaccinated with the commercial vaccine (group B). One isolate from each group was selected to perform an *in vivo* trial. A correlation between the serology test and the protection *in vivo* was observed. When the challenge was performed with the isolate from group A, the protection induced by the experimental vaccine was higher than that of the commercial vaccine. Opposite results were obtained when the challenge was performed with the isolate from group B.

Conclusion: Both phylogenetic studies and pan-genome calculations have revealed that *V. anguillarum* serotype O2A strains represent a very diverse group. As the results show, it is necessary to consider this diversity to create a vaccine that provides sufficient protection against vibriosis.

Keywords: *Vibrio anguillarum*, vaccine, rainbow trout

Funding: VAXFISK- GUDP.

314-P*

Mucosal nanoparticle immunisation against salmon alphavirus (SAV) in Atlantic salmon (*Salmo salar* L.)

Mckillen, John (United Kingdom)[1]; Savage, Paul (United Kingdom)[1]; Maxwell, Keith (United Kingdom)[1]; Welsh, Michael (United Kingdom)[2]; Gray, Darren (United Kingdom)[3]; Mooney, Mark (United Kingdom)[3]; Monaghan, Sean (United Kingdom)[4]; Yoon, Sohye (United Kingdom)[5]; Herath, Tharangani (United Kingdom)[6]; Secombes, Chris (United Kingdom)[5]; Adams, Sandra (United Kingdom)[4]; Allan, Gordon (United Kingdom)[3]

1 - Agri-Food and Biosciences Institute, Belfast, UK; 2 - Sisaf Ltd., Innovation Centre, Queen's Road, Belfast, UK; 3 - Institute for Global Food Security, Queen's University Belfast, Belfast, UK; 4 - Institute of Aquaculture, University of Stirling, Scotland, UK; 5 - School of Biological and Environmental Sciences, University of Aberdeen, Aberdeen, UK; 6 - Department of Animal Production, Welfare and Veterinary Sciences, Harper Adams University, Shropshire, UK

Introduction: Salmon alphavirus (SAV) causes pancreas disease (PD) in Atlantic salmon (*Salmo salar* L.) resulting in sudden inappetence and lethargy characterised by acute necrosis of exocrine pancreatic tissue and chronic myositis. Commercialised vaccines are available for PD based on inactivated SAV and have effectively reduced mortality, histopathological damage and improved growth rates in salmon compared to non-vaccinated fish. These vaccines are administered by intraperitoneal injection (IP) which is stressful to fish and sometimes causes side effects. Oral vaccination alleviates handling-stress and can provoke good mucosal immune responses. We have recently demonstrated strong immune responses following oral vaccination of nanoparticle (NP) bacterins in salmonids and here we investigated the potential of this antigen vehicle for vaccination against PD.

Methodology: Different vaccine administration routes were investigated using a commercial inactivated PD vaccine and novel NP formulated vaccine. The study included 6 groups of Atlantic salmon (n = 20 - 27) that received PD vaccination by different regimes: (1) single IP vaccination; (2) IP placebo followed by IP boost; (3) IP prime followed by NP boost; (4) NP prime followed by NP boost; (5) empty NP placebo, and (6) non-vaccinated control group. Fish were sampled at different time points post -prime, -boost and -challenge with SAV. qPCR and differential in-gel elecrophoresis (DIGE) was also used to determine immune response in the head kidney, spleen, pyloric caecae and hind gut. SAV viral load and histopathology was assessed from the heart and pancreas.

Results: Fewer SAV positive fish and lower SAV loads were detected in the groups receiving IP prime only and IP prime with NP boost. IP prime NP boost fish had significantly elevated IFN-γ and IL-6 by qPCR in the hind gut, and cathepsin M levels in the serum by DIGE compared to all other groups. IP prime NP boost fish also exhibited significantly lower pathology scores in the pancreas and heart.

Conclusion: Oral boost vaccination of nanoparticle incorporated SAV antigens may improve protection conveyed by conventional IP administration. Upregulation of Th1-like transcripts (e.g. IL-6 and IFN-γ) and serum cathepsin M may be associated with this protection.

Keywords: SAV, salmon alphavirus, nanoparticle vaccine, oral vaccination, booster vaccination

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Environmental and Toxicological Diseases

315-P

Environmental concentration of methamphetamine affects brown trout (*Salmo trutta m. fario*)

Sancho Santos, Maria Eugenia (Czech Republic)[1]; Grabicová, Kateřina (Czech Republic)[1]; Steinbach, Christoph (Czech Republic)[1]; Schmidt-Posthaus, Heike (Switzerland)[2]; Kolářová, Jitka (Czech Republic)[1]; Vojs Staňová, Andrea (Czech Republic)[1]; Grabic, Roman (Czech Republic)[1]; Randák, Tomáš (Czech Republic)[1]

1 - University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Vodňany, Czech Republic; 2 - Centre for Fish and Wildlife Health, Department for Infectious Diseases and Pathobiology, University of Bern, Bern, Switzerland

Introduction: Methamphetamine (METH) has been detected in water in concentrations ranging from nanograms to micrograms per liter due to inadequate removal in sewage treatment plants. METH as well as other psychoactive compounds, are suspected to produce effects in multiple aquatic organisms due to the evolutionary conserved drug targets.

Methodology: Brown trout were exposed at environmental (1 µg/L) and high (50 µg/L) concentrations of METH under laboratory conditions for 35 days, following a 4 day depuration phase. Following this test period, ventilation rate was recorded along with liver and heart histological analysis. Concentration of METH and its metabolite amphetamine (AMPH) was analyzed in plasma, muscle, brain, kidney and liver at the beginning of the experiment and after 4, 12, 20, 28, 35 days of exposure and after depuration phase.

Results: Ventilation rate was significantly increased in high concentration compared to control and environmental group. After depuration phase, no differences were found among groups. Histological analyses revealed changes in heart and liver at environmental and high concentration, but in different patterns and intensity of appearance. The main change in liver was cytoplasmatic vacuolation of hepatocytes; and microvascular injuries, and infiltration and fibrosis in heart. METH and AMPH were found in all the tissues and times, the concentration of the metabolite being higher than the parent compound. Bioconcentration levels followed the order kidney > liver > brain > muscle > plasma. AMPH remained in tissues even after depuration phase, but the concentration had tendency to decrease.

Conclusion: Histological effects in heart and liver were observed even at environmental concentration, and changes were similar to those observed previously in studies with mammals. Parent compound and metabolite can bioconcentrate in all fish tissues. Brown trout showed a tendency of adaptation under METH exposure due to chemical and histological findings. This study showed METH impacting fish at low (environmental) levels, indicating the possible detrimental effect in the aquatic environment.

Keywords: breathing rate, drug, histology, bioconcentration, perviti

Funding: The Czech Science Foundation (project No. 16-06498S) and the Grant Agency of the University of South Bohemia in Ceske Budejovice (No. 102/2019/Z).

316-P

Nephrocalcinosis in rainbow trout reared in recirculation aquaculture system – a case study

Papezikova, Ivana (Czech Republic)[1,2]; Palikova, Miroslava (Czech Republic)[1,2]; Minarova, Hana (Czech Republic)[3]; Syrova, Eva (Czech Republic)[1]; Mares, Jan (Czech Republic)[2]

1 - University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic; 2 - Mendel University in Brno, Czech Republic; 3 - Veterinary Research Institute

Introduction: In spring 2018, long-term low growth rates and sporadic mortalities were observed in rainbow trout reared in indoor recirculation aquaculture system. Similar symptoms were observed in all fish stocks independently of their age and origin.

Methodology: Blood samples were taken for hematological and biochemical examination and the sampled fish were killed and dissected. Based on pathoanatomical findings, kidney and gill samples were taken for histological examination and kidney smears were made to exclude bacterial kidney diseae (BKD). Water chemistry analysis was performed and the results were compared with results obtained at reference farm.

Results: The main pathological findings included enlarged body cavity, pale patches on gills, swollen kidneys and ureters and enlarged Stannius bodies. Hematological and biochemical blood examination revealed anaemia (decreased hemoglobin and erythrocyte counts) and compensated alkalosis. Histopathological examination of kidneys shown congestion, pigment deposits and crystal-like basophilic aggregates. Special Von Kossa staining of kidney sections proved the presence of large tubular calcium deposits which are pathognomic for nephrocalcinosis. Water chemistry analysis revealed significantly higher CO_2 level than at reference farm (10.68 mg/l vs. 1.05 mg/l).

Conclusion: Pathogenesis of nephrocalcinosis in fish is not fully understood. It was reported that nephrocalcinosis in intensive aquaculture is caused by a complex of environmental conditions with long-term increased CO_2 level in water as one of the most important factors. Lowering water CO_2 levels by pH adjustment and by reduction of stocking density is one of the measures recommended to manage the disease.

Keywords: nephrocalcinosis, CO_2, kidney, rainbow trout

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317-P

Transgenerational impact of glyphosate on the behavior and biometric parameters of juvenile rainbow trout *Oncorhynchus mykis*

Saliou, Florian (France)[1]; Le Du-Carree, Jessy (France)[1]; Weeks, Shannon (France)[2]; Cachot, Jérôme (France)[2]; Dory, Daniel (France)[3]; Danion, Morgane (France)[1]; Morin, Thierry (France)[1]

1 - French Agency for Food, Environmental and Occupational Health & Safety (Anses), Ploufragan-Plouzané-Niort Laboratory, Unit Viral Diseases of Fish, National Reference Laboratory (NRL) for listed fish diseases, Bretagne Loire University, Plouzané, France; 2 - Laboratory EPOC UMR CNRS 5805, University of Bordeaux, Talence, France; 3 - Anses, Ploufragan-Plouzané-Niort Laboratory, Unit Viral Genetic and Biosecurity, Ploufragan, France

Introduction: Glyphosate is an Active Substance (AS) known for its herbicidal properties. Commercial formulations available on the market associate this AS with adjuvants in products defined as "glyphosate based herbicide (GBH)". The massive use of GBH these past years made of glyphosate one of the most commonly chemical compounds found in aquatic environments. Several studies have shown deleterious effects in aquatic organisms following exposure to glyphosate. In the context of the renewal of its authorization in European Union, toxicity associated to some adjuvants is also a subject of debate.

Methodology and Results: As part of a global and multiparametric study, this work aims to bring new data about the effect of a transgenerational exposure with glyphosate on rainbow trout (RT) juveniles derived from genitors chronically contaminated. Adult RT were daily exposed during 8 months to a dose of glyphosate representative of environmental concentrations (around 1 µg/L) using AS alone or two GBH (i.e. Roundup Innovert® and Viaglif Jardin®). In November 2018, these genitors gave birth to an Offspring (F1-2018). At the post-larval stage, the swimming activity (measurement of distance traveled, mobility and speed), biometric characteristics and morphological malformations (poor yolk sac resorption and craniofacial deformities) of these fish were estimated.

Conclusion: The first results obtained suggest that GBH at an environmental concentration could have an effect on larval development by inducing an increase in the frequency of malformations and a change in swimming behavior. These first results, which must be confirmed by analyzing the totality of the data collected, will be supplemented by other observations on the capacity of resistance to diseases, the evolution of certain blood parameters or the activity of the antioxidant defenses.

Keywords: glyphosate, rainbow trout, transgenerational impact, swimming activity, malformations

Funding: Région Bretagne, Département des Cotes d'Armor, Saint Brieuc Armor Agglomération.

318-P

Variation in lipid content and fatty acid composition in baltic flounder as an indicator of the health status (preliminary results)

Góra, Agnieszka (Poland)[1]; Szlinder-Richert, Joanna (Poland)[1]

1 - National Marine Fisheries Research Institute, Gdynia, Poland

Introduction: Lipids are the main source of metabolic energy and fatty acids that play an important physiological role in fish health, development, growth and reproduction. Individuals with larger reserves of nutrients may have a better chance of survival and higher reproductive success. A relation between condition, fertility and fatty acids composition was observed in sprat and herring from the Baltic Sea. The decline of the fish condition was related to an increase in the amount of fatty acids 20:4 (n-6) (ARA) and 22:6 (n-3) (DHA). In addition, impact of the environmental pollution on fatty acids metabolism in aquatic invertebrates has been indicated. For this reason, in this study an assessment of lipid content and fatty acids composition of flounder (*Platichthys flesus*) from the Puck Bay, an area exposed to high anthropogenic pressure, and from a reference site was carried out.

Methodology: Flounders caught in April 2018, were subjected to basic ichthyologic analysis, age and digestive tract content evaluation and determination of lipid and fatty acids content in muscle tissue, livers and gonads. The relationship between lipid and fatty acids content and biometric indices reflecting the nutritional status (condition index-CF, hepato-somatic index-HSI) and gonad development (gonado-somatic index-GSI) were investigated.

Results: In case of females in the late resting ovary phases, no statistically significant differences in biometric indices, lipid content and fatty acid amounts in muscles and livers were found while comparing individuals from the Puck Bay and from a reference site. However, in relation to females in spent ovary phases differences were observed in terms of fat content and fatty acids pattern. Females from reference area indicated higher liver fat content. In addition, higher levels of ARA and DHA were noted in all the examined fish tissues sampled in the area subjected to high anthropogenic pressure. Differences detected during the study might be the result of a diet with different composition or can be caused by adverse environmental impact.

Conclusion: Our results indicate that fatty acids patterns can be considered a unique proxies for the health status of marine organisms.

Keywords: fatty acids, flounder, Baltic Sea, fish condition, pollution

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AUTHOR CONTACT DETAILS

Paper Reference	Submitting Author	Email Address
	Oral Presentations	
001-O	Nowak, Barbara	b.nowak@utas.edu.au
002-O	Mahony, Kate E.	katemahony@ucc.ie
003-O	Kett, Gary	gary.kett@ucc.ie
004-O	Arapi, Elissavet	arapie@cardiff.ac.uk
005-O	Park, Youngjin	park.youngjin@nord.no
006-O	Pietsch, Constanze	constanze.pietsch@zhaw.ch
007-O	Silayeva, Oleksandra	o.silayeva@uq.edu.au
008-O	Graf, Joerg	joerg.graf@uconn.edu
009-O	Moreira, Márcio	marcio.moreira@ipma.pt
010-O	Monaghan, Sean	sjm27@stir.ac.uk
011-O	Pavlinec, Željko	pavlinec@veinst.hr
012-O	Strem, Rosa Ines	rosastrem@yahoo.com
013-O	Frisch, Kathleen	kathleen.frisch@cermaq.com
015-O	Poynton, Sarah	spoynton@jhmi.edu
016-O	Bott, Nathan	nathan.bott@rmit.edu.au
017-O	Botwright, Natasha	natasha.botwright@csiro.au
018-O	Blindheim, Steffen H	steffen.blindheim@ilab.no
019-O	Botwright, Natasha	natasha.botwright@csiro.au
020-O	Gorgoglione, Bartolomeo	bartgorg@msu.edu
021-O	Adamek, Mikolaj	mikolaj.adamek@tiho-hannover.de
022-O	Olesen, Niels Jørgen	niven@aqua.dtu.dk
023-O	Cano, Irene	irene.canocejas@cefas.co.uk
024-O	Soto, Esteban	sotomartinez@ucdavis.edu
025-O	Beck, Benjamin	benjamin.beck@ars.usda.gov
026-O	Bang Småge, Sverre	sverre.smage@cermaq.com
027-O	Jones, Simon	simon.jones@dfo-mpo.gc.ca
028-O	Suarez, Rudy	rudy.suarez@ciba.cl
029-O	Brosnahan, Cara	cara.brosnahan@mpi.govt.nz
031-O	Castrillo, Pedro Antonio	pedroantonio.castrillo@usc.es
032-O	Hansen, Haakon	haakon.hansen@vetinst.no
034-O	Pavanelli, Gilberto	pavanelli@nupelia.uem.br
035-O	Sándor, Diána	sandor.diana.elte@gmail.com
038-O	Bang Jensen, Britt	britt-bang.jensen@vetinst.no
039-O	Bernhardt, Lisa-Victoria	lisa-victoria.bernhardt@vetinst.no
040-O	Markussen, Turhan	turhan.markussen@nmbu.no
041-O	Petersen, Petra E.	pep@hfs.fo

043-O	Christiansen, Debes H.	debesc@hfs.fo
044-O	Bang Jensen, Britt	britt-bang.jensen@vetinst.no
045-O	Einarsdottir, Thorbjorg	thorbje@hi.is
047-O	Alonso, M. Carmen	mdalonso@uma.es
048-O	Vázquez-Salgado, Lucía	l.vazquez.salgado@usc.es
049-O	Volpe, Enrico	enrico.volpe2@unibo.it
050-O	Toffan, Anna	atoffan@izsvenezie.it
051-O	Gallagher, Michael	m.gallagher-10@sms.ed.ac.uk
052-O	Aksnes, Ida	idaak@nmbu.no
055-O	Ní Dhufaigh, Kerrie	kerrie.nidhufaigh@research.gmit.ie
056-O	Cano, Irene	irene.canocejas@cefas.co.uk
057-O	Watanabe, Yuho	y.aqua.w@gmail.com
058-O	Sitjà Bobadilla, Ariadna	ariadna.sitja@csic.es
059-O	Muñoz, Pilar	pilarmun@um.es
060-O	Mladineo, Ivona	mladineo@izor.hr
061-O	Le Bouquin, Sophie	sophie.lebouquin-leneveu@anses.fr
062-O	James, Joanna	jo.james@environment-agency.gov.uk
063-O	Karlsbakk, Egil	egil.karlsbakk@uib.no
064-O	Okamura, Beth	b.okamura@nhm.ac.uk
065-O	Hansen, Haakon	haakon.hansen@vetinst.no
066-O	Godoy, Marcos	marcos.godoy@uss.cl
067-O	Malik, Muhammad Salman	muma@nmbu.no
068-O	Mohr, Peter	peter.mohr@csiro.au
069-O	Vakharia, Vikram	vakharia@umbc.edu
070-O	Panzarin, Valentina	vpanzarin@izsvenezie.it
071-O	Abbadi, Miriam	mabbadi@izsvenezie.it
072-O	Rice, Annabel	annabel.rice@cefas.co.uk
073-O	Øvergård, Aina-Cathrine	aina-cathrine.overgard@uib.no
074-O	Ambuali, Aisha	hala@squ.edu.om
075-O	Mladineo, Ivona	mladineo@izor.hr
077-O	Lages, Marta A.	marta0lages@gmail.com
078-O	Adamek, Mikolaj	mikolaj.adamek@tiho-hannover.de
079-O	Noguera, Patricia	patrusita@gmail.com
080-O	Godoy, Marcos	marcos.godoy@uss.cl
081-O	Constenla, Maria	maria.constenla@uab.cat
082-O	Nowak, Barbara	b.nowak@utas.edu.au
083-O	Schmidt, Jacob G.	jacsc@aqua.dtu.dk
084-O	Lewisch, Eva	eva.lewisch@vetmeduni.ac.at
085-O	Morin, Thierry	thierry.morin@anses.fr
086-O	Jordan, Lisa Katharina	lisa.jordan@fau.de

087-O	Delrez, Natacha	natacha.delrez@uliege.be
089-O	Siwicki, Andrzej	siwicki@uwm.edu.pl
090-O	Lieke, Thora	lieke@igb-berlin.de
091-O	Midtlyg, Paul J.	paul.midtlyng@nmbu.no
092-O	Goncalves, Ana Teresa	ateresagoncalves@gmail.com
093-O	Smith, Peter	peter.smith@nuigalway.ie
094-O	Baron, Sandrine	sandrine.baron@anses.fr
096-O	Baron, Sandrine	sandrine.baron@anses.fr
097-O	Buba, Elizabeth	elizabeth.buba1@stir.ac.uk
098-O	Desbois, Andrew	ad54@stir.ac.uk
099-O	Menanteau-Ledouble, Simon	menanteaus@staff.vetmeduni.ac.at
100-O	Saticioglu, Izzet B.	iburcinsat@gmail.com
104-O	Bass, David	david.bass@cefas.co.uk
105-O	Bailey, Christyn	christyn.bailey@vetsuisse.unibe.ch
107-O	Haenen, Olga	olga.haenen@wur.nl
108-O	Adamek, Mikolaj	mikolaj.adamek@tiho-hannover.de
109-O	Nadanasabesan, Nimalan	nimalan.nadanasabesan@nord.no
111-O	Xue, Qinggang	qxue@zwu.edu.cn
112-O	Lee, Sukkyoung	lskshiny@hotmail.com
114-O	Peña, Andrea	andrea.pena@pathovet.cl
116-O	Rodger, Hamish	hamishrodger@eircom.net
117-O	De Carvalho Soares, Silvia	silvia.soares@gov.scot
119-O	McLoughlin, Marian	marian.mcloughlin@fishvetgroup.com
120-O	Gagné, Nellie	nellie.gagne@dfo-mpo.gc.ca
121-O	Testerman, Todd	todd.testerman@uconn.edu
122-O	Kleingeld, Dirk Willem	dirk.kleingeld@laves.niedersachsen.de
123-O	Vercauteren, Maaike	maaike.vercauteren@ugent.be
124-O	Jung-Schroers, Verena	verena.jung-schroers@tiho-hannover.de
126-O	Knopf, Klaus	klaus.knopf@igb-berlin.de
127-O	Jung-Schroers, Verena	verena.jung-schroers@tiho-hannover.de
128-O	Holt, Corey C.	corey.holt@cefas.co.uk
129-O	Peña, Andrea	andrea.pena@pathovet.cl
130-O	Soleto, Irene	soleto.irene@inia.es
131-O	Garcia Cabrera, Jose Antonio	yosoyloren10@hotmail.com
132-O	Martín-Martín, Alba	alba.martin@inia.es
135-O	Shirato, Makoto	mako27.com@gmail.com
136-O	Morin, Thierry	thierry.morin@anses.fr
137-O	Bermúdez, Roberto	roberto.bermudez@usc.es
139-O	Soto, Esteban	sotomartinez@ucdavis.edu
140-O	Zupičić, Ivana Giovanna	zupicic@veinst.hr

141-O	Bigarré, Laurent	laurent.bigarre@anses.fr
143-O	O'Toole, Ciar	ciar.otoole@marine.ie
144-O	Cheslett, Deborah	deborah.cheslett@marine.ie
145-O	Midorikawa, Yuki	ccm.penaei@gmail.com
146-O	Davies, Charlotte	c.e.davies@swansea.ac.uk
147-O	Hooper, Chantelle	chantelle.hooper@cefas.co.uk
149-O	Colunga Ramirez, Graciela Esmeralda	gcolunga@uabc.edu.mx
150-O	Monaghan, Sean	sjm27@stir.ac.uk
151-O	Barnes, Andrew	a.barnes@uq.edu.au
152-O	Jung, Tae Sung	jungts@gnu.ac.kr
153-O	Nishiya, Kohei	brko18506@g.nihon-u.ac.jp
154-O	Steinhagen, Dieter	dieter.steinhagen@tiho-hannover.de
156-O	Dopazo, Carlos P.	carlos.pereira@usc.es
157-O	Chambers, Edel	edel.chambers@cefas.co.uk
158-O	Jansson, Eva	eva.jansson@sva.se
159-O	Guðmundsdóttir, Sigríður	siggag@hi.is
162-O	Moussa-Pouly, Mirna	mirna.moussa.pouly@ifremer.fr
163-O	Kawahara, Miku	39mirai-2869mk@g.ecc.u-tokyo.ac.jp
164-O	Kristmundsson, Árni	arnik@hi.is
165-O	Correia, Simão	simaocorreia@ua.pt
166-O	Huchon, Dorothee	huchond@tauex.tau.ac.il
167-O	Faber, Marc Nicolas	marc.faber1@abdn.ac.uk
168-O	Lisnerová, Martina	jedlickovamarti@seznam.cz
169-O	Kyslík, Jiří	jiri.kyslik@paru.cas.cz
171-O	Bass, David	david.bass@cefas.co.uk
172-O	Albuixech-Marti, Sara	saraalbuixechmarti@ucc.ie
173-O	Novoa, Beatriz	beatriznovoa@iim.csic.es
176-O	Hooper, Chantelle	chantelle.hooper@cefas.co.uk
177-O	Arzul, Isabelle	isabelle.arzul@ifremer.fr
180-P	Kunttu, Heidi Mt	heidi.kunttu@jyu.fi
181-O	Donati, Valentina Laura	valdo@aqua.dtu.dk
183-O	Serra, Cláudia R.	rafaela.santos@ciimar.up.pt
184-O	Santana Sepúlveda, Paula Andrea	paula.santana@uautonoma.cl
185-O	Holzer, Astrid	astrid.holzer@paru.cas.cz
186-O	Kosakyan, Anush	anna.kosakyan@gmail.com
187-O	Korytář, Tomáš	tkorytar@frov.jcu.cz
188-O	Bartošová-Sojková, Pavla	bartoska81@gmail.com
189-O	Hartigan, Ashlie	a.hartigan@nhm.ac.uk
190-O	Picard Sánchez, Amparo	amparo.picard@csic.es

191-O	Ronza, Paolo	paolo.ronza@usc.es
192-O	Arzul, Isabelle	lydie.canier@ifremer.fr
193-O	Arzul, Isabelle	isabelle.arzul@ifremer.fr
194-O	Delmotte, Jean	jeandelmotte0392@gmail.com
195-O	Prado-Álvarez, María	mprado@iim.csic.es
196-O	Morga, Benjamin	benjamin.morga@ifremer.fr
197-O	Costello, Katie Ellen	katiecostello@ucc.ie
198-O	Morin, Thierry	thierry.morin@anses.fr
199-O	Haugland, Gyri T.	gyri.haugland@uib.no
202-O	Novoa, Beatriz	beatriznovoa@iim.csic.es
203-O	Fernandez, Carolina	carolina.fernandez@stir.ac.uk
204-O	Taylor, Richard S.	richard.taylor@csiro.au
205-O	Sudhagar, Arun	arun.sudhagar@vetmeduni.ac.at
206-O	Schmidt-Posthaus, Heike	heike.schmidt@vetsuisse.unibe.ch
207-O	Kumar, Gokhlesh	gokhlesh.kumar@vetmeduni.ac.at
208-O	Atkinson, Stephen	atkinsos@oregonstate.edu
209-O	Bartholomew, Jerri	bartholj@science.oregonstate.edu
210-O	Alama-Bermejo, Gema	gema.alama@gmail.com
211-O	Byadgi, Omkar	omkarcof1@gmail.com
212-O	Gislefoss, Elisabeth	elisabeth.gislefoss@nmbu.no
213-O	Ellul, Rebecca Marie	rebecca.ellul@uib.no
214-O	Erkinharju, Toni	toni.erkinharju@vetinst.no
217-O	Andrews, Melanie	melanie.andrews@nmbu.no
218-O	Midtlyng, Paul J.	paul.midtlyng@nmbu.no
219-O	Fast, Mark	mfast@upei.ca
220-O	Kogiannou, Dimitra	dkogiannou@hcmr.gr
221-O	Power, Cecilia	cecilia.power@rmit.edu.au
224-O	Fiala, Ivan	fiala@paru.cas.cz
225-O	Cavaleiro, Bárbara	barbara.cavaleiro@mare-centre.pt
227-O	Hanche-Olsen, Ragnhild	ragnhild.hancheolsen@zoetis.com
228-O	Herath, Tharangani	therath@harper-adams.ac.uk
229-O	Kawasaki, Minami	minami.kawasaki@uqconnect.edu.au
230-O	Lange, Miles	miles.lange@ars.usda.gov
233-O	Lorenzen, Niels	nilo@aqua.dtu.dk
234-O	Castro, Dolores	dcastro@uma.es
235-O	Surachetpong, Win	fvetwsp@ku.ac.th
236-O	Bergmann, Sven M.	sven.bergmann@fli.de
237-O	Klafack, Sandro	sandro.klafack@fli.de
239-O	Johnson, Stewart	stewart.johnson@dfo-mpo.gc.ca
240-O	Vogelbein, Wolfgang	wolf@vims.edu

241-O	Morin, Thierry	thierry.morin@anses.fr
242-O	Isaksen, Trond Einar	tris@norceresearch.no
243-O	Saraiva, Marcia	marcia.saraiva@abdn.ac.uk
245-O	Díaz, Seila	seiladiazcostas@gmail.com
246-O	Gorgoglione, Bartolomeo	bartgorg@msu.edu
247-O	Noguera, Patricia	patrusita@gmail.com
248-O	Jones, Simon	simon.jones@dfo-mpo.gc.ca
249-O	Birlanga, Victor B.	v.birlanga1@nuigalway.ie
250-O	Magray, Aqib R.	rehman4aaqib@gmail.com
251-O	Paley, Richard K.	richard.paley@cefas.co.uk
252-O	Wiegertjes, Geert F.	geert.wiegertjes@wur.
	Poster Presentations	
001-P	Rintamäki, Päivi	paivi.h.rintamaki@gmail.com
002-P	Karataş, Süheyla	skaratas@istanbul.edu.tr
003-P	Dios, Sonia	sdios@iim.csic.es
004-P	Seitz, Andreas	andreas.seitz@zhaw.ch
006-P	Viljamaa-Dirks, Satu	satu.viljamaa-dirks@ruokavirasto.fi
007-P	Midtlyng, Paul J.	paul.midtlyng@nmbu.no
008-P	Ramos Pinto, Lourenço	lourenco.pinto@ciimar.up.pt
010-P	Costas, Benjamín	bcostas@ciimar.up.pt
012-P	Machida, Yuichiro	yumachida04@gmail.com
013-P	Larsen, Ingrid Sofie	ingrid.sofie.larsen@acdpharma.com
014-P	Munro, Eann	eann.munro@gov.scot
015-P	Ellul, Rebecca Marie	rebecca.ellul@uib.no
016-P	Roalkvam, Irene	irene.roalkvam@uib.no
017-P	Pajdak-Czaus, Joanna	joanna.pajdak@uwm.edu.pl
019-P	Lim, Jongwon	myobong@naver.com
021-P	Yáñez, Alejandro	ayanez@uach.cl
022-P	Lagadec, Erwan	erwan.lagadec@uib.no
023-P	Parshukov, Aleksey	aleksey.nik.parshukov@gmail.com
024-P	Brevik, Øyvind Jakobsen	oyvind.brevik@cermaq.com
025-P	Avendaño-Herrera, Ruben	reavendano@yahoo.com
026-P	Tapia-Cammas, Diana	reavendano@yahoo.com
027-P	Delghandi, Seyed Mohammad Reza	01543011@students.vetmeduni.ac.at
028-P	Ribeiro, Nuno	nuno.ribeiro@aqualifeservices.com
030-P	Stefánsson, Snorri Már	sms33@hi.is
032-P	Jung-Schroers, Verena	verena.jung-schroers@tiho-hannover.de
036-P	Haussmann, Denise	denisehaussmannbi@santotomas.cl
037-P	Figueroa, Jaime	jefiguerpa@uach.cl

039-P	Pastorino, Paolo	paolo.pastorino@izsto.it
040-P	Pastorino, Paolo	paolo.pastorino@izsto.it
041-P	Olsen, Anne Berit	anne-berit.olsen@vetinst.no
043-P	Santos, Paulo	paulo.santos@ciimar.up.pt
044-P	Torres, Beatriz S.	bs.torres@usc.es
045-P	Guevara Soto, Maricruz	mguevarasoto@gmail.com
046-P	Mori, Misato	misato15mor@gmail.com
047-P	Reed, Cecile	cecile.reed@uct.ac.za
048-P	Podolska, Magdalena	mpodolska@mir.gdynia.pl
051-P	Hunt, Rhi	huntrc1@cardiff.ac.uk
054-P	Kvinnsland, Herman Høgenes	herman@fom-as.no
055-P	Pawlak, Joanna	jpawlak@mir.gdynia.pl
056-P	Gomes, Tiago	tiago_alexandre_gomes@hotmail.com
057-P	Quaglio, Francesco	alberto.perolo@phd.unipd.it
058-P	Fernandez, Carolina	carolina.fernandez@stir.ac.uk
060-P	Moreira, Márcio	marcio.moreira@ipma.pt
061-P	Cech, Gábor	cech.gabor@agrar.mta.hu
063-P	Muñoz, Pilar	pilarmun@um.es
064-P	Cho, Young-Ghan	youngghan@jejunu.ac.kr
065-P	Cho, Young-Ghan	youngghan@jejunu.ac.kr
066-P	Čolak, Slavica	slavica.colak@cromaris.hr
067-P	Šarić, Tomislav	tosaric@unizd.hr
068-P	Muñoz, Pilar	pilarmun@um.es
069-P	Urawa, Shigehiko	urawa@affrc.go.jp
070-P	Erkinharju, Toni	toni.erkinharju@vetinst.no
071-P	Jung-Schroers, Verena	verena.jung-schroers@tiho-hannover.de
072-P	Pastorino, Paolo	paolo.pastorino@izsto.it
073-P	Rodríguez, Helena	helenard@iim.csic.es
074-P	Rodríguez, Helena	helenard@iim.csic.es
075-P	Rodríguez, Helena	helenard@iim.csic.es
076-P	Rodríguez, Helena	helenard@iim.csic.es
077-P	Pétursson, Hrólfur S	hsp5@hi.is
078-P	Ní Dhufaigh, Kerrie	kerrie.nidhufaigh@research.gmit.ie
080-P	James, Joanna	jo.james@environment-agency.gov.uk
081-P	Mladineo, Ivona	mladineo@izor.hr
082-P	Viljamaa-Dirks, Satu	satu.viljamaa-dirks@ruokavirasto.fi
083-P	Duman, Muhammed	mduman@uludag.edu.tr
084-P	Trumbić, Željka	ztrumbic@unist.hr
085-P	Mladineo, Ivona	mladineo@izor.hr
087-P	Hofmann, Arndt Christian	arndtchristian.hofmann@fli.de

088-P	Teixeira Alves, Mickael	mickael.teixeiraalves@cefas.co.uk
089-P	Hafstað Ármannsdóttir, Edda Björk	eba@hi.is
090-P	Jung-Schroers, Verena	verena.jung-schroers@tiho-hannover.de
091-P	Tavornpanich, Saraya	saraya.tavornpanich@vetinst.no
093-P	Bjornsdóttir, Thorunn S.	thorunnsoley@hi.is
094-P	Schmidt-Posthaus, Heike	heike.schmidt@vetsuisse.unibe.ch
095-P	Avenant-Oldewage, Annemarie	aoldewage@uj.ac.za
096-P	Bitchava, Konstantina	bitchava@vri.gr
097-P	Cuenca, Argelia	arcun@aqua.dtu.dk
098-P	James, Joanna	jo.james@environment-agency.gov.uk
099-P	Stachnik, Magdalena	magdalena.stachnik@piwet.pulawy.pl
100-P	Stachnik, Magdalena	magdalena.stachnik@piwet.pulawy.pl
101-P	Leandro, Margarida	margaridagl@hotmail.com
104-P	Pojezdal, Lubomir	pojezdal@vri.cz
105-P	Bigarré, Laurent	laurent.bigarre@anses.fr
106-P	Sano, Motohiko	msano00@kaiyodai.ac.jp
107-P	Kim, Hyoung Jun	hjkim1882@korea.kr
108-P	Bandín, Isabel	isabel.bandin@usc.es
109-P	Pajdak-Czaus, Joanna	joanna.pajdak@uwm.edu.pl
110-P	Veenstra, Kimberly	kimberly.veenstra@fli.de
112-P	Cho, Miyoung	mycho69@korea.kr
113-P	Qadiri, Syed Shariq Nazir	shariqqadiri@gmail.com
114-P	Qadiri, Syed Shariq Nazir	shariqqadiri@gmail.com
115-P	Eriksson-Kallio, Anna Maria	annamaria.eriksson-kallio@ruokavirasto.fi
116-P	Panzarin, Valentina	vpanzarin@izsvenezie.it
117-P	Tighe, Andrew	andrew.tighe@ucdconnect.ie
118-P	Jordan, Lisa Katharina	lisa.jordan@fau.de
119-P	Jordan, Lisa Katharina	lisa.jordan@fau.de
120-P	Golomaou, Eleni	dedeloudis@gmail.com
121-P	Joiner, Claire	claire.joiner@cefas.co.uk
122-P	Ito, Takafumi	takafumi@affrc.go.jp
124-P	Toffan, Anna	atoffan@izsvenezie.it
125-P	Thierry, Morin	thierry.morin@anses.fr
126-P	Adamek, Mikolaj	mikolaj.adamek@tiho-hannover.de
127-P	Ortega, Cesar	cos_mx@hotmail.com
128-P	Allshire, Rosa	rosa.allshire@stir.ac.uk
130-P	Doszpoly, Andor	doszpoly.andor@agrar.mta.hu
131-P	Adamek, Mikolaj	mikolaj.adamek@tiho-hannover.de
132-P	Cuenca, Argelia	arcun@aqua.dtu.dk

133-P	Adamek, Mikolaj	mikolaj.adamek@tiho-hannover.de
134-P	Adamek, Mikolaj	mikolaj.adamek@tiho-hannover.de
136-P	Dopazo, Carlos P.	carlos.pereira@usc.es
137-P	Weli, Simon	simon.weli@vetinst.no
138-P	Adamek, Mikolaj	mikolaj.adamek@tiho-hannover.de
139-P	Namba, Aki	mano.nobuhiro@nihon-u.ac.jp
140-P	Borzym, Ewa	ewa.borzym@piwet.pulawy.pl
141-P	Dahl, Maria M.	mmd@hfs.fo
142-P	Stachnik, Magdalena	magdalenastachnik@piwet.pulawy.pl
146-P	Heavyside, Rebecca	rebecca.heavyside@skretting.com
148-P	Van West, Pieter	p.vanwest@abdn.ac.uk
149-P	Hooper, Patrick	patrick.hooper@cefas.co.uk
150-P	Hooper, Patrick	patrick.hooper@cefas.co.uk
151-P	Galeotti, Marco	masyeso.michela@spes.uniud.it
152-P	Correia, Simão	simaocorreia@ua.pt
154-P	Bušelić, Ivana	buselic@izor.hr
155-P	Paley, Richard	richard.paley@cefas.co.uk
157-P	Solovyev, Mikhail	yarmak85@mail.ru
159-P	Avenant-Oldewage, Annemarie	aoldewage@uj.ac.zaaoldewage@uj.ac.za
160-P	De Sales-Ribeiro, Carolina	carolina.sales101@alu.ulpgc.es
161-P	Mathews Delgado, Patrick	patrickmathews83@gmail.com
163-P	Hermida, Margarida	margarida.hermida@mare-centre.pt
164-P	Jung-Schroers, Verena	verena.jung-schroers@tiho-hannover.de
165-P	Magi, Gian Enrico	gianenrico.magi@unicam.it
166-P	Ortega, Cesar	cos_mx@hotmail.com
167-P	Losada, Ana	anapaula.losada@usc.es
169-P	Schmidt-Posthaus, Heike	heike.schmidt@vetsuisse.unibe.ch
170-P	Hoitsy, Márton	hoitsym@gmail.com
171-P	Girons, Albert	albert.girons@ictiovet.com
172-P	Magi, Gian Enrico	gianenrico.magi@unicam.it
173-P	Orioles, Massimo	momorioles@gmail.com
174-P	Siwicki, Andrzej	aksiw@infish.com.pl
175-P	Villalba, Antonio	antonio.villalba.garcia@xunta.gal
179-P	Terech-Majewska, Elżbieta	etam@uwm.edu.pl
180-P	Terech-Majewska, Elżbieta	etam@uwm.edu.pl
182-P	González-Stegmaier, Roxana	isabel.aguirre@uach.cl
183-P	Lee, Jehee	jeheedaum@hanmail.net
184-P	Kim, Gabin	gabin125@naver.com
186-P	García, Ana	anagarcia_93@hotmail.com
187-P	Chambers, Edel	edel.chambers@cefas.co.uk

188-P	Landor, Lotta	lotta.landor@abo.fi
189-P	Jung-Schroers, Verena	verena.jung-schroers@tiho-hannover.de
190-P	Verner-Jeffreys, David	david.verner-jeffreys@cefas.co.uk
192-P	Ciulli, Sara	sara.ciulli@unibo.it
193-P	Palikova, Miroslava	palikovam@vfu.cz
194-P	Aguirre-Gil, Isabel	isabel.aguirre@uach.cl
198-P	Benedicenti, Ottavia	tafalla@inia.es
199-P	Fernández-Boo, Sergio	sboo@ciimar.up.pt
201-P	Baptista, Teresa	teresa.baptista@ipleiria.pt
202-P	Haugland, Gyri Teien	gyri.haugland@uib.no
203-P	Yang, Hyerim	yhr930@gmail.com
207-P	Lee, Sukkyoung	lskshiny@hotmail.com
208-P	Lee, Jehee	jeheedaum@hanmail.net
209-P	Lee, Jehee	jeheedaum@hanmail.net
210-P	Jeong, Tae Hyug	hmntsj@hanmail.net
212-P	Lee, Jehee	jeheedaum@hanmail.net
213-P	Palikova, Miroslava	palikovam@vfu.cz
214-P	Perdiguero, Pedro	perdiguero.pedro@inia.es
215-P	Chun, Jin Hong	hilanamang@naver.com
216-P	Ashfaq, Hassan	hasan.ashfaq@uvas.edu.pk
217-P	Minarova, Hana	minarova@vri.cz
218-P	Haugland, Gyri T.	gyri.haugland@uib.no
219-P	Chen, Shih-Chu	scchen@mail.npust.edu.tw
220-P	Adamek, Mikolaj	mikolaj.adamek@tiho-hannover.de
221-P	Carriero, Mateus	mmcarriero@gmail.com
222-P	Novoa, Beatriz	beatriznovoa@iim.csic.es
223-P	Figueras, Antonio	antoniofigueras@iim.csic.es
224-P	Lazarte, Jassy Mary	jassylazarte@yahoo.com
225-P	Jung, Tae Sung	jungts@gnu.ac.kr
226-P	Adamek, Mikolaj	mikolaj.adamek@tiho-hannover.de
230-P	Paździor, Ewa	ewa.pazdzior@piwet.pulawy.pl
231-P	Bigarré, Laurent	laurent.bigarre@anses.fr
232-P	Toffan, Anna	atoffan@izsvenezie.it
233-P	Toffan, Anna	atoffan@izsvenezie.it
234-P	Kim, Hyoung Jun	hjkim1882@korea.kr
236-P	Haenen, Olga	mirjam.boonstra@wur.nl
237-P	Yousaf, Muhammad Naveed	muhammad.naveed@skretting.com
238-P	Labrut, Sophie	sophie.labrut@labocea.fr
239-P	Østevik, Liv	liv.ostevik@fishvetgroup.com
240-P	Pastorino, Paolo	paolo.pastorino@izsto.it

242-P	Hansen, Haakon	haakon.hansen@vetinst.no
243-P	Erkinharju, Toni	toni.erkinharju@vetinst.no
244-P	Chérif, Nadia	nadia.cherif@instm.rnrt.tn
245-P	Jung-Schroers, Verena	verena.jung-schroers@tiho-hannover.de
246-P	Adamek, Mikolaj	mikolaj.adamek@tiho-hannover.de
247-P	Jung-Schroers, Verena	verena.jung-schroers@tiho-hannover.de
248-P	Menanteau-Ledouble, Simon	menanteaus@staff.vetmeduni.ac.at
249-P	Pacorig, Valentina	pacorig.valentina@spes.uniud.it
250-P	Hansen, Haakon	haakon.hansen@vetinst.no
251-P	Cano, Irene	irene.canocejas@cefas.co.uk
252-P	Losada, Ana	anapaula.losada@usc.es
254-P	Hodkovicova, Nikola	hodkovicova@vri.cz
257-P	Rhyner, Nicola	nicola.rhyner@zhaw.ch
258-P	Wahli, Thomas	thomas.wahli@vetsuisse.unibe.ch
259-P	Fernández-Boo, Sergio	sboo@ciimar.up.pt
260-P	Bjerke, Hanna	hanna@fom-as.no
261-P	Garcia Muñoz, Lorenzo	yosoyloren10@hotmail.com
262-P	Drønen, Karine	karine.dronen@uib.no
264-P	Steinhagen, Dieter	dieter.steinhagen@tiho-hannover.de
265-P	Jung-Schroers, Verena	verena.jung-schroers@tiho-hannover.de
266-P	Pastorino, Paolo	paolo.pastorino@izsto.it
268-P	Vercauteren, Maaike	maaike.vercauteren@ugent.be
269-P	Ovalle, Loreto	loreto.ovalle@ifop.cl
270-P	Cruz, Andreia	andreia.cruz@oceano-fresco.pt
273-P	Fioravanti, Maria Letizia	marialeti.fioravanti@unibo.it
275-P	López, Carmen	carmen.lopez.gomez@xunta.gal
276-P	Hallmann, Anna	anna.hallmann@gumed.edu.pl
278-P	Prado-Álvarez, María	mprado@iim.csic.es
279-P	Prado-Álvarez, María	mprado@iim.csic.es
280-P	Smolarz, Katarzyna	oceksm@ug.edu.pl
281-P	Smolarz, Katarzyna	oceksm@ug.edu.pl
282-P	Madsen, Lone	loma@aqua.dtu.dk
283-P	Cano, Irene	irene.canocejas@cefas.co.uk
284-P	Eszterbauer, Edit	eszterbauer.edit@agrar.mta.hu
285-P	Eszterbauer, Edit	eszterbauer.edit@agrar.mta.hu
287-P	Adriano, Edson	edapadrian@gmail.com
288-P	Wahli, Thomas	thomas.wahli@vetsuisse.unibe.ch
290-P	Okamura, Beth	jnaldoni@gmail.com
292-P	Andrews, Melanie	melanie.andrews@nmbu.no
293-P	Jin, Yeonhwa	yeonhwa.jin@fli.de

294-P	Zusková, Eliška	zuskova@frov.jcu.cz
295-P	Becker, Anna M.	anna.maria.becker@fau.de
296-P	Christie, Lyndsay	lyndsay.christie@cefas.co.uk
297-P	Christie, Lyndsay	lyndsay.christie@cefas.co.uk
298-P	Naumowicz, Karolina	karolina.naumowicz@uwm.edu.pl
299-P	Fioravanti, Maria Letizia	marialeti.fioravanti@unibo.it
300-P	Mathews Delgado, Patrick	patrickmathews83@gmail.com
301-P	Lim, Jae-Woong	yowoo98@gmail.com
302-P	Galeotti, Marco	marco.galeotti@uniud.it
303-P	Dopazo, Carlos P.	carlos.pereira@usc.es
304-P	Kang, So Young	sykang1@chonnam.ac.kr
305-P	Tschesche, Claudia	claudia.tschesche@stir.ac.uk
306-P	Tschesche, Claudia	claudia.tschesche@stir.ac.uk
307-P	Ben Hamed, Said	benhamed_med@yahoo.fr
308-P	Hoare, Rowena	rh30@stir.ac.uk
309-P	Serra, Claudia	cserra@ciimar.up.pt
310-P	Yang, Hyerim	yhr930@gmail.com
311-P	Gausdal Tingbø, Monica	monica.gausdal-tingbo@zoetis.com
312-P	Olsen, Rolf Hetlelid	rolf.hetlelid-olsen@zoetis.com
313-P	Lorenzen, Niels	dsep@aqua.dtu.dk
314-P	Monaghan, Sean	sjm27@stir.ac.uk
315-P	Sancho Santos, Maria Eugenia	esanchosantos@frov.jcu.cz
316-P	Papezikova, Ivana	papezikovai@vfu.cz
317-P	Morin, Thierry	thierry.morin@anses.fr
318-P	Góra, Agnieszka	agnieszka.gora@mir.gdynia.pl

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