Article

The effects of *Aphanius iberus* predation on an aquatic community: diel changes and the role of vegetation

Stéphanie Gascón[1, *], **Xavier Llopart**[1], **Ana Ruiz-Navarro**[2], **Jordi Compte**[1], **David Verdiell-Cubedo**[2], **Dani Boix**[1], **Francisco J. Oliva-Paterna**[2], **Xavier D. Quintana**[1] and **Mar Torralva**[2]

With 5 figures and 3 tables

Abstract: The predation effects of a cyprinodontid fish (*Aphanius iberus*) on the aquatic invertebrate community structure of a Mediterranean shallow system with vegetation were investigated. Field studies were performed in March and July of 2009 in a pond adjacent to the salterns of San Pedro del Pinatar (Murcia, Spain) to evaluate two distinct settings of *A. iberus* population dynamics. In March, the *A. iberus* population had a low density, mainly dominated by large fish, whereas in July, the population had a higher density, mainly dominated by small, young-of-the-year fish. To analyse the fish, vegetation and possible diel effects on the invertebrate community, we compared the aquatic invertebrate body size distribution (biomass size spectra) found under the following conditions: 1) during different seasons with different fish population structures to include possible ontogenic shifts, 2) in vegetated and non-vegetated areas to include sites with different resource availability, and 3) under day and night conditions to investigate possible diel cycles. We hypothesise that 1) because the main predator (*A. iberus*) has a high preference for vegetated areas and is expected to show a reduced mobility out of these areas, no diel differences in the aquatic invertebrate community structure would be apparent; 2) differences in the aquatic invertebrate size spectra would be found when comparing the non-vegetated area to the vegetated area (an area with higher resource availability but also higher predation pressure); and 3) differences in the aquatic invertebrate size spectra would be apparent when comparing different seasons due to an ontogenic shift in the *A. iberus* diet. Our results showed that in the vegetated areas, where fish were much more abundant, the assemblages of aquatic invertebrates had a characteristic size spectrum, showing a higher size diversity and a higher total biomass than the assemblages observed in the non-vegetated areas. Moreover, the observed differences were not affected by diel changes because none of the analysed parameters showed significant differences between the day and night samplings.

Key words: saline ponds, refuge, predation, plankton, benthos, Mediterranean.

Introduction

Fish predation pressure has been identified as a key factor that causes biomass, size structure, spatial distribution and species composition changes in invertebrate communities (Nyström et al. 2001, Sagrario & Balseiro 2003, Okun & Mehner 2005). The effects of predator fish depend on their population density (Jeppesen et al. 1997, Jakobsen et al. 2003, Badosa et al. 2007) and on the species of fish (Burks et al. 2001). Ontogenic changes in the fish diet are common, so potential prey items may change according to fish population size structure (Werner & Gilliam 1984, Alcaraz & Garcia-Berthou 2007). This change has been

Authors' addresses:

[1] Institute of Aquatic Ecology, University of Girona, Girona, Spain

[2] Dept. Zoology and Physical Anthropology, University of Murcia, Murcia, Spain

[*] Corresponding author; stephanie.gascon@udg.edu

largely disregarded in studies focused on fish predation effects; the size structure of fish populations is often not considered and only information about fish abundance is incorporated.

Aquatic invertebrates use various strategies to avoid or reduce predation (Pierce & Hinrichs 1997, Paukert & Willis 2003). One of these strategies is based on diel migrations. Although diel migration involves biological and metabolic cost (Hays 2003, Liu et al. 2003), it is widely accepted that diurnal vertical migration is advantageous to the invertebrate biota with respect to predation (Stich & Lampert 1981, Neill 1990, Ringelberg 1993). However, in shallow waters where diel vertical migration is most likely less advantageous, pelagic zooplankters may migrate into vegetated littoral zones during the day, resulting in horizontal diel migration. This type of diel migration should be favoured when the abundance of macrophytes is high (which may reduce planktivory) and when the abundance of piscivores in the littoral zone is sufficient to reduce planktivores (Burks et al. 2002). Thus, macrophytes play a key role in predator-prey interactions (Lauridsen et al. 1996, Blanco et al. 2003). However, the role of vegetation is still controversial. In some situations, vegetation provides refuge (e.g., Paukert & Willis 2003), but in other situations, such as when predation is mediated by small predators that can live and prey within vegetation, it does not (e.g., Meerhoff et al. 2007, Brucet et al. 2010, Jeppesen et al. 2010). Therefore, comparing the aquatic invertebrate composition in vegetated and non-vegetated sites during the day and at night will increase the understanding of invertebrate responses to fish presence in vegetated shallow waters.

Fish communities of the Mediterranean shallow coastal wetlands are usually subjected to high salinity fluctuations, have low species richness and are dominated by a few species (Blanco et al. 2003, Franco et al. 2006, Maci & Basset 2009, Brucet et al. 2010). However, the potential fish prey range in these wetlands is large due to the dominant omnivore strategy (e.g., Blanco et al. 2003). Consequently, these systems are complicated to study because it is necessary to examine the entire aquatic community (including planktonic and benthic organisms) to accurately assess the effects of fish predation. The Iberian toothcarp, *Aphanius iberus* (Valenciennes, 1846), is an endangered fish that can be locally abundant in salt exploitation sites (Oliva-Paterna et al. 2006). Its diet is based on both planktonic and benthonic invertebrates (Alcaraz & Garcia-Berthou 2007), which indicates that it is capable of altering the structure of the aquatic invertebrate community (Badosa et al. 2007, Compte et al. 2012). Recent studies have suggested that benthic organisms are more affected than planktonic ones by the presence of *A. iberus* (Compte et al. 2011). However, the previous studies were conducted only with females of similar sizes and thus did not allow an assessment of the effects of immature stages, which are expected to be different due to the ontogenic shift in the diet of this fish (Ramos-Jiliberto et al. 2011). Additionally, a high predation effect inside vegetation mats was expected due to the existing positive relationship between vegetation and density in this species (Alcaraz et al. 2008, Oliva-Paterna et al. 2009). Nevertheless, no information exists on the diel behaviour of the Iberian toothcarp, and this knowledge is fundamental to understanding its effects on the aquatic community.

The present study aims to investigate the effects of *A. iberus* on the entire aquatic invertebrate community in a Mediterranean shallow coastal wetland during a diel cycle, while at the same time comparing vegetated and non-vegetated sites. Moreover, the comparison is performed during two distinct phases of the fish population temporal dynamic: 1) a situation characterised by a fish population with a size structure dominated by large fish, just before the reproductive period; and 2) a situation in which the fish population is dominated by small sizes, during the recruitment period, when there is a massive emergence of young-of-the-year specimens. Therefore, this study also accounts for the variability observed in fish population size structure and possible ontogenic shifts in their diet. Body size appears to be a determinant of trophic interactions in the Mediterranean shallow coastal wetland (Compte et al. 2012). Thus, a body size approach is used in the present study. We compare the aquatic invertebrate body size distribution (biomass size spectra) found under different conditions: 1) during different seasons with different fish population structures to incorporate possible ontogenic shifts; 2) in vegetated and non-vegetated areas to include sites with different refuge characteristics and resource availability; and 3) under day and night conditions to investigate possible diel cycles. Thus, the shapes of the spectra are compared to try to identify irregularities among the different conditions. We hypothesise that 1) because the main predator (*A. iberus*) has a high preference for vegetated areas and is expected to show a reduced mobility out of these areas, no diel differences in the aquatic invertebrate community structure will be apparent; 2) differences in the aquatic invertebrate size spectra will be found when comparing the non-vegetated and the vegetated areas (a vegetated area has higher resource

availability but also higher predation pressure); and 3) differences in the aquatic invertebrate size spectra will be apparent when comparing different seasons as a response to the ontogenic shift of the *A. iberus* diet.

Material and methods

Study site, sampling design and processing

The study was performed in a small pond adjacent to a saltern located inside of the "Salinas y Arenales de San Pedro del Pinatar" Natural Park in south-eastern Spain (Fig. 1). The fish assemblages included *A. iberus* and a species from the Atherinidae family (*Atherina boyeri* Risso, 1810), but they were dominated by the eurythermic and euryhaline *A. iberus* (98 %). *A. iberus* is a cyprinodontid (approx. maximum total length < 60 mm) that can become locally abundant in salt exploitation sites (Oliva-Paterna et al. 2006, 2009).

Six sampling sites were distributed in the studied pond (Fig. 1). Three of them were located inside vegetated areas, and the other three were located outside of these areas. Two sampling surveys coinciding with two distinct phases of the *A. iberus* population dynamic were performed (Oliva-Paterna et al. 2009) and characterised by significant differences in size structure: the first survey was dominated by large fish (March 2009), and the second survey, during the recruitment period, was dominated by small fish (July 2009). In each survey, the samples were taken at midday (approx. from 10:00 AM to 14:00 PM) and at midnight (approx. from 11:00 PM to 03:00 AM). Both of these samplings were performed on the same day.

The fish sampling technique consisted of quickly throwing a square enclosure trap into the water (sides: 0.8 m; high: 0.5 m; mesh size: 2 mm) and then removing all of the fish that were contained within the trap using a dip net (Jordan et al. 1997). Physico-chemical parameters were measured at each enclosure trap site. Zooplankton samples (obtained by filtering 5 L of water through a 50 μm mesh) and zoobenthos samples (obtained using an Ekman Grab 225 cm^2) were collected in the same traps. Once the sampling was completed, the enclosure traps were extracted, but the location was marked with a stick that we fixed to the bottom of the pond so that the trap could be reset at the next sampling near the position of the first enclosure trap.

The zooplanktonic organisms were identified, counted and measured (at least 25 individuals were randomly chosen from each identified taxon) using a stereomicroscope and an inverted microscope. The zoobenthos sample allowed us to obtain macrozoobenthos organisms by sorting them directly from the sediment (organisms were retained in a 1 mm mesh sieve), and we were able to obtain meiozoobenthos after resuspending the organisms in a sucrose-water solution (1:1) for 3 h (organisms were retained in a 0.2 mm mesh sieve). The meiozoobenthic organisms were stained with Rose Bengal and preserved in 4 % formalin. The zoobenthic organisms were identified, counted and measured (at least 25 individuals were randomly chosen from each identified taxon) under a stereomicroscope. In total, 6776 individuals were identified and measured, corresponding to 16 faunal groups: Rotifera, Ciliates, Copepoda nauplii, Calanoida, Cyclopoida, Harpacticoida, Cladocera, Ostracoda, Hydracarina, Nematoda, Oligochaeta, Polychaeta, Gastropoda, Bivalvia, Chironomidae and Coleoptera. Organism biomass was
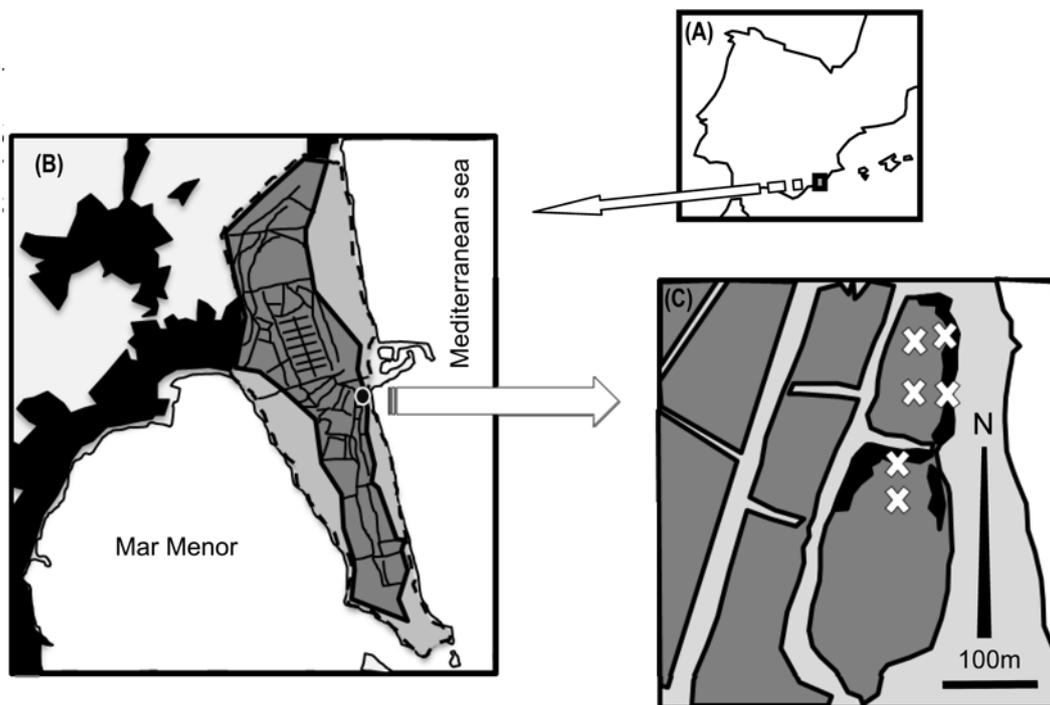

Fig. 1. Map indicating **(A)** the location of the study site on the Iberian Peninsula; and **(B)** the regional natural park vicinity (boundaries are indicated by the discontinuous line), the limits of the salt exploitation area (indicated by a continuous line) and the study site (indicated by a solid circle), which is inside this salt exploitation area. Urbanised areas are schematically indicated with black polygons; **(C)** detail of the study site indicating the vegetated areas (in black) and the sampling site locations (crosses).

estimated using published size-biomass equations (see Compte et al. (2011) and Gascón et al. (2005) for detailed information on the size-biomass equations).

Macrophytes were extracted from the first sieve (>1 mm mesh size), identified, separated, dried at 60 °C for 48 h, and weighed to estimate their biomass. The fish captured inside of

Table 1. Generalised additive models used in the normalised biomass size spectra analyses. In the model description, the main terms (factors), the smoothing term (covariate), and the interaction term included in each model are listed. Factor levels appear in brackets: March (M), July (J), Day (D), Night (N), Vegetated (V), and Non-vegetated (nV).

Model	Model description	Interpretation of the interaction One pattern (NBSS) for each combination of factor levels
Full	**Main terms**: Sampling survey (M;J) Day time (D;N) Vegetation (V;nV) **Smooth term**: Size class **Interaction term**: Sampling survey × Day time × Vegetation (MDV;MNV; MDnV;MNnV;JDV;JNV;JDnV;JNnV)	
Model 1; Update of full model merging day and night samples	**Main terms**: Sampling survey (M;J) Day time (D;N) Vegetation (V;nV) **Smooth term**: Size class **Interaction term**: Sampling survey × Vegetation (MV;MnV; JV;JnV)	
Model 2; Update of model 1 suppressing day time as factor	**Main terms**: Sampling survey (M;J) Vegetation (V;nV) **Smooth term**: Size class **Interaction term**: Sampling survey × Vegetation (MV;MnV; JV;JnV)	
Model 3; Update of model 2 merging sampling surveys	**Main terms**: Sampling survey (M;J) Vegetation (V;nV) **Smooth term**: Size class **Interaction term**: Vegetation (V;nV)	
Model 4; Update of model 2 merging vegetation areas	**Main terms**: Sampling survey (M;J) Vegetation (V;nV) **Smooth term**: Size class **Interaction term**: Sampling survey (M;J)	
Model 5; Update of model 2 without interaction	**Main terms**: Sampling survey (M;J) Vegetation (V;nV) **Smooth term**: Size class	

the enclosure traps were extracted using a hand net (quadrangular hand net 40 × 40 cm; 1 mm mesh size) until no more fish were captured. The captures obtained with this hand net were used to assess fish abundance and population size structure assuming similar efficiencies between habitats. In agreement with Alcaraz et al. (2008), the objective of our study was not to obtain absolute estimates of fish abundance but to compare relative density and size structure. The captured fish were identified to species level, counted, measured, sexed and released alive outside of the enclosure traps. The fish biomass was estimated using existing length-weight relationships (Verdiell-Cubedo et al. 2006).

Data analyses

The zooplankton and zoobenthos information was pooled to obtain information about the entire aquatic invertebrate assemblage. We calculated the biomass-size spectrum of each aquatic invertebrate sample. The biomass-size spectrum provides an integrative measure to quantify variations in the structure of aquatic invertebrate communities (Kerr & Dickie 2001, Brucet et al. 2006). The identification of irregularities in the shape of the biomass-size spectrum is ecologically relevant because the shape of the spectrum has shown sensitivity to both biotic factors (such as predation; Badosa et al. 2007, Brucet et al. 2010) and abiotic factors (such as different environmental conditions; Rodríguez et al. 1987, Gaedke 1992, Rodríguez 1994). To identify these irregularities, we used the normalised biomass size spectra (NBSS). The NBSS is obtained by plotting organisms' abundances (Y axis) against their sizes, which are organised in $\log_2$ classes (X axis). The NBSS is preferable to the non-normalised biomass size spectra because it minimises scaling problems due to the width of the size classes in which organisms' abundances are grouped (Blanco et al. 1994). The shape of the NBSS was assessed by adjusting a generalised additive model (GAM) to better detect nonlinear structures (De Eyto & Irvine 2007). The aquatic invertebrate abundances (response variable) were modelled using size class, vegetation (vegetated vs. non-vegetated areas), time of day (day vs. night samples), and sampling survey (season) as explanatory variables. Size class was included as a smoothing term, while the rest of the explanatory variables were included as nominal variables (factors). The interaction between size class and the combination of the three factors studied were also included in the model (Full model; Table 1). Determining this interaction is necessary to analyse possible changes, the so-called irregularities, in the

NBSS shape among the factors analysed. To do so, we first applied the most complex model (previously described as the Full model), and then we applied a simpler model, merging the day and night samples (Model 1; Table 1), suppressing the time of day factor (Model 2; Table 1), merging the sampling surveys samples (Model 3; Table1), or merging the vegetated and non-vegetated samples (Model 4; Table 1). Finally, we also calculated a model without any interaction (Model 5; Table 1). All of the models were compared (likelihood test; Table 2), keeping the simplest model only if simplification did not cause a significant loss of information. We followed the suggestions made by Burnham & Anderson (2002) to select the best model among the ones tested. Consequently, for each model we calculated the following: 1) Akaike's information criterion of the second order (AICc); 2) the AICc differences among the tested models; and 3) the AICc weights. Thus, the best model corresponds to the one with the lowest AICc, with the AICc differences below 2, and with the highest AICc weight. GAM models were applied using the "gam" function available in the "mgcv" package (Wood 2011), and the AICc, the AICc differences and the AICc weights were obtained using the function "model.sel" available in the "MuMIn" package (Barton 2012). Both packages were written in R language (R Development Core Team 2007).

Additionally, three parameters were calculated to characterise the structure of the aquatic invertebrate assemblages: size diversity, the biomass geometric mean and total biomass. Size diversity is analogous to Shannon diversity, but it is based on organisms' body sizes instead of their taxonomic identity (Quintana et al. 2008). Size diversity provides a unique value per size distribution, providing information about the relative size distribution along the size range and has the advantage of an intuitive interpretation of its ecological meaning because the concept of diversity is well established (Quiroga et al. 2005, Brucet et al. 2006, Quintana et al. 2008). The size diversity was calculated following the methodology described in Quintana et al. (2008), using free software available at http://limnolam.org. The biomass geometric mean was obtained from the same software and provided information about the mean size of the organisms observed in each sample. The total biomass was the sum of the biomass of all of the organisms found in a sample. The significance of the factors analysed (sampling survey, time of day, vegetation) and their interactions were tested using three-way ANOVA performed separately for each parameter. Similarly, differences in fish density and biomass among sampling surveys, vegetation areas and time of day were also tested using three-way ANOVA. Finally, possible vegetation differ-

Table 2. Results of the generalised additive models used in the analyses of the normalised biomass size spectra. See Table 1 for the model description. The degrees of freedom (DF), proportion of variance explained (R^2) and several measures of model fitting are shown: explained deviance, Akaike's Information Criterion (AICc), AICc differences (Δ AICc), and Akaike weights (ω_i). The model with the best fit that corresponds to the one with the lowest AICc, with AICc differences below 2 and with the highest AICc weight, is shown in bold.

Model	DF	R^2	Explained deviance (%)	AICc	Δ AICc	ω_i
Full	58.61	0.62	65.8	2034.6	39.2	0.000
Model 1	34.13	0.62	64.4	1996.0	0.64	0.421
Model 2	**33.17**	**0.62**	**64.3**	**1995.3**	**0.00**	**0.579**
Model 3	19.13	0.56	58.0	2047.3	51.95	0.000
Model 4	18.70	0.53	54.9	2083.3	88.00	0.000
Model 5	11.39	0.50	51.3	2106.8	111.44	0.000

ences (in species composition and total biomass) due to sampling survey (season) were tested with one-way ANOVA. All of the ANOVAs were calculated using the "aov" function available in R, starting with the most complex model, including the triple interaction. Then, the model was simplified by removing the non–significant interactions ($p > 0.05$) to increase the statistical power, which would otherwise be seriously compromised. A similar methodology was used to test for differences in the number of individuals for each aquatic invertebrate taxon separately.

Results

The vegetated areas were mainly composed of *Ruppia* sp. and *Cladophora* sp. in both seasons. The importance of both groups did not change (there was no significant interaction between the vegetation type (*Cladophora* sp. or *Ruppia* sp.) and sampling survey; $F_{1,38} = 0.58$; $p = 0.449$). However, significant differ-

ences in total vegetation biomass were detected when comparing seasons ($F_{1,38} = 7.30$; $p = 0.010$), and they were higher in July.

Although the fish biomass was not significantly different between seasons ($F_{1,22} = 0.69$; $p = 0.415$), the fish population size structure differed. Thus, the fish population was dominated by large size classes (mainly size classes 16 and 17, corresponding to individuals within 2.1 and 3.0 cm total length) in March and by smaller sizes in July (mainly size class 13, corresponding to individuals within 1.3 and 1.4 cm; Fig. 2). Moreover, the fish density (number of individuals) was significantly different between seasons ($F_{1,19} = 49.15$; $p < 0.001$), and it was almost undetectable in March (fewer than 5 individuals were caught per trap). Nevertheless, the fish distribution patterns observed between the vegetated areas and the time of day could be considered similar between seasons because
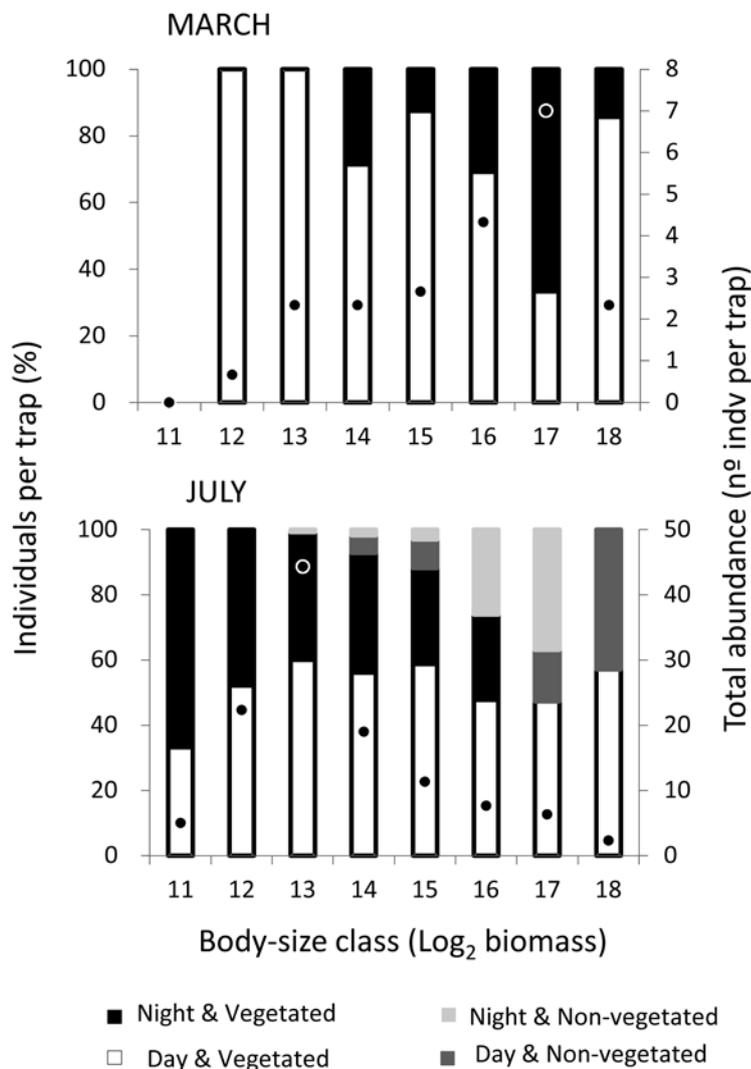

Fig. 2. Body size structure of the fish populations in the two studied periods. The plots show the percentage of individuals found in each sampling survey (areas), as well as the total abundance (dots) of the $\log_2$ biomass size classes.
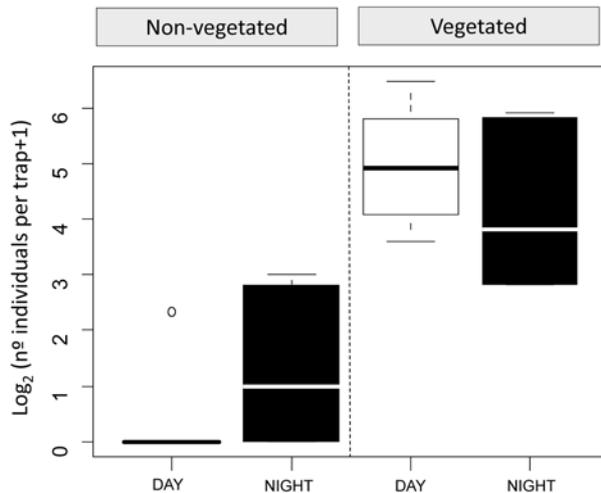
Fig. 3. Box plot showing the fish captures (expressed as the number of individuals per trap; y-axis) in the vegetated and the non-vegetated areas during the day and the night samplings. The data correspond to the pooled data (sum of individuals) of both sampling surveys (March and July).

no significant interaction term was observed either for the vegetation ($F_{1,17} = 0.80$; $p = 0.383$) or for the time of day ($F_{1,18} = 3.28$; $p = 0.087$). Therefore, the fish were predominately found in the vegetated areas regardless of the season. Similarly, the fish density was always higher in the vegetated areas regardless of the time of day. In contrast, in the non-vegetated areas, the fish density increased at night (Fig. 3). Consequently, there was a significant interaction between the vegetation and the time of day ($F_{1,17} = 9.54$; $p = 0.007$).

Nine invertebrate groups were responsible for more than 99 % of the total invertebrate abundance (measured by the number of individuals per trap): Ostracoda (37.17 %), Gastropoda (24.11 %), Harpacticoida (14.58 %), Nematoda (10.29 %), Cyclopoida (5.88 %), Chironomidae (3.93 %), Copepoda nauplii (1.61 %), Bivalvia (1.13 %), and Rotifera (0.69 %). None of these groups showed significant differences when the day and the night samples were compared. Instead, most of them showed significant differences ($p < 0.05$) when the vegetated areas and the non-vegetated areas were compared (Copepoda nauplii $F_{1,20} = 6.54$; Rotifera $F_{1,19} = 9.10$; Nematoda $F_{1,22} = 9.23$; Harpacticoida $F_{1,22} = 7.62$; Cyclopoida $F_{1,21} = 5.42$; Gastropoda $F_{1,22} = 46.72$; Chironomidae $F_{1,21} = 16.48$). All of these groups showed higher abundances in the vegetated areas. The observed differences between the vegetated and the non-vegetated areas were seasonally independent because the interaction between the vegetation and the sampling survey was not significant ($p > 0.05$), with the exception of Copepoda nauplii ($F_{1,20} = 7.33$; $p = 0.013$), which had lower abundance values in the non-vegetated areas during the July survey when the fish population was dominated by small sizes. Some taxa also showed significant differences ($p < 0.05$) in abundance when the sampling surveys were compared (Cyclopoida $F_{1,21} = 6.54$; Chironomidae $F_{1,21} = 49.06$; Bivalvia $F_{1,22} = 43.10$). Most of them had higher abundances in July, except Bivalvia, which followed an inverse pattern. Moreover, chironomids had larger-sized individuals dominating the non-vegetated areas, where the fish were less abundant (Fig. 4).

Table 3. Likelihood test results indicating which models are compared in each test. See the model description in Table 1.

| Likelihood test	Models compared	DF	Deviance	F	p	Meaning
Test1	Full Model vs. Model 1	–24.47	–49.87	0.76	0.786	Model simplification does not entail a significant loss of information
Test2	Model 1 vs. Model 2	–0.97	3.91	1.53	0.216	Model simplification does not entail a significant loss of information
Test3	Model 2 vs. Model 3	–14.04	–223.41	6.03	< 0.001	Model simplification entails a significant loss of information
Test4	Model 2 vs. Model 4	–14.47	–334.92	8.77	< 0.001	Model simplification entails a significant loss of information
Test5	Model 2 vs. Model 5	–21.77	–461.46	8.03	< 0.001	Model simplification entails a significant loss of information
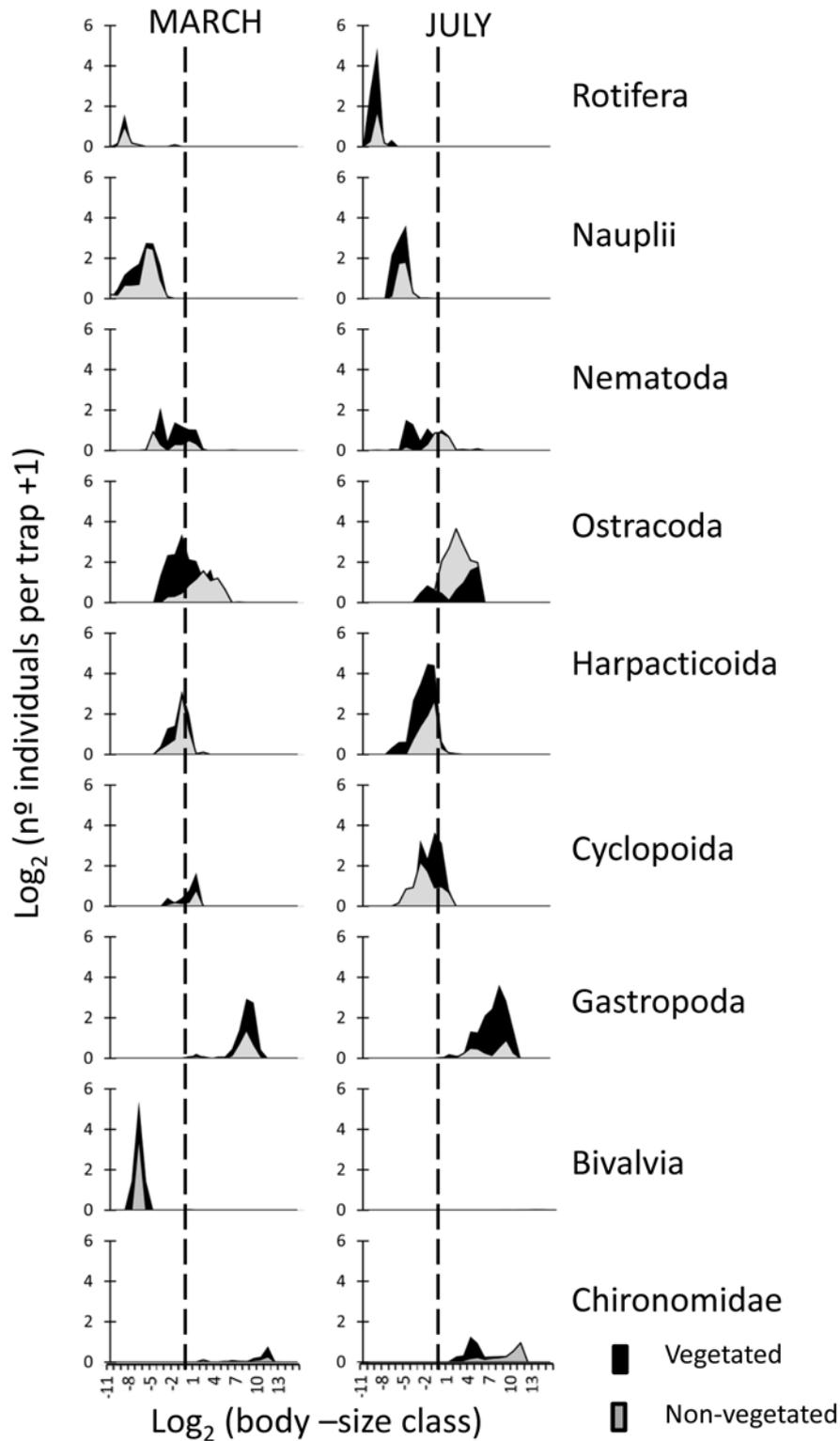
Fig. 4. Organisms' body size distribution represented by the means of a layered area chart. The information is given for each sampling survey and only for organism groups with abundances > 1 %. Vertical dashed lines indicate the location of the 0 body size class, plotted as a reference.
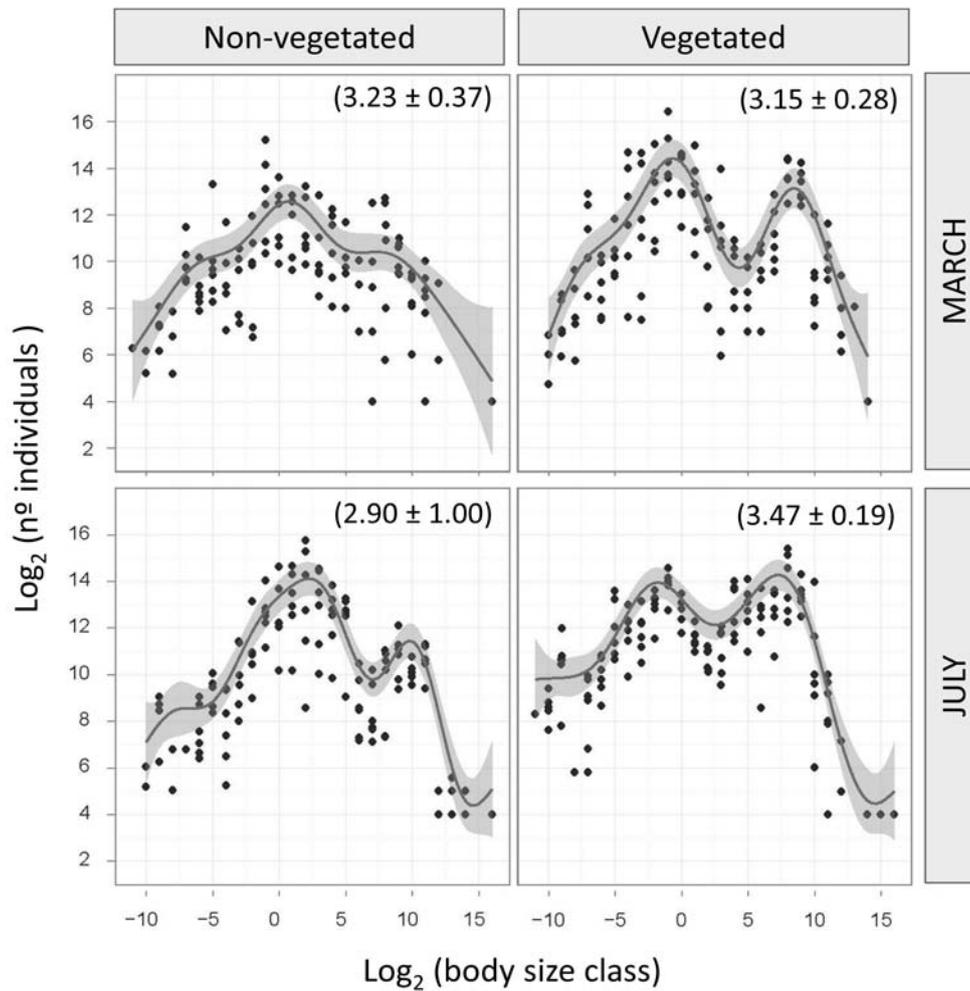
Fig. 5. Normalised biomass size spectra (NBSS) of the aquatic invertebrates. Only significantly different shapes of the NBSS are shown for each sampling survey. The line represents the fitted GAM model, the grey area corresponds to the 95 % confidence intervals, and the points are the observed values (n° of individuals) for each body size class (x-axis). The mean size diversity values ± standard deviation are shown in brackets.

Although some variability was observed, in general, the rest of the groups had similar size ranges when seasons and the vegetated vs. the non-vegetated areas were compared.

To analyse this variability in depth, we used the normalised biomass size spectra (NBSS) of the entire aquatic invertebrate community. We found significant differences in the NBSS of the aquatic invertebrates according to the fish population structure (Tables 2 and 3). Thus, NBSS were significantly different between the vegetated and the non-vegetated areas and between seasons (Fig. 5) but not between the day and the night samples (Tables 2 and 3). Thus, the shape of the biomass size spectra was significantly different when the vegetated areas and season were compared, but not when the day and the night samples were compared (Table 2: best model = Model 2). The size diversity

values were also significantly different when the vegetated and the non-vegetated areas were compared, and again, these differences were season-dependent due to the significance of the interaction between the season and the vegetation ($F_{1,20} = 5.90$; $p = 0.025$). In this sense, the vegetated areas had higher size diversity values than the non-vegetated areas, which had the lowest size diversity values during the July survey. Similarly, the total invertebrate biomass was higher in the vegetated areas than in the non-vegetated areas ($F_{1,21} = 65.50$; $p < 0.001$), and it was higher in the July survey vs. the March survey ($F_{1,21} = 8.69$; $p = 0.007$); however, in this case, no significant interaction was found. No significant differences of the biomass geometric mean were found between the vegetated and the non-vegetated areas. Instead, significant differences were found between the seasons ($F_{1,22} = 5.54$;

$p = 0.028$), with higher geometric means observed in the July samples. Moreover, the lack of a significant result for the interaction between season and the vegetation indicates that the aquatic invertebrates had a larger body size in July, in both the vegetated and the non-vegetated areas. In summary, no diel differences were apparent from size diversity, biomass geometric mean, total biomass, or NBSS.

Discussion

No diel differences of aquatic invertebrate structure between the vegetated and the non–vegetated areas were observed, and therefore, no diel horizontal migration was detected. Although it has been suggested that diel horizontal migration (DHM) may be favoured when macrophyte abundance is high (Burks et al. 2002), it is also true that DHM is expected to be less prevalent when predation pressure is exerted by a small fish species that inhabits and preys within vegetation mats (Brucet et al. 2012). *A. iberus* was mainly found in the vegetated areas regardless of the time of day, indicating that its predation pressure does not change due to diel movements. Consequently, the effect of *A. iberus* predation appears to be centred in the vegetated areas. Moreover, many studies dealing with DHM have focused on *Daphnia* movements (e.g., Burks et al. 2001, Meerhoff et al. 2006, Tavşanoğlu et al. 2012). At our sampling site, no large cladocerans (i.e., *Daphnia*) were found (we only detected some individuals of smaller species, such as Chydoridae), and zooplanktonic taxa were mainly represented by small taxa such as Rotifera and Cyclopoida. The lack of large zooplanktonic taxa is congruent with the existing negative relationship found between zooplankton body size and salinity (Brucet et al. 2009). It is important to note that DHM is not completely applicable to all taxa because some taxa are highly linked to vegetation and do not show DHM (Lauridsen et al. 1996). Accordingly, we did not find evidence of DHM, suggesting that the fauna inhabiting these systems are highly habitat-dependent.

The *A. iberus* population size structure changed between the sampling surveys, and consequently, we expected a differential predation effect because ontogenic changes in its diet have been already reported (Alcaraz & Garcia-Berthou 2007, Ramos-Jiliberto et al. 2011). In agreement with these studies, we found that the aquatic invertebrate total biomass and the body size geometric mean changed according to the observed differences in the fish size structure. Thus,

we found larger sizes of aquatic invertebrates when the fish size structure was dominated by young-of-the-year individuals (small size classes). This finding could be the result of predation pressure exerted by the smaller individuals that mainly prey on small organisms (Alcaraz & Garcia-Berthou 2007). However, our study did not allow us to accurately uncouple this effect from a possible seasonal pattern that was not due to predation. In fact, the change in the fish size structure coincided with an increase in macrophyte biomass; this increase may support greater habitat complexity and food resource abundance, which may ultimately influence aquatic invertebrate communities (e.g., McAbendroth et al. 2005, Lucena-Moya & Duggan 2011).

It is well known that aquatic vegetation influences invertebrate community structure not only because it provides food resources (e.g., Burdett & Watts 2009) but also because it provides physical refuge from fish predation (e.g., Paukert & Willis 2003). Nevertheless, in our study, macrophytes did not appear to provide a strong refuge from predation because the main predatory fish (*A. iberus*) inhabiting the studied vegetation mats was a small bodied species that usually lives within the vegetation (e.g., Alcaraz et al. 2008); this finding was also noted in our data. Therefore, the differences observed between the vegetated and the non-vegetated areas may result from a combination of three factors: 1) a high resource availability in the vegetated areas, 2) a high habitat specificity of the invertebrate species and 3) a stronger predation effect in the vegetated areas. In fact, the higher total invertebrate biomass in the vegetated areas was expected based on the higher resource availability (e.g., Watkins II et al. 1983, Diehl & Kornijów 1998). Furthermore, some of the invertebrates that we identified are strongly habitat-dependent and appear only in benthic habitats; these invertebrates include nematodes, chironomids, ostracods and gastropods, which mainly appear in benthic and/or vegetated habitats. Regarding predation, a priori we expected a higher predation pressure in the vegetated areas where the *A. iberus* density is especially high. However, we found higher values of the total biomass of aquatic invertebrates there, mainly due to the higher abundances of Rotifera, Cyclopoida, Copepoda nauplii, Nematoda, Harpacticoida, Gastropoda and Chironomidae. The size diversity values were also higher in the vegetated areas, contrasting with the results obtained previously, in which size diversity decreased with increasing predation pressure (Badosa et al. 2007, Brucet et al. 2010, Compte et al. 2012). Moreover, the fish density found in our study

was much higher than the threshold of 4 ind. m^{-2}, which denotes fish predation effects on invertebrate composition and size structure and has been noted in some experimental studies (Jeppesen et al. 1997, Jakobsen et al. 2003). Nevertheless, the cited studies were mainly focused on planktonic organisms. In contrast, a previous study analyzing macroinvertebrate predation effect on the benthic community (Gascón et al. 2009) found similar results to the ones reported in the current study. Another plausible explanation is that vegetation acts as a refuge for invertebrates against visual predators such as fish (Cook & Streams 1984). Therefore, invertebrates would be less predated upon in vegetated areas despite fish proximity because they would be more difficult to detect visually. Additionally, it is also probable that the higher resource availability of the vegetated areas counterbalanced *A. iberus* predation pressure. On the other hand, open water is a barren environment for invertebrates, with scarce resources and a high risk of visual detection by fish predators.

In summary, our results indicate that vegetation is highly important for the community structure of a shallow coastal wetland and suggest that the observed differences are not diel-affected. Higher resource availability (food and habitat complexity) makes vegetation mats an advantageous habitat for invertebrates, even if predation is mainly mediated by small fish such as *A. iberus*.

Acknowledgments

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