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Assessment of fish assemblages in coastal lagoon habitats: Effect of sampling method

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ABSTRACT

The structure of fish assemblages accounted for by different sampling methods (namely fyke net, seine nets, visual census) applied to vegetated and unvegetated lagoon habitats was investigated in terms of species composition, functional groups (ecological and trophic guilds), and fish size distribution. Significant differences were detected among methods, even among similar ones (seine nets). Visual census and fyke net detected more easily pelagic species, allowing the sampling of larger fish, whereas seine nets targeted more efficiently benthic-demersal species, with a dominance of 2–10 cm size classes in the fish catches. Differences were detected also among habitats, reflecting the different fish assemblages associated to vegetated and unvegetated habitats in coastal lagoons and transitional waters. However a different ability of discriminating between habitat-associated fish assemblages was recorded for the sampling methods. The different selectivity and functioning of the tested sampling methods confirm the importance of considering the targeted scale at which the research is being carried out, as well as the method that will be used to assess the ecological status of lagoon fish assemblages when choosing the most appropriate sampling method. A cross-validation of fish sampling methodologies in transitional waters is necessary to cope with the mandatory of the Water Framework Directive of standardization and comparability of monitoring methods.

1. Introduction

Not even the simplest of the ecosystems can be known in its totality, with all its individuals and species, their distribution and their relationships. Therefore, the knowledge of an ecosystem depends on taking a number of samples, and from them to infer the characteristics of the whole community. Sampling design for the study of animal assemblages relies on a series of decisions which regard for example the number and location of samples, the number of replicates, the sampling strategy for the distribution of the stations (random, systematic, stratified, hierarchical) according to the distribution and mobility of species (Andrew and Mapstone, 1987; Gaston and McArdle, 1994). Furthermore, when designing a sampling program, it is also important to consider which sampling technique may provide the most accurate information on the target assemblage, given also the final aim of the research (Watson et al., 2010).

Fish assemblages comprise many different groups representing different niches, hence the capture of all components of a fish assemblage requires different complementary methods (Elliott and Hemingway, 2002). Most of the commonly used methods in coastal and transitional environments are based on traditional fishing gears (trap nets, trammel nets, gill nets, long lines, trawling or seine nets and traps). Other methods have been developed, such as underwater visual censuses, or video recording, as they are relatively quick, non-destructive, repeatable and cost effective, though needing clear waters to effectively operate (St. John et al., 1990; Watson et al., 1995; Thompson and Mapstone, 1997; García-Charton et al., 2000). Most of the fish sampling methods are semi-quantitative methodologies which need the catch data to be

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standardized over a sampling effort unit, measured either by sampling area (e.g. for seine nets and visual census) or by other parameters such as number of boats, tonnage, horsepower of the engines, number of fishermen per boat, length of nets, number of traps deployed, number of hooks, the time spent fishing, etc. These effort measures have a nonlinear relationship with absolute density or abundance, because numerous factors affect catch rates, such as for example changes in species distribution (e.g. hyperaggregation) or in gear effectiveness (Xia and Boonstra, 1992; Gaston and McArdle, 1994; Harley et al., 2001; Maunder et al., 2006). Therefore, these techniques provide only indirect estimates of abundance, making difficult the comparison between different studies, and measures of variability can be seriously biased (Gaston and McArdle, 1994; Maunder et al., 2006).

The European Community established the Water Framework Directive (WFD) (Directive, 2000/60/EC) to protect surface waters (including inland, transitional and coastal waters) and groundwater and it aims at achieving a good ecological quality status for all water bodies by 2015 (European Union, 2000). The evaluation of the ecological quality status is based upon biological, hydromorphological and physico-chemical quality elements, and fishes are one of the biological quality elements which need to be assessed in transitional waters. Member States have to monitor fish communities on a systematic and comparable basis throughout the community using standardized methods of monitoring, sampling and analysis. All fish sampling methods are selective to some degree (e.g. with respect to fish size or species, morphological type or life stage, habitat type or place in the water column) (see Elliott and Hemingway, 2002 for a review). Hence, a cross-validation of methods is necessary to cope with the mandatory of the WFD of standardization and comparability of monitoring methods.

Thus, by all of the above, this work aims to perform a comparison of the main sampling methods used in the study of fish assemblages in European coastal lagoons, by investigating the structure of fish assemblages accounted for by the different methods in terms of species composition, ecological and trophic guilds, and fish size distribution. To fulfil this aim, a simultaneous sampling exercise was developed in the same localities and communities in different habitats of the Mar Menor lagoon, Spain. The methods assessed include fishing gears such as fyke nets and beach seine nets. Different gear settings were also considered as regards seine nets, in order to account for the variability of the method in coastal lagoons, depending on the lagoon and research groups using the net. In addition, the water conditions in the study site allowed applying also visual census techniques, which are usually restricted to coral reefs lagoons and some oligotrophic lagoons (Pérez-Ruzafa, 1989; Pérez-Ruzafa et al., 2006). This method has been included in the study besides its limited applicability in Mediterranean coastal lagoons, as the general comparison among sampling methods is considered of wider value to fish ecology studies. Several works have critically compared different techniques before, most of them focussing on either different fishing gears (Rozas and Minello, 1997) or different visual census techniques (De Martini and Roberts, 1982; Harmelin-Vivien et al., 1985; Bortone et al., 1986, 1989, 1991). However, very few works compared sampling gears such as fyke net versus seine nets or different fishing gears and visual census (Connell et al., 1998; Willis et al., 2000), especially in coastal lagoons.

2. Materials and methods

2.1. Study site

The Mar Menor is a large coastal lagoon (135 km^2), located in a semi-arid region of the SW Mediterranean, along the Spanish coasts (Fig. 1). More than twenty cataclinal watercourses are present in its watershed, most of them discharging into the southern basin of the lagoon with a sporadic and torrential rainfall regime. An exception is the El Albujon watercourse, the main collector in the drainage basin, which, due to changes in agricultural practises and related phreatic rising (Pérez-Ruzafa and Aragón, 2002), maintains a regular but low flux of water of around 0.02 m^3 s^{-1} rising in short peaks during storm events to 10.5 m^3 s^{-1} (Garcia-Pintado et al., 2007). The nutrient inputs associated to this influx (Pérez-Ruzafa et al., 2002; Velasco et al., 2006; Garcia-Pintado et al., 2007) have promoted important changes in the trophic webs in the lagoon with jellyfish proliferation (Pérez-Ruzafa et al., 2002) with consequences in water quality in some areas. Water temperature shows a regular seasonal cycle, with maximum records registered in August (30.0 °C) and minimum in February (11.2 °C). Salinity shows heterogeneous spatial and temporal distribution depending on season, rainfall, runoff and Mediterranean influence through the main inlets, with a minimum of 38.1 and a maximum of 51 (Pérez-Ruzafa et al., 2005a). In general, hydrographical conditions permit to differentiate three different zones or sub-basins (Pérez-Ruzafa et al., 2005a,b) that are also supported by biological assemblages structure and species composition (Pérez-Ruzafa et al., 2004, 2007). The lagoon has a mean depth of 3.6 m, with maximum of 6 m at the centre of the basin and shallow areas at the borders. Muddy bottoms characterize the central area, with dense macroalagal coverage of *Caulerpa prolifera* (Forsskål). Muddy sheltered shallow areas house patches of the seagrass *Ruppia cirrhosa* (Petagna) Grande. Along the lagoon margins, sandy bottoms are present with

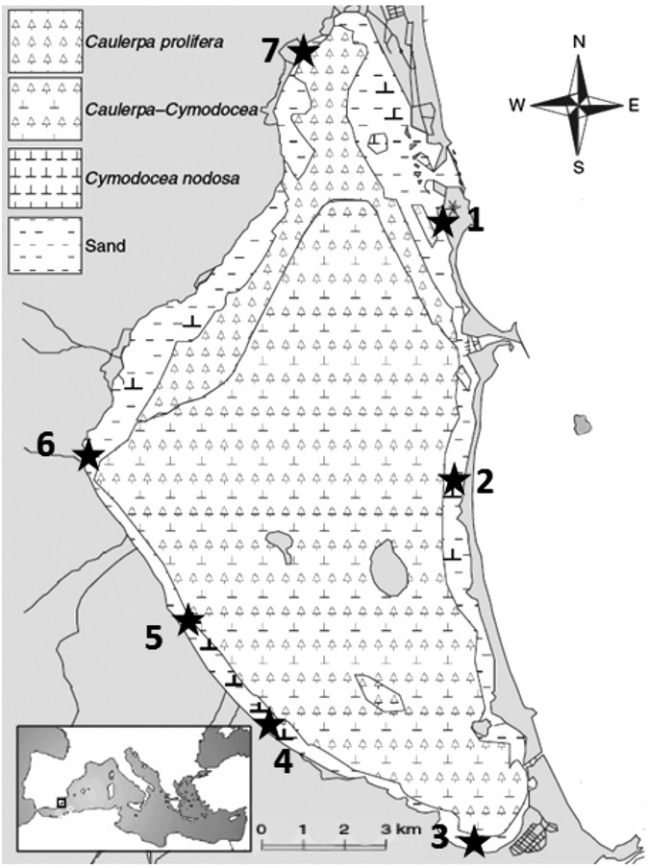

Fig. 1. Habitat distribution and sampling sites in Mar Menor, SE Spain.

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sparse patches of *Cymodocea nodosa* (Ucria) Asch (Pérez-Ruzafa et al., 1989, 2008) (Fig. 1).

The lagoon maintains a diverse fish community and supports important commercial fisheries, primarily of Anguillidae (*Anguilla anguilla*), Sparidae (*Sparus aurata* and *Diplodus* spp.), Mugilidae (*Mugil cephalus* and *Liza* spp.) and Atherinidae (*Atherina boyeri*) (Pérez-Ruzafa et al., 2005a; Andreu-Soler et al., 2006). Moreover, shallow littoral areas of the lagoon are highly productive and serve as nursery and feeding grounds for numerous fish species (Oliva-Paterna et al., 2006; Verdiell-Cubedo, 2009).

2.2. Sampling methodologies

Sampling was carried out in 7 stations located in the shallow waters along the lagoon's shore (Fig. 1). Sandy bottoms characterized all sites with the presence of patches of seagrass vegetation, except for sites 4 and 6, located at the mouth of El Beal and El Albujon watercourses, respectively. Stations 4 and 6 have muddy bottoms and small patches of *R. cirrhosa* were present in site 4. In each station fish were sampled in two habitats, vegetated by seagrass (when available) and unvegetated, and using three sampling methodologies: visual census (VC), fyke net (FN) and seine nets (SN). The details on the sampling methodologies (including three types of seine nets differing both on net structure and on the way the net was hauled) are provided in Table 1. Water depth was also recorded at each sampling occasion.

2.3. Data analysis

Fish were identified and their abundance standardized as catch per unit effort (CPUE), i.e. number of individuals per 100 m^2 sampling area for visual census and seine nets, and number of individuals per trap per day for fyke nets.

Fishes were allocated to 8 different size classes according to their body size: <1 cm, 1–2 cm, 2–3 cm, 3–5 cm, 5–10 cm, 10–20 cm, 20–50 cm, >50 cm. Fish species were also allocated to functional groups according to their habitat use and feeding modes, following the classification of Franco et al. (2008). The balance among relative abundance of the habitat use and feeding groups in the fish assemblage (namely, ecological and trophic balance) was calculated following the method proposed by Jordan and Vaas (2000), varying in theory between 1, when only one functional group is present, and $\sqrt{n}$ when the abundance is evenly distributed among n functional groups composing the fish assemblage.

Data were analysed by a factorial design with habitat and sampling method as fixed factors through the distance-based pseudo-F statistics, Permanova (9999 permutations, using Bonferroni's correction for multiple comparisons). The interaction between the two factors was tested first, and, if interactions exist then comparisons among methods were performed for each habitat level, and those between habitats were carried out for each method. In case of no significant interactions, we considered the main effects of the two factors overall (Underwood, 1997). Such a procedure was applied for both multivariate and univariate analyses, by using Bray–Curtis similarity and Euclidean distance matrices, respectively.

Multivariate analysis was performed on the fish assemblage species composition (presence-absence) and structure (% abundance), on the functional group structure (% number of taxa and % abundance per guild), and on the body size structure(% of fish abundance per size class).

Univariate analysis was performed i) on the total number of taxa, ii) on the total CPUE (FN excluded, given the different effort measure), iii) on the ecological and trophic balance, iv) on individual functional groups, both in terms of % number of taxa and % abundance, and v) on % abundance of size classes. Regarding the total number of taxa, its relationship with sampling area and water depth was explored by linear regression analysis, for seine nets and visual census. As sampling area and water depth are two sources of variability which might affect the results of tests on method and habitat differences, these tests were also carried out by introducing sampling area and water depth, both log-transformed, as covariates in the Permanova analysis, excluding fyke net samples. Simper analysis was also carried out on multivariate matrices to help identifying the taxa characterizing the catches from different habitats and of different sampling methods, and principal coordinate analysis (PCO) on similarity matrices was conducted to visualize Permanova and Simper significant results.

These analyses were done using the Primer 6 software package (Clarke and Warwick, 2001). Data will be presented as means, and standard deviation will be used as a measure of data dispersion.

Table 1
Main characteristics of the sampling methodologies applied in Mar Menor lagoon. Q4

Method Code	Method characteristics	Deployment/sampling	Sampling stations
VC	Visual census Transect method (1 m width × 50–75 m length, depending on vegetated patch size)	2 scuba divers × 2 replicated transects	All available habitats (vegetated, unvegetated) in all sites except for sites 4 and 6 (due to high water turbidity)
FN	Fyke net One trap, lead net of 18 m length, two 8 m long folded wings forming the first chamber of the trap Mesh size: 6 mm (stretch mesh)	Net deployed perpendicularly to the shore 2 replicates (min 75 m apart) Sampling duration 21–24 h	Site 3 (vegetated habitat) Sites 5 and 6 (unvegetated habitat) Site 1 (vegetated and unvegetated habitat, 1 replicate per habitat)
SN1	Seine net, trawl shaped 12 m length × 2 m height Mesh size: 6 mm on the wings, 2 mm on the central bag	Net fastened on the shore 2 replicate hauls parallel to the shore Sampling area 120–400 m^2*	All available habitats (vegetated, unvegetated) in all sites except for site 4 (unvegetated habitat only was sampled)
SN2	Seine net, bag-like type 12 m length × 2 m height Mesh size: 2 mm	Net not completely stretched during the hauling (forming a bag in the centre) Net fastened in the water, on the habitat 2 replicate hauls parallel to the shore Sampling area 63–140 m^2*	All available habitats (vegetated, unvegetated) in all sites except for vegetated habitat in site 4 (1 haul only, due to low vegetation coverage)
SN3	Seine net 10 m length × 2 m height Mesh size: 2 mm	Net fastened towards the shore, where the catches are retrieved 2 replicate hauls parallel to the shore Sampling area 160 m^2*	All available habitats (vegetated, unvegetated) in all sites

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3. Results

3.1. Overall analysis

A total of 38 taxa were sampled in Mar Menor shallow habitats, with a minimum of 16 (VC), and a maximum of 24 (FN) (Table 2). Fish were identified at the species level in most of cases, except for all the grey mullets recorded by visual census and some of those sampled with fyke nets (indicated as Mugilidae n.i.). Due to this difference in the taxonomical classification level allowed by the different sampling methods, all the analyses based on taxonomical identities were performed by grouping together all mugilids (Mugilidae).

3.2. Fish assemblage taxonomic richness and composition

The mean number of taxa in the samples increased significantly from VC (mean 3.2 ± 1.7 S.D.) to FN (8.4 ± 2.4), with intermediate values in seine net samples (pseudo-F = 22.71, $p < 0.001$) (Fig. 2). A significant main effect was also observed between habitats (pseudo-F = 7.21, $p < 0.01$), with a higher mean number of taxa recorded in vegetated than in unvegetated habitat (5.6 ± 1.9 and 4.4 ± 2.5, respectively). For all SN types and VC, the total number of taxa was related to sampling area ($n = 114$, beta = 0.40, $F = 21.66$, $p < 0.001$) and water depth ($n = 115$, beta = −0.44, $F = 27.42$, $p < 0.001$).

As regards sampling area, a significant interaction between habitat and sampling method occurred (pseudo-F = 2.67, $p < 0.05$), hence the method effect was explored in the two habitats. Significant differences were detected in both habitats (Vegetated: pseudo-F = 48.04, $p < 0.001$; Unvegetated: pseudo-F = 140.6, $p < 0.001$), with VC showing the lowest sampling area (62.5 m^2 in both vegetated and unvegetated habitat), and SN3 the highest (160 m^2 in both vegetated and unvegetated habitat). Habitat differences were detected only for SN2 (pseudo-F = 3.01, $p < 0.01$), with sampling area higher in unvegetated than in vegetated habitat (136.9 and 108.3 m^2, respectively). As regards water depth, the method effect only resulted significant (pseudo-F = 41.48, $p < 0.001$), with the highest mean value in VC (97.5 cm) and the lowest one in SN3 (50.2 cm). When including sampling area and water depth as covariates in the analysis of total number of taxa (excluding FN samples, as no area and depth measures were available for this case), no sensible changes were detected for the pattern among sampling methods (pseudo-F = 8.64, $p < 0.001$) and between habitats (pseudo-F = 29.64, $p < 0.001$).

As regards species assemblage composition, the sampling method effect was highly significant (pseudo-F = 5.18, $p < 0.001$), with Mugilidae and *Sarpa salpa* distinguishing the records of VC

Table 2
Species sampled in unvegetated (U) and vegetated habitat (V) by using the different sampling methods (visual census, VC; fyke net, FN; three types of seine nets, SN1, SN2 and SN3). Species allocation to habitat use and feeding mode functional groups is also indicated (residents, R; marine migrants, MM; marine stragglers, MS; strictly benthivores, Bv; detritivores, DV; herbivores, HV; planktivores, PL; hyperbenthos-zooplancton feeders, HZ; hyperbenthos-fish feeders, HP; fish showing an ontogenetic change in feeding preference from HZ to HP, HZ-HP, or from microbenthos to HP, Bmi-HP; omnivores, Ov).

Family	Species	Habitat use	Feeding modes	VC		SN1		SN2		SN3		FN	
				U	V	U	V	U	V	U	V	U	V
Anguillidae	Anguilla anguilla	MM	HP									X	X
Apogonidae	*Apogon imberbis*	MS	–									X	
Atherinidae	*Atherina boyeri*	R	HZ	X	X	X	X	X	X	X	X	X	X
Blenniidae	*Lipophrys dalmatinus*	R	OV		X		X	X	X	X	X	X	
	Salaria pavo	R	OV	X	X	X	X	X	X	X	X	X	X
Callionymidae	*Callionymus pusillus*	R	Bv	X		X		X					
	Callionymus risso	R	Bv	X	X	X		X	X				
Carangidae	*Trachinotus ovatus*	MS	–							X			
Clupeidae	*Sardina pilchardus*	MM	PL							X	X		
Cyprinodontidae	*Aphanius iberus*	R	Bv			X		X	X	X	X		
Engraulidae	*Engraulis encrasicolus*	MM	PL		X	X	X	X			X	X	X
Gobiidae	*Gobius cobitis*	R	Bmi, HP		X		X		X		X		X
	Gobius niger	R	Bmi, HP	X	X	X	X	X	X	X	X	X	X
	Pomatoschistus marmoratus	R	Bv	X	X	X	X	X	X	X	X	X	X
Labridae	*Symphodus cinereus*	R	Bv		X	X	X	X	X		X		X
	Symphodus ocellatus	R	Bv		X								
Moronidae	*Dicentrarchus labrax*	MM	HZ, HP			X							X
	Dicentrarchus punctatus	MM	HZ, HP							X			
Mugilidae	*Liza aurata*	MM	DV							X	X	X	
	Liza ramada	MM	DV			X	X	X	X	X		X	
	Liza saliens	MM	DV			X	X	X	X	X	X	X	
	Mugil cephalus	MM	DV			X	X	X	X	X	X	X	X
	Mugilidae n.i.	MM	DV	X	X							X	
Mullidae	*Mullus barbatus*	MM	Bv	X								X	X
	Mullus surmuletus	MM	Bv		X			X	X		X		
Poeciliidae	*Gambusia holbrooki*	R	OV									X	
Pomatomidae	*Pomatomus saltatrix*	MS	–					X				X	
Soleidae	*Pegusa impar*	R	Bv									X	
	Solea senegalensis	MM	Bv			X		X				X	
	Solea solea	MM	Bv								X		
Sparidae	*Diplodus annularis*	MM	OV				X						
	Diplodus puntazzo	MM	OV			X	X	X	X	X	X		
	Lithognathus mormyrus	MM	Bv			X		X					
	Sarpa salpa	MM	HV		X		X						X
	Sparus aurata	MM	Bv									X	X
Syngnathidae	*Syngnathus abaster*	R	Bv		X	X	X	X	X	X	X	X	X
	Syngnathus acus	R	Bv				X						
	Syngnathus typhle	R	HZ						X		X	X	

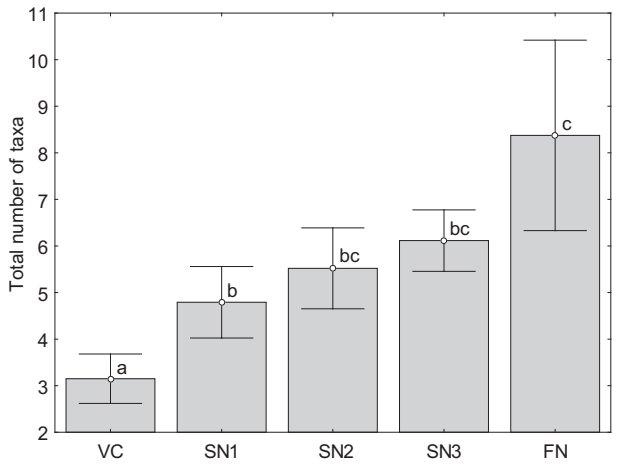

Fig. 2. Mean number of taxa sampled by the different methods (visual census, VC; fyke net, FN; three types of seine nets, SN1, SN2 and SN3). Vertical bars denote 0.95 confidence intervals; results of pair-wise comparisons are reported by using letters.

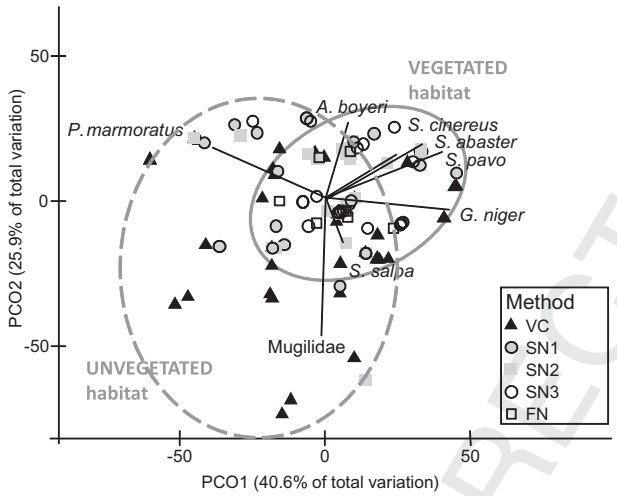

Fig. 3. Principal coordinates ordination plot of the samples, according to their species assemblage composition. Symbols distinguish sampling methods (visual census, VC; fyke net, FN; three types of seine nets, SN1, SN2 and SN3), while large circles distinguish habitats (vegetated and unvegetated). Vectors represent the main species affecting the observed differences, according to SIMPER analysis.

from the catches of SN, where *A. boyeri*, *Syngnathus abaster*, *Pomatoschistus marmoratus*, *Salaria pavo* and *Symphodus cinereus* were more frequent (Fig. 3). Also the habitat effect was significant (pseudo-F = 13.42, $p < 0.001$), with *S. pavo*, *Gobius niger*, *S. abaster* and *S. cinereus* characterizing the fish samples from vegetated habitat, and *P. marmoratus* and Mugilidae spp. typifying assemblages from unvegetated habitat (Fig. 3).

3.3. Fish assemblage taxonomic CPUE and structure

Given the different measure of sampling effort for FN (number of traps and deployment time) with respect to the other methods (sampling area), total CPUE values were expressed with different measurement units (Table 3). Hence comparisons on the mean total catches were carried out excluding data from FN sampling. No significant interaction or habitat effect, were detected on the mean total CPUE. In turn, a significant difference among methods occurred (pseudo-F = 4.05, $p < 0.01$), with VC showing lower fish abundance than seine nets, particularly SN2 and SN3 (Table 3).

Fish assemblage taxonomical structure (based on species % abundance) showed a significant interaction between habitat and method factors (pseudo-F = 1.95, $p < 0.05$). In the vegetated habitat a significant difference among methods was detected (pseudo-F = 5.34, $p < 0.001$), with VC differing from all the other methods for the higher contribution of *G. niger*, *S. salpa* and Muglidae (the latter with respect to seine nets only) to the overall catches, and the lower relative abundance of *S. abaster* (Fig. 4). A significant difference resulted also between SN1 and SN3, with higher relative abundance of *S. pavo*, *G. niger* and Mugilidae in SN1, and of *S. abaster* and *A. boyeri* in SN3 (Fig. 4). In turn, no differences were detected in the unvegetated habitat. When considering habitat differences, highly significant results ($p < 0.001$) were obtained for VC and all SN types, with higher relative abundance of *G. niger*, *S. pavo*, *S. abaster* and *S. salpa* in the vegetated habitat, and of Mugilidae and *P. marmoratus* in the unvegetated one. No habitat difference was detected in FN samples.

3.4. Habitat use functional group structure

Three functional groups were identified according to species habitat use: residents (R), including those species spawning in the lagoon, where they maintain stable populations; marine migrants (MM), including marine euhaline species (i.e. spawning at sea) entering the lagoon on a regular basis mainly for feeding and shelter; marine stragglers (MS), including marine stenohaline species entering the lagoon on an occasional basis.

Most of taxa (>50%) were resident in the lagoon (e.g. *A. boyeri*, *G. niger*, *S. abaster*, *P. marmoratus*), whereas very few taxa were marine straggles (<4%; *Apogon imberbis*, *Trachinotus ovatus*, *Pomatomus saltatrix*) (Fig. 5a). No differences among sampling methods were detected, whereas a significant habitat effect was observed (pseudo-F = 5.88, $p < 0.05$), mainly ascribed to the lower contribution of marine taxa (either migrants and stragglers, $p < 0.05$) and the higher contribution of residents ($p < 0.05$) to the total species richness in vegetated than in unvegetated habitat (Fig. 5a).

Habitat effect was highly significant also when considering the functional groups % abundance (pseudo-F = 12.72, $p < 0.001$), mainly due to the higher values of % abundance of residents and the lower values for marine migrants in vegetated than unvegetated habitat ($p < 0.001$ for both groups) (Fig. 5b). Functional structure differed significantly also among sampling methods (pseudo-

Table 3
Mean CPUE (±S.D.) of catches from different sampling methods (visual census, VC; fyke net, FN; three types of seine nets, SN1, SN2 and SN3) and habitats (vegetated and unvegetated). The number of sampling replicates (n) is reported in brackets.

Method	Measurement unit	Unvegetated	Vegetated	Total mean
VC	no. indiv 100 m^{-2}	22.7 ± 23.9 ($n = 20$)	54.9 ± 44.6 ($n = 20$)	38.8 ± 38.9 ($n = 40$)
SN1	no. indiv 100 m^{-2}	108 ± 173 ($n = 10$)	40 ± 20.1 ($n = 14$)	79.7 ± 135.1 ($n = 24$)
SN2	no. indiv 100 m^{-2}	73.2 ± 79.9 ($n = 11$)	131.8 ± 66.5 ($n = 14$)	99 ± 78.6 ($n = 25$)
SN3	no. indiv 100 m^{-2}	115.4 ± 147.3 ($n = 12$)	101.3 ± 50.9 ($n = 14$)	108.9 ± 111.7 ($n = 26$)
FN	no. indiv $trap^{-1}$ day^{-1}	1888.8 ± 2958.5 ($n = 4$)	571 ± 782.9 ($n = 6$)	1394.6 ± 2375.3 ($n = 10$)

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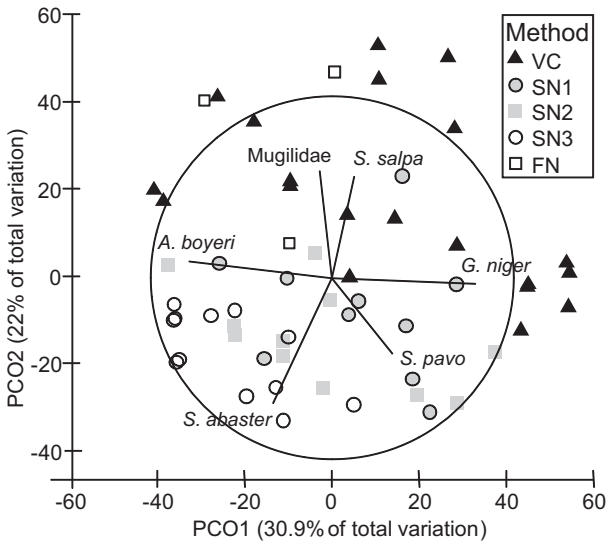

Fig. 4. Principal coordinates ordination plot of the samples taken in vegetated habitat, according to their species assemblage structure. Symbols distinguish sampling methods (visual census, VC; fyke net, FN; three types of seine nets, SN1, SN2 and SN3) and vectors represent the main species affecting the observed differences, according to SIMPER analysis.

F = 2.63, $p < 0.05$), particularly between SN2 and FN, due to the lower % abundance of resident species in FN catches (Fig. 5b).

No significant differences were detected in terms of ecological balance either between habitats or among methods, with an overall mean value of this variable of 1.17 ± 0.14, the theoretical range of variability of this parameter being 1–1.7.

3.5. Feeding mode functional group structure

Nine groups were identified according to the feeding preferences of the resident and migrant species only: detritivores (Dv); herbivores (Hv); planktivores (PL, feeding mainly on zooplankton); strictly benthivores (Bv), grouping together micro- and macro-benthivores; hyperbenthos-zooplancton feeders (HZ); hyperbenthos-fish feeders (HP); fish showing an ontogenetic change in feeding preference, from HZ to HP (HZ-HP) or from microbenthos to HP (Bmi-HP); omnivores (Ov).

Benthivorous (Bv) and hyperbenthivorous species (either HZ, HP, HZ-HP or Bmi-HP) were dominant in the fish assemblage in terms of both species number (71%) and abundance (65%) (Fig. 6). Highly significant differences were detected in the assemblage composition between habitats (pseudo-F = 7.07, $p < 0.001$) and among sampling methods (pseudo-F = 2.71, $p < 0.01$). The habitat effect was mainly ascribed to the lower contribution of detritivores ($p < 0.01$) and benthivores (particularly for VC samples, $p < 0.01$), and the higher contribution of benthos, hyperbenthos and fish feeders ($p < 0.01$) and of omnivores (particularly for VC and SN1 catches, $p < 0.001$) to the overall species richness in vegetated than in unvegetated habitat (Fig. 6a). Differences among sampling methods, in turn, were mainly ascribed to planctivores (more represented in FN catches than in SN2 ones, $p < 0.05$), to benthivores (particularly in vegetated habitat, where this group was less represented in VC records, $p < 0.05$), to hyperbenthos-fish feeders (more represented in FN samples, $p < 0.05$), and to omnivores (more represented in SN3 than in VC records, $p < 0.05$) (Fig. 6a).

As regards the trophic functional structure of fish assemblages, a highly significant interaction was detected between habitat and sampling method factors (pseudo-F = 2.95, $p < 0.001$). Habitat differences were observed for all sampling methods, except for FN. Such differences were mainly ascribed to detritivores (more represented in unvegetated than vegetated habitat, $p < 0.05$), strictly benthivores (more represented in unvegetated habitat for VC records, $p < 0.01$, and in vegetated habitat in SN3 and SN2 catches, $p < 0.001$), benthivores to hyperbenthos-fish feeders and omnivores (both more represented in vegetated habitat for VC, SN2 and SN1 samples, $p < 0.05$) (Fig. 6b). Differences among sampling methods were detected in vegetated habitat only (pseudo-F = 4.90, $p < 0.001$), and were mainly ascribed to strictly benthivores (more represented in SN2 and SN3 catches than in other methods, $p < 0.001$), benthivores to hyperbenthos-fish feeders (with lower relative abundance in SN3 (4%) than in VC and SN2 samples (33 and 20% respectively), $p < 0.01$) and omnivores (more represented in SN1 (33%) than in VC and SN3 samples (9 and 7% respectively), $p < 0.01$) (Fig. 6b).

As regards trophic balance, significant differences were detected both between habitats (pseudo-F = 17.82, $p < 0.001$) and among sampling methods (pseudo-F = 5.17, $p < 0.001$). A higher trophic balance resulted in vegetated (1.74 ± 0.21) than in unvegetated habitat (1.50 ± 0.28), and in SN2 (1.74 ± 0.21) and SN3 catches (1.60 ± 0.20) than in VC records (1.48 ± 0.32), where the minimum balance was observed, the theoretical range of variability of this parameter being 1–3.

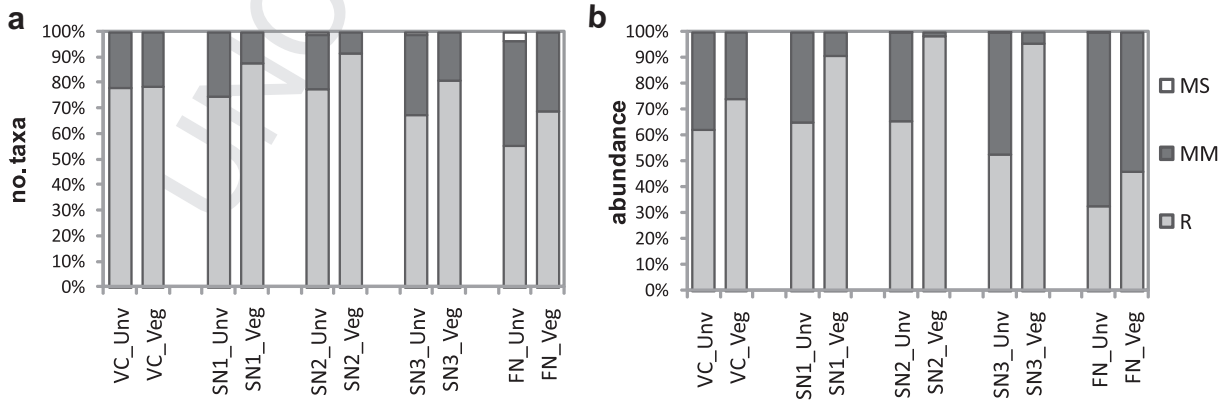

Fig. 5. Functional composition (average % number of taxa) (a) and structure (average % abundance) (b) in terms of habitat use functional groups (R, residents; MM, marine migrants; MS, marine stragglers) in catches from different habitats and sampling methods. X axis reports sampling method (visual census, VC; fyke net, FN; three types of seine nets, SN1, SN2 and SN3) by habitat (vegetated, Veg; unvegetated, Unveg).

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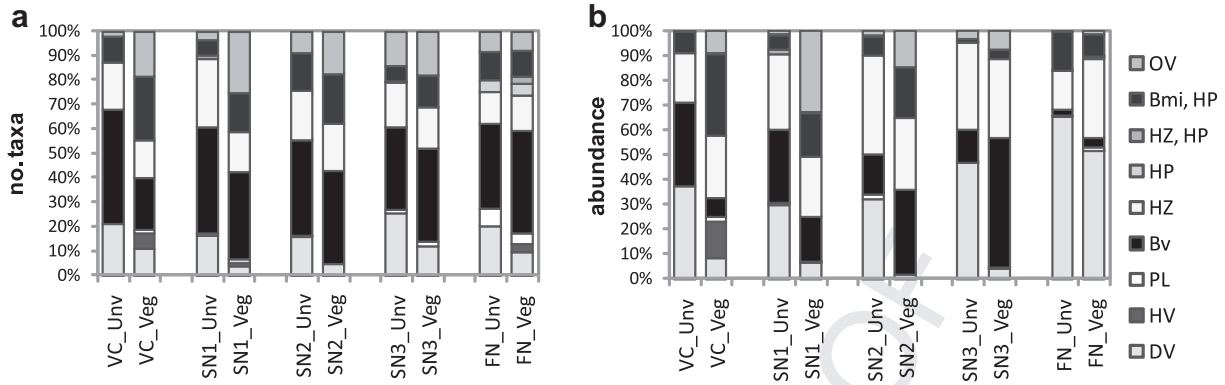

Fig. 6. Functional composition (average % number of taxa) (a) and structure (average % abundance) (b) in terms of feeding mode functional groups in catches from different habitats and sampling methods (DV, detritivores; HV, herbivores; PL, planctivores; Bv, benthivores; HZ, hyperbenthivores-zooplanctivores; HP, hyperbenthivores-piscivores; OV, omnivores). X axis reports sampling methods (visual census, VC; fyke net, FN; three types of seine nets, SN1, SN2 and SN3) by habitat (vegetated, Veg; unvegetated, Unveg).

3.6. Size structure

Intermediate-higher size classes (from 2 to 20 cm) dominate fish assemblages in the samples (over 80%), with significant differences in the overall size structures observed between habitats (pseudo-$F = 8.53$, $p < 0.001$) and among sampling methods (pseudo-$F = 6.20$, $p < 0.001$). Habitat differences were mainly ascribed to the higher average % abundance of smaller size classes in unvegetated habitat, namely classes 1–2 cm ($p < 0.01$ in SN3 catches only) and 2–3 cm ($p < 0.01$), and of size class 5–10 cm in vegetated habitat ($p < 0.05$ for all sampling methods except for FN catches, where no differences between habitats were detected) (Fig. 7).

Differences among sampling methods were ascribed to four size classes: i) 1–2 cm in unvegetated habitat ($p < 0.01$), where the highest average % abundance, around 35%, was detected in SN3 catches, and the lowest, <2%, in SN2 catches, whereas the other methods showed intermediate values; ii) 3–5 cm, with a higher % abundance, around 50% on average, in SN2 catches ($p < 0.001$); iii) 5–10 cm in vegetated habitat ($p < 0.05$), where SN1 and FN showed the highest % abundances, 57% and 55%, and SN3 the lowest values, about 13%, and in unvegetated habitat ($p < 0.001$), where FN catches showed the highest average % abundance, around 50%, than the other methods, all <15%; iv) 10–20 cm ($p < 0.001$), more represented in VC records, with an average value of 28%, than in the other catches, where average values were lower than 5%. Also larger size classes, 20–50 cm and >50 cm, contributed to differentiate sampling methods and habitats, due to their presence in FN (both classes) and VC samples (20–50 cm size class only), and their higher % abundance in unvegetated habitat (Fig. 7).

4. Discussion

The choice of the sampling method is one of the most important steps to be undertaken when planning a study on nekton assemblages in coastal and transitional environments (Elliott and Hemingway, 2002). The importance of the sampling method consists in acquiring as accurate and precise information as possible on the species assemblage (Rozas and Minello, 1997).

The selection of a fish sampling method is also critical in coastal and transitional environments, as these ecosystems are dynamic and complex due to the interaction of marine and freshwater. In these environments, shallow water habitat provides spawning and/or nursery area, foraging opportunities and facilities and refuge from predation for fish species (Deegan et al., 2000; Elliott and

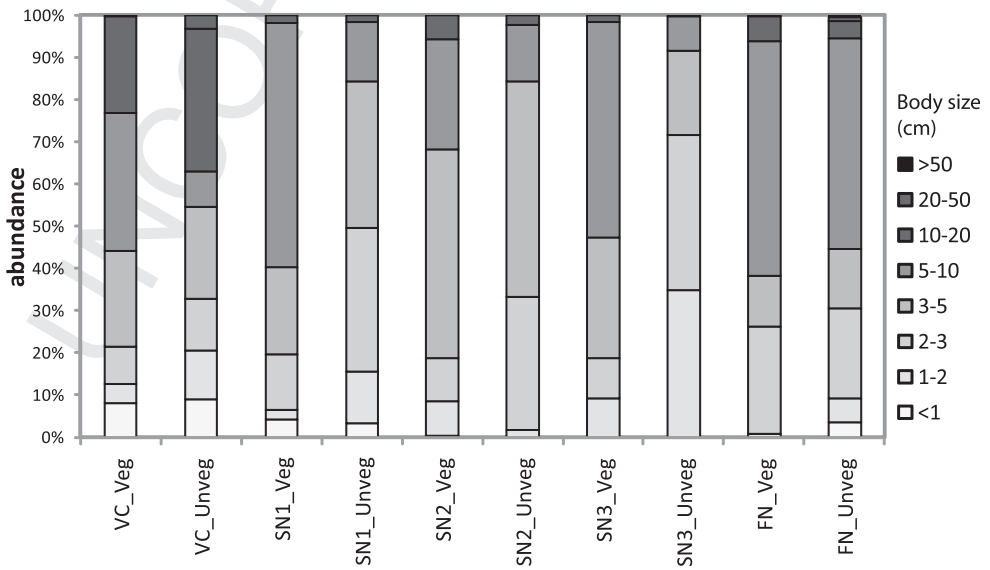

Fig. 7. Size structure (% abundance by size class, measured in cm) of fish assemblages sampled in different habitats (vegetated, Veg; unvegetated, Unveg) and by different sampling methods (visual census, VC; fyke net, FN; three types of seine nets, SN1, SN2 and SN3).

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Hemingway, 2002). Several habitats can be identified in coastal and transitional environments, each one of them being characterized by peculiar biotic and abiotic features, and supporting also functionally different fish assemblages (Mathieson et al., 2000; Elliott and Hemingway, 2002; Franco et al., 2006, 2008).

All fish sampling methods used in coastal and transitional environments are selective in some degree, and the catch efficiency of any sampling gear changes in response to several factors, such as the species present in the area, their behaviour and body size, and the habitat type. In particular, a single sampling gear cannot be used in all the habitats present in these ecosystems, and, even if it can be used in different habitats, its catch efficiency changes significantly with the habitat type (Elliott and Hemingway, 2002). Hence, the choice of the sampling methodology must take into account the aims of the study, as well as the characteristics of the habitat being surveyed.

Structural and functional attributes of fish community have been widely employed to monitor the ecological quality of estuarine or transitional water ecosystems (Deegan et al., 1997; Whitfield and Elliott, 2002; Harrison and Whitfield, 2004, 2006; Breine et al., 2007; Coates et al., 2007; Delpech et al., 2010). Most of fish based methods employed to assess the ecological status of coastal and transitional water ecosystems relies on an array of metrics, as species richness and diversity measures, fish abundance, composition or structure of the fish assemblage in ecological or trophic guilds (Bilkovic et al., 2004; Harrison and Whitfield, 2004; Franco et al., 2009). The choice of sampling method should then be aimed at providing a proper measuring of these metrics. Furthermore, the optimal sampling method should allow to relate catches to a measurable sampling area (or equivalent sampling effort), should be easy to use and have a low variability in catch efficiency (Elliott and Hemingway, 2002). In particular, the sampling gear should have comparable catch efficiency in the main habitat types present in coastal and transitional environments.

Although several studies on transitional water fish assemblages have employed different sampling methods, few of them compared different methodologies (Elliott and Hemingway, 2002). In particular, no comparative studies have been carried out before in Mediterranean coastal or transitional water environments. In the present study five different sampling methods (underwater visual census, fyke nets and three different types of seine nets) were used for sampling fish assemblages in Mar Menor lagoon. Furthermore, each of these methods was used in two shallow water habitats with depths lower than 2 m, either non vegetated (sand or mixed mud-sand bottom) and vegetated (bottom covered by seagrass meadows, mainly *Cymodocea*). These are among the most frequent and common habitats in Mediterranean coastal lagoon environments (Franco et al., 2008).

Significant differences were detected among methods, both regarding taxonomical and functional aspects. Visual census and fyke nets detected more easily pelagic species, whereas seine nets targeted more efficiently benthic-demersal species. Differences in fish body size were also detected, with visual census and fyke nets allowing the sampling of larger fish (>5 cm), whereas seine nets catches were dominated by 2–10 cm size classes. Differences were detected also among habitats, with fish size structure skewed towards larger size classes in vegetated habitat with respect to unvegetated one. This could be the result of the abundance of pipefish (characterized by an elongated body shape) in seagrass habitat, particularly for seine net catches, and of the lower visibility of smaller fish in the vegetation as regards visual census record. However, this is not valid for the 2 top-high size classes (>20 cm), recorded only in fyke net and visual census catches (i.e. *A. anguilla, Dicentrarchus labrax*), with higher abundance in unvegetated habitat.

The observed structural differences among habitats seem to reflect the different fish assemblages associated to vegetated and unvegetated habitats in coastal lagoons and transitional waters (Mathieson et al., 2000; Franco et al., 2006, 2008; Franzoi et al., 2010). Different habitats support also functionally different assemblages. Seagrass vegetation provides a relatively stable habitat for highly specialized residents (e.g. pipefish) and a feeding ground for strictly benthivorous fishes (e.g. *S. abaster*, *S. cinereus*) as well as for hyperbenthos-zooplankton (e.g. *Syngnathus typhle*) and fish feeders (e.g. *G. niger*). Unvegetated shallow areas provide a habitat suitable to smaller sized fishes, either juveniles or small sized species, better supporting nursery for marine migrants and providing important benthic and detritus food sources (Franco et al., 2009). However, fyke net seems not to catch this habitat variability, since no habitat differences for this method in terms of taxonomical, trophic or size structure were observed, and not surprisingly, given the functioning of such sampling method. It is designed to catch high efficiency moving fishes, as migrants, thus integrating the assemblages of a wider area and is not strictly representative of the habitat where the fyke net was deployed. Moreover because the fyke nets were deployed for 24 h the catch is representing day and night fish activity. However, the wider scale at which this net operates with respect to visual census and seine nets makes this gear a good sampling method for assessing the lagoon as a whole, but without considering habitat differences. Seine nets and visual census techniques, in turn, allow investigating fish assemblages at a finer spatial scale, hence distinguishing habitats, but giving site specific results leading to a major difficulty in catching the overall wider picture.

Underwater visual census methods (VC) include different sampling techniques (transects, point counts, rapid visual censuses, timed counts) (Harvey et al., 2004), and all can be referred to a sampling area. VC is mainly used in coral reefs (Barans and Bortone, 1983; Bohnsack and Bannerot, 1986; Kingsford, 1998), but is widely used also in the Mediterranean coasts (Harmelin-Vivien et al., 1985; Francour, 1997; García-Charton et al., 2000; García-Charton and Pérez-Ruzafa, 2001). As already underlined in reef habitats (García-Charton et al., 2000), VC is a highly site and habitat specific technique which has the advantage of being non extractive, thus reducing the "environmental costs" (in terms of fish killed) and being particularly desirable in protected areas. Contrarily to the other sampling methods tested in this work, VC is the only one that can provide information on fish assemblages also from hard bottom habitats (e.g. rocky bottoms, oyster and/or mussel bed, dams or breakwaters) and at depths higher than 2 m. In addition, VC allows integrating observations at different spatial scales (micro-, meso- and macrohabitat) (Elliott and Hemingway, 2002). VC is also cost effective in terms of both money and time saving, in that it requires limited equipment and personnel compared to other methods, and it is also low time consuming in the post-processing of the samples as identification of species and body size recording are performed at the same sampling time. However, VC also has limitations and several sources of bias and error (Harmelin-Vivien et al., 1985; Harvey et al., 2002). In coastal lagoons, visual census techniques are difficult to apply at very shallow areas (lower than 0.5 m) where some species reach higher densities. Furthermore, VC is highly dependent on water quality and has limitations with cryptic species (mainly Syngnathids and in very dense vegetated areas). When visibility is reduced, the detection of fish requires very short distance between the diver and the fish, giving it the opportunity to escape before being detected. At the same time, with low species richness and fish density, large sampling areas are required to cope with the organisms' spatial variability. From the point of view of size structure, VC shows the most balanced distribution being relatively more effective than the

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nets in detect very small fishes (<1 cm), which can escape through the mesh as well as larger fishes (20–50 cm) that can avoid seine nets. Very large fishes are however underestimated due to their scarcity and sampling area limitations. Also medium-low sized fishes appear underestimated by this method with respect to seine net catches, particularly when considering those nets with a smaller mesh size (SN2 e SN3), hence suggesting that the apparent most balanced size distribution of the catches in visual census records might not be representative of the effective size distribution of the lagoon fish assemblage. VC seems also to have underestimated fish species richness and total fish abundance, particularly with respect to the fry beach seines, SN2 and SN3, whereas the comparison with fyke net is not possible in this case. Furthermore, VC did not allow the identification of certain taxa at the species level (e.g. Mugilidae). This limit, though not having an effect on the sampled assemblage functional structure, as the different mugilid species belong to the same ecological (marine migrants) and trophic groups (detritivores), influences the species richness and the number of taxa of the analysed functional groups. In addition, the different species of Mugilidae are characterized by different tolerances and preferences towards abiotic conditions (temperature, salinity, granulometry), which affect their distribution in coastal and transitional environments (Lasserre and Gallis, 1975; Cardona, 2000; Koutrakis, 2004; Maio et al., 2004; Mićković et al., 2010).

Beach seine net is considered to be a very effective technique for sampling in shallow waters, especially in lagoon ecosystems (Pierce et al., 1990; Říha et al., 2008). The efficiency of seine nets has been studied by various authors who ascribed it to the species and size composition of a fish community, and to the environmental conditions of the littoral area (Lyons, 1986; Parsley et al., 1989; Pierce et al., 1990; Allen et al., 1992; Holland-Bartels and Dewey, 1997; Bayley and Herendeen, 2000). Moreover, it is also pointed out that seine technique and design affects the efficacy of this method (Lyons, 1986). Říha et al. (2008) pointed out that the main disadvantage of the seine net is that the use of seining requires specific conditions such as a bottom surface with a slight slope but without obstructions like extensive amounts of mud or submerged macrophytes. The seine nets, if compared with visual census and fyke net, resulted particularly efficient in sampling the fraction of lagoon fish assemblages made up of small lagoon resident species (i.e. *A. boyeri, P. marmoratus, Aphanius iberus, S. abaster, S. pavo*) and juvenile marine migrants. In turn, the catch efficiency of these nets is very low when size classes >10 cm are considered, if compared with the other methods. Significant differences were also detected among different seine nets. Although fish abundance (CPUE) did not differed among beach seines overall, lower abundance could be detected in SN1 catches in vegetated habitat. This seine net also resulted to be the least selective towards fish body sizes <10 cm, whereas no differences between seines could be observed in terms of average species number. Moreover, differences observed in size structure among seine nets may be related to differences in water depth hauling, since catches of smaller size classes (1–2 cm) were higher in SN3 than in the other seine nets. This pattern could be ascribed to the capture of very small individuals of mugilid species which form shoals that stay close to the shore at very shallow waters (<10 cm depth).

The results of this study confirm the high relevance of the choice of sampling methodology which should be based on the targeted scale at which the analysis will be carried out. When considering monitoring programmes fulfilling the WFD requirements, this choice should take into account the assessment method that will be used (depending on the weight of the metrics included in it) as well as the management designs applied by the different Member States with particular regard to water bodies identification in lagoon basins. Hence, it is anticipated that the use of fyke net sampling will be more suitable particularly whereas the whole lagoon basin has been assigned a single water body, as for example French lagoons. In these cases, the assessment is carried out at an integrative, larger scale, either temporal or spatial (Delpech et al., 2010). In turn, seine netting and visual census (where allowed by turbidity conditions) are anticipated as more suitable sampling methods for the ecological status assessment of coastal lagoons whereas water bodies have been identified at a smaller spatial scale than the whole lagoon basin, including different water body types. This is the case, for example, of the Venice lagoon, Italy, with its 5 water body types and 14 water bodies. Given fish mobility across different water bodies within the lagoon, the use of fishing gear operating across large spatial scales, as fyke nets, may not allow measurement of fish metrics specific to a single water body. Furthermore, the uneven distribution of seagrass vegetation among water bodies, even of a same type, might bias the use of a single type-specific reference condition and assessment tool, hence the utility of habitat specific sampling methods and assessment tools (like those proposed by Franco et al., 2009).

Unlike other biological quality elements, the ecological quality assessment methods based on fish fauna highly rely on specific sampling methodologies, which may vary locally, particularly among different Mediterranean lagoon areas. Different assessment tools are applied (e.g. Franco et al., 2009; Uriarte and Borja, 2009; Delpech et al., 2010), which account for the shortcomings of the different sampling methodologies, although not avoiding completely the related potential bias in the assessment of fish assemblages. The use of different tools, combined with the different management designs applied by Member States highlighted above, leads to the need of harmonising the ecological status assessment in the area. The present study represents a first step in this intercalibration process in Mediterranean lagoons, which, far from relying on the sharing of existing databases, like for some other biological quality elements (e.g. benthos), will need the application of an approach similar to that one undertaken in this study (i.e. the simultaneous application of different sampling and assessment methods to a same water body), like it was undertaken for intercalibration of North East Atlantic region fish indices in estuaries.

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