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An evaluation of sampling methodology for assessing settlement of temperate fish in seagrass meadows

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Abstract

All demersal fish with planktonic larvae settle at some point early in life, generally around the transformation from larvae to juveniles or soon after. Sampling pre-settlement or very young, settled fish is challenging due to spatial concretions within the habitat and the pulsed, rapid nature of the settlement process. There is a lack of robust sampling methods, but information on the settlement, which represents a mortality bottleneck, is crucial for the follow-up of populations for fisheries and conservation purposes. Empirical evaluation of sampling methods focusing on settling fish has not been conducted in temperate habitats. Here, we compare six different sampling methods to collect pre- and post-settlement stages of fish and determine the best combination of techniques to utilise in *Posidonia oceanica*, an endemic Mediterranean seagrass that provides a key nursery habitat for coastal fish. We used three types of pelagic nets (bongo net, neuston net and ring net), two types of light-traps (Quatrefoil and Ecocean CARE®) to sample pre-settled stages and a low-impact epibenthic trawl for recent settlers. Our results show a significantly different size-spectrum for each method, with a continuous range of sizes from 2 mm to 200 mm. The smallest sizes were collected by the bongo net, followed by the ring net, the neuston net, the Quatrefoil, the Ecocean and finally the epibenthic trawl. Our results suggest that an appropriate strategy for collecting and estimating the abundance of key littoral fish species around settlement size is the combination of the Ecocean light trap and the epibenthic trawl.

Keywords: Fish settlement, *Posidonia oceanica,* fish sampling, Mediterranean Sea, light traps, epibenthic trawl.

Introduction

The prominent role of seagrasses as nursery areas (i.e. juvenile habitats that contribute with more fish recruits per unit habitat to the adult population than the average juvenile habitats used by the species, Beck *et al.,* 2001) has been demonstrated for temperate zones worldwide (Rozas & Minello, 1998; Guidetti & Bussotti, 2000). Despite this key ecological role, methods that provide robust estimates of the nursery function are lacking. A key bottleneck in the survival of most fishes associated to seagrasses is settlement (Levin, 1994), which refers to the shift from the pelagic stages to the (relatively sedentary) benthic stages (Reñones *et al*., 1995; Jenkins *et al*., 1998; Vigliola *et al*., 1998; Leis, 2006; Alós & Cabanellas-Reboredo, 2012). The settlement separates largely different ecophysiological processes including mortality drivers and rates (Robertson *et al*., 1988; Holbrook & Schmitt, 2002). These rates are most variable around settlement in temperate species (Nash & Geffen, 2012). Knowledge about the effect of spatial/temporal settlement variability on fish population dynamics is crucial for fisheries management and conservation (Victor, 1986; Doherty & Fowler, 1994), but the spatial and temporal processes that determine settlement patterns in coastal benthic habitats are still poorly understood (Hixon, 2011; Nash & Geffen, 2012; Félix-Hackradt *et al*., 2013a, b), principally due to a lack of robust sampling methods (Carassou *et al*., 2009).

Conventional methods for sampling pre-settlement fish (pelagic late-larvae) concentrate on plankton and neuston nets of different mesh size, but when operated during daylight, they tend to subsample pre-settlement phases (Leis, 1982; Chícharo *et al*., 2009), which have a high avoidance of towed nets (Brander & Thompson, 1989). For this reason, there are few instances (large nets, night/fast tows) when they are effectively used to sample fish close to the settlement phase. Other sampling methods based on light-traps (Doherty, 1987) have been used extensively in the last two decades to sample fish just before settlement. Different light-baited trap designs (Secor & Hansbarger, 1992; Marchetti *et al*., 2004; Kehayias *et al*., 2008; Vilizzi *et al*., 2008; Nakamura *et al*., 2009) have been tested in past years to improve sampling efficiencies. However, most of these studies have been conducted in coral reefs, where fish diversity and behaviour are thought to account for the high catches in traps compared to temperate areas (Hickford & Schiel, 1999). Some recent studies in the Mediterranean have also demonstrated the efficiency of one type of light-based device (Lecaillon *et al*., 2012; Félix-Hackradt *et al*., 2013a, b). However, in temperate areas there are no comparative studies exploring the effectiveness of such methods versus classical methods (e.g. net-based). Light traps are known to underestimate the abundance of early larval stages and taxonomic diversity (Hickford & Schiel, 1999; Chícharo *et al*., 2009) by attracting individuals that have considerable swimming abilities and show positive phototaxis. One advantage of light traps is that they collect developmental phases close to settlement (henceforth, pre-settlers), thus providing empirical links between egg production and recruitment (including spatial resolution) that overcome the large uncertainty attributed to pelagic mortality of larval fishes.

Methods for collecting fish just after settlement range from manual collection techniques (Raventós & Macpherson, 2005; Strydom, 2008; Fontes *et al*., 2010; Félix-Hackradt *et al*., 2013a), such as hand-operated epibenthic sleds (e.g. Rooker *et al*., 1998) to experimental beam trawls (e.g. Deudero *et al*., 2008). Although scuba-based visual censuses have provided invaluable information with a high degree of spatial resolution, the spotting/collection of samples using divers may be inefficient as regards the detection of early settlers in seagrass meadows with high canopies or when large areas must be sampled (Franco *et al*., 2012). The use of hand-operated net devices (i.e. beach seines, lift nets, epibenthic light sleds) tends to lack spatial resolution at greater depths and is not useful when sampling large distances and collecting large sample sizes. Experimental light beam trawls have been used to analyse coastal demersal assemblages over *Posidonia oceanica* meadows but, in general, do not capture the smallest size fractions that have just settled in benthic habitats (Deudero *et al*., 2008).

The proven inadequacies of the current methods used to sample individuals on either side of the settlement period led a recent study in tropical areas (Carassou *et al*., 2009) to suggest that an optimal approach should combine different sampling methods. The use of two combined methods has been successfully used for sciaenids in seagrasses of subtropical areas where they appear in relatively large concentrations (Herzka *et al*., 2002), but the potential use of a combined approach is lacking for *P. oceanica* meadows. Approximately 300 species inhabit the littoral zone in the Mediterranean, 100 of which live in shallower than 50 metres waters and are associated with seagrass meadows (Whitehead *et al*., 1986; Reñones *et al*., 1995). Most of these species settle a few metres below the surface in well-defined habitats (Macpherson & Zika, 1999; Bussotti & Guidetti, 2011), usually in spring and summer (Tsikliras *et al*., 2010; Bussotti & Guidetti, 2011). The objective of this study was to compare six different sampling methods (three pelagic nets, two light traps and one epibenthic trawl) in terms of different criteria (size spectra, abundance, usefulness of the information provided) to serve as a foundation for the design of future surveys requiring the sampling of both pre- and post-settlement temperate littoral fish.

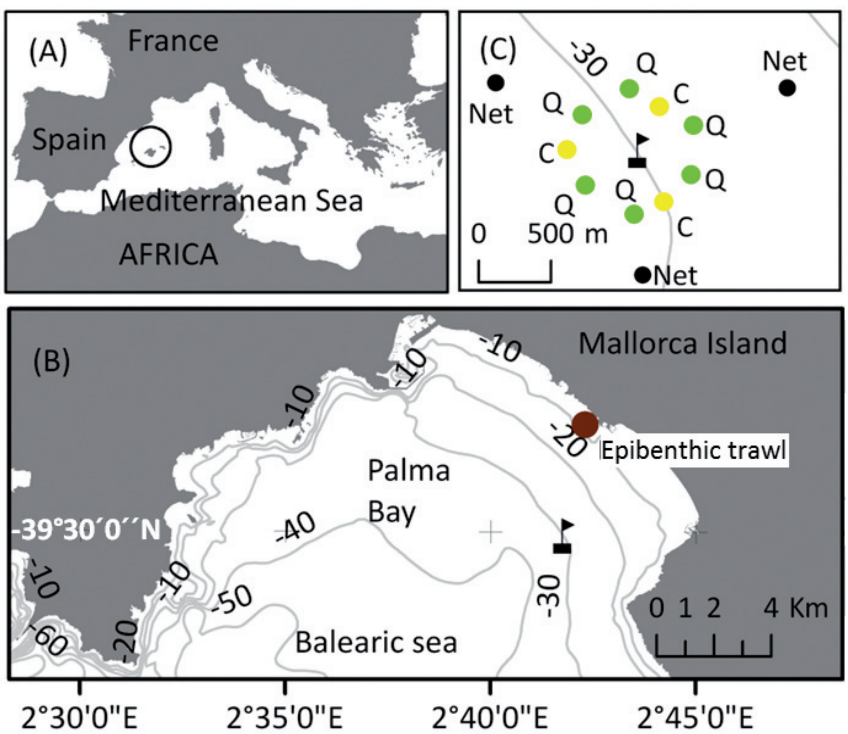

Fig. 1: Sampling area and field design. (A), general location. (B), position of the oceanographic buoy (flag) and light-weight epibenthic trawl area (dot). In (C), the position of the traps and net tows, where Net=net sampling (see text). C, Ecocean light trap; Q, Quatrefoil light trap.

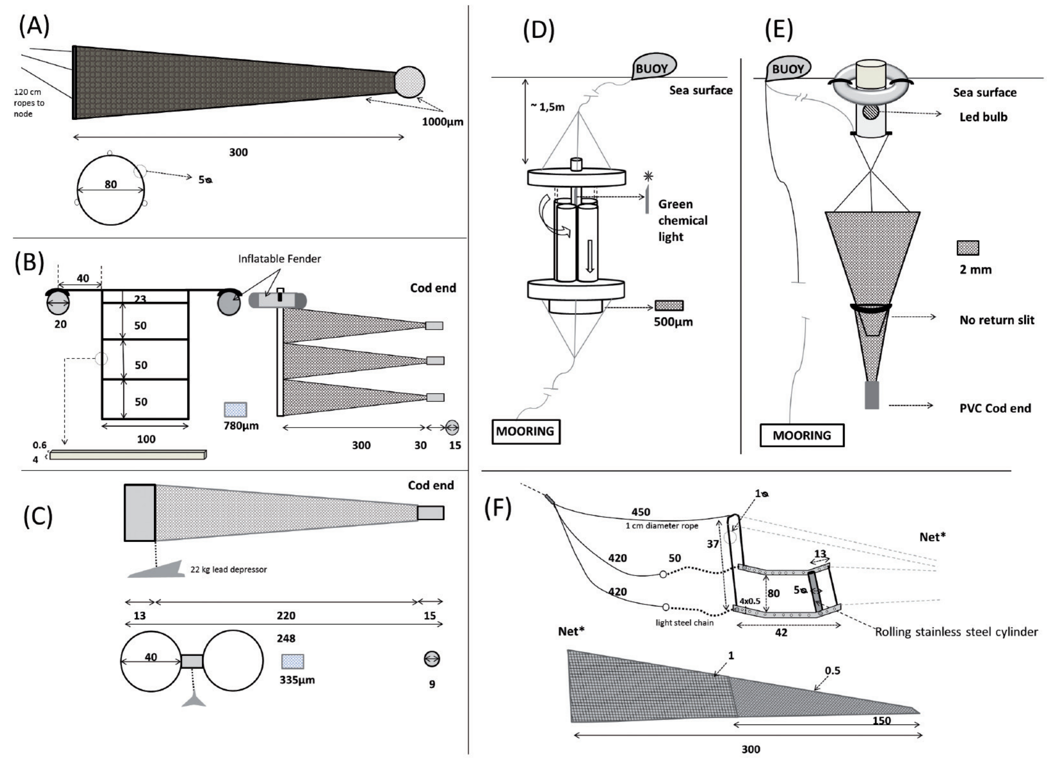

Fig. 2: Capturing methods employed. (A), ring net. (B), neuston net. (C), bongo net. (D), Quatrefoil light trap (modified from Floyd *et al.,* 1984). (E), Ecocean CARE® trap. (F), Epibenthic trawl. All measurements are in cm unless otherwise specified. Lengths, weights and materials of D and E are specified in their original description (see material and methods). Collectors B and C are made of PVC and the frames of A, B, C and F are made of stainless steel, all weighing <20 kg. All nets are made of Nytex nylon except for F, which is diamond polyester.

Materials and Methods

This study was conducted in Palma Bay (Mallorca Island, NW Mediterranean, Fig. 1). Palma Bay is characterized by bottom habitats dominated by sea grass meadows of *P. oceanica* (in areas shallower than 35 m depth) mixed with rocky and sandy bottoms and is a typical example of an open Mediterranean Bay disconnected from riverine inputs. We optimised our sampling design so that each set of methods (six) was tested as simultaneously as possible (all devices used in the same night in a precise area) to minimise the known daily variability in pre-settlement pulses. The whole sampling set was repeated twice (see further in the text). Pelagic sampling for pre-settlement stages was conducted around a fixed oceanographic buoy anchored within a *P. oceanica* meadow at ca. 27 m depth. This is close to the minimum depth recommended by the design of some of our light-based devices (Ecocean, see further) to avoid possible interferences of a visible seafloor in the choice of a settling individual. Post-settlement sampling was also conducted over *P. oceanica* in a nearby area (Fig. 1 A).

Sampling procedures: Pre-settlement

Pre-settlement fish were sampled using five different methods; three types of pelagic-nets towed from a 12 m boat and two types of light-traps (Fig. 2). Sampling was performed during two nights in May (24-25th) and June (13-14th) 2012, coinciding with the spawning/recruitment peak for many littoral species in the area (Tsikliras *et al*., 2010; Álvarez *et al*., 2012). To optimise the catch, sampling was performed as close as possible to the new moon period (Milicich, 1994). The first pelagic net was an 80 cm diameter ring net equipped with a 1000 µm mesh built for the collection of relatively developed larval fish (Fig. 2 A). Each sampling night, one circular tow around each replicated net station (Fig. 1 C) was taken just below the surface for approximately 20 min. The second net was a neuston net (Fig. 2 B) in which three vertical levels were discriminated (0-0.5 m, 0.5-1 m and 1-1.5 m) but pooled for the purpose of this work. The towing operation was the same as for the ring net. Additionally, a 40 cm bongo net (Fig. 2 C) was deployed in oblique stratified hauls from 15 m to the surface lasting approximately 12 min (equal time stops at bottom, 10 m and subsurface). In all cases, a flowmeter (General Oceanics, GO) was mounted in the centre of the nets, and the number of fish collected was standardised to the number of individuals per cubic metre. In addition to the pelagic nets, two types of light-traps were used. The first type was a Quatrefoil light-trap (Floyd *et al*., 1984), illuminated with a green chemical light stick, as recommended by Kawamura *et al*. (1996) (Fig. 2 D). Six of these traps were moored to the bottom and positioned at the surface around the oceanographic buoy at distances over 250 m apart to avoid light contamination among traps (Fig. 1C). The second type was the Ecocean CARE® (hereforth “Ecocean”, Fig. 2 E) trap. Each Ecocean trap consisted of a buoyant watertight block containing a 12 V battery and a 55 W 90 LED light, under which a 2 m conical net of 2 mm mesh size with a narrow mesh funnel in the middle was attached vertically (Lecaillon, 2004). This trap is based on the pre-settlers’ tendency to search for a substratum (the illuminated net) at settlement time, which impels them to explore the illuminated mesh. These traps were anchored around the oceanographic buoy no closer than 250 m to each other and to the Quatrefoil traps (Fig. 1C). Both types of light traps were left overnight for a minimum of 7 hours and collected the next morning before sunrise. Captures were referred to as effective sampling hours: an effective sampling hour was assumed to be between 30 minutes after sunset and 30 minutes before sunrise. The operational procedure for pre-settlement sampling (for one given night) consisted of *i*) light-trap deployment, *ii*) net towing (3 methods x 3 sampling points) during the night and *iii*) trap-recovery at dawn. All fish samples were preserved in 4% formaline buffered with sodium tetraborate. Fish were brought to the laboratory, photographed, identified to the lowest possible taxonomic level and measured for length using ImageJ software (Schneider *et al*., 2012).

Sampling procedures: Post-settlement

Post-settlers were collected during two consecutive nights just after the pre-settlement sampling period: May (26-27th) and June (15-16th) 2012. Fish were collected at night in shallow water (3-6 m depth, Fig. 1B) over *P. oceanica, interspersed with small sand patches, using* two identical experimental light-weight (ca. 10 kg) epibenthic trawls (Fig. 2 F) towed in parallel with a mouth diameter of ca. 80 cm. Both nets were composed of two parts separated by a no-return conical mesh. The boat described an ellipse of 400-700 m at a speed of approximately 1.1 knots for a minimum of 20 min. For each sampling tow, the catches of both nets were combined. Three tows (consisting of two nets each) were undertaken in May and five tows in June due to weather constraints in May. Each paired tow was conducted at nearby positions, and the data from both nets within a given position was combined. We excluded the use of other post-settlement collection methods (e.g. scuba-based) due to the night-based design and obvious lack of visual resolution. Samples were frozen at -20°C, and abundances were standardised to square metres of tow. Individuals were processed for length measurements as described above.

Data analysis

For each method, the relative abundance (% N) was calculated as the percentage contribution of a given taxon to the total number of individuals from all taxa caught by a given method. The relative occurrence (% O) of each taxon was calculated as the number of samples in which a given taxon was caught relative to the total number of samples for a given method (Table 1). Two species (*Scorpaena porcus* and *Syngnatus acus*) were excluded from the analyses as only adults were captured. The capture properties of each method were explored through the comparison of total catches and standardised catches (to volume, surface or time units). Due to the presence of zero captures in some gears and the limited number of replicates (usually 3) per sampling date, comparisons of the above descriptors were performed through inspection of quartile ranges per method and date. For the epibenthic trawl, the towed area (m^2) was calculated using the beam trawl opening size and distance towed.

The multimodal distribution of sizes of some methods (even after $\log_{10}$ transformation) and the lack of a balanced experimental design precluded the use of parametric techniques. Therefore, comparisons of pooled sizes were performed through non-parametric tests (Mann-Whitney (M-W), Kruskall-Wallis (K-W)) followed by multiple comparisons of mean ranks according to Siegel & Castellan (1988). In some comparisons, the sampling date (of the two possible) was not considered as a factor due to insufficient sample size for some methods and a descriptive approach was adopted. To analyse the multivariate contribution of sizes, taxa and yield by each method, a series of matrices (*i*: presence-absence data, *ii*: fourth-root transformed abundances and *iii*: percentage of captures standardised by sample) was first built on the samples vs. size-structured taxonomic composition. Each selected taxon (see Table 1) was subdivided into 5 length classes corresponding to *i*) newly hatched larvae (<3 mm standard length, SL), *ii*) larger larvae with some (assumed) degree of swimming abilities (3-6 mm SL), *iii*) individuals with a high probability of being close to settlement (6-12 mm SL, (Harmelin-Vivien *et al*., 1995; Vigliola & Harmelin-Vivien, 2001; Ishihara & Tachihara, 2011;), *iv*) early settlers (12-30 mm SL) and *v*) larger individuals. Samples with no data were discarded. We first compared the three distance-matrices (Bray-Curtis (B-C) similarity) through permutation-based rank correlation analyses and found that the multivariate structure remained exactly the same (*Rho* values >0.99 in all cases, permutations n=10000). The latter result suggested that size and taxa composition were responsible for the multivariate structure, and relative or absolute abundance provided almost no information for final ordination. We thus tested the possible differences between sampling methods using a permutation-based approach (permANOVA coupled with a multivariate dispersion analysis through *Betadisper* and *Adonis* functions in package *vegan*, R, permutations=4999, Oksanen *et al*., 2013.). We selected a fourth-root transformed abundance B-C dissimilarity

Table 1. Relative abundance and relative occurrence (in each cell, %N; %O) for each taxon and gear type over the sampling period. For each gear, the five most representative taxa in % N and % O are represented in bold. *: Taxon excluded from the multivariate analysis (see Materials and Methods). **: abundance data not available. NI: not identified, YSL: Yolk-sac larvae.

		Pelagic nets			Light traps		Trawl
Order&family	**genus/species**	**Bongo**	**Ring**	**Neuston**	**Quatrefoil**	**Ecocean**	**Epibenthic trawl**
Aulopiformes							
Paralepididae	*Lestidiops jayakari*	0.1; 16.7					
Clupeiformes							
Clupeidae	*Sardinella aurita*	0.8; 66.7	1.4; 33.3	**5.9**; **50.0**			
Engraulidae	*Engraulis encrasicolus*	1.5; **100.0**	**2.7**; **66.7**	**5.1**; **66.7**			
Gadiformes							
Gadidae	*Gaidropsarus mediterraneus*						1.1; 6.7
	NI		0.7; 16.7				
Gobiescociformes							
Gobiescocidae	*NI*	2.0; 66.7	0.7; 16.7				
Perciformes							
Bleniidae	*NI*	1.9; 83.3	0.7; 16.7	3.4; 50.0		**17.7**; **83.3**	
Callionymidae	*Callionymus spp.*	**2.1**; **100.0**	0.7; 16.7	**5.1**; **50**			
Carangidae	*Trachurus mediterraneus*	0.6; 50.0	0.7; 16.7				
	Trachurus trachurus					**26.5**; **66.7**	
Cepolidae	*Cepola sp.**	0.1; 16.7					
Gobiidae	*Gobius ater*						2.2; 13.3
	Gobius NI						1.1; 6.7
	Pomatochistus spp.						**16.5**; **33.3**
	NI	**46.0**; **100.0**	**54.8**; **83.3**	**30.5**; **66.7**			2.2; 13.3
Labridae	*Coris julis*	0.5; 16.7					
	Symphodus melops						4.4; 6.7
	Symphodus ocellatus						2.2; 13.3
	Symphodus roissali						**9.9** ; **33.3**
	Symphodus rostratus						**9.9**; **20.0**
	Symphodus tinca						2.2; 13.3
	Symphodus spp.	**2.9**; 83.3	**3.4**; **50.0**	0.9; 16.7			3.3; 13.3
	NI	1.8; 33.3		1.7; 33.3			
Mullidae	*Mullus barbatus*		0.7; 16.7	0.9; 16.7			1.1; 6.7
	Mullus surmuletus				**25.0**; 8.3	**14.7**; **16.7**	
	Mullus spp.	0.4; 50.0					
Pomacentridae	*Chromis chromis*						3.3; 20.0
Serranidae	*Serranus hepatus*	0.5; 33.3		0.9; 16.7			
	Serranus scriba						**11.0**; **40.0**
	Serranus scriba/cabrilla	1.7; 33.3					
Sparidae	*Diplodus annularis*						**15.4**; **53.3**
	Diplodus vulgaris						8.8; 26.7
	Pagrus pagrus		0.7; 16.7				
	NI	**11.5**; **100.0**	**24.0**; **100.0**	**28.8**; **83.3**	**75.0**; 25.0	**39.7**; **50.0**	
Trachinidae	*Trachinus draco*	0.6; 66.7					
Myctophiformes							
Myctophidae	*Hygophum sp.**	0.1; 16.7					
	Ceratoscopelus maderensis	0.3; 33.3					
	Myctophum punctatum			0.9; 16.7			
	NI						1.10; 6.7
Ophidiformes							
Ophididae	*Parophidion vassali**						2.2; 13.3
Pleuronectiformes							
Bothidae	*Arnoglossus sp*	0.6; 50.0		0.9; 16.7			
Soleidae		0.1; 33.3		3.4; 33.3			
Scorpaeniformes							
Scorpaenidae	*Scorpaena porcus*						**
Stomiiformes							
Gonostomatidae	*Cyclothone spp.*	0.8; 50.0	0.7; 16.7	2.5; 33.3			
Sternoptychidae	*Maurolicus muelleri*	0.1; 16.7		0.9; 16.7			
Syngnathiformes							
Syngnathidae	*Hyppocampus sp.**	0.1; 16.7	0.7; 16.7	1.7; 33.3			
	*Syngnathus acus**						**
YSL	*NI*	**21.1**; **100.0**	1.4; 16.7	0.9; 16.7			
NI	*NI*	1.8; 66.7	**6.2**; **83.3**	1.7; 33.3		**1.5**; **16.7**	

matrix and used the factor "method" as fixed variable. We excluded the Quatrefoil from this analysis due to low sample number. Furthermore, we excluded the factor "period" due to possible lack of independence between samples in the epibenthic trawl. We used Cluster analysis (Unweighted Paired Group Method with Arithmetic Mean, UPGMA) and non-metric Multidimensional Scaling (nMDS) to explore further the multivariate structure with respect to methods and periods. We defined broad groups from the cluster following permutational tests (SIMPROF test through 10 000 permutations, $p<0.05$, Clarke *et al*., 2008). The resulting groups were analysed for species composition and species contribution to within-group similarities through SIMPER analysis (Clarke & Ainsworth, 1993) using Primer v.6, which was also used for ordination methods.

Results

Taxonomic composition

Overall, the 62 samples included 1894 individuals from 24 families, from which 10 genera and 27 species could be discerned (Table 1). The bongo net caught 1467 individuals (77.46%), the ring net 146 individuals (7.71%), the neuston net 118 individuals (6.23%), the epibenthic trawl 91 individuals (4.80%), the Ecocean light-trap 68 individuals (3.59%) and the Quatrefoil trap 4 individuals (0.21%). Only two families, Sparidae and Mullidae, were caught by all six methods. Generally, the most abundant species (as the five highest % N) were also the most frequently (the five highest % O) caught (Table 1). Gobiidae was the most abundant family in the towed devices, whereas Sparidae was the most abundant in the light traps. In general, the light traps produced the lowest number of taxa (two at family level, two at species level and unidentified specimens), with only 50% of the catches being classified to species level. Pelagic nets, in contrast, caught the highest number of taxa (14-20 families), and 60-70% could be classified with higher precision. The highest precision in the classification was attained in the epibenthic trawl, with over 80% of the 11 families being classified to species level (Table 1).

Catch descriptors

Pelagic nets tended to catch a higher number of individuals, with the bongo net showing the highest values (Table 2). The epibenthic trawl caught approximately one order of magnitude less than the bongo net. All devices tended to yield positive samples except for the Quatrefoil light trap, which showed several zero catches in the different deployments (up to 83% of samples). In comparison, the Ecocean light trap clearly performed better than the Quatrefoil. Variability in catches between sampling periods was evident but was rather consistent between methods (e.g. all types of pelagic nets tended to catch more in May than in June), although in some cases, the values were too low to further interpret these results.

Size spectra

The size spectra of fish captured by the different methods ranged from approximately 2 mm to over 200 mm SL (Fig. 3). Significant differences in median size were detected between methods (all samples for each method pooled, K-W, $H_{5,1158} = 594.3$, $p < 0.001$). The smallest median sizes were represented by the bongo

Table 2. Descriptors of total catch (25 and 75% quantiles in number of individuals), standardized catch and percentage of zero catches per sampling set (single tow or trap) and by capture method.

Method (units)		Total catch	Standardized catch	% zero catches
Pelagic nets (standardized catch: Ind.100m^{-3})	*Neuston net*			
	May	4-55	13-15	0
	June	4-16	15-52	0
	Ring net			
	May	28-58	76-139	0
	June	8-9	20-22	0
	Bongo net			
	May	193-441	162-374	0
	June	91-253	93-295	0
Trawl (standardized catch: Ind. 100 m^{-2})	*Epibenthic trawl*			
	May	4-11	0.7-2.2	0
	June	3-6	0.5-13.2	0
Light traps (standardized catch: Ind. h^{-1})	*Quatrefoil*			
	May	<0.1-<0.1	<0.1-<0.1	83
	June	0-1	0-0.1	80
	Ecocean			
	May	2-11	0.2-1.3	0
	June	10-26	1.2-3.2	0

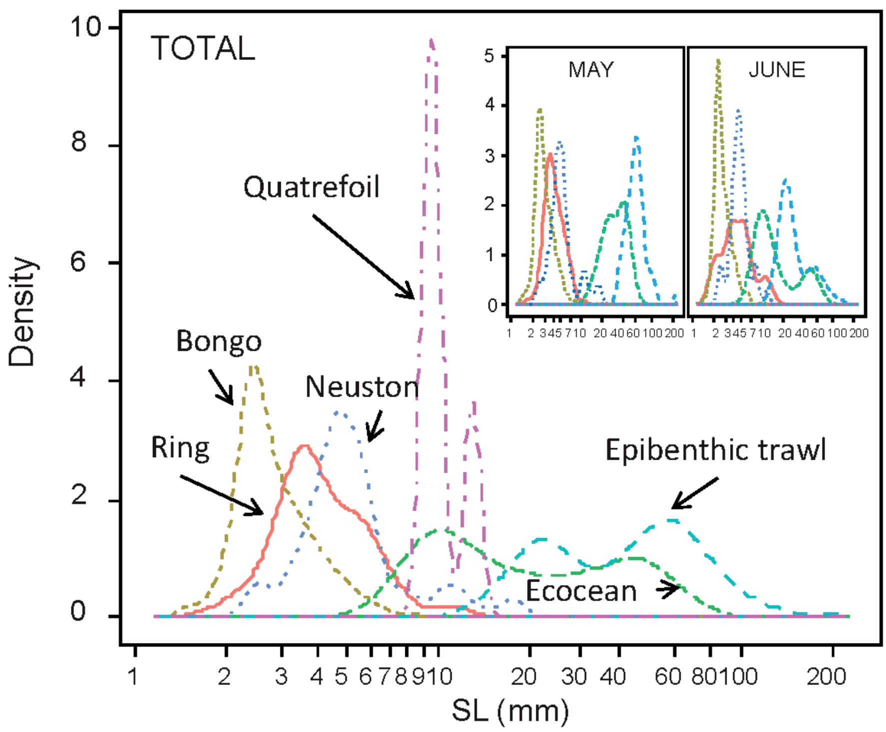

Fig. 3: Fish size distribution for each method according to an empirical kernel (density distribution of mass) based on 512 points/method (note that Density does not reflect abundance). In the main figure (TOTAL), both sampling days are pooled and disaggregated density plots (without the Quatrefoil samples) are shown in the inner panels for each period. The x axis is in log10 scale. Scorpaenidae and *Syngnathus acus* were excluded from the epibenthic trawl data set.

net (median = 2.7 mm, the only method having significantly different mean ranked sizes from any other method, post hoc rank-tests, not shown). Median size increased subsequently as follows: the ring net (median = 3.8 mm), the neuston net, (median = 4.85 mm), the Quatrefoil trap (median = 9.7 mm), the Ecocean trap (median = 16.3 mm) and the epibenthic trawl (median = 46.9 mm). Furthermore, the between-methods structure of size spectra was maintained in both the May and June samplings (Fig. 3 inner panel). The Quatrefoil trap only captured one individual in May, and it was therefore not plotted in the monthly comparison. The comparison of sizes among methods for two of the most representative teleost families inhabiting *P. oceanica* showed similar trends (Fig. 4 A). Significant differences between methods were detected for Sparidae (K-W; $H_{5,245} = 174.8$, $p < 0.001$) and Labridae (K-W; $H_{2,101} = 62.3$, $p < 0.001$). Hence, in these groups the smallest sizes were taken by the bongo net and the largest taken by the epibenthic trawl. Other groups (i.e. Gobiidae, Serranidae) followed a similar pattern.

Multivariate analyses

The multivariate permutation-based ANOVA showed that there were significant differences between methods ($F_{4,26} = 5.55$, $Pr > F < 0.001$). Multivariate dispersion analyses coupled with pairwise post-hoc tests for dispersion showed that there were significant differences in dispersion ($F_{4,26} = 3.04$, $Pr > F < 0.03$), and that the epibenthic trawl was responsible for these differences (Tukey HSD tests within *betadisper* function, *vegan*, not shown). Therefore, we repeated the analyses only for net-based methods and Ecocean trap and showed that there were no significant differences in the multivariate dispersion ($F_{3,19} = 1.82$, $Pr > F < 0.17$) and there were still significant differences between methods ($F_{3,19} = 5.38$, $Pr > F < 0.001$). The nMDS and cluster analysis (Fig. 4 B, C) evidenced a clear distinction between three broad sampling categories (epibenthic trawl, clearly different from the rest of the methods, pelagic nets and light traps). Although a finer distinction could not be formally established, the SIMPROF tests (red dashed lines in the cluster, indicating no significant structure in the station groups under their first black node, Fig. 4 C) suggested that sampling period may explain the catch structure in the epibenthic trawl and possibly in some net-based methods. In general, it was clear that the period of collection was less important than the method of collection for at least the three large groups presented in Fig.4 (B, C). We selected three main groups within the cluster at a 4% similarity. The contribution of the size-per-taxa classes to these groups analysed through SIMPER analysis (Fig. 4 C) showed the importance of size in the composition of the groups, with sparids being captured by all methods.

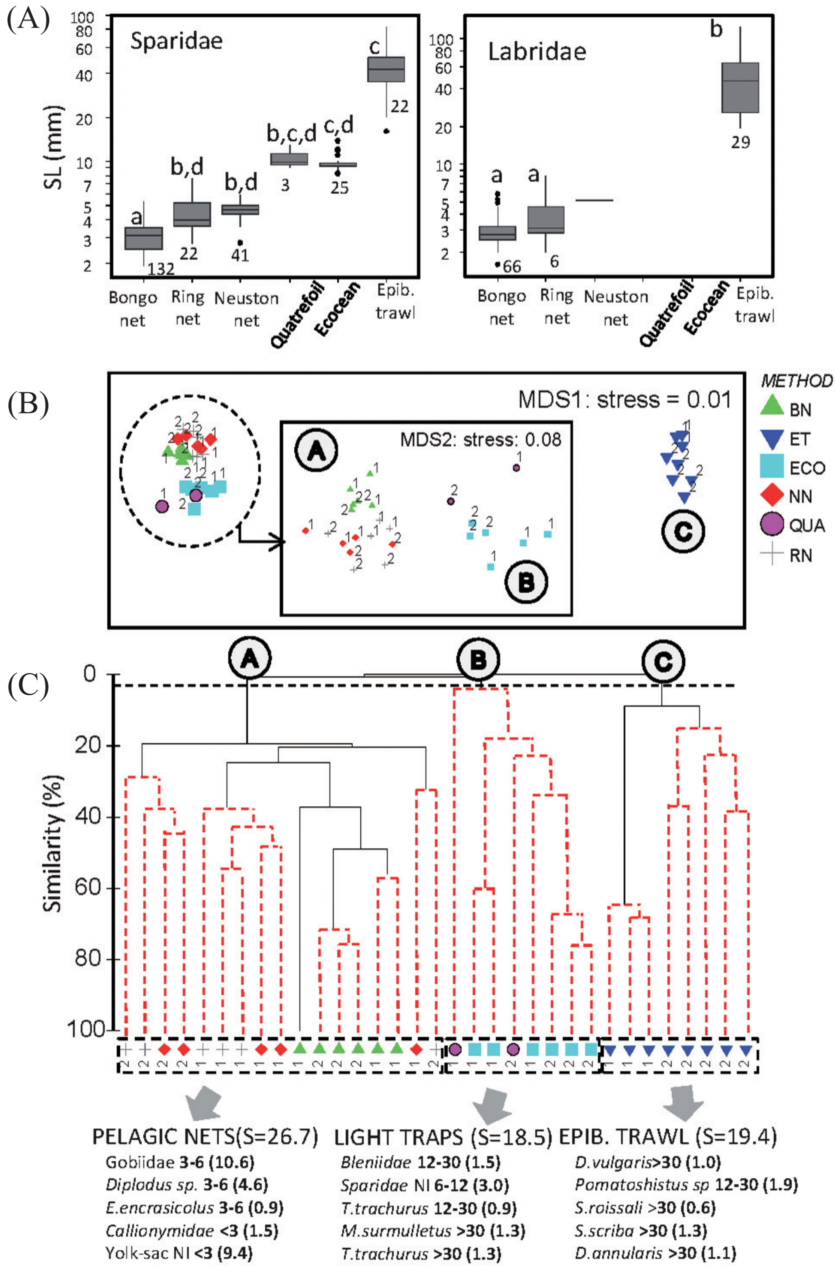

Fig. 4: (A), Example of size differences between capture methods for two abundant fish families in seagrass beds (*P.oceanica*). Both sampling dates and replicates are pooled due to low abundances (N). Within each family, a common letter among methods indicates no significant differences (after multiple K-W comparisons). (B), non-metric Multidimensional scaling of samples (MDS1). A second analysis (MDS2) was performed to show differences between pelagic nets and light traps. (C), Group average cluster (UPGMA) on the fourth-root transformed Bray-Curtis similarity matrix of taxa/sizes. Three main groups (A-C) were defined after SIMPROF test. Red dashed lines under a solid node depict groups which do not differ in multivariate structure. The top five taxa of each method in terms of their contribution to within-group similarity are shown, with indication of their size-range in mm (in bold) and average abundance (in brackets). S=average within-group similarity. Scorpaenidae and *Syngnathus acus* were excluded from the analysis. In B and C, numbers 1&2 are first and second sampling period and are assumed to be a replicate. Colour symbols are: BN, bongo net; ET, epibenthic trawl; ECO, Ecocean trap; NN, Neuston net; QUA, Quatrefoil trap; RN, ring net.

Discussion

Sampling of recent settlers has been successfully accomplished in several studies on seagrasses in subtropical areas associated with estuarine habitats, where presumably the estuarine nursery function promotes high settlement concentrations (e.g. Rooker *et al*.,1998; Herzka *et al*., 2002). Most of the information available on the early-life stages of fish inhabiting *P. oceanica* focuses on either pelagic larval stages or relatively advanced juvenile forms or adults, whereas studies on individuals who are about to settle or that have recently settled are still scarce (García-Rubíes & Macpherson, 1995; Macpherson & Raventós, 2005; Félix-Hackradt *et al*., 2013a, b). This is not due to a lack of interest (Planes *et al*., 2000) but rather to the difficulty in sampling and identifying settling individuals (Irisson & Lecchini, 2008).

Although multiple sets of our multi-gear experimental units (Fig. 1) should have been used to accommodate for spatial and temporal differences, it was logistically not possible to deploy simultaneous multi-gear sampling units at several sites. We therefore aimed to compare key factors, such as size spectra, for analysing settlement processes. Other variables such as variation in abundance, peak appearance or taxa composition of larvae, post-larvae and large settlers over time have been published separately for some of these (and other) methods used in the Mediterranean (e.g. Álvarez *et al*., 2012; Félix-Hackradt *et al*., 2013a).

Our comparative results suggest that sampling close to the settlement size (usually 1-3 cm for many species) in *P. oceanica* seagrass meadows is best achieved through the combination of Ecocean traps and small epibenthic trawls. Our work also found that precision in species identification is compromised by the lack of complete identification guides for Atlantic and Mediterranean temperate waters, especially for those stages close to settlement (many individuals collected by the light traps were difficult to identify). Overall, the present study demonstrates which methods are best suited for the collection of certain size ranges (and taxa) and provides a comparative background to design future experiments aiming to study settlement on seagrasses and other shallow coastal habitats. The size spectrum gathered by each method differs, suggesting that each method will provide different information on the settlement process, which supports the results of a recent comparative study conducted in a tropical area by Carassou *et al*. (2009). It is important to note that the size spectrum differences among methods are not only associated with scape ability but also with gear design, mesh and operational constraints. Moreover, several points of overlap in fish size exist between certain methods (e.g. Ecocean and epinenthic trawls, or Bongo and Neuston nets, Fig. 3). Nonetheless, we contend that our results on sampling methodology will be useful for the future design of sampling surveys. For example, it could be possible to follow individual fish cohorts during the pre- and post-settlement periods. Experiments of this type may provide precise estimates of growth rate and possibly indications of mortality rates. However, more information will be needed according to the specific survey objective (e.g. sampling efficiency over different depths and gear type, day-night differences between epibenthic tows).

Pelagic net sampling methods provide samples whose size structure and taxonomic composition depend on mouth diameter, towing speed, mesh size and sampling time/date (Barkley, 1972). Some of these methods such as plankton and neuston nets are known to underestimate the abundance of certain early-life stages (Choat *et al*., 1993; Chícharo *et al*., 2009). The present study supports the latter observation on littoral larval assemblages, as all three pelagic nets, including small (bongo 40) to mid-range nets (neuston nets), failed at sampling relatively large and mobile post-larvae even when towed at night at different depths (horizontal and oblique hauls) and using various mesh sizes (335 µm, 780 µm and 1000 µm, See Fig. 2). Advanced larval stages (although not necessarily from the same species) were present at that time in the water column, as revealed by the light traps. It is possible, however, that despite the relatively large volumes of water filtered through the nets, advanced stages were too sparse to be captured. Sampling with towed nets also damages individuals, which can increase the level of inaccuracy in the taxonomic classification and body metrics (Chícharo, 2009). Additionally, samples obtained using a fine-meshed collector could be large and sorting difficult due to zooplankton abundance, especially in the bongo nets. Despite the bias associated with pelagic nets, they offer invaluable information for the analysis of settlement processes, including the possibility of analysing the early dynamics of a larger number of species than other methods. For example, pelagic nets allow the identification of spawning sites and spawning stock size through the analysis of egg abundance and distribution.

In contrast to pelagic nets, light-trap devices (Quatrefoil and Ecocean) are passive sampling methods that rely on the swimming ability and the phototactic behaviour of pre-settlers, which are captured alive and relatively clean from plankton and debris (reducing sorting time). Again, identification presents a problem due to a lack of good diagnostic keys recognised elsewhere (Félix-Hackradt *et al*., 2013a). However, this method offers the possibility of rearing the captured individuals in aquaria until a clear phenotype helps in identification (Lecaillon *et al*., 2012). Our results also revealed that the Ecocean light-trap captured over one order of magnitude more individuals than the Quatrefoil light trap, which in most cases captured zero individuals (Table 2). The low efficiency of Quatrefoil light-traps could be due to various reasons including *i)* unwanted predation inside the trap, as suggested by Vilizzi *et al*. (2008), *ii)* lack of efficiency as catch relies on the chance that individuals find the slot to enter the trap (Lecaillon & Lourié, 2007) and *iii)* cases in which the chemical stick did not provide enough attracting power (Gehrke, 1994) for the species inhabiting this area compared to the battery-powered Ecocean design. The Ecocean trap combines the traditional light-trap strategy with an artificial reef. This design, proven to work in tropical regions, has been recently used to analyse settlement patterns in the SW Mediterranean (Félix-Hackradt *et al*., 2013a, b). We provide comparative evidence that this is the best performing gear of those tested in this study for capturing pre-settlement post-larvae in temperate waters.

In addition to the yield/sizes provided by each method, the captured species are one key variable to be accounted for when selecting a gear method for sampling fish just before (or after) settlement. The goal of this work was to compare methods to sample seagrass-specific species in temperate areas. The most important fish groups in *P. oceanica*, in terms of biomass are Sparidae, Serranidae and Labridae (Deudero *et al*., 2008). The Ecocean captured pre-settlers of the group Sparidae, most likely identified as the species *D. annularis* according to meristics and the time of the year (Félix-Hackradt *et al*., 2013b). Definite classification would require rearing until the species can be recognised or the use of genetic differentiation tools. Our sampling period of May and June may partly account for the absence of these species in the samples, but a longer time series using only Ecocean traps shows that Serranidae (in *P. oceanica* mainly *S. scriba*) and Labridae appear rarely (Félix-Hackradt *et al*., 2013a). Nevertheless, monthly to weekly surveys covering extensive meadows of *P. oceanica* would be required to understand the species-specific catch properties of this device. In general, the Ecocean traps caught a significant number of *Trachurus trachurus* individuals, most likely because Carangidae are phototactic and exhibit a pelagic-demersal exploratory (related to feeding) behaviour in their juvenile phase (I. Palomera pers. comm., ICM-CSIC, Barcelona). These high catches agree with the observations in the SW Mediterranean using the same device (Félix-Hackradt *et al*., 2013b). The Ecocean traps did not catch any Clupeiforms, despite this fish group being recorded in the pelagic nets. The trap is designed so that the catchability of phototactic non-settling species is low. However, one study showed that high abundances of post larval clupeiform fish have been found in the Ecocean traps in late spring-summer (Félix-Hackradt *et al*., 2013b), which sounds a note of caution as regards the claimed light trap properties. The presence of red mullet (*Mullus surmuletus*) in the light traps contrasts with their absence in the pelagic nets. Red mullet as large as 65 mm TL have been observed in the water column in previous studies (Machias *et al*., 1998; Deudero, 2002); thus, we speculate that this species either settles at a larger size than most other littoral species or, like *Trachurus* spp., uses the water column at night. An additional advantage of the Ecocean design is that, due to rapid deployment time, it allows simultaneous sampling at different locations; thus, such devices are particularly appropriate for the investigation of the spatial distribution of pre-settlers (e.g. Félix-Hackradt *et al*., 2013a).

We demonstrate, on the other hand, that the small-scale epibenthic trawl may be particularly appropriate to describe the community of settlers. Although tows were performed at night for consistency with the other methods, the diel performance of the epibenthic trawl should be tested through a specific experiment. The epibenthic trawl captured the main species inhabiting *P. oceanica* meadows and at sizes (for those with coinciding settlement periods) approximately 1 cm (particularly high catches of *D. annularis* were observed), which are representative of settlement (see references in Félix-Hackradt *et al*., 2013b). Moreover, due to its low towing speed (1 knot), the epibenthic trawl used in this study has been shown to *i*) have little impact on the seagrass, with no substrate collected in the nets and *ii*) provide live individuals (2 cm *D. annularis* survived at least 3 weeks in the lab). The high catches of *D. annularis* settlers (and very few other sparids) are an indication that this species most likely formed the bulk of the unidentified sparids in the light traps.

We contend that the use of Ecocean light traps may be particularly valuable for connectivity studies in littoral areas, as the presence of pre-settlers indicates the endpoint of both transport and behavioural processes occurring before the shift to a demersal lifestyle. Recruitment will, however, be further determined by high mortality during the first hours/days and complex density-dependent processes that may include juvenile dispersion at different scales (Almany, 2003; Doherty *et al*., 2004; Nash & Geffen, 2012). Once settled, the use of lightweight epibenthic trawling is proposed as a method for fish collection. Both methods enabled the provision of high-quality samples for both classification and further experiments using live individuals. This work is to be regarded as a foundation for future surveys investigating the settlement process associated with *P. oceanica*. However, future surveys will need to further explore the properties of these methods, including the efficiency of both gears in terms of, for example, catches at different depths and over time and the volume of water integrated by light traps.

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