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# Mediterranean juvenile white seabream rely on phytal fauna as primary food source in coastal nursery areas

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### Abstract

Nursery areas are essential fish habitats due to their relevance in the survival of early stages of fish populations. They are also considered as of high priority in marine conservation strategies. Here, we investigated the diet of white seabream [*Diplodus sargus* (Linnaeus, 1758)] settlers in six nursery areas located in the shallow waters of coves in Minorca Island (Balearic Islands, Spain). Our aim was to characterize the food sources at different stages of juvenile development and their site-related variability in order to discern the importance of trophic links in cove selection during settling. The gut contents of 101 juveniles captured at different coves, three to the north of the island (N), and three in the southern coast (S), revealed a marked preference for feeding on crustaceans and, in particular, on harpacticoid copepods (>90% of gut contents). Copepods represented the main food source ( $80 \pm 4.4\%$ ; mean  $\pm S.E.$ ) in younger seabream individuals (10 - 15 mm length). A higher diversity in prey items was observed in the larger size-classes (s2: 16 - 23 and S3: 24 - 30 mm, respectively), which incorporated other prey items such as amphipods, isopods, foraminiferans or ostracods. Diet composition did not vary between the two surveyed locations (North *vs.* South of the island), but it did show significant differences among the six coves (p<0.001). Comparison between cove sediment infaunal composition and gut contents revealed that predation on sediment communities was scarce. Instead, diet was typically of phytal origin. Our results highlight the importance of the algal component of shallow coastal areas as a foraging habitat. In particular, harpacticoid copepods were key for survival during early development phases. The potential use of harpacticoid copepods to track ontogenic shifts in habitat use by juvenile fish is discussed.

Keywords: essential fish habitats; coastal habitats; nursery habitats; fish settlement; juvenile fish diet; macroalgae; harpacticoid copepods; *Diplodus sargus*.

### Introduction

Essential fish habitats (EFHs) are considered high priority areas for conservation and management, given their fundamental role in guaranteeing important ecosystem services, including a continuous supply of food for humans, as well as ecosystem stability (Lindeman *et al.*, 2000; Ståhl *et al.*, 2007; Tugores *et al.*, 2019). Among EFHs, those acting as nursery or settlement zones in coastal areas have been recognised as paramount for the survival of many fish species (Beck *et al.*, 2001; Kraufve-lin *et al.*, 2016; Cheminée *et al.*, 2021).

Nursery habitats play a fundamental role in coastal fish recruitment (Sundblad *et al.*, 2013), since they may affect development, and can regulate the year-class strength, size, and condition of parental stocks, as well as their population size (Sale *et al.*, 1984a, b; Richards & Lindeman, 1987; Scharf, 2000). Because of their importance in a key life stage of fish populations, they should be considered priority areas for conservation. However, nursery areas and their influence in fish population dynamics are often neglected in fisheries management schemes (Rishworth *et al.*, 2015; Kraufvelin *et al.*, 2016; Cheminee *et al.*, 2017a).

Beck *et al.* (2001) define a nursery as a region where juvenile fish occur at higher densities, avoid predation more successfully, grow at a faster rate than in other habitats and so provide a greater relative contribution to adult recruitment, than other areas. Although real consensus regarding what makes a specific habitat a nursery has not been reached so far (e.g., Beck, 2001; Dahlgren *et al.*, 2006; Litvin *et al.*, 2018), it is generally agreed that the combination of suitable food for juveniles and protection from predation are central to these essential habitats (Harmelin-Vivien *et al.*, 1995; Garcia-Rubies & Macpherson, 1995; Biagi *et al.*, 1998; Kraufvelin *et al.*, 2016; Lefcheck *et al.*, 2019).

An abundant provision of food for the juvenile fish, should enable a successful settlement, and subsequent ontogenetic shifts to their adult habitats, thus guaranteeing the survival of the fish populations, and is hence an important value for a nursery habitat (Dahlgren & Eggleston, 2000; Beck *et al.*, 2001; Stål *et al.*, 2007; Abrantes *et al.*, 2015). However, despite the importance of diet studies to understand habitat preference and dependency of juvenile fish species in coastal habitats, it is only recently that dietary information is being used to describe fish-habitat associations in these essential ecosystems (Macreadie *et al.*, 2010 a, b; Jenkins *et al.*, 2011; Abrantes *et al.*, 2015; Cheminée *et al.*, 2017b; Hinz *et al.*, 2019).

Harpacticoid copepods have been found to be an important food source for many juvenile fish in most benthic habitats (Gee, 1989; Sogard, 1984; Aarnio et al. 1996; Schükel et al., 2012), and especially in phytal ones such as seagrass meadows (Tipton & Bell, 1988; Jenkins et al., 2011), algae on rocky reefs (Hinz et al., 2019), or even algal turf covering coral assemblages (Kramer et al., 2013), where they tend to be the dominating invertebrate taxon (Hicks & Coull, 1983; Arroyo et al. 2004). Harpacticoids are mainly herbivorous, feeding on microphytobenthos but also bacteria degrading larger macrophytes and are thus a fundamental trophic link between primary and secondary producers across habitats (Hicks & Coull, 1983; Hyndes & Lavery, 2005). They are highly energetic, with a natural high fatty acid content (Gee, 1989) and with sizes during most of their life stages that are suitable as prey for the juveniles of many species settling in coastal areas, so it is not surprising that they are a preferred food item, also under optimal foraging theory terms (Pyke et al., 1977; Nordström et al., 2015). Moreover, they are a diverse and abundant taxon and several morphotypes have evolved in different benthic habitats, allowing to identify the putative type of habitat from where prey items proceed (Hicks & Coull 1983; Arroyo et al., 2006).

In the present study, we investigated the diet of juvenile white seabream [Diplodus sargus (Linnaeus, 1758)] in six settling coves located in Minorca Island (Balearic Islands, Spain). The white seabream is a widely distributed Mediterranean coastal necto-benthic fish species. In the Mediterranean Sea, white seabreams spawn in spring (from the end of April to July in Minorca island, Cuadros et al., 2018), their pelagic larvae dispersing in surface waters during 15-28 days until settlement occurs at lengths about 10 mm after approximately 28 days (Vigliola, et al. 1998; Macpherson & Raventos, 2006; Di Franco & Guidetti; 2011; Di Franco et al., 2015). Their settlement areas typically consist of shallow (< 2m deep) mixed bottoms of sand, pebbles and rocks (García-Rubies & Macpherson 1995, Bussotti & Guidetti, 2011), in moderately exposed coves (Cuadros et al. 2017, 2018).

As of August, juveniles begin to leave the nursery areas to enter the adult population (Cuadros et al., 2018). In these areas, juveniles have been mainly found to feed on harpacticoid copepods, amphipods, polychaetes and algae (Christensen, 1978; Dias et al., 2014), though the feeding habits of settlers (1 - 4 cm in TL) have been only investigated scarcely. Adult Diplodus sargus (10-50 cm) are omnivores (Karachle & Stergiou, 2017; Stergiou & Karpouzi, 2002), feeding on algae and invertebrates (sea urchins, crustacean, polychaeta and gastropoda present in hard substrata (Sala & Ballesteros, 1997; Leitao et al. 2007; Figueiredo et al. 2005; Osman & Mahmoud, 2009; Daban, 2022), while larvae feed on copepods and cladocerans (Sánchez-Velasco & Norbis, 1997). It is a commercially and recreationally exploited species and has an important role as predator, controlling sea-urchin populations which decimate seaweed assemblages along coastal areas (Hereu et al., 2005).

Our aim was to identify the main prey items, their origin, and site variations, as well as diet changes during juvenile development. We hypothesized that the composition and diversity of prey would differ among coves and size classes, following on the one hand, variations in prey availability among coves and ontogenetic changes in food preference, on the other. Due to their special morphotypes, adapted to the biotopes they inhabit, harpacticoid copepods were used to discern the origin of the gut contents. Evidence regarding such changes should provide important information that will help discern the role of trophic links in cove selection during settling.

### **Material and Methods**

### Study area and field sampling procedure

The study was conducted at six randomly selected shallow site-coves distributed in two different geographic areas located on the northeast and the southwest coast of Minorca, in the NW Mediterranean Sea (hereafter referred to as N and S areas respectively, Fig. 1). These two areas are different in terms of landscape and geological history causing variations in cove morphology and exposure (wider and shallower coves in the north exposed to northern dominating winds vs. smaller more protected coves of karstic morphology in the south, Sanuy & Díaz, 2002), which may cause variations in food availability and composition for the juvenile sparids. All the coves were characterized by having gentle (1-2 %) seafloor slopes and both sandy and rocky bottoms (see Cuadros et al., 2018). At each cove, three sediment cores of 125 ml (plastic vials, 57 x 73 mm) were taken haphazardly (separated 50 cm) for granulometric and infauna analyses and pooled into a plastic bag. Samples for granulometry analyses were stored at -20 °C in pre-weighed plastic scintillation vials until analysis. Triplicate thawed sub-samples for each cove were washed, organic matter removed, oven dried and sieved through 0-2000 µm sieves (Cuadros et al., 2017). After analysis using the Gradistatv8 programme (Blott & Pie, 2001), the sediment



*Fig. 1:* Location of the six coves sampled during the study, located in two distinct sampling areas to the north and south of the island. Coves included in the North Area: 1= "Calderer", 2= "S'Enclusa", 3= "Mongofre"; and in the South Area: 4= "Talaier", 5= "Turqueta", 6= "Macarella".

of all coves was classified as sandy, but differences in grain size occurred, most locations presenting well sorted coarse and very coarse sandy sediments except for Turqueta, which was characterized by finer sand (Table 1). Moreover, there was a clear predominance of pebbles and rocks in Calderer, compared to other locations (Authors personal observations).

Thirty juvenile fish ranging from 10 to 30 mm TL were sampled by hand nets at each cove by wading through the cove in shallow water. Sampling was conducted twice per month between May and July 2012. Further details on fish sampling are given in Cuadros *et al.*, 2017.

### Infauna and gut content analyses

Samples destined to identify infaunal organisms were preserved in 4% buffered formalin. Once at the laboratory, five sediment samples taken randomly from all locations/dates were elutriated six times and the resulting water filtered through a 63  $\mu$ m sieve prior to examination under the stereomicroscope. The sieved meiofauna were counted and identified to higher taxa and processed.

When possible, ten fish from each cove and sampling date were haphazardly selected and the gut contents analysed. Fish were first measured, and the guts were subsequently dissected and examined under the stereomicroscope and all identifiable contents identified to the lowest taxonomic level possible and counted. On occasions, gut contents were composed of remnants of crustacean parts or debris, so it was difficult to quantify the exact number of individuals. When this occurred, given the small size of the guts, separate crustacean parts were checked to ascertain they belonged to the same taxon, identified as belonging to one single individual and counted as such. Algal fragments were considered as "presence" of algae. Entoprocts were comprised of colonial species of the *Pedicellina* type, but all individuals were counted separately. Harpacticoids from the 5 sediment samples analysed and from five juvenile guts per cove, were selected for the analysis. One hundred harpacticoid specimens were picked from each sediment sample and, when possible, from each gut sample, cleared in lactic acid and mounted in polyvinyl lactophenol for their subsequent identification under the microscope.

### Statistical analyses

We examined the relative importance of the various prey items in the diet of 3 size-classes (s1: 10-15 mm TL, s2: 16-23 mm TL and s3: 24-30 mm TL) of juvenile white seabream. To do so, we calculated the Frequency of Occurrence (O<sub>i</sub>) of the observed prey items in each of the classes, following the formula:

$$Oi = Ji/P$$

where, Ji is number of fish containing prey i and P is the number of fish with food in their stomach.

Levins' measure (B) was used for calculating niche breadth of each size class of *D. sargus* using the following equation (Krebs, 1989):

$$B = 1/\sum_{i=1}^{n} p_i^2$$

where pi is the proportion of each prey category i in the diet and n is the total number of prey categories in the diet of *D. sargus* juveniles. The standardized Levins' index [B est = (B - 1) / (n - 1)] was used to express niche breadth on a scale from 0 (a narrow niche breadth) to 1 (a broad niche breadth).

Additionally, prey item assemblage composition was studied through a multivariate exploratory approach using non-metric multi-dimensional scaling (nMDS) ordination (Clarke & Gorley, 2006). Dissimilarity measure

		FOLK AND WARD METHO	DD (µm)			
SAMPLE	SAMPLE TYPE:	SEDIMENT NAME	MEAN	SORTING	SKEW- NESS	KURTO- SIS
Calderer1	Bimodal, Very Well Sorted	Very Well Sorted Very Coarse Sand	1282.9	1.200	-2.308	0.782
Calderer2	Bimodal, Well Sorted	Well Sorted Very Coarse Sand	1197.1	1.279	-1.729	0.659
Calderer3	Bimodal, Well Sorted	Well Sorted Very Coarse Sand	1155.6	1.310	-1.469	0.531
S'enclusa1	Bimodal, Moderately Well Sorted	Moderately Well Sorted Coarse Sand	662.5	1.502	0.063	1.102
S'enclusa2	Bimodal, Well Sorted	Well Sorted Very Coarse Sand	1147.1	1.368	-2.359	0.549
S'enclusa3	Bimodal, Well Sorted	Well Sorted Very Coarse Sand	1092.6	1.359	-2.148	0.684
Mongofre1	Bimodal, Moderately Well Sorted	Moderately Well Sorted Coarse Sand	581.6	1.576	0.055	1.063
Mongofre2	Bimodal, Moderately Well Sorted	Moderately Well Sorted Coarse Sand	601.0	1.515	0.075	1.081
Mongofre3	Bimodal, Moderately Well Sorted	Moderately Well Sorted Coarse Sand	740.5	1.566	0.090	1.100
Talaier1	Bimodal, Moderately Well Sorted	Moderately Well Sorted Coarse Sand	527.0	1.497	-0.010	0.926
Talaier2	Bimodal, Moderately Sorted	Moderately Sorted Coarse Sand	578.1	1.702	-0.011	1.083
Talaier3	Bimodal, Moderately Well Sorted	Moderately Well Sorted Medium Sand	487.1	1.544	-0.022	0.925
Turqueta1	Unimodal, Moderately Well Sorted	Moderately Well Sorted Fine Sand	193.7	1.513	0.023	0.959
Turqueta2	Unimodal, Moderately Well Sorted	Moderately Well Sorted Fine Sand	214.5	1.516	0.013	0.955
Turqueta3	Bimodal, Moderately Sorted	Moderately Sorted Coarse Sand	777.2	1.709	0.124	0.965
Macarella1	Unimodal, Moderately Well Sorted	Moderately Well Sorted Medium Sand	312.7	1.615	0.001	0.939
Macarella2	Unimodal, Moderately Well Sorted	Moderately Well Sorted Medium Sand	264.8	1.517	0.024	0.962
Macarella3	Unimodal, Moderately Well Sorted	Moderately Well Sorted Fine Sand	243.1	1.546	0.025	0.956

 Table 1. Results of the grain-size analysis performed with Gradistatv8 for the three samples analysed at each cove. Results of the Folk and Ward method are presented.

matrices (binomial deviance scaled, as suggested by Anderson & Millar, 2004) were calculated from the initial data matrix containing for each sample (one gut content) the abundance of items for each prey taxon.

A PERmutational multivariate ANalysis of VAriance (PERMANOVA; Anderson, 2001), which is free from the assumptions of normality and homoscedasticity of residuals was used to test our hypotheses and help to interpret the ordination plot. The test was performed on the same dissimilarity matrices used for the nMDS–(Anderson, 2001). A model was fit to prey item assemblage densities to test their response to geographic area, cove, and sizeclass. Factor *area* with two levels (north and south) and factor *size-class* with three levels (s1: 10-15 mm TL, s2: 16-23 mm TL and s3: 24-30 mm TL) were fixed; while factor *cove* with 6 levels (the six coves) was random and nested in *area*. The PERMDISP routine was applied to the same model to compare dispersion ranges of diet data around their median values (Anderson *et al.*, 2008).

All Data treatment and analyses were performed using the R Statistical Software (v2.15.0; R Core Team 2021) and the PERMANOVA+ add on package for PRIMER software (Anderson *et al.*, 2008; Clarke & Gorley, 2006).

### Results

A total of 104 juvenile white seabream guts from the 6 coves were examined, 30 belonging to size one, 62 to size two and 7 to size 3. The distribution of guts per size and cove is shown in Table 2. Gut content analysis revealed that, in general, the diet of white seabream juveniles varied scarcely (Table 3). Juveniles preferred feeding on harpacticoid copepods (ca. 80% of total prey items identified) at all sampled sites and size-classes examined. Other prey such as ostracods (2%), entoporocts (2%), foraminifera (2%), amphipods (1.8%) or nauplii (2%), were also often found among the gut contents.

# Variations in gut content between geographic areas and among coves

Statistical analysis revealed that the diet composition did not vary between geographic areas (N vs. S), but it did show significant differences among coves (PERMANO-VA, P<0.001, Table 4, Fig. 2). At Calderer, Turqueta andS'Enclusa harpacticoids represented on average 92%, 85% and 75% of the diet, respectively, while at Mongofre Nou, and especially at Macarella these percentages were notably lower (Fig. 3, Table 3). At these two latter sites, other taxa such as amphipods and isopods (at Macarella), and nauplii (at Mongofre Nou) showed a higher representation among the juveniles' gut contents (Fig. 3, Table 3). Regardless of these differences, harpacticoids were always the preferred prey item at all locations.

### Variations among size classes

Gut content composition did not differ among the various size-classes (PERMANOVA, P>0.1, Table 4), but diet diversity (i.e., the dispersion of points on the ordination plot) was higher for size-class s2 (16-23 mm TL) than for size-classes s1 (10-16 mm TL) or s3 (24 - 30 mm TL)(PERMDISP, pair-wise tests, p<0.001, Table 4, Fig. 4). Indeed, while the diet of size-class s1 was dominated by copepods (harpacticoids accounting for >80% of the gut contents), the diet of juveniles in size-class s2 showed a more varied composition, still with harpacticoids as the main prey item  $(73\pm4\%)$ , but including amphipods, foraminifers and ostracods in larger numbers (Fig. 5). The variety of diet items was lower in those individuals in the largest size-class (s3: 24 - 30 mm), where the mean percentage contribution of harpacticoids decreased in favour of other taxa such as insects (probably eaten from the water surface) and larger prey such as isopods (Table 3, Fig. 5).

The frequency of occurrence of the various prey items varied among size-classes, but again, harpacticoids were always the most frequent prey found in all of them, accounting always for >80% of Oi in all size classes and reaching 97% and 95% in size-classes s1 and s2, respectively (Fig. 6). Other well represented taxa were algae, amphipods, ostracods, entoprocts and foraminifers, which were present in the diet of the 3 size-classes (Fig. 6).

Similarly, Levin's index was low for all size categories, Best= 0.05, 0.04, and 0.077 for Size classes 1, 2 and 3, respectively, indicating a high specialization on harpacticoid copepods in all cases.

### Comparison between sediment and gut-content harpacticoids

Sediment communities showed the typical composition of meiobenthic fauna, dominated by nematodes and harpacticoid copepods, with other taxa such as polychaetes, or oligochaetes, showing varying abundances depending on the cove of origin (Table 5). Sediment meiofauna differed substantially among locations (Table 5), probably as a result of the aforementioned different sediment composition at each site. These variations were mirrored in the meiobenthic composition found in the samples, Turqueta presenting a striking predominance of nematodes, with practically no other taxa and all other locations showing higher numbers of harpacticoids, and a more varied representation of other taxa (Table 5).

Comparison of sediment communities and gut contents showed a discrepancy between harpacticoid families found in the stomach contents. Stomach items belonged to families typical of phytal habitats, e.g.: Thalestridae,

Table 2. Distribution of size classes of the juveniles examined from the 6 coves sampled at Minorca Island.

Stomach contents analysed per cove/date	Cove Orientation	Size 1	Size 2	Size 3	Total
S'Enclusa	Ν	11	22		33
Calderer	Ν		8	1	9
Mongofre Nou	Ν	8	5		13
Es Talaier	S	3	7	2	12
Macarella	S	1	7	2	10
Turqueta	S	7	13	2	23
Total		30	62	7	104

				North					-		Sot	ith				
	Calderer	.	Mongof	re Nou	SE	Inclusa			<b>Furqueta</b>		E	s Talaier			Macarella	
Fish Size class	2	3	1	2	1	2	e	1	2	e	1	2	e	-	2	3
Algae	1				1	1		1	0		1	1			1	1
Amphipods	$1.75 \pm 0.48$	1	2	$1.33 \pm 0.33$	1	3.66±1.45		1	$1.5 {\pm} 0.5$		ŝ	2.25±0.48	1	-	l.33±0.33	1
Bivalves	2	1		1	1	1			1							
Calanoids					1	$3.5 {\pm} 0.5$					1					
Chironomids				1		2			1	2.	66±1.66	1.66±0.66	1			
Copepoda indet					4											
Crustacea indet	1		1	1		1					1	1			1	1
Crustacean	1															
Docured leaves													-			
Decapou larvae					e	¢					¢	¢	1			
Entoprocta	$3\pm 1$			$16\pm 15$	7	7		$1.66 \pm 0.66$	$1.33 \pm 0.33$		7	7		1		
Fish larvae					1											
Foraminifera	4	2	1		$1.5 \pm 0.29$	4.75±1.65			6.25±3.94						$1.5 {\pm} 0.5$	
Gastropod			1	$2.5 \pm 1.5$		1			$2\pm 1$		4	1	1			
larvae																
Harpacticoida	111.75±27.58	137	12.375±5.05	27.2±8.92 1	6.55±3.22	$50.44 \pm 8.66$	81	12.43±3.43	6.77±1.51 65	5.5±47.5 22	.33±6.69	19±8.60	l 8.5±14.5	3	1.25±0.25	.5±0.5
Hirudinea	1				1	1		1	3.66±1.41			1				
Indet larvae	L					1										
Insects	1					2±0.77			1							3
Isopods	$1.2 \pm 0.2$							2	1		1	$1.5 {\pm} 0.5$	1		1	٢
Mites					1	1										
Nauplii				50	2							2				
Nematodes	2				1	2				2		2				
Ostracods	$3.83 \pm 1.07$	2	$1.2 \pm 0.2$	1.5	2	3.75±1.48		4	$1.5 {\pm} 0.5$		1	1.66±0.33				
<b>Polychaete</b>	ŝ				1	1										
larvae																
Polychaetes	1							1			7	1	1			
Siphonostoma- toids	7															
Spiders																7
Terrestrial mites									1							
Unident eggs				1								2				





Fig. 2: nMDS ordination plot of stomach contents of Diplodus sargus sargus juveniles (10-30 mm TL) at the six coves sampled.



## % contribution

*Fig. 3:* Percentage contribution (mean  $\pm$  standard error) of the various prey items in the diet of the six coves sampled. Only those prey items accounting for a mean contribution higher than 1% are represented.



Fig. 4: nMDS ordination plot of the stomach content of the different size classes of Diplodus sargus juveniles (10 - 30 mm).



*Fig. 5:* Percentage contribution (mean  $\pm$  standard error) of the various prey items in the diet of the three size classes. Only those prey items accounting for a mean contribution higher than 1% are represented.

*Fig. 6:* Frequency of occurrence  $(O_i)$  of the various prey items in each of the size classes examined.

Table 4. Permutational Multivariate Analysis of variance table of results for Diplodus sargus stomach content in juveniles (1	0-
30mm TL) in each area and by size class of the fish: s1 (10-16 mm TL), s2 (16-23 mm TL) and s3 (24-30) mm TL).	

Source	Df	MS	Pseudo-F	P(perm)
area	1	9.3563	0.856	0.539
size class	2	2.7347	0.68564	0.699
cove (area)	5	22.887	4.4902	0.001**
area x size class	2	2.5644	0.64296	0.735
cove(area) x sizeclass	6	3.4872	0.68416	0.868
Res	82	5.097		
Total	98			

ns: not significant;  $P \le 0.05$ ; \*\*  $P \le 0.01$ ; \*\*\*  $P \le 0.001$ . P-values were obtained by 999 permutations of residuals under a reduced model.

Peltidiidae, Porcelidiidae, Harpacticidae, Tegastidae. Contrastingly, families occurring in the sediment samples (e.g.: Paramesochridae, Ameiridae, Leptastacidae), were, as expected, typical of sediment habitats (Table 6). These results were consistent regardless of the cove of origin.

### Discussion

The settling habitat of juvenile sparids in the Mediterranean is well established, consisting in shallow (<2 m depth) gently sloping coves with heterogeneous substrata of sand, pebbles and rocky bottoms colonized by algae (Garcia-Rubies & Macpherson, 1995; Harmelin-Vivien *et al.* 1995, Biagi *et al.*, 1998; Bussotti & Guidetti, 2011). Our study stresses the importance of the algal habitats in these coastal environments for sustaining juvenile fish, in detriment of bare sandy seabeds. During their post-settlement period in the spring – summer season juvenile white seabream settling in coves in Minorca mainly relied on phytal fauna as main food source, and more specifically, on harpacticoid copepods (80%).

Table 5. Sediment meiofauna found at each of the analysed coves. Numbers are abundance per 50 ml.

			Coves		
		North		Soi	ıth
Taxa (ii per core, 50ml)	Calderer	Mongofre Nou	S'Enclusa	EsTalaier	Turqueta
Amphipods	4				
Calanoids				2	
Ciliates				2	
Cyclopoids	31	10			
Gastropods	1			10	2
Gastrotrichs		6		1	
Harpacticoids	432	129	82	491	
Indet. copepods				1	
Indet. fauna	7	1	1	5	
Isopods	1				
Mites	3	1	2	1	
Nauplii	53	74	22	19	
Nematodes	325	344	51	485	1039
Oligochaetes	44	10	4	194	
Ostracods	3	3	65	1	
Polychaete larvae		66	7	4	1
Polychaetes	17	125	23	220	7
Tardigrades				7	
Turbellarians	15	76	10	30	

		SEDIN	MENT			GU	T CONT	TENTS	
HARPACTICOID FAMILY	CAL	TAL	MN	S'ENC	CAL	TAL	MN	S'ENC	TUR
Canthocamptidae								14	
Ameiridae	72	34	54	17	5				
Cletodidae		36	2						
Copepodites indet.	6		5			1			
Cyclopoida	4		9		1				
Diosaccidae	1	7	15	1	5	12	4	2	
Ectinosomatidae	4			1	4	2		1	
Harpacticidae					2		4	12	1
Harpacticoida Indet.			1	3	33		5	9	
Laophontidae	1			2	25		2	4	1
Leptastacidae		15	13	26					
Paramesochridae		17	22	18					
Peltidiidae					1				
Porcellididae					1				
Tegastidae					3				
Thalestridae		1			18	11	2	3	
Tisbidae					4		3	1	1
Grand Total	88	110	121	68	102	26	20	46	3

 

 Table 6. Harpacticoid families found in the sediment cores collected at each of the analysed coves and in the gut contents of juvenile fish collected in the same locations. CAL=Calderer, TAL=Talaier, MN= Mongofre Nou, S'ENC= S'Enclusa, TUR=Turqueta.

Several studies have highlighted the importance of vegetated areas within estuaries (Beck et al., 2001) or coastal areas (Hinz et al., 2019) as nurseries for fish. The fact that they support much higher densities of organisms than any other habitat within coastal areas favours juvenile survival and development (Guidetti, 2000; Christie et al., 2009; Cheminée et al., 2013). Within these vegetated areas, seagrass meadows and mangrove forests have received most attention when it comes to examining the nursery value of littoral zones (Heck & Orth 1980; Orth et al. 1984; Bell & Pollard 1989; Jenkins & Wheatley 1998; Reñones et al., 1995; Jenkins et al. 1997; Nagelkerken et al., 2000). Despite increasing studies showing higher juvenile fish abundances in seaweed than in neighbouring seagrass habitats (Eggersten et al., 2017; Tano et al., 2017), macroalgae covered substrates have been often overlooked. However, growing evidence is proving the importance of these nearshore habitats as settling and nursery areas for fish in coastal areas (Cheminée et al., 2013; Cheminée et al., 2017a), mainly due to the combined effect of providing refuge and abundant food sources for juveniles (Hinz et al., 2019, Maidanou et al., 2021). Moreover, macroalgae often colonize a variety of substrates (i.e.: rocks, coralligenous reefs or other calcareous bio-concretions), adding heterogeneity to marine coastal seascapes and acting as ecological engineers (Jones et al., 1994; Coleman et al. 2002). This is especially relevant in the light of new conservation targets aimed at prioritizing habitat connectivity in coastal areas

(Afonso *et al.*, 2008; Nagelkerken *et al.*, 2015; Tano *et al.*, 2017; Litvin *et al.*, 2018), where different seaweed species may provide continuous food and refuge, offering corridors that guarantee safe ontogenetic habitats shifts for many species.

### Variations among coves

In our study, significant variations in prey composition were found among the various coves but not between geographic areas, indicating that local variations in cove substrate, topography or conformation, and thus habitat availability, were more important in defining prey availability than broader acting factors such as cove orientation to wave exposure (i.e., sheltered vs. exposed coves), which ultimately also condition large-scale hydrodynamic effects. For instance, the larger number of harpacticoids found in the gut contents of juveniles caught in Calderer, may be explained by the wider presence of rocky and pebble habitats colonised by macroalgae, as opposed to other coves, were sandy and even finer sediments were more abundant. However, the number of individuals of the various sizes present at each cove was quite unbalanced (Table 2), and this might have had a bearing in these results, which should be thus, interpreted with caution.

Small variations in topography and habitat heterogeneity, can induce large variations in the abundance and diversity of the associated fauna, and this may have a bearing in food and space availability both for fish juveniles and their prey (Cheminee *et al.*, 2017b; Cuadros *et al.*, 2017; Hinz *et al.*, 2019). This is especially true when it comes to macrophytic systems, were the composition and abundance of the associated fauna have been found to depend on micro-habitat architecture and size, respectively (Arroyo *et al.*, 2004; Christie *et al.*, 2009).

### Variations among size-classes

Our data reveal that harpacticoid copepods were the dominating prey item in all size-classes (>80%), which has also been found for other juvenile sparids (Christensen, 1978; Rodriguez-Ruiz et al., 2002; Dias et al., 2014; Altin et al., 2015). The lack of significant differences among size-classes, in all the parameters and indices examined could be explained by the fact that the examined specimens were grouped within a narrow range of sizes (i.e.: 10 - 30 mm TL). Important shifts in prey size and type may occur further during ontogeny. For example, Hinz et al., 2019, found that the three fish species they analysed consumed very similar prey (mainly harpacticoids and amphipods) in size ranges below 60 mm. Christensen (1978) reports the most marked change in white seabream diet in the 35 to 50 mm size class, where fish fed little on harpacticoid copepods, their diet being largely composed of amphipods. His smallest size classes (5 - 15 mm and 16 - 20 mm) also fed mainly on harpacticoids and amphipods, while algae and polychaetes were increasingly taken in the larger ranges he examined. Dias et al. (2014), who examine larvae and juvenile white seabream diet from rockpools in Portugal also found harpacticoid copepods to be the numerically dominant prey items in fish smaller than 30mm, with a shift to larger and more varied prey items in larger specimens. We also recorded larger prey items such as amphipods and isopods and others as polychaetes or seaweed in the larger specimens, but this was not mirrored in a significant shift in overall diet composition, harpacticoids remaining as the main prey item for juvenile seabream during the study period and the size ranges examined.

Seasonal variations in prey consumption have been reported and attributed to seasonal variations in abundance/availability of the main prey items, as well as to competition with other sparid species (Christensen, 1978; Hinz et al., 2019), so we cannot discard variations in the diet of juvenile seabream with respect to what we found in other seasons. However, seabream settling in the Mediterranean Sea occurs mainly during spring, which is also the time when the highest abundances of macrophyte associated fauna are normally found (Hinz et al., 2019). Our study reinforces the evidence on the important role of harpacticoids as grantors of food for the juveniles of several coastal species during settlement. Phytal harpacticoids in particular, are usually larger in size than sediment associated ones, and good swimmers (Hicks & Coull, 1983), so they are probably readily detected and eaten by the fish juveniles. Some of the genera/species observed in the gut contents in this study, have been found previously among intertidal algal species in the Balearic Islands, such as *Corallina* spp. and *Jania* spp. (Arroyo, *pers. Obs.*), as well as associated with *Posidonia oceanica* (L.) De Lille, blades and their epiphytes (Arroyo *et al.*, 2013), which confirms their phytal origin and their presence among Balearic macrophytic stands.

### Harpacticoids as tool in habitat-fish relationship studies

Our study highlights the potential use of harpacticoid copepods as a tool to identify the foraging habitat of juvenile fish species. Their varied morphotypes associated to the different habitats they occupy makes them a good tool to identify the exact microhabitats where the fish are feeding. Tipton & Bell (1988) stress the need to identify harpacticoids at species level for a detailed understanding of benthic food selection by fish. However, we showed that identification at family level may already shed light on the foraging habitat of juvenile fish and on the explicit use of specific habitat patches in nursery or settling areas. Their identification can also help disentangle ontogenetic shifts in habitat use within seascapes and understand the fundamental link between habitats as corridors to final recruiting areas (Litvin et al., 2018). Further studies, including analyses of the fauna inhabiting the various habitats configuring the seascape from settling to recruiting areas in conjunction with juvenile gut-content analyses, are needed to analyse the specific association/preference of the various ontogenetic phases to specific algal species and their associated fauna, and whether selection of the best habitat is indicative of their success as recruits. This will help to ascertain the nursery role of seaweed habitats in coastal areas, and contribute to highlight the need to protect, preserve and restore them and the fundamental ecosystem services they provide across coastal seascapes.

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