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# Potential retention effect at fish farms boosts zooplankton abundance

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#### ABSTRACT

Coastal aquaculture activities influence wild macrofauna in natural environments due to the introduction of artificial structures, such as floating cages, that provide structural complexity in the pelagic system. This alters the abundance and distribution of the affected species and also their feeding behaviour and diet. Despite this, the effects of coastal aquaculture on zooplankton assemblages and the potential changes in their abundance and distribution remain largely unstudied. Traditional plankton sampling hauls between the farm mooring systems entail some practical difficulties. As an alternative, light traps were deployed at 2 farms in the SW Mediterranean during a whole warm season. Total zooplankton capture by traps at farms was higher than at control locations on every sampling night. It ranged from 3 to 10 times higher for the taxonomic groups: bivalvia, cladocera, cumacea, fish early-life-stages, gastropoda, polychaeta and tanaidacea; 10-20 times higher for amphipoda, chaetognatha, isopoda, mysidacea and ostracoda, and 22 times higher for copepoda and the crustacean juvenile stages zoea and megalopa. Permutational analysis showed significant differences for the most abundant zooplankton groups (copepoda, crustacean larvae, chaetognatha, cladocera, mysidacea and polychaeta). This marked incremental increase in zooplankton taxa at farms was consistent, irrespective of the changing environmental variables registered every night. Reasons for the greater abundance of zooplankton at farms are discussed, although results suggest a retention effect caused by cage structures rather than active attraction through physical or chemical cues.

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## 1. Introduction

Over the last three decades, fish-farming cages have rapidly developed throughout the world (FAO, 2004; Belias et al., 2007). In the Mediterranean Sea, gilthead seabream (Sparus aurata) and European seabass (*Dicentrarchus labrax*) are intensively farmed in most of the countries (FAO, 2004; Magill et al., 2006). It is well known that fish farming interacts with the marine environment at various spatial and temporal scales and generates variable shifts in composition of benthic (Karakassis et al., 2000; Mirto et al., 2010) and pelagic assemblages (Dempster et al., 2002). These changes are related to the organic enrichment derived from excess of uneaten food and fish excretions, chemical pollution from medicines and antifouling products, genetic effects and non-native species introductions (Dempster et al., 2002; Holmer et al., 2007; Borja et al., 2009; Fernandez-Gonzalez and Sanchez-Jerez, 2011).

Moreover, the deployment of these massive artificial structures in the pelagic environment may provoke severe changes in the wild biota composition, from phytoplankton (Dalsgaard and Krause-Jensen, 2006) to macrofauna (Carss, 1990; Franks, 2000; Dempster et al., 2002) and megafauna (Díaz López and Bernal Shirai, 2007; Arechavala-Lopez et al., 2014, 2015). Complex artificial structures drive changes in the behaviour or physiology of affected species (Fernandez-Jover et al., 2007a) but in turn, adult species aggregated to the fish farm environment may alter chemical or nutrient dynamics in the pelagic (Fernandez-Jover et al., 2007b) or benthic systems (Katz et al., 2002). It is noteworthy that the influence of coastal fish farms on ichthyofauna is not strictly limited to adult fish, since juvenile fish from several different families generally use farm structures as settlement grounds, with potential consequences for their physiology and growth (Fernandez-Jover et al., 2009; Fernandez-Jover and Sanchez-Jerez, 2014). The forces driving this behaviour have already been investigated, like for instance the food availability for juvenile fish in the water column around farms. It was found that resources may be at least as accessible as they are in traditional settlement environments such







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as natural shallow rocky shores. The main prey of aggregated juvenile fish are typical zooplankton taxa, *e.g.* adult and juvenile copepods, cladocerans, nauplius larvae or amphipods (Fernandez-Jover et al., 2009).

In the SW Mediterranean, it has already been corroborated using light traps that European seabass and gilt-head bream farms favour the presence (among others) of holoplanktonic amphipods in the pelagic environment. In this way, Fernandez-Gonzalez et al. (2014) detected an abundant community of planktonic amphipods at farms when compared to environments where these structures were absent, comprising strictly pelagic species and also benthic and fouling-community species that apparently undertake incursions into the pelagic zone at night. Therefore, the higher presence of a common prey may act as an enhancing factor favouring the abundance of early life-stages of different fish species. In this sense, farm nutrients release is also thought to increase plankton communities in oligotrophic environments (Tsagaraki et al., 2013).

Light devices have been traditionally used for capturing early life-stages of fish (Faber, 1981; Floyd et al., 1984; Doherty, 1987), but also with the objective of studying zooplankton communities (Miller and Shanks, 2004; Shaw et al., 2007; Tor et al., 2010; Fernandez-Gonzalez et al., 2014; Sigurdsson et al., 2014). Furthermore, the relationship between artificial light attraction and zooplankton has already been studied at farms; McConell et al. (2010) detected a higher presence of zooplankton communities at salmon farms illuminated during the whole night, finding that abundances of invertebrates, like bivalves or gastropods, as well as some larval and juvenile fish species, were greater at night-lit farms. However, the zooplankton communities at nonilluminated farms were not compared with areas not influenced by aquaculture activities, including the potential prey availability for early life-stages of fish.

Consequently, we relied on light traps to achieve four main objectives, to: i) assess their suitability for the study of zooplankton and early life-stages of fish at sites where traditional sampling tools such as plankton hauls are difficult to employ, and to determine if zooplankton taxa abundances vary in response to a fish farm environment, ii) evaluate changes through time in zooplankton taxonomic composition at two farms during a whole warm season, and finally iii) estimate if the abundance and family composition of early life-stages of fish are different at farms compared to control locations.

## 2. Material and methods

### 2.1. Study area and sampling effort

This study was carried out in coastal waters, in Guardamar del Segura bay (Alicante, Spain: 38° 5′ 7.45″ N; 0° 35′ 51.40″ W) from 12th June to 10th October 2012, the warm period in the Western Mediterranean. Sampling was conducted at two fish farms (Fig. 1A) producing seabass (Dicentrarchus labrax) and seabream (Sparus aurata), and two control areas, on 16 arbitrarily chosen nights. Control samples were also taken randomly within the bay with the condition that they were at least 2 km away from the nearest fish farm and at a minimum depth of 23 m, which was reached at least 3 km away from the shore. All four localities (2 control and 2 farms) were located 3–4 km offshore at depths ranging from 23 to 30 m. Each farm consisted of 18 rings with a diameter of 19 or 25 m and cage nets reaching depths from 12 to 15 m, enclosing a cage volume up to 7400 m<sup>3</sup>. Changes in abundances and species composition in the plankton population were investigated by sampling farm and control areas with light traps.

Light-trap design used in this study was a modification of that

employed by Floyd et al. (1984) and Kissick (1993), which consisted of a plexiglas collection chamber measuring  $40 \times 40 \times 40$  cm, with eight panels forming four funnel-shaped entrances 3 mm wide. The light source was a hand diving-torch (Led Lenser D14, 150 lumen) coupled to a white plastic container that produced a diffuse point of illumination.

The light-trap technique provides selective sampling, since results are biased towards photophilic species. However, it has traditionally been used for various purposes, generally aimed at capturing zooplankton species, most frequently early life-stages of fish (e.g. Floyd et al., 1984; Doherty, 1987). Additionally, it is useful in studies at places with difficult access or where habitual sampling methods such as plankton hauls are inconvenient. Specifically, oblique hauls may become logistically problematic. Researchers that still decided to deploy nets between the cages had to limit sampling to vertical hauls or small purse seines (McConell et al., 2010); light traps thus seem an appropriate alternative for sampling in logistically difficult habitats (Chicharo et al., 2009).

Traps were suspended at approx. 20 m above the sea bottom, at 4 m below an anchored buoy (Fig. 1B). They were deployed after sunset for approximately 1 h, recording deployment and retrieval times to the nearest minute (for later standardisation to individuals per traps per hour), and their contents then removed. Due to logistical constraints we were only able to sample one site during one single night (i.e. all samples from Control 1 and Farm 1 were sampled on one specific night and Control 2 and Farm 2 on a different night). Every night two traps were deployed approximately at the same time at the cages and two at control site and every one of them was retrieved three times during the whole night, making a total of six control and six farm samples considering each as one replicate. Traps were moved 20-30 m after retrieval, and a period of at last 30 min was allowed prior to next deployment. At recovery time, traps were raised slowly to allow filtration of the chamber content through the 250 µm-mesh bottom of the collection cup. Material retained was preserved in 4% formalin seawater solution. In the laboratory, samples were sorted, counted and the main plankton groups identified. Fish individuals were measured to the nearest 0.1 mm and identified to family level using published literature (Russell, 1976; Sabatés, 1988; Arias and Drake, 1990; Fahay, 2007; Ré and Meneses, 2008; Lecaillon et al., 2012).

Environmental variables were obtained or measured in situ in order to include them in the design as covariables with the objective of inferring if their fluctuations had a significant influence on the zooplankton assemblages studied, and thus cope with the environmental variability inherent to a study that spanned five months. They were: Water temperature, Day of lunar month (DLM), Moon illumination, State of the sea (wave height in m), Time to moonrise. Time since sunset. Time between sunset and moonrise, Time from the nearest high tide, and Cloud cover. The exact rising and setting times for the moon and sun and the percentage of moon illumination were taken from http://www. timeanddate.com/. Current direction and velocity were also added as predictor variables. The average direction and velocity during the previous 24 h before every sampling night was obtained from the historical data recorded by the national government in the region (http://www.puertos.es). Hourly current data, which was provided as magnitude and direction vectors were averaged for the previous 24 h prior to sampling and then simplified into four vectors corresponding to main current directions NNE-SSW, ENE-WSW, ESE-WNW and SSE-NNW, taking positive and negative values for every direction (e.g. positive values for currents with direction NNE, between 45 and 90°, and negative for currents towards SSW between 180 and 225°).



Fig. 1. Sampling design of control and farm sites, showing the sampling days at each site (left) and diagram of light trap deployment (right). The design was repeated 3 times, making a total of 6 replicates each night. Each cross represents a light trap.

#### 2.2. Plankton hauls

To assess the suitability and potential biases of light traps when sampling zooplankton and early life-stages of fishes, plankton hauls were performed. A conical plankton net 0.6 m in diameter and 250 mm mesh was connected to a flowmeter (model 2030 General Oceanics), and towed at a depth of 1–5 m for 4 min at low speed (3 knots). Four double-oblique plankton hauls were taken each sampling night in order to cover a similar depth to the light traps. At the end of each trawl, the net was washed down with seawater and the retained material preserved in 4% formalin seawater solution. Plankton net samples were standardised to the number of individuals collected per 100 m<sup>3</sup>. Light trap selectivity was estimated according to the formula:  $E=(r_i - p_i)/(r_i + p_i)$ , based on Ivlev's index (E; Ivlev, 1961), where  $r_i$  is the percentage of the species i in the trap and p<sub>i</sub> the percentage of the species i in the environment (plankton tows). This index varies from +1.0 to -1.0, where positive values indicate attraction and negative values avoidance.

#### 2.3. Data analysis

Light trap samples were standardised to catch per unit effort (CPUE, *i.e.* individuals caught per hour and trap). Periodic environmental variables such as those related to the lunar cycle (Days of the lunar month, Time to moonrise and Time from the nearest high tide) were transformed using both the sine and cosine of the independent variable (Bell et al., 1995 and references therein). The circular periods were 29.53 d for the lunar cycle, 24.83 h for the lunar day and 12.42 h for the tidal period. The nominal zero for the lunar cycle was considered at new moon.

In order to evaluate the influence of floating aquaculture

facilities on nocturnal abundances, data from trap captures were analysed according to a 3-factor hierarchical design: 'C-F' (fixed; two levels: Control and Farm); 'Site' (random; two levels) and 'Day' (random; eight levels), with six replicates for each treatment. Due to bad weather conditions, three replicates on day 1 and eight on day 5 could not be sampled. Consequently, data were analysed using PERMANOVA, which is robust even when there are unequal numbers of replicate samples within each factor level of the design (i.e. unbalanced designs; Anderson et al., 2008). The analysis was performed over the Bray-Curtis dissimilarities matrix (Euclidean distance matrix in the case of univariate analyses) of the transformed data, applying a log (x+1) transformation (Anderson, 2001a; McArdle and Anderson, 2001) using 4999 random permutations of residuals under a reduced model (Anderson, 2001b), with appropriate units as required by the design (Anderson and ter Braak, 2003).

Previously, the distance-based linear model (DistLM) was used to search for the group of environmental variables that best explained the distance matrix based on the overall taxa assemblage data and each taxonomic group separately, in a way comparable to multiple regression (Anderson et al., 2008). The Akaike information criterion (AIC) and  $R^2$  were used to choose the best model from all possible combinations of variables. Statistical significance (after 4999 permutations) and percentage contribution of each variable alone, ignoring all other variables, were obtained from marginal tests. This routine showed that environmental variables explained a very low proportion of variability found in the traps (never more than 0.6%). Despite this, a few variables exerted a significant influence on the planktonic assemblages and were therefore included as covariables in the permutational multi- and univariate analyses of variance (PERMANOVAs) explained in the previous paragraph. Consequently, the covariables included were: Htidal, Temperature, DLM, Sea State, Sunset/Rise, TM(h) and Current Directions NNE-SSW and NNW-SSE. Statistical analyses were performed using PRIMER-E software (PRIMER software; Clarke and Gorley, 2006) with the add-on package PERMANOVA+ (Anderson et al., 2008).

#### 3. Results

Comparing plankton hauls and light-trap captures, Ivlev's selectivity index showed that traps, when compared to plankton tows, tended to overestimate the presence of isopoda, polychaeta, mysidacea, ostracoda and zoea larvae with *E* values that ranged between 0.92 and 0.57 (Fig. 2). On the other hand, results pointed to an underestimation of such taxa as pteropoda, appendicularia, larvae planula, bivalves, cnidaria, cladocerans, tunicates, larvae nauplii and gasteropoda, with values between -0.75 and -0.37. Finally, for the groups amphipoda, copepoda, chaetognatha, fish and tanaidacea, values were close to 0 (between +0.10 and -0.21), showing results very similar to those found in plankton nets. Regarding fish, light traps tended to capture juvenile individuals, while plankton nets mainly captured preflexion and flexion larvae, thus not allowing the calculation of the selectivity index for osteichthyes.

Sea current direction varied mainly between ENE and WSW during the whole sampling period. Light traps captured a total number of zooplankton individuals of 526 ± 117 ind trap<sup>-1</sup> h<sup>-1</sup> (mean ± standard deviation) at control sites versus 12044 ± 2400 ind trap<sup>-1</sup> h<sup>-1</sup> in light traps deployed at fish farms (averaging all control and all farm samples). The higher abundance of total zooplankton individuals in the light traps situated at farms was consistent at the two control and two farm sites (Table 1). In order, the most abundant taxonomic groups were copepoda, with an abundance of  $392 \pm 92.7$  ind  $\cdot$  trap<sup>-1</sup> h<sup>-1</sup>at control sites and  $9235 \pm 2023$  ind trap<sup>-1</sup> h<sup>-1</sup> at farms, and larval crustacea including zoea and megalopa stages, with 100 ± 26.6 and  $2343 \pm 432$  ind trap<sup>-1</sup> h<sup>-1</sup> at control sites and farms respectively. Additional groups were found with tens of individuals per hour, like

cladocera, mysidacea, chaetognatha, polychaeta, ostracoda, isopoda and pteropoda (Table 1). Relatively, copepods reached 71.9% and 85.9% of total captured individuals at control and farm locations respectively, followed by larval crustacea (19.0% at control and 17.8% at farms) and cladocerans with 5.1% and 1.3% at control and farm locations respectively.

The maximum number of individuals captured at a single trap during one haul was 148,735, due to an especially high abundance of copepoda and zoea larvae during the 10th of July at farm site 1. In contrast, it was notable that only 14 ind trap<sup>-1</sup>  $h^{-1}$  were found inside a light trap on the 10th of October at a control site.

This higher abundance of total individuals at farm sites was supported by the multivariate analysis (PERMANOVA) of the taxonomic composition of the assemblage. The environmental features that stood out in the DistLM analysis were added as covariables, in order to control this source of variability (see Material and Methods section). The PERMANOVA showed a significant differentiation between farm and control sites (Table 2, *p*-value < 0.01). The high variability between days (*p*-value < 0.01) did not impede the detection of significant differences for the main factor.

The differences between the amount of individuals captured in control and farm areas were consistent throughout the study period. Every sampled day, the average total capture was higher in the traps situated near fish farm structures (Fig. 3; Table 1). On evaluating separately the different taxonomic components of the zooplankton assemblage, this pattern was also coherent for the most abundant taxa. Abundance at farms was on average 3 to 10 times higher for the taxonomic groups: bivalvia, cladocera, cumacea, fish, gastropoda, polychaeta and tanaidacea; 10 to 20 times higher for amphipoda, chaetognatha, isopoda, mysidacea and ostracoda, and notably, 22 times higher for copepoda and the crustacean juvenile stages zoea and megalopa (Fig. 3; Table 1).

On every sampling night, abundance was always higher for copepods and crustacean larvae at farms. For the rest of the taxonomic groups this pattern was quite similar, since only during a single sampling day, and not always the same day, more individuals



**Fig. 2.** Ivlev's Index (E) showing light trap selectivity estimated according to the formula:  $E = (r_i - p_i)/(r_i + p_i)$ , where  $r_i$  is the percentage of the species i in the trap and  $p_i$  the percentage of the species i in the environment (plankton tows). This index varies from +1.0 to -1.0, positive values indicate selectivity and negative values avoidance.

#### Table 1

Total abundance (individuals trap<sup>-1</sup> h<sup>-1</sup> ± standard error) in the light traps deployed at control and farm sites.

	Control sites		Farm sites			
	Site 1	Site 2	Site 1	Site 2		
Amphipoda	$0.69 \pm 0.22$	$0.43 \pm 0.12$	17.4 ± 8.8	2.6 ± 0.58		
Apendicularia	5.1 ± 1.5	$4.6 \pm 0.94$	$2 \pm 0.45$	$4.5 \pm 1.2$		
Bivalvia	$0.08 \pm 0.06$	$0.25 \pm 0.13$	$0.35 \pm 0.18$	$0.19 \pm 0.14$		
Cephalopoda		$0.03 \pm 0.03$				
Cladocera	22.5 ± 4.3	$20.8 \pm 3.7$	$230 \pm 98.4$	$170 \pm 41.6$		
Cnidaria	$0.49 \pm 0.24$	$0.56 \pm 0.21$	$0.06 \pm 0.04$	$0.15 \pm 0.07$		
Copepoda	163 ± 39.9	581 ± 181	13833 ± 3823	$4458 \pm 969$		
Cumacea	0.37 ± 0.11	3.3 ± 1.3	$4.2 \pm 0.84$	$7.2 \pm 2.2$		
Echinoderm larvae		$0.02 \pm 0.02$				
Planula larvae	$0.02 \pm 0.02$					
Veliger larvae			$0.02 \pm 0.02$			
Fish eggs	$0.25 \pm 0.09$	$0.24 \pm 0.09$	$0.24 \pm 0.1$	$0.12 \pm 0.06$		
Total fish	$0.88 \pm 0.47$	$0.52 \pm 0.22$	$0.95 \pm 0.17$	$1.68 \pm 0.38$		
Gasteropoda	$0.28 \pm 0.12$	$0.76 \pm 0.47$	0.31 ± 0.16	$4.2 \pm 3.7$		
Isopoda	$3.3 \pm 0.95$	$2.6 \pm 1.5$	30 ± 18.3	27.8 ± 10.6		
Mysidacea	$3.9 \pm 0.98$	9.9 ± 3.6	32.7 ± 8.3	115 ± 82.3		
Nauplius larvae	$0.02 \pm 0.02$	$0.02 \pm 0.02$		$0.02 \pm 0.02$		
Nematoda	$0.02 \pm 0.02$		$2.2 \pm 0.8$	$0.48 \pm 0.18$		
Non-identified	$0.05 \pm 0.05$	$0.03 \pm 0.03$		$0.03 \pm 0.02$		
Ostracoda	$2.7 \pm 1.4$	2.8 ± 1.1	$20.4 \pm 6.4$	44.2 ± 18.3		
Polychaeta	$17.1 \pm 6.3$	$2.5 \pm 0.55$	53.9 ± 21.3	29 ± 7.8		
Pteropoda	$0.3 \pm 0.14$	$0.17 \pm 0.14$	$1.8 \pm 1.0$	17.5 ± 13.5		
Chaetognata	$6.1 \pm 2.2$	$3.8 \pm 0.72$	37.8 ± 9.3	$72.6 \pm 20.5$		
Salpidae	$0.43 \pm 0.27$	$0.29 \pm 0.12$		$0.14 \pm 0.06$		
Tanaidacea	$0.02 \pm 0.02$	$0.18 \pm 0.14$	$0.76 \pm 0.35$	$0.28 \pm 0.12$		
Zoea and megalopa larvae	$30.3 \pm 6.0$	$171 \pm 50.3$	$2691 \pm 666$	1945 ± 533		
Total individuals	$257 \pm 44$	$806 \pm 219$	$16943 \pm 4471$	$6900 \pm 1447$		

#### Table 2

Permutational analysis of variance (PERMANOVA) of the multivariate taxonomic group composition (Total Fauna Composition and Fish Family Composition) and of the univariate analysis of the rest of the taxonomic groups and fish families Engraulidae and Sparidae. Environmental variables included in each model (indicated in Table 2), have been selected from a previous PERMANOVA test. Abbreviations used are: C·F.: Control/Farm, Res: residual, df: degrees of freedom, MS: Mean Squares, Pseudo-F: statistical F value as obtained in PERMANOVA (PRIMER software) analysis and P (perm): *p*-value obtained through 4999 permutations.

		df	MS	Pseudo-F	P(perm)			df	MS	Pseudo-F	P(perm)
Total Fauna Composition	C.F	1	31130	13.42	0.0008	Mysidacea	C.F	1	21071	37807.00	0.0056
	Site(C.F)	2	2102	10.84	0.374		Site(C.F)	2	536.37	0.42	0.7188
	Day(Site(C.F))	22	1798	47.06	0.0002		Day(Site(C.F))	27	1243.7	37109.00	0.0002
	Res	147	381.99				Res	147	335.16		
	Total	180					Total	180			
Copepoda	C.F	1	15311	17.91	0.0222	Polychaeta	C.F	1	9878.7	10259	0.0752
	Site(C.F)	2	827.98	15.41	0.217		Site(C.F)	2	935.05	25445	0.0876
	Day(Site(C.F))	26	514.23	43.55	0.0002		Day(Site(C.F))	26	354.2	27506	0.0004
	Res	148	118.07				Res	149	128.77		
	Total	180					Total	180			
Zoea and Macrura	C.F	1	28826	21.61	0.0136	Total Fish	C.F	1	2688.9	97.86	0.0876
	Site(C.F)	2	1262.7	14.54	0.2244		Site(C.F)	2	274.04	0.39	0.6862
	Day(Site(C.F))	25	836.28	49.56	0.0002		Day(Site(C.F))	28	697.39	29.76	0.0002
	Res	149	168.73				Res	147	234.31		
	Total	180					Total	180			
Cladocera	C.F	1	6165.1	75.01	0.0012	Fish Family Composition	C.F	1	2688.9	97.86	0.0876
	Site(C.F)	2	81.73	703.81	0.9822		Site(C.F)	2	274.04	0.39	0.6862
	Day(Site(C.F))	26	1270.3	50.48	0.0002		Day(Site(C.F))	28	697.39	29.76	0.0002
	Res	148	251.64				Res	147	234.31		
	Total	180					Total	180			
Chaetognatha	C.F	1	26616	14.63	0.0346	Engraulidae	C.F	1	1829.8	73.76	0.1122
	Site(C.F)	2	1819.4	0.75	0.4888		Site(C.F)	2	247.77	0.42	0.6696
	Day(Site(C.F))	27	2338.2	89.75	0.0002		Day(Site(C.F))	28	587.25	29.45	0.0002
	Res	148	260.54				Res	149	199.39		
	Total	180					Total	180			

were obtained at control sites for polychaetes, chaetognaths and mysidaceans and for two days only for cladocera (Fig. 3). As for fish, differences in the total amount of captured individuals were not that evident. Nonetheless, on 13 out of 16 days, captures at farms outnumbered those at control sites (Fig. 3). On applying PERMA-NOVA to every single taxonomic group, these patterns were reinforced by showing significant differences between the two levels of the main factor –farm and control– in the experimental design (Table 2). Specifically, chaetognaths, cladocerans, copepods, crustacean larvae, mysidaceans and polychaetes were found at significantly higher abundances at farms. All of the PERMANOVA analyses included the covariables found to significantly influence the variability of zooplankton abundance.

A high variability was found depending on the sampling night;



Fig. 3. Average abundance ± standard error of the total zooplankton abundance and the main taxonomic groups found in the light traps at farm and control locations during a warm season in two SW Mediterranean fish farms. Note the different scales of the y-axis for each subpanel.

considering the averaged count within single dates, the difference between the day with the lowest zooplankton abundance and the highest ranged between 64.6  $\pm$  24.2 to 3861  $\pm$  1165 ind trap<sup>-1</sup> h<sup>-1</sup> sites for the samples taken at control and 90.6  $\pm$  30.6-67979  $\pm$  16048 ind trap<sup>-1</sup> h<sup>-1</sup> at farm sites. This marked variability among days was reflected in the PERMANOVA test, since this factor (Day) appeared as significantly different for all the analysed groups. This variability, however, was not an impediment for detecting the differences at Farm vs. Control level. Additionally, regarding time within each sampling night, the Timesince-sunset variable was not identified as significant by the DistLM analysis, thus showing a probably steady concentration of zooplankton during every night.

Taking into account the size of trapped fish, probably only the individuals with a sufficient swimming capacity to surpass currents and actively enter the traps were found inside them. This was inferred from the mean size of captured individuals;  $21.99 \pm 1.43$ and  $18.43 \pm 6.8$  mean standard length (mm SL) at control and farm locations respectively. The most abundant family was Engraulidae with 22 vs 71 fish captured at control and farm locations respectively. They presented an average size of 21.67  $\pm$  0.76 mm SL at control and  $19.64 \pm 0.40$  mm SL at farm locations (Fig. 4), finding no significant differences between treatments at this level. Thus, Engraulis encrasicolus was the most abundant species with a presence of 0.41  $\pm$  0.12 ind trap<sup>-1</sup> h<sup>-1</sup> and 0.93  $\pm$  0.19 ind trap<sup>-1</sup> h<sup>-1</sup> at control and farm traps respectively, followed by sparids, with an average capture of 0.16  $\pm$  0.15 and 0.15  $\pm$  0.04 ind trap<sup>-1</sup> h<sup>-1</sup>, at control and farms. Other fish families were found in the traps, such as atherinids, blenniids, carangids, clupeids, mugilids and pomatomids, but with very low numbers that never exceed 0.08 ind trap<sup>-1</sup> h<sup>-1</sup>.

### 4. Discussion

A higher abundance of zooplankton in the pelagic zone around



**Fig. 4.** Boxplot of the standard length of *Engraulis encrasicolus* individuals captured at the four sampling sites. Boxes indicate 1st and 3rd quartiles, horizontal line shows median values, whiskers mark extreme values and points represent the outliers.

coastal facilities farming seabass and seabream in the SW Mediterranean was detected through the deployment of light trap devices. This pattern was consistent at two different farms and throughout the whole study period. Analysis of the different taxonomic groups in the zooplankton community revealed that this is a generalised effect for the vast majority of plankton groups, since abundances were several times higher around cages compared to control locations without aquaculture influence. Particularly, copepods and crustacean larva abundance was more than 20 times higher around farms than at control locations.

Analysis of results showed that certain groups had an augmented photophilic behaviour when comparing their proportional abundance with that of plankton tows, assuming the latter would reflect a taxonomic composition of zooplankton closer to reality. Therefore, it was concluded that traps overestimated taxa like isopoda, polychaeta, mysidacea or zoea and underestimated others like pteropoda or apendicularia. These groups were found in low numbers, accounting for less than 1% of the total faunal composition. An exception to this was of course zoea larvae, which were the second most abundant taxa and one of the groups responsible for the differences between farm and control locations. Consequently, results obtained using the light trap model used in this work should always be interpreted carefully, bearing in mind the potential biases regarding these taxa. However, it is clear that this bias occurred likewise at both control and farm locations and therefore the generalised pattern of a higher abundance at farm sites for all the taxonomical groups is consistent irrespective of the sampling methodology. In the case of fish captures, the number of individuals captured by the plankton nets was 3.5 times higher than that of traps but of very different size, since traps tended to capture juvenile fish and plankton nets caught larval individuals. Consequently, light traps may not be an appropriate tool for monitoring early life-stages of fish at farms, with the potential exception of families abundant in the Mediterranean like engraulidae or sparidae. More representative surveys might be obtained if the trapping effort were increased through a higher number of light traps or longer illumination periods and battery life. Other studies have previously used various light-trap models to study the plankton community, with similar results to this work regarding the groups captured. For instance, the crustaceans zoea and megalopa usually appear in high numbers inside light traps (Miller and Shanks, 2004; Sigurdsson et al., 2014). Furthermore, the relationship between light and farms has been studied at salmon farms in British Columbia, Canada, where some farms are illuminated during the night in order to suppress gonadal development (Hay et al., 2004). Specifically, through the use of plankton hauls and purse seines, McConell et al. (2010) assessed the zooplankton dynamics at illuminated farms, detecting markedly higher abundances of gastropods and bivalves, but also copepods, polychaetes (mainly Spionidae) and nauplius larvae as well as 5 species of larval fish and 2 of juvenile fish. However, the higher abundance of zooplankton at farms is not only restricted to illuminated facilities, because it has been corroborated elsewhere that the presence of pelagic invertebrates is also greater in the water column at nonilluminated farms compared to control locations. In this vein, Fernandez-Gonzalez et al. (2014) concluded that the abundant concentration of planktonic amphipods at farms is the combined result of the input from strictly pelagic species, individuals from fouling communities living on the farm structures, and migrant amphipods from soft sediments. Daily vertical migration from nearby benthic communities could be one of the driving forces that increase abundance of invertebrates around fish farms (Sanchez-Jerez et al., 1999). Bearing this in mind, the lack of significant differences for total fish composition and the Engraulidae family could be due to an actual lack of differences, to the low efficacy of our survey design to capture fish or to a general low abundance of fish in the region and time of sampling. Nonetheless, these low capture numbers are not surprising considering the relative low abundance of larval and juvenile fish in oligotrophic regions like the SW Mediterranean (Sabatés et al., 2003; Kehayias et al., 2008; López-Sanz et al., 2009; Tor et al., 2010; Félix-Hackradt et al., 2013), when compared to more productive regions (Carassou et al., 2009; Shaw et al., 2007; López-Sanz et al., 2009; Sabatés et al., 2003; Tor et al., 2010).

Nevertheless, high presence and diversity of juvenile fish closely associated with the farms have already been demonstrated at different SW Mediterranean farms, including those studied in this work (Fernandez-Jover et al., 2009; Fernandez-Jover and SanchezJerez, 2014). The reasons behind the selection of these artificial habitats by fish as settlement sites remain unclear, but some of the consequences have been outlined, for instance a change in the fatty acid profile of several fish species (Fernandez-Jover et al., 2009), as well as potential effects on fish growth noticed through otolith analysis (Fernandez-Jover and Sanchez-Jerez, 2014). Additionally, the higher zooplankton abundance at the cages may also promote the attraction and permanence of juvenile fish at farm sites, given that the different species of juvenile fish settled at farms actively feed on it (Fernandez-Jover et al., 2009; Fernandez-Jover and Sanchez-Jerez, 2014). Further studies should be carried out to elucidate the reasons for this higher abundance of zooplankton at farms, and its repercussions. However, we suggest that attraction by chemical cues may be of little importance due to the limited swimming capacity of the zooplankton detected. It is more likely the result of the physical retention of plankton dragged towards the farms by currents (see Klebert et al., 2013).

The structural framework of the farms, including nets, mooring systems and tons of cultivated fish, modifies the local oceanographic dynamics by reducing current velocity, and consequently favours retention of particles like plankton. This hydrodynamic effect of aquaculture structures occurs both at fish cages (Panchang et al., 1997; Madin et al., 2010; Klebert et al., 2013) and mussel farms (Plew et al., 2005; O'Donncha et al., 2013; Cranford et al., 2014). This could raise the concentration of pelagic zooplankton, also promoting the rapid colonisation of farm structures by a rich diverse fouling community (Greene and Grizzle, 2007; Madin et al., 2009). Additionally, zooplankton taxon diversity at control sites did not substantially differ from those at farms (because differences were mainly due to the relatively higher abundances at the aquaculture facilities but not to differences in groups composition). This also supports the hypothesis of plankton retention by farm structures rather than a selective attraction by chemical or physical cues. Various authors have proposed a rapid transfer of nutrients up the food web at farms, which could also have influenced the present results. In this way, in the Aegean Sea, Pitta et al. (2009) undertook dialysis bag experiments near fish farms in order to selectively withdraw grazers from some of these bioassays, concluding that the usual lack of detection of high levels of chlorophyll a in oligotrophic waters around farms may be a consequence of rapid transfer of nutrients up the food web, reinforced by intense grazing activity. Our results showing a notable abundance of zooplankton around farms would support this conclusion. Nutrients originating at farms may also stimulate the development of an abundant zooplankton community, due to the greater food availability in the form of particulate organic matter (POM) derived from aquaculture wastes (Koppelmann et al., 2009). This POM is consumed by zooplankton, since specific distinguishable fatty acids in the food pellets are incorporated into the trophic web, as detected via analysis of the lipid profile of zooplankton and juvenile fish (Fernandez-Jover et al., 2009). Thus, pelagic communities may be assimilating and taking advantage of POM in the same way as found for fouling species (Gonzalez-Silvera et al., 2015). The present data indicate a sharp rise in the population of zooplankton groups around aquaculture cages in SW Mediterranean coastal waters, including a tendency towards higher fish larva numbers in the case of engraulids and sparids. To our knowledge, the main driving factor of this enhanced abundance could be a general retention of the plankton particles as a result of modified hydrodynamics at farms, but other synergistic factors such as the action of physical and chemical cues or efficient flow of nutrients up the food web may also be involved. Modification of planktonic communities at farms may entail consequences for nutrient cycling, rapid development of fouling and its associated fauna on the farm structures, and also for trophic relationships between the components of the

food chain.

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