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D. Fernandez-Jover, K. Toledo-Guedes, J.M. Valero-Rodríguez, V. Fernandez-Gonzalez, P. Sanchez-Jerez

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#### 1 Potential retention effect at fish farms boosts zooplankton abundance

2 Fernandez-Jover, D.\*, Toledo-Guedes, K., Valero-Rodríguez, J.M., Fernandez-Gonzalez, V.,

3 Sanchez-Jerez, P.

4 Department of Marine Sciences and Applied Biology. University of Alicante. P.O. BOX 03690

5 Alicante, Spain.

6 **\*Corresponding author:** <u>jover@ua.es</u>; +34965903400

#### 7 ABSTRACT:

8 Coastal aquaculture activities influence wild macrofauna in natural environments due to the 9 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 10 11 their feeding behaviour and diet. Despite this, the effects of coastal aquaculture on 12 zooplankton assemblages and the potential changes in their abundance and distribution remain largely unstudied. Traditional plankton sampling hauls between the farm mooring 13 14 systems entail some practical difficulties. As an alternative, light traps were deployed at 2 15 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 16 10 times higher for the taxonomic groups: bivalvia, cladocera, cumacea, fish early-life-stages, 17 18 gastropoda, polychaeta and tanaidacea; 10 to 20 times higher for amphipoda, chaetognatha, 19 isopoda, mysidacea and ostracoda, and 22 times higher for copepoda and the crustacean 20 juvenile stages zoea and megalopa. Permutational analysis showed significant differences for 21 the most abundant zooplankton groups (copepoda, crustacean larvae, chaetognatha, 22 cladocera, mysidacea and polychaeta). This marked incremental increase in zooplankton taxa 23 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 24 25 results suggest a retention effect caused by cage structures rather than active attraction 26 through physical or chemical cues.

#### 27 Introduction

Over the last three decades, fish-farming cages have rapidly developed throughout the world (FAO 2014, 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 2014, 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).

38 Moreover, the deployment of these massive artificial structures in the pelagic environment 39 may provoke severe changes in the wild biota composition, from phytoplankton (Dalsgaard 40 and Jensen 2006) to macrofauna (Carss 1990, Franks 2000, Dempster et al. 2012) and megafauna (Díaz López and Bernal Shirai 2007, Arechavala-Lopez et al. 2014, 2015). Complex 41 42 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 43 44 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 45 farms on ichthyofauna is not strictly limited to adult fish, since juvenile fish from several 46 47 different families generally use farm structures as settlement grounds, with potential 48 consequences for their physiology and growth (Fernandez-Jover et al. 2009, Fernandez-Jover 49 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 50 51 found that resources may be at least as accessible as they are in traditional settlement 52 environments such as natural shallow rocky shores. The main prey of aggregated juvenile fish 53 are typical zooplankton taxa, e.g. adult and juvenile copepods, cladocerans, nauplius larvae or amphipods (Fernandez-Jover et al. 2009). 54

55 In the SW Mediterranean, it has already been corroborated using light traps that European 56 seabass and gilt-head bream farms favour the presence (among others) of holoplanktonic 57 amphipods in the pelagic environment. In this way, Fernandez-Gonzalez et al. (2014) detected 58 an abundant community of planktonic amphipods at farms when compared to environments 59 where these structures were absent, comprising strictly pelagic species and also benthic and 60 fouling-community species that apparently undertake incursions into the pelagic zone at night. 61 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 62 63 is also thought to increase plankton communities in oligotrophic environments (Tsagaraki et al. 64 2013).

65 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 66 67 communities (Miller and Shanks 2004; Shaw et al. 2007; Tor et al. 2010; Fernandez-Gonzalez et 68 al. 2014; Sigurdsson et al. 2014). Furthermore, the relationship between artificial light 69 attraction and zooplankton has already been studied at farms; McConell et al. (2010) detected 70 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 71 72 larval and juvenile fish species, were greater at night-lit farms. However, the zooplankton 73 communities at non-illuminated farms were not compared with areas not influenced by 74 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.

#### 82 Material and methods

#### 83 Study area and sampling effort

This study was carried out in coastal waters, in Guardamar del Segura bay (Alicante, Spain: 38° 84 5' 7.45" N; 0° 35' 51.40" W) from 12th June to 10th October 2012, the warm period in the 85 86 Western Mediterranean. Sampling was conducted at two fish farms (Fig. 1A) producing 87 seabass (Dicentrarchus labrax) and seabream (Sparus aurata), and two control areas, on 16 88 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 89 90 depth of 23 m, which was reached at least 3 km away from the shore. All four localities (2 91 control and 2 farms) were located 3-4 km offshore at depths ranging from 23 to 30 m. Each 92 farm consisted of 18 rings with a diameter of 19 or 25 m and cage nets reaching depths from 93 12 to 15 m, enclosing a cage volume up to 7400 m<sup>3</sup>. Changes in abundances and species 94 composition in the plankton population were investigated by sampling farm and control areas 95 with light traps.

96 Light-trap design used in this study was a modification of that employed by Floyd et al. (1984) 97 and Kissick (1993), which consisted of a plexiglas collection chamber measuring 40 x 40 x 40 98 cm, with eight panels forming four funnel-shaped entrances 3 mm wide. The light source was a 99 hand diving-torch (Led Lenser D14, 150 lumen) coupled to a white plastic container that 100 produced a diffuse point of illumination.

101 The light-trap technique provides selective sampling, since results are biased towards 102 photophilic species. However, it has traditionally been used for various purposes, generally 103 aimed at capturing zooplankton species, most frequently early life-stages of fish (e.g. Floyd et 104 al. 1984; Doherty 1987). Additionally, it is useful in studies at places with difficult access or 105 where habitual sampling methods such as plankton hauls are inconvenient. Specifically, 106 oblique hauls may become logistically problematic. Researchers that still decided to deploy 107 nets between the cages had to limit sampling to vertical hauls or small purse seines (McConell 108 et al. 2010); light traps thus seem an appropriate alternative for sampling in logistically 109 difficult habitats (Chicharo et al. 2009).

110 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 111 retrieval times to the nearest minute (for later standardisation to individuals per traps per 112 hour), and their contents then removed. Due to logistical constraints we were only able to 113 114 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 115 116 traps were deployed approximately at the same time at the cages and two at control site and 117 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 to 30 m 118 119 after retrieval, and a period of at last 30 min was allowed prior to next deployment. At 120 recovery time, traps were raised slowly to allow filtration of the chamber content through the 121 250 μm-mesh bottom of the collection cup. Material retained was preserved in 4% formalin 122 seawater solution. In the laboratory, samples were sorted, counted and the main plankton 123 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, 124 125 Fahay 2007, Ré and Meneses 2008, Lecaillon et al. 2012).

126 Environmental variables were obtained or measured *in situ* in order to include them in the 127 design as covariables with the objective of inferring if their fluctuations had a significant 128 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 129 130 of lunar month (DLM), Moon illumination, State of the sea (wave height in m), Time to 131 moonrise, Time since sunset, Time between sunset and moonrise, Time from the nearest high 132 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 133 134 direction and velocity were also added as predictor variables. The average direction and 135 velocity during the previous 24 hours before every sampling night was obtained from the 136 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 137 for the previous 24 hours prior to sampling and then simplified into four vectors corresponding 138 139 to main current directions NNE-SSW, ENE-WSW, ESE-WNW and SSE-NNW, taking positive and 140 negative values for every direction (e.g. positive values for currents with direction NNE, 141 between 45-90<sup>o</sup>, and negative for currents towards SSW between 180-225<sup>o</sup>).

#### 142 Plankton hauls

143 To assess the suitability and potential biases of light traps when sampling zooplankton and 144 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), 145 and towed at a depth of 1 to 5 m for four minutes at low speed (3 knots). Four double-oblique 146 147 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 148 149 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 150 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 151 152 the percentage of the species i in the trap and  $p_i$  the percentage of the species i in the 153 environment (plankton tows). This index varies from +1.0 to -1.0, where positive values indicate attraction and negative values avoidance. 154

155 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, 162 163 data from trap captures were analysed according to a 3-factor hierarchical design: 'C-F' (fixed; 164 two levels: Control and Farm); 'Site' (random; two levels) and 'Day' (random; eight levels), with 165 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, 166 which is robust even when there are unequal numbers of replicate samples within each factor 167 168 level of the design (*i.e.* unbalanced designs; Anderson et al. 2008). The analysis was performed 169 over the Bray–Curtis dissimilarities matrix (Euclidean distance matrix in the case of univariate 170 analyses) of the transformed data, applying a log (x+1) transformation (Anderson 2001a, 171 McArdle & Anderson 2001) using 4999 random permutations of residuals under a reduced 172 model (Anderson 2001b), with appropriate units as required by the design (Anderson & ter 173 Braak 2003).

174 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 175 assemblage data and each taxonomic group separately, in a way comparable to multiple 176 177 regression (Anderson et al., 2008). The Akaike information criterion (AIC) and R<sup>2</sup> were used to 178 choose the best model from all possible combinations of variables. Statistical significance 179 (after 4999 permutations) and percentage contribution of each variable alone, ignoring all 180 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 181 182 0.6%). Despite this, a few variables exerted a significant influence on the planktonic 183 assemblages and were therefore included as covariables in the permutational multi- and univariate analyses of variance (PERMANOVAs) explained in the previous paragraph. 184 185 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 186 performed using PRIMER-E software (PRIMER software; Clarke & Gorley 2006) with the add-on 187 package PERMANOVA+ (Anderson et al. 2008). 188

#### 189 Results

190 Comparing plankton hauls and light-trap captures, lvlev's selectivity index showed that traps, 191 when compared to plankton tows, tended to overestimate the presence of isopoda, 192 polychaeta, mysidacea, ostracoda and zoea larvae with *E* values that ranged between 0.92 and 193 0.57 (Fig. 2). On the other hand, results pointed to an underestimation of such taxa as 194 pteropoda, appendicularia, larvae planula, bivalves, cnidaria, cladocerans, tunicates, larvae

195 nauplii and gasteropoda, with values between -0.75 and -0.37. Finally, for the groups 196 amphipoda, copepoda, chaetognatha, fish and tanaidacea, values were close to 0 (between 197 +0.10 and-0.21), showing results very similar to those found in plankton nets. Regarding fish, 198 light traps tended to capture juvenile individuals, while plankton nets mainly captured 199 preflexion and flexion larvae, thus not allowing the calculation of the selectivity index for 200 osteichthyes.

201 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  $\pm$  117 ind  $\cdot$  trap<sup>-1</sup>  $\cdot$  h<sup>-1</sup> 202 (mean  $\pm$  standard deviation) at control sites versus 12044  $\pm$  2400 ind  $\cdot$  trap<sup>-1</sup>  $\cdot$  h<sup>-1</sup> in light traps 203 deployed at fish farms (averaging all control and all farm samples). The higher abundance of 204 205 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 206 copepoda, with an abundance of 392  $\pm$  92.7 ind  $\cdot$  trap<sup>-1</sup>  $\cdot$  h<sup>-1</sup>at control sites and 9235  $\pm$  2023 ind 207  $\cdot$  trap<sup>-1</sup>  $\cdot$  h<sup>-1</sup> at farms, and larval crustacea including zoea and megalopa stages, with 100 ± 26.6 208 and 2343  $\pm$  432 ind  $\cdot$  trap<sup>-1</sup>  $\cdot$  h<sup>-1</sup> at control sites and farms respectively. Additional groups were 209 210 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 211 212 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 213 control and farm locations respectively. 214

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  $10^{\text{th}}$  of July at farm site 1. In contrast, it was notable that only 14 ind  $\cdot$  trap<sup>-1</sup>  $\cdot$  h<sup>-1</sup> were found inside a light trap on the 10<sup>th</sup> 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).

235 On every sampling night, abundance was always higher for copepods and crustacean larvae at 236 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 were obtained at control 237 238 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. 239 240 Nonetheless, on 13 out of 16 days, captures at farms outnumbered those at control sites (Fig. 241 3). On applying PERMANOVA to every single taxonomic group, these patterns were reinforced 242 by showing significant differences between the two levels of the main factor -farm and 243 control- in the experimental design (Table 2). Specifically, chaetognaths, cladocerans, copepods, crustacean larvae, mysidaceans and polychaetes were found at significantly higher 244 245 abundances at farms. All of the PERMANOVA analyses included the covariables found to significantly influence the variability of zooplankton abundance. 246

A high variability was found depending on the sampling night; considering the averaged count 247 248 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  $\cdot$  trap<sup>-1</sup>  $\cdot$  h<sup>-1</sup> for the samples 249 taken at control sites and 90.6  $\pm$  30.6 to 67979  $\pm$  16048 ind  $\cdot$  trap<sup>-1</sup>  $\cdot$  h<sup>-1</sup> at farm sites. This 250 marked variability among days was reflected in the PERMANOVA test, since this factor (Day) 251 252 appeared as significantly different for all the analysed groups. This variability, however, was 253 not an impediment for detecting the differences at Farm vs. Control level. Additionally, 254 regarding time within each sampling night, the Time-since-sunset variable was not identified as 255 significant by the DistLM analysis, thus showing a probably steady concentration of 256 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. 261 262 They presented an average size of  $21.67 \pm 0.76$  mm SL at control and  $19.64 \pm 0.40$  mm SL at 263 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  $\cdot$  trap 264 265  $^{1} \cdot h^{-1}$  and 0.93 ± 0.19 ind  $\cdot$  trap  $^{-1} \cdot h^{-1}$  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  $\cdot$  trap<sup>-1</sup>  $\cdot$  h<sup>-1</sup>, at control and 266 267 farms. Other fish families were found in the traps, such as atherinids, blenniids, carangids, 268 clupeids, mugilids and pomatomids, but with very low numbers that never exceed 0.08 ind ·  $trap^{-1} \cdot h^{-1}$ . 269

#### 270 Discussion

271 A higher abundance of zooplankton in the pelagic zone around coastal facilities farming 272 seabass and seabream in the SW Mediterranean was detected through the deployment of light 273 trap devices. This pattern was consistent at two different farms and throughout the whole 274 study period. Analysis of the different taxonomic groups in the zooplankton community 275 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 276 277 aquaculture influence. Particularly, copepods and crustacean larva abundance was more than 278 20 times higher around farms than at control locations.

279 Analysis of results showed that certain groups had an augmented photophilic behaviour when 280 comparing their proportional abundance with that of plankton tows, assuming the latter 281 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 282 283 underestimated others like pteropoda or apendicularia. These groups were found in low 284 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 285 286 responsible for the differences between farm and control locations. Consequently, results 287 obtained using the light trap model used in this work should always be interpreted carefully, 288 bearing in mind the potential biases regarding these taxa. However, it is clear that this bias 289 occurred likewise at both control and farm locations and therefore the generalised pattern of a 290 higher abundance at farm sites for all the taxonomical groups is consistent irrespective of the 291 sampling methodology. In the case of fish captures, the number of individuals captured by the 292 plankton nets was 3.5 times higher than that of traps but of very different size, since traps 293 tended to capture juvenile fish and plankton nets caught larval individuals. Consequently, light

294 traps may not be an appropriate tool for monitoring early life-stages of fish at farms, with the 295 potential exception of families abundant in the Mediterranean like engraulidae or sparidae. 296 More representative surveys might be obtained if the trapping effort were increased through a 297 higher number of light traps or longer illumination periods and battery life. Other studies have 298 previously used various light-trap models to study the plankton community, with similar 299 results to this work regarding the groups captured. For instance, the crustaceans zoea and 300 megalopa usually appear in high numbers inside light traps (Miller and Shanks 2004; 301 Sigurdsson et al. 2014). Furthermore, the relationship between light and farms has been 302 studied at salmon farms in British Columbia, Canada, where some farms are illuminated during 303 the night in order to suppress gonadal development (Hay et al. 2004). Specifically, through the 304 use of plankton hauls and purse seines, McConell et al. (2010) assessed the zooplankton 305 dynamics at illuminated farms, detecting markedly higher abundances of gastropods and 306 bivalves, but also copepods, polychaetes (mainly Spionidae) and nauplius larvae as well as 5 307 species of larval fish and 2 of juvenile fish. However, the higher abundance of zooplankton at 308 farms is not only restricted to illuminated facilities, because it has been corroborated 309 elsewhere that the presence of pelagic invertebrates is also greater in the water column at 310 non-illuminated farms compared to control locations. In this vein, Fernandez-Gonzalez et al. 311 (2014) concluded that the abundant concentration of planktonic amphipods at farms is the 312 combined result of the input from strictly pelagic species, individuals from fouling communities 313 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 314 315 abundance of invertebrates around fish farms (Sanchez-Jerez et al. 1999). Bearing this in mind, 316 the lack of significant differences for total fish composition and the Engraulidae family could be 317 due to an actual lack of differences, to the low efficacy of our survey design to capture fish or 318 to a general low abundance of fish in the region and time of sampling. Nonetheless, these low 319 capture numbers are not surprising considering the relative low abundance of larval and 320 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 321 322 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). 323

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 Sanchez-Jerez 2014). The reasons behind the selection of these artificial habitats by fish as settlement sites remain

328 unclear, but some of the consequences have been outlined, for instance a change in the fatty 329 acid profile of several fish species (Fernandez-Jover et al. 2009), as well as potential effects on 330 fish growth noticed through otolith analysis (Fernandez-Jover and Sanchez-Jerez 2014). 331 Additionally, the higher zooplankton abundance at the cages may also promote the attraction 332 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-333 334 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 335 336 attraction by chemical cues may be of little importance due to the limited swimming capacity 337 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). 338

339 The structural framework of the farms, including nets, mooring systems and tons of cultivated 340 fish, modifies the local oceanographic dynamics by reducing current velocity, and consequently favours retention of particles like plankton. This hydrodynamic effect of aquaculture structures 341 342 occurs both at fish cages (Panchang et al. 1997, Madin et al. 2010, Klebert et al. 2013) and 343 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 344 345 structures by a rich diverse fouling community (Green and Grizzle 2007, Madin et al. 2009). 346 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 347 348 aquaculture facilities but not to differences in groups composition). This also supports the 349 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 350 351 food web at farms, which could also have influenced the present results. In this way, in the 352 Aegean Sea, Pitta et al. (2009) undertook dialysis bag experiments near fish farms in order to 353 selectively withdraw grazers from some of these bioassays, concluding that the usual lack of 354 detection of high levels of chlorophyll a in oligotrophic waters around farms may be a 355 consequence of rapid transfer of nutrients up the food web, reinforced by intense grazing 356 activity. Our results showing a notable abundance of zooplankton around farms would support 357 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 358 359 particulate organic matter (POM) derived from aquaculture wastes (Koppelmann et al. 2009). 360 This POM is consumed by zooplankton, since specific distinguishable fatty acids in the food 361 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 362 363 assimilating and taking advantage of POM in the same way as found for fouling species 364 (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 365 366 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 367 368 of the plankton particles as a result of modified hydrodynamics at farms, but other synergistic 369 factors such as the action of physical and chemical cues or efficient flow of nutrients up the 370 food web may also be involved. Modification of planktonic communities at farms may entail 371 consequences for nutrient cycling, rapid development of fouling and its associated fauna on 372 the farm structures, and also for trophic relationships between the components of the food 373 chain.

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- 545 Figure 1. Sampling design of control and farm sites, showing the sampling days at each site (A) and diagram of light trap deployment (B). The design in Figure A was 546 repeated 3 times, making a total of 6 replicates each night. Each cross represents a light trap.



- Figure 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.



558 Figure 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 559 during a warm season in two SW Mediterranean fish farms. Note the different scales of the y-axis for each subpanel.

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



567 Table 1. Total abundance (individuals  $\cdot$  trap<sup>-1</sup>  $\cdot$  h<sup>-1</sup> ± standard error) in the light traps 568 deployed at control and farm sites.

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

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Table 2. Permutational analysis of variance (PERMANOVA) of the multivariate 574 575 taxonomic group composition (Total Fauna Composition and Fish Family Composition) and of the univariate analysis of the rest of the taxonomic groups and 576 577 fish families Engraulidae and Sparidae. Environmental variables included in each model (indicated in Table 2), have been selected from a previous PERMANOVA test. 578 Abbreviations used are: C.F.: Control/Farm, Res: residual, df: degrees of freedom, 579 MS: Mean Squares, Pseudo-F: statistical F value as obtained in PERMANOVA 580 (PRIMER software) analysis and P (perm): p-value obtained through 4999 581 permutations. 582

	10 10	df	MS Pse	eudo-F P(	perm)			df	MS P	seudo-F P	(perm)
	C.F	Ч	31130	13.42	0.0008		C.F	Ч	21071	37807.00	0.0056
The second second	Site(C.F)	2	2102	10.84	0.374		Site(C.F)	2	536.37	0.42	0.7188
Iotal Fauna	Day(Site(C.F))	22	1798	47.06	0.0002	Mysidacea	Day(Site(C.F))	27	1243.7	37109.00	0.0002
Composition	Res	147	381.99				Res	147	335.16		
	Total	180					Total	180			
	CF	Ч	15311	17.91	0.0222		C.F	Н	9878.7	10259	0.0752
	Site(C.F)	2	827.98	15.41	0.217		Site(C.F)	2	935.05	25445	0.0876
Copepoda	Day(Site(C.F))	26	514.23	43.55	0.0002	Polychaeta	Day(Site(C.F))	26	354.2	27506	0.0004
	Res	148	118.07				Res	149	128.77		
	Total	180					Total	180			
	CF	L	28826	21.61	0.0136		C.	Н	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
zoea and	Day(Site(C.F))	25	836.28	49.56	0.0002	<b>Total Fish</b>	Day(Site(C.F))	28	697.39	29.76	0.0002
Macrura	Res	149	168.73				Res	147	234.31		
	Total	180					Total	180			
	CF	Г	6165.1	75.01	0.0012		C.	Н	2688.9	97.86	0.0876
	Site(C.F)	2	81.73	703.81	0.9822	Party Party Party	Site(C.F)	2	274.04	0.39	0.6862
Cladocera	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			Composition	Res	147	234.31		
	Total	180					Total	180			
	CF	Ч	26616	14.63	0.0346		C.F	Н	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
Chaetognatha	Day(Site(C.F))	27	2338.2	89.75	0.0002	Engraulidae	Day(Site(C.F))	28	587.25	29.45	0.0002
	Res	148	260.54				Res	149	199.39		
	Total	180					Total	180			/