

1 **Life strategies of cephalopod paralarvae in a coastal upwelling system**  
2 **(NW Iberian Peninsula): insights from zooplankton community and**  
3 **spatio-temporal analyses**

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12  
13 **Running title:** Life strategies of planktonic cephalopod larvae

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15 **ABSTRACT**

16 Early life stages of cephalopods -octopods, squids, sepiolids and ommastrephids- are  
17 uncommon in zooplankton samples and little is known about their life strategies.  
18 Accordingly, cephalopod paralarvae were examined in the upwelling ecosystem of the  
19 Ría de Vigo (NW Spain) at night from 2008-2010. Multivariate analyses and  
20 generalized linear models (GLMs) were used to explore relationships between  
21 cephalopod paralarvae and the zooplankton communities that they inhabited in 2008. In  
22 addition, the foraging strategy and prey preferences of *Octopus vulgaris* paralarvae  
23 within these communities were determined. Multivariate and GLM results showed a  
24 strong association of cephalopod paralarvae with coastal and frontal zooplankton  
25 communities. *Octopus* paralarvae were shown to be specialist predators with a strong  
26 preference for decapod zoeae in each of the communities examined. Using the three  
27 years of sampling, GLM analyses of paralarval spatio-temporal variations in relation  
28 with the upwelling strength showed a positive relationship with upwelling intensity for  
29 *O. vulgaris* and sepiolids, as well as contrasting temporal, horizontal and vertical  
30 distributions for the different paralarvae analysed. Under strong upwelling events  
31 *Octopus* paralarvae were more abundant in surface waters, whereas abundance of  
32 loliginids and sepiolids was higher in the water column. This vertical behaviour in  
33 conjunction with the physical conditions of the Western Iberian Upwelling ecosystem  
34 suggest the coexistence of two different life strategies: a coastal strategy displayed by  
35 loliginid and sepiolid paralarvae that are retained over the shelf, and an oceanic strategy  
36 displayed by *O. vulgaris* paralarvae that are dispersed far from the shelf.

37 **KEY WORDS:** cephalopod paralarvae, zooplankton communities, trophic ecology,  
38 early life strategies, vertical behaviour, NW Iberian upwelling.

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## 40 INTRODUCTION

41 Early life history stages of cephalopods are scarce in plankton samples due to their high  
42 growth rates, patchy distributions and seasonal life cycles. Their scarcity combined with  
43 a short-life span and the absence of overlap between successive generations brings  
44 about drastic inter-annual fluctuations in recruitment, mostly driven by environmental  
45 variability (Boyle and Boletzky, 1996; Boyle and Rodhouse, 2005). The NW coast of  
46 the Iberian Peninsula has been surveyed since the mid 1990s to understand the  
47 variability of early life stages of cephalopods in relation to the coastal oceanographic  
48 conditions (e.g. Rocha *et al.*, 1999; González *et al.*, 2005; Moreno *et al.*, 2009; Otero *et*  
49 *al.*, 2009). In this region, northerly winds promote seasonal coastal upwelling from  
50 March-April to September-October, however the upwelling season appears as a  
51 succession of wind stress/relaxation cycles of periods lasting 10 to 20 days (Álvarez-  
52 Salgado *et al.*, 2003). Early hatchlings of *Octopus vulgaris* Cuvier, 1797 have been  
53 studied in daylight hours, revealing that changes in abundance were linked to the  
54 hydrographic conditions (Otero *et al.*, 2009), and that the environmental forcing  
55 affecting the paralarval stage was related to the variability found in later adult catches  
56 (Otero *et al.*, 2008). Furthermore, Moreno *et al.* (2009) studied the distribution patterns  
57 of several neritic and oceanic paralarvae along the western Iberia upwelling system,  
58 showing that temperature and upwelling were the most important variables affecting the  
59 spatio-temporal distribution of neritic cephalopod paralarvae.

60 All the studies previously mentioned investigated abiotic influences on paralarvae  
61 abundance. Few studies have addressed the interactions between cephalopod paralarvae  
62 and zooplankton communities, likely due to the inherent difficulties in obtaining  
63 zooplankton data. The studies that did address these interactions revealed relevant  
64 ecological traits: like coastal community preferences in the long fin inshore squid -  
65 *Doryteuthis pealei*- paralarvae (Vecchione and Grant, 1983), changes in diet during the

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66 development of oceanic cephalopod paralarvae (Passarella and Hopkins, 1991),  
67 migration patterns in the Chokka squid hatchlings *Loligo reynaudii*- towards areas of  
68 copepod concentration on the eastern Agulhas Bank (Roberts, 2005), or retention at  
69 seamounts that favours local recruitment (Diekmann *et al.*, 2006). The only study  
70 carried out in the waters off NW Iberian Peninsula addressed the diet of wild *Octopus*  
71 *vulgaris* hatchlings in the zooplankton of the Ría de Vigo in 2008, revealing that  
72 octopus paralarvae were mainly feeding on crustaceans (Roura *et al.*, 2012). The  
73 authors suggested that *O. vulgaris* hatchlings were specialist predators and detected this  
74 by comparing the prey detected within hatchlings against the averaged abundances of  
75 zooplankton organisms in the Ría de Vigo.

76       The mesozooplankton communities present at night in the Ría de Vigo in 2008  
77 were recently described by Roura *et al.* (2013). Three mesozooplankton communities -  
78 named as coastal, frontal and oceanic- were identified depicting a coastal-oceanic  
79 gradient, according to the following ecological descriptors: holoplankton-meroplankton  
80 ratio, species richness and total abundance. These communities changed qualitatively  
81 and quantitatively from summer to autumn and, consequently, six mesozooplankton  
82 communities were identified in the Ría de Vigo. These findings provide a unique  
83 opportunity to explore the relationships of the cephalopod paralarvae with the  
84 zooplankton communities they inhabit. Moreover, knowing the composition of the  
85 different zooplankton communities permits testing of the hypothesis that *O. vulgaris*  
86 hatchlings are specialist predators on each community from which they were collected.  
87 This will enable testing of whether *O. vulgaris* hatchlings were selective in their feeding  
88 preferences and whether their foraging tactics differed on each community. The need of  
89 studies linking development at the planktonic stage and ecology in cephalopods is  
90 advocated, since it helps to deepen our comprehension of the different life strategies  
91 displayed by the paralarvae (Robin *et al.*, 2014).

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2 92 Specifically, the aims of this work are 1) to understand the short-term spatio-  
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4 93 temporal distributions of the cephalopod paralarvae found at night in the Ría de Vigo in  
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6 94 2008 and their relationships with the mesozooplankton community; 2) to study the  
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8 95 trophic ecology of *O. vulgaris* hatchlings within the zooplankton communities; and 3) to  
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10 96 examine the inter-annual spatio-temporal variability of cephalopod paralarvae collected  
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12 97 in the Ría de Vigo from 2008 to 2010 at night in relation to the upwelling index. These  
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14 98 three aims explore different aspects of the ecology of cephalopod paralarvae during  
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16 99 their planktonic stage and will increase our understanding of their life strategies in this  
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18 100 productive coastal upwelling ecosystem.  
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## 102 MATERIAL AND METHODS

### 103 *Mesozooplankton sampling and cephalopod paralarvae sorting*

104 Thirty surveys to collect zooplankton and hydrographic data were undertaken at night in  
105 the Ría de Vigo (NW Spain, 42°12.80'N, 09°00'W) onboard RV “Mytilus” from 2008  
106 to 2010 (Fig. 1). Ten surveys per year were performed in early summer (four in July)  
107 and early autumn (six in September-October), coinciding with the peaks of  
108 mesozooplankton and *O. vulgaris* paralarvae hatchlings (Otero *et al.*, 2009; Moreno *et*  
109 *al.*, 2009). Four stations (T2, T3, T4 and T5, Fig. 1) parallel to the coast were sampled  
110 in each survey following an onshore-offshore depth gradient (26, 68, 85 and 110 m,  
111 respectively), and two samples were collected on each station. The samplings carried  
112 out in 2008 and 2009 employed a 75 cm diameter bongo net of 375  $\mu\text{m}$  mesh. At a ship  
113 speed of 2 knots, the bongo net was first lowered and stabilised close to the bottom for a  
114 period of 5-15 min depending on the amount of salps present and subsequently hauled  
115 up at 0.5  $\text{m s}^{-1}$ . We considered this sample as “column” since the zooplankton was  
116 captured throughout the water column. Then, the net was cleaned on board and a second  
117 sample was taken from the surface to 5 meters depth and named “surface sample”. The  
118 volume of filtered water was determined from a calibrated flow meter attached to the  
119 mouth of the bongo. Zooplankton samplings carried out in 2010 employed a Multinet  
120 type maxi (Hydro-Bios) with a mouth opening of 70 x 70 cm and 200  $\mu\text{m}$  mesh.  
121 Samples were collected at the same stations, collecting two samples per station: one at  
122 the surface (0 to 5 m) and other close to the bottom (mean depths 25, 30, 60 and 90 m,  
123 in stations T2 to T5 respectively). The water filtered for each sample was approximately  
124 200  $\text{m}^3$  (average = 202.8  $\text{m}^3$ ).

125 Samples were fixed with 96% ethanol and stored at -20°C, to allow DNA  
126 preservation for genetic analyses. All cephalopod paralarvae were sorted and identified

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2 127 to the lowest taxon according to Sweeney *et al.* (1992) and our own reference  
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4 128 collections. The dorsal mantle length (DML) of each paralarvae collected in 2008 was  
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6 129 recorded to the nearest 0.001 mm using an image analysis system NIS-Elements 3.0  
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8 130 connected to a digital camera (Nikon DXM1200F) under a binocular microscope  
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10 131 (Nikon SMZ800).

#### 132 *Oceanographic and meteorological data*

133 Wind (10 m above sea level) and surface current velocities (3 m depth) were provided  
134 by the Seawatch buoy of Puertos del Estado ([www.puertos.es](http://www.puertos.es)) located off Cape Silleiro  
135 (42°7.8'N, 9°23.4'W, Fig. 1). Due to the lack of data from this buoy in 2009, we also  
136 obtained wind data from Ons Islands meteorological station (42°22.8'N, 8°55.8'W, Fig.  
137 1). Continuous records of water temperature at 4 m depth at the Rande bridge (inner part  
138 of the Ría de Vigo, 42°17.4'N, 8°39.6'W; Fig. 1) were provided also by Meteogalicia  
139 ([www.meteogalicia.es](http://www.meteogalicia.es)). The sampling area was situated between these three  
140 observatories, thus providing valuable information of the environmental conditions  
141 before, during and after the mesozooplankton surveys. Daily upwelling index ( $-Q_x$ , in  
142  $\text{m}^3 \text{s}^{-1} \text{km}^{-1}$ ) was calculated using the wind data obtained from Cape Silleiro buoy and  
143 the Ons Islands station following methods described in Bakun (1973).

#### 144 *Cephalopod paralarvae and zooplankton communities*

145 The mesozooplankton communities present off the Ría de Vigo in 2008 were  
146 characterized with multivariate techniques using PRIMER6 and PERMANOVA+  
147 software (Anderson *et al.*, 2008), as detailed in Roura *et al.* (2013). Briefly, prior to  
148 analysis the database was screened to select those taxa that appeared at least in 10% of  
149 the stations and then transformed using the natural logarithm (a constant of 1 was added  
150 before taking the log) (Legendre and Legendre 1998). The Bray–Curtis similarity matrix  
151 was used to calculate the resemblance matrix among samples, and principal coordinate

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152 analysis (PCO) ordination was then used to visualise the natural groupings of the  
153 samples. The communities emerging from the PCO plot were first analysed with  
154 PERMDISP, based on distances to centroids, to examine the dispersion among groups.  
155 Subsequently, a non-parametric permutational ANOVA (PERMANOVA) analysis was  
156 used to test for statistical differences in the communities found in the multidimensional  
157 space. Overall, six mesozooplankton communities identified in the Ría de Vigo in 2008  
158 (Roura *et al.*, 2013) were used to analyse the short-term spatio temporal interactions of  
159 cephalopod paralarvae on them: summer coastal (SC), summer frontal (SF), summer  
160 oceanic (SO), autumn coastal (AC), autumn frontal (AF) and autumn oceanic (AO).

161 In order to study the association between cephalopod paralarvae and the  
162 zooplankton communities identified off the Ría de Vigo, the abundance of  
163 ommastrephid paralarvae had to be added to the matrix since they were present in less  
164 than 10% of the samples and were excluded from the original zooplankton analysis.  
165 Then, the scores of PCO1 and 2 for the different cephalopod paralarvae were used to  
166 plot the vectors that define the strength and direction of the association between the  
167 paralarvae and the different mesozooplankton communities. RELATE analysis was  
168 carried out to test if the spatial pattern of the cephalopod paralarvae assemblage  
169 matched with the spatial pattern of the mesozooplankton communities, by correlating  
170 the matching entries of their resemblance matrices based on the Spearman rank  
171 correlation. PERMANOVA analysis was carried out with the different cephalopod  
172 paralarvae to test differences in abundance among zooplankton communities.  
173 PERMANOVA was run for 999 permutations using the “unrestricted permutation of  
174 raw data” and type III sum of squares, which yields an exact test for one-way design  
175 (Anderson *et al.*, 2008).

176 Cephalopod paralarvae abundance was further related with the main descriptors of  
177 the mesozooplankton communities (summarized in Table 1) by means of fitting



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2 178 generalized linear models (GLMs) to the cephalopod counts. The paralarvae data was  
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4 179 overdispersed, i.e., variances were greater than the mean (not shown), thus we assumed  
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6 180 that cephalopod counts for a given sample  $i$  ( $Y_i$ ) were negative binomial distributed with  
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8 181 mean  $\mu_i$  and shape parameter  $k$ . The variance was parameterised as  $\mu_i + \mu_i^2 / k$  (Venables  
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10 182 and Ripley 2002). The ln-transformed water volume filtered was used as an offset and  
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12 183 the following variables were used as potential explanatory variables:  
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14 184 holoplankton/meroplankton ratio (H/M), species richness (S), species diversity ( $H'$ ),  
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16 185 zooplankton total abundance (ind 1000 m<sup>-3</sup>), Simpson's index ( $\lambda$ ), and evenness ( $J'$ ). In  
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18 186 order to account for short-term spatio-temporal effects, the strata (two categories:  
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20 187 surface and water column) and month of sampling (three categories: July, September  
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22 188 and October) were added to the models. Collinearity among mesozooplankton  
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24 189 descriptors was assessed by means of calculating variance inflation factors and  
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26 190 subsequently dropping those above a cut-off value of 3 (Zuur *et al.*, 2007). Model fitting  
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28 191 was performed with R 3.0.1 language (R Development Core Team, 2013) and using the  
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30 192 "MASS 7.3-26" package (Venables and Ripley, 2002).  
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### 36 193 *Trophic ecology of O. vulgaris hatchlings within the zooplankton communities*

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39 194 The diet of 18 *O. vulgaris* hatchlings collected in 2008 was determined with molecular  
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41 195 techniques and up to 20 different prey were identified (Roura *et al.*, 2012). In order to  
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43 196 test whether octopus prey preferences remain the same with changing prey fields –i.e.  
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45 197 within different zooplankton communities- we analysed the linear index of food  
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47 198 selection (L). This food selection index avoids the bias observed for other indices like  
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49 199 Ivlev's Electivity index and the forage ratio when the sizes of the prey samples in the  
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51 200 gut and in the environment are unequal (Strauss, 1979). This index is obtained for every  
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53 201 prey detected with the formula:  
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$$57 \quad 202 \quad L = r_i - p_i$$

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2 203 resulting from the unweighted difference in relative proportions of prey item  $i$  in the gut  
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4 204 ( $r_i$ ) and in the community ( $p_i$ ).  $L$  ranges from -1 to +1, with positive values indicating  
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6 205 preference, negative values indicating avoidance or inaccessibility, and zero values  
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8 206 showing random feeding. Accordingly, we calculated  $L$  for all the prey detected in *O.*  
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10 207 *vulgaris* hatchlings, which allowed assessment of prey preferences of each octopus  
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12 208 paralarvae within the different communities where they were collected: 4 in the SC, 3 in  
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14 209 SF, 4 in AC and 7 in AF.

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17 210 Furthermore, we examined the trophic niche breadth for every octopus paralarvae using  
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19 211 the Czekanowski's Index (CI) also called Proportional Similarity Index (Feinsinger *et*  
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21 212 *al.*, 1981). Trophic niche breadth was calculated with the formula:

$$22 \quad 213 \quad CI = 1 - 0.5 \sum_i |p_i - q_i|$$

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24 214 where  $p_i$  and  $q_i$  are the relative abundance of resource item  $i$  eaten by the paralarvae ( $p_i$ )  
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26 215 and in the zooplankton ( $q_i$ ). Values of CI range from 1 for the broadest possible niche (a  
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28 216 population uses resources in proportion to their availability) to  $[\min q_i]$  for the  
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30 217 narrowest possible niche (a population is specialized exclusively on the rarest resource).  
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32 218 CI relies on how objectively the available resources for the predator are defined.  
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34 219 Accordingly, all the zooplankton organisms not likely to be consumed by the octopuses  
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36 220 according to their ecology were removed (reviewed in Villanueva and Norman, 2008).  
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38 221 These included salps, cnidarians, siphonophores and appendicularians, as well as small-  
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40 222 sized organisms like platyhelminthes or harpacticoids. When the available resources are  
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42 223 objectively chosen, CI index is appropriate not only for measuring niche breadths but  
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44 224 also for quantifying foraging patterns on individual animals (Feinsinger *et al.*, 1981).  
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46 225 Therefore, individual values of CI for each paralarvae were compared among the  
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48 226 mesozooplankton communities, in order to test whether the foraging tactics of *O.*  
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50 227 *vulgaris* changed in summer and autumn communities (t-test).  
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2 228 *Cephalopod paralarvae variability and its relationship with the upwelling between*  
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7 230 Finally, the spatio-temporal variability of cephalopod paralarvae collected in the Ría de  
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9 231 Vigo from 2008 to 2010 was evaluated using as input predictors into the GLMs, the  
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11 232 strata (two categories: surface and water column), the station (four categories: T2, T3,  
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13 233 T4 and T5), the month sampled (three categories: July, September and October), and the  
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15 234 upwelling index during the day preceding the surveys. The upwelling index was chosen  
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17 235 because it is one of the most important forcing factors in this region (e.g. Piedracoba *et*  
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19 236 *al.*, 2005). Model fitting was performed as outlined above.

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238 **RESULTS**239 *Hydrography and dynamics off the Ría de Vigo*

240 Coastal wind speed ( $W_x$ ,  $W_y$ , Fig. 2a), upwelling index ( $-Q_x$ , Fig. 2b), and sea surface  
241 temperature recorded at Rande observatory (Fig. 2c) showed that in summer 2008,  
242 surveys 1 to 4 (from July 2–11) were conducted under downwelling conditions,  
243 characterised by weak coastal winds of variable direction and a progressive increase of  
244 sea surface temperature, with the exception of the strong downwelling-favourable winds  
245 recorded on July 4 (Fig. 2a). Conversely, surveys 5 to 10 (conducted from September 26  
246 to October 14) were characterised by upwelling-favourable winds (Fig. 2a), which  
247 cooled the surface layer sharply (Fig. 2c). The only exception occurred in October 14,  
248 with the presence of weak southerly winds. In summer 2009 (surveys 11–14, conducted  
249 from July 15–24) all samples were collected under downwelling conditions, although an  
250 upwelling event occurred in between surveys 12 and 13, producing a transient decrease  
251 in temperature that was appreciable up to the innermost side of the Ría (Fig. 2c).  
252 Autumn samples (surveys 15–20, from September 23 to October 10), were first  
253 collected under weak upwelling conditions (surveys 15–16) produced by northerly  
254 winds of  $<4 \text{ m s}^{-1}$  that provoked a steep decrease of surface water temperature up to  
255 September 27 and a subsequent increase accompanying wind relaxation (surveys 17–  
256 18). The last two surveys (19–20) were carried out after a strong downwelling event that  
257 pushed warm oceanic waters inshore. In summer 2010 (surveys 21–24, from July 13–  
258 22) the first two surveys were carried out under weak downwelling conditions, while  
259 the last two surveys (23 and 24) were carried out under upwelling conditions. Autumn  
260 samplings (surveys 25–30, from September 22 to October 15) took place under  
261 upwelling-relaxation conditions (surveys 25, 26 and 28), while upwelling conditions  
262 forced by moderate to strong northerly winds dominated surveys 27, 29 and 30 (Fig. 2).

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2 263 *Short-term spatio-temporal interactions between cephalopod paralarvae and*  
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4 264 *mesozooplankton communities*

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7 265 The main characteristics of the six mesozooplankton communities described in the Ría  
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9 266 de Vigo in 2008 are summarized in Table 1. Briefly, the summer coastal community  
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11 267 (SC) showed the greatest abundance and diversity as a result of the great contribution of  
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13 268 the meroplankton fraction (Fig. 3). The main groups were copepods, larval stages  
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15 269 (calytopis and furcilia) of the euphausiid *Nyctiphanes couchii*, larval stages of  
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17 270 echinoderms and salps. The meroplankton fraction was less important in the summer  
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19 271 frontal community (SF), which was dominated by larval stages of *N. couchii*, copepods  
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21 272 and salps. The summer oceanic community (SO) was dominated by the holoplankton  
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23 273 fraction (salps and copepods) with the lowest values of homogeneity and biodiversity.  
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25 274 The autumn coastal community (AC) showed the highest abundance, with echinoderm  
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27 275 larval stages (echinopluteus and ofiopluteus) contributing the most to the overall  
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29 276 abundance, followed by larval stages of *N. couchii*, salps, copepods and cirriped larvae.  
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31 277 The autumn frontal community (AF) was dominated by larval stages of *N. couchii*  
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33 278 followed by copepods and salps. Finally, the autumn oceanic community (AO) had the  
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35 279 lowest abundance values, with an equitable contribution of both copepods and salps,  
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37 280 and followed by larval stages of *N. couchii*.

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42 281 Regarding cephalopod paralarvae, a total of 2039 individuals were captured from  
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44 282 2008 to 2010 off the Ría de Vigo (Table 2). *Octopus vulgaris* was the most abundant  
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46 283 species (64.4, 61.5, and 70.4%, collected from 2008 to 2010 respectively), followed by  
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48 284 loliginids (22.7, 29.4, and 23.9%), sepiolids (12.3, 8.7 and 5.1%) and ommastrephids  
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50 285 (0.6, 0.4 and 0.6%). With the exception of *O. vulgaris*, cephalopod paralarvae were  
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52 286 classified at the family level due to the lack of accurate descriptions for loliginid,  
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54 287 sepiolids and ommastrephid paralarvae below this level of classification. All  
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56 288 ommastrephid paralarvae (n=11) were found in the column sample, mainly in autumn  
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2 289 (only two were collected in summer). With the exception of three ommastrephids that  
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4 290 remained inside the egg covered with chromatophores, all other ommastrephid  
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6 291 individuals were considered as “rhynchoteuthions” because of the fusion of the tentacles  
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8 292 into a proboscis. All *O. vulgaris* captured had three suckers per arm, with DML ranging  
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10 293 from 1.04 to 2.18 mm that was not significantly different among the mesozooplankton  
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12 294 communities. Regarding loliginids and sepiolids, the DML of these groups of species  
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14 295 was more variable ranging from 1.12 to 7.91 and from 1.24 to 3.82 mm, respectively,  
15  
16 296 though their average DML neither varied significantly among communities, sampling  
17  
18 297 periods or strata.

19  
20  
21 298 Cephalopod paralarvae were unevenly distributed among the mesozooplankton  
22  
23 299 communities (Fig. 3), which was confirmed with the PERMANOVA results (Table 3).  
24  
25 300 A decreasing gradient from coastal to oceanic communities was observed, with the  
26  
27 301 exception of the autumn frontal community. *Octopus vulgaris* showed higher  
28  
29 302 abundances in the frontal and coastal communities throughout the sampled period, with  
30  
31 303 a maximum of 395 ind / 1000 m<sup>3</sup> in the autumn frontal community at the surface (Fig.  
32  
33 304 3). Loliginids and sepiolids were more abundant in the coastal and frontal communities  
34  
35 305 in both sampling periods, being almost absent in the oceanic samples. Finally, all the  
36  
37 306 ommastrephid paralarvae were found in the water column samples collected in autumn,  
38  
39 307 three belonging to the coastal and one to the oceanic community.

40  
41  
42 308 The direction of the vectors shown in Fig. 4 show that loliginids and sepiolids  
43  
44 309 were mainly associated with the coastal-frontal communities (represented by positive  
45  
46 310 values of PCO1 axis) found in summer (as shown by the positive values of PCO2 axis).  
47  
48 311 *Octopus vulgaris* was associated with the coastal community (positive value of PCO1  
49  
50 312 axis) found in autumn (negative value of PCO2). Ommastrephids were associated with  
51  
52 313 autumn frontal communities. The length of the vectors displayed in Fig. 4 evidenced  
53  
54 314 that the contribution of cephalopod paralarvae to the discrimination of the zooplankton  
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1  
2 315 communities identified in the Ría de Vigo in 2008 was very low, owed to their low  
3  
4 316 abundance in the zooplankton. RELATE analysis showed that the spatial pattern of the  
5  
6 317 cephalopod assemblage, did not match the spatial pattern of the mesozooplankton  
7  
8 318 resemblance matrix (Spearman rank correlation,  $S_r = 0.171$ ,  $N = 79$ ,  $P > 0.2$ ), thus  
9  
10 319 evidencing the uneven distribution of the cephalopod paralarvae among the  
11  
12 320 mesozooplankton communities.

13  
14  
15 321 The relationships found between cephalopod paralarvae abundance and the  
16  
17 322 descriptors of the mesozooplankton communities complete the above description and  
18  
19 323 are summarized in Table 4. Ommastrephids were excluded from the analysis due to  
20  
21 324 their low abundance. It can be drawn that all cephalopods were inversely correlated with  
22  
23 325 the holoplankton-meroplankton ratio and the evenness, especially *O. vulgaris* and  
24  
25 326 loliginids. This implied that there were more chances of finding cephalopod paralarvae  
26  
27 327 mainly in the coastal communities where the meroplankton fraction was higher and the  
28  
29 328 community was evenly distributed (Table 1). The short-term spatio-temporal analysis  
30  
31 329 showed that *O. vulgaris* was more abundant in September > October > July,  
32  
33 330 preferentially at the surface though this was not significant. Loliginids were more  
34  
35 331 abundant in September, preferentially in the water column though this was not  
36  
37 332 significant. Concerning sepiolids, they were more abundant in the water column than at  
38  
39 333 the surface, with no statistical differences in the month sampled.

#### 334 *Octopus vulgaris* trophic interactions

335 The eighteen *O. vulgaris* hatchlings analysed in the work of Roura *et al.* (2012) were  
336 collected from four different mesozooplankton communities identified off the Ría de  
337 Vigo in 2008 (Table 5). At least one decapod species was found within the digestive  
338 tract of every single paralarvae, a diverse taxonomic group that is found in very low  
339 abundance within the different communities (Table 1). Linear food selection indices (L)

1  
2 340 calculated for the different decapods (Brachyura, Processidae, Alpheidae, Paguridae,  
3  
4 341 Crangonidae, Thalassinidae and Porcellanidae) were positive and even 1, thus showing  
5  
6 342 a clear preference for these organisms in all the communities analysed (Table 5). In the  
7  
8 343 case of krill, octopus showed preference for this item in the AC community (positive  
9  
10 344 value), but not in the AF community (negative), since krill abundance greatly increased  
11  
12 345 in the latter (Table1). Octopus also showed preference for fish larvae or eggs (positive),  
13  
14 346 since they consumed them despite their low abundance in the AF community (Table1).

15  
16  
17 347 The trophic niche breadth (CI) estimated for each paralarva ranged from 0.042 to  
18  
19 348 0.374. These low values show that *O. vulgaris* foraging tactics are prey-specific and do  
20  
21 349 not depend on the prey available in the different communities. In summer, octopuses  
22  
23 350 feeding on the coastal community showed significantly higher CI mean values  $0.11 \pm$   
24  
25 351  $0.019$  than those of the frontal community  $0.056 \pm 0.008$  (t-test, *p-value* = 0.033, *n* = 7).  
26  
27 352 However, this change did not reflect a change in prey niche but rather it reflected the  
28  
29 353 decrease of decapods in the frontal community compared to the coastal community  
30  
31 354 (Table 1). CI values were on average lower in summer  $0.087 \pm 0.032$  than those  
32  
33 355 obtained in autumn  $0.13 \pm 0.12$ , although not significantly (t-test, *p-value* = 0.617, *n* =  
34  
35 356 18) showing that paralarvae display similar foraging strategies in both seasons. Mean CI  
36  
37 357 values for autumn were  $0.144 \pm 0.096$  and  $0.10 \pm 0.142$  for the coastal and frontal  
38  
39 358 communities respectively, showing no statistical differences (t-test, *p-value* = 0.214, *n* =  
40  
41 359 11). The increase found for CI values in autumn was due to the fact that three octopuses  
42  
43 360 (one from AC and two from AF communities, Table 5) feed on krill, which was evenly  
44  
45 361 distributed in those communities (specially in AF community, Table 1).

#### 362 *Spatio-temporal distribution of cephalopod paralarvae from 2008-2010*

363 The abundance of all the paralarvae collected between 2008 and 2010 at the different  
364 strata sampled is shown in Fig. 5. This figure show the different vertical distribution of



1  
2 365 the cephalopod paralarvae, which is more evident in 2010 when the Multinet was used  
3  
4 366 to sample the bottom layer. GLMs showed inverse spatio-temporal relationships for *O.*  
5  
6 367 *vulgaris* compared to those found for loliginids and sepiolids, except for the upwelling  
7  
8 368 index (Table 6). *Octopus vulgaris* abundance increased under upwelling-favourable  
9  
10 369 conditions the day preceding the survey, and it was significantly higher in September, at  
11  
12 370 the surface layer and East of the Cíes Islands (Fig. 6a). The modelling of loliginid and  
13  
14 371 sepiolid paralarvae revealed the same trend for both groups, indicating that abundance  
15  
16 372 of loliginids (Fig. 6b) and sepiolids (Fig. 6c) was higher in July and decreased in  
17  
18 373 autumn, mainly in October. Moreover, both paralarvae were mostly found in the water  
19  
20 374 column/bottom layer and West of Cíes Islands (T3, Fig. 1), with loliginids almost  
21  
22 375 absent in the westernmost station (T5, Fig. 1). Abundance of these two paralarvae  
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24 376 increased during upwelling-favourable conditions, though this was not significant for  
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26 377 loliginids.  
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378

379 **DISCUSSION**

380 With the exception of *Octopus vulgaris*, the identification of the cephalopod paralarvae  
381 was restricted to family level due to the lack of accurate descriptions for ommastrephid,  
382 loliginid and sepiolid paralarvae. Currently, the only reliable method to identify these  
383 groups is genetic barcoding. Indeed, the cephalopod paralarvae collected during a cruise  
384 in shelf waters off NW Iberian Peninsula –including the outer part of the Ría de Vigo–  
385 were identified to species level using the cytochrome oxidase subunit I gene, COI  
386 (Roura 2013, CEPAR project on iBOLD). The paralarval assemblage was comprised by  
387 *O. vulgaris*, three loliginid species (*Alloteuthis media*, *A. subulata* and *Loligo vulgaris*),  
388 two sepiolids (*Sepiola atlantica* and *S. tridens*), and three ommastrephids (*Illex*  
389 *coindetii*, *Todaropsis eblanae* and *Todarodes sagittatus*). Accordingly, the conclusions  
390 drawn herein about the planktonic ecology of cephalopod paralarvae in the Ría de Vigo  
391 are accurate for *O. vulgaris*, but are generalisations for loliginids and sepiolids due to  
392 the fact that our data on these taxa might be comprised of multiple species in both cases.

393 *Mesozooplankton communities and cephalopod paralarvae*

394 Cephalopod paralarvae were unevenly distributed in the mesozooplankton communities  
395 found in the Ría de Vigo, and suggests that the cephalopod paralarvae actively choose  
396 the communities they inhabit as observed in other ecosystems (Vecchione *et al.*, 1986;  
397 Roberts, 2005). Multivariate analysis suggests different associations of the cephalopod  
398 paralarvae with the zooplankton communities despite their low abundance: loliginids  
399 and sepiolids were associated with the coastal-frontal summer community, whilst *O.*  
400 *vulgaris* was lightly associated with the coastal community during autumn, and  
401 ommastrephids with the autumn frontal community (Fig. 4). Common octopus and  
402 loliginid paralarvae were negatively related to the holoplankton/meroplankton ratio  
403 (H/M). This variable was the best descriptor of the mesozooplankton communities

1  
2 404 accounting for 40% of the variability present in the zooplankton similarity matrix  
3  
4 405 (Roura *et al.*, 2013). H/M ratio summarizes both the spatial variability (from coast to  
5  
6 406 ocean, with more meroplankton close to the coast and more holoplankton in the ocean)  
7  
8 407 as well as the differences in the zooplankton assemblage. Accordingly, smaller values of  
9  
10 408 the H/M ratio would indicate a greater contribution of the meroplankton in the samples,  
11  
12 409 an inherent characteristic of coastal communities (e.g. Blanco-Bercial *et al.*, 2006). The  
13  
14 410 observed lack of relationship between sepiolid abundance and the zooplankton  
15  
16 411 descriptors might be due to the presence of different species present in the Ría de Vigo,  
17  
18 412 as suggested by Moreno *et al.* (2009) and indicated above.  
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21  
22 413 The association found with the cephalopod paralarvae and the coastal and frontal  
23  
24 414 communities may be driven by optimal trophic conditions present within these  
25  
26 415 environments. Several reasons might support this hypothesis. Coastal communities  
27  
28 416 showed the greatest zooplankton abundances increasing the chances for successful  
29  
30 417 feeding even for recently hatched paralarvae, as it was observed for *Loligo pealei*  
31  
32 418 (Vecchione, 1981) or for capelin larvae (Fossheim *et al.*, 2006). Moreover, besides  
33  
34 419 abundance, the taxonomic composition of the coastal and frontal communities would  
35  
36 420 meet the diet requirements of the different cephalopod paralarvae. Regarding loliginids  
37  
38 421 and ommastrephids, these are generalist paralarvae known to primarily feed on  
39  
40 422 copepods (Passarella and Hopkins 1991; Vecchione, 1991; Vidal and Haimovici, 1998;  
41  
42 423 Venter *et al.*, 1999, Hoving *et al.*, 2005) whose abundances peaked on coastal and  
43  
44 424 frontal communities in the Ría de Vigo (Table 1), thus providing an optimal prey  
45  
46 425 environment. These communities would also be appropriate rearing environments even  
47  
48 426 for the selective early hatchlings of *O. vulgaris*, since decapod zoeae are most abundant  
49  
50 427 in coastal communities (Table 1). Therefore, coastal and frontal communities off the Ría  
51  
52 428 de Vigo potentially satisfy the quantitative and qualitative zooplankton requirements for  
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54 429 the optimal development of cephalopod paralarvae. Indirect evidence of this is finding  
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1  
2 430 the wide range of sizes for loliginids and sepiolids, since these paralarvae are optimally  
3  
4 431 growing in the Ría de Vigo and their surroundings. However, all the *O. vulgaris*  
5  
6 432 collected in this study (n = 1297), as well as in previous works (n = 95 González *et al.*,  
7  
8 433 2005; n = 584 Otero *et al.*, 2009), had only three suckers per arm, meaning that all of  
9  
10 434 them were early hatchlings less than ten days old (Villanueva and Norman, 2008). This  
11  
12 435 fact suggests that the Ría de Vigo is optimal for the hatchlings of *O. vulgaris*, but sub-  
13  
14 436 optimal for further development as evidenced by the absence of older stages.

17 437 The specialist trophic ecology of *O. vulgaris* heavily contrasts with the generalist  
18  
19 438 diet of paralarvae of other cephalopod species (Passarella and Hopkins, 1991;  
20  
21 439 Vecchione, 1991; Vidal and Haimovici, 1998; Venter *et al.*, 1999; Hoving *et al.*, 2005).  
22  
23 440 Prey selection was consistent in four different mesozooplankton communities analysed  
24  
25 441 (Table 5), thus showing that *Octopus* paralarvae actively chose the prey, even though  
26  
27 442 they were very rare in the samples. This strategy suggests that the groups that were  
28  
29 443 targeted (mainly decapods) may meet the nutritional requirements of the paralarvae.  
30  
31 444 This suggestion is supported by captive studies where increased survival rates were  
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33 445 obtained when *Artemia* diets were complemented with decapod zoeae (reviewed in  
34  
35 446 Villanueva and Norman, 2008). The results observed for the planktonic paralarvae  
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37 447 contrasts with the diet of adult *O. vulgaris* that prey upon a wide range of items when  
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39 448 the whole population is considered, but where individual choices are markedly specific  
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41 449 (Anderson *et al.*, 2008; Mather *et al.*, 2012; Hernandez-Urcera *et al.*, 2014).

#### 47 *Spatio-temporal changes on paralarval abundance*

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49  
50 451 Our analysis showed that *O. vulgaris* paralarvae displayed contrasting spatio-temporal  
51  
52 452 patterns compared to those of sepiolids and loliginids. Considering the month sampled,  
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54 453 common octopus were more abundant in September and October, as observed earlier in  
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56 454 this area (Otero *et al.*, 2009) and for the whole western Iberian coast (Moreno *et al.*,  
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58 455 2009). A different pattern occurred with loliginids and sepiolids whose abundance  
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1  
2 456 peaked in July concurring with previous results (González *et al.*, 2005; Moreno *et al.*,  
3  
4 457 2009; Rodrigues *et al.*, 2011). This temporal change in abundance is likely to be due to  
5  
6 458 their different reproductive cycles. On one hand, loliginid spawning peaks occur in  
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8 459 December–January and, to a lesser extent, in June–July (Moreno *et al.*, 2002). Given  
9  
10 460 that the embryonic development lasts for 40–47 days on average (Villanueva *et al.*,  
11  
12 461 2003), two peaks of hatchlings are expected around March and August. Regarding  
13  
14 462 sepiolids, spawning occurs between March and July (Rodrigues *et al.*, 2011a) with an  
15  
16 463 early development lasting for around 62-23 days at 13-18°C (Rodrigues *et al.*, 2011b)  
17  
18 464 and hatching occurring between May and September (Rodrigues *et al.*, 2012). In  
19  
20 465 contrast, *O. vulgaris* has an extended embryonic development of up to 4 months  
21  
22 466 (Mangold and Boletzky, 1973). Taking into account that spawning occurs between  
23  
24 467 March and June (Sieiro *et al.*, 2014) the main hatching peak would occur at the end of  
25  
26 468 summer (July) and early autumn (September-October, Otero *et al.*, 2009).

27  
28  
29  
30 469 Clear differences were evident in the vertical position of the paralarvae. *Octopus*  
31  
32 470 *vulgaris* were more abundant in surface waters, while loliginids and sepiolids were  
33  
34 471 more abundant in the water column layer (Table 6, Fig. 6). These results indicate  
35  
36 472 contrasting vertical distributions at night-time with common octopus reaching the  
37  
38 473 surface layer (0-5 m) and the other cephalopod paralarvae presenting a clear affinity for  
39  
40 474 water column/bottom layers. This vertical pattern can be observed straightforward in the  
41  
42 475 cephalopods collected in 2010 with the Multinet (Fig. 5). Previous results in diel  
43  
44 476 vertical distribution of loliginid paralarvae showed a widespread distribution between 5  
45  
46 477 and 40 m at night (Moreno *et al.*, 2009) agreeing with our observation that loliginids are  
47  
48 478 spread throughout the water column. Horizontal distribution was also slightly different  
49  
50 479 among the different cephalopods. Recently hatched *O. vulgaris* mainly appeared East of  
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52 480 the Cíes Islands and displayed a coastal–ocean gradient decreasing towards station 5;  
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1  
2 481 whereas sepiolids and loliginids were more abundant West of the Cíes Islands also  
3  
4 482 decreasing towards the ocean.

5  
6 483 The abundance of all cephalopods increased with the upwelling strength the day  
7  
8 484 preceding the sampling, which is a reasonable lag for the Ría de Vigo whose subtidal  
9  
10 485 circulation responds immediately to the shelf winds forcing (Piedracoba *et al.*, 2005).  
11  
12 486 The probability of positive captures of cephalopod paralarvae with higher upwelling  
13  
14 487 strength has been previously described for the Western Iberian coast (Moreno *et al.*,  
15  
16 488 2009) and other upwelling areas (e.g. California Current: Zeidberg and Hamner, 2002;  
17  
18 489 and South African waters: Roberts, 2005). Most benthic organisms with planktotrophic  
19  
20 490 larvae found in the coastal area of the Atlantic Iberian Peninsula match their  
21  
22 491 reproductive cycle with the upwelling season, which is the main forcing factor of the  
23  
24 492 region (Queiroga *et al.*, 2007). This oceanographic feature increases primary production  
25  
26 493 due to the cold nutrient-rich upwelled waters that sustains the high zooplankton  
27  
28 494 production observed from early spring to late autumn (Santos *et al.*, 2007).

#### 29 30 31 32 33 34 495 *Cephalopod paralarval life strategies in the Ría de Vigo*

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36  
37 496 The intermittency of upwelling/downwelling events coupled with the vertical migration  
38  
39 497 patterns is an important mechanism for larvae retention in inshore waters (Santos *et al.*,  
40  
41 498 2004; Shanks and Brink, 2005; Marta-Almeida *et al.*, 2006; Queiroga *et al.*, 2007; dos  
42  
43 499 Santos *et al.*, 2008). However, this pattern is species-specific. For instance, inshore  
44  
45 500 crustacean larvae (e.g. *Necora puber* or *Carcinus maenas*) experience larger along-  
46  
47 501 shore rather than cross-shelf transport, retaining the larvae within 10 km off the  
48  
49 502 Portuguese coastal shelf (dos Santos *et al.*, 2008), while shelf species (*Atelecyclus*  
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51 503 *rotundatus*, *Liocarcinus* spp. or *Anapagurus* spp.) appear widely distributed over the  
52  
53 504 shelf (10–20 km) suggesting a more active cross-shelf rather than along-shore transport  
54  
55 505 (dos Santos *et al.*, 2008). Accordingly, the underlying mechanism of the observed  
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1  
2 506 cephalopod paralarvae distribution might result from the interaction between their  
3  
4 507 vertical behaviour and the residual circulation in the Ría de Vigo. During upwelling  
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6 508 (downwelling) conditions a coastal surface jet enters (leaves) the Ría through the  
7  
8 509 northern mouth and leaves (enters) through the southern mouth (Gilcoto *et al.*, 2007).

10 Previous works carried out mostly during day-time suggested that early  
11  
12 511 cephalopod paralarvae of neritic species might be retained in a cell over the shelf  
13  
14 512 avoiding offshore transport through a combination of behavioural and physical  
15  
16 513 processes (Rocha *et al.*, 1999; Zeidberg and Hamner, 2002; González *et al.*, 2005;  
17  
18 514 Roberts, 2005; Otero *et al.*, 2009; Moreno *et al.*, 2009). However, this hypothesis  
19  
20 515 assumes similar vertical behaviours for the different cephalopod paralarvae within these  
21  
22 516 cells. Notwithstanding, we found two different vertical behaviours in the cephalopod  
23  
24 517 paralarvae at night (Table 6): recently hatched *O. vulgaris* were mostly present in the  
25  
26 518 surface under upwelling conditions, while loliginids and sepiolids of variable sizes were  
27  
28 519 associated to the water column. Consequently, it might be plausible to suggest two  
29  
30 520 different life strategies in the coastal waters of the Iberian Peninsula, driven by changes  
31  
32 521 in the vertical distribution of the paralarvae: 1) we postulate that loliginids and sepiolids  
33  
34 522 display a coastal retention strategy through the avoidance of surface offshore currents,  
35  
36 523 which is in agreement with the shelf-cell retention hypothesis; 2) we postulate an  
37  
38 524 oceanic strategy for *O. vulgaris*, hatching near the coast and then selecting offshore  
39  
40 525 superficial currents under upwelling conditions to be transported far from the shelf for  
41  
42 526 later development.

48 We suggest that the separation between both life strategies would be the  
49  
50 528 continental shelf (~200 m depth), which greatly modulates the circulation patterns in the  
51  
52 529 Western Iberian Upwelling System by complex mesoscale processes (Relvas *et al.*,  
53  
54 530 2007). One of the most important mesoscale features is originated by the blocking effect  
55  
56 531 of the Iberian Poleward Current that acts as a barrier for the shelf-ocean exchange of  
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1  
2 532 upwelled waters (Castro *et al.*, 1997; Álvarez-Salgado *et al.*, 2003; Santos *et al.*, 2007).  
3  
4 533 Indeed, this feature has been recognised as an important retention mechanism for fish  
5  
6 534 and crustacean larvae over the shelf (Santos *et al.*, 2004; dos Santos *et al.*, 2008).  
7

8 535 The occurrence of loliginid and sepiolid paralarvae with different sizes and their  
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10 536 presence in the water column under upwelling conditions off the Ría de Vigo, supports  
11  
12 537 their coastal distribution favoring an alongshore dispersion rather than a cross-shelf  
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14 538 transport, as observed in an larger study through the Western Iberian Peninsula (Moreno  
15  
16 539 *et al.*, 2009) and other upwelling areas such as in California (Zeidberg and Hamner,  
17  
18 540 2002) or South Africa (Roberts, 2005). Our data support the hypothesis suggested by  
19  
20 541 Bello and Biagi (1995) that sepiolids of the subfamily Sepiolinae display a merobenthic  
21  
22 542 -i.e. distributed in the water column and close to the bottom-, instead of a holobenthic -  
23  
24 543 i.e. real benthic- life cycle (sensu Boletzky, 2003). The alongshore transport during the  
25  
26 544 juvenile stage would also help to explain why sepiolids, a group with limited  
27  
28 545 displacement capability, is able to maintain genetic connectivity across a wide area of  
29  
30 546 distribution (Reid and Jereb 2005; Groenenberg *et al.*, 2009; Roura, 2013).  
31  
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35 547 It has been assumed that a circulatory cell would retain *O. vulgaris* paralarvae  
36  
37 548 close to the coast (Rocha *et al.*, 1999; Faure *et al.*, 2000; González *et al.*, 2005; Otero *et*  
38  
39 549 *al.*, 2009). Alternatively, Moreno *et al.* (2009) suggested that *O. vulgaris* paralarvae  
40  
41 550 could be retained within the double frontal system present in the wide NW continental  
42  
43 551 shelf of the Iberian Peninsula (Peliz *et al.*, 2002) as shown for other organisms (Castro  
44  
45 552 *et al.*, 1997; Santos *et al.*, 2004). However, if octopus paralarvae were retained over the  
46  
47 553 shelf, then one would expect to find octopus paralarvae of different sizes, but to date,  
48  
49 554 not a single *O. vulgaris* with more than three suckers has ever been found over the  
50  
51 555 Iberian shelf in 4673 zooplankton samples analysed (Rocha *et al.*, 1999; González *et al.*,  
52  
53 556 2005; Otero *et al.*, 2009; Moreno *et al.* 2009). Larger octopus paralarvae have been  
54  
55 557 caught in other areas though. Rees and Lumby (1954) found some individuals in the  
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1  
2 558 English Channel, the northernmost limit of distribution of the species, and other  
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4 559 specimens counting more than three suckers per arm occurred in Japanese waters  
5  
6 560 (Takeda 1990; Sakaguchi *et al.*, 1999). It is important to note that the octopus that  
7  
8 561 occurs in Japan is genetically distinct from *O. vulgaris* (Guerra *et al.*, 2010; Amor *et al.*,  
9  
10 562 2014) and may represent a sibling species within the *O. vulgaris* complex, with a  
11  
12 563 different life strategy.

13  
14 564 Therefore, the affinity of early stages of *O. vulgaris* for surface waters at night  
15  
16 565 under upwelling conditions suggests an offshore displacement far from the continental  
17  
18 566 shelf into the ocean, offering an explanation for the absence of large individuals in the  
19  
20 567 coastal region. To this end the occurrence of upwelling filaments (Barton *et al.*, 1993)  
21  
22 568 would be a plausible mechanism for such offshore transport. Indeed, during a drifter  
23  
24 569 experiment following the offshore displacement of upwelled waters within a filament,  
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26 570 *O. vulgaris* paralarvae were found far from the shelf and increasing in size towards  
27  
28 571 oceanic waters (Roura, 2013). In particular, up to 30 *O. vulgaris* paralarvae with more  
29  
30 572 than three suckers per arm (from 4 to 12) were found over the continental slopes of both  
31  
32 573 the Iberian Peninsula and Morocco (from 600 to 3100 m water depth).

33  
34 574 In conclusion, our study suggests that the spatio-temporal distributions of  
35  
36 575 cephalopod paralarvae observed herein might be driven by two contrasting early life  
37  
38 576 strategies: a coastal-shelf strategy in loliginids and sepiolids, and an oceanic strategy in  
39  
40 577 *O. vulgaris*. Nevertheless, more detailed sampling combined with biophysical models is  
41  
42 578 needed to accurately resolve the vertical, along-shore and cross-shelf distribution of the  
43  
44 579 cephalopod paralarvae in order to confirm the contrasting life strategies proposed.  
45  
46 580 Furthermore, molecular analyses are also needed to clarify the diversity of loliginid,  
47  
48 581 sepiolids and ommastrephid larvae found in coastal environments to elucidate their  
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50 582 different life history strategies.

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599 **REFERENCES**

- 600 Álvarez-Salgado, X.A., Figueiras, F.G., Pérez, F.F., Groom, S., Nogueira, E., Borges,  
601 A.V., Chou, L., Castro, C.G., Moncoiffé, G., Ríos, A.F., Miller, A.E.J., Frankignoulle,  
602 M., Savidge, G., Wollast, R. (2003) The Portugal coastal counter current off NW Spain:  
603 new insights on its biogeochemical variability. *Prog. Oceanogr.* **56**: 28-321.
- 604 Amor, M.D., Norman, M.D., Cameron, H.E., Strugnell, J.M. (2014) Allopatric  
605 speciation within a cryptic species complex of australasian octopus. *PLOS ONE.* **9**:  
606 e98982.
- 607 Anderson, M.J., Gorley, R.N., Clarke, K.R. (2008) PERMANOVA+ for PRIMER:  
608 Guide to software and statistical methods. PRIMER-E, Plymouth, UK.
- 609 Anderson, R.C., Wood, J.B., Mather, J.A. (2008) *Octopus vulgaris* in the Caribbean is a  
610 specializing generalist. *Mar. Ecol. Prog. Ser.* **371**: 199-202.
- 611 Bakun, A. (1973) Coastal upwelling indices, west coast of North America, 1946-71.  
612 *U.S. Dep. Commer. NOAA Tech. Rep.*, NMFS SSRF-671, pp 103.
- 613 Barton, E.D., Haynes, R., Pilling, I. (1993) Development, Persistence, and Variability of  
614 Upwelling Filaments off the Atlantic Coast of the Iberian Peninsula. *J. Geophys. Res.*  
615 **98**: 681-692.
- 616 Bello, G., Biagi, V. (1995) How benthic are sepiolids? In: Boletzky S (ed)  
617 *Mediterranean Sepiolidae*. Musée Océanographique, Monaco pp 57-61.
- 618 Blanco-Bercial, L., Álvarez-Marqués, F., Cabal, J.A. (2006) Changes in the  
619 mesozooplankton community associated with the hydrography off the northwestern  
620 Iberian Peninsula. *ICES J. Mar. Sci.* **63**: 799-810.
- 621 Boletzky, Sv. (2003) Biology of early life stages in cephalopod molluscs. *Adv. Mar.*  
622 *Biol.* **44**: 143-203.
- 623 Boyle, P.R., Boletzky, Sv. (1996) Cephalopod populations: definition and dynamics.  
624 *Phil. Trans. R. Soc. Lond. B.* **351**: 985-1002.

- 1  
2 625 Boyle, P.R., Rodhouse, P. (2005) Cephalopods. Ecology and fisheries. Blackwell,  
3  
4 626 Oxford.  
5  
6 627 Castro, C.G., Álvarez-Salgado, X.A., Figueiras, F.G., Fraga, F., Pérez, F.F. (1997)  
7  
8 628 Transient hydrographic and chemical conditions affecting microplankton populations in  
9  
10 629 the coastal transition zone of the Iberian upwelling system (NW Spain) in September  
11  
12 630 1986. *J. Mar. Res.* **55**: 321-352.  
13  
14 631 Collins, M.A., Yau, C., Boyle, P.R., Friese, D., Piatkowski, U. (2002) Distribution of  
15  
16 632 cephalopods from plankton surveys around the British Isles. *Bull. Mar. Sci.* **71**: 239-  
17  
18 633 254.  
19  
20 634 Diekmann, R., Nellen, W., Piatkowski, U. (2006) A multivariate analysis of larval fish  
21  
22 635 and paralarval cephalopod assemblages at Great Meteor Seamount. *Deep-Sea Res.* **53**:  
23  
24 636 1635-1657.  
25  
26 637 dos Santos, A., Santos, A., Conway, D., Bartilotti, C., Lourenço, P., Queiroga, H.  
27  
28 638 (2008) Diel vertical migration of decapod larvae in the Portuguese coastal upwelling  
29  
30 639 ecosystem: implications for offshore transport. *Mar. Ecol. Prog. Ser.* **359**: 171-183.  
31  
32 640 Faure, V., Inejih, C.A., Demarcq, H., Cury, P. (2000) The importance of retention  
33  
34 641 processes in upwelling areas for recruitment of *Octopus vulgaris*: the example of the  
35  
36 642 Arguin Bank (Mauritania). *Fish. Oceanogr.* **9**: 343-355.  
37  
38 643 Feinsinger, P., Spears, E.E., Poole, R.W. (1981) A simple measure of niche breadth.  
39  
40 644 *Ecology.* **62**: 27-12.  
41  
42 645 Fossheim, M., Tande, K.S., Semenova, T., Timonin, A. (2006) Capelin larvae (*Mallotus*  
43  
44 646 *villosus*) and community structure of zooplankton off the coast of northern Norway. *J.*  
45  
46 647 *Plank. Res.* **28**: 585-595.  
47  
48 648 Gilcoto, M., Pardo, P.C., Álvarez-Salgado, X.A., Pérez, F.F. (2007) Exchange fluxes  
49  
50 649 between the Ría de Vigo and the shelf: A bidirectional flow forced by remote wind. *J.*  
51  
52 650 *Geophys. Res. Oceans.* **112**: C06001.  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2 651 González, A.F., Otero, J., Guerra, A., Prego, R., Rocha, F., Dale, A.W. (2005)  
3  
4 652 Distribution of common octopus and common squid paralarvae in a wind-driven  
5  
6 653 upwelling area (Ria of Vigo, northwestern Spain). *J. Plank. Res.* **27**: 271-277.  
7  
8 654 González, A.F., Otero, J., Pierce, G.J., Guerra, A. (2010) Age, growth, and mortality of  
9  
10 655 *Loligo vulgaris* wild paralarvae: implications for understanding of the life cycle and  
11  
12 656 longevity. *ICES J. Mar. Sci.* **67**: 1119-1127.  
13  
14 657 Groenenberg, D.S.J., Goud, J., De Heij, A., Gittenberger, E. (2009) Molecular  
15  
16 658 phylogeny of North Sea Sepiolinae (Cephalopoda: Sepiolidae) reveals a overlooked  
17  
18 659 *Sepiolo* species. *J. Mollus. Stud.* **75**: 361-369.  
19  
20 660 Guerra, A., Roura, A., González, A.F., Pascual, S., Cherel, Y., Pérez-Losada, M. (2010)  
21  
22 661 Morphological and genetic evidence that *Octopus vulgaris* Cuvier, 1797 inhabits  
23  
24 662 Amsterdam and Saint Paul Islands (souther Indian Ocean). *ICES J. Mar. Sci.* **67**: 1401-  
25  
26 663 1407.  
27  
28 664 Hernández-Urcera, J., Garci, M.E., Cabanellas-Reboredo, M., Roura, A., González,  
29  
30 665 A.F., Guerra, A., Morales-Nin, B. (2014) Cannibalistic behaviour of *Octopus vulgaris*  
31  
32 666 in the wild. *J. Comp. Psychol.* **128**: 427-430.  
33  
34 667 Hoving, H.J.T., Venter, J.D., Worst, D., Lipinski, M. (2005) Adaptation of an  
35  
36 668 immunodot assay for multiple prey identification of squid paralarvae in field trials. *J.*  
37  
38 669 *Mar. Biol. Assoc. UK.* **5**; 1499–1501.  
39  
40 670 Mangold, K., Boletsky, Sv. (1973) New data on reproductive biology and growth of  
41  
42 671 *Octopus vulgaris*. *Mar. Biol.* **19**: 7-12.  
43  
44 672 Mather, J.A., Leite, T.S., Batista, A.T. (2012) Individual prey choices of octopuses: Are  
45  
46 673 they generalist or specialist? *Current. Zool.* **58**: 597-603.  
47  
48 674 Moreno, A., Pereira, J., Arvanitidis, C., Robin, J.P., Koutsoubas, D., Raya, C.P., Cunha,  
49  
50 675 M.M., Balguerías, E., Denis, V. (2002) Biological variation of *Loligo vulgaris*  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2 676 (Cephalopoda: Loliginidae) in the Eastern Atlantic and Mediterranean. *Bull. Mar. Sci.*  
3  
4 677 **71**: 515-534.  
5  
6 678 Moreno, A., dos Santos, A., Piatkowski, U., Santos, A.M.P., Cabral, H. (2009)  
7  
8 679 Distribution of cephalopod paralarvae in relation to the regional oceanography of the  
9  
10 680 western Iberia. *J. Plank. Res.* **31**: 73-91.  
11  
12 681 Marta-Almeida, M., Dubert, J., Peliz, A., Queiroga, H. (2006) Influence of vertical  
13  
14 682 migration pattern on retention of crab larvae in a seasonal upwelling system. *Mar. Ecol.*  
15  
16 683 *Prog. Ser.* **307**: 1-19.  
17  
18 684 Otero, J., Álvarez-Salgado, X.A., González, A.F., Miranda, A., Groom, S.B., Cabanas,  
19  
20 685 J.M., Casas, G., Wheatley, B., Guerra, A. (2008) Bottom-up control of common octopus  
21  
22 686 *Octopus vulgaris* in the Galician upwelling system, northeast Atlantic Ocean. *Mar.*  
23  
24 687 *Ecol. Prog. Ser.* **362**: 181-192.  
25  
26 688 Otero, J., Álvarez-Salgado, X., González, A., Gilcoto, M., Guerra, A. (2009) High-  
27  
28 689 frequency coastal upwelling events influence *Octopus vulgaris* larval dynamics on the  
29  
30 690 NW Iberian shelf. *Mar. Ecol. Prog. Ser.* **386**: 123-132.  
31  
32 691 Passarella, K.C., Hopkins, T.L. (1991) Species composition and food habits of the  
33  
34 692 micronektonic cephalopod assemblage in the eastern Gulf of México. *Bull. Mar. Sci.*  
35  
36 693 **49**: 638-659.  
37  
38 694 Peliz, Á., Rosa, T.L., Santos, A.M.P., Pissarra, J.L. (2002) Fronts, jets, and counter-  
39  
40 695 flows in the Western Iberian upwelling system. *J. Mar. Syst.* **35**: 61-77.  
41  
42 696 Piedracoba, S., Álvarez-Salgado, X.A., Rosón, G., Herrera, J.L. (2005) Short-timescale  
43  
44 697 thermohaline variability and residual circulation in the central segment of the coastal  
45  
46 698 upwelling system of the Ría de Vigo (northwest Spain) during four contrasting periods.  
47  
48 699 *J. Geophys. Res.* **110**: C03018.  
49  
50 700 Queiroga, H., Cruz, T., dos Santos, A., Dubert, J., González-Gordillo, J.I., Paula, J.,  
51  
52 701 Peliz, Á., Santos, A.M.P. (2007) Oceanographic and behavioural processes affecting  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2 702 invertebrate larval dispersal and supply in the western Iberia upwelling ecosystem.  
3  
4 703 *Prog. Oceanogr.* **74**: 174-191.  
5  
6 704 R Development Core Team. 2013. R: A language and environment for statistical  
7  
8 705 computing. In: R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-  
9  
10 706 900051-07-0, URL <http://R-project.org>  
11  
12 707 Rees, W.J., Lumby, J.R. (1954) The abundance of *Octopus* in the English Channel. *J.*  
13  
14 708 *Mar. Biol. Assoc. UK.* **33**: 515-536.  
15  
16 709 Reid, A., Jereb, P. (2005) Family Sepiolidae. In: *Cephalopods of The World. An*  
17  
18 710 *Annotated and Illustrated Catalogue of Species Known to Date. Chambered Nautilus*  
19  
20 711 *and Sepioids (Nautilidae, Sepiidae, Sepiolidae, Sepiadariidae, Idiosepiidae and*  
21  
22 712 *Spirulidae)*. Jereb P, Roper CFE (eds) FAO Species Catalogue for fishery Purposes,  
23  
24 713 Rome.  
25  
26 714 Relvas, P., Barton, E.D., Dubert, J., Oliveira, P.B., Peliz, A., da Silva, J.C.B., Santos,  
27  
28 715 A.M.P. (2007) Physical oceanography of the western Iberia ecosystem: Latest views  
29  
30 716 and challenges. *Prog. Oceanogr.* **74**: 149-173.  
31  
32 717 Roberts, M.J. (2005) Chokka squid (*Loligo vulgaris reynaudii*) abundance linked to  
33  
34 718 changes in South Africa's Agulhas Bank ecosystem during spawning and the early life  
35  
36 719 cycle. *ICES J. Mar. Sci.* **62**: 33-55.  
37  
38 720 Robin, J.P., Roberts, M., Zeidberg, L. et al. (2014) Transitions during cephalopod life  
39  
40 721 history: the role of habitat, environment, functional morphology and behaviour. *Adv*  
41  
42 722 *Mar. Biol.* **67**: 361-437.  
43  
44 723 Rocha, F., Guerra, A., Prego, R., Piatkowski, U. (1999) Cephalopod paralarvae and  
45  
46 724 upwelling conditions off Galician waters (NW Spain). *J. Plank. Res.* **21**: 21-33.  
47  
48 725 Rodrigues, M., Garci, M.E., Troncoso, J.S., Guerra, Á. (2011a) Seasonal abundance of  
49  
50 726 the Atlantic bobtail squid *Sepiola atlantica* in Galician waters (NE Atlantic). *Mar. Biol.*  
51  
52 727 *Res.* **7**: 812-819.  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2 728 Rodrigues, M., Guerra, A., Troncoso, J.S. (2011b). The embryonic phase and its  
3  
4 729 implication in the hatchling size and condition of Atlantic bobtail squid *Sepiola*  
5  
6 730 *atlantica*. *Helgol. Mar. Res.* **65**: 211-216.  
7  
8 731 Rodrigues, M., Guerra, Á., Troncoso, J.S. (2012) Reproduction of the Atlantic bobtail  
9  
10 732 squid *Sepiola atlantica* (Cephalopoda: Sepiolidae) in northwest Spain. *Invertebr. Biol.*  
11  
12 733 **131**: 30-39.  
13  
14 734 Roura, Á (2013) Ecology of planktonic cephalopod paralarvae in coastal upwelling  
15  
16 735 systems. PhD thesis, University of Vigo, 227pp.  
17  
18 736 Roura, Á., González, Á., Redd, K., Guerra, Á. (2012) Molecular prey identification in  
19  
20 737 wild *Octopus vulgaris* paralarvae. *Mar. Biol.* **159**: 1335-1345.  
21  
22 738 Roura, Á., Álvarez-Salgado, X.A., González, A.F., Gregori, M., Rosón, G., Guerra, A.  
23  
24 739 (2013) Short-time meso-scale variability of mesozooplankton communities in a coastal  
25  
26 740 upwelling system (NW Spain). *Prog. Oceanogr.* **109**: 18-32.  
27  
28 741 Sakaguchi, H., Hamano, T., Nakazono, A. (1999) Occurrence of planktonic juveniles of  
29  
30 742 *Octopus vulgaris* in Eastern Iyo-Nada of the Seto Inland Sea, Japan. *Bull. Jap. Soc.*  
31  
32 743 *Fish. Oceanogr.* **63**: 181-187.  
33  
34 744 Santos, A.M.P., Peliz, A., Dubert, J., Oliveira, P.B., Angélico, M.M., Ré, P. (2004)  
35  
36 745 Impact of a winter upwelling event on the distribution and transport of sardine (*Sardina*  
37  
38 746 *pilchardus*) eggs and larvae off western Iberia: a retention mechanism. *Contin. Shelf*  
39  
40 747 *Res.* **24**: 149-165.  
41  
42 748 Santos, A.M.P., Chícharo, A., dos Santos, A., Moita, T., Oliveira, P.B., Peliz, A., Ré, P.  
43  
44 749 (2007) Physical-biological interactions in the life history of small pelagic fish in the  
45  
46 750 Western Iberia Upwelling ecosystem. *Prog. Oceanogr.* **74**: 192-209.  
47  
48 751 Shanks, A.L., Brink, L. (2005) Upwelling, downwelling, and cross-shelf transport of  
49  
50 752 bivalve larvae: test of a hypothesis. *Mar. Ecol. Prog. Ser.* **302**: 1-12.  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



- 1  
2 753 Sieiro, P., Otero, J., Guerra, A. (2014) Contrasting macroscopic maturity staging with  
3  
4 754 histological characteristics of the gonads in female *Octopus vulgaris*. *Hydrobiologia*.  
5  
6 755 **730**: 113-125.  
7  
8 756 Strauss, R.E. (1979) Reliability estimates for Ivlev's electivity index, the forage ratio  
9  
10 757 and a proposed linear index of food selection. *Trans. Amer. Fish. Soc.* **108**: 344-352.  
11  
12 758 Sweeney, M.J., Roper, C.F.E., Mangold, K., Clarke, M.R., Boletzky, Sv. (1992)  
13  
14 759 'Larval' and juvenile cephalopods: a manual for their identification. *Smithson. Contrib.*  
15  
16 760 *Zool.* **513**.  
17  
18 761 Takeda, R. (1990) The distribution of planktonic juveniles of *Octopus vulgaris* Cuvier  
19  
20 762 in Harima Nada. *Suisanzoshoku.* **38**: 183-190.  
21  
22 763 Vecchione, M. (1981) Aspects of early life history of *Loligo pealei* (Cephalopoda:  
23  
24 764 Myopsida). *J. Shell. Res.* **1**: 171-180.  
25  
26 765 Vecchione, M., Grant, G.C. (1983) A multivariate analysis of planktonic molluscan  
27  
28 766 distribution in the Middle Atlantic Bight. *Contin. Shelf. Res.* **1**: 405-424.  
29  
30 767 Vecchione, M., Roper, C.F.E., Lu, C.C., Sweeney, M.J. (1986) Distribution and relative  
31  
32 768 abundance of planktonic cephalopods in the Western North Atlantic. *Am. Malacol. Bull.*  
33  
34 769 **4**: 101.  
35  
36 770 Vecchione, M. (1991) A method for examining the structure and contents of the  
37  
38 771 digestive tract in paralarvae squids. *Bull. Mar. Sci.* **49**: 300-308.  
39  
40 772 Venables, W.N. and Ripley, B.D., 2002. Modern applied statistics with S. Springer,  
41  
42 773 New York, USA.  
43  
44 774 Venter, J.D., Wyngaardt, S., Verschoor, J.A. (1999) Detection of zooplankton prey in  
45  
46 775 squid paralarvae with Immunoassay. *J. Immunoassay.* **20**: 127-149.  
47  
48 776 Vidal, E.A.G., Haimovici, M. (1998) Feeding and the possible role of the proboscis and  
49  
50 777 mucus cover in the ingestion of microorganisms by rhynchoteuthion paralarvae  
51  
52 778 (Cephalopoda: Ommastrephidae). *Bull. Mar. Sci.* **63**: 305-316.  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2 779 Villanueva, R., Arkhipkin, A., Jereb, P., Lefkaditou, E., Lipinski, M.R., Raya, C.P.,  
3  
4 780 Riba, J., Rocha, F. (2003) Embryonic life of the loliginid squid *Loligo vulgaris*:  
5  
6 781 comparison between statoliths of Atlantic and Mediterranean populations. *Mar. Ecol.*  
7  
8 782 *Prog. Ser.* **253**: 197-208.  
9  
10 783 Villanueva, R., Norman, M.D. (2008) Biology of the planktonic stages of benthic  
11  
12 784 octopuses. *Oceanogr. Mar. Biol. Annu. Rev.* **46**: 105-202.  
13  
14 785 Zeidberg, L.D., Hamner, W.M. (2002) Distribution of squid paralarvae, *Loligo*  
15  
16 786 *opalescens* (Cephalopoda: Myopsida), in the Southern California Bight in the three  
17  
18 787 years following the 1997-1998 El Niño. *Mar. Biol.* **141**: 111-122.  
19  
20 788 Zuur, A.F., Ieno, E.N., Smith, G.M. (2007) Analysing ecological data. Springer. New  
21  
22 789 York.  
23  
24  
25  
26 790  
27  
28  
29  
30  
31  
32  
33  
34  
35  
36  
37  
38  
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791 **Table 1.** Ecological descriptors and taxonomic composition (ind.1000m<sup>-3</sup>) of the  
 792 mesozooplankton communities characterized off the Ría de Vigo in 2008, expressed as  
 793 mean values. Asterisks show prey detected in *O. vulgaris* paralarvae. The abundances of  
 794 the different decapod groups detected in the gut of *O. vulgaris* paralarvae are detailed  
 795 below.  
 796

Descriptors	Summer						Autumn					
	Coastal (n = 12)		Frontal (n = 12)		Oceanic (n = 8)		Coastal (n = 27)		Frontal (n = 11)		Oceanic (n = 9)	
Richness (S)	42		26		21		36		30		21	
Diversity (H')	2.25		1.61		1.31		1.98		1.85		1.70	
Uniformity (J)	0.42		0.34		0.30		0.38		0.38		0.39	
Simpson Index ( $\lambda$ )	0.19		0.33		0.44		0.25		0.29		0.28	
Total Abundance	1665502		1314714		387248		1952373		538493		168932	
Holo/Meroplankton	4.27		51.71		557.31		2.74		47.4		418.66	
Taxa	Abundance	%	Abundance	%	Abundance	%	Abundance	%	Abundance	%	Abundance	%
Copepoda	399617.1	24.000	443349.7	33.723	146886.9	37.906	205950.9	10.549	112934.4	20.972	53745.0	31.800
Euphausiacea *	381745.8	22.927	707947.7	53.849	18593.8	4.798	408420.2	20.919	326348.8	60.603	31617.9	18.708
Echinodermata	369368.7	22.184	103.9	0.008	-	-	656140.8	33.607	6725.6	1.249	-	-
Urochordata	230985.8	13.873	120264.7	9.148	213801.5	55.174	247044.7	12.654	40856.9	7.587	61478.5	36.375
Decapoda *	107870.2	6.479	22291.4	1.696	1546.4	0.399	100729.6	5.159	5422.2	1.007	396.7	0.235
Cladocera	96835.0	5.816	3890.9	0.296	574.6	0.148	39702.0	2.034	616.3	0.114	-	-
Cirripedia	25626.2	1.539	1332.8	0.101	-	-	135228.6	6.926	5161.1	0.958	59.2	0.035
Gasteropoda	19886.8	1.194	3711.1	0.282	247.3	0.064	21447.4	1.099	1440.9	0.268	12.5	0.007
Chaetognatha	11020.9	0.662	4384.5	0.333	2838.0	0.732	76317.7	3.909	32060.3	5.954	16780.6	9.929
Cnidaria	9933.4	0.597	1427.3	0.109	117.9	0.030	51543.1	2.640	3029.5	0.563	698.9	0.414
Chordata *	4949.5	0.297	1982.7	0.151	231.0	0.060	6296.5	0.323	370.5	0.069	76.5	0.045
Misidacea	4284.9	0.257	3189.0	0.243	2190.3	0.565	1400.6	0.072	2668.6	0.496	3691.8	2.184
Bivalvia	867.5	0.052	-	-	-	-	161.4	0.008	-	-	-	-
Platemintha	860.4	0.052	115.0	0.009	-	-	363.0	0.019	32.3	0.006	25.5	0.015
Polychaeta	495.6	0.030	2.8	0.000	25.3	0.007	368.6	0.019	341.4	0.063	106.7	0.063
Amphipoda	321.9	0.019	228.6	0.017	255.2	0.066	596.0	0.031	308.2	0.057	292.5	0.173
Cumacea	205.2	0.012	89.3	0.007	46.4	0.012	110.6	0.006	88.7	0.016	8.1	0.005
Cephalochordata	76.5	0.005	-	-	-	-	-	-	-	-	-	-
Ostracoda	56.1	0.003	-	-	-	-	-	-	-	-	-	-
Isopoda	21.4	0.001	1.6	0.000	1.4	0.000	41.6	0.002	28.2	0.005	10.5	0.006
Stomatopoda	0.4	0.000	357.9	0.027	147.8	0.038	-	-	-	-	5.2	0.003

Briozoa	-	-	-	-	-	-	478.5	0.025	-	-	-	-
<i>Octopus vulgaris</i>	8.650	0.000	4.191	0.000	1.940	0.000	19.201	0.000	59.490	0.000	3.596	0.000
Loliginidae	5.302	0.000	15.304	0.000	-	-	7.164	0.000	4.532	0.000	2.008	0.000
Sepiolidae	5.207	0.000	3.674	0.000	0.151	0.000	1.748	0.000	3.511	0.000	-	-
Ommastrephidae	-	-	-	-	-	-	0.236	0.000	-	-	-	-
Decapoda												
Brachyura *	63520.0	3.814	17732.3	1.349	918.3	0.237	31089.2	1.592	2252.4	0.418	113.6	0.067
Porcellanidae *	28471.8	1.710	1047.4	0.080	310.5	0.080	56116.2	2.874	184.2	0.034	-	-
Paguridae *	8681.0	0.521	1632.1	0.124	-	-	5983.4	0.306	1375.2	0.255	60.4	0.036
Alpheidae *	1792.4	0.108	1273.0	0.097	79.0	0.020	525.6	0.027	173.3	0.032	52.3	0.031
Processidae *	1445.6	0.087	318.9	0.024	121.6	0.031	3973.8	0.204	370.8	0.069	51.2	0.030
Thalassinidae *	571.3	0.034	-	-	-	-	64.7	0.003	-	-	-	-
Crangonidae *	375.4	0.023	85.3	0.006	-	-	714.2	0.037	76.9	0.014	-	-

797

798 **Table 2.** Number of cephalopod paralarvae caught in the Ría de Vigo (NW Spain) at  
 799 night from 2008 to 2010.  
 800

Species	Station	2008		2009		2010		Total
		Surface	Column	Surface	Column	Surface	Bottom	
<i>O. vulgaris</i>	T2	69	58	215	114	44	4	<b>504</b>
	T3	139	44	67	28	18	15	<b>311</b>
	T4	40	36	113	63	80	0	<b>332</b>
	T5	9	11	46	36	48	0	<b>150</b>
Loliginids	T2	12	25	2	54	6	19	<b>118</b>
	T3	24	51	29	107	3	26	<b>240</b>
	T4	7	19	26	79	11	6	<b>148</b>
	T5	1	5	7	22	1	0	<b>36</b>
Sepiolid	T2	2	9	1	13	1	3	<b>29</b>
	T3	9	26	2	44	3	2	<b>86</b>
	T4	6	16	0	18	1	5	<b>46</b>
	T5	1	8	1	17	0	1	<b>28</b>
Ommastrephids	T2	0	0	0	2	0	0	<b>2</b>
	T3	0	2	0	0	0	1	<b>3</b>
	T4	0	1	0	1	0	1	<b>3</b>
	T5	0	1	0	2	0	0	<b>3</b>
Total		<b>319</b>	<b>312</b>	<b>509</b>	<b>600</b>	<b>216</b>	<b>83</b>	<b>2039</b>
		<b>631</b>		<b>1109</b>		<b>299</b>		

801

802 **Table 3.** PERMANOVA results after 999 permutations to test differences in the  
 803 abundance of *O. vulgaris*, loliginid and sepiolid paralarvae among the zooplankton  
 804 communities (Abbreviated as: SC, summer coast; SF, summer front; SO, summer  
 805 ocean; AC, autumn coast; AF, autumn front; AO, autumn ocean). •  $p < 0.1$  marginally  
 806 significant, values in bold are significant at  $p < 0.05$ .

807

Tests	Level	Cephalopod paralarvae	<i>Octopus vulgaris</i>	Loliginidae	Sepiolidae
Main test	Community	<b>0.001</b>	<b>0.027</b>	<b>0.037</b>	<b>0.04</b>
pair-wise	SC-SF	0.206	0.112	0.981	0.951
	SC-SO	<b>0.001</b>	0.091 •	<b>0.002</b>	0.068 •
	SC-AC	0.182	0.273	0.63	0.239
	SC-AF	0.089 •	0.585	0.293	0.692
	SC-AO	<b>0.003</b>	0.088 •	<b>0.019</b>	<b>0.038</b>
	SF-SO	<b>0.003</b>	0.672	<b>0.01</b>	<b>0.022</b>
	SF-AC	<b>0.014</b>	<b>0.008</b>	0.659	0.135
	SF-AF	0.16	0.127	0.439	0.588
	SF-AO	<b>0.002</b>	0.664	0.07 •	<b>0.003</b>
	SO-AC	<b>0.007</b>	<b>0.021</b>	<b>0.016</b>	0.139
	SO-AF	0.112	0.163	0.087 •	0.165
	SO-AO	0.734	1	0.494	0.418
	AC-AF	0.322	0.803	0.529	0.548
AC-AO	<b>0.002</b>	<b>0.011</b>	0.056 •	0.067 •	
AF-AO	0.116	0.133	0.256	0.102	

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810 **Table 4.** Results of the generalized linear methods (GLMs) fitted to the abundance of  
811 *O. vulgaris*, loliginids and sepiolids using the descriptors of the mesozooplankton  
812 communities found in the Ría de Vigo in 2008 as explanatory variables. The GLMs  
813 assumed a negative binomial distribution and used the ln-transformed water volume  
814 filtered as an offset. Shown is the estimate ( $\pm$ S.E.) for each parameter. Note that the  
815 reference values were the water column and July for the categorical factors strata and  
816 month, respectively. ns: non-significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

817

Parameter	<i>O. vulgaris</i>	Loliginids	Sepiolids
Intercept	-2.77 (0.85)***	-2.63 (0.75)***	-4.52 (1.18)*
Surface	0.47 (0.29) <sup>ns</sup>	-0.44 (0.27) <sup>ns</sup>	-0.85 (0.41)*
September	2.03 (0.49)***	0.90 (0.43)*	0.06 (0.70) <sup>ns</sup>
October	0.85 (0.33)**	-0.12 (0.30) <sup>ns</sup>	-0.64 (0.45) <sup>ns</sup>
Ln (H/M)	-0.22 (0.08)**	-0.19 (0.08)*	-0.08 (0.11) <sup>ns</sup>
Evenness	-5.27 (1.85)**	-5.46 (1.62)***	-1.39 (2.57) <sup>ns</sup>

818

819 **Table 5** Prey preferences of eighteen *O. vulgaris* paralarvae (Oc 1 - Oc 18) in the  
 820 different mesozooplankton communities calculated with the linear food selection index  
 821 for each zooplankton group. Czekanowski's index (CI) is shown for each paralarvae to  
 822 elucidate changes on foraging tactics depending on the community.

823

Community		Brachyura	Processidae	Alpheidae	Paguridae	Crangonidae	Thalassinidae	Porcellanidae	Krill larvae	CI
SC	Oc 1	0.46		0.50						0.118
	Oc 2	0.30		0.33	0.33					0.123
	Oc 3		0.33	0.33			0.33			0.081
	Oc 4	0.96								0.117
SF	Oc 5	0.32		0.33		0.33				0.061
	Oc 6		1.00							0.047
	Oc 7	1.00								0.060
AC	Oc 8	0.32		0.67						0.093
	Oc 9	0.38	0.40	0.20						0.095
	Oc 10	0.23	0.25	0.25	0.25					0.098
	Oc 11		0.50					0.29		0.288
AF	Oc 12		0.50						-0.11	0.288
	Oc 13		0.50						0.50	0.042
	Oc 14	0.33		0.67						0.045
	Oc 15			0.67					-0.27	0.374
	Oc 16	0.50						0.50		0.045
	Oc 17	0.33	0.33						0.33	0.046
	Oc 18	0.50							0.50	0.045

824



825 **Table 6.** GLM results fitted to the abundance of *O. vulgaris*, loliginids and sepiolids  
 826 using the spatio-temporal variables and the upwelling strength the day preceding the  
 827 sampling date from 2008 to 2010 as explanatory variables. The GLMs assumed a  
 828 negative binomial distribution and used the ln-transformed water volume filtered as an  
 829 offset. Shown is the estimate ( $\pm$ S.E.) for each parameter. Note that the reference values  
 830 are July, water column and T2 for month, strata and station, respectively. ns: non-  
 831 significant, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

832

Parameter	<i>O. vulgaris</i>	Loliginids	Sepiolids
Intercept	-5.06 (0.25)***	-4.73 (0.21)***	-5.95 (0.29)***
September	0.68 (0.24)**	-0.03 (0.22) <sup>ns</sup>	-0.29 (0.28) <sup>ns</sup>
October	0.46 (0.24) <sup>ns</sup>	-0.74 (0.23)**	-0.93 (0.31)**
Surface	1.12 (0.19)***	-0.82 (0.18)***	-1.46 (0.26)***
T3	-0.52 (0.27) <sup>ns</sup>	0.59 (0.23)*	0.98 (0.32)**
T4	-0.51 (0.27) <sup>ns</sup>	0.02 (0.24) <sup>ns</sup>	0.31 (0.34) <sup>ns</sup>
T5	-1.11 (0.28)***	-1.51 (0.30)***	-0.36 (0.37) <sup>ns</sup>
- $Q_x$	1.08 (0.27)***	0.46 (0.26) <sup>ns</sup>	0.79 (0.34)*

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835 **Fig. 1.** Sampling area showing the four stations (T2–T5) where mesozooplankton  
836 samples and cephalopod paralarvae were collected off the Ría de Vigo (NW Iberian  
837 Peninsula). Hydrographical data was obtained from Rande bridge and meteorological  
838 data was obtained from Silleiro buoy and from Ons Island meteorological station.

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839 **Fig. 2** Meteorological conditions recorded around the sampled area. a Wind speed and  
840 direction ( $W_x$ ,  $W_y$ ,  $\text{m s}^{-1}$ ), b upwelling index ( $-Q_x$ ,  $\text{m}^3 \text{s}^{-1} \text{km}^{-1}$ ), and c sea surface  
841 temperature ( $^{\circ}\text{C}$ ) obtained from Rande observatory for 2008–2010 samplings. Vertical  
842 bars indicate the sampling days, which are numerated sequentially.

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843 **Fig. 3** PCO plot showing the distribution and abundance (ind.  $1000 \text{ m}^3$ ) of *Octopus*  
844 *vulgaris* (a,b), loliginid (c,d) and sepiolid (e,f) paralarvae at the surface and in the  
845 integrated water column samples within the different mesozooplankton communities  
846 identified off the Ría de Vigo in 2008. Abbreviations: SC, summer coast; SF, summer  
847 front; SO, summer ocean; AC, autumn coast; AF, autumn front; AO, autumn ocean.

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848 **Fig. 4** PCO plot showing the strength and direction of the associations between the  
849 cephalopod paralarvae collected in 2008 and the mesozooplankton communities found  
850 off the Ría de Vigo. The blue circle represents the maximum correlation with PCO axis.  
851 Abbreviations: lol, loliginidae; oct, *Octopus vulgaris*; oma, ommastrephidae; sep,  
852 sepiolidae.

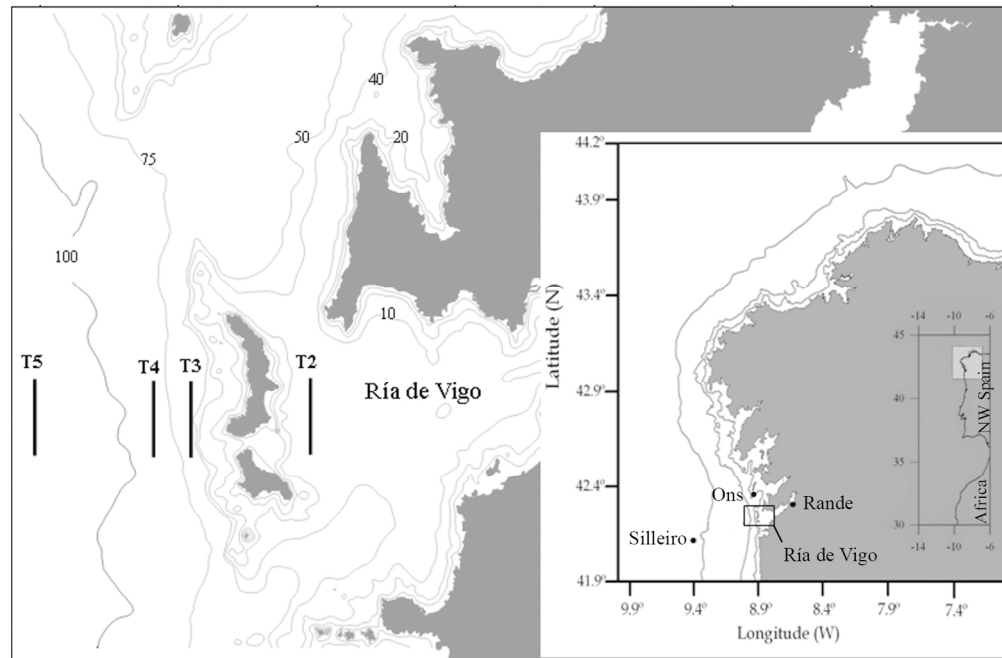
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853 **Fig. 5** Mean cephalopod paralarval abundance (ind  $1000 \text{ m}^3$ ) collected at the different  
854 strata during the 30 samplings carried out in the Ría de Vigo between 2008 and 2010.  
855 Samples were collected with a bongo net in 2008 and 2009 (column samples) and with a  
856 Multinet in 2010 (bottom sample).

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857 **Fig. 6** Predicted number of (a) *O. vulgaris* at station 2, and (b) loliginids and (c)  
858 sepiolids at station 3 as a function of the upwelling index the previous day of the  
859 capture, the strata and month sampled. The expected values are the result of a negative

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860 binomial GLM fitted to the abundance of cephalopod paralarvae collected from 2008 to  
861 2010 (see Table 6). Values were standardized to 1000 m<sup>3</sup>.

For Peer Review



29 Sampling area showing the four transects (T2–T5) where mesozooplankton samples and cephalopod  
30 paralarvae were collected off the Ría de Vigo (NW Iberian Peninsula). Hydrographical data was obtained  
31 from Rande bridge and meteorological data was obtained from Silleiro buoy and from Ons Island  
32 meteorological station.

33 142x93mm (300 x 300 DPI)

Review

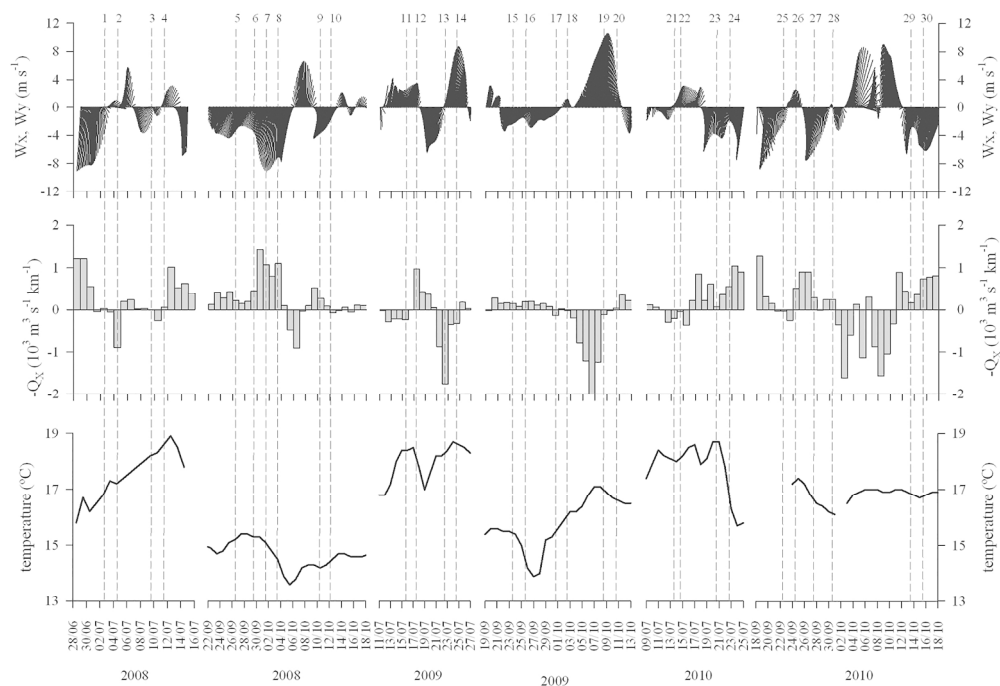
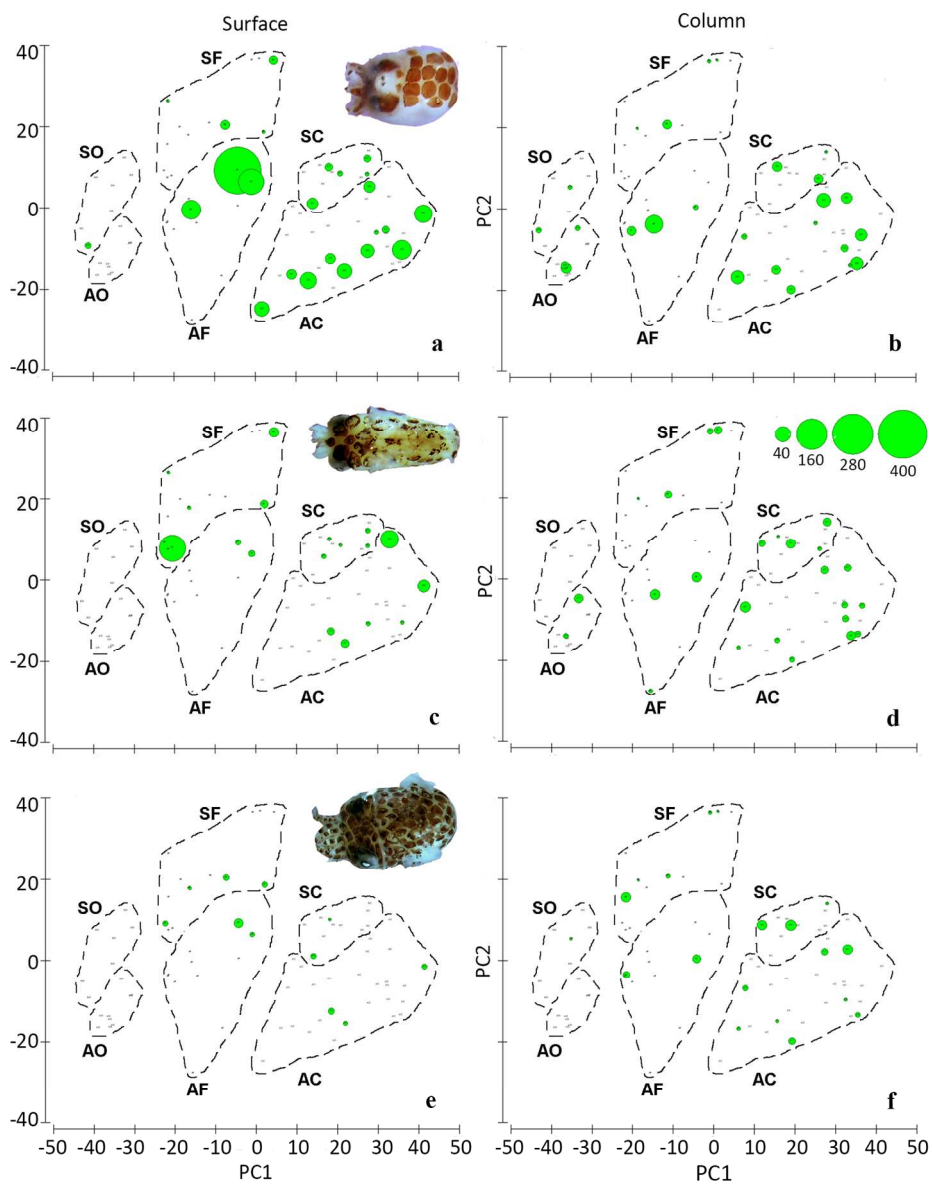


Fig. 2 Meteorological conditions recorded around the sampled area. a Wind speed and direction ( $W_x$ ,  $W_y$ ,  $m\ s^{-1}$ ), b upwelling index ( $-Q_x$ ,  $m^3\ s^{-1}\ km^{-1}$ ), and c sea surface temperature ( $^{\circ}C$ ) obtained from Rande observatory for 2008–2010 samplings. Vertical bars indicate the sampling days, which are numerated sequentially.

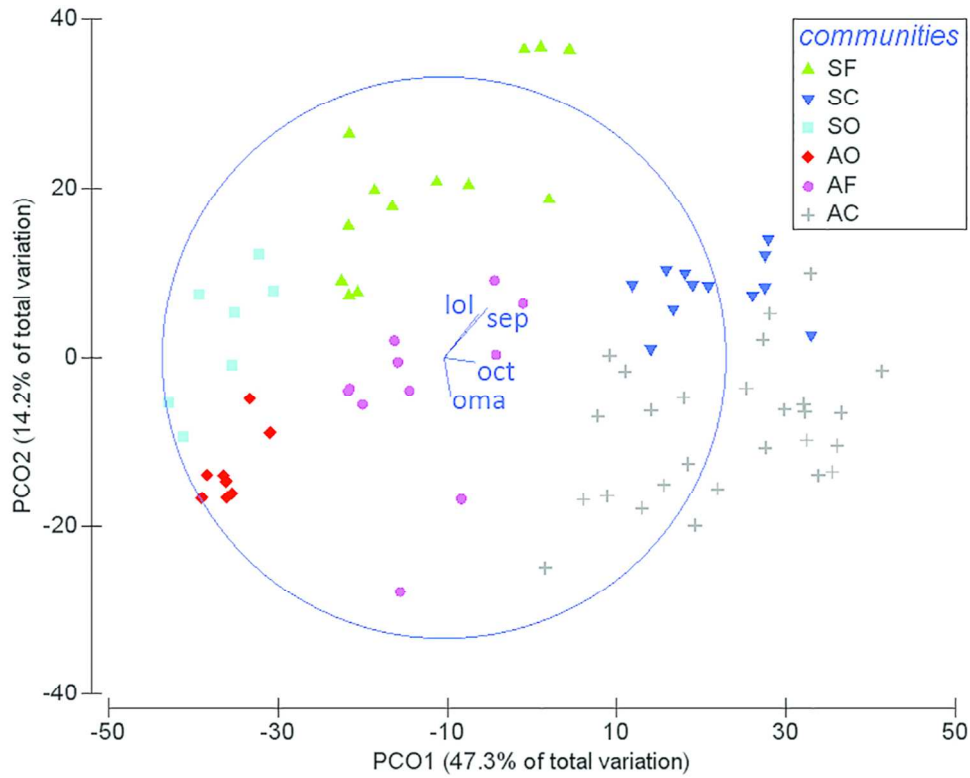
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PCO plot showing the distribution and abundance (ind. 1000 m<sup>3</sup>) of *Octopus vulgaris* (a,b), loliginid (c,d) and sepiolid (e,f) paralarvae at the surface and in the integrated water column samples within the different mesozooplankton communities identified off the Ría de Vigo in 2008. Abbreviations: SC, summer coast; SF, summer front; SO, summer ocean; AC, autumn coast; AF, autumn front; AO, autumn ocean.

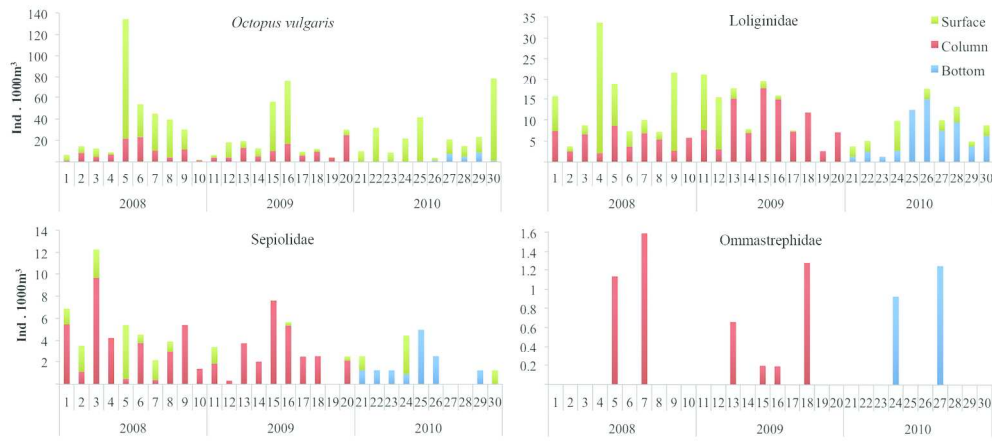
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PCO plot showing the strength and direction of the associations between the cephalopod paralarvae collected in 2008 and the mesozooplankton communities found off the Ría de Vigo. The blue circle represents the maximum correlation with PCO axis. Abbreviations: lol, loliginidae; oct, *Octopus vulgaris*; oma, ommastrephidae; sep, sepiolidae.

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Mean cephalopod paralarval abundance (ind 1000 m<sup>3</sup>) collected at the different strata during the 30 samplings carried out in the Ría de Vigo between 2008 and 2010. Samples were collected with a bongo net in 2008 and 2009 (column samples) and with a Multinet in 2010 (bottom sample).  
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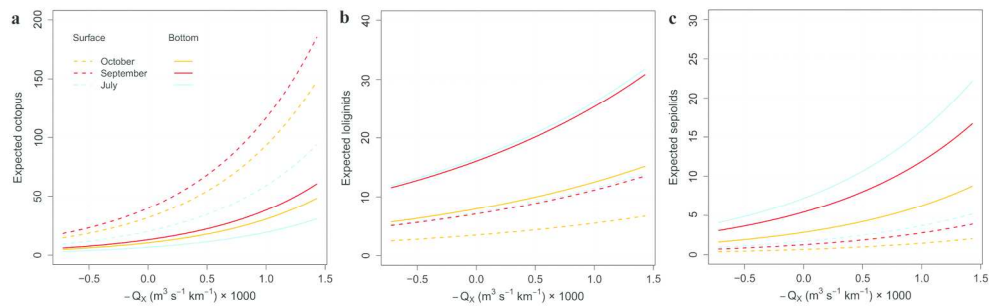


Fig. 6 Predicted number of (a) *O. vulgaris* at station 2, and (b) loliginids and (c) sepiolids at station 3 as a function of the upwelling index the previous day of the capture, the strata and month sampled. The expected values are the result of a negative binomial GLM fitted to the abundance of cephalopod paralarvae collected from 2008 to 2010 (see Table 6). Values were standardized to 1000 m<sup>3</sup>.  
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