

1 Immigration and early life stages recruitment of the European flounder (*Platichthys flesus*) to
2 an estuarine nursery: the influence of environmental factors

3 Eva Amorim^{1,2,3*}, Sandra Ramos^{2,3}, Michael Elliott³, Adriano. A. Bordalo^{1,2}

4 1. Institute of Biomedical Sciences, University of Porto (ICBAS-UP), 4050-313 Porto,
5 Portugal

6 2. CIIMAR – Interdisciplinary Centre for Marine and Environmental Research, Rua dos
7 Bragas, 289, 4050-123 Porto, Portugal

8 3. Institute of Estuarine & Coastal Studies, University of Hull, Hull, HU6 7RX, UK

9 * Corresponding author: ecamorim@icbas.up.pt; Tel.: 00351220428195

10 Abstract

11 Connectivity between coastal spawning grounds and estuarine nurseries is a critical step in the
12 life cycle of many fish species. Larval immigration and transport-associated physical-biological
13 processes are determinants of recruitment success to nursery areas. The recruitment of the
14 European flounder, *Platichthys flesus*, to estuarine nurseries located at the southern edge of
15 the species distribution range, has been usually investigated during its juvenile stages, while
16 estuarine recruitment during the earlier planktonic life stage remains largely unstudied. The
17 present study investigated the patterns of flounder larval recruitment and the influence of
18 environmental factors on the immigration of the early life stages to the Lima estuary (NW
19 Portugal), integrating data on fish larvae and post-settlement individuals (<50 mm length),
20 collected over 7 years. Late-stage larvae arrived at the estuary between February and July and
21 peak abundances were observed in April. Post-settlement individuals (<50 mm) occurred later
22 between April and October, whereas newly-settled ones (<20 mm) were found only in May
23 and June. Variables associated with the spawning, survival and growth of larvae in the ocean
24 (sea surface temperature, chlorophyll *a* and inland hydrological variables) were the major
25 drivers of flounder occurrence in the estuarine nursery. Although the adjacent coastal area is
26 characterized by a current system with strong seasonality and mesoscale variability, we did not
27 identify any influence of variables related with physical processes (currents and upwelling) on
28 the occurrence of early life stages in the estuary. A wider knowledge on the influence of the
29 coastal circulation variability and its associated effects upon ocean-estuarine connectivity is
30 required to improve our understanding of the population dynamics of marine spawning fish
31 that use estuarine nurseries.

32

33 Keywords: *Platichthys flesus*; estuarine recruitment; larvae; juveniles; physical-biological
34 processes

35 1. Introduction

36 Estuaries function as temporary habitats for early life stages of many fishes, a critical period in
37 the life cycle of marine fishes (Elliott et al., 2007; Potter et al., 2015). Although the passage of
38 early life stages through estuarine environments can present a 'bottleneck' on the abundance
39 of adult populations, there is still a poor understanding of these events (Able and Fahay, 2010).
40 The connectivity between spawning areas and nurseries is one of the major determinants of
41 the dynamics of fish populations (Cowen and Sponaugle, 2009). Processes such as pelagic
42 larval dispersal and supply depend on the biophysical regulators of offspring production and
43 availability, as well as on the interactions between local/regional hydrodynamics, larval
44 delivery into estuaries, and the behavioural capabilities of the individual larvae (Cowen and
45 Sponaugle, 2009; Sale et al., 2010; Potter et al., 2015). During the dispersal stage, eggs and
46 larvae experience high mortality, which strongly influences recruitment variability (Houde,
47 2008). Furthermore, population connectivity depends on additional processes, since effective
48 transfer requires successful recruitment to the juvenile receiving population (Sale et al., 2010).
49 For example, the availability of suitable settlement and nursery habitat, or the processes that
50 affect the transition from larvae into benthic juveniles, such as larval condition and benthic
51 predation, will influence recruitment (Cowen and Sponaugle, 2009).

52 The European flounder, *Platichthys flesus* (Linnaeus, 1758), is a widely-distributed flatfish
53 along the northeastern Atlantic coast and widely regarded as having an estuarine resident or
54 semi-catadromous life stage (Elliott et al., 2007). This species reproduces in winter/early spring
55 in marine waters (Campos et al., 1994; Dando et al., 2011; Grioche et al., 1997; Koubi et al.,
56 2006) and migrates during the early life stages to nursery grounds (e.g. Bos, 1999; Jager, 2001;
57 Martinho et al., 2008; Summers, 1979). Little is known about *P. flesus* settlement patterns
58 (Jager 2001; Bos and Thiel, 2006; van der Veer et al., 1991) and its favoured settlement habitat
59 (estuary vs. coastal areas) (Daverat et al., 2012). In general, for flatfishes, it has been
60 emphasized that the processes affecting recruitment variability are more relevant during the
61 pelagic phase, although post-settlement processes seem to regulate juvenile abundance (Bolle
62 et al., 2009; Geffen et al., 2007; van der Veer et al., 2000). However, and although *P. flesus* has
63 been described to enter estuaries during the larval phase (Bos, 1999; Jager, 1998; Ramos et al.,
64 2010), most studies concerning estuarine nurseries for this species are mainly focused on post-
65 settlement juveniles (Amara et al., 2009; Cabral et al., 2007; Freitas et al., 2009; Jager, 2001;

66 Martinho et al., 2008; Vasconcelos et al., 2010). Consequently, the supply of *P. flesus* early-life
67 stages to estuaries, and associated environmental constraints on recruitment variability, have
68 been investigated mainly in juveniles. These studies show that the abundance of juvenile
69 flounder in estuaries may be related to the average seawater temperature of the previous year
70 (Henderson and Seaby, 1994; Vinagre et al., 2009) and with river runoff, precipitation, and
71 wind prior to estuarine colonization (Martinho et al., 2009). Once in the estuary,
72 environmental constraints vary between larval and juvenile phases, with *P. flesus* larvae
73 showing a strong seasonal structure mainly regulated by biological features such as the
74 spawning season (timing and duration), while juveniles are markedly controlled by site-specific
75 characteristics such as sediment structure, distance from the river mouth, and salinity regime
76 (Ramos et al., 2009a). Therefore, any natural or anthropogenic events that affect either the
77 quality of the nursery areas, the delivery of young to those areas, or the passage of the young
78 from the nursery areas, will affect success of the recruitment to the adult population.

79 Given the demonstrated effects that the pre-settlement processes may have upon year-class
80 strength, it is important to investigate the connectivity between ocean and estuaries and the
81 processes associated with the supply of the pelagic larvae to the primary nursery grounds
82 (Cowen and Sponaugle, 2009). The Lima estuary, located in the North-Western (NW) Iberian
83 Peninsula, has been identified as a nursery habitat for European flounder (Ramos et al., 2010)
84 and because the NW Iberian Peninsula represents the southern edge of its geographical range,
85 environmental processes become particularly relevant, as the species becomes more
86 vulnerable to environmental fluctuations at its distributional edges (Miller et al., 1991).

87 Therefore, this study aimed to: (i) document the patterns of the recruitment of European
88 flounder to the Lima estuary, using pelagic-larval and post-settlement juvenile flounder data
89 describing 7 years within a 12-year period, and (ii) investigate the associated environmental
90 factors, following the hypothesis that estuarine recruitment at the larval phase is influenced by
91 the physical and biological processes of ocean and estuarine dynamics.

92 **2. Material and Methods**

93 **2.1 Study area**

94 The Lima estuary (Portuguese NW Atlantic coast of the Iberian Peninsula) has a catchment of
95 2446 km² (Fig. 1) and a discharge regime with an annual average flow of 70 m³ s⁻¹ mainly
96 controlled by two hydroelectric power stations located upstream. The tidal regime, the main
97 hydrodynamic forcing action of the estuary (Falcão et al., 2013), is mesotidal and semidiurnal
98 with a range of 3.7 m during spring tides and an upstream influence that reaches 20 km. The

99 Lima estuary can be divided into three areas with distinct geomorphological features: the
100 lower estuary (0-3 km from the mouth) is a narrow, deep and navigational channel with
101 artificial banks; the middle estuary (3-7 km) is a broad shallow zone with salt marshes and tidal
102 sandy islands; and the upper estuary (7-20 km) is a shallow and narrow channel with small
103 sandy islands (Ramos et al., 2010). Despite seasonal hydrological fluctuations in the Lima
104 estuary (Ramos et al., 2006a), during most of the year salinity ranges between 35 at the river
105 mouth and 0 in the uppermost section of the estuary (17 km).
106 The adjacent coastal area is characterized by a complex current system subjected to strong
107 seasonality and mesoscale variability, with reversing patterns between summer and winter
108 (Ambar and Fiúza, 1994; Barton, 1998). This area is located near the northernmost limit of the
109 North Atlantic Upwelling System region (43° N), being exposed to strong upwelling events
110 occurring during spring and summer (Wooster et al., 1976; Fiúza et al. 1982). Other major
111 features of the coastal circulation are the Portugal Current, the Iberian Poleward Current (IPC)
112 and the Western Iberia Buoyant Plume (WIBP) (Fig. 1b). The Portugal Current is a broad and
113 slow current generally flowing south and extending approximately between 10° W to 24° W
114 (off the continental slope) (Martins et al., 2002). The IPC is a current with warm and saline
115 waters that extends to the shelf edge and outer shelf mainly during the downwelling season in
116 the winter (Frouin et al., 1990; Haynes and Barton, 1990; Peliz et al., 2005). The WIBP low-
117 salinity buoyant plume is generated by the discharge of NW Iberian rivers (Peliz et al., 2005).
118 The WIBP has a rapid response to changes in the wind conditions, and spreads offshore over
119 the shelf extending its influence to the IPC during upwelling events (Ribeiro et al., 2005; Otero
120 et al., 2008).

121 2.2 Sampling and data acquisition

122 *Platichthys flesus* data

123 Larval and juvenile flounder data were gathered over many years from the Lima estuary using
124 consistent methodologies. Fish larvae were collected with plankton tows that were conducted
125 in a circular path performed with a planktonic 1 m diameter, 3 m long and 500 µm mesh-size
126 net, equipped with a Hydro-Bios (Kiel-Altenholz, Germany) flowmeter (model 438 110).
127 Sampling effort varied between 9 to 11 tows, always covering the lower, middle, and upper
128 sections of the estuary. The frequency of the sampling varied between fortnightly (April 2002-
129 April 2004 (Ramos et al., 2006b, 2010) and May-October 2013) to monthly (April 2009 and
130 September 2009-October 2010) (Table 1). Samples were fixed in 4% buffered formalin (pH 8)
131 immediately after collection, and *P. flesus* larvae were preserved in 96% ethanol after sorting.

132 Notochord length was measured for pre-flexion larvae and standard length was measured for
133 flexion and post-flexion larvae. Additionally, the ontogenic development stage of each larva
134 was determined as: stage I – newly hatched larva; stage II - pigmented eyes, yolk sac may still
135 be present; stage III - yolk sac totally absorbed, the urostyle flexes upward, and rays of the
136 caudal and dorsal fins are formed; stage IV - beginning of eye migration (loss of bilateral
137 symmetry); stage V - ending of eye migration (Ramos et al., 2010).

138 Juvenile surveys were conducted monthly between October 2003 and September 2005, with a
139 1 m beam trawl (Ramos et al., 2009a, 2010), September 2009 and October 2010 (2 m beam
140 trawl), and May - October 2013 (2 m beam trawl and otter trawl) (Table 1). The mesh-sizes
141 were 5 mm and 20 mm for the beam trawl and otter trawl, respectively. Samples were
142 collected throughout the estuary (10 to 12 tows), always covering the lower, middle, and
143 upper sections of the Lima estuary and reaching 10 km upstream from the river mouth
144 between 2003 and 2005, and 17 km upstream between 2009 and 2013. All fish caught were
145 refrigerated and transported to the laboratory where they were immediately frozen until
146 sorting. Fishes were identified, counted and measured (total and standard length). According
147 to Hutchinson and Hawkins (2004), who used laboratory observations to describe the
148 developmental sequence of the flounder beginning at hatching, the juvenile stage is fully-
149 formed at between 44.50 and 46.75 mm in total length; therefore, post-settlement flounder
150 less than 50 mm (total length) were considered for this study as post-settlement juveniles.

151 Environmental data

152 Several inland and oceanographic variables may influence fish larval stages while immigrating
153 to estuarine nurseries: precipitation (P), river flow (RF), chlorophyll *a* (Chl*a*), sea surface
154 temperature (SST), surface currents (horizontal vector, *u*; vertical vector, *v*), and upwelling
155 index (UI). All oceanographic data, except for the UI, were assessed at the scale of the area of
156 the coastal front of the Lima estuary, 1° in latitude (0.5° to the north and south of the estuary
157 mouth) and approximately 0.5° in longitude (between the coast line and limit of the
158 continental shelf).

159 Precipitation (mm) and river flow ($\text{m}^3 \text{s}^{-1}$) data were obtained from APA (Agência Portuguesa
160 do Ambiente, <http://snirh.pt>). Ocean Chl*a* concentration (mg m^{-3}) was obtained from the ESA
161 Ocean Colour Climate Change Initiative (<http://www.esa-oceancolour-cci.org/>), which
162 comprises merged and bias-corrected MERIS (Medium Resolution Imaging Spectrometer),
163 MODIS (Moderate-resolution Imaging Spectroradiometer) and SeaWiFS (Sea-Viewing Wide
164 Field-of-View Sensor) data, with a horizontal resolution of ~ 4 km/pixel. Chl*a* data for 2013

165 were acquired from the MODIS-Aqua products of the NASA project Ocean Color
166 (<http://oceancolor.gsfc.nasa.gov/>). Current speeds and SST were acquired from
167 Copernicus Marine Environment Monitoring Service (<http://marine.copernicus.eu/>). Currents,
168 both northward (v) and eastward velocity (u) components (m s^{-1}), were obtained with a
169 horizontal resolution of $1/12^\circ$, and with two vertical levels (surface and ~ 1.5 m depth). SST ($^\circ\text{C}$)
170 was obtained with a horizontal resolution of 0.05° . Upwelling Index time series data were
171 provided by the Instituto Español de Oceanografía (www.indicedeafloramiento.ieo.es) and
172 were generated using sea level pressure of the FNMOC (<http://www.usno.navy.mil/FNMOC/>)
173 WXMAP atmospheric model, at a position of 42°N latitude by 10°W longitude. The upwelling
174 index summarises the volume of oceanwards flow of surface waters per km of coastline (m^3s^{-1}
175 km^{-1}), with positive index representing upwelling events.

176 2.3 Data analysis

177 Larval abundance data were standardised into the number of individuals per 100 m^3 of water
178 filtered. Post-settlement individuals (<50 mm) abundance data were standardised into the
179 number of individuals per 1000 m^2 .

180 Relationships between the environmental data and the occurrence (presence-absence) of the
181 early life stages of flounder within the estuary were explored using boosted regression trees
182 (BRTs), a machine learning method composed of two algorithms: regression trees and boosting
183 (Elith et al., 2008; Friedman, 2002, Hastie et al., 2001). Tree models explain the variation of a
184 response variable by recursive binary partitions of the data (De'ath and Fabricious, 2000;
185 Hastie et al., 2001). In boosting, the models are fitted iteratively in a forward stage-wise
186 manner, progressively adding simple trees to the model, with each tree fitted to the residuals
187 of the existing set of trees (Elith et al., 2008; Leathwick et al., 2006). BTRs have been used
188 increasingly in ecology showing evidence of strong predictive performance (e.g. Elith et al.,
189 2006; França and Cabral, 2015; Leathwick et al., 2006; Soykan et al., 2014).

190 Two BRT models were built, one for the larval phase and the other for the post-settlement
191 juvenile phase (<50 mm), using presence-absence data within the estuary (where pooling all
192 sampling sites within one survey counted as an observation). The larvae dataset was
193 composed of the fortnightly surveys conducted between 2002 and 2004, and the survey
194 conducted in April 2009. The post-settlement juvenile dataset was composed of the monthly
195 surveys conducted between 2003-2005, 2009-2010 and 2013. All the environmental variables
196 were tested as predictors at different times relative to collection; the time of the collection
197 was considered as time=0, and intervals prior to the collection were then tested (for example:

198 one month before, one and a half months before, etc.). These environmental variables were
199 referenced with a subscript number indicating their time lag relative to the estuarine surveys;
200 for example, SST_0 = sea surface temperature at time 0; SST_1 = sea surface temperature at time
201 1 month prior to the estuarine collection. For the larval data set, environmental variables were
202 tested up to 3 months before the larval collection (fortnightly and monthly averages). For
203 post-settlement data, variables were tested up to 4 months prior to post-settlement-individual
204 collection (monthly and bimonthly averages).

205 These relationships were tested following two main assumptions, namely (i) the sampling
206 methodology for the flounder was efficient, i.e., 0 represented the absence of individuals in
207 the estuary rather than a methodological problem; (ii) post-settlement flounder (<50 mm) is a
208 proxy of larval recruitment.

209 BRT models were built in R-software, version 3.1.0 (R Core Team, 2014), using the 'gbm'
210 package (Ridgeway 2006), and the supplement functions provided in the 'dismo' package (Elith
211 et al., 2008). During preliminary analyses, models were fitted with the combinations of the
212 following settings: learning rates between 0.005-0.0005, bag fraction (bg) of 0.5 and 0.75, tree
213 complexity (tc) of 1 and 2, and k-fold cross validation of 5 and 10. After finding the optimal
214 settings, two full models were fitted and then simplified by removing non-informative
215 variables based on the decrease of deviance (using the routine `gbm.simplify`). Moreover, to
216 confirm that all the variables of the final models were valuable for modelling the response
217 variable, a random number was added as a predictor variable (Soykan et al. 2014). Therefore,
218 when evaluating the relative influence of each variable to the model, only the variables that
219 performed better than random numbers were retained. Also, the rule-of-thumb for variables
220 correlated at $|r| > 0.7$ was applied (Dormann et al., 2013), and the variables of the correlated
221 pair with the lower relative contribution were removed. The optimal final models were chosen
222 based on their statistical performance, parsimony and use. Model performance was assessed
223 by the amount of cross-validated deviance explained, cross-validated correlation between
224 model predictions and observed data, and the area under the Receiver Operating
225 Characteristic curve (AUC) score. AUC score ranges from 0 to 1; a value of 1 indicates a perfect
226 discrimination of probabilities between presence and absence, and a value of 0.5 indicates
227 that model discrimination is no better than a random result (Elith et al., 2006).

228 **3. Results**

229 **3.1 Estuarine recruitment**

230 Flounder larvae were collected in the estuary between February and July (Fig. 2) and peaks
231 occurred mostly in April (Fig. 2 and 3). Ontogenic development stages ranged between II and
232 IV and stages IV and III represented 88.6 % and 10% respectively of the larvae collected. Only
233 1.4% of the larvae collected were in stage II of development and they occurred only in
234 February (Fig. 3). Larvae were collected throughout the estuary, but highest abundances were
235 observed in the middle saltmarsh and upper sections of the Lima estuary (Fig. 4a).

236 The peak abundances of post-settlement juveniles (<50 mm) were observed in May and June
237 (Fig. 2). Newly-settled flounder (< 20 mm) were absent from the estuary after July (Fig. 3). Few
238 individuals >30 mm were found in August and October. Post-settlement juveniles were
239 predominantly found in the upper section of the estuary (> 95% of the total catch), with 60.6%
240 being found at the most-upstream station, approximately 17km from the mouth of the
241 estuary (Fig. 4b).

242 3.2 Environmental variables

243 Throughout the studied years, river flow showed a typical pattern of increased values during
244 the autumn/winter period and a decrease in late spring and summer (Fig. 5a). As for annual
245 variability, the year of 2005 had the lowest annual mean ($35.1 \text{ m}^3 \text{ s}^{-1}$), whereas 2003 had the
246 highest annual mean ($77.2 \text{ m}^3 \text{ s}^{-1}$). Similarly, precipitation showed a general decrease from
247 January to summer months (Fig. 5b). The lowest cumulative precipitation was 2005 with a total
248 of 915.5 mm, whereas 2013 had the highest cumulative precipitation, with 1650.0 mm.

249 SST showed a seasonal curve for all of the years studied (Fig. 5c), with the minimum
250 temperatures registered in late winter and increasing until mid-summer. Overall, average
251 minimum temperatures occurred in February for all years, with the exception of 2013 in which
252 the minimum occurred in March. Maximum temperatures occurred between July and October.

253 *Chla* increased from January to March, when the spring peak occurred. The summer peak,
254 generally lower than the spring peak, was registered in August (Fig. 5d). Despite this general
255 pattern, an expected interannual variability occurred, with annual peaks registered in February
256 (2004 and 2009), March (2003 and 2010), August (2002 and 2005) and September (2013).

257 The horizontal (u) component of the surface current velocities increased towards offshore
258 from January to July, with an increase in February (Fig. 5e). The vertical component (v) was
259 predominantly towards the north during the winter months, and reversed direction in April,
260 when it started increasing until July (Fig.5f). The upwelling index, in general, registered positive
261 and increasing values from April until September (summer upwelling) (Fig. 5g). Although the

262 first trimester of each year typically registered a negative index, positive mean upwelling
263 indices were recorded for one or two of the months during 2005, 2009 and 2013 (winter
264 upwelling). The upwelling period was also identifiable as modifications of the current
265 behaviour observed from April to September (Fig. 5e, 5f, 5g). As a consequence of the
266 northerly winds, the superficial vertical component of the currents was reversed towards the
267 south in April (Fig. 5f), leading to an increase of the horizontal component of the currents
268 towards offshore due to the Ekman transport (Fig. 5e). This process caused the coastal
269 upwelling shown in Figure 5g.

270 3.3 Influence of environmental variables

271 The final BTR models retained four variables (see next paragraph). The larval model had the
272 best overall fit (Table 2), explaining 63.3 % of the total deviance and a mean correlation
273 between predicted and observed data of 0.80. The post-settlement juvenile model had a
274 lower fit, explaining 39.0 % of the total deviance and had a mean correlation between
275 predicted and observed data of 0.71. The AUC score was also higher for the larval model.

276 The presence of larvae and post-settlement juveniles, was influenced by SST, followed by $Chl a$
277 and finally by the hydrological inland variables, precipitation and river flow (Fig. 6). The two
278 model results showed consistency, identifying the same predictors. The variable contribution
279 ranking of the larvae model matched with the variable contribution ranking of the post-
280 settlement model. SST was, in both models, the strongest contributor to the presence-absence
281 of the young flounder. Overall, low sea surface temperatures increased the probability of
282 occurrence of flounder early life stages in the estuary. $Chl a$ was the second-most important
283 variable in both models, with higher concentrations improving the probability of flounder early
284 life stage occurrence. Hydrological variables showed the weakest relationship with the
285 response variable, and only precipitation was present in both models.

286 Larvae were strongly influenced by the SST_2 , accounting for more than 55.8 % of the relative
287 contribution to the response variable (Fig. 6a). In general, the probability of larval occurrence
288 in the estuary decreased sharply with an increase in temperature (SST_2) from 13.8 °C to 14.6
289 °C. $Chl a_{1.5}$ contributed 22.4 % to the presence of larvae in the estuary, with the probability of
290 occurrence increasing with higher concentrations, above 1.2 mg m⁻³. Precipitation during the
291 2nd month prior to the larval collection (P_2) showed an influence of 15.4 % and had a positive
292 effect on the presence of larvae in the estuary. RF_3 was the weakest contributor (6.5%). RF
293 between 40 and 100 m³ s⁻¹ increased the probability of larval occurrence (Fig. 6a). Interactions
294 fitted for the larval model (Table 2) indicated a weak interaction between SST_2 and $Chl a_{1.5}$, and

295 a strong interaction between SST_2 and P_2 : there were peaks of occurrence when periods of
296 high P_2 and low SST_2 occurred simultaneously (Fig. 7).

297 For the post-settlement model, the most influential variable was SST, namely the average
298 temperature of the 3rd and 4th months prior to juvenile collection (SST_{3-4}), with a relative
299 contribution of 49.9 % (Fig. 6b). The probability of occurrence of post-settlement individuals
300 decreased with increases of SST_{3-4} . $Chla_2$ and $Chla_3$ contributed 25.6 % and 19.6 %, respectively,
301 to the response variable, and higher concentrations increased the probability of
302 post-settlement flounder occurrence in the estuary. P_3 had the lowest impact on post-
303 settlement occurrence, contributing 5 % and showing a positive influence. Interactions
304 indicated a peak of occurrence with higher P_3 and $Chla_2$ and with higher P_3 and lower SST_{3-4}
305 (Table 2).

306 **4. Discussion**

307 The location of the spawning grounds, jointly with transport and supply to the nursery areas of
308 *P. flesus*, still remain unclear at the NW Iberian coast, the species southern limit of distribution.
309 While the species is accepted as being semi-catadromous (Elliott et al., 2007), nearshore
310 spawning grounds are difficult to detect and may be relatively small (Bos et al., 1995; Grioche
311 et al., 1997). Spawning timing is described to occur between January and March (Sobral, 2008;
312 Martinho et al., 2013) with the pelagic stage lasting approximately 30 to 50 days (Martinho et
313 al., 2013), during which the larvae should reach the nursery areas. Our results indicate that
314 recruitment to the Lima estuary occurred early during the larval stages, prior to settlement.
315 Although larval and juvenile collections were not carried out in the same years, it was possible
316 to observe a synchronised pattern: the highest larval abundances were recorded in April,
317 followed by the presence of the newly-settled individuals (<20 mm) in the two following
318 months, May and June. Hence, flounder settlement seemed to have occurred inside the
319 estuary, in agreement with that reported for the Dollard (Ems estuary) (Jager, 1998) and for
320 the Elbe River (Bos, 1999). Nevertheless, fluctuations in the timing of arrival were observed,
321 with larvae arriving as early as February, and until July, whereas the post-settlement
322 individuals (<50 mm) were present between April and October.

323 Recent studies suggest that *P. flesus* might not only spawn in coastal areas, but also inside the
324 estuary (Morais et al., 2011; Daverat et al., 2012). Ferreira and Labarta (1988) reported a high
325 abundance of flounder eggs and high larval densities throughout a nearby estuary, the Ria de
326 Vigo. Although our study did not include the egg stages, only one pre-flexion larva (stage II)
327 was observed in the estuary, and most collected larvae were post-flexion (stages III and IV).

328 Vertical migrations appear to begin at stage III (e.g. Grioche et al., 1997, 2000; Khoubi et al.,
329 2006). Therefore, most of the larvae caught in the Lima were already able to regulate their
330 position in the water column, a capability which allowed them to use retention mechanisms,
331 such as selective-tidal-stream-transport (STST). This mechanism, which enables larvae to
332 migrate upstream the estuary and to avoid being flushed out to the ocean, has been
333 documented for several species (e.g., Forward et al., 1999; Hare et al., 2005; Islam et al., 2007;
334 Jager, 1999).

335 In the 2010 and 2013 ichthyoplankton surveys, lower abundances were registered and larvae
336 were caught after the abundance peak of post-settlement organisms. In 2013, sampling
337 started in May, probably after the main recruitment to the estuary and, in 2010, monthly
338 sampling was unable to detect the larval arrival into the estuary. The lowest abundances of
339 2010 emphasize the need to conduct at least fortnightly surveys to be able to detect the
340 recruitment pulse of larvae. In the western Wadden Sea, Van der Veer (1985) observed that
341 the immigration of *P. flesus* larvae started in April and lasted for one month, in contrast to the
342 plaice (*Pleuronectes platessa*, 2 months), but where the shorter supply period was
343 compensated with higher abundances.

344 Results identified a relationship between the occurrence of *P. flesus* early life stages and sea
345 surface temperature, chlorophyll *a*, and inland hydrological processes. The consistency shown
346 between the results of the two models indicated that post-settlement flounder (<50 mm)
347 could represent a proxy for larval recruitment because it corroborated the variables identified
348 by the larval model. On the other hand, the fit and predictive performance of the post-
349 settlement model were lower relative to the larval model. This was expected due to two
350 reasons: (i) a wider size range of post-settlement individuals, which might correspond to a
351 wider age range and consequently a distinct timing of nursery arrival, and (ii) post-settlement
352 individuals having been in the estuary for longer (at least a month), thus being more exposed
353 to estuarine conditions. Although the processes responsible for recruitment variability are
354 more relevant during the pelagic phase, density-dependent processes within nursery areas
355 (such as growth or predation) may dampen the variability generated during the pelagic phase
356 (van der Veer et al., 2000; Geffen et al., 2007; Bolle et al., 2009). Thus, the existence of
357 additional estuarine environmental constraints might potentially mask the identification of
358 variables that acted previously during the oceanic life stage of the flounder.

359 The successful arrival of early life stages to the estuarine nursery areas results from the
360 interaction between several processes. By taking advantage of local physical (e.g. larval

361 dispersal/transport/retention) and biological (e.g. survival and growth) conditions, organisms
362 optimize dispersal success and reduce mortality (Able and Fahay, 2010; Bailey et al., 2008;
363 Boehlert and Mundy, 1988, Duffy-Anderson et al., 2014).

364 Temperature is known to have a greater effect on the larval supply to temperate estuaries
365 (Able and Fahay, 2010), acting directly or indirectly to control biological processes (Houde,
366 2008). Temperature is a determinant factor at several stages of a fish life cycle, including
367 gonadal development and spawning (Pawson et al., 2000; Fincham et al., 2013; Sims et al.,
368 2004) and survival and growth of early life stages prior to nursery recruitment (Hutchinson and
369 Hawkins, 2004; Henderson and Seaby, 1994; Vinagre et al., 2009). The minimum annual SST of
370 the surveyed years coincided with the documented spawning timing (January to March).
371 According to the larval model, the time lag between the occurrence of flounder in the estuary
372 and the SST variables was consistent with that time of spawning: i.e. approximately 2 months
373 before the arrival of the larvae. As a limiting factor for reproductive success, temperature
374 becomes a particularly important feature at the geographic limit of the flounder distribution,
375 with regard to climate change. Teixeira et al. (2014) reported a decreasing trend in flounder
376 fishery landings along the Portuguese coast over the past 40 years, with landings being lower
377 in warmer years. Also, and according to historical data, the abundance decreased along the
378 Portuguese coast (Tagus estuary) (Cabral et al., 2001; Vinagre et al. 2009) and also in the Bay
379 of Biscay (Hermant et al., 2010).

380 The relationship between Chl a levels and the presence of *P. flesus* in the estuary highlights the
381 importance of larval fish encountering suitable prey abundance as a prerequisite for survival,
382 as proposed by many population recruitment hypotheses (Peck et al., 2012), such as the
383 'match-mismatch' phenomenon (Cushing, 1990). The ability to find and eat a sufficient
384 quantity of suitable prey enhances the survival of a larva, and the lack of synchrony, both
385 temporal and spatial, between prey and predators is considered to be one of the causes for
386 recruitment variability (Cushing, 1990; Durant et al., 2007). Although very few studies have
387 investigated the prey of *P. flesus* larvae, there is evidence that although diet is dominated by
388 zooplankton, they also feed on phytoplankton at the time of 'first-feeding' (Last, 1978; Engell-
389 Sørensen et al., 2004). Moreover, phytoplankton spring blooms are coupled with productivity
390 of zooplankton, which are the main prey of larvae before entering the estuary.

391 Inland hydrological processes, such as precipitation regime and river flow regulate the
392 freshwater input to estuaries and coastal areas. These variables may have a negative effect on
393 the entrance of marine fish larvae into the estuary (Ramos et al., 2006a, 2012), and also reduce

394 larval survival in the estuary (Chícharo et al., 2001). Conversely, Martinho et al. (2009) found
395 that precipitation and river flow in the months preceding the arrival of the flounder to the
396 estuary appear to positively influence abundance of young of the year class. In fact,
397 continental freshwater plumes have been linked to the growth and survival of fish larvae in the
398 sea (Sabatés et al., 2001; Lloret et al., 2004), as well as acting as a cue to estuarine location
399 (Leis et al., 2011; Sullivan et al., 2006). Hence, an understanding of estuarine ecological
400 processes is only possible with an adequate understanding of the interlinked physical
401 processes, the emerging field of estuarine ecohydrology (Wolanski and Elliott, 2015). For
402 example, along the NW Iberian coast, the nutrient-rich river discharges are responsible for the
403 presence of the Western Iberia Buoyant Plume; which, when associated with weak winter
404 upwelling events occurring in this area (e.g. Fiúza, 1982; Santos et al., 2004), enhance the
405 growth and concentration of phytoplankton (Ribeiro et al. 2005). These processes have been
406 identified as creating a suitable environment for the survival of sardine (*Sardina pilchardus*)
407 larvae (Santos et al., 2004, 2007; Ramos et al., 2009b). On the other hand, the increase in
408 intensity and frequency of upwelling events during the winter spawning season of small
409 pelagic species off the coast of Portugal was found to have a negative impact on recruitment,
410 limiting their survival success (Santos et al., 2001). Although winter upwelling events did not
411 occur during the current study years, the presence of the flounder in the estuary was not
412 influenced by upwelling or by currents acting prior to the arrival of the larvae into the estuary.

413 The Lima estuarine recruitment of *P. flesus* was related to oceanic temperature and
414 chlorophyll *a*, a common feature of several marine spawning fish species. During its earlier
415 stages, fish larvae have no or very poor swimming capabilities being more susceptible to
416 physical transport derived from oceanographic events, such as currents, upwelling, fronts and
417 eddies. Indeed, the dispersal of eggs and early larvae by advective processes is crucial to
418 guarantee the successful colonization of the nursery areas (Cowen and Sponaugle, 2009;
419 Duffy-Anderson et al., 2014). Despite all of this, our results showed that the environmental
420 variables associated with physical processes were not considered relevant to the recruitment
421 of flounder to the Lima estuary. However, elsewhere upwelling events have been associated
422 with the occurrence and density of estuarine larval fish assemblages (Able and Fahay, 2010).
423 The recruitment pulses of the English sole, an estuarine-dependent flatfish species that uses
424 onshore Ekman transport to arrive to an estuarine zone (Boehlert and Mundy 1987), have
425 been found to be negatively correlated with the upwelling index (Boehlert and Mundy, 1998).
426 In the case of the NW Iberia coast, the transition from winter downwelling to summer
427 upwelling typically occurs in April, with the persistent upwelling conditions prevailing until

428 September (Fiúza, 1983, Ramos et al., 2013). During our study period, the highest abundance
429 of larvae arrived at the estuary in April, when the summer upwelling events begin to occur.
430 This transition period is characterized by large variability in circulation (Torres and Barton,
431 2007), which might represent an additional constraint to flounder arrival at the Lima estuary.
432 Given that *P. flesus* is a late winter spawning species, one would expect that eggs and early
433 larvae were subjected to fluctuations in the transport patterns introduced by the seasonal and
434 annual variability of local oceanographic factors. However, our results showed that the
435 estuarine occurrence of larvae is mainly linked to variables regulating spawning, survival and
436 growth, suggesting some ability of the larvae to cope with local oceanographic circulation.
437 Therefore, it is necessary to locate the spawning areas in order to additionally determine how
438 the early life stages deal with local oceanographic variation. Increased knowledge on larval
439 behaviour and transport pathways will allow a better understanding of how deviations from
440 usual conditions will affect the ocean-estuary connectivity and, ultimately, the southern *P.*
441 *flesus* population.

442 **5. Conclusions**

443 The European flounder, *P. flesus* recruited to the Lima estuary early in life. Larval stages
444 arrived in spring and post-settlement juveniles (<50 mm) were found in the upper estuary one
445 or two months later. The larval model identified variables that had previously influenced the
446 immigration from the oceanic spawning grounds to the estuarine nursery as the major drivers
447 of *P. flesus* occurrence in the Lima estuary. These findings were corroborated by the post-
448 settlement juveniles (<50 mm) model. The study emphasized the relative importance of
449 environmental variables (SST, Chl α , P and RF) associated with the biological processes
450 (responsible for the spawning, and growth and survival of larvae), over the variables
451 responsible for larval dispersal, transport and retention (currents and upwelling). Hence the
452 study gives a good background for further research to understand how oceanographic
453 conditions control the estuarine recruitment of the European flounder.

454 **6. Acknowledgements**

455 This research was partially supported by the European Regional Development Fund (ERDF)
456 through the COMPETE - Operational Competitiveness Programme and national funds through
457 FCT – Foundation for Science and Technology, under the project “PEst-C/MAR/LA0015/2011”.
458 FCT also funded a PhD scholarship to Eva Amorim (SFRH/BD/75962/2011) and a Post-doctoral
459 fellowship (SFRH/BPD/26782/2006) to Sandra Ramos. The authors also would like to thank all

460 the people who helped in the field-work. The anonymous referees, as well as the International
461 Flatfish Symposium Guest Editor, are acknowledged for their valuable and constructive
462 comments which have improved the final manuscript.

463 **7. References**

- 464 Able, K.W., Fahay, M.P., 2010. Ecology of estuarine fishes: temperate waters of the western
465 North Atlantic. Johns Hopkins University Press.
- 466 Amara, R., Selleslagh, J., Billon, G., Minier, C., 2009. Growth and condition of 0-group European
467 flounder, *Platichthys flesus* as indicator of estuarine habitat quality. *Hydrobiologia* 627, 87-98.
- 468 Ambar, I., Fiúza, A., 1994. Some features of the Portugal current system: a poleward slope
469 undercurrent, an upwelling-related summer southward flow and an autumn–winter poleward
470 coastal surface current, Proceedings of the Second International Conference on Air–Sea
471 Interaction and on Meteorology and Oceanography of the Coastal Zone. American
472 Meteorological Society, pp. 286-287.
- 473 Bailey, K.M., Abookire, A.A., Duffy-Anderson, J.T., 2008. Ocean transport paths for the early life
474 history stages of offshore-spawning flatfishes: a case study in the Gulf of Alaska. *Fish and*
475 *Fisheries* 9, 44-66.
- 476 Barton, E.D., 1998. Eastern boundary of the North Atlantic: northwest Africa and Iberia.
477 Coastal segment (18, E), in: Inc, J.W.S. (Ed.), *The sea*, pp. 633-657.
- 478 Boehlert, G.W., Mundy, B.C., 1987. Recruitment dynamics of metamorphosing English sole,
479 *Parophrys vetulus*, to Yaquina Bay, Oregon. *Estuarine, Coastal and Shelf Science* 25, 261-281.
- 480 Boehlert, G.W., Mundy, B.C., 1988. Roles of behavioral and physical factors in larval and
481 juvenile fish recruitment to estuarine nursery areas, American Fisheries Society Symposium,
482 pp. 1-67.
- 483 Bolle, L.J., Dickey-Collas, M., van Beek, J.K.L., Erftemeijer, P.L.A., Witte, J.I.J., van der Veer,
484 H.W., Rijnsdorp, A.D., 2009. Variability in transport of fish eggs and larvae. III. Effects of
485 hydrodynamics and larval behaviour on recruitment in plaice. *Marine Ecology Progress Series*
486 390, 195-211.
- 487 Bos, A., Thiel, R., Nellen, W., 1995. Distribution and transport mechanisms of the upstream
488 migrating flounder larvae, *Pleuronectes flesus* Linnaeus, 1758, in the tidal Elbe River, Germany.
489 ICES CM 41, 1-10.

490 Bos, A.R., 1999. Tidal transport of flounder larvae (*Pleuronectes flesus*) in the Elbe River,
491 Germany. *Archive of Fishery and Marine Research* 47, 47-60.

492 Bos, A.R., Thiel, R., 2006. Influence of salinity on the migration of postlarval and juvenile
493 flounder *Pleuronectes flesus* L. in a gradient experiment. *Journal of Fish Biology* 68, 1411-1420.

494 Cabral, H.N., Costa, M.J., Salgado, J.P., 2001. Does the Tagus estuary fish community reflect
495 environmental changes? *Climate Research* 18, 119-126.

496 Cabral, H.N., Vasconcelos, R., Vinagre, C., França, S., Fonseca, V., Maia, A., Reis-Santos, P.,
497 Lopes, M., Ruano, M., Campos, J., Freitas, V., Santos, P.T., Costa, M.J., 2007. Relative
498 importance of estuarine flatfish nurseries along the Portuguese coast. *Journal of Sea Research*
499 57, 209-217.

500 Campos, W.L., Kloppmann, M., von Westernhagen, H., 1994. Inferences from the horizontal
501 distribution of dab *Limanda limanda* (L.) and flounder *Platichthys flesus* (L.) larvae in the
502 southeastern North Sea. *Netherlands Journal of Sea Research* 32, 277-286.

503 Chícharo, L., Chícharo, M.A., Esteves, E., Andrade, J.P., Morais, P., 2001. Effects of alterations
504 in freshwater supply on the abundance and distribution of *Engraulis encrasicolus* in the
505 Guadiana estuary and adjacent coastal areas of south Portugal. *Ecohydrology & Hydrobiology*
506 1, 341-345.

507 Cowen, R.K., Sponaugle, S., 2009. Larval Dispersal and Marine Population Connectivity. *Annual*
508 *Review of Marine Science* 1, 443-466.

509 Cushing, D., 1990. Plankton production and year-class strength in fish populations: an update
510 of the match/mismatch hypothesis. *Advances in Marine Biology* 26, 249-293.

511 Dando, P.R., 2011. Site fidelity, homing and spawning migrations of flounder *Platichthys flesus*
512 in the Tamar estuary, South West England. *Marine Ecology Progress Series* 430, 183-196.

513 Daverat, F., Morais, P., Dias, E., Babaluk, J., Martin, J., Eon, M., Fablet, R., Pecheyran, C.,
514 Antunes, C., 2012. Plasticity of European flounder life history patterns discloses alternatives to
515 catadromy. *Marine Ecology Progress Series* 465, 267-280.

516 De'ath, G., Fabricius, K.E., 2000. Classification and regression trees: a powerful yet simple
517 technique for ecological data analysis. *Ecology* 81, 3178-3192.

518 Dormann, C.F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J.R.G., Gruber,
519 B., Lafourcade, B., Leitão, P.J., 2013. Collinearity: a review of methods to deal with it and a
520 simulation study evaluating their performance. *Ecography* 36, 27-46.

521 Duffy-Anderson, J.T., Bailey, K.M., Cabral, H.N., Nakata, H., van der Veer, H.W., 2014. The
522 planktonic stages of flatfishes: physical and biological interactions in transport processes, in:
523 Gibson R.N., N.R.D.M., Geffen A.J., van der Veer H.W. (Ed.), *Flatfishes: Biology and*
524 *Exploitation*, Second Edition ed. Blackwell Oxford, London, pp. 132-170.

525 Durant, J.I.M., Hjermmann, D.Ã., Ottersen, G., Stenseth, N.C., 2007. Climate and the match or
526 mismatch between predator requirements and resource availability. *Climate Research* 33, 271-
527 283.

528 Elith, J., H. Graham, C., P. Anderson, R., Dudík, M., Ferrier, S., Guisan, A., J. Hijmans, R.,
529 Huettmann, F., R. Leathwick, J., Lehmann, A., Li, J., G. Lohmann, L., A. Loiselle, B., Manion, G.,
530 Moritz, C., Nakamura, M., Nakazawa, Y., McC. M. Overton, J., Townsend Peterson, A., J.
531 Phillips, S., Richardson, K., Scachetti-Pereira, R., E. Schapire, R., Soberón, J., Williams, S., S.
532 Wisz, M., E. Zimmermann, N., 2006. Novel methods improve prediction of species'
533 distributions from occurrence data. *Ecography* 29, 129-151.

534 Elith, J., Leathwick, J.R., Hastie, T., 2008. A working guide to boosted regression trees. *Journal*
535 *of Animal Ecology* 77, 802-813.

536 Elliott, M., Whitfield, A.K., Potter, I.C., Blaber, S.J.M., Cyrus, D.P., Nordlie, F.G., Harrison, T.D.,
537 2007. The guild approach to categorizing estuarine fish assemblages: a global review. *Fish and*
538 *Fisheries* 8, 241-268.

539 Engell-Sørensen, K., Støttrup, J.G., Holmstrup, M., 2004. Rearing of flounder (*Platichthys flesus*)
540 juveniles in semiextensive systems. *Aquaculture* 230, 475-491.

541 Falcão, A.P., Mazzolari, A., Gonçalves, A.B., Araújo, M.A.V.C., Trigo-Teixeira, A., 2013. Influence
542 of elevation modelling on hydrodynamic simulations of a tidally-dominated estuary. *Journal of*
543 *Hydrology* 497, 152-164.

544 Ferreiro, M., Labarta, U., 1988. Distribution and abundance of teleostean eggs and larvae on
545 the NW coast of Spain.

546 Fincham, J.I., Rijnsdorp, A.D., Engelhard, G.H., 2013. Shifts in the timing of spawning in sole
547 linked to warming sea temperatures. *Journal of Sea Research* 75, 69-76.

548 Fiúza, A., DeMacedo, M., Guerreiro, M., 1982. Climatological space and time-variation of the
549 Portuguese coastal upwelling. *Oceanologica Acta* 5, 31-40.

550 Fiúza, A.F.G., 1983. Upwelling patterns off Portugal, in: Suess, E., Thiede, J. (Ed.), *Coastal*
551 *Upwelling: Its Sediment Record*. Plenum, New York, pp. 85–98.

552 Forward, Jr., Reinsel, Peters, Tankersley, Churchill, Crowder, Hettler, Warlen, Green, 1999.
553 Transport of fish larvae through a tidal inlet. *Fisheries Oceanography* 8, 153-172.

554 França, S., Cabral, H.N., 2015. Predicting fish species richness in estuaries: Which modelling
555 technique to use? *Environmental Modelling & Software* 66, 17-26.

556 Freitas, V., Costa-Dias, S., Campos, J., Bio, A., Santos, P., Antunes, C., 2009. Patterns in
557 abundance and distribution of juvenile flounder, *Platichthys flesus*, in Minho estuary (NW
558 Iberian Peninsula). *Aquatic Ecology* 43, 1143-1153.

559 Friedman, J.H., 2002. Stochastic gradient boosting. *Computational Statistics & Data Analysis*
560 38, 367-378.

561 Frouin, R., Fiúza, A.F., Ambar, I., Boyd, T.J., 1990. Observations of a poleward surface current
562 off the coasts of Portugal and Spain during winter. *Journal of Geophysical Research: Oceans*
563 (1978–2012) 95, 679-691.

564 Geffen, A.J., van der Veer, H.W., Nash, R.D.M., 2007. The cost of metamorphosis in flatfishes.
565 *Journal of Sea Research* 58, 35-45.

566 Grioche, A., Harlay, X., Koubbi, P., Lago, L.F., 2000. Vertical migrations of fish larvae: Eulerian
567 and Lagrangian observations in the Eastern English Channel. *Journal of Plankton Research* 22,
568 1813-1828.

569 Grioche, A., Koubbi, P., Sautour, B., 1997. Ontogenic migration of *Pleuronectes flesus* larvae in
570 the eastern English Channel. *Journal of Fish Biology* 51, 385-396.

571 Hare, J.A., Thorrold, S.R., Walsh, H.J., Reiss, C.S., Valle-Levinson, A., Jones, C.M., 2005.
572 Biophysical mechanisms of larval fish ingress into Chesapeake Bay. *Marine Ecology Progress*
573 *Series* 303, 294-310.

574 Hastie, T., Tibshirani, R., Friedman, J., Hastie, T., Friedman, J., Tibshirani, R., 2009. The
575 elements of statistical learning. Springer-Verlag, New York. 745 pages.

576 Haynes, R., Barton, E.D., 1990. A poleward flow along the Atlantic coast of the Iberian
577 Peninsula. *Journal of Geophysical Research: Oceans* (1978–2012) 95, 11425-11441.

578 Henderson, P.A., Seaby, R.M.H., 1994. On the factors influencing juvenile flatfish abundance in
579 the lower Severn Estuary, England. *Netherlands Journal of Sea Research* 32, 321-330.

580 Hermant, M., Lobry, J., Bonhommeau, S., Poulard, J.-C., Le Pape, O., 2010. Impact of warming
581 on abundance and occurrence of flatfish populations in the Bay of Biscay (France). *Journal of*
582 *Sea Research* 64, 45-53.

583 Houde, E.D., 2008. Emerging from Hjort's shadow. *Journal of Northwest Atlantic Fishery*
584 *Science* 41, 53-70.

585 Hutchinson, S., Hawkins, L.E., 2004. The relationship between temperature and the size and
586 age of larvae and peri-metamorphic stages of *Pleuronectes flesus*. *Journal of Fish Biology* 65,
587 448-459.

588 Islam, M.S., Hibino, M., Tanaka, M., 2007. Tidal and diurnal variations in larval fish abundance
589 in an estuarine inlet in Ariake Bay, Japan: implication for selective tidal stream transport.
590 *Ecological Research* 22, 165-171.

591 Jager, Z., 1998. Accumulation of flounder larvae (*Platichthys flesus* L.) in the Dollard (Ems
592 estuary, Wadden Sea). *Journal of Sea Research* 40, 43-57.

593 Jager, Z., 1999. Selective Tidal Stream Transport of Flounder Larvae (*Platichthys flesus* L.) in the
594 Dollard (Ems Estuary). *Estuarine, Coastal and Shelf Science* 49, 347-362.

595 Jager, Z., 2001. Transport and retention of flounder larvae (*Platichthys flesus* L.) in the Dollard
596 nursery (Ems estuary). *Journal of Sea Research* 45, 153-171.

597 Koubbi, P., Loots, C., Cottonnec, G., Harlay, X., Grioche, A., Vaz, S., Walkey, M., Carpentier, A.,
598 2006. Spatial patterns and GIS habitat modelling of *Solea solea*, *Pleuronectes flesus* and
599 *Limanda limanda* fish larvae in the eastern English Channel during the spring. *Scientia Marina*,
600 147-157.

601 Last, J.M., 1978. The food of four species of pleuronectiform larvae in the eastern English
602 Channel and southern North Sea. *Marine Biology* 45, 359-368.

603 Leathwick, J., Elith, J., Francis, M., Hastie, T., Taylor, P., 2006. Variation in demersal fish species
604 richness in the oceans surrounding New Zealand: an analysis using boosted regression trees.
605 *Marine Ecology Progress Series* 321, 267-281.

606 Leis, J.M., Siebeck, U., Dixon, D.L., 2011. How Nemo Finds Home: The Neuroecology of
607 Dispersal and of Population Connectivity in Larvae of Marine Fishes. Integrative and
608 Comparative Biology, icr004.

609 Lloret, J., Palomera, I., Salat, J., Sole, I., 2004. Impact of freshwater input and wind on landings
610 of anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*) in shelf waters
611 surrounding the Ebre (Ebro) River delta (north-western Mediterranean). Fisheries
612 Oceanography 13, 102-110.

613 Martinho, F., Dolbeth, M., Viegas, I., Teixeira, C.M., Cabral, H.N., Pardal, M.A., 2009.
614 Environmental effects on the recruitment variability of nursery species. Estuarine, Coastal and
615 Shelf Science 83, 460-468.

616 Martinho, F., Leitão, R., Neto, J.M., Cabral, H., Lagardère, F., Pardal, M.A., 2008. Estuarine
617 colonization, population structure and nursery functioning for 0-group sea bass (*Dicentrarchus*
618 *labrax*), flounder (*Platichthys flesus*) and sole (*Solea solea*) in a mesotidal temperate estuary.
619 Journal of Applied Ichthyology 24, 229-237.

620 Martinho, F., van der Veer, H.W., Cabral, H.N., Pardal, M.A., 2013. Juvenile nursery
621 colonization patterns for the European flounder (*Platichthys flesus*): A latitudinal approach.
622 Journal of Sea Research 84, 61-69.

623 Martins, C.S., Hamann, M., Fiúza, A.F.G., 2002. Surface circulation in the eastern North
624 Atlantic, from drifters and altimetry. Journal of Geophysical Research: Oceans 107, 3217.

625 Miller, J.M., Burke, J.S., Fitzhugh, G.R., 1991. Early life history patterns of Atlantic North
626 American flatfish: Likely (and unlikely) factors controlling recruitment. Netherlands Journal of
627 Sea Research 27, 261-275.

628 Morais, P., Dias, E., Babaluk, J., Antunes, C., 2011. The migration patterns of the European
629 flounder *Platichthys flesus* (Linnaeus, 1758) (Pleuronectidae, Pisces) at the southern limit of its
630 distribution range: Ecological implications and fishery management. Journal of Sea Research
631 65, 235-246.

632 Otero, P., Ruiz-Villarreal, M., Peliz, A., 2008. Variability of river plumes off Northwest Iberia in
633 response to wind events. Journal of Marine Systems 72, 238-255.

634 Pawson, M.G., Pickett, G.D., Witthames, P.R., 2000. The influence of temperature on the onset
635 of first maturity in sea bass. Journal of Fish Biology 56, 319-327.

636 Peck, M.A., Huebert, K.B., Llopiz, J.K., 2012. Chapter 3 - Intrinsic and Extrinsic Factors Driving
637 Match–Mismatch Dynamics During the Early Life History of Marine Fishes, in: Guy Woodward,
638 U.J., Eoin, J.O.G. (Eds.), *Advances in Ecological Research*. Academic Press, pp. 177-302.

639 Peliz, Á., Dubert, J., Santos, A.M.P., Oliveira, P.B., Le Cann, B., 2005. Winter upper ocean
640 circulation in the Western Iberian Basin—Fronts, Eddies and Poleward Flows: an overview.
641 *Deep Sea Research Part I: Oceanographic Research Papers* 52, 621-646.

642 Potter, I.C., Tweedley, J.R., Elliott, M., Whitfield, A.K., 2015. The ways in which fish use
643 estuaries: a refinement and expansion of the guild approach. *Fish and Fisheries* 16, 230-239.

644 Queiroga, H., Cruz, T., dos Santos, A., Dubert, J., González-Gordillo, J.I., Paula, J., Peliz, Á.,
645 Santos, A.M.P., 2007. Oceanographic and behavioural processes affecting invertebrate larval
646 dispersal and supply in the western Iberia upwelling ecosystem. *Progress in Oceanography* 74,
647 174-191.

648 R Core Team (2014). R: A language and environment for statistical computing. R Foundation
649 for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>

650 Ramos, A.M., Pires, A.C., Sousa, P.M., Trigo, R.M., 2013. The use of circulation weather types
651 to predict upwelling activity along the Western Iberian Peninsula coast. *Continental Shelf*
652 *Research* 69, 38-51.

653 Ramos, S., Amorim, E., Elliott, M., Cabral, H., Bordalo, A.A., 2012. Early life stages of fishes as
654 indicators of estuarine ecosystem health. *Ecological Indicators* 19, 172-183.

655 Ramos, S., Cowen, R.K., Paris, C., Ré, P., Bordalo, A.A., 2006a. Environmental forcing and larval
656 fish assemblage dynamics in the Lima River estuary (northwest Portugal). *Journal of Plankton*
657 *Research* 28, 275-286.

658 Ramos, S., Cowen, R.K., Ré, P., Bordalo, A.A., 2006b. Temporal and spatial distributions of
659 larval fish assemblages in the Lima estuary (Portugal). *Estuarine, Coastal and Shelf Science* 66,
660 303-314.

661 Ramos, S., Ré, P., Bordalo, A.A., 2009a. Environmental control on early life stages of flatfishes
662 in the Lima Estuary (NW Portugal). *Estuarine, Coastal and Shelf Science* 83, 252-264.

663 Ramos, S., Ré, P., Bordalo, A.A., 2009b. New insights into the early life ecology of *Sardina*
664 *pilchardus* (Walbaum, 1792) in the northern Iberian Atlantic. *Scientia Marina* 73, 449-459.

665 Ramos, S., Ré, P., Bordalo, A.A., 2010. Recruitment of flatfish species to an estuarine nursery
666 habitat (Lima estuary, NW Iberian Peninsula). *Journal of Sea Research* 64, 473-486.

667 Ribeiro, A.C., Peliz, Á., Santos, A.M.P., 2005. A study of the response of chlorophyll-a biomass
668 to a winter upwelling event off Western Iberia using SeaWiFS and in situ data. *Journal of*
669 *Marine Systems* 53, 87-107.

670 Ridgeway, G., 2006. Generalized boosted regression models. Documentation on the R Package
671 'gbm', version 1 • 5 7.

672 Sabatés, A., Salat, J., Olivar, M.P., 2001. Advection of continental water as an export
673 mechanism for anchovy, *Engraulis encrasicolus*, larvae. *Scientia Marina* 65, 77-88.

674 Sale, P., Van Lavieren, H., Lagman, M.A., Atema, J., Butler, M., Fauvelot, C., Hogan, J., Jones, G.,
675 Lindeman, K., Paris, C., 2010. Preserving reef connectivity: A handbook for marine protected
676 area managers. Connectivity Working Group. Coral Reef Targeted Research & Capacity Building
677 for Management Program, UNU-INWEH.

678 Santos, A.M.P., Chícharo, A., Dos Santos, A., Moita, T., Oliveira, P.B., Peliz, Á., Ré, P., 2007.
679 Physical–biological interactions in the life history of small pelagic fish in the Western Iberia
680 Upwelling Ecosystem. *Progress in Oceanography* 74, 192-209.

681 Santos, A.M.P., de Fátima Borges, M., Groom, S., 2001. Sardine and horse mackerel
682 recruitment and upwelling off Portugal. *ICES Journal of Marine Science: Journal du Conseil* 58,
683 589-596.

684 Santos, A.M.P., Peliz, A., Dubert, J., Oliveira, P.B., Angélico, M.M., Ré, P., 2004. Impact of a
685 winter upwelling event on the distribution and transport of sardine (*Sardina pilchardus*) eggs
686 and larvae off western Iberia: a retention mechanism. *Continental Shelf Research* 24, 149-165.

687 Sims, D.W., Wearmouth, V.J., Genner, M.J., Southward, A.J., Hawkins, S.J., 2004. Low-
688 temperature-driven early spawning migration of a temperate marine fish. *Journal of Animal*
689 *Ecology* 73, 333-341.

690 Sobral, M., 2008. Aspectos relativos à biologia reprodutiva da solha, *Platichthys flesus*
691 (Linnaeus, 1758), da Ria de Aveiro e litoral adjacente. Relatórios científicos e técnicos do
692 IPIMAR, série digital, 31.

693 Soykan, C.U., Eguchi, T., Kohin, S., Dewar, H., 2014. Prediction of fishing effort distributions
694 using boosted regression trees. *Ecological Applications* 24, 71-83.

695 Sullivan, M.C., Able, K.W., Hare, J.A., Walsh, H.J., 2006. *Anguilla rostrata* glass eel ingress into
696 two, U.S. east coast estuaries: patterns, processes and implications for adult abundance.
697 *Journal of Fish Biology* 69, 1081-1101.

698 Summers, R., 1979. Life cycle and population ecology of the flounder *Platichthys flesus* (L.) in
699 the Ythan estuary, Scotland. *Journal of Natural History* 13, 703-723.

700 Teixeira, C., Gamito, R., Leitão, F., Cabral, H., Erzini, K., Costa, M., 2014. Trends in landings of
701 fish species potentially affected by climate change in Portuguese fisheries. *Regional*
702 *Environmental Change* 14, 657-669.

703 Torres, R., Barton, E.D., 2007. Onset of the Iberian upwelling along the Galician coast.
704 *Continental Shelf Research* 27, 1759-1778.

705 Van der Veer, H., 1985. Impact of coelenterate predation on larval plaice *Pleuronectes platessa*
706 and flounder *Platichthys flesus* stock in the western Wadden Sea. *Marine ecology progress*
707 *series. Oldendorf* 25, 229-238.

708 van der Veer, H.W., Bergman, M.J., Dapper, R., Witte, J., 1991. Population dynamics of an
709 intertidal 0-group flounder *Platichthys flesus* population in the western Dutch Wadden Sea.
710 *Marine Ecology Progress Series* 73.

711 Van der Veer, H.W., Geffen, A.J., Witte, J.I., 2000. Exceptionally strong year classes in plaice
712 *Pleuronectes platessa*: are they generated during the pelagic stage only, or also in the juvenile
713 stage? *Marine Ecology Progress Series* 199, 255-262.

714 Vasconcelos, R.P., Reis-Santos, P., Maia, A., Fonseca, V., França, S., Wouters, N., Costa, M.J.,
715 Cabral, H.N., 2010. Nursery use patterns of commercially important marine fish species in
716 estuarine systems along the Portuguese coast. *Estuarine, Coastal and Shelf Science* 86, 613-
717 624.

718 Vinagre, C., Santos, F.D., Cabral, H.N., Costa, M.J., 2009. Impact of climate and hydrology on
719 juvenile fish recruitment towards estuarine nursery grounds in the context of climate change.
720 *Estuarine, Coastal and Shelf Science* 85, 479-486.

721 Wolanski, E., Elliott, M., 2015. *Estuarine Ecohydrology: an introduction*. Elsevier Science,
722 Amsterdam. (due out September 2015)

723 Wooster, W., Bakun, A., McLain, D., 1976. The seasonal upwelling cycle along the eastern
724 boundary of the North Atlantic. *Journal of Marine Research* 34, 130-141.

Figure 1. Location of the Lima estuary in the NW Iberian Peninsula (Portugal): a) Lima estuary (41.68° N; 8.84° W); b) Western Iberian Peninsula, with the main oceanographic features represented (adapted from Queiroga et al., 2007 and Peliz et al., 2005). The isobaths of 200 m, 1000 m and 3000 m are presented in grey. * represents the location of the Lima estuary.

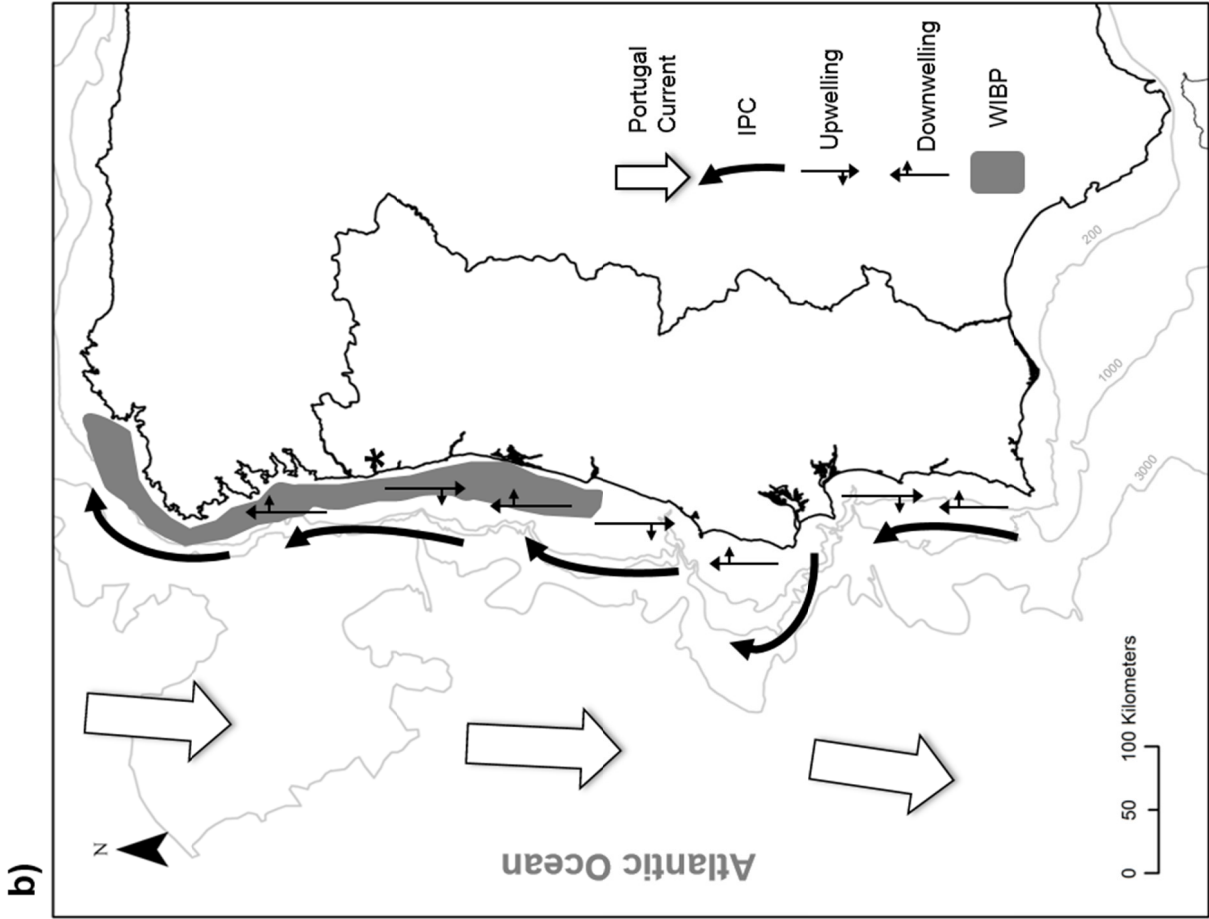
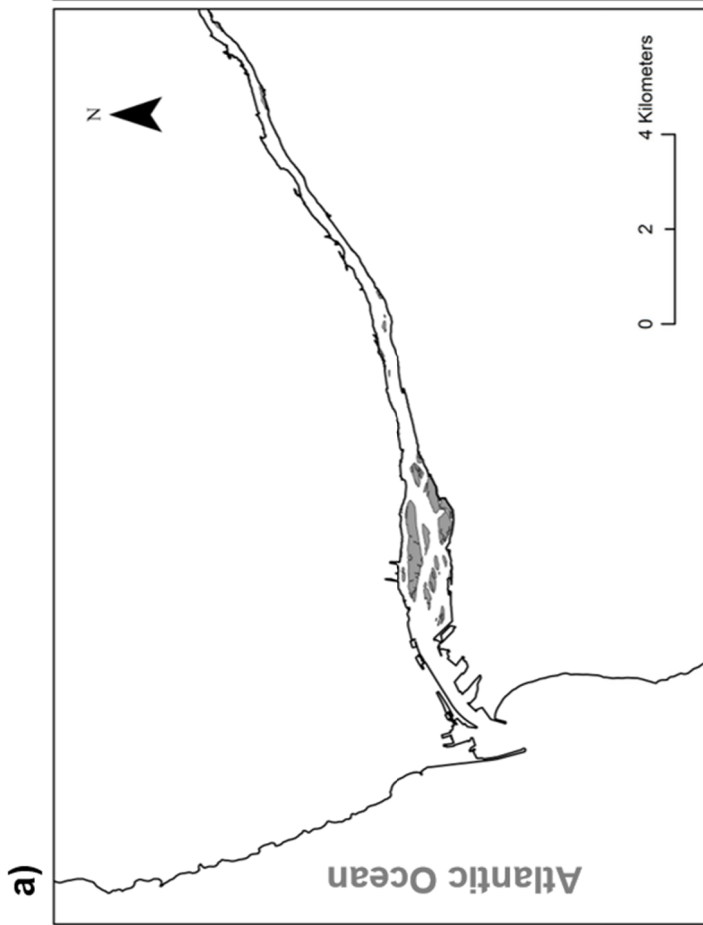


Figure 2. Temporal variation of the occurrence of the early life stages of *Platichthys flesus* in the Lima estuary for the studied years. Squares represent larvae and circles represent post-settlement individuals (<50 mm). Size is proportional to estuarine abundance. The legend represents the minimum and maximum observed abundances. Open circles represent when samples were taken but no individuals were collected.

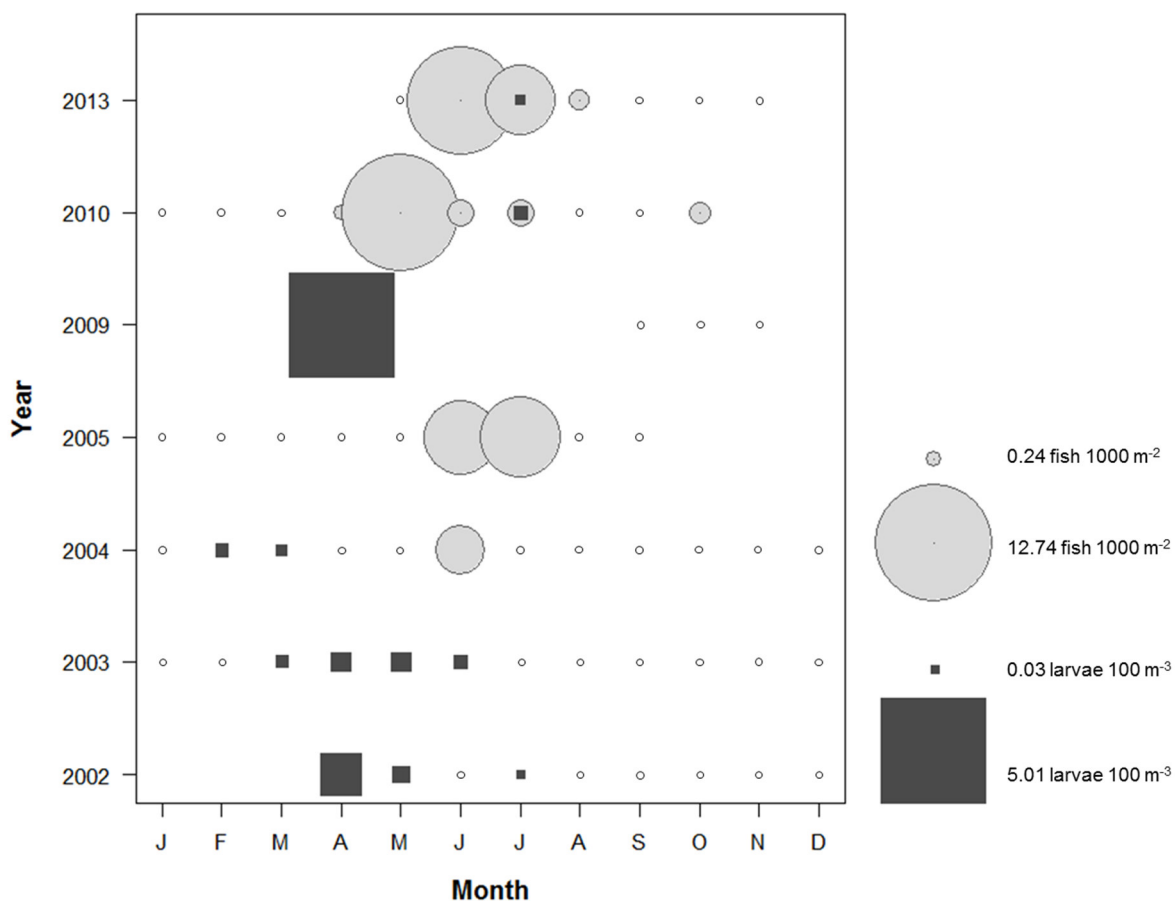


Figure 3. Monthly length frequencies of larval and post-settlement (<50 mm) *Platichthys flesus* for all the studied years.

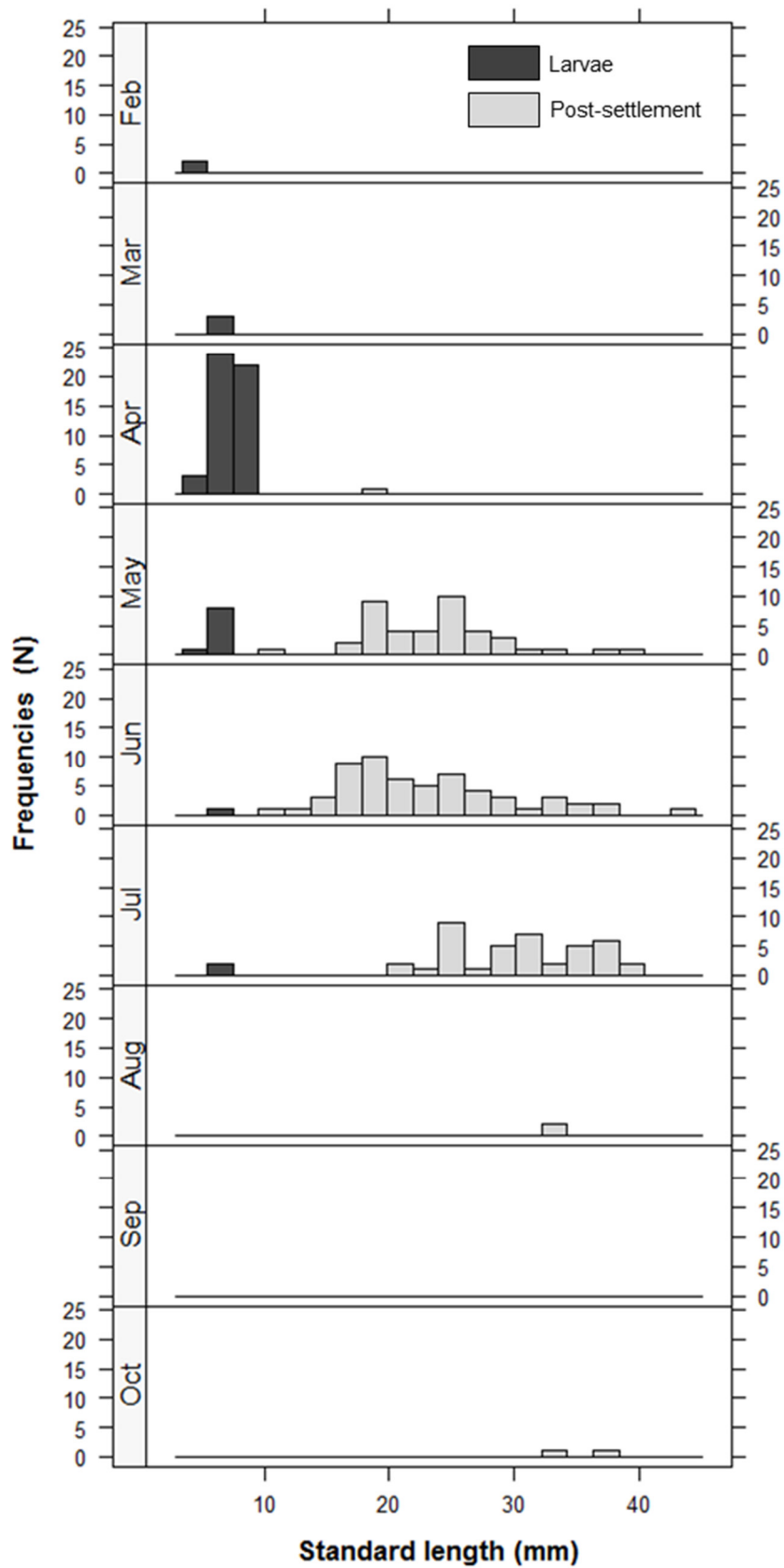


Figure 4. Map of the distribution of *Platichthys flesus* in the Lima estuary for all the studied years: a) larvae, b) post-settlement individuals (<50 mm). Dashed lines identify the limits of the different geomorphological zones: lower, middle and upper estuary.

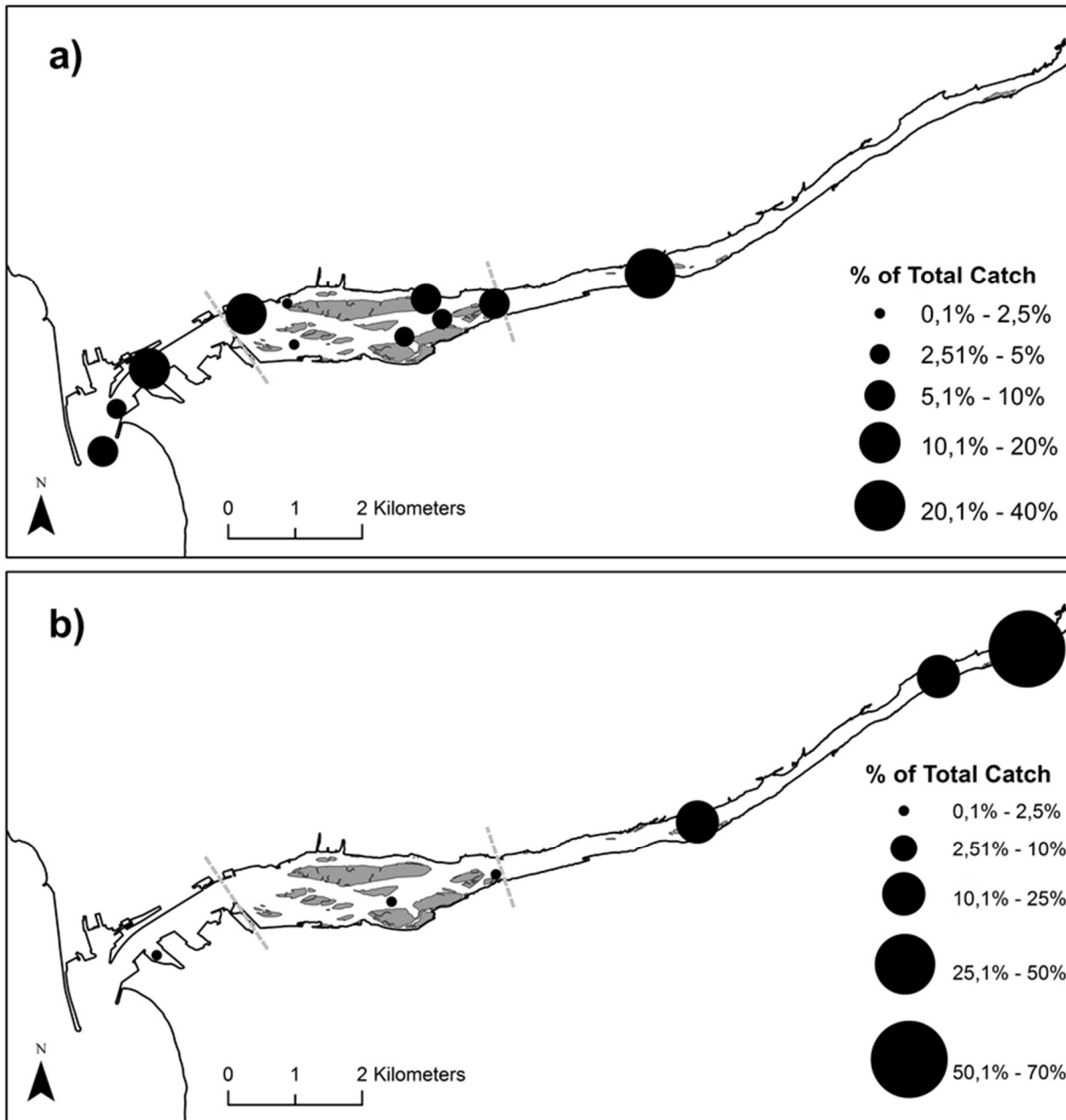


Figure 5. Monthly variation of environmental variables for the studied years: a) river flow; b) precipitation; c) sea surface temperature; d) chlorophyll *a*; e) horizontal component of the current velocity (positive and negative values indicate onshore and offshore flows, respectively); f) vertical component of the current velocity (positive and negative values indicate northward and southward flows, respectively); g) upwelling index (positive values indicate upwelling events).

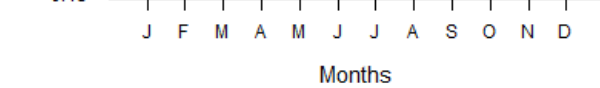
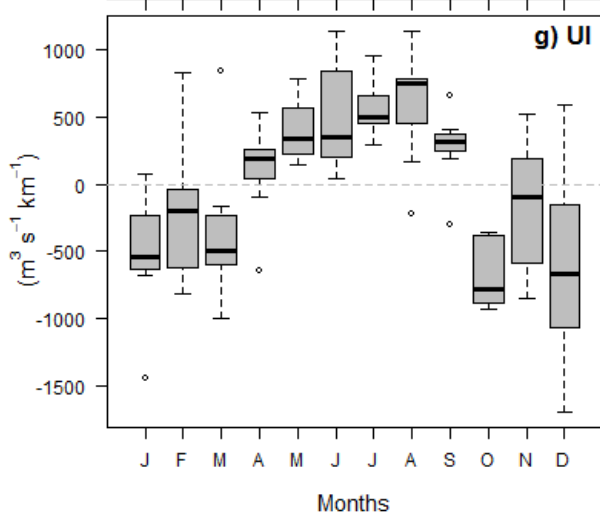
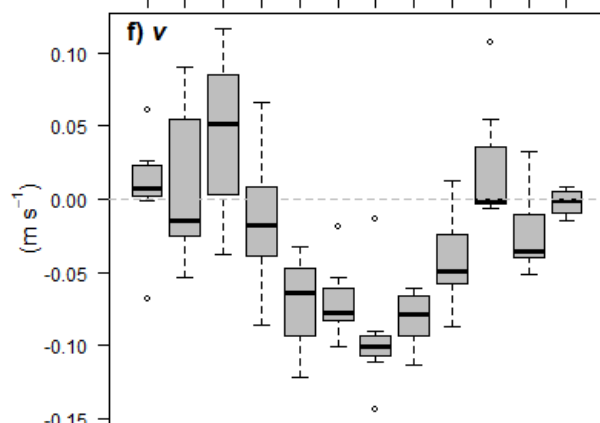
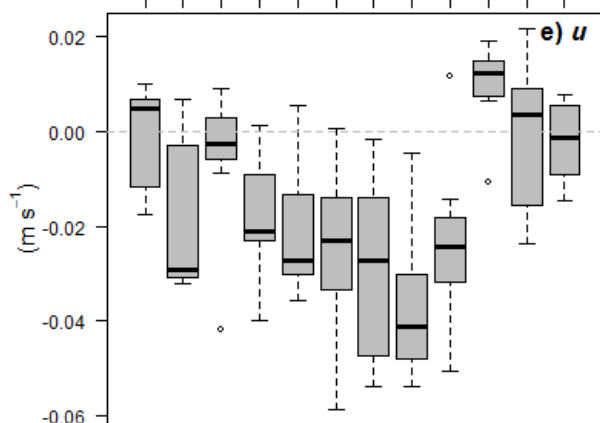
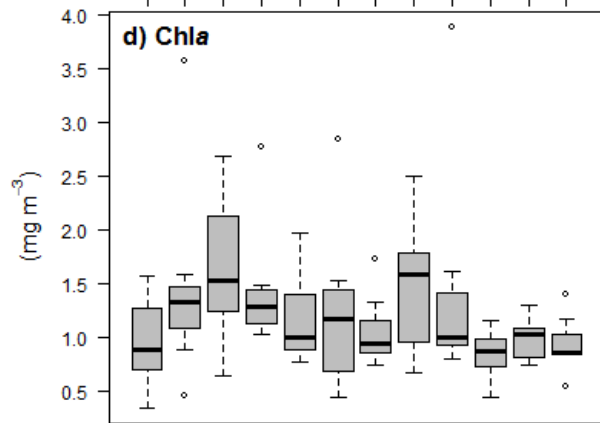
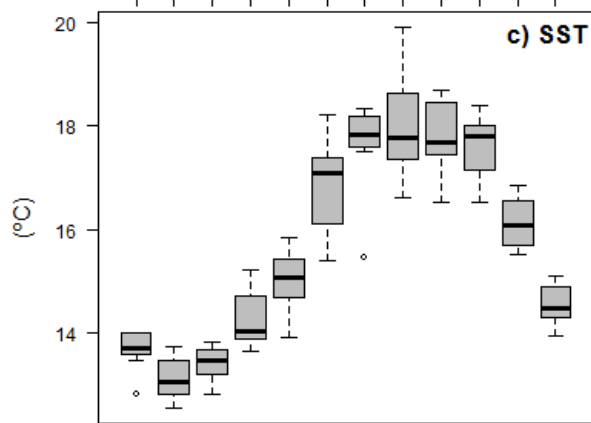
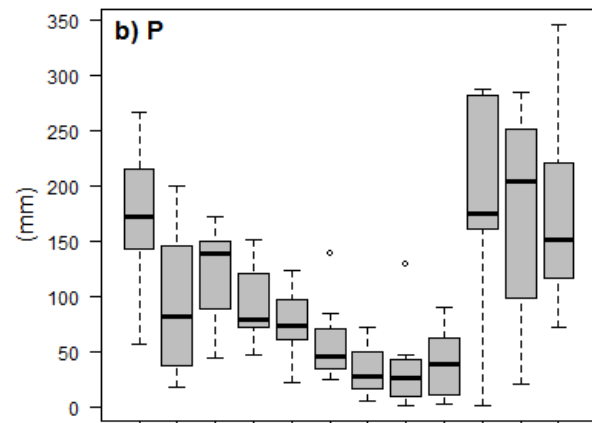
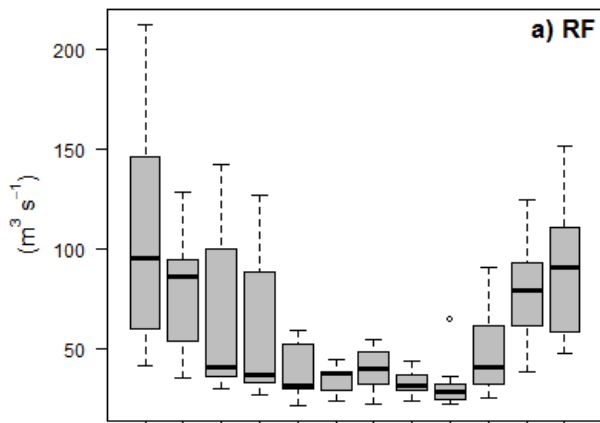


Figure 6. Partial plots of the functions fitted for the final models obtained by Boosted Regression Trees: a) larval model; b) post-settlement model. The relative contribution of each predictor variable is shown in brackets. Y axes are on the logit scale. Predictor variables are referenced with a subscript number indicating their time lag (in months) relative to the estuarine surveys. SST – sea surface temperature; Chla – chlorophyll a; P – precipitation; RF – river flow.

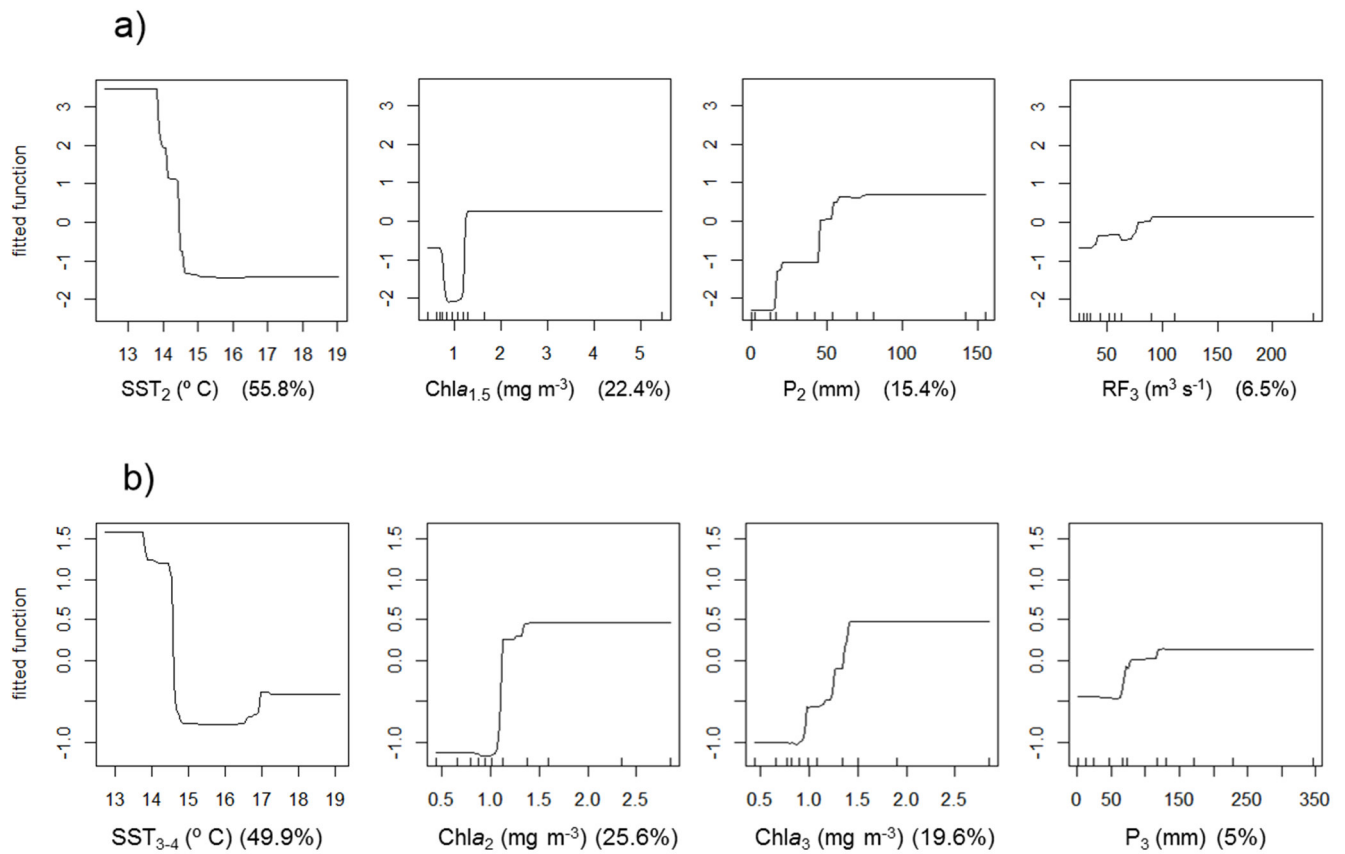


Figure 7. Three dimensional partial dependence plot for the interaction effects between SST_2 and P_2 in the larval model; all variables except those graphed are held at their means. Predictor variables are referenced with a subscript number indicating their time lag (in months) relative to the estuarine surveys. SST – sea surface temperature; P – precipitation.

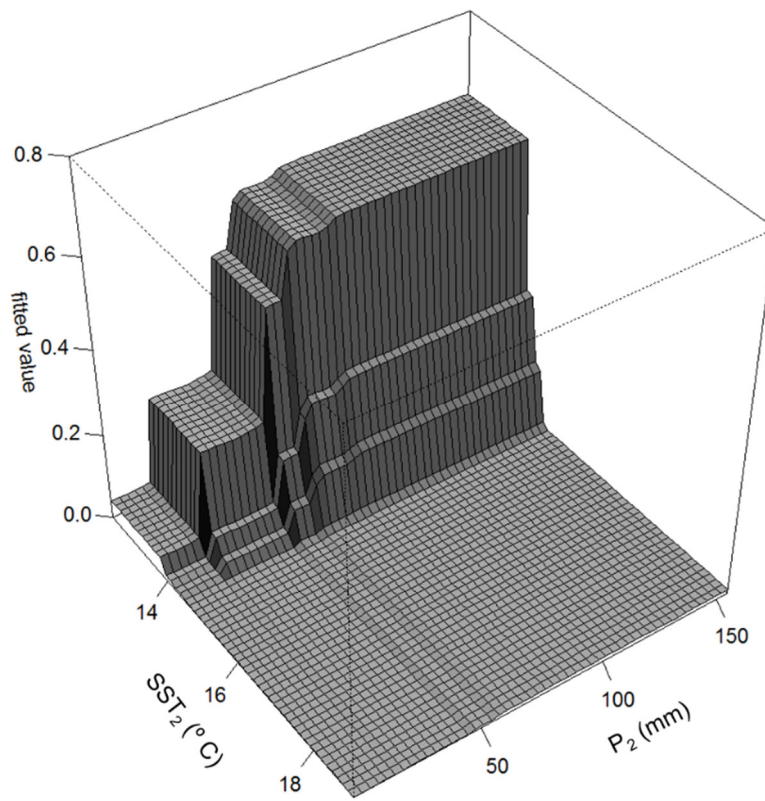


Table 1. Sampling frequency of larval and post-settlement founder (n/a: no surveys were conducted).

Years	Larvae			Post-settlement		
	Start	End	Frequency	Start	End	Frequency
2002	April	December	fortnightly	<i>n/a</i>	<i>n/a</i>	<i>n/a</i>
2003	January	December	fortnightly	October	December	monthly
2004	January	April	fortnightly	January	December	monthly
2005	<i>n/a</i>	<i>n/a</i>	<i>n/a</i>	January	September	monthly
2009	April	April	1 survey			
	September	December	monthly	September	December	monthly
2010	January	October	monthly	January	October	monthly
2013	May	November	fortnightly	May	October	monthly

Table 2. Predictive performance of the final models developed for larvae and post-settlement flounder and pairwise interactions between predictor variables. The interaction size specifies the relative degree of departure from a purely additive effect: a value of zero indicates that no interaction is present. Final settings: bag fraction - 0.75; tc – 2; no. folds – 5; lr (larvae) – 0.001; lr (post-settlement) - 0.0005.

	Larvae	Post-settlement
Number of trees	5600	6050
Deviance explained (%)	63.3	39.0
CV correlation (proportion of total)	0.804	0.713
AUC	0.958	0.907
Pairwise interactions	SST ₂ ; P ₂ - 29.00	P ₃ ; Chl _{a2} - 1.03
(Variable 1; Variable2 – Interaction size)	SST ₂ ; Chl _{a1.5} - 1.15	P ₃ ; SST ₃₋₄ - 0.25

Highlights

The estuarine recruitment of the European flounder early life stages was investigated.

The influence of oceanic physical-biological variables and estuarine dynamics was studied.

SST, ocean Chl a and precipitation were major drivers of larval occurrence in the estuary.

Oceanographic variables had no influence on larval occurrence in the estuary.