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

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Abstract

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

1. Introduction

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

The European flounder, *Platichthys flesus* (Linnaeus, 1758), is a widely-distributed flatfish along the northeastern Atlantic coast and widely regarded as having an estuarine resident or semi-catadromous life stage (Elliott et al., 2007). This species reproduces in winter/early spring in marine waters (Campos et al., 1994; Dando et al., 2011; Grioche et al., 1997; Koubi et al., 2006) and migrates during the early life stages to nursery grounds (e.g. Bos, 1999; Jager, 2001; Martinho et al., 2008; Summers, 1979). Little is known about *P. flesus* settlement patterns (Jager 2001; Bos and Thiel, 2006; van der Veer et al., 1991) and its favoured settlement habitat (estuary vs. coastal areas) (Daverat et al., 2012). In general, for flatfishes, it has been emphasized that the processes affecting recruitment variability are more relevant during the pelagic phase, although post-settlement processes seem to regulate juvenile abundance (Bolle et al., 2009; Geffen et al., 2007; van der Veer et al., 2000). However, and although *P. flesus* has been described to enter estuaries during the larval phase (Bos, 1999; Jager, 1998; Ramos et al., 2010), most studies concerning estuarine nurseries for this species are mainly focused on post-settlement juveniles (Amara et al., 2009; Cabral et al., 2007; Freitas et al., 2009; Jager, 2001;
Martinho et al., 2008; Vasconcelos et al., 2010). Consequently, the supply of *P. flesus* early-life stages to estuaries, and associated environmental constraints on recruitment variability, have been investigated mainly in juveniles. These studies show that the abundance of juvenile flounder in estuaries may be related to the average seawater temperature of the previous year (Henderson and Seaby, 1994; Vinagre et al., 2009) and with river runoff, precipitation, and wind prior to estuarine colonization (Martinho et al., 2009). Once in the estuary, environmental constraints vary between larval and juvenile phases, with *P. flesus* larvae showing a strong seasonal structure mainly regulated by biological features such as the spawning season (timing and duration), while juveniles are markedly controlled by site-specific characteristics such as sediment structure, distance from the river mouth, and salinity regime (Ramos et al., 2009a). Therefore, any natural or anthropogenic events that affect either the quality of the nursery areas, the delivery of young to those areas, or the passage of the young from the nursery areas, will affect success of the recruitment to the adult population.

Given the demonstrated effects that the pre-settlement processes may have upon year-class strength, it is important to investigate the connectivity between ocean and estuaries and the processes associated with the supply of the pelagic larvae to the primary nursery grounds (Cowen and Sponaugle, 2009). The Lima estuary, located in the North-Western (NW) Iberian Peninsula, has been identified as a nursery habitat for European flounder (Ramos et al., 2010) and because the NW Iberian Peninsula represents the southern edge of its geographical range, environmental processes become particularly relevant, as the species becomes more vulnerable to environmental fluctuations at its distributional edges (Miller et al., 1991). Therefore, this study aimed to: (i) document the patterns of the recruitment of European flounder to the Lima estuary, using pelagic-larval and post-settlement juvenile flounder data describing 7 years within a 12-year period, and (ii) investigate the associated environmental factors, following the hypothesis that estuarine recruitment at the larval phase is influenced by the physical and biological processes of ocean and estuarine dynamics.

2. **Material and Methods**

2.1 Study area

The Lima estuary (Portuguese NW Atlantic coast of the Iberian Peninsula) has a catchment of 2446 km$^2$ (Fig. 1) and a discharge regime with an annual average flow of 70 m$^3$ s$^{-1}$ mainly controlled by two hydroelectric power stations located upstream. The tidal regime, the main hydrodynamic forcing action of the estuary (Falcão et al., 2013), is mesotidal and semidiurnal with a range of 3.7 m during spring tides and an upstream influence that reaches 20 km. The
Lima estuary can be divided into three areas with distinct geomorphological features: the lower estuary (0-3 km from the mouth) is a narrow, deep and navigational channel with artificial banks; the middle estuary (3-7 km) is a broad shallow zone with salt marshes and tidal sandy islands; and the upper estuary (7-20 km) is a shallow and narrow channel with small sandy islands (Ramos et al., 2010). Despite seasonal hydrological fluctuations in the Lima estuary (Ramos et al., 2006a), during most of the year salinity ranges between 35 at the river mouth and 0 in the uppermost section of the estuary (17 km).

The adjacent coastal area is characterized by a complex current system subjected to strong seasonality and mesoscale variability, with reversing patterns between summer and winter (Ambar and Fiúza, 1994; Barton, 1998). This area is located near the northernmost limit of the North Atlantic Upwelling System region (43° N), being exposed to strong upwelling events occurring during spring and summer (Wooster et al., 1976; Fiúza et al. 1982). Other major features of the coastal circulation are the Portugal Current, the Iberian Poleward Current (IPC) and the Western Iberia Buoyant Plume (WIBP) (Fig. 1b). The Portugal Current is a broad and slow current generally flowing south and extending approximately between 10° W to 24° W (off the continental slope) (Martins et al., 2002). The IPC is a current with warm and saline waters that extends to the shelf edge and outer shelf mainly during the downwelling season in the winter (Frouin et al., 1990; Haynes and Barton, 1990; Peliz et al., 2005). The WIBP low-salinity buoyant plume is generated by the discharge of NW Iberian rivers (Peliz et al., 2005). The WIBP has a rapid response to changes in the wind conditions, and spreads offshore over the shelf extending its influence to the IPC during upwelling events (Ribeiro et al., 2005; Otero et al., 2008).

2.2 Sampling and data acquisition

*Platichthys flesus* data

Larval and juvenile flounder data were gathered over many years from the Lima estuary using consistent methodologies. Fish larvae were collected with plankton tows that were conducted in a circular path performed with a planktonic 1 m diameter, 3 m long and 500 µm mesh-size net, equipped with a Hydro-Bios (Kiel-Altenholz, Germany) flowmeter (model 438 110). Sampling effort varied between 9 to 11 tows, always covering the lower, middle, and upper sections of the estuary. The frequency of the sampling varied between fortnightly (April 2002-April 2004 (Ramos et al., 2006b, 2010) and May-October 2013) to monthly (April 2009 and September 2009-October 2010) (Table 1). Samples were fixed in 4% buffered formalin (pH 8) immediately after collection, and *P. flesus* larvae were preserved in 96% ethanol after sorting.
Notochord length was measured for pre-flexion larvae and standard length was measured for flexion and post-flexion larvae. Additionally, the ontogenic development stage of each larva was determined as: stage I – newly hatched larva; stage II - pigmented eyes, yolk sac may still be present; stage III - yolk sac totally absorbed, the urostyle flexes upward, and rays of the caudal and dorsal fins are formed; stage IV - beginning of eye migration (loss of bilateral symmetry); stage V - ending of eye migration (Ramos et al., 2010).

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

Environmental data

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

Precipitation (mm) and river flow (m³ s⁻¹) data were obtained from APA (Agência Portuguesa do Ambiente, http://snirh.pt). Ocean Chl a concentration (mg m⁻³) was obtained from the ESA Ocean Colour Climate Change Initiative (http://www.esa-oceancolour-cci.org/), which comprises merged and bias-corrected MERIS (Medium Resolution Imaging Spectrometer), MODIS (Moderate-resolution Imaging Spectroradiometer) and SeaWiFS (Sea-Viewing Wide Field-of-View Sensor) data, with a horizontal resolution of ~4 km/pixel. Chl a data for 2013...
were acquired from the MODIS-Aqua products of the NASA project Ocean Color (http://oceancolor.gsfc.nasa.gov/). Current speeds and SST were acquired from Copernicus Marine Environment Monitoring Service (http://marine.copernicus.eu/). Currents, both northward (v) and eastward velocity (u) components (m s$^{-1}$), were obtained with a horizontal resolution of 1/12°, and with two vertical levels (surface and ~1.5 m depth). SST (°C) was obtained with a horizontal resolution of 0.05°. Upwelling Index time series data were provided by the Instituto Español de Oceanografía (www.indicedeafloramiento.ieo.es) and were generated using sea level pressure of the FNMOC (http://www.usno.navy.mil/FNMOC/) WXMAP atmospheric model, at a position of 42°N latitude by 10°W longitude. The upwelling index summarises the volume of oceanwards flow of surface waters per km of coastline (m$^3$s$^{-1}$km$^{-1}$), with positive index representing upwelling events.

### 2.3 Data analysis

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

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

Two BRT models were built, one for the larval phase and the other for the post-settlement juvenile phase (<50 mm), using presence-absence data within the estuary (where pooling all sampling sites within one survey counted as an observation). The larvae dataset was composed of the fortnightly surveys conducted between 2002 and 2004, and the survey conducted in April 2009. The post-settlement juvenile dataset was composed of the monthly surveys conducted between 2003-2005, 2009-2010 and 2013. All the environmental variables were tested as predictors at different times relative to collection; the time of the collection was considered as time=0, and intervals prior to the collection were then tested (for example:
one month before, one and a half months before, etc.). These environmental variables were
referenced with a subscript number indicating their time lag relative to the estuarine surveys;
for example, \( \text{SST}_0 = \) sea surface temperature at time 0; \( \text{SST}_1 = \) sea surface temperature at time
1 month prior to the estuarine collection. For the larval data set, environmental variables were
tested up to 3 months before the larval collection (fortnightly and monthly averages). For
post-settlement data, variables were tested up to 4 months prior to post-settlement-individual
collection (monthly and bimonthly averages).

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

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

3. Results

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

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

3.2 Environmental variables

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

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

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

The horizontal (u) component of the surface current velocities increased towards offshore from January to July, with an increase in February (Fig. 5e). The vertical component (v) was predominantly towards the north during the winter months, and reversed direction in April, when it started increasing until July (Fig.5f). The upwelling index, in general, registered positive and increasing values from April until September (summer upwelling) (Fig. 5g). Although the
first trimester of each year typically registered a negative index, positive mean upwelling
indices were recorded for one or two of the months during 2005, 2009 and 2013 (winter
upwelling). The upwelling period was also identifiable as modifications of the current
behaviour observed from April to September (Fig. 5e, 5f, 5g). As a consequence of the
northerly winds, the superficial vertical component of the currents was reversed towards the
south in April (Fig. 5f), leading to an increase of the horizontal component of the currents
towards offshore due to the Ekman transport (Fig. 5e). This process caused the coastal
upwelling shown in Figure 5g.

3.3 Influence of environmental variables

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

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

Larvae were strongly influenced by the SST\textsubscript{2}, accounting for more than 55.8 % of the relative
contribution to the response variable (Fig. 6a). In general, the probability of larval occurrence
in the estuary decreased sharply with an increase in temperature (SST\textsubscript{2}) from 13.8 °C to 14.6
°C. Chlα\textsubscript{1.5} contributed 22.4 % to the presence of larvae in the estuary, with the probability of
occurrence increasing with higher concentrations, above 1.2 mg m\textsuperscript{-3}. Precipitation during the
2\textsuperscript{nd} month prior to the larval collection (P\textsubscript{2}) showed an influence of 15.4 % and had a positive
effect on the presence of larvae in the estuary. RF\textsubscript{3} was the weakest contributor (6.5%). RF
between 40 and 100 m\textsuperscript{3} s\textsuperscript{-1} increased the probability of larval occurrence (Fig. 6a). Interactions
fitted for the larval model (Table 2) indicated a weak interaction between SST\textsubscript{2} and Chlα\textsubscript{1.5}, and

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a strong interaction between SST$_2$ and P$_2$: there were peaks of occurrence when periods of
high P$_2$ and low SST$_2$ occurred simultaneously (Fig. 7).

For the post-settlement model, the most influential variable was SST, namely the average
temperature of the 3rd and 4th months prior to juvenile collection (SST$_{3-4}$), with a relative
contribution of 49.9 % (Fig. 6b). The probability of occurrence of post-settlement individuals
decreased with increases of SST$_{3-4}$. Chl$_a_2$ and Chl$_a_3$ contributed 25.6 % and 19.6 %,
respectively, to the response variable, and higher concentrations increased the probability of
post-settlement flounder occurrence in the estuary. P$_3$ had the lowest impact on post-
settlement occurrence, contributing 5 % and showing a positive influence. Interactions
indicated a peak of occurrence with higher P$_3$ and Chl$_a_2$ and with higher P$_3$ and lower SST$_{3-4}$
(Table 2).

4. Discussion

The location of the spawning grounds, jointly with transport and supply to the nursery areas of

$P. flesus$, still remain unclear at the NW Iberian coast, the species southern limit of distribution.

While the species is accepted as being semi-catadromous (Elliott et al., 2007), nearshore
spawning grounds are difficult to detect and may be relatively small (Bos et al., 1995; Grioche
et al., 1997). Spawning timing is described to occur between January and March (Sobral, 2008;
Martinho et al., 2013) with the pelagic stage lasting approximately 30 to 50 days (Martinho et
al., 2013), during which the larvae should reach the nursery areas. Our results indicate that
recruitment to the Lima estuary occurred early during the larval stages, prior to settlement.

Although larval and juvenile collections were not carried out in the same years, it was possible
to observe a synchronised pattern: the highest larval abundances were recorded in April,
followed by the presence of the newly-settled individuals (<20 mm) in the two following
months, May and June. Hence, flounder settlement seemed to have occurred inside the
estuary, in agreement with that reported for the Dollard (Ems estuary) (Jager, 1998) and for
the Elbe River (Bos, 1999). Nevertheless, fluctuations in the timing of arrival were observed,
with larvae arriving as early as February, and until July, whereas the post-settlement
individuals (<50 mm) were present between April and October.

Recent studies suggest that $P. flesus$ might not only spawn in coastal areas, but also inside the
estuary (Morais et al., 2011; Daverat et al., 2012). Ferreiro and Labarta (1988) reported a high
abundance of flounder eggs and high larval densities throughout a nearby estuary, the Ria de
Vigo. Although our study did not include the egg stages, only one pre-flexion larva (stage II)
was observed in the estuary, and most collected larvae were post-flexion (stages III and IV).
Vertical migrations appear to begin at stage III (e.g. Grioche et al., 1997, 2000; Khoubi et al., 2006). Therefore, most of the larvae caught in the Lima were already able to regulate their position in the water column, a capability which allowed them to use retention mechanisms, such as selective-tidal-stream-transport (STST). This mechanism, which enables larvae to migrate upstream the estuary and to avoid being flushed out to the ocean, has been documented for several species (e.g., Forward et al., 1999; Hare et al., 2005; Islam et al., 2007; Jager, 1999).

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

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

The successful arrival of early life stages to the estuarine nursery areas results from the interaction between several processes. By taking advantage of local physical (e.g. larval
dispersal/transport/retention) and biological (e.g. survival and growth) conditions, organisms optimize dispersal success and reduce mortality (Able and Fahay, 2010; Bailey et al., 2008; Boehlert and Mundy, 1988, Duffy-Anderson et al., 2014).

Temperature is known to have a greater effect on the larval supply to temperate estuaries (Able and Fahay, 2010), acting directly or indirectly to control biological processes (Houde, 2008). Temperature is a determinant factor at several stages of a fish life cycle, including gonadal development and spawning (Pawson et al., 2000; Fincham et al., 2013; Sims et al., 2004) and survival and growth of early life stages prior to nursery recruitment (Hutchinson and Hawkins, 2004; Henderson and Seaby, 1994; Vinagre et al., 2009). The minimum annual SST of the surveyed years coincided with the documented spawning timing (January to March).

According to the larval model, the time lag between the occurrence of flounder in the estuary and the SST variables was consistent with that time of spawning: i.e. approximately 2 months before the arrival of the larvae. As a limiting factor for reproductive success, temperature becomes a particularly important feature at the geographic limit of the flounder distribution, with regard to climate change. Teixeira et al. (2014) reported a decreasing trend in flounder fishery landings along the Portuguese coast over the past 40 years, with landings being lower in warmer years. Also, and according to historical data, the abundance decreased along the Portuguese coast (Tagus estuary) (Cabral et al., 2001; Vinagre et al. 2009) and also in the Bay of Biscay (Hermant et al., 2010).

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

Inland hydrological processes, such as precipitation regime and river flow regulate the freshwater input to estuaries and coastal areas. These variables may have a negative effect on the entrance of marine fish larvae into the estuary (Ramos et al., 2006a, 2012), and also reduce...
larval survival in the estuary (Chicharo et al., 2001). Conversely, Martinho et al. (2009) found that precipitation and river flow in the months preceding the arrival of the flounder to the estuary appear to positively influence abundance of young of the year class. In fact, continental freshwater plumes have been linked to the growth and survival of fish larvae in the sea (Sabatés et al., 2001; Lloret et al., 2004), as well as acting as a cue to estuarine location (Leis et al., 2011; Sullivan et al., 2006). Hence, an understanding of estuarine ecological processes is only possible with an adequate understanding of the interlinked physical processes, the emerging field of estuarine ecohydrology (Wolanski and Elliott, 2015). For example, along the NW Iberian coast, the nutrient-rich river discharges are responsible for the presence of the Western Iberia Buoyant Plume; which, when associated with weak winter upwelling events occurring in this area (e.g. Fiúza, 1982; Santos et al., 2004), enhance the growth and concentration of phytoplankton (Ribeiro et al. 2005). These processes have been identified as creating a suitable environment for the survival of sardine (Sardina pilchardus) larvae (Santos et al., 2004, 2007; Ramos et al., 2009b). On the other hand, the increase in intensity and frequency of upwelling events during the winter spawning season of small pelagic species off the coast of Portugal was found to have a negative impact on recruitment, limiting their survival success (Santos et al., 2001). Although winter upwelling events did not occur during the current study years, the presence of the flounder in the estuary was not influenced by upwelling or by currents acting prior to the arrival of the larvae into the estuary.

The Lima estuarine recruitment of P. flesus was related to oceanic temperature and chlorophyll a, a common feature of several marine spawning fish species. During its earlier stages, fish larvae have no or very poor swimming capabilities being more susceptible to physical transport derived from oceanographic events, such as currents, upwelling, fronts and eddies. Indeed, the dispersal of eggs and early larvae by advective processes is crucial to guarantee the successful colonization of the nursery areas (Cowen and Sponaugle, 2009; Duffy-Anderson et al., 2014). Despite all of this, our results showed that the environmental variables associated with physical processes were not considered relevant to the recruitment of flounder to the Lima estuary. However, elsewhere upwelling events have been associated with the occurrence and density of estuarine larval fish assemblages (Able and Fahay, 2010). The recruitment pulses of the English sole, an estuarine-dependent flatfish species that uses onshore Ekman transport to arrive to an estuarine zone (Boehlert and Mundy 1987), have been found to be negatively correlated with the upwelling index (Boehlert and Mundy, 1998). In the case of the NW Iberia coast, the transition from winter downwelling to summer upwelling typically occurs in April, with the persistent upwelling conditions prevailing until
September (Fiúza, 1983, Ramos et al., 2013). During our study period, the highest abundance of larvae arrived at the estuary in April, when the summer upwelling events begin to occur. This transition period is characterized by large variability in circulation (Torres and Barton, 2007), which might represent an additional constraint to flounder arrival at the Lima estuary. Given that *P. flesus* is a late winter spawning species, one would expect that eggs and early larvae were subjected to fluctuations in the transport patterns introduced by the seasonal and annual variability of local oceanographic factors. However, our results showed that the estuarine occurrence of larvae is mainly linked to variables regulating spawning, survival and growth, suggesting some ability of the larvae to cope with local oceanographic circulation. Therefore, it is necessary to locate the spawning areas in order to additionally determine how the early life stages deal with local oceanographic variation. Increased knowledge on larval behaviour and transport pathways will allow a better understanding of how deviations from usual conditions will affect the ocean-estuary connectivity and, ultimately, the southern *P. flesus* population.

5. Conclusions

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

6. Acknowledgements

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**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\textsubscript{2} and P\textsubscript{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).

<table>
<thead>
<tr>
<th>Years</th>
<th>Start</th>
<th>End</th>
<th>Frequency</th>
<th>Start</th>
<th>End</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>2002</td>
<td>April</td>
<td>December</td>
<td>fortnightly</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
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<tr>
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<td>January</td>
<td>December</td>
<td>fortnightly</td>
<td>October</td>
<td>December</td>
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<tr>
<td>2004</td>
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<tr>
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<td>n/a</td>
<td>January</td>
<td>September</td>
<td>monthly</td>
</tr>
<tr>
<td>2009</td>
<td>April</td>
<td>April</td>
<td>1 survey</td>
<td>September</td>
<td>December</td>
<td>monthly</td>
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<tr>
<td>2010</td>
<td>January</td>
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<td>January</td>
<td>October</td>
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</tr>
<tr>
<td>2013</td>
<td>May</td>
<td>November</td>
<td>fortnightly</td>
<td>May</td>
<td>October</td>
<td>monthly</td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th></th>
<th>Larvae</th>
<th>Post-settlement</th>
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<tr>
<td><strong>Number of trees</strong></td>
<td>5600</td>
<td>6050</td>
</tr>
<tr>
<td><strong>Deviance explained (%)</strong></td>
<td>63.3</td>
<td>39.0</td>
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<tr>
<td><strong>CV correlation (proportion of total)</strong></td>
<td>0.804</td>
<td>0.713</td>
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<tr>
<td><strong>AUC</strong></td>
<td>0.958</td>
<td>0.907</td>
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<tr>
<td><strong>Pairwise interactions</strong></td>
<td>SST; P2; 29.00</td>
<td>P3; Chla2; 1.03</td>
</tr>
<tr>
<td></td>
<td>SST2; Chla1.5; 1.15</td>
<td>P3; SST3.4; 0.25</td>
</tr>
</tbody>
</table>

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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 Chla and precipitation were major drivers of larval occurrence in the estuary.

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