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Larval fish dispersal along an estuarine-ocean gradient

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Abstract

The present study investigated the larval fish dispersal along an estuarine-ocean gradient to explore connectivity between ocean and estuaries. During spring 2009, a combined ocean-estuarine survey was conducted along the Lima estuarine salinity gradient and in two transects off the adjacent coast (NW Iberian Peninsula), until the 100m isobaths. Salinity, TPM, POM, TDC, DOC reached higher values at the ocean, chlorophyll *a* and nutrients increased at the estuary. From the total 56 taxa identified, 14 were present along the gradient, including estuarine species (ES), marine stragglers (MS) and migrants (MM). CCA analysis showed that species were separated along the gradient according to their ecological functional classification. MM associated with high salinity were separated from ES correlated with lower salinities and high chlorophyll *a* concentrations of inner estuary. Flounder showed a typical spatial gradient of MM, with abundance increasing from the ocean towards inner estuary. The dispersal of larvae along the Lima estuarine-ocean gradient was indicative of connectivity between habitats, emphasizing the need to consider this feature in management plans, mainly for species exploited by commercial fisheries.

Key-words: fish larvae; dispersal; estuarine-ocean gradient; nursery function

1. Introduction

Dispersal of living organisms implicates departure from the initial site, movement between sites and arrival in a new site (Clobert et al. 2009), and can be defined as the process by which living organisms expand actively or passively the space or range where they live (Cote et al. 2010). Dispersal is a fundamental life-history trait and a process fundamental to the population dynamics (Schludermann et al. 2012) of spatially structured populations (Cote et al. 2010). The exchange of individuals among geographically separated groups, or connectivity (Cowen et al. 2000) is a major driver of population replenishment (Bignami et al. 2013). Knowledge on connectivity of marine populations is fundamental to establish marine species spatio-temporal dynamics and the links between larval dispersal and supply, juvenile abundance, survival, and contribution to adult stocks (Vasconcelos et al. 2011a), has important applications for management and conservation of ecosystems (Cowen and Sponaugle 2009).

Most marine fish species experience a planktonic larval phase during which they are vulnerable to passive transport by currents or a combination of currents and swimming behavior that ends on dispersing fish larvae through long distances from the initial spawning grounds. During this dispersal phase, many environmental and biological features control larval survivorship, namely the high mortality rates typical of this early development stage (Houde 2008; Miller and Kendal 2009; Johnson et al. 2014; Garrido et al. 2015); or a successful dispersal of fish larvae to suitable nursery areas to ensure the development to the following juvenile phase (Able and Fahay 2010; Sale et al. 2010). After spawning, planktonic fish stages (eggs and larvae) may be advected and passively transported by the water currents (Wolanski 2016 and references therein) and therefore in order to reach an estuarine nursery area, the fish larvae may need directional swimming and competency to overcome coastal and tidal counter currents. (Wolanski 2016). The pelagic larval phase is dependent on biophysical characteristics related with reproduction strategies, as well as on the interactions between hydrodynamics and behavioral capabilities of individual larvae to reach and settle in favorable habitats (Cowen and Sponaugle 2009; Sale et al. 2010; Amorim et al. 2016; Wolanski 2016). Eastern boundary coastal waters are naturally highly dynamic and populated by transient structures such as river plumes, eddies and wind driven currents that contribute to a rather complex environment which could be very challenging for early life stages survival playing a role in dispersal, feeding conditions and exposure to predation (Relvas et al. 2007).

Connectivity between ocean and estuaries is vital for fish species with complex life cycles, such as migratory species and species dependent on coastal or estuarine habitats as nursery grounds (Harris et al. 2001; Elliott et al. 2007). These species, whose adults inhabit marine environments, have larvae or early juveniles that migrate to coastal or estuarine nursery grounds, where they remain until they grow to subadult stages to later join the marine adult populations (Vasconcelos et al. 2011b). Assuring a good connectivity between the different habitats that a species uses during its life-history is necessary to allow species to access resources (e.g. nursery habitat, food, protection) (Teodósio et al. 2016), promoting the resilience of that population and, consequently of the entire ecosystem (Gawarkiewicz et al. 2007; Mumby and Hastings 2008). Therefore, management strategies would benefit from considering the continuum between all the habitats used by the species, and that requires an understanding of the links between those habitats

(Wolanski 2016). In particular, considering fishes' connectivity it is essential to implement novel management strategies as the ecosystem-based fisheries management. According to this strategy, management focuses not only on the target species, but also contemplates the ecosystem (Pikitch et al. 2004), considering ecological and biological features associated with the target species as nursery grounds and temporarily fish habitats.

Larval dispersal is of crucial relevance not only to further perceive population dynamics of marine fish populations, but also to help design and implement efficient management strategies to protect fish species and marine ecosystems, nonetheless data on larval and juvenile dispersal of coastal fishes are still scarce (Di Franco et al. 2012). The comprehension of the links between estuarine and coastal environments is still a challenge, and the majority of the studies on larval dispersal are focused on marine invertebrates and coral reef species (e.g. Cowen et al. 2000; Kinlan and Gaines 2003; Shanks et al. 2003; Almany et al. 2007). Thus, the present study aims to investigate the larval fish dispersal along a temperate NE Atlantic estuarine-ocean gradient by combining simultaneous oceanic and estuarine plankton surveys to specifically: (i) characterize the spatial trends of environmental parameters and ichthyoplankton along an estuarine- ocean gradient; and (ii) investigate the influence of environmental drivers on structural and functional features of the ichthyoplankton assemblages.

2. Material and Methods

2.1 Study area

The present study investigated the dispersal of fish larvae along an estuarine-ocean gradient off the NW Iberian Peninsula, located at the northern end of the Canary Current Coastal Province. As for other eastern boundary current systems the regional oceanography is largely affected by the seasonal migration of the trade wind belt that drives the seasonal upwelling which is most intense during the summer period (Longhurst 2007). The pelagic ecosystem is therefore considerably controlled by vertical transport of nutrients into the euphotic zone. The occurrence of several estuaries and rias off the western Iberian shoreline further contributes to the region´s productivity. The region, laying in between the influence of the sub-polar and sub-tropical central waters (Mason et al. 2006), typically hosts a variety of fish species with small pelagics being very relevant. The estuaries available offer feeding opportunities and protection to offshore advection therefore are used as nursery grounds by many marine species. The present study focused on one of those estuaries, the Lima estuary, a protected area by the Habitats Directive (1992) and the EU Natura 2000 that, in spite of the major anthropogenic modifications at the outer estuary, still encompasses important intertidal saltmarsh areas and natural banks (Ramos et al. 2015) and functions as nursery area for some fish species amongst which are economically valuable resources (Ramos et al. 2010). The Lima estuary, located off the NW coast is an essential fish habitat and jointly with other estuaries in the vicinity (Cabral et al. 2007; Vasconcelos et al. 2011b) functions as nursery ground for many species, some of them with high economic importance (Ramos et al. 2010).

The temperate Lima River is an open estuary, with a semidiurnal and mesotidal regime (3.7 m), with an annual average river flow of 59 m^3s^{-1} and salt intrusion extends to 12 km upstream, with an average flushing rate of 0.5 m s^{-1} and a residence time of 9 days (Ramos et al. 2006a). The Lima estuary can be divided into three geomorphological regions: the polyhaline lower estuary, a deep dredged channel, highly urbanized and modified, sheltering a commercial harbor and a shipyard; the middle estuary, a shallow large area with several tidal islands and salt marsh; and the upper estuary, a narrow channel with natural banks and few tidal islands.

2.2 Fish larvae sampling

During the spring of 2009 (in April), the combined ocean-estuarine survey was conducted in six sampling sites along the entire salinity gradient of the Lima estuary (from the river mouth up to 10 km upstream) and in two transects off the adjacent coastal zone extending approximately 20 km offshore to the 100m depth isoline (Figure 1). In the Lima estuary, stations depth was on average 6 m, while in the ocean the average station depth was 48 m. A total of fifteen oceanic stations were occupied on the two transects: seven stations north and eight stations south of the river mouth (Figure 1), onboard IPMA´s RV, during a pelagic fish acoustics campaign. In order to sample the same water mass, the estuarine survey was performed in the following flood tide after the oceanic survey, i.e. all samples were taken in a total period of 24 hours, between 31st March and 1st April. Spring was chosen as many winter/spring marine spawning species colonize northern Portuguese estuaries during this season (Ramos et al. 2006), including important marine resources as sardine (Ramos et al. 2009) and flounder (Ramos et al. 2010).

Environmental surveying consisted of measurements of water column physical and chemical parameters, namely temperature, salinity and oxygen saturation with a YSI 6820 CTD in the estuarine stations and CTDF (salinity, temperature, depth and fluorescence) casts in the marine stations. Water samples were collected, in the estuary, with a Van Dorn bottle for further analytical determination of chlorophyll a, nutrients (nitrate, nitrite, ammonium, phosphate and silicate), total particulate matter (TPM) and particulate organic matter (POM), total dissolved carbon (TDC) and dissolved organic carbon (DOC). At sea, water samples were collected from two depths, surface and below the river plume. Water samples were transported to the laboratory in refrigerated ice chests and processed immediately.

Estuarine larval fish assemblages were collected with subsurface (1-2m depth) tows performed at a constant velocity of ca. 1 ms^{-1} for 5 min, with a 500 µm mesh size plankton net. In the coastal region, samples covering the entire water column, were obtained through oblique towing of a Bongo system with 335 µm mesh size nets. All nets were fitted with flowmeters (Hydro-Bios) for filtered water volume estimation. The volume of water filtered was on average 154 m^3 in the estuarine stations and 106 m^3 in the marine stations. All the plankton samples were immediately fixed in 4% buffered formalin (pH=8) and after sorting, fish larvae were preserved in 95% ethanol.

2.3 Laboratorial processing

All the analytical analyses of water parameters were performed in triplicate. The concentration of chlorophyll a was determined spectrophotometrically after extraction with 90% acetone (Parsons et al. 1984) with cell homogenization, using the SCOR-UNESCO (1966) trichromatic equation. Dissolved orthophosphate, nitrite, ammonium and silicate concentrations were quantified by the

Grasshoff et al. (1983) methods, and nitrate was analyzed by an adaptation of the spongy cadmium reduction technique (Jones 1984), subtracting nitrite from the total. For TPM and POM assessment, samples were previously filtered through precombusted GF/F glass-fibre filters, which were dried at 105ºC (TPM) and then incinerated at 500ºC (POM), according to APHA (1992). TDC and DOC were determined using a Shimadzu Instruments TOC-VCSN analyzer following Magalhães et al. (2008).

Fish larvae were sorted and identified to the highest possible taxonomic classification, to species level whenever possible. For the most abundant taxa, the total and standard length and the ontogenetic development stage were recorded. Abundance was standardized to the number of larvae per 100 m^3 of water filtered.

2.4 Data analyses

Environmental variables, larval fish assemblages descriptors (abundance, diversity and species richness), as well as abundance patterns of *Platichthys flesus* and *Sardina pilchardus* along the Lima estuarine-ocean gradient were mapped using ArcGIS 10.2 (ESRI, Redlands, CA). To characterize the spatial patterns of each environmental variable, continuous layer maps were created using a deterministic method, the inverse distance weighting (IDW) interpolation.

The diversity of larval fish assemblages was expressed by the Shannon-Wiener index (Shannon and Weaner 1963) and assemblage equitability was measured by Pielou's evenness index (J') (Pielou 1966). Each fish species were assigned to an ecological guild derived from estuarine use pattern, according to Franco et al. (2008): estuarine residents (ES), marine migrants (MM; spawn at sea and regularly enter estuaries in large numbers, including marine species using estuaries as nursery grounds), marine stragglers (MS; spawn at sea and enter estuaries accidentally in low numbers), freshwater species (F) and catadromous species (CA).

Differences in water and larval fish composition parameters between estuarine and marine habitats were investigated by the non-parametric test Kruskal-Wallis ANOVA analysis, with habitat (estuary/ocean) as fixed factors. The distribution of the larval fish assemblages along the environmental estuary-coastal gradient was investigated by canonical correspondence analysis (CCA) (Ter Braak 1986), using the software CANOCO (version 4.5, Microcomputer Power, Ithaca, NY). Larval abundances were transformed [log (x+1)] and downweighting of rare species was performed. Only species with frequency of occurrence higher than 1% were included in the analyses avoiding any undue effect of rare species. The option used for CCA was triplot scaling with focus on interspecies distances. Significance of the canonical model was given by a Monte Carlo test (Ter Braak and Smilaeur 2002). Inter-set correlation coefficients were used to assess the importance of the environmental variables, and when inter-set $\geq |0.4|$ variables were considered to be biologically important (Rakocinski et al. 1996). Environmental variables were added in their standardized form, namely: mean temperature and salinity of the water column; mean chlorophyll a, nitrate, nitrite, ammonium, phosphate, TPM, POM, TDC and DOC of surface and bottom samples; and depth of the water column.

3. Results

3.1 Environmental conditions

The spatial salinity pattern clearly showed the horizontal salinity gradient along the estuary, with salinity decreasing from the euhaline (>30) to the oligohaline range (<0.05) (Figure 2). Along the study area, salinity of the water column ranged between 0.3 (uppermost estuarine station) and 35.8 (marine station), significantly decreasing from the oceanic stations towards inland stations ($H=12.3$ $p<0.01$) (Table 1). In contrast, the water temperature did not vary between the ocean and estuary (Table 1), and the minimum (11.1 °C) and maximum (13.2 °C) values were both registered in the Lima estuarine stations (Figure 2). TPM ranged between 4.8 mg L^{-1} (estuarine station) and 65.8 mg L^{-1} (oceanic station) and POM varied between 2.4 mg L^{-1} (estuary) and 12.6 mg L^{-1} (ocean). Both TPM and POM reached higher concentrations in coastal northern and southern stations (Figure 2), decreasing offshore and mainly along the estuarine stations. Significantly higher concentrations of TPM ($H=11.4$ $p<0.01$) and POM ($H=7.4$ $p<0.01$) were observed at marine stations (Table 1). In the Lima estuary, higher TPM and POM concentrations were associated with the salt marsh area (Figure 2). A similar scenario was observed for the dissolved carbon (Table 1), with significantly higher TDC ($H=11.4$ $p<0.01$) concentration at oceanic stations, mainly at the most offshore stations (Figure 2). Although the organic fraction of dissolved carbon was also more concentrated at the most offshore stations (Figure 2), DOC reached significantly higher concentration in the Lima estuary ($H=5.1$ $p<0.05$) (Table 1). In the Lima estuary chlorophyll a significantly decreased from the estuarine stations towards offshore ($H=12.3$ $p<0.01$) (Figure 3). In fact, in the Lima estuary chlorophyll a ranged between 2.3-3.8 mgL^{-1} in comparison with marine stations that in average registered a chlorophyll a concentration of 0.7± 0.2 mgL^{-1}. Nutrients concentration also differed along the estuarine-ocean gradient (Figure 3). In average there were higher nutrients concentrations in the Lima estuary (Table 1), mainly nitrates ($H=11.4$ $p<0.01$), nitrites ($H=3.9$ $p<0.05$) and silica ($H=11.4$ $p<0.01$).

3.2 Larval fish assemblages

A total of 1226 fish larvae collected during the study corresponding to 56 taxa identified, from which 16 taxa were collected within the Lima estuary and 54 at the oceanic stations (Table A-supplementary data). A total of 14 taxa were spread along the estuarine-oceanic gradient (Table 2). There was a tendency for these common species to reach higher abundances at the ocean, namely Clupeidae ni (ni – not identified) that was significantly more abundant at the ocean than at Lima estuary ($H= 10.7$ $p<0.01$). In contrast, the common goby *Pomatoschistus microps* and flounder *Platichthys flesus* were significantly more abundant at the Lima estuary ($H= 8.6$ $p<0.01$; $H= 9.7$ $p<0.01$, respectively).

The total larval fish abundance varied along the gradient (Figure 4), increasing from the upper estuary towards offshore. Fish larvae were significantly ($H= 7.0$ $p<0.01$) more abundant at the ocean (Table 2), where abundance varied from a minimum of 21.7 larvae 100 m^{-3} at the northernmost coastal station until a maximum of 196.3 larvae 100 m^{-3} observed at the southern (Figure 4). Such high abundances observed closely to the Lima river mouth (Figure 4), were mainly composed by Clupeiforms (34%) and Labridae (28%). In the Lima estuary, the larval fish assemblage ranged between 6.1-58.5 fish larvae 100 m^{-3}, and the highest abundances were observed in salt marsh area (Figure 4) and were dominated by *P. microps*.

The larval fish assemblages showed a tendency to include more species and became more diverse from the upstream estuarine stations towards offshore (Figure 3). In fact, the Shannon Wienner index as well as the species richness reached significantly higher values at marine stations than in the Lima estuary ($H= 10.7$ $p<0.01$; $H= 8.9$ $p<0.01$, respectively) (Table 2).

From the 56 taxa identified, only six taxa were not assigned to an ecological guild, and 48% of the taxa were classified as MS, 23% as MM and 18% as ES. The coastal larval fish assemblages included five ecological guilds, but only three were observed in the Lima estuary, namely MS, MM and ES, whose relative abundance varied between the Lima estuary and the sea (Figure 5). The spatial distribution of each of these functional groups showed that estuarine species (ES) were more abundant within the Lima estuary, mainly in the saltmarsh zone (Figure 6), representing more than 75% of the assemblage. In fact, this group of species were significantly more abundant at the Lima estuary than in the marine stations ($H= 9.7$ $p<0.05$). In contrast, marine straggler species (MS) that were only observed in the lower section of the Lima estuary (Figure 6) reached significantly higher abundances in marine stations ($H= 5.5$ $p<0.01$).

The spatial distribution of estuarine dependent species (MM) showed that although these species occurred along the gradient without significant differences between marine and estuarine stations ($H=0.55$ $p>0.05$), they tended to concentrate in the middle and upper sections of the Lima estuary (Figure 6). Focusing on the most abundant MM species that occurred along the gradient, the spatial distribution showed that flounder abundance, gradually increased from offshore towards the upper estuary (Figure 7), where flounder reached significantly higher abundances ($H= 9.7$ $p<0.01$), overreaching 25 larvae $100m^{-3}$. On the other hand, sardines, *Sardina pilchardus*, were the second most abundant MM species, and they were more abundant at the marine stations, and were only present in small numbers in the lower sections of the Lima estuary (Figure 7).

3.3 Environmental influence

Canonical correspondence analysis showed that species were distributed along the first two CCA axes. The first CCA axis (eigenvalue = 0.6) and the second CCA axis (eigenvalue = 0.4) exhibited a high species–environment correlation (0.9) and the effect of the environmental variables on explained distribution of the CCA axes was significant (F= 1.5 p< 0.01, Monte Carlo permutation test). According to the inter-set correlation coefficients chlorophyll a, nitrates and DOC were positively related with first CCA axis, while depth, salinity, TPM and TDC were negatively correlated with the first CCA axis (Table 3). Samples clustered according to their origin, with estuarine and oceanic samples being separated along the first CCA axis. Estuarine samples with higher concentrations of chlorophyll a, nitrates and DOC clustered on the positive side of the ordination plot, while oceanic samples characterized by high salinity and TPM and TDC concentrations clustered on the negative side of first CCA axis (Figure 8a). Oceanic samples were separated along the second CCA axis that was negatively correlated with depth of the water column (Table 3). In fact, samples with less than 50 m depth clustered on the positive part of second CCA axis, while deeper samples located offshore of the 50 m isobaths were associated with the negative part of the second CCA axis (Figure 8a). The species classification in ecological guilds showed that functional groups were distributed along the first CCA axis, with MS tending to cluster in the negative part of first CCA axis, associated with high salinity. In contrast, ES showed a wider distribution along the estuarine-ocean gradient (Figure 8b) and were associated with lower salinities and high Chlorophyll a and nitrates concentrations. MM species occurred in between these two functional groups along the estuarine gradient.

4. Discussion

4.1 Larval fish dispersal according to species functional traits

The present study showed for the first time the dispersal of larval fish assemblages along the Lima estuarine-ocean gradient. The coordinated plankton collection in the ocean and estuary allowed to verify a mixture of estuarine and marine species occurring along a gradient of 30 km from the 100m isobaths offshore until the upper section of the Lima estuary. The species collected in this study are frequently observed in planktonic studies of the region, and the abundances registered were within the range of previous studies for the same time of the year (e.g. Azeiteiro et al. 2006; Ramos et al. 2006a; Garrido et al. 2009). These evidences support the representativeness of the data collected during this study and constitutes valuable baseline information to help understanding the connectivity between the ocean and the Lima estuary.

A major finding of the present study was to show that species distribution along the Lima estuarine-ocean gradient were in accordance with their ecological traits relative to species use of estuarine environments. Overall, each ecological guild group exhibited the expected spatial distribution along the gradient: estuarine species (ES) were more abundant in the Lima estuary, mainly in the salt marsh zone, while marine stragglers (MS) were associated with the ocean and restricted to the lower section of the Lima estuary, and finally marine migrants (MM) were spread along the gradient, with higher abundances in the middle and upper sections of the Lima estuary. The ecological guild classification is based on all life-cycle of the species (Elliott et al. 2007), and this study emphasized the importance of early larval stages for the determination of the species traits. One example was the European flounder *P. flesus*, whose larvae presented a typical spatial gradient of a marine migrant species, since its abundance gradually increased from offshore (spawning areas) towards the upper estuary where abundance peaked. This species, a typical user of coastal/estuarine nursery areas (Elliott et al. 2007), reproduces in winter/early spring in marine waters (e.g. Campos et al. 1994; Dando et al. 2011; Koubbi et al. 2006) and migrates during the early life stages to nursery grounds (e.g. Jager 2001; Martinho et al. 2008). The spatial pattern of flounder larvae observed in this study (i.e. abundance increasing from offshore towards the estuary) was in accordance with the previous studies that proposed the Lima estuary as a nursery area (Ramos et al. 2010; Amorim et al. 2016). According to those studies, *P. flesus* recruitment to estuary occurs early during the larval phase, with larvae migrating from the offshore spawning grounds to the estuarine nursery area. The present results further reinforce the evidence of connectivity between the ocean and the Lima estuary for a marine migrant species as *P. flesus*.

On the other hand, sardine *S. pilchardus* larvae were more abundant at the sea and were only present in small numbers in the lower sections of the estuary. Such spatial distribution is typical of marine stragglers, although *S. pilchardus* was classified as marine migrant species, in accordance with the classification proposed by Franco et al. (2008) and also corroborating previous studies in the Lima estuary during which high abundances of *S. pilchardus* larvae were observed in the inner sections of the estuary (Ramos et al. 2009). European sardine larvae tend to dominate the ichthyoplankton community in the Western Iberian upwelling ecosystem, particularly during colder months of the year (Garrido et al. 2009) and are thought to be limited to coastal areas (e.g. John et al. 1996; Chícharo et al. 1998; Olivar et al. 2003; Santos et al, 2004), and that is in agreement with the results from this work, since higher *S. pilchardus* abundance were observed at the oceanic stations. However, the observed abundances within the estuarine stations (0.5-3.2 sardine larvae 100 m^{-3}) were lower than those found in prior studies in the Lima estuary, where *S. pilchardus* larval abundance reached 60.8 larvae 100 m^{-3} (Ramos et al. 2006a; 2009). The comparatively lower abundances of the sardine larvae observed during this work (also quite restricted in time) might reflect the inter-annual variability of estuarine recruitment, derived from variability in the sardine densities (Massé et al. 2016) and inter-annual variation of oceanographic and estuarine hydrological conditions (Ramos et al. 2009; Amorim et al. 2016). This study gives support to the need of further research in understanding the sardine early life history and ascertaining the importance of estuarine habitats for this pelagic species.

4.2 ***Environmental drivers of larval dispersal***

The spatial distribution of the environmental variables showed that the study area covered two distinct water masses, and some variables varied greatly along the estuarine-ocean gradient, namely S, Chla, TPM, POM, TDC, DOC, $NO_{3,}$ NO_2 and Si. The Lima estuary was characterized by lower salinity and higher concentrations of chlorophyll *a*, nitrates, nitrites and silica, typical features for the time of the year (April) (Ramos et al. 2006b; Amorim et al. 2016). At the oceanic stations, temperature and salinity values (Massé et al. 2016) and chlorophyll *a* and nutrients concentrations (Moita 2001; Cabrita et al. 2015) were within the ranges commonly observed in the region during spring. Coastal salinity was considerably higher, as expected and presented higher concentrations of particulate matter, including the organic fraction and total carbon. The present study showed that the adjacent northern coastal stations presented higher values of particulate matter (TPM and POM), what is an unusual pattern, since estuaries are typically more turbid than coasts. However, the Lima estuary is characterized by clear waters with reduced turbidity levels (Ramos et al. 2006b; Ramos et al. 2009). Also, the observed higher TPM and POM concentrations in the northern adjacent coast may be associated with the presence of several small estuaries located northerly of the Lima river mouth, whose run-off is advected southwards due to the prevailing northern-southern currents (Amorim et al. 2016). The water characteristics varied less at the ocean in comparison with estuarine stations. Estuaries are interface ecosystems functioning as boundaries between rivers and the ocean, where abrupt changes in salinity, temperature, oxygen and turbidity occur due to the influence of tides and the mixing of marine and fresh waters (e.g. Elliott and Wollanski 2015). In this study, the extreme and steep gradients observed in many physical and chemical variables were derived from the mixing of the oceanic water mass with the freshwater inflow, since the sampling survey was conducted during the flood tide. Not many species can cope with the physiological stress induced by the environmental variability of estuarine habitats (Elliott and Hemingway 1995; Elliott et al. 2007), and as result, estuaries are characterized by comprising less species than the adjacent coastal areas. In fact, our results illustrated this feature, since the species richness and assemblage diversity were lower in the estuary in comparison with the oceanic stations.

Larval fish dispersal contemplates passive and active transport mechanisms (e.g. Harris et al. 2001; Schulderman et al. 2012), controlled by hydrodynamic conditions and by water characteristics as temperature, salinity, turbidity (e.g. Grouthes and Cowen 1999; Harris et al. 2001; Santos et al. 2004; Ramos et al. 2006b; Amorim et al. 2016). According to the canonical correspondence analysis results, salinity, chlorophyll a, nitrates, and depth were the most relevant environmental variables correlated with the larval fish assemblages of the Lima estuarine-oceanic gradient. In fact, these water parameters have been usually associated with the occurrence of abundance fluctuations of larval stages of fishes. Salinity and depth (which also reflect location) have been widely identified as important environmental drivers of larval fish assemblages (Harris et al. 2001; Ramos et al. 2006b and references therein), controlling the species composition of ichthyoplankton assemblages in function of the species tolerance to salinity gradients. Chlorophyll *a* has also been identified as an important environmental control of larval fish assemblages, since spring peaks of chlorophyll *a* derived from phytoplankton blooms have been associated with estuarine peaks of larval fish abundance (e.g. Livingston et al. 1997; Garcia et al. 2003; Amorim et al. 2016). In fact, some authors consider this synchronization as a strategy following the 'match-mismatch' hypothesis (Cushing 1990), according to which the temporal and spatial overlap between peaks in food resources (e.g., phytoplankton and subsequently zooplankton) and larval abundance regulates survival of larval fishes and subsequent recruitment (Cushing 1990; Chick and Van Den Avyle 1999).

The first canonical axis, which represented the spatial Lima estuarine–ocean gradient, separated typical marine species associated with high salinity from estuarine resident species as *P. microps* and *P. minutus* and estuarine-dependent species as *P. flesus*. Interestingly, species were more or less separated along the spatial gradient accordingly to their ecological functional classification. Results showed that MS species were positively correlated with salinity and were associated with marine stations. On the other hand, ES species showed a wider distribution and were associated with lower salinities and high chlorophyll a concentrations of the inner Lima estuarine stations. ES were more abundant in the Lima estuary, mainly in the salt marsh zone, where species like *P. microps* and *P. minutus* tend to concentrate (Ramos et al. 2006a; and data not published). Marine migrant species were distributed along the estuarine-ocean gradient, with some species positively correlated with salinity as sea bass *Dicentrarchus labrax*. Others as *P. flesus* were negatively correlated with salinity and associated with high concentrations of chlorophyll a. Actually, chlorophyll *a* has been identified as a major environmental driver of the occurrence of *P. flesus* larvae in the Lima estuary (Amorim et al. 2016). The second canonical axis was negatively correlated with depth, and represented a second environmental gradient separating shallow coastal stations from deep offshore stations. Species were also separated along this coastal-offshore gradient, and species like *Centrolabrus exoletus, Labrus merula,* and *Lipophrys trigloides* were negatively correlated with depth, since they are typical coastal species associated with shallow habitats (Whitehead et al. 1984). In contrast, larval stages of demersal species as *Ciliata mustela* or bathypelagic species as *Micromessistius poutassou* were positively correlated with depth and clustered associated with the deepest stations. Hence, the results of this study clearly showed the importance of the water characteristics in controlling the spatial patterns and dispersal of the larval fish species along an estuarine-oceanic gradient.

4.3 Importance of larval fish dispersal and connectivity to management

Processes occurring during the pelagic larval phase of fish life are well acknowledged to influence the spatial distribution of fish populations (e.g. McGilliard and Hilborn 2008; Schludermann et al. 2012), and ultimately the strength of annual recruitment (Cowen and Sponaugle 2009; Vasconcelos et al. 2011b) and abundance of adult populations (Able and Fahay 2010). Connectivity between marine and estuarine environments is fundamental for several fish species (Cowen and Sponaugle 2009), in some particular phase of their life cycle (Elliott et al. 2007; Franco et al. 2008). Larval dispersal is then essential to marine species to reach suitable coastal/estuarine nursery areas, where early development stages of marine fishes can growth faster and thus increasing their probability of survivorship before joining the adult populations. The results of this study showed that larval stages of species commercially exploited, as sardine and flounder, were dispersed along the Lima estuary-ocean corridor, indicative of the connectivity between the habitats. Particularly for these species is mandatory that human activities do not compromise the connectivity between ocean and estuarine habitats, what could pose additional pressures to the stocks. Thus, larval dispersal and connectivity with nursery areas should not be forgotten in management plans and the scientific research needs to continue increasing our understanding of the population’s movements which then will help in the conservation and preservation of the marine ecosystems. Knowing that larval fish dispersal is fundamental to the efficiency of governance practices as MPA (McGilliard and Hilborn 2008; Di Franco et al. 2012), the present study contributed to give empirical evidences of estuarine-ocean connectivity and, in the future it will be interesting to integrate estuarine stations in the current stock monitoring plans for some fisheries. Given that the Atlanto-Iberian sardine stock has reached historically minimum values of population abundance and recruitment strength (ICES 2015; Massé et al. 2016), the relevance of studies as the present one is important to foster comprehensive understanding of estuarine-ocean connectivity (and should be replicated in other larger estuaries), what has been acknowledged as having important applications for management and conservation of ecosystems (Cowen and Sponaugle, 2009).

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Figures Captations

Figure 1. Location of the sampling stations of the northern (⦿) and southern (●) transects, and estuarine (★) stations along the Lima estuary-ocean gradient.

Figure 2. Spatial variation of a) salinity (psu); b) temperature (ºC); c) total particulate matter (TPM: mg L^{-1}); d) particulate organic matter (POM: mg L^{-1}); e) total dissolved carbon (TDC: mg L^{-1}); and f) dissolved organic carbon (DOC: mg L^{-1}) along the Lima estuarine-ocean gradient in April 2009.

Figure 3. Spatial variation of a) chlorophyll a (mg m^{-3}); and nutrients (µM L^{-1}) (b) NH_4- ammonium; c) NO_3- nitrates; d) NO_2-nitrites; e) PO_4- phosphates; f) Si- silica) concentrations along the Lima estuarine-ocean gradient in April 2009.

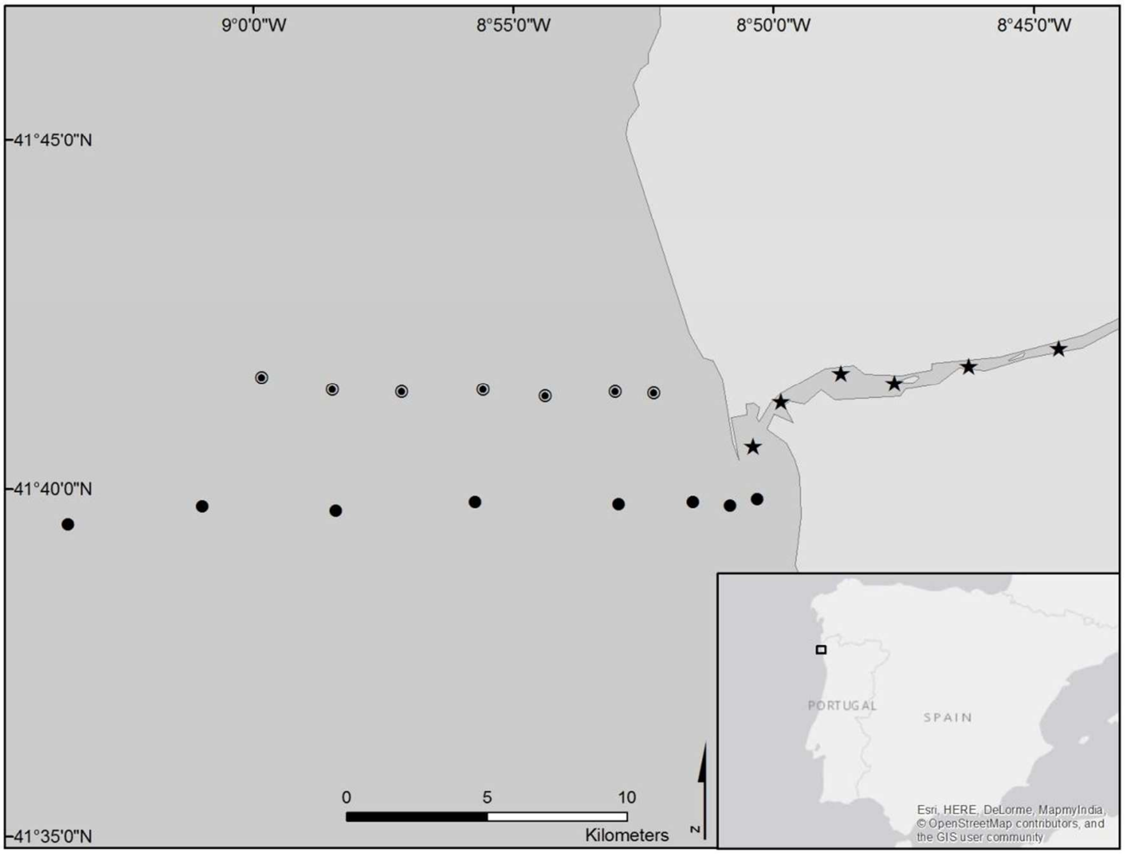
Figure 4. Spatial variation of a) larval fish abundances (no. larvae 100 m^{-3}), b) diversity (H') and c) species richness (no. species) along the Lima estuarine-ocean gradient in April 2009.

Figure 5. Relative abundance (%) of each ecological guilds of the Lima estuary (estuary) and marine larval fish assemblages, considering all species collected. ES- estuarine residents; MM-marine migrants; MS marine stragglers; other (species without an ecological guild assigned).

Figure 6. Spatial variation of the relative abundance (in %) of each functional groups of the larval fish assemblages along the Lima estuarine-ocean gradient in April 2009. ES- estuarine residents; MS marine stragglers; and MM-marine migrants.

Figure 7. Spatial variation of flounder (*Platichthys flesus*) and sardine (*Sardina pilchardus*) larval fish abundance (no. larvae 100 m^{-3}) along the Lima estuarine-ocean gradient in April 2009.

Figure 8. Ordination diagrams for the first two canonical correspondence axes of the canonical correspondence analysis: a) triplot between larval fish species, environmental variables and sampling stations (blue-ocean; green-estuarine); and b) biplot between environmental variables and larval fish species classified accordingly to their ecological guild classification in terms of estuarine use (green-estuarine species (ES); blue-marine stragglers (MS); and yellow- marine migrant species (MM). S- sanility; T- temperature; Depth-depth of the water column; TPM- total particulate matter; POM- particulates organic matter; TDC- total dissolved carbon; DOC- dissolved organic carbon (DOC); NH_4- ammonium; NO_3- nitrates; NO_2-nitrites; PO_4- phosphates; Chla- chlorophyll a concentration. For species codes please see Table 2 and Table A-supplementary data.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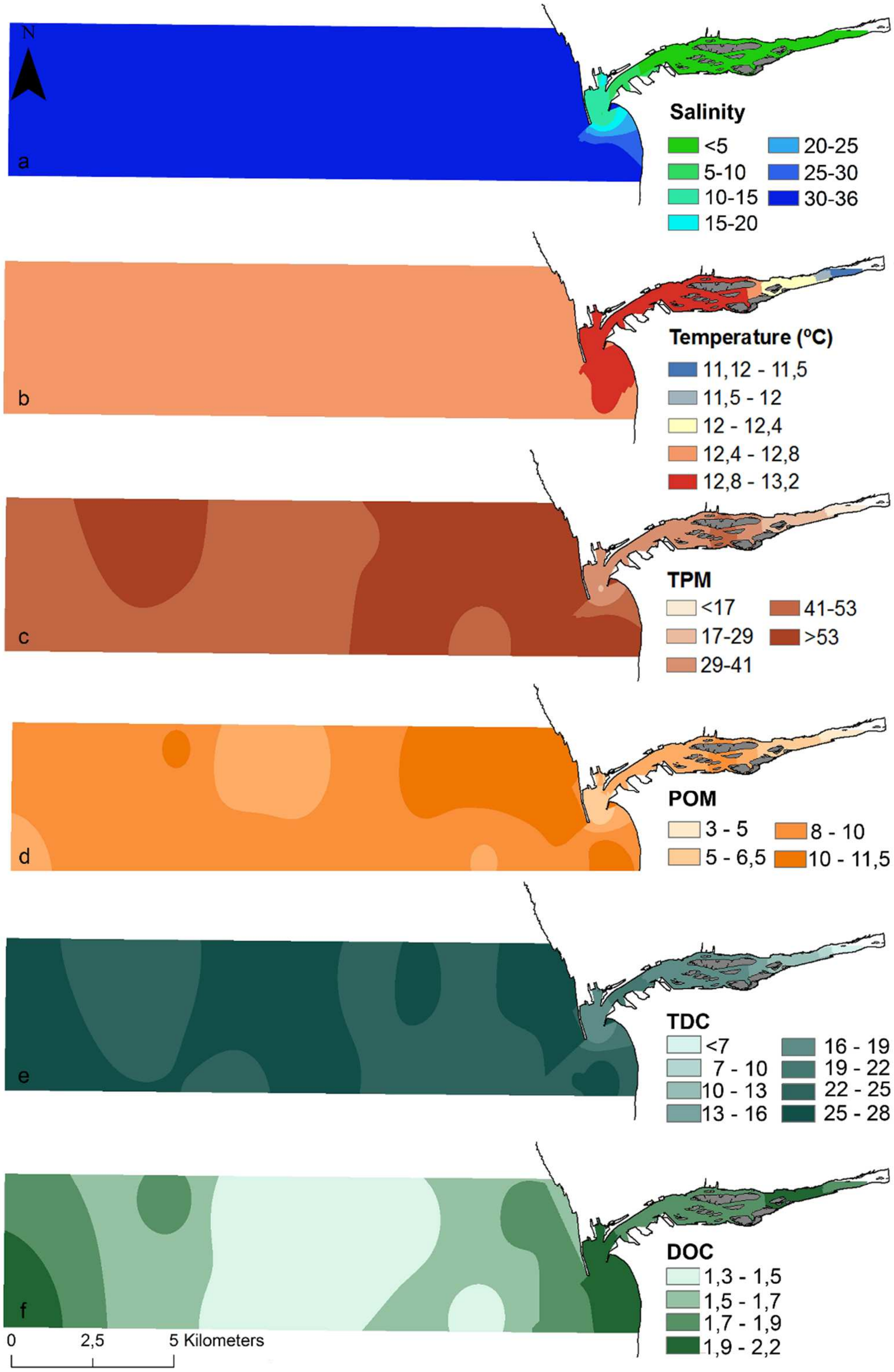
Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community

Figure 1.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30-36
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Temperature (ºC)
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12 - 12,4
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12,8 - 13,2
c
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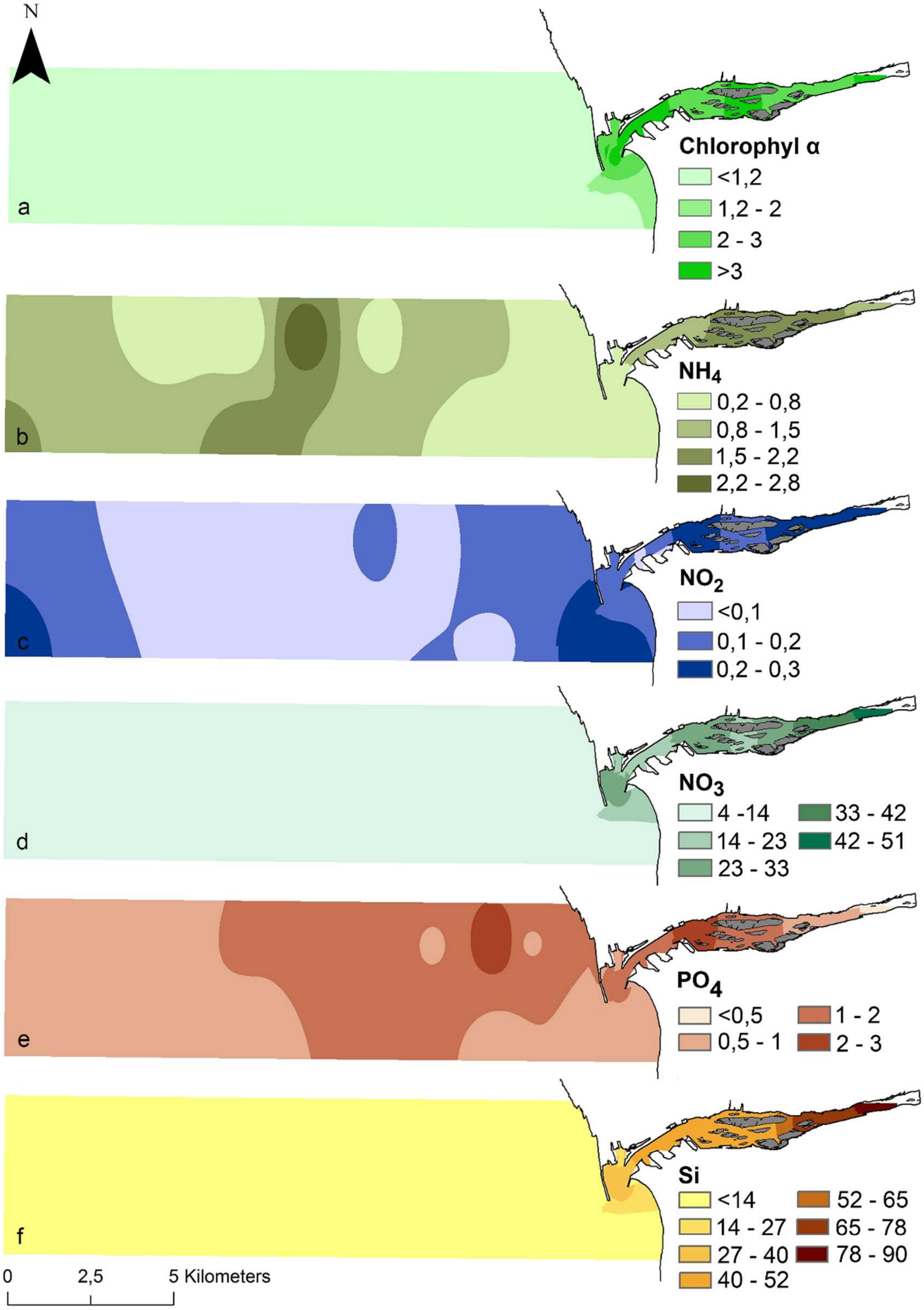
Figure 2.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2,2 - 2,8
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NO_2
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0,2 - 0,3
d
NO_3
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14 - 23
23 - 33
33 - 42
42 - 51
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Figure 3.

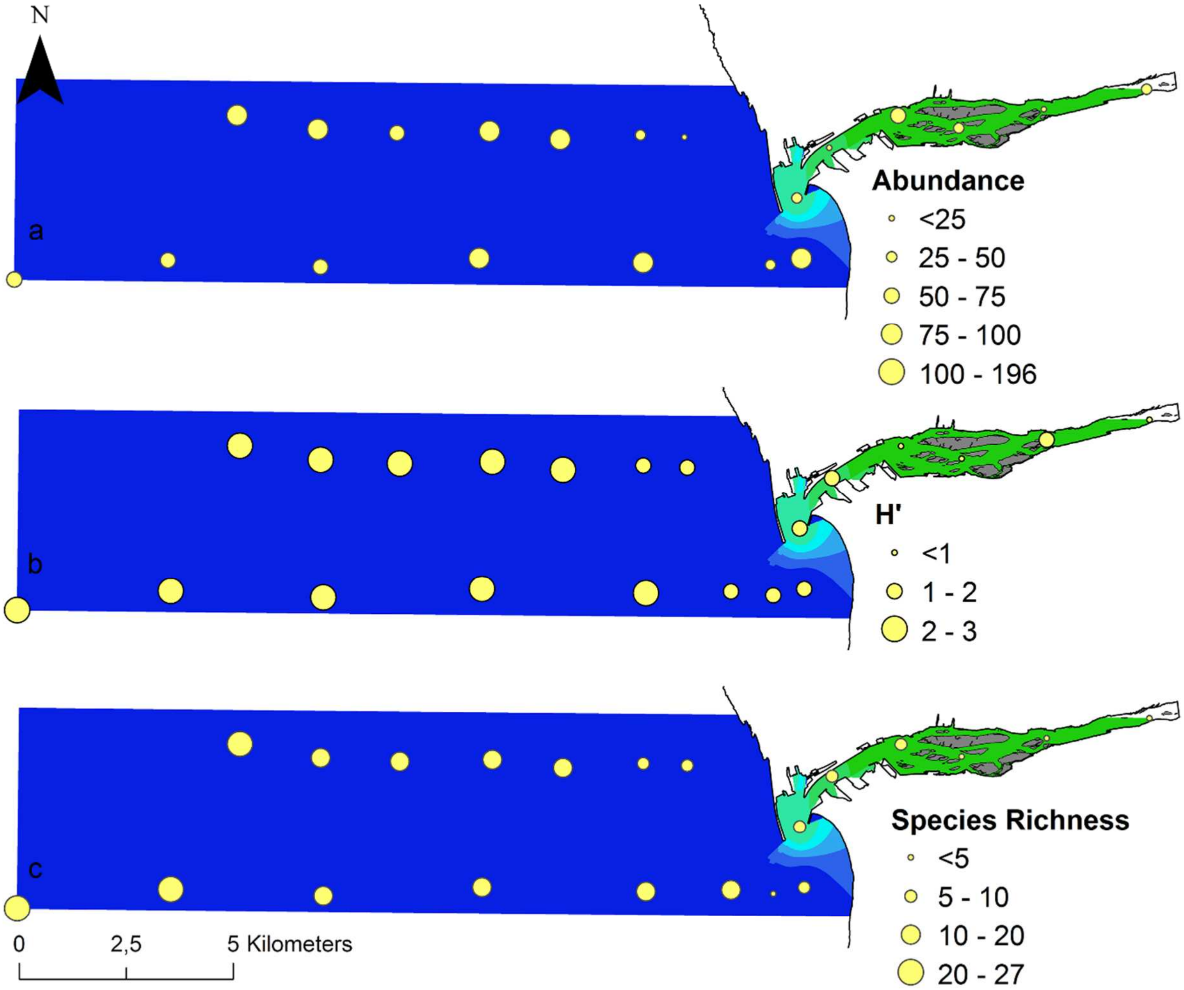
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Figure 4.

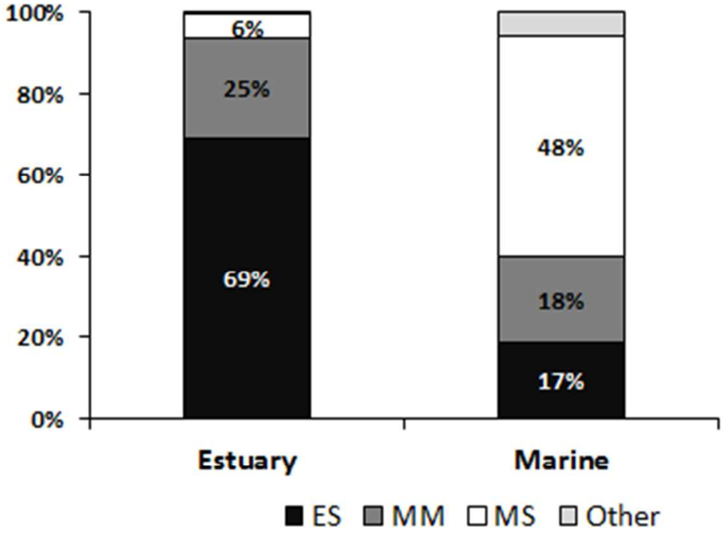
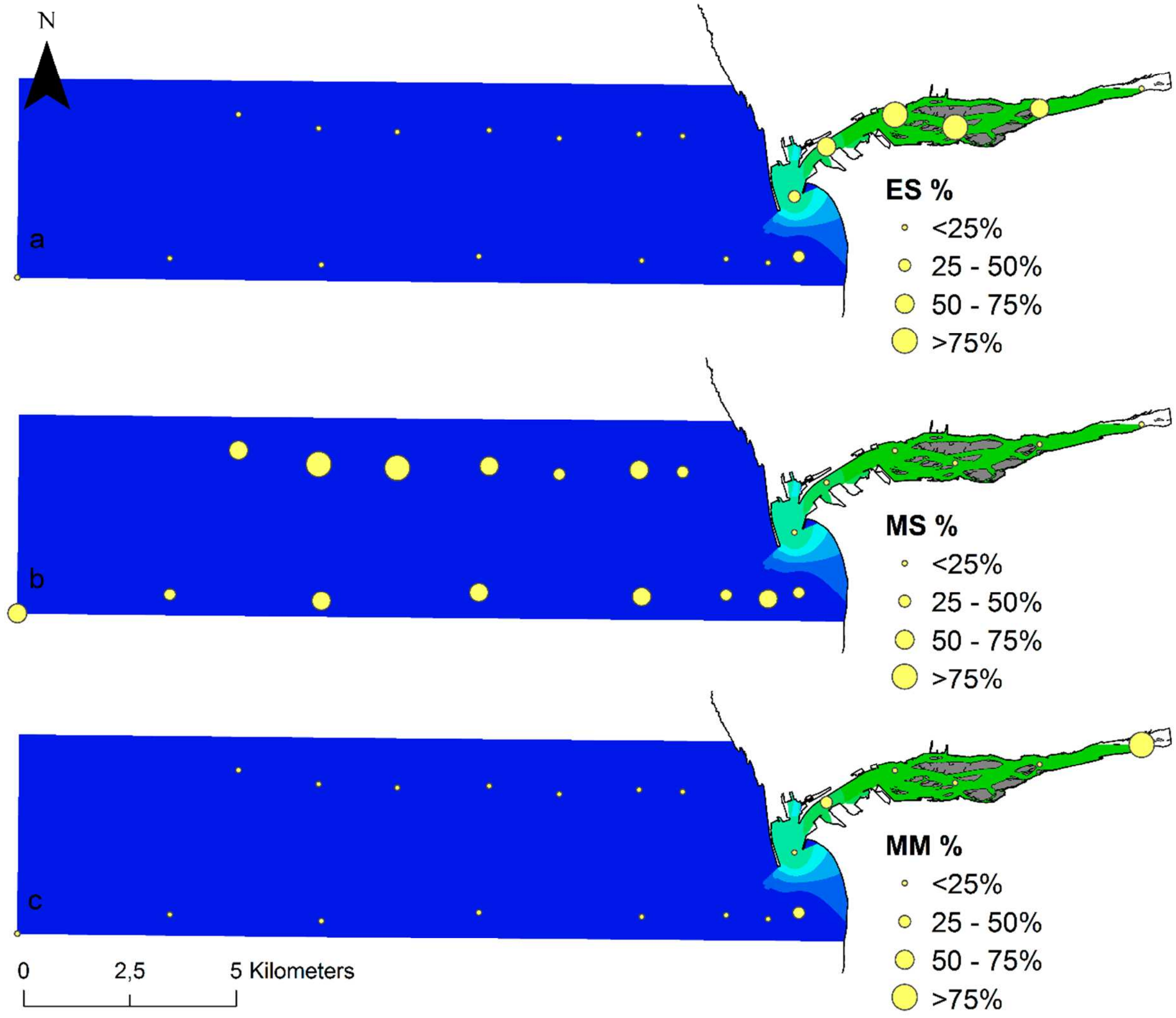


Figure 5.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5 Kilometers

Figure 6.

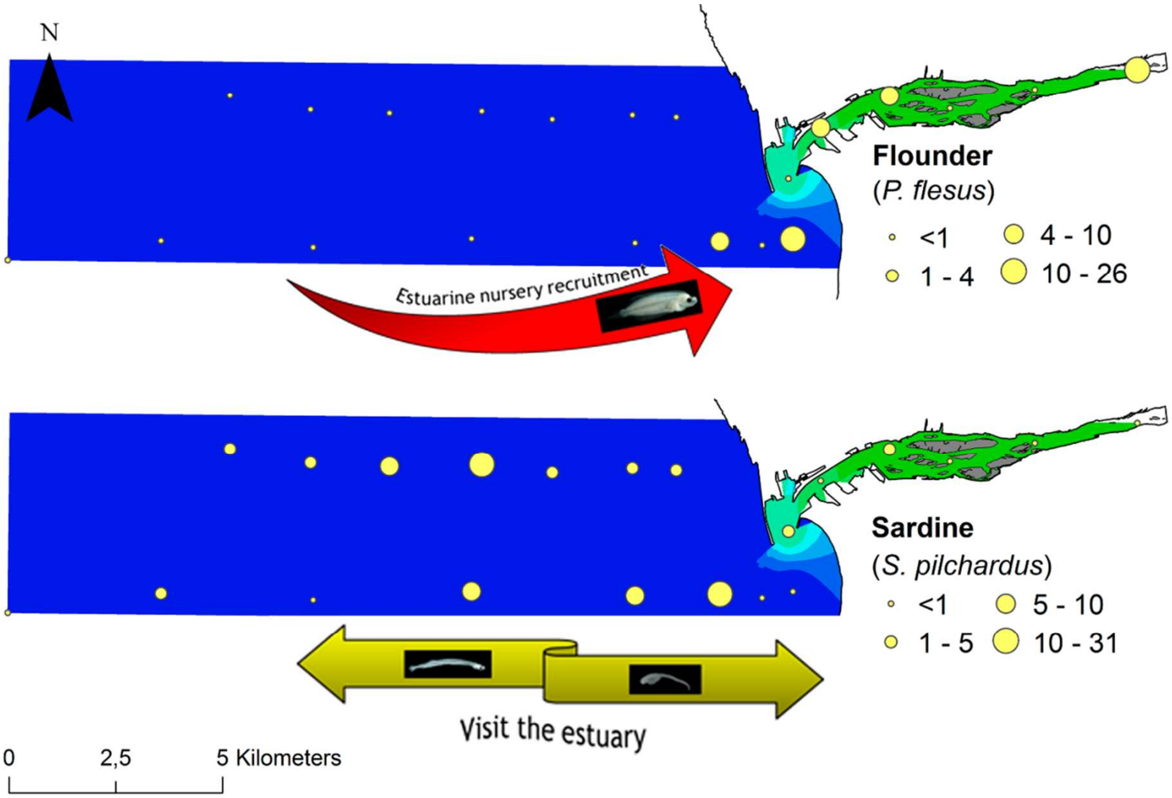
N
Flounder
(*P. flesus*)
<1
1 - 4
4 - 10
10 - 26
Estuarine nursery recruitment
Sardine
(*S. pilchardus*)
<1
1 - 5
5 - 10
10 - 31
Visit the estuary
0
2,5
5 Kilometers

Figure 7.

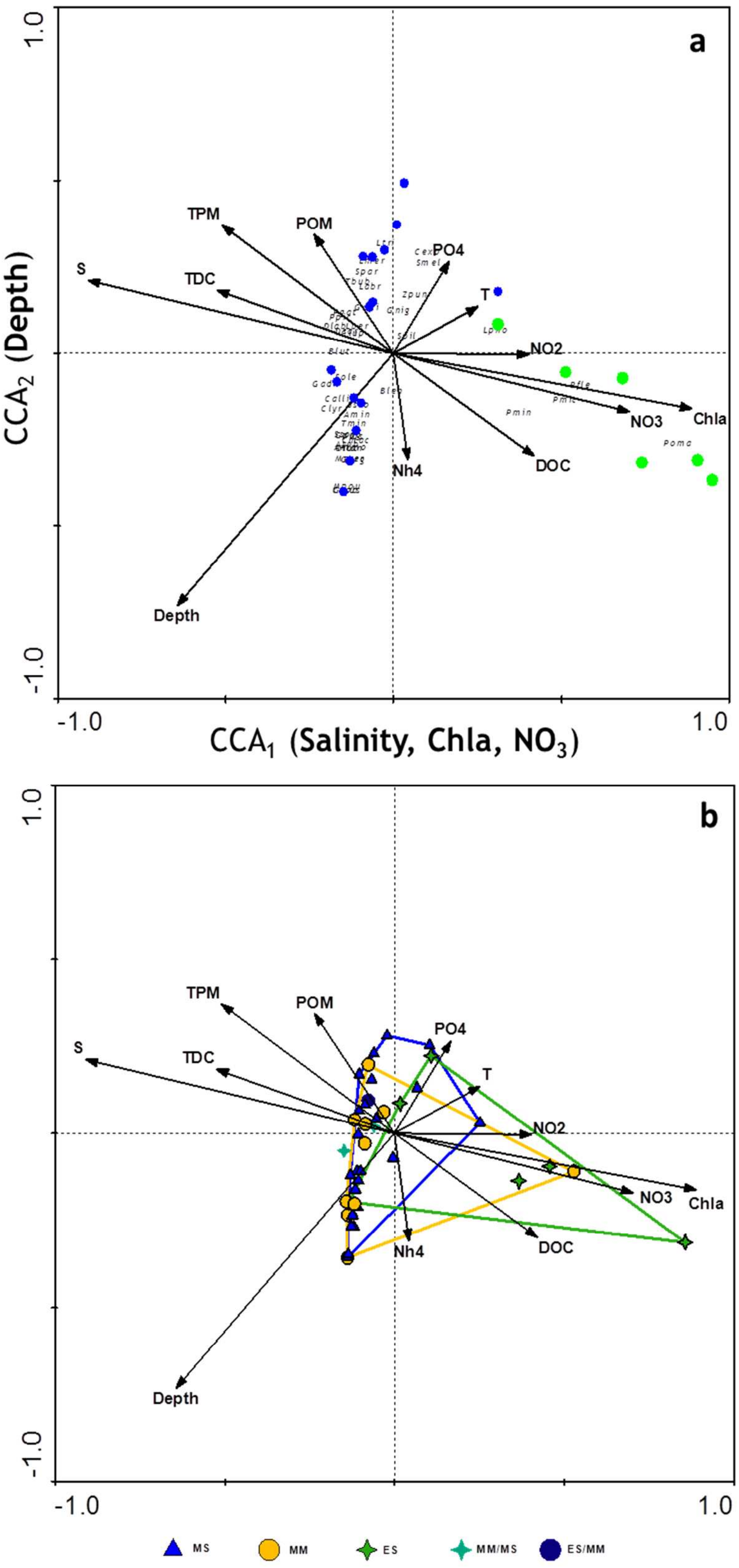
a
1.0
-1.0
CCA$_2$ (Depth)
CCA$_1$ (Salinity, Chla, NO$_3$)
TPM
POM
PO4
S
TDC
T
NO2
NO3
Chla
DOC
Nh4
Depth
b
MS
MM
ES
MM/MS
ES/MM

Figure 8.

Tables

Table 1. Water parameters of the Lima estuary and adjacent coastal zone along the water column.

	Lima estuary		Ocean	
	Average	SD	Average	SD
Temperature (ºC)	12.60	0.69	12.56	0.09
Salinity (psu)	13.00	15.73	35.69	0.09
Chlorophyll a (mg m^{-3})	3.20	1.50	0.65	0.16
TPM (mg L^{-1})	28.88	23.06	54.22	5.90
POM (mg L^{-1})	6.07	3.33	9.18	1.52
TDC (mg L^{-1})	15.01	8.80	25.25	1.93
DOC (mg L^{-1})	1.95	0.19	1.67	0.28
Nh_4 (µM L^{-1})	1.28	0.60	0.94	0.83
NO_3 (µM L^{-1})	29.79	18.68	6.56	1.28
NO_2 (µM L^{-1})	0.20	0.06	0.15	0.06
PO_4 (µM L^{-1})	1.24	0.94	1.05	0.71
Si (µM L^{-1})	54.62	38.16	3.69	1.75

Table 2. Abundance (no. larvae 100 m^{-3}), Shannon Wienner index (H') and species richness (no. of species) of the larval fish assemblages of Lima estuary and coastal area, and the ecological guild classification and abundance of the fourteen fish larvae species common along the estuarine-ocean gradient.

Species	CCA code	EG	Lima estuary Average	Lima estuary SD	Ocean Average	Ocean SD
Centrolabrus exoletus	*Cexo*	MS	0.33	0.81	3.46	7.29
Cupeidae ni	*Clup*	MS	0.08	0.20	**7.89**	**8.68**
Gobius niger	*Gnig*	ES	0.43	1.06	0.70	2.08
Labridae ni	*Labr*	MS	0.16	0.25	2.25	2.73
Labrus bergylta	*Lber*	MS	0.27	0.66	1.74	2.50
Lipophrys pholis	*Lpho*	MS	0.28	0.69	0.65	1.60
Platichthys flesus	*Pfle*	MM	5.84	9.96	1.58	5.29
Pomatoschistus microps	*Pmic*	ES	**15.39**	**18.25**	0.41	0.89
Pomatoschistus minutus	*Pmin*	ES	1.03	1.70	0.12	0.34
Pomatoschistus pictus	*Ppic*	MS	0.08	0.19	1.51	2.74
Sardina pilchardus	*Spil*	MM	0.95	1.36	5.33	7.91
Sparidae ni	*Spar*	MM	0.08	0.20	1.17	2.69
Symphodus melops	*Smel*	ES	1.49	2.42	5.59	9.47
Zeugopterus punctatus	*Zpun*	MS	0.43	1.06	0.46	1.35
Total abundance			30.45	23.46	73.23	39.49
Diversity (H')			0.97	0.95	2.25	0.44
Species richness			5.17	4.89	14.47	6.32

Table 3. Inter-set correlations of environmental variables with the first two CCA axes, based on the log-transformed abundance of larval fish assemblages of the estuarine-coastal gradient.

Environmental variables	CCA_1	CCA_2
Depth (m)	**-0.64***	**-0.71***
Temperature (ºC)	0.25	0.13
Salinity (psu)	**-0.90***	0.20
Chlorophyll a (mg m^{-3})	**0.88***	-0.16
TPM (mg L^{-1})	**-0.51***	0.36
POM (mg L^{-1})	-0.23	0.33
TDC (mg L^{-1})	**-0.52***	0.18
DOC (mg L^{-1})	**0.42***	-0.29
Nh_4 (µM L^{-1})	0.04	-0.30
NO_3 (µM L^{-1})	**0.70***	-0.16
NO_2 (µM L^{-1})	0.39	0.00
PO_4 (µM L^{-1})	0.16	0.26

* inter-set ≥ |0.4| corresponding to biologically important variables.