

# Temporal and spatial distributions of larval fish assemblages in the Lima estuary (Portugal)

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## Abstract

The Lima estuary (NW Portugal) is at the end of an international watershed, whose potential role as a spawning and nursery habitat for local fish populations has not been previously examined. To address this knowledge gap, fortnightly plankton surveys were conducted between April 2002 and April 2004. A total of 12,903 larvae, belonging to 20 families and 50 taxa were collected, with a mean abundance of 8 individuals per 100 m<sup>3</sup>. Gobiidae was the most abundant family comprising 71% of the total catch, followed by Clupeidae with 12% of the total. The top six abundant taxa (*Pomatoschistus* spp., *Sardina pilchardus*, *Ammodytes tobianus*, unidentified Clupeidae, *Symphodus melops* and *Solea senegalensis*) represented 91% of the total catch. Fish larvae showed a seasonal trend with abundances increasing during spring and summer. Diversity was generally low ( $H' = 0.65$ ) with high dominance of very few taxa. Near the ocean, the larval fish assemblage was more diverse due to the presence of marine species. In the lower estuary Channel zone, abundance was lower than in the two upstream salt marsh zones (North and South zones) and no statistical differences in abundance or diversity values were found within the latter zones. ANOSIM results demonstrated seasonal differences in the species composition, mainly during the second winter period which was typified by a pelagic species *A. tobianus*. The community in the Channel zone was more diverse in comparison with the other zones, which were highly dominated by the most abundant species. The spatial and temporal trends of the most abundant species were typical for Iberian estuaries, with the exception of the low abundance of anchovy larvae and the unusually high numbers and frequency of *S. pilchardus*, usually mentioned as accidental in estuarine systems. Overall results suggest that the Lima estuary larval fish assemblage has a strong seasonality and affinity to the salt marsh zones. It seems that spawning seasonality controlled the presence of temporary estuarine residents, while environmental aspects controlled the general abundance trend of the resident species.

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## 1. Introduction

Estuaries are nearshore coastal ecosystems where the ocean is diluted by freshwater (Dyer, 1997). They serve important economic functions including transport, industry and tourism but also drainage of waste from domestic, industrial and

agriculture activities (Heip and Herman, 1995; Raz-Guzman and Huidobro, 2002). Simultaneously, these ecosystems offer protection, not only for resident species, but also for a wide range of marine taxa (Weinstein, 1985; Weisberg et al., 1996; Cowley and Whitfield, 2002; McLusky and Elliott, 2004) which provide high fishery yields and important economic opportunities (Houde and Rutherford, 1993).

As boundary systems between watersheds and the sea, estuaries exhibit characteristic environmental gradients that favor the recruitment of a variety of species with diverse physical and trophic structures (Sánchez and Raz-Guzman, 1997; Harris

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et al., 2001; Kimmerer et al., 2001). Fishes play an important part in estuaries as they constitute permanent and temporary community components, with marine species visiting these habitats for feeding, reproduction, growth and protection (Raz-Guzman and Huidobro, 2002). Three general categories of fish are found in estuaries: marine fishes that use estuaries seasonally (temporary estuarine residents), those that complete their entire life history within the estuarine system (residents), and those that enter the estuary on rare occasions or are found in low numbers near inlets (occasional) (Lenanton and Potter, 1987).

The highly productive nature of estuarine habitats (Nixon et al., 1986; Day et al., 1989) and their role as nursery areas to fish in many life history stages are well documented for temperate (Powles et al., 1984; Elliott et al., 1990; Kennish, 1990; Drake and Arias, 1991; Szedlmayer and Able, 1996; Whitfield, 1999; Blaber, 2000; Shackell and Frank, 2000; Elliott and Hemingway, 2002) and tropical estuarine habitats (Raynie and Shaw, 1994; Sanvicente-Añorve et al., 2000; Harris et al., 2001; Cowley and Whitfield, 2002; Franco-Gordo et al., 2003). Since early life stages are a particularly vulnerable phase, it is hypothesized that marine fish larvae and juveniles migrate into estuaries to make use of the high food abundance and refuge against predators, in order to maximize survival (Frank and Legget, 1983; Kennish, 1990; Van der Veer et al., 2001). Therefore, a greater understanding of ichthyoplankton dynamics in estuaries would facilitate the further development of hypotheses about estuarine nursery function (Rakocinski et al., 1996). Estuarine fish larvae assemblages are variable both in terms of species composition and distribution patterns (Harris et al., 1999). These assemblages change continually in time and space, according to reproductive seasons of the species and also due to the environmental fluctuations (Whitfield, 1994; Harris and Cyrus, 1995; Hettler and Hare, 1998; Garcia et al., 2003). However, there seem to be a general tendency for estuarine fish larvae to peak in abundance during spring and summer (Talbot and Able, 1984; Cowan and Birdsong, 1985; Whitfield, 1989; Harris et al.,

1999; Young and Potter, 2003). Furthermore, temperate estuarine ichthyoplankton assemblages have been shown to be dominated by resident species belonging to Gobiidae family or seasonally by estuarine spawners, such as species of Clupeidae and Engraulidae family (Talbot and Able, 1984; Drake and Arias, 1991; Monteleone, 1992; Blaber et al., 1997; Ré, 1999; Harris and Cyrus, 2000; Strydom et al., 2003).

The Lima estuary is located in northwest Portugal. The lower part is heavily modified, including a large shipyard, commercial sea-port, and a fishing harbour. Upstream the shallow salt marsh and tidal islands are much less disturbed (Fig. 1). Little is known about the water quality of the Lima River estuarine area, although it receives urban runoff and the navigation channel is heavily dredged. On the other hand, the estuary still supports a locally important fishery (*Platichthys flesus*, *Dicentrarchus labrax* and some seasonal species such as *Petromyzon marinus*) though few data pertaining to its fish assemblage are available. The only study known was undertaken by Valente and Alexandrino (1988) that suggested that the south bank of the salt marsh area was used as nursery area for juveniles of some marine fishes. The composition of the larval ichthyofauna found in the Lima estuary is not known (Ré, 1999). The present study was undertaken in order to fill this knowledge gap: (1) by characterizing spatial and temporal trends of the larval fish assemblages of the Lima estuary and (2) by providing baseline data on the use of an estuarine system in the NW Iberian Peninsula by early life stages of fishes.

## 2. Material and methods

### 2.1. Study area

The Lima River is an international water body, draining into the Atlantic Ocean at 41° 40' N and 8° 50' W in the vicinity of the 32,000 inhabitant city of Viana do Castelo. Its watershed covers 2446 km<sup>2</sup>, of which 1143 km<sup>2</sup> are located in Portugal. The Lima estuary (Fig. 1) has a semidiurnal and

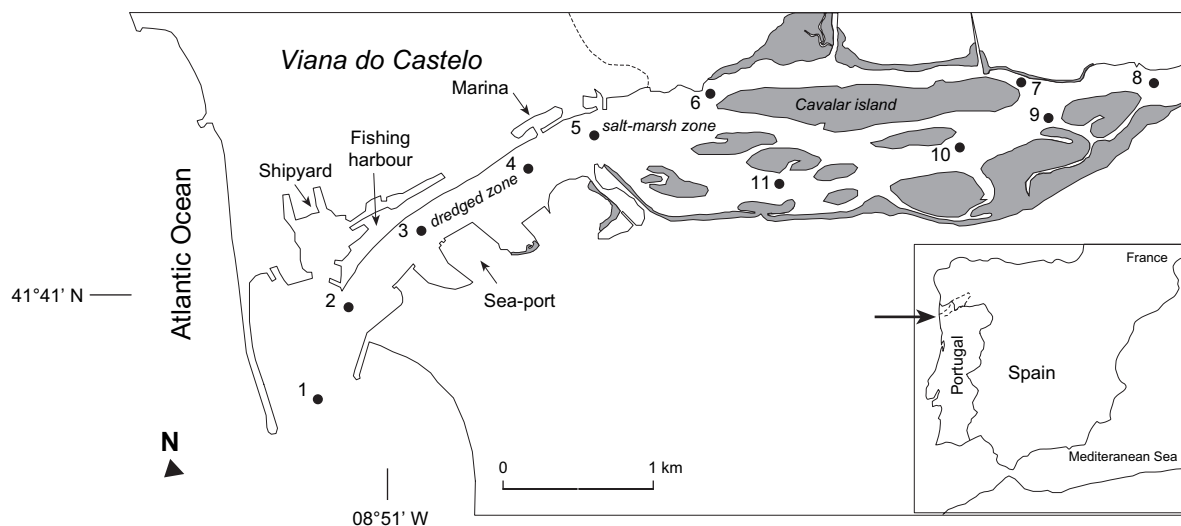


Fig. 1. Lima estuary and location of the sampling stations.

mesotidal regime. Salt intrusion extends up to 20 km upstream, with an average flushing rate of  $0.40 \text{ m s}^{-1}$  and a residence time of 9 days. The river mouth is partially obstructed by a 2-km-long jetty, deflecting the river flow to the south. The study area, located in the lower 7 km stretch of the estuary has an initial navigation channel and an upstream shallow salt marsh zone, mainly colonized by *Juncus* spp., with a large longitudinal sandy island (Cavalari Island) (Fig. 1). The study area was divided into three geomorphological distinct zones, according to water depth and the presence–absence of salt marsh. Channel zone (stations 1–4), located in the initial 2.5 km, is a narrow, 10-m-deep navigational channel, highly industrialized with walled banks. The zone to the North of Cavalari Island (stations 5–7) is a narrow tidal channel where two small streams drain. During high tide, mean depth is 4 m, but this zone is almost completely drained during low tide. The South zone includes the remaining stations (stations 8–11), and is a complex of small tidal island south of Cavalari Island, and has a mean depth of 3.7 m during high tide. This region encompasses the main course of the river and a large salt marsh bank.

## 2.2. Field methods

Between April 2002 and April 2004, a total of 46 fortnight sampling surveys were conducted in the Lima estuary. Eleven stations were sampled with a conical 1 m diameter, 3 m long and 500  $\mu\text{m}$  mesh size net. Subsurface circular tows were performed at a constant velocity of ca.  $1 \text{ m s}^{-1}$  for 5 min. Tows were performed during the slack phase of spring tides (i.e. 2 h before and after high tide). Geographical location of the sampling stations was provided by a GPS, version Magellan 315. A flowmeter (Hydro-Bios) was attached to the net in order to determine the volume of the water filtered. At each sampling station, vertical temperature ( $^{\circ}\text{C}$ ), salinity and turbidity (NTU) profiles were obtained by means of a YSI 6820 CTD.

## 2.3. Sample processing

After each tow, samples were immediately fixed in 4% buffered formalin (pH = 8). Three days after the survey, samples were sorted and fish larvae were preserved in 95% alcohol. Individuals were identified to the lowest taxonomic separation, using the appropriate literature (Macer, 1967; Nichols, 1971, 1976; Demir, 1976; Fives, 1976, 1986; Russell, 1976; Camus and Besseau, 1986; Ré, 1999). Numbers of individuals per taxa were counted from the entire sample and then standardized to number of fish larvae per  $100 \text{ m}^3$ .

## 2.4. Data analysis

Diversity of the larval fish assemblage was expressed by the Shannon–Wiener index (Shannon and Weaver, 1963) and equitability was measured by Pielou's evenness index ( $J'$ ) (Pielou, 1966). Seasons were aggregated into three-month groups: spring (Sp) comprised March, April and May; summer (S) June, July and August; autumn (A) September, October

and November and winter (W) December, January and February. Since the study covered 46 surveys, the first 24 surveys, from April 2002 to the first survey of April 2003, are therefore considered as the “first year” (seasons Sp<sub>1</sub>, S<sub>1</sub>, A<sub>1</sub>, W<sub>1</sub> and Sp<sub>2</sub>) and the remaining surveys are the “second year” (seasons Sp<sub>2</sub>, S<sub>2</sub>, A<sub>2</sub>, W<sub>2</sub> and Sp<sub>3</sub>).

Temporal and spatial 2D-maps of total ichthyoplankton abundance and Shannon–Wiener index were obtained with SURFER software using kriging as the interpolation method. ANOVA type III was used to investigate the effect of year, season and zone on temperature, salinity and turbidity of the subsuperficial layer (1–2 m depth) of the water column, with year, season and zone as fixed factors (Sokal and Rohlf, 1995; Zar, 1996). In order to ascertain the effect of zones and seasons on the density and the diversity of the assemblage, the analysis of variance ANOVA type II was used, with seasons and zones as fixed factors (Sokal and Rohlf, 1995; Zar, 1996). Temperature, salinity and turbidity data were log transformed [ $\ln(x)$ ], as were the raw abundance data [ $\log_{10}(x + 1)$ ], in order to stabilize the variance and to fit data to a normal distribution, fulfilling one of the ANOVA assumptions. Homogeneity of variance was tested with a Cochran test and whenever variance was still heterogeneous, conclusions from ANOVA results were only accepted for those cases where significance levels were less than 0.01. Furthermore, in the event of significance, an a posteriori Tukey HSD for unequal sample sizes was used to determine which means were significantly different at a 0.05 level of probability (Spjøtvoll and Stolone, 1973).

Multivariate analyses were performed with the software package PRIMER (Plymouth Routines Multivariate Ecological Research) (Clarke and Warwick, 1994). One-way analysis of similarity (ANOSIM) (Clarke and Warwick, 1994) was used to determine the significance of spatial and temporal trends in the structure of the assemblage. The tests were based on a Bray–Curtis rank similarity matrix, calculated using square root transformed data. Similarity percentages (SIMPER) (Clarke, 1993) were used to reveal the percentage contribution of each *taxon* to the average dissimilarity between samples of the various season and region pair combinations. Hierarchical agglomerative clustering with group-averaging linking and non-metric multi-dimensional scaling (nMDS) was performed to investigate similarities among species (Clarke and Warwick, 1994). Analyses were based on the Bray–Curtis similarity measure (Bray and Curtis, 1957). Only species with more than 0.2% of the total catch were included in the analysis avoiding any undue effect of rare species. Species abundance data were standardized without transformation (Clarke and Warwick, 1994).

## 3. Results

### 3.1. Environmental conditions

Temperature exhibited similar trends in all three zones of the estuary. The maximum mean temperature occurred during the A<sub>1</sub> period, while the lowest temperature values were recorded during W<sub>1</sub> (Fig. 2). During the first year the warmer season was A<sub>1</sub>, while in the second year it was S<sub>2</sub>. The low temperatures

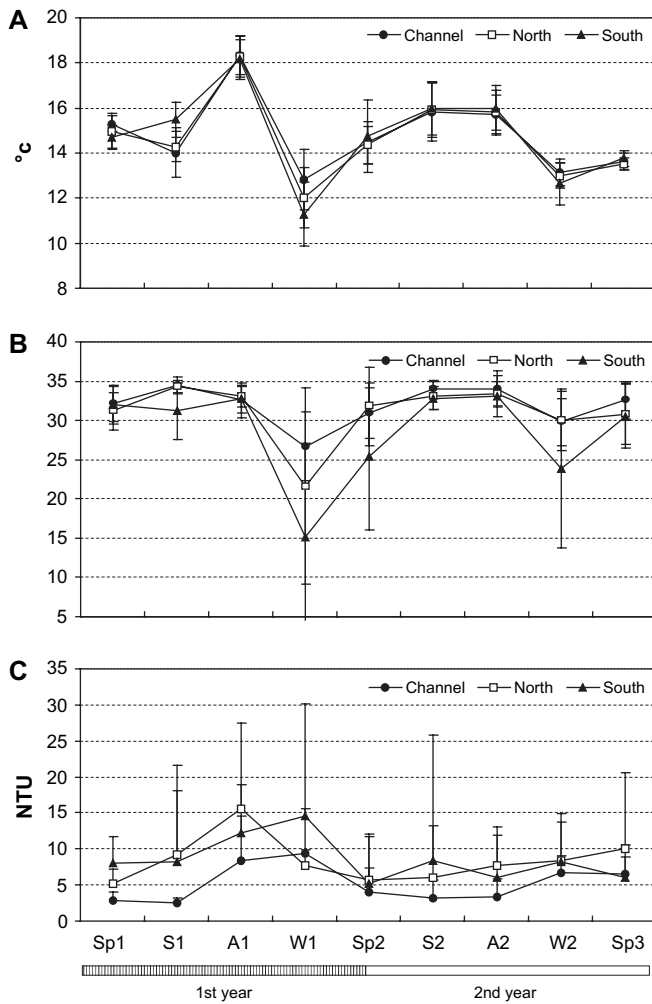


Fig. 2. Seasonal average values and standard deviation (error bars) for temperature (A), salinity (B) and turbidity (C) in the 11 surveyed stations in Lima estuary by zones.

recorded in  $S_1$  were a consequence of upwelling phenomena present in the first study year which was absent in the second year, preventing the summer temperature decrease. In the second year, temperature exhibited seasonality typical of temperate northern hemisphere environments, where the warmest period was the summer and the coldest was the winter (Fig. 2).

During most of the year, surface salinity was greater than 30, except during the winter and early spring (Fig. 2). During these freshest months, salinity decreased significantly ( $F = 40.51$ ,  $P < 0.01$ ), particularly in the South zone, where mean salinity reached 15 in  $W_1$  and 22 and  $W_2$ . During the winter seasons, salinity fluctuations were more evident in both zones of the salt marsh area (North and South zones) than in the channel zone.

Turbidity was significantly higher in  $A_1$  and  $W_1$  ( $F = 30.40$ ,  $P < 0.01$ ) than during the other seasons, namely spring and summer, when the minimum mean values were registered (Fig. 2). Sub-surface water layer was significantly more turbid in the South zone than in the other zones of the study area ( $F = 25.14$ ,  $P < 0.01$ ). Turbidity showed high sample variability, especially the salt marsh area, exhibiting high standard deviations for each season.

### 3.2. Species composition

During the study period, a total of 12,903 fish larvae were collected in the Lima estuary, with a mean abundance of 8 individuals per  $100 \text{ m}^3$ . The fish larvae assemblage included 20 families distributed through 50 taxa, where 38 could be assigned to species, 4 to genus and 7 to family (Table 1). The majority of unidentified individuals (2.55% of the total catch) were yolk-sac larvae (Table 1). Gobiidae was the most abundant family (71.0% of the total catch), followed by Clupeidae (12.2%), Ammodytidae (5.3%), Labridae (3.0%), Blenniidae (1.7%) and Soleidae (1.4%). At the species level, *Pomatoschistus* spp. reached 71.0% (Table 1) of the total fish larvae collected during this study, representing the most abundant taxon. *Sardina pilchardus* (8.3%), *Ammodytes tobianus* (5.1%), unidentified Clupeidae (3.3%), *Symphodus melops* (2.0%) and *Solea senegalensis* (1.2%) were the other abundant species (i.e. >1% of the total catch) (Table 1). Thus, these six species comprised 91% of the total catch. Thirty-three of the 50 taxa were found in all estuarine zones, whereas the remaining were confined either to the channel zone or to the salt marsh zone. From the 39 species of teleost identified, 22 are considered as occasional marine species, 15 as temporary estuarine resident and 2 as resident (Table 1).

### 3.3. Temporal and spatial patterns of abundance

A clear seasonal signal in larval abundance was evident, with significantly higher abundance values ( $F = 32.20$ ,  $P < 0.001$ ) during the spring and summer seasons relative to the other periods of the year (Fig. 3A). The highest abundance values were found at least 3 km upstream of the river mouth (Fig. 3A). Abundances in North and South zones were significantly higher than in the Channel zone ( $F = 16.94$ ,  $P < 0.001$ ).

After the spring–summer peak of abundance, the number of fish larvae decreased to very low values, especially during the winter (Fig. 3A). In  $W_2$  this period was shorter than during  $W_1$  due to the presence of increased numbers of *Ammodytes tobianus*, caught mainly in January 2004 in the upstream zones of the estuary (Fig. 4C). When temperature started to increase in the following spring periods ( $Sp_2$  and  $Sp_3$ ), fish larvae abundance also increased. During these periods, *Pomatoschistus* spp. were more abundant and concentrated in the North and South zones (Fig. 4A). However, during the  $Sp_2$  and  $S_2$  their abundance was lower in comparison with the same period of the previous year, thus the overall abundance of the assemblage did not exceed 200 fish larvae per  $100 \text{ m}^3$ . Also, the presence of high numbers of *Sardina pilchardus* larvae during the  $Sp_2$  and  $S_2$  period, mainly in the two first kilometer of the estuary (Fig. 4B), marked the difference between the two warmer periods of the study.

### 3.4. Temporal and spatial trends of diversity

The Shannon–Wiener diversity index ranged from 0 to 2.07 and Pielou's evenness varied between 0.03 and 1 (Fig. 3B,C). During the study period, the number of taxa and

Table 1

Numbers of species caught in the Lima estuary between April 2002 and April 2004 and relative contribution to the total catch in the whole estuary and by zone. The relative contributions were calculated from numbers in each sample after adjustment to constant volume of 100 m<sup>3</sup>. The 18 most abundant species also include the code used for multivariate analyses. — indicates species not caught in that region. MD: mean density of each taxa; O: occasional; TR: temporary estuarine resident; R: resident

Family	Species	Total catch			Sections		
		Habitat	MD (larvae/100 m <sup>3</sup> )	(%)	Channel	North zone	South zone
Gobiidae	<i>Pomatoschistus</i> spp. (Pom)	R	30.24	70.95	39.29	81.86	80.04
Clupeidae	<i>Sardina pilchardus</i> (Spil)	O	5.83	8.29	19.53	4.16	5.22
Ammodytidae	<i>Ammodytes tobianus</i> (Ato)	TR	7.44	5.06	5.73	4.23	5.28
Clupeidae	Unidentified (Clupeidae ni)	O	4.82	3.27	7.43	2.07	1.92
Not identified	Not identified		0.03	2.55	5.63	1.81	1.23
Labridae	<i>Symphodus melops</i> (Smel)	TR	2.77	1.91	3.11	0.98	1.92
Soleidae	<i>Solea senegalensis</i> (Ssen)	TR	1.75	1.19	2.13	1.01	0.83
Labridae	<i>Labrus bergylta</i> (Lber)	TR	1.66	0.66	1.31	0.66	0.32
Clupeidae	<i>Spratus spratus</i> (Sspra)	O	1.92	0.65	1.70	0.38	0.29
Blenniidae	<i>Parablennius gattorugine</i> (Pgatto)	O	1.09	0.60	1.67	0.25	0.27
Blenniidae	<i>Lipophrys pholis</i> (Lpho)	TR	1.34	0.53	1.08	0.35	0.36
Atherinidae	<i>Atherina presbyter</i> (Apre)	TR	2.56	0.50	2.03	0.03	0.02
Gadidae	<i>Trisopterus luscus</i> (Tlus)	O	1.67	0.33	0.85	0.13	0.20
Blenniidae	<i>Blennius ocellaris</i> (Bocce)	O	1.25	0.29	0.75	0.20	0.10
Gobiesocidae	<i>Diplecogaster bimaculata</i> (Dbim)	O	1.45	0.26	0.92	0.03	0.07
Gobiesocidae	<i>Lepadogaster lepadogaster</i> (Llepa)	O	1.27	0.23	0.79	0.03	0.07
Pleuronectidae	<i>Platichthys flesus</i> (Pfle)	TR	1.20	0.23	0.26	0.28	0.17
Ammodytidae	<i>Hyperoplus lanceolatus</i> (Hlan)	TR	2.24	0.22	0.79	0.03	0.05
Syngnathidae	<i>Syngnathus acus</i> (Sacus)	TR	1.06	0.20	0.10	0.23	0.24
Labridae	Not identified	TR	2.01	0.19	0.62	0.05	0.07
Trachinidae	<i>Trachinus draco</i>	O	2.12	0.19	0.62	0.05	0.07
Labridae	<i>Ctenolabrus rupestris</i>	O	1.32	0.18	0.36	0.15	—
Blenniidae	<i>Coryphoblennius galerita</i>	O	1.58	0.15	0.29	0.05	0.14
Sparidae	Not identified	O	0.96	0.12	0.23	—	0.07
Sparidae	<i>Spondyliosoma cantharus</i>	O	1.36	0.12	0.36	0.08	0.02
Soleidae	<i>Solea lascaris</i>	O	1.16	0.11	0.29	0.10	0.02
Carangidae	<i>Trachurus trachurus</i>	O	1.03	0.09	0.10	0.10	0.09
Bleniidae	Not identified	O	1.12	0.09	0.26	0.05	0.02
Callionymidae	<i>Callionymus lyra</i>	O	1.58	0.08	0.07	0.05	0.10
Engraulidae	<i>Engraulis encrasicolus</i>	TR	0.83	0.07	0.10	0.10	0.03
Labridae	<i>Centrolabrus exoletus</i>	O	1.11	0.06	0.16	—	0.15
Moronidae	<i>Dicentrarchus labrax</i>	TR	1.03	0.06	0.10	—	0.09
Liparidae	<i>Liparis montagui</i>	TR	3.83	0.06	0.23	—	0.02
Soleidae	Not identified		1.45	0.06	0.10	0.15	0.03
Callionymidae	<i>Callionymus</i> spp.	O	0.80	0.05	0.16	—	0.03
Gadidae	<i>Ciliata mustela</i>	TR	1.10	0.05	0.03	0.08	0.05
Trachinidae	<i>Echichthys vipera</i>	O	1.00	0.05	0.13	0.03	0.02
Gadidae	Not identified	O	1.49	0.05	0.07	0.08	0.02
Soleidae	<i>Solea vulgaris</i>	TR	1.46	0.05	0.10	0.03	0.03
Mugilidae	<i>Liza</i> spp.	TR	1.04	0.04	0.10	0.05	—
Syngnathidae	<i>Syngnathus</i> spp.		0.82	0.03	0.07	—	0.03
Sparidae	<i>Diplodus sargus</i>	O	0.80	0.02	0.03	—	0.03
Syngnathidae	<i>Entelurus aequoreus</i>	TR	0.85	0.02	0.07	0.03	—
Syngnathidae	<i>Nerophis ophidion</i>	O	0.87	0.02	—	0.03	—
Bothidae	<i>Zeugopterus punctatus</i>		1.21	0.02	0.10	—	—
Gobiidae	<i>Crystalllogobius linearis</i>	O	1.38	0.02	0.07	—	—
Syngnathidae	<i>Syngnathus abaster</i>	R	0.93	0.02	—	0.03	0.02
Soleidae	<i>Buglossidium luteum</i>	O	0.68	0.01	0.03	—	—
Soleidae	<i>Microchirus variegatus</i>	O	1.22	0.01	0.03	—	—
Syngnathidae	<i>Nerophis lumbriciformis</i>	O	0.76	0.01	0.07	0.03	—
Pleuronectidae	Pleuronectidae ni	TR	1.19	0.01	—	0.03	—
No. of larvae			12 903		3057	3969	5877
No. of species			50		48	41	41

Shannon–Wiener index increased significantly ( $F = 30.23$ ,  $P < 0.01$ ) from the first year (41 taxa and  $H' = 0.43$ ) to the second year (47 taxa and  $H' = 0.68$ ). Diversity increased during the warmer periods, A<sub>1</sub> in the first year and S<sub>2</sub> during the second

year (c.f. Figs. 2 and 3B). However, occasionally, high diversity levels were found in winter, as in February 2004 (Fig. 3B). The Channel zone exhibited significantly higher diversity values ( $F = 11.23$ ,  $P < 0.01$ ) than the North and South zones.

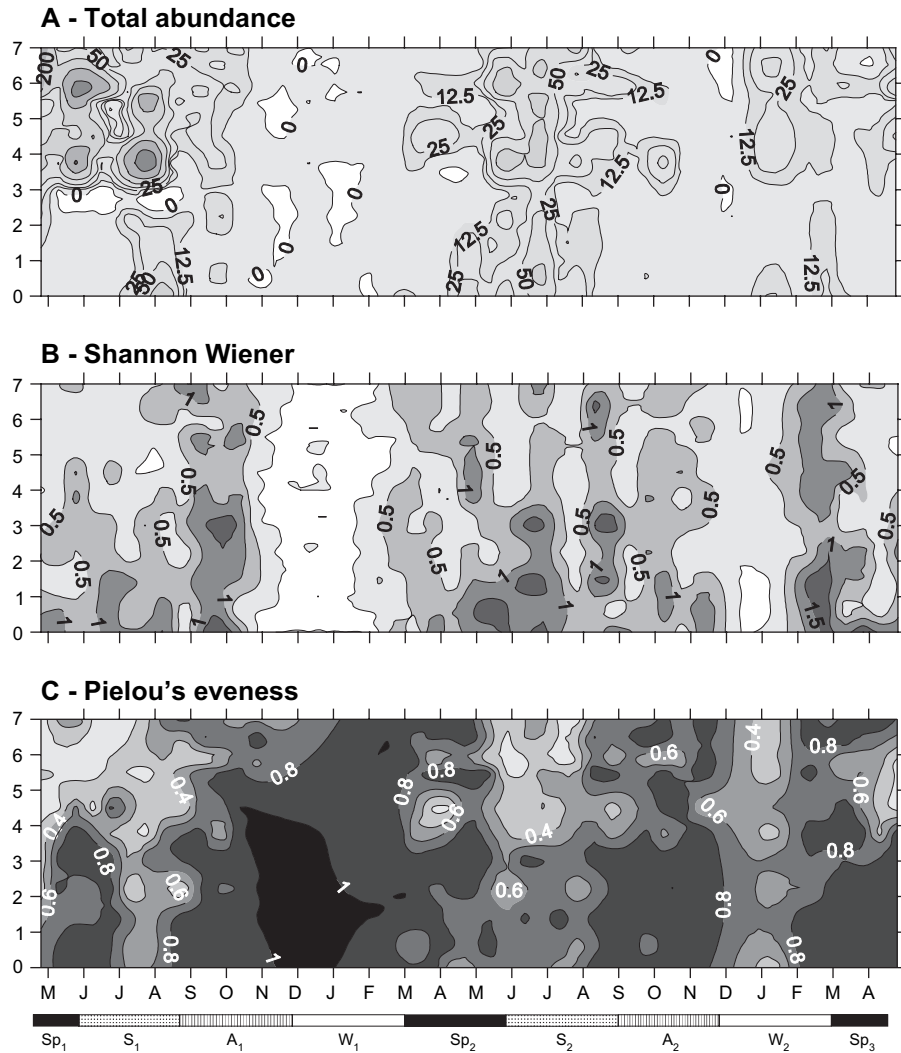


Fig. 3. Temporal and spatial dynamics of (A) total abundance (number of fish larvae per  $100\text{ m}^3$ ); (B) Shannon–Wiener index and (C) evenness of the Lima larval fish assemblage.

The fish larvae assemblage showed low Pielou's evenness values, with the exception of freshet periods (Fig. 3C). The peaks of fish larvae detected in this study, mainly in the first year, were dominated by single taxa *Pomatoschistus* spp. (c.f. Figs. 3A and 4A), affecting the low diversity values found during this period (Fig. 3B).

### 3.5. Assemblage composition

According to ANOSIM results (Table 2) there were significant differences between seasons ( $P < 0.05$ ), although groups were not clearly separated ( $R < 0.5$ ). In spite of *Pomatoschistus* spp. being identified as the discriminating species of the majority of the season groups, the structure of the assemblage differed along time (Table 3). After the period dominated by *Pomatoschistus* spp. ( $Sp_1$  and  $S_1$ ), new species appeared (i.e. *Solea senegalensis*) during  $A_1$ , leading to an increase in the diversity of the assemblage (Table 3). After the low abundance period ( $W_1$ ), *Pomatoschistus* spp. started to dominate the

assemblage ( $S_2$ – $A_2$ ), until the next freshet period ( $W_2$ ). This latter period was dominated by, *Ammodytes tobianus* (Table 3).

The spatial trend of community structure significantly varied from the Channel zone to the other two zones of the estuary (Table 4). In North and South zones, the assemblage was highly dominated by *Pomatoschistus* spp., responsible for 82 and 87% of the similarity within each zone, respectively (Table 5). The assemblage in the Channel zone was more diverse, with seven species being identified by SIMPER analysis as discriminating the larval fish assemblage structure in that zone of the estuary (Table 5).

Cluster analysis showed no marked tendency of species to be separated in compact groups. At a similarity of 15%, three species groups were obtained, whereas 5 of the total 18 species remained isolated (Fig. 5A). Since nMDS stress was higher than 0.1, cluster results were superimposed on 2D-multidimensional ordination. The three clusters separate out more or less according to the type of eggs and also to the temporal distribution of the species in the Lima estuary. Groups 1 and 3 clustered species

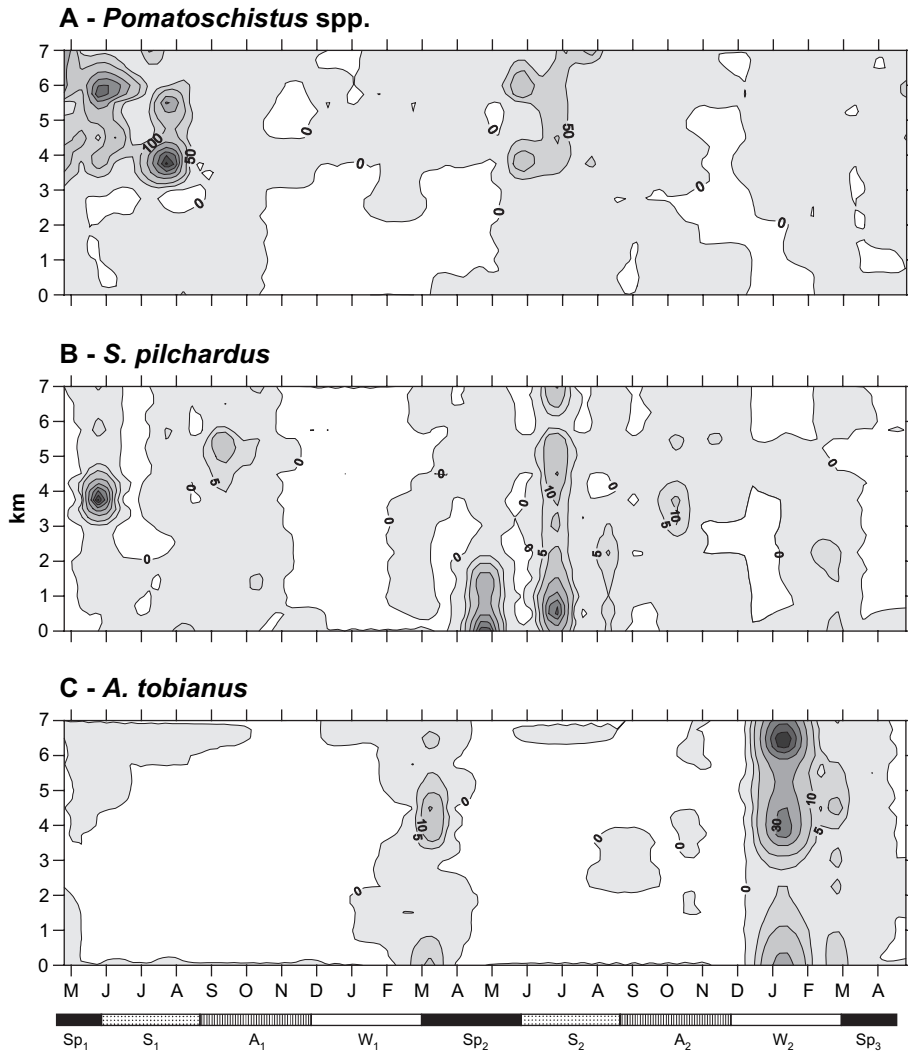


Fig. 4. Temporal and spatial distributions of abundance of the three dominant species (A, *Pomatoschistus* spp.; B, *S. pilchardus*; C, *A. tobianus*).

derived from demersal eggs, while Group 2 comprised species with pelagic eggs (Fig. 5B). Species from Group 1 (*Diplecogaster bimaculata* and *Lepadogaster lepadogaster*) were separate from Group 3 due to their low abundance and their restricted

presence to warmer periods and the Channel zone of the estuary. In contrast, species of Group 3 were captured in all three zones of the estuary from late winter until early autumn, with the exception of *Pomatoschistus* spp., which had a broad distribution throughout the study period. Group 2 included species with pelagic eggs that decreased sharply during the winter season and were more abundant in the Channel zone. The only species with direct development present in the top 18 species was *Syngnathus acus*, an estuarine–marine species, which was always represented by a single individual in each sample.

Table 2  
Results of one-way ANOSIM (*R* values and significance levels) and SIMPER analysis on the abundance of the top 18 most abundant species from seasonal groups

Groups	ANOSIM		SIMPER		
	<i>R</i>	<i>P</i>	Average dissimilarity (%)	Discriminating species	Contribution (%)
Sp <sub>1</sub> vs S <sub>1</sub>	0.121	0.005 <sup>a</sup>	59.7	<i>Pomatoschistus</i> spp.	52.1
S <sub>1</sub> vs A <sub>1</sub>	0.306	0.001 <sup>a</sup>	69.5	<i>Pomatoschistus</i> spp.	38.8
A <sub>1</sub> vs W <sub>1</sub>	0.356	0.001 <sup>a</sup>	86.4	<i>Pomatoschistus</i> spp.	28.1
W <sub>1</sub> vs Sp <sub>2</sub>	0.180	0.001 <sup>a</sup>	85.6	<i>Pomatoschistus</i> spp.	30.3
Sp <sub>2</sub> vs S <sub>2</sub>	0.125	0.001 <sup>a</sup>	74.4	<i>Pomatoschistus</i> spp.	33.3
S <sub>2</sub> vs A <sub>2</sub>	0.229	0.001 <sup>a</sup>	70.2	<i>Pomatoschistus</i> spp.	35.9
A <sub>2</sub> vs W <sub>2</sub>	0.402	0.001 <sup>a</sup>	76.2	<i>A. tobianus</i>	35.7
W <sub>2</sub> vs Sp <sub>3</sub>	0.271	0.001 <sup>a</sup>	78.3	<i>A. tobianus</i>	32.0

<sup>a</sup> Significant differences.

#### 4. Discussion

The larval fish assemblage found in the Lima estuary is composed of a few species with high abundance and a large number of rare species, a common feature of estuarine populations (Gaughan et al., 1990; Harrison and Whitfield, 1990; Drake and Arias, 1991; Harris and Cyrus, 1995; Whitfield, 1999). The number of taxa found in this study (41° N) (50 taxa, 20 families) was higher than in other estuaries of the western Iberian Peninsula. For example, in the Mondego

Table 3  
Discriminating species of each season using the SIMPER analysis

Average similarity (%)	Sp <sub>1</sub> (37.6)	S <sub>1</sub> (46.9)	A <sub>1</sub> (33.1)	W <sub>1</sub> (23.4)	Sp <sub>2</sub> (21.5)	S <sub>2</sub> (38.0)	A <sub>2</sub> (37.2)	W <sub>2</sub> (41.9)	Sp <sub>3</sub> (26.2)
Contribution (%)									
<i>Pomatoschistus</i> spp.	80.8	89.0	49.2	55.5	52.5	73.7	56.1	24.4	77.2
<i>A. tobianus</i>				39.6	7.1			58.5	8.9
<i>S. pilchardus</i>			29.5		16.5	8.0	25.0	11.0	
<i>S. melops</i>	10.2	4.9			6.4	5.0			
<i>S. senegalensis</i>			6.9						
Unidentified Clupeidae			6.7		5.9		11.3		
<i>T. luscus</i>					6.7				
<i>L. bergylta</i>						3.7			
Labridae ni									5.5
Total	91.0	94.0	92.3	95.2	95.2	90.5	92.39	93.92	91.62

estuary further south (40° N), only 28 taxa (16 families) were found (Ribeiro, 1991). In the Tagus estuary (38° N), 32 taxa and 20 families were found (Ré, pers. comm.). Finally, in the Guadiana estuary (37° N), only 20 taxa and 12 families were encountered (Chícharo, pers. comm.). These comparisons must be tempered by differences in sampling gear and effort. Moreover, each estuarine system may have a different abiotic environment (Blaber, 1997), resulting from the tidal range, freshwater input, geomorphology and human pressure (Dyer, 1997; McLusky and Elliott, 2004).

During the study period, no freshwater specimens were collected, probably due to the fact that the Lima estuary is a euhaline system during most of the year. This is consistent with the fact that most freshwater species are usually not capable of osmoregulating in salt water and consequently tend to be found in estuaries only when salinities decline to very low levels during periods of heavy freshwater discharge (Potter and Hyndes, 1999). The lowest mean salinity encountered in this study, 15, was likely too high for freshwater species.

The typical decrease of the number of species in the upstream direction (Steffe and Pease, 1988; Neira et al., 1992; Ré, 1999) was observed in the Lima estuary. The Channel zone was significantly more diverse than the salt marsh zones. This was a consequence of the relatively high number of species with marine origin, captured in this zone, where a connection with the adjacent coastal area exists. In the Channel zone,

turbidity was significantly lower than in the other zones, contributing to the presence of marine species, which have been described as more related to clear waters (Blaber et al., 1997). Moreover, the absence of freshwater species contributed to the low number of species caught in the upstream zones (North and South zones) of the study area.

Overall, diversity of the fish larvae assemblage in the Lima estuary was low, a common feature of these assemblages in temperate systems (Shenker and Dean, 1979; Claridge et al., 1986; Drake and Arias, 1991; Ré, 1999), due to the dominance by a single resident taxa *Pomatoschistus* spp. Several studies in different regions have reported resident species dominance in estuarine ichthyoplankton (Talbot and Able, 1984; Neira et al., 1992; Thayer et al., 1999; Strydom et al., 2003).

*Pomatoschistus* spp. was more abundant in the North and South zones, contributing to significantly higher abundances in the salt marsh zones. However, their wider distribution makes *Pomatoschistus* spp. the representative taxon of all the three study zones (Table 5). This led us to conclude that the differences in their abundance were responsible for spatial differences found between the structure of the larval fish assemblage community in the Channel (low abundances of *Pomatoschistus* spp.) and North and South zones (high abundances of *Pomatoschistus* spp.). At the same time, seasonal differences, such as the dominance of the *Ammodytes tobianus*, during W<sub>2</sub>, induced differences in the assemblage structure. This species was almost absent during the first

Table 4  
Results of one-way ANOSIM (*R* values and significance levels) and SIMPER analysis on the abundance of the top 18 most abundant species from three zones of the estuary (Ch: channel; NZ: North zone; SZ: South zone)

Groups	ANOSIM		SIMPER		
	<i>R</i>	<i>P</i>	Average dissimilarity (%)	Discriminating species	Contribution (%)
Ch vs NZ	0.048	0.001 <sup>a</sup>	78.5	<i>Pomatoschistus</i> spp.	33.7
Ch vs SZ	0.143	0.001 <sup>a</sup>	78.8	<i>Pomatoschistus</i> spp.	33.8
NZ vs SZ	0.005	0.335	66.2	<i>Pomatoschistus</i> spp.	40.9

<sup>a</sup> Significant differences.

Table 5  
Discriminating species of each zone of the estuary (Ch: channel; NZ: North zone; SZ: South zone) using the SIMPER analysis

Average similarity (%)	Ch (17.7)	NZ (32.9)	SZ (34.5)
Contribution (%)			
<i>Pomatoschistus</i> spp.	39.3	81.89	86.6
<i>S. pilchardus</i>	25.5	7.29	5.8
Unidentified Clupeidae	7.3	3.35	
<i>S. melops</i>	6.0		
<i>A. tobianus</i>	5.9		
<i>P. gattorugine</i>	4.5		
<i>S. senegalensis</i>	3.0		
Total	91.6	92.5	92.4



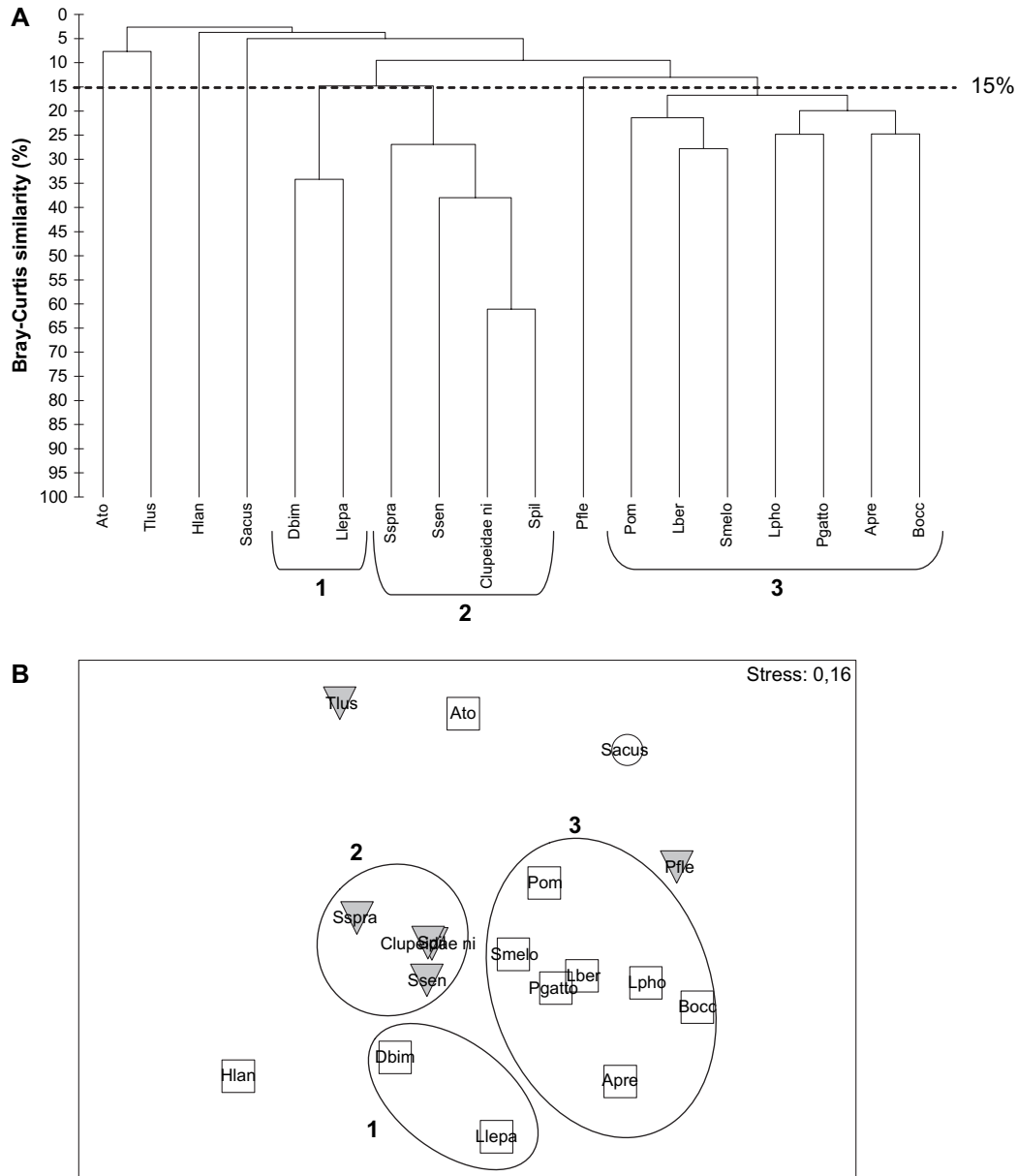


Fig. 5. Dendrogram (A) showing species clustering analysis based on Bray–Curtis similarity matrix of most abundant 18 species, and the ordination in 2-dimensions (B) using MDS on the same similarity matrix. Clusters 1–3 were superimposed on the ordination, being represented by circles (see Table 1 for translation of species codes). White squares, species with demersal eggs; grey triangles, species with pelagic eggs; white circle, brooders species.

year (Fig. 4C), possibly due to interannual variation in the initial arrival time from the spawning area and/or to environmental variability of the estuarine habitat. A similar effect was observed for other species with a short recruitment period such as *Sparus aurata* and *Dicentrarchus labrax*, in Cadiz Bay (Drake and Arias, 1991).

Sardines, which have a coastal spawning pattern (Ré et al., 1990), are not typical users of estuaries in the Iberian Peninsula and their presence is generally considered accidental and largely restricted to mouth portion of estuaries, as a result of tidal transport of coastal water from the adjacent coastal areas (Ré, 1999). In contrast, sardines were caught in all sampling stations of the Lima estuary, throughout the study period, and were the second most abundant species caught in this

study (Fig. 4B). Thus, *Sardina pilchardus* was not an occasional species of the Lima estuary, nor was it restricted to the mouth of the system. The overall high salinity of the estuary could be the possible explanation of this feature, allowing this typical coastal species to extend throughout the Lima estuary. If this high salinity condition is stable, this habitat could be used as a spawning area for *S. pilchardus*.

Most fish larvae were caught in the salt marsh North and South zones, the shallow and less disturbed stretch of the estuary, which could be a way of larvae avoiding getting flushed out to less productive and inhospitable offshore areas where they subsequently die from starvation (Melville-Smith et al., 1981). The upstream zones of Lima estuary were dominated by species derived from demersal eggs (*Pomatoschistus* spp.

and *Ammodytes tobianus*). This reproductive strategy (demersal eggs) has been described as a successful adaptation to estuarine environments by reducing mortality risks associated with the uncontrolled dispersal of eggs and early larvae out of the estuary (Whitfield, 1990). Besides the broad distribution of *Pomatoschistus* spp. along the study area, they were not abundant in the Channel zone, suggesting an affinity to the shallow waters in the salt marsh zones. Alternatively, the low abundance of demersally spawned larvae within the Channel zone might be due to mortality of the eggs, as the channel is heavily dredged. This activity could reinforce the spatial trend of these taxa, once that Channel zone does not constitute an appropriate habitat for species with this type of reproduction strategy.

The Lima estuarine larval fish assemblage was systematically more abundant during spring and summer periods and in the upstream regions (North and South zones) of the estuary. Similar trends were found in other Portuguese estuaries, such as Mondego and Tejo (Ré, 1999). However, in these systems, spring and summer densities were mainly due to the presence of a typical estuarine spawner, *Engraulis encrasicolus*, whose larval stages were caught in high numbers, becoming one of the representative taxa of those habitats (Ribeiro, 1991; Ré, 1999). In Lima estuary, this species was present only occasionally and in low numbers (<1%). Therefore, it is unlikely that this species uses the Lima estuary for spawning. This is an atypical feature, since estuaries along the west coast of Iberian Peninsula were considered as preferential spawning grounds for *E. encrasicolus* (Ré, 1986, 1987; Ribeiro, 1989, 1991). The abundance and distribution of anchovy early life stages seem to be closely dependent on abiotic parameters, namely temperature and salinity (Ré et al., 1983; Ribeiro et al., 1996). Comparing the physical parameters during the spring months, it was observed that the surface water was colder and salinity was higher in Lima estuary than in other estuaries of the same latitude. Dekhnik and Sinyoukova (1964 in Demir, 1974) suggest that European anchovy only forms large spawning shoals in waters of low salinity (7–20). According to these authors, when salinity is similar to the sea water, which was the case of the Lima estuary during the spring and summer seasons, this species tends to spawn in coastal waters.

As in other estuaries (e.g. Whitfield, 1989; Loneragan and Potter, 1990; Drake and Arias, 1991; Barletta-Bergan et al., 2002; Young and Potter, 2003), seasonality was the most general feature observed among the different parameters used to study the larval fish assemblage. In general, differences between seasons are more pronounced than between years. According to Lam (1983), the seasonal water variability in the spawning area has an important influence on the spawning activity, and consequently, on the strong seasonal pattern observed in environments with a nursery function (McErlean et al., 1973). In the Lima estuary, environmental influence was apparently most extensive during the winter, when water parameters, such as temperature and salinity sharply decreased. Probably, the increase in the river flow during the winter freshet period prevents the recruitment of marine species, leading to the decrease in diversity. In this study, the seasonality of the environmental conditions (Fig. 2) co-occurred

with the major variations of the fish larvae assemblage. Seasonal variations occurred not only on total abundance and diversity, but also on the structure of the larval fish assemblage of Lima estuary. Besides seasonal variations, the assemblage also exhibited a defined spatial pattern. Resident species were more abundant in the shallow salt marsh zones, while temporary estuarine residents were primarily caught in the Channel zone, which is immediately connected to the adjacent coastal area. The presence of these species seems to be more related with the spawning seasonality (spring and summer), and also with the clarity of the water. In contrast, the general abundance trends of the resident species were controlled by environmental conditions of the estuary. The winter decrease of *Pomatoschistus* spp. could have been a consequence of the increase in flushing rate and/or due to a decreased food supply. Notwithstanding, the forcing control of environmental factors on the Lima estuarine ichthyoplankton should be assessed with more detail in future research.

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