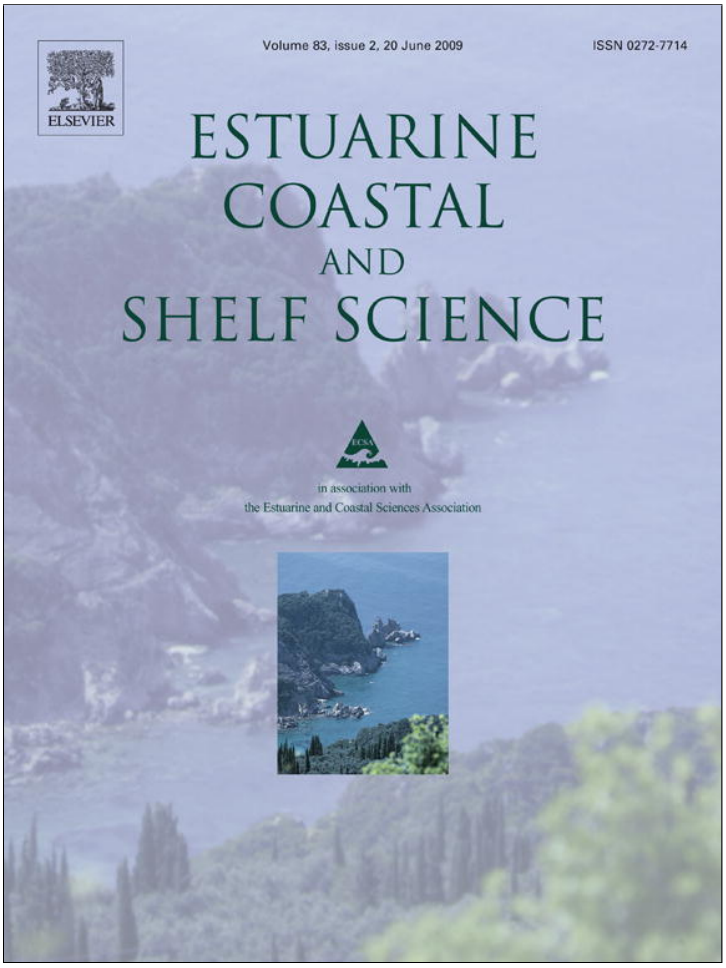
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Environmental control on early life stages of flatfishes in the Lima Estuary (NW Portugal)

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A B S T R A C T

Several flatfishes spawn in oceanic waters and pelagic larvae are transported inshore to settle in the nursery areas, usually estuaries, where they remain during their juvenile life. Nursery areas appear as extremely important habitats, not only for juveniles but also for the earlier planktonic larval fish. Yet, the majority of nursery studies tend to focus only on one development stage, missing an integrative approach of the entire early life that fishes spent within a nursery ground. Thus, the present study assessed the influence of environmental parameters on the dynamics of the larval and juvenile flatfishes, throughout their nursery life in the Lima Estuary. Between April 2002 and April 2004, fortnightly subsurface ichthyoplankton samples were collected and juveniles were collected from October 2003 until September 2005. Larval assemblages comprised nine flatfish species, while only six were observed among the juvenile assemblages. *Solea senegalensis* and *Platichthys flesus* were the most abundant species of both fractions of the Lima Estuary flatfishes. Larval flatfish assemblages varied seasonally, without relevant differences between lower and middle estuary. *Platichthys flesus* dominated the spring samples and summer and autumn periods were characterized by an increase of overall abundance and diversity of larval flatfishes, mainly *S. senegalensis*, associated with temperature increase and reduced river flow. On the contrary, during the winter abundance sharply decreased, as a consequence of higher river run-off that might compromised the immigration of incompetent marine larvae. Juvenile flatfishes were more abundant in the middle and upper areas of the estuary, but the species richness was higher near the river mouth. Sediment type, distance from the river mouth, salinity, temperature and dissolved oxygen were identified as the main environmental factors structuring the juvenile flatfish assemblages. Juveniles were spatially discrete, with the most abundant species *S. senegalensis* and *P. flesus* associated with the middle and upper estuary, while the remaining species were associated with the lower estuarine areas. The larval fraction exhibited distinct dynamics from the juvenile estuarine flatfish community. Larval flatfishes showed a strong seasonal structure mainly regulated by biological features as the spawning season and also by seasonal variations of water characteristics. On the other hand, juvenile flatfishes were markedly controlled by site specific characteristics such as sediments structure, distance from the river mouth and salinity regime. The present study emphasized the idea that the environmental control varies throughout the ontogenetic development, stressing the importance of integrating all the early life of a species in flatfish nursery studies.

1. Introduction

The habitats of fishes vary temporally and spatially, therefore fishes must survive, grow and reproduce in a changing balance of biotic and abiotic environmental conditions (Yamashita et al., 2001). Flatfishes occur throughout the world seas from the subartic to the tropics (Pauly, 1994) with a wide range of spawning seasons, habitat requirements and life history strategies (Minami and Tanaka, 1992; Gibson, 1994). Several flatfishes spawn in marine waters, and their pelagic larvae are transported inshore to settle in the nurseries of shallow coastal and estuarine habitats. Ontogenetic migrations vary among species, and are largely dependent on biological features such as spawning strategies, age and size at metamorphosis, larval behavior (e.g. vertical movement, timing of settlement), and physical conditions such as local topography,

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currents, tide and temperature (Sogard et al., 2001; Bailey et al., 2005).

During metamorphosis, pelagic flatfish larvae undergo substantial changes in their morphology, physiology and ecology, emerging as benthic juveniles. This irreversible shift from pelagic to benthic habitats is considered the most pronounced ecological change of metamorphosis (Inui et al., 1995; Tanaka et al., 1996). According to Yamashita et al. (2001), development of organs, osmeoregulation, behavioural changes and size at metamorphosis are regulated by environmental factors. Settlement should occur in areas where food is abundant and predation risk is low (Gibson, 1999), in order to maximize growth and survival (Gibson, 1994). Thus, the recruitment to a suitable nursery area is crucial for the survivorship of young flatfishes that form the basis of many commercial fisheries (Van der Veer et al., 2001; Reichert, 2003). Thus, environmental factors may be crucial in determining the recruitment to nursery grounds via their effects on growth and mortality during the metamorphosis-settlement period (Yamashita et al., 2001).

Estuaries are essential fish habitats, compiling several functions such as nursery grounds, migration routes and refuge areas for a variety of fish species (Cattrijsse and Hampel, 2006). Estuarine and coastal areas have been recognized as nurseries habitat for many flatfish species (e.g. Able and Fahay, 1998; Le Pape et al., 2003; Gilliers et al., 2004). Estuaries are dynamic environments, where abrupt changes in salinity, temperature, oxygen and turbidity occur due to the influence of tides and the mixing of marine and fresh waters (Vernberg, 1983; Dyer, 1997). Actually, several variables such as temperature (Henderson and Seaby, 1994; Power et al., 2000), salinity (Elliott et al., 1990; Henderson, 1998), dissolved oxygen (Scholz and Waller, 1992; Petersen and Pihl, 1995), turbidity (Marshall and Elliott, 1998; Hostens, 2000), depth and sediment type (Gibson and Robb, 1992; Norcross et al., 1997) have been identified as the dominant environmental factors influencing juvenile flatfish distribution and abundance within nursery grounds.

Nursery areas are extremely important habitats, not only for juveniles but also for the earlier planktonic larval fish. Some species colonize the nursery areas early in life, still as undeveloped larvae, settling within the nursery habitat and remaining there throughout their juvenile phase (Gibson, 1973). Yet, the nursery studies traditionally focus on the juvenile stages, based on the fact that the juvenile phase is often considered to be most dependent on nearshore habitats for use as nurseries (Fodrie and Levin, 2008). Besides the recent efforts on the estimation of the ecological and commercial importance of nurseries to the adult stock management, an integrative approach for all initial development stages, including larval and juvenile fish has still not been contemplated. Accordingly, the present study represents a multispecies comprehensive approach of the species–environmental relationships' changes during the life-cycle, by assessing the influence of environmental parameters on early life stages of flatfishes within a nursery ground. Thus, our aim was to investigate the influence of selected environmental parameters on the dynamics of the larval and juvenile flatfish assemblages associated with the Lima estuarine nursery habitat.

2. Material and methods

2.1. Study area

The Lima Estuary, located in the NW Atlantic coast of Portugal, has a semidiurnal and mesotidal regime (the tidal range is 3.7 m) with an average flushing rate of 0.4 m s^{-1} and an annual average river flow of 70 $m^3 s^{-1}$. Due to the present geomorphology of the system, the lower estuary is highly urbanized, comprising a deep, narrow channel and the river mouth partially obstructed by a 2 km long jetty, deflecting the river flow to the south. Middle estuary is a shallow saltmarsh zone, mainly colonized by sea rush *Juncus* spp., enclosing several sand islands and intertidal channels. The upper estuary is a narrow channel with some intertidal areas and undisturbed banks.

2.2. Sampling strategy

2.2.1. Larval stages

Larval fish assemblages of the Lima Estuary were investigated fortnightly between April 2002 and April 2004 along the initial 7 km stretch of the Lima Estuary. A total of eleven sampling stations within the lower and middle estuary were established for fish larvae (Fig. 1). The first four stations were located in the deep lower estuary (stations 1–4), while the former stations were distributed through the middle estuary (stations 5–11). Subsurface circular tows were performed at a constant velocity of ca. 1 m s^{-1} for 5 min, with a conical 1 m diameter, 3 m long and 500 μm mesh size net. A

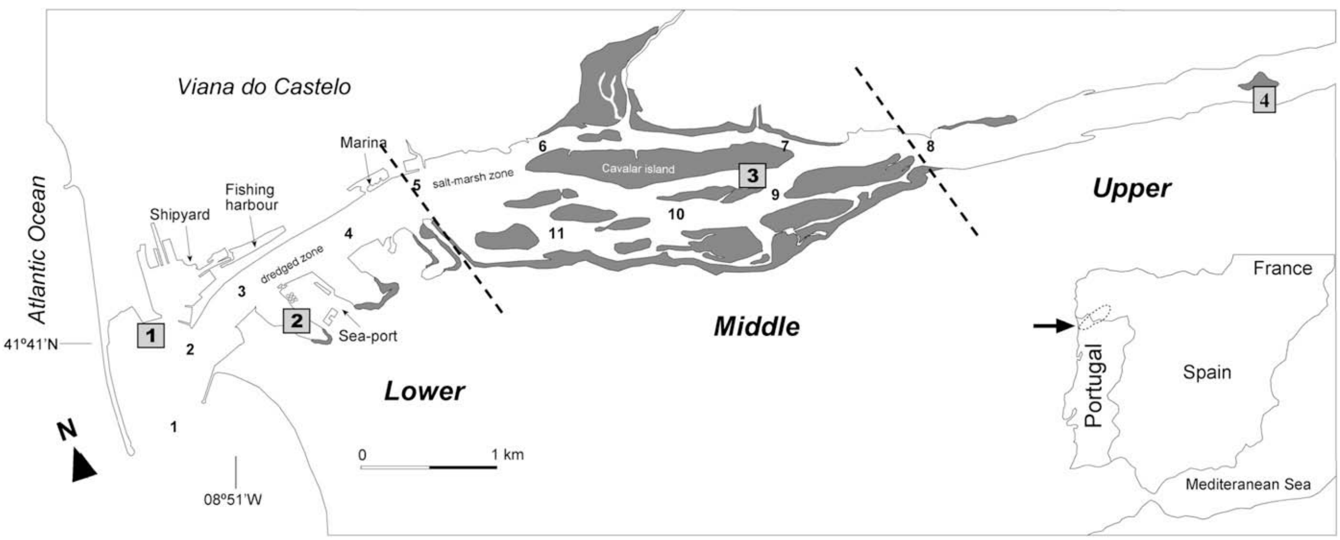

Fig. 1. Lima River estuary and location of the sampling stations were larval (small numbers 1–11) and juvenile flatfishes (numbers in grey boxes 1–4) were collected. Shaded area represents sand islands and saltmarshes.

flowmeter (Hydro-Bios) was attached to the net in order to determine the volume filtered. At each location, vertical profiles of temperature, salinity, oxygen saturation, pH and turbidity were obtained with a YSI 6820 CTD. River flow and precipitation data were collected from the Portuguese Water Institute (http://snirh.inag.pt). Flatfish larvae were sorted and identified to the lowest possible taxon. Abundance data were standardized to larvae 100 m^{-3}.

2.2.2. Juvenile stages

Juveniles were studied at four sampling locations identified from a preliminary survey (data not shown). Station 1 was located in the lower estuary, 0.8 km upstream of the river mouth and near a shipyard (Fig. 1). This area is rather deep (10 m), subjected to frequent dredging. Station 2 was located within a small artificial embayment in the south bank at about 1.5 km from the mouth. Station 3 was located 5 km upstream at the vicinity of a tidal creek in the shallow middle estuary. Station 4 was located in the upper estuary, 10 km upstream of the river mouth and near a tidal sand island. Monthly surveys were conducted between October 2003 and September 2005 with a 1-m beam-trawl with a mesh size of 5 mm and a tickler chain. Hauls were made during daylight at flood tides at an average velocity of 0.8 m s^{-1} for 3 min (each trawl covered in average 140 m^2 of bottom). Samples were refrigerated in boxes with ice and transported to the laboratory where they were immediately frozen until sorting. Due to logistic problems, Stations 3 and 4 during winter 2004/2005 and Station 4 during spring 2005 were not possible to sample. As above environmental parameters were obtained with a YSI 6820 CTD. After sorting, flatfishes were identified to species level and abundance was expressed as fishes 1000 m^{-2}. Geographic location of the sampling stations and distance traveled during each tow was measured by a Magellan 315 GPS.

In November 2004, sediment composition at each sampling station was analyzed. Three sediment samples were taken in each sampling area using a Petit Ponar grab and were sorted for grain size. Sediments were dried at 100 °C and grain size determination was performed by wet (fraction <0.063 mm) and dry (other fractions) sieving (CISA Sieve Shaker Mod. RP.08) of samples previously treated with hydrogen peroxide. The sediments were divided into four fractions: silt and clay (<0.063 mm), fine sand (0.063–0.250 mm), sand (0.250–1.000 mm) and gravel (>1.000 mm). Each fraction was weighed and expressed as a percentage of the total weight.

2.3. Data analyses

Monthly data were averaged by season: autumn (A), winter (W), spring (Sp) and summer (S). Two-way crossed analysis of similarity (ANOSIM) (Clarke and Warwick, 2001) was used to determine the significance of spatial and temporal trends in the structure of the flatfish larval and juvenile assemblages, with season and station as factors. The null hypotheses were that there was no significant difference between flatfish larval and juvenile assemblages between seasons (spring, summer autumn and winter) and sampling stations (lower versus middle estuary for larval flatfish and Stations 1, 2, 3 and 4 for juvenile flatfishes). The tests were based on a Bray–Curtis rank similarity matrix, calculated using log($x+1$) transformed data. Similarity percentages (SIMPER) (Clarke, 1993) were used to reveal the percentage contribution of each species to the average dissimilarity between samples of the various season and station pair combinations. Non-metric multidimensional scaling (MDS), based on Bray–Curtis similarity matrix (Bray and Curtis, 1957) was carried out using log($x+1$) transformed data. Only species with frequency of occurrence higher than 1% were included in the analyses avoiding any undue effect of rare species. Multivariate analyses were performed with the software package PRIMER (Plymouth Routines Multivariate Ecological Research, version 6) (Clarke and Warwick, 2001).

The influence of environmental variables on the flatfish assemblages was assessed with a canonical correspondence analysis (CCA), a multivariate method of direct gradient analysis (Ter Braak, 1986), using the software CANOCO (version 4.5, Microcomputer Power, Ithaca, NY). Flatfish larval abundance was averaged by month and by sampling area (lower and middle estuary). A taxon "No Fish" was created to prevent CANOCO from eliminating samples containing no fish larvae (Grothues and Cowen, 1999). Larval and juveniles 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. For the larval flatfish assemblage the following environmental variables were used: mean temperature, salinity, oxygen saturation, pH, turbidity of the subsurface water layer (0.9–2.1 m); river flow and precipitation; depth of the water column. Considering that all flatfish species caught in the Lima Estuary were spring spawners, spring months were used as spawning season qualitative variable. For juvenile assemblages the environmental variables used were: mean temperature, salinity, oxygen saturation, pH and turbidity of the bottom water layer (1 m above the maximum depth); river flow and precipitation; distance from the river mouth; depth of the water column and the percentage of gravel, sand, fine sand and silt and clay sediments.

3. Results

3.1. Larvae

3.1.1. Environmental conditions

Between April 2002 and April 2004, monthly precipitation varied between 1 and 285 mm, increasing during autumn–winter months, especially in 2002, a highly wet period with a mean precipitation of 160 ± 18 mm. River flow was generally below 50 m^3 s^{-1}, increasing during the winter months, mainly in 2002, when river flow overreached 100 m^3 s^{-1}. Salinity of the subsurface water layer revealed that Lima Estuary was generally polyhaline–euhaline (> 18), with an average salinity of 30.6 ± 6.5, except during the wet winter of 2002, when salinity decreased below 10. Temperature ranged between 9.4 and 19.6 °C, with an average of 14.8 ± 1.9 °C, and exhibited a typical seasonal trend, decreasing during the winter months and increasing during the spring and summer months. Water was well oxygenated with an average saturation of 101.2 ± 10.7% and 8.7 ± 1.1 mg l^{-1} of dissolved oxygen, but >120% during the spring months. The mean pH of 7.8 ± 0.4 tended to decrease during the freshet periods. Turbidity was lower than 7 NTU (mean: 6.8 ± 7.4 NTU), except during the wet winter 2002, when the transparency of the water decreased related to increased run-off.

3.1.2. Temporal and spatial patterns of larval flatfish assemblages

The larval flatfish assemblage included nine taxa, where seven could be assigned to species and two to family. Soleidae, which represented 87% of the total flatfishes, included five species:

Buglossidium luteum, *Microchirus variegates*, *Solea lascaris*, *Solea senegalensis* and *Solea solea* and a taxon that was not identified further, Soldeidae ni. Pleuronectidae comprised 11% of the total flatfish larvae and included *Platichthys flesus* and a taxon that was not identified further, Pleuronectidae ni. Scopthalmidae represented by *Zeugopterus punctatus* totalized 2% of the flatfish larvae assemblage. On a species level, *S. senegalensis* was the most abundant species (71% of the flatfish larvae) and frequently caught (frequency of occurrence of 17% in 489 ichthyoplankton trawls). The second most abundant was *P. flesus* reaching 10% of the total flatfish larvae and was collected with a frequency of 4%. *Solea lascaris* and *S. solea*, which comprised 7% and 4% of the total flatfish larvae, with a frequency of 2% and 1%, respectively. The remaining species were considered rare with frequency of occurrence lower than 1%, and not individually representing more than 4% of the flatfish larval assemblage.

Flatfish larvae were present in 22% of the total ichthyoplankton trawls performed between April 2002 and April 2004. In average flatfish larvae were more abundant during summer 2003 (1.0 ± 1.5 larvae 100 m^{-3}), and in the lower estuary where abundance averaged 0.5 ± 1.4 larvae 100 m^{-3} (Fig. 2). Species composition varied throughout the study period, without relevant differences between lower and middle estuary (Fig. 2), although occasional species such as *Buglossidium luteum*, *Microchirus variegates* were only observed in the lower estuary, contributing to a higher species richness of that area of the Lima Estuary.

MDS plot revealed that samples were seasonally discriminated (Fig. 3a). Samples containing no flatfish larvae, mainly winter samples, were isolated from the remaining samples, which overlapped in a single group (Fig. 3a). In order to visualize this overlapped group, we use a sub-set of MDS that separated those samples according to their sampling season (Fig. 3b). Spring samples clustered on the opposite side of summer and autumn samples. In addition, ANOSIM results showed that larval flatfish assemblages varied significantly between seasons ($R = 0.3$, $p < 0.01$), but without significant differences between the lower and the middle estuary ($R = 0.03$, $p > 0.21$). Larval assemblage of spring months significantly differed from other seasons (Table 1). Spring samples, characterized by high abundances of *Platichthys flesus* (Fig. 3c) were isolated from summer and autumn samples, characterized by higher abundances of the other flatfish species, including *Solea senegalensis* (Fig. 3d). According to SIMPER analysis, the high spring abundance of *P. flesus* was responsible for 26%, 24% and 51% of the average dissimilarity between spring and summer, autumn and winter, respectively. During winter, larval assemblages were also significantly different from the remaining periods of the year (Table 2), what according to SIMPER analysis was due to the sharp decrease of the larval flatfishes, mainly *S. senegalensis*, which was responsible for 25%, 77% and 95% of the average dissimilarity between winter and spring, summer and autumn, respectively.

3.1.3. Relationships with environmental factors

The first CCA axis (eigenvalue = 0.5) and the second CCA axis (eigenvalue = 0.3) exhibited a high species–environment correlation (0.7). These two axes explained 88% of the cumulative percentage variance of species–environment relation and therefore, the latter two CCA axes (CCA_3 and CCA_4) were not interpreted further. The effect of the environmental variables on explained distribution of the CCA axes was significant ($F = 2.6$, $p < 0.01$, Monte Carlo permutation test). Inter-set correlation coefficients showed

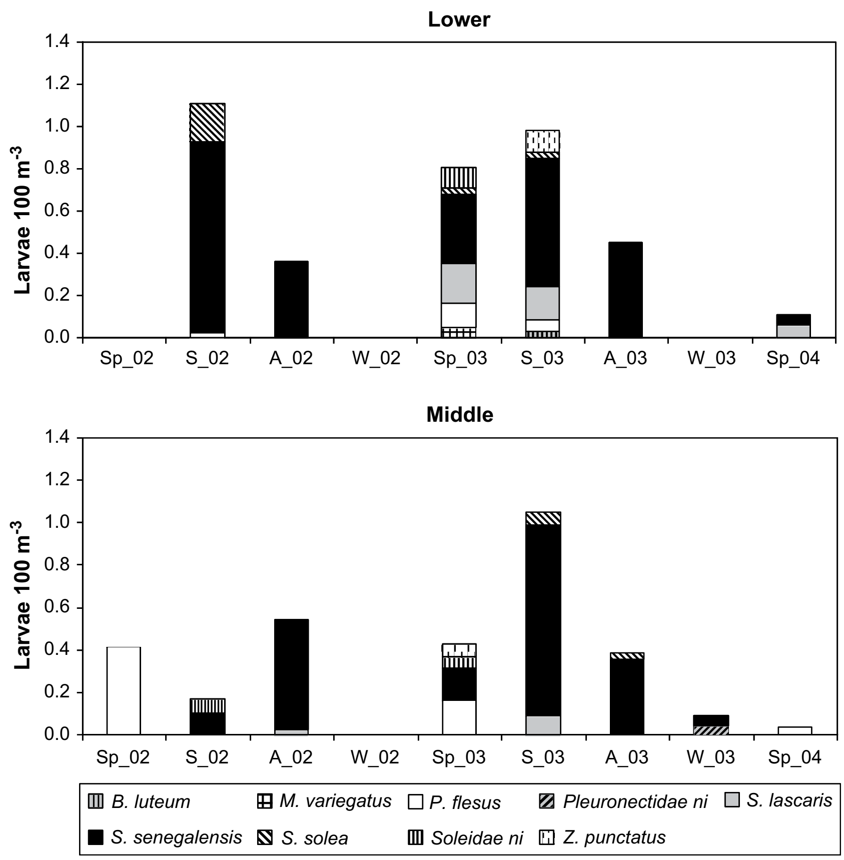

Fig. 2. Seasonal variation of the larval flatfishes in the lower and middle area of Lima Estuary. Sp_02 – spring 2002; S_02 – summer 2002; A_02 – autumn 2002; W_02 – winter 2002/2003; Sp_03 – spring 2003; S_03 – summer 2003; A_03 – autumn 2003; W_03 – winter 2003/2004; Sp_04 – spring 2004.

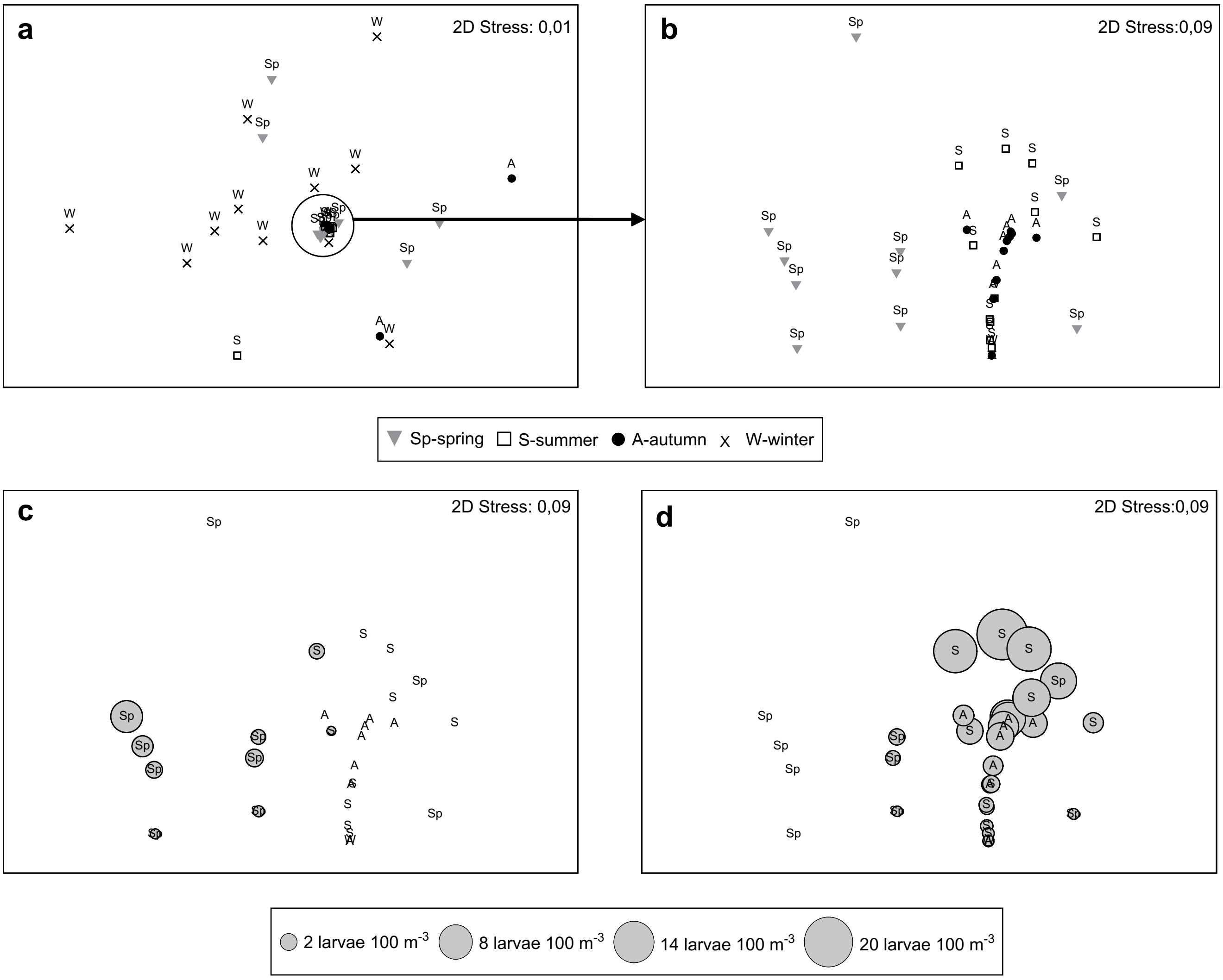

Fig. 3. MDS plot of all larval flatfish samples, showing the cluster of positive samples (samples containing flatfish larvae) (a); MDS sub-set plot of the positive samples (b); superimposition of the abundance bubble of *P. flesus* (c) and *S. senegalensis* (d) on the MDS plot of positive samples.

that river flow and turbidity were positively related with CCA_1, while temperature was negatively correlated with the first CCA axis (Table 2). Samples clustered more or less according to their respective season, with winter and summer samples being separated along the CCA_1. Winter samples with high river flow and consequently high turbidity clustered on the right side of the ordination plot, while summer samples characterized by high temperature clustered on the opposite side of the plot (Fig. 4). Spring and autumn samples clustered in between those two former seasons, and were separated along the CCA_2. Spawning season was positively correlated with CCA_2 (Table 2). Spring samples, with high oxygen saturation clustered in the positive part of CCA_2, while autumn samples with higher precipitation and temperature clustered on the negative part of the CCA_2. The absence of flatfish larvae

Table 1
R-values derived from the two-way crossed ANOSIM test for seasonal and spatial differences between larval flatfish assemblages of the Lima Estuary. **Denotes a significant difference at $p < 0.01$.

Season ($R = 0.3$)**	Spring	Summer	Autumn	Station ($R = 0.03$)
Summer	0.28**			
Autumn	0.32**	0.01		
Winter	0.28**	0.67**	0.54**	

(NO) was associated with the freshet winter period, when river flow increased leading to a high run-off that increased the turbidity of the water. All species clustered on the left side of the plot associated with higher temperatures and lower river flow, and were separated along the second canonical axis. *Solea senegalensis* and *S. solea* were correlated with higher temperatures of summer and autumn samples and therefore clustered on the negative part of CCA_2. The former species clustered on the positive part of CCA_2. *Platichthys flesus*, highly correlated with spawning season and spring samples was isolated from *Zeugopterus punctatus* and *Solea lascaris*, correlated with high oxygen saturation values observed during spring and some summer samples.

3.2. Juveniles

3.2.1. Environmental conditions

Between October 2003 and September 2005, precipitation varied between 1.3 and 286.8 mm, and maximum values were observed during autumn months of 2003 and 2004, when average precipitation overreached 100 mm. On the contrary, the driest periods were winter 2004 and summer 2005, with average precipitation lower than 50 mm. River flow was generally below

Table 2
Inter-set correlations of environmental variables with the first two CCA axes, based on the log-transformed abundance of larval flatfish assemblages of the Lima River estuary. *Inter-set ≥ |0.4| corresponding to biologically important variables.

Environmental variables	CCA_1	CCA_2
Temperature (°C)	−**0.61***	−0.06
Salinity	−0.39	−0.14
Oxygen saturation (%)	−0.26	0.23
pH	−0.21	−0.08
Turbidity (NTU)	**0.41***	−0.05
River flow ($m^3 s^{-1}$)	**0.58***	−0.07
Precipitation (mm)	0.39	−0.13
Depth (m)	−0.05	−0.13
Spawning season	−0.02	**0.57***

$30 m^3 s^{-1}$ and increased during autumn 2003 and winter 2004, reaching the maximum of $79 m^3 s^{-1}$.

During the study period, the typical estuarine horizontal salinity gradient was observed in the bottom water layer. Salinity decreased towards upstream, from the euhaline range of the lower estuary until the oligohaline range (< 5) in the upper section of the estuary. Salinity was seasonally stable at the lower estuarine stations (34.0 ± 4.2 at Station 1 and 28.3 ± 6.6 at Station 2). However, at the inner stations, salinity decreased during autumn–winter period, to a mean of 9.7 ± 9.2 at Station 3 and 0.7 ± 1.0 at Station 4, as a consequence of the increased freshwater influence. Temperature averaged 15.9 ± 2.9 °C, ranging from 8.4 °C (during winter 2003 at Station 4) and a maximum of 23.1 °C during summer 2005 at Station 3. Bottom water temperature exhibited the typical seasonal variation, cooling during winter and warmed up during the spring–summer period, with higher amplitudes in the inner Stations 3 and 4. In general, water was well oxygenated, with a mean of 102 ± 15.3% of dissolved oxygen saturation. The typical spring-summer increase was observed, especially in the inner stations, where dissolved oxygen saturation over reached 120%. On average, pH was seasonally stable, increasing towards upstream, from an average of 7.5 ± 0.3 at Station 1 until 8.1 ± 0.7 at Station 4. Turbidity varied throughout the study, with a tendency for an autumn–winter increase, mainly near the river mouth (Station 1). Although, bottom water was less transparent at middle and upper stations, it was in general <10 NTU, with a mean of 6 ± 3.4 NTU.

Sediments composition varied between sampling stations, although sediments were mainly composed by sand (>50%), except Station 2, which exhibited the most equilibrated sediment composition, with the highest silt and clay fraction (15%). On the other hand, Station 1 exhibited the highest fraction of fine sand (32%) and silt and clay fraction was almost absent (0.1%). Stations 3 and 4 exhibited similar sediment composition, with high percentage of gravel 33% at Station 3 and 44% at uppermost Station 4.

3.2.2. Temporal and spatial patterns of juvenile flatfish assemblages

From a total of 128 trawls, six juvenile flatfish species, belonging to four families were identified. Soleidae represented 55% of the total catch and included two species *Solea senegalensis* and *S. solea*. *Solea senegalensis* was the most abundant, representing 48% of the total catch with a mean abundance of 4.9 ± 9.9 fishes 1000 m^2 and frequency of occurrence of 32%. *Solea solea* achieved 14% of the total catch, being captured with a mean abundance of 1.4 ± 4.0 fishes 1000 m^2 and 13% of occurrence. Pleuronectidae included *Platichthys flesus*, which was the second most abundant reaching 24% of the total catch, and a mean abundance of 2.3 ± 7.3 fishes 1000 m^2 and frequency of occurrence of 13%. Bothidae represented by *Arnoglossus laterna* totalized 8% of the total juveniles and were captured with frequency of 6% and a mean abundance of 1.7 ± 4.8 fishes 1000 m^2. Only one exemplar of *Arnoglossus thori* was collected, with a mean abundance of 0.1 ± 0.7 fishes 1000 m^2.

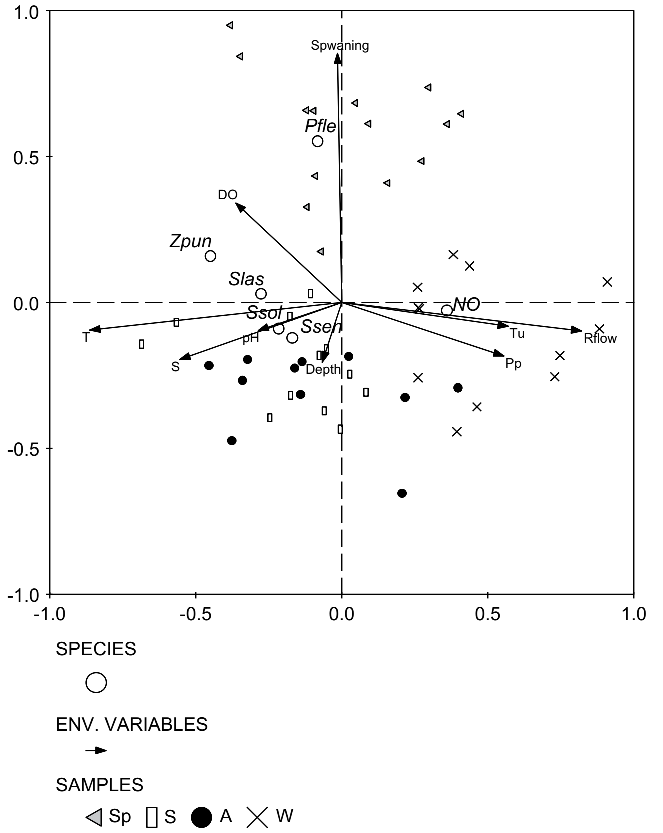

Fig. 4. Ordination diagram for the first two canonical correspondence axes of the canonical correspondence analysis performed to larval fish abundance data. Sp – spring; S – summer; A – autumn; W – winter; *Pfle* – *P. flesus*; *Slas* – *S. lascaris*; *Ssen* – *S. senegalensis*; *Ssol* – *S. solea*; *Zpu* – *Z. punctatus*; NO – no fish. Environmental variables (arrows): T – temperature; S – salinity; DO – oxygen saturation; Tu – turbidity; PP – precipitation; RF – river flow; Depth – depth of the water column; Spawning – spawning season.

Scophtalmidae included *Scophthalmus rhombus*, which represented 6% of the total flatfishes captured in the Lima Estuary and presented a mean abundance of 0.6 ± 2.5 fishes 1000 m^2 and a frequency of occurrence of 7%.

Juvenile flatfish assemblages varied throughout the study period and also between sampling station (Fig. 5). On average, flatfishes were more abundant at the upstream Station 4 (18.0 ± 19.4 fishes 1000 m^{-2}) and the most abundant season was summer of 2005 (18.9 ± 19.5 fishes 1000 m^{-2}), the driest period throughout the study. There was a trend for an upstream loss of species richness, since only three species were observed at the upper Station 4, the most abundant species, *Solea senegalensis*, *Platichthys flesus* and *Solea solea* (Fig. 5).

MDS showed that samples were separated along the horizontal axis of the plot, according to their sampling station and following the estuarine horizontal gradient from lower station 1, to station 2, followed by middle station 3 and finally the uppermost station 4 (Fig. 6). In fact, ANOSIM results showed that juvenile assemblages varied significantly between sampling stations ($R = 0.4$, $p < 0.01$). Upstream Station 4 significantly differed from the remaining areas of the estuary, especially those from the lower estuary, Stations 1 and 2 (Table 3). This station exhibited the highest abundance values, with an average of 18.0 ± 19.4 fishes 1000 m^{-2}, which tended to increase during the summer periods, mainly during 2005, when a maximum of 29.8 ± 22.6 fishes 1000 m^{-2} was observed. SIMPER analysis identified the high abundances of *Platichthys flesus* as responsible for the 33%, 39% and 60% of the average dissimilarity

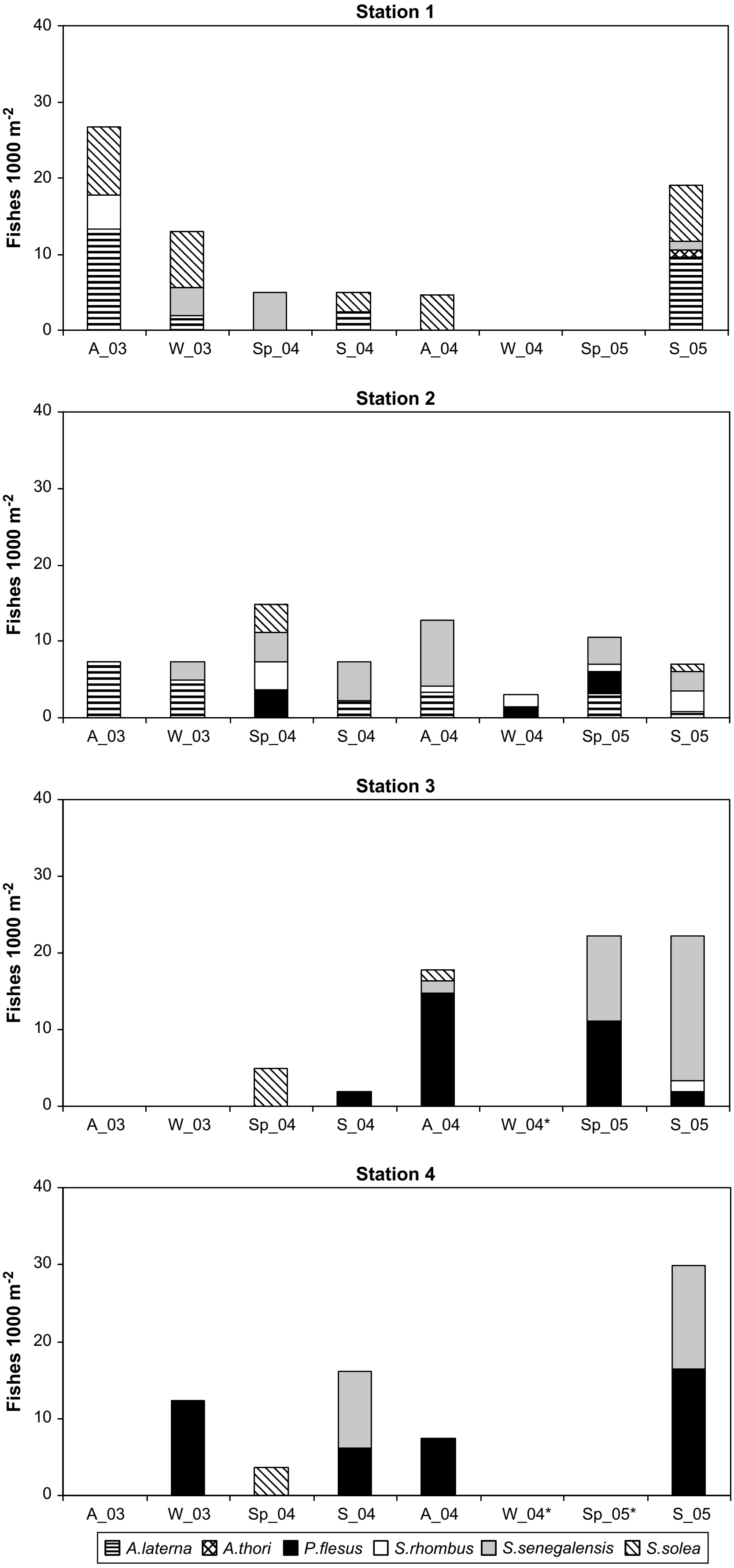

Fig. 5. Seasonal variation of the juvenile flatfish species at each sampling station of the Lima Estuary. A_03 – autumn 2003; W_03 – winter 2003/2004; Sp_04 – spring 2004; S_04 – summer 2004; A_04 – autumn 2004; W_04 – winter 2004–2005; Sp_05 – spring 2005; S_05 – summer. *Without data.

between Station 4 and Station 1, Station 2 and Station 3, respectively. In addition, the high abundances of *Solea senegalensis* at the upper estuary were also responsible for 27%, 25% and 21% of the average dissimilarity between Station 4 and Station 1, Station 2 and Station 3, respectively. The high abundance of *Solea solea* at Station 1 and low abundance at the upper Station 4, contributed with 33% to the averaged dissimilarity between Station 4 and Station 1. ANOSIM results also revealed significant differences between season ($R = 0.3$, $p < 0.01$). During summer the juvenile flatfish assemblages differed significantly from the assemblages during the rest of the year (Table 3). According to SMPER results, that was a consequence of the high abundances of *S. senegalensis* observed during the summer, which was responsible for 36%, 35% and 45% of the average dissimilarity between summer and spring, autumn and winter, respectively.

3.2.3. Relationships with environmental factors

From the original set of 14 environmental variables, depth of the water column and gravel sediment were eliminated from the model due to multicolinearity problems (inflation factor > 20).

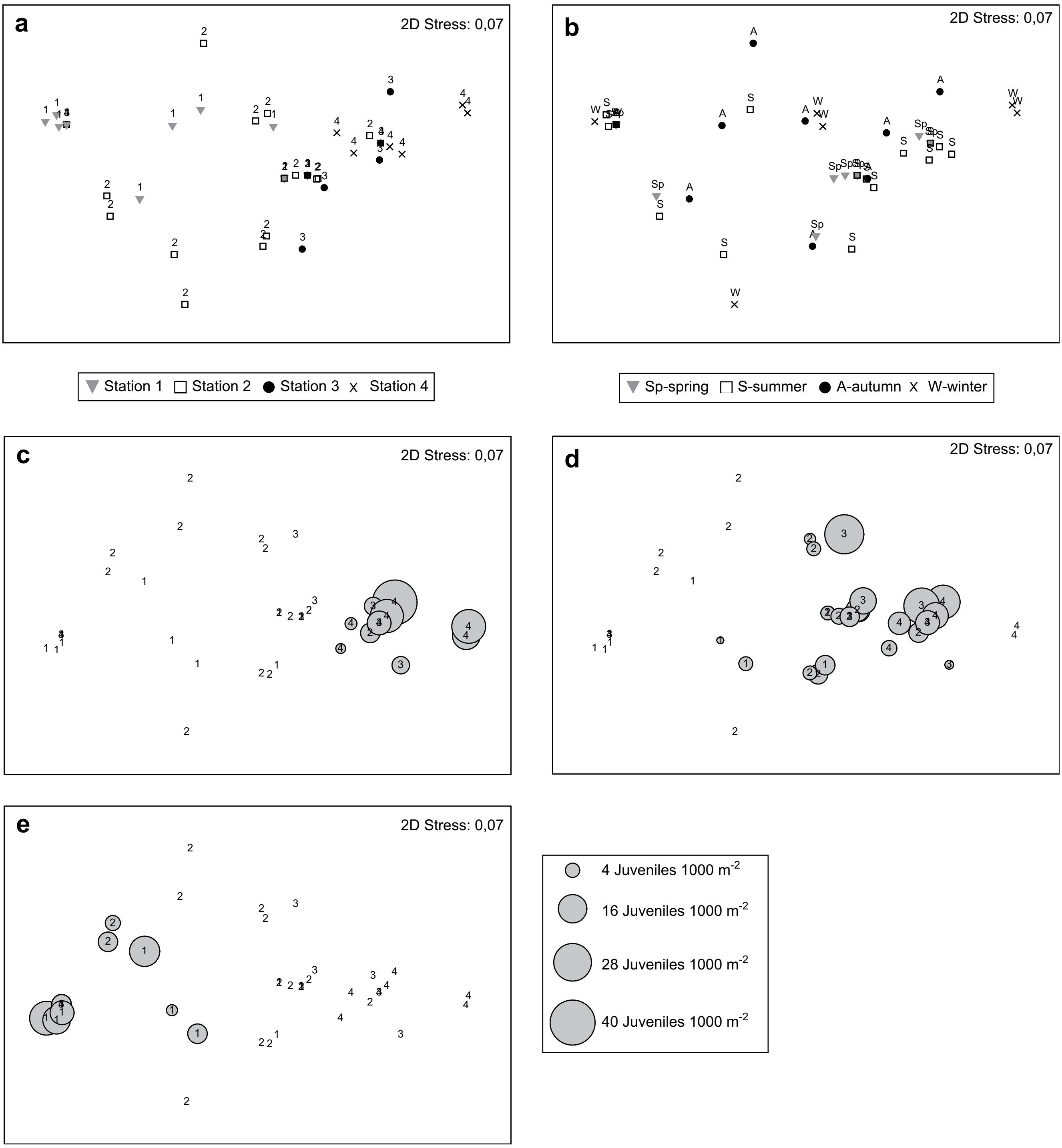

Fig. 6. MDS plot of juvenile flatfish samples according to sampling station (a) and season (b); superimposition of the abundance bubble of *P. flesus* (c), *S. senegalensis* (d) and *S. solea* (e) on the MDS plot.

Table 3
R-values derived from the two-way crossed ANOSIM test for seasonal and spatial differences between juvenile flatfish assemblages of the Lima Estuary. *Denotes a significant difference at $p < 0.05$. **Denotes a significant difference at $p < 0.01$.

Season ($R = 0.3$)**	Spring	Summer	Autumn	Station ($R = 0.4$)**	Station 1	Station 2	Station 3
Summer	0.44*			Station 2	0.08		
Autumn	−0.03	0.28*		Station 3	0.74*	0.18	
Winter	0.21	0.33*	−0.25	Station 4	0.87**	0.39**	0.45*

The first CCA axis (eigenvalue = 0.5), and the second CCA axis (eigenvalue = 0.3) exhibited a high species–environment correlation (0.8 and 0.7, respectively). These two axes explained 90% of the cumulative percentage variance of species–environment relation and therefore, the latter two CCA axes (CCA_3 and CCA_4) were not interpreted further. The effect of the environmental variables on explained distribution of the CCA axes was significant ($F = 14.1$, $p < 0.01$, Monte Carlo permutation test). According to the inter-set correlation coefficients, fine sand and salinity were positively correlated with CCA_1 and distance from the river mouth, dissolved oxygen saturation and temperature were negatively correlated with the first CCA axis (Table 4). Thus, CCA_1 represented the horizontal estuarine gradient, with salinity decreasing with the increase of the distance from the river mouth. Samples clustered more or less according to their respective sampling station. Two different samples' clusters were separated along the CCA_1 (Fig. 7). Samples from the brackish inner stations 3 and 4, where temperature, oxygen saturation reached higher values, clustered on the negative part of the CCA_1. *Platichthys flesus* and *Solea senegalensis* were associated with this group of samples. *Platichthys flesus* was positively correlated with the increase of the river mouth distance and associated with samples of the most upstream Station 4 (Fig. 7). *Solea senegalensis* associated with Station 3 was positively correlated with the increase of temperature. The other group of samples clustered on the positive part of CCA_1 axis representing the lower marine Station 1 with the highest fraction of fine sand (32%). Species that clustered on the positive part of CCA_1 revealed higher influence of the adjacent coastal waters, being positively correlated with salinity and negatively with temperature and dissolved oxygen (Fig. 7). *Arnoglossus laterna* and *Soela solea* were associated with Station 1 samples and correlated with cold and marine waters. On the other hand, *Scophthalmus rhombus* jointly with Station 2 clustered on the negative part of CCA_2. CCA_2, which explained 30% of the explained variance was positively correlated with silt and clay sediments and negatively correlated with sand. Station 2 samples clustered on the positive part of the CCA_2 since at this station silt and clay reached the highest percentage of the sediment composition.

Table 4
Inter-set correlations of environmental variables with the first two CCA axes, based on the log-transformed abundance of juvenile flatfish assemblages of the Lima River estuary. *Inter-set ≥ |0.4| corresponding to biologically important variables.

Environmental variables	CCA_1	CCA_2
Temperature (°C)	**−0.47***	0.14
Salinity	**0.49***	0.08
Oxygen saturation (%)	**−0.49***	−0.09
pH	−0.36	−0.08
Turbidity (NTU)	−0.05	0.12
Distance from the river mouth (km)	**−0.60***	−0.27
River flow ($m^3 s^{-1}$)	0.21	0.00
Precipitation (mm)	0.14	−0.06
% Sand	0.26	**−0.51***
% Fine sand	**0.70***	−0.04
% Silt and clay	0.13	**0.50***

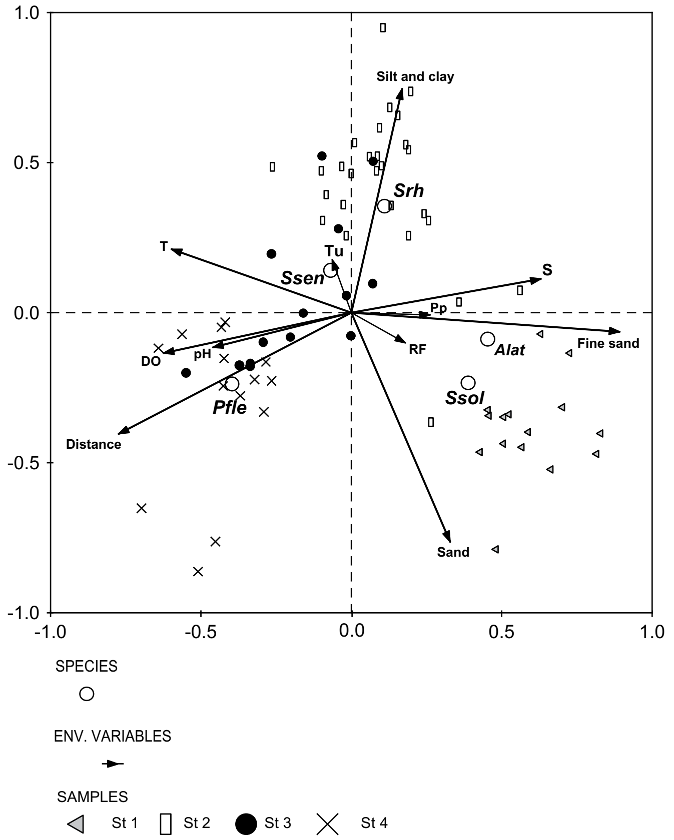

Fig. 7. Ordination diagram for the first two canonical correspondence axes of the canonical correspondence analysis performed to juvenile flatfish abundance data. St1 – Station 1; St2 – Station 2; St3 – Station 3; St4 – Station 4; *Alat* – *A. laterna*; *Pfle* – *P. flesus*; *Srh* – *S. rhombus*; *Ssen* – *S. senegalensis*; *Ssol* – *S. solea*; Environmental variables (arrows): Distance – distance from the river mouth; S – salinity; T – temperature; DO – oxygen saturation; Tu – turbidity; PP – precipitation; RF – river flow.

4. Discussion

The Lima Estuary was used by marine spawning species, whose larvae or juveniles migrated to the estuary, mainly during the spring–summer period. A total of nine flatfish species used the estuary during their pelagic life, while only six species habited the estuary during their juvenile phase. However, from the nine taxa observed, four larval species were considered rare, with frequency of occurrence lower than 1%. Therefore, their presence in the Lima Estuary can be interpreted as accidental and caused by the tidal excursion. There were also observed differences in the species composition of the larval and juvenile assemblages. In fact, only three species were commonly present as larvae and also as juveniles, the two most abundant of both fractions *Solea senegalensis* and *Platichthys flesus* and also *Solea solea*. Although, *S. solea* was not abundant among the larval assemblages, this species was the third most abundant between juvenile flatfishes. The lower larval abundances can be interpreted as a consequence of indirect settlement, meaning that *S. solea* immigrated to the Lima nursery after settlement, already as juveniles. This indirect settlement could have also been the reason for the absence of *Arnoglossus laterna* and *Scophthalmus rhombus* among the larval fraction. On the other hand, the lack of *Solea lascaris* and *Zeugopterus punctatus* among the juveniles corroborates their typical marine habitat. Since these species do not use estuaries as nurseries, the occurrence of their larvae within the Lima Estuary might be interpreted as a consequence of tidal excursion that advected the larvae from the adjacent coastal area to the lower estuary, where these species were more abundant (Fig. 2). So, only the most abundant species, *S. senegalensis* and *P. flesus*, settled within the Lima Estuary, remaining there throughout their juvenile phase.

Larval and juvenile flatfish assemblages presented distinct temporal and spatial patterns. Larval flatfishes exhibited a strong seasonality associated with high abundances over the spring–summer months, in opposition to the drastic abundance decreased observed during winter freshet periods (Fig. 2). The temporal variations of the most abundant species were responsible for this seasonal structure. After a period of larval scarcity observed during the winter, the Lima Estuary was colonized by *Platichthys flesus* larvae during spring (Fig. 3c) and followed by *Solea senegalensis*, mainly during summer–autumn periods (Fig. 3d). In addition, there were also observed some interannual variations among the larval flatfish assemblages. During the second study year, the larval assemblages were in general more abundant and more diverse, mainly in the spring–summer period due to the occurrence of new species such as *Solea lascaris*, the third most abundant species of the larval assemblages (Fig. 2). Previous studies also notified interannual variations in the Lima estuarine ichthyoplankton (Ramos et al., 2006b), associated with interannual climate and hydrodynamic variations (Ramos et al., 2006a). The structure of the juvenile flatfish community also varied throughout time, with high interannual variations on the abundance pattern of each species (Fig. 5). These temporal variations have also been reported in other estuaries (e.g. Marchand, 1988; Potter et al., 2001; Le Pape et al., 2003). Moreover, in the Portuguese Tagus and Sado estuaries, *P. flesus*, *S. solea* and *S. senegalensis* showed a high interannual variability in abundance without a clear trend (Cabral et al., 2007). Interannual variation on spawning and recruitment might be the major cause for those differences between years (Rijnsdorp et al., 1992). The larval supply to the estuarine habitats appears to be of major importance for the control of the abundance of juveniles on a seasonal basis. Adult stocks of many of these species are located over the continental shelf, where spawning takes place (Rijnsdorp et al., 1985; Koutsikopoulos and Lacroix, 1992) and planktonic larvae and or juveniles tend to migrate to coastal areas in order to reach suitable nursery areas (Bailey et al., 2003). Moreover, the environmental conditions at each sampling station also varied between years, which might have intensified the interannual variations of the flatfish community. According to Lam (1983), the seasonal shift of the water characteristics in the spawning area has an important influence on the spawning activity, and consequently, the seasonal pattern observed in environments with a nursery function.

Juvenile flatfishes demonstrated a strong spatial structure, with species exhibiting preferences for specific areas within the estuary. The affinity of *Platichthys flesus* and *Solea senegalensis* with the upper and middle estuary, respectively and also the almost exclusive use of the lower estuary by *Solea solea* were determinant on the spatial structure of juvenile flatfishes (Fig. 6). CCA identified two main groups of species: one represented by the two most abundant species captured mainly in inner areas, namely *S. senegalensis* and *P. flesus* and the other included species that occurred mainly in lower polyhaline estuarine area (Fig. 7). The lower estuary was markedly influenced by the adjacent coastal zone, with an overall salinity higher than 30 (euhaline), with temporal oscillations of the water parameters. This high influence of the adjacent coastal area, allowed coastal species, such as *Arnoglossus laterna* to extend their marine habitat through the lower polyhaline estuary. The exclusive use of the lower estuarine stations by this species is thus understandable (Fig. 5). The upper estuary hosted a different juvenile assemblage (Table 3), and appeared extremely attractive to abundant species, mainly *P. flesus*, which was almost restricted to this section of the estuary (Fig. 6).

Henderson (1989) suggested that discrete communities may be linked with different combinations of salinity and substrate, whereas temperature is responsible for a continuous change in the community. Temperature was one of the most important ecological factors influencing the dynamics of early life stages of flatfishes inhabiting the Lima Estuary (Tables 2 and 4). Actually, temperature has been identified as one the best predictors of abundance of estuarine fish assemblages (Thiel et al., 1995; Marshall and Elliott, 1998), playing a structural and a moderating role in determining flatfish abundances (Power et al., 2000). The influence of temperature derives from endogenous seasonal migrations of larvae, postlarvae and juveniles into the nursery areas that lead to seasonal variations in communities (Potter et al., 1986). *Solea senegalensis*, the most abundant species was positively correlated with temperature. Indeed, the abundance peaks of this species coincided with periods of warmer water, both for the larval as well as for the juveniles. Moreover, both larvae (Fig. 4) and juveniles (Fig. 7) were highly correlated with temperature. According to García-López et al. (2006), the spawning activity, which is regulated by temperature, seems to be maximized at temperature range of 15–21 °C. In fact, peaks of larval abundance were only observed when water temperature overreached 15 °C (Fig. 8). During the study, *S. senegalensis* larvae tended to be more abundant during summer months, but during summer 2002 abundance peaks started latter from August until October 2002 (Fig. 2). This might have been due to the lower water temperatures of June–July, due to strong summer upwelling observed during 2002 (Ramos et al., 2006a). During August–October 2002, several *S. senegalensis* were young larvae still presetting yolk, with less than 30 days (data not shown), what is not in accordance with the spring spawning season described for this species (García-López et al., 2006; Vinagre et al., 2007). This finding accents the need to improve the scientific knowledge of the *S. senegalensis* larval ecology studies.

The distribution and movements of several juvenile flatfish species have been correlated with the salinity regime (Riley et al., 1981; Marchand, 1993). The present study showed that juvenile flatfish assemblages were spatially segregated along the horizontal estuarine salinity gradient (Figs. 6 and 7). Thus, salinity emerged as an important agent for the spatial structure of juvenile flatfishes of Lima Estuary (Table 4), as has been also observed for other estuarine environments (e.g. Kerstan, 1991; Cabral, 2000; Amara et al., 2004). However, the influence of salinity on each species demonstrated some particularities. Results obtained for the Douro estuary, south of the Lima Estuary, showed that *Platichthys flesus* and *Solea solea* were particularly abundant in mesohaline waters, rather than in oligohaline or fresh waters (Vinagre et al., 2005). Contrarily, in

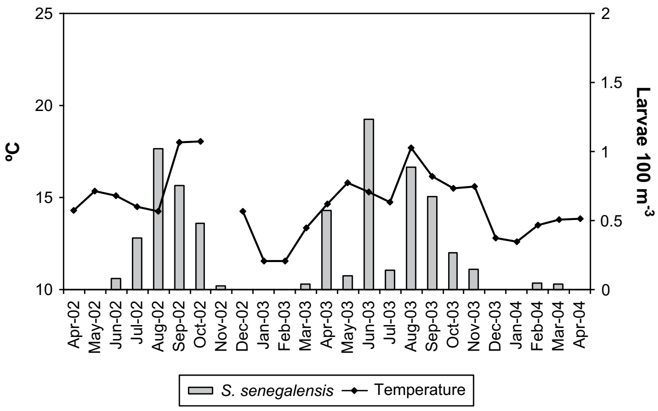

Fig. 8. Monthly distribution of water temperature and *S. senegalensis* larval abundance in the Lima Estuary. There was no temperature data for November 2002.

the Lima Estuary, juveniles of *P. flesus* were restricted to the upstream oligohaline area of the estuary and almost absent from the middle mesohaline zone (i.e. Station 3). In addition, there were also differences on the spatial distribution of *S. solea*, a typical user of estuarine nursery grounds. This species has been associated with inner and consequently less saline areas of Douro (Vinagre et al., 2005), Tagus (Cabral and Costa, 1999) and Sado (Cabral, 2000) estuaries in the W. Portuguese coast. However, in the Lima Estuary, this species was more abundant at the lower estuary, namely Station 1 (Fig. 5), being positively correlated with sand and salinity (Fig. 7). These results are in accordance with other studies that showed that 0-group *S. solea* were associated with areas with a salinity range of 10–33 (Riley et al., 1981), and sandy and muddy sediments (Marchand, 1988; Dorel et al., 1991; Jager et al., 1993). The preference of the lower Station 1 might have been related to the affinity of this species with fine sand sediments that dominated that location. The influence of salinity might have been indirect and a consequence of preferences for particular sediment type (Riley et al., 1981). In fact, CCA results showed that salinity and fine sand were correlated (Fig. 7), which corroborate the indirect influence of salinity.

In estuary-dependent species, salinity conditions, often correlated with sediment structures (fine vs. coarse grain size), have been suggested to be important factors controlling the immigration, settlement and distributions of larvae and juveniles (Burke et al., 1995). Moreover, sediments have been identified as important factors influencing the distribution of juvenile flatfishes, with species associated with a particular sediment type (Gibson and Robb, 1992; Gibson, 1994). The dependence of flatfish on sediments is generally related both to the ability to burying behavior to avoid predators and also to the distribution of suitable epi- and endobenthic prey abundance (Amaral and Cabral, 2004; Walsh et al., 2004). Several authors highlighted the role of sediments composition for the structuring of flatfish communities. For example, Riley et al. (1981) reported the *Solea solea* preferences for sandy and muddy sediments. For this species, Dorel et al. (1991) and Rogers (1992) attributed the patchy distribution within a nursery area to the sedimentary discontinuity. Thus, differences of substrate within each sampling station could induce difference of preys abundance and consequently on flatfish community. Cabral and Costa (1999) also noticed that *Solea solea* and *Solea senegalensis* abundances in the Tagus Estuary were related to sediment and prey abundance. The observed spatial distribution of the Lima juvenile flatfish community could be related to different sediment composition of each local and, consequently, different prey abundances. In fact, the abundance and structure of the Lima macroinvertebrate community, considered as the main food supply for young flatfish (e.g. Costa, 1986; Marchand, 1993; Vinagre et al., 2005) vary between the lower and the middle estuary (Sousa et al., 2006). However, further investigations of the species food habitats are necessary in order to corroborate the hypothesis of the association of flatfishes with a particular area as a consequence of specific prey availability.

Despite the recognized importance of salinity for the structure of estuarine ichthyoplankton assemblages (Rakocinski et al., 1996), salinity was not identified as a relevant environmental control of the larval flatfish assemblages (Table 2), similarly to the general larval fish assemblages of the Lima Estuary (Ramos et al., 2006a). In fact, during the study period, salinity did not exhibited relevant variations between the lower and the middle estuary, always being in the polyhaline–olygohaline range. This lack of spatial salinity variations may justify the absence of spatial differences of the larval flatfish assemblages. Therefore, the overall high salinity profile of the lower (euhaline) and middle (polyhaline) estuary allowed the extension of the marine flatfish larvae throughout the study area. On the other hand, salinity varied seasonally, during the freshet winter of 2002, when salinity decreased below 10, what coincided with the absence of flatfish larvae (Fig. 2). Season oscillations of salinity have been associated with temporal variations on the species composition of estuarine larval fish assemblages, conditioning the presence of salinity-dependent species. For example the salinity reduction as a consequence of the increase of river flow, will change the species composition of the estuarine larval fish assemblages, by restraining the number of marine species and at the same time allowing the occurrence of freshwater species (Barletta-Bergana et al., 2002; Strydom et al., 2003). This was the case of the Lima Estuary, where salinity regime was highly controlled by river flow (Fig. 4). During the winter 2002, river flow overreached 100 $m^3\ s^{-1}$ and conducted to the previously described salinity decreased and also turned a noticeable vertical salinity stratification of the water column (Ramos et al., 2006a). The high run-off observed during this period was associated with the absence of flatfish larvae within the Lima Estuary. During the winter there was an increase of precipitation and river flow, which led to a higher freshwater input in the estuary and consequently an overall decrease of the mean salinity and transparency. According to Ramos et al. (2006a), variations of the river flow affected the hydrological processes that control water exchange between the Lima River estuary and the adjacent coastal area. Taking into account that the spawning of the Lima flatfish species occurs in the ocean, and considering that flatfish larvae migrate passively from the ocean into the estuary with the tide, the high run-off might have compromised this mechanism of immigration into the estuary, especially near the surface. Thus, in periods of high river flow and turbidity, such as the winter of 2002, flatfish larvae were not able to migrate into the estuary. During the following winter (2003), the river run-off was lower (Ramos et al., 2006a), allowing the appearance of few flatfish larvae in the estuary (Fig. 2). River flow was identified as one of the most important environmental controls of the Lima estuarine flatfish larval assemblage (Table 2), and also of the entire Lima estuarine larval assemblages (Ramos et al., 2006a). According to the authors, the increase of the river run-off was responsible for the decrease of the entire larval fish assemblage, including resident species that were exported to the coastal area, and also marine species, which were not capable of immigrate into the estuary due to the hydrodynamic shift caused by the increase of river flow. River flow has been pointed as an important controller of the estuarine nursery recruitment of marine species. Although the high run-off of winter 2002 compromised the penetration of marine larvae inside the estuary, it could also be responsible for the high abundance and diversity observed in the following spring season (spring 2003). One positive effect of river flow deals with the role of river plumes as indicators of the proximity of nursery areas for fish. The extension of river plumes throughout the adjacent coastal area might attract larvae from sea to the estuarine nurseries. Vinagre et al. (2007) highlighted the importance of river drainage on sole colonization of Tagus estuary, possibly due to the existence of chemical cues used by larvae for movement orientation. Ramos et al. (2009) also stressed the importance of river drainage increase of the winter 2002, for the unusual estuarine colonization of *S. pilchardus*. Thus, on the other hand, river flow might also have a parallel negative effect. High river flow drainage can compromise the estuarine penetration of incompetent marine larvae, rendering the estuarine colonization.

Moreover, the larval flatfish assemblages were negatively correlated with turbidity (Fig. 4), which was identified by CCA analysis as ecologically relevant parameter for the larval flatfish dynamics (Table 2). In fact, the absence of flatfish larvae was associated with the high turbidity values, observed during the freshet winter periods (Fig. 4). However, the influence of turbidity on the larval flatfish dynamics should be considered indirect, since the increase of turbidity was a consequence of the increase run-off that compromised the passive tidal immigration of the flatfish larvae. In comparison with the general ichthyoplankton assemblages of the Lima Estuary, which included resident and marine species, the larval flatfish assemblages exhibited a similar seasonal environmental control, with assemblages being more abundant when temperature increased (spring–summer periods) and sharply decreasing during the freshet winter periods, as a consequence of the increase river run-off (Ramos et al., 2006a,b). However, the larval flatfishes showed some particularities, such as being negatively correlated with the turbidity increase caused by the higher river run-off, and being highly correlated with the spring spawning season, that was associated with the first occurrence of larvae coming from the marine spawning ground.

The species–environmental relationships change during the life-cycle (Koubbi et al., 2006). The present study reinforced this idea, because the environmental control of larval flatfishes differed from the juvenile flatfishes. Despite temperature, which was considered an important environmental control of both larval and juvenile flatfish assemblages, each fraction was influenced by different abiotic characteristics associated with each type of environment (Tables 2 and 4). Thus, pelagic larval flatfishes were mainly controlled by seasonal variations of river flow, turbidity and temperature (Table 2); while juveniles were markedly controlled by site specific characteristics, such as type of sediment, distance from the river mouth and subsequently the salinity regime of the benthic environment (Table 4).

5. Conclusions

The present study showed that the species–environmental relationships may vary throughout the ontogenetic development of flatfishes, stressing the importance of the drastic habitat shift that flatfishes undergo throughout their life changing from a pelagic to a benthic habitat. Pelagic larvae revealed a strong seasonal pattern, controlled by the spawning season and also by the temporal variations of water column characteristics, namely river flow, turbidity and temperature. On the contrary, juveniles were temporally more stable, but demonstrated a clear spatial structure, highly correlated with site specific characteristics, mainly the sediment structure and the salinity regime of the habitat. Young larval stages were highly susceptible to temporal habitat oscillations, while juveniles, less vulnerable, already demonstrated habitat preferences. In conclusion, this study highlighted the importance of integrating all the ontogenetic development stages of a species on the nursery flatfish studies.

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References

Able, K.W., Fahay, M.P., 1998. The First Year in the Life of Estuarine Fishes in the Middle Atlantic Bight. Rutgers University Press, New Brunswick, NJ, 342 pp.

Amara, R., Mahé, K., LePape, O., Desroyc, N., 2004. Growth, feeding and distribution of the solenette *Buglossidium luteum* with particular reference to its habitat preference. Journal of Sea Research 51, 211–217.

Amaral, V., Cabral, H., 2004. Ecology of the whiskered sole in the Sado Estuary, Portugal. Journal of Fish Biology 64, 460–474.

Bailey, K., Brown, E., Duffy-Anderson, J., 2003. Aspects of distribution, transport and recruitment of Alaska plaice (*Pleuronectes quadrituberculatus*) in the Gulf of Alaska and eastern Bering Sea: comparison of marginal and central populations. Journal of Sea Research 50, 87–95.

Bailey, K.M., Nakata, H., Van der Veer, H.W., 2005. The planktonic stages of flatfishes: physical and biological interactions in transport process. In: Gibson, R.N. (Ed.), Flatfishes, Biology and Exploitation. Blackwell, Oxford, pp. 94–119.

Barletta-Bergana, A., Barletta, M., Saint-Paula, U., 2002. Structure and seasonal dynamics of larval fish in the Caeté river estuary in North Brazil. Estuarine, Coastal and Shelf Science 54, 193–206.

Bray, J.R., Curtis, J.T., 1957. An ordination of the upland forest communities of southern Wisconsin. Ecology Monographs 27, 325–349.

Burke, J.S., Tanaka, M., Seikai, T., 1995. Influence of light and salinity on behaviour of larval Japanese flounder (*Paralichthys olivaceus*) and implications for inshore migration. Netherlands Journal of Sea Research 34, 59–69.

Cabral, H.N., 2000. Distribution and abundance patterns of flatfishes in the Sado Estuary, Portugal. Estuaries 23, 351–358.

Cabral, H.N., Costa, M.J., 1999. Differential use of nursery areas within the Tagus estuary by sympatric soles, *Solea solea* and *Solea senegalensis*. Environmental Biology of Fishes 56, 389–397.

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

Cattrijsse, A., Hampel, H., 2006. European intertidal marshes: a review of their habitat functioning and value for aquatic organisms. Marine Ecology Progress Series 324, 293–307.

Clarke, K.R., 1993. Non parametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18, 117–143.

Clarke, K.R., Warwick, R.M., 2001. Change in Marine Communities. An Approach to Statistical Analysis and Interpretation, second ed. Primer-E, Plymouth, U.K., 144 pp.

Costa, M.J., 1986. Les poisson de l'estuaire du Tage. Cybium 10, 57–75.

Dorel, D., Koutsikopoulos, C., Desaunay, Y., Marchand, J., 1991. Seasonal distribution of young sole (*Solea solea* (L.)) in the nursery ground of the Bay of Vilaine (Northern Bay of Biscay). Netherlands Journal of Sea Research 27, 297–306.

Dyer, K.R., 1997. Estuaries. A Physical Introduction, second ed. John Wiley and Sons, Chichester, 195 pp.

Elliott, M., O'Reilly, M.G., Taylor, C.J.L., 1990. The forth estuary: a nursery and overwintering area for North Sea fishes. Hydrobiologia 195, 89–103.

Fodrie, F.L., Levin, L.A., 2008. Linking juvenile habitat utilization to population dynamics of California halibut. Limnology and Oceanography 53, 799–812.

García-López, Á., Pascual, E., Sarasquete, C., Martínez-Rodríguez, G., 2006. Disruption of gonadal maturation in cultured Senegalese sole *Solea senegalensis* Kaup by continuous light and/or constant temperature regimes. Aquaculture 261, 789–798.

Gibson, R., Robb, L., 1992. The relationship between body size, sediment grain-size and the burying ability of juvenile plaice *Pleuronectes platessa* L. Journal of Fish Biology 40, 771–778.

Gibson, R.N., 1973. The intertidal movements and distribution of young fish on a sandy beach with special reference to the plaice (*Pleuronectes platessa* L.). Journal of Experimental Marine Biology and Ecology 12, 79–102.

Gibson, R.N., 1994. Impact of habitat quality and quantity on the recruitment of juvenile flatfishes. Netherlands Journal of Sea Research 32, 191–206.

Gibson, R.N., 1999. The ecology of the early life stages of the plaice, *Pleuronectes platessa* L. A review. Bulletin of Tohoku Regional Fisheries Research Laboratory 62, 17–50.

Gilliers, C., Amara, R., Bergeron, J.P., Le Pape, O., 2004. Comparison of growth and condition indices of juvenile flatfish in different coastal nursery grounds. Environmental Biology of Fishes 71, 189–198.

Grothues, T.M., Cowen, R.K., 1999. Larval fish assemblages and water mass history in a major faunal transition zone. Continental Shelf Research 19, 1171–1198.

Henderson, P.A., 1989. On the structure of the inshore fish community of England and Wales. Journal of the Marine Biological Association of the United Kingdom 69, 145–163.

Henderson, P.A., 1998. On the variation in dab *Limanda limanda* recruitment: a zoogeographic study. Netherlands Journal of Sea Research 40, 131–142.

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

Hostens, K., 2000. Spatial patterns and seasonality in the epibenthic communities of the Westerschelde (Southern Bight of the North Sea). Journal of the Marine Biological Association of the United Kingdom 80, 27–36.

Inui, Y., Yamano, K., Miwa, S., 1995. The role of thyroid hormone in tissue development in metamorphosing flounder. Aquaculture 135, 87–98.

Jager, Z., Kleef, H.L., Tydeman, P., 1993. The distribution of 0-group flatfish in relation to abiotic factors on the tidal flats in the brackish Dolard (Ems Estuary, Wadden Sea). Journal of Fish Biology 43, 31–43.

Kerstan, M., 1991. The importance of rivers as nursery grounds for 0- and 1-group flounder (*Platichthy flesus* L.) in comparison to the Wadden Sea. Netherlands Journal of Sea Research 27, 353–366.

Koubbi, P., Loots, C., Cotonnec, G., Harlay, X., Grioche, A., Vaz, S., Martin, C., Walkey, M., Carpentier, A., 2006. Spatial patterns and GIS habitat modelling of *Solea solea*, *Pleuronectes flesus* and *Limanda limanda* fish larvae in the eastern English Channel during the spring. Scientia Marina 70, 147–157.

Koutsikopoulos, C., Lacroix, N., 1992. Distribution and abundance of sole (*Solea solea* L.) eggs and larvae in the Bay of Biscay between 1986 and 1989. Netherlands Journal of Sea Research 32, 321–330.

Lam, T.J., 1983. Environmental influences of gonodal activity in fish. In: Hoar, W.S., Randall, D.J., Donaldson, E.M. (Eds.), Fish Physiology, vol. 9. Academic Press, London, pp. 65–116.

Le Pape, O., Chauvet, F., Desaunay, Y., Guerault, D., 2003. Relationship between interannual variations of the river plume and the extent of nursery grounds for the common sole (*Solea solea* L.) in Vilaine Bay. Effects on recruitment variability. Journal of Sea Research 50, 177–185.

Marchand, J., 1988. Seasonal distribution, growth and ecological role of the juvenile sole, *Solea solea* L., population in the Loire estuary, France. Journal of Fish Biology 33, 229.

Marchand, J., 1993. The influence of seasonal salinity and turbidity maximum variations on the nursery function of the Loire estuary (France). Netherlands Journal of Aquatic Ecology 27, 427–436.

Marshall, S., Elliott, M., 1998. Environmental influences on the fish assemblage of the Humber estuary, UK. Estuarine Coastal and Shelf Science 46, 175–184.

Minami, T., Tanaka, M., 1992. Life history cycles in flatfish from the northwestern Pacific, with particular reference to their early life histories. Netherlands Journal of Sea Research 29, 35–48.

Norcross, B.L., Muter, F., Holladay, B.A., 1997. Habitat models for juvenile pleuronectids around Kodiak Island, Alaska. Fishery Bulletin 95, 504–520.

Pauly, D., 1994. A framework for latitudinal comparisons of flatfish recruitment. Netherlands Journal of Sea Research 32, 107–118.

Petersen, J.K., Pihl, L., 1995. Responses to hypoxia of plaice, *Pleuronectes platessa*, and dab, *Limanda limanda*, in the south-east Kattegat: distribution and growth. Environmental Biology of Fishes 43, 311–321.

Potter, I.C., Bird, D., Claridge, P., Clarke, K., Hyndes, G., Newton, L., 2001. Fish fauna of the Severn Estuary. Are there long-term changes in abundance and species composition and are the recruitment patterns of the main marine species correlated? Journal of Experimental Marine Biology and Ecology 258, 15–37.

Potter, I.C., Claridge, P.N., Warwick, R.M., 1986. Consistency of seasonal changes in an estuarine fish assemblage. Marine Ecology Progress Series 32, 217–228.

Power, M., Attrill, M., Thomas, R., 2000. Environmental factors and interactions affecting the temporal abundance of juvenile flatfish in the Thames Estuary. Journal of Sea Research 43, 135–149.

Rakocinski, C.F., Lyczkowski-Shultz, J., Richardson, S.L., 1996. Ichthyoplankton assemblage structure in Mississippi sound as revealed by canonical correspondence analysis. Estuarine Coastal and Shelf Science 43, 237–257.

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

Ramos, S., Cowen, R.K., Ré, P., Bordalo, A.A., 2006b. Temporal and spatial distribution of larval fish assemblages in the Lima estuary (Portugal). Estuarine Coastal and Shelf Science 66, 303–313.

Ramos, S., Ré, P., Bordalo, A.A., 2009. New insights into the early life ecology of Sardina pilchardus (Walbaum, 1792) in the Northern Atlantic. Scientia Marina 73, 449–459.

Reichert, M.J.M., 2003. Diet, consumption, and growth of juvenile fringed flounder (*Etropus crossotus*); a test of the "maximum growth/optimum food hypothesis" in a subtropical nursery area. Journal of Sea Research 50, 97–116.

Rijnsdorp, A.D., Van Beek, F.A., Flatman, S., Millner, R.N., Riley, J.D., Giret, M., De Clerck, R., 1992. Recruitment of sole stocks, *Solea solea* (L.), in the northeast Atlantic. Netherlands Journal of Sea Research 29, 173–192.

Rijnsdorp, A.D., van Stralen, M., Van der Veer, H.W., 1985. Selective tidal transport of North Sea plaice larvae *Pleuronectes platessa* in coastal nursery area. Transactions of the American Fisheries Society 114, 461–470.

Riley, J.D., Symonds, D.J., Woolner, L., 1981. On the factors influencing the distribution of 0-group demersal fish in coastal waters. Rapport Process-verbaux Réunion. Conseil International pour l'Exploration de la Mer 178, 223–228.

Rogers, S.I., 1992. Environmental factors affecting the distribution of sole (*Solea solea* (L.)) within a nursery area. Netherlands Journal of Sea Research 29, 153–161.

Scholz, U., Waller, U., 1992. The oxygen requirements of three fish species from the German Bight: cod *Gadus morhua*, plaice *Pleuronectes platessa*, and dab *Limanda limanda*. Journal of Applied Ichthyology 8, 72–76.

Sogard, S., Able, K., Hagan, S., 2001. Long-term assessment of settlement and growth of juvenile winter flounder (*Pseudopleuronectes americanus*) in New Jersey estuaries. Journal of Sea Research 45, 189–204.

Sousa, R., Dias, S., Antunes, J.C., 2006. Spatial subtidal macrobenthic distribution in relation to abiotic conditions in the Lima estuary, NW of Portugal. Hydrobiologia 559, 135–148.

Strydom, N.A., Whitfield, A.K., Wooldridge, T.H., 2003. The role of estuarine type in characterizing early stage fish assemblages in warm temperate estuaries, South Africa. African Zoology 38, 29–43.

Tanaka, M., Kawai, S., Seikai, T., Burke, J.S., 1996. Development of the digestive organ system in japonese flounder in relation to metamorphosis and settlement. Marine and Freshwater Behaviour and Physiology 28, 19–31.

Ter Braak, C.J.F., 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. Ecology 67, 1167–1179.

Ter Braak, C.J.F., Smilaeur, P., 2002. CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (Version 4.5). Microcomputer Power, Ithaca, 500 pp.

Thiel, R., Sepúlveda, A., Kafemann, R., Nellen, W., 1995. Environmental factors as forces structuring the fish community of the Elbe estuary. Journal of Fish Biology 46, 47–69.

Van der Veer, H.W., Dapper, R., Whitte, J.I., 2001. The nursery function of the intertidal areas in the western Wadden Sea for 0-group sole *Solea solea* (L.). Journal of Sea Research 45, 271–279.

Vernberg, W.B., 1983. Responses to estuarine stress. In: Ketchum, B.H. (Ed.), Estuaries and Enclosed Seas. Elsevier, Amsterdam, pp. 43–60.

Vinagre, C., Franca, S., Costa, M.J., Cabral, H.N., 2005. Niche overlap between juvenile flatfishes, *Platichthys flesus* and *Solea solea*, in a southern European estuary and adjacent coastal waters. Journal of Applied Ichthyology 21, 114–120.

Vinagre, C., Costa, M.J., Cabral, H.N., 2007. Impact of climate and hydrodynamics on sole larval immigration towards the Tagus estuary, Portugal. Estuarine Costal and Shelf Science 75, 516–524.

Walsh, S.J., Simpson, M., Morgan, J.M., 2004. Continental shelf nurseries and recruitment variability in American plaice and yellowtail flounder on the Grand Bank: insights into stock resiliency. Journal of Sea Research 51, 271–286.

Yamashita, Y., Tanaka, M., Miller, J.M., 2001. Ecophysiology of juvenile flatfish in nursery grounds. Journal of Sea Research 45, 205–218.