

Environmental forcing and larval fish assemblage dynamics in the Lima River estuary (northwest Portugal)

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This study investigated the potential control of selected abiotic parameters on an estuarine larval fish assemblage from the Lima River. Surveys were done fortnightly during spring tides, from April 2002 until April 2004, at 11 stations distributed along the estuary from the mouth to 7 km upstream. The surveys consisted of subsurface plankton tows of 5-min duration using a 1-m diameter, 500- μ m mesh net and coupled with vertical profile measurements of temperature, salinity, dissolved oxygen, pH and turbidity. The Lima River estuary exhibited seasonal vertical stratification of salinity during the winter period, when salinity sharply increased with depth and a layer of fresh water was sometimes present at the surface. Temperature was always vertically stratified. Cooler water was typically found near the bottom of the water column, except during winter, when a thermal inversion occurred. A seasonal decrease in abundance and diversity of the larval assemblage was observed during winter, when fish larvae were almost absent from the plankton collections. Canonical correspondence analysis (CCA) results showed that the first axis represented a temporal gradient and the second axis represented a spatial gradient. Seasonal variations on temperature and precipitation were responsible for the temporal differences on the fish larval assemblages. This study reinforced the concept that interannual climate and hydrodynamic variations have a strong influence on estuarine ichthyoplankton and, consequently, on the recruitment of marine coastal fish populations.

INTRODUCTION

The success of individuals in the early life stages is crucial to the natural equilibrium of the fish adult stocks. Initial development stages of fishes are highly dependent on physical and biological processes. Local hydrological conditions associated with transport processes, seasonal variability, prey and predator densities, and the spawning patterns of adult fishes are identified as factors responsible for the survival and distribution of early life stages of fishes (Gray, 1993; Franco-Gordo *et al.*, 2002). Distribution patterns of the larval stages of teleosts are primarily influenced by spawning time and location (Rakocinski *et al.*, 1996). However, environmental forcing and larval behaviour can produce distinctive ichthyoplankton patterns (Cowen *et al.*, 1993; Sanvicente-Añorve *et al.*, 2000; Hare *et al.*, 2001).

Environmental features may affect communities indirectly by influencing physiological and behavioural responses of organisms and directly by affecting the distribution and abundance patterns of individual species (Moser and Smith, 1993; Pearcy *et al.*, 1996). Salinity and temperature have been shown to play an important role in the occurrence, density and growth of the larval stages of fishes (Haedrich, 1983; Day *et al.*, 1989; Houde, 1989; Rakocinski *et al.*, 1996; Strydom *et al.*, 2003).

Estuaries are ecosystems characterized by environmental fluctuations, 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; McLusky and Elliott, 2004). As such, estuaries are highly dynamic and diverse regions of high productivity, with fish faunas

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that exhibit variable abundance and composition (Gunter, 1961; Haedrich, 1983; Day *et al.*, 1989).

Variation across spatial and temporal scales (Robertson and Duke, 1990; Ferrell *et al.*, 1993; Santos and Nash, 1995), with habitats (West and King, 1996; Jenkins *et al.*, 1997; Peterson and Whitfield, 2000) or with environmental variables (Gelwick *et al.*, 2001; Howell and Simpson, 1994; Griffiths, 2001) creates the distinct larval fish assemblages associated with the dynamic nature of estuaries (Drake and Arias, 1991; Rakocinski *et al.*, 1996; Garcia *et al.*, 2003). Furthermore, it has been shown that other water characteristics also have an important influence on the dynamics of estuarine larval fish assemblages (Bardin and Pont, 2002; Garcia *et al.*, 2003; Berasategui *et al.*, 2004).

The Lima River estuary is located in northwest Portugal. The lower part is heavily modified and includes a large shipyard, a 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 estuary's physical and chemical characteristics or how they affect planktonic organisms. The aim of this study was to investigate the influence of selected environmental parameters on estuarine fish larval assemblages associated with the Lima River estuary.

METHOD

Study area

The Lima River drains 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 km²,

of which 1143 km² are located in Portuguese territory. The temperate Lima River has a small open estuary (Fig. 1), with a semidiurnal and mesotidal regime (3.7 m), with an average flushing rate of 0.40 m s⁻¹ and river flow of 70 m³ s⁻¹ and a hydraulic residence time of 9 days. During spring tides, salt intrusion can extend until 20 km upstream. 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 deep navigation channel and an upstream shallow saltmarsh zone with many longitudinal sandy islands (Fig. 1).

Sampling and sample processing

Between April 2002 and 2004, eleven sampling stations distributed along the study area (Fig. 1) were sampled fortnightly during spring tides. The first four stations (1–4) were located in the navigation channel while the remaining stations (5–11) were distributed in the shallow saltmarsh zone (stations 5–11) (Fig. 1). Subsurface circular tows were performed at a constant velocity of ~1 m s⁻¹ for 5 min, with a conical 1-m diameter, 3-m long and 500-mm mesh size net. A flowmeter (Hydro-Bios) was attached to the net in order to determine the volume of the filtered water. Geographical locations of the sampling stations were provided by a GPS (Magellan 315). At each sampling station, vertical profiles of temperature, salinity, oxygen saturation and concentration, pH and turbidity were obtained by means of a YSI 6820 CTD. River flow and precipitation data were obtained from the Portuguese Water Institute (INAG, 2005). River flow data for the first year were not available. Fish larvae were sorted from the 489 ichthyoplankton

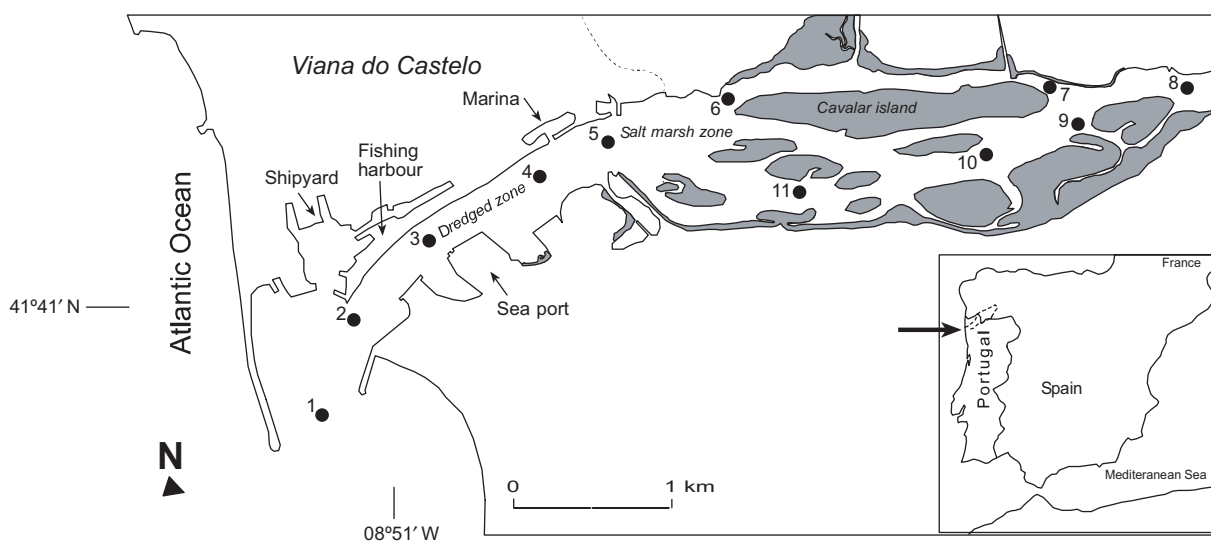


Fig. 1. River Lima estuary and location of the 11 sampling stations. Shaded area represents sand islands and saltmarshes.

samples and identified to the lowest possible taxon. Abundance data were standardized into number of larvae per 100 m³.

Data analysis

Diversity of the larval fish assemblages was expressed using the Shannon Wiener Index (Shannon and Weaver, 1963). Sampling months were aggregated into four conventional seasons: spring (Sp) included March, April and May; summer (S), June, July and August; autumn (A), September, October and November and winter (W), December, January and February. Since 46 surveys were conducted, the first 24 surveys, from April 2002 to the first survey of April 2003, were considered the first year (seasons Sp₁, S₁, A₁, W₁ and Sp₂) and the remaining surveys were the second year (seasons Sp₂, S₂, A₂, W₂ and Sp₃).

The effect of sampling location and season on the subsurface water layer characteristics (0.9- to 2.1-m depth) was analysed using a type II MANOVA with season and station as fixed factors (Sokal and Rohlf, 1995; Zar, 1996). Data were log transformed [Ln(x)], and conclusions from MANOVA results were only accepted for those cases where significance levels were <0.01. Furthermore, in the event of significance, a *posteriori* Tukey HSD for unequal sample sizes was used to determine which means were significantly different at a 0.01 level of probability (Spjøtvoll and Stoline, 1973). Due to a CTD malfunction, no environmental data were available for November 2002.

Species were classified according to their habitat (Russell, 1976; Whitehead *et al.*, 1984; Ré, 1999) into three categories: (i) residents (R)—estuarine species that spend the total life cycle inside the estuary; (ii) seasonal estuarine residents (SR)—marine species that use the estuary as a temporary habitat during a particular period of their life cycle and in a specific time of the year and (iii) occasional species (O)—marine species that enter occasionally in the estuary due to the tidal excursion, being present in low numbers and restricted to the adjacent coastal zone of the estuary.

The effects of environmental variables on larval fish assemblages were analysed 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). The median temperature, salinity, oxygen saturation and concentration, pH, turbidity and water density of the subsurface water layer (0.9–2.1 m) were used as environmental variables. Additionally, monthly river flow and precipitation, distance from the river mouth, water column depth and season (spring, summer, autumn and winter) were also used as environmental

variables. All the 51 taxa were used in the CCA in order to have a comprehensive picture of ichthyoplankton distributions. A taxon 'No Fish' was created to prevent CANOCO from eliminating samples containing no fish larvae (Grothues and Cowen, 1999). Larval abundances were transformed [$\log(x+1)$], and downweighting of rare species was performed. The option used for CCA was biplot scaling with focus on interspecies distances. Forward selection was performed in order to test the statistical significance of environmental variables that contributed most strongly to the canonical model. Selected environmental variables were added to the model when their *F*-ratios were >5% of the simulated *F*-values generated by 99 Monte Carlo permutations (i.e. $P < 0.05$) (Ter Braak and Smilauer, 2002).

RESULTS

Environmental characteristics

Precipitation and river flow exhibited similar temporal trends, with high precipitation and river flow occurring during the winter period. The driest period was during the summer, when river flow decreased dramatically (Fig. 2). During most of the 2-year study period, the Lima River estuary was at least partially mixed (lacked a vertical and horizontal salinity gradient) and euhaline (salinity >30; Fig. 3A). However, during winter and early freshet spring periods, salinity decreased in the upper layers, leading to vertical stratification with a freshwater surface layer (Fig. 3A).

Temperature was always vertically stratified; the strongest gradient occurred during summer and in shallow areas (Fig. 3B). During winter, a thermal inversion occurred, with temperature increasing with depth. During summer of the first study year, a cold and saline water mass entered the Lima River estuary (Fig. 3), probably as a result of an upwelling event in the coastal area. This feature was not detected in the following summer, a non-upwelling year, when temperature reached the highest values of the second study year.

In the subsurface water layer there were temporal (Wilks' lambda = 0.07; $P < 0.01$) and spatial (Wilks' lambda = 0.66; $P < 0.01$) effects on the measured water characteristics (Fig. 4). Salinity, with a mean of 32.7, differed significantly ($P < 0.01$) from zero during W₁ in saltmarsh stations (Fig. 4) and was significantly lower in uppermost station 8. The warmest periods were A₁ (with upwelling) and S₂ (without upwelling). Temperature decreased significantly ($P < 0.01$) during the winter months (W₁ and W₂), reaching a minimum of 9.5°C. Oxygen saturation was, in general, higher than 80%, with a median concentration of 8.6 mg L⁻¹ (Fig. 4).

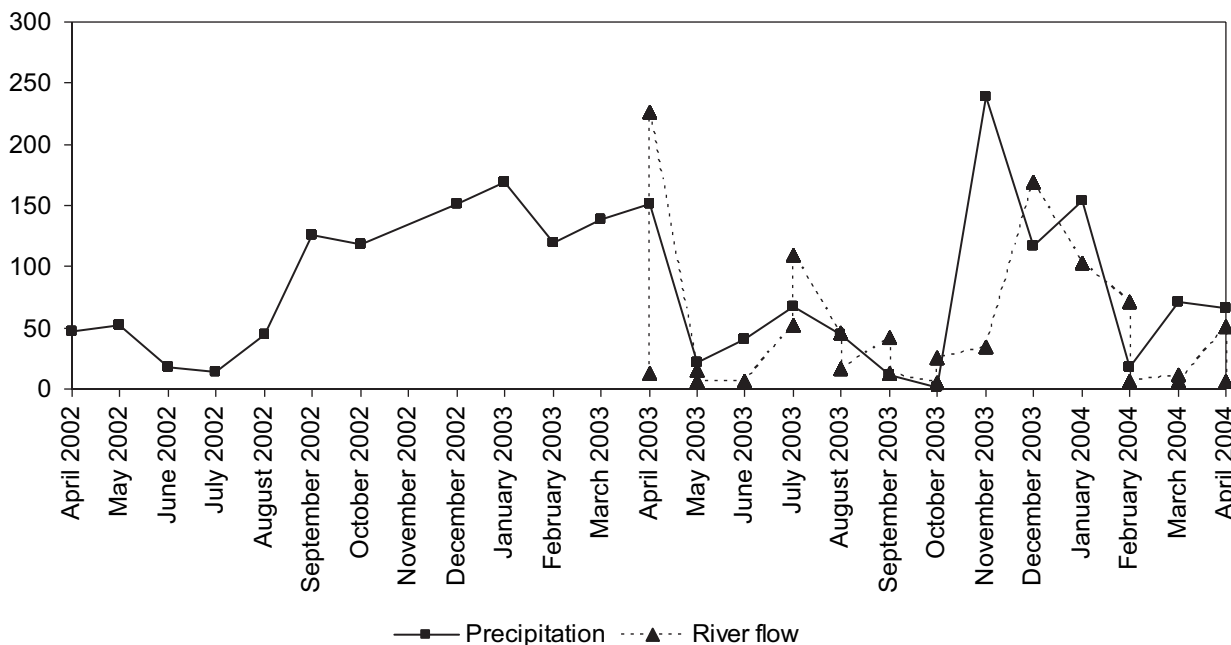


Fig. 2. Precipitation (mm) and river flow (m³ s⁻¹) in the Lima River estuary between April 2002 and 2004.

Lower oxygen values were measured in the deep channel (stations 1–4) during the warmer periods of the year. Median pH was 7.9 and was significantly lower ($P < 0.01$) in the first sampling station and during the freshest period of the second sampling year (W₂ and Sp₃). At station 11, turbidity was significantly higher ($P < 0.01$) than in the deep first four stations and increased significantly ($P < 0.01$) during A₁ and W₁.

Species composition

During the study period, a total of 12 903 fish larvae were collected in the Lima River estuary, with a mean abundance of eight individuals per 100 m³. Twenty fish families were collected. Fifty taxa were identified: 39 taxa were assigned to species, 4 to genus and 7 to family. *Pomatoschistus* spp. was the most abundant and frequent taxa, accounting for 71% of the total fish larvae caught, followed by *Sardina pilchardus* (8.3%), *Ammodytes tobianus* (5.1%), unidentified Clupeidae (3.3%), *Symphodus melops* (2.0%) and *Solea senegalensis* (1.2%) (Table I). These six species comprised 91% of the total catch. From the 39 teleost species identified, 22 were considered to be occasional species, 15 to be seasonal estuarine residents and 2 to be residents (Table I). Monthly mean abundance varied from 0.1 larvae 100 m⁻³ (December 2003) to 139.7 larvae 100 m⁻³ (April 2002), and the Shannon Wiener diversity index ranged from 0 (December 2002, January and December 2003) to 1.2 (February 2004) (Fig. 5). Larval fish abundance showed similar seasonal

trends between the two study years, increasing during spring until summer and then decreasing to the lowest values during the winter period (Fig. 5). Monthly mean diversity also varied, increasing during the warmer periods.

Relationship between fish assemblages and environmental variables

From the original 15 environmental variables, only nine contributed significantly to the explanation of species distribution according to Monte Carlo test of F -ratios ($P < 0.05$) (Table II). The effect of the combined nine variables on explained distribution of the CCA axes was significant as well ($P < 0.01$, Monte Carlo permutation test; Table III). The first CCA axis (Eigenvalue = 0.315) alone modelled 51% of the total explained variance, demonstrating a high species–environment correlation (0.762) (Table III). The second axis represented 24.4% of the explained variance (Table III), while the third and fourth axis additionally explained >16% of the variance each. Since the first two CCA axes explained 75% of the cumulative percentage variance of species–environment relation (Table III), the latter two CCA axes (CCA₃ and CCA₄) were not interpreted further.

The analysis of the ordination diagram (Fig. 6A) showed that the seasonal patterns of temperature and precipitation, highly correlated with the first CCA axis (Table II), represented a temporal gradient. Winter samples (high precipitation and low temperature) clustered

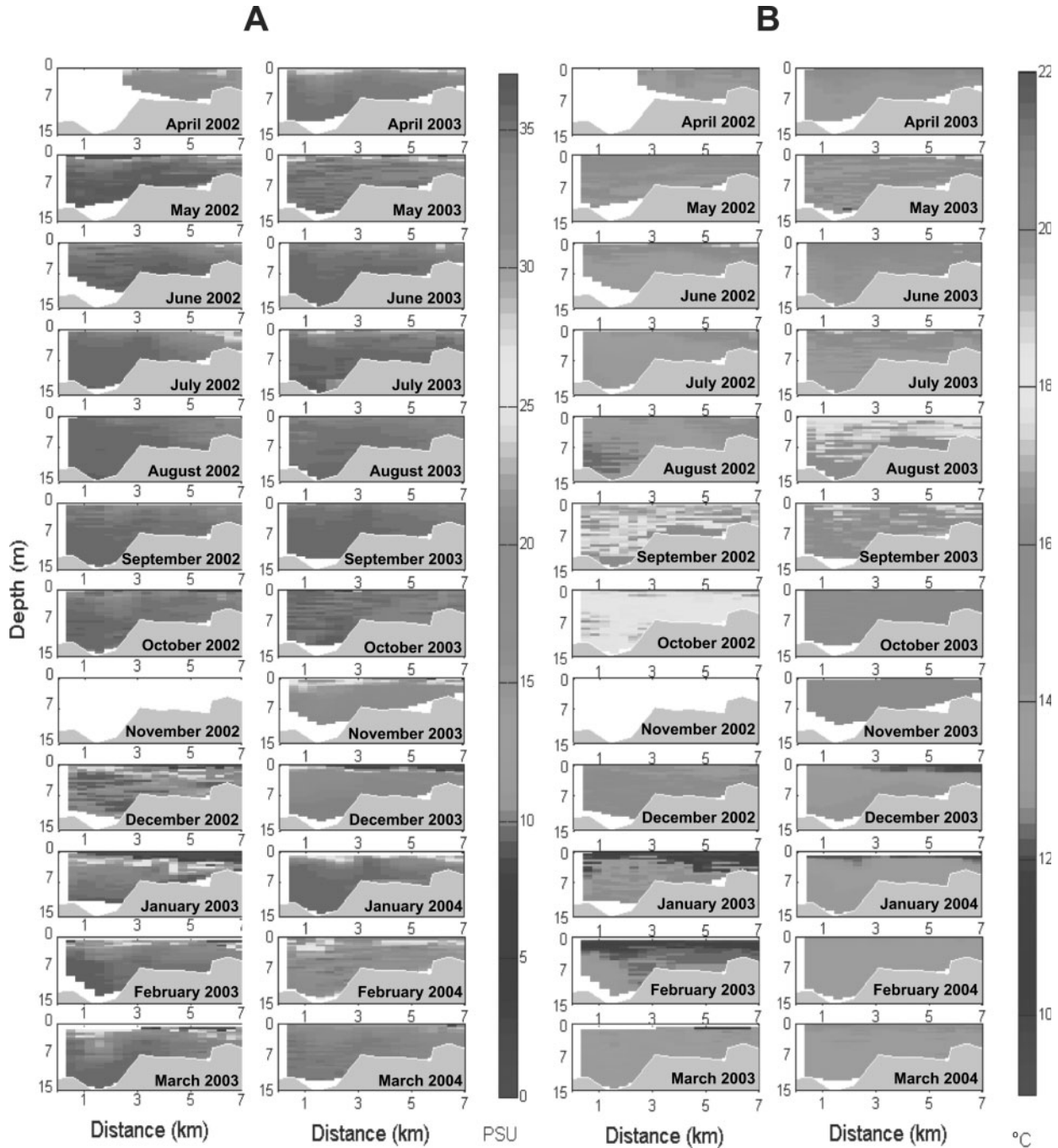


Fig. 3. Vertical profiles of salinity (A) and temperature (B) in the Lima River estuary between April 2002 and March 2004. In November 2002, there were no data. A colour version of Fig. 3 can be found online as supplementary data at <http://plankt.oxfordjournals.org>.

on the right side of the ordination plot, and summer samples (low precipitation and higher temperatures) clustered on the opposite side of the plot (Fig. 6A). The second axis represented a spatial gradient, with distance from the river mouth showing a high correlation with

this axis (Table II). Therefore, samples collected in the upstream area of the estuary (more distant from the river mouth) clustered in the bottom half of the plot, and samples collected near the ocean clustered in the top half of the plot. River flow was also correlated with the

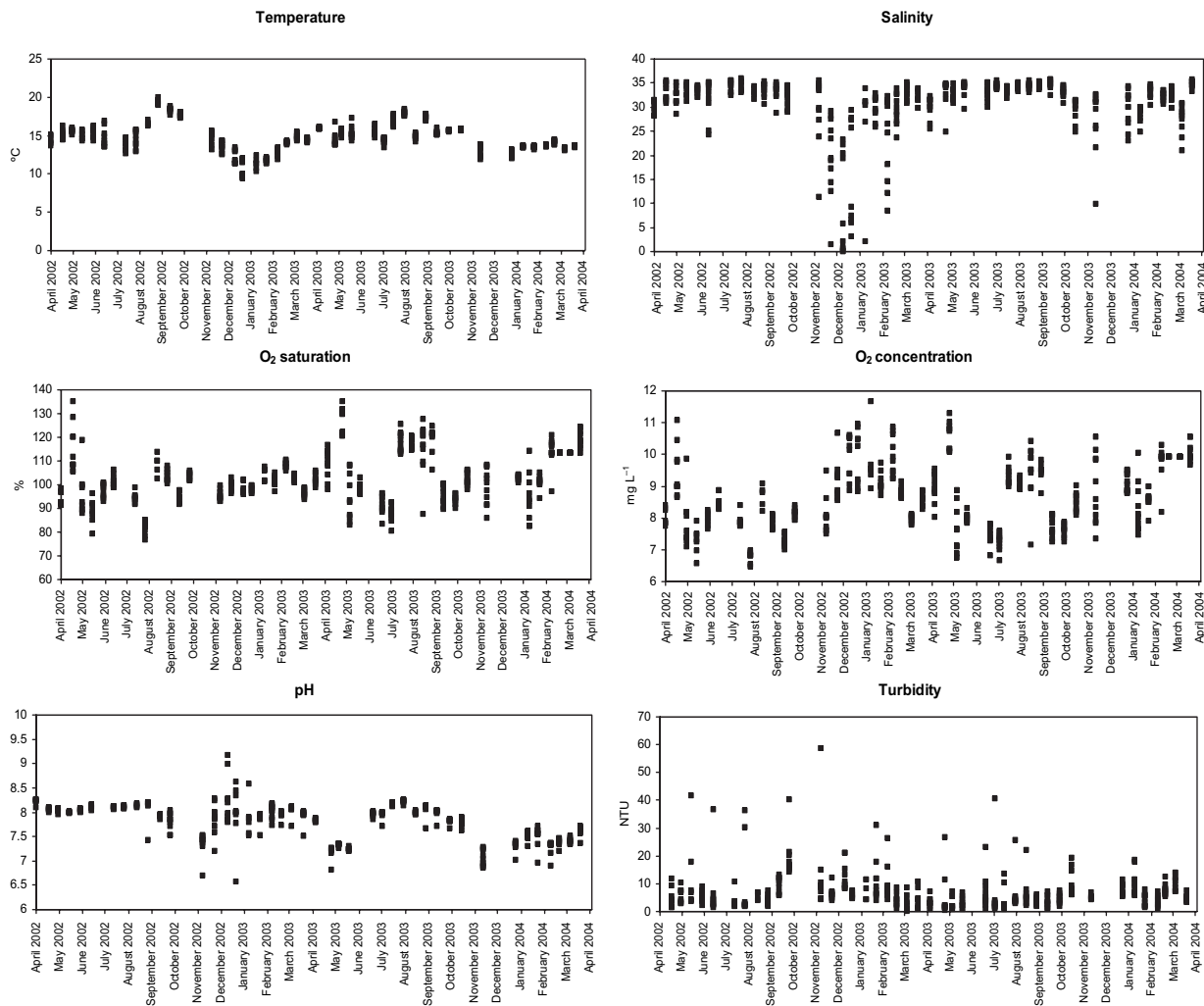


Fig. 4. Monthly results for median temperature, salinity, oxygen saturation and concentration, pH and turbidity observed in the subsurface water layer (0.9- and 2.1-m depth) for all 11 sampling stations together.

second CCA axis (Table II). Spring and autumn samples were distributed along the second axis (CCA₂).

Resident species (*Pomatoschistus* spp. and *Syngnathus abaster*) were correlated with the distance from the river mouth, being more abundant in the upper sampling stations (5–11). Moreover, in periods of low precipitation and moderate water temperature, the abundance of resident species was higher than in other periods (Fig. 6B). The majority of seasonal estuarine resident species was collected in the middle of the 7-km stretch. They were distributed randomly among the four seasons and clustered at the origin of the diagram, reflecting their high association with the grand mean of each environmental variable. Occasional marine species were more concentrated in the upper half of the plot (i.e. near the adjacent coastal area) and never penetrated far into the river. The winter species group was spatially separated, with Pleuronectidae and *Diplodus*

sargus in the stations located in the channel area (1–4) while *A. tobianus* and *Nerophis lumbriciformis* were more abundant in the upstream saltmarsh area (5–11). Clupeidae representatives (*S. pilchardus*, Clupeidae and *Sprattus sprattus*) were typical spring species, occurring in moderate precipitation and water temperature. These species were negatively correlated with distance from the river mouth, since their abundance was higher in the terminal area of the estuary.

DISCUSSION

According to the classification of estuarine zones proposed by McLusky and Elliott (McLusky and Elliott, 2004), the study area did not include the head of the estuary (salinity <5) and only reached the upper section of the estuary (salinity 5–18). During most of the study period, salinity differences between the top and bottom

Table I: Abundance (number larvae $\times 100\text{ m}^{-3}$) and occurrence (number positive hauls) of the 50 taxa used in the canonical correspondence analysis (CCA), from a total of 438 planktonic hauls

Taxa	CCA code	Habitat	Abundance	Occurrence
<i>Pomatoschistus</i> spp.	<i>Pom</i>	R	6137	293
<i>Sardina pilchardus</i>	<i>Spi</i>	O	817	154
<i>Ammodytes tobianus</i>	<i>Ato</i>	SR	494	69
Clupeidae ni	<i>Clu</i>	O	320	96
<i>Symphodus melops</i>	<i>Sme</i>	SR	215	100
<i>Solea senegalensis</i>	<i>Sse</i>	SR	132	71
<i>Labrus bergylta</i>	<i>Lbe</i>	SR	69	39
<i>Lipophrys pholis</i>	<i>Lph</i>	SR	60	43
<i>Parablennius gattorugine</i>	<i>Pga</i>	O	59	53
<i>Sprattus sprattus</i>	<i>Ssp</i>	O	54	27
<i>Blennius ocellaris</i>	<i>Boc</i>	O	36	27
<i>Atherina presbyter</i>	<i>Apr</i>	SR	36	12
<i>Ciliata mustela</i>	<i>Cmu</i>	SR	35	5
<i>Trisopterus lucus</i>	<i>Tlu</i>	O	33	22
<i>Nerophis lumbriciformis</i>	<i>Nlu</i>	O	33	1
<i>Diplecogaster bimaculata</i>	<i>Dbi</i>	SR	26	17
<i>Syngnathus acus</i>	<i>Sac</i>	O	21	23
<i>Platichthys flesus</i>	<i>Pfl</i>	SR	20	17
<i>Coryphoblennius galerita</i>	<i>Cga</i>	SR	19	13
<i>Liparis montagui</i>	<i>Lmo</i>	SR	19	5
<i>Trachinus draco</i>	<i>Tdr</i>	O	18	8
<i>Ctenolabrus rupestris</i>	<i>Cru</i>	O	16	12
<i>Solea lascaris</i>	<i>Sla</i>	O	14	12
<i>Lepadogaster lepadogaster</i>	<i>Lle</i>	O	13	10
Sparidae ni	<i>Spni</i>	O	12	12
<i>Trachurus trachurus</i>	<i>Ttr</i>	O	11	11
<i>Hyperoplus lanceolatus</i>	<i>Hla</i>	O	11	8
<i>Spondyllosoma cantharus</i>	<i>Sca</i>	SR	11	7
<i>Solea vulgaris</i>	<i>Svu</i>	O	10	6
Blenniidae ni	<i>Bni</i>	SR	9	7
<i>Dicentrarchus labrax</i>	<i>Dla</i>	O	7	7
<i>Callionymus lyra</i>	<i>Cly</i>	O	7	6
<i>Centrolabrus exoletus</i>	<i>Cex</i>		7	6
Soleidae ni	<i>Soni</i>	SR	7	5
<i>Engraulis encrasicolus</i>	<i>Een</i>	O	6	7
<i>Callionymus</i> spp.	<i>Cani</i>	SR	6	7
<i>Liza</i> spp.	<i>Liz</i>	O	5	5
<i>Echiichthys vipera</i>	<i>Evi</i>	SR	5	5
Labridae ni	<i>Lni</i>	O	4	13
<i>Syngnathus</i> spp.	<i>Syn</i>	O	4	4
Gadidae ni	<i>Gni</i>		4	3
<i>Nerophis ophidion</i>	<i>Nop</i>	O	3	3
<i>Diplodus sargus</i>	<i>Das</i>	O	2	3
<i>Zeugopterus punctatus</i>	<i>Zpu</i>		2	2
<i>Entelurus aequoreus</i>	<i>Eae</i>	SR	2	2
<i>Syngnathus abaster</i>	<i>Sab</i>	R	2	2
<i>Crystallogobius linearis</i>	<i>Cli</i>	O	1	1
<i>Microchirus variegatus</i>	<i>Mva</i>	O	1	1
Pleuronectidae ni	<i>Pni</i>	SR	1	1
<i>Buglossidium luteum</i>	<i>Blu</i>	O	1	1

Species were classified according to their habitat (Russell, 1976; Whitehead *et al.*, 1984; Ré, 1999). R, estuarine permanent resident species; SR, seasonal estuarine resident; O, occasional marine species; ni, non-identified further.

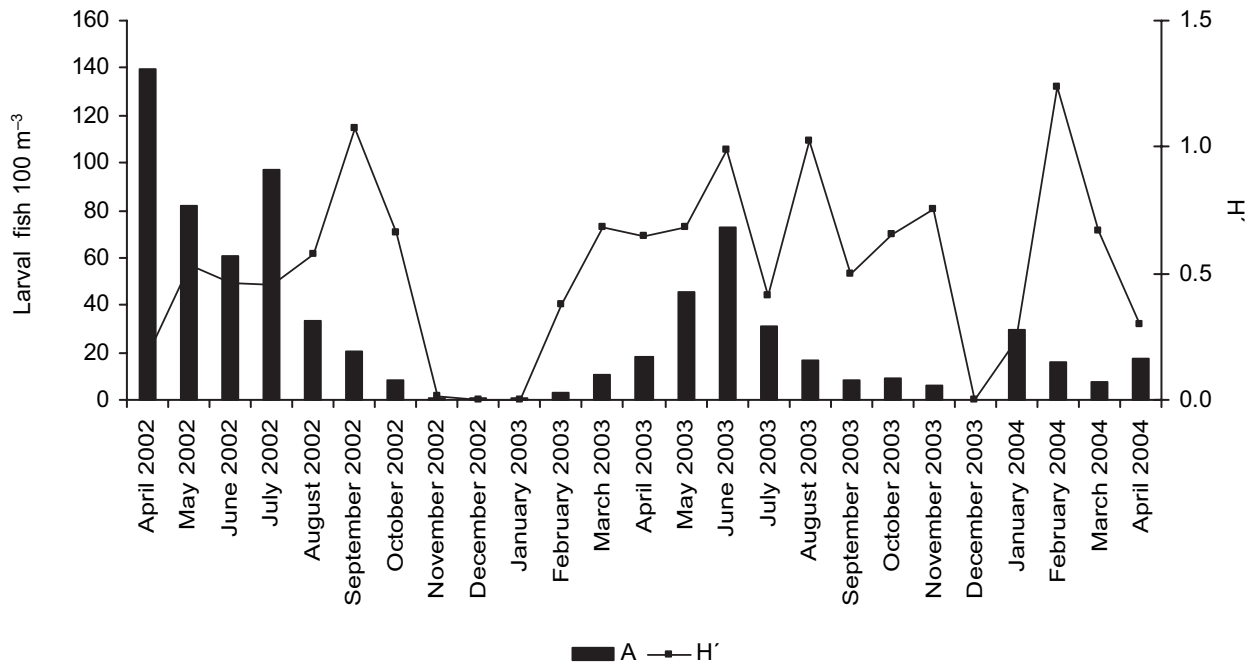


Fig. 5. Monthly mean abundance (A) and Shannon Wiener diversity index (H') of the fish larvae collected in the Lima River estuary, from April 2002 to 2004.

Table II: Biplot scores of the nine significant environmental variables with canonical correspondence analysis (CCA) axes

Name	AX1	AX2	AX3	AX4
River flow	0.150	0.339	0.285	-0.039
Precipitation	0.398	0.375	0.256	0.260
Temperature	-0.659	0.379	0.179	-0.024
Oxygen saturation (DO)	0.038	-0.046	-0.299	-0.285
Spring	0.051	-0.265	-0.391	0.652
Summer	-0.466	-0.200	-0.106	-0.586
Autumn	-0.238	0.507	0.517	0.116
Winter	0.870	0.071	0.095	-0.176
Distance	-0.058	-0.653	0.649	0.011

of the water column were small, indicating that the Lima River estuary was partially mixed, where the tidal range (3.7 m) influence was larger than that of the river flow (annual mean = $70 \text{ m}^3 \text{ s}^{-1}$). However, during winter, an increase of precipitation and consequently of river flow, led to vertical salinity stratification. Dyer (Dyer, 1997) describes this common feature for periods of high river flow, when partially mixed estuaries become highly stratified, and the intensity of the mean circulation diminishes.

Table III: Results of the canonical correspondence analysis (CCA) based on the density-standardized, log transformed, occurrence of 50 taxa in 438 collections from the Lima River estuary

Axes	1	2	3	4
Eigenvalues	0.315	0.151	0.065	0.038
Species–environment correlations	0.762	0.631	0.516	0.495
Cumulative percentage variance				
Of species data	5.4	8.1	9.2	9.8
Of species–environment relation	50.7	75.1	85.5	91.6
Sum of all unconstrained Eigenvalues (total inertia)				5.793
Sum of all canonical Eigenvalues				0.621
Summary of Monte Carlo test				
Test of significance of first canonical axis				
Eigenvalue	0.315			
F-ratio	21.44			
P-value	0.002			

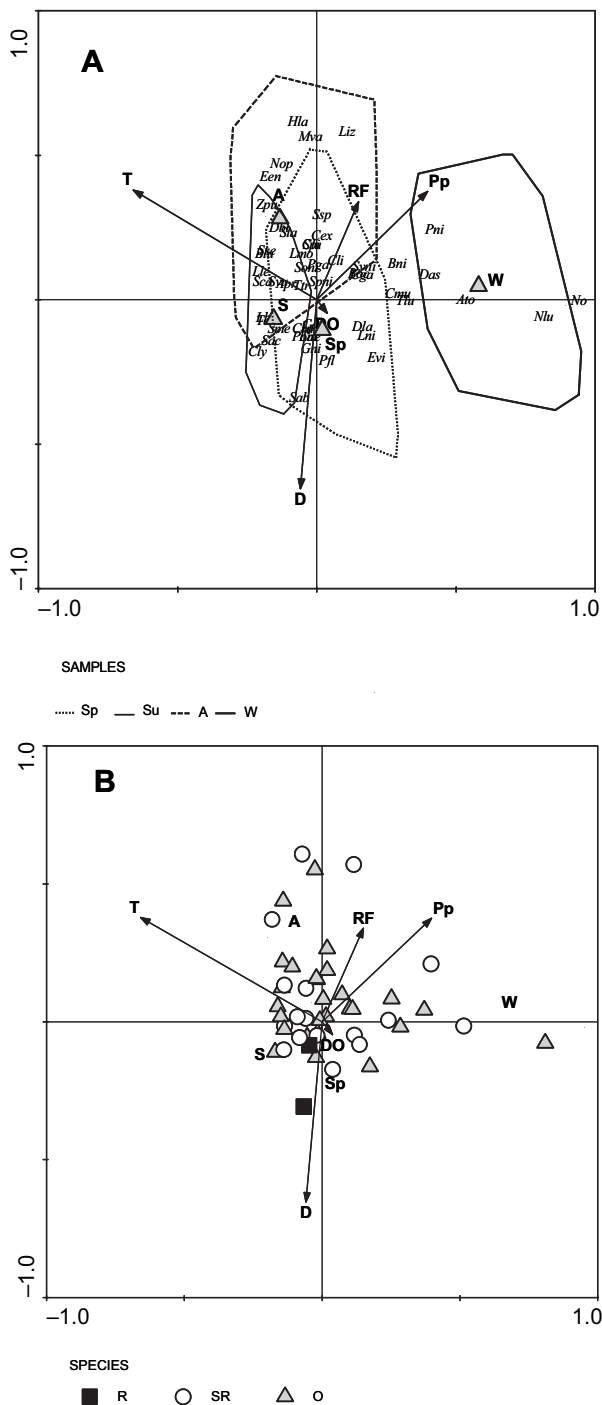


Fig. 6. Canonical correspondence analysis of the larval fish assemblages in Lima River estuary—scores of environmental variables, samples and species in the plane of the first two axes of the canonical correspondence analysis (CCA) ordination. (A) Samples were classified in four seasons (Sp, spring; S, summer; A, autumn and W, winter). (B) Species were classified by habitat (see Table I for species and habitats codes). Environmental variables (arrows): T, temperature; RF, river flow; Pp, precipitation; DO, oxygen saturation and D, distance from the river mouth.

In this study, the typical longitudinal gradient was only detected during winter periods, with fresh water occasionally occurring at the surface of the upstream stations (Fig. 3A). This could be a consequence of the high salt intrusion during periods of average river flow. Additionally, the presence of a deep dredged channel in the terminal area of the estuary could facilitate the penetration of a superior amount of marine water into the estuary, leading to the presence of marine water farther into the estuary.

The Iberian Peninsula Atlantic coast lies at the northern limit of the east central Atlantic coastal upwelling system, which is seasonal (April to September) in this area (Fiúza, 1982). Typically, upwelling takes place along the west coast of Portugal in response to cycles of northerly winds (Fiúza, 1983). During the study period, the upwelling signal was found only in the first study year, in July and August 2002, when cold marine water (salinity of 35 and 12.5–14.0°C) was observed in the estuary (Fig. 3A). During upwelling events, coastal waters are usually pushed offshore, which could delay the recruitment of marine species into the estuary, causing the lower diversity observed in S₁ compared to S₂ (Fig. 5).

Winter samples were totally separated from the remaining seasons (Fig. 6). Winter periods were characterized by a decrease in temperature at the top of the water column, high precipitation and subsequently stronger river flow (Figs 2 and 3B). Concurrently, larval fish abundance decreased drastically (Fig. 5). According to CCA results, temperature, precipitation and river flow were the most important variables affecting seasonal patterns of the larval fish assemblages. Nevertheless, species can be affected differently by the environment, in accordance with their ecological guilds (Drake and Arias, 1991; Strydom *et al.*, 2003). In Lima River estuary, abiotic parameters had different influences on seasonal resident species and on resident species. Since variations of the precipitation regime directly influenced the river flow, precipitation also affected the hydrological processes that control water exchange between the Lima River estuary and the adjacent coastal area. Considering that seasonal species migrate passively from the ocean into the estuary with the tide, the high run-off may have rendered this mechanism of immigration into the estuary ineffective, especially near the surface. Such effects were also found in subtropical estuaries of South America (Barletta-Bergan *et al.*, 2002; Garcia *et al.*, 2003).

During the winter months, the abundance of resident species such as *Pomatoschistus* spp. also decreased. Considering that these taxa can spawn during the entire year (Whitehead *et al.*, 1984), spawning periodicity was not the cause of this abundance reduction. Thus, we hypothesized three different causes for this abundance

reduction that could have worked either separately or in synergy. The first possible cause was the increase in river flow, which could have flushed incompetent fish larvae out of the estuarine habitat. CCA results showed that *Pomatoschistus* spp. were negatively correlated with precipitation and river flow (Fig. 6). Furthermore, greater precipitation during Sp₂ could have been the cause of the low abundances of resident species compared to Sp₁. This is particularly relevant since river flow was extremely high during Sp₂. These taxa peaked in periods of both low precipitation and river flow [i.e. spring and summer during the first study year and summer during the second year (Ramos *et al.*, in press)]. In fact, the highest *Pomatoschistus* spp. abundance (i.e. 739 larvae 100 m⁻³) was recorded in July 2002 (Ramos *et al.*, in press), when precipitation was low (i.e. 13.6 mm; Fig. 3). The second possible cause of low abundances was a downward vertical migration of fish larvae to avoid the cold and less saline surface waters. Because collections were always performed at 1- to 2-m depth, such a migration could explain the absence of *Pomatoschistus* spp. from the winter samples. Several studies have shown that fish larvae can perform vertical migrations, keeping them from being flushed out of the estuarine environment (Weinstein *et al.*, 1980; Rowe and Epifanio, 1994; Schultz *et al.*, 2000). This behaviour of modulating horizontal transport through timed vertical migration has been described for *Pomatoschistus* spp. in a Mediterranean lagoon (Bardin and Pont, 2002). The third cause could have been that larvae died from starvation due to a decrease of food supply. During winter in temperate ecosystems, temperature and light intensity decrease and, consequently, phytoplankton and zooplankton biomass decrease to very low levels (Grindley, 1981; Kennish, 1986). In several estuarine ecosystems, peaks in larval fish abundance seem to be correlated with peaks in phytoplankton production and biomass (Martin *et al.*, 1992; Livingston *et al.*, 1997; Garcia *et al.*, 2003). In the Lima River estuary, maximum abundances of fish larvae were observed during the spring–summer months. During these periods, oxygen saturation in the Lima River estuary reached the highest values (>120%; Fig. 4). According to Bohme (Bohme, 1994), dissolved oxygen has a predictable seasonal pattern with increased supersaturation during spring phytoplankton blooms. Therefore, we can infer that during the spring–summer surface supersaturation months, primary production and phytoplankton biomass increased. This high production would have stimulated the growth and survival of resident and seasonal estuarine species, a pattern especially apparent during the first study year.

Besides the temporal gradient observed in the community structure (e.g. seasonal changes of the water

characteristics and their direct or indirect influences on Lima estuarine larval fish assemblages), a spatial gradient was also evident. Distance from the sea was highly correlated with the second axis of the CCA plot (Table II; Fig. 6), along which spring and autumn samples were distributed. Spring samples clustered in the bottom half of the plot because this season was characterized by high abundances of resident species (e.g. *Pomatoschistus* spp.). Also, a typical estuarine dependent species, *Platichthys flesus* (spawning occurs in the sea, but larvae and juveniles migrate into estuaries, remaining there till the spawning migration), was present in spring samples and in the upstream stations. Conversely, autumn samples were predominantly clustered in the top half of the plot. These samples were dominated by opportunistic and seasonal estuarine resident species, which occurred predominantly in the terminal area of the estuary. Resident and some seasonal resident species were correlated with the distance from the river mouth, revealing an affinity to the upstream stations, located in the saltmarsh area (Fig. 6B). The nursery role of these habitats, usually referred as shelter and food habitats, have been widely described around the world (e.g. Shenker and Dean, 1979; Talbot and Able, 1984; Sogard and Able, 1991).

Some species, such as *Trachinus draco*, *Callionymus byra* and *Trisopterus luscus*, representing >1% of the total catch, were positively correlated with increasing distance from the sea. This suggests that those species used the Lima River estuary as a temporary habitat, acting as seasonal estuarine residents (*T. draco* and *C. byra* during the summer and *T. luscus* during the spring). The findings contradict the ‘occasional’ classification referred to in the literature (Ré, 1999).

The Lima River estuarine larval fish assemblage included a high number of marine species which utilized the Lima River estuary seasonally. In addition, during spring and summer when temperature increased and river flow dropped, several marine species penetrated inside the estuary as a result of greater salt water intrusion. Numerically, the larval fraction of the estuarine fish fauna was dominated by a single resident taxa, *Pomatoschistus* spp. Seasonal trends of temperature, precipitation and river flow were the major forcing of the temporal variations of the Lima estuarine larval fish community. Furthermore, larval fish assemblages were spatially discrete, with a higher number of marine species (seasonal estuarine resident and occasional species) in the vicinity of the river mouth versus few, but highly abundant, resident species in the shallow saltmarsh area of the estuary.

These results suggest that abiotic conditions regulated the larval fraction of the Lima River estuarine ichthyofauna. The environmental control could have acted in a

direct way, by the direct influence that water parameters have on the development rate, growth and mortality of individuals. On the other hand, these parameters could have operated in an indirect way, through their effect on food availability. This study reinforced the concept that interannual climate and hydrodynamic variations have a strong influence on estuarine ichthyoplankton and, consequently, on the recruitment of marine coastal fish populations.

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REFERENCES

- Bardin, O. and Pont, D. (2002) Environmental factors controlling the spring immigration of two estuarine fishes *Atherina boyeri* and *Pomatoschistus* spp. into a Mediterranean lagoon. *J. Fish Biol.*, **61**, 560–578.
- Barletta-Bergan, A., Barletta, M. and Saint-Paula, U. (2002) Structure and seasonal dynamics of larval fish in the Caeté river estuary in North Brazil. *Est. Coast. Shelf Sci.*, **54**, 193–206.
- Berasategui, A. D., Acha, E. M. and Fernández Araoz, N. C. (2004) Spatial patterns of ichthyoplankton assemblages in the Río de la Plata Estuary (Argentina-Uruguay). *Est. Coast. Shelf Sci.*, **60**, 599–610.
- Bohme, M. (1994) Release and consumption of oxygen by a phytoplankton dominated community of an eutrophic lowland river. In Sládecková, A. (ed.), *Congress in Barcelona 1992*. Vol. XVI. E. Schweizerbart Science Publishers, Stuttgart, Germany, pp. 1585–1589.
- Cowen, R. K., Hare, J. A. and Fahay, M. P. (1993) Beyond hydrography: can physical processes explain larval fish assemblages within the middle Atlantic Bight? *Bull. Mar. Sci.*, **53**, 567–587.
- Day, J. W. Jr., Hall, C. A. S., Kemp, W. M. *et al.* (1989) *Estuarine Ecology*. John Wiley and Sons, New York.
- Drake, P. and Arias, A. M. (1991) Composition and seasonal fluctuations of the ichthyoplankton community in a shallow tidal channel of Cadiz Bay (S.W. Spain). *J. Fish Biol.*, **39**, 245–263.
- Dyer, K. R. (1997) *Estuaries. A Physical Introduction*, 2nd edn. John Wiley and Sons, Chichester, UK.
- Ferrell, D. J., McNeill, S. E., Worthington, D. G. *et al.* (1993) Temporal and spatial variation in the abundance of fish associated with the seagrass *Posidonia australis* in south-eastern Australia. *Aust. J. Mar. Freshw. Res.*, **44**, 881–899.
- Fiúza, A. F. G. (1982) The Portuguese coastal upwelling system. In Fiúza, A. F. (ed.), *Proceedings of the Symposium on Actual Problems of Oceanography in Portugal*. JNICT/NATO Marine Science Panel, Lisboa, pp. 45–71.
- Fiúza, A. F. (1983) Upwelling patterns off Portugal. In Suess, E. and Thiede, J. (eds), *Coastal Upwelling. Its Sediment Record*. Plenum Press, New York, pp. 85–98.
- Franco-Gordo, C., Godínez-Domínguez, E. and Suárez-Morales, E. (2002) Larval fish assemblages in waters off the central Pacific coast of Mexico. *J. Plankton Res.*, **24**, 775–784.
- Garcia, A. M., Vieira, J. P. and Winemiller, K. O. (2003) Effects of 1997–1998 El Niño on the dynamics of the shallow-water fish assemblage of the Patos Lagoon Estuary (Brazil). *Est. Coast. Shelf Sci.*, **57**, 489–500.
- Gelwick, F. P., Akin, S., Arrington, B. A. *et al.* (2001) Fish assemblage structure in relation to environmental variation in a Texas Gulf Coastal Wetlands. *Estuaries*, **24**, 285–296.
- Gray, C. A. (1993) Horizontal and vertical trends in the distribution of larval fishes in coastal waters off central New South Wales Australia. *Mar. Biol.*, **116**, 649–666.
- Griffiths, S. P. (2001) Factors influencing fish composition in an Australian intermittently open estuary. Is stability salinity-dependent? *Est. Coast. Shelf Sci.*, **52**, 739–751.
- Grindley, J. R. (1981) Estuarine plankton. In Day, J. H. (ed.), *Estuarine Ecology: With Particular Reference to Southern Africa*. A.A. Balkema, Rotterdam, pp. 117–146.
- Grothues, T. M. and Cowen, R. K. (1999) Larval fish assemblages and water mass history in a major faunal transition zone. *Cont. Shelf Res.*, **19**, 1171–1198.
- Gunter, G. (1961) Some relations of estuarine organisms to salinity. *Limnol. Oceanogr.*, **6**, 182–190.
- Haedrich, R. L. (1983) Estuarine Fishes. In Ketchum, B. H. (ed.), *Estuaries and Enclosed Seas. Ecosystems of the World*, 26. Elsevier Scientific, New York, pp. 183–207.
- Hare, J. A., Fahay, M. P. and Cowen, R. K. (2001) Springtime ichthyoplankton of the Slope Sea: larval assemblages, relation to hydrography and implications for larval transport. *Fish. Oceanogr.*, **10**, 164–192.
- Houde, E. D. (1989) Comparative growth, mortality, and energetics of marine fish larvae: temperature and implied latitudinal effects. *Fish. Bull.*, **87**, 471–495.
- Howell, P. and Simpson, D. (1994) Abundance of marine resources in relation to dissolved oxygen in Long Island Sound. *Estuaries*, **17**, 394–402.
- INAG (2005) <http://www.inag.pt>.
- Jenkins, G. P., May, H. M. A., Wheatley, M. J. *et al.* (1997) Comparisons of fish assemblages associated with seagrass and adjacent unvegetated habitats of Port Philip Bay and Corner Inlet, Victoria, Australia. With emphasis on commercial species. *Est. Coast. Shelf Sci.*, **44**, 59–588.
- Kennish, M. J. (1986) *Ecology of Estuaries. Vol. II. Biological Aspects*. CRC Press, Boca Raton, Florida.
- Livingston, R. J., Niu, X., Lewis, F. G. *et al.* (1997) Freshwater input to a gulf estuary: long-term control of trophic organization. *Ecol. Appl.*, **7**, 277–299.
- Martin, T. J., Cyrus, D. P. and Forbes, A. T. (1992) Episodic events: the effects of cyclonic flushing on the ichthyoplankton of St. Lucia estuary on the southern coast of Africa. *Neth. J. Sea Res.*, **30**, 273–278.

- McLusky, D. S. and Elliott, M. (2004) *The Estuarine Ecosystem*. John Wiley and Sons, New York.
- Moser, H. G. and Smith, P. E. (1993) Larval fish assemblages of the California current region and their horizontal and vertical distributions across a front. In advances in the early life history of fishes. *Bull. Mar. Sci.*, **53**, 645–691.
- Pearcy, W. G., Fisher, J. P., Anma, G. *et al.* (1996) Species associations of epipelagic nekton of the North Pacific Ocean, 1978–1993. *Fish. Oceanogr.*, **5**, 1–20.
- Peterson, A. W. and Whitfield, A. K. (2000) Do shallow water habitats function as refugia for juvenile fishes? *Est. Coast. Shelf Sci.*, **51**, 359–364.
- Rakocinski, C. F., Lyczkowski-Shultz, J. and Richardson, S. L. (1996) Ichthyoplankton assemblage structure in Mississippi sound as revealed by canonical correspondence analysis. *Est. Coast. Shelf Sci.*, **43**, 237–257.
- Ramos, S., Cowen, R. K., Ré, P. *et al.* (2005) Temporal and spatial distribution of larval fish assemblages in the Lima estuary (Portugal). *Est. Coast. Shelf Sci.*, in press.
- Ré, P. (1999) Ictioplâncton estuarino da Península Ibérica. Guia de identificação dos ovos e estados larvares planctónicos. In *Câmara Municipal de Cascais*, Cascais, 163 pp.
- Robertson, A. I. and Duke, N. C. (1990) Mangrove fish-communities in tropical Australia: spatial and temporal patterns in densities, biomass and community structure. *Mar. Biol.*, **104**, 369–379.
- Rowe, P. M. and Epifanio, C. E. (1994) Tidal stream transport of weakfish larvae in Delaware Bay, USA. *Mar. Ecol. Prog. Ser.*, **110**, 105–114.
- Russell, F. S. (1976) *The Eggs and Planktonic Stages of British Marine Fishes*. Academic Press, London.
- Santos, S. R. and Nash, R. D. M. (1995) Seasonal changes in a sandy beach fish assemblage at Porto Pim, Faial, Azores. *Est. Coast. Shelf Sci.*, **41**, 579–591.
- Sanvicente-Añorve, L., Flores-Coto, C. and Chiappa-Carrara, X. (2000) Temporal and spatial scales of ichthyoplankton distribution in the southern Gulf of Mexico. *Est. Coast. Shelf Sci.*, **51**, 463–475.
- Schultz, E. T., Cowen, R. K., Lwiza, K. M. *et al.* (2000) Explaining advection: do larval bay anchovy (*Anchoa mitchilli*) show selective tidal-stream transport? *ICES J. Mar. Sci.*, **57**, 360–371.
- Shannon, C. E. and Weaver, W. (1963) *The Mathematical Theory of Communications*. University of Illinois Press, Urbana, Illinois, 125 pp.
- Shenker, J. M. and Dean, J. M. (1979) The utilization of an intertidal salt marsh creek by larval and juvenile fishes: abundance, diversity and temporal variation. *Estuaries*, **2**, 154–163.
- Sogard, S. M. and Able, K. W. (1991) A comparison of eelgrass, sea lettuce macroalgae, and marsh creeks as habitats for epibenthic fishes and decapods. *Est. Coast. Shelf Sci.*, **33**, 501–519.
- Sokal, R. R. and Rohlf, F. J. (1995) *Biometry*, 3rd edn. W.H. Freeman, New York, 887 pp.
- Spjøtvoll, E. and Stolne, M. R. (1973) An extension of the T-method of multiple comparison to include the cases with unequal sample sizes. *J. Am. Stat. Assoc.*, **68**, 976–978.
- Strydom, N. A., Whitfield, A. K. and Wooldridge, T. H. (2003) The role of estuarine type in characterizing early stage fish assemblages in warm temperate estuaries, South Africa. *Afr. Zool.*, **38**, 29–43.
- Talbot, C. W. and Able, K. W. (1984) Composition and distribution of larval fishes in New Jersey high marshes. *Estuaries*, **7**, 434–443.
- 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. and Smilaeur, P. (2002) *CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination*. Version 4.5. Microcomputer Power, Ithaca, New York, 500 pp.
- Vernberg, W. B. (1983) Responses to estuarine stress. In Ketchum, B. H. (ed.), *Estuaries and Enclosed Seas*. Ecosystems of the World, 26. Elsevier Scientific, New York, pp. 43–60.
- Weinstein, M. P., Weiss, S. L., Hodson, R. G. *et al.* (1980) Retention of three taxa of postlarval fishes in an intensively flushed tidal estuary, Cape Fear River, North Carolina. *Fish. Bull.*, **78**, 419–436.
- West, R. J. and King, R. J. (1996) Marine, brackish and freshwater fish communities in the vegetated and bare shallows of an Australian coastal river. *Estuaries*, **19**, 31–41.
- Whitehead, P. J., Bauchot, M. L., Hureau, J. C. *et al.* (1984) *Fishes of the North-Eastern Atlantic and the Mediterranean*. UNESCO, Paris, 1473 pp.
- Zar, J. H. (1996) *Biostatistical Analysis*. Prentice Hall, International Editions, New Jersey, California, 662 pp.