

New insights into the early life ecology of *Sardina pilchardus* (Walbaum, 1792) in the northern Iberian Atlantic

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SUMMARY: In the Atlantic Iberian estuaries, sardines tend to be a minor component of the ichthyoplankton assemblages, with rare occurrence largely restricted to estuarine mouths. However, this was not the case of the Lima estuary (NW Portugal), where sardines were the most abundant and frequently occurring marine taxon of the larval fish assemblages. Abundance of sardines collected during a two-year survey (2002-2004) ranged from 0.5 to 60.8 larvae/100 m³, with the majority still showing yolk and unpigmented eyes. Moreover, 80% of sardines were 8 days old or less and the total length ranged from 2 to 16 mm. Seasonal variations of sardine occurrence in the Lima estuary correlated with temporal variations of salinity and temperature in the water column. The unusually high river-flow observed during the winter of 2002-2003, which preceded the spring-summer abundance peak, seemed to act as a signal for the estuarine recruitment that was encountered. In addition, the estuarine occurrence of sardine larvae was also correlated with the intensity of summer upwelling, which was responsible for coastal depletion of sardine larvae resulting in estuarine low catches during the summer of 2002. The Lima estuary also revealed another peculiarity related to the lack of temporal synchrony between coastal spawning activity and occurrence of larvae within the estuary. The abundance peaks of newly-hatched larvae (autumn 2002 and spring-summer 2003) did not coincide with the winter-spring spawning pattern described earlier for the northern Iberian coast. Modifications of the spawning strategy of the northern Iberian sardine stock are discussed based on these new insights into the early life of *S. pilchardus*.

Keywords: *Sardina pilchardus*, larval stages, spawning strategy, environmental forcing, upwelling, Lima River estuary.

RESUMEN: NUEVOS ASPECTOS SOBRE LA ECOLOGÍA DE LOS ESTADOS INICIALES DE DESARROLLO DE *SARDINA PILCHARDUS* (WALBAUM, 1792) EN EL NORTE DEL ATLÁNTICO IBÉRICO. – Las sardinas tienden a ser un componente menor del ictioplankton de los estuarios atlánticos ibéricos. Su aparición es rara y restringida, en gran parte, a las desembocaduras de los ríos. Sin embargo, éste no fue el caso del estuario del río Lima (NW Portugal), donde las sardinas fueron la especie marina más abundante del ictioplankton estuarino. Las sardinas fueron recolectadas durante un período de dos años (2002-2004), y su abundancia varió entre 0.5-60.8 larvas/100 m³. La mayoría de dichas larvas todavía presentaban saco vitelino y ojos no pigmentados. Por otra parte, el 80% de las sardinas tenían 1 semana o menos de vida y su longitud total varió entre 2 y 16 mm. Las variaciones estacionales en la aparición de larvas de sardina en el estuario del río Lima se correlacionaron con fluctuaciones temporales de salinidad y de temperatura de la columna de agua. Los elevados caudales del río observados durante el invierno 2002-2003, que precedieron el pico de abundancia del primavera-verano, parecieron actuar como señal para el reclutamiento al estuario. Además, la aparición en el estuario de larvas de sardina también fue controlada por la intensidad del afloramiento de verano, responsable de la disminución de abundancia de larvas de sardina en la costa, lo que dio lugar a bajas capturas en el estuario durante el verano 2002. Además la inusual aparición de larvas de sardina, dentro del estuario del río Lima reveló otra particularidad relacionada con la carencia de sincronía temporal entre la actividad de freza y la aparición de larvas dentro del estuario. Los picos de abundancia de larvas recién eclosionadas (otoño 2002 y período de primavera-verano de 2003) no coincidieron con la época de puesta invernal previamente descrita para la costa norte de la península ibérica. Estos nuevos datos sobre la ecología de los estados iniciales de desarrollo de la sardina se discuten a la luz de las modificaciones de la estrategia reproductiva del stock de sardinas del norte del Atlántico ibérico.

Palabras clave: *Sardina pilchardus*, estados larvarios, estrategia reproductiva, control ambiental, afloramiento, estuario del río Lima.

INTRODUCTION

The European sardine (*Sardina pilchardus* Walbaum, 1792) is a planktivorous species with a wide distribution in both NE Atlantic waters (from Mauritania to the North Sea) and the Mediterranean Sea (Porteiro *et al.*, 1996). In Atlantic waters, it is a target species for the Moroccan, Spanish and Portuguese fishing fleets (Pestana, 1989). In Portugal, this fishery represents more than 40% of the total annual national landings, highlighting its socioeconomic importance. However, in the past few decades, sardine landings off the western Iberian Peninsula have been decreasing, concomitantly with a decline in overall small pelagic fish productivity (Guisande *et al.*, 2001; Santos *et al.*, 2001; Borges *et al.*, 2003). It has been reported that the decreasing trends of sardine observed in NW Iberia are associated with variations in prevailing winds and upwelling patterns (Santos *et al.*, 2001; Borges *et al.*, 2003). In the case of upwelling, the main effect is the transport of eggs and larvae offshore by northern winds (Guisande *et al.*, 2001). Thus, strong upwelling events during the recruitment season decrease the probability of survival of sardine larvae as they are dispersed to outer shelf and oceanic zones (Bode *et al.*, 2007).

The sardine is a batch spawner, since females spawn partially on several occasions within a single season (Andreu and Pinto, 1957; Blaxter and Hunter, 1982; Quintanilla and Pérez, 2000). On the Iberian Atlantic coast, two main spawning areas have been identified, one along the Portuguese coast (Cunha and Figueiredo, 1988), and one in the Cantabrian Sea (García *et al.*, 1998). In Portugal spawning occurs primarily off the northern part of the continental shelf in November-April, with two main peaks of intensity (November to January and April/May) (Ré, 1986; Figueiredo and Santos, 1989; Pestana, 1989; Ré *et al.*, 1990; Borges *et al.*, 2003). In contrast, in the Cantabrian Sea, further north, spawning starts earlier in October and extends until July (Lago de Lanzós *et al.*, 1988; Solá *et al.*, 1992). In European Atlantic waters, spawning is restricted to the shelf when temperatures are within the 12-17°C range (Bernal *et al.*, 2007). The spring peak in spawning activity is temporally broader off northern Spain, as is the winter peak off the western and southern Iberian Peninsula (Stratoudakis *et al.*, 2007).

Estuaries worldwide are recognised as important nursery habitats for early life history stages of

fishes (Powles *et al.*, 1984; Whitfield, 1999; Blaber, 2000; Elliott and Hemingway, 2002). The early life stages of European sardines are thought to be limited to coastal areas (Ré *et al.*, 1990; John *et al.*, 1996; Chícharo *et al.*, 1998; Olivar *et al.*, 2003; Coombs *et al.*, 2004; Santos *et al.*, 2004), because they seem to avoid the high turbidity and low salinity typical of North Atlantic estuaries. Therefore, the presence of larvae of *S. pilchardus* in Atlantic Iberian estuaries has been considered rare and restricted to the adjacent coastal zone off the estuaries (Ré, 1999). This was not the case of the Lima estuary, where sardines were the most abundant and frequently occurring marine taxon in the larval fish assemblages (Ramos *et al.*, 2006a). Due to the high economic importance of sardines in European fisheries, the presence of sardine larval stages in the Lima estuary is of interest. This work reports on the unusual abundance of sardine early life within an estuarine system and investigates the association of environmental factors with the spatial and temporal patterns of larval sardine age groups in the Lima estuary.

MATERIALS AND METHODS

Study area

The Lima River is an international water body draining into the Atlantic Ocean at 41°40'N and 8°50'W near Viana do Castelo (Fig. 1). Its watershed covers 2446 km². The river mouth is partially obstructed by a 2-km-long jetty, deflecting the river flow to the south. The Lima estuary has a semidiurnal, mesotidal regime. Salt intrusion extends to 12 km upstream, with an average flushing rate of 0.5 m s⁻¹ and a residence time of 9 days (Ramos *et al.*, 2006 a). The study area, located in the lower 7 km stretch of the estuary, includes an initial dredged and narrow navigation channel and an upstream shallow and wide saltmarsh zone, mainly colonised by *Juncus* spp., with a large longitudinal sandy island (Cavalari Island). The first four stations (1-4) were located within the navigation channel, while the remaining stations (5-11) were distributed within the shallow saltmarsh zone. According to the morphology of the estuary and the salt intrusion, the initial polyhaline dredged navigation channel was considered as *lower* estuary, while the brackish shallow and wider saltmarsh area was designated as *middle* estuary (Fig. 1).

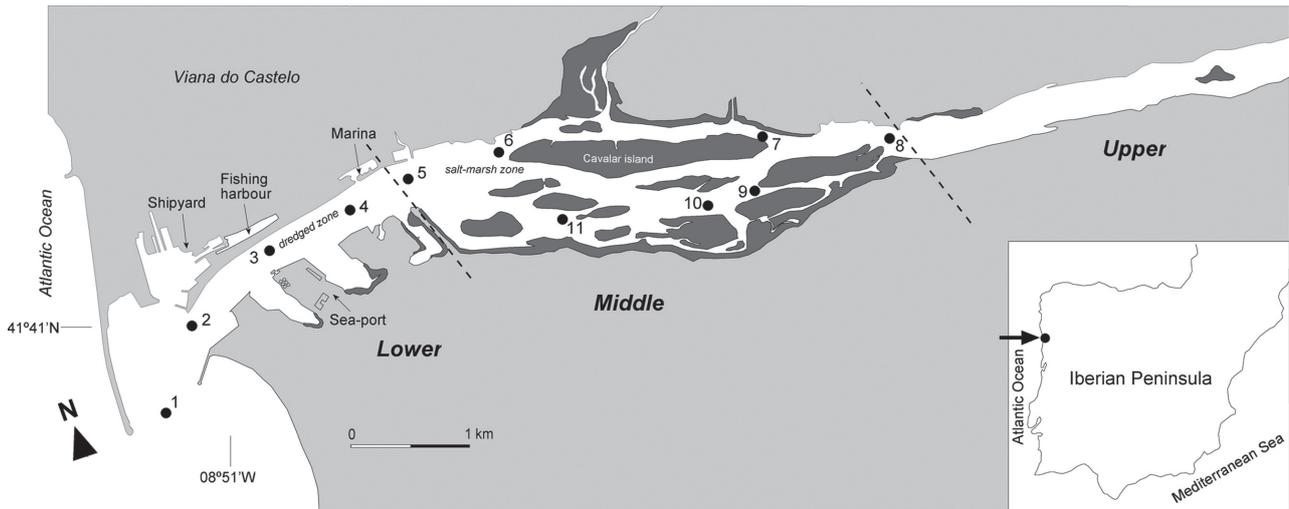


FIG. 1. – The Lima River estuary and location of the 11 sampling stations. Shaded area represents land (light grey) and sand islands and saltmarshes (dark grey). Dotted lines separate the three estuarine areas: lower, middle and upper estuary.

Data collection

Sardine larvae were collected during a major ichthyoplankton survey conducted in the Lima estuary between April 2002 and April 2004 (Ramos *et al.*, 2006a). Sampling surveys were conducted fortnightly during spring tides in the Lima River estuary. Circular planktonic tows were performed with a conical net 1 m in diameter, 3 m long and with a 500 μm mesh size in the subsurface water layer (1-2 m depth) at a constant velocity of ca. 1 m s^{-1} for five minutes. The volume of water sampled was recorded by a flowmeter (Hydro-Bios) attached to the net. At each sampling station, temperature, salinity, dissolved oxygen saturation, pH and turbidity of the subsurface water layer (0.9 and 2.1 m depth) were obtained by means of a YSI 6820 CTD. Precipitation and river flow data were obtained from the Portuguese Water Institute (<http://snirh.pt/>). Upwelling index and sea surface temperature (SST) data between April 2002 and April 2004 relate to a station located at 41.5°N, 8.5°W and were retrieved from the Live Access to ERD Environmental Data (http://las.pfeg.noaa.gov/las6_5/servlets/constrain?var=1634). The Atlantic northwest coast of the Iberian Peninsula has a north-south orientation and, hence, northern winds produce offshore transport of surface coastal waters, which are replaced by deep upwelled water. Positive values of the index indicate upwelling events and consequently water transport offshore and potential indicators of offshore advection of larvae during the spawning season (Riveiro *et al.*, 2004).

Sample processing

Ichthyoplankton samples were fixed in 4% buffered formalin (pH=8). After sorting, sardine larvae were preserved individually in 95% alcohol. Abundance was standardised as the number of sardine larvae/100 m^3 . Total lengths (TL) of fish larvae were measured to the nearest 0.01 mm using an image analyser (Leica Microsystem DJ DC480, software LEICA QWin 32), and then corrected for shrinkage according to Theilacker (1980). Since sardine hatch with a total length of 3-4 mm (Ré, 1999), samples with a total length of less than 3 mm were considered to be pre-hatched individuals that had broken out of the egg capsule (disrupted capsules). Sardine larvae were assigned to one of the three development categories: pre-hatch (total length <3 mm), yolk-sack larvae (presence of yolk) and preflexion-flexion stages (without yolk and eyes completely pigmented). Age of the larvae was estimated through the conversion of body lengths into ages, using the larval length versus otolith growth increments linear equation (Ré, 1984). According to Russell (1976) and Ré (1999), the delay between fertilisation and hatching is 2.7 days and that between hatching and first-feeding is 4 days in an environment with average temperatures similar to those experienced in the Lima estuary (15°C). A total of 6.7 days was therefore added to the estimated age. Individuals were aggregated into four age groups: G_1 (<8 days old), G_2 (between 8 and 14 days old), G_3 (between 15 and 21 days old) and G_4 (>21 days old). Considering the pelagic larval

duration of *S. pilchardus* to be of 40 days, G_1 was considered as newly-hatched larvae.

Data analyses

Monthly data were averaged by season: 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. The temporal and spatial variations in sardine larvae abundance were assessed using a two way-ANOVA, with seasons and sampling stations as fixed factors (Sokal and Rohlf, 1995; Zar, 1996). Whenever necessary, raw data were log-transformed to meet the ANOVA assumptions of homogeneous variance and normally-distributed data. Homogeneity of variance was tested with Cochran's test. Whenever variance was still heterogeneous, conclusions from ANOVA results were only accepted for those cases in which significance levels were less than 0.01. Furthermore, in the event of significance, an a posteriori Tukey HSD for unequal sample sizes was used to determine which means were significantly different at a 0.01 probability level (Spjøtvoll and Stoline, 1973).

The environmental conditions associated with the spatial and temporal age patterns of sardines were investigated by means of canonical correspondence analysis (CCA), a multivariate method of direct gradient analysis (Ter Braak, 1986), using the CANOCO software (version 4.5, Microcomputer Power, Ithaca, NY). An environmental set of nine variables was used: water parameters (mean temperature, salinity, oxygen saturation, pH and turbidity of the subsurface water layer); monthly river flow and precipitation; upwelling index; and the temperature gradient between the sea surface temperature and the estuarine temperature. Samples were averaged into 3-month groups (i.e. seasons). Samples without sardine larvae were removed from the analysis. The option used for CCA was triplot scaling with a focus on interspecies distances, and species abundances were transformed (square root). Significance of the canonical model was given by a Monte Carlo test (Ter Braak and Smilauer, 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).

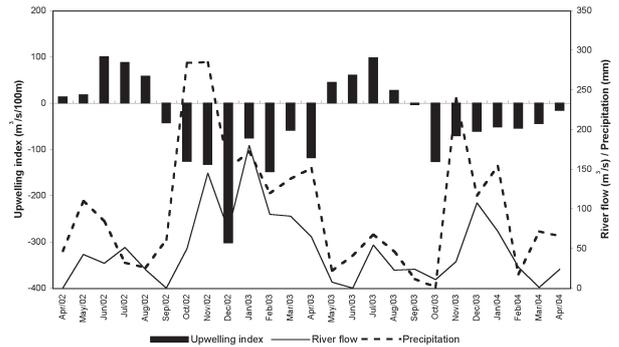


FIG. 2. – Monthly precipitation (mm), average monthly river flow ($\text{m}^3 \text{s}^{-1}$) and upwelling index ($\text{m}^3 \text{s}^{-1} 100 \text{m}^{-1}$) in the Lima estuary between April 2002 and April 2004. Positive values of the index indicate upwelling events.

RESULTS

Environmental conditions

During the study period, monthly precipitation varied between 1 and 285 mm, and river flow ranged from 0 to $180 \text{ m}^3 \text{ s}^{-1}$ (Fig. 2). Although precipitation and river flow fluctuated greatly throughout the study period, there was a general trend for an autumn-winter increase in both parameters. The wet period of the first study year was considerably longer and intense, with precipitation and river flow registering the highest values of the study. The monthly distribution of the upwelling index (Fig. 2) indicated that spring-summer upwelling events occurred in both years (positive values of index), although in the summer 2002 the intensity and duration of upwelling was demonstrably higher.

The physical and chemical characteristics of the subsurface water layer varied seasonally, and only a minor spatial variability between the lower and middle estuary was found (Fig. 3). Temperature ranged between 9.4 and 19.6°C , with a mean of $14.8 \pm 1.9^\circ\text{C}$, and exhibited the expected seasonal trend. It decreased during the winter months and increased during the spring-summer months. Nevertheless, in 2002 the summer temperature was lower than expected due to the above-mentioned strong upwelling, shifting the warmest period of the year to the autumn season. The Lima estuary was generally euhaline, with an average salinity above 30 (30.6 ± 6.5). The only exception was the wet winter of 2002-2003, when salinity decreased below 10 due to river flow increase. Water was in general well oxygenated, with an average oxygen saturation of $102.8 \pm 10.8\%$, and values tended to

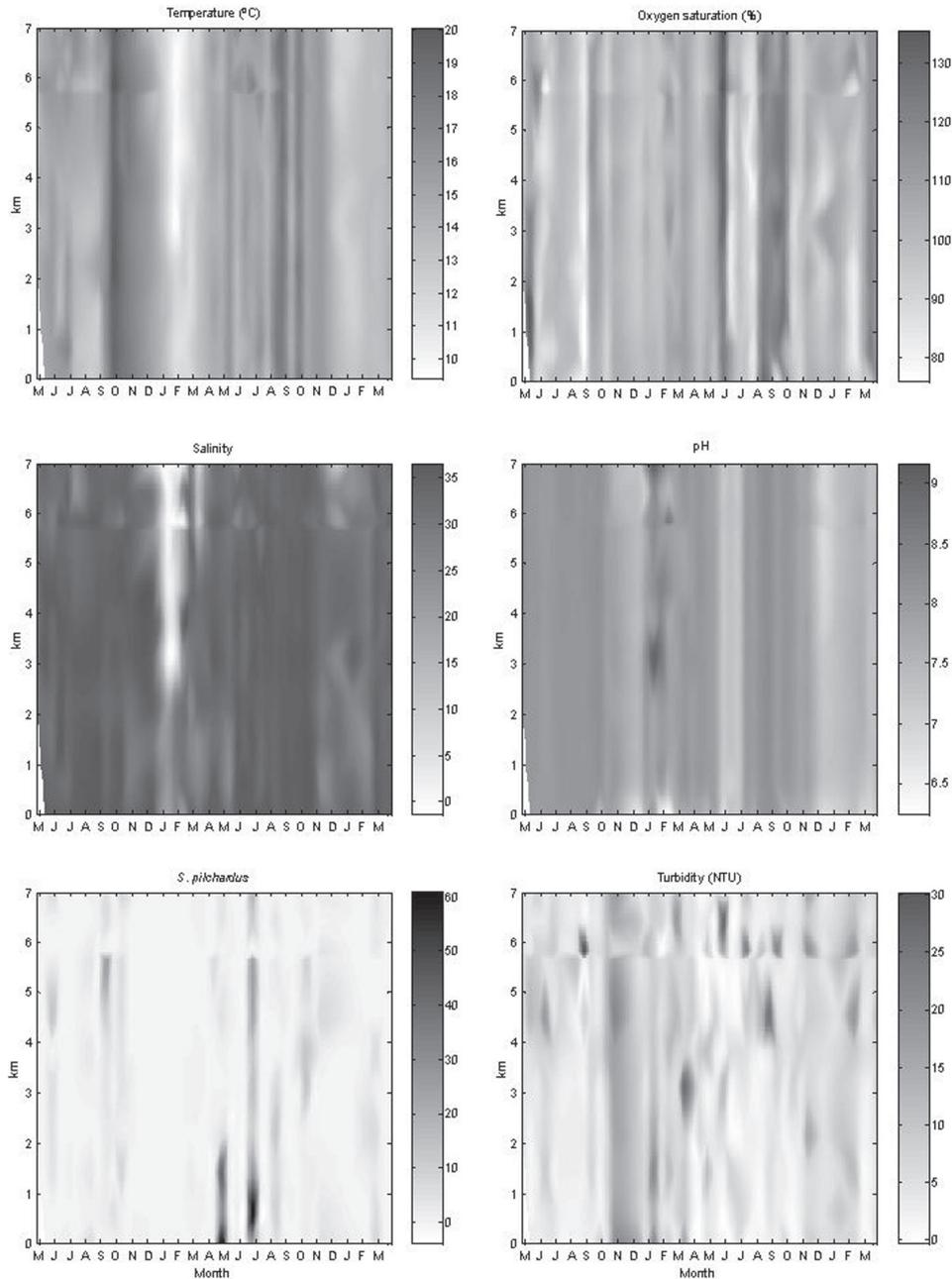


FIG. 3. – Interpolation plots of temperature, salinity, oxygen saturation, pH, turbidity of the subsurface water layer (0.9-1.2 m depth) and sardine larvae abundance (larvae/100 m³) along the time (x-axis) and distance from the river mouth (y-axis) in the Lima estuary during April 2002 and April 2004. km - distance from the river mouth.

increase during the freshet periods (winter and early spring months). Conversely, pH (mean: 7.8 ± 0.4) tended to decrease during the freshet periods, which was particularly evident in the second sampling year. Mean seasonal turbidity was lower than 7 NTU (mean: 6.8 ± 7.4 NTU), except during the freshet period of the first study year, when the transparency of the water decreased due to increased runoff.

Seasonal occurrence of sardine larvae

During the study period, a total of 1064 sardine larvae were captured in the Lima estuary. Sardine larvae occurred in 31% of the total 489 plankton trawls. Abundance ranged from 0.5 to 60.8 larvae/100 m³, with an average of 1.8 ± 6.1 larvae/100 m³.

Three distinct development stages were observed: i) the most frequent and abundant stage (56%) was

TABLE 1. – Total length (mm), age (days), abundance (larvae/100 m³), frequency of occurrence (F.O.) and relative contribution to the total catch (%) of the sardine larvae caught in the Lima estuary from April 2002 until April 2004.

Ontogenetic stages		Total length (mm)	Age (days)	Abundance (larvae/100 m ³)	F.O.	%.
Pre-hatch		2.63 ± 0.27	2.20 ± 0.48	0.05 ± 0.31	4%	3%
Yolk-sack		4.22 ± 0.55	5.01 ± 0.97	1.01 ± 3.51	22%	56%
Preflexion and flexion		7.18 ± 2.71	10.26 ± 4.81	0.73 ± 3.78	21%	41%

that of yolk-sack larvae with unpigmented eyes and a total length of 3.0 to 6.1 mm, corresponding to ages from 3 to 7 days (Table 1); ii) larvae in preflexion and flexion stages represented 41% of the total sardines collected, and included all individuals without yolk, with highly variable lengths, from 3.9 to 16.5 mm TL corresponding to ages from 4.4 to 26.7 days; and iii) just 3% of larvae were identified as pre-hatching stages, younger than 3 days (Table 1).

The occurrence of sardine larval stages in the Lima estuary varied significantly between seasons ($F=6.99$, $P<0.001$), with a distinct seasonality in each of the study years (Fig. 3). In 2002/2003, sardines were collected with a 25% frequency of occurrence, which increased to 38% during the second study year (2003/2004). Additionally, abundance peaked during autumn 2002 of the first year, and in the second year higher abundances were observed during the spring-summer period. Moreover, in 2002/2003 the lowest abundance period was in the winter, but in 2003/2004 it was early spring, when only 3 and 5%, respectively, of the planktonic trawls included sardine larvae.

Apart from the absence of significant differences between the lower and middle estuary ($F=0.9$, $P>0.52$), or interaction between seasons and sampling stations ($F=0.68$, $P>0.98$), a slight decrease in sardine larval abundance upstream was found. Mean abundance was higher in the lower estuary (2.9 ± 8.7 sardines/100 m³), than in the middle estuarine area (1.4 ± 3.5 sardines/100 m³) (Fig. 3).

Age patterns

The total length of the 1064 sardine larvae collected ranged from 1.8 to 16.5 mm TL, correspond-

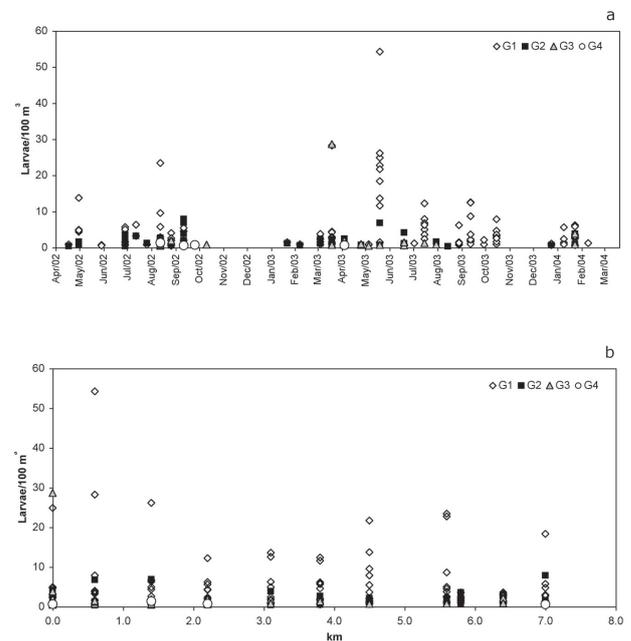


FIG. 4. – Monthly (a) and spatial (b) patterns of the four weekly age groups of sardine larvae collected in the Lima estuary between April 2002 and April 2004. G₁, 1 week old; G₂, 2 weeks old; G₃, 3 weeks old; and G₄, 4 weeks old.

ing to an age of 1–27 days. Sardines less than 8 days old occurred at a density of 1.5 ± 4.9 larvae/100 m³. Abundance of those newly hatched larvae varied significantly between seasons ($F=5.3$, $P<0.001$), but not between sampling stations ($F=0.8$, $P>0.62$), and no interaction between seasons and stations was found ($F=0.6$, $P>0.99$). Among age groups, G₁ was the most abundant and frequent, representing 80% of the positive collections (i.e. samples containing sardine larvae), and peaked during summer 2003, when abundance reached 54 larvae/100 m³ (Fig. 4). The 2-week old sardines represented 12% of the total and were observed in 35% of the

positive collections, with a mean abundance of 0.2 ± 0.9 larvae/100 m³. Abundance of G₂ group varied significantly between seasons ($F=3.8$, $P<0.001$), with a peak in autumn 2002. However, they were absent during spring 2004 (Fig. 4). Sampling station and the interaction between sampling station and season did not have significant effects on the abundance of G₂ larvae ($F=1.66$ and 0.85 , $P>0.09$ and $P>0.86$, respectively). Three week old larvae (G₃) were present in 18% of the positive collections, representing 7% of the total number of sardines, with a mean abundance of 0.1 ± 1.4 larvae/100 m³. As for the G₁ and G₂ age groups, seasons had a significant effect on the abundance of the G₃ age group ($F=2.99$, $P<0.001$), unlike sampling stations ($F=1.52$, $P>0.13$), and interactions between season and station ($F=0.87$, $P>0.76$), which had no significant effect. G₃ sardines were only found during the higher abundance periods of each year (i.e. autumn 2002 and spring-summer 2003), but also during winter 2003/2004 (Fig. 3). Older sardines aged 22 or more days (G₄) were rare, representing 0.4% of the total sardines with a mean abundance of 0.01 ± 0.1 larvae/100 m³. This age group was only observed during autumn 2002 and spring 2003 (Fig. 4), and did not show any significant seasonal ($F=2.14$, $P>0.03$) or spatial ($F=0.64$, $P>0.78$) variations.

Responses to environmental forcing

From the original set of nine environmental variables, pH, turbidity, precipitation and temperature gradient were excluded due to the multicollinearity problems (inflation factor >20). The effect of the combined five variables on explained distribution of the CCA axes was significant according to the Monte Carlo permutation test ($P<0.05$). The first CCA axis (eigenvalue=0.12), and the second CCA axis (eigenvalue=0.07) explained together 97% of the cumulative percentage variance of species-environment relation. Therefore the following two CCA axes (CCA₃ and CCA₄) were not interpreted further. According to inter-set correlations, river flow, temperature, salinity and upwelling index were considered to be biologically significant (Table 2). CCA₁ was positively correlated with upwelling index and negatively correlated with river flow. Temperature and salinity were negatively correlated with CCA₂. The first canonical axis separated seasons with high river flow and low upwelling index such as autumn

TABLE 2. – Inter-set correlations of environmental variables with the first two CCA axes. * inter-set $\geq |0.4|$ corresponding to biologically important variables.

Environmental variables	CCA ₁	CCA ₂
River flow (m ³ /s ¹)	-0.9*	-0.1
Temperature (°C)	-0.3	-0.8*
Salinity	-0.1	-0.7*
Oxygen saturation (%)	-0.2	0.2
Upwelling index (m ³ /s ¹ 100 m)	0.7*	0.3

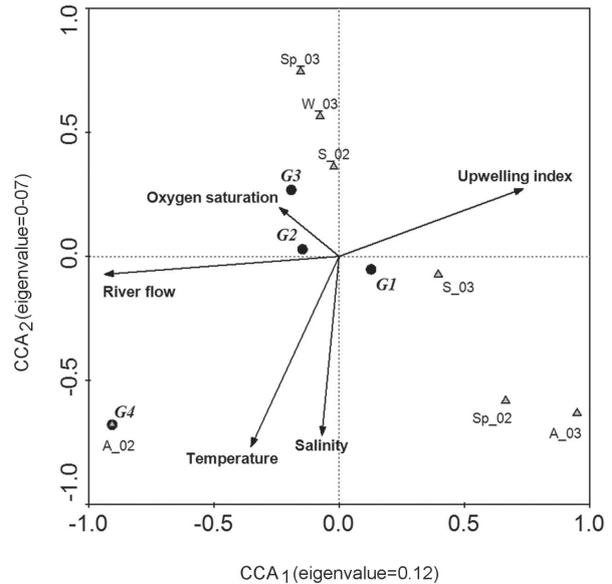


Fig. 5. – Sardine age groups and environmental variables in a triplot ordination diagram using the first two canonical correspondence axes. G₁, age group 1; G₂, age group 2; G₃, age group 3; G₄, age group 4; NO, no sardine. Sp_02, spring 2002; S_02, summer 2002; A_02, autumn 2002; W_02, winter 2002/2003; Sp_03, spring 2003; S_03, summer 2003; A_03, autumn 2003; W_03, winter 2003/2004; Sp_04, spring 2004.

2002 from seasons with reduced river flow such as spring 2002 and autumn 2003 (Fig. 5). The remaining seasons were separated along CCA₂ by temperature and salinity since seasons whose mean temperature and salinity were below the 15°C and 33, respectively, clustered in the positive part of CCA₂ (Fig. 5). Sardine age groups were separated along the two first CCA axes. G₁ was correlated with upwelling events, since this age group peaked during the summer of 2003 (c.f. Fig. 4) and, therefore, clustered in the positive part of the CCA₁ axis. The remaining age groups merged in the negative part of the CCA₁ axis and were separated along CCA₂. G₄ clustered with the autumn of 2002 (Fig. 5), associated with high temperature and salinity values (Fig. 3). G₂ and G₃ were associated with an increase in river flow and with the lower temperatures and salinities of summer 2002 and spring and winter 2003.

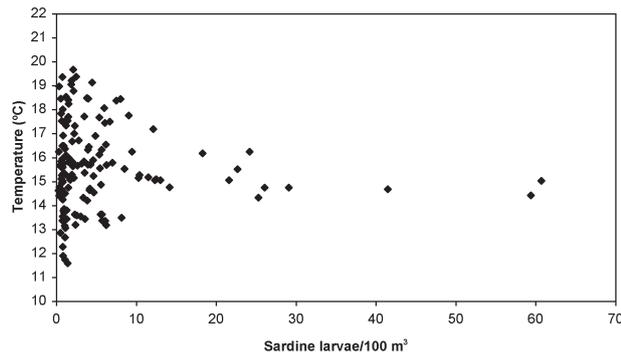


FIG. 6. – Sardine larval abundance versus temperature in the Lima estuary between April 2002 and April 2004.

DISCUSSION

According to a two-year-long study, *S. pilchardus* was the second most abundant species in the ichthyoplankton assemblage of the Lima estuary (Ramos *et al.*, 2006a). This pattern has not been described before for Atlantic Iberian estuaries, where sardines have been considered a minor component of the estuarine ichthyoplankton assemblage, generally dominated by *Engraulis encrasicolus*, another clupeid species (Ribeiro, 1991; Ré, 1996; Ré, 1999; Faria *et al.*, 2006; Marques *et al.*, 2006). Although larval stages of sardines were considered rare in Iberian estuaries (Ré, 1999), in the present study, sardine larvae were frequently observed in the Lima estuary independently of the season. None of the sardine age groups revealed any specificity to a particular zone of the estuary owing to their rather good distribution along the initial 7 km of the Lima estuary. These findings contradict previous studies that reported sardine larvae as an occasional visitor restricted to the terminal zone of Iberian estuaries (Ré, 1999), providing new insight into the early life ecology of *S. pilchardus* along the Northern Iberian coast.

During the 25-month study period, sardines were frequently observed in the Lima estuary, exhibiting a clear seasonality that varied between years (Fig. 3). Two periods of low abundances emerged, in winter 2002 and spring 2004, followed by two peaks of high abundances, in autumn 2002 and spring-summer 2003 (Fig. 3). According to Olivar *et al.* (2001; 2003), environmental variations can modify not only the distribution but also the abundance of sardine larvae, as was the case in the Lima estuary. Seasonal variations in the abundance of sardines followed temporal variations in water characteristics.

For example, the freshet period of winter 2002, when river flow exceeded $100 \text{ m}^3 \text{ s}^{-1}$ and salinity decreased sharply (below the 25), coincided with the absence of sardine larvae (Fig. 3), and a decrease in larval fish assemblages (Ramos *et al.*, 2006b). Thus, increased run-off limits the penetration of marine larvae into estuarine habitats. In winter 2003, river flow was lower and therefore average salinity did not decrease below 25 (Figs. 2 and 3). As a result, sardine larval abundance was higher than in the winter of 2002 (Fig. 3). The present data seem to indicate that whenever the salt intrusion was favourable (i.e. when the system was euhaline), larvae penetrated into the Lima estuary. Thus, the occurrence of sardine larval stages in the estuary was correlated with the salinity regime of the system, which was, in turn, controlled by river flow. Overall, salinity and river flow were identified as important regulatory environmental parameters of the occurrence of sardine larvae in the Lima estuary (Table 2).

A second period of sardine scarcity was observed during spring 2004. During this season, river flow registered the lowest values of the study period (Fig. 2). Considering that high recruitment levels should be expected for intensive river flow (Costa *et al.*, 2007), the reduced abundance during spring 2004 could have been a consequence of the lower precipitation and river flow observed since the preceding winter (2003/2004). Therefore, one can hypothesise that the unusually high river-flow observed during winter 2002-2003 (Fig. 2), which preceded the spring-summer abundance peak, could have acted as a signal for the estuarine recruitment. Actually, physical and chemical stimuli of freshwater are usually required as a clue to drive migratory processes (Champalbert and Koutsikopoulos, 1995), leading to the strong positive impact of river discharges on coastal fisheries (Loneragan and Bunn, 1999; Chícharo *et al.*, 2006a; Chícharo *et al.*, 2006b; Meynecke *et al.*, 2006) driven by the nutrient upload of river plumes.

The typical pelagic fish life-history strategy in areas of coastal upwelling favours spawning when the offshore transport is minimal and later feeding stages are able to exploit increases in productivity due to summer coastal upwelling (Cushing, 1982; Roy *et al.*, 1989). This spawning strategy can serve as an adaptive mechanism to avoid offshore transport in upwelling areas and associated loss of eggs and larvae from the coastal nursery habitat (Parrish *et al.*, 1981). The low abundances of sardine lar-

vae observed during a typical summer upwelling, as was the case of summer 2002 (Fig. 2), supports the importance of such oceanographic events on the recruitment of small pelagic fishes such as sardines in the coastal zone. Upwelling intensity was shown to affect sardine recruitment off the NW Iberian Atlantic both positively and negatively (Dickson *et al.*, 1988; Roy *et al.*, 1995). During the summer of 2003, when the upwelling index did not approach the levels observed in 2002, sardine larvae reached the highest abundances (Fig. 4). Moreover, CCA revealed that upwelling index was an environmental control of sardine larvae abundance, namely that of the newly-hatched (G_1) larvae (Fig. 5).

Sardina pilchardus spawning activity is temperature-dependent, occurring mainly when surface water temperatures range from 14 to 16°C (Ré *et al.*, 1990). In addition, Muiño *et al.* (2003) identified temperature as the major environmental factor responsible for temporal variations in sardine larvae abundance along the coast of northern Spain. In the Lima estuary, periods of maximum sardine abundance coincided with periods of water temperatures higher than 17°C (Fig. 3). These results are in agreement with the recent study by Bernal *et al.* (2007), who reported sardine spawning off the European Atlantic to occur at a temperature range of 12-17°C. Similarly, peaks in abundance of sardine larvae inside the estuary (autumn 2002 and spring-summer 2003), were associated with increased temperatures (Fig. 6). Indeed, temperature was also indentified as an important environmental determinant of sardine larvae in the Lima estuary (Table 2 and Fig. 5). Thus, not only is temperature an important variable for coastal spawning, but it also has an indirect effect on the temporal patterns of sardine larvae distribution in the Lima estuarine habitat.

Sardine larvae were captured throughout the study period and were mainly composed of newly-hatched sardine larvae (i.e. sardines that were 1-week old), indicating highly frequent spawning activity in this region. Since spawning occurs in the coastal area (Bernal *et al.*, 2007), the temporal pattern of sardine larvae abundance in the estuary should reflect this activity. In fact, the maximum abundance values recorded (60 larvae/100 m³), were similar to those reported for the coastal area during the spawning season (Ré *et al.*, 1990; John *et al.*, 1996; Santos *et al.*, 2004). However, the maximum sardine abundance in the Lima estuary during autumn 2002 and spring, summer and autumn 2003

did not coincide with the adult spawning season described off northern Portugal, which occurs from November to April (Ré *et al.*, 1990; Zwolinski *et al.*, 2001; Stratoudakis *et al.*, 2003; Bernal *et al.*, 2007). This lack of temporal synchrony between previously reported spawning activity and occurrence of larvae inside the estuary may indicate a change in the spawning strategy of the northern Iberian sardines stock. Several scenarios can be hypothesised: (i) the high abundances of sardines during summer suggest that sardine populations could be extending their spawning season through late spring and summer; (ii) the typical coastal nursery ground is being extended throughout the study area, as observed further north in the Rias Baixas, NW Spain by Muino *et al.* (2003); and (iii) alternative estuarine spawning is taking place. These hypotheses require further investigation of the temporal abundance patterns of sardine eggs in the estuary as well as in the surrounding coastal waters. Moreover, the actual nursery role of the Lima estuary for *S. pilchardus* needs to be evaluated. According to Beck *et al.* (2001), a juvenile habitat should be considered a nursery ground whenever its contribution to the adult stock is high in comparison with other areas. The present study did not allow the assessment of the real contribution of the Lima estuary to the adult stocks of *S. pilchardus*. Although several authors defend the criteria of Beck *et al.* (2001) as fundamental for proving the nursery role of an habitat (Cattrijsse and Hampel, 2006), recruitment success depends not only on the survival and growth of juveniles spent in the nursery grounds, but also on the highly variable larval phase (Van der Veer, 1986; Beverton, 1995; Andersen *et al.*, 2005). Thus, the unusual high abundance of sardine larvae within the Lima estuary is a crucial finding for the recruitment assess of *S. pilchardus* in the northern Iberian Atlantic. Due to the economic importance of sardines in European fisheries, the unusually high abundance and frequency of sardine larvae in an estuarine habitat is key information for the management and sustainability of this typically coastal species. Beare *et al.* (2004) emphasised that the use of estuaries can involve many difficult problems for fisheries managers monitoring these environments. Thus, assessing the effective role of the Lima estuary for this economically and socially important species is necessary, requiring future research to evaluate the contribution of the Lima estuary to the recruitment of the northern Iberian *S. pilchardus* stock. Though this study provides new

insights into the early life ecology of sardines in the NW Iberian Atlantic, the use of the Lima estuary as a frequent spawning and nursery area for sardines requires further investigation.

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