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Recruitment of flatfish species to an estuarine nursery habitat (Lima estuary, NW Iberian Peninsula)

Sandra Ramos^{a,*}, Pedro Ré^b, Adriano A. Bordalo^{a,c}

^a Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR); Rua dos Bragas 289, 4050-123 Porto, Portugal

^b Faculdade de Ciências da Universidade de Lisboa, Laboratório Marítimo da Guia, Estrada do Guincho, 2750-374 Cascais, Portugal

^c Laboratory of Hydrobiology, Institute of Biomedical Sciences, University of Porto, Largo Professor Abel Salazar, no. 2, 4099-003 Porto, Portugal

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ABSTRACT

One of the present concerns of fish biologists involves defining and identifying nursery habitats in the context of conservation and resource management strategies. Fish nursery studies usually report upon nursery occupation during the latter juvenile stages, despite the fact that recruitment to nurseries can start early in life, during the larval phase. Here we investigated the use of a temperate estuarine nursery area, the Lima estuary (NW Portugal), by initial development stages of flatfish species before and after metamorphosis, integrating the larval and juvenile phases. The Lima estuarine flatfish community comprised twelve taxa, seven of which were present as pelagic larvae, six as juveniles and three as adults. There was a general trend of increasing spring–summer abundance of both larvae and juveniles, followed by a sharp winter decrease, mainly of larval flatfishes. The Lima estuary was used by *Solea senegalensis*, *Platichthys flesus* and *Solea solea* as a nursery area, with direct settlement for the two first species. In contrast, indirect settlement was suggested for *S. solea*, with metamorphosis occurring outside the estuarine area. Estuarine recruitment of *S. senegalensis* varied between years, with young larvae occurring in the estuary throughout a prolonged period that lasted 6–9 months, corroborating the protracted spawning season. *P. flesus*, the second most abundant species, exhibited a typical spring estuarine recruitment, without inter-annual variations. Developed larvae arrived in the estuary during spring, whereas the 0-group juveniles emerged in the following summer period. The present study contributes new insight to our understanding of the economically important *S. senegalensis*, and highlights the importance of integrating the planktonic larval phase into traditional flatfish nursery studies.

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1. Introduction

Many species of marine fishes employ a life-history strategy in which the larvae take up a pelagic lifestyle and the adults are closely associated with the benthos. Perhaps no group of fishes has committed to this strategy so completely as flatfishes, whose morphological transformation from a symmetrical pelagic larva to the ultimate bottom mimic is an unparalleled feat of ontogeny (Fuiman, 1997). Metamorphosis, characterized by severe morphological (e.g. eye migration and a 90° rotation in posture), anatomical and physiological transformations, enables the transition from a pelagic to a benthic lifestyle (Able and Fahay, 1998). This transition is usually denominated at settlement. Some flatfish species colonize their nursery areas early in life, during the pelagic larval phase, employing a direct settlement strategy (Gibson, 1973). In contrast, when nursery recruitment occurs after settlement, during the juvenile phase, it is considered indirect settlement. Recruitment success

depends on survival and growth of recruits, both through the highly variable larval stages and during the juvenile phase spent in the nursery grounds (Van der Veer, 1986; Beverton, 1995; Andersen et al., 2005). Thus, the settlement of planktonic larvae on a suitable nursery habitat is one of the most important features of flatfish population dynamics. In fact, migrations of metamorphosing larvae to the proper nursery habitats have been recognized as an important control of recruitment strength (Bell et al., 2001; Warlen et al., 2002; Garcia et al., 2003). For species that spawn offshore but are dependent upon inshore nurseries, larvae that enter the estuary on flood tide but fail to settle during some critical period of time may be subsequently flushed back into coastal waters, where they are presumably lost to the population (Amara et al., 2000; Curran and Able, 2002; Cabral, 2003). Thus, the dynamics of nursery areas play a major role in determining overall population size and, hence, their study contributes towards understanding recruitment variability (Rijnsdorp et al. 1995; Iles and Beverton, 2000; Van der Veer et al., 2000; Walsh et al., 2004).

Nursery areas may be described as a restricted area where juveniles of a species spend a limited period of their life cycle, during which they are spatially and temporally separated from the adults and

* Corresponding author. Tel.: +351 222062285; fax: +351 222062284.
E-mail address: ssramos@icbas.up.pt (S. Ramos).

where their survival is enhanced through optimal conditions for feeding, growth, and/or predation refuge (Bergman et al., 1988; Pihl et al., 2002). Since survival may also be optimized in other adjacent habitats, recent frameworks defend that a habitat only functions as a nursery when it is able to yield relatively more adult recruits per unit of area than other juvenile habitats used by a species (Beck et al., 2001; Cattrijsse and Hampel, 2006). However, this definition can be limited, because it can omit habitats that have a small per unit area contribution to adult recruitment, but may be essential for sustaining adult populations (Dahlgren et al., 2006). Thus, the traditional nursery concept that defines nurseries simply as habitats supporting high juvenile densities should not be abandoned and still retains values in the context of discussing conservation and restoration strategies.

Flatfishes are usually abundant within estuarine and coastal fish assemblages (Yamashita et al., 2001; Bailey et al., 2003; Le Pape et al., 2003). Along the northeast Atlantic and Mediterranean margins, several flatfish species use estuarine and coastal areas as nursery habitats (Henderson and Seaby, 1994; Nash and Santos, 1998; Watts and Johnson, 2004; Vinagre et al., 2005). The coastal zones of the North Sea and the Wadden Sea have been extensively studied, since those areas comprise important nursery areas for several flatfish species of commercial interest, namely *Pleuronectes platessa*, *Limanda limanda*, *Platichthys flesus* and *Solea solea*. Within the Iberian Peninsula, the NE Bay of Biscay has been identified as an important nursery area for *S. solea* (Amara and Paul, 2003; Le Pape et al., 2003; Desaunay et al., 2006; Gilliers et al., 2006). Some southern Iberian estuaries are also used as nursery grounds for *S. solea* and *S. senegalensis* (e.g. Costa and Bruxelas, 1989; Drake and Arias, 1991; Andrade, 1992; Cabral, 2000b; Cabral et al., 2002; Drake et al., 2002). The Minho and Douro estuaries, located along the northern coast of Portugal, have recently been recognized as nursery areas for *P. flesus* and *S. solea* (Cabral et al., 2007; França et al., 2009; Freitas et al.), but based solely upon the estuarine juvenile phase. In fact, a common feature of most fish nursery studies is to deal with only one particular phase of ontogenetic development, typically the latter stages of nursery occupation. Thus, the majority of flatfish nursery studies tend to focus only on the benthic juvenile phase (e.g. Hostens, 2000; Power et al., 2000; Gilliers et al., 2004), indirectly inferring information about larval stages (Vinagre et al., 2007; Vinagre et al., 2008) due to the lack of empirical data. Accordingly, further studies are required in order to incorporate early larval stages into flatfish nursery studies, thereby combining the pelagic larval and benthic juvenile stages into a more comprehensive assessment of the importance of nurseries to different

phases of the life cycle of each species of interest. For example, a recent study of the Lima estuarine flatfish community emphasized the concept that environmental control varies throughout ontogenetic development, stressing the importance of integrating all the early life of a species in flatfish nursery studies (Ramos et al., 2009).

The present study forms part of a comprehensive analysis of the use of an estuarine nursery by early life stages of flatfishes, including both the larval and juvenile stages. Presently, it is known that several flatfish, namely *S. senegalensis*, *S. solea* and *P. flesus*, utilize the Lima estuary as planktonic larvae (Ramos et al., 2006b) and also among the benthic community (Ramos et al., 2009). Apart from the economic importance of these species, several biological and ecological aspects of their early life history are still unknown, namely their mechanisms of nursery recruitment and the specific habitats that they use (Vinagre et al., 2008; Freitas et al., 2009). Thus, our aim was to investigate the estuarine recruitment of flatfishes to the Lima estuary, through the temporal and spatial patterns of larval and juvenile abundances over a 2-year period.

2. Material and methods

2.1. Study area

The Lima River is an international water body with headwaters in northwestern Spain, that flows westward through Portugal and 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, northern Portugal (Fig. 1). Its watershed covers 2446 km² of which 47% is located in Portuguese territory. The temperate Lima River has a small open estuary, with a semidiurnal and mesotidal regime (3.7 m), with an average flushing rate of 0.40 m s⁻¹, a river flow of 70 m³ s⁻¹, and a hydraulic residence time of 9 days. The Lima estuary is a partially mixed system, exhibiting seasonal vertical stratification of salinity during the winter period, during which salinity sharply increases with depth. A layer of fresh water is sometimes present at the surface, associated with periods during which river discharges increase. Temperature is vertically stratified, with cooler water typically found near the bottom of the water column, except during wintertime thermal inversions (Ramos et al., 2006a). Due to the geomorphology of the system, the lower estuary is highly urbanized and composed of a narrow channel dredged to a depth of 10 m, and with hardened shorelines. The river mouth is partially obstructed by a 2 km long jetty that deflects the river flow to the south (Fig. 1). The middle estuary is a shallow

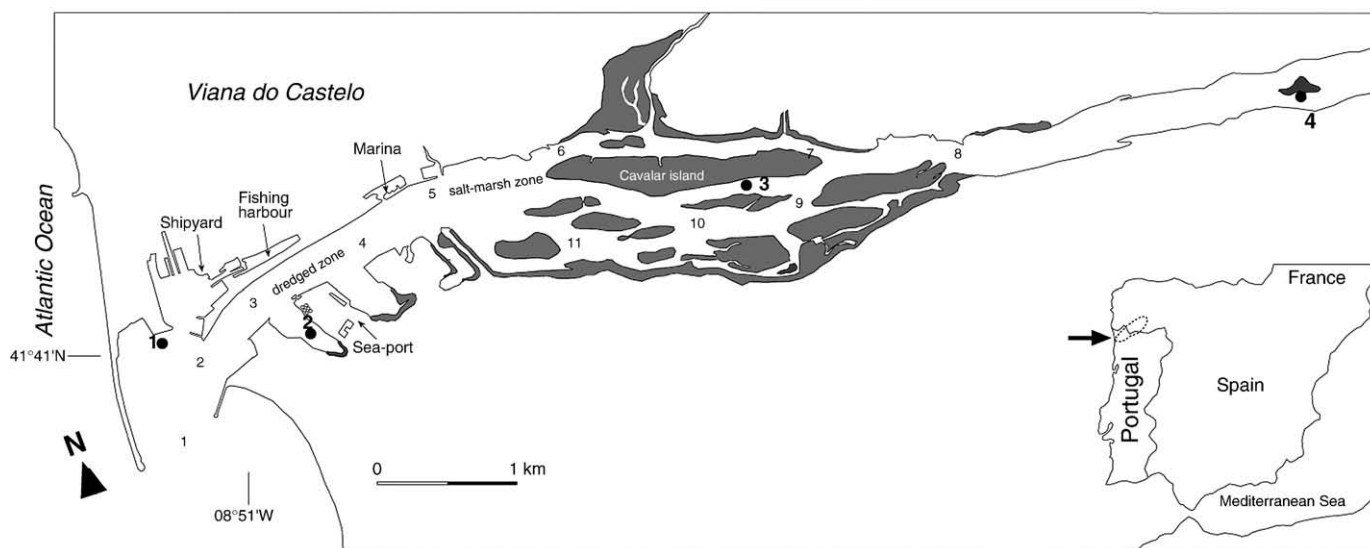


Fig. 1. Lima River estuary and location of the sampling stations where larval (small numbers 1–11) and juvenile flatfishes (numbers in bold 1–4) were collected. Shaded area represents sand islands and saltmarshes.

saltmarsh zone, mainly colonized by sea rush *Juncus* spp., and enclosing several sand islands and intertidal channels. The upper estuary is a narrow shallow channel, where depth decreases upstream, with natural banks and some tidal islands.

2.2. Sampling strategy

The sampling strategy included ichthyoplankton and beam trawl surveys, in order to collect pelagic larvae and benthic juveniles, respectively. Flatfish larvae were collected between April 2002 and April 2004, as part of a broad ichthyoplankton study performed in the Lima estuary (Ramos et al., 2006b). Ichthyoplankton surveys were performed at 11 sampling stations distributed along the initial 7-km stretch of the Lima estuary, corresponding to the lower and the middle estuary (Fig. 1). Fortnightly subsurface circular tows were

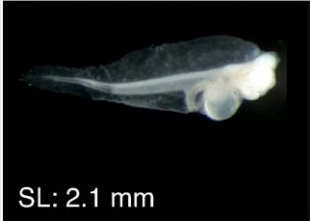





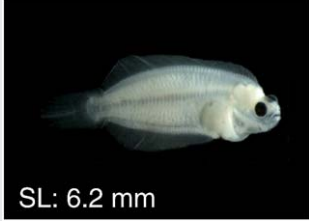

carried out during daytime, at a constant velocity of ca. 1 m s⁻¹ for 5 min, using a conical net that was 1-m diameter, 3-m long and 500-µm mesh size. A flowmeter (Hydro-Bios) was attached to the net, enabling calculation of the volume of water filtered. Samples were immediately fixed in 4% buffered formalin (pH = 8) and after sorting, fish larvae were preserved in 95% ethanol. Flatfish larvae were separated from the remaining fish larvae and identified to the lowest possible taxon. Abundance data were standardized into the number of larvae per 100 m³ of water filtered.

Juvenile flatfish abundance was investigated from October 2003 through September 2005, in the lower, middle and upper sections of the Lima estuary (Fig. 1). Stations 1 and 2 were located in the lower estuary, Station 3 in the middle estuary, and 10 km upstream of the river mouth Station 4 was located, in the upper estuary. Monthly beam trawl (1-m beam trawl net with a mesh size of 5 mm and one

Table 1

Ontogenetic stages of *S. senegalensis* and *P. flesus* larvae. SL—standard length.

Adapted from Fernández-Díaz et al. (2001), Osse and Van den Boogaart (1997), Edwards (1996) and Nichols (1971).

Description	<i>Solea senegalensis</i>	<i>Platichthys flesus</i>
Stage 1 Newly hatched larvae; yolk sac larvae; unpigmented eyes	 SL: 2.1 mm	
Stage 2 Pigmented eyes; yolk sac may still be present; formation of hypural plates	 SL: 3.4 mm	 SL: 5 mm
Stage 3 Yolk sac totally absorbed; the urostyle flexes upward; rays from caudal and dorsal fins are formed; larvae with bilateral symmetry	 SL: 4.4 mm	 SL: 6.3 mm
Stage 4 Beginning of eye migration (loss of bilateral symmetry): the left eye still didn't cross the top of the head	 SL: 4.7 mm	 SL: 6.2 mm
Stage 5 Ending of eye migration: the left eye crossed the top of the head (more than 50% visible on the right side of the larvae)	 SL: 5.1 mm	

tickler chain) collections were performed at the four sampling stations, during daytime flood and spring tides, with the net towed at an average velocity of 0.8 m s^{-1} for 3 min (each trawl covered in average 140 m^2). Geographic location of the sampling stations and distance travelled during each tow were monitored using a Magellan 315 GPS unit. Due to logistic problems, Stations 3 and 4 during winter 2004/2005 and Station 4 during spring 2005 were not sampled. Samples were refrigerated in boxes with ice and transported to the laboratory where they were immediately frozen until sorting. Flatfishes were counted and identified to the species level. Trawl opening (1 m) and distance travelled (determined by GPS) were used to estimate the sampled area and densities were standardized as the number of individuals per 1000 m^2 swept.

2.3. Data analyses

Recruitment to the Lima estuary was investigated for the most abundant flatfish species. The temporal and spatial patterns of occurrence for different length larval and juvenile stages of each species were investigated. For larval stages, notochord length was determined for pre-flexion larvae and standard length (SL) for flexion and post-flexion larvae. Those measurements were performed under a binocular microscope with Leica QWin 32 image acquisition software. No correction for shrinkage was performed. In addition, the ontogenetic development stage of each larva was identified (Table 1). For juveniles, the total and standard lengths were determined with 1 mm precision. Due to several observations of damage to the caudal fin, standard length (SL) was measured directly and was then used to estimate the total length (TL). The percent of juveniles occurring in each sample was calculated according to length at first maturation available in the literature for each flatfish species.

Monthly samples were aggregated into three month groups: autumn (A), composed of September, October and November; winter (W) = December, January and February; spring (Sp) = March, April and May and summer (S) = June, July and August.

The temporal and spatial abundance patterns of each larval flatfish species were assessed by 2 way-ANOVA, with seasons and estuarine location (i.e. lower versus middle estuary) as fixed factors (Sokal and Rohlf, 1995; Zar, 1996). For juvenile data, 1 way-ANOVA was used, with seasons or sampling locations as fixed factors. In order to fulfil the ANOVA assumptions (stabilize the variance and fit data to a normal distribution), data were log transformed [$\text{Log } 10(x + 1)$]. Homogeneity of variance was tested with a Cochran test and whenever variance was still heterogeneous following transformation, conclusions from ANOVA results

were only accepted for those cases where significance levels were <0.01 . Additionally, in the event of significance, a *posteriori* Tukey HSD test was used to determine which means were significantly different at a 0.05 level of probability.

3. Results

3.1. Species composition

The Lima estuarine flatfishes included four families, distributed among twelve taxa, where ten could be assigned to species and two only to family (Table 2). The larval fraction comprised nine taxa while six species were identified among the benthic flatfishes. Soleidae was the most abundant family comprising five species, *Buglossidium luteum*, *Microchirus variegatus*, *Solea lascaris*, *S. senegalensis* and *S. solea* and a taxon that was not identified further, Soleidae ni. All of those taxa were found in the larval stage but only *S. senegalensis* and *S. solea* were also found to be part of the juvenile fraction. Pleuronectidae included larvae, juveniles and adults of *P. flesus* and a larval taxon that was not identified further, Pleuronectidae ni. Scopthalmidae contributed two species, one in the larval stage (*Zeugopterus punctatus*) and *Scopthalmus rhombus* as juveniles. Bothidae was also represented by two species: *Arnoglossus laterna* and *A. thori*, both of which were present as juveniles and adults.

Only *S. senegalensis*, *S. solea* and *P. flesus* occurred as planktonic larvae and benthic juveniles, while the remaining flatfish taxa observed in the Lima estuary were only present in one of the two early life-history stages studied. *S. senegalensis* was the most abundant species, achieving 71% of total flatfish larvae and 41% of benthic flatfishes. *P. flesus* was the second most abundant flatfish species, representing 10% of total flatfish larvae and 26% of benthic flatfish catch. *S. solea* was not abundant as larvae, representing only 4% of the total larvae, but as juveniles was the third most abundant species, totalling 12% of the benthic catch.

3.2. Estuarine recruitment of most abundant species

3.2.1. *S. senegalensis*

S. senegalensis larval abundance ranged between 0.0 and $9.1 \text{ larvae } 100 \text{ m}^{-3}$. Larval stages of this species were observed along the initial 7 km stretch of the estuary without significant differences ($F = 0.6$ $p > 0.4$) between the lower and middle estuarine zones (Fig. 2). Still, abundance revealed significant variations between seasons ($F = 7.4$ $p < 0.01$), decreasing during winter–spring periods, mainly in spring and winter 2002 when *S. senegalensis* disappeared from the Lima estuarine fish larvae assemblages (Fig. 2). A total of 149 *S. senegalensis*

Table 2
Mean abundance of larval (number of individuals $100 \text{ m}^{-3} \pm$ standard deviation), juvenile and adult flatfish (number of individuals $1000 \text{ m}^{-2} \pm$ standard deviation) and frequency of occurrence of positive hauls (i.e. hauls with flatfishes) in the Lima estuary.

Family	Species	Larval		Juvenile		Adult	
		Abundance	FO	Abundance	FO	Abundance	FO
		Mean \pm SD		Mean \pm SD		Mean \pm SD	
Bothidae	<i>A. laterna</i>	–	–	1.74 ± 4.80	10%	0.94 ± 3.54	15%
Bothidae	<i>A. thori</i>	–	–	–	–	0.06 ± 0.65	1%
Pleuronectidae	<i>P. flesus</i>	0.04 ± 0.27	15%	2.29 ± 7.30	21%	0.43 ± 2.44	6%
Pleuronectidae	Pleuronectidae ni	–	1%	–	–	–	–
Scopthalmidae	<i>S. rhombus</i>	–	–	0.64 ± 2.50	11%	–	–
Scopthalmidae	<i>Z. punctatus</i>	0.01 ± 0.13	4%	–	–	–	–
Soleidae	<i>B. luteum</i>	–	1%	–	–	–	–
Soleidae	<i>M. variegatus</i>	–	1%	–	–	–	–
Soleidae	<i>S. lascaris</i>	0.03 ± 0.19	11%	–	–	–	–
Soleidae	<i>S. senegalensis</i>	0.29 ± 0.90	75%	4.86 ± 9.91	50%	–	–
Soleidae	<i>S. solea</i>	0.02 ± 0.20	6%	1.39 ± 4.05	20%	–	–
Soleidae	Soleidae ni	0.01 ± 0.16	5%	–	–	–	–

– <0.01 larvae 100 m^{-3} .

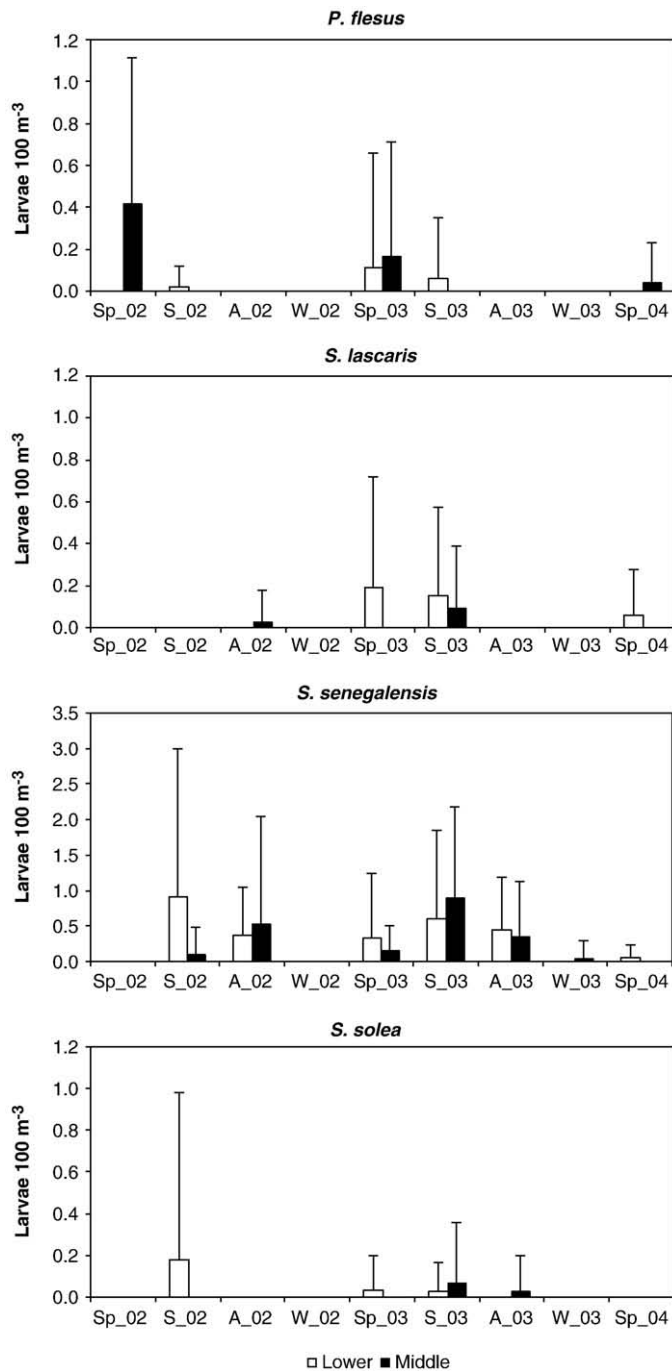


Fig. 2. Seasonal and spatial trends of abundance (mean \pm standard deviation) of the top four larval flatfish species caught in the Lima estuary between April 2002 and April 2004, in the lower and middle Lima estuary. Sp_02: spring 2002; S_02: summer 2002; A_02: autumn 2002; W_02: winter 2002–2003; Sp_03: spring 2003; S_03: summer 2003; A_03: autumn 2003; W_03: winter 2003–2004; Sp_04: spring 2004.

larvae were measured and SL ranged between 1.7 and 5.1 mm. All five ontogenetic stages were observed (Table 4). Stage 2 was the most abundant, representing 72% of *S. senegalensis* larvae. These young larvae were collected along the entire length of the study area, but on average were more abundant in the lower estuary (within 2.2 km of the river mouth) than farther upriver. Similarly, newly hatched larvae (Stage 1) were also more abundant in the lower stretch of the estuary (Fig. 3). Older larvae, including metamorphosing larvae (Stage 5), were not abundant, achieving only 17% of the total larvae and were mainly observed in the shallow middle saltmarsh area (Fig. 3).

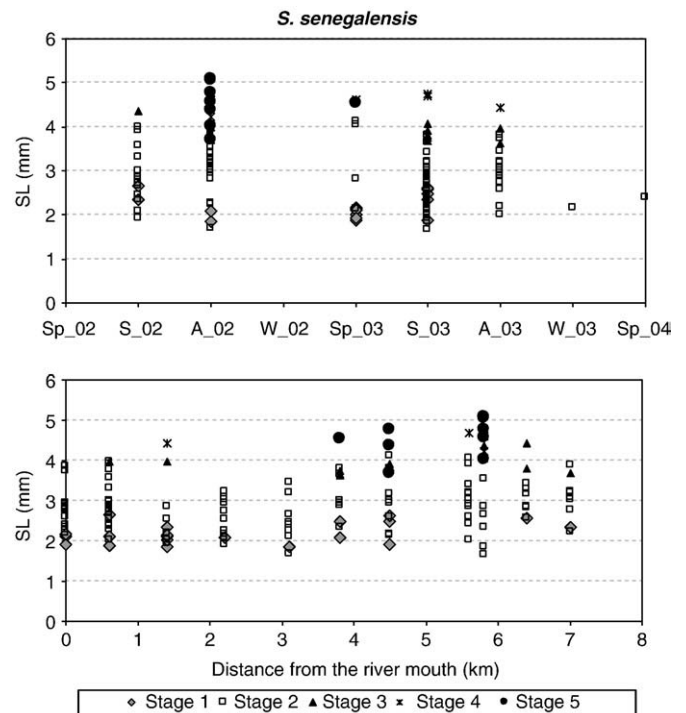


Fig. 3. Seasonal variation of the standard length (SL) by ontogenetic stage of *S. senegalensis* larvae collected in the initial 7 km stretch of the Lima estuary. Sp_02: spring 2002; S_02: summer 2002; A_02: autumn 2002; W_02: winter 2002–2003; Sp_03: spring 2003; S_03: summer 2003; A_03: autumn 2003; W_03: winter 2003–2004; Sp_04: spring 2004.

Seasonal variation in the SL of the larvae revealed different patterns in the two study years. Young larvae (Stages 1 and 2), which were the first to arrive in the estuary, first occurred in June during 2002 but arrived considerably earlier in 2003, during early March (Fig. 3). Their occurrence extended until late October in 2002 and November in 2003, resulting in totals of 5 and 9 consecutive months of residence in 2002 and 2003, respectively. Older larvae appeared 3–4 months later than young larvae. For example, in 2002 metamorphosing larvae first appeared in October, whereas in the following year those larvae were observed earlier, in May, consistent with that year's much earlier appearance of Stages 1 and 2 larvae (Fig. 3).

Regarding the benthic fraction, *S. senegalensis* was captured at all sampling stations, being most frequently observed at Station 4 (present in 34% of samples), but on average more abundant at Station 3 (7.8 ± 13.7 fishes 1000 m^{-2}). The peak abundance of this species was observed in July 2005, when a maximum of 66.7 fish 1000 m^{-2} was registered at Station 3. Abundance did not vary significantly between seasons ($F=2.1$ $p>0.05$), but it tended to decrease during winter periods (Fig. 4). *S. senegalensis* total length ranged 33–156 mm, with an average of 113 ± 58 mm TL. Despite the wide range of lengths, only juveniles were captured (Table 3) and 44% of juveniles measured less than 100 mm. Temporal and spatial distributions of the total length of *S. senegalensis* juveniles showed differences between the two study years (Fig. 5). Small individuals (TL < 50 mm), which represented 11% of total *S. senegalensis* abundance, were most abundant during the spring–summer period of the second year of the study. In this year, they were occasionally observed in distant months (February, June and September 2004). During the second year they were more abundant and continuously observed from April through June. In fact, during spring and summer 2005, the *S. senegalensis* population was dominated by small individuals (TL < 100 mm) (Fig. 5). Focusing on that period, small individuals first colonized the lower polyhaline Station 2 and progressively moved upstream as they grew, initially passing through Station 3 and later

reaching Station 4. Older fish (TL > 200 mm) were not abundant and appeared later than young soles: during late spring–summer months, and in the lower estuary only (Fig. 5).

3.2.2. *P. flesus*

P. flesus larval abundance varied between 0 and 3.3 larvae 100 m⁻³, with an average of 0.04 ± 0.3 larvae 100 m⁻³. The occurrence of these larvae in the Lima estuary showed a typical seasonal pattern that was observed during both study years. *P. flesus* larvae occurred mainly in spring months, predominantly during spring 2002 when their abundance was significantly highest ($F = 3.4$ $p < 0.01$) (Fig. 2). *P. flesus* larvae did not reveal any particular affinity to the lower or middle estuary. However, during spring 2002 in the middle estuary, abundance reached a significant maximum of 0.3 ± 0.7 larvae 100 m⁻³ ($F = 3.0$ $p < 0.001$). Metric analyses of the 29 larvae collected showed that all were in Stage 2 through Stage 4 of development with SLs that ranged between 4.9 and 7.2 mm (Table 4). Stage 4 represented 72% of the *P. flesus* larvae, followed by 20% in Stage 3 and only one larva that was classified as Stage 2. Seasonal variation in mean SL revealed similar patterns of occurrence in the two study years (Fig. 6). The first larvae observed in the estuary were already relatively old (Stage 4), and late-stage larvae continued to arrive in the estuary until July in 2002 and until June in 2003 (Fig. 6). The younger exemplar of *P. flesus* (Stage 2) was collected in February 2004 in the lower estuary.

With respect to the monthly beam trawl data, *P. flesus* occurred in 32% of the positive hauls (i.e. samples containing flatfishes). Total length varied between 27 and 325 mm, corresponding to 73% juvenile and 27% adult (Table 3). On average, juveniles (2.3 ± 7.3 fish 1000 m⁻²) were more abundant than adults (0.4 ± 2.4 fish 1000 m⁻²). This species was not very abundant in the lower estuary, being absent near the river mouth (Station 1). Occasionally, older fish were observed at Station 2, mainly during the winter–spring period of 2005 (Fig. 4). Juveniles tended to concentrate upstream at Station 4, where abundance was significantly highest ($F = 14.7$ $p < 0.001$) and peaks of 44.4 fishes 1000 m⁻² were observed in June and July 2005, a very dry summer. Small individuals (TL < 50 mm), which represented 34% of all *P. flesus* captured, were only observed in early summer (June–July) during both study years, and only upstream at Station 4 (Fig. 5). Older juveniles were frequently captured throughout the study period, but disappeared from November 2004 until April 2005 (Fig. 5). Those juveniles exhibited a broad spatial distribution, being captured at all sampling stations except Station 1.

3.2.3. *S. solea*

S. solea larvae displayed a lower frequency of occurrence than the other species and were most abundant overall in the lower estuary and during the second study year (2003/2004). However, short-term peak abundance was observed in summer of 2002, when it reached a maximum of 3.60 larvae 100 m⁻³ (Fig. 2). A total of six larvae of *S. solea* were measured and SL ranged between 1.85 and 3.31 mm (Table 4). All larvae caught were young: three recently hatched (Stage 1) and three that already presented pigmented eyes (Stage 2).

S. solea represented 12% of the benthic flatfishes and were significantly most abundant at Station 1 ($F = 7.8$ $p < 0.001$), where abundance 22.2 fish 1000 m⁻² in December 2003. On average, *S. solea* was most abundant during autumn–winter 2003 (Fig. 4), but no statistical differences in abundance were detected on a seasonal basis ($F = 0.7$ $p > 0.6$). All *S. solea* individuals collected within the beam trawl surveys were juveniles. The majority of them (70%) measured more than 100 mm TL. Older individuals were mainly observed at Station 1. In contrast, small *S. solea* (TL < 50 mm) were only observed at Stations 2 through 4 and during a specific time: spring 2004 (Fig. 5).

3.3. Other flatfish species

Recruitment patterns were not possible to investigate for other species, due to their relative low abundances. Yet, some relevant information about their use of the system, including temporal and spatial trends of abundance is described. Besides *S. senegalensis* and *P. flesus*, the larval component of the Lima flatfishes also included *S. lascaris*, representing 7% of total flatfish larvae, collected mainly in the lower estuary. These larvae were significantly ($F = 3.8$ $p < 0.001$) most abundant during the summer months of 2003 (Fig. 2). The other larval taxa were considered rare, since their frequency of occurrence was lower than 5% and each species individually did not represent more than 4% of the flatfish larval assemblage (Table 2).

The benthic Lima flatfishes included species that were not present among the larval fish assemblages (Table 2). *A. laterna* was the most abundant, representing 15% of the total catch and present in 24% of positive hauls. Abundance varied significantly among sampling stations ($F = 5.8$ $p < 0.001$), because the presence of *A. laterna* was restricted to the lower estuarine stretch (Fig. 4). Total length ranged between 33 and 156 mm, corresponding to 56% juvenile and 44% adult (Table 3). Juveniles were observed at both lower estuarine stations, being on average more abundant at Station 1 (2.2 ± 6.2 fishes 1000 m⁻²), than at Station 2 (0.8 ± 2.9 fishes 1000 m⁻²). Juveniles were observed during autumn–winter 2003, autumn 2004 and summer 2005 (Fig. 5) and peaked in October 2003, with a maximum of 26.7 fishes 1000 m⁻². During autumn 2003, juveniles were significantly more abundant than during the other seasons ($F = 2.9$ $p < 0.001$). Small individuals (TL < 50 mm) were rare (7%) and only captured during summer periods (Fig. 5). Adults did not appear to exhibit any particular preference for either one or the other of the two lower stations, although older individuals were more frequently observed at Station 2 (Fig. 5). *S. rhombus* was infrequently observed, with abundance that peaked in September 2005 at Station 2 (17.8 fish 1000 m⁻²). Abundance did not vary significantly between seasons ($F = 0.7$ $p > 0.7$), but greatest abundance was observed during autumn 2003 (Fig. 4). *S. rhombus* was not captured in the upper estuary at Station 4 and was more abundant (1.2 ± 3.6 fishes 1000 m⁻²) and frequently captured (11%) at lower estuarine Station 2. Only one fish of this species, captured in April 2005 at Station 2, measured less than 50 mm. The remaining *S. rhombus* measured > 150 mm TL (Fig. 5). During summer 2005, one adult of *A. thori* (147 mm TL) was captured at Station 1 (Table 3).

4. Discussion

4.1. Larval and juvenile flatfish assemblages

The Lima flatfish larval and juvenile assemblages included twelve taxa; nine were collected as pelagic larvae and the benthic flatfish fraction comprised six species. The species composition of the Lima flatfish larval assemblage was similar to that reported for other Portuguese estuaries, but the species richness was slightly higher than in central Portuguese estuaries such as the Mondego (4 taxa; Ribeiro, 1991) and Tagus (5 taxa; Ré, 1984). Nevertheless, recent studies revealed that in the south of Portugal, the Guadiana estuary ichthyoplankton assemblage is composed of more and different flatfish species (Faria et al., 2006) than is the Lima. The species richness of benthic flatfishes observed in the present study (6 species) was similar to several other European estuaries (Elliott and Dewailly, 1995; Mathieson et al., 2000; Thiel et al., 2003), including Iberian ecosystems (Costa and Bruxelas, 1989; Andrade, 1992; Costa et al., 1994; Fariña et al., 1997; Cabral, 2000a; Cabral et al., 2002; Drake et al., 2002; Pombo et al., 2002; Thiel et al., 2003; Vinagre et al., 2005), but relatively lower when compared to other areas, namely the northern Bay of Biscay (8 species; Serrano et al., 2006), the Sado

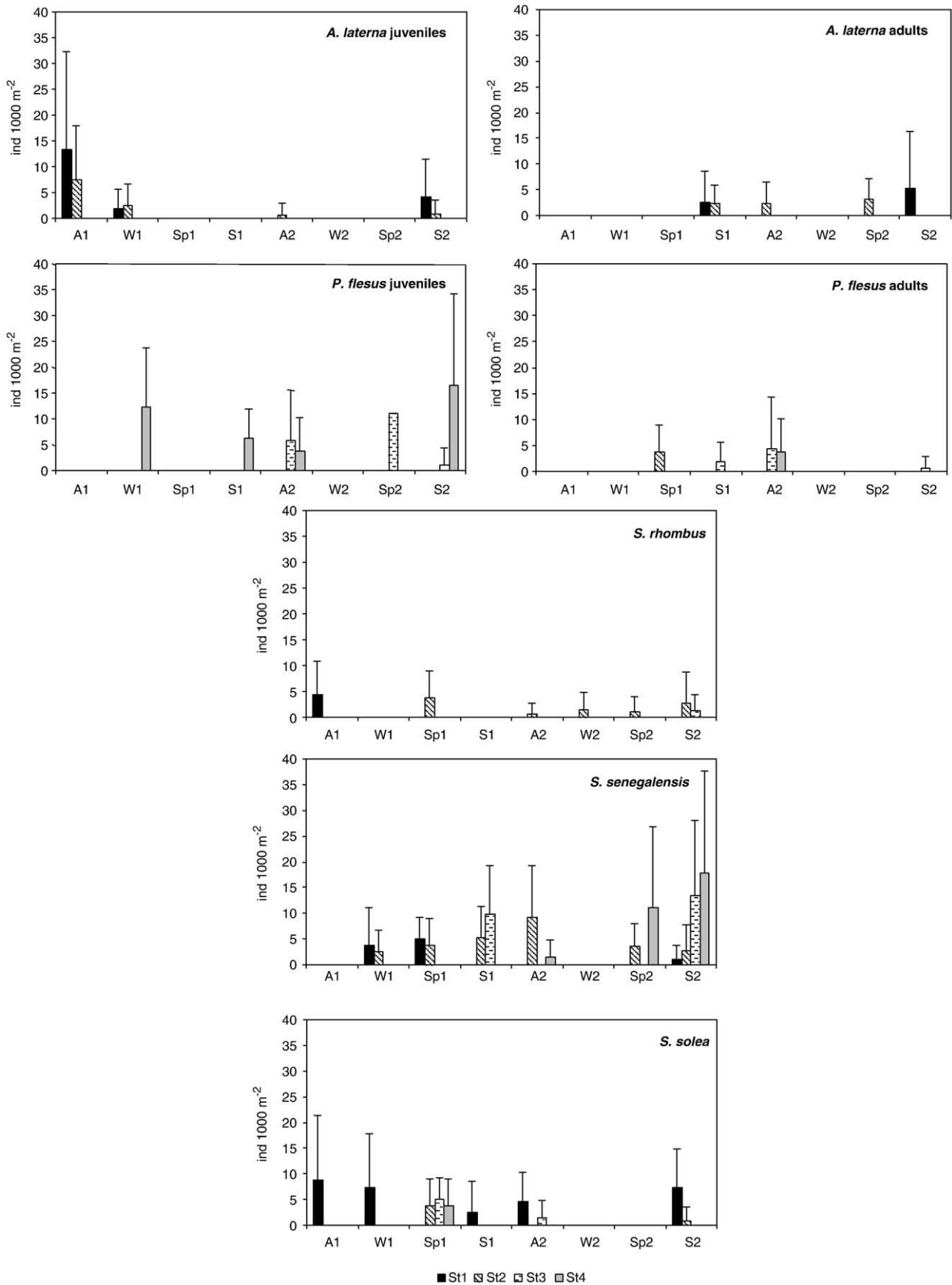


Fig. 4. Seasonal and spatial trends of abundance (mean ± standard deviation) of the top five benthic flatfish species caught in the Lima estuary between October 2003 and September 2005, at the four sampling stations. St1—station 1; St2—station 2; St3—station 3; St4—station 4; A_03—autumn 2003; W_03—winter 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.

Table 3

Mean and standard deviation (Mean \pm SD), minimum (Min) and maximum (Max) of total length (TL) of each flatfish species caught in the Lima estuary between October 2003 until September 2005. Length at first maturation and percentage of juveniles caught (Juv %).

Family	Species	Total length (mm)			Length at first maturation	Juv %
		Mean \pm SD	Min	Max		
Bothidae	<i>A. laterna</i>	100 \pm 33	33	156	110 ^a	50%
	<i>A. thori</i>	147	–	–	120 ^b	0%
Pleuronectidae	<i>P. flesus</i>	136 \pm 87	27	325	200 ^c	70%
Scophthalmidae	<i>S. rhombus</i>	227 \pm 59	32	227	310 ^d	100%
Soleidae	<i>S. senegalensis</i>	113 \pm 58	15	276	300 ^c	100%
	<i>S. solea</i>	110 \pm 46	24	170	320 ^c	100%

^a Deniel (1984).

^b Deniel (1990).

^c Dinis (1986).

^d Quéro (1984).

estuary (18 species; Cabral, 2000a) and Ria Formosa (8 species; Andrade, 1992), the last two of which are in southern Portugal.

Due to its geographical position, the Portuguese west coast lies between three important biogeographic areas: the cold temperate Atlantic, the warm temperate Atlantic and the Mediterranean. This constitutes an important transition zone where marine species with boreal, temperate and subtropical affinities occur in sympatry (Marques et al., 2006). Flatfish species diversity seems to be distributed along latitudinal gradients with higher richness in tropical and subtropical areas, and decreasing towards the boreal zone (Miller et al., 1991; Minami and Tanaka, 1992). The Lima estuarine flatfish assemblages comprised species typically observed in northern European estuaries, but also species associated with warmer waters. Actually, the Portuguese coast constitutes the northern distribution limit of *S. senegalensis* and the southern distribution limit of *P. flesus* (i.e., flounder) within the Atlantic Ocean (Marques et al., 2006). *S. senegalensis* is a species typical of southern European estuaries (Andrade, 1992; Cabral and Costa, 1999; Cabral, 2000a), while flounder is commonly observed at higher latitudes (Kerstan, 1991; Jager et al., 1995; Grioche et al., 1997; Andersen et al., 2005). According to Costa and Cabral (1999), flounder used to frequently use the Tagus estuary as a nursery, but since the 1990s this species has disappeared altogether from the estuarine flatfish community. Nowadays, their southern geographic limit seems to be the northern Atlantic Iberian coast. The simultaneous presence of *S. senegalensis* and *P. flesus* reinforces the importance of the northern Portuguese margin in functioning as a boundary, or mixing-zone, for the distributional range of several flatfish species. The absence of *P. platessa* from the Lima estuarine flatfish assemblages corroborates the hypothesis that Bay of Biscay is close to the southern geographical limit of this species (Desaunay et al., 2006). Yet, these results should be interpreted with caution, since species' geographic distribution limits are dynamic and susceptible to variations, in particular, as they respond to environmental constraints.

Nursery areas for juvenile fishes emerge as an important link between early life-history stages and adult populations (Rooper et al., 2006). According to Beck et al. (2001), a juvenile habitat should be considered a nursery ground whenever its contribution to the adult stock is higher in comparison with other areas. The present study did not allow for an assessment of the real contribution of the Lima estuary to the adult stocks of the coastal flatfish community. However, a comparison of the maximum yields of the most abundant species reported for the Lima estuary to other nurseries areas (Table 6), demonstrates that the Lima abundance values for juveniles are similar or even higher than elsewhere, suggesting that the Lima estuary is indeed a nursery area for *P. flesus*, *S. solea* and *S. senegalensis*. Interestingly, these were the only species that were collected both as larvae and also as juveniles, highlighting the importance of the Lima estuary to early life-history stages of those species.

4.2. Estuarine recruitment

P. flesus recruitment to estuarine habitat was clear, with larvae entering the Lima estuary during spring–summer and newly settled juveniles observed during summer months. This synchrony was observed in two consecutive years of study, for both larvae and juveniles (Figs. 5 and 6), and was consistent with the species' spring spawning season (Table 5). Moreover, the absence of adults during the winter periods (Fig. 5) also conforms with the offshore reproductive migration described to occur during that period of the year (Deniel, 1984). *P. flesus* spawns offshore and its eggs and larvae subsequently disperse into shallow coastal nurseries where they undergo metamorphosis and settle in spring to early summer (Gibson, 1994; Modin and Pihl, 1996; Koubbi et al., 2006). The absence of yolk sac larvae within the estuary is in accordance with this offshore spawning location. Moreover, the majority of larvae were older individuals in post-flexion stage (Table 4) which, according to Koubbi et al. (2006), is the developmental stage in which *P. flesus* larvae start to perform vertical migrations from surface waters to the bottom in order to reach coastal waters and ultimately their shallow estuarine nurseries grounds (Riley et al., 1981; Kerstan, 1991; Raffaelli and Hawkins, 1996). Apart from being captured throughout the initial 7 km stretch of the estuary, *P. flesus* larvae tended to concentrate in the inner area (Fig. 6), which was coincident with the high abundances of newly settled juveniles (TL < 50 mm) that were encountered in the upper estuarine area (Fig. 5). Thus, the spatial and temporal patterns of utilization of the Lima estuary by *P. flesus* can be inferred, as illustrated in Fig. 7. During the winter, adults migrate offshore to spawn. Older larvae arrived at the Lima estuary during spring and into early summer, following a long migration from the offshore spawning ground(s). Pre-metamorphosing larvae tended to concentrate in the inner shallow sections of the estuary; then, metamorphosing larvae underwent settlement within the shallow areas of the upper estuary, where young juveniles emerge and live. As these juveniles grew, their distribution expanded until the middle estuary, where they begin to co-occur with adults.

S. senegalensis was the most abundant flatfish species of the Lima estuary, with respect to larvae and juveniles. *S. senegalensis* larvae appeared in the Lima estuary during spring/summer periods and continued to be encountered until autumn (Fig. 3), what is in agreement with the described spawning season (Table 5). The high abundance of young larvae (Stages 1 and 2, Table 4) indicates that spawning took place near the Lima estuary. There was a trend for older individuals, including metamorphosing larvae, to concentrate in the shallow intertidal saltmarsh area (Fig. 3), suggesting that settlement occurred somewhere in that zone of the estuary. Indeed, young juveniles (TL < 50 mm) were more abundant in the inner intertidal stations than elsewhere (Fig. 5). Vinagre et al. (2006) suggested that *S. senegalensis*, similarly to *S. solea* (Van der Veer et al., 2001), might settle in the intertidal areas. Moreover, juveniles of the species have been associated with intertidal areas, which seem to function as their major feeding grounds, and therefore represent a key factor governing the species' distribution (Cabral, 2000a). In the present study, there was high inter-annual variability in the first appearance of *S. senegalensis* larvae and juveniles within the Lima estuary. Nursery colonization processes are dependent on several factors, namely river flow, tidal cycle and wind regime, which may not be synchronized, inducing several pulses of new recruits over time (Marchand, 1991). This protracted estuarine colonization may also be induced by a prolonged spawning period and by the production of several batches of larvae during the spawning season; this has been demonstrated for *S. senegalensis* (Dinis, 1986; Andrade, 1992). Apart from these temporal variations, the use of Lima estuary was also illustrated for *S. senegalensis* based on the temporal and spatial distributions of larvae and juveniles (Fig. 8). During the spawning season, several pulses of young larvae migrated from the continental

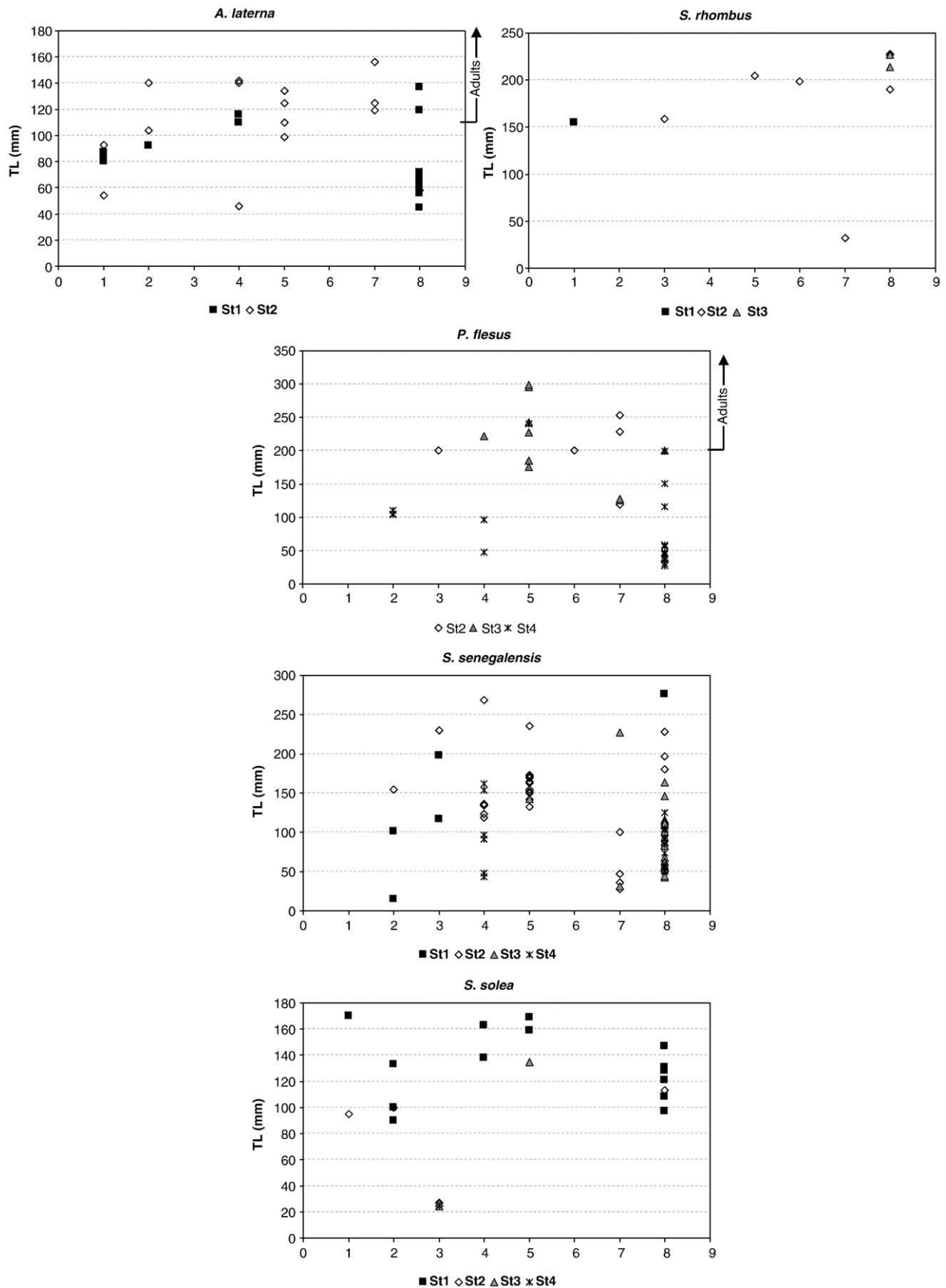


Fig. 5. Seasonal and spatial variations of the total length (TL) of *A. laterna*, *P. fesus*, *S. rhombus*, *S. senegalensis* and *S. solea* collected in the Lima estuary, between October 2003 and September 2005. St1—station 1; St2—station 2; St3—station 3; St4—station 4; A_03—autumn 2003; W_03—winter 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.

Table 4
Mean and standard deviation (Mean ± SD), minimum (Min) and maximum (Max) standard length of each ontogenetic stage of *S. senegalensis*, *P. flesus* and *S. solea* larvae caught in the Lima estuary between April 2002 and April 2004.

Ontogenetic stage	Standard length (mm)											
	<i>S. senegalensis</i>				<i>P. flesus</i>				<i>S. solea</i>			
	N	Mean ± SD	Min	Max	N	Mean ± SD	Min	Max	N	Mean ± SD	Min	Max
Stage 1	16	2.1 ± 0.2	1.9	2.5	0	–	–	–	3	2.0 ± 0.1	1.9	2.1
Stage 2	108	2.8 ± 0.5	1.7	4.1	1	5.0	–	–	3	2.5 ± 0.7	1.9	3.3
Stage 3	7	3.9 ± 0.1	3.7	4.1	4	5.7 ± 0.4	5.3	6.3	–	–	–	–
Stage 4	9	4.5 ± 0.2	4.2	4.8	24	6.0 ± 0.6	4.9	7.2	–	–	–	–
Stage 5	9	4.5 ± 0.5	3.7	5.1	0	–	–	–	–	–	–	–

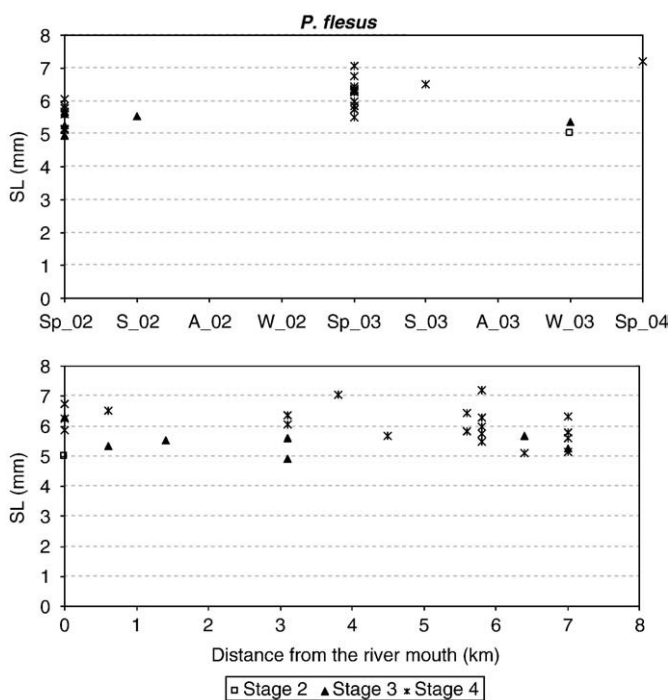


Fig. 6. Seasonal variation of the standard length (SL) by ontogenetic stage of *P. flesus* larvae collected in the initial 7 km stretch of the Lima estuary. Sp_02: spring 2002; S_02: summer 2002; A_02: autumn 2002; W_02: winter 2002–2003; Sp_03: spring 2003; S_03: summer 2003; A_03: autumn 2003; W_03: winter 2003–2004; Sp_04: spring 2004.

shelf into the estuary and dispersed along the lower and middle estuary. With time, those larvae developed and tended to concentrate in the shallow saltmarsh area, where they settled and later emerged as young juveniles. Those young juveniles started to disperse along the inner intertidal areas of the middle and upper sections of Lima estuary and as these juveniles grew, they started to disperse more broadly over the Lima estuary, eventually reaching the lower estuary.

The presence of pelagic larvae of *P. flesus* and *S. senegalensis* is a sign that direct settlement is likely within the Lima estuary. According to this mechanism, estuarine nursery colonization occurs early, during the pelagic larval phase, as metamorphosed larvae settle directly into their estuarine nurseries (Gibson, 1973; Lockwood, 1974). However, our observation of a relatively small number of newly settled fishes may compromise this hypothesis. The low number of very small (newly settled) individuals might be indicative of a methodological problem related to the gear used and/or spatial mismatch between first settlement and the chosen sampling locations. A low yield of very small (0-group) flatfishes has been identified as one of the main problems of similar studies, resulting from the fact that juvenile flatfishes live in shallow intertidal areas where it is not always possible to effectively fish (Riley et al., 1981; Rogers and Millner, 1996). The beam trawl used in the present study, is similar to those used in the Wadden Sea and is able to capture recently-settled *P. platessa*, *P. flesus* and *S. solea* (Van der Veer, 1986; Van der Veer and Bergman, 1987; Van der Veer et al., 2001). Yet, this gear might not be the most appropriate to collect new settled flatfishes in the Lima estuary. In future research, additional fishing gears could be used in order to increase the yield of very small flatfishes.

In contrast to direct settlement, the indirect settlement mechanism presupposes that fish settlement occurs in oceanic habitat and juveniles then migrate into the estuarine nursery areas. This might be the case for *S. solea*, since few pelagic larvae were observed in the Lima

Table 5
Life-history traits of the flatfish species present in the Lima estuary, according to literature.

Species	Distribution	Habitat	Spawning period	Nurseries
<i>A. laterna</i>	Eastern Atlantic from 65°N to 21°N and Mediterranean ^a	Coastal areas, mainly between 10 and 60 m, occasionally in estuaries ^a	From April to August ^{a,b}	Coastal areas ^b
<i>P. flesus</i>	Eastern Atlantic from 70°N to 35°N and western Mediterranean ^c	Shallow coastal areas. Common in estuaries and also found in freshwaters ^c	February to June ^{c,b}	Coastal areas and estuaries ^b
<i>S. rhombus</i>	Eastern Atlantic: 64°N to Morocco and Mediterranean and Black Sea ^d	Shallow coastal waters, Scarce in brackish water ^d	March to August ^d	No information
<i>S. senegalensis</i>	Eastern Atlantic, from Bay of Biscay to Senegal, and western Mediterranean (rare) ^b	From coastal marine areas and estuaries to 100 m ^b	May to August ^e April to July ^{f,g}	Mainly estuaries ^{e,f,g}
<i>S. solea</i>	Eastern Atlantic, from 65°N to Senegal, and Mediterranean ^b	From the shore down to 200 m ^b	April to June (North Sea) ^b December to May (South Europe) ^{e,f,g}	Coastal areas, mainly estuaries ^{e,f,g}

^a Nielsen (1986a).
^b Quéro et al. (1986).
^c Nielsen (1986b).
^d Bauchot (1987).
^e Deniel (1984).
^f Andrade (1992).
^g Dinis (1986).

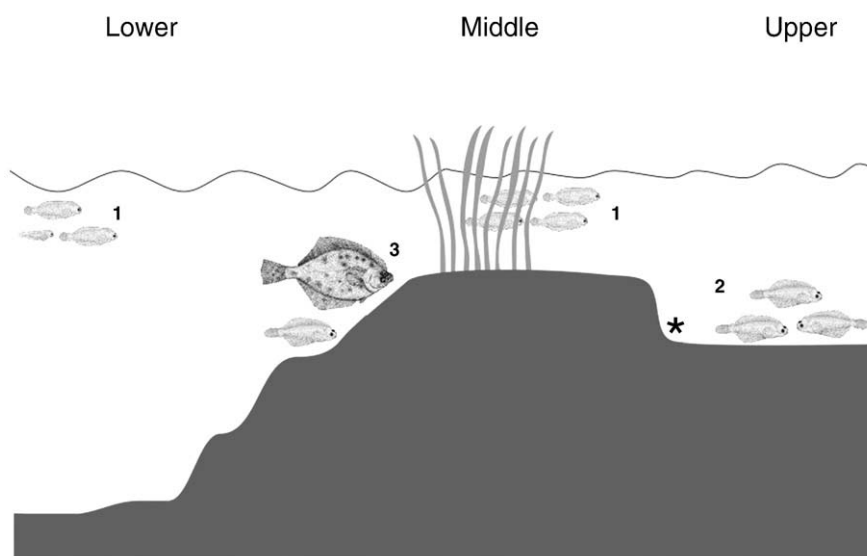


Fig. 7. Summary of the early life of *P. flesus* in the lower, middle and upper Lima estuarine sections. 1—metamorphosing larvae; 2—new settled juveniles and 3—adults; *—possible settlement area.

estuary (Table 2). *S. solea* nursery grounds have been reported from marine coastal areas as well as in estuarine systems (Pihl, 1989; Dorel et al., 1991; Marchand, 1993; Rogers, 1994). Juveniles of *S. solea* concentrate in estuaries and in bays for a period of about two years post-settlement (Koutsikopoulos et al., 1989). Cabral and Costa (1999) described a seasonal pattern of juvenile *S. solea* distribution characterized by an inshore spring migration. The present results are also consistent with the spring inshore migration, since the smallest individuals arrived at the Lima estuary during spring (Fig. 5), after the described winter spawning (Table 5).

The overall patterns observed here of habitat usage by the Lima estuarine flatfish assemblages are partially in accordance with those described for neighboring NW Portuguese estuaries, namely the Minho and Douro estuaries (Cabral et al., 2007). The presence of *P. flesus* and *S. solea* nursery grounds seems to be a characteristic of the northern Portuguese estuaries (Table 6). However, *S. solea* was not

very abundant in the Lima, representing only 12% of the flatfish assemblages. The volume of available nursery habitat will also affect the abundance of species. The amount and type of habitat available can influence survival (Fernandez et al., 1993; Lipcius et al., 1997), growth (Tupper and Boutilier, 1995) and distribution (Norcross et al., 1997; Howell et al., 1999) of juvenile fishes and crustaceans (Rooper et al., 2004). The reduced dimensions of the Lima estuary may not be able to support the coexistence of two similar and competitive species such as *S. solea* and *S. senegalensis* at high abundance, contrary to larger estuarine systems such as the Tagus and Sado, further to the south (Table 6). It has also been demonstrated that density-dependent factors may induce high levels of juvenile mortality (Gibson, 1994; Rogers, 1994). High abundance of *S. senegalensis* was another unique nursery feature of the Lima that has not been reported for other northern Portuguese estuaries, where this species is not very abundant (Cabral et al., 2007).

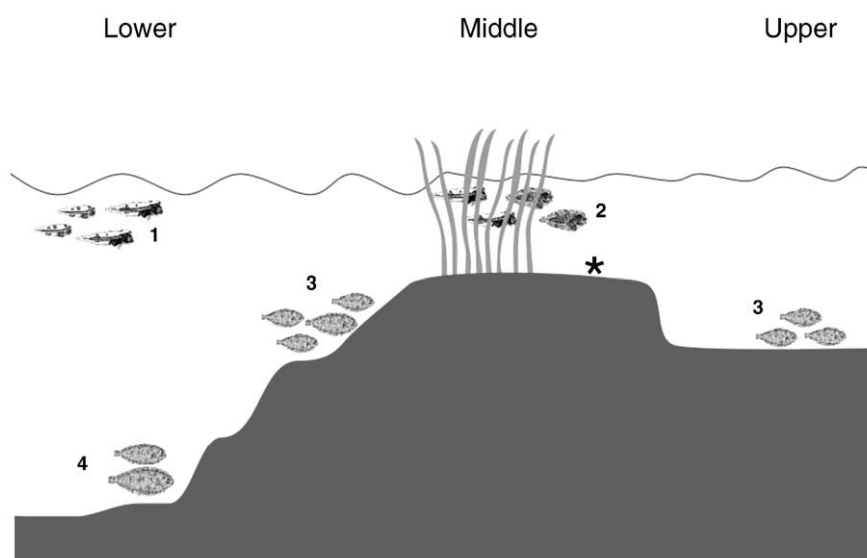


Fig. 8. Summary of the early life of *S. senegalensis* in the lower, middle and upper Lima estuarine sections. 1—young larvae; 2—metamorphosing larvae; 3—new settled juveniles and 4—older juveniles; *—possible settlement area.

Table 6
Peak densities (number of individuals 1000 m⁻²) reported for *P. flesus*, *S. solea*, *S. senegalensis* in the Lima estuary (present study*) and in several estuarine systems along the Portuguese coast (data source Cabral et al. 2007).

Estuary	Lima*	Minho	Douro	Ria de Aveiro	Mondego	Tejo	Sado	Mira	Ria Formosa	Guadiana
Total area (km ²)	5.8	23	7.8	96.5	9.8	325	174.7	4.1	72.2	20.3
<i>P. flesus</i>	44.4	236	9.1	4.7	30	0	0	0	0	0
<i>S. solea</i>	22.2	67	8	2.2	23.1	25.9	24.5	15.7	0	0
<i>S. senegalensis</i>	66.7	0	0	1.9	0	61.6	3.1	0	4.1	0.5

4.3. Conclusions

The Lima estuarine flatfish assemblage comprised twelve taxa, but only *S. senegalensis*, *P. flesus* and *S. solea* occurred in the estuary during both the planktonic larval and benthic juvenile phases of life. In fact, only these species were observed to use the Lima estuary as nursery area. Results indicated that *S. senegalensis* and *P. flesus* colonized the Lima nursery area earlier, during the pelagic larval phase, while *S. solea* migrated into the estuary later in life, during the juvenile phase. Moreover, results also indicated that *S. senegalensis* and *P. flesus* used different microhabitats of the Lima estuary as nursery grounds, namely the intertidal saltmarsh and the upper estuary, respectively. In conclusion, this study contributed to increase the scientific knowledge of *S. senegalensis* early life history, and highlights the importance of integrating the early planktonic larval phase into traditional flatfish nursery studies.

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