

ICHTHYOPLANKTON OF THE LIMA ESTUARY (NW PORTUGAL): ECOLOGY OF THE EARLY LIFE STAGES OF PLEURONECTIFORMES

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Dissertação de Doutoramento em Ciências do Meio Aquático

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PORTUGAL): ECOLOGY OF THE EARLY LIFE STAGES OF
PLEURONECTIFORMES**

Dissertação de Candidatura ao grau de Doutor em
Ciências do Meio Aquático, submetida ao Instituto de
Ciências Biomédicas de Abel Salazar da Universidade
do Porto.

Orientador – Professor Doutor Adriano Bordalo e Sá

Categoria – Professor Associado com Agregação

Afiliação – Instituto de Ciências Biomédicas Abel
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Co-orientador – Professor Doutor Pedro Miguel Alfaia
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Categoria – Professor Associado com Agregação

Afiliação – Faculdade de Ciências da Universidade de
Lisboa.

Aos meus pais e avó.
E ao Nuno.

Fundo do mar

*“No fundo do mar há brancos pavores,
Onde as plantas são animais
E os animais são flores.*

*Mundo silencioso que não atinge
A agitação das ondas.
Abrem-se rindo conchas redondas,
Baloíça o cavalo-marinho.
Um polvo avança
No desalinho
Dos seus mil braços,
Uma flor dança,
Sem ruído vibram os espaços.*

*Sobre a areia o tempo poisa
Leve como um lenço.*

*Mas por mais bela que seja cada coisa
Tem um monstro em si suspenso.”*

Sophia de Mello Breyner Andresen

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Resumo

O estuário do Rio Lima (NW Portugal) é a parte terminal de uma bacia hidrográfica internacional cujo potencial como local de desova e de viveiro para peixes marinhos nunca foi avaliado. O principal objectivo desta tese foi estudar, pela primeira vez, os estados iniciais de desenvolvimento de peixes que ocorrem no estuário do Rio Lima, com especial destaque para aspectos da Ecologia de estados iniciais de desenvolvimento de Pleuronectiformes. Para além do ictioplâncton estuarino, este estudo também investigou as comunidades de peixes bentónicos, com o objectivo final de analisar os estados juvenis e adultos de Pleuronectiformes e assim, analisar a função de viveiro do estuário do Rio Lima.

O estudo iniciou-se com a caracterização dos padrões temporais e sazonais das comunidades durante um período de 2 anos. A abundância média foi de 8 indivíduos 100 m^{-3} e 50 taxa pertencentes a 20 famílias foram identificados. As seis espécies mais abundantes (*Pomatoschistus* spp., *Sardina pilchardus*, *Ammodytes tobianus*, *Clupeidae* não identificado, *Symphodus melops* e *Solea senegalensis*) totalizaram 91% do total de larvas recolhidas. Em geral, a diversidade foi baixa ($H' = 0.65$), com uma grande dominância de poucos taxa, nomeadamente *Pomatoschistus* spp. O padrão sazonal revelou que ocorreu um importante decréscimo da abundância e diversidade das comunidades ictioplânctónicas durante o Inverno, altura em que quase não foram recolhidas estados larvares de teleósteos. Segundo os resultados da análise canónica efectuada, as variações sazonais da temperatura, precipitação e consequentemente do caudal do rio, foram responsáveis pelas variações temporais das comunidades, enquanto que a distância à barra e o consequente regime de salinidade do estuário, foram responsáveis pelos padrões espaciais da comunidade de larvas de peixe do estuário do Lima. Este estudo reforçou o conceito de que as alterações climáticas inter-anuais têm uma grande influência no ictioplâncton estuarino. Por outro lado, a elevada frequência e abundância de larvas de *S. pilchardus* observada foi analisada, uma vez que os estados larvares desta espécie são geralmente considerados como ocasionais e acidentais em ambientes estuarinos.

A maioria das sardinhas recolhidas foram larvas pouco desenvolvidas, apresentando ainda vitelo e com uma semana de idade. Estas larvas recém-eclodidas foram observadas continuamente, durante o período de estudo, indicando que a desova ocorreu ao longo de todo o ano. A abundância de larvas de sardinhas foi mais elevada no fim da Primavera e no Verão, o que não coincide com o Inverno, descrito como a época de maior intensidade de postura do stock de sardinhas do norte de Portugal. As variações sazonais e diárias da ocorrência das larvas de sardinha no estuário do Lima foram controladas por factores ambientais, principalmente a salinidade e a temperatura. Por outro lado, alguns fenómenos oceanográficos, nomeadamente o *upwelling*, podem ter controlado a presença de larvas de sardinha na zona costeira e, conseqüentemente, no interior do estuário.

A segunda parte desta tese focou aspectos referentes à comunidade de peixes bentónicos que habitam o estuário do Lima. No total, 29 espécies distribuídas por 17 famílias de teleósteos foram observadas ao longo de todo o estuário. Destas espécies, treze foram classificadas como espécies residentes, seis como espécies marinhas casuais, seis como juvenis residentes, uma como migrador sazonal marinho e outra como catádroma. A comunidade de peixes bentónicos apresentou-se temporalmente estável, mas distribuída de forma heterogénea ao longo do estuário. Factores como a salinidade, distância à barra, tipo de sedimento e aspectos qualitativos de cada local, nomeadamente a presença de sapal, de dragagens e a profundidade dos locais foram os responsáveis pelas variações encontradas. Duas espécies residentes de góbios dominaram as capturas: *Pomatoschistus microps* e *P. minutus*. Estas duas espécies, largamente distribuídas pela área de estudo, totalizaram 95% das capturas. Várias espécies marinhas, algumas de elevado interesse económico, estiveram presentes apenas no estado juvenil, como foi o caso de certas espécies de Pleuronectiformes. No estuário do Lima foram observados 12 taxa de Pleuronectiformes, distribuídos por quatro famílias (Bothidae, Pleuronectidae, Scopthalmidae and Soleidae). Na fracção juvenil foram encontrados nove taxa e seis espécies na fracção bentónica. A abundância tanto de larvas como de juvenis apresentou uma tendência para aumentar nos períodos de Primavera-Verão. Contudo, a dinâmica da fracção larvar diferiu da dinâmica dos estados juvenis bentónicos. Variações sazonais de temperatura e salinidade foram identificados como os principais factores ambientais reguladores da dinâmica dos estados larvares de Pleuronectiformes. Por outro lado, os juvenis e adultos bentónicos estiveram relacionados com características específicas de cada local, tais como o tipo de sedimento, distância à barra e subsequente regime de salinidade do estuário. O recrutamento de estados iniciais de desenvolvimento de

Pleuronectiformes para o estuário do Lima foi controlado por factores ambientais, nomeadamente a época de desova e o hidrodinamismo do estuário. Neste estudo foram capturados estados larvares, e também juvenis, de três espécies de Pleuronectiformes, *Platichthys flesus*, *S. senegalensis* and *S. solea*. *S. senegalensis* foi a espécie mais abundante, tanto na fracção larvar como na fracção juvenil da comunidade de Pleuronectiformes do estuário. Não foi possível identificar um padrão temporal no aparecimento de larvas de *S. senegalensis*, uma vez que estados larvares foram observados durante um longo período de tempo compreendido entre a Primavera e o Outono. *P. flesus*, a segunda espécie mais abundante, apresentou um padrão sazonal que não variou entre os dois anos de estudo. As larvas em elevado estado de desenvolvimento começaram a aparecer no estuário durante a Primavera e os primeiros juvenis do ano foram observados no Verão seguinte. Os estados larvares de *S. solea*, não foram muito abundantes, mas os juvenis representaram 12% do total de Pleuronectiformes bentónicos. O estuário do Lima foi utilizado como local de viveiro por *S. senegalensis*, *P. flesus* and *S. solea*, cujo *settlement* foi directo para as duas primeiras espécies e indirecto para *S. solea*. Assim, o estuário do Lima pode ser considerado como o limite Norte para viveiro de *S. senegalensis*. O presente estudo evidenciou algumas particularidades do ecossistema estuarino do Rio Lima, assim como a importância potencial para espécies não consideradas como dependentes de estuários. Apesar das grandes pressões a que o estuário está sujeito, continua a representar um importante habitat na zona costeira do nordeste Atlântico.

Abstract

The Lima River estuary (NW Portugal) is the end member of an international watershed, whose potential role as spawning and nursery habitat for fish populations has not been previously examined. The main purpose of this study was to investigate for the first time the early life stages of fishes occurring in the Lima estuary, with special emphasis on the ecological aspects of Pleuronectiformes early life history. In addition to the ichthyoplankton, the overall benthic fish fauna was also characterized, with the main purpose of investigate the juveniles and adults of Pleuronectiformes, and ultimately assess the potential nursery role of Lima River estuary.

This thesis started with the characterization of seasonal and spatial patterns of the estuarine ichthyoplankton over a 2-year period. A total of 20 families and 50 *taxa* were collected, with a mean abundance of 8 individuals 100 m^{-3} . The top six abundant *taxa* (*Pomatoschistus* spp., *Sardina pilchardus*, *Ammodytes tobianus*, unidentified Clupeidae, *Symphodus melops* e *Solea senegalensis*) represented 91% of the total catch. Diversity was generally low ($H' = 0.65$) with high dominance of very few *taxa*, namely *Pomatoschistus* spp. A seasonal decrease in abundance and diversity of the larval assemblage was observed during winter, when fish larvae abundance dramatically decreased. According to results of the canonical correspondence analysis, seasonal variations of temperature, precipitation and consequently river flow were responsible for the temporal differences of the fish larval assemblages, while distance from the river mouth and the salinity regime were responsible for the encountered spatial trends of the larval fish assemblages. The present study reinforced the concept that interannual climate and hydrodynamic variations have a strong influence on estuarine ichthyoplankton. The unusual high numbers and frequency of *S. pilchardus*, usually mentioned as accidental in estuarine systems was also investigated. The majority of the sardines collected were young 1 week old larvae, still presenting yolk. These newly-hatched sardines were continuously observed during both study years, indicating that hatching took place throughout the entire year, contradicting the winter-spring spawning season described for the northern Portuguese

coast. Moreover, the abundance peaks of sardine larvae within the estuary did not coincide with the reported spawning peaks of this northern stock. Seasonal and diel variations of sardine occurrence in the Lima River estuary were controlled by environmental features, mainly salinity and temperature. In addition, oceanographic features such as upwelling events could have influenced the coastal presence of sardine larvae, and ultimately controlled its estuarine presence.

The benthic fish fauna of the Lima River estuary was the focus of the second part of this thesis. A total of 29 species of 17 teleost families were captured within the estuary. Thirteen were classified as estuarine resident species, six as marine adventitious visitor species, six as marine juvenile resident species, one as marine seasonal migrant and one was catadromous. The Lima River benthic fish assemblage was seasonally stable but heterogeneously distributed among the different types of habitats, as a function of salinity, distance to mouth, sediment characteristics as well as qualitative habitats features, such as presence of saltmarsh, dredging activity and depth of the water column. Assemblages were dominated by two resident Gobiidae species *Pomatoschistus microps* and *P. minutus* that represented 95% of the total catches and were collected throughout the study area. However, several economically important marine species were only captured as juveniles, as was the case of some flatfish species. The Lima River estuarine flatfish assemblage comprised 12 taxa distributed by four families (Bothidae, Pleuronectidae, Scopthalmidae and Soleidae). The flatfish larval fraction comprised nine taxa and six species were identified among the benthic flatfishes. There was a general trend for a spring-summer abundance increase of both larvae and juveniles flatfishes. The pelagic larval fraction exhibited a distinct dynamics from the benthic estuarine flatfish community. Flatfish larvae were mainly regulated by seasonal variations of temperature and salinity of the estuarine habitat, while benthic flatfishes were markedly controlled by site-specific characteristics such as sediments structure, distance from the river mouth and the salinity regime. The estuarine recruitment of flatfishes was mainly controlled by the spawning season and the hydrodynamics of the estuary. Three species, *Platichthys flesus*, *S. senegalensis* and *S. solea* were captured both as larvae and juveniles. *S. senegalensis* was the most abundant flatfish species of both larval and juvenile fractions of the Lima River estuarine flatfish community. Estuarine recruitment of *S. senegalensis* did not reveal a clear temporal pattern, with young larvae occurring in the estuary throughout a prolonged period from spring to autumn. *P. flesus* was the second most abundant species and larvae, which estuarine recruitment revealed a clear temporal pattern. Developed larvae arrived in the estuary during spring whereas the 0-group juveniles

started to appear in the following summer period. *S. solea* was not an abundant component of the flatfish larval assemblage, but juveniles reached 12% of the total flatfish benthic catch. The estuary was used by *S. senegalensis*, *P. flesus* and *S. solea* as a nursery area with direct settlement for the two first species, while *S. solea* was suspected to undergo an indirect settlement. The Lima River estuary emerged as the northern nursery area for *S. senegalensis* in the west Iberian margin. The present study highlighted some unique aspects of this estuarine ecosystem and its potential importance for non typical estuarine dependent species. In spite of the high level of estuarine area reclamation for urban and industry, the Lima River estuary remains an ecologically and economically important habitat in the coastal NE Atlantic zone.

Résumé

L'estuaire du fleuve Lima (NW Portugal) est le membre d'un bassin hydrographique international, dont le rôle potentiel de frai et de l'habitat pour les poissons marins n'a pas été précédemment examinées. L'objectif principal de cette étude était d'étudier pour la première fois les stades initiales de la vie des poissons qui utilisent l'estuaire du Lima, en mettant particulièrement l'accent sur l'écologie des Pleuronectiformes. À part du ichthyoplancton, l'ensemble de la faune benthique de poissons a été également caractérisé, avec l'objectif principal de l'enquête sur les jeunes et les adultes des Pleuronectiformes et, à terme, évaluer le potentiel rôle de pépinière estuaire de la rivière Lima.

Cette thèse a commencé avec la caractérisation des variations saisonnières et spatiales de l'ichthyoplancton estuarien au cours d'une période de 2 ans. Un total de 20 familles et 50 taxons ont été recueillis, l'abondance avec une moyenne de 8 personnes à 100 m³. Les six plus abondantes taxons (*Pomatoschistus* sp., *Sardina pilchardus*, *Ammodytes tobianus*, *Clupeidae* non identifié, *Symphodus melops* et *Solea senegalensis*) ont représenté 91% du total des captures. La diversité était généralement faible ($H' = 0,65$) à forte dominance de peu taxons, comme les *Pomatoschistus* sp. Une baisse saisonnière dans l'abondance et la diversité des larves de l'assemblage a été observé au cours de l'hiver, quand l'abondance des larves de poissons a diminué de façon spectaculaire. Selon les résultats de l'analyse de correspondance canonique, les variations saisonnières de température, de précipitations et, par conséquent, le débit fluvial sont responsables de la différence temporelle des poissons larvaires des assemblages, alors que la distance l'embouchure de la rivière et la salinité sont responsables pour les tendances spatiales des larves de poissons. La présente étude a renforcé l'idée que l'hydrodynamique interannuelle du climat et de variations ont une forte influence sur l'ichthyoplancton. Le nombre élevé d'inhabituel et de la fréquence de *S. pilchardus*, le plus souvent mentionné comme accidentelle dans les systèmes estuariens a également été étudié. La majorité des sardines était des larves d'une semaine qui présentant toujours le

jaune. Ces nouvelles éclos de sardine ont été observés en permanence au cours des deux années d'études, en indiquant que l'éclosion a eu lieu pendant toute l'année, contredisant la saison hiver-printemps de frai décrits pour le nord de la côte portugaise. En outre, les pics d'abondance des larves de sardines dans l'estuaire ne coïncidaient pas avec les sommets de frai du stock de sardines du Nord du Portugal. Les variations journalières et saisonnières des larves de sardine ont été contrôlées par des caractéristiques environnementales, notamment la salinité et de température. En outre, les caractéristiques océanographiques telles que la remontée des événements pourraient avoir influé sur la présence des larves de sardine côtières, et finalement contrôlé sa présence estuariens.

La faune benthique de poissons de l'estuaire du fleuve Lima a fait l'objet de la deuxième partie de cette thèse. Un total de 29 espèces de téléostéens et 17 familles ont été capturés dans l'estuaire. Treize ont été classés comme larves d'espèces résidentes dans l'estuaire, six comme visiteur accidentelle d'espèces marines, six comme mineurs résidant espèces marines, une espèce marine comme saisonniers migrants et un autre a été cathadrome. L'assemblage des poissons benthiques était saisonnièrement stable, mais hétérogènement répartie entre les différents types d'habitats, en fonction de la salinité, la distance à l'embouchure, les caractéristiques des sédiments, ainsi que des caractéristiques qualitatives, comme la présence de marais salés, de l'activité de dragage et de la profondeur de la colonne d'eau. Les assemblages ont été dominés par deux espèces résidant Gobiidae *Pomatoschistus microps* et *P. minutus* qui ont représenté 95% du total des captures et ont été recueillies tout au long de la zone d'étude. Toutefois, plusieurs espèces marines économiquement importantes ont été capturées en tant que mineurs seulement, comme cela a été le cas de certaines espèces de poissons plats. La rivière Lima estuariens plats d'assemblage a compté 12 taxons distribués par quatre familles (Bothidae, Pleuronectidae, Scopthalmidae et Soleidae). La fraction juvénile comprenait neuf taxons et six ont été identifiés parmi les espèces benthiques. Il y avait une tendance générale d'une augmentation de l'abondance des larves et des jeunes poissons plats pendant la période printemps - été. La dynamique des fractions de larves a été différente de la dynamique de la communauté benthique des poissons plats. Larves des poissons plats sont principalement régis par les variations saisonnières de température et de salinité des estuaires l'habitat, tandis que les poissons plats benthiques ont été nettement contrôlé par les caractéristiques spécifiques des sites tels que la structure des sédiments, la distance de l'embouchure de la rivière et de la salinité régime. Le recrutement des poissons plats estuariens est

essentiellement contrôlé par la saison du frai et par l'hydrodynamique de l'estuaire. Trois espèces, *Platichthys flesus*, *S. senegalensis* et *S. solea* ont été capturés en tant que larves et juvéniles. *S. senegalensis* a été la plus abondante des deux espèces de poissons plats dans les fractions larvaire et juvénile. Le recrutement de *S. senegalensis* dans l'estuaire n'a révélé une nette évolution temporelle, car les jeunes larves se produisent dans l'estuaire tout au long d'une longue période allant du printemps à l'automne. *P. flesus* la deuxième espèce la plus abondante a présenté un comportement temporel clair qui s'est répété pendant toute la durée de l'étude. Les larves bien développées sont arrivées dans l'estuaire au printemps alors que le groupe des jeunes de 0 à commencé à apparaître dans la période estivale. *S. solea* n'était pas une composante de l'abondance des larves d'assemblage des plats, mais des jeunes atteignent 12% du total des captures Pleuronectiformes benthiques. L'estuaire a été utilisé par *S. senegalensis*, *P. flesus* et *S. solea* comme une pépinière avec règlement direct pour les deux premières espèces, tandis que *S. solea* était soupçonné de se soumettre à une colonisation indirecte. L'estuaire du fleuve Lima apparue comme le secteur nord de pépinière pour *S. senegalensis*, de la marge ibérique ouest. La présente étude a mis en lumière certains aspects uniques de cet écosystème estuarien et son importance potentielle pour les espèces dépendantes des estuaires. En dépit du niveau élevé des pressions sur l'estuaire du Lima, la région demeure un habitat écologiquement et économiquement important habitat dans la zone côtière du nord-est de l'Atlantique.

List of papers

This thesis is based on the following papers:

1. Ramos, S., Cowen, R. K., Ré, P. and Bordalo, A. A., 2006. Temporal and spatial distribution of larval fish assemblages in the Lima estuary (Portugal). *Estuarine Coastal and Shelf Science*, 66, 303-314.
2. Ramos, S., Cowen, R. K., Paris, C., Ré, P. and Bordalo, A. A., 2006. Environmental forcing and larval fish assemblage dynamics in the Lima River estuary (NW Portugal). *Journal of Plankton Research*, 28(3), 275-286.
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6. Ramos, S., Ré, P. and Bordalo, A. A. Environmental control on early life stages of flatfishes within a nursery area (Lima estuary, NW Portugal). Submitted to *Estuarine Coastal and Shelf Science*.

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CHAPTER 1

General introduction

This dissertation has been designed to improve the scientific knowledge of the early life stages of fishes in the Lima estuary, with special attention to the ecological aspects of the early life of pleuronectiformes. Based on the temporal and spatial trends of larval and juvenile fish assemblages, the ecological role of the Lima estuary for the initial developmental stages of flatfishes was addressed for the first time. Thus, a gap in scientific knowledge was filled and, at the same time, these studies provided a base line for future research, particularly for evaluating the condition of an estuarine ecosystem using fish larvae.

1.1 Estuaries as unique environments

Historically, people have always lived near estuaries all over the world, using them as a gateway for mobility, trade and food retrieval. These ecosystems have been classified as the most productive and valuable aquatic systems on earth (Costanza et al.1997) and are recognized worldwide as an important component of continental coasts in terms of their biological importance and utilization by humans (Elliott and McLusky 2002; Yáñez-Arancibia and Day 2004).

Since the earliest attempts to study estuaries in a comprehensive fashion, several definitions of those systems have been proposed according to geomorphology, hydrography, sediments and fauna. One of the first definitions was proposed by Odum

(1959), which described an estuary as a “river mouth where tidal action brings about a mixing of salt and fresh water”. Later, Pritchard (1967) developed the concept and defined an estuary as “a semi-enclosed body of water which has a free connection with the open sea and within which sea water is measurably diluted with fresh water derived from land drainage”. This definition does not take into account the influence of the tide, which can be important even in the tidal reaches of the river above the saline intrusion. More recently, Dyer (1997) proposed a definition of estuary based on the Pritchard's physical definition, but with mention to the tidal influence: “an estuary is a semi-enclosed coastal body of water which has a free connection to the open sea, extending into the river as far as the limit of the tidal influence, and within which sea water is measurably diluted with fresh water derived from land drainage”.

Characteristics of estuaries vary greatly and are determined by the shape of the estuary, the volume of river flow at the head of the estuary, and the range of tides at the estuaries mouth. The mixing of waters of different densities, the rise and fall of tides, and the flow of the incoming river all combine with the local characteristics of wind, temperature, precipitation and currents to result in a complex pattern of water circulation. Thus, according to the magnitude of salt water circulation there are different types of estuaries: *Salt wedge* - when the river output greatly exceeds marine input, there is little mixing, and therefore a sharp contrast between fresh surface water and saline bottom water; *partially mixed* – the river output and marine input are more or less even and turbulence induces more mixing of salt water; *vertically mixed* – here the marine input exceeds the river output, once the freshwater contribution is negligible, salinity only varies longitudinally and is similar between the top and bottom of the water column. There is also the *inverse estuary*, which occurs in regions with high evaporation, where there is no freshwater input and in fact salinity increases inland. In some regions of the globe, there are estuaries that vary dramatically depending on freshwater input called *intermittent estuaries*. These are capable of changing from a wholly marine embayment to any of the other estuary types.

Estuaries are transitional waters, as defined in the Water Framework Directive (WFD 2000), and work as boundaries between rivers and ocean. Consequently, extreme and steep gradients in many physical and chemical variables, including salinity, temperature, pH, dissolved oxygen, redox potential, nutrients and quality and quantity of particles may occur. Those gradients exist along the longitudinal axis of an estuary, i.e. between the river and sea but also laterally, between banks. Moreover, in stratified and partially stratified estuaries such as salt wedge and fjords (Dyer 1997), vertical gradients arise along the water column, between the surface and the bottom.

Estuaries are not a single habitat, but rather a complex and interrelated web of habitats of great ecological value, including unvegetated habitats like sand and mudflats, and vegetated habitats, namely saltmarshes, oyster and seagrass beds (McLusky and Elliott 2004). Salt marshes involve areas with alluvial sediments deposited on the shore by the sea and subjected to tidal or weather-effected inundation by more or less diluted seawater. Salt marshes occur on intertidal shores in mid- and high latitude regions worldwide, since mangroves, which dominate the tropical zones, cannot survive in lower temperatures (Kennish 2001). Estuarine intertidal mudflats are very important in the functioning of estuarine systems, and it is generally recognized that they have a disproportionately high productivity when compared to subtidal areas (Elliott and Dewailly 1995).

Estuarine environments are typically considered to be rich environments, since primary productivity is often extraordinarily high, when compared to the coastal zone. High rates of primary production within estuaries are generally associated with nutrient loadings from freshwater input, and overall high rates of productivity are maintained by a complex of emergent vegetation, benthic algae and phytoplankton, which efficiently utilize these introduced nutrients within the estuary (Odum 1959; Day et al. 1989). Decomposition of fast-growing, salt-tolerant plants and macroalgae provides the raw material for the large and complex food webs and rapid nutrient turnover that are characteristic of these communities. According to Odum (1959), estuaries behave as nutrient traps where complex reactions occur in order to make nutrients available to primary producers. However, active nutrient removal from the water column can occur within the system, namely by benthic and hard surface biofilms (Magalhães et al. 2005). On the other hand, since major cities are usually installed within the estuarine boundaries, sewage and urban runoff may also be a major source of organic matter and nutrients (Bordalo 1993), turning the water column heterotrophic, i.e. where respiration surpasses primary production, a characteristic of most estuaries (Azevedo et al. 2006).

Estuarine fish assemblages

Although estuaries are transitional, unstable environments, they form the temporary or permanent habitat for many animals and plants (McLusky and Elliott 2004). The estuarine fish fauna includes both resident and transient components and a wide range of sizes and ages, creating a great diversity in life-history stages and a more limited diversity of species (Able and Fahay 1998). The number of species found in estuaries is much fewer

than the number found on the adjacent continental shelf. Despite the high productivity of estuarine habitats, a reduced number of species (most of which are small in body size) comprises a large percentage of ichthyofauna, both in numbers and biomass (Elliott et al. 1990; Whitfield 1994b). The reduced number of species reflects the fact that only a small number of species have successfully adapted to estuarine conditions. This feature has been justified as a consequence of the high physiological stress induced in organisms by estuarine environmental oscillations (McLusky and Elliott 2004) or as a consequence of the relatively short lifetime of estuaries from a geological perspective (Whitfield 1994b). In addition to supporting their own resident fish communities, estuaries are nursery grounds, migration routes and refuge areas for a variety of fish species (Boesch and Turner 1984; Whitfield 1990; Whitfield 1998; Potter and Hyndes 1999; Cattrijsse and Hampel 2006). Therefore, there are transient species that temporarily inhabit estuaries, including migratory diadromous and catadromous species as well as primarily marine species that spawn at sea but whose early developmental stages use estuaries as nurseries.

Estuaries are used temporarily by species with different life history patterns (Haedrich 1983). Juveniles of some fish species sometimes occur at high densities in localized areas, and the ecological niches of several similar morphological types (e.g. species of Soleidae, Mugilidae, Gobiidae) overlap within these communities (Day et al. 1989). Therefore, the spatial and temporal distribution and abundance patterns of fish species, as well as resource partitioning within estuarine fish assemblages, are important and interesting issues for understanding the structure and dynamics of these communities. Many studies have dealt with the species composition of fish communities and their seasonal and spatial variation within estuaries (e.g. Yáñez-Arancibia 1985; Potter et al. 1997; Marshall and Elliott 1998; Blaber 2000; Akin et al. 2005).

Despite persistent fluctuations in both the biotic and abiotic environment in estuaries, the basic ichthyofaunal structure appears to have an underlying stability, which seems to be governed by factors such as the dominance of eurytopic taxa within estuarine assemblages and the robust nature of food webs within these systems (Whitfield 1999). According to this author, the predictability arises from factors such as the seasonality associated with estuarine spawning cycles and juvenile fish recruitment patterns. The fish communities inhabiting estuaries have been studied worldwide, and there have been several attempts to indicate the common features of those communities in order to use those criteria in all different types of estuaries around the world (e.g. Whitfield 1994a; Mathieson et al. 2000; Elliott and Hemingway 2002; Nordlie 2003; Able 2005). Recently, studies have focused on a functional analysis of community structure, in which the

species present are assigned to groupings or guilds, each of which denotes certain attributes. A guild can be defined as a group of species that exploit the same class of environmental resources in a similar way (Root 1967). The concept of guilds has been used for several decades (Perkins 1974; Haedrich 1983) and is currently extended to include descriptions of the migration, feeding, reproduction and habitat preferences of species (Potter et al. 1990; Elliott and Dewailly 1995; Thiel et al. 2003). The latest classification proposed by Elliot et al (2007), used a guild approach and increased the understanding of estuarine use by fishes, their interactions and connectivity with adjacent areas, and the estuarine resources required by fishes. In addition, this study provided a global perspective on estuarine fish fauna categorization by presenting new or refined definitions for the fish categories, listing the synonyms from the literature, and illustrating the concepts using examples from geographical areas covering north and central America, north and southern Europe, central and southern Africa, Australia and the Indo-Pacific. Briefly, those authors categorized the estuarine fishes into ten functional groups:

- *marine stragglers* - species that spawn at sea and typically enter estuaries only in low numbers, occurring most frequently in the lower reaches where salinities are approximately 35 PSU. These species are often stenohaline and associated with coastal marine waters;
- *marine migrants* - species that spawn at sea and often enter estuaries in large numbers, particularly as juveniles. Some of these species are highly euryhaline and move throughout the full length of the estuary. This group includes two sub-groups: marine estuarine-opportunistic and marine estuarine-dependent species;
- *estuarine species* - this category was subdivided into estuarine residents (estuarine species capable of completing their entire life cycle within the estuarine environment) and estuarine migrants (estuarine species that have larval stages of their life cycle completed outside the estuary or are also represented by discrete marine or freshwater populations);
- *anadromous* - species that undergo their greatest growth at sea and which, prior to reaching maturity, migrate into rivers where spawning subsequently occurs;
- *semi-anadromous* - species whose spawning runs from the sea extends only as far as the upper estuary rather than going into freshwater;
- *catadromous* - species that spend all of their trophic life in freshwater and subsequently migrate out to sea to spawn;
- *semi-catadromous* - species whose spawning runs extends only to estuarine areas rather than the marine environment;

- *amphidromous* - species which migrate between the sea and freshwater and for which neither migration is related to reproduction;
- *freshwater migrants* - freshwater species found regularly and in moderate numbers in estuaries and whose distribution can extend beyond the oligohaline sections of these systems;
- *freshwater stragglers* - freshwater species found in low numbers in estuaries and whose distribution is usually limited to the low salinity, upper reaches of estuaries.

The nursery function

A nursery 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 where their survival is enhanced through optimal conditions for feeding, growth, and/or predation refuge (Bergman et al. 1988; Pihl et al. 2002). These areas have been proven to be “better” in an evolutionary sense than other areas, because they provide conditions that maximize growth and survival of the young individuals (Jager 1999). Since survival may also be optimized in other adjacent habitats. Beck et al. (2001) highlighted that a habitat only functions as a nursery when, on average, from that habitat more juveniles per unit area recruit to the adult population than from other habitats. According to Cattrijsse and Hampel (2006), the existence of this criterion proves the nursery role.

The use of estuarine habitats has been attributed to factors such as food supply and refuge from predation in turbid estuarine waters (Blaber and Blaber 1980; Power et al. 2000). Estuarine habitats can also function as an overwintering refuge (Elliott et al. 1990; Attrill and Power 2002). All of these characteristics make estuaries attractive as nursery grounds for a variety of marine organisms, and their use has been well-documented around the world (e.g. Elliott et al. 1990; Blaber et al. 1995; Guindon and Miller 1995; Valesini et al. 1997; Able et al. 1998; Hostens 2000; Cabral and Costa 2001; Fujita et al. 2002; Smith and Parrish 2002; Ikejima et al. 2003; Ross 2003; Strydom et al. 2003; Sinovcic 2004). Moreover, experimental investigations have also identified some of the mechanisms thought to make estuaries a preferred habitat, including increased food availability, refuge from predation, increased living space, and habitat richness (Leber 1985; Miranda et al. 2005).

Fishes that use estuaries as nursery areas employ a range of life history strategies in order to settle into a favourable habitat. Several marine species whose spawning takes place at the sea utilize shallow coastal areas, such as estuaries as nursery grounds during their early life. Pelagic fish larvae settle into these habitats and grow from juveniles to sub-adults or even adults, leaving these habitats by means of post-settlement migrations (Blaber 2000). However, planktonic larvae and/or juveniles initially need to migrate to coastal areas in order to reach suitable nursery areas within estuaries (Bailey et al. 2003). A major problem associated with this coastal migration is the net seaward flow of estuarine waters combined with tidal flux, which may affect transport into or away from estuaries (Boehlert and Mundy 1988). Some of these species have an extended larval phase and experience a wide range of physical processes before reaching an estuarine area. Larval transport can be one of the most important factors influencing recruitment; if settlement does not occur within a given estuary, larvae of species which spawn offshore and utilize inshore nurseries can be flushed out into coastal waters during low tide, where they are presumably lost to the population (Bailey 1981; Amara et al. 2000; Curran and Able 2002; Cabral 2003). Moreover, migrations of metamorphosing larvae to the proper nursery habitats appear to act as an important control of recruitment (Bailey 1981; Hannan and Williams 1998; Bell et al. 2001; Warlen et al. 2002; Garcia et al. 2003a). For those species residing in estuaries through their entire life cycle (estuarine residents), the problem of export for early life history stages is sometimes alleviated by production of large, demersal eggs and young with short early life history stages (Kneib 1997). Some species brood their young within a pouch (Syngnathidae) (Dando 1984) or seek more protected habitats near the margins of estuaries.

Nursery areas for juvenile fishes form an important link between early life history stages and adult populations. In fish species where juvenile habitat differs from the habitat at the spawning ground, dispersal of pelagic eggs and larvae to an appropriate nursery area is an especially important process. Survival through the pelagic egg and larval stages can be influenced by oceanic conditions, including prevailing current patterns (Iles and Sinclair 1982), the physical character of the water column (Lasker 1975), and the abundance of predators (Bailey and Houde 1989) or prey species in the water column (Cushing 1975). The size of the 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; Rooper et al. 2004) of juvenile fishes and crustaceans.

Environmental forcing control

Due to the tidal nature of estuaries, their habitats are usually characterized as stressful environments, where organisms deal with drastic environmental shifts within a tidal cycle. In particular, the often abrupt changes in salinity, water temperature, dissolved oxygen and turbidity place considerable physiological demands on the fishes that utilize estuaries. These factors have a major influence on the density, diversity and biomass of fishes that can be supported in these systems (Whitfield 1996; Marshall and Elliott 1998; Power et al. 2000). Many environmental variables are assumed to be responsible for the distribution of fish populations (e.g. Morin et al. 1992; Tzeng and Wang 1993; Gibson et al. 1996; Able et al. 1998). The main forcing variables structuring the fish communities are given in Figure 1.1.

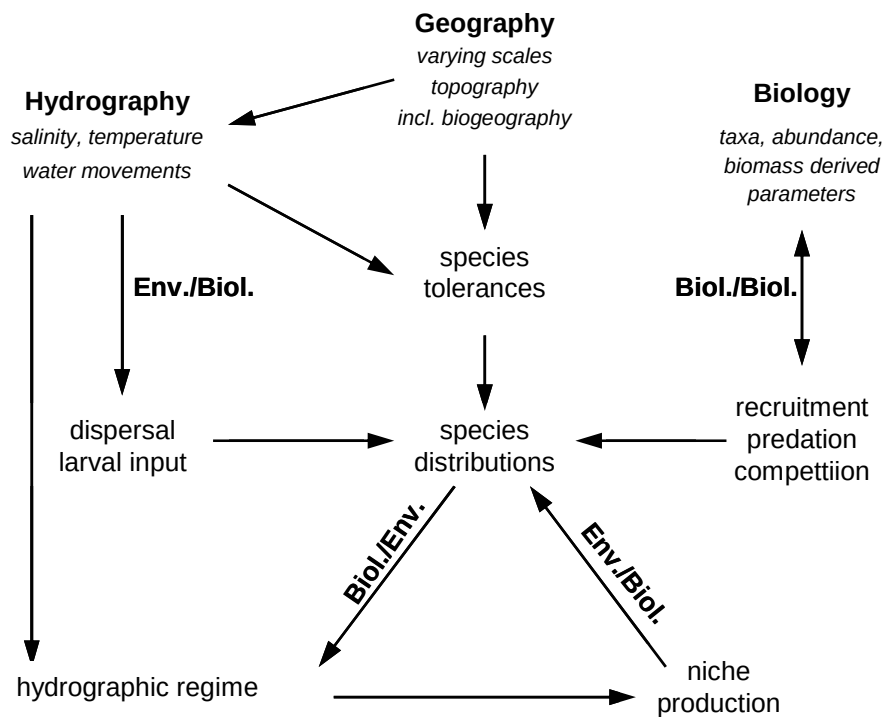


Figure 1.1. Main forcing variables that structure fish communities (from Elliott and Hemingway, 2002)

The forcing variables that affect estuarine fish communities can be grouped into three categories: environmental variables that affect biology, the interactions between biological variables, and the means by which biological variables can affect the environment (Marshall and Elliott 1998). River flow, and consequently salinity regime, temperature, dissolved oxygen and turbidity are indicated as major abiotic environmental variables,

whereas food supply and predation are the most important biological variables responsible for the structure of fish communities.

Temperature is presumably the most pervasive abiotic factor governing the growth of fishes, as it affects food intake, respiration, digestion and assimilation (Jobling 1994; Kestemont and Baras 2001; Baras et al. 2002). Temperature, defined by Fry (1971) as a controlling factor, exerts its influence in all environments and latitudes by directly affecting organism physiology, and it is unevenly distributed in heterogeneous space. Accordingly, temperature is a characteristic feature of any animal habitat (Magnuson et al. 1979) and for ectothermic organisms, such as fish, temperature can determine survival and fitness (Attrill and Power 2004). Temperature can be lethal when limits for physiological control are exceeded (e.g., maturation, feeding and growth) or when it influences habitat preference (Magnuson and Destasio 1996). Several studies of estuarine fish ecology have demonstrated the importance of temperature in determining fish abundance (e.g. Marshall and Elliott 1998; Power et al. 2000; Power and Attrill 2002). Temperature (i.e. habitat at any given temperature) is an ecological resource for which organisms compete to maximise growth and other measures of fitness (Attrill and Power 2004).

River discharge can influence the geomorphology, salinity, and nutrient distribution and processes of rivers and estuaries. These relationships affect the distribution and abundance of river and estuarine dependent biota (Drinkwater and Frank 1994). In addition, the extent of the river plume seems to influence the larval supply as well as the size and biotic capacity of habitats in estuarine nursery grounds, ultimately determining the number of resulting juveniles (Le Pape et al. 2003a).

Community-based studies have linked spatial organisation in estuarine fish communities to salinity and substrate (Henderson 1989; Maes et al. 1998) and temporal structure to sequential seasonal migrations (Potter et al. 1986; Elliott et al. 1990; Potter et al. 1997). Salinity plays an important role in the determination of estuarine community size and spatial structure. The incidence of marine and freshwater species in estuaries is highly dependent on the salinity (Elliott et al. 1990), yet salinity seems to have less influence on temporal fluctuations in abundance than on spatial patterns (Power et al. 2000).

Dissolved oxygen will affect species composition and abundance directly through intra- and inter-species differences in physiological tolerances and therefore has been justified as a suitable environmental quality measure for estuaries (Elliott et al. 1988). Declines in dissolved oxygen are known to be associated with stress in marine fish at levels below 4.5

mg dm⁻³ (Poxton and Allouse 1982) and to be associated with reductions in flatfish growth (Petersen and Pihl 1995). Although low oxygen levels affect species composition through differential tolerances, there are indications of spatial gradients and facultative adjustments to periodic low-oxygen conditions in estuarine habitats (Maes et al. 1998).

Variations in turbidity have been shown to correspond closely with observed species distribution patterns in estuaries and have been suggested to be a critical factor in reducing interspecific and intraspecific predation risks through the reduction of light intensity in nursery areas (Blaber and Blaber 1980; Marchand 1993; Maes et al. 1998; Power et al. 2000). Estuarine areas of turbidity maxima are characterized by higher prey concentrations and low predatory pressure, because predators of larval fishes are generally less frequent in these regions (Parrish 1989). Therefore, those areas offer increased survival rates for larval and juvenile fishes, which tend to aggregate within turbid waters (North and Houde 2003).

Environmental conditions at each ontogenetic stage can have different effects on growth, survival, and subsequent recruitment to the adult population. Growth during the early life stages often regulates recruitment of larval fishes to the adult population (Werner and Gilliam 1984). Survival through ontogeny depends on becoming sufficiently large to consume increasingly larger prey items, avoid predation, and survive competitive interactions (Werner and Gilliam 1984). Consequently, food limitation during the larval stage has been hypothesized as the primary factor influencing survival of larval fishes. The critical-period hypothesis suggests that availability of food to first-feeding fishes (i.e., exogenous feeding) controls year-class strength (Hjort 1914). Recently, the match–mismatch hypothesis has expanded the critical-period hypothesis to include not only the first-feeding stage, but the entire larval period (Cushing 1990). Specifically, the hypothesis states that temporal and spatial overlap between peaks in food resources (e.g., zooplankton) and larval abundance regulates survival of larval fishes and subsequent recruitment (Cushing 1990; Chick and Van Den Avyle 1999). Because the ability to reach a large size is important for piscivores, hatching chronology should be matched to the availability of suitable food resources at each ontogenetic stage (Li and Mathias 1982). In natural systems, ontogeny of fishes has been patterned by the long evolutionary history with the physical, chemical, and biological characteristics of the system (Werner and Gilliam 1984). Population dynamics during early life stages are strongly influenced by the environmental conditions experienced by planktonic fish eggs and larvae (Megrey et al. 2005). Predation intensity is dynamic throughout the course of fish ontogeny. For example, the suite of predators capable of feeding on early-stage fish changes as a

function of fish growth and thus body size-at-age (Taylor 2003). Moreover, continuous development of fish may result in age and/or size-specific habitat requirements, consequently exposing individuals to different predator types (Werner et al. 1983; Taylor 2005). Predation on the early life history of fish is an important factor regulating year-class strength. Specifically, in benthic fish species with a pelagic larval phase, the vulnerability to predation is high during and shortly after settlement (Van der Veer 1986).

Early life stages of Pleuronectiformes

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 the Pleuronectiformes (i.e. 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, anatomical and physiological transformations (e.g. eye migration and a 90° rotation in posture), enables the transition from a pelagic to a benthic lifestyle (Able and Fahay 1998).

Flatfishes occur throughout the world seas from the sub-Arctic to the tropics (Pauly 1994) and have a wide range of spawning seasons, habitat requirements and life history strategies (Minami and Tanaka 1992; Gibson 1994). Flatfish are usually abundant within estuarine and coastal fish assemblages (Allen and Baltz 1997; Van der Veer et al. 2000; Yamashita et al. 2001; Bailey et al. 2003; Le Pape et al. 2003b; Cabral et al. 2007). Several flatfishes spawn in oceanic waters, producing pelagic larvae that are transported inshore to settle in suitable nursery areas, usually shallow coastal and estuarine habitats. Estuarine and coastal areas have been recognized worldwide as nursery habitats for many flatfish species (Rogers 1993; Guindon and Miller 1995; Allen and Baltz 1997; Able and Fahay 1998; Le Pape et al. 2003a; Gilliers et al. 2004).

It is a widely accepted fact in flatfish ecology that nursery areas play an important role towards understanding variability in recruitment and determining overall population size (Rijnsdorp et al. 1995; Iles and Beverton 2000; Van der Veer et al. 2000; Walsh et al. 2004). Food, predators, temperature, dissolved oxygen and turbidity have been suggested to be the most important factors contributing to the habitat quality for each flatfish species (Gibson 1994). However, in estuary-dependent species, salinity conditions highly dependent on river flow and often correlated with sediment structures (fine vs. coarse

grain size) have been suggested to be important factors controlling the immigration, settlement and distributions of Pleuronectiform larvae and juveniles (Burke et al. 1998).

Simultaneous with the ontogenetic habitat shift is a change in predator species composition. Larval flatfish are often susceptible to coelenterate and pelagic fish predators (Bailey and Batty 1984; Van der Veer 1986), and juvenile flatfish are often susceptible to decapod crustaceans, demersal fish and avian piscivores (Manderson et al. 1999; Fairchild and Howell 2000; Taylor 2003). For several species of flatfish, year-class size is determined at the post-settlement stage and shortly thereafter (Zijlstra et al. 1982; Van der Veer and Bergman 1987). The transition in habitat utilization following metamorphosis and the subsequent change in predator prey dynamics at the post- settlement stage may be critical in determining year-class strength and recruitment success of flatfish (Bailey and Houde 1989; Bailey 1994).

1.2 Study area

The Lima River is an international water body, with a source at about 950 m altitude in the Sierra S. Mamede, located in the province of Orense–Spain (Figure 1.2). After 108 km (of which 67 km are in Portuguese territory), the river drains ENE–WSW into the Atlantic Ocean at 41° 40' N and 8° 50' W, in the vicinity of Viana do Castelo, NW Portugal. Viana do Castelo is a small town in the northern coast of Portugal with approximately 32,000 inhabitants.

The international Lima River basin, located in the northern region of the Iberian Peninsula, covers approximately 2,480 km² and 1,177 km² (47%) are located in Portuguese territory. The basin is bordered by the Minho River watershed in the north, the Cávado and Âncora Rivers in the south, and the Douro River watershed in the east. The Lima River basin is oriented lengthwise along a Northwest-Southwest direction and passes through granite outcrops with some schist and sand deposits. There are four main tributaries, two on the left side (Faramantoes-105 km² and Salas-206 km²) and two on the right side (Anteia Lagoon-206 km² and Vez-263 km²).

The average basin altitude is 447 m, and the Northern Peneda Mountain (1,416 m) and the Southern Yellow mountain (1,361 m) constitute the highest places in the region. The presence of those mountains as well as the proximity to the Atlantic Ocean determines the climate of the region, which is highly wet, mesothermal and usually described as a maritime, Atlantic façade (PBHL 2002). Therefore, the Lima River basin is located in one of the rainiest regions of Portugal, and consequently the Lima River valley is considered to be one of the richest regions in terms of hydrological resources. Precipitation is, on average, higher than 2,000 mm per year and is lower in the littoral region (usually under 1,400 mm per year). Temperature increases downstream, with a mean annual temperature of 9°C in the upper mountain sector and 14°C in the lower sector.

In the last decade (1995-2005), the water quality of the Lima River has been classified as intermediate to good, (INAG 2007). The classification has been made accordingly to the water parameters as total and fecal coliforms, pH, Kjeldahl nitrogen and dissolved oxygen saturation. However, no data are available for the estuarine stretch.



Figure 1.2. Map of the Portuguese section of the Lima River and its two dams, Alto do Lindoso and Touvedo.

The Lima River basin is mainly used for water supply, agricultural irrigation and hydropower generation. Two large hydroelectric dams (Alto do Lindoso and Touvedo) have been in operation since 1992. A storage volume of 274 hm³ is possible with these two dams. The most industrialized areas are concentrated at the vicinity of the Lima estuary, in Viana do Castelo, and also further upstream near Ponte de Lima and Ponte da Barca.

The Lima basin has an overall moderate to high ecological importance, which is recognized in terms of relevant ecosystems and important biodiversity. There are several areas designated within the Natura 2000 network, Corine biotop and Protected Areas criteria, as well as areas classified accordingly to the Habitats Directive (92/43/EEC), Annex I. In terms of vegetation, the Lima River exhibits an important biodiversity, mainly along the banks and also in terms of aquatic vegetation. According to the existing Hydrographic Basin Plan (PBHL 2002), the ichthyological community comprises several vulnerable and threatened species, such as eel, shad, Twaite shad and sea trout.

The Lima estuary

The Lima River has a small open estuary expanding East-West, where the river mouth was partially obstructed by a 2 km long jetty that deflects the river flow to the south. This thesis study was undertaken throughout the initial 10 km stretch of the Lima River estuary, with an approximate area of 6 km². This area comprises a high variety of habitats, such as rocky substrata (essentially represented by sea walls in the lower part of the estuary), constructed and natural embayments, sandy-muddy intertidal banks, intertidal saltmarshes, sandy islands and small streams. For this study, the Lima estuary was divided into three geomorphologically distinct zones: lower, middle and upper estuary. These designations were based on morphology, bathymetry, salinity range and the presence/absence of saltmarsh (Figure 1.3).

The lower estuary, located in the initial 2.5 km, is a narrow, deep navigational channel that is highly industrialized, with walled banks and includes a large shipyard, commercial sea-port, and a fishing harbour. The river mouth is an artificial and deep channel with two protection piers. The average depth of 10 m is maintained by constant dredging activity. During this study period (2002-2005), salinity was generally higher than 30 psu, and despite the depth, the water column exhibited slight vertical stratification. Generally, the water characteristics were temporally stable. However, due to the increase river run-off during the freshet winter periods, there was a slight decrease in salinity and temperature and an increase in turbidity. Overall, the water was well oxygenated, with the typical spring increase a possible consequence of phytoplankton blooms. This section is the sea water zone of the estuary.

The middle estuary, located upstream of the railway bridge, represents the largest part of the estuary. This zone encompasses a broad shallow saltmarsh zone, mainly colonized by the common rush (*Juncus* spp.), with a large longitudinal sandy island (Cavalar Island). The zone to the north of Cavalar Island is a narrow tidal channel where two small streams drain. During high tide, mean depth is 4 m, but this zone is almost completely drained during low tide. The south zone is a complex of small tidal islands south of Cavalar Island and has a mean depth of 3.7 m during high tide. This region encompasses the main course of the river and a large saltmarsh bank. This saltmarsh area is an important wetland that provides food and shelter to vertebrates such as mammals, birds, reptiles, amphibians and fishes (PBHL 2002). Within this mixing zone, salinity decreases towards the oligohaline range (18-30 psu). Water parameters such as temperature exhibit

seasonal trends that are similar to those of the lower estuary but with higher amplitudes. In general, the water transparency was slightly higher than in the lower section.

Farther upstream, the estuary continues as a narrow and shallow channel (3 m), where depth decreases upstream. This sector still remains in an almost natural state with undisturbed banks. There are some intertidal banks and a few sand islands. This upper section differed from the remaining sectors based on the water parameters. Salinity is near the lower limit of the oligohaline range (5-18 psu). The seasonality was strong in this section, with drastic differences between summer and winter. During summer the water warmed and the sea water reached this estuarine section. On the contrary, the increased river run-off during winter led to a decrease in water temperature and the salt wedge did not penetrate into this section. Another particularity of this sector was the summer peak in oxygen saturation, which peaked in the spring in the two downstream sectors.

The Lima estuary has a semidiurnal and mesotidal regime (3 m tidal amplitude on average), and downstream current velocity reached a maximum of 1.03 m s^{-1} during ebb tide. The maximum current velocity seems to occur 3 hours after the slack of the high and low tides, indicating that the Lima estuary tide is dominated by a stationary wave (see below, Chapter 3-Part I). The estuary is a partially mixed system, exhibiting seasonal vertical stratification in salinity during the winter period, when salinity sharply decreases at the top of the water column due to increased freshwater discharge. A layer of fresh water is sometimes present at the surface. Temperature is vertically stratified, with cooler water typically found near the bottom of the water column (corresponding to higher salinities); an exception occurs during winter as a result of a thermal inversion (Ramos et al. 2006b) when freshwater temperature is considerably lower than the temperature of the coastal water.

The river discharge into the estuary is controlled by Touvedo Reservoir (Figure 1.2), located 43 km upstream of the river mouth. According to the existing Hydrographic Basin Plan the mean annual river flow is $70 \text{ m}^3 \text{ s}^{-1}$; however during the study period (2002-2005) the mean annual river flow decreased to $40 \text{ m}^3 \text{ s}^{-1}$ (INAG 2007). The calculated hydraulic residence time is 9 days (Ramos et al. 2006b), which seems to be in accordance with the 1 week estimate produced by hydrodynamic models (MARETEC 2007; Saraiva et al. 2007). Both models considered the residence time of the Lima estuary long enough to enable ecological processes to take place inside the estuary. Nutrients or light were identified as the major limiting factors since temperature is not usually a strong factor at these latitudes. The MARETEC model (MARETEC 2007) also estimated that the estuary

is a productive system that exports biomass and consumes a portion of the nutrients entering the system. Yet the estuary is nutrient limited, therefore nutrient availability seems to be the most important limiting condition for pelagic primary production. For that reason, the areas with higher phytoplankton abundance are located in the tidal freshwater zone, the most productive area, whereas the seawater zone is the least productive (MARETEC 2007).

The upstream limit of the estuary is not well known since the salt intrusion can extend to different areas. During spring tides, the salt intrusion can extend up to 20 km upstream (Alves 1996). In fact, Sousa et al. (2006) found 1.1 psu near the bottom at about 15 km away from the river mouth during a summer high tide survey. However, according to the 2D- hydrodynamic model proposed by Pinho and Vieira (2007), the salinity intrusion reaches a river section located about 12 km upstream of the river mouth during spring tides and low flow river discharges ($< 35 \text{ m}^3 \text{ s}^{-1}$). For larger river flows, the salinity gradients occur mainly downstream (5 km from the river mouth). However, these simulations did not consider the eventual vertical stratification, which occurs when the river flow increases (see below – Chapter 3-Part I). Despite the uncertainty relative to the upstream limit of the salt intrusion, there are several indications that the tidal freshwater zone extends up to Ponte de Lima, approximately 22 km upstream of the river mouth (Alves 1996; Sousa et al. 2006; MARETEC 2007; Pinho and Vieira 2007).

Over several decades, the Lima estuary has been negatively affected by several heavy-modification construction projects that were designed to serve commercial navigation and fisheries in this important Portuguese harbour. The Lima estuary suffers from several sources of disturbance, such as a cellulose factory in the upper estuary, as well as input of agricultural run off and urban and industrial sewage, which discharges nutrients and other substances that are transported from domestic, industrial and agricultural areas into the estuarine area. There is also constant dredging of the navigational channel within its first 2.5 km, which seems to promote erosion of the banks and sand islands and can also compromise the foundations of emerged structures, such as the bridge pillars (Alves 1996). All of these interventions have modified the physical nature of the lower part of the estuary and altered the water quality. Although the Portuguese Water Institute (INAG) maintains a water quality monitoring program in different zones within the watershed, little is known about the water quality of the estuary. During this thesis research, the fecal contamination of the Lima estuary was analyzed fortnightly between Spring 2002 and Spring 2004 (for details of the method see Bordalo (1994)). Results showed that sewage contamination was present but generally under the maximum amount for allowed

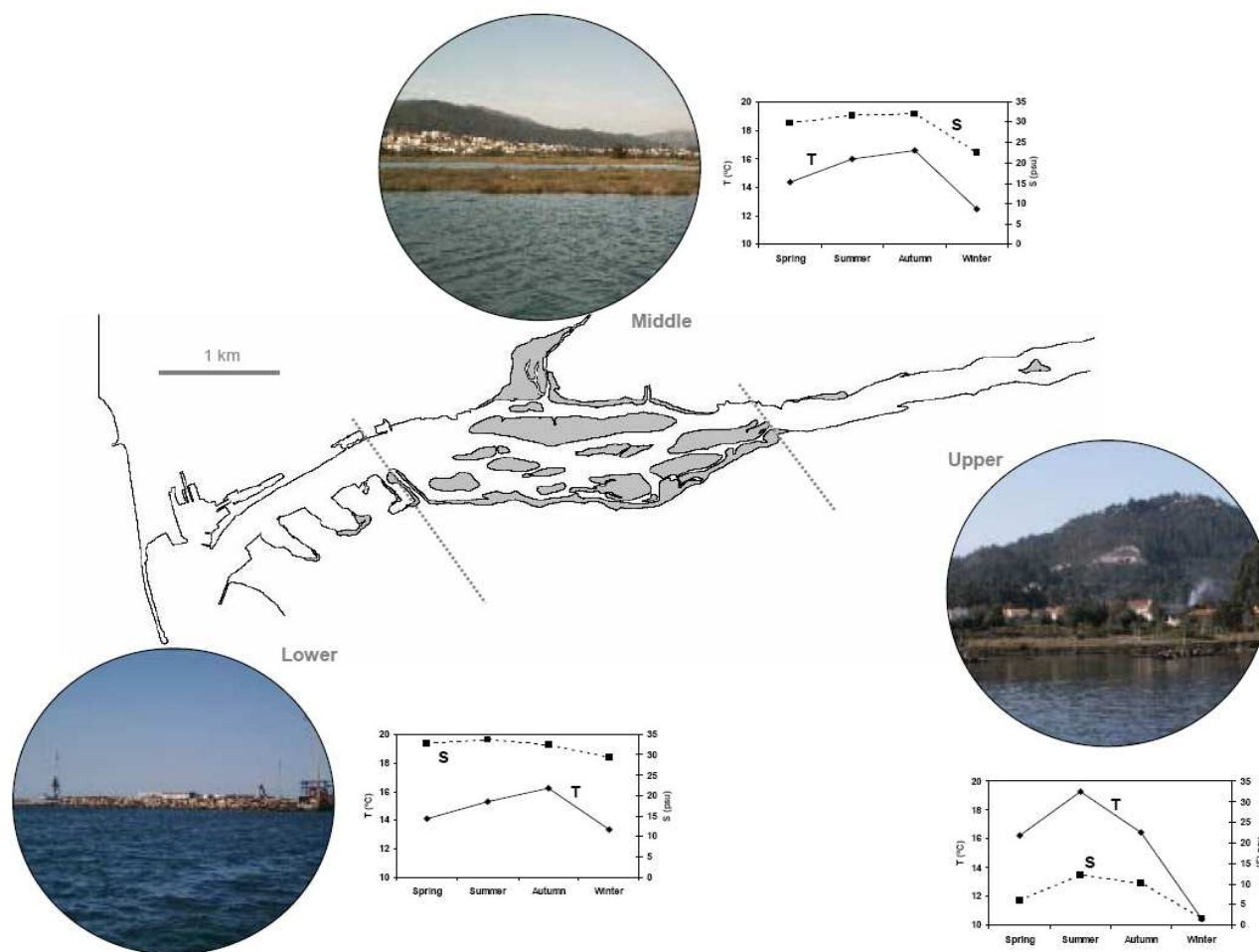


Figure 1.3. Seasonal salinity and temperature regimes throughout the three geomorphologic zones of the Lima River estuary (lower, middle and upper estuary). Data from vertical profiles performed between 2002-2005 were averaged by season.

recreational water (2, 000 cfu fecal coliforms 100 mL⁻¹) (Figure 1.4). However, there was a clear trend towards an increase in contamination during the rainy winter periods as a consequence of the increased urban run-off.

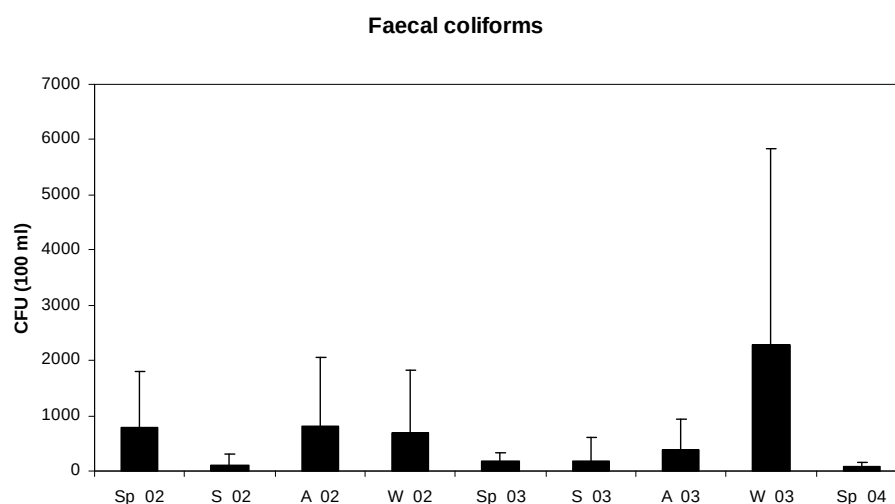


Figure 1.4. Fecal contamination expressed by number of CFU units per 100 ml of water in the Lima estuary. Fortnight data was averaged seasonally $\pm$ standard deviation (Sp – spring; S – summer; A – autumn; W – winter).

Overall, there is still little information about the impacts of these human interventions on the ecology of the estuary. There are indications of male urogenital papilla feminisation (UGPF) in the sand goby *Pomatoschistus minutus*, a major component of the estuarine ichthyofauna, due to high levels of estrogenic compounds, associated with sewage discharges (Rodrigues et al. 2006). In addition, the high levels of organic matter caused by the cellulose industry may have promoted the invasion of an exotic bivalve species *Corbicula fluminea* (Sousa et al. 2006).

The estuary still supports local commercial fisheries, where flounders, soles and sea bass are the main target species. There is also an important seasonal fishery of highly market-priced migratory species such as the anadromous lamprey (*Petromyzon marinus*), shads (*Alosa alosa* and *A. fallax*) and the catadromous eel (*Anguilla anguilla*). The estuarine fish fauna was estimated at 41 fish species, which include several marine species with commercial importance, such as the sea bass *Dicentrarchus labrax* and sole *Solea solea* (Antunes and Dias 2005). Before the large-scale construction that led to the actual configuration of the estuary, Valente and Alexandrino (1988) suggested that the south bank of the salt marsh area was used as nursery area for juveniles of some marine fishes.

The macrozoobenthic community is dominated by few but highly abundant opportunistic species (Sousa et al. 2007). Nevertheless, it is also exploited by recreational and commercial bait diggers, mainly in the shallow saltmarshes and sandy banks of the lower southern bank.

The few biological studies carried out in this estuary were mainly performed in the 1980s and the early 1990s, prior to the latest construction projects at the terminal area of the estuary. Those studies described the ecology of some species and characterized the main physical, chemical, biological and human processes which modify the structure and dynamics of biological communities such as the phyto- and zooplankton (Guimarães and Galhano 1987; Guimarães and Galhano 1988; Guimarães and Galhano 1989; Da Silva 1990; Coelho 1997) and macroinvertebrates (Fontoura 1984; Fontoura and Moura 1984; Sousa et al. 2006; 2007). Estuarine fish communities have received less attention. Before the present configuration of the estuary, Valente and Alexandrino (1988) have studied species composition of a restricted area on the south bank of the salt marsh area. More recently, Antunes and Dias (2005) studied the faunal composition of the euryhaline section of the estuary. Nevertheless, the ichthyoplankton assemblages of the estuary were never fully investigated, and this thesis is the first scientific characterization of the estuarine larval fish dynamics.

1.3 Motivation

Estuaries are among the most productive ecosystems. Yet they are also some of the ecosystems that are most threatened by conflicting human activities, which damage their ecological functions, including their nursery role for many fish species. Estuaries also support economic and recreational activities. Many estuaries no longer have their full measure of biotic integrity, because they receive wastewater discharges that may have depleted oxygen levels (Eddy 2005), or they their natural services were impaired by heavy modifications. On the European continent, humans have reclaimed large intertidal vegetated ecosystems since the early Middle Ages, and estuaries have changed completely due to anthropogenic influences. Some of these activities, such as land reclamation or the construction of dikes (Mitsch et al. 1994), destroyed vast areas of intertidal wetlands, while others changed the functioning of the estuary. Habitat loss through physical removal, the deterioration of quality, or the prevention of use by faunal species poses serious threats within and across estuaries (Elliott and Hemingway 2002). These processes are still present along all European coasts, although conservation attempts have largely put a stop to the reclaiming of marshlands (Elliott and Hemingway 2002). While direct destruction of intertidal marshes is currently under very strict restrictions, physical alterations of estuaries still continue and result in loss of intertidal area. Anthropogenic alterations have removed more than 7,200 km² of estuarine habitats during the 20th century alone (Cattrijsse and Hampel 2006). Estuaries exhibit a wide array of human impacts that can compromise their ecological function threatening the long term viability and health of these important ecotones (Goldberg 1995; Kennish 2001; McLusky and Elliott 2004).

There is growing concern about sustainable exploitation of estuaries, in particular how economic and natural environment interests can be reconciled in the face of increasingly large-scale developments accompanied by accelerating rates of change in the global environment (Prandle 2004). This is particularly important where fishes are concerned due to their economic but also environmental importance. Given the decrease trend of the fisheries worldwide during the last decades (Worm et al. 2006), the identification and conservation of the spawning and nursery areas of the exploitable fish species is of crucial importance. Therefore, fisheries management should include conservation strategies for

estuarine nursery habitats, since these habitats maximize the survivorship of the early life stages of many marine species (Frank and Leggett 1983; Van der Veer et al. 2001).

Knowledge of estuarine fish communities and the biotic and abiotic factors influencing spatial and temporal patterns is increasing as more long-term studies of estuaries are performed (Maes et al. 1998; Marshall and Elliott 1998; Thiel and Potter 2001). Although Portuguese estuaries are still poorly studied and understood and only few have historical data series (Vasconcelos et al. 2007). Several estuaries have already been identified as important habitats for early stages of fishes, namely Ria de Aveiro (Rebelo 1992; Pombo et al. 2005), Tagus estuary (Costa and Bruxelas 1989; Cabral and Costa 1999; Vinagre et al. 2006b), Ria Formosa (Andrade 1992) and Guadiana estuary (Chícharo et al. 2006). However, little information is available for the northern estuaries of Portugal, such as the Lima estuary.

Information on estuarine fish community structure and functioning is important to understand the biological features of estuaries, but it is becoming increasingly important to classify and categorize those estuarine fauna as an aid to the understanding and management of the effects of human activities in estuaries (Whitfield and Elliott 2002; McLusky and Elliott 2004). For example, the WFD (2000) presently being implemented in Europe, relies on the ability to detect a change in estuarine faunae from a defined reference condition (Apitz et al. 2006; Breine et al. 2007; Coates et al. 2007). Despite the absence of reference conditions for several Portuguese estuaries, including the Lima River, Portugal is forced to achieve good ecological status for the Lima River by 2015. Therefore, this present study can provide a baseline, enhancing the ecological knowledge of the Lima estuary.

1.4 Objectives of the thesis

The main purpose of this study was to investigate the early life stages of fishes occurring in the Lima estuary, with special emphasis on the ecology of an important and typical estuarine group, the Pleuronectiformes. Since this thesis is the first study of the Lima larval fish assemblages, there was the need to perform a baseline characterization of the estuarine ichthyoplankton, based on the temporal and spatial trends of the larval fish assemblages and also on their relationships with the major environmental forcing control. The study of the ecological aspects of early life of Pleuronectiformes included the study of the pelagic larvae and also the benthic juveniles and adults inhabiting the Lima estuary. The overall benthic fish fauna was initially characterized and later the juvenile flatfishes inhabiting the Lima estuary was investigated with the ultimate purpose of assessing the potential nursery role of the Lima estuary. Therefore, the present thesis is comprised of two distinct parts, the first refers to the ichthyoplankton assemblages, and the second is dedicated to the benthic fish assemblages. Thus, the specific objectives of this thesis are to:

1. characterize the ichthyoplankton of the Lima estuary by describing the temporal and spatial patterns in abundance, diversity and species composition and the structure of the larval fish assemblages;
2. analyse the environmental forcing control of the major structuring environmental variables on the larval fish dynamics;
3. investigate the undescribed presence of a typical coastal species (*Sardina pilchardus*) among the Lima estuarine ichthyoplankton;
4. characterize the spatial and temporal patterns of the benthic fish assemblages along an estuarine salinity gradient in order to assess the general tendencies of the benthic fish fauna associated with the Lima estuary;
5. characterize the early life stages of the flatfish community inhabiting the estuary;
6. evaluate the abiotic and biotic influences on larval and juvenile flatfishes dynamics within the Lima estuary.

The present thesis is organised in two main parts, comprising three chapters each, in a total of eight chapters:

Chapter 1 starts with a general introduction to the thesis theme, where the state of the science is presented, as well as the motivation and objectives of the present study.

Part I refers to the ichthyoplankton studies that describe the structural patterns in larval fish assemblages, both in time and space. Chapters 2 to 4 are included in Part I and contain results obtained to address the objectives and their respective discussion. Chapter 2 is dedicated to the temporal and spatial patterns of the estuarine ichthyoplankton; Chapter 3 describes the environmental forcing control of those larval fish assemblages and Chapter 4 brings new insights into the early life ecology of *S. pilchardus*, investigating its unusual presence among a northern Portuguese estuarine habitat.

Part II is dedicated to the benthic fish fauna inhabiting the Lima estuary. Results concerning the temporal and spatial dynamics of the benthic estuarine fish fauna are presented in Chapter 5. Chapters 6 and 7 are dedicated to early life stages of flatfishes. Chapter 6 describes the estuarine flatfish community and investigates the potential nursery role of the Lima estuary on a species basis. Chapter 7 evaluates the influence of abiotic and biotic parameters on larval and juvenile estuarine flatfishes.

Chapter 8 includes the main conclusions of the study and provides directions for future research.

PART I: Estuarine Ichthyoplankton

CHAPTER 2

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

2. 1 Introduction

Estuaries are nearshore coastal ecosystems where the ocean is diluted by freshwater (Dyer 1997). They serve important economic functions including transport, industry and tourism but also drainage of waste from domestic, industrial and agriculture activities (Heip and Herman 1995; Raz-Guzman and Huidobro 2002). Simultaneously, these ecosystems offer protection, not only for resident species, but also for a wide range of marine taxa (Weinstein 1985; Weisberg et al. 1996; Cowley and Whitfield 2002; McLusky and Elliott 2004) which provide high fishery yields and important economic opportunities (Houde and Rutherford 1993).

The contents of this chapter were originally published in Ramos, S., Cowen, R. K., Ré, P. and Bordalo, A. A., 2006. Temporal and spatial distribution of larval fish assemblages in the Lima estuary (Portugal). *Estuarine Coastal and Shelf Science*, 66, 303-314.

As boundary systems between watersheds and the sea, estuaries exhibit characteristic environmental gradients that favor the recruitment of a variety of species with diverse physical and trophic structures (Sánchez and Raz-Guzman 1997; Harris et al. 2001; Kimmerer et al. 2001). Fishes play an important part in estuaries as they constitute permanent and temporary community components, with marine species visiting these habitats for feeding, reproduction, growth and protection (Raz-Guzman and Huidobro 2002). Three general categories of fish are found in estuaries: marine fishes that use estuaries seasonally (temporary estuarine residents), those that complete their entire life history within the estuarine system (residents), and those that enter the estuary on rare occasions or are found in low numbers near inlets (occasional) (Lenanton and Potter 1987).

The highly productive nature of estuarine habitats (Nixon et al. 1986; Day et al. 1989) and their role as nursery areas to fish in many life history stages are well documented for temperate (Powles et al. 1984; Elliott et al. 1990; Kennish 1986; Drake and Arias 1991; Szedlmayer and Able 1996; Whitfield 1999; Blaber 2000; Shackell and Frank 2000; Elliott and Hemingway 2002) and tropical estuarine habitats (Raynie and Shaw 1994; Sanvicente-Añorve et al. 2000; Harris et al. 2001; Cowley and Whitfield 2002; Franco-Gordo et al. 2003). Since early life stages are a particularly vulnerable phase, it is hypothesized that marine fish larvae and juveniles migrate into estuaries to make use the high food abundance and refuge against predators, in order to maximize survival (Frank and Leggett 1983; Kennish 1986; Van der Veer et al. 2001). Therefore, a greater understanding of ichthyoplankton dynamics in estuaries would facilitate the further development of hypotheses about estuarine nursery function (Rakocinski et al. 1996). Estuarine fish larvae assemblages are variable both in terms of species composition and distribution patterns (Harris et al. 1999). These assemblages change continually in time and space, according to reproductive seasons of the species and also due to the environmental fluctuations (Whitfield 1994a; Harris and Cyrus 1995; Hettler and Hare 1998; Garcia et al. 2003b). However, there seem to be a general tendency for estuarine fish larvae to peak in abundance during spring and summer (Talbot and Able 1984; Cowan and Birdsong 1985; Whitfield 1989; Harris et al. 1999; Young and Potter 2003). Further, temperate estuarine ichthyoplankton assemblages have been shown to be dominated by resident species belonging to Gobidae family or seasonally by estuarine spawners, such as species of Clupeidae and Engraulidae family (Talbot and Able 1984; Drake and Arias 1991; Monteleone 1992; Blaber et al. 1997; Ré 1999; Harris and Cyrus 2000; Strydom et al. 2003).

The Lima estuary is located in northwest Portugal. The lower part is heavily modified, including a large shipyard, commercial sea-port, and a fishing harbour. Upstream the shallow salt marsh and tidal islands are much less disturbed (Figure 2.1). Little is known about the water quality of the Lima River estuarine area, although it receives urban runoff and the navigation channel is heavily dredged. On the other hand, the estuary still supports a locally important fishery (*Platichthys flesus*, *Dicentrarchus labrax* and some seasonal species such as *Petromyzon marinus*) though few data pertaining to its fish assemblage are available. The only study known was undertaken by Valente and Alexandrino (1988) that suggested that the south bank of the salt marsh area was used as nursery area for juveniles of some marine fishes. The composition of the larval ichthyofauna found in the Lima estuary is not known (Ré 1999). The present study was undertaken in order to fill this knowledge gap by: (i) characterizing spatial and temporal trends of the larval fish assemblages of the Lima estuary and (ii) providing baseline data on the use of an estuarine system in the NW Iberian Peninsula by early life stages of fishes.

2. 2 Material and Methods

2.2.1 Study area

The Lima River is an international water body, draining into the Atlantic Ocean at 41° 40' N and 8° 50' W in the vicinity of the 32,000 inhabitant city of Viana do Castelo. Its watershed covers 2, 446 km², of which 1,143 km² are located in Portuguese territory. The Lima estuary (Figure 2.1) has a semidiurnal and mesotidal regime. Salt intrusion extends up to 20 km upstream, with an average flushing rate of 0.4 m s⁻¹ and a residence time of 9 days. The river mouth is partially obstructed by a 2 km long jetty, deflecting the river flow to the south. The study area, located in the lower 7 km stretch of the estuary has an initial navigation channel and an upstream shallow saltmarsh zone, mainly colonized by *Juncus* spp., with a large longitudinal sandy island (Cavalar Island) (Figure 2.1). The study area was divided into three geomorphological distinct zones, according to water depth and the presence/absence of saltmarsh. Channel zone (stations 1- 4), located in the initial 2.5 km, is a narrow, 10 m deep navigational channel, highly industrialized with walled banks. The zone to the North of Cavalar Island (stations 5 - 7) is a narrow tidal channel where two small streams drain. During high tide, mean depth is 4 m, but this zone is almost

completely drained during low tide. The South zone includes the remaining stations (8-11), and is a complex of small tidal island south of Cavalar Island, and has a mean depth of 3.7 m during high tide. This region encompasses the main course of the river and a large saltmarsh bank.

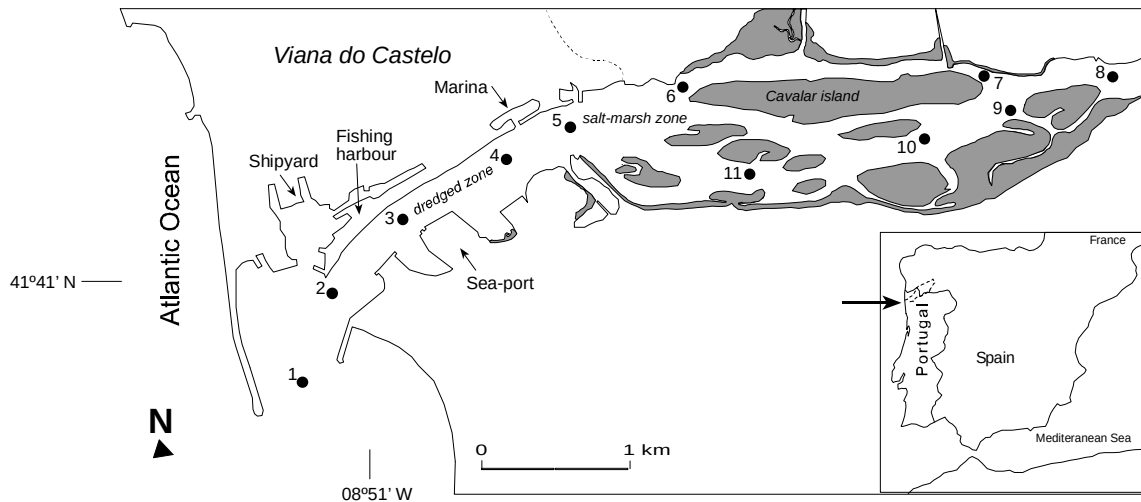


Figure 2.1. Lima estuary and location of the sampling stations.

2.2.2 Field Methods

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

2.2.3 Sample processing

After each tow, samples were immediately fixed in 4% buffered formalin (pH=8). Three days after the survey, samples were sorted and fish larvae were preserved in 95% alcohol. Individuals were identified to the lowest taxonomic separation, using the

appropriate literature (Macer 1967; Nichols 1971; Demir 1976; Fives 1976; Nichols 1976; Russell 1976; Camus and Besseau 1986; Fives 1986; Ré 1999). Numbers of individuals per taxa were counted from the entire sample and then standardized to number of fish larvae per 100 m³.

2.2.4 Data analysis

Diversity of the larval fish assemblage was expressed by the Shannon Wiener index (Shannon and Weaver, 1963) and equitability was measured by Pielou's evenness index (J') (Pielou 1966). Seasons were aggregated into three month groups: spring (Sp) comprised March, April and May; summer (S) June, July and August; autumn (A) September, October and November and winter (W) December, January and February. Since the study covered 46 surveys the first 24 surveys, from April 2002 to the first survey of April 2003, are therefore considered as the "first year" (seasons Sp₁, S₁, A₁, W₁ and Sp₂) and the remaining surveys are the "second year" (seasons Sp₂, S₂, A₂, W₂ and Sp₃).

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

Multivariate analyses were performed with the software package PRIMER (Plymouth Routines Multivariate Ecological Research) (Clarke and Warwick 1994). One-way analysis of similarity (ANOSIM) (Clarke and Warwick, 1994) was used to determine the

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

2.3 Results

2.3.1 Environmental conditions

Temperature exhibited similar trends in all three zones of the estuary. The maximum mean temperature occurred during the A_1 period, while the lowest temperature values were recorded during the W_1 (Figure 2.2). During the first year the warmer season was A_1 , while in the second year it was S_2 . The low temperatures recorded in S_1 were a consequence of upwelling phenomena present in the first study year which was absent in the second year, preventing the summer temperature decrease. In the second year, temperature exhibited seasonality typical of temperate northern hemisphere environments, where the warmest period was the summer and the coldest was the winter (Figure 2.2).

During most of the year, surface salinity was greater than 30, except during the winter and early spring (Figure 2.2). During these freshet months, salinity decreased significantly ($F=40.5$ $P<0.01$), particularly in the South zone, where mean salinity reached 15 in W_1 and 22 in W_2 . During the winter seasons, salinity fluctuations were more evident in both zones of the salt marsh area (North and South zones) than in the channel zone.

Turbidity was significantly higher in A_1 and W_1 ($F=30.4$ $P<0.01$) than during the other seasons, namely spring and summer, when the minimum mean values were registered

(Figure 2.2). Sub- surface water layer was significantly more turbid in the South zone than in the other zones of the study area ($F= 25.1$ $P<0.01$). Turbidity showed high sample variability, especially the salt marsh area, exhibiting high standard deviations each season.

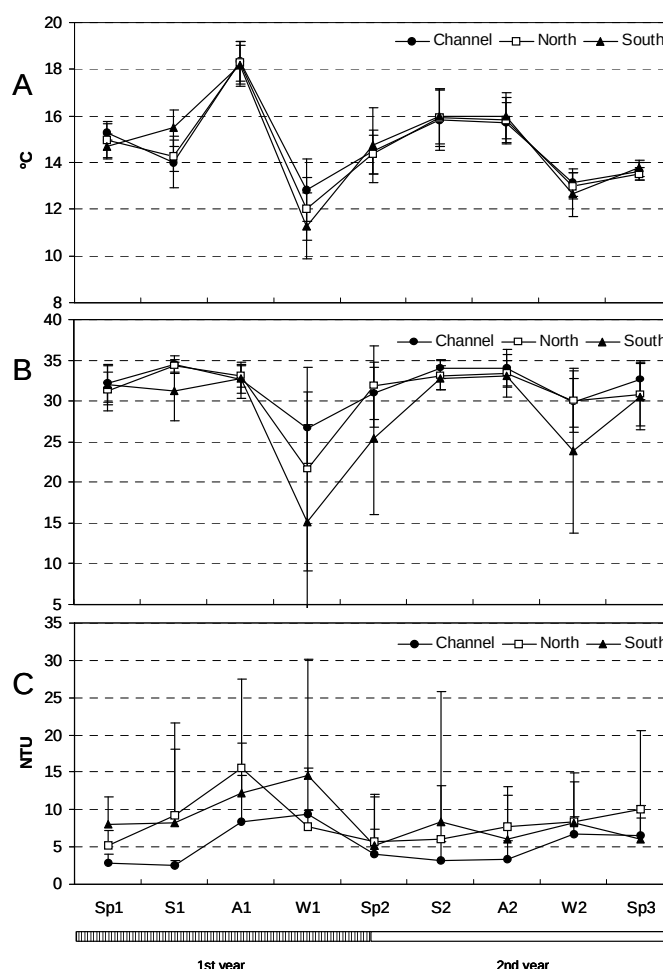


Figure 2.2. Seasonal average values and standard deviation (error bars) for temperature (A), salinity (B) and turbidity (C) in the eleven surveyed stations in Lima estuary by zones.

2.3.2 Species composition

During the study period, a total of 12,903 fish larvae were collected in the Lima estuary, with a mean abundance of eight individuals per 100 m³. The fish larvae assemblage included 20 families distributed through 50 taxa, where 38 could be assigned to species, 4 to genus and 7 to family (Table 2.1). The majority of unidentified individuals (2.55% of the total catch) were yolk-sac larvae (Table 2.1). Gobiidae was the most abundant family (71.0 % of the total catch), followed by Clupeidae (12.2 %), Ammodytidae (5.3%),

Labridae (3.0%), Blenniidae (1.7 %) and Soleidae (1.4 %). At the species level, *Pomatoschistus* spp. reached 71.0 % (Table 2.1) of the total fish larvae collected during this study, representing the most abundant *taxon*. *Sardina pilchardus* (8.3 %), *Ammodytes tobianus* (5.1 %), unidentified Clupeidae (3.3 %), *Symphodus melops* (2.0 %) and *Solea senegalensis* (1.2 %) were the other abundant species (i.e. > 1% of the total catch) (Table 2.1). Thus, these six species comprised 91 % of the total catch. Thirty three of the 50 taxa were found in all estuarine zones, whereas the remaining were confined either to the channel zone or to the salt marsh zone. From the 39 species of teleost identified, 22 are considered as occasional marine species, 15 as temporary estuarine resident and 2 as resident (Table 2.1).

2.3.3 Temporal and spatial patterns of abundance

A clear seasonal signal in larval abundance was evident, with significantly higher abundance values ($F= 32.2$ $P<0.001$) during the spring and summer seasons relative to the other periods of the year (Figure 2.3-A). The highest abundance values were found at least 3 km upstream of the river mouth (Figure 2.3-A). Abundances in North and South zones were significantly higher than in the Channel zone ($F= 16.9$ $P<0.001$).

After the spring-summer peak of abundance, the number of fish larvae decreased to very low values, especially during the winter (Figure 2.3-A). In W_2 this period was shorter than during W_1 due to the presence of increased numbers of *Ammodytes tobianus*, caught mainly in January 2004 in the upstream zones of the estuary (Figure 2.4-C). When temperature started to increase in the following spring periods (Sp_2 and Sp_3), fish larvae abundance also increased. During these periods, *Pomatoschistus* spp. were more abundant and concentrated in the North and South zones (Figure 2.4-A). However, during the Sp_2 and S_2 their abundance was lower in comparison with the same period of the previous year, thus the overall abundance of the assemblage did not exceed 200 fish larvae 100 m^{-3} . Also, the presence of high numbers of *S. pilchardus* larvae during the Sp_2 and S_2 period, mainly in the two first km of the estuary (Figure 2.4-B), marked the difference between the two warmer periods of the study.

2.3.4 Temporal and spatial trends of diversity

The Shannon Wiener diversity index ranged from 0 to 2.1 and Pielou's evenness varied between 0.03 and 1 (Figure 2.3 B-C). During the study period, the number of taxa and

Table 2.1. Numbers of species caught in the Lima Estuary between April 2002 and April 2004 and relative contribution to the total catch in the whole estuary and by zone (Ch- Channel; Nz- North zone and Sz- South zone). The relative contributions were calculated from numbers in each sample after adjustment to constant volume of 100 m³. The 18 most abundant species also include the code used for multivariate analyses. Dash indicates species not caught in that region. MD: mean density of each taxa (larvae/100m³); O: occasional; TR: temporary estuarine resident; R: resident.

Family	Species	Habitat	Total catch		Sections		
			MD	%	Ch	Nz	Sz
GOBIIDAE	<i>Pomatoschistus</i> spp. (Pom)	R	30.24	70.95	39.29	81.86	80.04
CLUPEIDAE	<i>Sardina pilchardus</i> (Spil)	O	5.83	8.29	19.53	4.16	5.22
AMMODYTIDA	<i>Ammodytes tobianus</i> (Ato)	TR	7.44	5.06	5.73	4.23	5.28
CLUPEIDAE	Unidentified (Clupeidae ni)	O	4.82	3.27	7.43	2.07	1.92
Not identified	Not identified		0.03	2.55	5.63	1.81	1.23
LABRIDAE	<i>Symphodus melops</i> (Smel)	TR	2.77	1.91	3.11	0.98	1.92
SOLEIDAE	<i>Solea senegalensis</i> (Ssen)	TR	1.75	1.19	2.13	1.01	0.83
LABRIDAE	<i>Labrus bergylta</i> (Lber)	TR	1.66	0.66	1.31	0.66	0.32
CLUPEIDAE	<i>Spratus spratus</i> (Sspra)	O	1.92	0.65	1.70	0.38	0.29
BLENNIIDAE	<i>Parablennius gattorugin</i> (Pgatto)	O	1.09	0.60	1.67	0.25	0.27
BLENNIIDAE	<i>Lipophrys pholis</i> (Lpho)	TR	1.34	0.53	1.08	0.35	0.36
ATHERINIDAE	<i>Atherina presbyter</i> (Apre)	TR	2.56	0.50	2.03	0.03	0.02
GADIDAE	<i>Trisopterus luscus</i> (Tlus)	O	1.67	0.33	0.85	0.13	0.20
BLENNIIDAE	<i>Blennius ocellaris</i> (Bocc)	O	1.25	0.29	0.75	0.20	0.10
GOBIESOCID	<i>Diplecogaster bimaculata</i> (Dbim)	O	1.45	0.26	0.92	0.03	0.07
GOBIESOCID	<i>Lepadogaster lepadogaster</i> (Llepa)	O	1.27	0.23	0.79	0.03	0.07
PLEURONECT	<i>Platichthys flesus</i> (Pfle)	TR	1.20	0.23	0.26	0.28	0.17
AMMODYTIDA	<i>Hyperoplus lanceolatus</i> (Hlan)	TR	2.24	0.22	0.79	0.03	0.05
SYNGNATHID	<i>Syngnathus acus</i> (Sacus)	TR	1.06	0.20	0.10	0.23	0.24
LABRIDAE	Not identified	TR	2.01	0.19	0.62	0.05	0.07
TRACHINIDAE	<i>Trachinus draco</i>	O	2.12	0.19	0.62	0.05	0.07
LABRIDAE	<i>Ctenolabrus rupestris</i>	O	1.32	0.18	0.36	0.15	--
BLENNIIDAE	<i>Coryphoblennius galerita</i>	O	1.58	0.15	0.29	0.05	0.14
SPARIDAE	Not identified	O	0.96	0.12	0.23	--	0.07
SPARIDAE	<i>Spondyllosoma cantharus</i>	O	1.36	0.12	0.36	0.08	0.02
SOLEIDAE	<i>Solea lascaris</i>	O	1.16	0.11	0.29	0.10	0.02
CARANGUIDA	<i>Trachurus trachurus</i>	O	1.03	0.09	0.10	0.10	0.09
BLENNIIDAE	Not identified	O	1.12	0.09	0.26	0.05	0.02
CALLIONYMID	<i>Callionymus lyra</i>	O	1.58	0.08	0.07	0.05	0.10
ENGRAULIDA	<i>Engraulis encrasicolus</i>	TR	0.83	0.07	0.10	0.10	0.03
LABRIDAE	<i>Centrolabrus exoletus</i>	O	1.11	0.06	0.16	--	0.15
MORONIDAE	<i>Dicentrarchus labrax</i>	TR	1.03	0.06	0.10	--	0.09
LIPARIDAE	<i>Liparis montagui</i>	TR	3.83	0.06	0.23	--	0.02
SOLEIDAE	Not identified		1.45	0.06	0.10	0.15	0.03
CALLIONYMID	<i>Callionymus</i> spp.	O	0.80	0.05	0.16	--	0.03
GADIDAE	<i>Ciliata mustela</i>	TR	1.10	0.05	0.03	0.08	0.05
TRACHINIDAE	<i>Echiichthys vipera</i>	O	1.00	0.05	0.13	0.03	0.02
GADIDAE	Not identified	O	1.49	0.05	0.07	0.08	0.02
SOLEIDAE	<i>Solea vulgaris</i>	TR	1.46	0.05	0.10	0.03	0.03
MUGILIDAE	<i>Liza</i> spp	TR	1.04	0.04	0.10	0.05	--
SYNGNATHID	<i>Syngnathus</i> spp.		0.82	0.03	0.07	--	0.03
SPARIDAE	<i>Diplodus sargus</i>	O	0.80	0.02	0.03	--	0.03
SYNGNATHID	<i>Entelurus aequoreus</i>	TR	0.85	0.02	0.07	0.03	--
SYNGNATHID	<i>Nerophis ophidion</i>	O	0.87	0.02	--	0.03	--
BOTHIDAE	<i>Zeugopterus punctatus</i>		1.21	0.02	0.10	--	--
GOBIIDAE	<i>Crystalllogobius linearis</i>	O	1.38	0.02	0.07	--	--
SYNGNATHID	<i>Syngnathus abaster</i>	R	0.93	0.02	--	0.03	0.02
SOLEIDAE	<i>Buglossidium luteum</i>	O	0.68	0.01	0.03	--	--
SOLEIDAE	<i>Microchirus variegatus</i>	O	1.22	0.01	0.03	--	--
SYNGNATHID	<i>Nerophis lumbriciformis</i>	O	0.76	0.01	0.07	0.03	--
PLEURONECT	<i>Pleuronectidae</i> ni	TR	1.19	0.01	--	0.03	--
No. of larvae			12 903		3 057	3 969	5 877
No. of species			50		48	41	41

Shannon Wiener index increased significantly ($F= 30.2$ $P<0.01$) from the first year (41 taxa and $H'=0.4$) to the second year (47 taxa $H'=0.7$). Diversity increased during the warmer periods, A_1 in the first year and S_2 during the second year (c.f. Figure 2.2 and Figure 2.3-B). However, occasionally, high diversity levels were found in winter, as in February 2004 (Figure 2.3-B). The Channel zone exhibited significantly higher diversity values ($F= 11.2$ $P<0.01$) than the North and South zones. The fish larvae assemblage showed low Pielou's evenness values, with the exception of freshet periods (Figure 2.3-C). The peaks of fish larvae detected in this study, mainly in the first year, were dominated by single taxa *Pomatoschistus* spp. (c.f. Figure 2.3-A and Figure 2.4-A), affecting the low diversity values found during this period (Figure 2.3-B).

2.3.4 Assemblage composition

According to ANOSIM results (Table 2.2) there were significant differences between seasons ($P<0.05$), although groups were not clearly separated ($R<0.5$). In spite of *Pomatoschistus* spp. being identified as the discriminating species of the majority of the season groups, the structure of the assemblage differed along time (Table 2.3). After the period dominated by *Pomatoschistus* spp (Sp_1 and S_1), new species appeared (i.e. *S. senegalensis*) during A_1 , leading to an increase on the diversity of the assemblage (Table 2.3). After the low abundance period (W_1), *Pomatoschistus* spp. started to dominate the assemblage (S_2 - A_2), until the next freshet period (W_2). This latter period was dominated by, *A. tobianus* (Table 2.3).

Table 2.2. Results of one-way ANOSIM (R values and significance levels) and SIMPER analysis on the abundance of the top eighteen most abundant species from seasonal groups.

Groups	ANOSIM		SIMPER		
	R	P	Average dissimilarity (%)	Discriminating species	% Contribution
Sp_1 vs S_1	0.121	0.005*	59.7	<i>Pomatoschistus</i> spp.	52.1
S_1 vs A_1	0.306	0.001*	69.5	<i>Pomatoschistus</i> spp.	38.8
A_1 vs W_1	0.356	0.001*	86.4	<i>Pomatoschistus</i> spp.	28.1
W_1 vs Sp_2	0.180	0.001*	85.6	<i>Pomatoschistus</i> spp.	30.3
Sp_2 vs S_2	0.125	0.001*	74.4	<i>Pomatoschistus</i> spp.	33.3
S_2 vs A_2	0.229	0.001*	70.2	<i>Pomatoschistus</i> spp.	35.9
A_2 vs W_2	0.402	0.001*	76.2	<i>A. tobianus</i>	35.7
W_2 vs Sp_3	0.271	0.001*	78.3	<i>A. tobianus</i>	32.0

* significant differences

The spatial trend of community structure, significantly varied from the Channel zone to the other two zones of the estuary (Table 2.4). In North and South zones, the assemblage was highly dominated by *Pomatoschistus* spp., responsible for 82 and 87 % of the similarity within each zone, respectively (Table 2.5). The assemblage in the Channel zone was more diverse, with seven species being identified by SIMPER analysis as discriminating the larval fish assemblage structure in that zone of the estuary (Table 2.5).

Cluster analysis showed no marked tendency of species to be separated in compact groups. At a similarity of 15 %, three species groups were obtained, whereas 5 of the total 18 species remained isolated (Figure 2.5-A). Since nMDS stress was higher than 0.1, cluster results were superimposed on 2d- multidimensional ordination. The three clusters separate out more or less according to the type of eggs and also to the temporal

Table 2.3. Discriminating species of each season using the SIMPER analysis.

	Sp1	S1	A1	W1	Sp2	S2	A2	W2	Sp3
Average similarity	(37.6)	(46.9)	(33.1)	(23.4)	(21.5)	(38.0)	(37.2)	(41.9)	(26.2)
<i>Pomatoschistus</i> spp.	80.8	89.0	49.2	55.5	52.5	73.7	56.1	24.4	77.2
<i>A. tobianus</i>				39.6	7.1			58.5	8.9
<i>S. pilchardus</i>			29.5		16.5	8.0	25.0	11.0	
<i>S. melops</i>	10.2	4.9			6.4	5.0			
<i>S. senegalensis</i>			6.9						
<i>Clupeidae</i> *			6.7		5.9		11.3		
<i>T. luscus</i>					6.7				
<i>L. bergylta</i>						3.7			
<i>Labridae</i> ni									5.5
Total	91.0	94.0	92.3	95.2	95.2	90.5	92.39	93.92	91.62

*unidentified Clupeidae

distribution of the species in the Lima estuary. Groups 1 and 3 clustered species derived from demersal eggs, while group 2 comprised species with pelagic eggs (Figure 2.5-B). Species from Group 1 (*Dipledogaster bimaculata* and *Lepadogaster lepadogaster*) were

Table 2.4. Results of one-way ANOSIM (*R* values and significance levels) and SIMPER analysis on the abundance of the top eighteen most abundant species from three zones of the estuary (Ch: channel; NZ: North zone; SZ: South zone).

Groups	ANOSIM		Average	SIMPER	
	<i>R</i>	<i>P</i>		Discriminant species	%
Ch vs NZ	0.048	0.001*	78.5	<i>Pomatoschistus</i> spp.	33.7
Ch vs SZ	0.143	0.001*	78.8	<i>Pomatoschistus</i> spp.	33.8
NZ vs SZ	0.005	0.335	66.2	<i>Pomatoschistus</i> spp.	40.9

* significant differences

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

Table 2.5. Discriminating species of each zone of the estuary (Ch: channel; NZ: North zone; SZ: South zone) using the SIMPER analysis.

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

2.4 Discussion

The larval fish assemblage found in the Lima estuary is composed of a few species with high abundance and a large number of rare species, a common feature of estuarine populations (Gaughan et al. 1990; Harrison and Whitfield 1990; Drake and Arias 1991; Harris and Cyrus 1995; Whitfield 1999). The number of taxa found in this study (41°N) (50 taxa, 20 families) was higher than in other estuaries of the western Iberian Peninsula. For example, in the Mondego estuary further south (40° N), only 28 taxa (16 families) were found (Ribeiro 1991). In the Tagus estuary (38° N), 32 taxa and 20 families were found (Ré, pers. comm.). Finally, in the Guadiana estuary (37° N), only 20 taxa and 12 families were encountered (Chícharo, pers. comm.). These comparisons must be tempered by differences in sampling gear and effort. Moreover, each estuarine system may have a different abiotic environment (Blaber 1997), resulting from the tidal range, freshwater input, geomorphology and human pressure (Dyer 1997; McLusky and Elliott 2004).

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

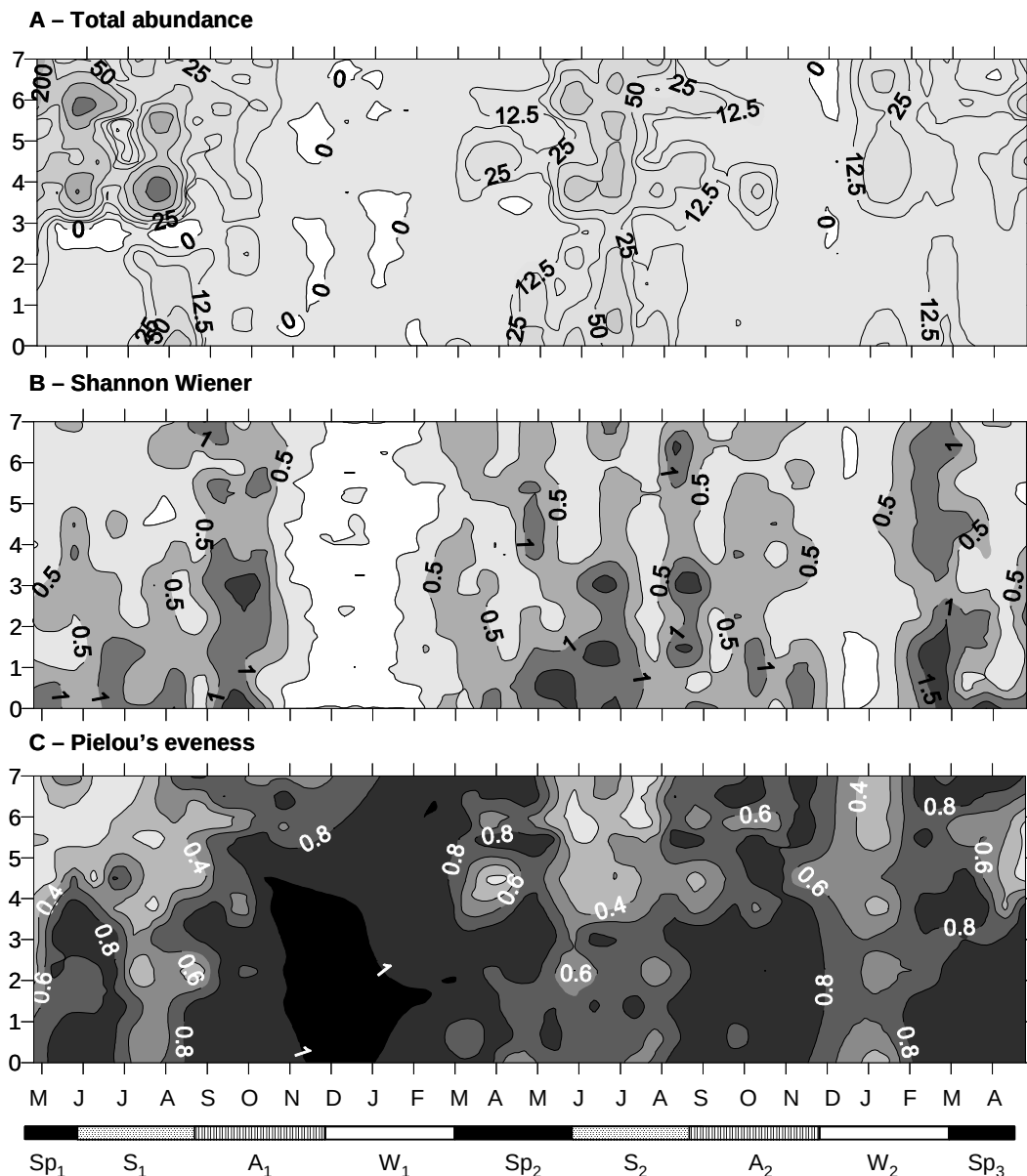


Figure 2.3. Temporal and spatial dynamics of: (A) total abundance (number of fish larvae 100m⁻³); Shannon Wiener index (B) and Evenness (C) of the Lima larval fish assemblage.

The typical decrease of the number of species in the upstream direction (Steffe and Pease 1988; Neira et al. 1992; Ré 1999) was observed in the Lima estuary. The Channel zone was significantly more diverse than the salt marsh zones. This was a consequence of the relatively high number of species with marine origin, captured in this zone, where a connection with the adjacent coastal area exists. In the Channel zone, turbidity was significantly lower than in the other zones, contributing to the presence of marine species, which have been described as more related to clear waters (Blaber et al. 1997). Moreover, the absence of freshwater species contributed to the low number of species caught in the upstream zones (North and South zones) of the study area.

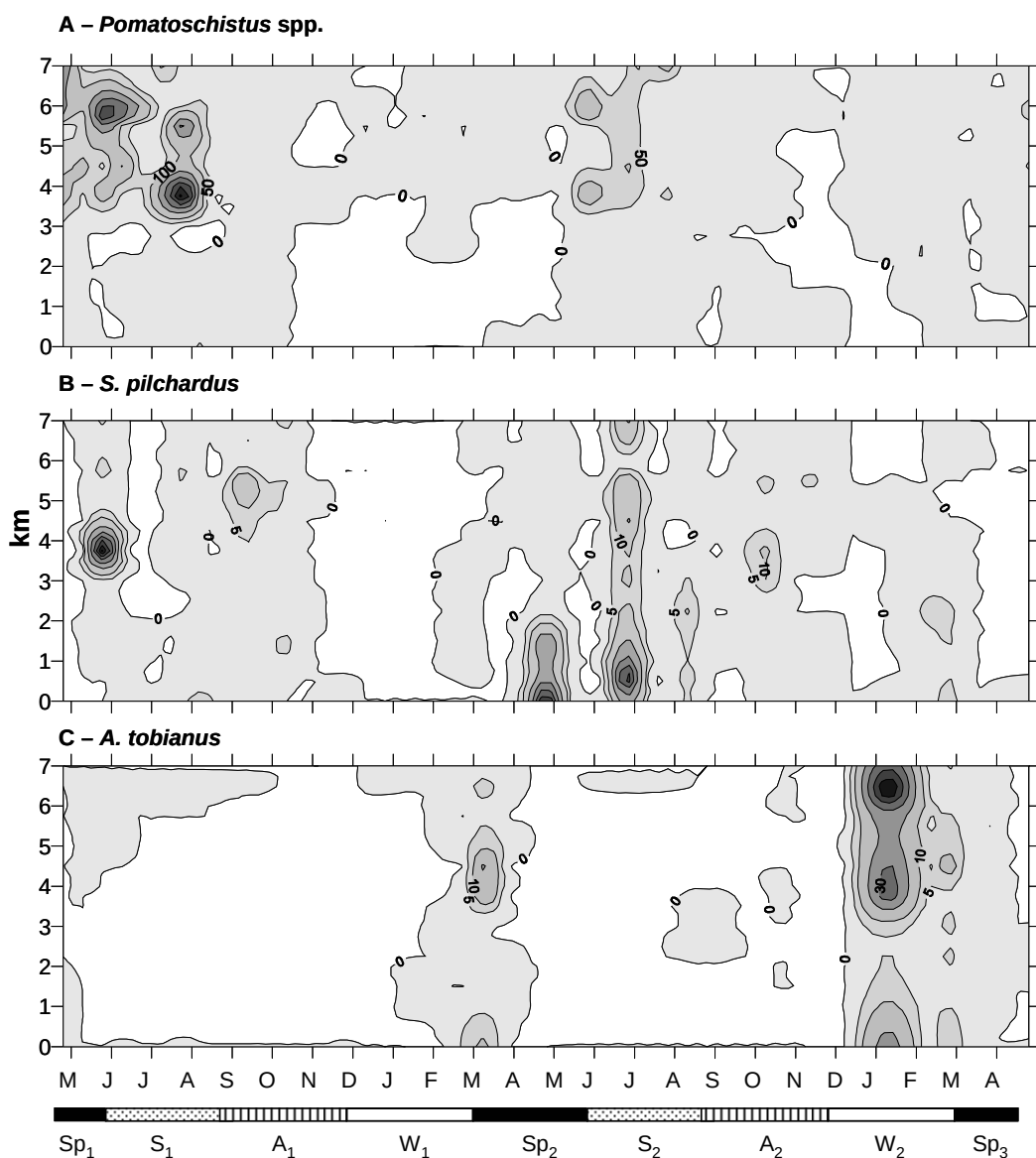


Figure 2.4. Temporal and spatial distributions of abundance of the three dominant species (**A-** *Pomatoschistus* spp.; **B-** *S. pilchardus*; **C-** *A. tobianus*).

Overall, diversity of the fish larvae assemblage in the Lima estuary was low, a common feature of these assemblages in temperate systems (Shenker and Dean 1979; Claridge 1986; Drake and Arias 1991; Ré 1999), due to the dominance by a single resident taxa *Pomatoschistus* spp. Several studies in different regions have reported resident species dominance in estuarine ichthyoplankton (Talbot and Able 1984; Neira et al. 1992; Thayer et al. 1999; Strydom et al. 2003). *Pomatoschistus* spp. was more abundant in the North and South zones, contributing to significantly higher abundances in the salt marsh zones. However, their wider distribution makes *Pomatoschistus* spp. the representative *taxon* of all the three study zones (Table 2.5). This led us to conclude that the differences in their abundance were responsible for spatial differences found between the structure of the larval fish assemblage community in the Channel (low abundances of *Pomatoschistus* spp.) and North and South zones (high abundances of *Pomatoschistus* spp.). At the same time, seasonal differences, such as the dominance of the *A. tobianus*, during W_2 , induced differences in the assemblage structure. This species was almost absent during the first year (Figure 2.4-C), possibly due to interannual variation in the initial arrival time from the spawning area and/or to environmental variability of the estuarine habitat. A similar effect was observed for other species with a short recruitment period such as *Sparus aurata* and *Dicentrarchus labrax*, in Cadiz Bay (Drake and Arias 1991).

Sardines, which have a coastal spawning pattern (Ré et al. 1990), are not typical users of estuaries in the Iberian Peninsula and their presence is generally considered accidental and largely restricted to mouth portion of estuaries, as a result of tidal transport of coastal water from the adjacent coastal areas (Ré 1999). In contrast, sardines were caught in all sampling stations of the Lima estuary, throughout the study period, and was the second most abundant species caught in this study (Figure 2.4-B). Thus, *S. pilchardus* was not an occasional species of the Lima estuary, nor was it restricted to the mouth of the system. The overall high salinity of the estuary could be the possible explanation of this feature, allowing this typical coastal species to extend throughout the Lima estuary. If this high salinity condition is stable, this habitat could be used as a spawning area for *S. pilchardus*.

Most fish larvae were caught in the salt marsh North and South zones, the shallow and less disturbed stretch of the estuary, which could be a way of larvae avoiding getting flushed out to less productive and inhospitable offshore areas where they subsequently die from starvation (Melville-Smith et al. 1981). The upstream zones of Lima estuary were dominated by species derived from demersal eggs (*Pomatoschistus* spp. and *A. tobianus*). This reproductive strategy (demersal eggs) has been described as a successful adaptation to estuarine environments by reducing mortality risks associated with the

uncontrolled dispersal of eggs and early larvae out of the estuary (Whitfield 1990). Besides the broad distribution of *Pomatoschistus* spp. along the study area, they were not abundant in the Channel zone, suggesting an affinity to the shallow waters in the saltmarsh zones. Alternatively, the low abundance of demersally spawned larvae within the Channel zone might be due to mortality of the eggs, as the channel is heavily dredged. This activity could reinforce the spatial trend of these taxa, once that Channel zone does not constitute an appropriate habitat for species with this type of reproduction strategy.

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

As in other estuaries (e.g. Whitfield 1989; Loneragan and Potter 1990; Drake and Arias 1991; Barleta-Bergan et al. 2002; Young and Potter 2003), seasonality was the most general feature observed among the different parameters used to study the larval fish assemblage. In general, differences between seasons are more pronounced than between years. According to Lam (1983), the seasonal water variability in the spawning area has an important influence on the spawning activity, and consequently, on the strong seasonal pattern observed in environments with a nursery function (McErlean et al. 1973).

In the Lima estuary, environmental influence was apparently most extensive during the winter, when water parameters, such as temperature and salinity sharply decreased. Probably, the increase of the river flow during the winter freshet period prevents the recruitment of marine species, leading to the decrease of diversity. In this study, the seasonality of the environmental conditions (Figure 2.2) co-occurred with the major variations of the fish larvae assemblage. Seasonal variations occurred not only on total abundance and diversity, but also on the structure of the larval fish assemblage of Lima

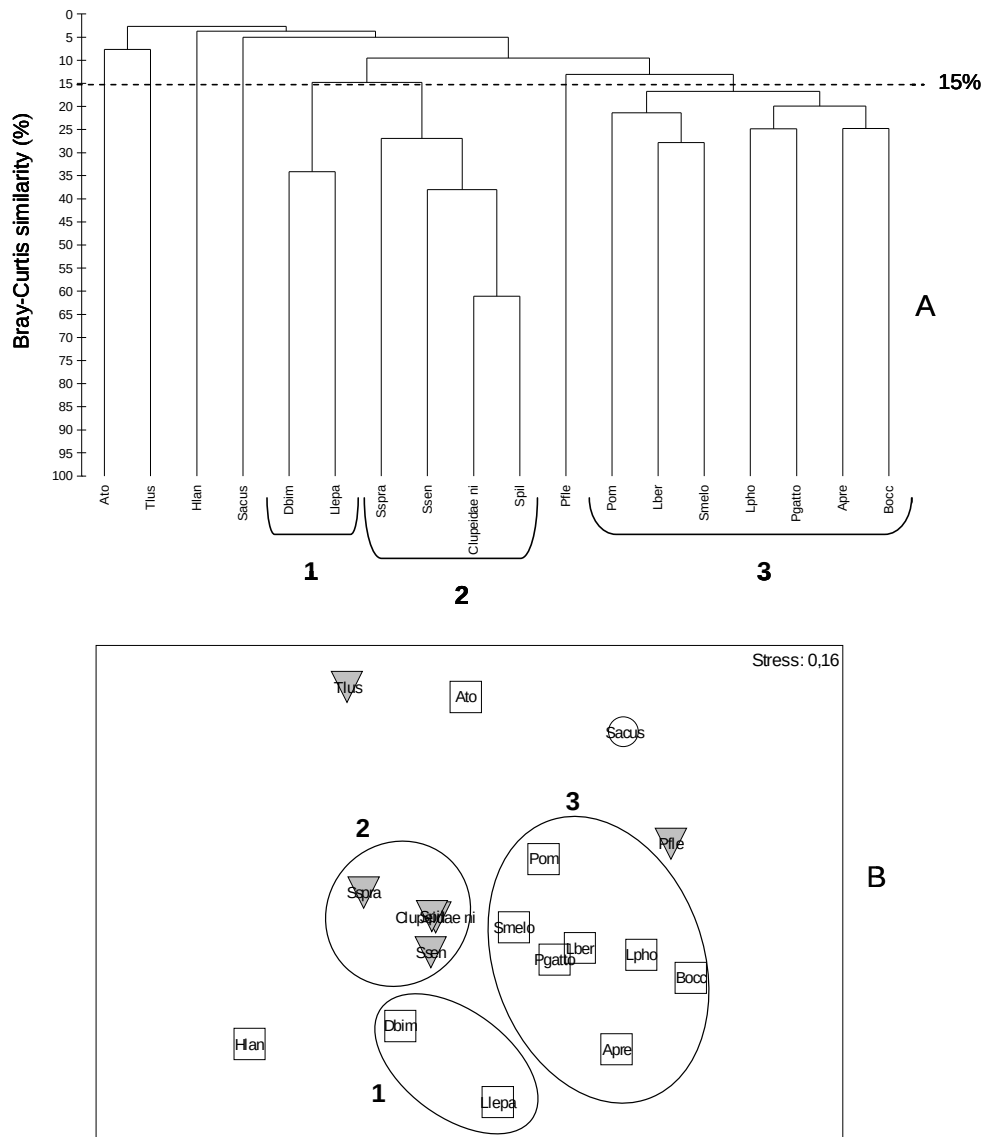


Figure 2.5. Dendrogram (A) showing species clustering analysis based on Bray-Curtis similarity matrix of most abundant 18 species, and the ordination in 2-dimensions (B) using MDS on the same similarity matrix. Clusters 1–3 were superimposed on the ordination, being represented by circles (see Table 2.1 for translation of species codes). White squares- species with demersal eggs; grey triangles- species with pelagic eggs; white circle- viviparous species.

estuary. Besides seasonal variations, the assemblage also exhibited a defined spatial pattern. Resident species were more abundant in the shallow saltmarsh zones, while temporary estuarine residents were primarily caught in the Channel zone, which is immediately connected to the adjacent coastal area. The presence of these species seems to be more related with the spawning seasonality (spring and summer), and also with the clarity of the water. In contrast, the general abundance trends of the resident species were controlled by environmental conditions of the estuary. The winter decrease of *Pomatoschistus* spp. could have been a consequence of the increase flushing rate and/or due to a decreased of food supply. Notwithstanding, the forcing control of environmental factors on the Lima estuarine ichthyoplankton should be assessed with more detail in future research.

CHAPTER 3

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

3.1 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).

Environmental features may affect communities indirectly by influencing physiological and behavioural responses of organisms and directly by affecting the distribution and

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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; Houde 1989; Dyer 1997; McLusky and Elliott 2004). As such, estuaries are highly dynamic and diverse regions of high productivity, with fish faunas 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 1997; Peterson and Whitfield 2000) or with environmental variables (Howell and Simpson 1994; Gelwick et al. 2001; 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. 2003b). 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. 2003b; 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 (Figure 3.1). Little is known about the estuary's physical and chemical characteristics or how they affect planktonic organisms. The aim of the present study was to investigate the influence of selected environmental parameters on estuarine fish larval assemblages associated with the Lima River estuary.

3.2 Method

3.2.1 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 2,446 km², of which

1,143 km² are located in Portuguese territory. The temperate Lima River has a small open estuary (Figure 3.1), with a semidiurnal and mesotidal regime (3.7 m), with an average flushing rate of 0.4 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 Lima estuary has been previously presented an initial deep navigation channel and an upstream shallow saltmarsh zone with many longitudinal sandy islands (Figure 3.1).

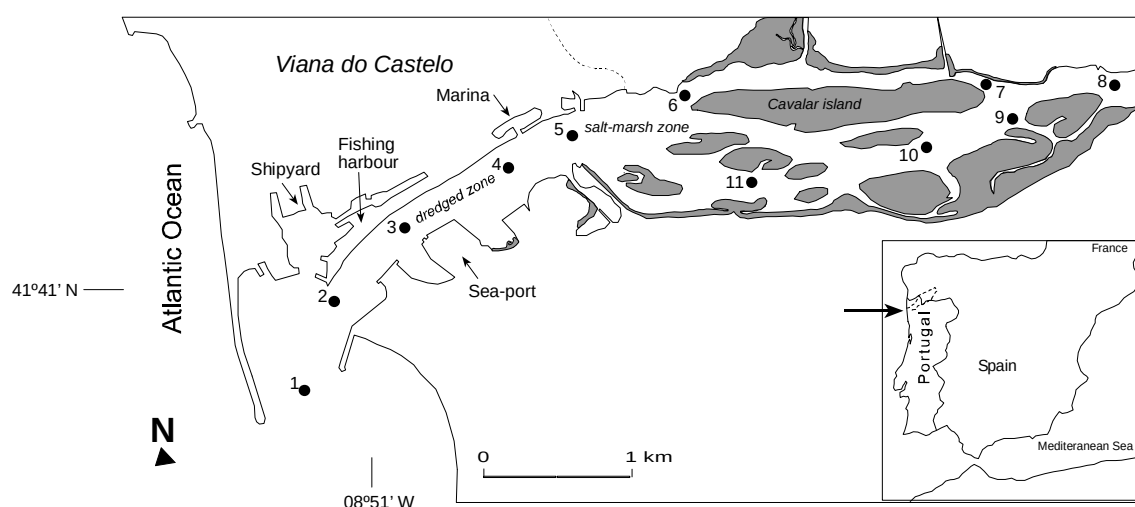


Figure 3.1. Lima River estuary and location of the 11 sampling stations. Shaded area represents sand islands and saltmarshes.

3.2.2 Sampling and sample processing

Between April 2002 and April 2004, eleven sampling stations, distributed along the study area (Figure 3.1) 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) (Figure 3.1). Subsurface circular tows were performed at a constant velocity of ca. 1 m s⁻¹ for five minutes, with a conical 1 m diameter, 3 m long and 500 µm 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

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

3.2.3 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 (0.9-2.1 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 less than 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 (Spjotvoll and Stoline 1973). Due to a CTD misfunction no environmental data was 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 analyzed 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 higher than 5% of the simulated *F*-values generated by 99 Monte Carlo permutations (i.e. $P < 0.05$) (Ter Braak and Smilauer 2002)..

3.3. Results

3.3.1 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 (Figure 3.2). During most of the two year study period, the Lima River estuary was at least partially mixed (lacked a vertical and horizontal salinity gradient) and euhaline (salinity > 30 ; Figure 3.3-A). However, during winter and early freshet spring periods, salinity decreased in the upper layers, leading to vertical stratification with a freshwater surface layer (Figure 3.3-A).

Temperature was always vertically stratified; the strongest gradient occurred during summer and in shallow areas (Figure 3.3-B). 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 (Figure 3.3), 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.

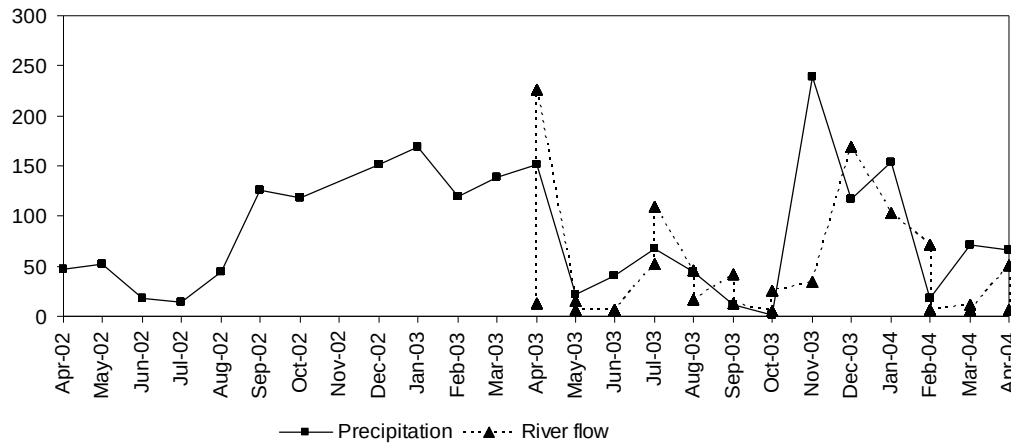


Figure 3.2. Precipitation (mm) and river flow (m³ s⁻¹) in the Lima River estuary between April 2002 and April 2004.

In the subsurface water layer there were temporal (Wilks' lambda = 0.1 $P < 0.01$) and spatial (Wilks' lambda = 0.7 $P < 0.01$) effects on the measured water characteristics (Figure 3.4). Salinity, with a mean of 32.7, differed significantly ($P < 0.01$) from zero during W_1 in saltmarsh stations (Figure 3.4) and was significantly lower in uppermost Station 8. The warmest periods were A_1 (with upwelling) and S_2 (without upwelling). Temperature decreased significantly ($P < 0.01$) during the winter months (W_1 and W_2), 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 (Figure 3.4). 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 freshet period of the second sampling year (W_2 and Sp_3). At Station 11 turbidity was significantly higher ($P < 0.01$) than in the deep first four stations and increased significantly ($P < 0.01$) during A_1 and W_1 .

3.3.2 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

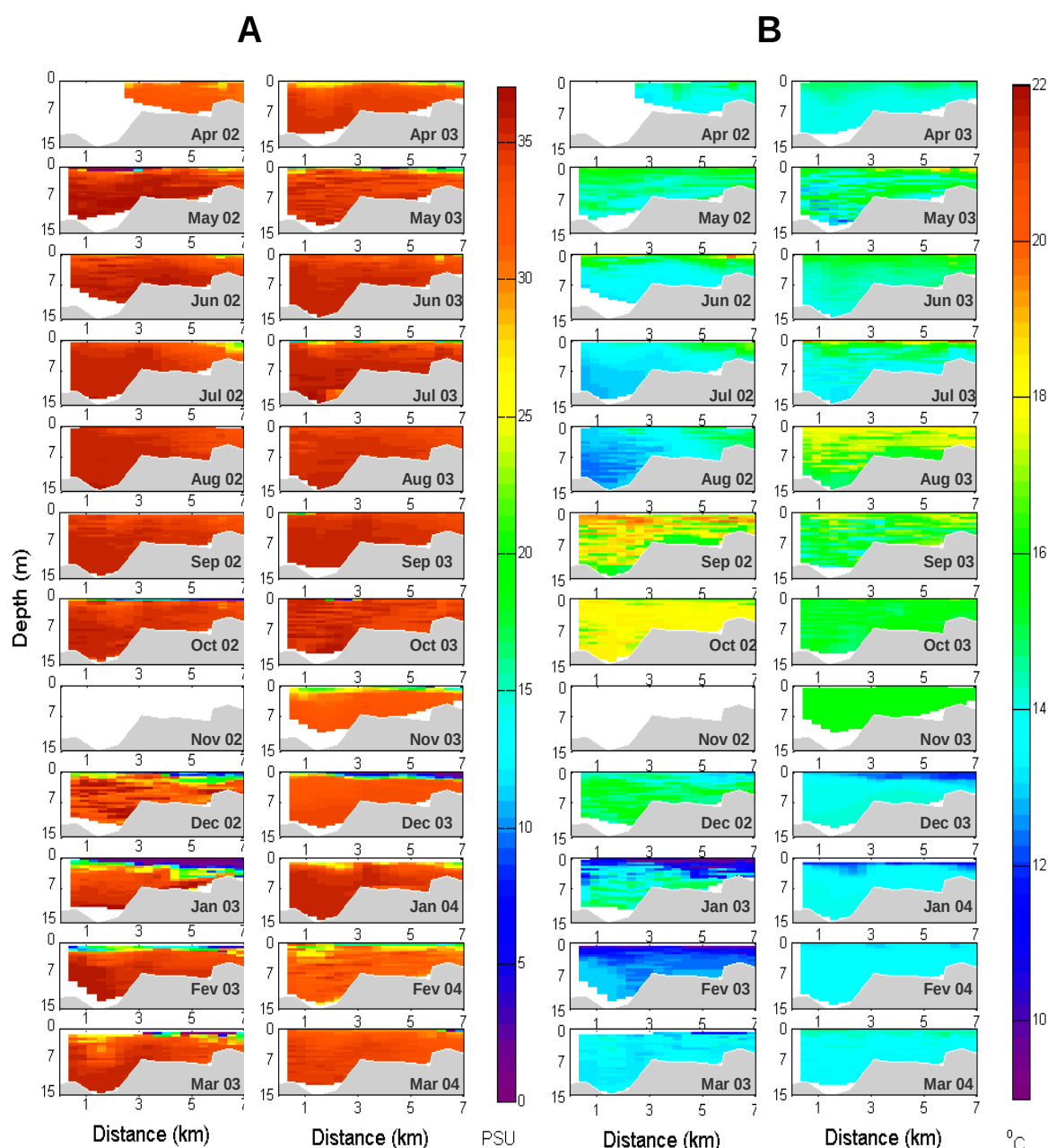


Figure 3.3. Vertical profiles of salinity (A) and temperature (B) in the Lima River estuary between April 2002 and March 2004. In November 2002 there were no data.

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 3.1). 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 3.1). 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) (Figure 3.5). 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 (Figure 3.5). Monthly mean diversity also varied, increasing during the warmer periods.

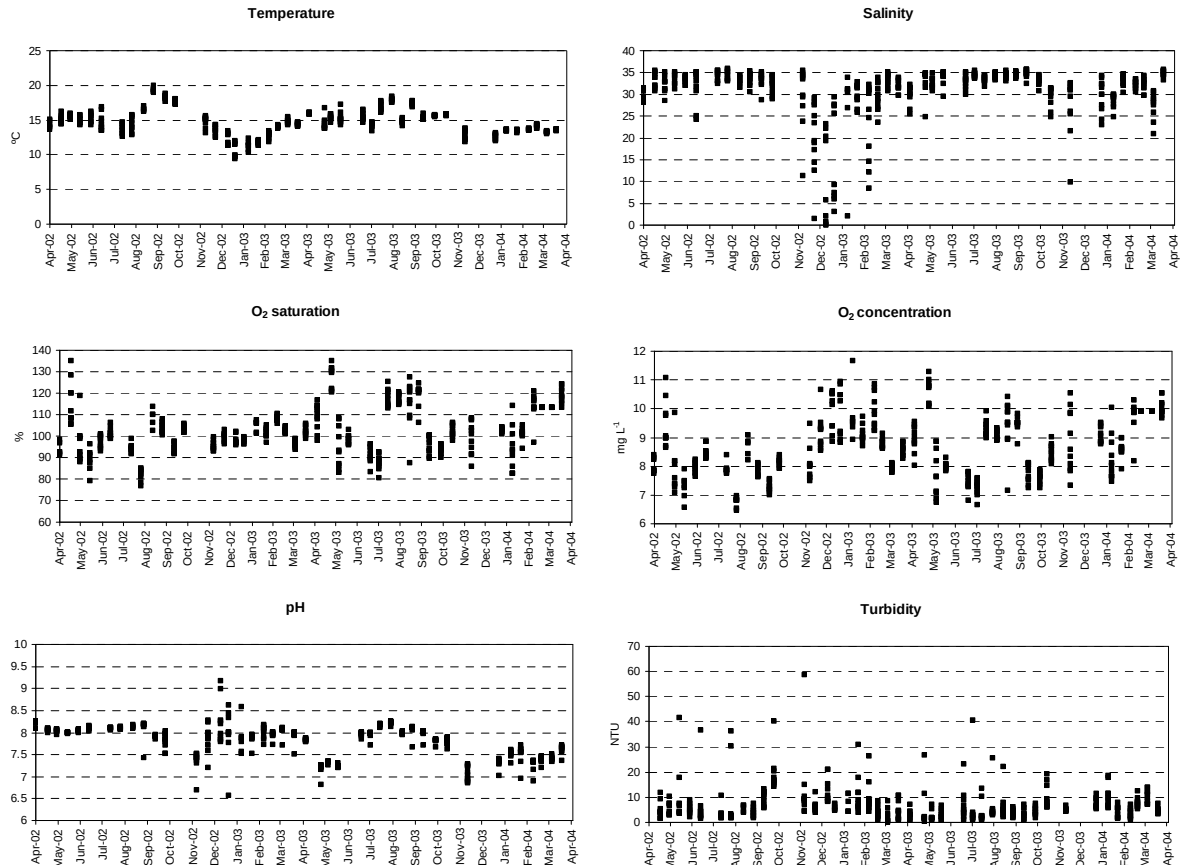


Figure 3.4. Monthly results for median temperature, salinity, oxygen saturation and concentration, pH and turbidity observed in the subsurface water layer (0.9 and 2.1 m depth), for all 11 sampling stations together.

3.3.3 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 3.3). The effect of the combined nine variables on explained distribution of the CCA

Tabela 3.1. Abundance (Number Larvae x 100 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. 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.

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

axes was significant as well ($P < 0.01$, Monte Carlo permutation test, Table 3.2). The first CCA axis (eigenvalue = 0.315) alone modeled 51 % of the total explained variance, demonstrating a high species–environment correlation (0.762) (Table 3.2). The second axis represented 24.4 % of the explained variance (Table 3.2), while the third and fourth axis additionally explained more than 16 % of the variance each. Since the first two CCA axes explained 75 % of the cumulative percentage variance of species–environment relation (Table 3.2), the latter two CCA axes (CCA₃ and CCA₄) were not interpreted further.

Table 3.2. Results of the 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
<i>Summary of Monte Carlo test</i>				
Test of significance of first canonical axis	Eigenvalue		0.315	
	F-ratio		21.44	
	P-value		0.002	

The analysis of the ordination diagram (Figure 3.6-A) showed that the seasonal patterns of temperature and precipitation, highly correlated with the first CCA axis (Table 3.3), represented a temporal gradient. Winter samples (high precipitation and low temperature) clustered on the right side of the ordination plot, and summer samples (low precipitation and higher temperatures) clustered on the opposite side of the plot (Figure 3.6-A). The second axis represented a spatial gradient, with distance from the river mouth showing a high correlation with this axis (Table 3.3). 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 second CCA axis (Table 3.3). Spring and autumn samples were distributed along the second axis (CCA₂).

Resident species (*Pomatoschistus* spp. and *S. 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 (Figure 3.6-B). 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

Tabela 3.3. Biplot scores of the nine significant environmental variables with 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

(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 *Ammodytes tobianus* and *Nerophis lumbriciformes* were more abundant in the upstream saltmarsh area (5-11). Clupeidae representatives (*S. pilchardus*, Clupeidae and *Spratus spratus*) 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.

3.4 Discussion

According to the classification of estuarine zones proposed by 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 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 (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.

In the present study, the typical longitudinal gradient was only detected during winter periods, with fresh water occasionally occurring at the surface of the upstream stations (Figure 3.3-A). 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.

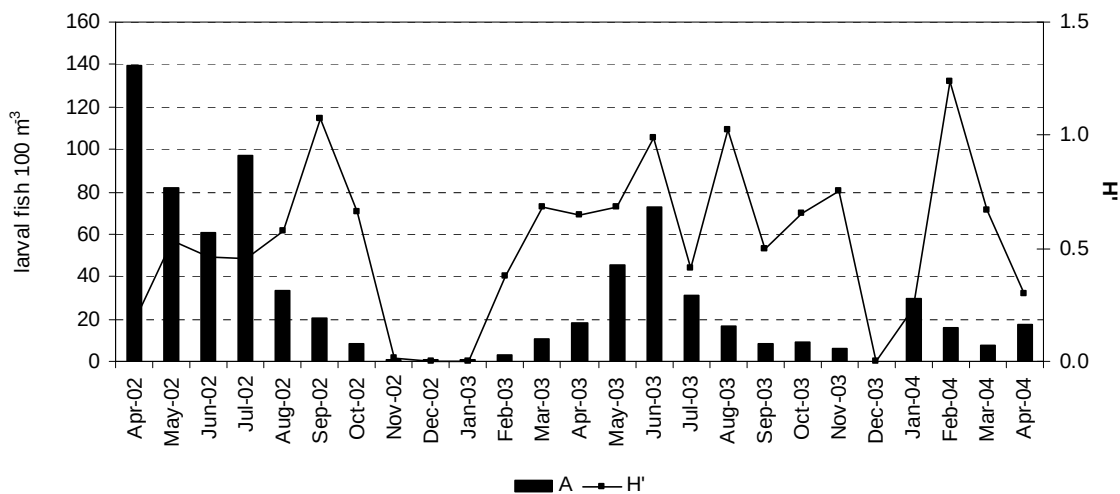


Figure 3.5. Monthly mean abundance (A) and Shannon Wiener diversity index (H') of the fish larvae collected in the Lima River estuary, from April 2002 to April 2004.

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 (Fiuza 1982). Typically, upwelling takes place along the west coast of Portugal in response to cycles of northerly winds (Fiuza 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 (Figure 3.3-A). 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 S_1 compared to S_2 (Figure 3.5).

Winter samples were totally separated from the remaining seasons (Figure 3.6). Winter periods were characterized by a decrease in temperature at the top of the water column, high precipitation and subsequently stronger river flow (Figure 3.2 and 3-B). Concurrently, larval fish abundance decreased drastically (Figure 3.5). 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 (Barleta-Bergan et al. 2002; Garcia et al. 2003b).

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 (Figure 3.6). 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. 2006a). In fact, the highest *Pomatoschistus* spp. abundance (i.e., 739 larvae 100 m⁻³) was recorded in July 2002 (unpublished data), when precipitation was low (i.e., 13.6 mm; Figure 3.3). 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 1-2m 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. 2003b). 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%, Figure 3.4). According to 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 3.3 and Figure 3.6), 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 also 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, who 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 (Figure 3.6-B). 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 lyra*, and *Trisopterus luscus*, representing more than 1% of the total catch, were positively correlated with increasing distance from the sea. This

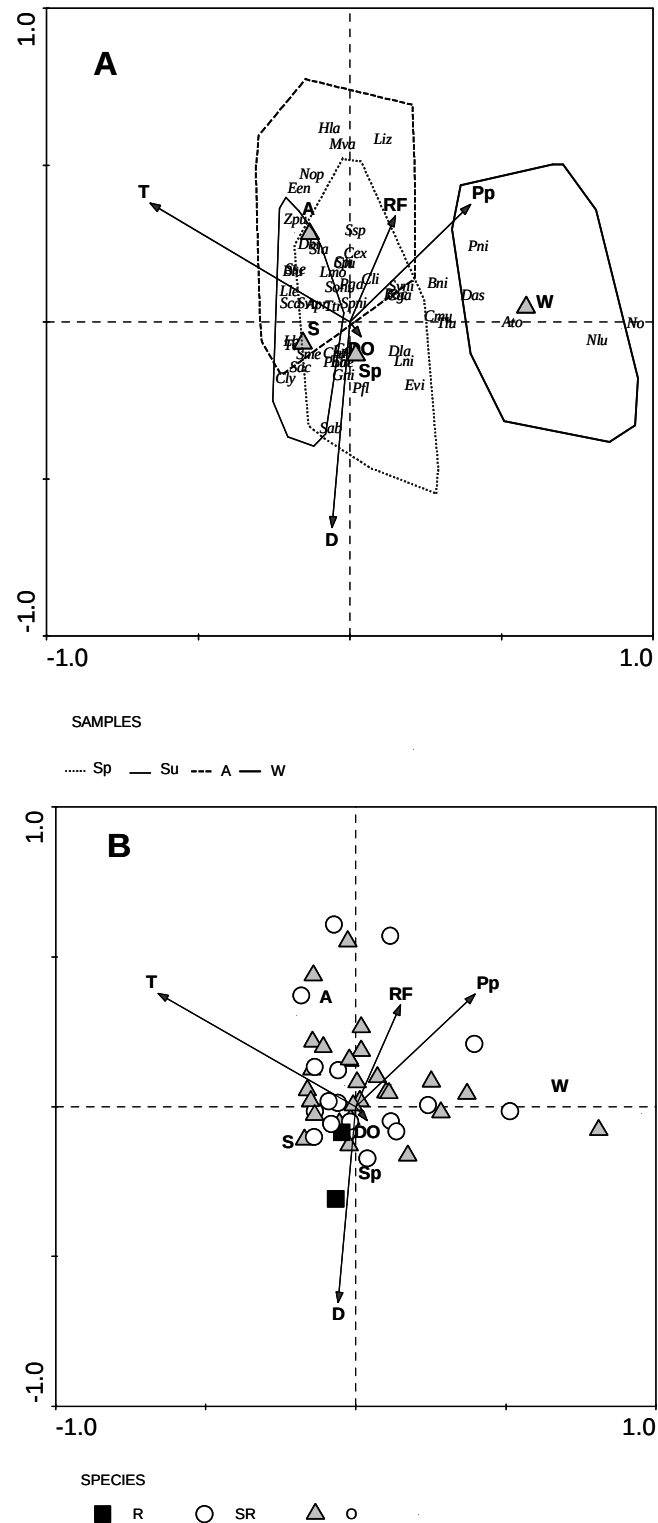


Figure 3.6. Canonical correspondence analysis of the larval fish assemblages in Lima River estuary- scores of environmental variables, samples and species in the plane of the first two axes of the CCA ordination. A- Samples were classified in four seasons (Sp- spring; S- summer; A- autumn and W- winter). B- Species were classified by habitat (see Table 3.1 for species and habitats codes). Environmental variables (arrows): T-temperature; RF- river flow; Pp- precipitation; DO- oxygen saturation; D- distance from the river mouth.

suggests that those species used the Lima River estuary as a temporary habitat, acting as seasonal estuarine residents (*T. draco* and *C. lyra* 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. The present 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.

CHAPTER 4

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

4.1 Introduction

The European sardine (*Sardina pilchardus* Walbaum, 1792) is a planktivorous species with a wide distribution around both NE Atlantic waters (from Mauritania up to the North Sea) and in 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, denoting its high socio-economic importance. Nevertheless, in the past few decades, sardine landings off the western Iberian Peninsula have been decreasing, concomitant with a decline in small pelagic fish productivity (Guisande et al. 2001; Santos et al. 2001; Borges et al. 2003). Major causes of change in stock sizes include a reduction in the lifespan of sardines as well as variations in their migration range (Lluch-Belda et al. 1989).

The contents of this chapter can be found in Ramos, S., Ré, P. and Bordalo, A. A. New insights into the early life ecology of *Sardina pilchardus* (Walbaum, 1792) in the Northern Atlantic. Submitted to Scientia Marina.

The sardine is a multiple spawner; females spawn partially on several occasions within a single season (Andreu and Pinto 1957; Blaxter and Hunter 1982). Two main spawning areas are reported in the Iberian Atlantic: along the Portuguese coast (Cunha and Figueiredo 1988) and in the Cantabrian sea (García et al. 1998). In Portugal, spawning occurs primarily off the Northern part of the continental shelf during November – April with two main peaks of intensity (November to January and April/May) (Figueiredo and Santos 1989; Pestana 1989; Ré et al. 1990; Borges et al. 2003). On the other hand, in the Cantabrian Sea, further north, the spawning season starts early in October and extends until July (Lago de Lanzós et al. 1988; Solá et al. 1992).

Estuaries worldwide are recognized as important nursery habitats for early life history stages of fishes (Powles et al. 1984; Whitfield 1999; Blaber 2000; Elliott and Hemingway 2002; McLusky and Elliott 2004). However, the initial developmental stages of sardines seem to avoid the high turbidity and low salinity typical of estuaries, at least during the winter period, and remain in the coastal areas during the larval phase. 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). Therefore, the presence of *S. pilchardus* larvae in Atlantic Iberian estuaries, has been considered accidental and restricted to the adjacent coastal zone of the estuaries, mainly due to tidal transport (Ré 1999). However, this was not the case in 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, it is important to further investigate the presence of sardine larval stages in the Lima estuary. The present study assessed the importance of the Lima estuary to this typical coastal species by investigating which environmental characteristics were associated with spatial and temporal age patterns of sardines in the lower and middle stretches of the Lima estuary.

4.2 Material and Methods

4.2.1 Study area

The study area, located in the lower 7 km stretch of the Lima River estuary, includes an initial dredged and narrow navigation channel and an upstream shallow and wide

saltmarsh zone, mainly colonized by *Juncus* spp., with a large longitudinal sandy island (Cavalar 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 *low* estuary, while the brackish shallow and wider saltmarsh area was designated as *middle* estuary.

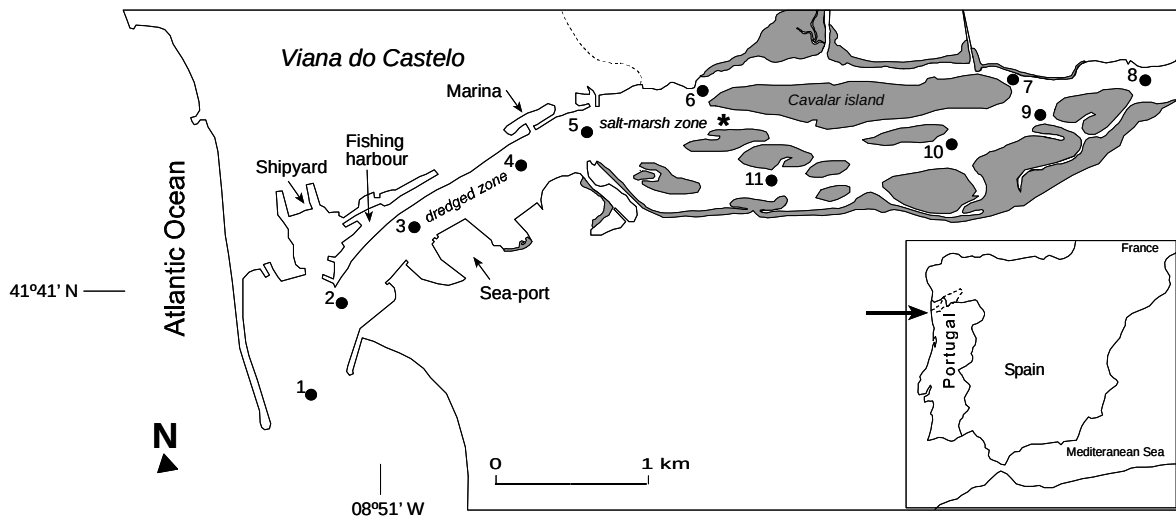


Figure 4.1. The Lima River estuary and location of the 11 sampling stations. Shaded area represents sand islands and saltmarshes. * corresponds to the location of the diel survey.

4.2.2 Data collection

4.2.2.1 Fortnightly surveys

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 1 m diameter, 3 m long and 500 μ m mesh size net 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 concentration, pH and turbidity of the subsurface water layer (0.9 and 2.1 m depth) were obtained by means of an YSI 6820 CTD. Precipitation data were obtained from the Portuguese Water Institute (INAG 2005).

4.2.2.2 Tidal survey

In addition to the fortnightly surveys, a diel survey was conducted on 3rd and 4th May 2004. Sampling targeted half of a tidal cycle, starting at 2:30 pm and finishing at 4:00 am for a total of 13:30 hours of study. The tidal cycle survey started approximately 30 minutes before the establishment of high tide and finished 60 minutes after the second high tide peak. Fish larvae were collected every 30 minutes at a sampling station located in the middle estuary (i.e. at the boundary between the initial channel and the upstream saltmarsh area, Figure 4.1). Samples were coded as either “day” (sampled during the light period, i.e. before 6:00 pm) or “night” (sampled during the dark period, i.e. between 6:00 pm and 4:00 am). Plankton tows were performed as previously described for the fortnightly surveys. Vertical profiles of temperature, salinity, dissolved oxygen concentration, pH and turbidity were performed and surface chlorophyll a was recorded by means of an YSI 6820 CTD. The depth of the water column was recorded using a pressure sensor (CTD YSI 6600 series) attached to a fixed buoy and corrected for tidal height. A current meter (Valeport) also attached to the buoy measured the surface current velocity.

4.2.3 Sample processing

Ichthyoplankton samples were fixed in 4% buffered formalin (pH=8). After sorting, sardine larvae were preserved individually in 95% alcohol. Abundance was standardized as the number of sardine larvae 100 m⁻³. Total lengths (TL) of larvae were measured to the nearest 0.01 mm using an image analyzer (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 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 stages: pre-hatch (total length < 3 mm), yolk-larvae (presence of yolk) and post-larvae (without yolk, eyes completely pigmented).

4.2.4 Data analyses

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; winter (W), December, January and February. Since 46 surveys were performed, the first 24 surveys, from the first survey of April 2002 to the first survey

of April 2003, were considered to be the first year (seasons Sp1, S1, A1, W1 and Sp2) and the remaining surveys were assigned to the second year (seasons Sp2, S2, A2, W2 and Sp3).

The temporal and spatial variations in larval sardine abundance were assessed using a two way-ANOVA, with seasons and sampling stations as fixed factors (Sokal and Rohlf 1995; Zar 1996). For the diel survey data, one-way ANOVA analyses were used to ascertain the influence of tidal phase and day/night period on sardine larvae abundance (Sokal and Rohlf 1995; Zar 1996). Whenever necessary, raw data was log-transformed to meet the ANOVA assumptions of homogeneous variance and normally-distributed data. Homogeneity of variance was tested with a Cochran test. Whenever variance was still heterogeneous, conclusions from ANOVA results were only accepted for those cases where significance levels were less than 0.01. Furthermore, in the event of significance, an *a posteriori* Tukey HSD for unequal sample sizes was used to determine which means were significantly different at a 0.01 probability level (Spjotvoll and Stoline 1973).

Age of the larvae was estimated through the conversion of body lengths into ages, using the Gompertz growth parameters determined by Ré (1984_a). According to Russell (1976) and Ré (1999), the delay between fertilization and hatching is 2.7 days and between hatching and first-feeding is 4 days, in an environment with average temperatures similar to those experienced in the Lima estuary (15 °C). Thus, a total of 6.7 days was added to the estimated age. Individuals were aggregated into four age groups: G₁ (< 8 days old), G₂ (between 8 and 14 days old), G₃ (between 15 and 21 days old) and G₄ (> 21 days old). Considering the *S. pilchardus* pelagic larval duration of 40 days, G₁ was considered a newly-hatched larvae.

The influence of environmental variables on the abundance of each of the four sardine age groups (G₁, G₂, G₃ and G₄) was analysed by means of 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, water density of the subsurface water layer (0.9-2.1 m) and monthly precipitation were used as environmental variables. In order to avoid multicollinearity problems, environmental variables with inflation factor >20 were removed from the original model (Ter Braak and Smilauer 2002). A *taxon* "No Fish" (NO) was created to prevent CANOCO from eliminating samples containing no fish larvae (Grothues and Cowen 1999). Samples were aggregated into months. The option used for CCA was Triplot scaling with focus on

interspecies distances, and species abundances were transformed [$\log (x+1)$]. 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).

Pearson correlations were used in order to ascertain the relationship between the environmental variables and sardine larvae abundance of the tidal survey data.

4.3 Results

4.3.1 Environmental conditions

During the study period, monthly precipitation varied between 28 and 239.3 mm, with a mean of 80 ± 58.6 mm. In a general way, precipitation was lower during spring-summer periods and increased during autumn-winter months. The first study year was particularly wet during A1, W1 and Sp2. The physical and chemical characteristics of the subsurface water layer (1-2 m depth) varied seasonally but with slight spatial variability between the lower and middle estuary (Figure 4.2). Temperature ranged 9.4 - 19.6 °C, with a mean of 14.8 ± 1.9 °C, and exhibited a typical seasonal trend, decreasing during the winter months and increasing during the spring and summer months. However, in 2002 temperature decreased during S1 due to upwelling, thus the warmest period of the year was A1. The Lima estuary was generally euhaline, with an average salinity above 30 psu (30.6 ± 6.5 psu). The only exception was the wet W1, when salinity decreased below 10 psu. The mean concentration of dissolved oxygen was 8.7 ± 1.1 mg L⁻¹, and values tended to increase during the freshet periods (W1, W2 and Sp3). Conversely, pH (mean: 7.8 ± 0.4) tended to decrease during the freshet periods, mainly in the second sampling year (W₂ and Sp3). Mean seasonal turbidity was lower than 7 NTU (mean: 6.8 ± 7.4 NTU), except during the A1-W1 freshet period, when the transparency of the water decreased due to increased precipitation and run-off.

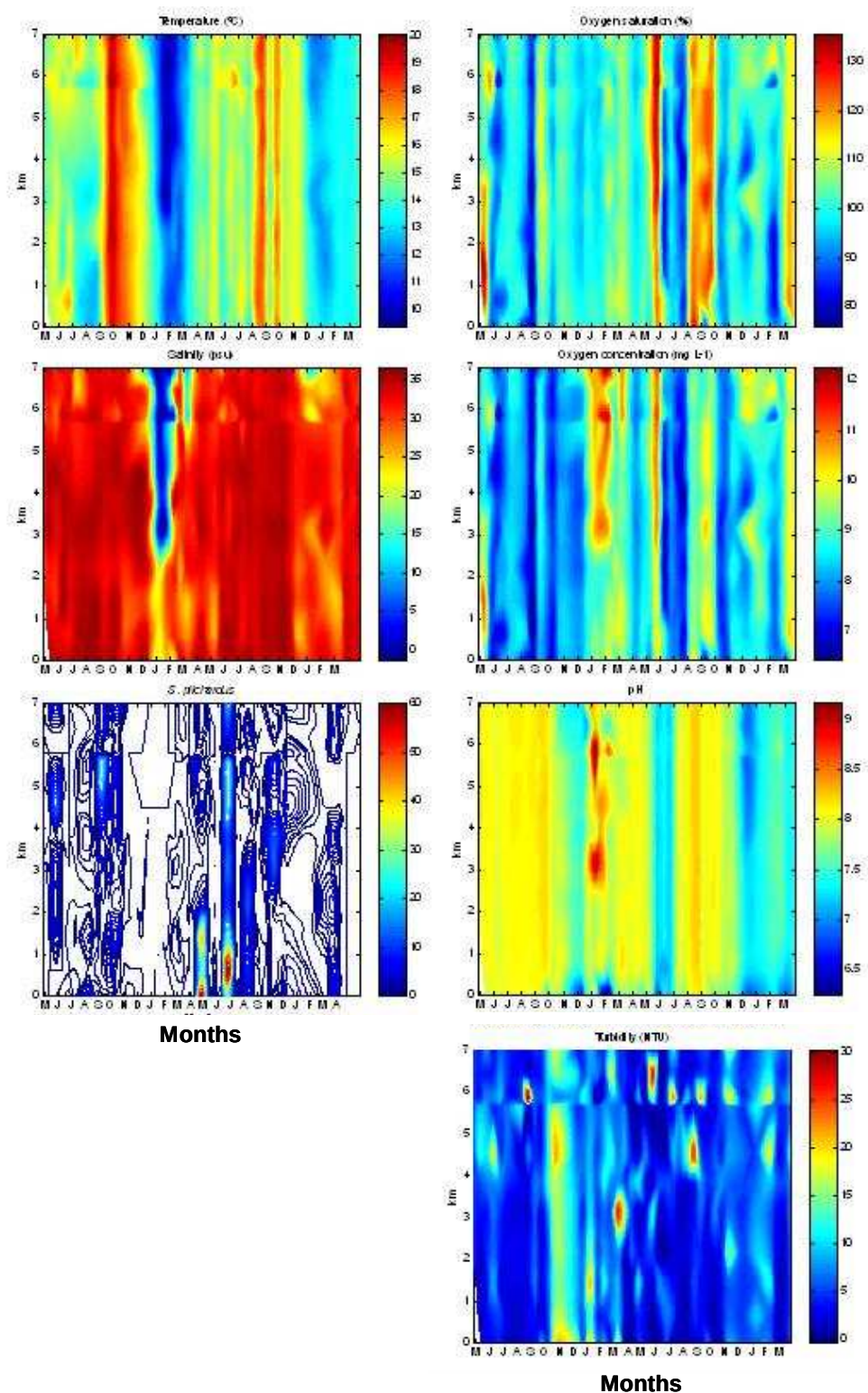


Figure 4.2. Temporal and spatial temperature, salinity, oxygen saturation and concentration, pH, turbidity and sardine larvae abundance (larvae 100 m⁻³) distribution in the Lima estuary during April 2002 and April 2004. km- distance from the river mouth.

4.3.2 Seasonal occurrence of sardine larvae

During the study period, a total of 1,069 *S. pilchardus* larvae were captured in the Lima estuary. Sardine larvae occurred in 31% of the total 489 plankton. Larval abundance ranged from 0.5 to 60.8 larvae 100 m⁻³, with an average of 1.8 ± 6.1 larvae 100 m⁻³.

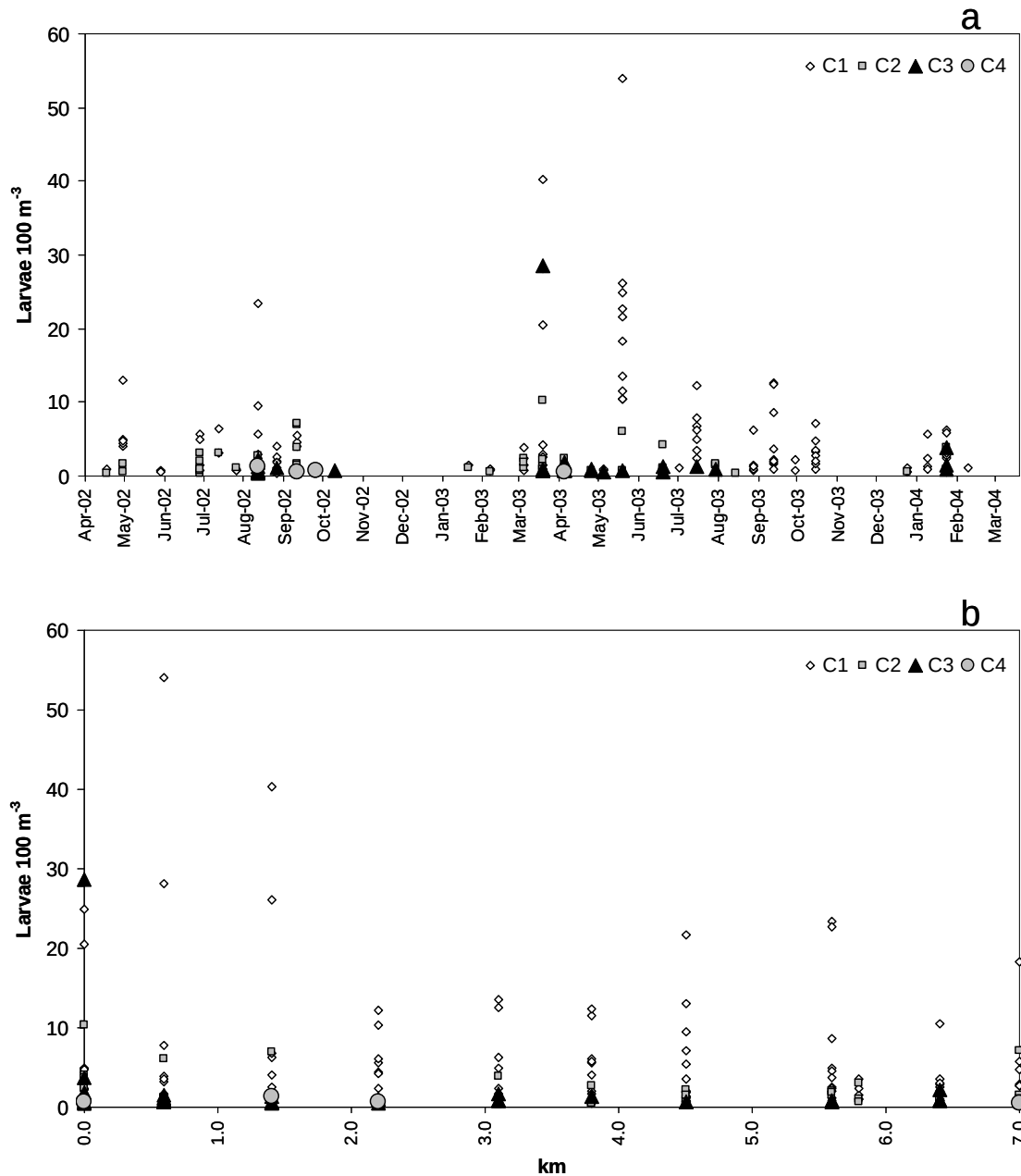


Figure 4.3. Monthly (a) and spatial (b) age patterns of sardine larvae collected in the Lima estuary between April 2002 and April 2004.

Three distinct development stages were observed: 56% were yolk-larvae, 41% were post-larvae and 3% were considered pre-hatch larvae. The majority of sardines had yolk and

unpigmented eyes. This ontogenetic stage was the most frequent and abundant, including sardines with total length ranging from 3.0 to 6.1 mm and corresponding to an age of 3 to 7 days (Table 4.1). Post-larval sardines were the second most abundant stage, corresponding to 41% of total sardines collected. Since this ontogenetic stage included all individuals without yolk, lengths were highly variable (TL range: 3.9 -16.5 mm; age range: 4.4 - 26.7 days). The pre-hatch larvae were younger than 3 days but were relatively more rare in the samples (Table 4.1).

Table 4.1. Ontogenetic stages, total length (mm), age (days), abundance (larvae 100 m⁻³) and frequency of occurrence (%) 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)	Frequency of occurrence (%)
Pre-hatch Presence of egg capsule		2.63 ± 0.27	0.73 ± 3.78	0.05 ± 0.31	4%
Yolk-larvae Yolk with an oil globule; unpigmented eyes		4.22 ± 0.55	5.01 ± 0.97	1.01 ± 3.51	22%
Post-larvae Yolk completely absorbed; pigmented eyes		7.18 ± 2.71	10.26 ± 4.81	0.73 ± 3.78	21%

The occurrence of *S. pilchardus* larval stages in the Lima estuary varied significantly between seasons ($F= 7.0$ $P<0.001$), with a distinct seasonality in each of the study years (Figure 4.3). In 2002/2003, sardines were collected with 25% frequency of occurrence, which increased to 38% during the second study year (2003/2004). Additionally, abundance peaked during A1 of the first year, and in the second year higher abundances were observed during spring-summer period (Sp2 and S2). Moreover, in 2002/2003 the period of lower abundance was W1 and in 2003/2004 was Sp3, 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 ($F= 0.7$ $P> 0.98$), a noticeable decrease in sardine larval abundance upstream was found. Mean abundance was higher in the lower estuary (2.9 ± 8.7 sardines 100 m⁻³) than in the middle estuarine area (1.4 ± 3.5 sardines 100 m⁻³) (Figure 4.2).

4.3.3 Age patterns

The total length of the 1,064 sardine larvae collected ranged 1.8-16.5 mm TL, corresponding to an age range of 1 - 27 days. Sardines with less than 8 days old were captured with a mean abundance of 1.5 ± 4.9 larvae 100 m^{-3} corresponding to 25% of frequency of occurrence. 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 were found ($F= 0.6$ $P>0.99$). G_1 was the most abundant and frequent age group, representing 80% of the positive collections (*i.e.* samples containing sardine larvae), and peaking during S2, when abundance reached 4 larvae 100 m^{-3} (Figure 4.3).

On the other hand, sardines that were 2 weeks old represented 12% of the total sardines and were observed in 35% of the positive collections, with a mean abundance of 0.2 ± 0.9 larvae 100 m^{-3} . G_2 abundance varied significantly between seasons ($F= 3.8$ $P<0.001$). Even though they occurred broadly, 2-weeks sardines were not captured during Sp3, and an abundance peak was observed in A1 (Figure 4.3). This age group did not vary significantly between sampling stations ($F= 1.7$ $P>0.09$), and no concurrent effect of season and stations was observed ($F= 0.8$ $P>0.86$).

Larvae with 3 weeks old (G_3) were present in 18% of the positive collections. Larvae with that age were less abundant, only representing 7% of the total number of sardines, with a mean abundance of 0.1 ± 1.4 larvae 100 m^{-3} . Similar to the two former age groups seasons had a significant effect on the abundance of this age group ($F= 3.0$ $P<0.001$), unlike sampling stations ($F= 1.5$ $P>0.13$), and no interactions between season and station were observed ($F= 0.9$ $P>0.76$). G_3 sardines were only found during the abundant periods of each year (*i.e.* A1 and Sp2-S2 periods), and also during W2 (Figure 4.3). Sardines aging 22 or more days old (G_4) were rare, representing 0.4% of the total sardines with a mean abundance of 0.01 ± 0.1 larvae 100 m^{-3} . This age group was only observed during A1 and Sp2 (Figure 4.3) and did not show significant seasonal ($F= 2.1$ $P>0.03$), or spatial ($F= 0.6$ $P>0.78$), variations.

4.3.4 Responses to environmental forcing

From the original set of nine environmental variables, dissolved oxygen saturation, pH, water density and precipitation were excluded due to the multicollinearity found (Inflation factor > 20). The first CCA axis (eigenvalue = 0.27), and the second CCA axis (eigenvalue

= 0.08), exhibited a high species–environment correlation (0.53 and 0.82, respectively). These two axes explained altogether 92% of the cumulative percentage variance of species–environment relation and therefore the latter two CCA axes (CCA₃ and CCA₄)

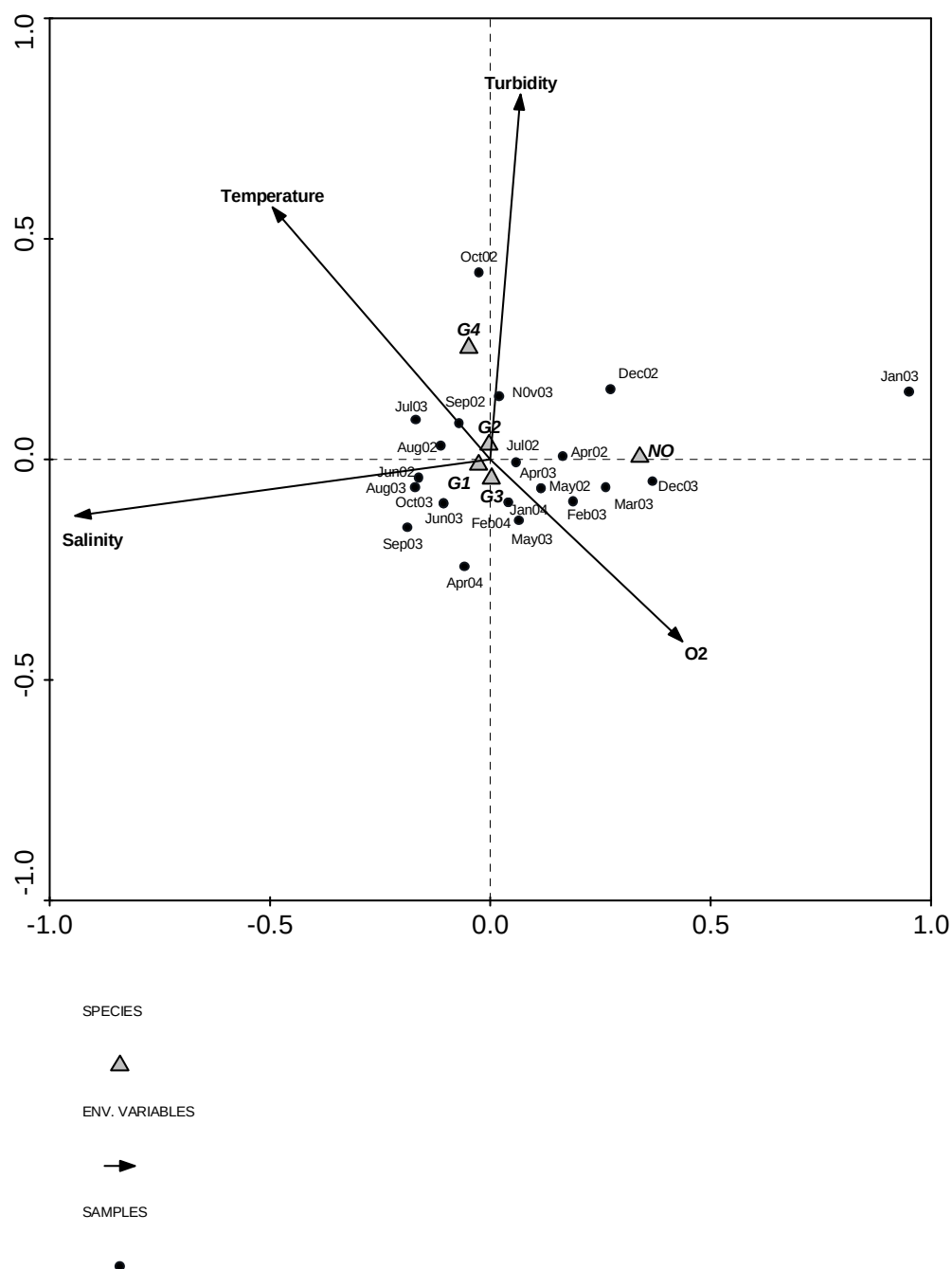


Figure 4.4. Sardine age groups and environmental variables in a triplot ordination diagram using the first two canonical correspondence axes. G₁- age group 1; G₂- age group 2; G₃- age group 3; G₄- age group 4; NO- no sardine. Arrows- environmental variables

were not interpreted further. According to inter-set correlations, temperature, salinity and turbidity were considered to be biologically important (Table 4.2). CCA₁ was negatively correlated with salinity, while temperature and turbidity were positively correlated with CCA₂. Months were separated along those two axes; those that clustered on the negative part of CCA₁ were linked with high salinity values. These months were also separated along the CCA₂ by temperature since warmer months clustered on the positive part of CCA₂. On the positive part of the CCA₁ mainly winter and early spring months clustered

Table 4.2. Inter-set correlations of environmental and supplementary variables with the first two CCA axes. * inter-set $\geq |0.4|$ corresponding to biologically important variables.

Environmental variables	CCA ₁	CCA ₂
Temperature (°C)	-0.26	0.47*
Salinity (psu)	-0.50*	-0.11
Oxygen saturation (%)	0.23	-0.34
Turbidity (NTU)	0.04	0.68*

with low salinity (Figure 4.4). December 2002 was also isolated, but it clustered in the positive part of CCA₂ due to temperatures higher than the remaining winter-spring months (Figure 4.2). G₁, G₂ and G₃ clustered at the centre of the plot, which indicated that either they do not show a strong relationship to any of the variables or they were found at mean environmental conditions (Figure 4.4). G₁ was found on the negative part of CCA₁, indicating a correlation with more saline waters. G₄ clustered in the positive part of CCA₂ (Figure 4.4), associated with October 2002 and characterized by higher temperature and salinity values (Figure 4.2). The absence of sardine larvae in the estuary was correlated with a drop in salinity, which is characteristic of wet winter months, such as January 2003 (Figure 4.2).

4.3.5 Tidal occurrence of sardine larvae

During the tidal cycle survey, sardine larvae were captured with a mean abundance of 0.8 ± 1.1 larvae 100 m⁻³ and a 43% frequency of occurrence in a total of 28 plankton trawls performed. All individuals collected were newly-hatched larvae with evidence of yolk and unpigmented eyes (TL range: 2.2 - 5.2 mm; age range: 2-7 days old). Only one larva in

the post-larval stage, without yolk and eyes pigmented (7.8 mm TL, 11 days old), was captured during the flooding tide. The abundance and age of sardines captured during the day were not significantly different than those observed during the night (abundance: $F=0.3$ $P>0.5878$; age: $F=0.2$ $P>0.654$).

During the surveyed period, the water column height varied between 0.6 m at the low tide and 3.5 m at the high tide, with amplitude of ca. 3 m, demonstrating the mesotidal regime of the Lima estuary. The maximum current velocity was observed 3 hours after the slack of the high and low tides, indicating that the Lima estuary tide is dominated by a stationary wave (Figure 4.5).

During the tidal cycle two different water masses were observed in the estuary: a cold coastal water mass (temperature < 14 °C, salinity ≥ 25 psu) and a warmer brackish water mass (temperature ≥ 14 °C, salinity < 25 psu). The cold and saline water was observed during the first half of the ebbing tide and the last half of the flooding tide. Sardine larvae were exclusively associated with these water masses; therefore no larvae were captured when salinity decreased below 25 psu during the other tidal stages (Figure 4.6). Pearson correlations corroborated this feature, since a significant positive correlation with salinity ($r = 0.5$ $P<0.01$), a negative correlation with temperature ($r = -0.5$ $P<0.01$), and with Chla ($r = -0.5$ $P<0.01$), were found. However, no significant variability in sardine abundance was found with respect to tide ($F=0.2$ $P>0.642$), and average abundance was 1.3 larvae 100 m^{-3} during the flooding tide and 0.3 larvae 100 m^{-3} during the ebbing tide.

4.4 Discussion

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 the Atlantic Iberian estuaries, where sardines are considered a residual component of the estuarine ichthyoplankton assemblage, generally dominated by another clupeid species *Engraulis encrasicolus* (Ribeiro 1991; Queiroga 1996; Ré 1996; Ré 1999; Faria et al. 2006; Marques et al. 2006b). Although larval stages of sardines are considered accidental in Iberian estuaries (Ré 1999), sardine larvae were frequently observed in the Lima estuary throughout all studied seasons (Figure 4.2). None of the sardine age groups revealed any specificity to a particular zone of the estuary since they were well distributed along the initial 7 km of the Lima estuary. Thus, the present study revealed that *S.*

pilchardus was not an occasional visitor or restricted to the terminal zone of the Lima estuary, providing new insight into the early life ecology of *S. pilchardus* along the Northern Iberian coast.

Sardine larvae were captured throughout the study period and 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, the temporal pattern of sardine larvae abundance in the estuary should reflect that spawning activity. In fact, maximum abundance values recorded in this study (60 larvae 100 m⁻³), were similar

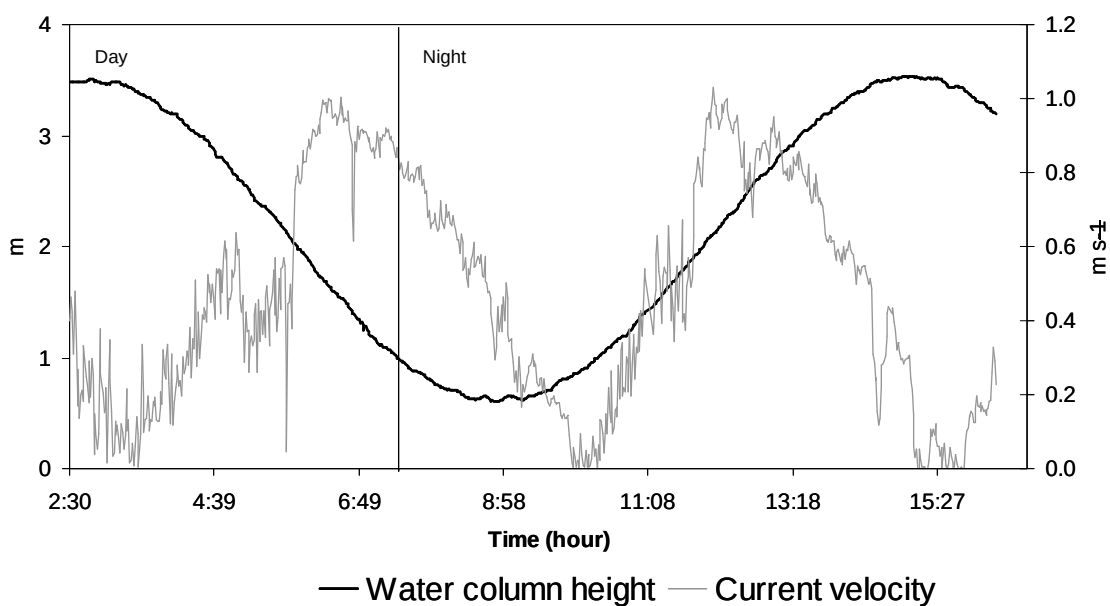


Figure 4.5. Water column height (m) and current velocity (m s⁻¹) during the diel survey.

to those reported for the coastal area during the spawning season (Ré et al. 1990; John et al. 1996). However, the maximum sardine abundance in the Lima estuary during A1, Sp2, S2 and A2 did not coincide with the described adult spawning season off northern Portugal, which occurs from November to April (Ré et al. 1990; Zwolinski et al. 2001; Stratoudakis et al. 2003). This lack of temporal synchrony between spawning activity and occurrence of larvae inside the estuary might be a consequence of several factors. The high abundances of sardines during summer suggest that sardine populations could be extending their spawning season through late spring and summer. Another possible explanation for the asynchrony is the existence of alternative estuarine spawning, which may explain the presence of high numbers of sardine eggs in the ichthyoplankton samples (data not shown). However, this theory requires further investigation of the temporal abundance patterns of sardine eggs inside and outside the estuary.

Since spawning is known to occur in the coastal area adjacent to the estuary, the present study indicates that *S. pilchardus* extended their typical coastal nursery ground throughout the study area (Muiño et al. 2003). This might be interpreted as a consequence of the favourable environmental conditions of the Lima estuary. During the study period, the Lima estuary was a euhaline system ($25 < \text{salinity} < 35$), exhibiting water characteristics similar to that of the adjacent coastal water with clear water even in the shallow saltmarsh area.

During the 25-month study period, sardines were frequently observed in the Lima estuary, exhibiting a clear seasonality that varied between years (Figure 4.2). There were two distinct periods of low abundances, W1 and Sp3, and two peaks of high abundances, A1 and Sp2-S2 (Figure 4.2). According to Olivar et al. (2001; 2003) environmental variations can modify both distribution and abundance of sardine larvae as was the case in the Lima estuary. Seasonal variations in abundance of sardines accompanied temporal variations in the water characteristics. For example, the freshet period of W1 was characterized by a sharp salinity decrease (below the 25 psu), which coincided with the absence of sardine larvae (Figure 4.2). Similarly, the Lima estuarine larval fish assemblages also decreased during W1 as a consequence of the increased river run-off and reduction of saltwater intrusion, which led to a vertical stratification of salinity (Ramos et al. 2006b). The increased run-off made it difficult marine larvae to penetrate into the estuarine habitat. In the second winter period (W2), median salinity did not decrease below 25 psu and sardine larval abundance was higher than in W1 (Figure 4.2). Even on a diel/tidal scale, sardines were almost entirely restricted to the euhaline stretch and were almost absent when salinity decreased below 25 (Figure 4.6). The present data seems 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 within the estuarine boundaries was directly controlled by the salinity regime of the system, which was in turn controlled by the tide on a diel scale and by precipitation on a seasonal and annual scale. Overall, salinity was one of the major environmental controls on the occurrence of sardine larvae in the Lima estuary (Table 4.2).

The other period of sardine scarcity (Sp3) was not associated with a salinity reduction. Therefore a factor or factors other than salinity were responsible for this decline. According to the CCA results (Table 4.2), temperature and turbidity were also identified as environmental determinants of the presence of sardine larvae. During Sp3, temperature and turbidity were similar to those observed in W1 (i.e. mean temperature $< 15^{\circ}\text{C}$ and some samples with turbidity > 10 NTU). However, other periods of even higher sardine

larvae abundance (e.g. W2) exhibited similar temperature and turbidity values (Figure 4.2). Thus, with the present data set is not possible to assess the cause of the low abundances observed during Sp3.

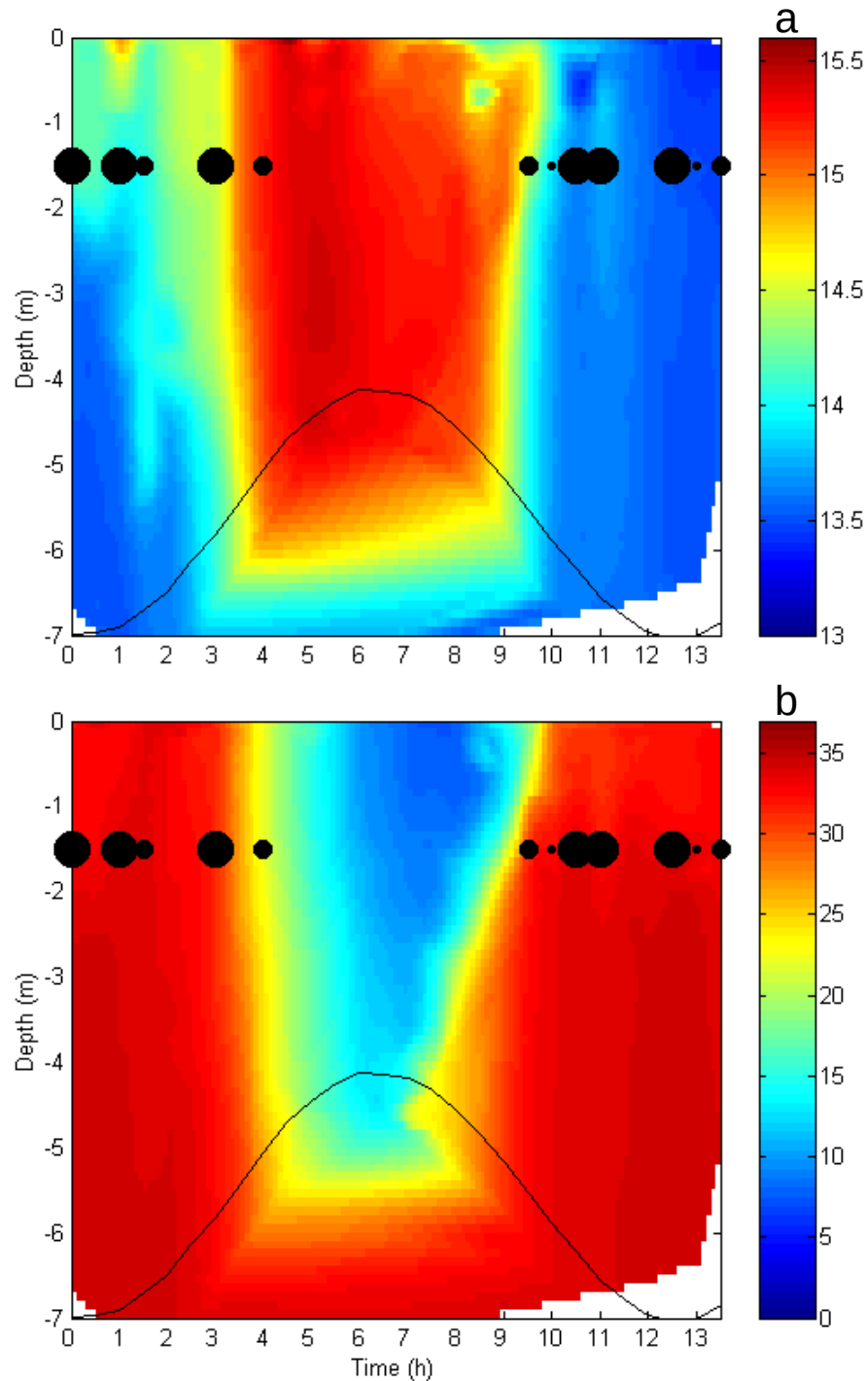


Figure 4.6. Vertical distribution of temperature (a) and salinity (b) along the 13.5 hours of the diel survey. The dark line corresponds to the water column depth (without correction for the hydrographic zero) and circles correspond to sardine larval abundance (small circles: < 1 sardine 100 m^{-3} ; medium circles: 1-2 larvae 100 m^{-3} ; big circles: > 2 sardine 100 m^{-3}).

The typical pelagic fish life-history strategy in areas of coastal upwelling favours spawning when the offshore transport is minimal and feeding at a later time to profit from the increase 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 larvae during a typical summer upwelling, as was the case during S1 (Ramos et al. 2006b), corroborates a spawning strategy that avoids the upwelling periods. In fact, during the summer of the following year, when no upwelling signal was observed, sardine larvae reached the highest abundances during the study period (Figure 4.2). Upwelling is responsible for the offshore transport of coastal waters, which can include surface coastal species such as sardine larvae (John et al. 1996). High wind speed generates strong turbulence that desegregates food and larval patches (Peterman and Bradford 1987; Roy et al. 1989), promoting larval offshore transport, creating a strong mixed layer and a light-limited phytoplankton population, and therefore reducing primary production and zooplankton biomass (Huntsman and Barber 1977). This offshore water transport can have a negative effect on larval survival when eggs and larvae are transported to areas with low food availability (Bailey 1981; Parrish et al. 1981). Several studies demonstrated that winter upwelling seems to have a significant negative correlation on the sardine recruitment index (Santos et al. 2001; Borges et al. 2003). Thus, we can suggest that the depletion of sardine larvae during Sp3 could have been a consequence of the spring upwelling event. This is similar to what has been observed in northern Spain, where the spring upwelling event off the Galicia coast was associated with low sardine larval abundance (Riveiro et al. 2000).

Sardine spawning activity is temperature dependent, occurring mainly when surface water temperatures range between 14°C - 16°C (Ré et al. 1990). On the other hand, 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 (Figure 4.2). In fact, whenever the temperature increased, sardine larval abundance inside the estuary peaked (A1 and Sp2-S2 periods). Temperature was identified as an important environmental determinant of sardine larvae in the Lima estuary (Table 4.2 and Figure 4.4). Thus, just as temperature is an important variable for coastal spawning, it also had an indirect effect on the temporal patterns of *S. pilchardus* larvae in the Lima estuarine habitat.

The rare occurrence of older larvae could have been a consequence of net avoidance, since older larvae have better visualization and swimming capability that may have allowed them to avoid the net. Many studies collected larvae larger than 16 mm in other habitats using similar nets and trawling strategies (Ré 1984a; 1986; Chícharo 1998; Olivar et al. 2001; Santos et al. 2004), and the trawling velocity used in this study is considered to minimize net avoidance and extrusion of the larvae (Ré 1986). Therefore, the sampling strategy does not constitute an explanation for the reduced abundance of larger sardine larvae. In the Tagus estuary, Ré (1984b) captured larger larvae at night. Sardine larvae migrate along the water column, staying in deeper water during the day and migrating to the surface during the night. Based on the tidal cycle results, there was no evidence that sardine performed this vertical diel migration in the Lima estuary. Body lengths and ontogenetic stages did not vary between day and night, similar to observations made by (Andres et al. 1992) in the northern coast of Portugal. Larger larvae could have adopted a selective tidal stream transport as a retention mechanism by migrating up in the water column during flooding tidal currents and downward during ebbing tidal currents (Epifanio 1988). However, results of the diel survey are not consistent with selective tidal stream transport, since abundance did not vary between the flood and ebb tide. However, diel survey results should be interpreted with caution because data are from a single survey, conducted during an apparently non-abundant period for sardine larvae (Figure 4.2). Another plausible explanation for the absence of larger larvae is the offshore location of older individuals. Similar trends were observed by Olivar et al. (2003) in the Mediterranean Sea. Moreover, Santos et al. (2004) noticed that sardine larvae larger than 15 mm were found further offshore, between the 100 m and 200 m bathymetry line, while smaller individuals were associated with inshore areas. Smaller larvae were also found most frequently near a coastal lagoon in South Portugal (Chícharo 1998).

The high abundance and frequency of 1-week old sardine larvae in the Lima estuary indicated that there was a spawning area of *S. pilchardus* close by the Lima estuary and that the spawning season of this species was not restricted to the winter-spring months as previously described. Due to the economic importance of sardines in European fisheries, the unusually high abundance and frequency of sardine larvae in an estuarine habitat is important information for the management and sustainability of this typically coastal species. Beare et al. (2004) emphasized that the use of estuaries can present 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 importance of the Lima estuary for the recruitment of the northern Iberian *S. pilchardus* stock. Moreover, the present study

was not sufficient to conclusively determine whether sardine larvae actively migrated into the Lima estuary, or if their presence in the estuary was the result of passive tidal transport. Since only one tidal/diel survey was performed and abundances of older larvae were low, whether or not the Lima estuary is a frequent spawning and nursery area for sardines requires further investigation.

PART II: Benthic Fish Assemblages

CHAPTER 5

Temporal and spatial dynamics of benthic fish fauna in the temperate Lima River estuary (Northwest Portugal)

5.1 Introduction

Estuaries are both a fundamental part of the coastal zone and the riverine system and hence have been termed transitional waters under the EU Water Framework Directive (WFD 2000). The current management of estuaries relies on a good knowledge of their structure and functioning and of the way in which their habitat status, use, loss and gain has been influenced by human activities (Belfiore 2003; Elliott and Cutts 2004). Fishes play an important part in estuaries as they constitute permanent and temporary community components, with marine species visiting these habitats for feeding, reproduction, growth and protection and as migration routes, thus linking the coastal areas to the upstream catchments.

The contents of this chapter can also be found in Ramos, S., Cowen, R. K., Ré, P. and Bordalo, A. A. Temporal and spatial dynamics of benthic fish fauna in the temperate Lima River estuary (Northwest Portugal). Submitted to *Hydrobiologia*.

Estuarine fish assemblages suffer multiple sources of variations, as a result of physical and biological forcing. For example, apart from species-specific reproductive biology (Potter et al. 2001; Hagan and Able 2003), competition and predation have been reported as important biological factors responsible for the spatial and temporal patterns of estuarine fish populations (Holbrook and Schmitt 1989; Ogburn-Matthews and Allen 1993; Lankford Jr. and Targett 1994; Barry et al. 1996). Moreover, water characteristics such as salinity, temperature, turbidity and oxygen concentration are also important environmental controls for fish communities (Cyrus and Blaber 1992; Rakocinski et al. 1996; Whitfield 1999; Strydom et al. 2003). Additionally, estuarine fish assemblages can vary with habitat characteristics, such as sediments and vegetation (West and King 1996; Jenkins et al. 1997; Paterson and Whitfield 2000), and also with anthropogenic pressures such as pollution and habitat loss (McLusky and Elliott 2004).

Estuarine fish assemblages are composed of marine, estuarine and freshwater species and a variety of life history stages of estuary-associated species can be identified (Claridge 1986; Sánchez and Raz-Guzman 1997; Able and Fahay 1998; Whitfield 1998; Blaber 2000; Elliott and Hemingway 2002). Several classifications have been proposed in order to distinguish the different life history patterns of estuarine fishes. In what European estuaries are concerned, Elliott and Dewailly (1995) divided the estuarine fish assemblages into six different ecological guilds: estuarine residents, marine juvenile migrants, catadromous and anadromous migrants, marine seasonal users and freshwater and marine adventitious visitors. These patterns of estuary fish use seem to be similar across the world (Able 2005).

The ecological importance of estuaries to early life stages of fishes has been recognized worldwide (Blaber et al. 1995; Hostens 2000; Fujita et al. 2002; Weerts and Cyrus 2002; Ross 2003; Baker and Sheaves 2005), and is inherently linked to fishery yields and important economic opportunities (Houde and Rutherford 1993; Blaber 1997). Along the southern and central Portuguese coast (West Iberian Peninsula), several estuaries have already been identified as important habitats for early stages of fishes, namely Ria de Aveiro (Rebelo 1992; Pombo et al. 2005), Tagus (Costa and Bruxelas 1989; Cabral and Costa 1999), Ria Formosa (Andrade 1992) and Guadiana estuary (Chícharo et al. 2006). However, little information is available for the northern Iberian estuaries, such as the Lima estuary, which still supports a locally and regionally important fishery (flounder, seabass, lamprey and shad). This study aims to study, for the first time, the spatial and temporal patterns of the Lima estuarine benthic fish assemblage and to understand their relationships with key environmental parameters acting as controls.

5.2 Material and Methods

5.2.1 Study area

The Lima River is an international water body, draining into the Atlantic Ocean at 41° 40' N and 8° 50' W in the vicinity of the 32,000 inhabitant city of Viana do Castelo, Portugal. The Lima River has a rather small open estuary (Figure 5.1), with a semidiurnal and mesotidal regime. The river mouth is partially obstructed by a 2 km long jetty, deflecting the river flow to the south. The Lima estuary can be divided into three geomorphological distinct zones, according to water depth, width and the presence/absence of saltmarsh. The lower estuary is a narrow, 10 m deep dredged navigational channel that is highly urbanized with hardened shorelines. The middle estuary includes a broad shallow

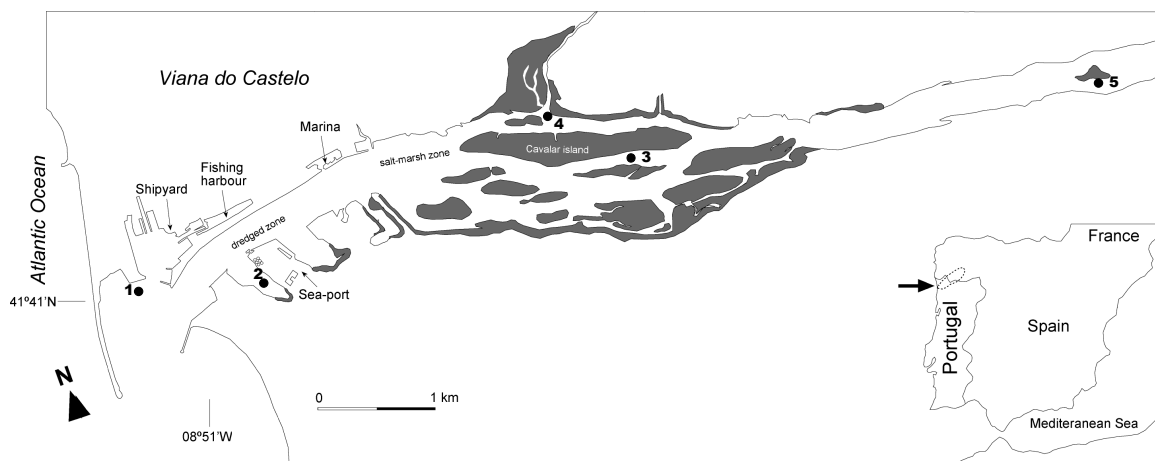


Figure 5.1. Lima River estuary and location of the 5 sampling stations. Shaded area represents sand islands and saltmarshes.

saltmarsh zone, mainly colonized by the common rush (*Juncus* spp.) with a large longitudinal sandy island (Cavalier Island). More upstream, the estuary continues as a narrow shallow channel with natural shorelines and few intertidal areas. Due to the high variety of habitats present in these three regions of the estuary (hardened and natural shorelines, constructed and natural embayments, areas with or without marsh or dredging activity), sampling stations were chosen according to results of preliminary surveys conducted at the onset of the study (September 2003), at different areas within each estuarine region. Thus, five major habitats were identified along the Lima estuary. In the lower urbanized estuary, Station 1 corresponded to a 10-m dredged and urbanized area

(near a large shipyard), with hardened shorelines located 0.9 km from the river mouth. Station 2 (1.5 km from the river mouth) represented a small artificial presently not dredged embayment, with hardened shorelines, located in the south bank of the lower estuary. Station 3 typified a shallow intertidal saltmarsh channel 5 km upstream the river mouth. Station 4, also in the middle estuary (4.5 km), represented a subtidal saltmarsh area at the vicinity of a small creak. According to the preliminary results, the habitat variability in the upper region of the estuary was low, and only one station was regularly visited. Thus, station 5 was located 9.8 km upstream of river mouth in a narrow and shallow (3 m) intertidal channel not dredged with natural shorelines.

5.2.2 Sampling methods

Monthly daytime surveys were conducted between October 2003 and November 2004 during the flood of spring tides. In order to minimize the variability associated to tides, sampling at each location was conducted roughly at the same tidal height. Fishes were collected by means of a 1-m beam trawl with one tickler chain and 5 mm stretched mesh size. Tows of 3 min duration with an average speed of 0.8 m s^{-1} were performed at each sampling station. Samples were preserved in ice and transported to the laboratory where they were immediately frozen until sorting. All teleosts were separated from the remaining material. Fishes were counted, identified to the species level and the total length was measured with 1 mm precision. The percent of juveniles was calculated according to the length at first maturation available in the literature.

Vertical profiles of temperature, salinity, dissolved oxygen concentration and saturation, pH and turbidity were obtained by means of a YSI 6820 CTD. Geographic location of the sampling stations and distance travelled for each tow was provided by a Magellan 315 GPS. River flow and precipitation data were obtained from the Portuguese Water Institute (INAG 2005).

During the final survey (November, 2004), triplicate sediment samples from each location were taken for grain size analysis at each sampling area using a Petit Ponar grab. Sediments were dried at 100°C . Grain size analysis was performed by wet (fraction $< 0.063 \text{ mm}$) and dry (other fractions) sieving (CISA Sieve Shaker Mod. RP.08), of samples previously treated with hydrogen peroxide. The sediments were divided into four fractions: silt and clay ($< 0.063 \text{ mm}$), fine sand ($0.063\text{-}0.5 \text{ mm}$), coarse sand ($0.5\text{-}2 \text{ mm}$), and gravel ($>2 \text{ mm}$). Each fraction was weighed and expressed as a percentage of the total weight.

5.2.3 Data analyses

Seasons were aggregated into three- month groups: autumn (A) consisted of September, October and November 2003, winter (W) of December 2003, January and February 2004, spring (Sp) of March, April and May 2004, summer (S) of June, July and August 2004, and the second autumn (A1) of September, October and November 2004.

The abundance of fishes was determined based on the time and velocity of each trawl and expressed as number of individuals per 1000 m². Diversity was expressed by the Shannon-Wiener index (Shannon and Weaver 1963). Each fish species was assigned to one of the five ecological guilds, according to the classification proposed by Elliott and Dewailly (1995), and distributed into truly estuarine residents (ER), marine adventitious visitors (MA), diadromous (catadromous or anadromous) migrant species (CA), marine seasonal migrant species (MS) and marine juvenile migrant species (MJ).

Two-way ANOVA was used to assess the effect of sampling stations and seasons on the environmental parameters and on fish density and diversity, with seasons and stations as fixed factors (Sokal and Rohlf 1995; Zar 1996). In order to stabilize the variance and to fit data to a normal distribution, fulfilling the ANOVA assumptions, environmental data were log transformed [$\log_{10}(x)$] as was the raw abundance and diversity data [$\log_{10}(x + 1)$]. Homogeneity of variance was tested with a Cochran test and whenever variance was still heterogeneous, conclusions from ANOVA results were only accepted for those cases where significance levels were < 0.01 . Additionally, in the event of significance, a posteriori Tukey HSD for unequal sample sizes was used to determine which means were significantly different at a 0.05 level of probability (Spjøtvoll and Stoline 1973).

The environmental control on fish assemblages was assessed by means of 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 and turbidity of the bottom water layer (1m above the maximum depth) were used as environmental variables. Additionally, river flow, distance from the river mouth, the percentage of gravel, coarse sand, fine sand, silt and clay were also used as environmental variables. Qualitative habitat characteristics such as the presence of saltmarsh, intertidal, shallow and dredging activity were also included in the model as nominal variables. Species with abundance lower than 1 fish 1000 m⁻² were excluded from the analysis. Species abundances were transformed [$\log(x+1)$] and downweighting of rare

species was performed. In order to avoid multicollinearity problems, environmental variables with inflation factor >20 were removed from the original model (Ter Braak and Smilauer 2002). The option used for CCA was triplot scaling with focus on interspecies distances. Significance of the canonical model was given by a Monte Carlo test (Ter Braak and 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). Samples and species were classified according to sampling station and ecological guilds, respectively.

Two-way crossed analysis of similarity (ANOSIM) (Clarke and Warwick 1994) was used to determine the significance of spatial and temporal trends in assemblage species composition. Tests were based on a Bray–Curtis rank similarity matrix, with log transformed $[\log_{10}(x + 1)]$ data (Clarke and Warwick 1994). For the data matrix without the two dominant species, data was not transformed. Groups were considered to vary significantly only when $R > 0.5$. Similarity percentages (SIMPER) (Clarke 1993) were used to reveal the percent contribution of each species to the average dissimilarity between seasons and stations. These analyses were performed with the software package PRIMER (Plymouth Routines Multivariate Ecological Research) (Clarke and Warwick 1994).

5.3 Results

5.3.1 Environmental characteristics

A marked horizontal salinity gradient was observed during the entire study period, with salinity decreasing significantly from the river mouth (average 34 psu) until 10 km upstream (average 3 psu) ($F=19.9$ $P<0.01$). Salinity showed little seasonal variability in the lower zone of the estuary, which was, on average 34 and 26 psu at Stations 1 and 2, respectively (Figure 5.2). The upper estuary was also salinity stable, but in the oligohaline range, with an average of 2.3 psu. However, in the middle estuary, salinity was in average 17.2 and 16.9 psu at Stations 3 and 4, respectively, and varied seasonally. The values decreased significantly during W and A1 ($F=7.2$ $P<0.01$), to values lower than 15 psu. At Station 3, no statistical interactions were observed between seasons and stations on the salinity regime of Lima estuary ($F=1.5$ $P>0.2$).

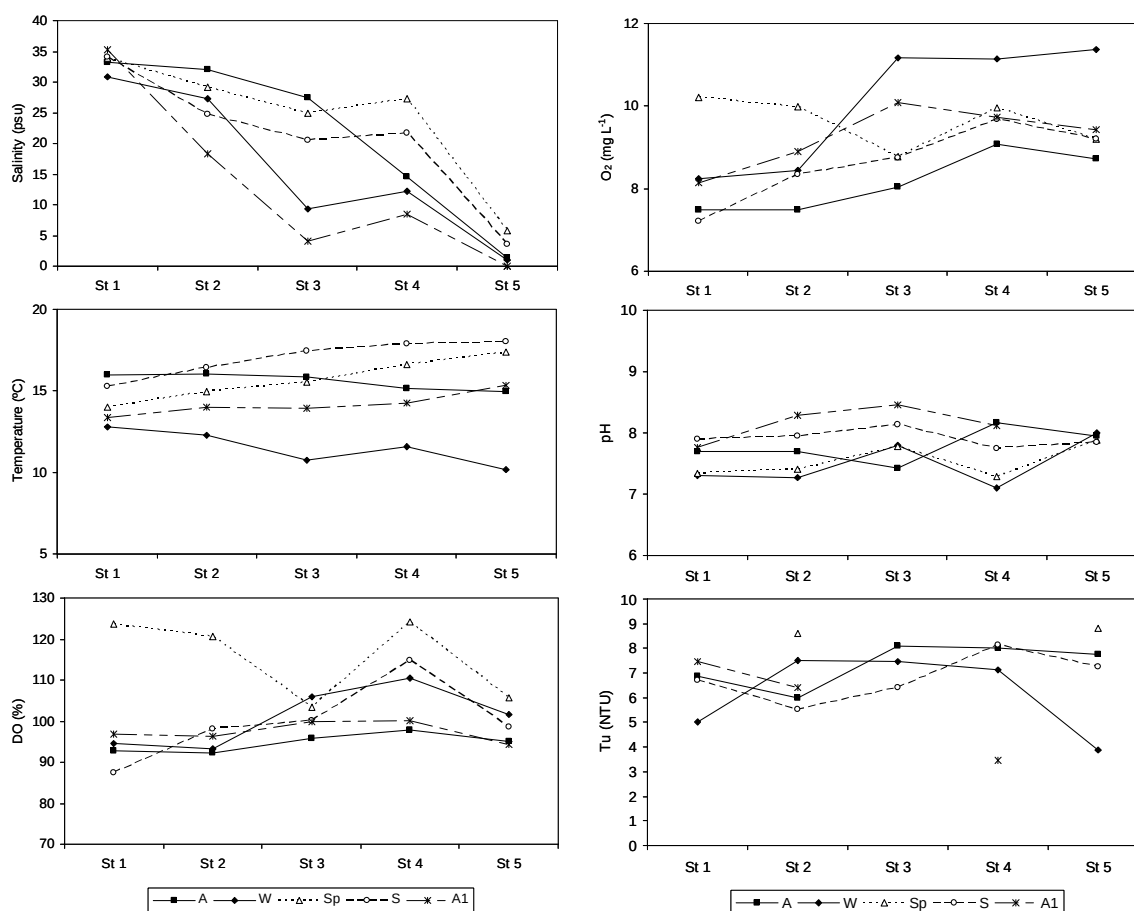


Figure 5.2. Seasonal distribution of the temperature (°C), salinity (psu), oxygen saturation (%) and concentration (mg L⁻¹), pH and turbidity (NTU) at the five sampling stations of the Lima estuary. A- autumn 2003; W- winter 2003/ 2004; Sp- spring 2004; S- summer 2004; A1- autumn 2004.

Temperature ranged from 8.4 - 21.7 °C, with an average of 14.7 °C. As expected, during the winter, temperature significantly decreased in all study areas ($F=22.3$ $P<0.001$), but mainly in the upper estuary due to the pronounced river influence. During spring-summer there was a thermal inversion of the gradient and temperature started to increase with distance from the river mouth due to the inflow of low salinity warmer water (Figure 5.2). Dissolved oxygen saturation was on average 100% (9.2 mg L⁻¹), excepting during Sp when supersaturation was observed, namely at Station 1 (Figure 5.2), probably due to phytoplankton blooms. Concentration of dissolved oxygen varied significantly throughout the study period ($F=4.8$ $P<0.01$), being higher in W and lower in S, as expected. An average of 7.7 pH was observed in the bottom layer of the Lima estuary, with a significant increase during A1 ($F=8.7$ $P<0.001$), in the majority of the areas, except Station 5 (Figure 5.2). During the study period, the bottom layer of the estuary was rather clear, with

an average turbidity of 6.5 NTU, ranging 1 to 10 NTU (Figure 5.2). Transparency of the water was spatially stable at the majority of the sampling stations and showed slight temporal variations.

Sediment in all sampling stations was mainly composed by fine sand, especially in the lower estuary (Station 1 and Station 2) (Table 5.1). However, upstream stations 4 and 5 exhibited higher percentage of silt and clay and also gravel compared to the other areas probably due to the geomorphology of the area.

Table 5.1. Sediment composition of the Lima estuary at the five sampling stations.

Zone	Station	Sediment (%)			
		Gravel	Coarse sand	Fine sand	Silt and clay
Lower	1	1.1%	11.0%	87.8%	0.1%
	2	0.2%	5.1%	94.3%	0.4%
Middle	3	0.2%	5.6%	93.8%	0.4%
	4	6.1%	14.6%	74.8%	4.5%
Upper	5	10.0%	20.0%	59.4%	10.7%

5.3.2 Temporal and spatial trends of benthic fishes

A total of 66 tows were performed in the Lima estuary throughout the study period, yielding 9,732 fishes of 29 species belonging to 17 families of teleosts (Table 5.2). *Pomatoschistus microps* and *P. minutus* were the most abundant species, representing 59% and 36% of the Lima benthic fish assemblage, respectively. Considering the number of species, the Lima estuarine benthic fish assemblages were composed of 41% estuarine resident species, 21% marine juvenile resident species, 17% marine adventitious species, and 7% marine seasonal species and 3% catadromous species. Estuarine residents (ER) dominated numerically (i.e. represented 97.3% of the total catch) and also in the number of species, comprising 12 of the 29 teleost species observed in the Lima estuary (Table 5.2).

This group of species (ER) was captured in all areas and at all surveys and was significantly less abundant at Station 1 ($F= 17.3$ $P<0.01$) (Figure 5.3). All resident species catches included juveniles and adults (Table 5.2). Marine species were captured in lower numbers, accounting for 2.3% of the total catch and included five adventitious visitors (MA-1.7%), six juvenile migrant residents (MJ-0.5%) and two seasonal migrant (MS-0.1%) (Table 5.2). Marine adventitious species (MA) did not vary significantly throughout the study period ($F=0.8$ $P>0.53$) and were significantly more abundant in the lower estuary

(Stations 1 and 2) ($F=12.1$ $P<0.01$) (Figure 5.3). Those species was composed by high percentage of juveniles. Marine juveniles (MJ) did not show significant differences between seasons ($F=0.2$ $P>0.96$), or stations ($F=2.0$ $P>0.20$) (Figure 5.3), and were composed by 100% juveniles.

Table 5.2. The Lima estuarine benthic fish assemblage. CCA codes, ecological guild classification (EG), total number of fishes (No. fishes), mean abundance (A), frequency of occurrence (FO) and total estuarine abundance (A) (Fishes 1000 m⁻²). Total length (TL) and size at first maturation (SFM) and respective literature reference (Source) considered to the percentage of juveniles calculation (Juv. %). *not classified due to the lack of information on their life cycle.

Family	Species	CCA codes	EG	No. fishes	A	FO	TL (mm)		SFM (mm)	Source	% Juv
							Min	Max			
Gobiidae	<i>Pomatoschistus microps</i>	Pmicro	ER	5,744	45,279.9	76%	10	50	35	Arruda et al. (1993)	63%
Gobiidae	<i>Pomatoschistus minutus</i>	Pmin	ER	3,486	27,021.2	88%	15	85	50	Arruda et al. (1993)	57%
Gobiidae	<i>Aphia minuta</i>	Amin	ER	76	570.4	14%	15	65	42	Árias and Drake (1990)	25%
Gobiidae	<i>Pomatoschistus pictus</i>	Ppict	MA	68	514.1	20%	20	45	-	-	-
Callionymidae	<i>Callionymus lyra</i>	Clyr	MA	55	437.0	21%	35	200	122	King et al. (1994)	95%
Gobiidae	<i>Gobius niger</i>	Gnig	ER	35	258.3	26%	30	130	60	Arruda et al. (1993)	12%
Gobiidae	<i>Gobius paganellus</i>	Gpaga	ER	29	216.8	17%	30	125	70	Santos et al. (1998)	79%
Trachinidae	<i>Echiichthys vipera</i>	Evip	MA	28	211.1	14%	20	130	95	Creutzberg and Witte (1989)	89%
Syngnathidae	<i>Syngnathus acus</i>	Sacus	ER	27	194.3	20%	20	195	-	-	-
Ammodontidae	<i>Ammodontes tobianus</i>	Ato	ER	22	155.6	6%	55	135	130	Dardenne (1975)	5%
Gobiidae	<i>Pomatoschistus sp. a</i>	Pom a	-	19	140.7	5%	20	40	30	-	-
Soleidae	<i>Solea solea</i>	Ssol	MJ	16	125.2	18%	20	180	320	Diniz (1986)	100%
Bothidae	<i>Arnoglossus laterna</i>	Alat	MA	15	109.6	15%	50	145	109	Deniel (1984)	60%
Soleidae	<i>Solea senegalensis</i>	Ssen	MJ	17	107.4	17%	15	205	300	Diniz (1986)	100%
Triglidae	<i>Trigla lucerna</i>	Tluc	MJ	13	96.3	8%	25	65	378	Baron (1985)	100%
Gobiidae	<i>Pomatoschistus sp. b</i>	Pom b	-	12	94.8	6%	20	65	-	-	-
Labridae	<i>Symphodus melops</i>	Smel	ER	12	90.4	9%	30	55	100	Árias and Drake (1990)	100%
Pleuronectidae	<i>Platichthys flesus</i>	Pfle	ER	11	85.2	12%	75	325	200	Diniz (1986)	73%
Syngnathidae	<i>Syngnathus abaster</i>	Saba	ER	11	63.3	8%	60	115	-	-	-
Lotidae	<i>Ciliata mustela</i>	Cmust	MS	7	51.9	8%	35	125	-	-	-
Syngnathidae	<i>Nerophis ophidion</i>	Noph	ER	6	45.9	6%	75	340	-	-	-
Anguillidae	<i>Anguilla anguilla</i>	Aang	CA	5	37.0	6%	135	255	600	Beverton and Holt (1959)	100%
Syngnathidae	<i>Nerophis lumbriciformes</i>	Nlum	ER	5	31.1	5%	75	115	-	-	-
Sparidae	<i>Diplodus sargus</i>	Dsar	MJ	4	29.6	3%	50	75	173	Erzini et al. (2001)	100%
Scophthalmidae	<i>Scophthalmus rhombus</i>	Srho	MJ	3	23.7	5%	25	210	310	Quéro et al. (1984)	100%
Mugilidae	<i>Liza aurata</i>	Laur	MS	2	14.8	2%	35	40	305	Campillo, A. (1992)	100%
Labridae	<i>Labrus bergylta</i>	Lber	MA	2	14.8	3%	80	-	152	Árias and Drake (1990)	100%
Bleniidae	<i>Parablennius gattorugine</i>	Pgato	*	1	8.9	2%	80	-	-	-	-
Atherinidae	<i>Atherina presbyter</i>	Apres	MJ	1	7.4	2%	65	-	65	Árias and Drake (1990)	100%
TOTAL				9,732	76,055.36						

The overall abundance of the Lima estuarine benthic fishes ranged between 15 and 6,622 fishes 1000 m⁻², with a mean of $1,130 \pm 1,262$ fishes 1000 m⁻². Abundance did not vary significantly between seasons ($F=2.24$ $P>0.08$), however maximum values were observed during W and the minimum during S (Table 5.3). Abundance varied significantly among stations ($F=22.7$ $P<0.01$), being significantly lower at Station 1 (Table 5.3).

Diversity varied between 0 and 1.9 H', with an overall mean of 0.7 and did not exhibited significant differences between seasons ($F=0.2$ $P>2.4$), or sites ($F=0.2$ $P>1.2$). Nevertheless, benthic fish assemblages were more diverse during the winter months and a general trend of an upstream decrease of the assemblages diversity was observed

(Table 5.3). In general, diversity increased in the lower estuary, being Station 1 the most diverse ($H' = 1 \pm 0.62$), but Station 2 ($H' = 0.8$) exhibited the maximum number of species recorded (24).

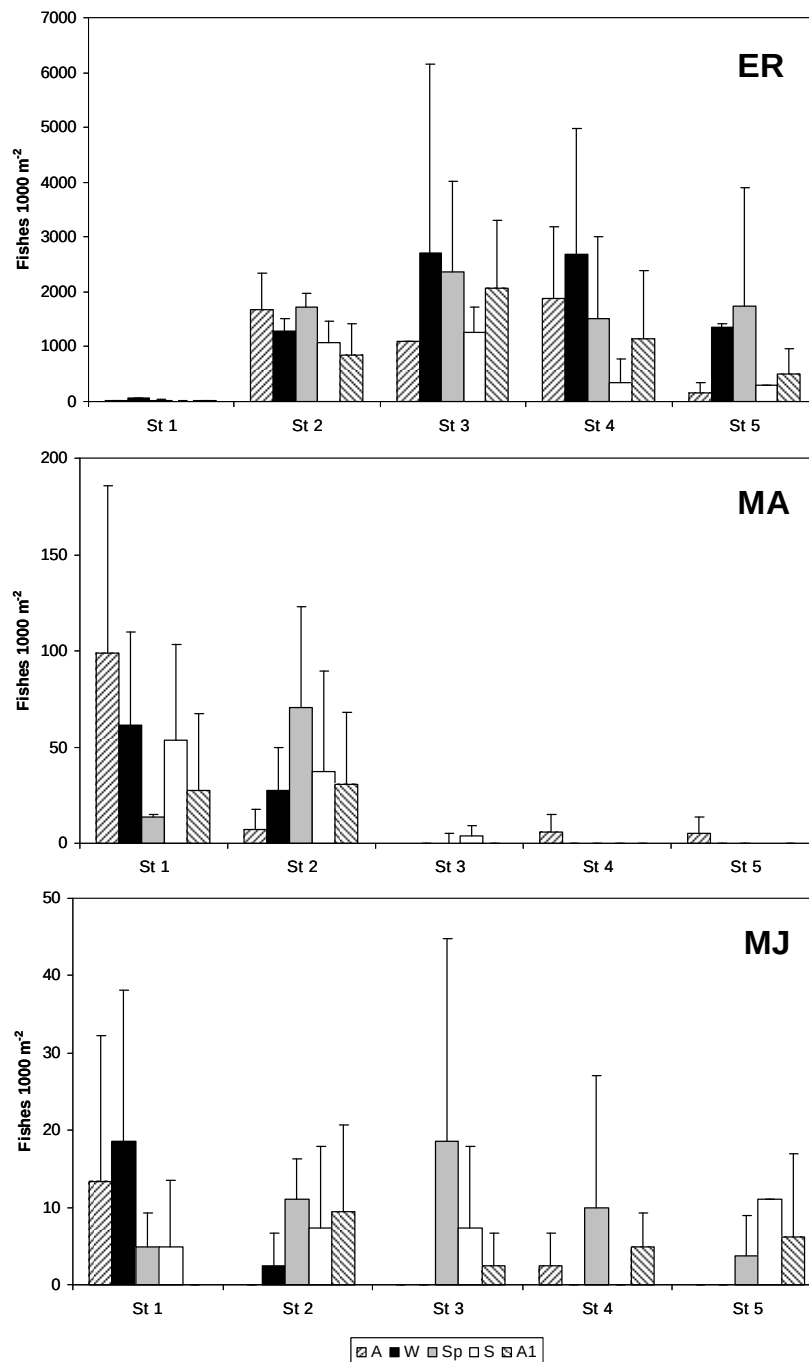


Figure 5.3. Seasonal (A- autumn 2003; W- winter 2003/2004; Sp- spring 2004; S- summer 2004; A1- autumn 2004) mean abundance of estuarine residents (ER), marine adventitious species (MA) and marine juveniles (MJ) captured in Lima estuary between October 2003 and November 2004, at 5 sampling stations (St1, St2, St3, St4 and St5).

According to ANOSIM results, the structure of the assemblages was temporally constant, without significant differences between seasons ($R = 0.01$ $P > 0.419$). Nevertheless, species composition varied between sampling stations, being Station 1 significantly different from the remaining areas ($R = 0.3$ $P < 0.01$).

Table 5.3. Average seasonal abundance (fishes 1000 m⁻²), Shannon-Wiener index (H') and number of species captured in Lima estuary between October 2003 and November 2004, at 5 sampling stations.

		Abundance (1000 m ²)	Number of species	Shannon-Wiener (H')
Station 1	A	147.4 ± 156.1	6	0.9 ± 1.3
	W	127.8 ± 48.9	5	1.3 ± 0.2
	Sp	54.9 ± 14.5	4	1.1 ± 0.6
	S	66.7 ± 71.4	3	0.6 ± 0.8
	A ₁	44.4 ± 31.4	3	0.8 ± 0.1
Station 2	A	1692.6 ± 654.7	5	0.7 ± 0.1
	W	1367.9 ± 322.9	9	1.1 ± 0.2
	Sp	1792.6 ± 188.6	8	0.9 ± 0.1
	S	859.3 ± 31.4	7	1.1 ± 0.5
	A ₁	884.0 ± 574.1	5	0.4 ± 0.3
Station 3	A	1111.1	2	0.6
	W	2718.5 ± 3427.7	3	0.7 ± 0.3
	Sp	2377.8 ± 1676.1	5	0.7 ± 0.1
	S	1096.3 ± 471.4	6	0.7 ± 0.3
	A ₁	1721.0 ± 1252.6	4	0.7 ± 0.1
Station 4	A	2622.2 ± 1202.2	4	0.2 ± 0.3
	W	2686.6 ± 2287.1	3	0.6 ± 0.2
	Sp	1538.3 ± 1512.7	4	0.7 ± 0.2
	S	344.4 ± 445.2	4	0.6 ± 0.1
	A ₁	1153.1 ± 1230.6	4	0.5 ± 0.3
Station 5	A	459.3 ± 175.5	6	0.5 ± 0.3
	W	1500.0 ± 73.1	3	0.5 ± 0.4
	Sp	970.4 ± 2147.5	5	0.5 ± 0.4
	S	1244.4	1	0.0
	A ₁	1656.8 ± 445.4	4	0.5 ± 0.1

According to SIMPER results, variations in abundance of the two most abundant species *P. microps* and *P. minutus* contributed in excess of 50% to the total dissimilarity between assemblages of Station 1 and assemblages of the middle and upper estuary (Stations 3, 4

and 5). The differences between the two stations of the lower estuary, namely Stations 1 and 2 were due to variations on *P. minutus* (33%) and *Echiichthys vipera* (17%) abundances.

5.3.3 Relationship between fish assemblages and environmental

From the original set of 16 environmental variables, fine sand and natural shorelines were eliminated from the model due to multicollinearity problems (Inflation factor > 20). The effect of environmental variables on explained distribution of the CCA axes was significant (Table 5.4). Since the first two CCA axes accounted for 70% of the cumulative variance in species-environment relation modelled by CCA (Table 5.4), the latter two CCA axes (CCA3 and CCA4) were not interpreted further.

According to the inter-set correlations, salinity, distance from the river mouth, gravel and silt and clay sediment fractions and the qualitative habitat characteristics (saltmarsh, intertidal, shallow) and dredging activity were considered as biologically important (Table 5.5). Samples clustered according to their sampling station and each cluster was separated along the two first CCA axes. The first CCA axis, which modelled 49% of the total explained variance and demonstrated a high species–environment correlation (0.88) (Table 5.4), was positively correlated with salinity and dredging activity and negatively correlated with shallow saltmarsh areas with higher percentage of silt and clay in the sediments and distance from the river mouth (Table 5.5). This axis represented the spatial downstream-upstream salinity gradient, separating three major groups of samples (Figure 5.4). Station 1 samples grouped in the right side of the plot, were characterized by high salinity values and a marine species *E. vipera*. Middle and upper estuary samples (Stations 3, 4 and 5), clustered on the left side of CCA1, correlated with low salinity and representing shallow saltmarsh areas with higher percentage of silt and clay sediments. These stations were separated along CCA2, which modelled 20% of the total explained variance (Table 5.4), and was positively correlated with gravel sediments, distance from the river mouth and intertidal areas (Table 5.5). Station 2 samples clustered in between those two groups, associated with intermediate values of salinity and distance from the river mouth. Two species were associated with this station, *Nerophis lumbriciformes* and *Diplodus sargus*, an estuarine resident and marine species, respectively. Species were separated along the first two canonical axes in accordance with their ecological guild (Figure 5.4). In general, marine species tended to cluster in the positive part of the CCA1,

associated to Stations 1 and 2. Conversely, estuarine resident species were associated with shallow and inner areas of the estuary (Stations 3, 4 and 5) (Figure 5.4).

Table 5.4. Results of the CCA based on the density-standardized, log-transformed, occurrence of 24 taxa in 66 collections from the Lima River estuary.

Axes	1	2	3	4
Eigenvalues	0.36	0.15	0.06	0.05
Species-environment correlations	0.88	0.80	0.57	0.67
Cumulative percentage variance				
of species data	14.4	20.5	22.8	24.8
of species-environment relation	48.9	69.4	77.2	83.8
Sum of all unconstrained Eigenvalues (Total inertia)				2.466
Sum of all canonical Eigenvalues				0.728
<i>Summary of Monte Carlo test</i>				
Test of significance of first canonical axis	Eigenvalue		0.728	
	F-ratio		1.851	
	P-value		0.002	

Since *P. microps* and *P. minutus* were major components of the fish assemblage (see above), a further detailed analysis was performed. A total of 5,744 *P. microps* were collected in the Lima estuary, with a mean abundance of 686.9 ± 1024.3 fishes 1000 m^{-2} . *P. microps* was captured throughout the study period but abundance was significantly

Table 5.5. Inter-set correlations of environmental variables with CCA axes, based on the density-standardized, log-transformed, occurrence of 24 taxa in 66 collections from the Lima River estuary. * inter-set $\geq |0.4|$ corresponding to biologically important variables.

Environmental variables	CCA code	CCA ₁	CCA ₂
Temperature (°C)	T	0.07	0.09
Salinity (psu)	S	0.47*	-0.28
Oxygen saturation (%)	DO	-0.14	0.02
Oxygen concentration (mg L^{-1})	O2	-0.29	0.14
pH	pH	-0.17	0.11
Turbidity (NTU)	Tu	-0.09	0.16
River flow ($\text{m}^3 \text{ s}^{-1}$)	RF	-0.08	-0.14
% Gravel	Gr	-0.35	0.42*
% Coarse Sand	CS	0.11	-0.31
% silt and clay	SC	-0.43*	0.29
Distance from the river mouth (km)	D	-0.43*	0.59*
Salt marsh	SM	-0.43*	0.26
Intertidal	IN	-0.38	0.53*
Shallow	SH	-0.40*	-0.18
Dredging	DG	0.83*	0.17

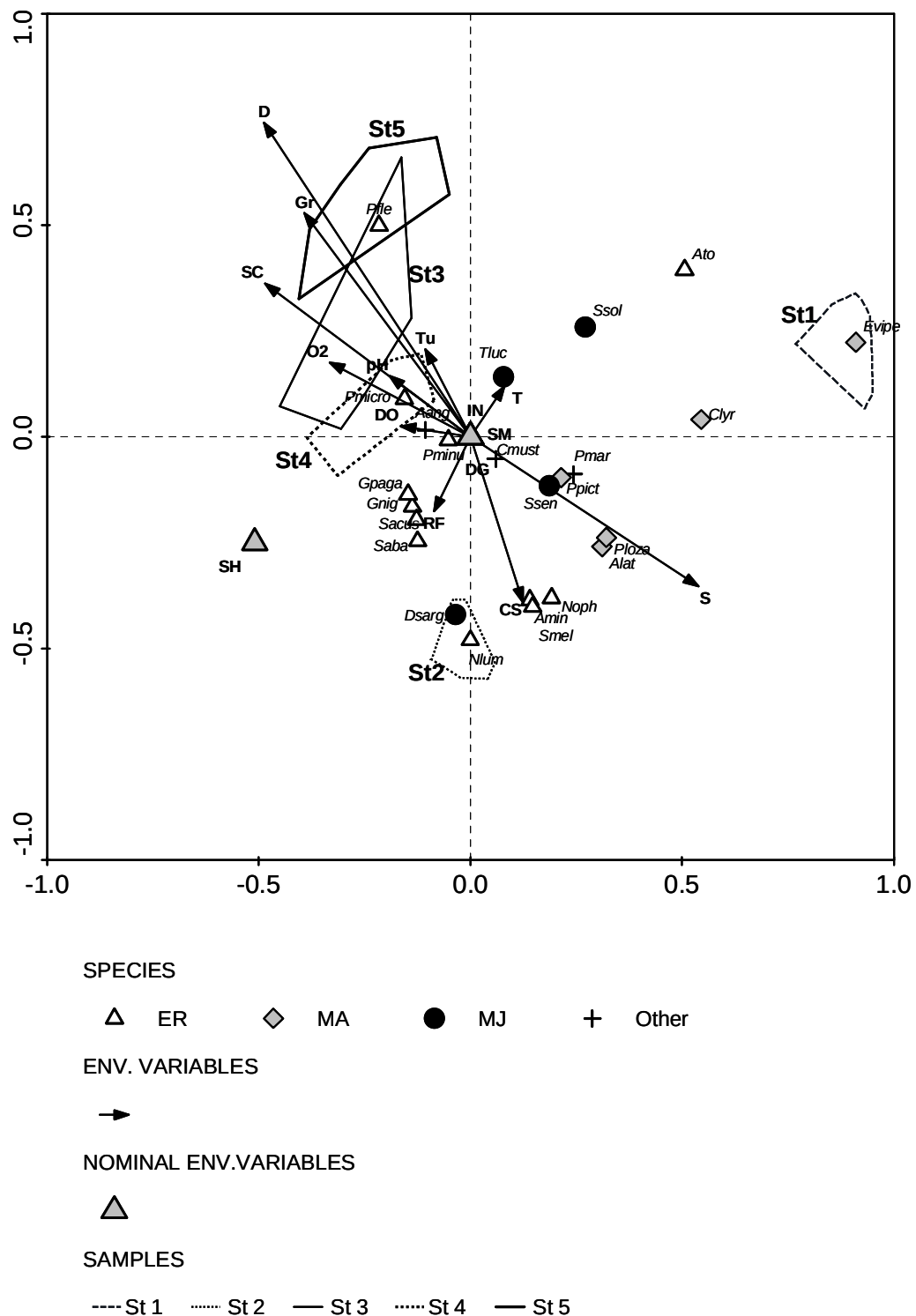


Figure 5.4. Canonical correspondence analysis of the 24 most abundant species of fish assemblages in Lima estuary- scores of environmental variables, samples and species in the plan of the first two axes of the CCA ordination (see Table 5.2 for species codes and Tables 5 and 7 for environmental variables codes-arrows). St1- Station 1; St2- Station 2; St3- Station 3 ; St4- Station 4; St5- Station 5); ER- estuarine resident species; MA- marine adventitious visitors; MJ- marine juvenile species.

lower at Station 1 ($F= 25.6$ $P<0.01$), close to the coastal zone, in general one order of magnitude lower than in other sampling stations (Figure 5.5). Highest abundances were observed at Stations 3 and 4 during the winter months, while an abundance peak occurred at the upstream station during the spring months. Abundance generally decreased during the summer months, as salinity increased as well as water temperature.

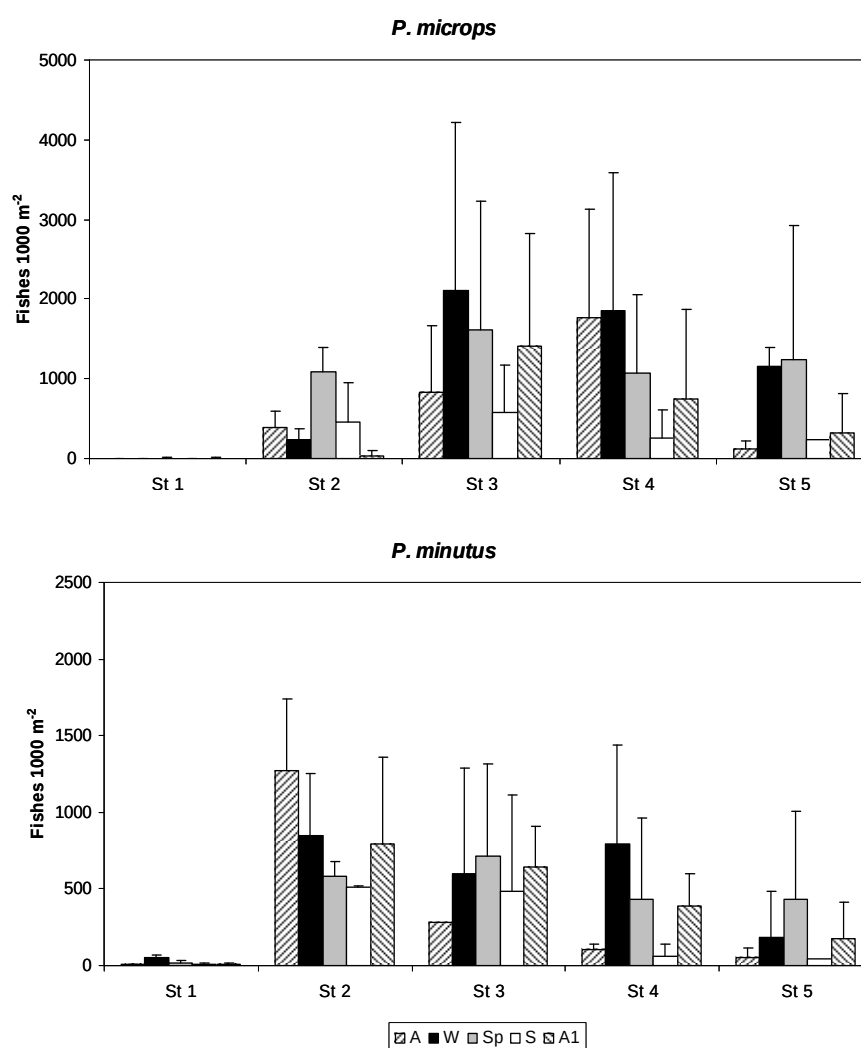


Figure 5.5. Mean seasonal abundance (standard deviation) of *Pomatoschistus microps* and *P. minutus* at five sampling stations of Lima estuary (St1, St2, St3, St4 and St5). A-autumn 2003; W-winter 2003/ 2004; Sp-spring 2004; S- summer 2004; A₁- autumn 2004.

Total length of *P. microps* varied between 10 and 50 mm and the majority of these gobies captured was juvenile (57%). The temporal distribution of the 1 mm length classes showed that juveniles (TL < 35 mm) were present in all sampling stations, especially in the middle estuary where highest abundances were noticed during the winter months (Figure

5.6). Small individuals (LT < 15 mm) were not very abundant, reaching the highest abundances at Station 4 during A1 (Figure 5.6). Older gobies (LT > 45 mm) were absent from Station 1 but peaked at Station 4, during autumn.

P. minutus was the most frequently caught species (Table 5.2), with a mean abundance of 409.4 ± 462.2 fishes 1000 m^{-2} . Abundance of this species did not vary between seasons ($F= 1.3$ $P>0.3$), however it was significantly higher at Station 2 ($F= 17.0$ $P<0.01$) (Figure 5.5). At each sampling station, abundance peaked at different seasons. For example, at Station 2 it occurred during A, while at Stations 3 and 5 it occurred during Sp and at Station 4 during W (Figure 5.5). Total length varied between 15 and 85 mm and 64% of gobies caught were juveniles. The temporal distribution of length classes showed that juveniles (TL < 50 mm) were captured at all sampling stations, but maximum abundances were observed at Station 3 during summer months (Figure 5.6). At Station 1, *P. minutus* abundances were one order of magnitude smaller than in the other stations. Small individuals (TL < 20 mm) were rare, only observed in A1 at Station 2 (Figure 5.6). Older gobies (TL > 80 mm) were not abundant and mainly observed during winter months (Figure 5.6).

In order to assess the influence of these two dominant species on the temporal and spatial patterns of the fish assemblages, all the previous analyses were performed without *P. microps* and *P. minutus* data. With the removal of the two dominant species, total abundance ($F= 1.8$ $P> 0.1$), Shannon Wiener index ($F= 0.2$ $P>0.9$) and assemblage structure ($R = 0.1$ $P>0.2$) showed no significant differences between seasons. However, on a spatial basis, fish assemblages at Station 2 became significantly more abundant ($F= 4.3$ $P< 0.01$), and diverse ($F= 7.1$ $P< 0.01$), than at the remaining locations. Moreover, ANOSIM results showed that without the dominance of *P. microps* and *P. minutus*, species composition at Station 2 differed significantly from others ($R=0.4$ $P<0.01$), mainly due to differences on abundance of *Aphia minuta*.

The weight of *P. microps* and *P. minutus* for the CCA ordination was high as their removal produced a different ordination diagram. Furthermore, CCA1 only explained 29.6 % of variance and had a high species–environment correlation (0.9), which was positively correlated with areas with dredging activity. On the other hand, CCA2 explained 25.2% of variance and was positively correlated with dissolved oxygen concentration, gravel and silt and clay sediments, distance from river mouth and saltmarsh and intertidal areas, but negatively with dredging, a disturbing factor (Tables 5.6 and 5.7). With the deletion of the

two resident species, Station 1 samples were less isolated from the remaining samples that clustered along the upstream – downstream salinity gradient that was shifted to CCA2 (Figure 5.7). Species continued to be ordinated according to their ecological guild, but in general tended to be closer to the centre of the plot. Marine species (MA and MJ) were separated along the CCA2. Furthermore, the majority of estuarine residents clustered in the negative part of CCA1 and were well separated along the upstream – downstream gradient.

Table 5.6. Results of the CCA based on the density-standardized, log-transformed, occurrence of 22 taxa in 66 collections from the Lima River estuary.

Axes	1	2	3	4
Eigenvalues	0.47	0.40	0.20	0.15
Species-environment correlations	0.85	0.81	0.76	0.66
Cumulative percentage variance				
of species data	7.9	14.7	17.9	20.5
of species-environment relation	29.6	54.8	67.2	76.7
Sum of all unconstrained Eigenvalues (Total inertia)				5.986
Sum of all canonical Eigenvalues				1.599
<i>Summary of Monte Carlo test</i>				
Test of significance of first canonical axis	Eigenvalue		1.599	
	F-ratio		1.419	
	P-value		0.002	

5.4 Discussion

Estuarine fish communities have been characterized by assemblages containing a few species of very high abundances and a large number of species with low abundances (Drake and Arias 1991; Whitfield 1999; Barletta et al. 2005), and the Lima estuary followed this pattern. The Lima estuarine benthic fish fauna was composed by two highly abundant estuarine species (*P. microps* and *P. minutus*) that represented 95% of the total catches and 27 fairly less abundant species (including estuarine and marine species). The high dominance of those species inflicted the obtained low diversity of the benthic fish assemblage (mean $H' = 0.7$). In fact, diversity was higher at Station1, where those two gobies were less abundant (Table 5.3 and Figure 5.5). The species richness (29 teleost species) was lower when compared to other North Atlantic temperate estuaries (Rebelo 1992; Elliott and Dewailly 1995; Mathieson et al. 2000; Thiel et al. 2003), what can be associated with overall reduce size of the Lima estuary, in comparison with the other transitional environments. According to Wootton (1990), the diversity of fish species within

an area is a function of the number of available niches and partly the area size. In fact, recent studies demonstrated that small Portuguese estuaries such as Mondego (40 °N) (Leitão et al. 2006) and Guadiana (37°N) (Chícharo et al. 2006), exhibited similar low estuarine species richness. In terms of ecological guilds, Lima estuarine benthic fish assemblages exhibited a higher percentage of estuarine resident species (45%) than expected for temperate European estuaries (24%, maximum 33%) (Elliott and Dewailly 1995). The heterogeneity of habitats available in the Lima estuary (saltmarsh, intertidal and subtidal areas, small creeks, artificial bays), leads to a greater number of niches that is associated to the abundant and diverse macroinvertebrate preys communities (Sousa et al. 2006). This allows a higher number of species to cohabit thus producing the higher percentage of estuarine resident species found.

Table 5.7. Inter-set correlations of environmental variables with CCA axes, based on the density-standardized, log-transformed, occurrence of 22 taxa in 66 collections from the Lima River estuary. * inter-set $\geq |0.4|$ corresponding to biologically important variables.

Environmental variables	CCA code	CCA ₁	CCA ₂
Temperature (°C)	T	0.11	0.12
Salinity (psu)	S	0.11	-0.58*
Oxygen saturation (%)	DO	-0.21	0.04
Oxygen concentration (mg L ⁻¹)	O2	-0.16	0.45*
pH	pH	-0.05	0.20
Turbidity (NTU)	Tu	0.11	0.24
River flow (m ³ s ⁻¹)	RF	-0.15	-0.04
% Gravel	Gr	0.15	0.49*
% Coarse Sand	CS	-0.27	-0.33
% silt and clay	SC	-0.16	0.43*
Distance from the river mouth (km)	D	0.25	0.70*
Salt marsh	SM	-0.23	0.45*
Intertidal	IN	0.18	0.63*
Shallow	SH	-0.37	0.30
Dredging	DG	0.58*	-0.47*

In various European estuaries, freshwater species constitute a reduced percentage of the estuarine fish fauna or are even absent from the benthic estuarine fish assemblage. (Elliott and Dewailly 1995; Mathieson et al. 2000; Thiel et al. 2003). During the study period, no freshwater species was observed at all, even in the most upstream sampling station where salinity was within the oligohaline range. However, due to the sampling strategy, the further upstream freshwater region was not covered. *Petromyzon marinus* constitute an important seasonal fishery in the Lima estuary. This diadromous species is more likely to be found in the water column than on the bed of the estuary, not being captured by the beam trawl and therefore absent from the present study. The sampling

net is also responsible for the absence of other pelagic species like mullets and seabass (Antunes and Dias 2005).

Benthic fish assemblages were numerically dominated by two resident goby species, *P. microps* and *P. minutus*, common components of other European estuaries (Hamerlynck and Cattrijsse 1994; Appleby 1996; Pampoulie et al. 1999). The two species peaked in autumn and winter months, a regular feature in other Portuguese estuarine habitats (Arruda et al. 1993; Leitão et al. 2006). During the study period, the two gobies tended to be spatially discrete along the salinity gradient, with *P. minutus* frequently abundant at the lower high salinity Station 2, while *P. microps* was associated with the middle brackish saltmarsh area (Figure 5.5). These results agree with other locations, where *P. microps* seems to be more common at brackish environments (Fonds 1973; Bouchereau et al. 1993). On the other hand, *P. minutus* is less tolerant to salinity and temperature variations, thus exhibiting more marine characteristics (Fonds and Van Buurt 1974; Pampoulie et al. 1999). The spatial separation can be interpreted as a survival strategy, creating resource partitioning and thus avoiding food or habitat competition. This was already observed in another estuary (Mondego estuary about 200 km to the south) and interpreted as a consequence of the similar feeding habits of sand and common gobies (Leitão et al. 2006).

The ecological importance of gobies in the estuarine ecosystems is especially related to their role as intermediate predators in the food-web connecting microbenthos with large predator fish (Raffaelli and Milne 1987; Raffaelli et al. 1989). Thus, gobies constitute an important group, reaching high abundances and comprising several species of estuarine fish fauna (e.g. Thorman and Wiederholm 1983; West and King 1996; Mathieson et al. 2000; Malavasi et al. 2004). The spatial distribution of the two most abundant species was a major cause of the isolation of the three different assemblages. At Station 1 *P. microps* and *P. minutus* were almost absent; *P. minutus* was more abundant at Station 2 and the remaining stations were dominated by *P. microps*. The absence of the two most abundant species at Station 1 was a factor for the isolation of this assemblage in the CCA ordination plot (Figures 5.4 and 5.7). Comparing the environmental parameters recognized as ecologically important by CCA between sampling stations, the overall higher salinity and presence of dredging activities were the unique aspects only observed at Station 1. Considering that *P. minutus* is a marine species, the high salinity would never be responsible for the reduce abundances at Station 1. The negative effect of dredging activities on benthic fauna (McLusky and Elliott 2004) particularly on small fishes such as

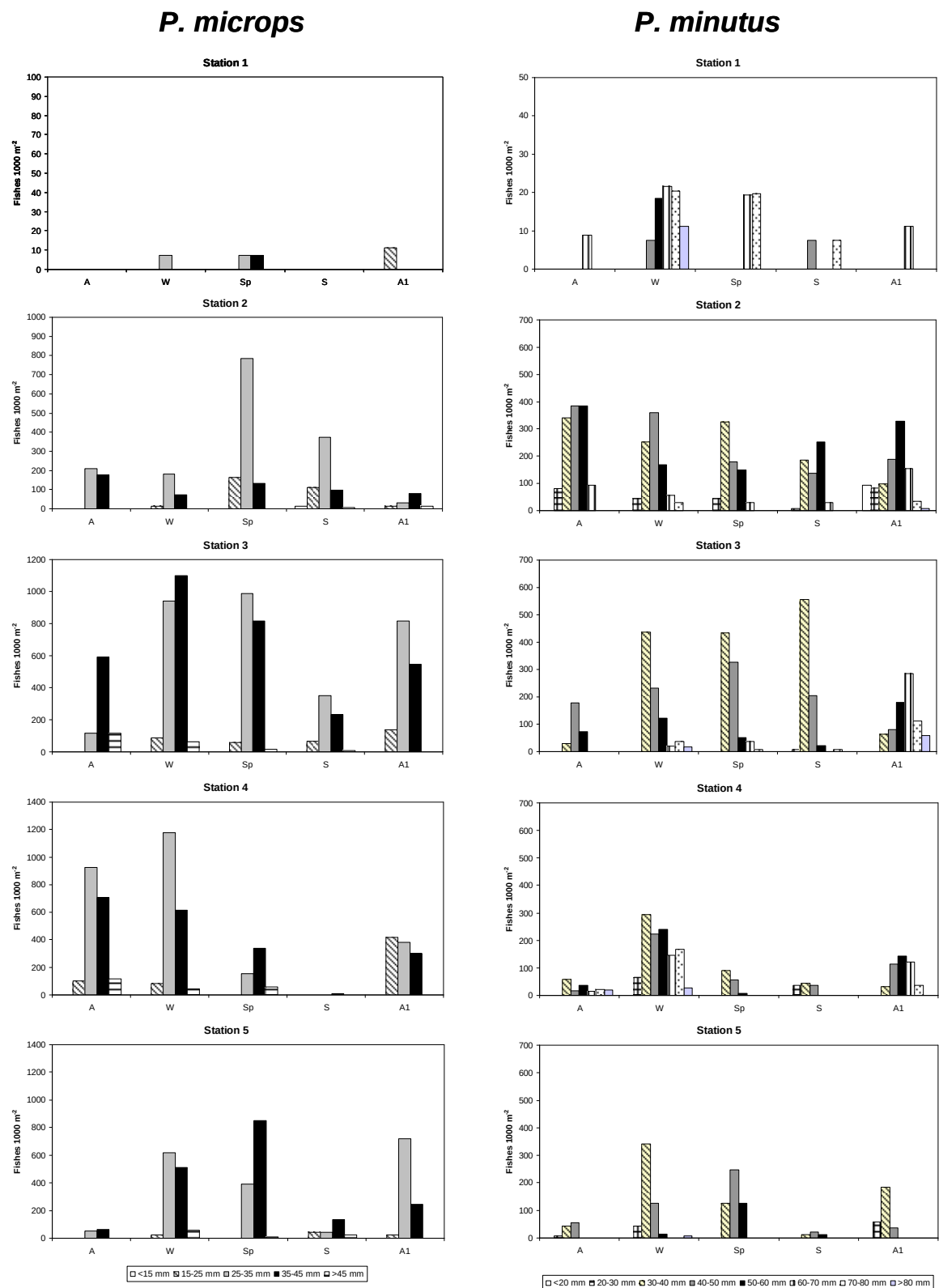


Figure 5.6. Seasonal variation of the length distributions (Total length mm) of *P. microps* and *P. minutus* at each sampling station.

gobies is easily understandable, especially dredging by suction, as in the case of the Lima estuary. In fact, this qualitative habitat characteristic was one of the most influential on the assemblage structure (Tables 5.5 and 5.7), and the two species were negatively correlated with it (Figure 5.4). Moreover, the anthropogenic stress observed at the lower and deeper estuary (Sousa et al. 2006) might be avoided by these two small benthic species. The absence of these two species at Station 1 revealed that they are ecological sensitive species, and thus may be indicative of the ecological status of the habitat, providing relevant information about the ecosystem status and health.

Contrary to several studies (Allen and Baltz 1997; Ellis et al. 2000; Attrill and Power 2004), seasonal environmental parameters such as temperature were not considered as biologically important for the environmental control of fish assemblages (Tables 5.5 and 5.7). All the univariate measures of the assemblages (i.e. total abundance and diversity), and also their structures did not appear to be sensitive to seasonal environmental fluctuations throughout the study. This apparent lack of seasonality might have been a consequence of the numerical dominance of *P. microps* and *P. minutus*. The presence year-round of the two gobies throughout the study period and their high abundances (Figure 5.5) could have masked the temporal variations of the total abundance and diversity of the assemblages. However, even after the removal of the two species, the density, diversity and also the assemblage structure did not show statistically robust seasonal differences. Moreover, temporal variations were not observed at an ecological guild level (Figure 5.3). In general, marine species (including MA and MJ) were captured throughout the study period, mainly in the lower estuary. At this polyhaline area, seasonality was little evident since water characteristics were temporal stable, revealing the strong influence of the adjacent coastal area. CCA analysis supported the observed slight seasonality, in that the lower estuarine samples clustered closer to each other, revealing little variation among them (Figures 5.4 and 5.7). This temporal stability might have favoured a constant use of lower estuary by marine species. Nevertheless, it is of note that seasonality could have been indicated at the species level and not on a larger scale such as species groups (e.g. ecological guild or total assemblage), as found. Due to the intrinsic specific rhythms (recruitment, migrations), the estuarine fish fauna varies seasonally, as a result of sequential immigration and emigration of certain fish species (Hyndes et al. 1999). However, in the Lima estuary the seasonal variation of each species did not led to temporal changes on the total assemblages, but rather to small seasonal variations in the abundance and composition of fish assemblages. On the other hand, and

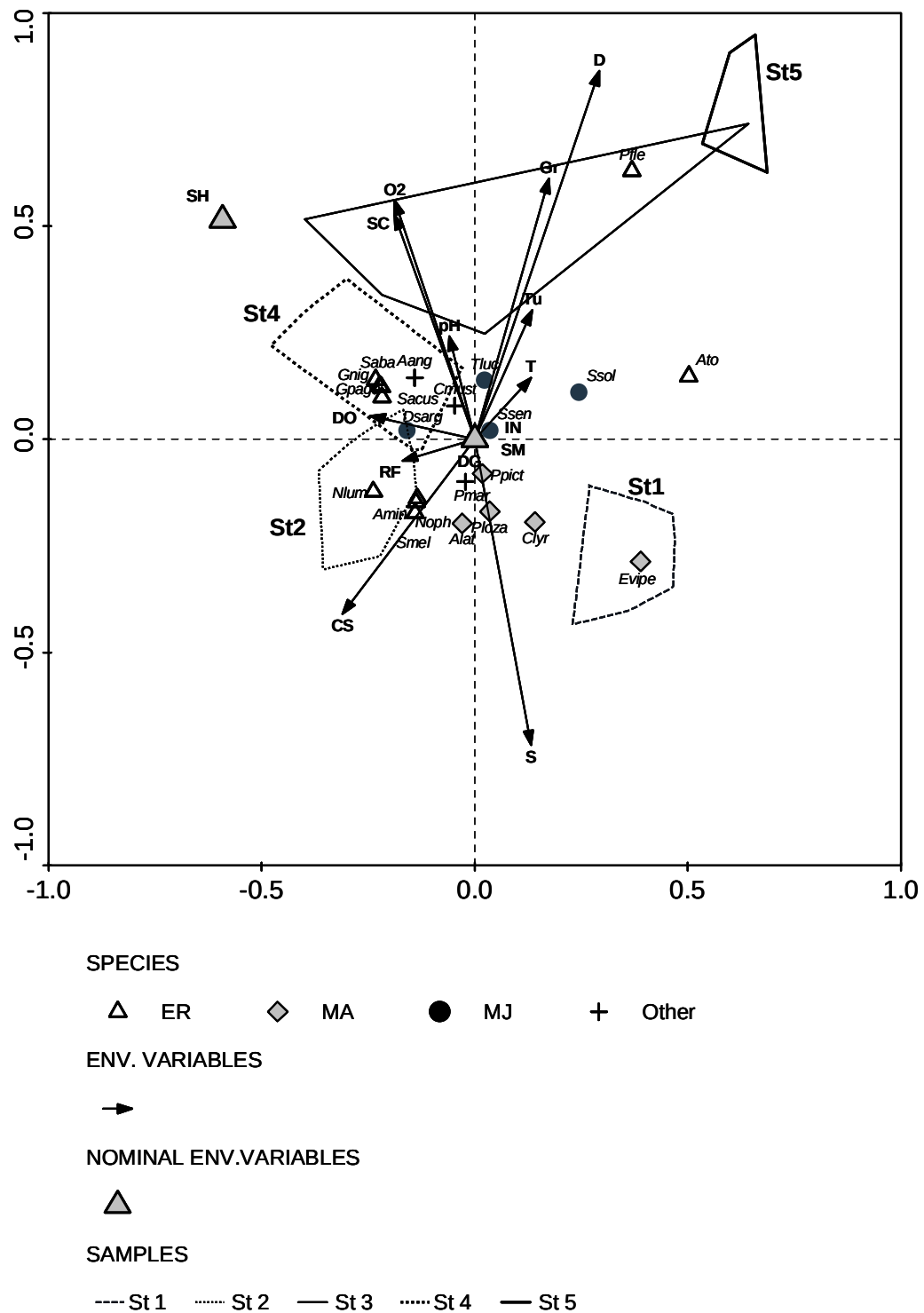


Figure 5.7. Canonical correspondence analysis of the 22 most abundant species (except *P. microps* and *P. minutus*) of fish assemblages in Lima estuary- scores of environmental variables, samples and species in the plan of the first two axes of the CCA ordination (see Table 5.2 for species codes and Tables 5 and 7 for environmental variables codes-arrows). St1- Station 1; St2- Station 2; St3- Station 3 ; St4- Station 4; St5- Station 5); ER- estuarine resident species; MA- marine adventitious visitors; MJ- marine juvenile species.

apart from the temporal differences at the species level, the seasonal scale was insufficient to observe temporal variability of the benthic fish assemblages, which might vary inter-yearly, as reported for other areas (Thiel and Potter 2001).

Fishes tend to select residence zones partially based on physicochemical factors and substratum characteristics (Araujo et al. 2000; Prista et al. 2003; Nobriga et al. 2005). In the Lima estuary, salinity emerged as biologically important by the CCA analysis (Tables 3 and 5). The importance of salinity as a controlling factor of the estuarine fish assemblages has been recognized in several other estuaries (e.g. Szedlmayer and Able 1996; Marshall and Elliott 1998; Wagner and Austin 1999; Power and Attrill 2002; Barletta et al. 2005). In the case of the Lima, the longitudinal salinity gradient could act as a physiological barrier for stenohaline marine and freshwater species. Thus, it is hypothesized that the large salinity fluctuations observed in the middle and upper sections of the estuary were responsible for the reduction of the number of species.

The composition of substratum has also been identified as an important factor for the establishment of communities directly associated with it, such as the macrobenthic invertebrates (Warwick et al. 1991; Meire et al. 1994; Bachelet et al. 1996; Nanami et al. 2005) and also benthic fishes (Cabral and Costa 1999; Wagner and Austin 1999; Nanami et al. 2005). In the Lima estuary, sediment composition was identified as a regulator of the associated macrobenthic community (Sousa et al. 2006). Considering that macrobenthic invertebrates are main food supply for benthic fishes, the influence of the sediment type on benthic fishes revealed by CCA results should be interpreted as an indirect relationship, especially as the type of sediment reflects variations in food availability.

In addition to salinity and sediment composition, the present study demonstrated that some qualitative habitat characteristics were key factors for the structuring of fish assemblages (Tables 5.5 and 5.7). In general, three benthic fish assemblages were identified along the study area. The euhaline urbanized Station 1 supported a diverse assemblage, mostly composed of marine species (MA and MJ), which were caught in reduced and relatively constant abundances throughout the study period (Figure 5.3). The low abundances and species richness of this station was similar to those encountered in the adjacent south coastal area (unpublished data). Thus, we can conclude that juveniles of marine species (e.g. *E. vipera* and *C. lyra*) used this area of the Lima estuary as an extension of their coastal habitat. The shallow artificial embayment supported the most diverse assemblage (in terms of number of species), comprising several estuarine

resident species and marine species (Figure 5.4). *P. minutus* dominated the catches and as shown by their length distribution, post-larvae (TL<20 mm) were only observed at this station (Figure 5.6), thus suggesting a spawning area for *P. minutus*. The temporal constancy of water parameters and absence of dredging seemed to attract a high number of marine species (e.g. *D. sargus*, *P. pictus*, *A. laterna*, *C. mustela*) and other estuarine resident species (e.g. *A. minuta*, *N. lumbriciformes*, *S. melops* and *N. ophidium*) to the shallow artificial polyhaline embayment. Despite the presence of different habitats, the middle and upper estuarine areas exhibited similar benthic fish assemblages (Figures 5.4 and 5.7). In the saltmarsh brackish middle and upper area, the fish assemblage was characterized by a high abundance of few estuarine resident species, such as *P. microps*, *P. minutus* and *P. flesus*. The temporal instability of water parameters, mainly salinity and temperature, limited the use of this area by other species, namely marine species. In fact, marine juveniles were only observed at that area during spring and summer months, when the saline intrusion extended upstream and temperature increased (Figure 5.2). Overall, the middle and upper stations were used by few but very abundant estuarine resident species.

All the estuarine resident species were captured as both juvenile and adult stages (Table 5.2), indicating their capability to complete their life cycle within the Lima estuary boundaries. Moreover, several of these species were also found as larvae in the Lima estuary (Ramos et al. 2006a), confirming their designation in the estuarine resident guild. The presence of a high number of juveniles indicates the nursery role of the habitats (Heck et al. 1989; Ayvazian et al. 1992; Gibson et al. 1993; Able et al. 1998; Lazzari et al. 2003). Following this approach, the present study highlights the function of Lima estuary as a nursery ground by marine species, such as *E. vipera*, *C. lyra*, *S. solea* and *S. senegalensis*. In an earlier study (Valente and Alexandrino 1988), reached the same conclusion although the study was restricted to a small tidal channel in the south bank. The present study reinforces the Lima estuary role as a nursery habitat for marine species, some of which have high economical importance such as flatfishes. Nevertheless, the seasonal stability of the fish assemblages needs to be confirmed in larger temporal scales, in order to assess the interannual variation on fish assemblages found in other estuaries (Thiel and Potter 2001).

The Lima estuary benthic fish fauna was composed by estuarine residents, distributed along a longitudinal salinity gradient, and by marine species, confined to the polyhaline stretch of the lower estuary. The assemblage was seasonally stable and heterogeneously distributed among the different types of habitats, according to salinity, distance to mouth,

sediments and qualitative habitats features. At the lower urbanized estuary, the benthic fish assemblage density was low and included several marine adventitious species. Several estuarine and marine juveniles were associated with a small constructed embayment. In the brackish shallow saltmarsh areas in the middle and upper estuary, the fish assemblage was almost entirely composed of estuarine residents with high abundances. The dominance of *P. microps* and *P. minutus* had a large influence on the spatial structure of fish assemblages, except near the river mouth, where these two species were a minor component of the assemblages, probably as a result of the anthropogenic stress of that area. Marine species were in low abundances, but several economically important species were captured only as juveniles, suggesting their use of Lima estuary as a nursery habitat.

CHAPTER 6

Is the Lima estuary (NW Iberian Peninsula) acting as a nursery habitat for flatfishes?

6.1 Introduction

Flatfishes exhibit a life-history strategy in which the egg and larval stages take up a pelagic lifestyle and the juveniles and adults are associated with the bottom. 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. The morphological transformation from a symmetrical pelagic larva to the ultimate bottom mimic is an unparalleled feature of ontogeny (Fuiman 1997). One of the most important features of flatfish population dynamics is the settlement of planktonic larvae on a suitable nursery habitat. Recruitment success depends on both survival and growth of recruits through the highly variable larval stages and during the juvenile phase spent in the nursery grounds (Van der Veer 1986; Beverton 1995;

The contents of this chapter can also be found in Ramos, S., Ré, P. and Bordalo, A. A. Is the Lima estuary (NW Iberian Peninsula) acting as a nursery habitat for flatfishes? Submitted to Estuarine Coastal and Shelf Science.

Andersen et al. 2005). Thus, nursery areas play a major role for the recruitment and determining overall population size (Gibson 1994; Rijnsdorp et al. 1995; Iles and Beverton 2000; Van der Veer et al. 2000; Walsh et al. 2004).

Several estuaries are described as flatfish nursery areas (Costa et al. 1994; Blaber et al. 1995; Able et al. 1998; Fujita et al. 2002; Ross 2003; Baker and Sheaves 2005), since these ecosystems provide physiologically suitable conditions for survival, such as abundant prey resources and/or reduced rates of predation, when compared to other coastal environments (Haedrich 1983; Boesch and Turner 1984; Elliott and Hemingway 2002). 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, namely *Pleuronectes platessa*, *Limanda limanda*, *Platichthys flesus* and *Solea solea* (Van der Veer and Bergman 1987; Hostens 2000; Power et al. 2000; Van der Veer et al. 2001; Gilliers et al. 2004). 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. 2003b; Desaunay et al. 2006; Gilliers et al. 2006). Some southern Iberian estuaries are also used as nursery grounds for *S. solea* and *S. senegalensis* (Costa and Bruxelas 1989; Drake and Arias 1991; Andrade 1992; Cabral 2000b; Cabral et al. 2002; Drake et al. 2002; Vinagre et al. 2006b). However, there is little information for the northern Iberian estuaries, where studies on habitat use by juvenile flatfishes are scarce (Vinagre et al. 2005; Cabral et al. 2007).

Along the northern Iberian margin, several estuaries support important local flatfish fisheries. The Lima estuary, located in the NW Atlantic Portuguese coast is one of those examples, where flatfishes are target of estuarine fisheries but there is no information on the potential nursery role of the estuary for flatfish species. In an earlier study, Valente and Alexandrino (1988) suggested that a salt marsh area of the middle southern estuarine bank acted as a nursery area for juveniles of some marine fishes. Presently, it is known that the Lima estuarine larval fish assemblages comprised several flatfish species, namely *S. senegalensis*, *S. solea* and *P. flesus* (Ramos et al. 2006a). The Senegal sole, *S. senegalensis*, is distributed from the Bay of Biscay to Senegal and Western Mediterranean (Quéro et al. 1986). Several aspects of their biology and ecology are still unknown and studies on this species are scarce and limited to the central and south of Portugal (e. g. Andrade 1992; Cabral and Costa 1999; Cabral 2000a; 2000b; Cabral 2003;

Vinagre et al. 2006a); and Mediterranean (Rodriguez and Rodriguez 1980; Ramos 1982; Bentuvia 1990; Borsa and Quignard 2001). On the other hand, *P. flesus* has been more intensively studied, mainly in the northern European coast (Kerstan 1991; Griocche et al. 1997; Marshall and Elliott 1998; Andersen et al. 2005). The present study aims to characterize for the first time the Lima estuarine flatfish community by 1) describing the temporal and spatial abundance patterns, 2) studying the recruitment of larval and juveniles flatfishes to the Lima estuary and 3) assess the potential nursery role of the Lima estuary for flatfishes.

6.2 Material and Methods

6.2.1 Study area

The Lima River is an international water body, draining into the Atlantic Ocean at 41° 40' N and 8° 50' W in the vicinity of the 32,000 inhabitant city of Viana do Castelo, northern Portugal. Its watershed covers 2, 446 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 0.4 m s⁻¹ and 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, when salinity sharply increases with depth. Eventually, a layer of fresh water is sometimes present at the surface, when river discharges increases. Temperature is vertically stratified, with cooler water typically found near the bottom of the water column, except during winter as a result of a thermal inversion (Ramos et al. 2006b). Due to the actual geomorphology of the system, the lower estuary is highly urbanized comprising a 10-m dredged and urbanized narrow channel, with hardened shorelines. The river mouth is partially obstructed by a 2 km long jetty, deflecting the river flow to the south (Figure 6.1). Middle estuary is a shallow saltmarsh zone, mainly colonized by *Juncus* spp., enclosing several sand islands and intertidal channels. Upper estuary is a narrow channel, with average depth and some tidal islands and natural banks.

6.2.2 Sampling strategy

Flatfishes hatch in pelagic larvae and after settlement juveniles undergo a benthic life habitat, which is maintain throughout the adult life. Therefore, 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. 2006a). Ichthyoplankton surveys were performed along the initial 7 km stretch of the Lima estuary, corresponding to lower and middle estuary. Subsurface circular tows were carried out during daytime, at a constant velocity of ca. 1 m s^{-1} for five minutes, with a conical 1 m diameter, 3 m long and 500 μm mesh size net. A flowmeter (Hydro-Bios) was attached to the net enabling the calculation of the volume of water filtered through the net. Flatfish larvae were separated from the remaining fish larvae and identified to the lowest possible *taxon*. Abundance data was standardized into number of larvae per 100 m^3 .

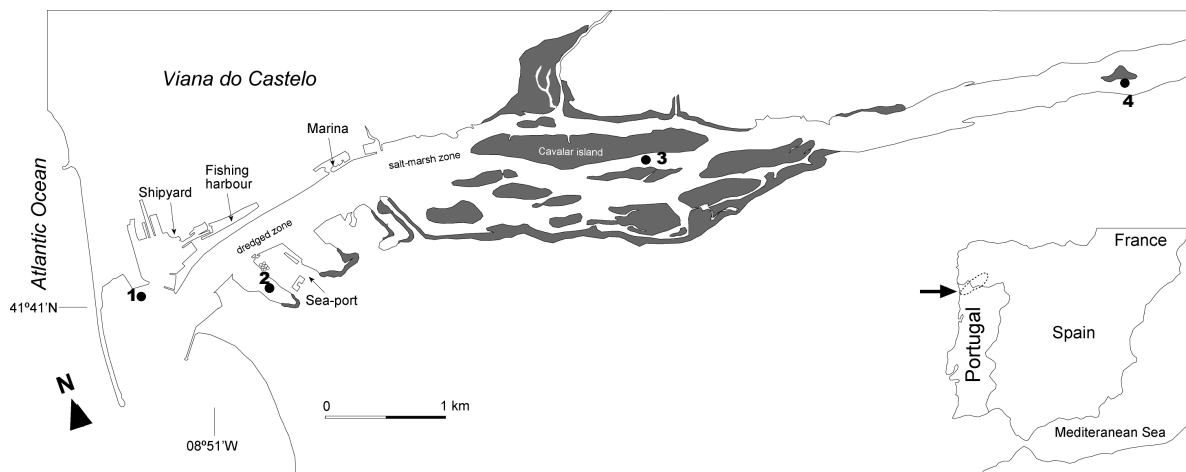


Figure 6.1. Lima River estuary and location of the 4 sampling stations where benthic flatfishes were collected. Shaded area represents sand islands and saltmarshes.

The juvenile fraction of the flatfishes was investigated between October 2003 and September 2005, in the lower, middle and upper sections of the Lima estuary. In the lower estuary two sampling stations were surveyed. Station 1 was a rather deep area due to frequent dredging activity, near a shipyard and distanced 0.8 km upstream from the river mouth. Station 2 was located within a small artificial embayment in the south bank at about 1.5 km from the mouth. Station 3 was located 5 km upstream at the vicinity of a tidal creek in the shallow middle estuary and near a tidal sand island in the upper estuary, more precisely 10 km upstream the river mouth was located Station 4. Monthly beam trawl (1-m beam-trawl net with a mesh size of 5 mm and one tickler chain) collections were

performed in the four sampling stations, during daytime and flood of spring tides, at an average velocity of 0.8 m s^{-1} during 3 min (each trawl covered in average 140 m^2). Geographic location of the sampling stations and distance traveled during each tow was provided by a Magellan 315 GPS. Samples were refrigerated in boxes with ice and transported to the laboratory where they were immediately frozen until sorting. Due to logistic problems, were not possible to sample Stations 3 and 4 during winter 2004/2005 and Station 4 during spring 2005. Flatfishes were counted, identified to the species level. Trawl opening (1 m) and distance traveled (given by GPS) were used to estimate trawled area and therefore to determinate the abundance of fishes, which was standardized as the number of individuals per 1000 m^2 .

The recruitment of flatfishes to the Lima estuary was investigated in the most abundant species. The temporal and spatial length patterns of larval and juveniles stages of each species were investigated. For larval stages, lengths recorded were notochord length for pre-flexion larvae and standard length (SL) for flexion and post-flexion larvae. Those measurements were performed under a binocular scope with image acquisition software Leica QWin 32. No correction for shrinkage was performed. In addition, the ontogenetic development stage of the larvae was identified according to Table 6.1, adapted from Fernández-Díaz et al.(2001), Osse e Van den Boogaart (1997), Edwards (1996), Nichols (1971; 1976) For juveniles, the total and standard lengths were measured with 1 mm precision. Due to several damages on the caudal fin, standard length (SL) was used to estimate the total length (TL). The percent of juveniles were calculated according to the length at first maturation available in the literature.

6.2.3 Data analyses

Seasons were aggregated into three month groups: autumn (A) comprised September, October and November; winter (W) December, January and February; spring (Sp) March, April and May and summer (S) June, July and August. The last survey, conducted in September 2005 was considered as a summer sample.

The temporal and spatial abundance patterns of 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 fulfill the ANOVA assumptions (stabilize the variance and fit data to a normal distribution), data were log

transformed $[\text{LN}(x + 1)]$. Homogeneity of variance was tested with a Cochran test and whenever variance was still heterogeneous, conclusions from ANOVA results were only accepted for those cases where significance levels were < 0.01 . Additionally, in the event of significance, a posteriori Tukey HSD for unequal sample sizes was used to determine which means were significantly different at a 0.05 level of probability.

Table 6.1. Ontogenetic stages of *Solea senegalensis* and *Platichthys flesus*. SL- standard length

	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	

6.3 Results

6.3.1 Species composition

The Lima estuarine flatfishes included four families, distributed by twelve taxa, where ten could be assigned to species and two to family (Table 6.2). 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 variegates*, *Solea lascaris*, *S. senegalensis* and *S. solea* and a taxon that was not identified further, Soleidae ni. All those taxa were found in the larval stage and only *S. senegalensis* and *S. solea* were also part of the juvenile fraction of the flatfishes. Pleuronectidae included larvae, juveniles and adults of *Platichthys flesus* and a larval taxon that was not identified further, Pleuronectidae ni. Scopthalmidae counted with two species, one in larval stage *Zeugopterus punctatus* and *Scophtalmus rombus* as juveniles. Bothidae was also represented by two species, *Arnoglossus laterna* and *A. thori*, present among the benthic flatfish assemblages.

Both fractions of the flatfish Lima estuarine assemblages were dominated by *S. senegalensis*. This species achieved 71% of the total flatfish larvae and 41% of the benthic flatfishes. *P. flesus* was the second most abundant flatfish species, representing 10% of the total flatfish larvae and 26% of the benthic flatfishes catch. *S. solea* was also present in the larval and juvenile fractions of the Lima estuarine flatfish assemblages. This species was not an important component of the flatfish larval assemblage, since represented only 4% of the total larvae. Concerning the benthic fraction of the flatfishes, *S. solea* represented 12% of the total catch, while the remaining flatfish taxa observed in the Lima estuary were only present in one of the two fractions studied.

Despite *S. senegalensis* and *P. flesus*, the larval component of the Lima flatfishes also included *S. lascaris*, which totalized 7% of the total flatfish larvae and was mainly collected in the lower estuary. These larvae were significantly ($F=3.8$ $P<0.001$) more abundant during summer months of 2003 (Figure 6.2). 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 6.2).

The benthic Lima flatfishes included other species that were not present among the larval fish assemblages (Table 6.2). *A. laterna* was the most abundant, totalizing 15% of the

total catch and were present in 24% of the positive hauls (i.e. samples containing flatfishes). Abundance varied significantly between sampling stations ($F= 5.8$ $P<0.001$) because the presence of *A. laterna* was restricted to the lower estuarine stretch (Figure 6.3). Total length ranged 33-156 mm, corresponding to 56% of juveniles and 44% of adults (Table 6.3). Juveniles were observed at both lower estuarine stations, being in 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 (Figure 6.4) and peaked in October 2003, with a maximum of 26.7 fishes 1000 m⁻². During autumn 2003, juveniles were significantly more abundant than in the other seasons ($F= 2.9$ $P<0.001$). Small individuals (TL < 50 mm) were rare (7%) and only captured during summer periods (Figure 6.4). Adults did not exhibit any particular preference for one of the two lower stations, though older individuals were more frequently observed at Station 2 (Figure 6.4).

Table 6.2. Flatfish species of the Lima estuary, mean abundance larval values (number of individuals 100 m⁻³ $\pm$ standard deviation), and frequency of occurrence of positive hauls (i.e. hauls with flatfishes).

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%	-	-	-	-
Scophthalmidae	<i>S. rhombus</i>	-	-	0.64 ± 2.50	11%	-	-
Scophthalmidae	<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

S. rhombus was not frequently observed and abundance peaked in September 2005 at Station 2 (17.8 fishes 1000 m⁻²). Similarly to the two previous species, abundance did not varied significantly between seasons ($F= 0.7$ $P>0.7$), but autumn 2003 was in average the most abundant season (Figure 6.3). *S. rhombus* was not captured in the upper Station 4 and was more abundant (1.2 ± 3.6 fishes 1000 m⁻²) and frequent (11%) at the lower

estuarine Station 2. Only one fish measured less than 50 mm and was captured in April 2005 at Station 2. The remaining *S. rhombus* measured > 150 mm TL (Figure 6.4). During summer 2005, one exemplar of *A. thori* was captured at Station 1 measuring 147 mm TL, being an adult fish (Table 6.3).

Table 6.3. Mean abundance values (number of individuals $1000\text{ m}^{-2} \pm$ standard deviation), minimum (Min) and maximum (Max) of total length (TL), length at first maturation (LFM) and percentage of juveniles (Juv %) of each flatfish species caught in the Lima estuary between October 2003 until September 2005.

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	69%
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 Queró et al. (1984)

6.3.2 Estuarine flatfishes recruitment

S. senegalensis

S. senegalensis larvae abundance ranged 0-9.1 larvae 100 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 lower and middle estuarine zone (Figure 6.2). 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 (Figure 6.2). A total of 149 *S. senegalensis* larvae were measured and SL ranged 1.7-5.1 mm. The all five ontogenetic stages were observed (Table 6.4). Stage 2 was the most abundant, representing 72% of the *S. senegalensis* larvae. These young larvae were collected along the study area, but in average they were more abundant at the lower estuary.

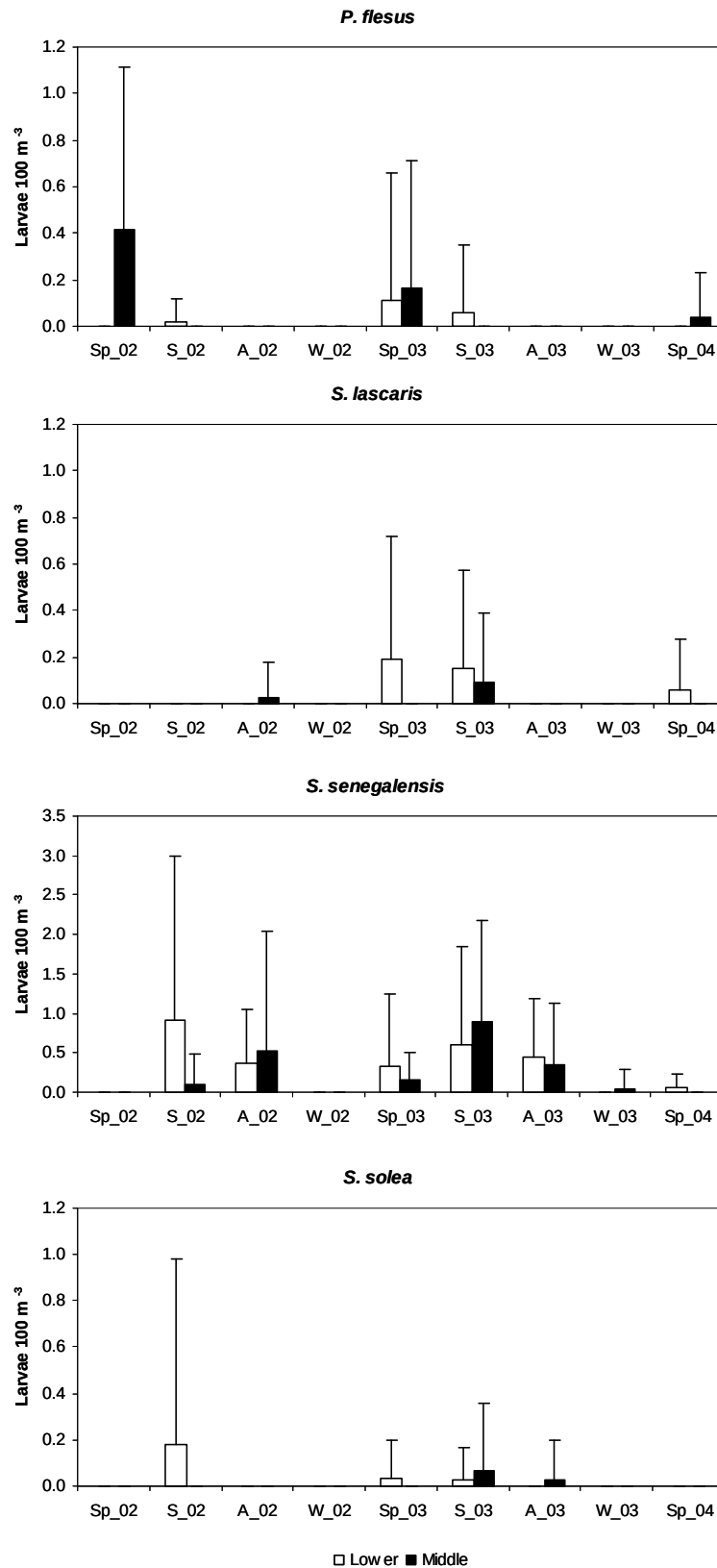


Figure 6.2. Seasonal abundance (mean $\pm$ standard deviation) of the larval flatfish species caught in the Lima estuary between April 2002 and April 2004, in the lower and middle Lima estuary. Sp- spring; S- summer; A- autumn and W- winter.

Similarly new-hatched larvae (Stage 1) were also more abundant in the lower stretch of the estuary (Figure 6.5). Older larvae, including metamorphosing larvae (Stage 5), were not abundant, achieving only 17% of the total larvae and were mainly observed at the shallow middle saltmarsh area (Figure 6.5). The seasonal variation of 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 at the estuary, occurred in June 2002 during the first study year and earlier in the second year, March 2003 (Figure 6.5). Their occurrence extended until late October in 2002 and November in 2003, in a total of 5 and 9 consecutive months, respectively. Older larvae appeared 3-4 months later than young larvae. For example, in 2002 metamorphosing larvae appeared in October 2002, while in the following year those larvae were observed earlier in May (Figure 6.5).

Regarding the benthic fraction, *S. senegalensis* was captured at all sampling stations, being more frequently observed at Station 4 (34%), but in average this specie was more abundant at Station 3 (7.8 ± 13.7 fishes 1000 m^{-2}). The abundance peak of that species was observed in July 2005 when a maximum of 66.7 fishes 1000 m^{-2} were registered at Station 3. Abundance did not varied significantly between seasons ($F= 2.1$ $P>0.05$), but it tended to decrease during winter periods (Figure 6.3). *S. senegalensis* total length ranged 33-156 mm, with an average of 113 ± 58 mm. Despite the wide range of lengths, only juveniles were captured (Table 6.3) and 44% of juveniles measured less than 100 mm.

Table 6.4. Mean and standard deviation ($M \pm SD$), minimum (Min) and maximum (Max) of standard length of each ontogenetic stage of *Solea senegalensis*, *Platichthys flesus* and *S. solea* larvae caught in Lima estuary between April 2002 and April 2004.

Ontogenetic stage	Stanard length (mm)											
	<i>S. senegalensis</i>				<i>P. flesus</i>				<i>S. solea</i>			
	N	M $\pm$ SD	Min	Max	N	M $\pm$ SD	Min	Max	N	M $\pm$ 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	-	-	-	-	-	-	-

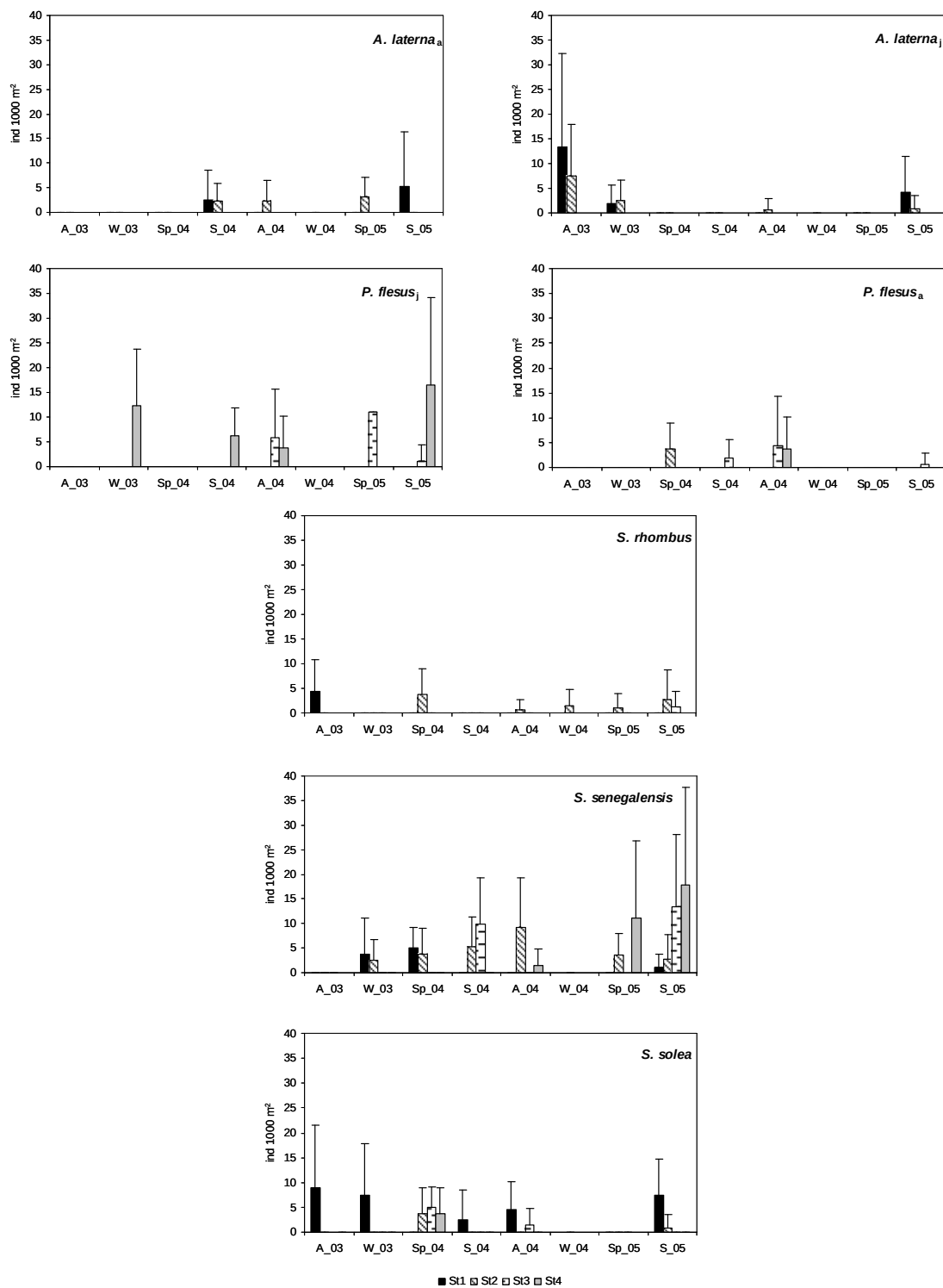


Figure 6.3. Seasonal abundance (mean ± standard deviation) of the benthic flatfish species caught in the Lima estuary between October 2003 and September 2005, at the four sampling stations. St1- station 1; St2- station 2; St3- station 3; St4- station 4; Sp-spring; S- summer; A- autumn and W- winter; a-adults and j- juveniles.

Temporal and spatial distribution of the total length of *S. senegalensis* juveniles showed differences between the two-study years (Figure 6.4). Small individuals (TL < 50 mm) that represented 11% of the total *S. senegalensis*, were more abundant during the spring-summer period of the second year. In the first 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) (Figure 6.4). Focusing on that period, small individuals firstly colonized the lower polyhaline Station 2 and as they grew progressively moved upstream, initially passing through Station 3 and latter reaching Station 4. Older fishes (TL > 200 mm), were not abundant and appeared latter than young soles during late spring - summer months and in the lower estuary (Figure 6.4).

P. flesus

P. flesus larvae abundance varied between 0 – 3.3 larvae 100 m⁻³, with an average of 0.04 ± 0.3 larvae 100 m⁻³. The occurrence of larvae in the Lima estuary showed a typical seasonal pattern that was observed during both study years. *P. flesus* larvae occurred almost exclusive during spring months, mainly Sp1 when the abundance was significantly higher ($F= 3.4$ $P<0.01$) (Figure 6.2). *P. flesus* larvae did not reveal particular affinity to the lower or middle estuary, however, during Sp1 in the middle estuary abundance reached the significant maximum of 0.3 ± 0.7 larvae 100 m⁻³ ($F=3.0$ $P<0.001$). The metric analyses of the larvae showed that the 29 individuals were in Stage 2 through Stage 4 of development and SL ranged 4.9 – 7.2 mm (Table 6.4). Stage 4 represented 72% of the *P. flesus* larvae, followed by 20% of Stage 3 and only one larval was classified as Stage 2. The seasonal variation of the SL revealed similar patterns of occurrence in the two study years (Figure 6). The first larvae observed in the estuary were already older larvae (Stage 4) that continued to arrive until July in 2002 and until June in 2003 (Figure 6). The younger exemplar of *P. flesus* (Stage 2) was collected in February 2004 at the lower estuary.

With respect to the monthly beam trawl data, *P. flesus* occurred in 32% of the positive hauls. Total length varied between 27 and 325 mm corresponding to 73% of juveniles and 27% of adults (Table 6.3). In average, juveniles (2.3 ± 7.3 fishes 1000 m⁻²) were more abundant than adults (0.4 ± 2.4 fishes 1000 m⁻²). This species was not very abundant in the lower estuary, being absent near the river mouth (Station 1). Occasionally, older fishes visited the Station 2, mainly during the winter-spring period of 2005 (Figure 6.3). Juveniles tended to concentrate at the upstream Station 4, where abundance was significantly higher ($F= 14.7$ $P<0.001$) and peaks of 44.4 fishes 1000 m⁻² were observed in June and

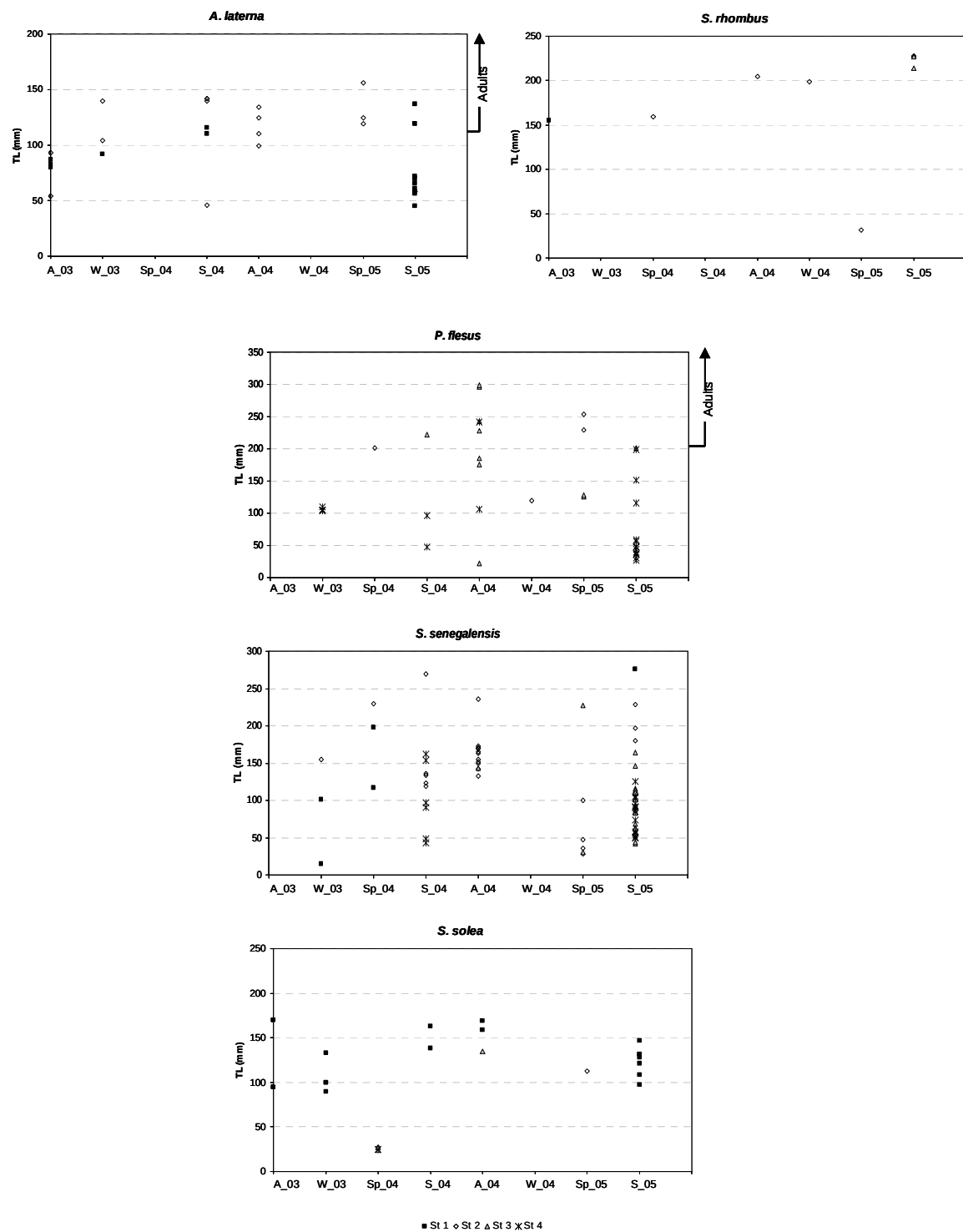


Figure 6.4. Seasonal and spatial variation of the total length (TL) of *A. laterna*, *P. flesus*, *S. rhombus*, *S. senegalensis* and *S. solea* collected in the Lima estuary, between October 2003 and September 2005. St1- station 1; St2- station 2; St3- station 3; St4- station 4; Sp-spring; S- summer; A- autumn and W-winter.

July 2005, a very dry summer. Small individuals (TL < 50 mm), which represented 34% of the total *P. flesus* captured, were only observed in early summer (June-July) of both study years and at the upstream Station 4 (Figure 6.4). Older juveniles were frequently captured throughout the study period, disappearing from November 2004 until April 2005 (Figure 6.4). Those juveniles exhibited a spread spatial distribution, being captured at all sampling stations excepting Station 1.

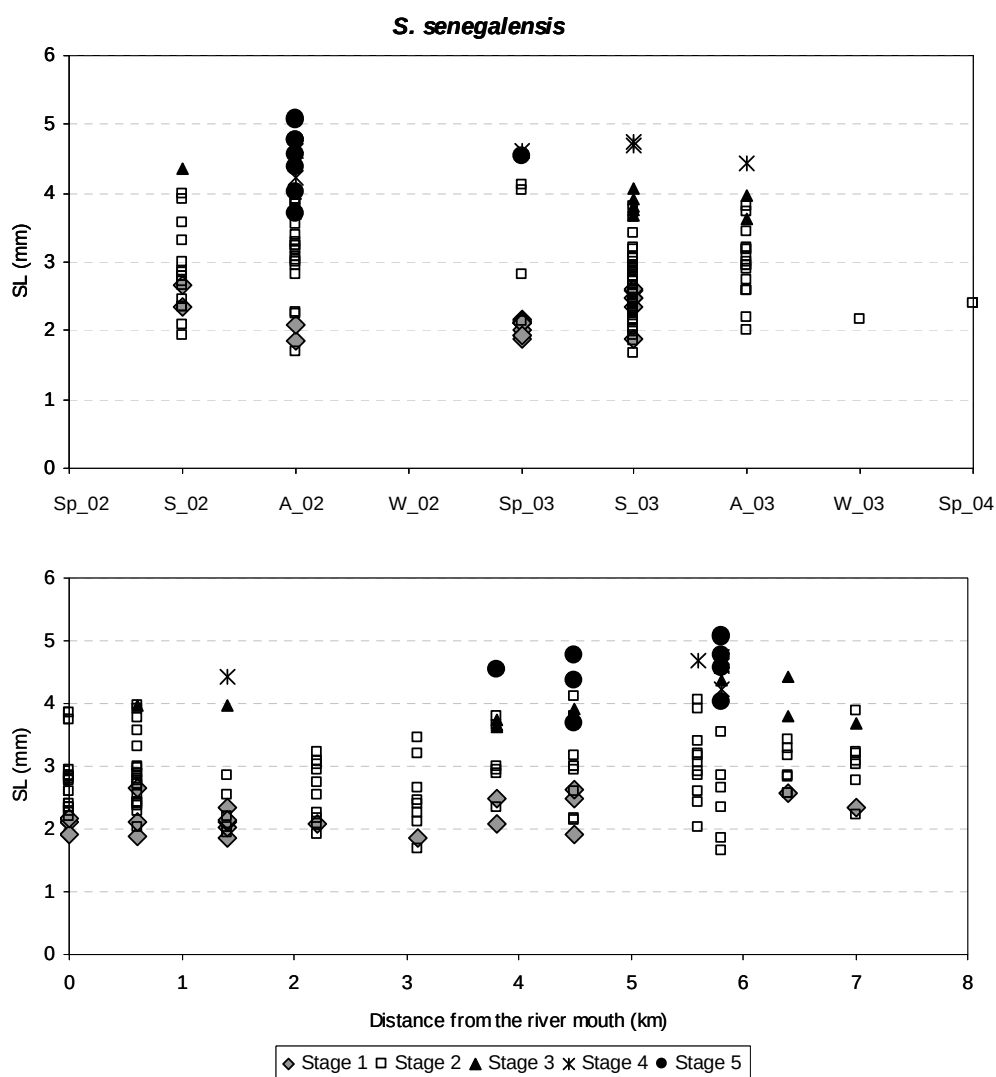


Figure 6.5. Seasonal variation of the standard length (SL) by ontogenetic stage of *S. senegalensis* larvae collected in the initial 7 km stretch of the Lima estuary. Sp_02: spring 2002; S_02: summer 2002; A_02: autumn 2002; W_02: winter 2002-2003; Sp_03: spring 2003; S_03: summer 2003; A_03: autumn 2003; W_03: winter 2003-2004; Sp_04: spring 2004.

S. solea

With a lower frequency of occurrence, *S. solea* larvae were more abundant in the lower estuary and during the second study year (2003/2004). Yet, abundance peaked in

summer of 2002, reaching a maximum of 3.60 larvae 100 m⁻³ (Figure 6.2). A total of six larvae of *S. solea* were measured and SL ranged 1.8 - 3.3 mm (Table 6.4). All larvae caught were young larvae, 50% were recently hatched (Stage 1) and the remaining larvae presented already pigmented eyes (Stage 2).

S. solea represented 12% of the benthic flatfishes and were significantly more abundant at Station 1 ($F=7.8$ $P<0.001$), where abundance reached the maximum of 22.2 fishes 1000 m⁻² in December 2003. In average, *S. solea* was more abundant during A1 and W1 (Figure 6.3), but no seasonal statistical differences in abundance were observed ($F=0.7$ $P>0.6$). All *S. solea* individuals collected within the beam trawl surveys were juveniles. The majority of them (70%) measured more than 100 mm TL. The temporal and spatial distribution of the total length showed that, contrarily to older individuals that were mainly observed at Station 1, small *S. solea* (TL < 50 mm) were only observed at Stations 2 though 4 and in a specific time of year, spring 2004 (Figure 6.4).

6.4 Discussion

The Lima flatfish larval and juvenile assemblages included twelve taxa, seven were collected as pelagic larvae and the benthic flatfish fraction comprised six species. The species composition of the Lima flatfish larvae was similar to other Portuguese estuaries, however the species richness was slightly higher than in central Portuguese estuaries, such as Mondego - 4 taxa; (Ribeiro 1991) and Tagus - 5 tax (Ré 1984b). Nevertheless, recent studies revealed that in the south of Portugal the Guadiana estuary ichthyoplankton assemblage comprised more and different flatfish species (Faria et al. 2006). The species richness of benthic flatfishes (6 flatfish 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) and the Sado estuary - 18 species (Cabral 2000a) and Ria Formosa - 8 species (Andrade 1992), both in the southern Portugal.

Due to its geographical position, the Portuguese west coast is located between three important biogeographic areas (the cold temperate Atlantic, the warm temperate Atlantic

and the Mediterranean), constituting an important transition zone where marine species with boreal, temperate and subtropical affinities occur in sympatry (Marques et al. 2006a). Flatfish species diversity seems to be distributed along latitudinal gradients with the richness higher 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 the northern European estuaries, but also species of warmer waters. Actually, the Portuguese coast constitutes the northern distribution limit

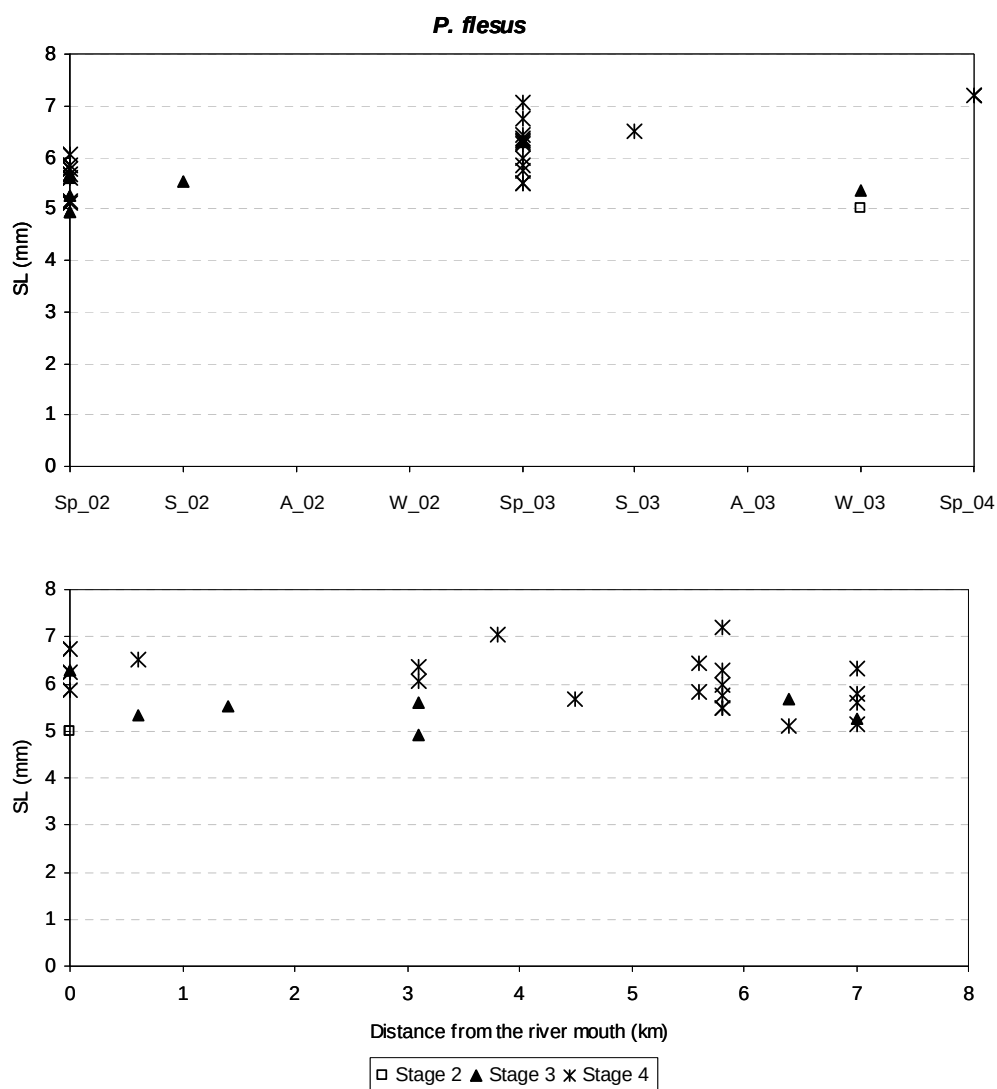


Figure 6.6. Seasonal variation of the standard length (SL) by ontogenetic stage of *P. flesus* larvae collected in the initial 7 km stretch of the Lima estuary. Sp_02: spring 2002; S_02: summer 2002; A_02: autumn 2002; W_02: winter 2002-2003; Sp_03: spring 2003; S_03: summer 2003; A_03: autumn 2003; W_03: winter 2003-2004; Sp_04: spring 2003.

of *S. senegalensis* and the southern distribution limit of *P. flesus* within the Atlantic Ocean (Marques et al. 2006a). *S. senegalensis* is a typical species of southern European

estuaries (Andrade 1992; Cabral and Costa 1999; Cabral 2000b), while flounder is commonly observed at higher latitudes (Kerstan 1991; Jager et al. 1995; Griocche et al. 1997; Amara and Paul 2003; Andersen et al. 2005). According to Costa and Cabral (1999), flounder used to be a frequent user of Tagus estuary as a nursery, but since the 90s this species disappeared altogether from the estuarine flatfish community. Nowadays, the southern geographic limit seems to be the northern Atlantic Iberian coast. The simultaneous presence of these two species reinforces the importance of the northern Portuguese margin, which function as a boundary for the distribution range of several flatfish species. The absence of *P. platessa* from the Lima estuarine flatfish assemblages corroborates the statement that Bay of Biscay seems to be closer to the southern limit of geographical distribution of this species (Desaunay et al. 2006).

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 the assessment of the real contribution of the Lima estuary to the adult stocks of the flatfish community that inhabit the estuary. However, comparing the maximum yields of the most abundant species reported to other nurseries areas (Table 6.6), the Lima abundance values for juveniles are similar or even higher, suggesting that Lima estuary is indeed a nursery area for *P. flesus*, *S. solea* and *S. senegalensis*. Interestingly, these were the only species that were collected as larvae and also as juveniles, highlighting the importance that the Lima estuary has on the early the life cycle of those species.

The *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 the two consecutive years of study for both larvae and juvenile (Figures. 6.4 and 6.6), and are consistent to the spring spawning season (Table 6.5). Moreover, the absence of adults during the winter periods (Figure 6.4) is also in conformity with the offshore reproductive migration described to occur in that period of the year (Deniel 1984). *P. flesus* spawns offshore and eggs and larvae disperse into shallow coastal nurseries where they undergo metamorphosis and settle in spring to early summer (Gibson 1994; Modin and Pihl 1996; Power et al. 2000; Koubbi et al. 2006). The absence of yolk larvae within the estuary is in accordance with this offshore spawning location. Moreover, the majority of larvae were older larvae in pos-flexion stage (Table 6.4), what according to Koubi et al. (2006) is the time when *P. flesus* larvae start to perform vertical migrations to the bottom in order to reach coastal waters and finally the

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, larvae tended to concentrate in the inner area (Figure 6.6), what is in agreement with the high abundances of new- juveniles (TL< 50 mm) encountered in the upper estuarine area (Figure 6.4). Therefore, a conceptual model for the *P. flesus* utilization of the Lima estuary can be designed. During the winter, adults migrate offshore to spawn. Older larvae arrived at the Lima estuary during spring until early summer, coming from a long migration from the offshore spawning place. Pre-metamorphosing larvae tended to concentrate in the inner shallow sections of the estuary; then, metamorphosing larvae undergo the settlement, which occurs within the shallow areas of the upper estuary, where young juveniles emerge and live. As long as they growth, older juveniles extend their distribution until the middle estuary, where they start to live together with adults.

S. senegalensis was the most abundant flatfish species of the Lima estuary, in what fish larvae and juveniles were concerned. *S. senegalensis* larvae appeared in the Lima estuary during spring/summer periods until autumn (Figure 6.5), what is in agreement with the described spawning season (Table 6.5). The high abundance of young larvae (Stages 1 and 2, Table 6.4) indicates that the 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 (Figure 6.5), suggesting that settlement occurred somewhere in that zone of the estuary. In fact, young juveniles (TL<50 mm) were more abundant in the inner intertidal stations (Figure 6.4). Vinagre et al. (2006a), suggested that *S. senegalensis*, similarly to *S. solea* (Van der Veer et al. 2001), might settled in the intertidal areas. This species has been associated with intertidal areas, which seem to function as the major feeding grounds for juveniles of *S. senegalensis*, working as key factor for the species distribution (Cabral 2000b). There was a high inter-annual variability on the first appearance of *S. senegalensis* larvae and juveniles within the Lima estuary. The nursery colonization processes are dependent on several factors, namely river flow, tidal cycle and wind regime that 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 during the spawning season, as it is the case of *S. senegalensis* (Dinis 1986; Andrade 1992). Apart from these temporal variations, and based on the temporal and spatial distribution of larvae and juveniles, a conceptual model of the use of Lima estuary was also designed for *S. senegalensis*. During the spawning season, several pulses of young larvae migrated from the continental 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 latter emerged as young juveniles. Those young juveniles started to disperse along the inner intertidal areas of the middle and upper section of Lima estuary. As far as juveniles grew, they started to spread over the Lima estuary, reaching the lower estuary.

Table 6.5. Life history traits of the flatfish species caught in Lima estuary between October 2003 and September 2005.

Species	Distribution	Habitat	Spawning Period	Nurseries
<i>A. laterna</i>	Eastern Atlantic from 65°N to 21°N and Mediterranean ¹	Coastal areas, mainly between 10 and 60 m, occasionally in estuaries ¹	From April to August ^{1,4}	Coastal areas ⁴
<i>P. flesus</i>	Eastern Atlantic from 70°N to 35°N and western Mediterranean ²	Shallow coastal areas. Common in estuaries and also found in freshwaters ²	February to June ^{2,4}	Coastal areas and estuaries ⁴
<i>S. rhombus</i>	Eastern Atlantic: 64°N to Morocco and Mediterranean and Black Sea ³	Shallow coastal waters, Scarce in brackish water ³	March to August ³	No information
<i>S. senegalensis</i>	Eastern Atlantic, from Bay of Biscay to Senegal, and western Mediterranean (rare) ⁴	From coastal marine areas and estuaries to 100 m ⁴	May to August ⁵ April to July ^{6,7}	Mainly estuaries ^{5,6,7}
<i>S. solea</i>	Eastern Atlantic, from 65°N to Senegal, and Mediterranean ⁴	From the shore down to 200 m ⁴	April to June (North Sea) ⁴ December to May (South Europe) ^{5,6,7}	Coastal areas, mainly estuaries ^{5,6,7}

¹ Nielsen (1986a); ² Nielsen (1986b); ³ Bauchot (1987); ⁴ Quérou et al. (1986); ⁵ Deniel (1984); ⁶ Andrade (1992); ⁷ Dinis (1986).

The presence of pelagic larvae of *P. flesus* and *S. senegalensis* is a sign of direct settlement. According to this mechanism, the estuarine nurseries colonization occurs early, during the pelagic larval phase, and metamorphosed larvae settle inside the estuarine nurseries (Gibson 1973; Lockwood 1974). However, the reduced number of new- settled fishes can compromise the theory of direct settlement. The reduce number of very small individuals (new- settled fishes) might be interpreted as a methodological problem related to the gear used and or with the sampling location. The reduced yield of very small (0-group) flatfishes has been identified as one of the main problems with these type of studies, because juveniles flatfishes live in shallow intertidal areas where it is not always possible to 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 recent 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 appropriated to collect new settled flatfishes in the Lima estuary. In future researches, additional engines should be used in order to increase the yield of very small flatfishes.

Contrarily to direct settlement, the indirect settlement mechanism presupposes that fishes settled in the ocean and then migrate to the nursery areas. This might be the case of *S. solea*, since few pelagic larvae were observed in the Lima estuary (Table 6.2). *S. solea* nursery grounds are reported to be located in marine coastal areas as well as in estuarine systems (Pihl 1989; Dorel et al. 1991; Marchand 1993; Rogers 1994; Cabral and Costa 1999). Juveniles of *S. solea* concentrate in estuaries and in bays for a period of about two years (Koutsikopoulos et al. 1989). The seasonal distribution and abundance of *S. solea* within nursery areas, and their relationship with environmental factors, namely depth, sediment type, salinity and temperature, have been studied in northern European estuaries and bays (e.g. Marchand 1988; Dorel et al. 1991; Rogers 1992; Jager et al. 1993; Henderson and Seaby 1994). Cabral and Costa (1999) described a seasonal pattern of juvenile *S. solea* distribution characterized by an inshore spring migration. The present results also confirm the spring inshore migration, since the smallest individuals arrived at the Lima estuary during spring (Figure 6.4), after the described winter spawning (Table 6.5).

The overall scenario of the Lima estuarine flatfish assemblages is partially in accordance with neighboring NW estuaries, namely Minho and Douro estuary (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 6.6). However, *S. solea* was not very abundant, representing only 12% of the Lima flatfish assemblages. The size 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 could not support the coexistence of two similar and competitive species such as *S. solea* and *S. senegalensis*, contrarily to larger estuarine systems such as the Tagus and Sado, further south (Table 6.6). It has also been demonstrated that density-dependent factors may have a high preponderance within nursery grounds, inducing high levels of juvenile mortality (Gibson 1994; Rogers 1994).

Another particularity of this northern estuary is the high abundances and use of the Lima as a nursery by *S. senegalensis*. This has not been reported to the other northern estuaries, where this species is not very abundant (Cabral et al 2007). Thus, along the Atlantic coast of Iberian Peninsula, the Lima estuary, a small estuarine system, shows some unique features, such as the northern nursery ground limit for *S. senegalensis*. This finding is of crucial importance, not only because is a contribution to the poor knowledge

of the ecology of this species, but also because this species has a high economical value. Thus, management and conservation practices the Lima estuarine habitat should play special attention to its nursery role for *S. senegalensis* that is on its northern limit of distribution and therefore more vulnerable to environmental variations, as loss of habitat, water and sediment quality.

Table 6.6. Peak densities (ind 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).

Estuary	Lima*	Minho	Douro	Ria de Aveiro	Mondego	Tagus	Sado	Mira	Ria Formosa	Guadiana
Total area (km²)	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

CHAPTER 7

Environmental control on early life stages of flatfishes within a nursery area (Lima estuary, NW Portugal)

7.1 Introduction

The habitats of fishes may vary temporally and spatially, therefore fishes must survive, growth and reproduce in conditions of a changing balance of biotic and abiotic environmental factors (Yamashita et al. 2001). Flatfishes occur throughout the world seas from the subarctic to the tropics (Pauly 1994), having a wide range of spawning seasons, habitat requirements and life history strategies (Minami and Tanaka 1992; Gibson 1994). Several flatfishes spawn in oceanic waters, being pelagic larvae transported inshore to settle in the nurseries areas, usually shallow coastal and estuarine habitats. The ontogenetic migrations vary among species, and are largely dependent on biological features such as spawning strategies, age and size at metamorphosis, larval behaviour

The contents of this chapter can also be found in Ramos, S., Ré, P. and Bordalo, A. A. Environmental control on early life stages of flatfishes within a nursery area (Lima estuary, NW Portugal). Submitted to Estuarine Coastal and Shelf Science.

(e.g. vertical movement, timing of settlement), and physical conditions such as local topography, currents, tide and temperature (Tanaka et al. 1989; Burke et al. 1998; Sogard et al. 2001; Bailey et al. 2005).

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

Estuarine and coastal areas have been recognized worldwide as nurseries habitats for many flatfish species (e.g. Rogers 1993; Guindon and Miller 1995; Allen and Baltz 1997; Able and Fahay 1998; Le Pape et al. 2003b; Gilliers et al. 2004). Estuaries are unstable environments, where abrupt changes in salinity, temperature, oxygen and turbidity occur due to the influence of tides and the mixing of marine and fresh waters (Vernberg 1983; Dyer 1997; McLusky and Elliott 2004). Actually, several variables such as temperature (Henderson and Seaby 1994; Power et al. 2000), salinity (Elliott et al. 1990; Henderson 1998), dissolved oxygen (Scholz and Waller 1992; Petersen and Pihl 1995), turbidity (Marchand 1993; Marshall and Elliott 1998; Hostens 2000), depth and sediment type (Gibson and Robb 1992; Norcross et al. 1999) have been identified as the dominant environmental factors influencing juvenile flatfish distribution and abundance within nursery grounds. Furthermore, food availability (Cabral 2000b; Cabral et al. 2002; Andersen et al. 2005) and predation pressure (Van der Veer and Bergman 1987; Ansell et al. 1999) are also important modellers of structure of flatfish assemblages in nursery areas. Ultimately, environmental shifts on the habitat characteristics of nurseries areas can induce important modifications on the year class strength and recruitment, ultimately affecting adult stocks.

The Lima estuary, located in the northern Atlantic coast of Portugal (NW Iberian Peninsula), comprises several flatfish species that are the basis of important local fisheries. Little is known about the link between Lima estuarine flatfishes and abiotic factors and how they affect the early life stages of flatfishes and therefore, no scientific sound management policy can be developed. The present study aims to investigate the influence of selected environmental parameters on temporal and spatial abundance patterns of the flatfish larval and juvenile assemblages of the Lima estuary.

7.2 Material and Methods

7.2.1 Study area

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

A total of eleven sampling stations within the lower and middle estuary were established for fish larvae (Ramos et al. 2006a). Benthic juveniles were studied in four sampling locations identified from a preliminary study (data not shown). Station 1 was located in the lower estuary, 0.8 km upstream of the river mouth and near a shipyard (Figure 7.1). This area is rather deep (10 m), submitted to frequent dredging. Station 2 was located within a small artificial embayment in the south bank at about 1.5 km from the mouth. Station 3 was located 5 km upstream at the vicinity of a tidal creek in the shallow middle estuary. Station 4 was located in the upper estuary, 10 km upstream the river mouth and near a tidal sand island.

7.2.1 Sampling strategy

7.2.1.1 Larval stages

Fish larvae assemblages of the Lima estuary were investigated between April 2002 and April 2004 along the initial 7 km stretch of the Lima estuary. Subsurface circular tows were performed at a constant velocity of ca. 1 m s^{-1} for five minutes, with a conical 1 m diameter, 3 m long and 500 μm mesh size net. A flowmeter (Hydro-Bios) was attached to the net in order to determine the volume of the filtered estuarine water. More details of the sampling strategy can be found in Ramos et al. (2006a). At each location, 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 collected from the Portuguese Water Institute (INAG 2005). River flow data for the first year were not available. Flatfish larvae were sorted and identified to the lowest possible *taxon*. Abundance data were standardized into number of larvae per 100 m^3 .

7.2.1.2 Juveniles and adults stages

Benthic flatfishes were monthly collected between October 2003 and September 2005, with a 1-m beam-trawl net with a mesh size of 5 mm and one tickler chain. Hauls were made during daylight at flood of spring tides at an average velocity of 0.8 m s^{-1} during 3 min (each trawl covered in average 140 m^2 of estuarine bottom), with a 1-m beam trawl net. Samples were refrigerated in boxes with ice and transported to the laboratory where they were immediately frozen until sorting. Due to logistic problems, Stations 3 and 4 during winter 2004/2005 and Station 4 during spring 2005 were not possible to sample. Vertical profiles of temperature, salinity, dissolved oxygen concentration and saturation, pH and turbidity of water column were obtained by means of a YSI 6820 CTD. After sorting, flatfishes were identified to the species level and fish abundance was expressed as the number of fishes per 1000 m^2 . Geographic location of the sampling stations and distance travelled during each tow was provided by a Magellan 315 GPS.

In November 2004, sediment composition at each sampling station was analyzed. Three sediment samples were taken in each sampling area using a Petit Ponar grab and were sorted for grain size purposes. Sediments were dried at $100 \text{ }^{\circ}\text{C}$ and grain size determination was performed by wet (fraction $< 0.063 \text{ mm}$) and dry (other fractions) sieving (CISA Sieve Shaker Mod. RP.08) of samples previously treated with hydrogen

peroxide. The sediments were divided into four fractions: silt and clay (< 0.063 mm), fine sand (0.063 - 0.250 mm), sand (0.250 - 1.000 mm) and gravel (>1.000 mm). Each fraction was weighed and expressed as a percentage of the total weight.

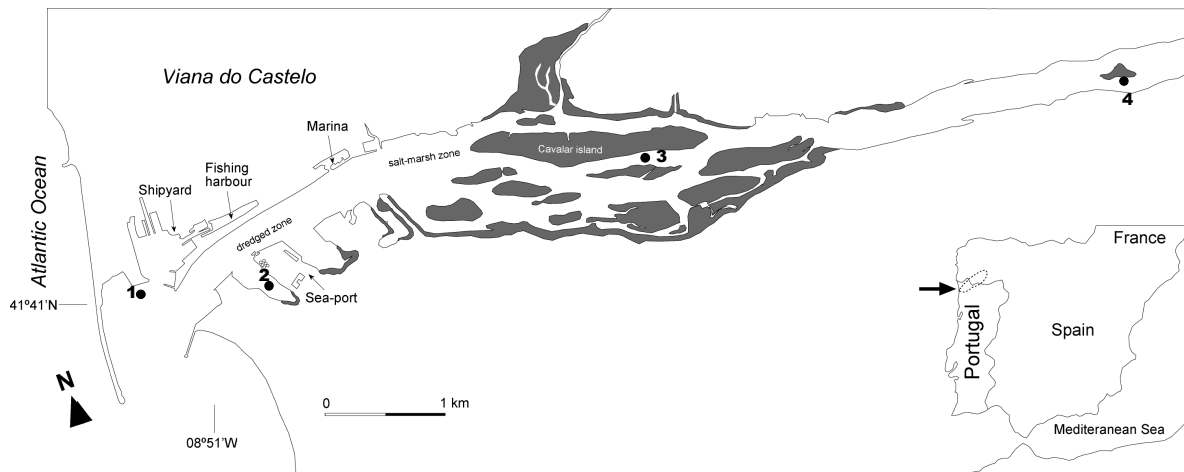


Figure 7.1. Lima River estuary and location of the 4 sampling stations where benthic flatfishes were collected. Shaded area represents sand islands and saltmarshes.

The abundance of the crab *Carcinus maenas* and the brown shrimp *Crangon crangon* was used to evaluate the predation pressure on the juvenile flatfish assemblages since these two invertebrate species have been identified as the main predators of other 0-group juvenile flatfishes (e.g. Pihl and Van der Veer 1992; Modin and Pihl 1996; Van der Veer et al. 2001; Vinagre et al. 2006a). Data on the abundance of predators covering the period of October 2003 until November 2004 were gently provided by Prof. Paulo Santos, J. Campos and V. Freitas. Data for the remaining surveys were obtained simultaneously with the flatfish surveys.

7.2.3 Data analyses

Monthly data were averaged by season: autumn (A), winter (W), spring (Sp) and summer (S). The last survey, conducted in September 2005 was considered as a summer sample. The temporal and spatial variations of environmental parameters of the bottom layer (median of data from 1 m above the maximum depth) were assessed by 1 way-ANOVA, with seasons or sampling stations as fixed factors (Sokal and Rohlf 1995; Zar 1996). In order to fulfil the ANOVA assumptions (stabilized variance and data fit to a normal distribution), data were log transformed [$\log(x)$]. Homogeneity of variance was tested with a Cochran test and whenever variance was still heterogeneous, conclusions from ANOVA

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

The influence of environmental variables on the flatfish assemblages was assessed with a canonical correspondence analysis (CCA), a multivariate method of direct gradient analysis (Ter Braak 1986), using the software CANOCO (version 4.5, Microcomputer Power, Ithaca, NY). Each larval species abundance data was averaged by month and by sampling area (lower and middle estuary). Flatfishes abundances were transformed [$\log(x+1)$] and downweighting of rare species was performed. A *taxon* "No Fish" was created to prevent CANOCO from eliminating samples containing no fish larvae (Grothues and Cowen 1999). The option used for CCA was triplot scaling with focus on interspecies distances. Significance of the canonical model was given by a Monte Carlo test (Ter Braak and 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). For the larval flatfish assemblage the following environmental variables were used: median temperature, salinity, oxygen saturation and concentration, pH, turbidity of the subsurface water layer (0.9 - 2.1 m); monthly river flow and precipitation; and lower or middle estuary as qualitative habitat characteristic. For juvenile assemblages the environmental variables used were: median temperature, salinity, oxygen saturation and concentration, pH and turbidity of the bottom water layer (1 m above the maximum depth); monthly river flow and precipitation; distance from the river mouth; the percentage of gravel, sand, fine sand and silt and clay; and abundance of predators (*C. maenus* and *C. crangon*). *Arnoglossus thori* was excluded from the analysis since only one specimen was caught. River flow and precipitation data were collected from the Portuguese Water Institute (INAG 2005).

7.3 Results

7.3.1 Larvae

7.3.1.1 Environmental conditions

Between April 2002 and April 2004, monthly precipitation varied between 28 and 239.3 mm, increasing during autumn-winter months, especially winter 2002 and the following spring. River flow ranged $4.9 - 226.1 \text{ m}^3 \text{ s}^{-1}$ and exhibited similar temporal trend. The water parameters of the sub-surface water layer revealed that Lima estuary was generally euhaline, with an average salinity above 30 psu (30.6 ± 6.5 psu), excepting during the wet period of winter 2002, when salinity decreased below 10 psu. Temperature ranged $9.4 - 19.6 \text{ }^\circ\text{C}$, with an average of $14.8 \pm 1.9 \text{ }^\circ\text{C}$, and exhibited a typical seasonal trend, decreasing during the winter months and increasing during the spring and summer months. Water was well oxygenated with an average saturation of $101.2 \pm 10.7\%$ and $8.7 \pm 1.1 \text{ mg L}^{-1}$ of dissolved oxygen. pH with a mean of 7.8 ± 0.4 tended to decrease during the freshet periods. Turbidity was lower than 7 NTU (mean: 6.8 ± 7.4 NTU), except during the wet period of winter 2002, when the transparency of the water decreased due to increased run-off, resulting from high precipitation.

7.3.1.2 Temporal and spatial patterns of flatfish larval assemblages

The flatfish larvae assemblage included nine taxa, where seven could be assigned to species and two to family. Soleidae, which represented 87% of the total flatfishes, included five species: *Buglossidium luteum*, *Microchirus variegates*, *Solea lascaris*, *S. senegalensis* and *S. solea* and a *taxon* that was not identified further, Soldeidae ni. Pleuronectidae reached 11% of the total flatfish larvae and included *Platichthys flesus* and a *taxon* that was not identified further, Pleuronectidae ni. Scopthalmidae represented by *Zeugopterus punctatus* totalized 2% of the flatfish larvae assemblage. On a species level, *S. senegalensis* was the most abundant species (71% of the flatfish larvae) and frequently caught (frequency of occurrence of 17% of 489 ichthyoplankton trawls). The second most abundant was *P. flesus* reaching 10% of the total flatfish larvae and was collected with a frequency of 4%. *S. lascaris* and *S. solea*, which totalized 7% and 4% of the total flatfish larvae, were collected with a frequency of 2% and 1%, respectively. The remaining species were considered rare with low frequency of occurrence lower than 1%, not individually representing more than 4% of the flatfish larval assemblage.

Flatfish larvae were present in 22% of the total ichthyoplankton trawls performed between April 2002 and April 2004. In average flatfish larvae were more abundant during summer 2003 (1.0 ± 1.5 larvae 100 m^{-3}), and in the lower estuary where abundance was in average 0.5 ± 1.4 larvae 100 m^{-3} . Species composition varied throughout the study period, without relevant variations between lower and middle estuary (Figure 7.2). Although,

occasional species such as *B. luteum*, *M. variegatus* were only observed in the lower estuary, contributing to a higher species richness of that area of Lima estuary.

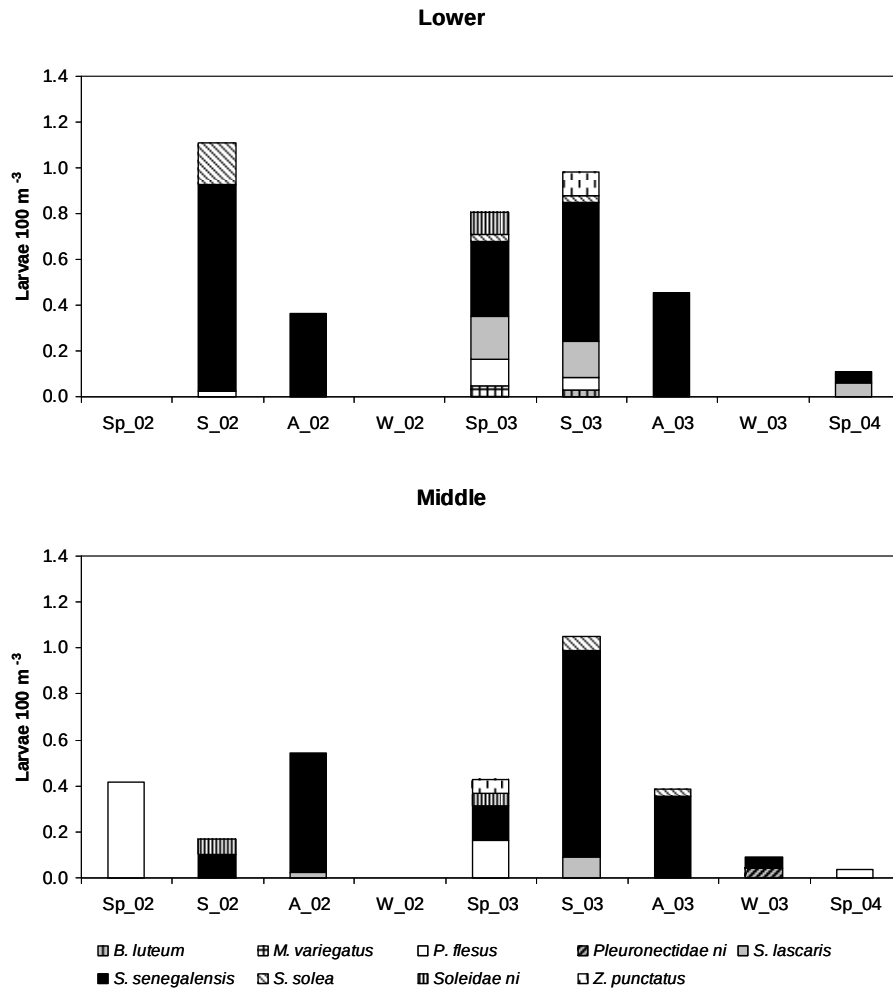


Figure 7.2. Seasonal variation of the flatfish larval assemblage structure in the lower and middle area of Lima estuary. Sp_02-spring 2002; S_02-summer 2002; A_02-autumn 2002 W_02-winter 2002/2003; Sp_03-spring 2003; S_03-summer 2003; A_03-autumn 2003 W_03-winter 2003/2004; Sp_04-spring 2004.

Flatfish larvae assemblages varied throughout the study period. There was a general trend for higher abundances and species richness during spring-summer periods, and a decrease during freshet winter periods, mainly in the lower estuary (Figure 7.2). However, during the first study year and in the middle estuary, the maximum abundances were observed during autumn months. As a rule, flatfish larvae were more frequent within the middle estuary, being present during all seasons, with the exception of the wet winter of 2002.

7.3.1.3 Relationships with environmental factors

The first CCA axis (eigenvalue = 0.5) and the second CCA axis (eigenvalue = 0.2) exhibited a high species–environment correlation (0.7 and 0.6, respectively). These two axes explained 80% of the cumulative percentage variance of species–environment relation and therefore, the latter two CCA axes (CCA3 and CCA4) were not interpreted further. The effect of the environmental variables on explained distribution of the CCA axes was significant ($F = 2.3$ $P < 0.01$, Monte Carlo permutation test).

Table 7.1. Inter-set correlations of environmental and supplementary variables with the first two CCA axes, based on the log-transformed density of flatfish larvae from the Lima River estuary. * inter-set $\geq |0.4|$ corresponding to biologically important variables.

Environmental variables	CCA ₁	CCA ₂
Temperature (°C)	-0.63*	0.03
Salinity (psu)	-0.42*	-0.09
Oxygen saturation (%)	-0.24	0.27
Oxygen concentration (mg L ⁻¹)	0.25	0.12
pH	-0.21	-0.04
Turbidity (NTU)	0.41*	-0.12
Precipitation (mm)	0.31	-0.17
River flow (m ³ s ⁻¹)	0.27	0.02
Lower	-0.10	-0.12
Middle	0.10	0.12

Inter-set correlation coefficients showed that temperature and salinity were negatively correlated with CCA1, while turbidity was positively correlated with the first CCA axis (Table 7.1). Samples clustered more or less according to their respective season. Winter samples with high turbidity and low temperature and salinity clustered on the right side of the ordination plot, while summer samples characterized by low turbidity and higher temperatures and salinity clustered on the opposite side of the plot (Figure 7.3). Spring and autumn samples clustered in between those two former seasons. Therefore, CCA1 represented a temporal gradient, separating seasons along it. The absence of flatfish larvae (NO) was associated with the freshet winter period, when river flow increased leading to a high run-off that increased the turbidity of the water. *P. flesus* associated with spring samples was also correlated with the middle estuarine area. Soleidae species grouped on the positive part of CCA1, correlated with higher temperatures of summer and autumn samples. This group of species was also correlated with higher salinities

7.3.2 Juveniles and adults

7.3.2.1 Environmental conditions

Between October 2003 and September 2005, precipitation varied between 1.3 and 286.8 mm and river flow ranged $10.9 - 78.9 \text{ m}^3 \text{ s}^{-1}$ with an average of $33.1 \pm 18.5 \text{ m}^3 \text{ s}^{-1}$. During autumn-winter there was an increase of precipitation, typical of the temperate Atlantic climate that led to an increase of the river flow. During the study period, the typical estuarine horizontal salinity gradient was always observed. Salinity decreased significantly from the river mouth until 10 km upstream ($F = 43.5$ $P < 0.01$). At the lower estuarine stations, salinity did not varied seasonally, being in average 34.0 ± 4.2 psu at Station 1 and 28.3 ± 6.6 psu at Station 2, further inside. However, at the inner stations a clear seasonal pattern emerged. Salinity decreased during autumn-winter period (Table 1), maybe as a consequence of the increased freshwater influence. At the bottom layer, temperature was in average 15.9 ± 2.9 °C, raging from 8.4 °C (during W1 at Station 4) and a maximum of 23.1° C (during S2 at Station 3). Temperature varied significantly between seasons ($F = 15.3$ $P < 0.001$), decreasing during winter and reaching the highest values during the spring-summer period (Table 7.2). A horizontal temperature gradient was always observed throughout the study period, which varied according to the time of the year. During winter, temperature decreased with distance from the river mouth whereas during summer an inversion of this spatial pattern occurred as temperature decrease towards downstream. There were significant differences in the water temperature between stations ($F = 6.9$ $P < 0.001$). In average, temperature reached higher values at Stations 3 and 4 than in the lower estuarine stations, where temperature of the water was temporally stable, with slight seasonal variations (Table 7.2). In general, water was well oxygenated, with a mean of $102 \pm 15.3\%$ and $8.8 \pm 1.5 \text{ mg L}^{-1}$ of saturation and concentration of dissolved oxygen, respectively. The typical spring increase was observed during the first year at the middle and upper estuary, where salinity was lower, while at the downstream stations this peak was observed early during the W1, coinciding with the lowest temperatures. During the second study year the spring increase of oxygen saturation ($F = 2.8$ $P < 0.01$) and concentration ($F = 2.9$ $P < 0.01$) was significant, especially at Stations 3 and 4, where oxygen saturation ($F = 3.3$ $P < 0.01$) and oxygen concentration ($F = 8.0$ $P < 0.001$) reached the highest values observed in this study (Table 7.2). In average, pH was seasonally stable, excepting during A2 when pH increased significantly reaching 8.3 ($F = 3.6$ $P < 0.001$). pH was in average 7.5 ± 0.3 at Station 1 and 8.1 ± 0.7 at Station 4,

Table 7.2. Mean values and standard deviation (SD) of bottom layer environmental parameters, per season, at the four sampling stations of the Lima estuary. (* without data.)

		Temperature	Salinity	Oxygen saturation	Oxygen concentration	pH	Turbidity
		(°C)	(psu)	(%)	(mg L ⁻¹)		(NTU)
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Station 1	A1	16.0 ± 0.5	33.3 ± 1.0	92.7 ± 3.0	7.5 ± 0.4	7.7 ± 0.0	6.9 ± 3.3
	W1	12.8 ± 1.0	30.9 ± 4.9	94.4 ± 3.6	8.3 ± 0.3	7.3 ± 0.1	5.0 ± 3.1
	Sp1	14.0 ± 0.9	33.9 ± 1.3	123.7	10.2	7.3 ± 0.0	*
	S1	15.8 ± 1.1	33.7 ± 0.8	96.2 ± 10.2	7.8 ± 0.6	7.6 ± 0.3	4.4 ± 2.0
	A2	14.3 ± 0.7	35.0 ± 0.6	92.1 ± 3.4	7.6 ± 0.4	7.8 ± 0.1	6.2 ± 1.5
	W2	11.8	24.7	122.1	11.3	*	9.4 -
	Sp2	15.0 ± 2.0	34.4 ± 0.4	103.4 ± 4.6	8.4 ± 0.0	7.3	5.7 ± 1.3
	S2	14.2 ± 0.7	37.9 ± 1.7	94.1 ± 14.1	7.6 ± 1.1	7.3 ± 0.2	5.1 ± 2.1
Station 2	A1	16.0 ± 0.5	31.9 ± 0.4	92.2 ± 1.6	7.5 ± 0.2	7.7 ± 0.4	6.0 ± 0.5
	W1	12.3 ± 1.3	27.3 ± 4.2	93.3 ± 6.3	8.4 ± 0.8	7.3 ± 0.2	7.5 ± 1.0
	Sp1	15.0 ± 1.6	29.1 ± 0.4	120.6	*	*	*
	S1	16.4 ± 0.8	28.1 ± 7.4	100.0 ± 8.3	8.3 ± 0.5	7.9 ± 0.1	4.8 ± 2.2
	A2	15.8 ± 1.9	21.9 ± 8.7	98.8 ± 3.4	8.6 ± 0.5	8.1 ± 0.4	5.8 ± 2.1
	W2	12.6 ± 0.8	31.3 ± 4.7	109.1 ± 6.2	9.6 ± 1.0	7.7 ± 0.5	8.1 ± 0.5
	Sp2	14.9 ± 1.3	31.0 ± 3.8	110.9 ± 19.6	9.2 ± 1.3	7.7 ± 0.6	7.3 ± 1.3
	S2	17.7 ± 1.4	31.0 ± 2.7	95.9 ± 19.1	7.6 ± 1.5	7.7 ± 0.2	4.8 ± 1.5
Station 3	A1	15.2 -	22.6	100.4	8.8	7.1	7.6
	W1	10.8 ± 1.7	9.3 ± 13.8	105.9 ± 12.4	11.2 ± 1.9	7.8 ± 0.6	7.5 ± 2.3
	Sp1	15.6 ± 3.6	24.9 ± 10.8	103.3	8.8	7.3 ± 0.0	*
	S1	17.5 ± 1.3	21.4 ± 10.3	101.5 ± 2.6	8.7 ± 0.6	8.0 ± 0.2	6.7 ± 0.4
	A2	16.4 ± 2.5	7.3 ± 5.0	107.0 ± 7.1	10.0 ± 0.4	8.5 ± 0.7	3.2 ± 3.1
	W2	*	*	*	*	*	*
	Sp2	16.7 ± 0.1	33.5 ± 0.2	152.9 ± 0.3	12.1 ± 0.0	8.5 ± 0.0	6.6 ± 1.7
	S2	18.5 ± 2.8	26.1 ± 8.8	103.7 ± 17.6	8.3 ± 1.4	7.7 ± 0.7	5.0 ± 1.6
Station 4	A1	15.0 ± 1.0	4.1 ± 4.0	95.1 ± 8.2	8.7 ± 2.2	7.9 ± 0.2	7.8 ± 1.6
	W1	10.2 ± 1.7	1.0 ± 1.5	101.6 ± 16.3	11.4 ± 1.9	8.0 ± 0.1	3.9 ± 1.5
	Sp1	17.4 ± 4.6	5.8 ± 0.9	105.7	9.2	7.9	8.8
	S1	18.0 ± 3.0	3.6 ± 3.2	98.7 ± 18.2	9.2 ± 2.3	7.9 ± 0.6	7.3 ± 0.2
	A2	17.7 ± 2.3	0.4 ± 0.3	106.4 ± 13.3	10.1 ± 0.8	9.2 ± 0.3	7.4 ± 0.4
	W2	*	*	*	*	*	*
	Sp2	*	*	*	*	*	*
	S2	20.5 ± 1.5	13.1 ± 8.7	119.7 ± 16.5	10.0 ± 1.3	7.9 ± 0.7	7.8 ± 1.3

increasing significantly with distance from the river mouth ($F= 5.9$ $P<0.001$), probably due to phytoplankton activity (Table 7.2). Turbidity did not exhibit any particular temporal or spatial pattern, being in general <10 NTU, with a mean of 6 ± 3.4 NTU.

Sediments composition varied between sampling stations (Figure 3), although the sand fraction represented always $>50\%$ of the composition in all locations. Silt and clay fraction was almost absent at the downstream Station 1 (0.1%), where fine sand was predominant. As expected, there was an upstream increase of the percentage of gravel from 3% by the river mouth to 44% at upermost Station 4. Station 2 exhibited the most equilibrated sediment composition (Figure 7.4).

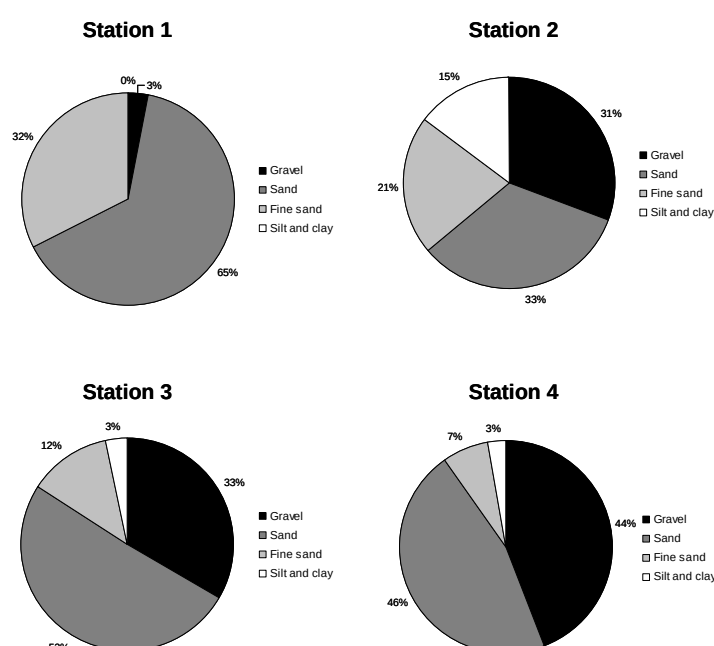


Figure 7.4. Sediments composition of each sampling station of the Lima estuary.

7.3.2.2 Temporal and spatial patterns of fish assemblages

From a total of 128 trawls, six flatfish species, belonging to four families were identified. Soleidae represented 55% of the total catch and included two species *S. senegalensis* and *S. solea*. *S. senegalensis* was the most abundant, representing 41% of the total catch with a mean abundance of 4.9 ± 9.9 fishes 1000 m^{-2} and frequency of occurrence of 32%. *S. solea* achieved 12% of the total catch, being captured with a mean abundance of 1.4 ± 4.0 fishes 1000 m^{-2} and 13% of occurrence. Pleuronectidae included *P. flesus*, which was the second most abundant reaching 26% of the total catch, and a mean abundance of 3.1 ± 8.2 fishes 1000 m^{-2} and frequency of occurrence of 20%. Bothidae represented by two

species *Arnoglossus laterna* and *A. thori*, represented 15% of the total catch. *A. laterna* totaled 15% of the total flatfishes and were captured with frequency of 24% and a mean abundance of 1.7 ± 4.8 fishes 1000 m^{-2} . Only one exemplar of *A. thori* was collected, with a mean abundance of 0.1 ± 0.7 fishes 1000 m^{-2} . Scophtalmidae included *Scophthalmus rhombus*, which represented 5% of the total flatfishes captured in the Lima estuary and presented a mean abundance of 0.6 ± 2.5 fishes $1,000\text{ m}^{-2}$ and a frequency of occurrence of 7%.

Flatfishes were captured along the Lima estuary, with a global frequency of occurrence of 64%. In average, flatfishes were more abundant at the upstream Station 4 (18.0 ± 19.4 fishes 1000 m^{-2}) and the most abundant season was summer of 2005 (18.9 ± 19.5 fishes 1000 m^{-2}), the driest period throughout the study. Species composition varied throughout the study period and also between sampling station (Figure 7.5). There was a trend for an upstream loss of species richness, since only three species were observed at the upper Station 4. At the lower Station 1, four flatfish species were observed, *A. laterna*, *A. thori*, *S. rhombus*, *S. senegalensis* and *S. solea*. Abundance decreased from a maximum of 26.7 ± 37.7 fishes 1000 m^{-2} in autumn 2003, until the total absence observed during winter and spring of 2004. In the following summer (2005) flatfishes reappeared at Station 1, with a mean abundance of 19.1 ± 12.7 fishes 1000 m^{-2} . Station 2 exhibited the most temporal stable flatfish assemblage, but with the lowest mean abundance (9.0 ± 8.4 fishes $1,000\text{ m}^{-2}$) in comparison with the other stations. However, species richness was higher since all flatfish species were observed, excepting *A. thori* (Figure 7.5). At the middle estuarine Station 3, four flatfish species were observed: *P. flesus*, *S. rhombus*, *S. senegalensis* and *S. solea*. Flatfishes started to appear in the middle estuary after winter 2003 and became more abundant and frequent in the following year. At the upper estuary, only three species were captured, *S. senegalensis*, *S. solea* and *P. flesus* which was the most abundant and frequent species. During autumn 2003, no flatfishes were observed at Station 4. In general, abundance tended to increase during summer periods, mainly during 2005, when a maximum of 29.8 ± 22.6 fishes 1000 m^{-2} was observed.

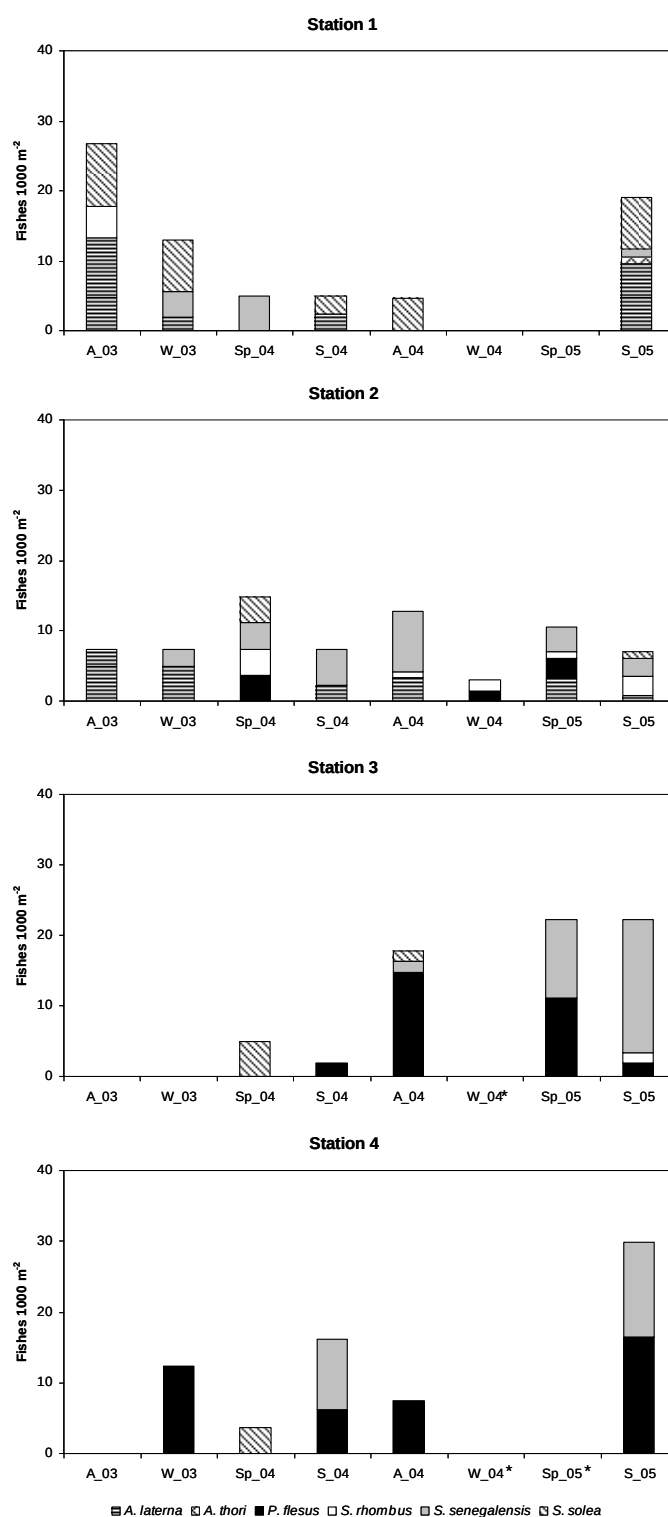


Figure 7.5. Seasonal variation of the benthic flatfishes at each sampling station of the Lima estuary. A_03-autumn 2003; W_03-winter 2003/ 2004; Sp_04-spring 2004; S_04- summer 2004; A_04- autumn 2004; W_04-winter 2004-2005; Sp_05-spring 2005; S_05- summer. * without data.

7.3.2.3 Relationships with environmental factors

From the original set of 15 environmental variables, the gravel fraction was eliminated from the model due to multicollinearity problems (Inflation factor > 20). The first (eigenvalue = 0.53) and the second CCA axis (eigenvalue = 0.25) exhibited a high species–environment correlation (0.81 and 0.65, respectively). These two axes explained 70% of the cumulative percentage variance of species–environment relation and therefore, the latter two CCA axes (CCA3 and CCA4) were not interpreted further. The effect of the environmental variables on explained distribution of the CCA axes was significant ($F=2.06$ $P<0.01$, Monte Carlo permutation test).

Table 7.3. Inter-set correlations of environmental and supplementary variables with the first two CCA axes, based on the log-transformed density of benthic flatfish taxa from the Lima River estuary. * inter-set $\geq |0.4|$ corresponding to biologically important variables.

Environmental variables	CCA ₁	CCA ₂
Temperature (°C)	*0.49*	*0.13
Salinity (psu)	0.56*	*0.01
Oxygen saturation (%)	*0.53*	0.00
Oxygen concentration (mg L ⁻¹)	*0.59*	0.04
pH	*0.40*	0.01
Turbidity (NTU)	*0.07	*0.08
Distance from the river mouth (km)	*0.69*	0.19
Precipitation (mm)	0.17	0.05
River flow (m ³ s ⁻¹)	0.11	0.04
% Sand	0.09	0.50*
% Fine sand	0.77*	0.09
% Silt and clay	0.33	*0.45*
<i>C. crangon</i>	*0.29	0.06
<i>C. maenus</i>	*0.20	*0.27

According to the inter-set correlation coefficients, fine sand sediments and salinity were positively correlated with CCA1, while distance from the river mouth, temperature, dissolved oxygen saturation and concentration and pH were negatively correlated with the first CCA axis (Table 7.3). Thus, CCA1 represented the horizontal estuarine gradient, with salinity decreasing with the increase of the distance from the river mouth. Samples clustered more or less according to their respective sampling station. Two different samples clusters were separated along the CCA1 (Figure 7.6). Samples from the brackish

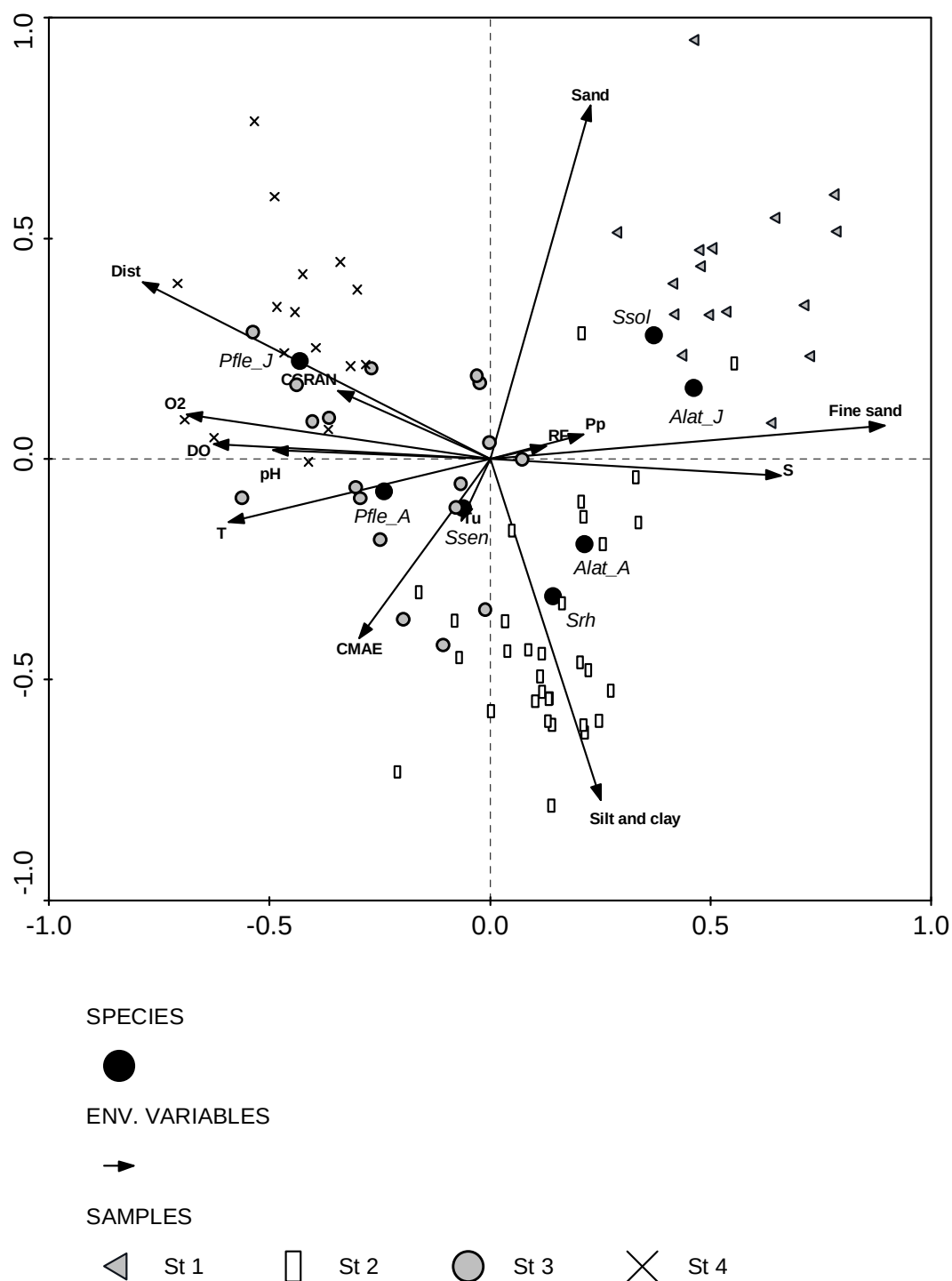


Figure 7.6. Ordination diagram for the first two canonical correspondence axes of the canonical correspondence analysis performed to sbenthic flatfish abundance data. St1- Station 1; St2- Station 2; St3- Station 3; St4- Station 4; *Alat_A*- *A. laterna* adults; *Alat_J*- *A. laterna* juveniles; *Pfle_A*- *P. flesus* adults; *Pfle_J*- *P. flesus* juveniles; *Srh*- *S. rhombus*; *Ssen*- *S. senegalensis*; *Ssol*- *S. solea*; NO- no fish. Environmental variables (arrows): Dist- distance from the river mouth; S- salinity; T- temperature; DO- oxygen saturation; Tu- turbidity; PP- precipitation; RF- river flow; CMAE- *C. maenus* and CCRAN- *C. crangon*.

inner stations 3 and 4, where temperature, oxygen saturation and concentration and pH reached higher values, clustered on the negative part of the CCA1. *P. flesus* and *S. senegalensis* were associated with this group of samples (Figure 7.6). *P. flesus* juveniles were separated on the ordination plot from the adults. Juveniles were positively correlated with the increase of the river mouth distance and associated with samples of the most upstream Station 4. On the other hand, adults were associated with brackish and warmer water of Station 3. *S. senegalensis* associated with Station 3 was positively correlated with the increase of temperature. The other group of samples clustered on the positive part of CCA1 axis, representing the lower marine Station 1 with the highest fraction of fine sand (32%). Species that clustered on the positive part of CCA1 revealed higher influence of the adjacent coastal waters, being positively correlated with salinity and negatively with temperature and dissolved oxygen (Figure 7.6). *A. laterna* juveniles and *S. solea* were associated with Station 1 samples and correlated with cold and marine waters. On the other hand, *A. laterna* adults and *S. rhombus* jointly with Station 2 clustered on the negative part of CCA2. CCA2, which explained 22 % of the explained variance was positively correlated with sand and negatively correlated with silt and clay sediments. Station 2 samples clustered on the negative part of the CCA2 since at this station silt and clay reached the highest percentage of the sediments composition (Figure 7.4).

7.4 Discussion

In general, the estuary behaved as a partially mixed waterbody (Ramos et al. 2006b), excepting during the winter when the increase of the river flow flected a noticeable vertical salinity stratification. On a horizontal basis, salinity decreased upstream. Lower estuary was markedly influenced by the adjacent coastal zone, with an overall salinity higher than 30 psu (polyhaline). The upper estuary was in general an oligohaline system (salinity <5 psu). Therefore, and according to Mclusky and Elliott (2004) the study area reached the head of the estuary (salinity < 5 psu). Occasionally, in some summer surveys, salt intrusion extended as far as 10 km upstream. The Lima estuary presented seasonal fluctuations of the water parameters, typical of temperate systems. Temperature increased during the spring-summer periods and oxygen peaked during spring, as a consequence of phytoplankton blooms. Although the Lima estuary was a rather clear system, turbidity increased during the freshet winter periods, as a consequence of higher estuarine run-off. Seasonal variations of the water parameters increased along the salinity

gradient towards upstream. Water parameters exhibited higher temporal oscillations in the middle and upper estuarine areas (Table 7.2).

The environmental control differed between pelagic larvae and benthic juveniles. Larval assemblage was mainly controlled by seasonal variations of temperature, salinity and turbidity (Table 7.1), leading to the separation of the species along the seasonal gradient represented by the first CCA axis (Figure 7.3). Two main groups of species emerged, Soleidae species in summer and *P. flesus* and *Z. punctatus* in winter. On the contrary, the benthic flatfish assemblage was markedly controlled by site specific characteristics, such as type of sediment, distance from the river mouth and subsequently the salinity regime (Table 7.3). In this case and according to the CCA, two main groups of species were identified: one included species that occurred mainly in lower polyhaline estuarine area and the other represented by the two most abundant species captured mainly in inner areas, namely *P. flesus* and *S. senegalensis* (Figure 7.6). As expected, the lower estuary revealed a high influence of the adjacent coastal area, allowing coastal species, such as *A. laterna* to extend their marine habitat through the lower polyhaline estuary. Thus, it is understandable the exclusive use of the lower estuarine stations by both juveniles and adults of this species (Figure 7.5).

Temperature was one of the most important ecological factors influencing the flatfish larval dynamics inhabiting the Lima estuary (Table 7.1). Actually, temperature has been identified as one of the best predictors of abundance of estuarine fish assemblages (Thiel et al. 1995; Marshall and Elliott 1998), playing a structural and a moderating role in determining flatfish abundances (Power et al. 2000). The influence of temperature derives from endogenous seasonal migrations of larvae, post-larvae and juveniles into the nursery areas that lead to seasonal variations in communities (Potter et al. 1986). Apart from the environmental control on the juvenile flatfish assemblages, larval supply to the estuarine habitats appears to be of major importance for the control of the abundance on a seasonal basis. Adult stocks of many of these species are located over the continental shelf, where spawning takes place (Rijnsdorp et al. 1985; Koutsikopoulos and Lacroix 1992) and planktonic larvae and or juveniles tend to migrate to coastal areas in order to reach suitable nurseries areas (Bailey et al. 2003). In fact, flatfish larvae clustered according to their spawning season, summer species *versus* spring species. Summer species included Soleidae species, whose spawning season occurs mainly during summer (Deniel 1984; Dinis 1986; Quéro et al. 1986; Andrade 1992), while for *P. flesus* and *Z. punctatus* the spawn season starts earlier, in late winter (Quéro 1984; Nielsen 1986b).

The distribution and movements of several flatfish species have been correlated with the salinity regime (Riley et al. 1981; Marchand 1993). Salinity has been identified as an important agent for the spatial structure of estuarine flatfish communities (e.g. Kerstan 1991; Marchand 1993; Cabral 2000a; Amara et al. 2004). Several authors outlined that both species showed a preference for low salinity waters (Jager 1998). Results obtained for the Douro estuary, south of the Lima estuary, showed that *P. flesus* and *S. solea* were particularly abundant in mesohaline waters, rather than in oligohaline or fresh waters (Vinagre et al. 2005). Contrarily, in the Lima estuary, juveniles of *P. flesus* were restricted to the upstream oligohaline area of the estuary and almost absent from the middle mesohaline zone (i.e. Station 3). In addition, there were also differences on the spatial distribution of *S. solea*, a typical user of estuarine nurseries grounds. This species has been associated with inner and consequently less saline areas of Douro (Vinagre et al. 2005), Tagus (Cabral and Costa 1999) and Sado (Cabral 2000a) estuaries in the W Portuguese coast. However, in the Lima estuary, this species was more abundant at the lower estuary, namely the Station 1 (Figure 7.5), being positively correlated with sand and salinity (Figure 7.6). These results are in accordance with other studies that showed that 0-group *S. solea* were associated with areas with a salinity range of 10-33 psu (Riley et al. 1981), and sandy and muddy sediments (Marchand 1988; Dorel et al. 1991; Jager et al. 1993). The preference of the lower Station 1 might have been related to the affinity of this species with fine sand sediments that dominated that location (Figure 7.4). The influence of salinity might have been indirect and a consequence of preferences for particular sediment type, typical of more sheltered and less saline areas (Riley et al. 1981). In fact, CCA results showed that salinity and fine sand were correlated (Figure 7.6), what corroborate the indirect influence of salinity.

The larval fraction of the Lima flatfishes was negatively correlated with salinity and positively with turbidity (Figure 7.3). CCA analysis identified salinity and turbidity as ecologically relevant parameter for the larval flatfish dynamics (Table 7.1). In fact, the absence of flatfish larvae was associated with lower salinities and high turbidity values, observed during the freshet winter periods (Figure 7.3). During the winter there was an increase of precipitation and river flow, which led to a higher freshwater input in the estuary and consequently an overall decreased of the mean water salinity and transparency of the water. According to Ramos et al. (2006b), variations of the river flow affected the hydrological processes that control water exchange between the Lima River estuary and the adjacent coastal area. Taking into account that the spawning of the Lima flatfish species occurs in the ocean, and considering that flatfish larvae migrate passively from the ocean into the estuary with the tide, the high run-off might have compromised

this mechanism of immigration into the estuary ineffective, especially near the surface. Thus, in periods of high river flow and turbidity, such as the winter of 2002, flatfish larvae were not able to migrate into the estuary. During the following winter (2003), the river run-off was much lower (Ramos et al. 2006b), allowing the appearance of few flatfish larvae in the estuary (Figure 7.2). In conclusion, the influence of salinity and turbidity on the larval flatfish dynamics should also be considered indirect, since the decreased of salinity and increased of turbidity were a consequence of the increase run-off that rendered the passive tidal migration of the flatfish larvae.

Sediments have been identified as important factors influencing the distribution of benthic flatfishes, with species associated with a particular sediment type (Gibson and Robb 1992; Gibson 1994). The dependence of flatfish on sediments is generally related both to the ability to burying behaviour to avoid predators and also to the distribution of suitable epi- and endobenthic prey abundance (Gibson and Robb 1992; Gibson 1994; Amaral and Cabral 2004; Walsh et al. 2004). Several authors highlighted the role of sediments composition for the structuring of flatfish communities. For example, Riley et al. (1981b) reported the *S. solea* preferences for sandy and muddy sediments. For this species, Dorel et al. (1991) and Rogers (1992) attributed the patchy distribution within a nursery area to the sedimentary discontinuity. Thus, differences on substrate within each sampling station could induce differences on preys abundance and consequently on flatfish community. Cabral and Costa (1999) also noticed that *S. solea* and *S. senegalensis* abundance in the Tagus Estuary was related to sediment and prey abundance. The observed spatial distribution of the Lima benthic flatfish community could have been due to different sediment composition of each local (Figure 7.4) and consequently different prey's abundances. In fact, the abundance and structure of the Lima macroinvertebrate community, considered as the main food supply for young flatfish (e.g. Costa 1986; Marchand 1993; Vinagre et al. 2005) vary between the lower and the middle estuary (Sousa et al. 2006). However, further investigations of the species food habitats are necessary in order to corroborate the hypothesis of the association of flatfishes with a particular area as a consequence of specific prey availability.

Food, predators and temperature have been suggested to be the most important factors contributing to the habitat quality for fish species (Gibson 1994). However, in estuary-dependent species, salinity conditions, often correlated with sediment structures (fine vs. coarse grain size), have been suggested to be important factors controlling the immigration, settlement and distributions of larvae and juveniles (Burke et al. 1995). Regardless of the sediments and salinity influence, CCA analysis also revealed the

influence of other environmental parameters on the benthic species structure (Table 7.3). Henderson (1989) suggested that discrete communities may be linked with different combinations of salinity and substrate, whereas temperature is responsible for a continuous change in the community. The structure of the benthic flatfish community varied throughout the time, with high inter-annual variations on the abundance pattern of each species (Figure 7.5). These temporal variations have also been reported in other estuaries (e.g. Marchand 1988; Potter et al. 2001; Le Pape et al. 2003a). Moreover, in the Portuguese Tagus and Sado estuaries, *P. flesus*, *S. solea* and *S. senegalensis* showed a high inter-annual variability in abundance without a clear trend (Cabral et al. 2007). Inter-annual variation on spawning and recruitment might be the major cause for those differences between years. (Rijnsdorp et al. 1992). Moreover, the environmental conditions at each sampling station also varied between years (Table 7.2), what might have intensified the inter-annual variations of the flatfish community. According to Lam (1983), the seasonal shift of the water characteristics in the spawning area has an important influence on the spawning activity, and consequently, on the seasonal pattern observed in environments with a nursery function

In addition, temperature influence might be indirect through interactions with other physico-chemical variables, such as dissolved oxygen (Marchand 1993). Dissolved oxygen was also considered as an important environmental influence for the benthic flatfish structure (Table 7.3). The effects of dissolved oxygen on flatfish abundances in the Thames operated indirectly through interactions with temperature and flow (Power et al. 2000). CCA analysis revealed that these two parameters were positively correlated with each other (Figure 7.6). During the study period, there was a trend for an increase of the oxygenation of the water during the warmer periods, mainly at the inner stations (Table 7.2). Temperature and dissolved oxygen were correlated with the CCA1 that separated the two main groups of species (Figure 7.6). *P. flesus* and *S. senegalensis* were positively correlated with both parameters (Figure 7.6). Actually, the abundance of those species peaked during summer, when temperature and oxygen of the water increased, which is in accordance with the preferences of *P. flesus* for higher temperatures (Jager et al. 1993; Van der Veer et al. 2001)

Predation on the early life history of fishes is also an important regulatory factor of the year-class strength. For several species of flatfish, year-class size is determined at the post-settlement stage and shortly thereafter (Edwards and Steele 1968; Zijlstra et al. 1982; Witting and Able 1995; Yamashita et al. 1996; Tarpgaard et al. 2005). Predation pressure by *C. maenus* and especially by *C. crangon* diminish with the fish growth, being

smaller sized fish consumed more intensively (e.g. Van der Veer and Bergman 1987; Ellis and Gibson 1995; Modin and Pihl 1996). Attending to the fact that the majority of juveniles recovered were post-settlement, these invertebrates might have worked as preys and not as predators. Therefore, it is understandable the weak influence of those biotic variables on the structure of flatfish community revealed by the CCA analysis (Table 7.3 and Figure 7.6). On the other hand, the low abundance of new settled fishes can be interpreted as a consequence of the high predation pressure by these two species, mainly on those flatfishes that were also captured in the larval stage, such as *S. senegalensis* and *P. flesus*.

The species-environmental relationships change during the life-cycle and sometimes, each developmental stage has a particular habitat that can be geographically separated from the adult habitat (Koubbi et al. 2006). Juveniles and adults of *P. flesus* tended to occur at different locations. Juveniles were mostly collected in the upstream Station 4, while adults exhibited a more widely distribution along the study area (Figure 7.5). This spatial segregation between juveniles and adults has been also reported for *P. platessa* and *S. solea* in the North Sea (Bolle et al. 2001). On the contrary, *A. laterna* juveniles and adults were only observed at the lower estuarine stations and had similar spatial distributions (Figure 7.5), what may indicate that this species does not have distinct nurseries. A similar pattern was found for *B. luteum* in the North Sea, where the distribution of juveniles and adults is broadly the same (Baltus and Van der Veer 1995).

The Lima estuarine flatfish assemblages comprised pelagic larvae, benthic juveniles and adults. Spring-summer period was the most relevant time of the year, when a higher number of flatfishes habited the estuary. This system was used by marine spawning species whose larval or juveniles migrated to the estuary. Larval and benthic flatfishes exhibited a particular dynamics, highly related with the specific habitat of each fraction. Pelagic larvae revealed a strong seasonal pattern, controlled by the seasonal variations of water column characteristics (e.g. temperature, salinity and turbidity) and also by the spawning season of each species. On the contrary, benthic juveniles and adults were more stable throughout the time, but demonstrated a clear spatial structure. They were highly correlated with site specific characteristics, mainly the sediment structure and the salinity regime of their habitat. Young stages were highly susceptible to environmental variations of their habitat, while older fishes were more dependent of the habitat site specific characteristics. The present study emphasized the concept that the environmental control varied throughout the ontogenetic development of flatfishes, what might be

interpreted as a consequence of the drastic habitat shift that flatfishes undergo throughout their life changing from a pelagic to a benthic habitat).

CHAPTER 8

General considerations and future directions

Nowadays, there is an increase interest for a sound knowledge of estuarine fish communities as an instrument for reliable management of estuaries. The ecological importance of estuaries for the life cycle of several economical important species fostered the worldwide interest on the scientific knowledge of the estuarine fish assemblages.

Estuarine habitats are used by various fish species, with different life strategies, including a great number of marine species, whose initial development stages as larval and/or juveniles inhabit temporarily these shallow ecosystems. The usual food abundance and sheltering areas offered by estuaries make them unique habitats, widely used as nursery areas for several marine fishes. Within the nursery, young fishes find favorable environmental conditions that promote growth and survival and, ultimately, enhance the recruitment to the adult stocks. Recruitment is a phenomenon of crucial importance for the ecological equilibrium of the population, especially when those populations are object of exploitable activities. Much of the recruitment variability in fishes seems to lie on variable survival in the first year of life, what reinforces the importance of the habitats used during the early life of fishes, namely the estuaries.

The study of the estuarine larval and juvenile fish assemblages has received the attention of the scientific community over the last decades. However, in Portugal little information is available for the northern estuaries, such as the Lima River estuary although that stretch of the Atlantic margin is responsible for a larger fraction of fish landings in Portugal. The Lima estuary is the end member of an international watershed, whose potential role as a

spawning and nursery habitat for local fish populations has not been previously examined. Therefore, the main contribution of this thesis deals with the fact that represents the first comprehensive investigation on the early life stages of fishes that inhabit the estuarine ecosystem, fulfilling a scientific knowledge gap over the Northern Iberian scenario. The fish larvae assemblages that inhabit the Lima estuary were investigated and the environmental control that they have experienced was also addressed, constituting the first part of this thesis. The second part of the thesis focused on ecological aspects of the early life stages of Pleuronectiformes within the Lima estuary. These fishes are common among the estuarine fish assemblages and potential users of the estuarine habitats as nurseries for their pelagic larvae and/or benthic juveniles. Thus, the second part of the thesis began with the general characterization of the temporal and spatial trends of the overall benthic fish fauna. After, the Ecology of early life stages of fishes (including pelagic larvae and benthic juveniles) were studied and finally a tentative assessment of the potential nursery role of Lima estuary was also carried out. This chapter deals with the general conclusions of the thesis and some several possible directions for future investigations are also presented.

Ichthyoplankton assemblages

This study began with the characterization of the seasonal and spatial patterns of the larval fish assemblages over a 2-year period. This first approach revealed that, surprisingly, the Lima estuary supported a high number of species, even when compared to larger Portuguese estuaries. A total of 50 taxa were identified, distributed by 20 families. The larval fish assemblage found in the Lima estuary included estuarine and marine species, but no freshwater specimens were collected, probably related to the overall high salinity of the water column, resulting from limited freshwater inputs. Gobiidae was the most abundant family comprising 71 % of the total catch, followed by Clupeidae with 12 % of the total. The top six most abundant taxa (*Pomatoschistus* spp., *Sardina pilchardus*, *Ammodytes tobianus*, unidentified Clupeidae, *Symphodus melops* and *Solea senegalensis*) represented 91% of the total catch. With an overall mean abundance of 8 larval fishes 100 m⁻³, the Lima ichthyoplankton was composed of a few species with high abundance and a large number of rare species. Thus, diversity was generally low, with high dominance of very few taxa, namely the estuarine residents *Pomatoschistus* spp.

The larval fish assemblages showed a typical and strong seasonal trend. Spring and summer were the periods of higher abundances, whereas winter was characterized by a drastic density reduction or even the absence of larval fishes in the estuarine environment. Seasonal variations of temperature, precipitation and river flow were identified as the most important variables affecting the temporal patterns of the larval fish assemblages. For example, during the winter 2002-03, larval fish decreased dramatically from the Lima estuary. This abundance decrease was associated with lower temperatures, high precipitation and subsequently, stronger river flow influence. The increase of the river flow seems to have affected the hydrological processes that control water exchange between the estuary and the adjacent coastal area, rendering the passive migrations of the coastal species into the estuary. Contrarily, during spring and summer, when temperature increased and river flow dropped, several marine species penetrated inside the estuary, as result of the jump in salinity related to higher salt water intrusion. During the winter resident species decreased, namely the most abundant taxa *Pomatoschistus* spp., which was attributed to three possible causes: (i) larvae could have been flushed out of the estuary, due to the high run-off; (ii) larvae could have vertically migrated downward; (iii) larvae could have died from starvation. Actually, during the winter of 2003-04, the river run-off was commensurably lower and the abundance of *Pomatoschistus* spp. increased.

The Lima estuarine larval fish assemblages also varied spatially. The assemblage structure of the lower estuary within the first 2.5 Km of a narrow dredged channel differed from the middle estuary, a wider stretch with abundant saltmarshes. In the channel of the lower estuary, larval fish assemblage was less abundant and more diverse, comprising a higher number of marine species, including the second most abundant taxa, *S. pilchardus*. In this transition area with the coastal zone, several marine species penetrated into the estuarine habitat, probably as a consequence of the tidal excursion. On the other hand, in the shallow saltmarsh zone (middle estuary), overall abundance reached higher values, but a reduce number of species were observed. This area was typified by high abundances of *Pomatoschistus* spp. leading to a high dominance of the assemblage. Larval fish assemblage was mainly composed by resident and also some marine species with an affinity to the saltmarsh area, namely *P. flesus*.

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, controlling the presence of incompetent larvae inside the estuary and also regulating the growth and mortality of individuals. On the other hand, environmental 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 species. Overall results suggest that the Lima estuary larval fish assemblage has a strong seasonality associated with the spawning seasonality, which controlled the presence of marine species, and also with the environmental aspects, whose main effect was observed in the general abundance trends of larval fish assemblages.

The spatial and temporal trends of the most abundant species were typical for Iberian estuaries, with the exception of the low abundance of anchovy larvae (*Engraulis encrasicolus*), and the unusually high numbers and frequency of *S. pilchardus*, usually mentioned as accidental in estuarine systems. Sardine eggs and larvae were previously not reported as a major component of estuarine ichthyoplankton along the Atlantic Iberian Peninsula. Their presence is generally considered accidental and largely restricted to the estuary mouth as a result of tidal transport from the adjacent coastal areas. The Lima estuary was an exception to this pattern, since sardine larvae were the second most abundant species of the estuarine ichthyoplankton assemblages, being the most abundant among the marine species. Therefore, *S. pilchardus* was not an occasional visitor or restricted to the terminal zone of the Lima estuary. Sardine larvae were captured throughout the study period and were mainly composed of newly-hatched sardine larvae (*i.e.* 1-week old sardines), indicating highly frequent spawning activity in this region. The seasonal age patterns also revealed that they peaked outside the known spawning periods of the northern Iberian stock. These data indicate that the adult stock extended the winter-spring spawning season throughout the summer and autumn periods. Seasonal and diel variations in occurrence of sardines within the Lima estuary were controlled by environmental features, such as salinity, temperature and turbidity. The occurrence of sardine larval stages within the estuary was directly controlled by the salinity regime of the system, which was in turn controlled by the tide on a diel scale and by precipitation on seasonal and annual scales. In addition, oceanographic features such as upwelling events could have been associated with coastal depletion of sardine larvae that resulted in estuarine low catches during summer 2002 and spring 2004. Catches of sardine larvae in the Lima estuary illustrate the significance of this feature during the early life of an important commercial species. This study reveals continuous spawning activity of the northern Iberian sardine stock, demonstrating new relevant aspects for its management and conservation and provides new insights into the ecology of *S. pilchardus* early life history along the Northern Iberian coast.

Benthic fish fauna

The benthic fish fauna of the Lima estuary in general and the juvenile fraction of the estuarine flatfishes were the focus of the second part of this thesis. This part of the study began with the description of the major temporal and spatial trends of the overall benthic fish fauna. Thus, the benthic fish fauna present in different habitats of the lower, middle and upper estuary was studied over a year. A total of 29 species distributed by 17 families were captured. Thirteen were classified as estuarine resident species, six as marine adventitious visitor species, six as marine juvenile resident species, one as marine seasonal migrant and one was catadromous. The environmental control of the Lima estuary was mainly based on spatial trends related with habitat affinity. On the other hand, seasonality was not an important descriptor of the Lima benthic fish assemblages. Benthic fish assemblage was seasonally stable and heterogeneously distributed among the different types of habitats, according to salinity, distance to mouth, sediment characteristics and qualitative habitats features, such as presence of saltmarsh, dredging activity and depth of the water column. Three major fish assemblages were identified in the Lima estuary. The lower dredged and polyhaline estuarine stretch was used mainly by juveniles of marine species as an extension of their adjacent coastal habitat; a shallow artificial embayment adjacent to the first area in the left bank acted as a refuge for marine juveniles and also for a wide diversity of estuarine resident species; the shallow saltmarsh areas in the middle and upper estuary were almost composed by few but abundant estuarine residents. Assemblages were dominated by two resident Gobiidae species *P. microps* and *P. minutus* that represented 95% of the total catches and were collected throughout the study area, except near the river mouth, probably as a consequence of the increased anthropogenic stress. Several economically important marine species (e.g. flatfishes) were only captured as juveniles, suggesting the use of Lima estuary as a nursery habitat.

Several flatfishes spawn in oceanic waters and pelagic larvae are transported inshore to settle in the nurseries areas. Estuaries are usually used as nursery grounds for pelagic larvae and/or benthic juveniles of several flatfish species, some of them of high economical importance. Early life stages of flatfishes are an important compound of the estuarine fish assemblages. Due to its geographical location, Portugal emerges as a confluent area for North Atlantic boreal and Southern Mediterranean flatfish species, acting as southern and northern geographical limit distribution of those species, respectively. The Lima estuarine flatfish assemblage comprised 12 taxa distributed by four

families (Bothidae, Pleuronectidae, Scopthalmidae and Soleidae) and ten species. The larval fraction comprised nine taxa while six species were identified among the benthic flatfishes. Soleidae was the most abundant family with five species *i.e.* *B. luteum*, *M. variegates*, *S. lascaris*, *S. senegalensis* and *S. solea* and a *taxon* that was not identified further (Soleidae ni). All those taxa were found in the larval stage. However, only *S. senegalensis* and *S. solea* were also part of the juvenile fraction of the flatfishes. Pleuronectidae included larvae, juveniles and adults of *P. flesus* and a larval *taxon* that was not identified further, (Pleuronectidae ni). Scopthalmidae counted with two species, one in larval stage (*Z. punctatus*) and *S. rombus* as juvenile. Bothidae was also represented by two species, *A. laterna* and *A. thori*, present among the benthic flatfish assemblages. In general, the larval fraction was dominated by two species (*S. senegalensis* and *P. flesus*), being the remaining species considered rare.

There was a general trend for a spring-summer abundance increase of both larvae and juveniles flatfishes. Flatfish larvae were more abundant during the spring-summer period, being almost absent during the freshet winter period. Species richness was higher at the lower estuary as a result of the visit of occasional marine species. The influence of selected environmental parameters on temporal and spatial abundance patterns of the flatfish larval and juvenile assemblages of the Lima estuary were examined. Temperature, salinity and turbidity were identified as the most relevant environmental factors for the flatfish larval assemblage. The structure of the flatfish larval assemblage was associated with the spawning season, with summer spawners (Soleidae species), being isolated from the spring species (*P. flesus* and *Z. punctatus*). The absence of flatfish larvae was associated with the freshet winter period, characterized by high river flow and turbidity of the water column. The estuarine recruitment was controlled by several aspects, namely the spawning season and the hydrodynamics of the estuary. Benthic flatfishes were more abundant in the middle and upper oligohaline areas of the estuary, but the species richness was higher near the river mouth. Sediment type, salinity, temperature and dissolved oxygen were identified as the main environmental factors structuring the benthic fraction of Lima flatfish assemblage. Two different groups of species were identified: *P. flesus* and *S. senegalensis* that were associated with the middle and upper estuary, while the remaining species were correlated with the otter estuarine areas. The pelagic larval fraction exhibited a distinct dynamics from the benthic estuarine flatfish community. Flatfish larvae were mainly regulated by seasonal variations of temperature and salinity of the estuarine habitat, while benthic flatfishes were markedly controlled by site specific characteristics such as sediments structure, distance to the river mouth and the salinity regime. The Lima estuary was used by *S. senegalensis*, *P. flesus* and *S. solea* as a

nursery area with direct settlement for the two first species, while *S. solea* was suspected to undergo an indirect settlement. Other flatfishes used the estuary as an extension of the adjacent coastal habitats such as *A. laterna*, while other species such as *S. rhombus* visited the estuary occasionally.

One of the main findings of this study concerns the fact that the Lima estuary emerged as the northern nursery area for *S. senegalensis*, in the west Iberian margin. *S. senegalensis* was the most abundant flatfish species of both larval and juvenile fractions of the Lima estuarine flatfish community. Among the pelagic larvae, the majority was young specimens, still presenting bilateral symmetry. These larvae were collected along the study area, but in average their abundant increased in the lower estuary. Older metamorphosing larvae were less abundant and revealed a preference for the shallow saltmarsh area. Benthic juveniles were observed throughout the Lima estuary, but tended to concentrate in the middle and upper estuarine sections. Older juveniles were not abundant and appeared latter than young soles during late spring - summer months and in the lower estuary. The use of the Lima estuary during the early life of *S. senegalensis* can be summarized on the scheme presented on Figure 8.1.

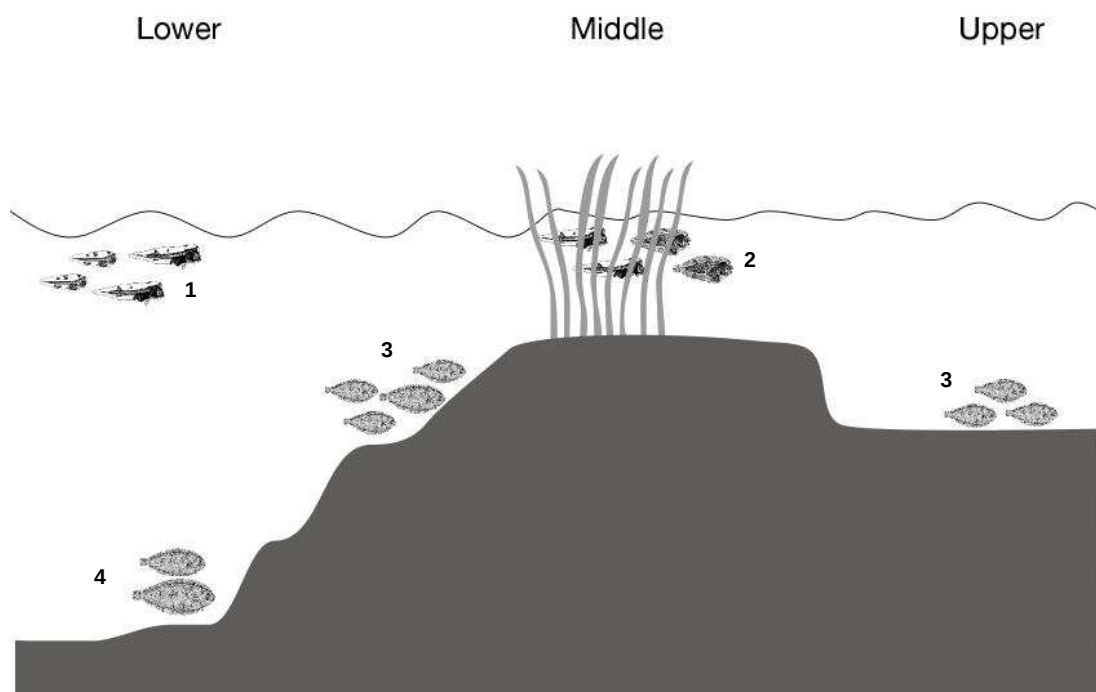


Figure 8.1. Summary of the early life of *S. senegalensis* in the lower, middle and upper Lima estuarine sections. 1- young larvae; 2- metamorphosing larvae; 3- new settled juveniles and 4- older juveniles.

P. flesus was the second most abundant species. Larvae, juveniles and adults were collected throughout the Lima estuary. Early life of *P. flesus* exhibited a similar temporal pattern without inter-annual differences. Developed larvae arrived in the estuary during spring whereas the 0-group juveniles started to appear in the following summer period. This species exhibited a high affinity with upstream zones of the estuary, since larvae tended to concentrate in the saltmarsh middle estuary and young juveniles in the upper estuarine section. Older juveniles and adults had a broader distribution, but only occasionally visited the small artificial embayment in the lower estuary. The early life of this species within the Lima estuary is resumed on Figure 8.2.

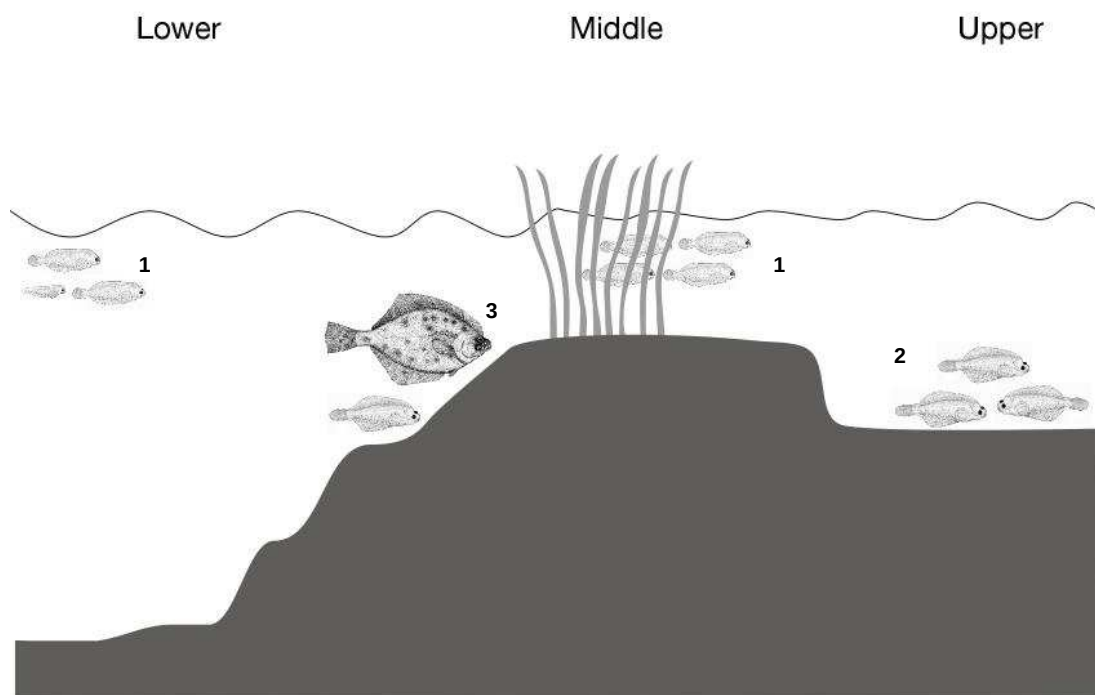


Figure 8.2. Summary of the early life of *P. flesus* in the lower, middle and upper Lima estuarine sections. **1-** metamorphosing larvae; **2-** new settled juveniles and **3-** adults.

According to the results of this study, the Lima estuarine fish fauna was characterized by the presence of:

- permanent residents such as Gobies and Syngnathiformes, with a high affinity to the shallow saltmarsh and upper regions of the estuary;
- developmental stages of marine migrant species that use the estuary as nursery, namely *P. flesus*, *S. senegalensis*, whose larvae settled into the estuary, probably in the saltmarsh and upper sections;
- migratory catadromous species, such as *Anguilla anguilla*;

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- and a wide range of young and/or adults of marine stragglers species that are not considered tied to estuaries as nursery grounds, but entered especially during periods of low rainfall when salinities were high, like *S. pilchardus*, *E. vipera* and *C. lyra*.

Overall, in terms of fishes, the Lima estuary comprised two distinct areas, the deep lower channel and the upstream shallow saltmarsh area, including the middle and upper estuary. In the highly disturbed lower estuary a high number of larval stages of marine species was found and was also used by older juveniles and/or adults as an extension of their coastal habitat. However, the artificial embayment that has not suffered any intervention for the last decade, was intensively used by juveniles and adults of marine species, highlighting the ecological importance of the lower estuary in spite of the human modifications. Upstream, the estuary retained natural features and the shallow saltmarsh in the middle stretch was the main habitat of larvae and juveniles of resident species, such as Gobies, but also of some marine species, mainly Pleuronectiformes. This area of the estuary (middle and upper sections) experienced higher environmental oscillations that seems not be tolerated by a high number of species, where resident species and flatfishes were the main compounds of the fish fauna. Nevertheless, whenever the hydrological regime allowed a higher salt water penetration, some marine species that usually were restricted to the lower estuary migrated towards upstream, habiting temporarily the saltmarsh area.

The present study emphasized the idea that the environmental control varies throughout the ontogenetic development and also that young stages are highly susceptible to environmental fluctuations of their habitat. In addition, this study reinforced the concept that inter-annual climate and hydrodynamic variations have a strong influence on estuarine ichthyoplankton and, consequently, on the recruitment of marine coastal fish populations. In spite of the high level of estuarine area reclamation for urban and industry, the Lima estuary remains an ecologically and economically important habitat in the coastal NE Atlantic zone. This study revealed some peculiarities of the Lima estuarine fish assemblages, namely the unusual presence of *S. pilchardus* larvae and the un-described northern Iberian nursery role for *S. senegalensis*. Thus, future management and conservation practices in the Lima estuarine habitat should pay special attention to those features of the estuarine environment, preserving habitats and water and sediment quality in order to attain a good ecological status of the estuary, the ultimate goal of the Water Framework Directive for transition (estuarine) waters.

Future directions

The present study was the first scientific approach to the Lima estuarine ichthyoplankton assemblages and also to the early life stages of flatfishes inhabiting the Lima estuary. This study focused mainly on the study of the temporal and spatial patterns of the assemblages, providing baseline data for future research, namely the development of an ecological indicator of the functionality of the estuary as a nursery habitat. The current management of estuaries relies on a good knowledge of their structure and functioning and of the way in which their habitat status, use, loss and gain has been influenced by human activities. Nowadays, there is a high need for indicators of those changes which are relevant to managers and policy makers and understandable by the layman. As the initial development stages of fishes (larvae and juveniles) are highly dependent on physical and biological processes, they may constitute a good indicator of the health and function of estuaries, what could also be a valuable asset for the existing Environmental Management Plan of the Viana do Castelo Port, as well as for the implementation of the Water Framework Directive.

As a consequence of being the first approach for the early life stages of fishes, the present study was not sufficiently conclusive in some aspects and several fundamental important questions arised. One major question that emerged, deals with the nursery role for *S. senegalensis*. Future research should investigate if survivorship is optimized within the estuary and also should try to estimate if the estuarine contribution to the adult stock is higher in comparison with other areas. In addition, another major question relies on the reduced abundances of newly settled flatfishes. Future research should also contemplate different methodologies, as the hyperbenthic sledge, and also investigate possible other estuarine areas, in order to find the settlement area for the most abundant species. Moreover, the research should also include other specific aspects such as inter and intraspecific competition, niche overlap, habitat preference, prey preferences and predation pressure.

A second major question that also emerged from the study is what is going on with the northern Iberian stock of sardines and what is the real role of the Lima estuary for *S. pilchardus*? The unusual high abundance of sardine larvae within an estuarine environment might be indicative of differences among the populations. Therefore, the

northern sardine stock should be investigated further in order to assess, if likely to other stocks, the northern Iberian stock is also changing their spawning strategy. In what the Lima estuary is concerned, the following central question remains unanswered: do sardine larvae actively migrated into the Lima estuary or their presence in the estuary was just the result of passive tidal transport? Since only one tidal/diel survey was performed and abundances of older larvae were low, whether or not the Lima estuary is a frequent spawning and nursery area for sardines requires further investigation. Thus, assessing the effective role of the Lima estuary for this economically and socially important species is necessary, requiring future research to evaluate the importance of the Lima estuary for the recruitment of the northern Iberian *S. pilchardus* stock.

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