Larval Fish Dynamics in the Mondego estuary: Linking early life stages and recruitment processes

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ABSTRACT

Estuaries present a great variety of habitats that, allied with their productivity, provide a high abundance of refugees and food supply for several species. These optimal conditions are crucial in their role as nursery areas for both estuarine and marine spawning fish species. For these species, estuarine habitats are essential to successfully complete their life cycle and their recruitment is strongly related with the nursery habitat suitability and with the environmental factors experienced during their early life. Since fish early life stages are particularly vulnerable phases, a greater understanding of both larvae and juvenile dynamics in these ecosystems would facilitate the further knowledge of the estuarine nursery function.

Mondego estuary as been previously mentioned as important nursery area for several fish, however information about its role during the larval stages of fish was limited and outdated. In this way, this thesis aimed to fulfil this gap and contribute to a better understanding of these assemblages and of the nursery role of the Mondego estuary.

The first study presented in this thesis (Chapter II) addresses spatial and seasonal patterns of the larval fish assemblages in the Mondego estuary and identify the main environmental factors structuring this community. Also, and taking advantage of a natural extreme event occurred during the studied period, ichthyoplankton responses to an extreme drought event were assessed. The larval fish assemblages of the Mondego estuary presented 31 taxa dominated by Pomatoschistus spp. Results showed two seasonal assemblages, spring/summer and autumn/winter, with the later presenting lower densities. Estuarine species were well distributed across the estuary while marine straggler and migrant species were higher at south arm downstream station. Temperature, chlorophyll *a*, and river flow were indicated as the main variation drivers observed. Interannual analysis showed that extreme drought events had no effect on ichthyoplankton community structure. However, spatial displacement of salinity gradient along the estuarine system led to analogous changes in marine species distribution.

In the chapter three, the dynamic of larvae ingress in the estuary and subsequent upstream transport were studied according to its seasonal, lunar-tide cycle, and tidal variation. Both seasonal and lunar-tide cycle represented an important influence structuring communities that arrive to the estuary and its following distribution. Ichthyoplankton entrance in the estuary seemed related to species spawning period, while its distribution within the estuary depends on in situ spawning as well as on the capacity of species to counteract currents and river flow.

In the chapter four, patterns of larvae ingress in the estuary were assessed by analysing fish larvae vertical distribution and migration pattern in relation to diel and tidal cycles. Harmonic regression identified main species larval densities variation associated with both cycles. Fish larvae density increased with receding tide and as night falls at both depths analysed. Thus, tidal and diel cycle did not influence larvae vertical distributions for the majority of the species. Pomatoschistus spp. and Gobius niger seem to be the exceptions, exploiting tides in order to maximize their upstream transport and retention in the estuary. Vertical patterns observed appear to be related to seasonal stratification and river flow, increasing amplitude during periods of less stratification and lower water currents.

In the chapter five, the integration of both benthic and pelagic phases was performed for a more comprehensive assessment of the nursery importance of Mondego estuary for estuarine dependent species, as flatfishes. In this study, flatfish larvae entrance mechanisms in the estuary and both larvae and juvenile seasonal and spatial distribution were analysed in order to investigate the life cycle of the main flatfish species in the estuary. Larvae ingress into the estuary occurred mainly during summer and autumn with no evidence for diel or tidal vertical stratification. Solea senegalensis larvae were mainly at downstream areas and presented low successful settlement and juveniles' densities inside the estuary. Conversely, Platichthys flesus and Solea solea larvae seemed to settle in nearby coastal areas and were mainly present in the estuary as juveniles. This study showed that the Mondego estuary nursery role differs along the flatfish life cycle and highlight the importance of integrate larval and juvenile stages of fish when assessing this important role of estuarine areas.

This thesis represents a progress in order to understand Mondego estuary as nursery habitat by adding valuable information about fish early life stages and its relationship with the adjacent coastal areas.

RESUMO

Os ecossistemas estuarinos apresentam uma grande variedade de habitats que, aliada à sua produtividade, proporcionam grande abundância de refúgio e alimento para muitas espécies. Estas condições são fundamentais para o desenvolvimento do seu papel como zonas de viveiro para espécies de peixe que desovam quer no mar, quer no estuário. Para estas espécies os habitats estuarinos são essenciais para completar o seu ciclo de vida e o seu recrutamento está estreitamente relacionado com a capacidade de viveiro destes habitats e com os fatores ambientais experienciados durante a fase inicial de vida dos peixes. Uma vez que os eus estádios de vida iniciais são extremamente vulneráveis, um melhor conhecimento da dinâmica larvar e dos juvenis nestes ecossistemas contribuirá para um melhor conhecimento da função de viveiro dos estuários.

O estuário do Mondego já foi anteriormente referido como uma importante zona de viveiro para muitas espécies de peixe, no entanto a informação acerca do seu papel durante os estádios larvares dos peixes é limitada e desatualizada. Desta forma, esta tese tem como objetivo preencher esta lacuna de informação e contribuir para um melhor conhecimento destas comunidades e do papel desenvolvido pelo estuário do Mondego como zona de viveiro.

O primeiro estudo apresentado nesta tese (Capítulo II) apresenta os padrões de distribuição espaciais e sazonais das comunidades de larvas de peixes do estuário do

Mondego, identificando os principais fatores ambientais responsáveis pela sua estruturação. Simultaneamente, e tirando partido de um evento natural ocorrido durante o período de estudo, foi também determinada a influência de um evento de seca extrema nas comunidades ictioplanctónicas. As comunidades larvares do estuário apresentaram 31 taxa dominadas pelo gobídeo *Pomatoschistus* spp. Os resultados obtidos mostram a existência de dois conjuntos de espécies sazonais, um de primavera/verão e outro de outono/inverno, apresentado este último densidades mais baixas. As espécies estuarinas encontram-se uniformemente distribuídas pelo estuário enquanto as espécies marinhas ocasionais e migratórias apresentaram maiores densidades na estação a jusante do braço sul. A temperatura, a concentração de clorofila *a* e o fluxo de água doce foram os principais factores ambientais responsáveis pela estruturação das comunidades. A análise interanual revelou que os eventos de seca extrema não apresentaram qualquer efeito na estrutura da comunidade de larvas de peixe. No entanto, a deslocação do gradiente de salinidade ao longo do sistema estuarino induziu alterações análogas na distribuição espacial de espécies de larvas marinhas.

No terceiro capítulo foi estudada a variação sazonal e do ciclo lunar e tidal da entrada das larvas de peixe e posterior transporte ao longo do estuário. A estrutura da comunidade larvar à entrada do estuário apresentou variações sazonais e de acordo com o ciclo lunar, bem como a sua posterior distribuição no estuário. A entrada de ictioplâncton no estuário parece estar relacionada com o período de desova das espécies, enquanto a sua distribuição no interior do estuário depende da desova local e da capacidade das espécies de contrariar as correntes e o fluxo de água doce.

No quarto capítulo foram estudados os padrões de entrada das larvas no estuário através da análise da distribuição vertical e dos padrões de migração das larvas ao longo do dia e do ciclo de maré. A regressão harmónica identificou variação na densidade larvar associada a ambos os ciclos. A densidade larvar à entrada do estuário aumentou com a vazante e com o final do dia, quer à superfície quer no fundo. Desta forma o ciclo de maré e diário não influenciaram a distribuição vertical das larvas da maioria das espécies. As exceções foram *Pomatoschistus* spp. e *Gobius niger* que parecem aproveitar as marés de forma a maximizar o seu transporte para montante e a sua retenção no estuário. Os padrões verticais observados parecem estar relacionados com variações sazonais de estratificação da coluna de água e do fluxo de água doce, aumentado durante períodos de estratificação reduzida e de fracas correntes.

No quinto capítulo foi feita a integração das fases pelágicas larvares e bentónicas de forma a determinar de forma mais abrangente a importância do estuário do Mondego como zona de viveiro para espécies dependentes do estuário, como os Pleuronectiformes. Neste estudo foram analisados os mecanismos de entrada no estuário das larvas de Pleuronectiformes e a sua distribuição espacial e sazonal enquanto larvas e juvenis de forma a explorar o seu ciclo de vida no estuário. A entrada das larvas no estuário ocorreu principalmente durante o verão e o outono sem qualquer evidência de estratificação vertical ao longo do dia ou de acordo com o ciclo de maré. As larvas de *Solea senegalensis* distribuíram-se nas zonas mais a jusante e apresentaram uma taxa de assentamento baixa, bem como de densidade de juvenis. As larvas de *Platichthys flesus* e *Solea solea* parecem assentar nas zonas costeiras adjacentes, estando presentes no estuário apenas como juvenis. Este estudo demonstra que o papel do estuário do Mondego como zona de viveiro varia ao longo do ciclo de vida dos Pleuronectiformes e salienta a importância de integrar estádios larvares e juvenis neste tipo de estudos.

Esta tese representa um progresso no conhecimento do papel do estuário do Mondego como zona de viveiro, das suas comunidades de estádios iniciais de vida dos peixes e da sua relação com as zonas costeiras adjacentes.

CHAPTER I

General Introduction

The importance of estuaries as nurseries for marine fish

Estuaries are among the most biologically productive and valuable ecosystems in the world (Costanza et al. 1997; Able, 2005) playing well-defined roles as nurseries, feeding and refuge areas for many invertebrate, fish, and bird species (Beck et al., 2001; Elliott and McLusky, 2002; Able, 2005). Rather than a single habitat, estuaries include a complex and interrelated web of habitats including sand, mudflats, saltmarshes, oyster beds, and seagrass meadows (McLusky and Elliot, 2004). Added to the high primary productivity, the habitat richness inside these ecosystems provides optimal settlement conditions for a great variety of species (Beck et al., 2001).

The nursery-role concept was first applied, nearly a century ago, to motile invertebrates and fishes with complex life cycles, in which larvae are transported to estuaries, metamorphose, grow to subadult stages, and then move to adult habitats offshore (Beck et al., 2001). The nursery role of estuaries is particularly important for marine species, mainly for those that live in coastal areas but require sheltered estuarine habitats to successfully complete their life cycle. For estuarine dependent species like the European sea bass (*Dicentrarchus labrax*), flounder (*Platichthys flesus*) and the common sole (*Solea solea*), which are among the most abundant in Portuguese estuaries (Cabral et al., 2007; Martinho et al., 2007a,b; Ribeiro et al., 2008), connectivity between estuarine and coastal areas is of great importance (Ray, 2005). Since these species usually have high commercial value, understanding the dynamics of recruitment variability is essential for an accurate fisheries management strategy.

Fish recruitment, i.e., the number of individuals from a specific year class attaining sexual maturity and joining to the reproductive component of a population, is strongly determined by the habitat that influence growth and survival of juveniles (Gibson, 1994). Recruitment variability has been attributed to factors acting either in estuarine waters or during the estuarine colonization phase (Van der Veer et al., 2000). Quantitative (nursery area) and qualitative factors (e.g. food supply, temperature, and mortality due to predation) have a major influence on recruitment level (overviews in Gibson, 1994; Van der Veer et al., 2000).

Tidal currents, local topography and upwelling events are known to influence larvae retention and transport patterns, which may influence the amount of potential settlers and ultimately recruitment levels (Rijnsdorp et al., 1985; Metcalfe et al., 2006). Also, hydrodynamics, mainly related with river runoff patterns, has been identified as critical for recruitment (e.g. Amara et al., 2000; Metcalfe et al., 2006; Martinho et al., 2009), as well as temperature (e.g. Van der Veer et al., 2001; Le Pape et al., 2003; Attrill and Power, 2004). More recently, large-scale oceanographic patterns have also been related with recruitment variability in marine fishes, largely due to their influence on sea surface temperature, wind and current patterns (Attrill and Power, 2004, Henriques et al., 2007).

Despite the several factors that may influence recruitment processes, it is believed that recruitment success and variability in marine fishes is largely regulated by the demographic changes that occur during the early life stages (Horwood et al., 2000; Brander et al., 2001; Islam et al., 2011). Therefore, a thorough understanding of early life stages is essential for predicting recruitment variability in fishes.

Estuarine ichthyoplankton

The term plankton refers to any small biota (from microns to centimetres) living in the water and drifting with the currents – ranging from bacteria to jellyfish (Rissik and Suthers, 2009). They may be classified either as holoplankton or meroplankton. Contrarily to holoplankton, meroplankton are temporary residents of the plankton community and include larvae of many benthic invertebrate such as polychaetes, molluscs, echinoderms, and decapods (Grantham et al., 2003). Ichthyoplankton comprise fish eggs and larvae that are also found among the meroplanktonic organisms in the water column. However, fish eggs are immotile, whereas larvae swim feebly after hatching, but became more motile as they develop (Moser and Watson, 2006).

The larval stage of fish usually lasts 3 to 4 weeks and the presence of fish larvae can indicate important spawning areas nearby (Suthers et al., 2009). Based on their life history, the majority of fish larvae caught in estuaries can be categorized as estuarine or marine opportunists, and the reduced numbers of larvae of freshwater and marine straggler species depends on the degree of input of marine or freshwater into the system (Potter et al., 1990). Fish early life stages are a particularly vulnerable phase and it is hypothesized that marine fish larvae and juveniles migrate into estuaries to make use of the high food abundance and refuge against predators, in order to maximize survival (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).

The abundance of larvae in estuaries usually shows a seasonal pattern (e.g. Barletta-Bergan et al., 2002; Ramos et al., 2006; Sabatés et al., 2007; Primo et al., 2011, 2012a). These patterns are mainly linked to reproductive strategies of adult population and life cycles (respiration, reproduction, embryonic and gonad development, mortality), which, in turn are often associated with environmental features (Hernández-Miranda et al., 2003). Several physical factors have been reported as influencing ichthyoplankton abundance, such as temperature, salinity, winds, and currents (e.g. Alemany et al., 2006; Azeiteiro et al., 2006; Boeing and Duffy-Anderson, 2008; Ramos et al., 2009; Rodríguez et al., 2009).

In addition to reproductive seasonality, larval fish may also show differences in their spatial patterns of abundance (e.g. with respect to bathymetry or distance to shore). These patterns are particularly strong in areas with ample tidal regimes, such as in estuaries (Kingsford and Suthers, 1996). Species diversity of fish larvae generally decreases with increasing distance from the mouth of the estuary. Although estuarine stations usually have a much lower diversity compared with marine stations, abundances of larvae of estuarine species are usually much higher than for larvae of marine species entering the estuary (Neira and Potter, 1994).

In well-mixed estuaries, much of the spatial distribution of their aquatic inhabitants can be explained by reference to a gradual salinity gradient along the estuary (Kimmerer, 2002). This is especially notable for young individuals of marine species, who temporally use estuaries as nursery and/or feeding grounds. This is because the extent of their penetration and the length of time they remain in estuaries depend on their osmoregulation capability (Fernandéz-Delgado et al., 2007). Salinity has been considered one of the main factors structuring estuarine ichthyoplankton assemblages (e.g. Barletta-Bergan et al., 2002; Faria et al., 2006; Drake et al., 2007; Primo et al., 2011). Freshwater flow is often pointed out as the responsible for the maintenance of the salinity gradient in estuarine ecosystems and thus extremely important in the advective processes occurring in these systems. It is known that management actions regarding freshwater flow influence the longitudinal position of the salinity gradient affecting species recruitment (e.g. Drake et al., 2007; Morais et al., 2009). Nevertheless, apart from anthropogenic action, river inflow in temperate estuaries varies substantially within and among years, and recent climate changes increased this variability, in turn affecting a wide range of estuarine communities.

Impact of climate change on estuarine systems

The way that marine ecosystems respond to overexploitation and changing climate is a principal concern in sustainability science, and understanding how these factors interact to affect marine populations is a challenge for global ecology (Molinero et al., 2009). Due to its sensitiveness in reflecting environmental perturbations, plankton may be a good indicator of climate change in the marine environment (Hays et al., 2005) and according to Boeing and Duffy-Anderson (2008), early life stages may be environmentally sensitive, prior to buffering through density-dependent mechanisms and community effects.

Recently, several global climatic patterns such as the North Atlantic Oscillation (NAO), sea surface temperature (e.g. Atrill and Power, 2002; Molinero et al., 2005; Lynam et al., 2004, 2005), and the El Niño-Southern Oscillation (e.g. Franco-Gordo et al., 2004; Brodeur et al., 2008) have been used to identify climate influence in marine ecosystems. In estuaries, impacts of climate change are often associated with variations in river flow, affecting habitats and organisms. Effects on organisms include mortality, changes in growth and development or organism dislocation (Gillanders and Kingsford, 2002). Seasonal and interannual shifts in the natural fluxes in the flow regime may influence the distribution and abundance of marine, estuarine and freshwater fish species (Hurst et al., 2004; Costa et al., 2007; Baptista et al., 2010). Also, extreme events linked to river flow variations, like floods and droughts, were also associated with shifts in abundance and species composition of estuarine and coastal fisheries landings (Erzini et al., 2005; Meynecke et al., 2006; Gillson et al., 2012). Under the current climate change pattern, extreme weather events are expected to increase in frequency over the next years being therefore essential to understand their influence in the ecosystems. Climatic related reduced river flow influence on communities may vary. Movement is a common response, mainly for motile species (e.g. Attrill and Power, 2000; Martinho et al., 2007a). On the other hand, sessile organisms tolerate or die, depending on the species vulnerability to the stress (e.g. Attrill and Power, 2000; Cardoso et al., 2008). Generally, planktonic organisms follow the salinity gradient displacement since they have low ability to counteract the currents (e.g. Drake et al., 2007; Marques et al., 2007; Primo et al., 2009, 2011). However, this passive nature is not always verified, since that despite their planktonic nature, several organisms, like fish larvae, are able to control their passive drift.

Fish larvae migration and transport patterns

Many planktonic animals can either swim reasonably well, or are able to control their position by selecting different depths and currents and by adjusting buoyancy (Redden et al., 2009). Diel vertical migration (DVM) is one of the most studied plankton migration processes and occurs in a wide range of both freshwater and marine zooplankton taxa, representing probably the biggest synchronized animal migration on the planet, in terms of biomass (Hays, 2003). Vertical migration patterns have been studied in a wide range of marine organisms such as decapods larvae (e.g. Queiroga et al., 2007; Tamaki et al., 2010), copepods and cladocerans (e.g. Haupt et al., 2009; Ueda et al., 2010), and fish larvae (e.g. Auth et al., 2007; Voss et al., 2007; Leis, 2010; Primo et al., 2012b). Diel migratory behaviour is triggered by changes in light intensity, and is largely an adaptation to avoid visually feeding predators, particularly fish (Redden et al., 2009). It has also been related with optimum light conditions and suitable prey concentrations (e.g. Brodeur and Rugen, 1994; Irigoien et al., 2004). The most common DVM pattern concerns ascending to the upper water column during night and descending into deeper water during the day (e.g. Tsukamoto et al., 2001; Auth et al., 2007). However, the reverse pattern has also been observed (e.g. Brodeur and Rugen, 1994; Bradbury et al., 2006).

Tidally timed migrations appear to be much less common than diel migrations, but recent studies made across different invertebrate and fish taxa indicate that many species that inhabit estuaries migrate in synchrony with the tidal cycle. Tidal vertical migration is a process generally linked to organisms' horizontal transport, retention or dispersion mechanisms (reviewed by Forward and Tankersley, 2001; Gibson, 2003). This behaviour can be critical to fish larvae survival, since it allows their entrance in estuaries where the abundance of food and refuges is higher, thus affecting the recruitment success of both estuarine and non estuarine-dependent species (Parrish et al., 1981).

Selective tidal-stream transport (STST) in one of the most common estuarine transport mechanisms, where organisms make use of strong tidal currents over one part of the tidal cycle (either ebb or flood) to transport them in the required direction (Forward and Tankersley, 2001). During flood tide transport, larvae ascend actively in the water column and return to the bottom when the tide turns. This behaviour allows larvae to move upstream in the estuary reaching their nursery areas (Jager, 1999; DiBacco et al., 2001). In ebb tide transport, larvae ascend in the water column during ebb allowing organisms to move downstream or leave the estuary and joining their adults' habitats (e.g. Barletta and Barletta-Bergan, 2009).

Migration patterns can be variable, and are known to differ with the sex and age of the species, habitat or season (Redden et al., 2009; Queiroga et al., 2007). Much research focuses on distinguishing which physical and biological signals induce these migratory behaviours (e.g. Strydom and Wooldridge, 2005; Garrido et al., 2009; Haslob et al., 2009), but these processes are often complicated by their multiple confounding interactions.

Study site: the Mondego estuary

The study presented in this thesis was carried out in the Mondego estuary (40°08'N; 8°50'W). The Mondego estuary is a mesotidal estuary located in the western Atlantic coast of Portugal, a warm temperate region (Fig. 1). The hydrological basin of the Mondego, with an area of 6670km^2, provides an average freshwater flow rate of 79m^{3}s^{-1} (Dolbeth et al., 2010). This well-mixed estuary consists of two channels, north and south, separated by an alluvium-formed island, the Murraceira Island (Fig. 1). The north arm is deeper (5-10m at the high tide), has a tidal range of 2-3m and constitutes the main navigation channel and the location of the Figueira da Foz harbour. It is subjected to regular dredging activities and connects directly to the Mondego River. The south arm is shallower (2–4 m at high tide), has a tidal range of 1–3m and is characterised by large areas of exposed intertidal flats during low tide with several seagrass meadows (*Zostera noltii*) and saltmarshes (*Scirpus maritimus*, *Spartina maritima*) at the downstream areas (Lillebø et al., 2004). Most of the freshwater discharge occurs through the north arm, while in the southern arm water circulation is mostly due to tides and to the freshwater input from a small tributary, the Pranto River, a small tributary artificially regulated by a sluice, according to the water needs of the surrounding rice fields. The estuary occupies an area of 8.6Km^2, where 75% corresponds to intertidal mudflats in the south arm, and less than 10% in the north arm.

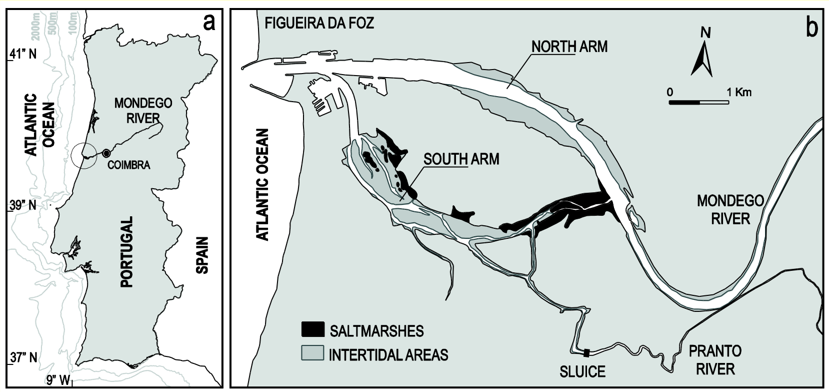

Figure 1. Location of the Mondego estuary on the west coast of Portugal (a); detailed scheme of the estuary showing the two arms and the intertidal and saltmarsh areas (b).

The Mondego estuary is a transitional system with intense and conflicting human activities. Over the period of 1990-1998, the ecological quality in south arm declined due to several human activities (harbour facilities, aquaculture farms, intensive agriculture with abusive fertilizer use in nearby rice fields, and salt-work) that contributed to an eutrophication process, which directly affected *Z. noltii* meadows (Dolbeth et al., 2011; Grilo et al., 2012). Presently, the south arm is gradually recovering from the negative effects imposed by organic pollution, due to the implementation of a restoration programme in 1998, whose major aim was to restore the original condition of the system. Application of physical restoration measures, coupled with public education, highlighting the ecological importance of conserving intertidal vegetation for the health and related socioeconomic activities of the estuary, resulted in: 1) significant nutrient loading reduction; 2) improvement of water dynamics and transparency, by reestablishment of the south arm riverhead connection; and 3) seagrass bed protection from human disturbance (Cardoso et al., 2008; Dolbeth et al., 2011; Grilo et al., 2012).

Over the last years, research conducted in the Mondego estuary provided a comprehensive dataset on several areas, such as nutrient dynamics (e.g. Coelho et al., 2004), intertidal and subtidal benthic invertebrates (e.g Cardoso et al., 2008; Verdelhos et al., 2005; Viegas et al., 2007), plankton (e.g. Marques et al., 2007; Primo et al., 2009, 2011) and fish (Dolbeth et al., 2008, 2010; Leitão et al., 2007; Martinho et al., 2007a,b; 2008). The Mondego estuary has been described as an active nursery area for marine fish (Leitão et al., 2007; Martinho et al., 2007a,b; 2008; Dolbeth et al., 2008), and identified as an important nursery origin for stocks off the central Portuguese coast (Vasconcelos et al., 2008).

In the last years, Portugal has experienced low precipitation values compared to the general climate pattern for the period of 1971-2000 (Fig. 2). Mainly, the hydrological year of 2004-2005, with precipitation values 45-60% below average and significant reduction in freshwater flow, originated the biggest drought of the last century (INAG – Portuguese Water Institute, http://www.snirh.inag.pt; IM – Portuguese Weather Institute, http://www.meteo.pt). Several of the recent studies in the estuary focused on these climate induced changes in the estuarine pelagic and benthic communities.

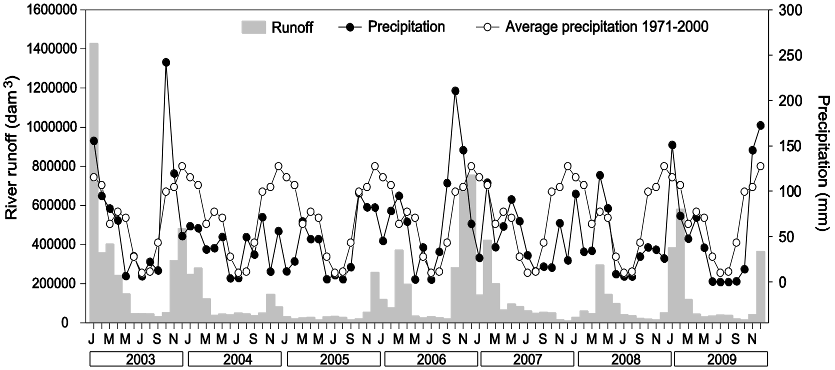

Figure 2. Monthly river runoff (dam^3) and average of precipitation (mm) compared with the average precipitation for the 1971-2000 period in Mondego estuary.

General Objectives

The main purpose of this study was to investigate the early life-stages of fishes occurring in the Mondego estuary. The importance of estuaries as fish nurseries increases during the initial phase of life cycle, since a successful recruitment depends on larval transport and settlement on suitable habitats (Bailey et al., 2008). Initial studies were conducted by Ribeiro (1991) but after that, only Marques et al. (2006) addressed very briefly those communities; thus, information about these assemblages and their dynamic is limited and outdated. In this way, it is crucial to understand the dynamics of fish larvae entrance in the estuary, as well as the main factors responsible for their spatial and temporal patterns. Further, the integration of both benthic and pelagic larval phases is essential for a more comprehensive assessment of the nursery importance of estuaries.

Considering this, several field studies were conducted and are presented in this thesis in the following four chapters. The first chapter aimed to characterize ichthyoplankton assemblages and evaluate the environmental influence in their structure. In addition, and taking advantage of the natural extreme weather events that occurred during the studied period, the consequences of drought events on estuarine fish larvae communities were evaluated (Chapter II). In the following chapter, the interface dynamics between the estuary and adjacent coastal area was investigated by determining the effect of lunar tidal cycle and season in fish larvae entrance, upstream transport and spatial distribution (Chapter III). Chapter IV focused on fish larvae migration patterns and on the influence of diel and tidal cycle on larval fish vertical distribution over one neap and one spring tide during two seasons (winter and summer). It describes how larval vertical distributions differed among species and which transport mechanisms could result from these distributions. Finally, chapter V integrates larval and benthic life-stages of the main flatfish species present in the estuary, in order to assess the potential nursery role of the Mondego estuary for flatfish species.

CHAPTER II

Impact of climate variability on ichthyoplankton communities in a small European temperate estuary

ABSTRACT

Recently, variations in precipitation regime across south Europe led to changes in river fluxes and salinity gradients in most of the European rivers and estuaries affecting biological communities. Since that, in the Mondego estuary, a sampling program was developed from January 2003 to December 2008 at five distinct sampling stations being spatial, seasonal and interannual distributions of fish larvae evaluated. Gobiidae was the most abundant family representing 80% of total catch and *Pomatoschistus* spp. was the most important taxon. Fish larvae community presented a clear seasonality with higher abundances and diversities during spring and summer seasons. Multivariate analysis reinforced differences among seasons but the same did not happen between years or sampling stations. In this way*, Atherina presbyter, Solea solea, Syngnathus abaster, Crystallogobius linearis* and *Platichthys flesus* presented higher abundances during spring/summer period while *Ammodytes tobianus, Callionymus sp., Echiichthys vipera* and *Liza ramada* were higher in autumn/winter*.* Temperature, chlorophyll *a* and river flow were indicated as the main variation drivers observed however, extreme drought events (year 2005) seemed not to affect ichthyoplankton community structure. Main changes were related to spatial displacement of salinity gradient along the estuarine system leading to analogous changes in marine species distribution.

Keywords: Ichthyoplankton; seasonality; environmental factors; drought; Mondego estuary.

Introduction

Nearshore estuarine and marine ecosystems serve many important functions in coastal waters. Often referred as nurseries, estuaries play an important role in many species lifecycles, including fish (Beck et al., 2001; Elliott and McLusky, 2002; Martinho et al., 2007a) providing food abundance and shelters to marine fish larvae and juveniles and therefore maximizing their survival (Whitfield, 1999; Elliott and McLusky, 2002).

Larval fish dynamics can contribute significantly to understand the biology and ecology of fish populations (Doyle et al., 2002) since it can be an indicator of the spawning-stock biomass and recruitment in adult fish stocks (Hsieh et al., 2005). Initial development stages of fishes are particularly vulnerable, depending on physical and biological processes. Indeed, several factors had already been related with survival and distribution of ichthyoplankton (e.g. hydrological conditions, transport processes, seasonal variability, spawning patterns of adults, food availability) (Franco-Gordo et al., 2002; Alemany et al., 2006; Sabatés et al., 2007; Isari et al., 2008). Effects of climate on fish populations can also be provided by long term trends in ichthyoplankton populations. Lower trophic level organisms should be more sensitive reflecting responses to environmental perturbations more quickly than higher trophic levels but early life stages may be environmentally sensitive prior to buffering through density-dependent mechanisms and community effects (Boeing and Duffy-Anderson, 2008). Knowledge of the ichthyoplankton community dynamics may represent an important role to understand changes in fish communities.

Recent studies pointed out Mondego estuary (40° 08’ N, 8° 50’ W) as an important nursery ground for several commercial fish species (e.g. *Dicentrarchus labrax*, *Platichthys flesus* and *Solea solea*) (Leitão et al., 2007; Martinho et al., 2007a). Studies about ichthyoplankton started with Ribeiro (1991) but recently only Marques et al. (2006) referred to these communities. Previous works focused in community assemblages but indication about environmental factors forcing community structure is still very limited. In addition, recently Portugal has been under varying precipitation regimes presenting values of 45–60% below average in hydrological year 2004/2005 resulting in the biggest drought of twentieth century (Portuguese Weather Institute: http://www.meteo.pt/en/index.html) providing a unique opportunity to investigate ichthyoplankton responses to extreme events. In this way, this study aimed to characterize ichthyoplankton assemblages, evaluate environmental influence in its structure and establish which consequences extreme events, such as droughts, might have on estuarine fish larvae communities. The tested hypothesis was that reduced river flow resulted from the decrease of precipitation mean levels lead to changes on community structure and longitudinal displacement of species according to salinity gradients.

Material and Methods

Study area

The Mondego estuary, located on the Atlantic coast of Portugal (40° 08’ N, 8° 50’ W), consists of two channels (northern and southern) with different hydrological characteristics separated by the Murraceira Island (Fig. 1). The north channel is deeper (4–8m depth at high tide) has lower residence times (<1 day) and constitutes the main navigation channel, while the south channel is shallower (2–4m deep, at high tide) has higher residence times (2–8 days) and is almost silted up in the upper areas. Most of the freshwater discharge is throughout the northern channel since it is directly connected with the Mondego River. In the southern channel, water circulation is mostly due to tides and the freshwater input from a small tributary, the Pranto River which is small and artificially regulated by a sluice. Previous studies demonstrated that distinct environmental factors provide a large variety of aquatic habitats for populations of marine, brackish and freshwater zooplankton species, mainly due to salinity and water temperature gradients (Azeiteiro et al., 1999; Marques et al., 2006; Primo et al., 2009).

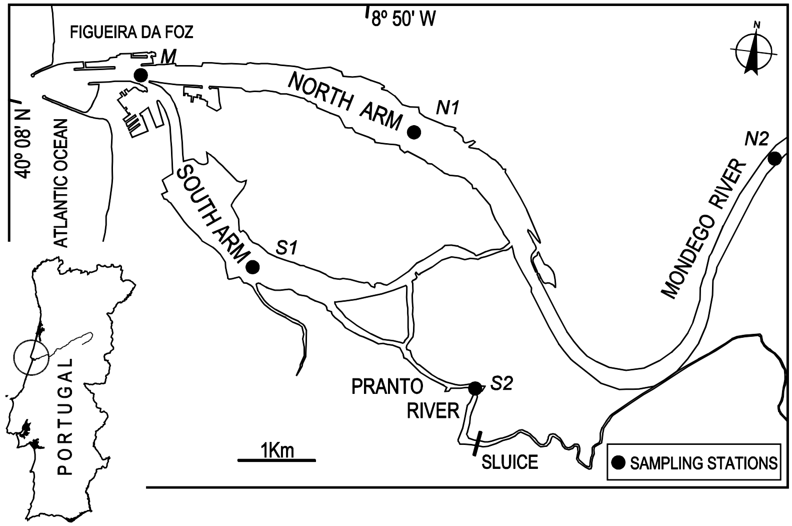

Figure 1. Map of the Mondego estuary, located on the western coast of Portugal. Sampling stations surveyed in this study are indicated (M, mouth station; S1 and S2, southern arm stations; N1 and N2, northern arm stations).

Sample collection

Sampling was carried out monthly during daylight at high tide, from January 2003 to December 2008 at five stations distributed throughout both arms (Fig. 1). Samples were collected by horizontal subsurface tows (Bongo net: mesh size 335 µm, mouth diameter: 0.5 m, tow speed: 2 knots), equipped with a Hydro-Bios flowmeter (volume filtered average 45 m^3) and preserved in a 4% buffered formaldehyde seawater solution. Additionally, at each site, salinity, water temperature (°C), dissolved oxygen (mg l^{-1}), pH and turbidity (Secchi disc depth, m) were also recorded. Subsurface water samples were also collected for subsequent determination in laboratory of chlorophyll *a* (mg m^{-3}) and total suspended solids (mg l^{-1}). In the laboratory, ichthyoplankton was sorted, enumerated (number of individuals per 100 m^3) and identified to lowest possible taxa (Petersen, 1919; Fives, 1970; Nichols, 1976; Demir, 1976; Russell, 1976; Ré, 1999; Ré and Meneses, 2008). Copepod densities (ind. m^{-3}) were also recorded. Monthly precipitation values were acquired from INAG – Instituto da Água (http://snirh.pt/) measured at the Soure 13 F/01G station and freshwater discharge from Mondego River was obtained from INAG station Açude Ponte Coimbra 12G/01AE.

Data analysis

Sampling months were combined into four conventional seasons: winter (W) included December, January and February; spring (S), March, April and May; summer (SM), June, July and August and autumn (A), September, October and November. Species were characterized in three main ecological guilds (adapted from Elliott et al., 2007): marine stragglers (MS - Species that spawn at sea and typically enter estuaries in low numbers occurring frequently in the lower reaches), marine migrants (MM - Species that spawn at sea and often enter estuaries in large numbers) and estuarine species (ES – including estuarine species capable of completing their entire life cycle within the estuarine environment and those with stages of their life cycle completed outside the estuary).

Salinity anomalies were calculated by subtracting the mean seasonal value from the mean value of the given time period. Differences between seasons and years in each sampling stations were tested by Analysis of Variance (ANOVA) for environmental factors. Log (x+1) transformation was performed and for pairwise multiple comparisons Holm-Sidak method was applied. Temporal and spatial ichthyoplankton distribution maps were obtained by Sigmaplot software as well as diversity, expressed by Shannon-Wiener Index (log 2).

PERMANOVA+ for PRIMER software (PRIMER v6 & PERMANOVA+ v1, PRIMER-E Ltd.) was used to perform a non-parametric permutational multivariate analysis of variance (PERMANOVA) to test for differences in the assemblage structure between years, seasons and sampling stations. Analysis was based on Bray-Curtis similarities between samples, after a fourth root transformation of abundance data, considering all the factors (year, season, station) as fixed and unrestricted permutation of raw data. When necessary, *a posteriori* multiple comparisons were used to test for differences between/within groups for pairs of levels of factors.

Effects of environmental variables on larval fish assemblage were analyzed with canonical correspondence analysis (CCA) using software CANOCO (version 4.5, Microcomputer Power). Environmental variables included salinity (Sal), water temperature (T) dissolved oxygen (O2), pH, turbidity (Secchi), chlorophyll *a* (Chl a), total suspended solids (TSS), copepod densities (Cop), freshwater discharge (Runoff) and precipitation (PP). Seasons were also included as nominal variables. All species were used and a new category coded as "no fish" was created to prevent CANOCO from eliminating samples containing no fish. "No fish" was assigned the minimum possible weight (density=0.001) to prevent an otherwise uniform concentration in samples from driving the ordination (Grothues and Cowen, 1999). Larval abundances were ln(2x+1) transformed and environmental variables were standardized by subtracting the mean and dividing by the standard deviation. A forward stepwise selection procedure of explanatory variables was applied and a CCA triplot scaling with focus interspecies distances was performed.

Results

Environmental conditions

In Mondego estuary was observed a typical seasonal pattern of precipitation and freshwater discharge along the six-year period with higher values during winter and lower during summer. However 2004, 2005 and 2008 years showed below-mean precipitation values and presented a low freshwater discharge, particularly the year of 2005 (Fig. 2).

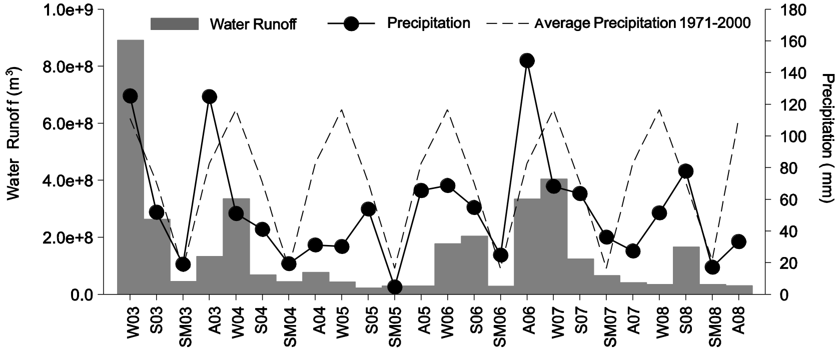

Figure 2. Seasonal water runoff (m³) and average of precipitation (mm) in Mondego estuary during the study period.

Salinity was highly variable between years with 2005 and 2008 presenting positive anomalies in all sampling sites during almost all seasons indicating higher salinity values than average (Fig. 3). In fact, salinity values recorded at sampling stations M and S2 in 2005 showed to be significantly higher than in 2006 (*post hoc* test $p<0.05$ and $p<0.01$, respectively). Also, upstream sampling station N2 presented significant higher salinity in 2005 and 2008 than in 2003 and 2006 (*post hoc* test $p<0.01$). Seasonally, only sampling stations M and N2 presented significant differences with winter presenting lower salinity than summer, autumn and spring (*post hoc* test $p<0.01$).

Water temperature showed a similar pattern across the years with higher values in summer and lower in winter (Fig. 3). All sampling stations showed significant differences between seasons with winter presenting lower values than summer, autumn and spring (*post hoc* test $p<0.001$). No significant differences were detected between years at any sampling station ($p>0.05$).

Chlorophyll *a* reached higher values in the most upstream sampling stations (Fig. 3), however no significant differences were detected between years ($p>0.05$) and only sampling station N2 presented significant higher chlorophyll *a* in summer than in autumn ($F=3.826$, $p<0.05$; *post hoc* test $p<0.01$).

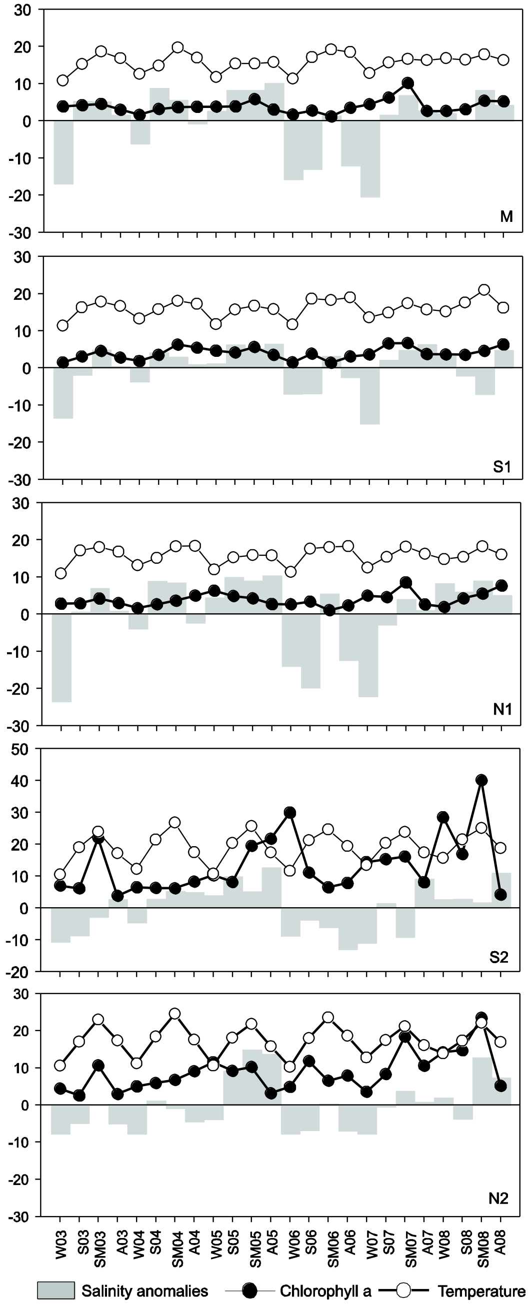

Figure 3. Seasonal average water temperature (°C), chlorophyll *a* (mg m^{-3}) and salinity anomalies in Mondego estuary during the study period.

Seasonal and spatial patterns of larval distribution

During the study period, a total of 7211 fish larvae were collected in Mondego estuary. Fish larvae were identified in 31 different *taxa* (Table I).

Table I. Mean Density (larvae 100m^{-3}) of species caught during the sampling period and relative contribution (%) to the total catch in whole estuary, in each season and sampling station. MD – Mean Density; W – winter; S – spring; SM – summer; A – autumn.

Family	**Species**	**CCA CODE**	**Ecological guild**	**Total catch**		**Season (%)**				**Sampling station (%)**				
				MD	**%**	**W**	**S**	**SM**	**A**	**M**	**S1**	**S2**	**N1**	**N2**
Gobiidae	*Pomatoschistus* spp.	Pspp	ES	70.65	63.40	54.42	58.26	68.52	65.39	3.19	18.52	16.35	7.74	17.59
Not identified	Not identified	-		9.52	8.75	6.69	13.54	4.20	12.31	3.82	2.20	1.01	1.50	0.22
Gobiidae	*Pomatoschistus microps*	Pmic	ES	6.63	6.02	3.44	9.92	3.20	6.76	0.05	0.98	2.61	1.39	0.98
Gobiidae	Gobiidae n. id.	Gobi	ES	5.37	4.65	15.29	4.49	3.23	3.09	0.74	0.40	2.56	0.50	0.46
Gobiidae	*Gobius niger*	Gnig	ES	3.79	3.57	1.22	2.85	5.59	0.62	0.06	1.62	1.24	0.29	0.36
Engraulidae	*Engraulis encrasicolus*	Eenc	MS	2.01	1.96	0.49	-	4.16	0.83	-	0.12	1.78	-	0.06
Blenniidae	*Parablennius pilicornis*	Ppil	MS	2.02	1.88	-	1.36	3.08	0.60	0.13	0.93	0.10	0.24	0.47
Gobiidae	*Gobius* spp.	Gspp	ES	2.49	1.86	1.17	2.05	1.63	2.52	0.07	0.90	0.73	0.11	0.04
Soleidae	*Solea senegalensis*	Ssen	MM	1.51	1.33	3.19	0.55	1.37	1.97	0.32	0.44	0.04	0.39	0.14
Soleidae	Soleidae n. id.	Solei		1.32	1.06	1.65	0.86	0.26	3.60	0.09	0.48	0.16	0.28	0.06
Syngnathidae	*Syngnathus acus*	Sacu	ES	0.96	0.85	-	0.37	1.55	0.40	0.04	0.17	0.16	0.24	0.24
Gobiidae	*Aphia minuta*	Amin	MS	0.61	0.59	-	1.73	-	-	-	0.08	0.51	-	-
Blenniidae	*Coryphoblennius galerita*	Cgal	MS	0.55	0.49	-	0.75	0.42	0.42	0.27	0.08	-	0.10	0.05
Clupeidae	*Sardina pilchardus*	Spil	MM	0.50	0.42	0.40	0.50	0.18	0.97	-	0.17	0.04	0.17	0.04
Blenniidae	*Lipophrys pholis*	Lpho	MS	0.38	0.38	2.38	0.44	-	0.22	0.12	0.15	-	0.08	0.03
Ammodytidae	*Ammodytes tobianus*	Atob	MS	0.35	0.32	3.86	-	-	-	-	0.27	-	0.06	-
Atherinidae	*Atherina presbyter*	Apres	ES	0.32	0.28	-	0.30	0.40	-	0.19	0.08	-	-	-
Atherinidae	*Atherina* spp.	Aspp	ES	0.44	0.27	-	0.12	0.52	-	-	0.15	-	0.12	-
Soleidae	*Solea solea*	Ssol	MM	0.26	0.26	-	0.15	0.48	-	-	0.26	-	-	-
Gobiidae	*Pomatoschistus minutus*	Pmin	ES	0.23	0.23	-	0.68	-	-	-	0.10	0.09	0.04	-
Syngnathidae	*Syngnathus* spp.	Sygn	ES	0.22	0.21	-	0.44	0.14	-	-	0.19	-	-	0.02
Blenniidae	Blenniidae n. id	Blenn		0.23	0.17	1.35	0.17	-	-	-	-	0.06	-	0.11
Mugilidae	*Liza ramada*	Lram	CA	0.20	0.16	1.07	-	0.17	-	0.07	-	0.09	-	-
Labriidae	*Symphodus melops*	Smel	MS	0.15	0.15	-	0.17	0.22	-	-	0.15	-	-	-
Syngnathidae	*Syngnathus abaster*	Saba	ES	0.26	0.13	-	-	0.30	-	-	0.07	-	-	0.06
Callionynidae	*Callionymus* spp.	Cspp	ES	0.17	0.11	1.35	-	-	-	0.06	-	-	0.06	-
Trachinidae	*Echiichthys vipera*	Evip	MS	0.17	0.11	1.35	-	-	-	0.06	-	-	0.06	-
Gobiidae	*Crystallogobius linearis*	Clin	MS	0.24	0.11	-	-	0.25	-	-	0.07	0.04	-	-
Pleuronectidae	*Platichthys flesus*	Pfle	MM	0.10	0.10	-	0.30	-	-	-	-	-	-	0.10
Ammodytidae	Ammodytidae n. id.	Amm		0.09	0.06	0.68	-	-	-	-	-	-	-	0.06
Bothidae	*Arnoglossus thori*	Atho	MS	0.05	0.05	-	-	0.12	-	-	-	0.05	-	-
Blenniidae	*Lipophrys* spp.	Lspp	MS	0.04	0.04	-	-	-	0.29	-	-	0.04	-	-
Total Number of Larvae				**7211**		**608**	**2449**	**3112**	**1042**	**669**	**2060**	**1995**	**965**	**1522**
Number of Species				**31**		**17**	**22**	**22**	**15**	**17**	**25**	**19**	**18**	**19**

Unidentified larvae represented 8.75% of total catch and were generally yolk-sac or damaged larvae. The most abundant family was Gobiidae (80%) followed by Blenniidae (3%), Soleiidae (3%) and Engraulidae (2%). *Pomatoschistus* spp. was the most abundant *taxon* accounting for 63.4% of all fish larvae caught, followed by *Pomatoschistus microps* (6.02%), unidentified Gobiidae (4.65%), *Gobius niger* (3.57%), *Engraulis encrasicolus* (1.96%) and *Parablennius pilicornis* (1.88%). All together, these species contributed for 81% of total catch and are present in almost every seasons and sampling stations. Summer and spring presented a higher number of species as well as sampling station S1 while autumn seemed to present lower diversity. Species like *Atherina presbyter*, *Solea solea*, *Symphodus melops*, *Syngnathus abaster*, *Crystallogobius linearis*, *Platichthys flesus* and *Arnoglossus thori* were exclusively captured during spring and summer. On the other hand, *Solea senegalensis*, *Ammodytes tobianus*, *Callionymus* spp., *Echiichthys vipera* and unidentified Ammodytidae were more abundant in winter.

Fish larvae density presented a clear sign of seasonality with higher densities during spring and summer (Fig. 4). According to ecological guilds, estuarine species showed greatest densities during all study period except for year 2008 where marine stragglers abundance reached maximum values. It was also observed that estuarine taxa showed peaks of densities during summer except in 2004 and 2005 where higher values occurred early in spring (Fig. 4).

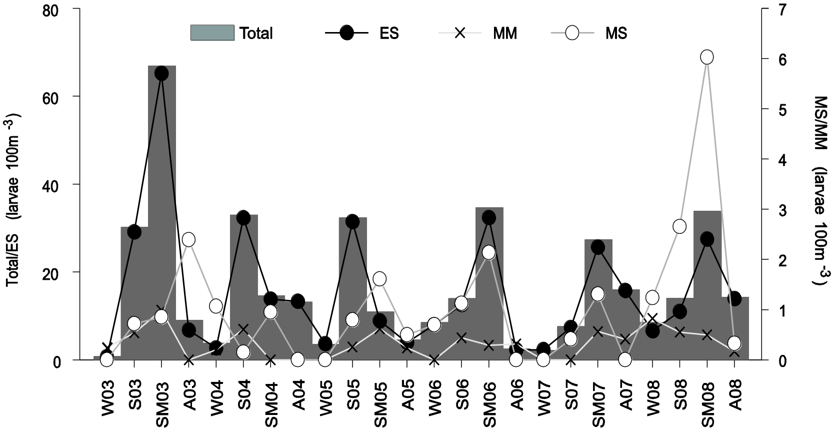

Figure 4. Seasonal density (larvae 100m^{-3}) of total fish larvae and of each main ecological guild. ES – Estuarine species; MS – Marine Stragglers; MM – Marine Migrants.

Generally, these species were well distributed along the estuary with highest densities in downstream south arm station (S1) and in upstream estuary (S2 and N2) (Fig.5A). On the other hand, marine stragglers occurred mainly in station S1 reaching upstream stations only in year 2008 (Fig. 5B). The same happened with marine migrant species that during 2005 and 2008 reached upper estuary (N1, N2 and S2) (Fig. 5C).

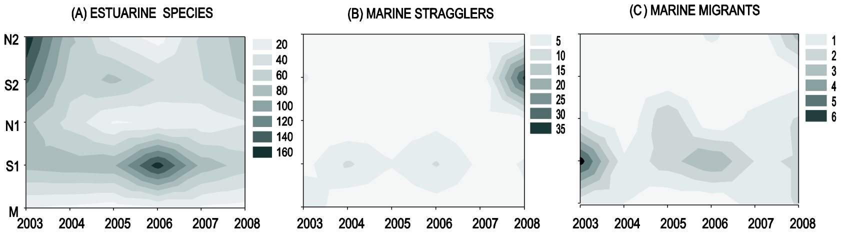

Figure 5. Interannual and spatial density (larvae 100m^{-3}) distribution of the three main ecological guilds. (A) Estuarine Species; (B) Marine Stragglers; (C) Marine Migrants.

Pomatoschistus spp. abundance and distribution mimic the total fish larvae pattern since it is the most important species in Mondego estuary (Fig. 6). Sampling station S1 seem to present higher abundances across the years. *Engraulis encrasicolus* attained higher densities during 2008 and was found especially in upper south arm (station S2) (Fig. 6). *Solea senegalensis* presented seasonal peaks mainly in summer and spring but occasionally also in winter and autumn (Fig. 6). Its spatial distribution is generally restricted to downstream sampling stations M and S1 but in 2005 and 2008 it reached high densities in upstream sampling stations S2 and N2. Seasonality was evident also in Shannon-Wiener diversity index showing higher values in spring/summer (Fig.6).

Community structure and relation with environmental variables

According to PERMANOVA results, community structure presented significant differences between years and seasons, as well as significant interaction between factors "year" and "season" (Pseudo F= 1.429, p(perm)<0.05). Pairwise *a posteriori* comparison revealed that in summer, year 2003 presented a distinct community from the remaining years (t<2.060, p(perm)<0.05). Autumn and winter presented no differences between years (p(perm)>0.05) and in spring only pairwise comparison 2003/2008 presented significant differences (t= 1.582, p(perm)<0.05). In adition 2003, 2006 and 2007 showed differences between seasons with summer presenting a distinct community from autumn and winter (p(perm)<0.05). In 2008 summer presented differences only from winter (p(perm)<0.01) and in 2004 and 2005 no seasonal differences were detected (p(perm)>0.05).

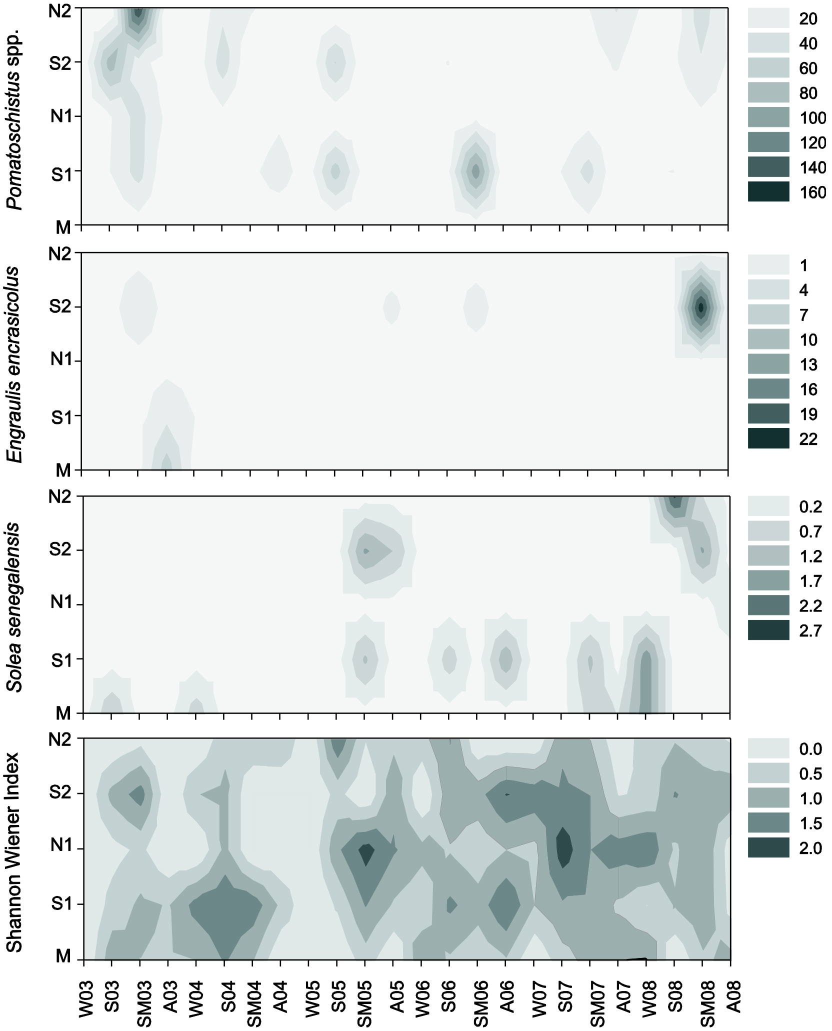

Figure 6. Temporal and spatial density (larvae 100m^{-3}) distributions of *Pomatoschistus* spp., *Engraulis encrasicolus*, *Solea senegalensis* and Shannon Wiener index (log2) in the Mondego estuary.

Community differences between sampling stations were also detected (Pseudo F= 2.003, p(perm)<0.01) but only between sampling station M and S1 (Pairwise *a posteriori* comparison t=2.186, p(perm)<0,001).

According to Monte Carlo test of F-ratio, only six environmental variables contributed significantly to the explanation of species distribution ($p<0.05$) (winter, salinity, temperature, chlorophyll *a*, summer and runoff). Together, environmental variables considered in the final CCA explained 8% of the total variation in fish larvae assemblages. The first two CCA axes accounted for 57% of the variability explained. First axis has tough correlation with winter while second axis is positively correlated with summer, temperature, chlorophyll *a* and salinity and negatively with runoff. The ordination diagram of the first two axes shows that down right side of ordination plot grouped winter samples characterized by low temperatures and high river flow (Fig. 7). Species like *Echiichthys vipera*, *Callionymus* spp. and *Liza ramada* were more correlated with winter. On the other hand, summer samples clustered in upper left side are more related with *Atherina presbyter*, *Crystallogobius linearis*, *Syngnathus abaster*, *Syngnathus acus* or *Solea solea* (Fig. 7).

Discussion

Larval fish assemblages of Mondego estuary presented 31 taxa dominated by *Pomatoschistus* spp. Dominance by few species and presence of a high number of rare species is a common feature observed in estuaries around the world either in larval or juvenile fish populations (e.g. Barletta-Bergan et al., 2002; Strydom et al., 2003; Selleslagh et al., 2009). *Pomatoschistus* spp. larvae dominance was already referred in other Portuguese estuaries (e.g. Faria et al., 2006; Ramos et al., 2006). Gobies success in estuarine environment may be related with their benthic reproductive strategy ensuring that eggs are not flushed out from the estuary and are less exposed to salinity and temperature fluctuations, which are more pronounced in surface waters (Ribeiro et al., 1996). Also, Mazzoldi and Rasotto (2001) suggested that in highly productive habitats with warm summers, long breeding season of short living species (such as *Pomatoschistus microps*) can give rise to more than one spawning peak in the breeding period, which may be the case of *P. microps* in the Mondego estuary as already been noticed by Dolbeth et al. (2007). In the Mondego estuary, *P. microps* and

Pomatoschistus minutus are among the most abundant in estuarine fish assemblages (Leitão et al., 2007; Martinho et al., 2007a) but, unfortunately, during early life stages these species cannot be easily differentiated.

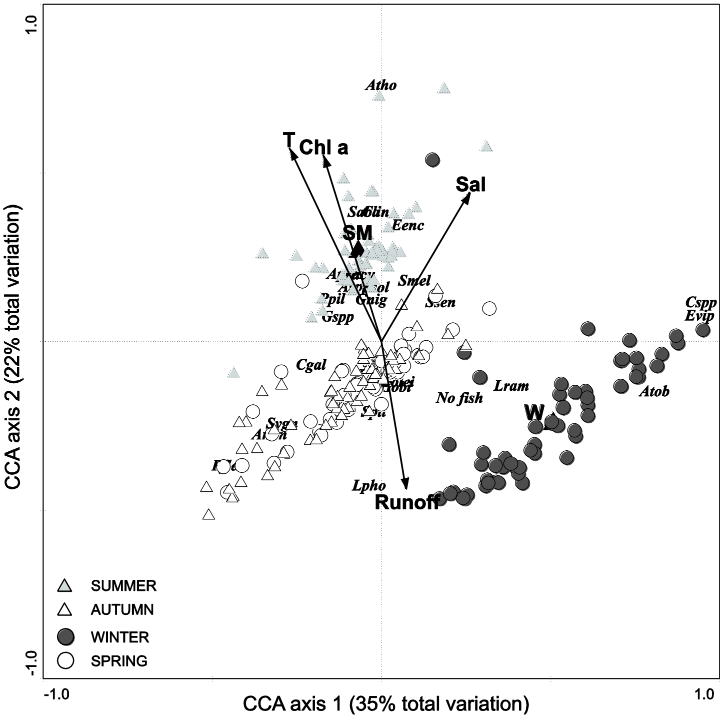

Figure 7. Triplot ordination diagram of the larval fish assemblages in Mondego estuary using the first two canonical correspondence axes. Samples were classified in winter, spring, summer and autumn. Significant environmental variables are plotted as arrows (T – temperature; Chl a – chlorophyll *a*; Sal – salinity; Runoff – river discharge) or nominal variables (W – winter; SM – summer). Species codes are presented in Table I.

According to Ribeiro and Gonçalves (1993) *Engraulis encrasicolus* captures in Mondego estuary accounted for 44% of total fish larvae while in present study the species only represents 2% of total capture. This decreasing tendency was also recently recorded in Guadiana estuary (Faria et al., 2006) and in Lima estuary where this species was present occasionally and in low numbers (<1%) (Ramos et al., 2006). Abundance and distribution of anchovy is closely related with environmental factors as temperature, turbidity, salinity or prey availability (Ribeiro et al., 1996, Chícharo et al., 2001; Drake et al., 2007). Probably, salinity gradient changes due to dry events stimulated anchovy to spawn in inner parts since that *E. encrasicolus* modify its spatial position in order to remain within limited intervals of salinity (Drake et al., 2007). Anchovy abundances increased in coastal adjacent areas of Guadiana estuary during high river flow periods and authors suggest that a reduction of inflow may have more negative consequences for eggs and larval stages that are more susceptible (Chícharo et al., 2001).

The majority of taxa displayed a seasonal pattern presenting higher abundances during spring and summer. Multivariate analysis confirmed distinct seasonal communities. However this seasonal sign decreases during years with low freshwater discharges and consequent higher salinity anomalies. Indeed differences among seasons were higher than across years or sampling stations. Several studies had already shown that temporal changes in composition and abundance are mostly related to spawning patterns of adult fishes (e.g. Barletta-Bergan et al., 2002; Ramos et al., 2006; Sabatés et al., 2007). In this way, *Atherina presbyter*, *Solea solea*, *Syngnathus abaster*, *Crystallogobius linearis* and *Platichthys flesus* presented higher abundances during spring/summer period while *Ammodytes tobianus*, *Callionymus* sp., *Echiichthys vipera* and *Liza ramada* were higher in autumn/winter. Spatial distribution indicated that fish larvae predominated in inner areas of estuary, mainly in the south arm probably due to the higher residence time thus avoiding being washed out by river flux. On the contrary, sampling station M, at the mouth of the estuary, presented a distinct fish larvae assemblage with lower number of species and abundances.

Seasonal changes in temperature, chlorophyll *a* and runoff seem to be the main factors forcing larval fish assemblages' distribution leading to a decrease in abundance and diversity in fish larval communities during colder months. Seasonal variations in environmental parameters seemed to influence the assemblage structure but the same did not happen considering inter-annual changes in the last six years. Mondego estuary has recently experienced periods of low precipitation regime resulting in reduced freshwater runoff and consequently changes in salinity gradients and on estuarine communities of different trophic levels (e.g. Marques et al., 2007; Martinho et al., 2007b; Cardoso et al., 2008). In 2004/2005 dry years was detected an increase in zooplankton density, a higher abundance and prevalence of marine species along the year and a replacement of the freshwater community by one predominantly dominated by estuarine organisms in most upstream areas (Marques et al., 2007; Primo et al., 2009). On the other hand, in juvenile fish assemblages the main drought-induced effects detected were related with a depletion of freshwater species and an increase in marine adventitious (Martinho et al., 2007b). Lack of close correlation between fish populations and environmental signals is a sign that species might show nonlinear responses to external forcing (Hsieh et al., 2005). In fact, drought seemed to have a similar impact in larval fish assemblages. Despite community structure remains relatively unchanged, as indicated by multivariate analysis, the main effects detected were observed in species distribution. During years with positive salinity anomalies (mainly 2005 and 2008) marine species (both stragglers and migrants) were able to reach upstream parts of estuary in higher densities. Short term fluctuations in larval abundances are mostly related with reproductive output or geographic shifts (Hsieh et al., 2005). According to Fernández-Delgado et al. (2007), temporal changes in freshwater discharge cause longitudinal displacement of estuarine salinity gradient leading to analogous changes in marine species distribution. Also, during 2004 and 2005 the seasonal peak occurred in spring regardless of summer, as recorded during all the other years. Early timing of seasonal peaks is an important response to climate change since it can influence trophic interactions leading to eventual ecosystem-level changes (Edwards and Richardson, 2004). Short-term drought events seem to have low influence in fish communities probably because fish species are characterized by slow response time to disturbance (Cabral et al., 2001). Also, number of influential factors is too large and individual species may differ too widely in their response. Nevertheless longer time-series should be crucial in order to detect more significant impacts and long term effects of climate change on larval fish assemblages in opposition to other trophic levels.

Mondego estuary fish larvae assemblages displayed a clear seasonal pattern presenting higher abundances and diversities during warmer months. Main effects of dry events seemed not to affect fish larvae community structure but changes in estuarine salinity gradient appears to lead to analogous changes in marine species distribution. Therefore, river flow played a key function in structuring ichthyoplankton assemblage representing also an important retention mechanism responsible for a successful larval development and recruitment.

Acknowledgments

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CHAPTER III

Seasonal, lunar and tidal control of ichthyoplankton dynamics at the interface between a temperate estuary and adjacent coastal waters (Western Portugal)

ABSTRACT

Influence of season, lunar-tide cycle and tide on ichthyoplankton at the interface between Mondego estuary and adjacent coast and on up-estuary transport was evaluated. Seasonal samples were collected at a fixed station at the mouth of the estuary during diel cycles, at neap and spring tides. Additionally, four sampling stations along the estuary were assessed. *Pomatoschistus* spp. was the main estuarine taxa, while *Sardina pilchardus* and *Parablennius pilicornis* were the most important marine species. Ichthyoplankton entrance and transport along the estuary presented a seasonal pattern with higher densities during summer. Lunar-tide cycle also represented an important influence structuring communities that arrive to the estuary and its subsequent distribution. *Solea senegalensis* and *Sardina pilchardus* seemed to take advantage from spring tides to enter in the estuary. *S. pilchardus* appear to be using tides to move upstream of the estuary. Ichthyoplankton entrance in the estuary seemed related to species spawning period, while its distribution within the estuary depends on *in situ* spawning as well as on the capacity of species to counteract currents and river flow. The present study provides a better understanding of ichthyoplankton dynamics at the interface of two coastal systems reinforcing knowledge of lunar-tide cycle influence on ichthyoplankton communities.

Keywords: Ichthyoplankton, larval ingress, tidal exchange, seasonality, lunar cycle, larval transport.

Introduction

Exchange between estuaries and the ocean is a key issue to coastal scientists because many species of fish and invertebrates move between the continental shelf and estuaries during their early life-history (Epifanio and Garvine, 2001). Transport to an estuarine inlet and subsequent up-estuarine movement are crucial for successful completion of the life cycle of several fish species (Islam et al. 2007). Many fishes using inshore nurseries are subject of commercially important fisheries. Therefore, evaluating where the pelagic larval stages disperse to and how they manage to successfully recruit back to adult populations is not only critical for understanding the evolution of early life history stages and the dynamics of marine populations, but also in the development and implementation of management and conservation efforts (Forrester and Swearer, 2002).

Means of cross-shelf transport and estuarine ingress are not well understood and are often complicated by species-dependent interactions of hydrography (e.g. DiBacco et al., 2001), behaviour (e.g. Jager, 1999; Queiroga et al., 2007) and ontogeny (e.g. Hare et al., 2005).

Tidal phase-related factors such as salinity, temperature, chemical substances, current velocity and hydrostatic pressure are reported to be important to plankton distribution (Hsieh et al., 2010). Spring–neap tidal cycle can also represent a strong impact on species distribution since that spring tide may difficult vertical stratification (Schultz et al., 2003). This lunar associated tidal cycle can also represent an important role in fish reproduction acting as cue for synchronization of reproductive events (Oliveira et al., 2009a; Takemura et al., 2010).

Vertical migration according to tidal cycle is a common behaviour linked to transport, retention or dispersion mechanisms. During flood-tide transport larvae settle on, or move close to the bottom during ebb tide and ascend in the water column during flood tide resulting in upstream transport (Forward and Tankersley, 2001). Additionally, getting out of seaward-moving water layers during ebb facilitate larval retention within an estuary (e.g. Jager, 1999). In ebb-tide transport, larvae settle during flood and ascend during ebb resulting in seaward transport (Queiroga et al., 2007). Retention mechanisms and selective tidal stream transport (STT) has been imputed to several species of zooplankton, larval benthic invertebrates (e.g. Queiroga et al., 2007; Jessopp and McAllen, 2008; Marques et al., 2009) and larval and juvenile fishes (Schultz et al., 2003; Miller and Shanks, 2004; Islam et al., 2007).

Mechanisms of fish larvae ingress and retention in nurseries areas are the focus of several studies across North America (Epifanio and Garvine, 2001; Hare et al., 2005; Rooper et al., 2006), Japan (Islam et al*.,* 2007), or Europe (Jager, 1999, 2001; Jessopp and McAllen, 2008). Little is known concerning the implications of endogenous rhythms associated with lunar-tide cycle on larval entrance and consequent up-estuary transport and horizontal distribution inside these nurseries areas.

The present study presents the results of a high frequency sampling, in which diel cycles were sampled seasonally, at neap and spring tide cycle, combined with spatial sampling inside the estuary. The main objectives of this study were: (1) to understand how lunar associated tidal cycle's control fish larvae entrance in the estuary; (2) to identify how this influence varies seasonally; and (3) determine to what extent it affects up-estuary transport and spatial distribution inside the estuary.

Material and Methods

Study site

The Mondego River estuary is a mesotidal system, located in the western Atlantic coast of Portugal (40°08'N, 8°50'W) (Fig. 1). The hydrological basin of the Mondego, with an area of 6 670 km^2, provides an average freshwater flow rate of 79 m^3 s^{-1} (Dolbeth et al., 2010). Mondego is a well-mixed estuary and consists of two channels divided by the Murraceira Island and converges again near the mouth. Here the influence of both the river flow and neritic waters is strong and the depth is around 6-13 m. The north arm is deeper, with 5–10 m depth at high tide, while the south arm is shallower, with 2–4 m depth at high tide. Tides in this system are semi-diurnal, and at the inlet the tidal range is 0.35–3.3 m.

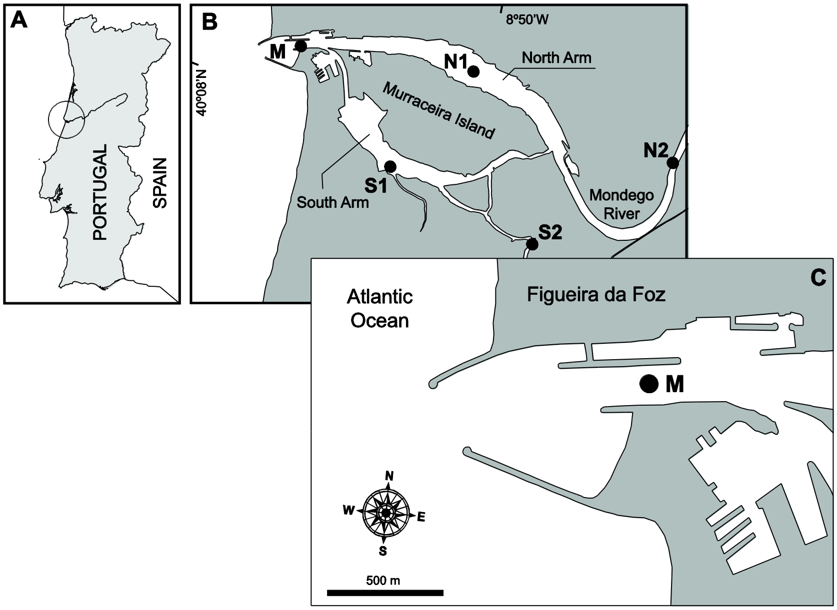

Figure 1. Mondego estuary location in west coast of Portugal (A). Sampling stations sampled inside the estuary (B) in both south (S1, S2) and north arm (N1, N2) and detailed location of sampling site M (high frequency sampling) (C).

Sample collection

In order to investigate fish larvae entrance and subsequent up-estuary transport two different approaches were used. For the high frequency sampling, ichthyoplankton was sampled at a fixed station located at the mouth of the estuary (M) (mean depth: 8m) (Fig. 1) at 1-hour intervals over a diel cycle. Seasonal sampling took place in June 2005 (summer), September and October 2005 (autumn), December 2005 (winter) and March and April 2006 (spring). In each season two diel cycles were made coinciding with neap and spring tides. Simultaneously, from June 2005 till April 2006, monthly spatial distribution inside the estuary was accessed. Spatial sampling was performed during flood tide by collecting samples at four stations distributed throughout both arms of the estuary (S1, S2, N1, and N2) (Fig. 1). Monthly samples were combined into four conventional seasons (summer: June05, July05 and August05; autumn: September05, October05 and November05; winter: December05, January06 and February06; spring: March06, April06 and May06) and classified according to lunar-tide cycle (neap and spring). Neap and spring tides were sampled at least once in each season.

At both surveys, ichthyoplankton was collected using horizontal subsurface tows (bongo net: mesh size 335μm, mouth diameter: 0.5m; tow speed: 2 knots, 3min.) equipped with a Hydro-Bios flowmeter and preserved in a 4% borax-buffered formalin seawater solution. Samples were sorted and ichthyoplankton identification to the species level was made whenever possible under a stereoscopic microscope (Primo et al., 2011). Densities estimates were standardized (individuals *per* 100 m^3). At each sampling period, salinity, water temperature (°C), dissolved oxygen (mg l^{-1}) and pH were recorded *in situ* with appropriate sensors. Sub-surface water samples (1.5L) were collected for the determination of chlorophyll *a* (Chl *a*) and suspended particulate matter (SPM) (Marques et al., 2006).

Data analysis

High frequency samples were grouped by season (summer, autumn, winter and spring), lunar-tide cycle (neap and spring tide) and tide cycle (ebb and flood tide). Species were grouped in three main ecological guilds adapted from Elliott et al. (2007): marine stragglers (MS - species that spawn at sea and typically enter estuaries in low numbers occurring frequently in the lower reaches), marine migrants (MM - species that spawn at sea and often enter estuaries in large numbers), and estuarine species (ES –estuarine species capable of completing their entire life cycle within the estuarine environment and those with stages of their life cycle completed outside the estuary).

Statistical analyses were made for each season separately. First, a SIMPER analysis was performed for each ecological guild in order to identify main species present in each season. Then, variation in environmental factors, guilds and main species densities was tested by a univariate PERMANOVA test. Univariate PERMANOVA tests included a two way design (Lunar-tide cycle/Tidal cycle) and were based on Euclidian distances between samples, after ln(x+1) transformation of density data, considering all the factors as fixed and unrestricted permutation of raw data.

Species multivariate analysis was performed using multivariate PERMANOVA. Multivariate tests focused on differences in the assemblage structure between seasons, lunar-tide and tide cycles (three way design: Season/Lunar-tide cycle/Tidal cycle) and were based on Bray-Curtis similarities between samples, after an ln(x+1) transformation of density data, considering all the factors as fixed and unrestricted permutation of raw data. For multivariate analysis, samples without larvae and larvae that could not be identified to at least the family level were excluded from the analysis.

Effects of environmental variables on larval fish assemblages were analyzed with canonical correspondence analysis (CCA) (CANOCO version 4.5, Microcomputer Power). Environmental variables included salinity (Sal), water temperature (T) dissolved oxygen (O2), pH, chlorophyll *a* (Chl *a*) and suspended particulate matter (SPM). Neap/spring and ebb/flood tides were also included as nominal variables. All species were used and a new category coded as “no fish” was created to prevent CANOCO from eliminating samples containing no fish larvae (Grothues and Cowen 1999). Larval densities were ln(x+1) transformed and environmental variables were standardized by subtracting the mean and dividing by the standard deviation. Each season was treated separately applying forward stepwise selection procedure of explanatory variables in order to identify significant variables ($p<0.05$). Finally, a total CCA biplot scaling with focus on inter-samples distances was performed using the former identified variables and the nominal variable seasons.

Monthly spatial distribution data was also grouped in seasons and spring/neap tides and species characterized in the same three main ecological guilds (Elliott et al., 2007). Variance in guilds and main specie abundance was tested by univariate PERMANOVA tests performed under on a two way design (Lunar-tide cycle/Sampling station) and based on Euclidian distances between samples, after ln(x+1) transformation of density data, considering all the factors as fixed and unrestricted permutation of raw data.

SIMPER, univariate and multivariate PERMANOVA tests were applied with PERMANOVA+ and PRIMER software (PRIMER v6 & PERMANOVA+ v1, PRIMER-E Ltd.) and significant results were investigated using *post-hoc* pairwise comparison tests.

Results

Hydrological conditions

Summer spring tide showed significantly lower values of temperature, salinity and chlorophyll *a*. At summer, hydrological conditions presented no differences between tides (Fig. 2, Table I).

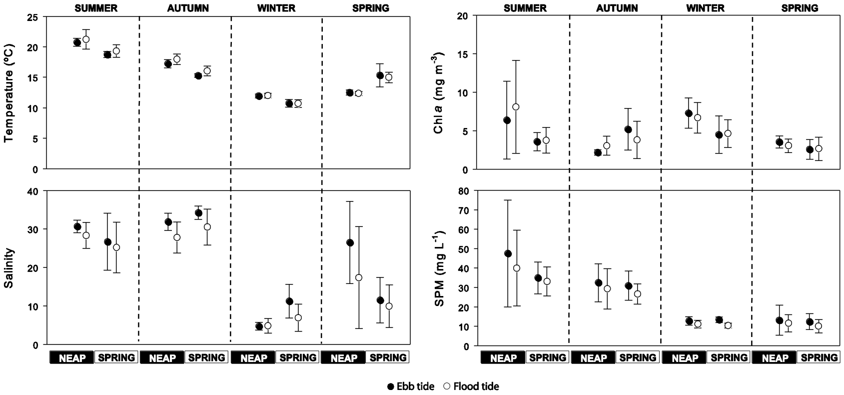

Figure 2. Seasonal variation (mean ± SD) in temperature (°C), salinity, chlorophyll *a* (mg m^3) and suspended particle matter (SPM, mg L^{-1}) measured during high frequency sampling period.

At autumn spring tide, temperature was also significantly lower however both salinity and chlorophyll *a* values were higher than at neap tide. During autumn ebb tides, temperature and chlorophyll *a* (mainly during autumn neap tide; PERMANOVA parwise comparison: t(neap tide)=2.26, $p<0.05$)) were lower than during flood while salinity was superior (Fig.2, Table I). During winter spring tide temperature and chlorophyll *a* were, again, lower but salinity continued higher than at neap tide. At this season higher salinity values were recorded during ebb spring tides (PERMANOVA parwise comparison: t(spring tide)=2.75, p<0.05) (Fig. 2, Table I). Finally, at spring spring tide, temperature was higher than at neap tides but salinity and chlorophyll *a* were lower. At spring, generally, salinity values were higher during ebb tide (Fig. 2, Table I). Suspended particulate matter was highly variable during seasonal diel periods with no significantly differences between lunar-tide cycle and tides, exception for winter ebb tide values that were significantly higher than during floods (Fig. 2, Table I).

Table I. Summary results (Pseudo-F values) from 2-way univariate PERMANOVA analysis on environmental and biological variables. SPM, suspended particulate matter; ES, estuarine species; MM, marine migrants; MS, marine stragglers. *p≤0.001; **p≤0.01; *p≤0.05.

	ENVIRONMENTAL VARIABLES				BIOLOGICAL VARIABLES			
	Temperature	Salinity	Chlorophyll	SPM	Total	ES	MM	MS
SUMMER	(n=50)							
Lunar	41.34***	5.44*	9.28**	3.82	2.85	4.06	0.61	1.97
Tide	2.94	1.55	0.64	0.90	2.02	1.81	0.03	1.18
LUxTi	0.02	0.07	0.43	0.32	0.45	0.52	1.56	2.23
AUTUMN	(n=50)							
Lunar	92.50***	6.68*	11.99**	0.74	2.86	0.06	1.24	4.80*
Tide	13.66***	15.99***	0.21	2.38	1.37	0.09	0.13	0.56
LUxTi	0.01	0.04	4.20*	0.05	0.09	0.96	2.16	0.80
WINTER	(n=47)							
Lunar	66.50***	23.05***	16.37**	0.04	7.03*	0.85	1.95	0.48
Tide	0.05	5.50*	0.15	20.90**	0.94	0.43	1.17	0.48
LUxTi	0.11	6.23*	0.41	1.63	0.17	0.33	1.10	3.17
SPRING	(n=50)							
Lunar	79.86***	17.55***	4.61*	0.57	48.78***	29.16***	11.16***	7.30*
Tide	0.73	3.99*	0.39	1.77	0.41	0.00	3.76	1.86
LUxTi	0.12	1.99	0.77	0.07	5.83*	1.21	3.50	5.81*

High frequency sampling: Seasonal and spring-neap tide effects

A total of 39 different taxa of fish larvae were collected in high frequency sampling at Mondego estuary. The number of fish larvae was highly variable (ranging from 1 to 1641 larvae 100 m^{-3}) and showed a strong seasonal variation with higher densities in summer, followed from autumn and spring and finally winter presenting the lowest larvae density (Fig. 3). Autumn and summer presented no significant differences between neap and spring tides however both spring and winter showed significantly higher densities during spring tide (Fig. 3, Table I). Also, there was no significant difference between flood and ebb tide larvae mean densities with exception to spring

spring tide which showed significantly higher values during flood tide (PERMANOVA parwise comparison: t(spring tide)=2.86, p<0.01).

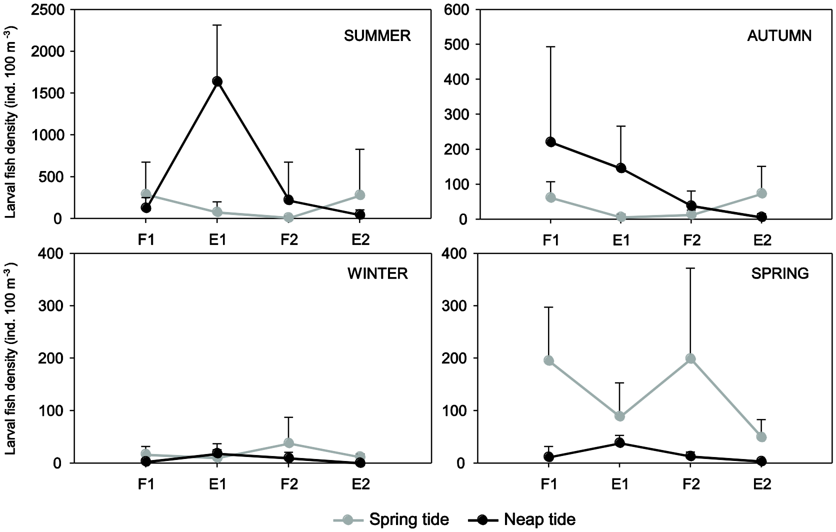

Figure 3. Total larval fish densities (mean+SD, ind. 100m^{-3}) at each season and lunar-tide cycle according to tide (F, Flood; E, Ebb).

Estuarine species represented the main ecological guild in almost all seasons except for winter where marine migrant species were higher (Fig. 4).

Marine migrant species increased during winter and spring, while marine stragglers were maximum during summer. Comparing lunar-tide cycles, estuarine and marine stragglers species were higher during summer and autumn neap tides, respectively. In contrast, in spring higher densities were reached during spring tides for the three main ecological guilds (Fig. 4, Table I). Tide was a significant factor only at spring spring tide (PERMANOVA parwise comparison: t(spring tide)=0.45, p<0.05) when marine stragglers species were significantly higher during floods.

SIMPER analysis revealed that *Pomatoschistus* spp. was the main estuarine species present in all the seasons with the lowest contribution observed in summer (70%) and the highest in winter (100%). *Sardina pilchardus* was the main marine migrant species present in summer (53%), winter (100%) and spring (99%) while *Solea senegalensis* presented the highest contribution in autumn (67%). Finally, *Parablennius pilicornis* was the main marine straggler species present in larval assemblage with contributions from 52% (spring) to 97% (summer). Note that marine stragglers species were absent during winter.

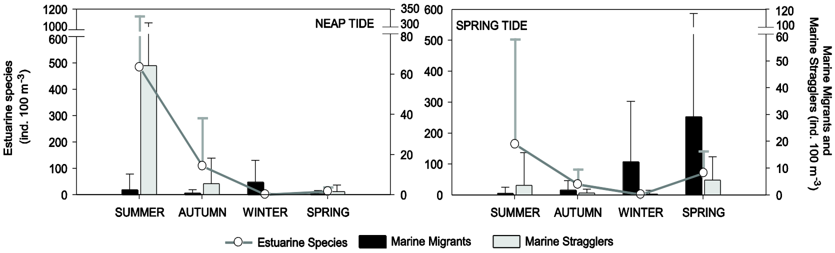

Figure 4. Seasonal larval fish densities (mean+SD, ind. 100 m^{-3}) of the three main ecological guilds at each lunar-tide cycle. Estuarine species (left axis); Marine Migrant and Marine Stragglers (right axis).

Pomatoschistus spp. dominated the ichthyoplankton assemblages in all seasons, except winter. This species density was higher during spring spring tide but no differences were detected between lunar or tide cycle for all the remaining seasons (Table II). The marine migrant *S. pilchardus* was the main species present in winter assemblages being evenly present both in spring and neap tide (Table II). Maximum densities of this species were found during flood in spring spring tide reaching significantly higher density than during spring neap flood tide (PERMANOVA parwise comparison: t(spring tide)=4.85, $p<0.05$) (Table II). *S. senegalensis* reaches higher densities in autumn spring tide with no distinction between tidal cycles (Table II). Concerning marine stragglers, *P. pilicornis* presence increased in summer with no distinction between lunar or tidal cycle. However, in autumn, densities were significantly higher at neap tides (Table II).

High frequency sampling: assemblage structure and its relationship with environmental variables

Assemblage structure revealed a clear seasonal pattern with differences between all the seasons (PseudoF= 18.96, p(perm)≤0.001). On the other hand, lunar-tide cycle influence on species assemblages varied according to season (PseudoF= 3.73, p(perm)≤0.001) being significant in summer (t=2.18, p(perm)<0.01), autumn (t=1.80, p(perm)<0.01) and spring (t=2.52, p(perm)≤0.001), but not in winter. Assemblages were not significantly different between ebb and flood tides.

Table II. Mean density (ind. 100 m^{-3}) of the main ichthyoplankton species recorded in Mondego estuary for each season, lunar-tide cycle (neap and spring) and tide cycle (ebb and flood) and results from 2-way univariate PERMANOVA (Pseudo-F values) analysis. Standard deviation indicated between brackets. n, sample size. *p≤0.001; **p≤0.01; *p≤0.05.

		NEAP		SPRING		Statistics		
		Ebb	Flood	Ebb	Flood	Lunar	Tide	LUxTI
SUMMER	n	13	12	12	13			
Pomatoschistus spp.		574.59 (641.53)	142.64 (284.78)	154.01 (364.41)	130.27 (255.70)	2.44	1.27	0.14
Sardina pilchardus		1.39 (5.00)			1.19 (2.97)	0.09	0.09	2.68
Parablennius pilicornis		112.90 (324.47)	2.15 (5.25)	4.82 (16.70)	2.47 (5.97)	1.88	1.27	2.36
AUTUMN	n	11	14	11	14			
Pomatoschistus spp.		59.59 (111.34)	144.92 (224.62)	34.24 (59.55)	33.90 (40.33)	2.07	4.07	0.28
Solea senegalensis			0.42 (1.53)	1.42 (1.98)	1.42 (2.98)	5.49*	0.0003	0.64
Parablennius pilicornis		9.79 (17.45)	0.61 (1.19)	0.26 (0.86)	0.41 (1.06)	4.85*	2.29	3.22
WINTER	n	12	10	12	13			
Pomatoschistus spp.		2.35 (4.98)	2.15 (3.90)	3.26 (3.52)	2.14 (2.80)	1.23	0.22	0.57
Sardina pilchardus		7.39 (12.65)	5.01 (8.42)	5.08 (6.62)	19.07 (29.48)	1.95	1.17	1.10
SPRING	n	12	13	13	12			
Pomatoschistus spp.		14.73 (16.88)	7.16 (12.25)	53.04 (44.61)	87.39 (90.39)	34.7***	0.44	1.86
Sardina pilchardus		0.33 (1.16)		2.98 (6.22)	56.77 (119.42)	13.31***	3.29	4.85*
Parablennius pilicornis				1.04 (1.81)	3.11 (4.31)	17.98	2.60	2.60

Forward stepwise analysis showed that in summer significant environmental variables were chlorophyll *a*, suspended particle matter, tide cycle and temperature. During autumn, lunar-tide cycle, salinity, temperature and chlorophyll *a* were the main variables responsible for structuring assemblages. Chlorophyll *a* and lunar-tide cycle played an important role also during winter while during spring temperature was the main factor determining distribution pattern of ichthyoplankton assemblages. The seven environmental variables considered in the final CCA (season, lunar-tide cycle, tide, salinity, temperature, chlorophyll *a* and SPM) explained 18% of the total variation in larval fish assemblages, with first axis accounting for 45% of species-environment relation. Canonical correspondence analysis illustrated at least three distinct groups corresponding to spring, winter and autumn/summer (Fig. 5). The distinction between spring/neap tides is also evident.

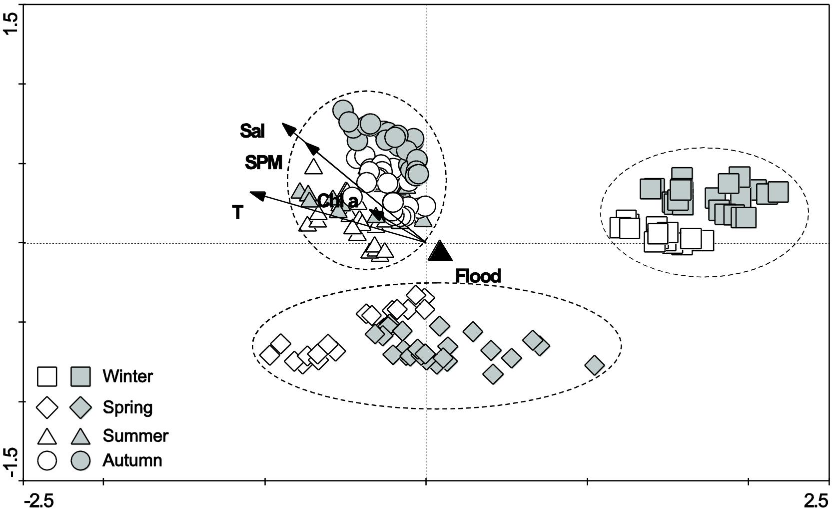

Figure 5. Biplot ordination diagram of the larval fish assemblages in Mondego estuary using the first two canonical correspondence axes. Samples were classified according to season (winter, spring, summer and autumn) and lunar-tide cycle (neap and spring). Significant environmental variables are plotted as arrows (T – temperature; Sal – salinity; SPM – suspended particle matter; Chl a – chlorophyll *a*). Tide cycle was also included as a nominal variable "Flood". White, neap tide; Grey, spring tide.

Seasonal and spring-neap tide effects in spatial distribution

Estuarine species were present mainly during neap tides except at summer in sampling stations S1 and N2 and during autumn in S1, N1 and N2 stations (Fig. 6). Marine migrants' density was also higher during neap tides, particularly at upstream areas of the estuary. However, downstream sampling stations S1 and N1 showed higher densities at spring tides mainly during autumn and summer (Fig. 6). Regarding marine stragglers species, high densities were recorded at upstream station N2 during autumn spring tides and in sampling station S1during spring spring tides (Fig. 6)

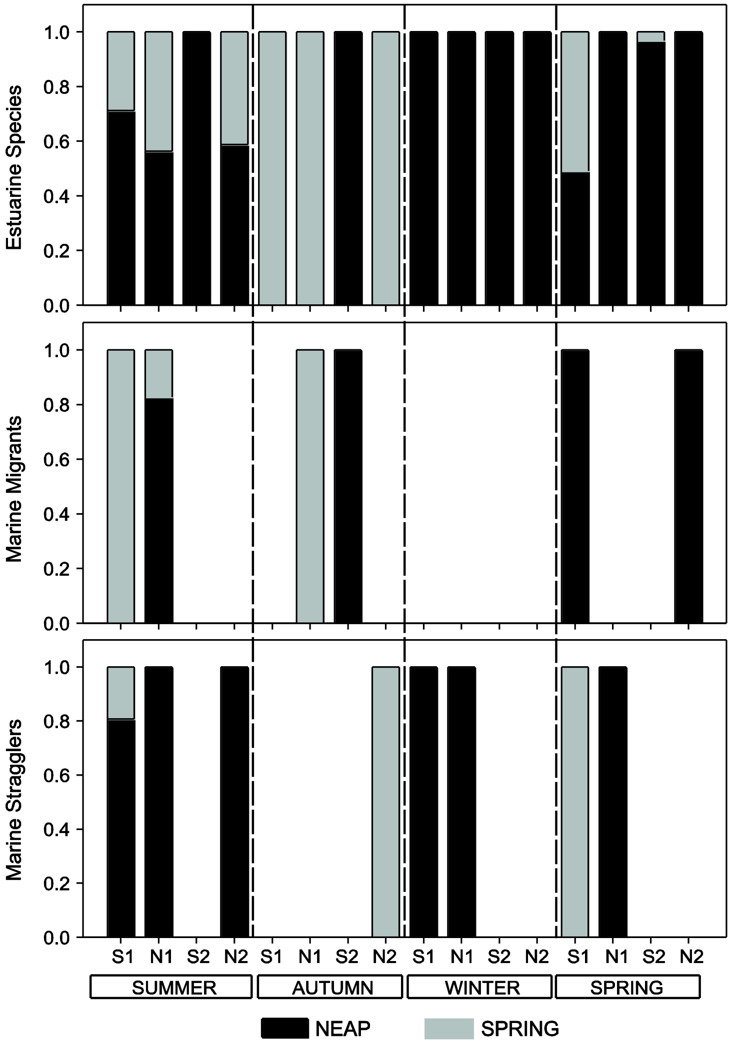

Figure 6. Seasonal and spatial relative density of the three main ecological guilds during the two lunar-cycle tides.

Pomatoschistus spp. represented the most abundant species in all sampling stations and seasons with higher densities during autumn and spring neap tides (Table III). Generally, its distribution was uniform across the estuary with sampling station S2 presenting the highest larval densities. Sampling station S1 presented significantly higher densities during autumn spring tides (PERMANOVA pairwise comparison: t(S1)=0.36, p≤0.05). In autumn spring tides, *Solea senegalensis* was present in downstream sampling station while *Sardina pilchardus* was not detected during spatial assessment (Table III). *Parablennius pilicornis* was particularly abundant during summer neap tides and in upstream station N2. In autumn spring tides also reached upstream sampling station N2 (Table III).

Table III. Mean density (ind. 100 m^{-3}) of the main ichthyoplankton species in each ecological guild identified in Mondego estuary for each season, lunar-tide cycle (neap and spring) and sampling station and results from 2-way univariate PERMANOVA (Pseudo-F values) analysis. Standard deviation indicated between brackets. n, sample size. *p≤0.001; **p≤0.01; *p≤0.05.

	NEAP					SPRING					Statictics		
	M	S1	N1	S2	N2	M	S1	N1	S2	N2	Lunar	Station	LUxST
SUMMER n	1	1	1	1	1	2	2	2	2	2			
Pomatoschistus spp.		21.22 (0)	5.83 (0)	15.00 (0)	19.91 (0)	7.34 (4.64)	8.49	6.06		13.93 (8.11)	0.97	0.86	1.31
Sardina pilchardus											not tested		
Parablennius pilicornis		5.95 (0)	5.83 (0)		9.95 (0)		1.41 (0)				23.05^{*}	6.14	4.43
AUTUMN n	1	1	1	1	1	2	2	2	2	2			
Pomatoschistus spp.				31.49 (0)		0.65 (0)	2.78 (0.28)	2.29 (0)		5.60 (0.89)	0.36	2.55	8.19^{*}
Solea senegalensis								1.77 (0)			not tested		
Parablennius pilicornis										3.12 (0)	not tested		
WINTER n	2	2	2	2	2	1	1	1	1	1			
Pomatoschistus spp.	6.29 (0)	26.92 (6.78)	7.59 (0)	11.97 (3.95)	4.76 (0)						6.18^{*}	0.29	0.29
Sardina pilchardus											not tested		
SPRING n	2	2	2	2	2	1	1	1	1	1			
Pomatoschistus spp.	5.68 (0)	16.98 (0)	2.80 (0)	36.76 (20.10)	8.15 (0)		10.54 (0)				1.81	0.48	0.49
Sardina pilchardus											not tested		
Parablennius pilicornis							7.02 (0)				not tested		

Discussion

Seasonal environmental characterization showed that in summer, salinity and temperature were higher, mainly during neap tide, which can indicate a stronger marine influence at this lunar cycle. Generally, the same occurred during spring, which presented higher salinity, but lower temperature, at neap tide. Autumn and winter showed the opposite pattern presenting higher salinity and lower temperature during spring tides. Higher river flow during these seasons could reduce the marine influence downstream in the estuary and therefore, being noticed mainly during the higher tidal amplitude of spring tides.

High frequency sampling of ichthyoplankton showed a clear seasonal distribution pattern. Higher densities during summer and lower in winter are characteristic temporal patterns usually related to spawning of adult fishes (e.g. Barletta-Bergan et al., 2002; Ramos et al., 2006; Sabatés et al., 2007). Seasonal pattern of fish larvae density was already reported for Mondego estuary (Primo et al., 2011).

Winter assemblages differed from the others mainly due to the lower densities of *Pomatoschistus* spp. and higher of marine migrant species *Sardina pilchardus*. Winter was already pointed as a preferred spawning season for *S. pilchardus* along the western and southern Iberian Peninsula Stratoudakis et al. (2007) or northwestern Mediterranean (Olivar et al., 2003; Palomera et al., 2007) and it seems to be triggered by the decrease in the sea surface temperature (Zarrad et al., 2008).

Marine migrant *S. pilchardus* was also an important contribute during spring as well as *Pomatoschistus* spp. In fact, *Pomatoschistus* spp. represented an important fraction of the assemblage also during autumn and summer. Dolbeth et al. (2007) already noticed that, in the Mondego estuary, *Pomatoschistus microps* presents an extended breeding season and several spawning periods and this is probably the main reason for *Pomatoschistus* spp. larvae importance in assemblage structure throughout the year. Also in summer, marine stragglers were a significant fraction of the ichthyoplankton assemblages mainly *Parablennius pilicornis*. This common rocky subtidal fish species has already recorded in Portuguese estuarine environments mainly in summer at upper and lower estuary (e.g. Faria et al., 2006). As a marine straggler species, *P. pilicornis* probably enters in the Mondego estuary only during short periods of its larval stage, since that its presence in the estuary as juvenile was not recorded (Leitão et al., 2007; Martinho et al., 2007).

Solea senegalensis higher larval densities during autumn can also be related with species spawning period. Spawning period of *S. senegalensis* is very variable and consists of two periods, from January to June and in autumn (Garcia-Lopez et al.,, 2006). Despite being a marine species, it is common in estuaries and several southern

Iberian estuaries were already referred as nursery grounds for *S. senegalensis* (e.g. Drake et al., 2002; Cabral et al., 2007).

Lunar-tide cycle influence represented an important role in structuring community assemblages, except for winter. However, inside the estuary, winter spatial distribution is affected since that during neap tides estuarine guild presented high densities of *Pomatoschistus* spp., conversely to spring tides when no fish larvae were caught. This is probable a consequence of higher river flow that flushed out larvae from the estuary as already been recorded in previous studies (Marques et al., 2006) or of the reduced spawning activity during this season. On the other hand, low river flow periods enable species to reach upstream parts of the estuary as happened at summer neap tide when marine stragglers larvae, as *P. pilicornis*, reached the upper limits in the estuary.

The importance of lunar-tide cycle, not only in fish larval exchange with the adjacent ocean, but also in spatial distribution of species within the estuary can be related to the planktonic nature of larvae that, unable to counteract strong water flow, increase in periods with weak hydrodynamics. Larvae swimming capacity may differ from species to species and according to development stage. Although present study lacks ontogenetic stage determination, larvae collected were small larvae (pre-flexion or flexion stage) and thus analyzed together.

Marine influence is expected to increase during higher amplitude tides influencing larvae ingress and settlement in the estuary. This is probably the case of marine species *S. senegalensis* and *S. pilchardus* which seemed to take advantage from spring tides to enter in the estuary. In small estuaries like Mondego, marine influence is highly dependent of river flow and thus, higher tide amplitude not always imply higher marine intrusion, which seemed to be the case of summer spring tides.

Lunar-tide cycle is often related to the spawning period of species. Takemura et al. (2010) observed that during the spawning season of several fishes, synchronous spawning can occur accordingly to a lunar, semi-lunar or a tidal cycle. Several studies demonstrated the effect of lunar cycle in the reproduction of *S. senegalensis* (Oliveira et al., 2009a) and gilt-head sea bream, *Sparus aurata* (Saavedra and Pousão-Ferreira, 2006). It is thought that lunar or tidal rhythms synchronize fish endocrine organs and induce the beginning of gonad maturation and time of spawning (Oliveira et al., 2009b). Lunar cycle influence seems to be species-specific and related to photoperiod and intensity of moonlight varying according to species diurnal/nocturnal behaviour and season (Pankhurst and Porter 2003; Oliveira et al., 2009b; Takemura et al., 2010).

Several studies have shown that fish larvae are able to actively disperse with floods mainly owing to vertical movements with the tidal cycle (Grioche et al., 2000; Aceves-Medina et al., 2008). The present study considered only sub-surface samples and the vertical migration pattern could not be confirmed. Moreover, differences between tidal cycles were only recorded for *S. pilchardus* with superior densities during spring spring tide flooding. This can indicate that these species could be using tides to move upstream of the estuary by means of selective tidal-stream transport. Flood-tide transport implies that organisms are abundant in the water column during flood tide and absent or reduced during ebb tide. It is more common in estuarine dependent species which moves up the estuary to nursery or adults habitats (Forward and Tankersley, 2001).

Assemblage structure demonstrated a weak relation with environmental factors suggesting that other physical variables associated with the spring-neap and ebb-flood tidal cycle could affect fish larval exchange with the ocean. Factors as wind speed/direction, intensity/direction of currents were not recorded but may play a critical role in these exchanges (Miller and Shanks, 2004; Hare et al., 2005; Queiroga et al., 2007). Temperature and salinity showed a strong association with assemblage structure and seasonality. This seasonality can be related to species synchronizing reproduction with the seasonal increase of food (Sims et al., 2004; Sabatés et al., 2007). Clorophyll *a* and SPM were also important factors in the flux of larvae and both can be associated with turbidity that is often pointed as an environmental cue for exchanges between estuaries and adjacent coasts (Strydom and Wooldridge, 2005; Drake et al., 2007). Tidal flux of larvae is associated with several related factors including current speed, salinity (as affected by river discharge and magnitude of tidal mixing and exchange), temperature, olfactory cues, turbidity, substrate composition and lunar phase (Strydom and Wooldridge, 2005).

Present study reinforces knowledge of lunar-tide cycle influence on ichthyoplankton entrance and posterior distribution throughout the estuaries. However, these results could benefit with replicated sampling in each season and lunar-tide cycle and at different depths. Future studies should also include determination of larvae ontogenetic stage in order to have a more comprehensive understanding about fish larvae entrance and distribution pattern in the estuaries.

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CHAPTER IV

Vertical patterns of ichthyoplankton at the interface between a temperate estuary and adjacent coastal waters: seasonal relation to diel and tidal cycles

ABSTRACT

Vertical distribution and migration pattern of ichthyoplankton assemblage in the Mondego estuary were investigated in relation to diel and tidal cycle. Summer and winter communities were sampled, at surface and bottom, over a diel cycle during spring and neap tides at a fixed station at the mouth of the estuary. Summer presented higher larvae density mainly of *Pomatoschistus* spp., *Gobius niger* and *Parablennius pilicornis*. Main species in winter assemblages were *Pomatoschistus* spp. and *Sardina pilchardus*. There were no differences between depth stratums across diel or tide cycle. Nevertheless, main species larval densities showed significant periodic variation associated with tide (M2) and diel (K1) cycles presenting generally, higher density at night and around low tide. Conversely, vertical patterns observed could not be related with diel or tidal cycle. Tough, main species presented some extent of vertical migration. Vertical patterns observed appear to be related to seasonal stratification and river flow, increasing amplitude during periods of less stratification and lower water currents. Present study provides a better understanding of ichthyoplankton vertical movement patterns and of small scale dynamics at the interface of two coastal European systems.

Keywords: Diel migrations; ichthyoplankton; selective tidal-stream transport; vertical distribution; Mondego estuary; Portugal.

Introduction

For many marine species, recruitment success requires transport from open-ocean to estuarine nursery habitats during early life and larvae must therefore adopt strategies for successful ingress into estuarine nursery grounds (Islam et al., 2007). Estuary ingress strategies are often related with larvae vertical distribution patterns. These patterns are complex and can be actively altered depending biotic and abiotic factors, involving behaviours that can change from species to species and depending on factors such as endogenous rhythms or development stage (e.g. Irigoien et al., 2004; Barletta and Barletta-Bergan, 2009; Leis, 2010; Tamaki et al., 2010).

Recently, there has been a growing interest in vertical distribution and migration by decapods larvae (e.g. Queiroga et al., 2007; Tamaki et al., 2010), copepods (e.g. Ueda et al., 2010) and fish larvae (e.g. Auth et al., 2007; Voss et al., 2007; Barletta and Barletta-Bergan, 2009; Leis, 2010). Despite their planktonic nature, fish larvae can control their vertical position in the water column. This vertical migration seems to have a main role that allows the larvae to reach or stay in certain favourable areas and control passive drift (Aceves-Medina et al., 2008; Tamaki et al., 2010). Moreover, knowledge of the vertical distribution of larvae is critical to understand the structure and ecological interactions of ichthyoplankton communities (Gray, 1998). Larvae often use active upward or downward swimming and passive sinking coupled with both circadian and tidal rhythms (Tamaki et al., 2010).

Diel vertical migrations are common within larval phase of fish. The most common is movement into the upper water column during night and into deeper water during the day (e.g. Tsukamoto et al., 2001; Auth et al., 2007). However, the reverse pattern has also been observed (e.g. Brodeur and Rugen, 1994; Bradbury et al., 2006). Some species do not show diel variations but may form aggregations during the daytime and disperse at night (e.g. Jensen et al., 2003). Diel vertical migrations have been related to optimum light conditions, predator avoidance and suitable prey concentrations (e.g. Brodeur and Rugen, 1994; Irigoien et al., 2004).

Tidal vertical migration is also a common mechanism in estuarine species living in areas dominated by tidal currents. While diel patterns are widely associated with predation-prey relationships, tidal cycles of vertical migration are linked to horizontal transport, retention or dispersion mechanisms. The most common is selective tidal-stream transport (STST), during which larvae are supposed to ascend actively in the water column during flood and return to the bottom when the tide turns (e.g. Forward and Tankersley, 2001; Gibson, 2003). In this way, organisms can be advected to upstream parts of the estuary during floods and, on the other hand, getting out of seaward-moving water layers during ebb could facilitate larval retention within an estuary (e.g. Jager, 1999, DiBacco et al., 2001). STST have been imputed to several species of zooplankton (e.g. Rawlinson et al., 2005), larval benthic invertebrates (e.g. Queiroga et al., 2007; Jessopp and McAllen, 2008) and larval and juvenile fishes (e.g., Schultz et al., 2003; Miller and Shanks, 2004; Islam et al., 2007). For fish larvae, this behaviour can be critical to survival since changes in environmental conditions may affect recruitment success of both estuarine and non estuarine-dependent species (Parrish et al., 1981).

Fish larvae vertical migration studies are often performed in coastal/offshore habitats (e.g. Auth et al., 2007; Voss et al., 2007; Garrido et al., 2009) or focused on tidal transport (e.g. Jager, 1999) however integrated work on the seasonal effect of the three cycles (tidal, diel and semi-lunar) in estuarine icthyoplankton is uncommun (e.g. Schultz et al., 2003). This study aimed to investigate the influence of diel and tidal cycle on larval fish vertical distribution over one neap and one spring tide during two opposite seasons (winter and summer) in a temperate small European estuary. It describes how larval vertical distributions differed among species and which mechanisms of transport could result from these distributions. Also, it is the first examination of diel/tidal variation in the vertical distributions of ichthyoplankton in the Mondego estuary and intended to supplement previous spatial and temporal analyses (e.g. Primo et al., 2011).

Material and methods

Study site

The Mondego River estuary is a mesotidal system, located in the western coast of Portugal (40°08’N, 8°50’W) (Fig. 1). The hydrological basin of the Mondego, with an area of 6 670 km^2, provides an average freshwater flow rate of 79 m^3 s^{-1} (Dolbeth et al., 2010). The well-mixed estuary consists of two channels divided by the Murraceira Island converging again near the mouth. Here the influence of both the river flow and neritic waters is strong and the depth is around 6-13 m. The north arm is deeper, with 5–10 m depth at high tide while the south arm is shallower, with 2–4 m depth at high tide. Tides in this system are semi-diurnal, and at the inlet the tidal range is 0.35–3.3m.

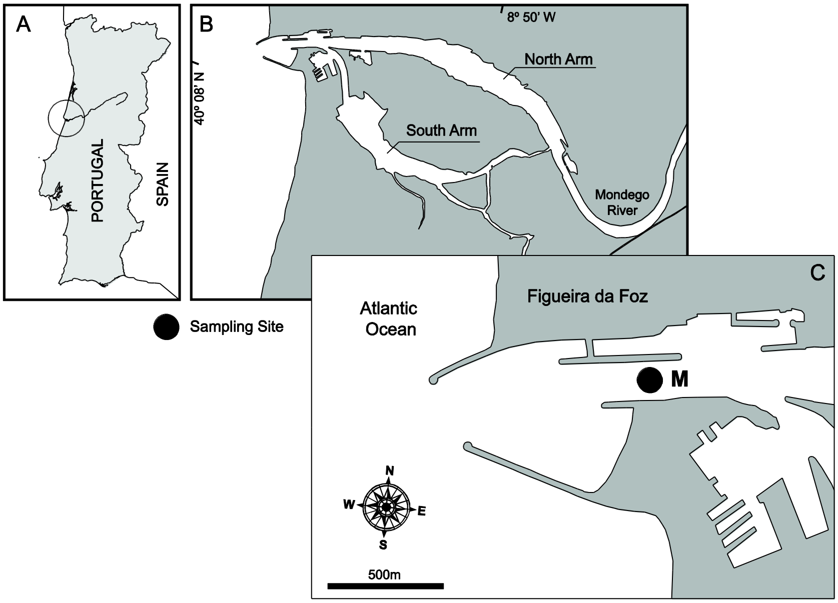

Figure 1. Map of the Mondego estuary and location of sampling site (M).

Sample collection

Plankton samples were collected from a single station (M) located at the mouth of the estuary (Fig. 1). Sampling cruises were performed both in summer (June 2005) and winter (December 2005). In each season, hourly samples were collected over two diel cycles coinciding with neap and spring tides. Samples were collected in the opposite direction of the current using horizontal tows (bongo net: mesh size 335µm, mouth diameter: 0.5 m; 3 min. tow; 2 knots) equipped with a Hydro-Bios flowmeter (average water filtered: 20m^3) and preserved in a borax-buffered formalin seawater solution. Each hour samples were collected from both subsurface and near (<1m) bottom. Salinity and water temperature (°C) were measured immediately after sampling using appropriate sensors. Samples were sorted in the laboratory and fish larvae were identified to the species level whenever possible under a stereoscopic microscope. Although restricted over the spatial scale, missing lateral gradients, this high frequency sampling allowed to detect changes in the main ichthyoplankton species density related to ebb-flood dynamics.

Data analysis

Larval density for each depth sampled was expressed as the number of larvae per 100 m^3. The samples were classified as day (from sunrise to sunset) and night periods. According to the expected moments of high- and low-water, samples were also classified as ebb or flood tides. Samples collected 1 hour before and after the expected moments of high or low tide were consider as collected during flood or ebb, respectively.

Diel and tidal fish larvae vertical distribution pattern were analysed separately. For that, main species mean density per depth was determined at each sampling moment. Also, weighted mean densities of dominant larval taxa at each sampling moment were calculated as $WMD=\sum(C_i d_i)/\sum(C_i)$, where C_i stands for larval density at depth d_i (i, depth layer number) (Pearre, 2003). The amplitude of diel migration (DVM) was calculated as the difference between the WMD at day and night. A positive value indicated movement towards the surface during the night and a negative value reverse vertical migration.

Due to the large variability of data and to unbalanced design, a univariate PERMANOVA test was applied to assess variation in environmental factors, fish larvae density and weighted mean depths of larvae. The PERMANOVA tests hypotheses for multi-factors in a great variety of designs using permutation methods. Analysis was run for each season/lunar cycle combination separately and was based on Euclidian distances between samples, considering all the factors as fixed and unrestricted permutation of raw data.

Salinity and temperature was tested for differences between depth (surface, bottom) and tidal cycle (flood, ebb) with a two-way design (Tide/Depth). Fish larvae density was tested to assess separate and interactive effects of depth (surface, bottom), diel (day, night) and tidal cycle (flood, ebb) with a two-way design (Diel/Depth or Tide/Depth). Both analysis were made on ln(x+1) transformed data matrix. Weighted mean depth was tested to find differences in median depth positions between diel (day/night) and tidal (ebb/flood) cycles. For that a two-way design (Diel/Tidal) was applied on un-transformed data matrix. PERMANOVA tests were applied with PERMANOVA+ for PRIMER software (PRIMER v6 & PERMANOVA+ v1, PRIMER-E Ltd.). Significant results were investigated using a *post-hoc* test and Bonferroni correction for multiple comparisons was applied to final significance value.

To identify periodic changes on larval vertical distribution, a harmonic regression (Batschelet, 1979) was performed. Harmonic regression is a simple adaptation of ordinary regression to situations where *x*-variables are cyclic or periodic. In practice it is a multiple regression carried out on trigonometric functions of angular transforms of periodic *x*-variables (Bell et al., 2001). The model was tested for 3 tidal constituents K1 (diurnal; time period = 23.9h), M2 (semi-diurnal; time period = 12.4h) and M4 (quarter-diurnal; time period = 6.2h) in order to determine diel and tidal variations of species mean density in each sampling moment. Analyses were performed in SAS® software and regression models were constructed according to Schultz et al. (2003).

Analysis was carried out on larval density at both depths and on weighted mean depth of main species.

Results

Hydrological conditions

During summer sampling, salinity values ranged from 28 to 33 at surface and 32 to 34 at bottom (Fig. 2). Neap tide presented differences in salinity values between surface and bottom samples while during spring tides no significant differences were detected (Table I). During winter, these differences were more evident with surface waters presenting salinity values ranging from 4 to 11 and bottom from 31 to 35 (Fig. 2, Table I). At winter spring tide, a significant interaction between depth and tide occurred with ebb bottom samples presenting higher salinity values than during flood (*post hoc* t=3.19, corrected p=0.002).

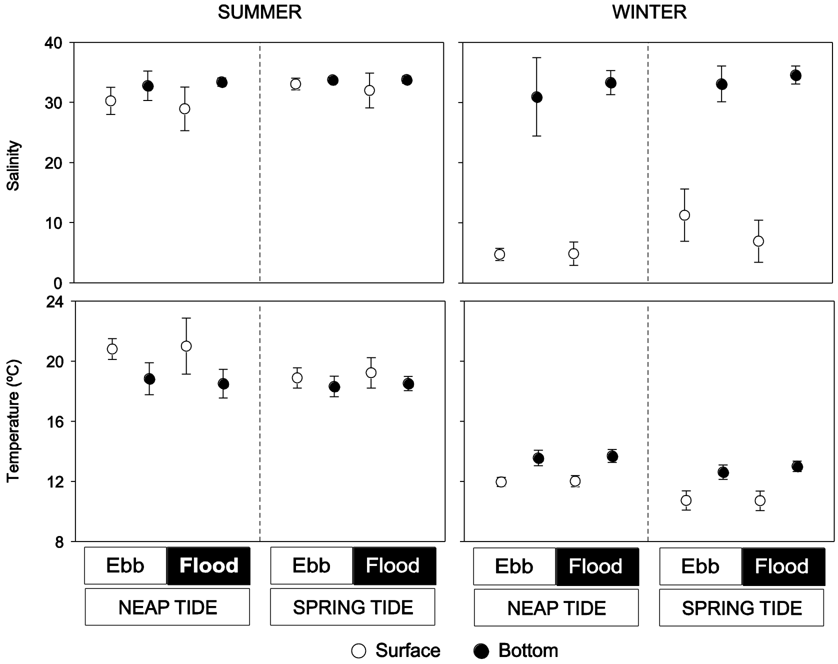

Figure 2. Salinity and temperature (°C) (mean+SD) recorded during each sampling period according to tide (ebb and flood).

Regarding temperature, summer samples showed significantly higher values at the surface while at winter temperature was higher at bottom (Fig. 2, Table I). Ebb and flood tide presented no significant differences both for salinity or temperature values (except for salinity winter spring tide) (Fig. 2, Table I).

Table I. Summary results of PERMANOVA analysis on salinity and temperature. p≤0.05*, **p≤0.01, ***p≤0.001.

		Summer Neap (n=50)	Summer Spring (n=50)	Winter Neap (n=44)	Winter Spring (n=50)
		$F_{1,1}$	$F_{1,1}$	$F_{1,1}$	$F_{1,1}$
Salinity	Tide (TI)	0.34	0.03	0.46	7.91 **
	Depth (DE)	22.93 ***	0.02	653.51 ***	340.76 ***
	TIxDE	2.13	0.07	0.57	11.87 **
Temperature	Tide (TI)	0.11	1.60	0.92	1.11
	Depth (DE)	43.34 ***	9.39 **	4.68 ***	171.70 ***
	TIxDE	0.48	0.08	0.78	1.37

Tidal and diel vertical pattern of larval distribution

A total of 8476 fish larvae were collected during the study period. In summer, densities reached 1753 ind. 100 m^{-3} during neap tide and 485 ind. 100 m^{-3} during spring tides. In winter density varied from 20 and 28 ind. 100 m^{-3} between neap and spring tides. *Pomatoschistus* spp., *Parablennius pilicornis* and *Gobius niger* the most abundant during summer and *Sardina pilchardus* and *Pomatoschistus* spp. during winter.

Species presented an even vertical distribution throughout the study period with exception for *Pomatoschistus* spp. which showed significantly higher densities in bottom, mainly during winter spring tide (Table II). At summer neap tide both *G. niger* and *P. pilicornis* showed higher density during ebb with no distinction between depths (Table II). Also, the majority of the species showed increased mean density standard deviation during ebb tides (Table II).

All the species were collected both at surface and bottom and during day and night period with the exception of *P. pilicornis* at summer spring tide and *Sardina pilchardus* at winter neap tide (Table II). Regarding diel cycle, species distribution was also uniform across the water column and, again, depth stratum was a significant factor only for *Pomatoschistus* spp. at winter spring tides (Table II). Regardless of season, the majority of species presented significantly higher density during night period. Also, mean density standard deviation is consistently higher during night periods (Table II). No significant interactions between depth and tide or diel cycle were detected (Table II).

Table II. Vertical tidal and diel pattern of mean total fish larvae density (ind. 100 m^{-3}) in each sampling period. Significant pairwise comparisons are listed from highest mean (left) to lowest mean (right). B, bottom; S, surface. E, Ebb; F, Flood; D, Day; N, Night. p≤0.05*, **p≤0.01, ***p≤0.001.

		Tidal cycle					Diel cycle				
		Flood		Ebb			Day		Night		
Sampling period	Species	B	S	B	S	Stat.	B	S	B	S	Stat.
Summer Neap	n	12	12	13	13		16	16	9	9	
	Pomatoschistus spp.	242.96 (352.47)	145.40 (283.34)	992.54 (1241.35)	630.16 (640.11)		201.67 (352.14)	143.84 (269.28)	1130.88 (1237.41)	686.11 (653.12)	ND***
	P. pilicornis	11.30 (13.96)	26.08 (25.14)	60.89 (94.75)	293.53 (499.57)	EF*	6.33 (2.58)	11.83 (6.82)	63.09 (93.72)	253.70 (457.35)	ND***
	Gobius niger	18.98 (20.37)	30.53 (40.43)	55.30 (27.98)	195.89 (285.64)	EF**	25.02 (19.24)	28.39 (32.36)	55.31 (31.99)	317.68 (328.31)	ND***
Summer Spring	n	13	13	12	12		15	15	10	10	
	Pomatoschistus spp.	168.23 (309.32)	211.70 (303.95)	157.36 (296.92)	184.81 394.94)		48.60 (52.93)	45.39 (91.38)	353.13 (427.50)	348.13 (443.64)	ND***
	P. pilicornis	10.59 (5.04)	10.71 (9.03)	9.90 (7.15)	57.84 (0)		9.97 (5.75)	0.00 (0)	10.34 (6.80)	22.49 (24.69)	ND**
	Gobius niger	30.95 (30.18)	24.43 (18.28)	37.15 (30.22)	31.12 (24.73)		26.09 (30.24)	30.63 (19.66)	41.12 (28.26)	26.91 (23.44)	ND*
Winter Neap	n	10	10	12	12		6	6	16	16	
	Pomatoschistus spp.	2.59 (0.83)	3.82 (1.67)	5.50 (3.41)	4.99 (3.51)		3.00 (1.03)	1.23 (0)	4.44 (3.15)	4.86 (2.47)	
	S. pilchardus	14.55 (16.55)	10.03 (9.84)	11.55 (11.31)	13.11 (13.69)		0.00 (0)	3.56 (0)	13.26 (14.11)	12.46 (11.67)	ND***
Winter Spring	n	13	13	12	12		10	10	14	14	
	Pomatoschistus spp.	10.18 (6.20)	4.34 (2.49)	7.51 (5.27)	4.49 (3.19)	BS**	5.47 (2.79)	2.20 (0.43)	10.09 (6.14)	5.03 (2.88)	ND*** BS**
	S. pilchardus	12.17 (13.62)	19.76 (30.24)	10.62 (12.88)	6.60 (4.57)		17.70 (15.97)	20.23 (34.31)	5.28 (3.50)	9.10 (6.15)	

At summer neap tide species weighted mean depth (WMD) ranged from 2.6 to 6.2m. *Parablennius pilicornis* presented, generally, higher WMD while *Gobius niger* showed a shallower distribution (Fig. 3). At summer spring tide, WMD ranged from 4.4 to 6m indicating a species distribution generally deeper than at summer neap tide. Main species analysed presented similar weighted mean depth (Fig. 3). During winter neap tide *Pomatoschistus* spp. WMD ranged from 4.7 to 5.4m while at spring tide was around 6m. *Sardina pilchardus* WMD ranged between 4.9 and 6 in neap tide and 4 and

5.1 in winter spring tide, reaching shallower layers of water column during flood (Fig. 3). *Pomatoschistus* spp. and *G. niger* were the only species presenting significant differences between flood and ebb WMD ($PseudoF_{1,1}=5.13$, $p<0.05$ and $PseudoF_{1,1}=6.57$, $p<0.05$, respectively) in summer neap tides. Both species presented higher WMD during floods (Fig. 3).

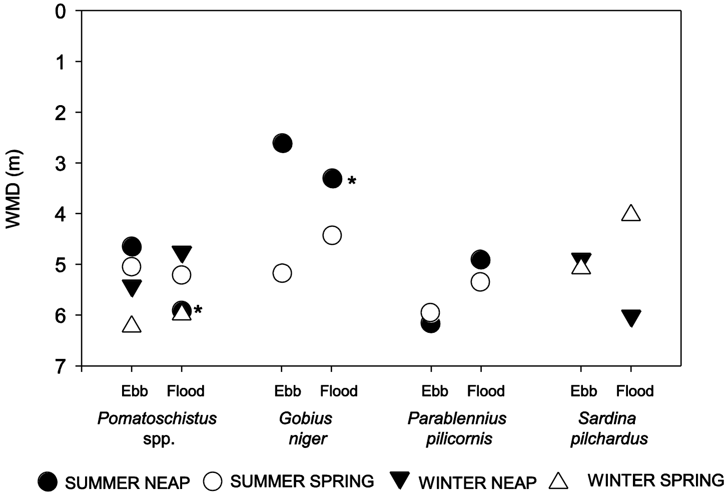

Figure 3. Tidal variation of weighted mean depth (m) of the main species at each sampling moment. Significantly differences between ebb and flood weighted mean depths are marked with (*).

Diel vertical migration amplitude varied from 0.72 to 5.34m with *S. pilchardus* reaching the highest value during winter neap tide (Fig. 4). In summer and winter spring tide, species showed a positive DVM indicating higher WMD during the day than at the night. At summer neap tide, *Pomatoschistus* spp. and *G. niger* presented a reverse pattern with higher WMD at night (Fig. 4). Significant differences between day and night WMD were detected for *G. niger* (-1.6m) and *Pomatoschistus* spp. (-1.20m) at summer neap tide ($PseudoF_{1,1}=6.57$, $p<0.05$ and $PseudoF_{1,1}=5.13$, $p<0.05$, respectively), for *P. pilicornis* (2.81m) at summer spring tide ($PseudoF_{1,1}=6.13$; $p<0.05$) and for *S. pilchardus* (-5.34m) at winter neap tide ($PseudoF_{1,1}=5.13$; $p<0.05$).

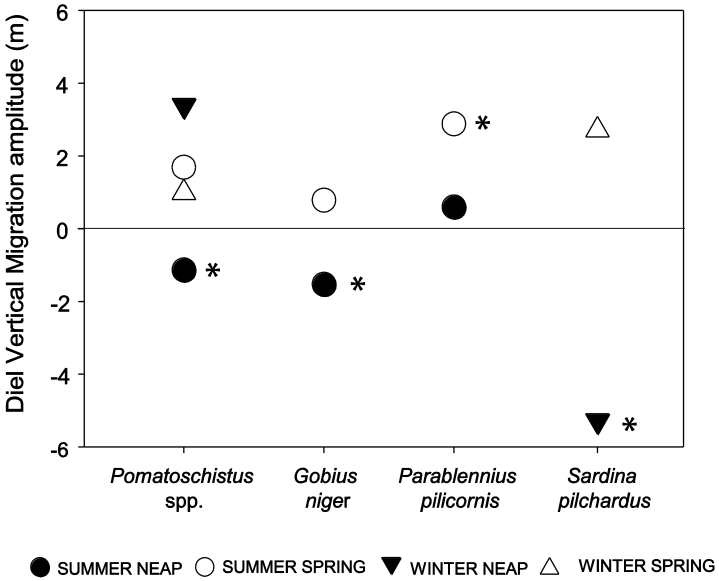

Figure 4. Amplitude of diel vertical migration (m) for the main species at each sampling moment. Significantly differences between day and night weighted mean depths are marked with (*). Negative values indicate reverse migration (deeper at night time than during the day).

Harmonic analysis of tidal and diel vertical pattern of larval distribution

Harmonic regression revealed periodic variability on most larval species analysed (Table III; Fig. 5). *Pomatoschistus* spp. mean density at surface and at bottom varied periodically according to K1 (day-night) and M2 tidal constituents during all the analysed periods except for winter neap tides. *Pomatoschistus* spp. density was generally higher at night period and around low tide (Fig. 5) and variability explained by the model was higher during summer neap tides at the surface (Table III). *Parablennius pilicornis* mean density presented periodic variability only during summer neap tide at bottom samples with predicted model explaining 54% of the variability. Again mean density varied according to K1 and M2 periodicity (Table III; Fig. 5). *P. pilicornis* density at bottom was higher during night around high tide (Fig. 5). Also at summer neap tide, the effect of K1 and M2 on *Gobius niger* larval mean density were significant and, together with M4 constituent explained 85% of the variability observed at the bottom samples. Higher densities were mainly collected during night at high tide (surface) and low tide (bottom). In summer spring tides *G. niger* variability explained by the model was lower, either for bottom or surface larval mean density (Table III; Fig. 5). K1 and M2 periodicity were significant also for *Sardina pilchardus* larvae density during winter neap and spring tides. However during winter spring tide effect was only significant in the bottom samples (Table III; Fig. 5). In winter neap tide, *S. pilchardus* density was higher at night around high tide while in spring tide larvae density maximum was observed at dusk high tide (Fig. 5).

Regarding larvae mean depth, periodic variability was only significant for *Pomatoschistus* spp. during summer spring tides with tidal predictor M2 explaining 63% of the variability observed (Table III; Fig. 5). Position in the water column was generally shallower around low tide (Fig. 5).

Table III. Coefficient of determination (R2) of harmonic regression adjusted to surface and bottom larvae densities and to weighted mean depth (WMD). Significant regression estimates are marked with $p\leq0.05^{*}$, $^{**}p\leq0.01$, $^{***}p\leq0.001$.

		Summer Neap		Summer Spring		Winter Neap		Winter Spring	
Pomatoschistus spp.	Surface	0.77^{***}	K1, M2	0.50^{*}	K1, M2	0.36		0.63^{**}	K1, M2
	Bottom	0.73^{***}	K1, M2	0.54^{*}	K1, M2	0.27		0.58^{**}	K1, M2
	WMD	0.39		0.48^{*}	M2	0.68		0.21	
P. pilicornis	Surface	0.32		0.26					
	Bottom	0.54^{*}	K1, M2	0.17					
	WMD	0.40		0.69					
Gobius niger	Surface	0.58^{**}	K1, M2	0.50^{*}	M2				
	Bottom	0.85^{***}	K1, M2, M4	0.50^{*}	K1, M2				
	WMD	0.23		0.63					
S. pilchardus	Surface					0.61^{**}	K1, M2	0.40	
	Bottom					0.71^{*}	K1, M2	0.63^{**}	K1, M2
	WMD					0.43		0.49	

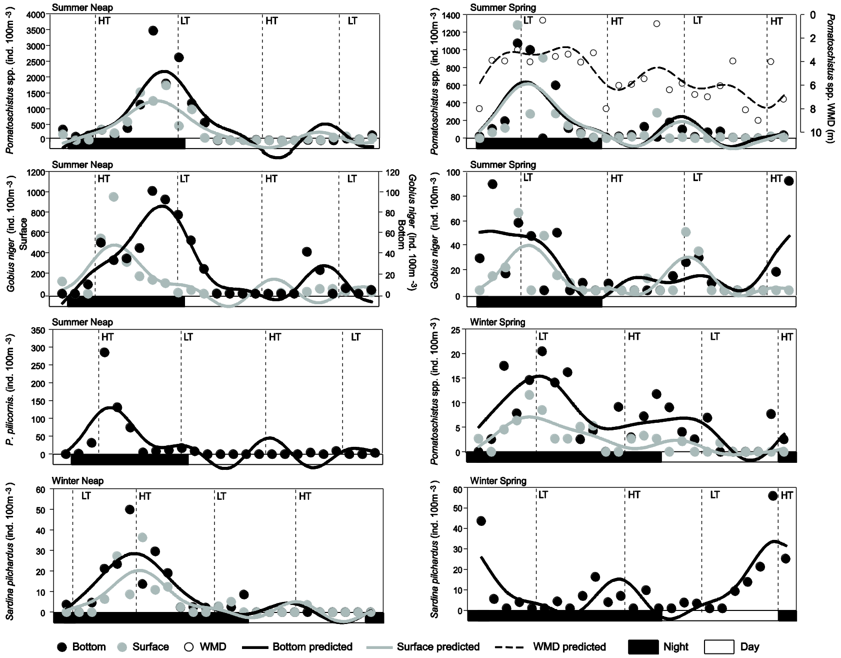

Figure 5. Variation in fish larvae density (ind. $100m^{-3}$) and weighted mean depth (m) at each season and lunar phase according to tidal cycle (LT, low tide; HT, high tide) and diel phase at each sampled depth. Symbols represent observed values and lines the fitted prediction based on harmonic regression. Only data relative to significant ($p \leq 0.05$) predicted models are presented.

Pomatoschistus spp. presented higher amplitude estimates than the other species reaching maximum values for bottom samples in summer neap tide (Table IV). Bottom larval densities showed generally higher amplitude estimates than at surface (Table IV). Also, species presented amplitude estimates generally higher during neap tides. Only *Sardina pilchardus* showed higher M2 amplitude during winter spring tide (Table IV).

Table IV. Amplitude estimates for each tidal constituent (K1, M2, M4). Estimates are presented only for significant (p≤0.05) fitted models.

		Summer Neap			Summer Spring			Winter Neap			Winter Spring		
		K1	M2	M4	K1	M2	M4	K1	M2	M4	K1	M2	M4
Pomatoschistus spp.	Surface	501.0	344.9		219.3	206.0					2.8	1.9	
	Bottom	838.9	576.1		199.7	213.4					4.89	4.61	
	WMD					1.2							
P. pilicornis	Surface												
	Bottom	43.1	37.0										
	WMD												
Gobius niger	Surface	168.5	134.1			15.9							
	Bottom	30.0	24.1	9.7	20.7	15.7							
	WMD												
S. pilchardus	Surface							7.65	5.92				
	Bottom							12.2	8.19		7.8	9.61	
	WMD												

Discussion

Hydrological conditions experienced during both studied periods indicated that ebb and flood tides presented similar conditions (salinity and temperature). During summer, the entire water column presented salinities over 30 while in winter, surface water presented visibly lower salinity leading to water column stratification. These patterns reflect the strong influence of river flow in small estuaries like Mondego estuary. In dry months, low river flow lead to an increase of tidal influence on the estuary resulting in high sea water incursion. Marine influence is expected to increase also during higher amplitude tides so, as expected, both spring tides showed generally higher salinities and lower temperatures, mainly at surface. It is expected that these different environmental conditions influence fish larvae vertical distribution.

Seasonal pattern of high fish larvae density in summer and lower in winter was already reported for Mondego estuary and is a common feature in several estuaries being probably related to spawning period of species (Primo et al., 2011). For this reason, it was chosen to analyse vertical distribution in each season separately.

Larval fish density showed no clearly preference in terms of vertical distribution, since differences between surface and bottom samples were not significant for the majority of the species. Also, no evidence of vertical stratification of larvae relative to diel cycle was found however species showed significant periodic variability in larval density related with K1 (day/night effect). Diel cycle seemed to have a strong influence on fish larvae density which presented, generally, a marked reduction in number during the day, both in summer and winter conditions. Diel changes in estuarine ichthyoplankton have been documented by several authors (e.g. Brodeur and Rugen, 1994; Auth et al., 2007; Islam et al., 2007; Aceves-Medina et al., 2008) and the differences between night and day periods were often attributed to factors as gear avoidance or diel vertical migration (Rodríguez et al., 2006; Auth et al., 2007). Gear avoidance is a common concern when sampling larval fishes, particularly the older developmental stages, as it is usually lower during night resulting in the misleading idea of higher larvae densities during nocturnal sampling. Also, rhythms of swim bladder inflation/deflation were often related with larvae vertical distribution (e.g. Ré, 1996; Santos et al., 2006). Swim bladder inflation/deflation rhythms seem to be synchronized with light cycle and vary according to larvae development stage (Santos et al., 2006).

Migration amplitude and vertical distribution patterns may vary with size, development stage and from species to species (Hays et al., 1994; De Robertis, 2002; Rodríguez et al., 2006). Although present study lacks ontogenetic stage determination, larvae collected were small larvae (pre-flexion or flexion stage) and thus analyzed together. Diel vertical migration is a common behaviour in estuarine fish and invertebrate larvae and seems to be related with light-dependent predation mortality (e.g. De Robertis, 2002; Hays, 2003; Irigoien et al., 2004). Though, low amplitude migrations observed are not expected to be light-mediated. The highest DVM amplitude was recorded for *Sardina pilchardus* (-5.34m) during winter neap tide which could be consistent with reverse diel vertical migration however, periodic analysis revealed no correlation between *S. pilchardus* WMD and day-night effect, similarly to all the remaining species. Reverse vertical migration is common and was previously found in copepods (e.g. Rawlinson et al., 2004) decapods (e.g. Tamaki et al., 2010) and fish larvae (e.g. Rodríguez et al., 2006). In the Catalan Sea, most of the mesopelagic larvae fish species were closer to the surface during the day than at night (Sabatés, 2004). Moreover Olivar et al. (2001) found the reverse DVM for *S. pilchardus*. One interpretation about adaptive significance for reverse DVM is avoidance of larger predatory invertebrates that perform normal DVM to escape from visual predators

(Hays, 2003). Furthermore, as visual predators fish larvae need an adequate level of light in the water column to detect and capture prey (Batty, 1987) and have a preferred light level that determines their diurnal distributions and diel changes in the vertical distribution result from the dispersal of larvae once the light stimulus disappears (Leis, 1991).

Similarly to DVM, tidal vertical migration has also been widely verified in several zooplankton and fish larvae species (e.g. Jager, 1999; Ueda et al., 2010). Again, larvae densities showed no evidence of vertical stratification relative to tide. Nevertheless, species showed significant periodic variability in larval density related with tide presenting generally higher densities around low tide. The increasing larval density during ebb may result in larval export from the estuary since this behaviour represents a typical ebb-tide transport. *Pomatoschistus* spp. preference for shallower waters during ebb confirmed at summer spring tide reinforces this idea. However, for estuarine resident species like *Pomatoschistus* spp. or *G. niger*, it would be disadvantageous. Though, during this season no water vertical stratification occurred neither in ebb or flood, as a result of the low river inflow experienced, resulting in an increased landward transport. In this way, deeper position in the water column during floods may be related with species exploiting stronger landward currents. Preference for deeper layers of water column was also found in Chesapeake Bay (Hare et al., 2005), Ariake Bay (Yamaguchi and Kume, 2007) and for gobies larvae in Hudson River estuary (Schultz et al., 2003). Residual currents are often referred as important mechanisms promoting upstream transport and the retention of planktonic larvae (Schultz et al., 2003; Hare et al., 2005; Islam et al., 2007). In estuaries and bays, flood-tide transport may depend on current flow at the sampling location since it can prevent larvae from successfully undertaking vertical migrations (Forward and Tankersley, 2001).

Tidal transport can also undergo diurnal changes and several authors refer an increased in flood transport during the night (e.g. Forward and Tankersley, 2001; Islam et al., 2007). Increased larvae density during ebb night time observed during this study may reflect an inverse tendency with higher downstream transport at night, particularly at winter which showed stronger seaward transport. This pattern was already mentioned for several invertebrate larvae (e.g. Queiroga et al., 1994; Forward and Tankersley, 2001).

The efficiency of larval transport on shallow depths seemed to depend on hydrodynamics condition of the estuary and inputs of river flow can be the main driven-force responsible for differences in larval distribution between the studied seasons. During summer a stronger landward transport occurred, both during ebb and flood tides while in winter, water column stratification lead to two different transport patterns, seaward on the surface and landward at the bottom. Therefore, *Pomatoschistus* spp. preference for bottom waters in winter was probably associated with retention and upstream transport in the estuary. Differences in physical transport processes within partially mixed and low-inflow estuaries may influence the dispersal and exchange of planktonic larvae (DiBacco et al., 2001). For instance, Lagadeuc et al*.* (1997) observed that the copepod *Temora longicornis* exhibited more significant vertical migrations in stratified water, but when water was mixed showed less significant migrations and a deeper distribution where vertical mixing was lowest. On the contrary, in Lough Hyne, the same species only undertook vertical migration under weakly stratified water, stopping when stratification was stronger (Rawlinson et al., 2005).

Despite vertical patterns observed could not be related with diel or tidal cycle, main species presented some extent of vertical migration. Also, spring-neap tidal cycle seemed to influence vertical distribution of fish larvae species. During summer, neap tide seemed to favour this behaviour with species presenting higher amplitude movements. At summer, spring tide conditions seemed to present a more uniform water column, concerning to environmental condition, which can be preventing species vertical migration. Often, during spring tides, faster currents and increased water turbidity occur, limiting vertical migrations (Schultz et al., 2003). In winter, stratification is probably higher during spring tides due to high river flow and increased tidal influence leading to a significant vertical density distribution, mainly for *Pomatoschistus* spp. The reverse pattern occurred in Hudson River estuary, where spring tide resulted in reduced stratification of the water column and more uniform distribution of larvae (Schultz et al., 2003). Species migration was, however, higher during neap tide, probably because of weaker currents. Some species seem to present an endogenous rhythm associated with spring-neap cycle in order to synchronise to specific tidal amplitude (e.g. Hough and Naylor, 1991; Queiroga et al., 1994).

Periodic regression advantages' over analysis of variance techniques have been previously reported (e.g. Bell et al., 2001, Schultz et al., 2003). In the present study, harmonic regression revealed patterns that analysis of variance approach could not identify reinforcing the merit of this analysis in this kind of studies.

Vertical distribution of larvae influences their transport to and across the estuary being crucial to larvae development. Seaward transport could result in exportation of to the adjacent coastal area while landward reflect larvae import from ocean or retention mechanisms within the estuary and transport to the upstream areas. For many species, reaching estuarine nursery areas is essential to successfully complete their life-cycle and understanding this process is fundamental to a more general knowledge of the population dynamics of the species involved.

Fish larvae seem to be evenly distributed throughout the water column in Mondego estuary. Also, tide and diel cycles seemed to be strongly related with fish larvae density which may influence their entrance in the estuary. Despite vertical migration observed could not be related with diel or tidal cycle, species showed variation in their position in the water column. The main feature associated with vertical patterns observed appear to be related with hydrological regime (inputs of freshwater flow) and consequently seasonal stratification, increasing amplitude during periods of less stratification and weaker water currents.

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CHAPTER V

Colonization and nursery habitat use patterns of larval and juvenile flatfish species in a small temperate estuary

ABSTRACT

Migrations between coastal and estuarine nursery areas are essential for successful completion of the life cycle of several marine fish. The present study evaluates the use of a small temperate estuary, the Mondego, Portugal, as a nursery habitat for several flatfishes during their early life stages. Data from seasonal and diel larval sampling at the mouth of the estuary and both larvae and juvenile monthly spatial distribution in the estuary (2005-2009) were gathered in order to investigate the life cycle of *Platichthys flesus*, *Solea solea* and *Solea senegalensis.* Larvae entrance in the estuary occurred mainly during summer and autumn with no evidence for diel or tidal vertical stratification. *S. senegalensis* larvae were present in all seasons at downstream areas presenting low successful settlement and juveniles' densities inside the estuary. Conversely, *P. flesus* and *S. solea* were mainly present as juveniles with upstream areas being preferred by flounder. Both species larvae seemed to settle in nearby coastal areas. The importance of the Mondego estuary for flatfishes differed according to the species, playing an important role mainly during the first year for all species. The present study highlights the importance of integrating larval and juvenile stages of fish to assess the very important role of estuaries as nursery areas.

Keywords: early life-cycle; estuarine nursery; *Platichthys flesus*; *Solea solea*; *Solea senegalensis*; Mondego estuary.

Introduction

Estuaries are recognized as nursery areas for several fish species worldwide, including flatfish (e.g. Amara et al., 2000; Able et al., 2005; Cabral et al., 2007). Most flatfishes spawn in marine waters and their pelagic larvae are transported to inshore nurseries, settling in shallow coastal and estuarine habitats (Ramos et al., 2010). The end of planktonic stage is a critical period during fish life cycle, and a successful recruitment depends on larval transport and settlement on suitable habitats (Bailey et al., 2008).

Fish larvae shoreward transport from spawning sites depends on more or less passive coastal processes (e.g. Rooper et al., 2006) but once in estuarine environments, many species present a more actively transport (Jager, 1999; Forward and Tankersley, 2001). Tidal vertical migration as mechanism of larval transport and/or retention has been verified in several fish species, including flatfish (e.g. Grioche et al., 1997, 2000; Bos, 1999; Jager, 1999; Lagardère et al., 1999). For upstream transport, larvae settle on, or move close to the bottom, during ebb tide and ascend in the water column during flood (Forward and Tankersley, 2001). Vertical movements may depend on development stage of larvae (e.g. Rowe and Epifanio, 1994; Grioche et al., 1997) and current flows at the estuarine location (Churchill et al., 1999).

A successful juvenile recruitment appears to be related with mortality processes operating during the early stage of fish life history (Van der Veer and Leggett, 2005), while abundance and distribution of juvenile flatfishes has been related to several biotic (e.g. predation, food availability) and abiotic factors (e.g. temperature, salinity, sediment type) (Able et al., 2005). Nevertheless, the nursery function of estuaries is defined by their capacity of producing more adult recruits per unit of area than other juvenile habitats used by a species (Beck et al., 2001) or by its contribution to adult populations, regardless of their unit area contribution (Dahlgren et al., 2006). The Mondego estuary as long been recognized as nursery area for several fish (e.g. Martinho et al., 2007, 2008) and its important contribution for flatfish coastal adult stocks has been recently confirmed (Vasconcelos et al., 2008, 2011). Despite the wide range of studies focusing on flatfish communities (e.g. Bos, 1999; Van der Veer, 2001;

Koubbi et al., 2006; Cabral et al., 2007; Martinho et al., 2008; Vasconcelos et al., 2008; Freitas et al., 2009), most are mainly centred in the juvenile or larval phases. Nevertheless, as proven previously, the integration of both benthic and pelagic phases is essential for a more comprehensive assessment of the nursery importance of estuaries for flatfishes (e.g. Jager, 2001; Ramos et al., 2010).

The present study aims at evaluating the larvae colonization mechanisms and the distribution of pelagic and juvenile stages of *P. flesus*, *S. solea* and *S. senegalensis*, the most abundant flatfish species in the estuary, assessing the importance of the Mondego estuary for each phase of flatfish early life history.

Material and methods

Study site

The Mondego River estuary is a mesotidal system, located in the western Atlantic coast of Portugal (40°08'N, 8°50'W) (Fig. 1). The hydrological basin of the Mondego, with an area of 6 670 km^2, provides an average freshwater flow rate of 79 m^3 s^{-1} (Dolbeth et al., 2010).

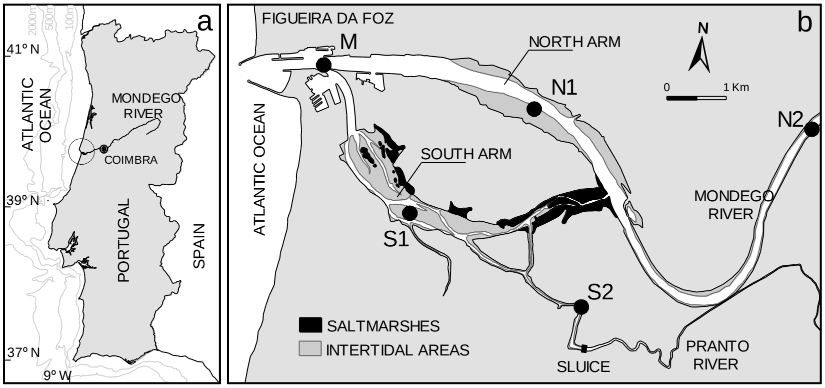

Figure 1. Location of the Mondego estuary in the west coast of Portugal (a) and sampling stations within the estuary (b).

This well-mixed estuary consists of two channels divided by the Murraceira Island, which converge again near the mouth. The north arm is deeper (5-10m at high tide), has a tidal range of 2-3m and constitutes the main navigation channel. The south arm is shallower (2–4 m at high tide), has a tidal range of 1–3m and is characterised by large areas of intertidal flats during low tide and, in the downstream areas, contains several seagrass meadows. At the mouth of the estuary, the influence of both river flow and neritic waters is strong and the depth ranges from 8 to 13 m. Tides in this system are semi-diurnal, and at the inlet the tidal range varies between 0.35–3.8m.

Sample collection

The sampling strategy included both ichthyoplankton sampling and beam trawl surveys in order to collect larval and juvenile stages, respectively.

Larvae collection was performed by combining two different approaches. A high frequency sampling programme was performed at a fixed station at the mouth of the estuary (M; Fig. 1). This sampling programme was carried out seasonally, at 1-hour intervals over a diel cycle. All hourly samples were collected from both subsurface and near bottom (<1m). Seasonal sampling took place in June 2005 (summer), September/October 2005 (autumn), December 2005 (winter) and March/April 2006 (spring), with two diel cycles performed in each season at neap and spring tides. For the spatial distribution approach, larvae were collected monthly, in spring tides whenever possible, between January 2005 and December 2009, during daytime and at flood tide, in five sampling stations (M, S1, S2, N1, N2; Fig. 1). In the spatial distribution approach, samples were collected only at subsurface. In both approaches, ichthyoplankton samples were collected using horizontal tows (bongo net: mesh size 335 µm, mouth diameter: 0.5 m; tow speed: 2 knots during 3 min) equipped with a Hydro-Bios flowmeter. Samples were fixed in a borax-buffered formalin seawater solution and preserved in 95% ethanol. Flatfish larvae were sorted and identified under a stereoscopic microscope to the lowest possible taxon and were staged as preflexion, flexion, and postflexion, based on the position of the posterior portion of the notochord and development of the hypural bones. Larvae abundance was standardized as number of larvae per 100 m^3.

Benthic juveniles were collected monthly between January 2005 and July 2007, and then every other month until December 2009. Fishing was carried out during the night at the same five sampling stations in larval surveys (M, N1, N2, S1, S2; Fig. 1), using a 2 m beam trawl with one tickler chain and 5 mm mesh size in the cod end. At each station, three tows of about 5 min at the speed of two knots were carried out, covering at least an area of 500 m^2. All fish caught were immediately frozen and transported to the laboratory for posterior sorting, identification, counting and measurement (Total length: TL). Juvenile densities were standardized as number of individuals per 1000 m2 and classified in two different categories (0-group: 0+; 1-group: 1+) according to maximum length at age 0+. Species maximum lengths at age 0+ were considered as 220 mm TL for Senegalese sole (*Solea senegalensis*), 170 mm TL for sole (*Solea solea*) and 150 mm TL for flounder (*Platichthys flesus*) and (Andrade et al., 1992; Martinho et al., 2007; Dolbeth et al., 2010).

Data analysis

High frequency sampling plankton data were grouped by season (according to sampled month) and lunar-tide cycle. Samples were classified as day (from sunrise to sunset) and night (from sunset to sunrise) periods. Samples were classified as ebb or flood tide: samples collected 1h before and after high tide were considered as flood tide, and samples collected 1h before and after low tide were considered as ebb tide. Data was used to investigate larvae seasonal and lunar tide-cycle associated distribution. In addition, diel and tidal vertical distribution patterns of larvae were studied for to the three main larval stages.

The seasonal and spatial patterns in Mondego estuary were investigated for the main flatfish species present both in larval and benthic surveys. In order to reduce interannual variability, overall seasonal means were calculated by aggregating monthly samples as follows: winter (December, January and February), spring (March, April and May), summer (June, July and August) and autumn (September, October and

November). Seasonal and spatial patterns of larval and juvenile distribution were assessed and significant differences tested by a univariate PERMANOVA analysis (PRIMER v6 & PERMANOVA+ v1, PRIMER-E Ltd.). Univariate PERMANOVA tests included a two way design (Season/Sampling station) and were based on Euclidian distances between samples, after a square root transformation of abundance data, considering all the factors as fixed and unrestricted permutation of raw data. Box and Whiskers plot were performed comprising the total length of all juvenile fish collected during benthic surveys.

Results

Larval surveys in the Mondego estuary collected five different flatfish larvae species: *S. senegalensis*, *S. solea*, *P. flesus*, *Pegusa lascaris* and *Buglossidium luteum*. However, the most common and abundant were soles *S. senegalensis* and *S. solea* and flounder *P. flesus*. *S. senegalensis* was the main species in plankton surveys, reaching 69% and 60% of total flatfish larvae density collected during high frequency sampling and spatial sampling, respectively. The second most abundant species was *S. solea* reaching 27% (high frequency sampling) and 34% (spatial sampling), while *P. flesus* represented only 4% and 6% of the flatfish larval density in the two surveys. The three species were also present in juvenile catches in different proportions, with *P. flesus* and *S. solea* reaching higher abundances (46% and 51%, respectively), while *S. senegalensis* totalized only 3% of the total juvenile catch.

Seasonal, diel and tidal larvae vertical distribution pattern

High frequency sampling of larvae revealed that Senegalese sole (*S. senegalensis*) was present in all seasons presenting higher densities during autumn (PseudoF=25.26, $p<0.01$, post hoc test, $p<0.01$), irrespectively of lunar tide cycle (Fig. 2A). For sole (*S. solea*), higher densities were found during summer (PseudoF=3.62, $p=0.01$, post hoc test, $p<0.05$) at neap tides (PseudoF=5.09, $p=0.02$) (Fig. 2B). Flounder (*P. flesus*) occurred in higher densities during spring neap tides (Fig. 2C); however, this result should be carefully interpreted since it was based only on five individuals (not tested).

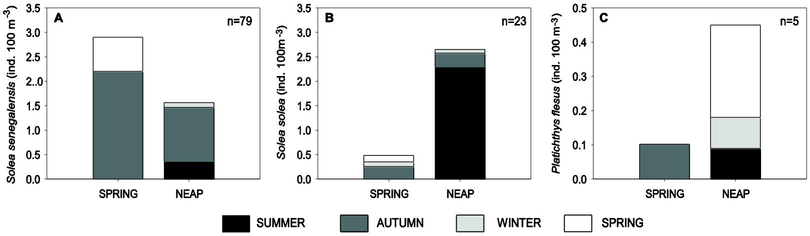

Figure 2. Seasonal and tidal variation of *Solea senegalensis* (A), *Solea solea* (B) and *Platichthys flesus* (C) larvae mean density (ind. 100m^{-3}) at the entrance of the Mondego estuary (station M) during high frequency approach. n, number of larvae collected.

Generally, flatfish larvae density was higher during the night (PseudoF=5.10, p=0.02) (Table I) with no distinction between depth (PseudoF=1.48, p=0.24) and tide (PseudoF=0.002, p=0.89).

Table I. Diel and tidal vertical distribution patterns of flatfish larvae at the Mondego estuary entrance during high frequency approach. Numbers indicate mean densities (ind. 100m^{-3}) ± standard deviation (in brackets). n, number of larvae collected.

		Solea senegalensis (n=79)				Solea solea (n=23)				Platichthys flesus (n=5)			
	Depth	Day	Night	Ebb	Flood	Day	Night	Ebb	Flood	Day	Night	Ebb	Flood
Preflexion	Surface	0.19 (±0.45)	0.24 (±0.44)	0.22 (±0.48)	0.21 (±0.41)	0.04 (±0.15)	0.13 (±0.37)	0.13 (±0.37)	0.04 (±0.15)				
	Bottom	0.19 (±0.36)	0.34 (±0.65)	0.33 (±0.64)	0.20 (±0.39)		0.05 (±0.20)		0.05 (±0.20)	0.05 (±0.18)	0.10 (±0.26)	0.09 (±0.25)	0.05 (±0.20)
Flexion	Surface	0.16 (±0.40)	0.19 (±0.43)	0.18 (±0.42)	0.18 (±0.41)	0.15 (±0.50)	0.68 (±1.84)	0.42 (±1.70)	0.41 (±0.95)				
	Bottom	0.24 (±0.44)	0.36 (±0.53)	0.35 (±0.59)	0.25 (±0.36)	0.03 (±0.09)	0.44 (±0.89)	0.29 (±0.73)	0.18 (±0.59)		0.06 (±0.25)		0.06 (±0.25)
Postflexion	Surface	0.06 (±0.17)			0.06 (±0.17)		0.04 (±0.16)		0.04 (±0.16)		0.07 (±0.29)		0.07 (±0.29)
	Bottom	0.10 (±0.29)	0.16 (±0.44)	0.16 (±0.44)	0.10 (±0.29)								

Postflexion stage of *S. senegalensis* larvae were generally less abundant that flexion and preflexion stage (PseudoF=3.27, p=0.04, *post hoc* test, p<0.05) while flexion stage *S. solea* larvae presented higher densities than other stages (PseudoF=6.02, p<0.01, post hoc test, $p<0.05$), reaching higher values at surface night samples (Table I). Both tidal and diel cycle seemed not to represent an influence in both sole vertical distributions (Table I). Again, regarding flounder, the low number of larvae collected prevents further conclusions.

Flatfish larvae and juvenile seasonal and spatial distribution

Larval stages

In the spatial distribution approach, *S. senegalensis* larvae density ranged between 0 and 0.4 ind. 100m^{-3}, being present in the Mondego estuary in all seasons (Fig. 3A). Mean densities presented were not significantly different between seasons (PseudoF=0.42, p=0.76). Also, no significant differences in mean density were detected between larval stages (PseudoF=1.25, p=0.28), despite that, preflexion larvae were more common. Generally, preflexion larvae densities were lower during winter months, while late larval stages were more abundant in spring/summer months (Fig. 3A). Spatially, Senegalese sole larvae were well distributed across the estuary (PseudoF=1.41, p=0.22), with preflexion stage larvae mainly present at sampling station S1 (post hoc test, $p<0.05$), while posflexion larvae showed higher densities at sampling stations N1 and N2 (Fig. 3B).

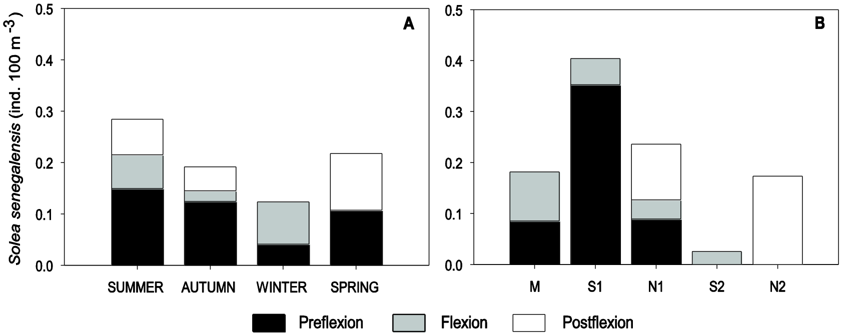

Figure 3. Seasonal (A) and spatial (B) variation of *Solea senegalensis* larvae mean density (ind. 100m^{-3}) by ontogenetic stage in the Mondego estuary during spatial distribution approach.

Average densities of S. solea larvae collected inside the Mondego estuary reached 0.2 ind. $100m^{-3}$ and were all at preflexion stage (Fig. 4). No significant differences between larvae mean density were observed for seasons (PseudoF=0.41, p=0.76) or for sampling stations (PseudoF=1.22, p=0.33). However, higher larvae densities were generally observed in autumn, winter and spring and at sampling stations S1 and N1 (Fig. 4A, B).

The few *P. flesus* larvae present in the samples were collected in autumn, at sampling station N1 (flexion stage; 0.13 larvae $100m^{-3}$) and N2 (postflexion stage; 0.25 larvae $100m^{-3}$).

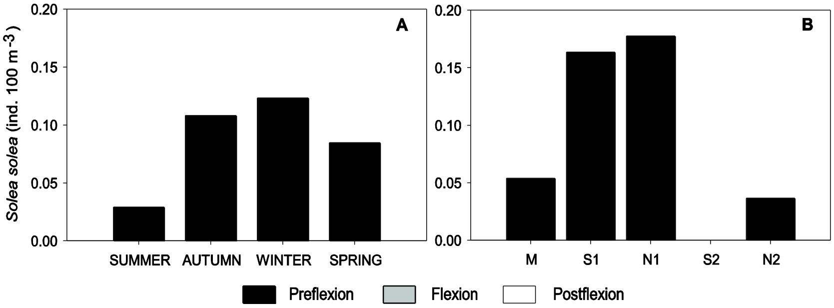

Figure 4. Seasonal (A) and spatial (B) variation of *Solea solea* larvae mean density (ind. $100m^{-3}$) by ontogenetic stage in the Mondego estuary during spatial distribution approach.

Juvenile stages

The Mondego estuary flatfish juvenile spatial approach showed that 0-group (0+) fish densities were generally higher than 1-group (1+), irrespectively of the species (Senegalese sole: PseudoF=16.23, p<0.01; Sole: PseudoF=27.73, p<0.01; Flounder: PseudoF=11.22, p<0.01) (Fig. 5).

Mean density of *S. senegalensis* juvenile ranged between 0 to 0.4 ind. $1000m^{-2}$ (Fig. 5A). Densities were not significantly different between seasons (PseudoF=0.33, p=0.82) (Fig. 5A). Spatial distribution varied significantly according to fish age group (PseudoF=2.96, p=0.03): both categories were absent from upstream sampling station

N2, however at N1 and S2 younger juveniles' densities were generally higher (post hoc test, $p<0.05$); older juveniles were only present at M and S1 (Fig. 5A).

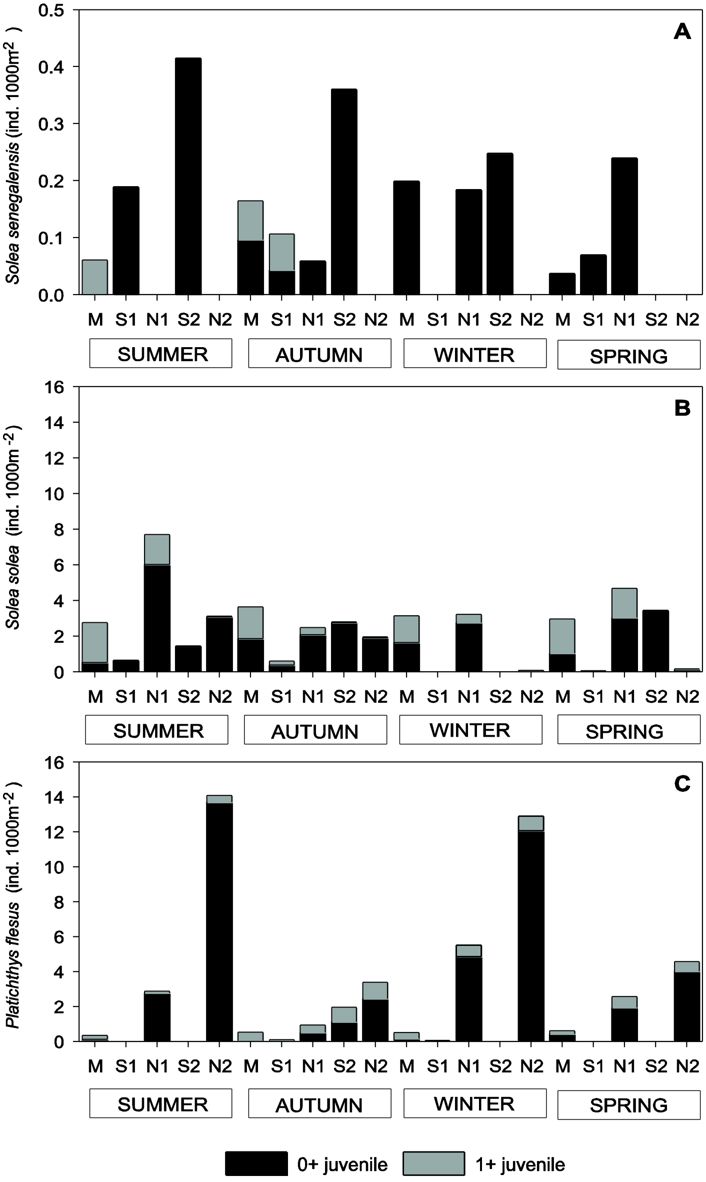

Figure 5. Seasonal and spatial variation of flatfish juvenile density (ind. $1000m^{-2}$) in Mondego estuary by different size groups during spatial distribution approach. (A) *Solea senegalensis*, (B) *Solea solea* and (C) *Platichthys flesus*.

Mean density of *S. solea* juvenile was considerably higher than those of *S. senegalensis* (maximum peak density: 8 ind. 1000 m^{-2}) (Fig. 5B). Seasonal pattern generally presented lower densities in the winter (PseudoF=3.74, p=0.01; post hoc test, p<0.05) (Fig. 5B). Sampling station S1 showed the lowest juvenile density and M and N1 the highest (PseudoF=20.15, p<0.01; *post hoc* test, p<0.05). Sole spatial distribution also differed between age groups (PseudoF=6.14, p<0.01): all sampling stations presented higher densities of younger juveniles, except for the M station, where significant differences were not detected (*post hoc* test, p<0.05) (Fig. 5B).

Mean density of *P. flesus* juveniles reached nearly 14 ind. 1000m^{-2} with peak densities recorded at the upstream station N2 during the summer (Fig. 5C). However, flounder mean abundance did not vary significantly between seasons (PseudoF=0.91, p=0.44). Sampling station N2 showed the highest densities of flounder juvenile (PseudoF=18.58, p<0.01; *post hoc* test, p<0.05) (Fig. 5C). Differences between age groups were not significant at the sampling stations M, S1 and S2 but N1 and N2 showed significantly higher 0+ flounder juveniles (PseudoF=7.13, p<0.01; *post hoc* test, p<0.05) (Fig. 5C).

Juvenile size-specific habitat use

Smaller juveniles of *S. senegalensis* were recorded in the summer, mainly at the south arm sampling stations (S1, S2) (Fig. 6). During the autumn and winter, Senegalese sole was well distributed along the estuary, while in spring juveniles were concentrated at downstream sampling stations (M, S1 and N1) (Fig. 6). Once completing their first year, fish were concentrated at the mouth of the estuary, where they remained until the autumn. Afterwards, older fish (1+) were not recorded in the estuary (Fig. 6).

First record of *Solea solea* 0-group fish occurred in the spring and mainly at the S2 and N1 sampling stations (Fig. 6). During the first summer, autumn and winter, juvenile sole were well distributed across the estuary (Fig. 6). After completing one year, juveniles moved downstream (M, N1) and remained there throughout next spring, summer, autumn and winter (Fig. 6). Larger fishes were observed in the summer and spring at the mouth of the estuary (Fig. 6).

Smaller individuals of *P. flesus* were mainly collected in the summer at sampling station N2 and during the first year, flounder juveniles were restricted to the north arm sampling stations (N1, N2) (Fig. 6). After that, fish moved downstream to the mouth of the estuary and older individuals (1+) were almost restricted to this area during the next year (Fig. 6).

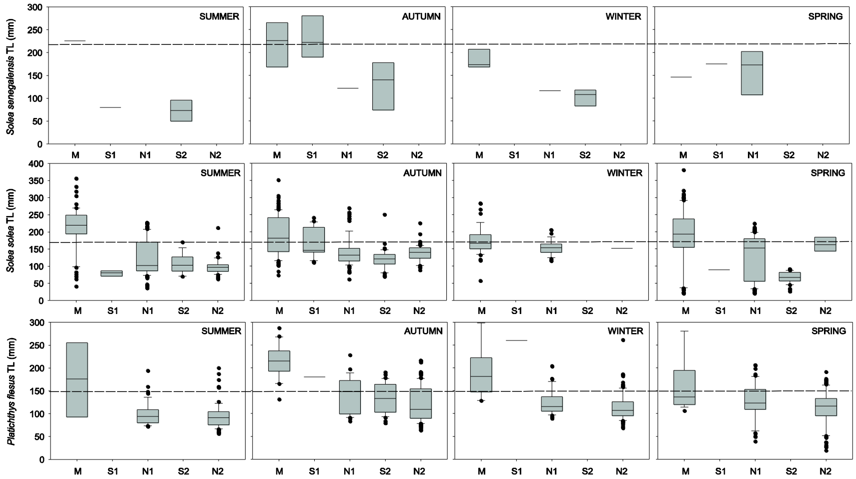

Figure 6. Box and Whiskers plot for seasonal and spatial variation of total mean length (TL) of *Solea senegalensis*, *Solea solea* and *Platichthys flesus* in Mondego estuary during spatial distribution approach. Boxes comprise 25th-75th percentiles marked with the median value. Whiskers (error bars) indicate the 90th and 10th percentiles. Filled circles represent outliers. Dashed line represents the maximum length of 0^+ juveniles (*Solea senegalensis*: 220mm; *Solea solea*: 170mm; *Platichthys flesus*: 150mm).

Discussion

The input of larvae is one of the factors determining the importance of an area as nursery for marine fish (Jager, 2001), and connectivity between coastal and estuarine nurseries is often critical to a successful completion of a species life cycle. Recruitment of larvae to nursery areas is linked to active behavioural responses on the part of larvae (Boehlert and Mundy, 1988). Grioche et al. (2000) found that for transport towards the coast in the English Channel, sole larvae were able to perform tidal and diel vertical migrations even during the youngest stages, while flounder larvae only begun their vertical migration at the stage of notochord flexion. In the present study, larvae densities showed no evidence of vertical stratification relative to tide. Also, vertical migrations seemed not to be triggered by diel period. In agreement with our results, Koutsikopoulos et al. (1990) and Champalbert and Koutsikopoulos (1995) did not find any evidence of a behavioural selection of tidal currents by the pelagic sole larvae in the Bay of Biscay, assuming that probably this behaviour may develop after settlement to the bottom (Koutsikopoulos et al., 1990). In addition, the stronger tidal pressure at the English Channel may enhance larvae vertical migrations (Grioche et al., 2000).

Amara et al. (2000) found that the time of initiation of sole estuarine colonization can vary enormously depending on the balance between sea and river water, regulated by a combination of direction and intensity of winds, river flow and tidal cycle. During the high frequency sampling period in the Mondego estuary, the salinity differences between surface and bottom waters were probably not sufficient to induce vertical migration in larvae. On the other hand, such migrations may occur in a posterior development phase resulting in a later entrance in the estuary. Mechanisms of vertical migration often vary with size, development stage and are species specific (e.g. Grioche et al., 2000), resulting in distinct patterns of nursery colonization.

Ecological information concerning *S. senegalensis* is scarce and mostly refers to the Portuguese coast (e.g. Andrade, 1992; Cabral et al., 2003, 2007; Vinagre et al., 2009). Despite that *S. senegalensis* is a typical southern Soleidae species, its distribution area has expanded further north (Desaunay et al., 2006) and it is expected to occur more frequently along the NE Atlantic, including Portuguese estuaries and coastal areas (Vasconcelos et al., 2008). In the Tagus estuary, *S. senegalensis* juveniles concentrate in large intertidal mudflats (Vinagre et al., 2009). Among the Portuguese estuaries, Tagus and Sado present higher Senegalese sole juvenile densities followed by Ria de Aveiro (Vasconcelos et al., 2010); further north, in the Lima estuary, *S. senegalensis* is also an abundant flatfish species both as larvae and juveniles. In this estuary, young juveniles disperse along the inner intertidal areas of the middle and upper sections, reaching the lower estuary as they grow (Ramos et al., 2010). In the Mondego estuary, Senegalese sole were more abundant during the pelagic phase, while the juvenile densities were usually low. In fact, *S. senegalensis* nursery grounds are limited to large estuarine systems with an extended intertidal area which can provide, for marine species like *S. senegalensis*, refuge from predators and favourable conditions for a rapid growth (Cabral et al., 2007; Vinagre et al., 2009). The Mondego estuary intertidal zone is mainly located at the south arm and may not be adequate as nursery for this species, particularly given its small size when comparing with larger estuaries. In addition, different predictors as depth, percentage of mud in the sediment, relative distance to the estuary mouth and macrozoobenthos density may explain *S. senegalensis* distribution (Vasconcelos et al., 2010), but the importance of each predictor needs further investigation.

Despite the low Senegalese sole juvenile densities, the Mondego estuary seemed to be largely used during this species' larval phase, since it was the most abundant and frequent flatfish in plankton samples. The presence of several larval stages in all seasons is an indication of a protracted spawning season which has been reported for other estuarine areas (e.g. Cabral et al., 2003; Fonseca et al., 2006; Ramos et al., 2010). The spawning period of *S. senegalensis* consists of two periods, from January to June and in autumn around October-November (Garcia-Lopez et al., 2006). The production of several batches of larvae during the spawning season has been previously demonstrated for *S. senegalensis* and explains the prolonged spawning period (Dinis, 1986; Andrade, 1992). In the present study, higher densities of larvae arrived to the estuary during summer/autumn period and the presence of high densities of early larvae (pre and flexion stage) may indicate a nearby spawning location.

Once they reach the estuary, larvae disperse mainly along downstream sampling stations (M, S1, N1) with early juveniles settling in the south arm during summer and distributed throughout the estuary as they grew (Fig. 7). Older juveniles (1+) were restricted to the mouth of the estuary and left during winter (Fig. 7). The use of estuarine areas during this initial phase of life may be crucial for *S. senegalensis* development and survival. The use of the estuary only during larval phase may indicate recruitment failure and larval mortality as a consequence of the low suitability of the estuary as a settlement area for the species. Otherwise, the larvae may be leaving the estuary before settlement (Fig. 7). Planktonic interval duration may also influence the settlement pattern of species and it is often related with the retention mechanisms of several fish species (Amara et al., 1998). Longer pelagic larval duration may increase species dispersion and transport to suitable coastal nursery and adult habitats. According to Cabral et al. (2003) *S. senegalensis* presents a high genetic flux between populations in the Portuguese coast, confirmed by coastal populations with nursery origins often far from the point they were collected (Vasconcelos et al., 2008). However, the extent of the relation between pelagic larval duration and retention mechanisms in *S. senegalensis* requires further research.

The Mondego estuary played a different role as nursery area for *S. solea*. Sole larvae abundances were generally low, but it was present in all seasons. In the spatial approach, all larvae collected were at preflexion stage. Such early developmental stage may indicate, once more, a nearby coastal spawning area. *Solea solea* has a shorter period of larval occurrence in the plankton (Dinis, 1986), which can explain the lack of latter larval stages in the samples collected inside the estuary. Also, larvae may accidentally enter in the estuary with the seawater incursion, remaining in estuarine areas during small periods of time. Larvae may then return to nearby coastal areas where they settle, returning to the Mondego estuary afterwards as benthic juveniles. This hypothesis can be corroborated by the presence of advanced sole larval stages only during the high frequency sampling approach in the mouth of the estuary, contrasting to the earlier larval stages collected inside the estuary where the distance to coastal settlement areas is higher. In the Vilaine estuary (Bay of Biscay), sole larvae begin to metamorphose before entering and complete the process by settling as late larvae or early juveniles (Lagardère et al., 1999; Amara et al., 2000). Most of the timing of coastal immigration of metamorphosing sole to the bays and estuaries of the northern Bay of Biscay appear ontogenetically determined by the initiation of metamorphosis (Lagardère et al., 1999).

Nursery areas of *S. solea* may be located in marine coastal areas as well as within estuarine systems (Cabral et al., 2007). Van der Veer (2001) found that sole larval settlement occurs not only in the intertidal flats, but also in deeper parts of the Wadden Sea. In the Mondego estuary, *S. solea* seemed to present also indirect settlement (recruitment to nurseries areas after settlement), a pattern observed also in nearby Lima estuary (Ramos et al., 2010). Spawning seemed to occur during winter/spring and after settling in coastal areas, small recently settled juveniles migrate to the south arm (S1, S2). As they grew, fish moved to the middle (N1) and upstream (N2) areas of the estuary (Fig. 7). Vinagre et al. (2008) has previously reported the winter/spring spawning season for *Solea solea* in several European estuaries. Again, in the Mondego estuary, juveniles moved to the mouth of the estuary after completing one year leaving during winter (Fig. 7). According to Vasconcelos et al. (2008, 2011), the Mondego estuary is an important source of successful sole recruits to coastal adults stocks.

Flounder presented a similar colonization pattern to the common sole. The number of larvae collected in the estuary was very low, indicating an offshore spawning. Recently, Morais et al. (2011) suggested that *P. flesus* appears to spawn not only in coastal areas, but also inside the Minho estuary (Portugal). On the contrary, Ramos et al. (2010) referred that *P. flesus* enters the Lima estuary (Portugal) in an advanced stage of the larval phase, as has also been reported for the Ems estuary in the Wadden Sea (Jager, 1998, 1999). Motility of early flounder larvae is relatively low and larvae are totally dependent of water movements (Grioche et al., 2000; Bos et al., 2006). At latter developmental stages, larvae start to perform vertical migrations from surface waters, allowing them to reach coastal areas transported by bottom currents (Grioche et al., 2000; Koubbi et al., 2006). In the English Channel, older larvae are found mainly in the coast, with higher abundances near estuaries (Grioche et al., 1997). Since late larvae are preferably in the bottom, the almost inexistence of larvae may be a consequence of surface sampling. However, neighbouring studies collected flounder larvae with a similar sampling procedure (e.g. Ramos et al., 2010) and therefore this seems not to be a limitation. In this way, the almost inexistence of larvae may indicate that flounder, as sole, enters in the Mondego estuary only after settlement. After spawning, larvae settle and early juveniles enter in the Mondego estuary, migrating to upstream freshwater areas (Fig. 7). The highest densities of younger juveniles (0+) were recorded during the summer and winter in this area. Salinity has been shown to be highly a determinant factor for the distribution of flounder within estuaries (e.g. Van der Veer et al., 1991; Jager, 1998; Freitas et al., 2009) being particularly important in the Mondego estuary (Martinho et al., 2007; Vasconcelos et al., 2010). Despite this recognized preference for freshwater habitats by 0-group flounder, it is less evident whether this preference already exists during larval stage, at metamorphosis or if it develops after settlement (Jager, 1998). According to Bos et al. (2006), salinity becomes important as a major factor affecting the behaviour of postlarval flounder during the settlement period, and becomes even more important for spatial distribution in early juvenile stages. This fact reinforces the idea that flounder only enters in the Mondego estuary after settlement, when salinity starts to become a major factor affecting flounder behaviour. Once within estuarine areas, flounder seemed to restrict its distribution to the north arm (M, N1, N2), since it has a higher freshwater input. After completing the first year in the estuary, 1+ juveniles moved to the downstream station, where they can reside until reach maturity (length at first maturation: 200mm; Dinis, 1986) (Fig. 7). According to Dolbeth et al. (2010), both *S. solea* and *P. flesus* can live up to two years in Mondego estuary, completely disappearing before the age of three years, only returning to the estuary sporadically (Martinho et al., 2008).

The nursery function of the Mondego estuary and its contribution for flatfish coastal assemblages has been previously confirmed (e.g. Martinho et al., 2007, 2008; Vasconcelos et al., 2008, 2011). However, the importance of this estuary for larval and juvenile life stages of flatfish was species-specific, being mainly used by juvenile stages of flounder and sole and by larval stages of Senegalese sole. As already mentioned, neighbouring estuaries (Lima and Minho) presented distinct colonization patterns from the Mondego, mainly regarding *S. senegalensis* and *P. flesus*. These patterns probably result from the characteristics of each potential nursery habitat. River plume is often pointed out as one of the major factors affecting estuarine colonization as suggested previously by the positive relationship between river flow and juvenile densities (Vinagre et al., 2007; Martinho et al., 2009). Forward and Tankersley (2001) referred that environmental cues (e.g. odour, temperature, salinity, turbidity) might be used by fish for initiating behavioural responses in order to enter in the estuarine nurseries. The extent of the river plume influences both the larval supply and the size and biotic capacity of habitats in estuarine nursery grounds (Le Pape et al., 2003). The relatively high river flow may provide the required cues for the larvae gathering around the estuary, but its low residence time may influence their retention. This seemed to be the case of flounder and sole larvae that successfully colonize the estuary only as juveniles, conversely to the Lima estuary in which, according to Ramos et al. (2010), flounder is an important component of the larval flatfish assemblages.

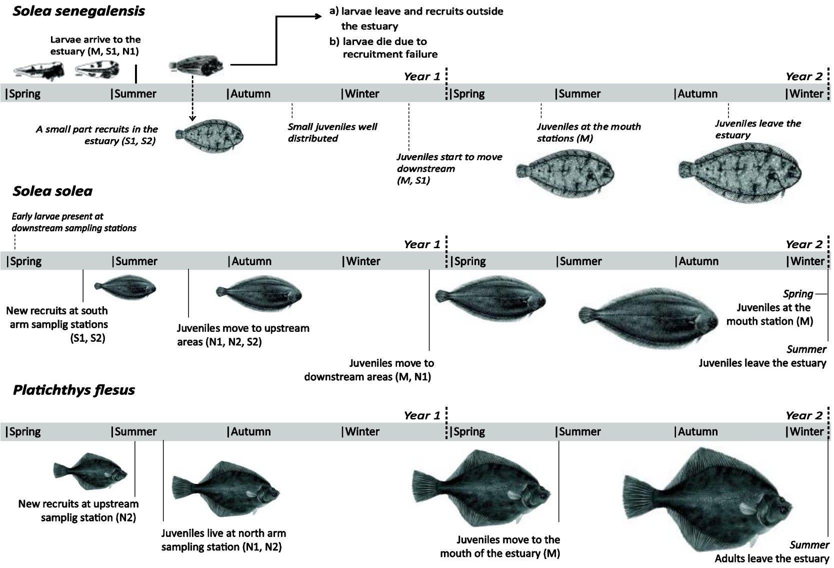

Figure 7. Schematic summary of *Solea senegalensis*, *Solea solea* and *Platichthys flesus* early life cycle in the Mondego estuary.

The importance of the Mondego estuary for the early stages of flatfishes life-cycle differed according to the species: *S. senegalensis* was present in the estuary mainly during larval phase, while *S. solea* and *P. flesus* entered after settlement. Despite that *P. flesus* juveniles were almost restricted to north arm, both flounder and sole were often present in the same microhabitats inside the estuary and both seemed to leave during their second years' winter in the estuary. The present study highlights the importance of estuaries as flatfish nurseries, mainly during their first year as well as the importance of integrating larval and juvenile stages of fish to assess the role of nursery areas.

Acknowledgments

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CHAPTER VI

General Discussion

Estuarine fish larvae assemblages: seasonal and spatial patterns

The Mondego estuary pelagic and benthic communities have been extensively studied (e.g. Marques et al., 2007; Cardoso et al., 2008; Martinho et al., 2008; Dolbeth et al., 2011; Grilo et al., 2012) and its role as an important nursery ground for pelagic and benthic marine fish widely recognized (Martinho et al., 2008; Vasconcelos et al., 2008, 2011). However, due to the scarcity of studies on fish early life stages, the present study represents one step forward in the assessment of the nursery function of the estuary.

Estuarine fish larvae assemblages are variable both in terms of species composition and distribution patterns. The larval fish assemblages of the Mondego estuary presented slightly lower number of taxa (31 taxa; 16 families) than other estuaries of Portugal. In the Lima estuary were recorded the highest number of taxa (50 taxa; 20 families) (Ramos et al., 2006), while in Tagus estuary were reported 32 taxa (20 families) (Ré, 1999) and in the Guadiana occurred 34 taxa (13 families) (Faria et al., 2006). The use of different sampling methodologies (sampling gear, period and area) must be taken in consideration when comparing these results. Moreover, each estuarine system has different abiotic environments that result from differences in the tidal range, freshwater input, geomorphology and human pressure (McLusky and Elliott, 2004), which influence species composition, larvae entrance and distribution, as well as habitat quality for settlement. Ultimately, these factors influence directly the nursery function of each estuary. Among environmental parameters, temperature, salinity and river flow are particularly important variables affecting ichthyoplankton communities in estuarine systems (e.g. Whitfield, 1999; Barletta-Bergan et al., 2002; Drake et al., 2007).

The first ichthyoplankton study in the Mondego estuary is outdated and mainly focused at south arm (Ribeiro, 1991) while Marques et al. (2006) described patterns only for the most abundant taxa (*Pomatoschistus* spp., *Engraulis encrasicolus* larvae and eggs). The present study constitutes a more comprehensive approach enclosing fish larvae spatial and seasonal patterns as well as environmental forcing structuring these assemblages at the Mondego estuary.

Spawning seasons are dictated by temperature since it is closely related to the adult's growth and reproductive cycle (Palomera, 1992; Ramos et al., 2006). In this way, seasonality becomes a common feature in Mondego estuary larval fish assemblages, with higher densities occurring mainly during late spring and summer. Added to the dominance of *Pomatoschistus* spp. throughout the year, the Mondego estuary summer/spring larval assemblages were constituted by *Atherina presbyter*, *Solea solea*, *Syngnathus abaster*, and *Crystallogobius linearis*, while *Ammodytes tobianus*, *Callionymus* sp., *Echiichthys vipera,* and *Liza ramada* were more abundant in the autumn/winter.

While temperature is the main driving force for temporal patterns, spatial preferences of organisms within estuaries are intrinsically related to the salinity gradient (e.g. Kimmerer et al., 2002; Drake et al., 2007). Spatial patterns revealed that fish larvae assemblages at south arm downstream sampling station (S1) presented generally higher diversity and density. South arm shallower depth, lower daily salinity changes, and hydrodynamics enhanced the establishment of larval fish assemblages. Fish larvae distribution in the Mondego estuary was closely related with the salinity gradient, with marine stragglers and migrant species mainly present in the downstream areas. This seemed to be primarily related with the planktonic nature of fish larvae that are incapable of counteracting currents, remaining in areas where marine water reaches.

Variations in freshwater supply will influence salinity gradient and species distribution, which may bring consequences to estuarine communities. At a short time scale, seasonal differences in river flow due to different precipitation regimes will affect communities, as observed in this work (Chapter II). Furthermore, under the global climate change scenario and recurrent extreme climatic events, it is fundamental to understand how these variations will affect estuarine communities at longer time scales.

Extreme climatic events impact on estuarine communities

Temporal and spatial variability in estuarine organisms occur mainly through seasonal and interannual variability in freshwater flow (Kimmerer, 2002). River discharge into estuaries is substantially altered for human use and may be extremely sensitive to climate change (Vörösmarty et al., 2000). Variation in river flow has important consequences within the estuary, leading to changes in organic matter input, salinity or water currents (Kimmerer, 2002; Vinagre et al., 2007; Martinho et al., 2007; Primo et al., 2009). During reduced freshwater flow periods, the estuarine salinity gradient changes, since marine waters push further upstream. In extended periods, these events may result in droughts which may lead to hypersaline conditions in the upstream regions and to the loss of aquatic habitat for many species (Pillay and Perissinotto, 2008). In the Mondego estuary, an extreme drought event affected the macrobenthic communities by reducing *Scrobicularia plana* densities (Dolbeth et al., 2011; Grilo et al., 2011), *Cyathura carinata* growth rates (Bordalo et al., 2011), *Zostera* *noltii* biomass and *Hydrobia ulvae* populations (Cardoso et al., 2008; Dolbeth et al., 2011). Also, fish assemblages suffered depletion in freshwater species, reduction of estuarine resident fish abundances and an increase in marine straggler species (Martinho et al., 2007; Baptista et al., 2010). During the drought period, a 15-45% reduction in the estuarine fish production was estimated, affecting the nursery function of the estuary (Dolbeth et al., 2008). Furthermore, the trophic level of the fish assemblage increased due to higher predators' densities, namely planktivorous and invertebrate feeders (Nyitrai et al., 2012). Drought effects observed in the larval fish assemblage of the Mondego estuary were mainly related to the species displacement within the estuary (Chapter II). Changes in the salinity gradient affected species distribution, allowing marine fish larvae to reach the most upstream areas of the estuary. In addition, a lack of seasonal variation on larvae species assemblage was observed during the dry years, leading to a stable community throughout the year. Similar responses occurred in other local planktonic organisms: together with increased mesozooplankton density, the dry period was also associated with a reduction in zooplankton seasonality and salinity-associated differences between upstream and downstream communities (Marques et al., 2006; Primo et al., 2009).

Recruitment seems to present a close relationship with river flow and large scale patterns such as the NAO, the main driving force influencing north Atlantic climate regime (e.g. Atrill and Power, 2002; Sims et al., 2004). River runoff is interrelated with the extension of river plumes to coastal areas, an important cue for orienting fish larvae towards estuarine nurseries, thus fundamental to recruitment process in estuaries (Amara et al., 2000; Forward and Takersley, 2001; Vinagre et al., 2007). In conformity, in the Mondego estuary, lower larvae abundance was recorded during drought, mainly when compared with 2003, a year with higher runoff. On the other hand, the higher prevalence of marine waters in the estuary led to an increase in marine species abundance in the estuary, mainly in 2008. A dualism also reported in the juvenile assemblages, where estuarine and marine nurseries species decreased (*Platichthys flesus*, *Solea solea*, *Dicentrarchus labrax*) but marine adventitious increased (e.g. *Solea lascaris*, *Arnoglossus laterna*).

Due to the increased occurrence of extreme events, like droughts, there has been a growing interest in climate variability and its effects on marine ecosystems (Parsons and Lear, 2001; Hays et al., 2005; Rijnsdorp et al., 2009). In this way, studies like this contribute to understanding how these events may affect estuarine communities worldwide. As for estuarine communities previously studied (e.g. Martinho et al., 2007; Primo et al., 2009; Dolbeth et al., 2011; Grilo et al., 2011), changes in freshwater flow in the Mondego estuary proved to affect larval fish seasonal and spatial patterns. The balance between fresh and marine water showed to be extremely important for fish larvae distributions, influencing also their entrance and recruitment in the estuary.

Fish larvae recruitment in the estuary: transport and migration patterns

Ontogenic and periodic seasonal migrations between reproductive, feeding and nursery areas mark the life cycle of fish populations (Joyeux, 2001). Ingress into and retention within an estuary is crucial for successful completion of many fish life cycle, but can be affected by several aspects of estuarine circulation (Islam et al. 2007). In the Mondego estuary, fish larvae assemblages entering in the estuary and their subsequent distribution were mainly related with each species' spawning period (Chapter III). Higher larvae densities in the estuary were recorded during summer, mainly composed of marine straggler species. For winter spawning species as *Sardina pilchardus*, maximum values were observed during the winter. The lunar cycle had higher influence in larval assemblage composition and spatial distribution inside the estuary. Due to higher seawater intrusion in the estuary at spring tides, it would be expected an increase in marine larvae entrance and in their ability to reaching most upstream areas. However, this was not always observed. Despite that several studies suggested that the lunar phase plays a role in recruitment patterns, the delivery of competent larvae to nursery areas may also be influenced by local hydrographic events (Thorrold et al., 1994; Vinagre et al., 2007). Environmental variables, such as river runoff, may also contribute to explain the differences observed during spring/neap tides. In fact, lunar associated cycles are species-specific and seem mainly related with each species' spawning period (Oliveira et al., 2009).

Tide represented low influence on seasonal patterns of larvae entering the Mondego estuary, either on assemblage composition or in abundance, a trend also noted by Roper (1986) and Trnski (2001). *Sardina pilchardus* was the only species showing differences between tides, with higher densities at flood during spring spring tides, indicating that this was an important period of larval entrance in the estuary. Due to their low ability to counteract the currents, fish larvae seemed to follow the salinity gradient displacement that occurs during rising and receding tide.

Mechanisms of estuarine recruitment and larval fish immigration into shallow estuaries include non-tidal flows, developing during episodic meteorological events, and selective tidal stream transport (STST), which presumes the existence of vertical migrations within the water column to take advantage of favourable currents (Joyeux, 1998). These migration patterns were also studied in the Mondego estuary by analysing diel and tidal cyclic variations of larvae density and mean depth (Chapter IV). The results showed that Mondego estuary presented low evidence of vertical migration according to diel or tidal cycles. Nevertheless, *Pomatoschistus* spp. during summer spring tides and *Gobius niger* during summer neap tides seemed to be the exceptions. The former showed tidal variation on its weighted mean depth, with deeper position in the water column during floods, probably for optimal exploitation of stronger landward transport. In addition to STST, residual bottom inflow is often referred as important mechanism promoting upstream transport and the retention of planktonic larvae (Schultz et al., 2003; Hare et al., 2005; Islam et al., 2007). On the other hand, *G. niger* showed a pattern consistent with flood tide transport (STST) showing inverse tidal pattern at the surface and bottom: increasing density at the surface during floods and decreasing during ebbs, while at the bottom larvae increased during ebb tide. This pattern enabled larvae to reach upstream areas of the estuary and avoiding being flushed out.

Often referred as a retention mechanism, tidal-rhythmic vertical migrations not always result in improved retention when compared with non-tidal vertical migrations (Joyeux, 2001). Despite the low influence of vertical migration, harmonic regression analysis identified periodic variations in larval density associated with tidal and diel cycles. Larvae were generally more abundant around low tide (increasing during ebb) and at night, both at surface and bottom. This pattern will result in larvae exportation (at surface) and retention (at bottom). Since densities were consistently higher at the bottom, larvae retention in the estuary should prevail.

Several studies reported the fish larvae migration patterns in estuarine environments (e.g. Trnski, 2001; Joyeux, 2001; Schultz et al., 2003; Strydom et al., 2005). Stronger diel than tidal variation in larval fish densities was previously recorded, mainly in estuaries with weak or no vertical stratification, and/or with strong tidal currents (e.g. Roper, 1986; Whitfield, 1989; Churchill et al., 1999; Forward et al., 1999; Trnski, 2001). Vertical stratification seemed to be an important factor for larval migrations also in the Mondego estuary. Thus, behaviour and retention mechanisms employed by larvae will depend on the prevalent hydrographic features of individual estuaries (Grioche et al., 2000; Hare et al., 2005).

Few studies report estuarine migration patterns recurring to larvae collected at different depths (e.g. Joyeux, 2001; Schultz et al., 2003) however, reporting vertical migration patterns with only surface samples should be interpreted with caution, since increased densities at surface during a specific tide do not imply that the opposite pattern is occurring at the bottom. Thus, depth preference could not be confirmed. Despite the low evidence of fish larvae migration according to tidal or diel cycles, larvae presented cyclic density variability. This affects larvae entrance and retention in the Mondego estuary, and thus, its suitability as a nursery area.

The Mondego estuary as nursery area for flatfish

The Mondego estuary has been previously recognized as an important contribution source for flatfish adult coastal stocks (Martinho et al., 2008, Vasconcelos et al., 2008, 2011). Nevertheless, this importance is species-specific and changes throughout the fish life cycle. *Platichthys flesus* and *Solea solea* occur in the estuary since early juvenile stages, while *Solea senegalensis* is found in the estuary mainly at the larval phase. Differences in the nursery use patterns between flatfishes seem to be related with the pelagic larval duration and mechanisms of estuarine colonization and transport. *S. solea* larvae were consistently more abundant at the surface which, added to the shorter larval duration, may difficult the ingress in the estuary. On the other hand, the longer larval period in *S. senegalensis* coupled with the preference for bottom layers enhances the transport from the spawning grounds into the estuary. The low densities in the juvenile phase seem to be mainly related with the lack of suitable settlement habitats in the Mondego estuary.

Able et al. (2006) found that several species inhabiting the Great Bay region (USA) are able to settle either in estuarine or coastal habitats, whereas others settle strictly in estuarine areas. Nearshore regions often accumulate larvae and serve as an important path to the estuaries (e.g. Hettler and Hare, 1998; Brown et al., 2004). The ability of larvae in the coastal assemblages to remain outside or enter estuaries has been related with their capacity to find their way in (Able et al., 2006). A combination of species-specific aptitudes and hydrodynamic factors when larvae arrive to coastal areas seem to determine whether larvae settle in coastal areas or ingress into estuarine nurseries to settle (reviewed by Forward and Tankersly, 2001). Results presented in this work (Chapter V) suggest that *S. solea* and *P. flesus* settle at coastal areas and then ingress into the Mondego estuary. This pattern seems to be related with species behaviours that initiate during the metamorphosis or due to salinity changes (e.g. coastal immigration, vertical migration) (Lagardère et al., 1999; Bos et al., 2006). In addition, Mondego estuary presented low suitability for the settlement and recruitment of *S. senegalensis* despite the species presence during its larval phase.

Larvae supply to estuaries can be related with juvenile abundance in these nurseries habitats. However, this is not always true. In the Beaufort Inlet, (East coast USA), larval supply influenced spot (*Leiostomus xanthurus*), pinfish (*Lagodon rhomboids*) and southern flounder (P*aralichthys lethostigma*) juvenile density, but not summer flounder (*Paralichthys dentatus*), Atlantic croaker (*Micropogonias undulates*) and Atlantic menhaden (*Brevoortia tyrannus*) (Taylor et al., 2009). The use of diverse habitats during their life cycle, as described *S. solea*, *S. senegalensis* and *P. flesus* in the Mondego estuary, may contribute to the differences observed.

The juveniles' estuarine residence allows them to grow and accumulate biomass before moving back to the coastal ocean. This ocean-estuary coupling represents an important export of energy and nutrients from the estuary to the coastal ocean. Exported adult fishes are clearly important high quality sources of estuarine productivity in the coastal area (Odum, 1980). Estuarine contribution to coastal adult stocks is fundamental for its definition as nursery areas (Beck et al., 2001; Dahlgren et al., 2006). The success of an estuary as a nursery area will depend on its suitability for larvae and juvenile settlement and growth. Overall, suitable estuarine grounds for juveniles are essential since growth and survival, hence recruitment into adult populations, are greatly determined by the quality of these nursery habitats (Gibson, 1994; Le Pape et al., 2003). Improving the management of fish stocks requires the identification of these essential habitats and the assessment of their suitability and quality (Levin and Stunz, 2005).

FINAL REMARKS

This work presents a comprehensive approach concerning the Mondego estuary ichthyoplankton assemblages. Fish larvae assemblages proved to be highly sensitive to hydrological changes resulted from different precipitation regimes, as those observed during the drought in Portugal during 2004-2005 period. River flow seemed to be a major factor affecting the larval fish community structure, as well as their position in the water column and, consequently, larvae ingress into the estuary. Fish larvae are transported from spawning sites towards the coast, and estuarine colonization seemed to be a species-specific mechanism. Species may enter as larvae or settle in nearby coastal areas and enter as early juveniles. Either way, the Mondego estuary plays an important nursery role for estuarine and marine migrant species.

This study represents a progress in order to understand the importance of the Mondego estuary as nursery habitat, by adding valuable information regarding fish early life stages and connectivity with the adjacent coastal areas. Due to its role in the life cycle of several species, it is fundamental to ensure the maintenance of monitoring programs, in order to identify further changes in these highly sensitive coastal ecosystems. In addition, a better knowledge of the suitability of these habitats during this critical period of the life cycle is fundamental for a better understanding of its nursery role and to the design of efficient management and conservation plans.

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CHAPTER II

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