



UNIVERSIDADE D
COIMBRA

Milene Alexandra Duarte Nobre Guerreiro

**IMPORTANCE OF NURSERIES AREAS FOR EARLY LIFE
STAGES OF FISH AT CENTRAL COAST OF PORTUGAL**

**Dissertação no âmbito do Mestrado em Ecologia orientada pela Doutora Ana
Lúcia Sequeira Primo e pelo Professor Doutor Miguel Ângelo do Carmo Pardal
apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências
e Tecnologia da Universidade de Coimbra.**

Junho de 2019

Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia
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Abstract

Most of marine fishes spawn offshore and the newly hatched larvae have to migrate into nursery areas. A successful recruitment into these areas is essential for their survival. Thus, the processes that affect its variability have been one of the major research items. Although estuaries are intensively studied, little is known about their adjacent coastal areas. This study aims to analyse and compare the importance of two nursery areas (an estuary and a marine coastal area) to the early life stages of fish. For this, larvae and juvenile fishes were sampled in the Mondego estuary and in the adjacent coastal zone from April 2018 to January 2019. The local, temporal and spatial pattern of the ichthyoplankton community was described and it was evaluated the influence of environmental factors in the species distribution. Then, the larval recruitment pattern was evaluated through the comparison with the composition of juvenile fish. Results show a seasonal and local variation of larvae density between and within these systems. At the coastal area, *Sardina pilchardus* was the most abundant species peaking during autumn while at the estuary, the most abundant species were *Pomatoschistus microps* and *Pomatoschistus minutus*, mainly during summer. Species as *Atherina presbyter* and *Pomatoschistus microps* seem to prefer estuarine areas while *Sardina pilchardus*, *Trisopterus luscus*, *Trachurus trachurus* recruit in coastal areas. On the other hand, for *Solea solea* both habitats are important, although at different stages of the life cycle. Local variation found in this study seems to be related with physiological constrains and species preferences while seasonal variation seems to be related with reproductive strategies and river runoff. Within each site, spatial distribution was related to seasonal oceanographic events or interaction between species as competition. The most important environmental factor structuring the community was temperature, probably due to its relationship with reproduction and food availability. The patterns observed show that different species and their initial stages of life use nursery areas differently, reinforcing the need of integrate the larval and juvenile stage in order to a better understanding of their life cycles and to make a correct management of fish stocks.

Keywords: Ichthyoplankton, Recruitment, Estuary, Marine coastal area, Environmental factors.

Resumo

A maioria dos peixes marinhos desova em alto mar e as larvas recém eclodidas tem de migrar para as zonas berçário. Um recrutamento bem-sucedido para estas áreas é essencial para a sua sobrevivência. Assim, os processos que afetam a sua variabilidade têm sido um dos principais itens de investigação. Embora os estuários estejam profusamente estudados, pouco se sabe sobre as suas áreas costeiras adjacentes. Este estudo tem como objetivo analisar e comparar a importância de duas zonas berçário (estuário e zona marinha costeira) para os estágios iniciais de vida dos peixes. Para este fim, larvas e peixes juvenis foram amostrados no estuário do Mondego e na zona costeira adjacente de abril de 2018 a janeiro de 2019. O padrão local, temporal e espacial de variação da comunidade ictioplantónica foi descrito e foi avaliada a influência de fatores ambientais na distribuição das espécies. Em seguida, o padrão de recrutamento larvar foi avaliado através da comparação com a composição de juvenis. Os resultados mostram uma variação sazonal e local da densidade de larvas entre e dentro dos sistemas. Na zona costeira, *Sardina pilchardus* foi a espécie mais abundante com um pico durante o outono, enquanto no estuário, as espécies mais abundantes foram *Pomatoschistus microps* e *Pomatoschistus minutus* principalmente durante o verão. Espécies como *Atherina presbyter* e *Pomatoschistus microps* parecem preferir áreas estuarinas enquanto *Sardina pilchardus*, *Trisopterus luscus* e *Trachurus trachurus* recrutam em áreas costeiras. Por outro lado, para *Solea solea* ambos os habitats são importantes, embora em diferentes fases do ciclo de vida. As variações locais encontradas neste estudo parecem estar relacionadas com restrições fisiológicas e preferências das espécies, enquanto as variações sazonais parecem estar relacionadas com estratégias reprodutivas e escoamento do rio. Dentro de cada local, a distribuição espacial foi relacionada com eventos oceanográficos sazonais e com interação entre espécies como competição. O fator ambiental mais importante na estruturação da comunidade ictioplantónica foi a temperatura, provavelmente devido à sua relação com a reprodução e a disponibilidade de alimentos. Os padrões observados mostram que diferentes espécies e os seus estágios iniciais de vida utilizam de forma diferente as zonas berçário, reforçando a necessidade de integrar o estágio larvar e juvenil, a fim de compreender melhor os seus ciclos de vida e fazer uma correta gestão dos stocks pesqueiros.

Palavras-chave: Ictioplâncton, Recrutamento, Estuário, Zona costeira marinha, Fatores ambientais.

1. Introduction



1.1. Marine fish life cycle

Fishes exhibit great diversity in ecology, morphology and life history patterns, being usually classified according to their adult characteristics (Costa et al., 2002). In most marine fishes (Osteichthyes – bony fishes) it is possible to distinguish 5 major periods of ontogenic development that are: the embryo, larva, juvenile, adult and senescent (Fig. 1) (Balon, 1984, 1986).

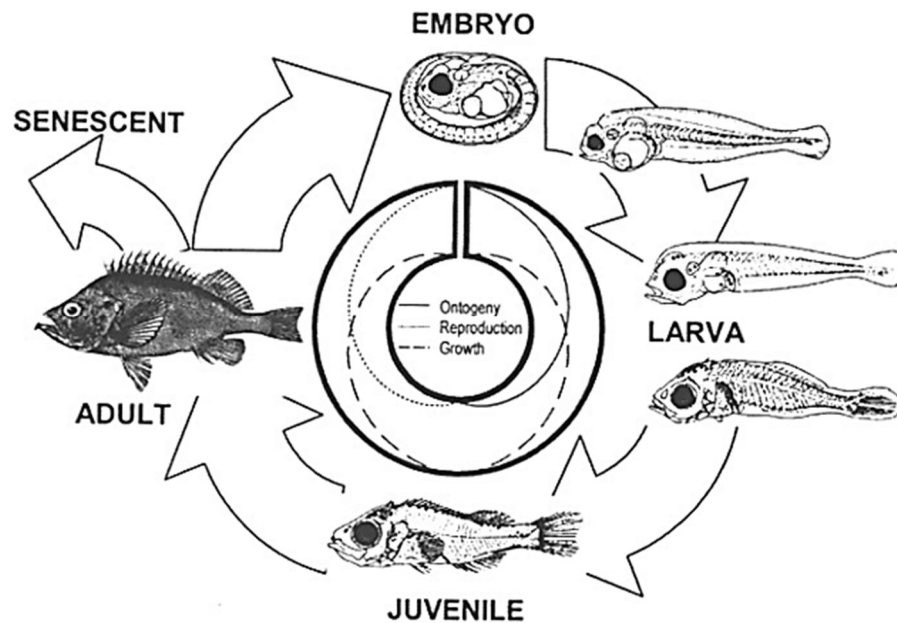


Figure 1 – Life cycle of marine fish (Osteichthyes). From: Bigelow and Welsh (1925).

In order to reproduce, adults carry out migrations from their feeding grounds to spawning areas, that are located more offshore (Beck et al., 2003; Jennings et al., 2001). At these sites, fishes release pelagic eggs that are fertilized externally and from which planktonic larvae will hatch. While eggs are completely immotile, larvae have some swimming capacity that develops as they grow. Both are part of the fraction of the plankton called ichthyoplankton (Bone and Moore, 2008; Moser and Watson, 2006). The duration of these stages depends on the species as well of the environmental factors to which they are subjected, such as temperature (Bone and Moore, 2008). Fish eggs can drift hundreds of kilometres before they hatch, and thereafter the larval period usually lasts 3 to 4 weeks (Jennings et al., 2001; Suthers and Rissik, 2009). These stages are particularly vulnerable phases of the life cycle since they are highly subjected to dispersion and mortality. At the end of the larval stage, the metamorphosis occurs, and the juvenile is formed. This transition is characterized by a remodelling of all organ systems and results in an individual similar to the adult, but smaller and immature. Sometimes the transformations are so complex that it can also lead to changes in the eating habits and in the habitats used (Bone and Moore, 2008; Helfman et al., 2009). When the fish reaches maturity is called adult and the cycle continues until the individuals cease to reproduce, grow old or die (Fig. 1).

1.2. Nursery areas

The success in survival, growth and development during early life stages depends on the larvae being able to reach areas suitable for their development, the nursery areas. These are defined as a subset of habitats where juveniles of one species occur at a higher level of productivity per unit of area than other juveniles' habitats. Nurseries areas are very productive areas that provide high food availability and favourable conditions of temperature and shelter required to the survival, development and rapid growth of larvae and juveniles. These habitats are located in shallow coastal marine areas and estuaries contributing with high abundances of fish to the adult populations in their habitats (Beck et al., 2001).

The importance of estuaries as nursery areas has been widely recognized. The high abundance of food resources as well as the richness of habitats (e.g. sand, mudflats, salt marshes, oyster beds) offer good conditions for the settlement of several species (Beck et al., 2001; McLusky and Elliott, 2004). Their importance has been especially emphasized for marine species with complex life cycles that live in the coastal area but depend on estuarine habitats to successfully complete their life cycle. (e.g *Dicentrarchus labrax*, *Platichthys flesus*, *Solea solea*) (Cabral et al., 2007; Martinho et al., 2007; Primo et al., 2013; Ribeiro et al., 2008). On the other hand, estuaries are very dynamic environments where the influence of tides and the mixture of freshwater lead to variability in factors such as salinity, oxygen, and turbidity, affecting the distribution and abundance of species (Dyer, 1997; Vernberg, 1983).

Coastal areas are also often referred as important nursery areas (Cabral et al., 2003; Castro et al., 2013; França et al., 2004). In British and Dutch waters flatfishes juveniles as *Pleuronectes platessa*, *Limanda limanda* and *Scophthalmus maximus* are common in nearshore sandy bays (McLachlan and Brown, 2006). These sites provide rich food resources and protection for the early life stages due to the shallowness and turbidity (Lenanton et al., 1982; Pessanha and Araújo, 2003; Robertson and Lenanton, 1984; Watt-Pringle and Strydom, 2003). However, as opposed to estuaries that have been intensively studied, not much is known about the nursery function of the coastal zone, especially in Portugal.

The connectivity between coastal zones and estuarine systems are vital for species with more complex life cycles, which depend on the estuaries as a nursery zone (Ramos et al., 2017; Ray, 2005). A better knowledge of the relation between larval dispersal and supply, juvenile abundance, survival, and contribution to adult stocks is fundamental to determine marine species spatio-temporal dynamics, thus to a better management and conservation of ecosystems (Cowen and Sponaugle, 2009; Ramos et al., 2017; Vasconcelos et al., 2011). Connectivity between coastal habitats is not only influenced by natural conditions (e.g. river flow) but also by anthropogenic factors (Able et al., 1999; Le Pape et al., 2007; Vasconcelos et al., 2007) and it is strongly related with larval transport mechanisms.

1.3. Fish migrations and transport patterns

For several marine fish species, there is an ontogenic habitat segregation throughout their life cycle: while adults live in the deeper offshore areas, larvae and juveniles accumulate in coastal and estuarine sites (Beck et al., 2001, 2003). Since spawning occurs offshore, sometimes at more than 100km, it is implied that the newly hatched larvae have to travel long distances to reach the coastal nursery areas (Beck et al., 2003; Helfman et al., 2009). This transport process includes 3 phases: (1) movement to the coast; (2) location and movement to nursery areas; and (3) retention in these areas, where the larvae must be able to fight the currents to avoid being dragged to offshore (Boehlert and Mundy, 1988; Miller, 1988; Norcross and Shaw, 1984). However, a pertinent question arise “ how the larvae with their limited swimming capabilities can cross such long distances?”. Active transport would be energetically expensive and too slow to travel long distances as well as to counteract strong currents. Thus, it has been suggested that this movement to the coast depends essentially on passive transport and larval behaviour, such as vertical migrations (Fisher, 2005; Helfman et al., 2009). In passive transport, larvae take advantage of the ocean currents, which are designated as the main dispersion controllers (Churchill et al., 1999; Rooper et al., 2006). The ability of larvae to mediate transport grows throughout their development since their sensorial acuity increases as well as the capacity to perform vertical migrations and to swim. This behaviour allows them to choose appropriate currents for their transport and also mediates the process of entry and retention within the nursery zones (Helfman et al., 2009; Norcross and Shaw, 1984). To increase the chances of successful migration, larvae are able to respond to environmental cues which indicates the proximity of a nursery area (Helfman et al., 2009). These include celestial (solar or stellar), geomagnetic, odour, sound and visual cues (Teodósio et al., 2016). In the case of estuaries it has been related to the river plumes due to the presence of chemical cues as e.g. salinity, temperature, potential primary production and odour (Amara et al., 2000; Martinho et al., 2009; Vasconcelos et al., 2008). Once at the coastal area, the larvae enter into the estuary by selective tidal-stream transport (STST), residual bottom inflow or through sites with slowed water velocity (e.g. margins) (Teodósio et al., 2016). During the selective tidal-stream transport (STST), the larvae are at the surface in the water column during the flood tide and move to the bottom during ebb tides (Forward and Tankersley, 2001). If the larvae use the bottom inflow strategy, they will enter the estuary by moving to the bottom to catch the dense current of seawater entering in these systems. (Grioche et al., 1997; Hare et al., 2005).

Larval dispersion is a fundamental feature of fish life history and an essential process in the dynamics of populations (Di Franco et al., 2012; Ramos et al., 2017) enabling the colonization of new habitats and gene flow and minimizing intraspecific competition. However, during this process, many biological and environmental factors control larval survival, and these dispersal

advantages can be diminished by the high mortality rates typical of these early stages of development (Bailey et al., 2005).

1.4. Recruitment

One of the main research items in fisheries biology has been the understanding of the processes that condition the variability of recruitment strength (Houde, 2008; Klein et al., 2018). This is defined by the entry of individuals (at any particular stage) in the next stage of development (Elliott and Hemingway, 2002). It is generally accepted that annual adult recruitment is determined during the early stages of the fish life cycle, particularly the planktonic phases (embryonic and larval) (Houde, 2008). These are the most sensitive phases of the life cycle with mortality rates that can range from 5 to 40% per day (Fig. 2) (Bailey et al., 2008; Bone and Moore, 2008), so a small variation in these rates has profound effects on subsequent abundances (Heath, 1992). The processes involved in recruitment variability are not yet fully identified and its success or failure may depend on several biological and physical factors (Houde, 2008; Ré, 1999). Still, it has been suggested that larvae starving, predation on eggs and transport to disadvantaged areas are the 3 main factors that determine the strength of recruitment to a large extent (Houde, 2008; Ré, 1999). Cushing et al. (1975), suggested that interannual variability in larval survival may be explained by the ‘match or mismatch’ hypothesis. If there is a mismatch in the time and space between the production cycle and the hatching of the larvae, they will not find enough food and can reach a point of no return of starvation death or, get too weak to find food, becoming more vulnerable to predators (Bailey and Houde, 1989). Thus, the strength of the match determines the amount and food available for the larvae.

Another hypothesis for the variation of recruitment was proposed by Sinclair (1988), the “member-vagrant” hypothesis, emphasized the importance of the relationship between spawning time and the oceanographic characteristics that transport and retain the larvae in favourable environments. Unfavourable oceanographic conditions may lead larvae to places that are not appropriate for their development or carried them to offshore. The coincidence of spawning and favourable environmental conditions for the transport of larvae and the production of prey has led to the evolution of specific spawning times. Year-to-year fluctuations in oceanographic factors and production cycles still account for much of the variation in recruitment success. The “member-vagrant” hypothesis emphasizes the role of physical and non-biological factors in spawning or success in recruitment. However, both physical and biological factors may be important and will interact (Heath, 1992).

During the colonization phase of the nursery areas, planktonic stages are mainly affected by density-independent factors (Fig. 2). On the other hand, during the juvenile phase within nursery zones, density-dependent processes such as variations in growth, predation, mortality, and habitat

quality and availability act influencing the success of recruitment to adult habitats (Fig. 2) (Van Der Veer et al., 2000). This recruitment variability determines the structure of adult populations and the amount of fish that can be captured.

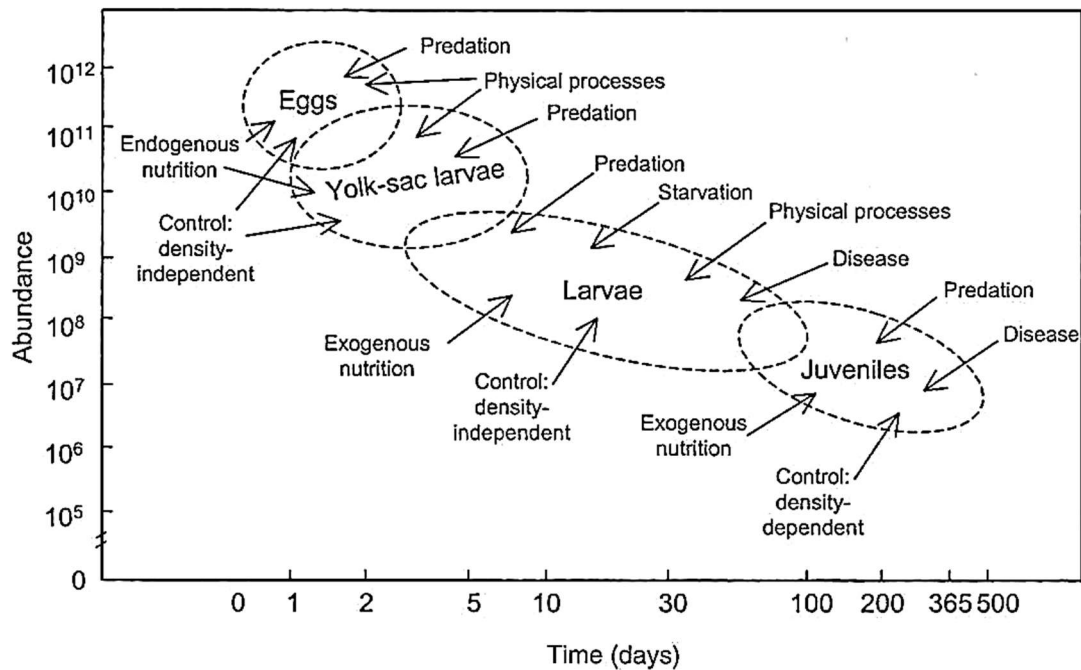


Figure 2 – Diagram showing the different processes that act on the early life stages of marine fishes (eggs, yolk-sac, larvae and juveniles). From Houde (1987).

1.5. Why study the early life stages of the life cycle?

Understanding the distribution of species and their life cycle is critical to comprehend how fish are affected by the environment and fisheries. In recent years there has been an increased concern about climate change and its impact on the ecosystem. Plankton may be a good indicator of climate change because of its sensitivity to reflect environmental disturbances (Hays et al., 2005). Furthermore, early life stages of fish can also be environmentally sensitive and reflect the effects on fish communities prior to buffering through density-dependent mechanisms (Boeing and Duffy-Anderson, 2008).

Fisheries play an important socio-economic role worldwide. Portugal, for example, is the world's third largest consumer of fish, consuming more than 55kg of fish per capita in one year, which is more than double the average of European citizens (EUMOFA, 2017). However, it has been observed a reduction in the catch: in 2018 fewer fish were transacted in the fishing auctions in comparison with 2016 and 2017, leading to an increase of the value of sales (INE, 2017, 2018, 2019). The most well-known case in Portugal is the reduction of the sardine (*Sardina pilchardus*) stocks, which is a species of great commercial interest. In 2017, 70 000 tonnes were captured in the

ICES zone, which involved the movement of about 28 million euros (ICES, 2018). Portugal and Spain have witnessed a sharp decrease in the stock of this species, which has been related not only with their exploitation but also with recruitment problems (Santos et al., 2012).

Studies on the early life stages of fish are thus of great importance since it allows to determine spawning times and locations, estimate population sizes, and understand what factors affect the dynamics of recruitment variability (Ré, 1999). This information is essential to an accurate fisheries management, once fish with different life histories can be differently affected by different stressors (Jennings et al., 2001). In addition, a better understanding of the ichthyoplankton dynamics in the different areas, for example in estuaries (Rakocinski et al., 1996) allows to develop hypothesis on nursery habitats and connectivity of marine populations.

1.6. Objectives

Ichthyoplankton and juvenile communities had previously been studied in the Mondego estuary (Leitão et al., 2007; Martinho et al., 2007; Primo et al., 2011) however, there is still a lack of integration between the two stages of the life cycle (Primo et al., 2013). Furthermore, although the estuary is relatively well studied, little is known about the nursery function of the adjacent marine coastal area and the dynamics between the two ecosystems. Studies in the Portuguese coastal zone are spaced in time and local or with special focus on the distribution and recruitment of sardine (*Sardina pilchardus*) (Santos et al., 2004, 2006). Thus, the objective of this study was to evaluate and compare the importance of these two areas as nursery grounds for the early life stages of fish. We hypothesize that both sites are important nursery grounds and communities could be influenced by seasonality, environmental factors and spatial segregation differently throughout the fish life cycle. In this way, additional objectives include:

1. Describe the spatial and temporal communities of ichthyoplankton on both systems;
2. Evaluate the influence of environmental factors in ichthyoplankton communities on both systems;
3. And evaluate the recruitment pattern through comparison with juveniles.

2. Materials and methods



2.1. Study site

The study was carried out on the western Atlantic coast of Portugal, in the Mondego estuary and adjacent coastal area of Figueira da Foz (40° 26' N to 40° 03' N), (Fig. 3). This area is located on the northern part of the subtropical anticyclone belt, which largely influence the climate and oceanographic features (Wooster et al., 1976; Santos et al., 2004). The seasonal evolution of the subtropical front and the migration of the Azores anticyclone gyre regulate the wind conditions. During spring and summer, the coastal area is subjected to strong upwelling events, induced by the prevalence of strong northerly winds (Wooster et al., 1976). During winter, due to changes in wind components, between northerly and southerly, both upwelling and downwelling events can occur (Santos et al., 2004). These upwelling pulses, as well as the existence of several estuaries and rias along the coast, contributed to the high productivity of the Portuguese coast. Other coastal oceanographic features include the Western Iberian Buoyant Plume (WIPB) and the Iberian Poleward current (IPC). The WIPB is a low salinity surface water originated by the discharge of several rivers in this region (Minho, Mondego, Douro, Lima and Vouga) (Peliz et al., 2002). While the IPC is a weak, warm and salty current that sometimes extends to the surface during winter (Peliz, 2003). The west Portuguese coast cover around 800 km and is considered as exposed to high waves from Cabo Mondego to north and moderately exposed to the south. It is mainly dominated by sand beaches with patches of rock shores and cliffs. It has a semi-diurnal and mesotidal regime, with a spring tidal approximately of 3.5 m (Bettencourt et al., 2004; Taveira Pinto, 2004).

The Mondego river estuary is a small intertidal system, well-mixed, composed by two channels (north and south) which join in their terminal zone. The north arm is deeper (5 to 10 m at high tide) with a low residence time (< 1day). This arm constitutes the main navigation channel and the access to the Figueira da Foz harbour, being subjected to constant dredging activities. In addition, it is directly connected to the river Mondego and therefore receives most of the freshwater discharge. On the other hand, the south arm is shallower (2 to 4 m at high tide) with a high residence time (4 - 8 days) (Primo et al., 2011). This arm is quite silted up in upstream areas and therefore, water circulation is mainly dependent on the tides and on minor freshwater flow from the Pranto river, a small tributary which is regulated by a sluice. In this study we only focus in the north arm. Despite having different hydrological characteristics, previous studies show that both arms have similar composition of fish species (Leitão et al., 2007; Primo et al., 2011).

2.2. Field sampling and laboratory procedures

Sampling program included larvae and juvenile fish collection in the Mondego estuary and the adjacent marine coastal zone (Fig. 3).

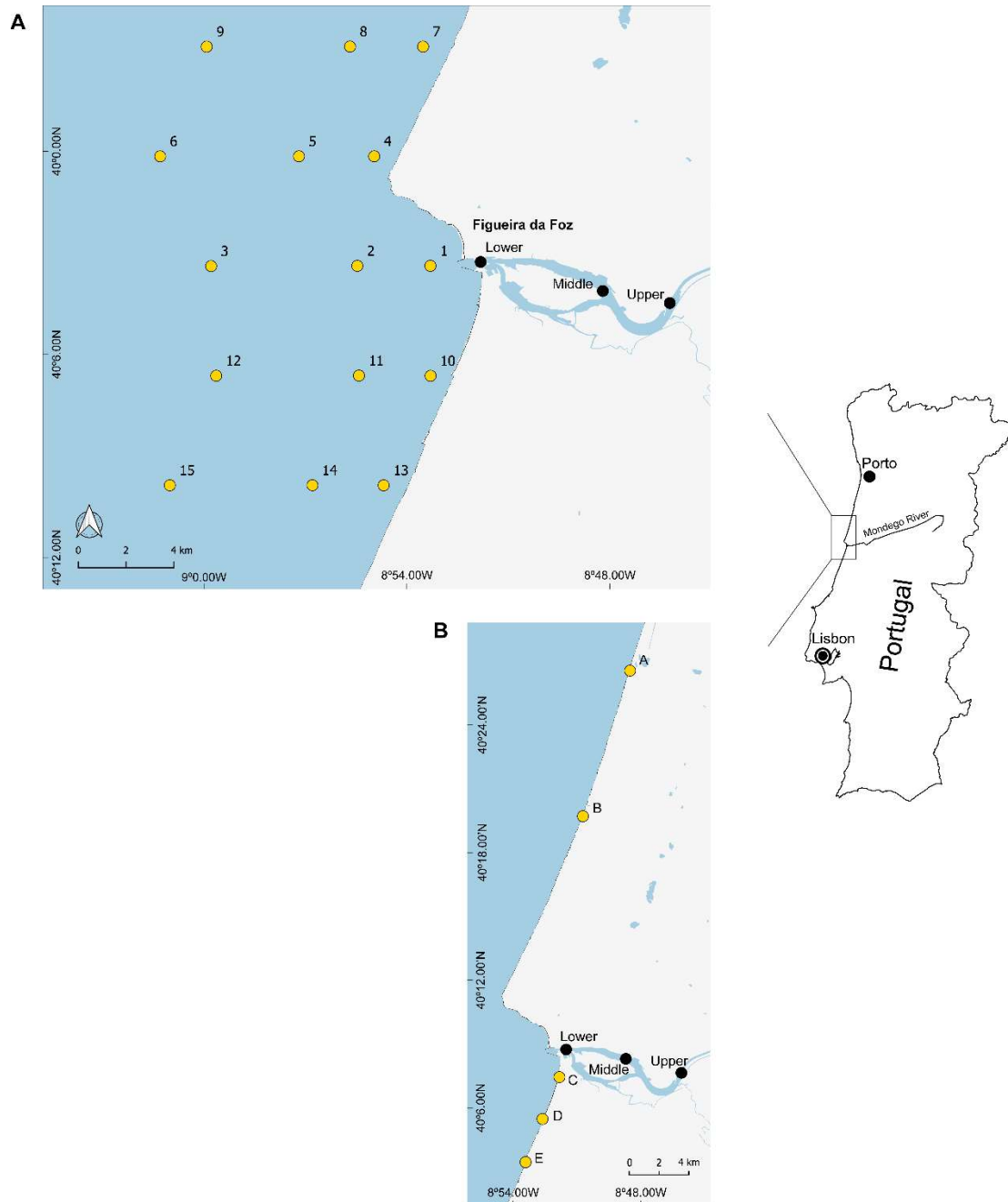


Figure 3 – Map of the study area on the western coast of Portugal. Sampling stations of (A) larvae and (B) juveniles in the Mondego estuary (black dots) and adjacent coastal area (yellow dots) are indicated.

2.1.1. Larval sampling

Larvae sampling occurred over a year, from April 2018 to January 2019. It was performed, in both systems, during daylight with a ring net WP3 of 500 μm mesh size, equipped with a Hydro-Bios flowmeter to determine the amount of water filtered. In the coastal area, sampling was carried out seasonally (spring, summer and autumn), along 4 transects defined perpendicular to the coast, each with 3 sampling points (1, 4 and 10 km from the coast) (Fig. 3). In order to sample the entire water column, stepped oblique tows were made during 3 - 5 minutes at 3 knots tow speed. In the estuary, sampling was carried out monthly, at high tide, in 3 points along the north arm by horizontal surface tows during 5 minutes at 2 knots tow speed (Fig. 3).

All samples were preserved in 95% ethanol. At the laboratory, organisms were sorted under a stereoscopic microscope, identified to the lowest possible taxon and staged as preflexion, flexion and postflexion. Stages were attributed based on the development of the hypural bones and position of the posterior portion of the notochord. The abundance of organisms was standardized as the number of individuals per 100 m^3 .

2.1.2. Juvenile sampling

Juveniles were collected from May to October of 2018 in both systems. In the estuary, monthly samplings were performed during the night at 3 sampling points along the north arm (Fig. 3). Fishing was carried using a 2 m beam trawl with a mesh size of 20 mm and 5 mm in the terminal bag. At each station, 3 trawls were towed for 3 min each at 2 knots tow speed, covering at least an area of 500 m^3 . All fish caught were immediately frozen and transported to the laboratory for further identification, measurement and counting. Only juvenile abundance was taken into account (Fish length < maturation size).

In the coastal area, the juvenile's abundances were calculated based on the catches of beach seine (Arte xávega). This is a traditional fishing method that still operates on some beaches of Portugal. In this, nets with a bag of approximately 30 m and with a mesh size of 20 mm are dropped by a small boat from the beach around an area up to 2 km. The leading rope is brought back to the shore and subsequently, the net is hauled back on the beach with the help of small trucks. Each trawl lasts approximately 60 minutes. Beach seine hauls were carried out on 5 beaches (Fig. 3), two north of estuary and two at the south of the estuary. Each haul was randomly sub-sampled and individuals were identified and measured in the field. Again, only juvenile abundance was taken into account and abundances in the haul were extrapolated from the analysed sub-sample.

The abundances of organisms from both systems were standardized under catches per unit effort (CPUE) as number of fish per minute.

2.1.3. Environmental factors

Simultaneously to larval sampling, at each point, the water temperature (°C), salinity and dissolved oxygen (mg l^{-1}) were recorded *in situ* (WTW Multi 3410 IDS) from two depths, surface and bottom. At the deepest coastal stations (> 30 m) environmental factors from the bottom waters were measured at 30 m depth. The pH was measured immediately upon arrival at the laboratory. Water samples from surface and bottom were also collected with a Van Dorn bottle for subsequent determination in the laboratory of chlorophyll *a* (Chl *a*, mg m^{-3}) and particulate organic matter (POM, mg m^{-3}) following Parsons et al. (1984) and APHA, (1995), respectively.

To determine the abundance of prey and potential predator's, zooplankton samples were also collected using a ring net WP2 with a mesh size of 200 μm . Stepped oblique tows were made at the coastal area and surface tows in the estuary, both with a duration of 3 minutes. Samples were preserved in a 4% buffered formaldehyde seawater solution. In laboratory, samples were transferred to an 80% ethanol solution and then sorted and identified under a stereomicroscope. The organisms were identified in five main groups: Copepoda, Cladocera, Cnidaria, Appendicularia and Chaetognatha. When necessary samples were fractionated through a Folsom plankton splitter. The total fraction was adjusted so that a minimum of 500 individuals was counted. The abundance of organisms was standardized as the number of individuals per m^3 .

2.3. Statistical analysis

2.3.1. Univariate analysis

PERMANOVA (non-parametric permutational multivariate analysis of variance) was used due to the high skewness of the data which did not allow the use of parametric approaches. So, seasonal and spatial patterns in water temperature were investigated by Univariate PERMANOVA. Analysis was performed on square root transformed data, based on Euclidean distance between samples, considering all the factors as fixed, with 999 random permutations, and permutations of residuals under the reduced model. First, it was used a two-way test design (local x season) and then, within each local, spatial and depth variations were tested by a three-way design (season x depth x zone). To test for differences between/within groups for pair levels of factors a posteriori multiple comparison was made.

Univariate PERMANOVA was also performed to test differences between locals and seasons for total larval abundance and for spatial distribution of the main species (*Sardina pilchardus*, *Ammodytes tobianus*, *Engraulis encrasicolus*, *Gobius niger*, *Pomatoschistus microps*, *Pomatoschistus minutus*, *Solea solea* and *Solea senegalensis*). Tests were performed for local x season factors, then within each local, spatial distribution were analysed. Due to the seasonality of some species, spatial PERMANOVA analysis was made only for the most abundant season. When

no seasonal pattern was detected, all seasons were considered in the analysis. All abundances (total and of the most important species) were fourth root transformed previously to the analysis. Then, analysis was run as previously described.

Temperature and spatial distribution of the main species were mapped along the estuary and on the marine coastal area using the software QGIS 3.4.3-Madeira. To characterize the spatial patterns of temperature, continuous layer maps were created with a processing tool Inverse Distance Weight, distance coefficient: 1, projected on the WGS 84 coordinate system.

2.3.2. Multivariate analysis

A SIMPER analysis (percentage similarity procedure) based on a Euclidean distance was used in order to identify the main species that contribute to differences between ecosystems (coastal area and estuary). Then, a multivariate PERMANOVA was used to test differences in the ichthyoplankton community between local, seasons and sampling zones. First analysis was performed for local x season factors, then within each local, spatial distribution and seasonality was analysed (season x zone). The analysis was run on fourth root transformed data, based on Euclidean distance and as previously described.

The relationship between environmental variables and the larval fish assemblages was examined using a redundancy analysis (RDA). Concurrently, a DISTLM (Distance based linear model) was also performed to select the best explanatory model. Only species that contribute to more than 10% of the community were considered in the analysis. Environmental variables included were: water temperature, salinity, pH, oxygen, chlorophyll *a*, particulate organic matter (POM) and abundances of Copepoda, Cladocera, Cnidaria, Chaetognatha and Appendicularia. Euclidean similarity measure was used, and estuary data were averaged by local/season. Ichthyoplankton abundances were fourth root transformed while no transformation was applied to environmental variables. The 3 best variable models were selected by applying the best subsets model selection routine, with R^2 as the selection criterion on 999 permutations.

Finally, to analyse the local recruitment of juveniles a principal component analysis (PCA) was carried out. Previously to the analysis juvenile's catches per unit of effort was fourth root transformed. PC1 scores were extracted and a Spearman correlation with species abundance was performed. The spearman correlation test was performed through the Software Sigma plot 12.0 while the remaining data analysis was carried out using the PRIMER v6 + PERMANOVA package (Anderson et al., 2008; Clarke and Gorley, 2015).

3. Results



3.1. Environmental factors

Estuarine water temperature was higher than at the coastal area (Pseudo-F (lo x se) = 30.79, $p(\text{perm}) < 0.01$). Also, it showed a clear seasonal pattern with higher values during summer and spring and lower in autumn (Pseudo-F = 39.61, $p(\text{perm}) < 0.01$, pairwise test $p < 0.05$) (Fig. 4).

At the coastal area, temperature patterns regarding distance to the coast varied according to season and depth (Pseudo-F (se x de x lo) = 2.68, $p(\text{perm}) < 0.01$). In summer, both depths showed no spatial differences, as well as autumn at the surface (pairwise test $p < 0.05$); in spring, inner stations were colder than outer stations at the surface while at the bottom the opposite pattern occurred (pairwise test $p < 0.05$); in autumn, at the bottom, temperature increased with distance from the coast, with inner stations showing colder temperatures (pairwise test $p < 0.05$) (Fig. 4). Only spring and summer showed differences between surface and bottom temperatures (Pseudo-F = 80.04, $p(\text{perm}) < 0.01$, pairwise test $p < 0.05$), with lower temperatures at the bottom (Fig. 4). Furthermore, summer showed increased water temperature at the north of the estuary (Pseudo-F = 3.39, $p(\text{perm}) < 0.01$, pairwise test $p < 0.05$) and surface samples showed spatial differences with north sampling stations showing, generally, higher temperatures (Pseudo-F = 3.70, $p(\text{perm}) < 0.05$, pairwise test $p < 0.05$) (Fig. 4).

At the estuary, water temperature was higher at the surface (Pseudo-F = 5.98, $p(\text{perm}) < 0.05$) in all seasons and zones. Furthermore, warmer temperatures were found at the upstream sampling stations in spring and summer, while autumn showed no spatial differences inside the estuary (Pseudo-F = 2.55, $p(\text{perm}) < 0.05$, pairwise test $p < 0.05$) (Fig. 4).

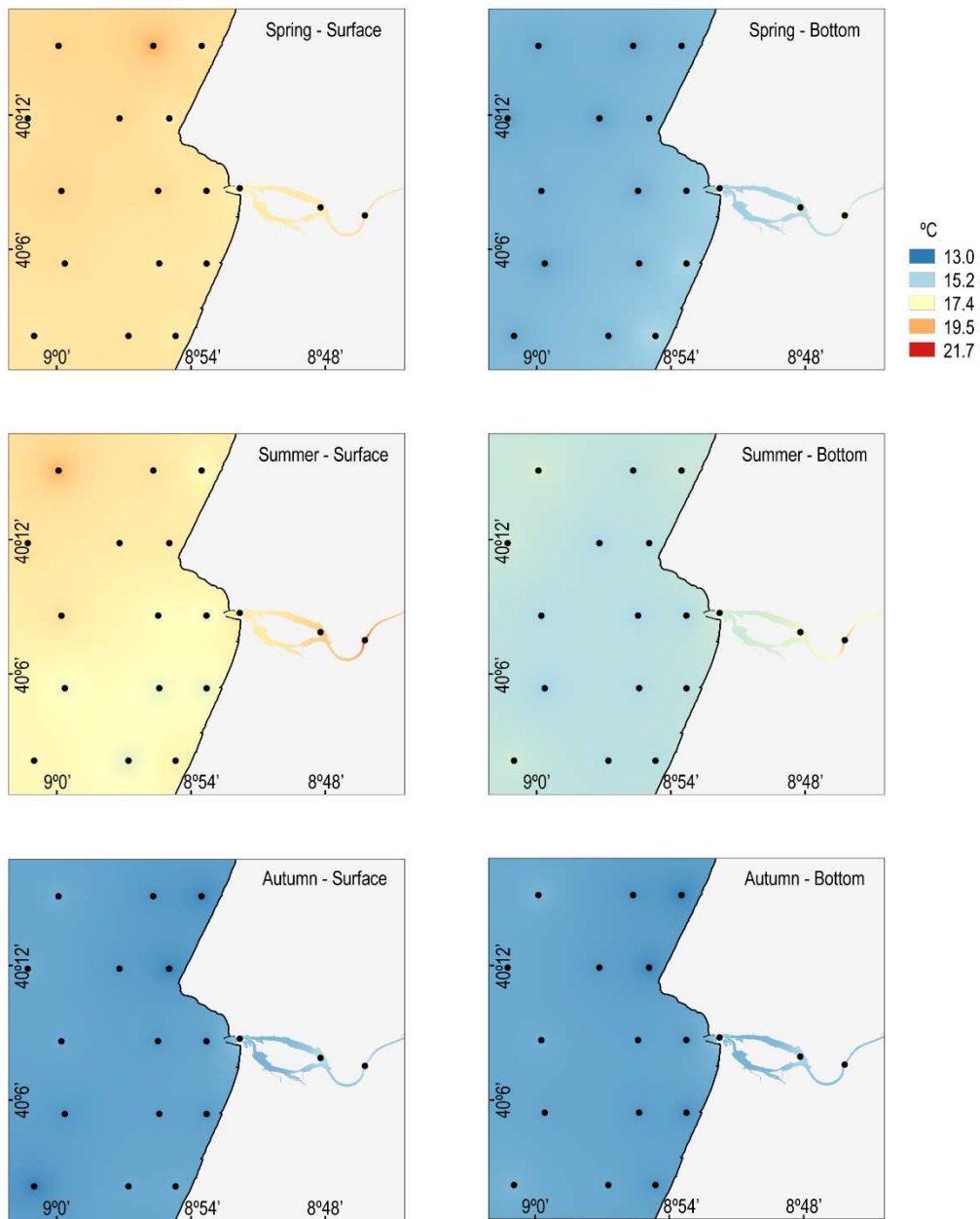


Figure 4 – Spatial variation of temperature (°C) along the coastal area and the Mondego estuary, according to depth (surface and bottom).

3.2. Larvae abundances and stages composition

A total of 5104 larvae were collected in the coastal area and in the estuary during the study period. Densities varied according to local and season (PERMANOVA, Pseudo-F(se x lo) = 12.80, $p(\text{perm}) < 0.01$). The coastal area showed higher abundances than estuary during autumn (pairwise test $p < 0.01$), while in summer, the opposite occurred (pairwise test $p < 0.01$) (Fig. 5). In spring, abundances were very similar in both systems. Throughout the year the abundance of larvae varied differently in each system. Coastal area showed significantly lower abundances during summer (pairwise test $p < 0.01$), whereas in the estuary it was in autumn (pairwise test $p < 0.01$) (Fig. 5).

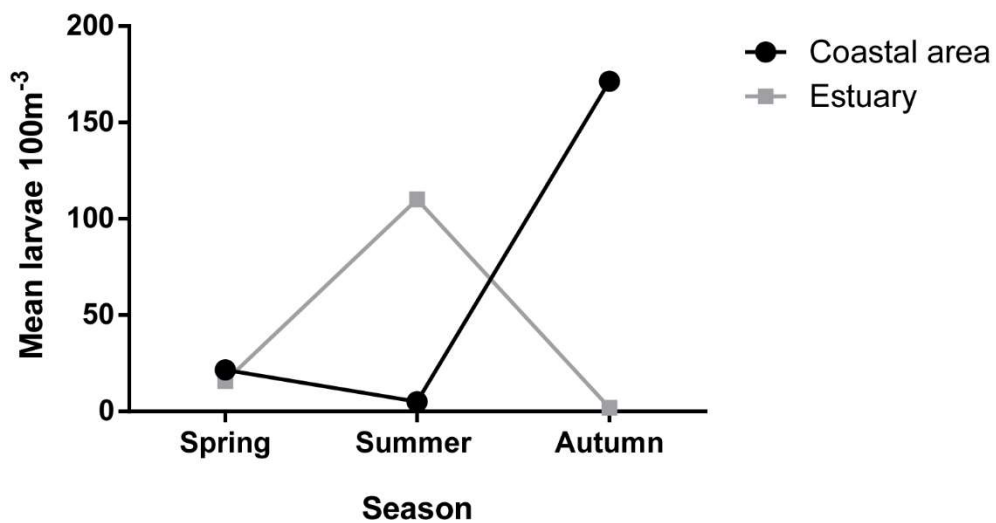


Figure 5 - Seasonal variation in mean density of larvae (100 m^{-3}) in the coastal area and the Mondego estuary.

The larval stages varied between the coastal area and the estuary. Preflexion larvae dominated in the coastal area (91%), followed by yolk-sac (7%). Very few larvae were found in flexion and postflexion (Fig. 6). In contrast, the dominant stages in the estuary were postflexion (59%) and flexion (20%) (Fig. 6A). These prevalence of these stages in the estuary is due to the high densities of *Pomatoschistus* spp. Without this species it is possible to verify a predominance of preflexion (42%) and yolk sac stages (39%) in the remaining community (Fig. 6B).

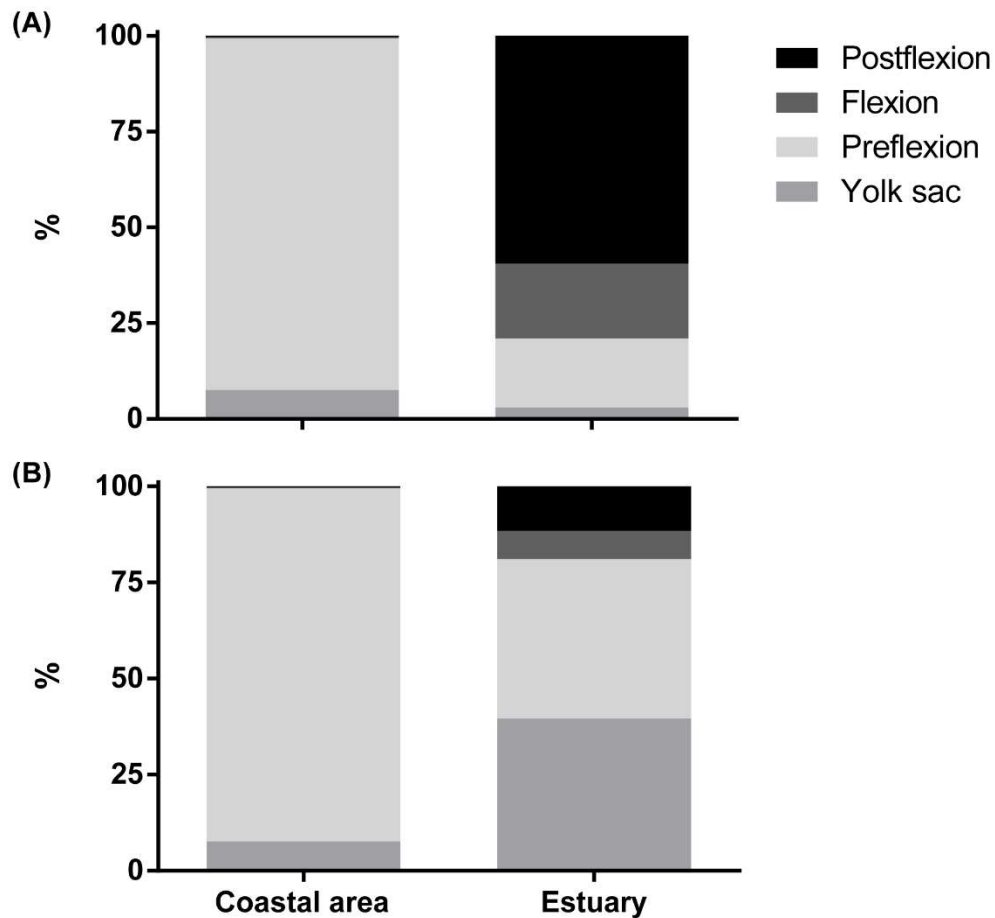


Figure 6 – Proportion of stages of larvae (%) in the coastal area and in the Mondego estuary. (A) Including all species collected and (B) without the contribution of *Pomatoschistus* species.

3.3. Larval fish community composition

Larvae belonging to 20 families and 33 species were identified in the estuary and in the coastal area, with only 13 common to both systems. The most frequent and abundant families were Clupeidae, Gobiidae, Ammodytidae, Engraulidae and Soleidae. *Sardina pilchardus* was the most abundant species in the study, followed by *Pomatoschistus minutus* and *Pomatoschistus microps* (Table I). Other abundant species were *Ammodytes tobianus*, *Engraulis encrasicolus*, *Gobius niger*, *Solea senegalensis* and *Solea solea*.

Univariate PERMANOVA revealed seasonality and differences in distribution of the main species between locals (Table II). *Ammodytes tobianus* was only recorded in the coastal area during the autumn (Table I). *Sardina pilchardus* was significantly more abundant in the coastal area during summer and autumn than in the estuary (pairwise $p(\text{perm}) < 0.05$). On both systems, autumn was the season with highest abundances of this species (pairwise $p(\text{perm}) < 0.01$) (Table I, II). In the coastal area, *Sardina pilchardus* was not captured during spring. *Pomatoschistus microps* showed significant higher abundances in estuary during summer (pairwise $p(\text{perm}) < 0.01$). While within the coastal area abundances was very low throughout all year. *Pomatoschistus minutus* showed higher abundances in the estuary in the spring and summer period (Table I, II). Within the coastal area *Pomatoschistus minutus* abundances recorded were very low during all year (Table I). *Engraulis encrasicolus* was significantly more abundant during spring in the coastal area (pairwise $p(\text{perm}) < 0.01$). In the estuary despite the higher values observed during summer, these were not statistically significant (Table I, II). The species *Solea senegalensis* and *Solea solea* only show local differences, the first was significantly more abundant in the estuary and the latter was significantly more abundant in the coastal area (Table I, II). Finally, the abundance of *Gobius niger* did not differed neither between seasons nor locals (Table II).

Table I – Mean density (larvae 100 m⁻³) of species caught in each sampling local (coastal area and Mondego estuary) per season.

Species	Coastal area				Estuary			
	Spring	Summer	Autumn	Total	Spring	Summer	Autumn	Total
Ammodytidae								
<i>Ammodytes tobianus</i>	0.00	0.00	19.47	19.47	0.00	0.00	0.00	0.00
Atherinidae								
<i>Atherina presbyter</i>	0.00	0.00	0.00	0.00	0.18	0.20	0.00	0.38
Blenniidae								
<i>Coryphoblennius galerita</i>	0.00	0.00	0.00	0.00	0.04	0.02	0.00	0.06
<i>Lipophrys pholis</i>	0.00	0.00	0.04	0.04	0.07	0.02	0.04	0.14
<i>Parablennius gattorugine</i>	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.34
<i>Parablennius pilicornis</i>	0.24	0.00	0.00	0.24	0.00	0.43	0.00	0.43
Bothidae								
<i>Arnoglossus</i> sp.	1.13	0.00	0.00	1.13	0.00	0.00	0.00	0.00
Carangidae								
<i>Trachurus trachurus</i>	0.38	0.00	0.00	0.38	0.00	0.00	0.00	0.00
Clupeidae								
<i>Sardina pilchardus</i>	0.00	1.76	142.91	144.67	0.10	0.11	0.50	0.71
Clupeidae/Engraulidae n. id.	1.84	0.23	0.00	2.07	0.71	0.95	0.04	1.69
Engraulidae								
<i>Engraulis encrasicolus</i>	13.43	0.06	0.00	13.48	0.39	1.56	0.00	1.95
Gadidae								
<i>Pollachius pollachius</i>	0.00	0.00	0.18	0.18	0.00	0.00	0.00	0.00
<i>Trisopterus luscus</i>	0.00	0.00	1.51	1.51	0.00	0.00	0.00	0.00
<i>Trisopterus minutus</i>	0.00	0.00	0.29	0.29	0.00	0.05	0.00	0.05
Gobiidae								
<i>Gobius niger</i>	0.08	0.09	0.00	0.18	0.29	0.46	0.00	0.75
<i>Gobius paganellus</i>	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.05
<i>Gobius</i> sp.	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.20
<i>Lebetus</i> sp.	1.28	0.11	2.00	3.39	0.00	0.00	0.00	0.00
<i>Pomatoschistus microps</i>	0.00	0.06	0.28	0.33	2.03	33.15	0.09	35.27
<i>Pomatoschistus minutus</i>	0.00	0.00	0.28	0.28	5.91	64.05	0.19	70.15
<i>Pomatoschistus pictus</i>	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.02
<i>Pomatoschistus</i> sp.	0.04	0.06	0.00	0.10	3.81	6.48	0.67	10.97
Gobiidae n. id.	0.00	0.00	0.00	0.00	0.00	0.44	0.00	0.44
Labridae								
<i>Centrolabrus exoletus</i>	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.02
<i>Labrus bergylta</i>	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.02
Lotidae								
<i>Ciliata mustela</i>	0.00	0.00	2.97	2.97	0.00	0.00	0.00	0.00
Moronidae								
<i>Dicentrarchus labrax</i>	0.00	0.00	0.00	0.00	0.11	0.00	0.00	0.11
Mugilidae								
Mugilidae n. id.	0.00	0.06	0.00	0.06	0.00	0.00	0.00	0.00
Pleuronectidae								
<i>Platichthys flesus</i>	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.17
Pleuronectidae n. id.	0.21	0.00	0.00	0.21	0.00	0.00	0.00	0.00
Scophthalmidae								
<i>Scophthalmus maximus</i>	0.00	0.00	0.09	0.09	0.00	0.00	0.00	0.00
Soleidae								
<i>Buglossidium luteum</i>	0.00	0.43	0.00	0.43	0.00	0.17	0.23	0.41
<i>Solea senegalensis</i>	0.00	0.26	0.00	0.26	0.76	0.48	0.10	1.33
<i>Solea solea</i>	2.23	1.72	0.18	4.14	0.11	0.04	0.00	0.15
Soleidae n. id.	0.00	0.04	0.00	0.04	0.00	0.06	0.00	0.06
Sparidae								
<i>Diplodus</i> sp.	0.00	0.03	0.00	0.03	0.11	0.08	0.00	0.19
Sparidae n. id.	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.02
Syngnathidae								
<i>Syngnathus abaster</i>	0.00	0.00	0.00	0.00	0.07	0.15	0.00	0.22
<i>Syngnathus acus</i>	0.00	0.00	0.13	0.13	0.07	0.35	0.00	0.42
<i>Syngnathus</i> sp.	0.00	0.00	0.04	0.04	0.00	0.16	0.00	0.16
Trachinidae								
<i>Trachinus draco</i>	0.00	0.04	0.00	0.04	0.00	0.00	0.00	0.00
Triglidae								
<i>Chelidonichthys lucerna</i>	0.00	0.08	0.47	0.55	0.00	0.00	0.00	0.00
Not identified	0.82	0.00	0.61	1.43	0.48	0.31	0.05	0.84
	21.66	5.03	171.43	198.12	15.70	110.11	1.92	127.73

Table II – Summary results (Pseudo-F values and p(perm)) from univariate temporal and spatial PERMANOVA analysis on densities of main species. Local - coastal area and ocean; Season - spring, summer and autumn; Zone 1 - inner, middle and outer areas; Zone 2 - north, south, estuary transects; Estuary - lower, middle and upper areas.

Species	Temporal analysis						Spatial analysis							
	Local		Season		Local x Season		Zone 1		Coastal area		Zone 1 vs Zone 2		Estuary	
	Pseudo-F	p(perm)	Pseudo-F	p(perm)	Pseudo-F	p(perm)	Pseudo-F	p(perm)	Pseudo-F	p(perm)	Pseudo-F	p(perm)	Pseudo-F	p(perm)
<i>Ammodytes tobianus</i>	21.59	0.001*	18.86	0.001	18.86	0.001	0.66	0.540	8.41	0.023	0.83	0.546	-	-
<i>Engraulis encrasicolus</i>	11.20	0.001	24.42	0.001	15.83	0.001	6.28	0.036	0.50	0.609	1.09	0.448	1.40	0.228
<i>Gobius niger</i>	1.02	0.324	1.64	0.199	0.32	0.733	-	-	-	-	-	-	0.38	0.682
<i>Pomatoschistus microps</i>	24.37	0.001	9.88	0.001	11.01	0.001	-	-	-	-	-	-	3.92	0.044
<i>Pomatoschistus minutus</i>	19.19	0.001	2.06	0.115	3.15	0.048	-	-	-	-	-	-	1.82	0.179
<i>Sardina pilchardus</i>	14.31	0.001	18.72	0.001	7.07	0.003	4.83	0.056	59.55	0.001	2.50	0.172	5.40	0.063
<i>Solea senegalensis</i>	6.02	0.017	2.69	0.066	0.04	0.961	0.03	0.968	0.68	0.553	0.74	0.622	1.22	0.317
<i>Solea solea</i>	12.76	0.001	2.72	0.060	1.45	0.250	1.34	0.265	0.47	0.607	0.59	0.653	-	-

Spatial differences within each system were only recorded for some species (Table II). In the coastal area the density of *Sardina pilchardus* and *Ammodytes tobianus* differed significantly between transects. *Sardina pilchardus* was significantly more abundant in front of the estuary (pairwise $p(\text{perm}) < 0.05$) unlike the *Ammodytes tobianus* which was absent in this transept (Table II, Fig. 7). Although no differences were detected in relation to the distance to the coast for distribution of *Sardina pilchardus* is possible to verify an increase from inner to outer areas along the estuary transept. Also, *Ammodytes tobianus* seemed to be more abundant in south stations near the coast. On the other hand, the density of *Engraulis encrasicolus* differed significantly with the distance to the coast (Table II), with the outer areas presenting higher density than the inner areas (pairwise $p(\text{perm}) < 0.05$) (Fig. 7). Within the estuary only *Pomatoschistus microps* presented spatial differences (Table II). Its abundance was significantly lower in the stations next to the mouth of the estuary (pairwise $p(\text{perm}) < 0.01$) (Fig. 7). For the remaining main species, no significant spatial differences were detected.

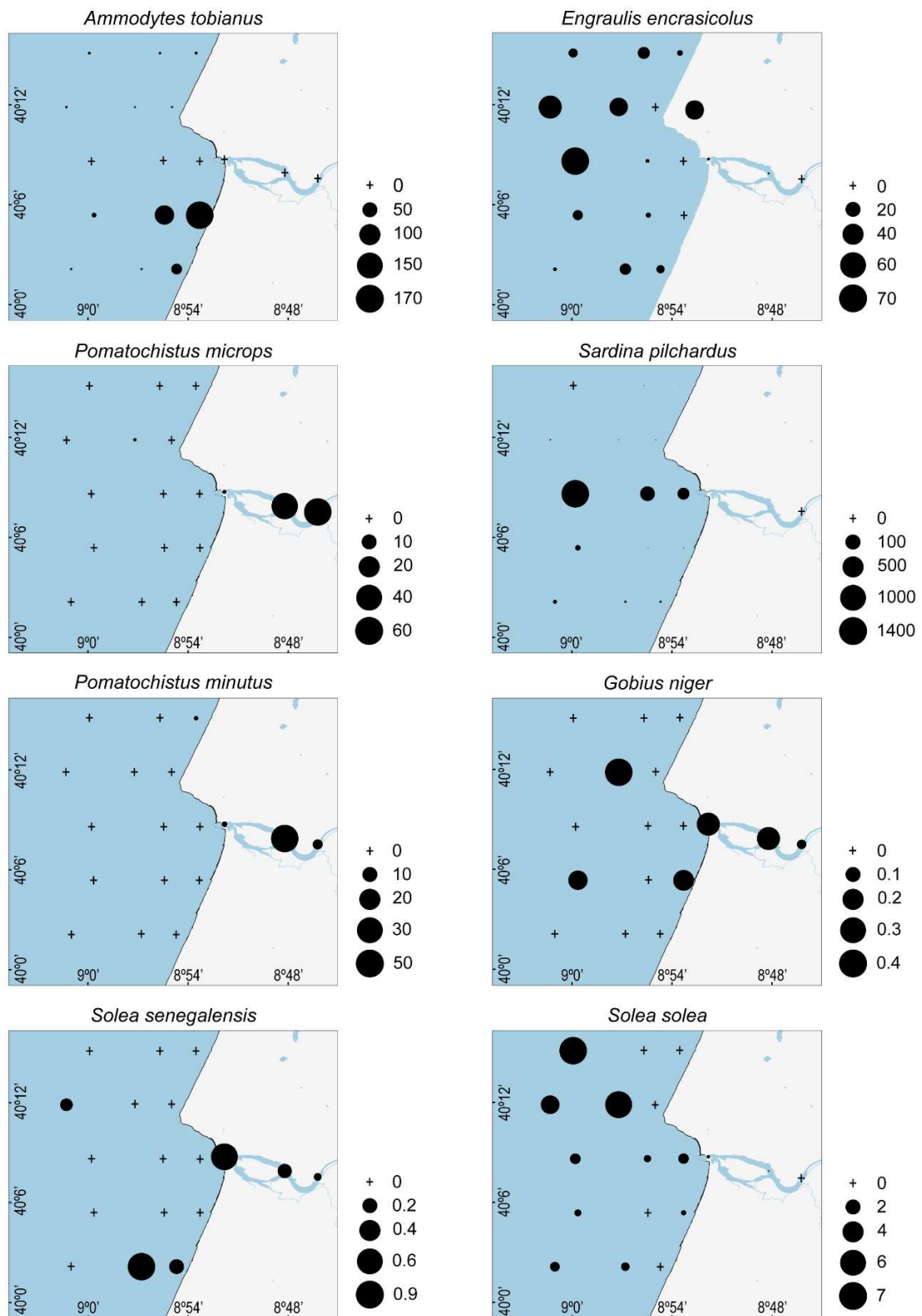


Figure 7 –Spatial distribution of the main species (larvae 100 m⁻³) in the coastal area and in the Mondego estuary. Species with seasonal pattern are represented in the most abundant season: *Ammodytes tobianus* and *Sardina pilchardus* in autumn; *Engraulis encrasicolus* in spring; and *Pomatoschistus microps* in summer. Mean annual values are represented for *Pomatoschistus minutus*, *Gobius niger*, *Solea senegalensis* and *Solea solea*.

3.4. Larval fish community structure

SIMPER analysis revealed that the species that most contribute to differences between systems were *Sardina pilchardus* (16.4%), *Pomatoschistus minutus* (14.6%), *Pomatoschistus microps* (13.1%), *Pomatoschistus* spp. (7.7%) and *Engraulis encrasicolus* (7.3%). In addition, other species appeared only in one of the systems: *Arnoglossus* sp., *Trisopterus luscus*, *Chelidonichthys lucerna*, *Trachurus trachurus*, *Pollachius pollachius*, *Scophthalmus maximus*, Mugilidae n. id., *Ciliata mustela*, *Lebetus* sp. and *Trachinus draco* in coastal area; *Atherina presbyter*, *Parablennius gattorugine*, *Syngnathus abaster*, *Gobius* sp., *Platichthys flesus*, *Dicentrarchus labrax*, *Coryphoblennius galerita*, *Gobius paganellus*, *Centrolabrus exoletus*, *Pomatoschistus pictus* and *Labrus bergylta* in the estuary.

The multivariate PERMANOVA results showed significant differences in community structure between locals (Pseudo-F = 12.99, $p(\text{perm}) < 0.01$) and seasons (Pseudo-F = 7.77, $p(\text{perm}) < 0.01$), as well as significant interactions between these factors (Pseudo-F = 5.33, $p(\text{perm}) < 0.01$). A pairwise *a posteriori* comparison revealed that all communities differ between them ($p(\text{perm}) < 0.01$). *Pomatoschistus* spp. has dominated all seasons throughout the year in the estuary while *Solea senegalensis* was mainly present in spring, *Engraulis encrasicolus* in summer and *Sardina pilchardus* in autumn (Table I). In the coastal area, *Engraulis encrasicolus* dominated in the spring community, *Solea solea* and *Sardina pilchardus* in summer and *Sardina pilchardus*, *Ammodytes tobianus* and *Ciliata mustela* in the autumn community.

Regarding the spatial analysis, the coastal area community showed significant differences between seasons (Pseudo-F = 18.104, $p(\text{perm}) < 0.01$), position in relation to estuary (north, estuary, south) (Pseudo-F = 3.6407, $p(\text{perm}) < 0.01$) as well as an interaction between these factors (Pseudo-F = 4.5257, $p(\text{perm}) < 0.01$). Seasonal variation was evident only at the sampling stations of north and south (pairwise $p(\text{perm}) < 0.05$). In the autumn all communities (north, estuary and south) differed (pairwise $p(\text{perm}) < 0.05$) and during spring only communities in front of the estuary and at the south transects differed (pairwise $p(\text{perm}) < 0.05$). During autumn *S. pilchardus*, *Trisopterus luscus* and *Lebetus* sp. predominated in front of the estuary, while *Ammodytes tobianus* and *Ciliata mustela* predominated at the south. At the north, species showed lower abundances compared to the other transects. Regarding to the spring, higher abundances occurred in front of the estuary with a predominance of *Arnoglossus* sp., *Engraulis encrasicolus* and *Lebetus* sp. than in the south. On the other hand, *Solea solea* appeared in the south transects and not in front of estuary.

3.5. Influence of environmental variables in the larval community

The best 3 subset model selected by the DISTLM analysis included the variables temperature, abundance of Cnidaria and Cladocera which explained 36% of the variation observed in the community. When considered alone, marginal testes showed that temperature explained 19%, Cnidaria 16%, and Cladocera 8% of the variability observed (Table III). In addition, although not included in the model, when considered alone, environmental factors such as oxygen, abundance of Appendicularia and Chaetognatha show a significant influence in the community, explaining 7% (Oxygen, Appendicularia) and 9% (Chaetognatha) of the variation observed (Table III).

Table III – Results of the marginal testes for the environmental variables included in DISTLM analysis. Temperature (°C); Oxygen (mg l⁻¹); POM, Particulate organic matter (mg m⁻³); Chl *a*, chlorophyll *a* (mg m⁻³); Appendicularia, Chaetognatha, Cladocera, Cnidaria, Copepoda abundances (ind. m⁻³).

Variable	Pseudo-F	<i>p</i>	Prop.
Temperature	8.11	0.001	0.19
Salinity	2.56	0.047	0.07
pH	2.24	0.054	0.06
Oxygen	2.52	0.042	0.07
POM	1.94	0.078	0.05
Chl <i>a</i>	2.39	0.069	0.07
Appendicularia	2.42	0.032	0.07
Chaetognatha	3.21	0.024	0.09
Cladocera	2.91	0.041	0.08
Cnidaria	6.27	0.001	0.16
Copepoda	1.36	0.185	0.04

The first two axes of the RDA explained 27.8% and 12.5% of the total variance, respectively. The first axis showed a strong seasonal separation with distinct communities during autumn and spring in the coastal area (Fig. 8). In the estuary, seasonality was not so evident. Summer communities of both coastal and estuarine areas presented similar structure. In the coastal area, spring communities were positively related to peaks of abundance of Cladocera and Cnidaria, while the opposite occurred in autumn. Higher temperatures were mainly found in the estuary (Fig. 8).

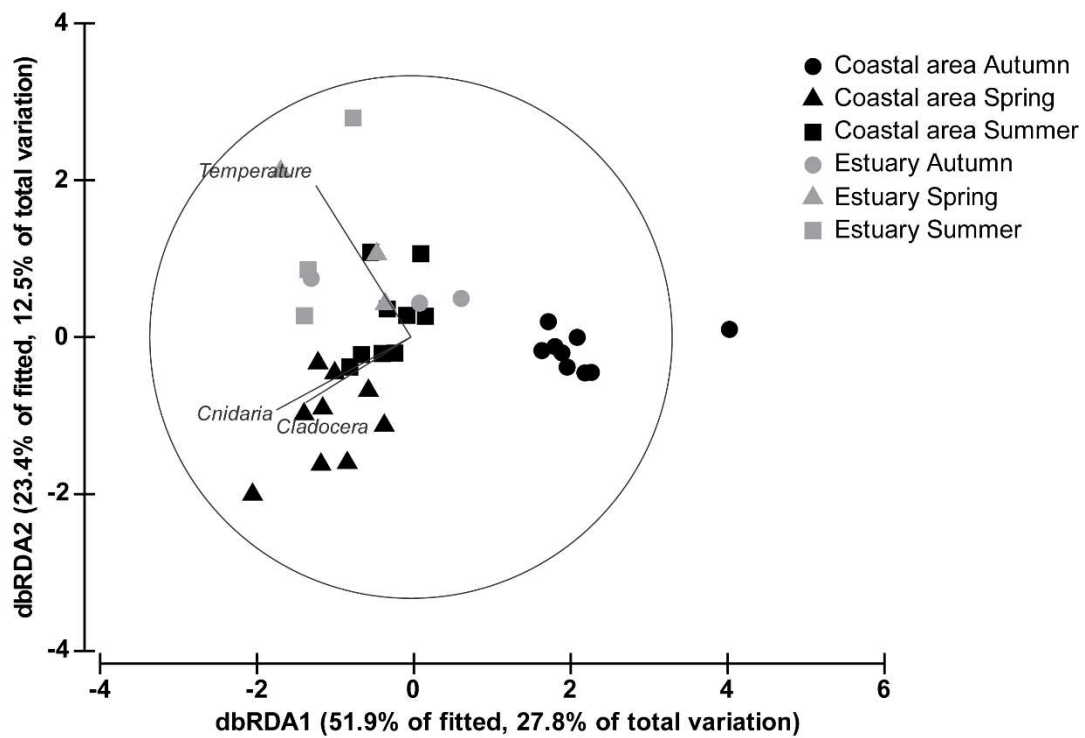


Figure 8 – RDA showing the relationship between environmental variables (best 3 subset model identified by DISTLM) and ichthyoplankton community.

3.6. Juveniles fish composition and recruitment

In this study, it were captured a total of 36 species of juveniles, 27 in the coastal area and 15 in the estuary. Of these, only 6 species were common to both systems: *Conger conger*, *Engraulis encrasicolus*, *Dicentrarchus labrax*, *Chelon auratus*, *Sparus aurata* and *Chelidonichthys lucerna*. Although *Engraulis encrasicolus*, *Sparus aurata* and *Chelidonichthys lucerna* have been more abundant in the coastal area and *Dicentrarchus labrax* in the estuary (Table IV).

The main species collected in the coastal area were *Trachurus trachurus*, *Trisopterus luscus*, *Sardina pilchardus* and *Scomber scombrus*, and in the estuary were *Pomatoschistus microps*, *Solea solea*, *Dicentrarchus labrax* and *Platichthys flesus* (Table IV). In the coastal area the values of CPUE were generally higher than those observed in estuary.

The results of the principal components analysis (PCA) of juvenile data allowed to discriminate species preferences for recruitment area. Analysis revealed that PC1 accounted for 68.9% of the variation and PC2 for 7.6%. PC1 showed a clear estuarine (positive) and oceanic (negative) distribution of samples along this axis (Fig. 9). There were 14 species positively correlated (adjusted $p < 0.05$) with PC1. *Pomatoschistus microps*, *Solea solea*, *Dicentrarchus labrax*, *Platichthys flesus*, *Anguilla anguilla*, *Pomatoschistus* sp., and *Atherina presbyter* seem to recruit in the estuary. On the other hand, *Trachurus trachurus*, *Trisopterus luscus*, *Scomber scomber*, *Sardina pilchardus*, *Alosa fallax*, *Microchirirus azevia* and *Mullus surmulletus* only appear as juveniles in the coastal area thus, recruitment was associated with this system.

Table IV – Catches per unit effort (CPUE) of juvenile species collected in each sampling local (coastal area and the Mondego estuary).

Species	Coastal area	Estuary
Anguillidae		
<i>Anguilla anguilla</i>	0.00	0.10
Atherinidae		
<i>Atherina boyeri</i>	0.00	0.02
<i>Atherina presbyter</i>	0.00	0.02
<i>Atherina sp.</i>	0.00	0.01
Callionymidae		
<i>Callionymus lyra</i>	<0.01	0.00
Carangidae		
<i>Trachinotus ovatus</i>	<0.01	0.00
<i>Trachurus trachurus</i>	83.97	0.00
Clupeidae		
<i>Alosa alosa</i>	0.01	0.00
<i>Alosa fallax</i>	0.51	0.00
<i>Sardina pilchardus</i>	3.37	0.00
Congridae		
<i>Conger conger</i>	<0.01	0.02
Engraulidae		
<i>Engraulis encrasicolus</i>	0.73	0.01
Gadidae		
<i>Trisopterus luscus</i>	20.31	0.00
Gobiidae		
<i>Pomatoschistus microps</i>	0.00	1.08
<i>Pomatoschistus sp.</i>	0.00	0.05
Lotidae		
<i>Ciliata mustela</i>	0.00	0.01
Moronidae		
<i>Dicentrarchus labrax</i>	0.04	0.69
<i>Dicentrarchus punctatus</i>	0.01	0.00
Mugilidae		
<i>Chelon auratus</i>	0.01	0.01
<i>Chelon ramada</i>	0.01	0.00
Mullidae		
<i>Mullus surmuletus</i>	0.35	0.00
Pleuronectidae		
<i>Platichthys flesus</i>	0.00	0.14
Sciaenidae		
<i>Argyrosomus regius</i>	<0.01	0.00
Scombridae		
<i>Scomber colias</i>	0.12	0.00
<i>Scomber scombrus</i>	2.84	0.00
Scophthalmidae		
<i>Scophthalmus maximus</i>	0.01	0.00
<i>Scophthalmus rhombus</i>	0.01	0.00
Soleidae		
<i>Microchirus azevia</i>	0.28	0.00
<i>Solea senegalensis</i>	<0.01	0.00
<i>Solea solea</i>	0.00	0.86
Sparidae		
<i>Diplodus sargus</i>	0.28	0.00
<i>Diplodus spp.</i>	0.12	0.00
<i>Diplodus vulgaris</i>	0.00	0.01
<i>Sparus aurata</i>	0.06	0.01
<i>Spondyliosoma cantharus</i>	0.02	0.00
Syngnathidae		
<i>Hippocampus hippocampus</i>	0.00	0.01
Trachinidae		
<i>Echiichthys vipera</i>	0.37	0.00
Triglidae		
<i>Chelidonichthys lucerna</i>	0.13	0.01
Zeidae		
<i>Zeus faber</i>	0.01	0.00
Total Geral	113.54	3.05

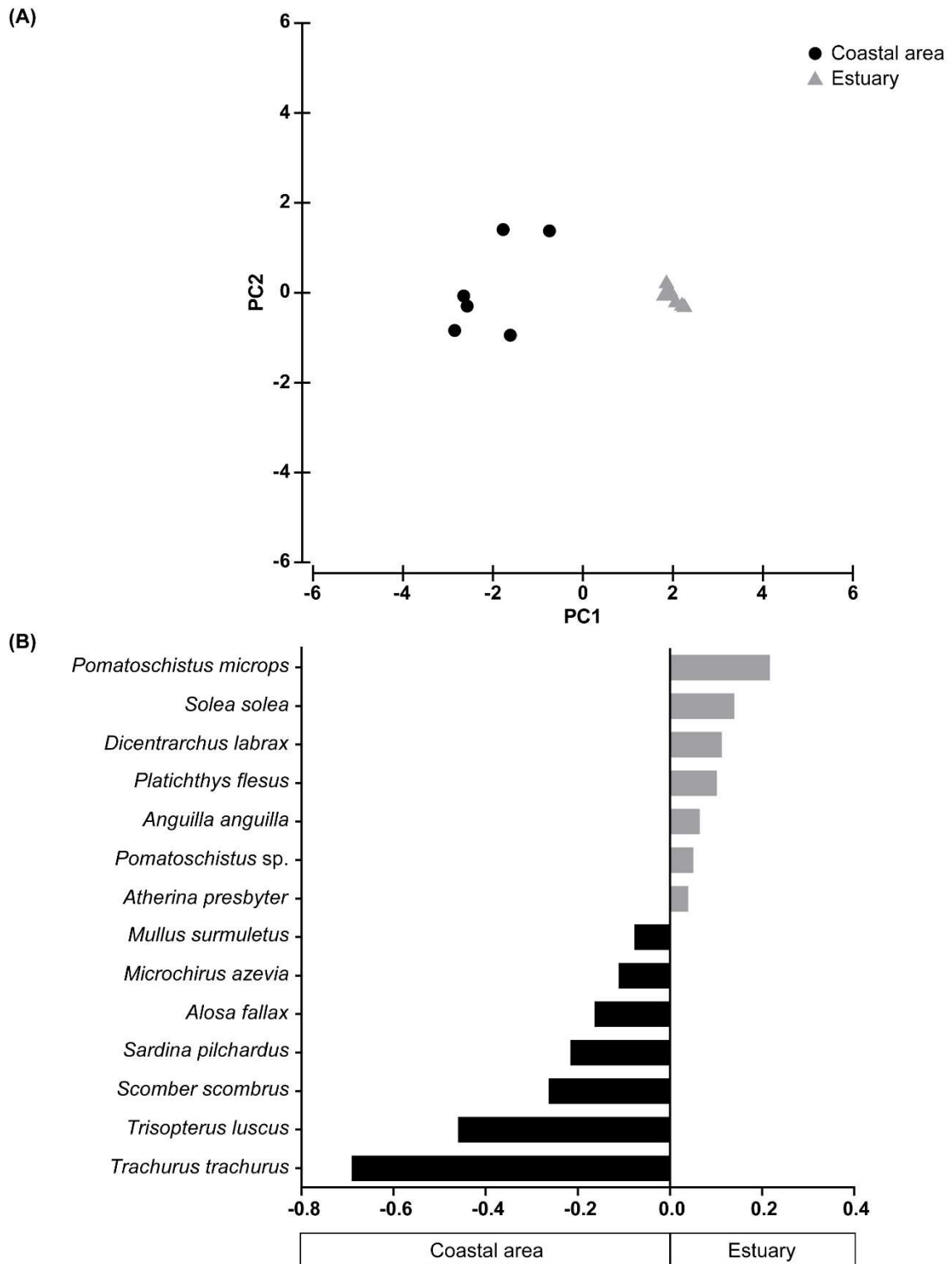
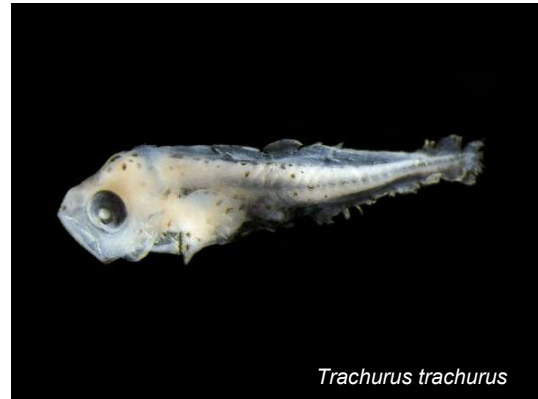


Figure 9 – (A) Results of PCA on juvenile species collected in the Mondego estuary and coastal area. (B) PC1 Species eigenvectors loadings. Only species which showed significant correlation with PC1 scores are presented. Negative values correspond to the coastal area and positive values to the estuary.

4. Discussion



4.1. Seasonal and spatial distribution of ichthyoplankton communities

The species of larvae and juveniles captured in this study are in agreement with the ones commonly found on the Portuguese coast and on the Mondego estuary (Garrido et al., 2009; Leitão et al., 2007; Primo et al., 2011). Results showed a dominance of few species over many other, a pattern previously observed in the Mondego estuary (Nyitrai et al., 2012; Primo et al., 2011) and characteristic of many estuaries and shelf-regions around the world, both in larval and juvenile populations (Hagan and Able, 2003; Rodriguez, 2008; Strydom et al., 2003).

Of the 33 species captured throughout the study, only 13 were common to both estuarine and coastal areas. Moreover, *Sardina pilchardus*, *Ammodytes tobianus*, *Engraulis encrasicolus* and *Solea solea* dominated in the coastal area, while *Pomatoschistus microps*, *Pomatoschistus minutus* and *Solea senegalensis* were more common in the estuary, although at different period of the year. The differences observed could be related to species salinity tolerance, which is one of the main factors influencing fish distribution (Grothues and Cowen, 1999; Ramos et al., 2017), as to the high environmental variability of estuaries that many species cannot support (Elliott et al., 2007).

Ichthyoplankton showed different abundances and community structures according to system and season. Temporal differences, are often related to the reproductive strategy of adults (Ramos et al., 2006; Sabatés et al., 2007). In this region, some pelagic species that spawn in the coastal area have a reproductive strategy adapted to upwelling systems. These species avoid the strong upwelling season to prevent being dragged to offshore by the displacement of water characteristic of these events. Thus, spawning occurs during cold months, ensuring shoreward transport and larval retention (Roy et al., 1989; Santos et al., 2001). That is the case of the *Sardina pilchardus*, which was the main responsible for the peak of abundance during autumn in the coastal area. This is the most abundant pelagic species spawning off the western coast of the Iberian Peninsula from November to April (Zwolinski et al., 2001). Overall, autumn was the season with higher larval abundance at the coastal area, in opposition to other studies in Portugal, which report higher abundances in spring and summer (Azeiteiro et al., 2006; Borges et al., 2007). However, these are focused on very nearshore zones and captured mainly intertidal species. Most of these lay demersal eggs and have strong retention mechanisms being less affected by advective processes and reducing the transport to offshore. Furthermore, Borges et al. (2007) only studied communities from May to October, leaving the potential autumn and winter patterns uncovered. Other autumn spawners were *Ammodytes tobianus*, *Ciliata mustela* and *Trisopterus luscus*.

At the estuary, species appear to spawn mainly during the warmer months (summer), as in the case of *Pomatoschistus minutus* and *Pomatoschistus microps*, the most abundant species and the main responsible for the peak of abundance during the summer. The dominance of these species during this time of year has been reported for some time, both in the Mondego estuary and in other Portuguese estuaries (Faria et al., 2006; Marques et al., 2008; Primo et al., 2011; Ramos et al.,

2006b) and it seems to be related with high temperatures and low river discharge. Temperature has been identified as the main factor controlling the reproduction and survival process of eggs of these short-lived species (Fonds and Van Buurt, 1974). In Mondego estuary previous studies estimated that the breeding season of *Pomatoschistus microps* is from January to June and that of *Pomatoschistus minutus* from February to August-September (Dolbeth et al., 2007; Leitão et al., 2006). Also, Ramos et al., (2006a) considered that these species reproduce throughout the year and that their decrease during autumn and winter is related with the larvae migration to the bottom in order to avoid the colder and less saline waters of the surface. Despite this extended breeding season, their abundance is always higher in warmer months. Species such as *Engraulis encrasicolus*, *Gobius niger*, *Parablennius pilicornis* and *Coryphoblennius galerita* also spawned during warmer months.

Seasonal variations in river discharge are known to have important implications in these communities. During summer, the low river runoff, prevents larvae from being dragged and allows an higher intrusion of sea water and the entrance of marine species in the estuary (Primo et al., 2011; Ramos et al., 2006a) thus increasing larval abundance. This is confirmed by our results which showed that during summer estuarine and coastal communities are similar. Consequently, during the autumn the opposite pattern occurs and larvae are washed out the estuary and marine species entrance is prevented (Ramos et al., 2006b). Despite, the importance of river plumes is well known acting as cues for larvae to enter into estuarine areas (Teodósio et al., 2016). For instance, the arrival of *Platichthys flesus* juveniles and its abundance in the Mondego seem to be related to the precipitation and river flow in the months before their arrival at the estuary (Martinho et al., 2009).

Spatial distribution of *Engraulis encrasicolus* in the coastal zone pointed out an increase of larvae in the sampling stations further away. Since the abundance of this species was higher in the spring, when upwelling events are common, this can be related to this water circulation pattern. The occurrence of upwelling events lead to an increase of larval abundances in the direction farther from the coast (Garrido et al., 2009; Santos et al., 2006). In addition, upwelling events has also been frequently related with the distribution of *Sardina pilchardus*. Some studies show that this species is more abundant in the mid-shelf (Garrido et al., 2009; Jonh et al., 1996) and nearly absent in the fresh upwelling waters during the spring (Jonh et al., 1996), which may justify the low number of sardines larvae during spring sampling in our results. Furthermore, in autumn, *Sardina pilchardus* was distributed essentially in the transept in front of the estuary, which could be related to the river plume. This species is known to be often found in association with Western Iberian Buoyant Plume (WIPB) (Garrido et al., 2009; Santos et al., 2006, 2004), a plume confined to inner-shelf areas from the Mondego mouth northward, in the absence of upwelling (Peliz et al., 2002). These are habitats that provide favourable conditions for the growth and survival of larvae and fish due to high food availability (Grimes and Finucane, 1991; Sabatés et al., 2001), stimulated by high concentration of

nutrients that increase the growth of phytoplankton (Ribeiro et al., 2005). Conversely, during the same period, *Ammodytes tobianus* was absent in the transect in front of the estuary which can be indicative of competition between the two species.

In the estuary the only species that showed a distinct distribution pattern was the *Pomatoschistus microps*, with low densities in the mouth of the estuary. This species has a higher tolerance to variations in temperature and salinity, exhibiting more estuarine characteristics than its conspecific *Pomatoschistus minutus* (Fonds and Van Buurt, 1974; Pampoulie, 2001). Previous studies of juveniles and adults have shown that *Pomatoschistus* spp. occupy different areas in the Mondego estuary with *Pomatoschistus microps* being more frequent in the upstream stations (Dolbeth et al., 2007; Leitão et al., 2006) and therefore their larvae may reflect the distribution of their progenitors.

The main environmental factors that seem to shape the larval community structure were temperature and abundance of Cladocera and Cnidarian. Temperature has been pointed out as one of the main factors that determines the distribution and abundance of many species of fish being related to the reproductive cycle of adults and their spawning, as well as with egg survival and development (Pankhurst and Munday, 2011; Rakocinski et al., 1996). For *Sardina pilchardus*, for example, spawning is triggered by the decrease in the sea surface temperature (Zwolinski et al., 2001), and for the species *Pomatoschistus minutus* temperatures above 25°C are not viable for the eggs (Fonds and Van Buurt, 1974). Temperature gradients may also be associated with other environmental variables such as dissolved oxygen concentration, which may be a limiting factor for some species of fish larvae (Neilson and Perry, 1990). Furthermore, temperature is also known to influence zooplankton communities. During spring, the increase in temperature together with upwelling events, which enrich the water column with nutrients, favour the increase of zooplankton abundances, which are the main food sources for larval fish. However, the high abundance of Cladocera and Cnidaria seemed to had a negative influence on the fish larvae communities, which showed a decrease. This can be due to the fact that in our study, Cladocera was not their preferred food. Other studies refer copepod and other heterotrophic microzooplankton as important prey items e.g. (de Figueiredo et al., 2007; Garrido et al., 2009; Pepin and Dower, 2007) however, these relationships were not observed/investigated. Also Cnidaria are known to be important predators of fish larvae (Frank and Leggett, 1985; Schneider and Behrends, 1994) contributing to the larval fish reduction.

4.2. Preferred recruitment habitats of fish juveniles

Atherina presbyter and *Pomatoschistus microps* appeared both as larvae and juveniles in the estuary. These species are among the most common families found in Portuguese estuaries (Nyitrai et al., 2012; Pombo et al., 2005; Ribeiro et al., 2008). Although *Atherina presbyter* is normally

associated with coastal ecosystems (Almada et al., 2017; Borges et al., 2007), in this study this species only appeared inside the estuary. In turn *Pomatoschistus microps* was one of the most important species in the estuary, captured in large amounts both as larvae and juvenile. Its dominance in Mondego estuary has been reported over the years (Leitão et al., 2007; Nyitrai et al., 2012), and its estuarine preference is reinforced by the occurrence of larvae in all stages of development. On the other hand, its conspecific *Pomatoschistus minutus* was highly abundant in the estuary as larvae, but juveniles were not captured. Some studies indicate that this species, due to inter and intraspecific competition, can disperse into the sea and only return later to reproduce (Dolbeth et al., 2007; Leitão et al., 2006). Both *Atherina presbyter* and *Pomatoschistus microps* are considered estuarine resident species at the Mondego estuary (Nyitrai et al., 2012), completing their entire life cycle there.

Trisopterus luscus and *Trachurus trachurus* were only present in the coastal zone, suggesting that these species have their entire life cycle outside the estuary, despite previous studies referring that *Trisopterus luscus* can also use the estuaries as nursery zones (Hamerlynck and Hostens, 1993). In Portugal this pattern was already observed by Costa and Bruxelles (1989) in the Tagus estuary, however, later studies showed a drastic reduction of this species there (Costa and Cabral, 1999). In the Lima estuary, *Trisopterus luscus* larvae seem to act as a seasonal estuarine resident species during spring (Ramos et al., 2006a), however, in the Mondego estuary only the juveniles were previously observed, in small numbers and during periods of higher sea water intrusion (Nyitrai et al., 2012). In turn, *Trachurus trachurus* seems to be restricted to coastal environments and the entry of larvae into the estuary seems to be an occasional event (Klein et al., 2018; Ramos et al., 2006b). Both *Trisopterus luscus* and *Trachurus trachurus* seem to use coastal zone as nursery grounds being frequently observed in this area (Cabral et al., 2003; Castro et al., 2013; França et al., 2004; Garrido et al., 2009). The early stages of *Sardina pilchardus* (larvae and juvenile) were also mainly associated with the coastal zone. Although this species is frequently found in the ichthyoplankton communities of estuaries (Faria et al., 2006; Ramos et al., 2009, 2006b), the abundances found in the coastal area are far higher. During this study no juveniles were present in the Mondego estuary but this species was previously considered estuarine opportunistic, regularly entering in the estuary in considerable numbers while juvenile (Nyitrai et al., 2012).

Solea solea revealed a more complex life cycle. This species was mainly captured as larvae in the coastal zone and as juvenile in the estuary. Primo et al. (2013) had already hypothesized that this species would be on the coastal zone as larvae and only enter in the estuary after settlement, once the abundance of larvae present inside the estuary was very low. Therefore, the present study reinforces that hypothesis. It was already known that the nursery areas of *Solea solea* could be in the coastal zones as well as in the estuaries (Cabral et al., 2007). In this region it seems to use both. This pattern has already been observed in the Lima estuary, although *Solea solea* was less abundant

compared to the Mondego (Ramos et al., 2010). Several authors have already emphasized the importance of the Mondego estuary as a nursery area for the juveniles of *Solea solea* and consequently for adult coastal stocks (Vasconcelos et al., 2008).

Platichthys flesus and *Dicentrarchus labrax* showed large numbers of juveniles in the estuary, but larval abundances in both systems were too low to determine their preference. Low abundances of *Platichthys flesus* in the Mondego estuary has been already indicated by Primo et al. (2013) who pointed out the same hypothesis of recruitment as for *Solea solea*. Several studies refer that flounder larvae found near the estuaries and within these were already in advanced stages of development which supports this hypothesis (Grioche et al., 1997; Ramos et al., 2010). Also, it is well known that salinity is a determinant factor in the distribution of this species, which has a preference for freshwater, mainly during the settlement and early juvenile stages (Bos and Thiel, 2006; Martinho et al., 2007). Our samplings also did not find the larvae in the coastal zone however, this may be due to the fact that sampling missed the species spawning period. According to Martinho et al., (2013), the species spawns during winter, unfortunately, adverse winter weather prevent us of sampling during this season. *Dicentrarchus labrax* spawns offshore and larvae migrates to the coastal zone within a month (Jennings et al., 1992), where they remain until reaching the juvenile stage (2 to 3 months) and only enter in estuaries when they receive the appropriate environmental cue (Pickett and Pawson, 1994). Several authors have reported that larvae below 20 mm total length (SL) are rarely seen, and usually only when tows are carried out in large estuaries (Dando and Demir, 1985; Jennings et al., 1992). In fact, in this work very few larvae were collected inside the estuary and all in latter stages of development (post-flexion), which may coincide with the settling period. Their larvae was not previously recorded in the Mondego estuary although, in the Lima estuary, their presence was reported in several studies (Ramos et al., 2017, 2006a). The low number of larvae can be related, again, with their preferred spawning season, the winter-early spring (Arias, 1980; Vinagre et al., 2009a) when adverse weather conditions prevented sampling campaigns. The Mondego estuary has also been reported as an important nursery area for juveniles stages of *Platichthys flesus* and *Dicentrarchus labrax* (Vasconcelos et al., 2008).

Solea senegalensis presented the inverse pattern, appearing in large quantities as larva in the Mondego estuary, but not as juvenile. In the coastal zone, juveniles were captured in very low abundances which prevents any conclusion about their preferred recruitment areas. The low number of larvae was previously reported by Primo et al. (2013) study which assumed that larvae leave the estuary or die, failing their juvenile recruitment. The populations of *Solea senegalensis* present a great genetic flow, sometimes with distant origins from where they were captured, which may reinforce that the larvae disperse to other areas (Vasconcelos et al., 2008). In fact, the nursery areas of this species has been associated with large estuaries with large intertidal areas, which function as their feeding grounds (Cabral et al., 2007; Vinagre et al., 2009b). This may explain the reason

why the Mondego estuary is not a suitable area for this species, once it is a small system in which the intertidal zones are located mainly in the south arm which was not analysed. However, the low abundances of juveniles of *Solea senegalensis* is confirmed by previous studies in the Mondego estuary (Leitão et al., 2007; Martinho et al., 2010). At the Lima estuary, despite its small size and high abundance of *Solea senegalensis*, *Solea solea* appears in low numbers, and authors suggest that the estuary may be too small to contemplate the two species (Ramos et al., 2010). We hypothesize that the same may be occurring at the Mondego estuary, but with the inverse pattern.

Engraulis encrasicolus was more abundant as larvae in the coastal area although it had also appeared inside the estuary. Previous studies have shown high abundances of larvae within the estuaries (Jonh and Ré, 1995; Ribeiro et al., 1996) however, it has been observed a decrease (Primo et al., 2011). In the present study, both larvae and juveniles seem to prefer the coastal zone although the results have not been shown to be significant for juveniles.

The results of this work showed that both the estuary and the adjacent coastal zone represent important nursery areas for different species of fish. For some of them, such as *Solea solea*, *Platichthys flesus* and *Dicentrarchus labrax*, the two habitats were evenly important, although at different stages of the life cycle. The type of information obtained in this work, helps to know the life cycle of the species and have important implications for the stock management and ecosystem conservation.

5. Final remarks



Ichthyoplankton communities' structure and abundance varied seasonally and according to locals (estuary and adjacent marine coastal area). Differences between locals could be attributed to salinity gradients, physiological constraints or/and to the species nursery habitat preference. On the other hand, seasonal differences seem to be linked to the reproduction strategies of species, which can be influenced by upwelling and temperature. In addition, in the estuary, the seasonal river runoff, also seems to have important implications in the species occurrence. Within each site, spatial distribution was mainly associated with seasonal events such as upwelling or river plume, or even related to competition between species. Temperature was the most important factor structuring the community probably due to its relationship with reproduction and food availability.

Both the Mondego estuary and the adjacent marine coastal area represent important nursery areas for fish. The estuary was important for *Pomatoschistus microps* and *Atherina presbyter*, two estuarine resident species, while the coastal zone seemed to be important for *Sardina pilchardus*, *Trisopterus luscus*, *Trachurus trachurus*, and *Engraulis encrasicolus*, although for the latter more evidence is needed. On the other hand, *Solea solea* seem to use the two habitats as a nursery area, although at different phases of their life cycle. For *Dicentrarchus labrax* and *Platichthys flesus*, although the number of larvae was not enough in this study to take conclusions, previous evidences suggest that these species mimics the habitat use patterns of *Solea solea*.

This study represents a preliminary work involving more than one nursery area and early life cycle stages. The patterns observed show a different use of nursery habitats by species highlighting the need to integrate larval and juvenile phases for a better understanding of life cycle, management of fish stocks and conservation of ecosystems.

6. References



- Able, K.W., Manderson, J.P., Studholme, A.L., 1999. Habitat quality for shallow water fishes in an urban estuary: The effects of man-made structures on growth. *Mar. Ecol. Prog. Ser.* 187, 227–235. doi:10.3354/meps187227
- Almada, F., Carpi, P., Carlos, F., Francisco, S.M., Faria, A.M., Lungu, R., Duarte-Coelho, P., Neto, J., Gonçalves, E.J., Robalo, J.I., 2017. Identification of adult and juvenile *Atherina presbyter* Cuvier, 1829 in a marine coastal area adjacent to Tagus estuary (NE Atlantic): A molecular tool for an ecological question. *J. Appl. Ichthyol.* 33, 168–173. doi:10.1111/jai.13296
- Amara, R., Lagardere, F., Desaunay, Y., Marchand, J., 2000. Metamorphosis and estuarine colonisation in the common sole, *Solea solea* (L.): Implications for recruitment regulation. *Oceanol. Acta* 23, 469–484. doi:10.1016/S0399-1784(00)00134-1
- American Public Health Association (APHA), 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington DC.
- Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth, UK. doi:10.13564/j.cnki.issn.1672-9382.2013.01.010
- Arias, A., 1980. Crecimiento, régimen alimentario y reproducción de la dorada (*Sparus aurata* L.) y del róbalo (*Dicentrarchus labrax* L.) en los esteros de Cádiz. *Investig. Pesq.* 44, 59–83.
- Azeiteiro, U.M., Bacelar-Nicolau, L., Resende, P., Gonçalves, F., Pereira, M.J., 2006. Larval fish distribution in shallow coastal waters off North Western Iberia (NE Atlantic). *Estuar. Coast. Shelf Sci.* 69, 554–566. doi:10.1016/j.ecss.2006.05.023
- Bailey, K.M., Abookire, A.A., Duffy-Anderson, J.T., 2008. Ocean transport paths for the early life history stages of offshore-spawning flatfishes: a case study in the Gulf of Alaska. *Fish Fish.* 9, 44–66. doi:10.1111/j.1467-2979.2007.00268.x
- Bailey, K.M., Houde, E.D., 1989. Predation on Eggs and Larvae of Marine Fishes and the Recruitment Problem. *Adv. Mar. Biol.* 25, 1–83. doi:10.1016/S0065-2881(08)60187-X
- Bailey, K.M., Nakata, H., Veer, H.W. Van Der, 2005. The planktonic stages of flatfishes: physical and biological interactions in transport processes, in: Gibson, R.N. (Ed.), *Flatfishes, Biology and Exploitation*. Blackwell Publishing, Oxford, pp. 94–119.
- Balon, E.K., 1986. Types of feeding in the ontogeny of fishes and the life-history model. *Environ. Biol. Fishes* 16, 11–24. doi:10.1007/BF00005156
- Balon, E.K., 1984. Reflections on Some Decisive Events in the Early Life of Fishes. *Trans. Am. Fish. Soc.* 113, 178–185. doi:10.1577/1548-8659(1984)113<178:ROSDEI>2.0.CO;2
- Beck, M., Heck, K., Able, K., Childers, D., Eggleston, D., Gillanders, B., Halpern, B., Hayes, C., Hoshino, K., Minello, T., Orth, R., Sheridan, P., Weinstein, M., 2003. The role of nearshore ecosystems as fish and shellfish nurseries. *Issues Ecol.* 11, 1–12.

- Beck, M.W., Heck, K.L., JR., Able, K.W., Childers, D.L., Eggleston, D.B., Gillanders, B.M., Halpern, B., Hays, C.G., Hoshino, K., Minello, T.J., Orth, R.J., Sheridan, P.F., Weinstein, M.P., 2001. The Identification, Conservation, and Management of Estuarine and Marine Nurseries for Fish and Invertebrates. *Bioscience* 51, 633–641. doi:10.1641/0006-3568(2001)051[0633:TICAMO]2.0.CO;2
- Bettencourt, A.M., Bricker, S.B., Ferreira, J.G., Franco, A., Marques, J.C., Melo, J.J., Nobre, A., Ramos, L., Reis, C.S., Salas, F., Silva, M.C., Simas, T., Wolff, W., 2004. Typology and Reference Conditions for Portuguese Transitional and Coastal Waters, Development of Guidelines for the Application of the European Union Water Framework Directive., INAG/IMAR. Lisbon, Portugal.
- Bigelow, H.B., Welsh, W.W., 1925. Fishes of the Gulf of Maine. *Bull. U.S. Bur. Fish.* 40, 1–567.
- Boehlert, G.W., Mundy, B.C., 1988. Roles of behavioral and physical factors in larval and juvenile fish recruitment to estuarine nursery areas. *Am. Fish. Soc. Symp.* 3, 51–67.
- Boeing, W.J., Duffy-Anderson, J.T., 2008. Ichthyoplankton dynamics and biodiversity in the Gulf of Alaska: Responses to environmental change. *Ecol. Indic.* 8, 292–302. doi:10.1016/j.ecolind.2007.03.002
- Bone, Q., Moore, R.H., 2008. Reproduction, and Life Histories, in: Owen, E. (Ed.), *Biology of Fishes*. Taylor & Francis Group, London, UK, pp. 217–250.
- Borges, R., Ben-Hamadou, R., Chícharo, M.A., Ré, P., Gonçalves, E.J., 2007. Horizontal spatial and temporal distribution patterns of nearshore larval fish assemblages at a temperate rocky shore. *Estuar. Coast. Shelf Sci.* 71, 412–428. doi:10.1016/j.ecss.2006.08.020
- Bos, A.R., Thiel, R., 2006. Influence of salinity on the migration of postlarval and juvenile flounder *Pleuronectes flesus* L. in a gradient experiment. *J. Fish Biol.* 68, 1411–1420. doi:10.1111/j.0022-1112.2006.01023.x
- Cabral, H., Duque, J., Costa, M.J., 2003. Discards of the beach seine fishery in the central coast of Portugal. *Fish. Res.* 63, 63–71. doi:10.1016/S0165-7836(03)00004-3
- Cabral, H.N., Vasconcelos, R., Vinagre, C., França, S., Fonseca, V., Maia, A., Reis-Santos, P., Lopes, M., Ruano, M., Campos, J., Freitas, V., Santos, P.T., Costa, M.J., 2007. Relative importance of estuarine flatfish nurseries along the Portuguese coast. *J. Sea Res.* 57, 209–217. doi:10.1016/j.seares.2006.08.007
- Castro, N., Costa, J.L., Domingos, I., Angélico, M.M., 2013. Trophic ecology of a coastal fish assemblage in Portuguese waters. *J. Mar. Biol. Assoc. United Kingdom* 93, 1151–1161. doi:10.1017/S0025315412001853
- Churchill, J.H., Forward, R.B., Luettich, R.A., Hensch, J.L., Hettler, W.F., Crowder, L.B., Blanton, J.O., 1999. Circulation and larval fish transport within a tidally dominated estuary. *Fish. Oceanogr.* 8, 173–189. doi:10.1046/j.1365-2419.1999.00025.x

- Clarke, K., Gorley, R., 2015. User Manual/Tutorial. PRIMER-E, Plymouth.
- Costa, M.J., Bruxelas, A., 1989. The structure of fish communities in the Tagus estuary, Portugal, and its role as a nursery for commercial fish species. *Sci. Mar.* 53, 2–3.
- Costa, M.J., Cabral, H.N., 1999. Changes in the Tagus nursery function for commercial fish species: Some perspectives for management. *Aquat. Ecol.* 33, 287–292. doi:10.1023/A:1009904621771
- Costa, M.J., Cabral, H.N., Drake, P., Economou, A.N., Fernandez-Delgado, C., Gordo, L., Marchand, J., Thiel, R., 2002. Recruitment and Production of Commercial Species in Estuaries, in: *Fishes in Estuaries*. Blackwell Science Ltd, Oxford, UK, pp. 54–123. doi:10.1002/9780470995228.ch3
- Cowen, R.K., Sponaugle, S., 2009. Larval Dispersal and Marine Population Connectivity. *Ann. Rev. Mar. Sci.* 1, 443–466. doi:10.1146/annurev.marine.010908.163757
- Dando, P.R., Demir, N., 1985. On the spawning and nursery grounds of bass, *dicentrarchus labrax*, in the plymouth area. *J. Mar. Biol. Assoc. United Kingdom* 65, 159–168. doi:10.1017/S0025315400060872
- de Figueiredo, G.M., Nash, R.D.M., Montagnes, D.J.S., 2007. Do protozoa contribute significantly to the diet of larval fish in the Irish Sea? *J. Mar. Biol. Assoc. United Kingdom* 87, 843–850. doi:10.1017/s002531540705713x
- Di Franco, A., Gillanders, B.M., de Benedetto, G., Pennetta, A., de Leo, G.A., Guidetti, P., 2012. Dispersal patterns of coastal fish: Implications for designing networks of marine protected areas. *PLoS One* 7, e31681. doi:10.1371/journal.pone.0031681
- Dolbeth, M., Martinho, F., Leitão, R., Cabral, H., Pardal, M.A., 2007. Strategies of *Pomatoschistus minutus* and *Pomatoschistus microps* to cope with environmental instability. *Estuar. Coast. Shelf Sci.* 74, 263–273. doi:10.1016/J.ECSS.2007.04.016
- Dyer, K.R., 1997. *Estuaries: a physical introduction*, 2nd ed. John Wiley & Sons, Chichester.
- Elliott, M., Hemingway, K., 2002. *Fishes in estuaries*, 1st ed. Blackwell Science Ltd, Oxford, UK.
- Elliott, M., Whitfield, A.K., Potter, I.C., Blaber, S.J.M., Cyrus, D.P., Nordlie, F.G., Harrison, T.D., 2007. The guild approach to categorizing estuarine fish assemblages: A global review. *Fish Fish.* 8, 241–268. doi:10.1111/j.1467-2679.2007.00253.x
- EUMOFA, 2017. *The EU Fish Market 2017 Edition*. Brussels. doi:10.2771/455963
- Faria, A., Morais, P., Chícharo, M.A., 2006. Ichthyoplankton dynamics in the Guadiana estuary and adjacent coastal area, South-East Portugal. *Estuar. Coast. Shelf Sci.* 70, 85–97. doi:10.1016/j.ecss.2006.05.032
- Fisher, R., 2005. Swimming speeds of larval coral reef fishes: Impacts on self-recruitment and dispersal. *Mar. Ecol. Prog. Ser.* 285, 223–232. doi:10.3354/meps285223

- Fonds, M., Van Buurt, G., 1974. The influence of temperature and salinity on development and survival of goby eggs (Pisces, Gobiidae). *Hydrobiol. Bull.* 8, 110–116. doi:10.1007/BF02254911
- Forward, R.B., Tankersley, R.A., 2001. Selective tidal-stream transport of marine animals. *Oceanogr. Mar. Biol. an Annu. Rev.* 39, 305–353.
- Franca, S., Vinagre, C., Costa, M.J., Cabral, H.N., 2004. Use of the coastal areas adjacent to the Douro estuary as a nursery area for pouting, *Trisopterus luscus* Linnaeus, 1758. *J. Appl. Ichthyol.* 20, 99–104. doi:10.1046/j.1439-0426.2003.00525.x
- Frank, K.T., Leggett, W.C., 1985. Reciprocal Oscillations in Densities of Larval Fish and Potential Predators: A Reflection of Present or Past Predation? *Can. J. Fish. Aquat. Sci.* 42, 1841–1849. doi:10.1139/f85-232
- Garrido, S., Santos, A.M.P., dos Santos, A., Ré, P., 2009. Spatial distribution and vertical migrations of fish larvae communities off Northwestern Iberia sampled with LHPR and Bongo nets. *Estuar. Coast. Shelf Sci.* 84, 463–475. doi:10.1016/J.ECSS.2009.07.018
- Grimes, C., Finucane, J., 1991. Spatial distribution and abundance of larval and juvenile fish, chlorophyll and macrozooplankton around the Mississippi River discharge plume, and the role of. *Mar. Ecol. Prog. Ser. Oldend.* 75, 109–119.
- Grioche, A., Koubbi, P., Sautour, B., 1997. Ontogenic migration of *Pleuronectes flesus* larvae in the eastern English Channel. *J. Fish Biol.* 51, 385–396. doi:10.1111/j.1095-8649.1997.tb06110.x
- Grothues, T.M., Cowen, R.K., 1999. Larval fish assemblages and water mass history in a major faunal transition zone. *Cont. Shelf Res.* 19, 1171–1198. doi:10.1016/S0278-4343(99)00010-2
- Hagan, S.M., Able, K.W., 2003. Seasonal changes of the pelagic fish assemblage in a temperate estuary. *Estuar. Coast. Shelf Sci.* 56, 15–29. doi:10.1016/S0272-7714(02)00116-6
- Hamerlynck, O., Hostens, K., 1993. Growth, feeding, production, and consumption in 0-group bib (*Trisopterus luscus* L.) and whiting (*Merlangius merlangus* L.) in a shallow coastal area of the south-west Netherlands. *ICES J. Mar. Sci.* 50, 81–91. doi:10.1006/jmsc.1993.1009
- Hare, J.A., Thorrold, S., Walsh, H., Reiss, C., Valle-Levinson, A., Jones, C., 2005. Biophysical mechanisms of larval fish ingress into Chesapeake Bay. *Mar. Ecol. Prog. Ser.* 303, 295–310. doi:10.3354/meps303295
- Hays, G.C., Richardson, A.J., Robinson, C., 2005. Climate change and marine plankton. *Trends Ecol. Evol.* 20, 337–344. doi:10.1016/j.tree.2005.03.004
- Heath, M.R., 1992. Field Investigations of the Early Life Stages of Marine Fish. *Adv. Mar. Biol.* 28, 1–174. doi:10.1016/S0065-2881(08)60039-5

- Helfman, G.S., Collette, B.B., Facey, D.E., Bowen, B.W., 2009. Early life history, in: *The Diversity of Fishes: Biology, Evolution, and Ecology*. Blackwell, pp. 129–147.
- Houde, E.D., 2008. Emerging from Hjort’s shadow. *J. Northwest Atl. Fish. Sci.* 41, 53–70. doi:10.2960/J.v41.m634
- Houde, E.D., 1987. Fish Early Life Dynamics and Recruitment Variability. *Am. Fish. Soc. Symposium* 2, 17–29.
- ICES, 2018. Report of the Working Group on Southern Horse Mackerel, Anchovy and Sardine (WGHANSA). Lisbon, Portugal.
- INE, 2019. Estatísticas da Pesca 2018. Lisbon, Portugal.
- INE, 2018. Estatísticas da Pesca 2017. Lisbon, Portugal.
- INE, 2017. Estatísticas da Pesca 2016. Lisbon, Portugal.
- Jennings, S., Jennings, S., Pawson, M.G., 1992. The origin and recruitment of bass, *dicentrarchus labrax*, larvae to nursery areas. *J. Mar. Biol. Assoc. United Kingdom* 72, 199–212. doi:10.1017/S0025315400048888
- Jennings, S., Kaiser, M.J., Reynolds, J.D., 2001. Fished species, life histories and distribution, in: *Marine Fisheries Ecology*. Blackwell Science, p. 417.
- Jonh, H.-C., Ré, P., 1995. Cross-shelf zonation, vertical distribution and drift of fish larvae off Northern Portugal during weak upwelling. *Arq. Mus. Bocage* 2, 607–632.
- Jonh, H.-C., Ré, P., Zuelicke, C., 1996. Sardine larvae in a spring-upwelling event off northern Portugal. *Ecol. Syst.* 16, 1021–1036.
- Klein, M., Beveren, E. Van, Rodrigues, D., Serrão, E.A., Caselle, J.E., Gonçalves, E.J., Borges, R., 2018. Small scale temporal patterns of recruitment and hatching of Atlantic horse mackerel (L.) at a nearshore reef area. *Fish. Oceanogr.* 27, 505–516. doi:10.1111/fog.12269
- Le Pape, O., Gilliers, C., Riou, P., Morin, J., Amara, R., Désaunay, Y., 2007. Convergent signs of degradation in both the capacity and the quality of an essential fish habitat: State of the Seine estuary (France) flatfish nurseries. *Hydrobiologia* 588, 225–229. doi:10.1007/s10750-007-0665-y
- Leitão, R., Martinho, F., Cabral, H.N., Neto, J.M., Jorge, I., Pardal, M.A., 2007. The fish assemblage of the Mondego estuary: composition, structure and trends over the past two decades. *Hydrobiologia* 587, 269–279. doi:10.1007/s10750-007-0688-4
- Leitão, R., Martinho, F., Neto, J.M., Cabral, H., Marques, J.C., Pardal, M.A., 2006. Feeding ecology, population structure and distribution of *Pomatoschistus microps* (Krøyer, 1838) and *Pomatoschistus minutus* (Pallas, 1770) in a temperate estuary, Portugal. *Estuar. Coast. Shelf Sci.* 66, 231–239. doi:10.1016/J.ECSS.2005.08.012
- Lenanton, R., Robertson, A., Hansen, J., 1982. Nearshore Accumulations of Detached Macrophytes as Nursery Areas for Fish. *Mar. Ecol. Prog. Ser.* 9, 51–57. doi:10.3354/meps009051

- Marques, S.C., Azeiteiro, U.M., Leandro, S.M., Queiroga, H., Primo, A.L., Martinho, F., Viegas, I., Pardal, M.Â., 2008. Predicting zooplankton response to environmental changes in a temperate estuarine ecosystem. *Mar. Biol.* 155, 531–541. doi:10.1007/s00227-008-1052-6
- Martinho, F., Cabral, H.N., Pardal, M.A., 2013. Juvenile nursery colonization patterns for the European flounder (*Platichthys flesus*): A latitudinal approach. *J. Sea Res.* 84, 61–69. doi:10.1016/J.SEARES.2013.07.014
- Martinho, F., Dolbeth, M., Viegas, I., Baptista, J., Cabral, H.N., Pardal, M.A., 2010. Does the flatfish community of the Mondego estuary (Portugal) reflect environmental changes? *J. Appl. Ichthyol.* 26, 843–852. doi:10.1111/j.1439-0426.2010.01486.x
- Martinho, F., Dolbeth, M., Viegas, I., Teixeira, C.M., Cabral, H.N., Pardal, M.A., 2009. Environmental effects on the recruitment variability of nursery species. *Estuar. Coast. Shelf Sci.* 83, 460–468. doi:10.1016/j.ecss.2009.04.024
- Martinho, F., Leitão, R., Neto, J.M., Cabral, H.N., Marques, J.C., Pardal, M.A., 2007. The use of nursery areas by juvenile fish in a temperate estuary, Portugal. *Hydrobiologia* 587, 281–290. doi:10.1007/s10750-007-0689-3
- McLachlan, A., Brown, A.C., 2006. *The Ecology of Sandy Shores*, 2nd ed, *The Ecology of Sandy Shores*. Academic Press, New York. doi:10.1016/B978-0-12-372569-1.X5000-9
- McLusky, D.D., Elliott, M., 2004. *The estuarine ecosystem: ecology, threats, and management*, 3rd ed. Oxford University Press, Oxford; New York. doi:10.1093/ISBN
- Miller, J.M., 1988. Physical processes and the mechanisms of coastal migrations of immature marine fishes, in: Weinstein, M.P. (Ed.), *Larval Fish and Shellish Transport Through Inlets*. American Fisheries Society, Bethesda, Maryland, pp. 68–76.
- Moser, H.G., Watson, W., 2006. Ichthyoplankton, in: Allen, L.G., Pondella, D.J., Horn, M.H. (Eds.), *The Ecology of Marine Fishes: California and Adjacent Waters*. University of California Press, pp. 269–319.
- Neilson, J.D., Perry, R.I., 1990. Diel Vertical Migrations of Marine Fishes: an Obligate or Facultative Process? *Adv. Mar. Biol.* 26, 115–168. doi:10.1016/S0065-2881(08)60200-X
- Norcross, B.L., Shaw, R.F., 1984. Oceanic and Estuarine Transport of Fish Eggs and Larvae: A Review. *Trans. Am. Fish. Soc.* 113, 153–165. doi:10.1577/1548-8659(1984)113<153:OAETOF>2.0.CO;2
- Nyitrai, D., Martinho, F., Dolbeth, M., Baptista, J., Pardal, M.A., 2012. Trends in estuarine fish assemblages facing different environmental conditions: Combining diversity with functional attributes. *Aquat. Ecol.* 46, 201–214. doi:10.1007/s10452-012-9392-1
- Pampoulie, C., 2001. Demographic structure and life history traits of the common goby *Pomatoschistus microps* (Teleostei, Gobiidae) in a Mediterranean coastal lagoon (Rhône River delta, France). *Acta Oecologica* 22, 253–257. doi:10.1016/S1146-609X(01)01127-4

- Pankhurst, N.W., Munday, P.L., 2011. Effects of climate change on fish reproduction and early life history stages. *Mar. Freshw. Res.* 62, 1015–1026. doi:10.1071/mf10269~
- Parsons, Ti.R., Maita, Y., Lalli, C.M., 1985. Determination of Chlorophylls and Total Carotenoids: Spectrophotometric Method, in: *A Manual of Chemical & Biological Methods for Seawater Analysis*. Pergamon Press, Oxford, pp. 101–104. doi:10.1016/B978-0-08-030287-4.50032-3
- Peliz, Á., 2003. Generation and unstable evolution of a density-driven Eastern Poleward Current: The Iberian Poleward Current. *J. Geophys. Res.* 108, 3268. doi:10.1029/2002jc001443
- Peliz, Á., Rosa, T.L., Santos, A.M.P., Pissarra, J.L., 2002. Fronts, jets, and counter-flows in the Western Iberian upwelling system. *J. Mar. Syst.* 35, 61–77. doi:10.1016/S0924-7963(02)00076-3
- Pepin, P., Dower, J.F., 2007. Variability in the trophic position of larval fish in a coastal pelagic ecosystem based on stable isotope analysis. *J. Plankton Res.* 29, 727–737. doi:10.1093/plankt/fbm052
- Pessanha, A.L.M., Araújo, F.G., 2003. Spatial, temporal and diel variations of fish assemblages at two sandy beaches in the Sepetiba Bay, Rio de Janeiro, Brazil. *Estuar. Coast. Shelf Sci.* 57, 817–828. doi:10.1016/S0272-7714(02)00411-0
- Pickett, G.D., Pawson, M.G., 1994. *Sea Bass. Biology, Exploitation and Conservation*, 1st ed. Chapman and Hall, London.
- Pombo, L., Elliot, M., Rebelo, J., 2005. Ecology, age and growth of *Atherina boyeri* and *Atherina presbyter* in the Ria de Aveiro, Portugal. *Cybium* 29, 47–55.
- Primo, A.L., Azeiteiro, U.M., Marques, S.C., Martinho, F., Baptista, J., Pardal, M.A., 2013. Colonization and nursery habitat use patterns of larval and juvenile flatfish species in a small temperate estuary. *J. Sea Res.* 76, 126–134. doi:10.1016/J.SEARES.2012.08.002
- Primo, A.L., Azeiteiro, U.M., Marques, S.C., Pardal, M.Â., 2011. Impact of climate variability on ichthyoplankton communities: An example of a small temperate estuary. *Estuar. Coast. Shelf Sci.* 91, 484–491. doi:10.1016/j.ecss.2010.11.009
- Rakocinski, C.F., Lyczkowski-Shultz, J., Richardson, S.L., 1996. Ichthyoplankton assemblage structure in Mississippi sound as revealed by canonical correspondence analysis. *Estuar. Coast. Shelf Sci.* 43, 237–257. doi:10.1006/ecss.1996.0067
- Ramos, S., Cowen, R.K., Paris, C., Ré, P., Bordalo, A.A., 2006a. Environmental forcing and larval fish assemblage dynamics in the Lima River estuary (northwest Portugal). *J. Plankton Res.* 28, 275–286. doi:10.1093/plankt/fbi104
- Ramos, S., Cowen, R.K., Ré, P., Bordalo, A.A., 2006b. Temporal and spatial distributions of larval fish assemblages in the Lima estuary (Portugal). *Estuar. Coast. Shelf Sci.* 66, 303–314. doi:10.1016/j.ecss.2005.09.012

- Ramos, S., Paris, C.B., Angélico, M.M., 2017. Larval fish dispersal along an estuarine–ocean gradient. *Can. J. Fish. Aquat. Sci.* 74, 1462–1473. doi:10.1139/cjfas-2016-0325
- Ramos, S., Ré, P., Bordalo, A.A., 2010. Recruitment of flatfish species to an estuarine nursery habitat (Lima estuary, NW Iberian Peninsula). *J. Sea Res.* 64, 473–486. doi:10.1016/j.seares.2010.01.010
- Ramos, S., Ré, P., Bordalo, A.A., 2009. New insights into the early life ecology of *Sardina pilchardus* (Walbaum, 1792) in the northern Iberian Atlantic. *Sci. Mar.* 73, 449–459. doi:10.3989/scimar.2009.73n3449
- Ray, G.C., 2005. Connectivities of estuarine fishes to the coastal realm. *Estuar. Coast. Shelf Sci.* 64, 18–32. doi:10.1016/j.ecss.2005.02.003
- Ré, P., 1999. Ictioplâncton estuarino da Península Ibérica : guia de identificação dos ovos e estados larvares planctónicos. Câmara Municipal de Cascais.
- Ribeiro, A.C., Peliz, Á., Santos, A.M.P., 2005. A study of the response of chlorophyll-a biomass to a winter upwelling event off Western Iberia using SeaWiFS and in situ data. *J. Mar. Syst.* 53, 87–107. doi:10.1016/j.jmarsys.2004.05.031
- Ribeiro, J., Monteiro, C.C., Monteiro, P., Bentes, L., Coelho, R., Gonçalves, J.M.S., Lino, P.G., Erzini, K., 2008. Long-term changes in fish communities of the Ria Formosa coastal lagoon (southern Portugal) based on two studies made 20 years apart. *Estuar. Coast. Shelf Sci.* 76, 57–68. doi:10.1016/j.ecss.2007.06.001
- Ribeiro, R., Reis, J., Santos, C., Gonçalves, F., Soares, A.M.V.M., 1996. Spawning of anchovy *engraulis encrasicolus* in the Mondego Estuary, Portugal. *Estuar. Coast. Shelf Sci.* 42, 467–482. doi:10.1006/ecss.1996.0030
- Robertson, A.I., Lenanton, R.C.J., 1984. Fish community structure and food chain dynamics in the surf-zone of sandy beaches: The role of detached macrophyte detritus. *J. Exp. Mar. Bio. Ecol.* 84, 265–283. doi:10.1016/0022-0981(84)90185-0
- Rodriguez, J.M., 2008. Temporal and cross-shelf distribution of ichthyoplankton in the central Cantabrian Sea. *Estuar. Coast. Shelf Sci.* 79, 496–506. doi:10.1016/j.ecss.2008.05.010
- Rooper, C.N., Gunderson, D.R., Hickey, B.M., 2006. An examination of the feasibility of passive transport from coastal spawning grounds to estuarine nursery areas for English sole. *Estuar. Coast. Shelf Sci.* 68, 609–618. doi:10.1016/j.ecss.2006.03.009
- Roy, C., Cury, P., Fontana, A., Belvèze, H., 1989. Stratégies spatio-temporelles de la reproduction des clupéidés des zones d’upwelling d’Afrique de l’Ouest. *Aquat. Living Resour.* 2, 21–29. doi:10.1051/alr:1989003
- Sabatés, A., Olivar, M.P., Salat, J., Palomera, I., Alemany, F., 2007. Physical and biological processes controlling the distribution of fish larvae in the NW Mediterranean. *Prog. Oceanogr.* 74, 355–376. doi:10.1016/j.pocean.2007.04.017

- Sabatés, A., Salat, J., Olivar, M.P., 2001. Advection of continental water as an export mechanism for anchovy, *Engraulis encrasicolus*, larvae. *Sci. Mar.* 65, 77–87. doi:10.3989/scimar.2001.65s177
- Santos, A.M.P., Borges, M.D.F., Groom, S., 2001. Sardine and horse mackerel recruitment and upwelling off Portugal. *ICES J. Mar. Sci.* 58, 589–596. doi:10.1006/jmsc.2001.1060
- Santos, A.M.P., Peliz, A., Dubert, J., Oliveira, P.B., Angélico, M.M., Ré, P., 2004. Impact of a winter upwelling event on the distribution and transport of sardine (*Sardina pilchardus*) eggs and larvae off western Iberia: A retention mechanism. *Cont. Shelf Res.* 24, 149–165. doi:10.1016/j.csr.2003.10.004
- Santos, A.M.P., Ré, P., dos Santos, A., Peliz, Á., 2006. Vertical distribution of the European sardine (*Sardina pilchardus*) larvae and its implications for their survival. *J. Plankton Res.* 28, 523–532. doi:10.1093/plankt/fbi137
- Santos, M.B., Gonzalez-Quirs, R., Riveiro, I., Cabanas, J.M., Porteiro, C., Pierce, G.J., 2012. Cycles, trends, and residual variation in the Iberian sardine (*Sardina pilchardus*) recruitment series and their relationship with the environment. *ICES J. Mar. Sci.* 69, 739–750. doi:10.1093/icesjms/fsr186
- Schneider, G., Behrends, G., 1994. Population dynamics and the trophic role of *Aurelia aurita* medusae in the Kiel Bight and western Baltic. *ICES J. Mar. Sci.* 51, 359–367. doi:10.1006/jmsc.1994.1038
- Sinclair, M., 1988. Marine populations, in: *An Essay on Population Regulation and Speciation*. University of Washington Press, Seattle, p. 252.
- Strydom, N.A., Whitfield, A.K., Wooldridge, T.H., 2003. The role of estuarine type in characterizing early stage fish assemblages in warm temperate estuaries, South Africa. *African Zool.* 38, 29–43. doi:10.1080/15627020.2003.11657192
- Suthers, I.M., Rissik, D., 2009. *Plankton: A Guide to Their Ecology and Monitoring for Water Quality*. Csiro Publishing, Melbourne, Australia.
- Taveira Pinto, F., 2004. The Practice of Coastal Zone Management in Portugal. *J. Coast. Conserv.* 10, 147–157. doi:10.2307/25098447
- Teodósio, M.A., Paris, C.B., Wolanski, E., Morais, P., 2016. Biophysical processes leading to the ingress of temperate fish larvae into estuarine nursery areas: A review. *Estuar. Coast. Shelf Sci.* 183, 187–202. doi:10.1016/j.ecss.2016.10.022
- Van Der Veer, H.W., Berghahn, R., Miller, J.M., Rijnsdorp, A.D., 2000. Recruitment in flatfish, with special emphasis on North Atlantic species: Progress made by the Flatfish Symposia. *ICES J. Mar. Sci.* 57, 202–215. doi:10.1006/jmsc.1999.0523

- Vasconcelos, R.P., Reis-Santos, P., Cabral, H.N., Costa, J.L., Costa, M.J., 2011. River-Coast Connectivity, Estuarine Nursery Function and Coastal Fisheries. *Treatise Estuar. Coast. Sci.* 81–107. doi:10.1016/B978-0-12-374711-2.01005-6
- Vasconcelos, R.P., Reis-Santos, P., Fonseca, V., Maia, A., Ruano, M., França, S., Vinagre, C., Costa, M.J., Cabral, H., 2007. Assessing anthropogenic pressures on estuarine fish nurseries along the Portuguese coast: A multi-metric index and conceptual approach. *Sci. Total Environ.* 374, 199–215. doi:10.1016/j.scitotenv.2006.12.048
- Vasconcelos, R.P., Reis-Santos, P., Tanner, S., Maia, A., Latkoczy, C., Günther, D., Costa, M.J., Cabral, H., 2008. Evidence of estuarine nursery origin of five coastal fish species along the Portuguese coast through otolith elemental fingerprints. *Estuar. Coast. Shelf Sci.* 79, 317–327. doi:10.1016/j.ecss.2008.04.006
- Vernberg, W.B., 1983. Responses to estuarine stress, in: *Estuaries and Enclosed Seas*. Elsevier, Amsterdam, pp. 43–60.
- Vinagre, C., Ferreira, T., Matos, L., Costa, M.J., Cabral, H.N., 2009a. Latitudinal gradients in growth and spawning of sea bass, *Dicentrarchus labrax*, and their relationship with temperature and photoperiod. *Estuar. Coast. Shelf Sci.* 81, 375–380. doi:10.1016/j.ecss.2008.11.015
- Vinagre, C., Maia, A., Reis-Santos, P., Costa, M.J., Cabral, H.N., 2009b. Small-scale distribution of *Solea solea* and *Solea senegalensis* juveniles in the Tagus estuary (Portugal). *Estuar. Coast. Shelf Sci.* 85, 479–486. doi:10.1016/j.ecss.2008.11.008
- Watt-Pringle, P., Strydom, N.A., 2003. Habitat use by larval fishes in a temperate South African surf zone. *Estuar. Coast. Shelf Sci.* 58, 765–774. doi:10.1016/S0272-7714(03)00183-5
- Wooster, W.S., Bakun, A., McLain, D.R., 1976. The seasonal upwelling cycle along eastern boundary of north-Atlantic. *J. Mar. Res.* 34, 131–141.
- Zwolinski, J., Stratoudakis, Y., Soares, E., 2001. Intra-annual variation in the batch fecundity of sardine off Portugal. *J. Fish Biol.* 58, 1633–1645. doi:10.1006/jfbi.2001.1573

Chapter imagens are photos taken to the fish larvae collected throughout this study.