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Effect of natural extreme events and human induced changes on the fish assemblage of a temperate estuarine system

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Supervision of Professor Doutor Miguel Ângelo Pardal, presented to the
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Abstract

The frequency and intensity of extreme natural events (e.g. droughts, floods and heat weaves) due to global climatic changes coupled with human induced changes (e.g. nutrient enrichment or regularisation of channels) prompted many studies, especially in terrestrial and open marine ecosystems. However, relatively little is known about the effects of global climate change on transitional waters, like estuaries, which have rapidly become the focus of European-wide policy through several EU directives (e.g. Water Framework Directive).

Estuaries are among the most productive ecosystems in the world, with important ecological functions for fauna and flora. For fish, estuaries are very important habitats and for many species serve as nursery areas, given the high productivity of macrofauna and a wide array of suitable habitats that provide optimum growth conditions. However, estuaries are highly impacted by human pressures and extreme natural events that are likely to increase in frequency and intensity worldwide, which may have considerable influences on estuarine fish assemblages.

Accordingly, the objectives of the present thesis were to evaluate the effects of environmental variations in the Mondego estuary fish assemblage, taking into account taxonomic and functional diversity; to estimate the responses of two estuarine resident fish species (*Pomatoschistus microps* and *Pomatoschistus minutus*) to different environmental variables, including local and large-scale climate patterns; and to evaluate the changes in community-based measures of ecological quality in the Mondego estuary over the study period, and to infer the interrelationship between them and the trends in anthropogenic pressures.

In general, species number, diversity, and evenness of the fish assemblage were higher in the dryer periods. In dry years, estuarine residents were the most abundant group, while in regular and rainy years, the marine estuarine-dependent species increased in abundance. Among the feeding guilds, the most abundant group was the invertebrate and fish feeders. Planktivorous and invertebrate feeders occurred in higher abundance in dry years, while the opposite was verified for omnivorous species. The mean trophic level of the fish assemblage increased during the drought period, constantly decreasing afterwards.

The two observed estuarine resident species appeared in high densities in the beginning of the study period, with subsequent occasional high annual density peaks, while their secondary production was lower in dry years. For *P. microps*, precipitation and NAO were the significant factors explaining interannual abundance variations, while for *P. minutus*, river runoff was the only significant predictor. Regarding ecological quality measures, single metric indices (species number, Shannon-Wiener H’, Pielou J’) were highly variable and neither concordant amongst themselves nor with the Estuarine Fish Assessment Index (EFAI). The EFAI was the only index significantly correlated with the Anthropogenic Pressure Index (API), indicating that higher ecological quality was associated with lower anthropogenic pressure. The obtained results reinforced that estuarine fish assemblages are suitable indicators of environmental changes and anthropogenic pressures.

Resumo

A frequência e intensidade de eventos naturais extremos (e.g. secas, cheias e ondas de calor) levou ao surgimento de muitos estudos, em especial os referentes a ecossistemas marinhos e terrestres, devido a alterações climáticas globais relacionadas com actividade antropogénica (e.g. aumento da disponibilidade de nutrientes ou regularização de cursos de água). No entanto, há ainda relativamente pouco conhecimento sobre os efeitos das alterações climáticas globais em sistemas de transição, como os estuários, que rapidamente se converteram num tema central de política à escala Europeia através de várias directivas da EU (e.g. Directiva-quadro da água).

Os estuários encontram-se entre os ecossistemas mais produtivos do globo terrestre, desempenhando funções ecológicas importantes para a fauna e flora. Para os peixes em geral, os estuários são importantes habitats, sendo inclusive zonas importantes de viveiro devido à elevada produtividade de macrofauna, além de que proporcionam uma vasta gama de habitats que permitem condições ideais de desenvolvimento. Contudo, os estuários são alvo de pressões humanas e eventos naturais extremos que tendem a aumentar em frequência e intensidade em todo o mundo, o que poderá afectar consideravelmente as comunidades de peixes estuarinos.

Os objectivos da presente tese centram-se na avaliação dos efeitos das alterações ambientais nas comunidades de peixes do estuário do Mondego, tendo em consideração a diversidade taxonómica e funcional; na estimativa de respostas de duas espécies estuarinas residentes (*Pomatoschistus microps* e *Pomatoschistus minutus*) em relação a diferentes variáveis ambientais, incluindo padrões climáticos locais e de grande escala; na avaliação de alterações de qualidade ecológica baseadas em medições nas comunidades no estuário do Mondego, inferindo assim a relação entre estas e as pressões antropogénicas.

No geral, o número de espécies, a diversidade e equitabilidade foram superiores nos anos mais secos. Durante este período, as espécies estuarinas residentes foram as mais abundantes, no entanto, em anos regulares e chuvosos, as comunidades marinhas estuarino-dependentes aumentaram em abundância. No que respeita às cadeias alimentares, os grupos mais abundantes foram os que se alimentam de invertebrados e de peixes. Os grupos que se alimentam de plâncton e de invertebrados, tiveram maiores abundâncias em anos secos, o oposto foi verificado para os grupos de espécies omnívoras. O nível trófico médio das comunidades de peixes aumentou durante os períodos de seca, demonstrando posteriormente um constante decréscimo.

As duas espécies estuarinas residentes observadas surgiram em densidades elevadas no início do período de estudo, com subsequentes picos ocasionais de densidades anuais elevados, enquanto que as suas produções secundárias foram mais baixas nos anos secos. Para *P. microps*, a precipitação e NAO foram os factores mais significativos que explicaram as variações interanuais de abundância, enquanto que para o *P. minutus*, o escoamento do rio foi o único indicador significativo. Em relação às medições de qualidade ecológica, os índices de métricas individuais (número de espécies, Shannon-Wiener H', Pielou J') foram muito variáveis entre eles e com o Estuarine Fish Assessment Index (EFAI). O EFAI foi o único índice significativamente correlacionado com o Anthropogenic Pressure Index (API), indicando que maior qualidade ecológica está relacionada com menor pressão antropogénica. Os resultados obtidos reforçam a utilidade das comunidades de peixes estuarinas como indicadores de alterações ambientais e pressões antropogénicas.

Table of Contents

CHAPTER

I

General Introduction

Estuarine ecosystems

Estuaries are highly productive ecosystems, characterised by relatively low species diversity but with high abundance of individual species (McLusky and Elliott, 2004; Dolbeth et al. 2007; Elliott and Whitfield, 2011). Their high productivity lies in the high primary production, acting also as detritus traps for the autochthonous and allochthonous material (Elliott and Whitfield, 2011). Moreover, these systems have an abundance of autochthonous producing fringing areas (e.g. reedbeds, seagrass meadows, mangroves and saltmarshes) and they also receive large amounts of organic material from riverine primary producers, the sea and also from anthropogenic waste (Abrantes and Sheaves, 2010; Howe and Simenstad, 2011). Moreover, wet climatic periods due to the North Atlantic Oscillation or El Niño/ La Niña can also deliver large amounts of nutrients into estuaries and the adjacent ocean, as well as flush accumulated nutrients and organic matter (Elliott and Whitfield, 2011). Therefore, estuaries provide rich supply of food resources for their inhabitants, either for direct or indirect consumption (McLusky and Elliott, 2004; Gamito et al. 2005).

The high food availability combined with the large diversity of habitats, providing shelter and protection, are the major attractions for invertebrate, fish and bird species (e.g. Cardoso et al. 2012; Martinho et al. 2012; Fonseca et al. 2013). Several species stay in the estuary during their complete life cycle, while many of them use it as nursery grounds, as migratory routes or as food supplier (McLusky and Elliott, 2004).

Estuaries can be characterized by the salinity gradient, from the brackish waters of the upper regions to the euhaline downstream areas. Indeed, salinity regimes and freshwater flow are the primary factors determining the distribution of organisms within estuarine waters (Leitão et al. 2007; Fonseca et al. 2011; Kantoussan et al. 2012). Furthermore, salinity and temperature can change considerably over tidal cycles, suggesting that estuarine organisms have a high resilience to change comparing with the situation in more stable aquatic environments (Elliott and Whitfield, 2011).

Estuarine ecosystems are highly threatened by global climatic changes and anthropogenic pressures as well. These include rising temperatures, altered precipitation patterns, changes in nutrient cycling, as well as habitat destruction by bank reclamation, reduction in water quality by domestic, agricultural and industrial effluent discharges and nutrient enrichment. Eutrophication, caused by nutrient enrichment is one of the major problems influencing the functioning of the estuarine ecosystems. In general, structural changes in local communities have been observed due to eutrophication, such as considerable reductions in species abundance and diversity (Cardoso et al. 2004, 2008; Schindler, 2006; Dolbeth et al. 2011).

Estuarine fish assemblages

Estuaries are essential habitats for many fish species, acting mainly as nursery areas, migration routes, feeding and shelter areas (McLusky and Elliott, 2004; Nicolas et al. 2010; Vasconcelos et al. 2012). Estuarine fish species present a wide diversity of biological cycles and ecological compartments, making them important indicators of changes in estuarine conditions at spatial and temporal scales as well (Whitfield and

Elliott, 2002). The life strategies of estuarine fishes related to their ecological use of estuarine habitats can reflect the functioning of estuaries (Elliott et al. 2007). The functional approach of estuarine fish assemblages has been increased in recent years, which method is based on functionality rather than taxonomic aspects (Elliott et al. 2007; Franco et al. 2008; Nicolas et al. 2010).

Functional attributes have been commonly used to study and describe estuarine fish assemblages (Claridge et al. 1986; Elliott et al. 2007; Martinho et al. 2007; Nyitrai et al. 2012). In this classification, the fish species that have similar characteristics in resource exploitation are assigned to the same functional group (Blondel, 2003). The functional approach reduces the complexity of fish assemblages and also provides the opportunity for the comparison of the functioning of transitional waters for fish along different geographical areas (Garrison and Link, 2000; Elliott et al. 2007). Elliott and Dewailly (1995) first introduced the concept of classifying fish species in functional guilds, and defined the typical European Atlantic seaboard estuarine fish assemblage, demonstrating that there were common patterns in estuarine usage by fishes. Afterwards, Elliott et al. (2007) reviewed the guild approach in categorizing estuarine fish species, in order to standardize their concepts and applications as follows: marine stragglers (MS), species that breed and spawn at sea and usually enter estuaries in low numbers, mainly stenohaline species; marine migrants (MM), species that breed and spawn at sea and often enter estuaries in large numbers, particularly as juveniles, some of them are highly euryhaline; this group can be divided in two categories: marine estuarine-opportunists (MMO), marine species that regularly enter estuaries in considerable numbers especially as juveniles but use nearshore marine waters as an alternative habitat, and marine estuarine-dependents (MMD), marine species that live along coasts but require sheltered estuarine habitats as juveniles therefore, these species depend on estuaries; estuarine residents (ER), estuarine species capable of completing their entire life cycle within the estuary; anadromous species (AN) that undergo their greatest growth at sea and which, prior to maturity, migrate into rivers where spawning subsequently occurs; catadromous species (CA) that spend all of their trophic life in freshwater and subsequently migrate to sea to spawn; freshwater stragglers (FS), freshwater species found in low numbers in estuaries and whose distribution is limited to areas of low salinity, upper reaches of estuaries. Besides the estuarine use functional group mentioned above, the feeding mode and reproductive mode functional groups were also defined by the previous authors.

In general, in most European estuaries the estuarine resident and the marine migrant species are the most abundant groups (e.g. Martinho et al. 2007; Nicolas et al. 2010; Vasconcelos et al. 2012). Several factors can determine the distribution of these species within estuarine waters, including substrate type, hydrological features and the presence of rooted vegetation, producing a complex mosaic of specific habitats (Pihl et al. 2002; França et al. 2009; Martinho et al. 2012). Most of the estuarine resident fishes, such as the common and sand goby have the plasticity and adaptability towards environmental changes that allows them to successfully occupy different biotopes, from brackish waters to euhaline areas (Leitão et al. 2006; Dolbeth et al. 2007, 2010). Regarding marine migrants, the marine estuarine-dependent species also have high tolerances to salinity variations, and can occur from euhaline areas to upper reaches of estuaries (Martinho et al. 2012; Vasconcelos et al. 2012). These species have complex life cycles, in which larvae are transported to estuaries, where they metamorphose and grow to subadult stages, and then move to adult habitats offshore (Beck et al. 2001). Marine migrant species are of high commercial value, as they are often important fisheries resources in the coastal environment, being the main focus of numerous studies worldwide (e.g. Fernández-Delgado et al. 2007; Taylor et al. 2010; Vasconcelos et al. 2012).

As a general trend, the species composition of estuarine fish assemblages in different biogeographic regions is relatively stable and the fish species have more or less predictable patterns of abundance and distribution (Whitfield, 1998). This can be attributed to the occurrence and distribution of specific ecological groups along environmental gradients such as salinity and temperature, the dominance of relatively few species, the physiological constraints imposed on estuarine fishes, the seasonal movements in and out of estuaries and the robust nature of food webs within estuaries (Kennish, 1990; Whitfield, 1998). In general, estuarine ichthyofauna is abundant but characterized by the dominance of a few species (Kennish, 1990), and the overall fish species richness in estuaries is lower than in the adjacent marine environment (Martino and Able, 2003). Moreover, a pattern of declining estuarine species diversity with increase in latitude has been observed (Blaber, 2000; Attrill, 2002). Previous studies described that individual temperate estuaries may have an average of 20-30 species (Potter et al. 1986; Pomfret et al. 1991; Elliott and Dewailly, 1995), warm temperate ones can have ~50-60 (Darnell, 1961; Lenanton and Hodgkin, 1985), and most subtropical and tropical estuarine areas have at least 100 species, with some reaching over 200 (Blaber, 2000). The composition of estuarine fish assemblages depends on many factors including estuary size, shape, depth and physical regimes such as salinity and turbidity, habitat-type availability, the nature and depth of adjacent marine waters, freshwater flow (e.g. Pasquaud et al. 2015), and the geographical location of the estuary in terms of latitude and also in relation to marine features such as ocean currents, canyons and reefs (Blaber, 2000).

Effects of global climatic changes on estuarine fish assemblages

Global climatic changes have considerable effects on estuarine fish assemblages, and their frequency and intensity have been increasing, and their occurrence is likely to increase worldwide (Mirza, 2003; Coumou and Rahmstorf, 2012; Rose and Allen, 2013). In estuarine areas, the main impacts of climate change are the variations in river flow, inducing flood or drought events. Furthermore, due to water circulation and oceanic volume changes, estuarine and coastal systems are predicted to experience a loss of marsh and intertidal habitat, a greater marine intrusion or freshwater plumes, and increased eutrophication, hypoxia, and anoxia as well (Kennedy, 1990; Ray et al. 1992; Schwartz, 1998). As many native organisms currently live near their tolerance limits, previous studies described that estuarine and coastal ecosystems will probably exhibit earlier responses to regional changes, including the loss of native species and increases in exotic species (Kennedy, 1990; Carlton, 1996).

Fish are intimately connected to their environment, and considerable changes in regional and global oceanic conditions will probably have direct and indirect effects on individuals, their populations, and communities as well (Roessig et al. 2004). Warmed marine and estuarine waters and consequent changes in dissolved gases, pH and salinity caused by global climatic changes are expected to influence many fish species and life stages (Roessig et al. 2004). Extremes in environmental factors, such as high water temperature, low dissolved oxygen or salinity, and pH, can have detrimental effects on fish species (Moyle and Cech, 2004). Suboptimal environmental conditions can decrease foraging, growth, and fecundity, alter metamorphosis, and influence endocrine homeostasis and migratory behaviour (Donaldson, 1990; Pörtner et al. 2001).

In Portugal, considerable variations in precipitation amounts were observed along the last twenty years, causing flood and drought events in consecutive years. Six of the last fifteen years have been classified among the driest and/or warmest since 1931 by the Portuguese Weather Institute (*http://www.ipma.pt*). Moreover, in 2005 a severe drought occurred, with significant reductions in precipitation and freshwater runoff, when compared to the long-term average values. The years of 2007 and 2008 were also registered as dry years with very low precipitation values specially in the autumns. Regarding flood events, in the winter of 2000/01 very high precipitation values were observed and it was considered the 3rd rainiest winter of the last 30 years, and also in the winter of 2009/10, when some of the highest precipitation levels were observed since 1970. Both events induced highly elevated freshwater runoff values, and consequent decrease in salinities. Moreover, in the autumn months in 2003 and 2006 precipitation values were also above the mean average.

The previous climatic events had considerable effects on the structure and composition of the Mondego estuary fish assemblage. As the climatic changes mentioned above are expected to increase in frequency over the next years, it is important to understand their effects on estuarine ecosystems including key species such as fish or decapods.

Study site – the Mondego estuary

The study was performed in the Mondego estuary, a small intertidal estuary located in the Atlantic coast of Portugal (40º08'N, 8º50'W), a warm temperate region with a continental temperate climate. The estuary has an area of 8.6km^2. It has two branches, north and south, with different hydrological features, separated by the Murraceira Island. The north branch is deeper (4-10m during high tide, tidal range 1-3m), and the freshwater input comes from the Mondego River, and it is also the main navigation channel and location of the Figueira da Foz harbour. The south branch is shallower (2-4m during high tide, tidal range 1-3m), characterized by an intertidal mudflat that occupies about 75% of the total area. Water circulation of the south branch is caused by tidal influence and the freshwater input from the Pranto River, a small tributary regulated by a dam according to the water needs of the surrounding rice fields. The upstream areas of the south branch exhibit *Scirpus maritimus* and the downstream areas exhibit *Spartina maritima* marshes and *Zostera noltii* beds (Lillebø et al. 2006).

There are several industries, saltworks, agricultural activities and aquacultures along the Mondego estuary. Moreover, the commercial harbour of Figueria da Foz city is the main industrial driver in this estuarine system. The increasing population has influenced the estuarine system, as about 60.000 habitants live in Figueira da Foz city (Pinto et al. 2010). The human activities coupled with favourable physical characteristics (high water residence time and shallowness) and climate conditions had imposed a high pressure on this system that culminated in an eutrophication process in the early 90's (Martins et al. 2001; Cardoso et al. 2004; Pardal et al. 2004; Leston et al. 2008; Dolbeth et al. 2011). The most important anthropogenic pressures are organic pollution, bank reclamation and shipping activities.

Mitigation measures were implemented in the estuary in 1998, in order to reduce the effects of eutrophication. These measures included the re-establishment of the south arm riverhead connection, to improve freshwater circulation, reduction of nutrient loading, essentially ammonium (Lillebø et al. 2005; Cardoso et al. 2010), protection of seagrass bed from human disturbance, and also public education about the ecological importance of intertidal vegetation for the health and related socioeconomic activities of the estuary. Since then, the system has gradually recovered from the effects of eutrophication, and showed improved water transparency and decreased nutrient loading (Lillebø et al. 2005; Cardoso et al. 2010).

In the Mondego estuary, a more than 20-year database on macrobenthic communities and a more than 10-year database on fish abundance and planktonic dynamics are available, providing highly valuable information to evaluate the effects of climatic changes and anthropogenic pressures on the estuarine ecosystem.

General Objectives

The primary objective of the present thesis was to evaluate the variations in the Mondego estuary fish assemblage from June 2003 to March 2010, taking into account taxonomic and functional diversity. Taxonomic attributes were used to evaluate the variations of the dominant species of the fish assemblage and to estimate the alterations among the less abundant species, while ecological and feeding attributes were used to evaluate functional changes in the estuary. A subsequent objective was to determine whether the changes in the structure and composition of the fish assemblage matched to the environmental variations that occurred along the study period.

The second objective was to evaluate the effects of climatic variations on two estuarine resident fish species, and to highlight the important role of estuarine resident fishes as indicators of environmental changes. More specifically, the main objectives were (1) to evaluate the abundance, growth and production patterns of *Pomatoschistus microps* and *Pomatoschistus minutus* over a nine year period (June 2003 to June 2011); (2) to estimate the responses of the two species to different environmental variables, including local and large-scale climate patterns; (3) to assess the distinct response patterns to the environmental variables by the different life stages of each species.

The third main objective was to evaluate the changes in community-based measures of ecological quality in the Mondego estuary over an eight-year period, and to infer the interrelationship between them and the trends in anthropogenic pressures in the same time frame. This objective was achieved by evaluating (1) changes in anthropogenic pressure over time; (2) changes in several fish-based diversity measures; and by (3) establishing relationships between diversity measures and anthropogenic pressures over time, based on the principle that higher stressed environments will result in lower diversity and ecological quality status.

CHAPTER

II

Trends in estuarine fish assemblages facing different environmental conditions: combining diversity with functional attributes

Abstract

Changes in the Mondego estuary (Portugal) fish assemblage were documented with a long-term monitoring program between June 2003 and March 2010, during which several extreme environmental events occurred, including severe droughts and heavy precipitation. The structure and composition of the fish assemblage was analyzed based on a set of indicators: dominance, diversity, evenness, and composition in functional groups (ecological and feeding guilds). Higher species number, diversity, and evenness were observed in the dry periods. Variations in the relative abundance of the ecological guilds were also observed: in dry years, estuarine residents were the most abundant group, while in typical and rainy years, the marine estuarine-dependent species increased in abundance. Among the feeding guilds, the most abundant group was the invertebrate and fish feeders. Planktivorous and invertebrate feeders occurred in higher abundance in dry years, while the opposite was verified for omnivorous species. The mean trophic level of the fish assemblage increased during the drought period, constantly decreasing afterward, which could be attributed to an increase in predators in dry years. The cumulative responses of estuarine fish assemblages to ongoing climate changes and discrete extreme weather events confirm their importance as indicators of environmental changes.

Keywords

Long-term approach; Mondego estuary; Fish assemblage; Extreme weather events
Indicators; Global climate change

Introduction

Estuaries play an important role in the life cycle of many fish, including marine, migratory, and estuarine species (Franco et al. 2008). These habitats provide sheltered areas and higher food availability, being used by several species as nursery grounds (Cabral et al. 2007; Martinho et al. 2007a). Moreover, many fish species spend their complete life cycle within the estuary (Elliott et al. 2007; Dolbeth et al. 2007, 2010). Estuarine fish assemblages are usually characterized by a relatively low diversity, but high abundances of individual taxa are present (Whitfield, 1999).

Estuarine fish assemblages are generally good indicators of environmental changes at both spatial and temporal scales (Whitfield and Elliott, 2002; Noble et al. 2007; Ecoutin et al. 2010), due to their wide diversity of biological cycles and ecological use of estuarine habitats, reflecting the functioning of estuaries (Elliott et al. 2007). In addition, most fish species are good indicators of long-term effects and large–scale habitat conditions (Moore et al. 1997). In this sense, several fish species are known to respond to changes in hydrologic regimes which, among other factors, are related with water quality (Attrill and Power, 2000) and with the extension of river plumes to adjacent coastal areas (Vinagre et al. 2008; Martinho et al. 2008). At a larger scale, variations in global oceanographic and climatic patterns, such as North Atlantic Oscillation (NAO), have been determined to exert an influence in the composition of fish assemblages, as well as in the abundance and growth of juvenile marine fish during estuarine residency (Attrill and Power, 2002; Genner et al. 2004; Henderson, 2007).

Global climate change has significant effects on the structure and functioning of coastal marine ecosystems (Houghton, 2005; Harley et al. 2006). These effects can be derived from extreme weather events such as drought and floods, whose frequency and intensity are likely to increase worldwide (Gleick, 2003; Mirza, 2003). As a result, changes in freshwater flow highly influence the biological processes and ecosystem development of estuaries (e.g. Attrill et al. 1999; Kimmerer, 2002; Flemer and Champ, 2006). In the Mondego estuary, Portugal, considerable changes occurred in precipitation and freshwater runoff over the past years, influencing the salinity ranges inside the estuary (Marques et al. 2007; Martinho et al. 2007b), due to variations between drought and short-term intense rainy periods.

In view of the climate events that occurred in recent years, the aim of the present study was to evaluate the variations in the Mondego estuary fish assemblage from June 2003 to March 2010, taking into account taxonomic and functional diversity. Taxonomic attributes were used to evaluate the variations of the dominant species of the fish assemblage and to estimate the alterations among the less abundant species, while ecological and feeding attributes were used to evaluate functional changes in the estuary. A subsequent aim was to determine whether the changes in the structure and composition of the fish assemblage matched to the environmental variations that occurred along the study period.

Materials and methods

Study site

The Mondego estuary is a small intertidal estuary with an area of 8.6 km^2, located on the western coast of Portugal (40° 08'N, 8° 50'W) (Fig. 1).

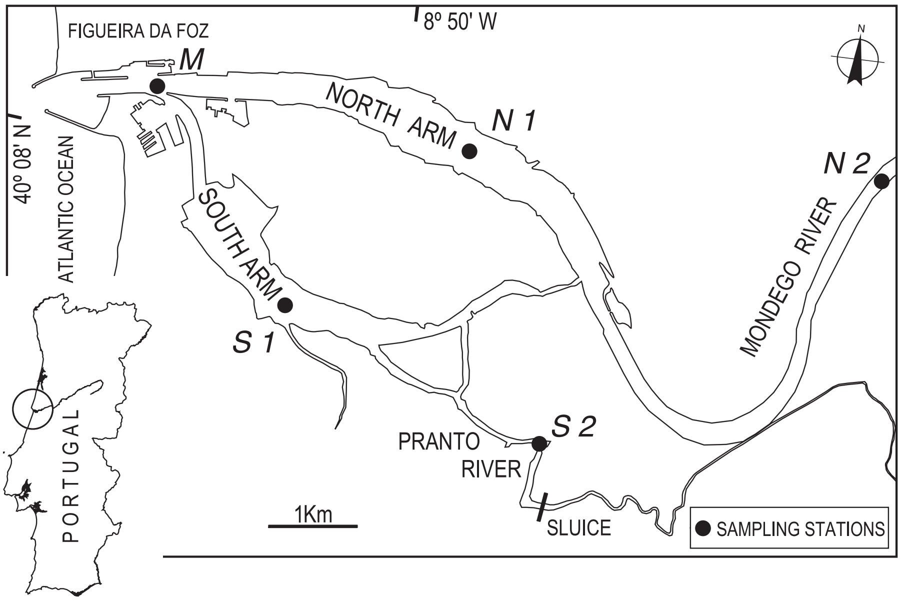

Figure 1 The Mondego estuary, with the location of the five sampling stations.

In the terminal part, the estuary is divided in two arms (north and south) at about 7 km from the coast joining again near the mouth. The north and south arm have quite different hydrologic characteristics: the north arm is deeper, with 5–10 m depth at high tide, with a tidal range of 2–3 m, and constitutes the main navigation channel and the location of the Figueira da Foz harbor. In this area, constant dredging and shipping occurs, causing physical disturbance of the bottom. The south arm is shallower, with 2–4 m during high tide with a tidal range of 1–3 m and about 75% of the total area consists of large intertidal mudflats. Freshwater flows mainly through the north arm, as the south arm is almost silted up in the upstream areas. The water circulation in the south arm is mainly dependent on the tides and on a small freshwater input from the Pranto River, which is a small tributary, regulated by a sluice according to the water needs in the surrounding rice fields. The connection between the two arms was enlarged in early 2006, enabling a higher water circulation through the south arm.

Sampling procedures

Sampling was carried out monthly from June 2003 until January 2007, and then bimonthly until March 2010 (except in July, September, October, and December 2004 and in October and November 2008, due to technical constraints or bad weather). Fishing took place during the night at five sampling stations (M, N1, N2, S1, and S2) (Fig. 1), at high water of spring tides, using a 2-m beam trawl with one tickler chain and 5-mm mesh size in the cod end. At each sampling station, three hauls at the speed of two knots were towed for an average of 3 min each, covering at least an area of 500 m^2. Samples were preserved in iceboxes and transported to the lab, where fish were sorted. All fish were identified, counted, measured (total length to nearest 1 mm), and weighted (wet weight, 0.01 g precision). Bottom water temperature and salinity were measured during the fishing campaigns.

Acquisition of hydrologic data

Hydrologic data were obtained from the Portuguese Water Institute (INAG 2010; http://snirh.inag.pt) and the Portuguese Weather Institute (IM, http://www.meteo.pt). Monthly precipitation (from June 2003 to March 2010) was obtained from the Soure 13F/01G station (INAG); the long-term average precipitation (1971–2000) for the city of Coimbra, located 40 km upstream the estuary (IM), was obtained in http://www.meteo.pt, and freshwater runoff was acquired from INAG station Açude Ponte Coimbra 12G/01A, near the city of Coimbra. Sea surface temperature (SST) data for the 1° Lat x 1° Long square that is located nearest to the Mondego estuary were obtained from the International Comprehensive Ocean–Atmosphere Data Set (ICOADS) online database (http://dss.ucar.edu/pub/coads, Slutz et al. 1985; 07.04.2011).

Data analysis

Monthly density data (individuals per 1,000 m^2) were calculated by averaging the total number of individuals of each species in relation to the surveyed area. Seasonal abundance was estimated by averaging monthly data as follows: winter (December, January, and February), spring (March, April, and May), summer (June, July, and August), and autumn (September, October, and November). The annual abundance data were also estimated by averaging the monthly data from each year (from January to December). Monthly, seasonal and annual biomass data were calculated by the same way. The number of dominant species that comprised 90% of the fish assemblage in terms of abundance was determined for each year, in order to evaluate variations in dominance throughout the study period.

The diversity of the fish assemblage was characterized using the total species number, Shannon-Wiener index, and Pielou index. Differences in assemblage seasonal abundance data per year were performed with analysis of similarities (ANOSIM) on a Bray-Curtis similarity matrix, after square root transformation of the raw data, to scale down the scores of the very abundant species (Clarke and Warwick, 2001). Afterward, ranked species abundance plots were computed for the seasonal abundance data (k-dominance plot), to clarify the interannual differences per season. These analyses were performed in PRIMER software package (version 5.0) (Clarke and Warwick, 2001).

The functional composition of the fish assemblage was analyzed using five ecological guilds, established from habitat use patterns, and four feeding guilds, according to Elliott et al. (2007). The five ecological guilds were (1) marine stragglers (MS), species that breed and spawn at sea and usually enter estuaries in low numbers; (2) marine migrants (MM), species that breed and spawn at sea and often enter estuaries in large numbers, particularly as juveniles; this group can be divided in two categories: marine estuarine-opportunists (MMO), marine species that regularly enter estuaries in considerable numbers especially as juveniles but use nearshore marine waters as an alternative habitat, and marine estuarine-dependents (MMD), marine species that live along coasts but require sheltered estuarine habitats as juveniles therefore, these species depend on estuaries; (3) estuarine residents (ER), estuarine species capable of completing their entire life cycle within the estuary; (4) catadromous species (CA) that spend all of their trophic life in freshwater and subsequently migrate to sea to spawn; (5) freshwater stragglers (FS), freshwater species found in low numbers in estuaries and whose distribution is limited to areas of low salinity, upper reaches of estuaries. Four feeding guilds were identified: (1) planktivorous (PS), species that feed predominantly on zooplankton (e.g., hydroids, planktonic crustaceans, fish eggs, and larvae) and phytoplankton; (2) invertebrate feeders (IS), species that feed predominantly on invertebrates associated with the substratum, including animals that live above the sediment (hyperbenthos), on the sediment (epifauna) or in the sediment (infauna); (3) feeding on invertebrates and fishes (IF), species that feed mainly on invertebrates and finfish; (4) omnivorous (OV) species, that feed mostly on filamentous algae, macrophytes, periphyton, epifauna, and infauna.

Mean trophic level (*TLm*) of the fish assemblage was calculated according to:
$$TL_m = \sum B_i * TL_i / \sum B_i$$

where B_i is the biomass per species and TL_i, the trophic level of the species obtained from Fishbase (www.fishbase.org; Froese and Pauly, 2007).

Results

Environmental characterization of the Mondego estuary

In general, 2003, 2006, and 2009 were considered as regular hydrologic years in terms of precipitation, while 2004, 2005, 2007, and 2008 were considered as dry years, when compared to climatic reference for 1971–2000 (Fig. 2a).

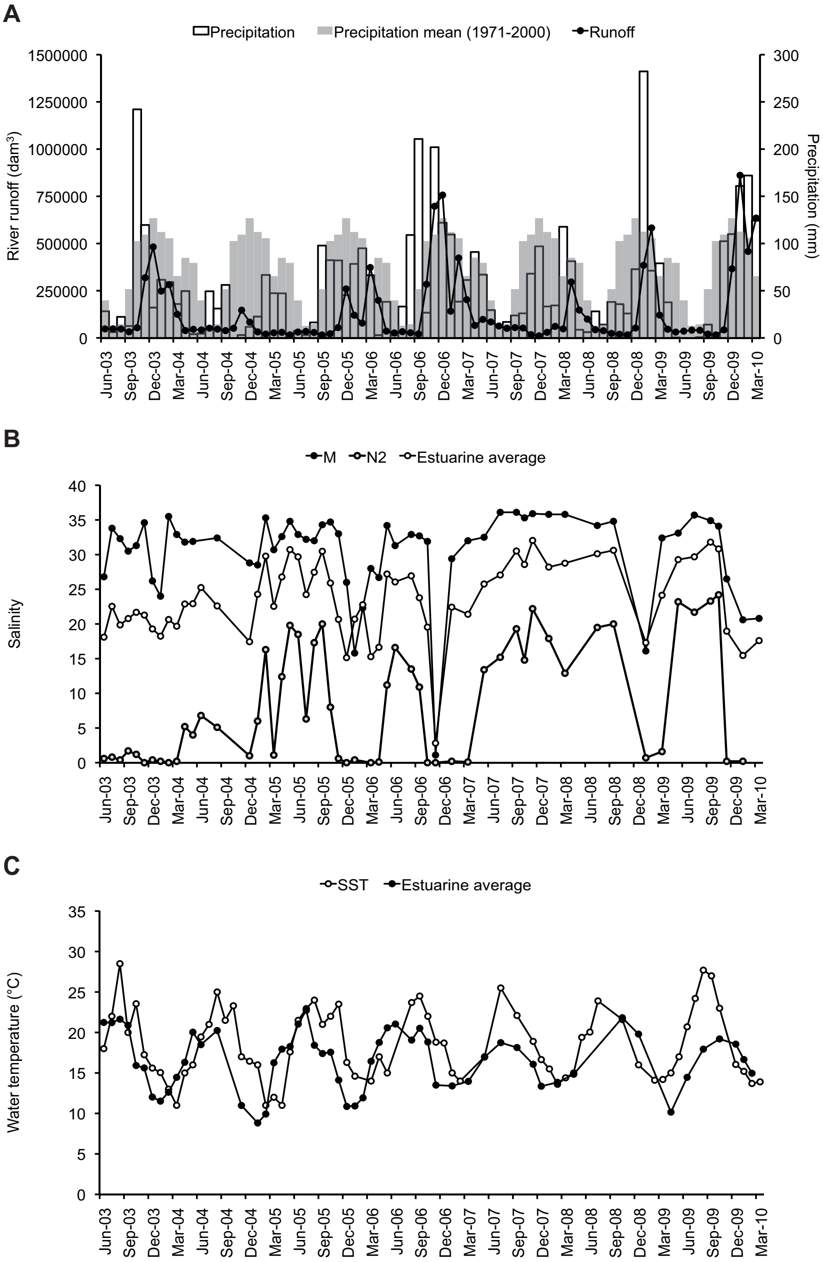

Figure 2 Monthly variation of (**a**) precipitation and river runoff (cubic decameter, dam^3) during the study period and average precipitation values during the period of 1971–2000 in the Mondego river basin; (**b**) salinity at stations M (farthest downstream station), N2 (farthest upstream station) and estuarine average salinity values; and (**c**) estuarine average temperature in the Mondego estuary and sea surface temperature (SST) in the adjacent coastal area.

Among the dry years, 2005 was considered as extreme drought, since precipitation values were far below the long-term average (classified as the worst drought since 1931 by the Portuguese Weather Institute—IM). In this year, precipitation values were the lowest during the study period, which induced lower freshwater runoff (Fig. 2a) and a higher salinity incursion into the most upstream areas (Fig. 2b). Precipitation was the highest in the autumns of 2003 and 2006 and by the end of 2009, when some of the highest levels were observed since 1970, causing an abrupt increase in river runoff and consequent decrease in salinities. Salinity values were highly variable inside the estuary during the whole study period (Fig. 2b). The mean salinity values were higher during the drought periods and lower during rainy periods (Fig. 2b).

The mean water estuarine temperature was slightly lower than sea surface temperature (SST) in the adjacent coastal area, varying between 9 and 23°C, compared to 11 and 29°C, respectively (Fig. 2c). Higher estuarine temperature was observed in 2005 (Fig. 2c).

Structure and composition of the fish assemblage

During the study period, a total of 15,273 individuals belonging to 43 species and 23 families were captured in the Mondego estuary (Table 1). Species richness was the highest in 2005 and showed a tendency to decrease in the following years (35 species observed and highest H’ value) (Fig. 3). Evenness (J’) was also the highest in 2005, followed by a decrease in 2006. From 2007 on, evenness values increased again to similar values of those observed in 2003 and 2004 (Fig. 3). The increase in species number in 2005 was driven by the occurrence of the marine species *Arnoglossus laterna*, *Buglossidium luteum*, *Dicologlossa hexophthalma*, *Gaidropsarus mediterraneus*, and *Pegusa lascaris*.

Table 1 Species list of the Mondego estuary fish assemblage, with respective family, ecological guild (MS—marine straggler species; MMO—marine estuarine-opportunist species; MMD— marine estuarine-dependent species; ER—estuarine resident species; CA—catadromous species; FS—freshwater stragglers), and feeding guild (PS—planktivorous species; IS—invertebrate feeders; IF—invertebrate and fish feeders; OV—omnivorous species) throughout the study period.

Species	Ecological guild	Feeding guild	Species	Ecological guild	Feeding guild
Ammodytidae			**Mugilidae**		
Ammodytes tobianus	MS	PS	*Chelon labrosus*	MMO	OV
Anguillidae			*Liza aurata*	MMO	OV
Anguilla anguilla	CA	IS	*Liza ramada*	CA	OV
Atherinidae			*Mugil cephalus*	MMO	OV
Atherina boyeri	ER	IS	**Mullidae**		
Atherina presbyter	ER	IS	*Mullus surmuletus*	MMO	IS
Blenniidae			**Pleuronectidae**		
Parablennius gattorugine	MS	IS	*Platicthys flesus*	MMD	IF
Callionymidae			**Poeciliidae**		
Callionymus lyra	MS	IS	*Gambusia holbrooki*	FS	IS
Clupeidae			**Scophthalmidae**		
Sardina pilchardus	MMO	PS	*Arnoglossus laterna*	MS	IF
Congridae			*Scophthalmus rhombus*	MMO	IF
Conger conger	MS	IF	**Soleidae**		
Cyprinidae			*Buglossidium luteum*	MS	IS
Barbus bocagei	FS	IS	*Dicologlossa hexophthalma*	MMO	IS
Carassius auratus	FS	OV	*Pegusa lascaris*	MS	IS
Engraulidae			*Solea senegalensis*	MMO	IS
Engraulis encrasicolus	MS	PS	*Solea solea*	MMD	IS
Gadidae			**Sparidae**		
Ciliata mustela	MMO	IF	*Diplodus vulgaris*	MMO	IS
Gaidropsarus mediterraneus	MS	IF	*Sparus aurata*	MMO	OV
Trisopterus luscus	MS	IF	*Spondyliosoma cantharus*	MS	OV
Gobiidae			**Syngnathidae**		
Aphia minuta	MS	PS	*Nerophis lumbriciformis*	ER	IS
Gobius niger	ER	IF	*Syngnathus abaster*	ER	IS
Pomatoschisus microps	ER	IS	*Syngnathus acus*	ER	IS
Pomatoschistus minutus	ER	IS	*Syngnathus typhle*	ER	IS
Labridae			**Trachinidae**		
Symphodus bailloni	MS	IS	*Echiichthys vipera*	MS	IS
Moronidae			**Triglidae**		
Dicentrarchus labrax	MMD	IF	*Chelidonichthys lucerna*	MMO	IF

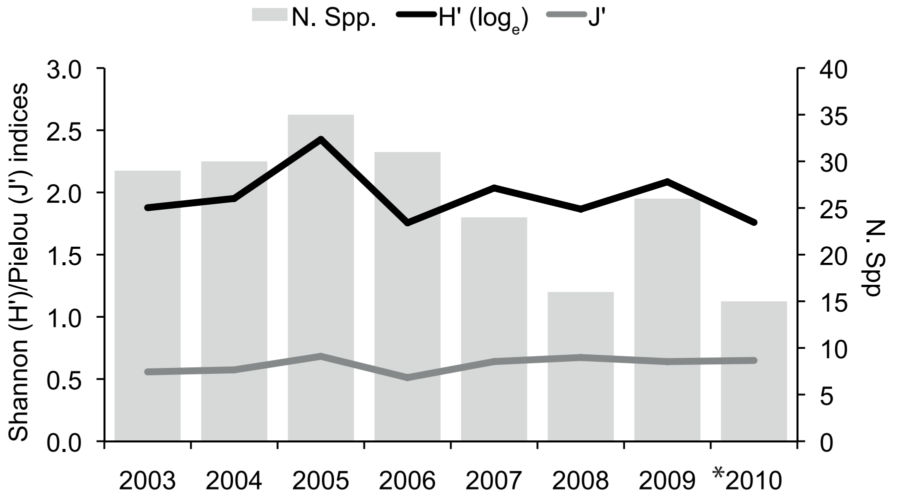

Figure 3 Shannon (H'), Pielou (J') indices and total number of species (N. Spp) per year. * only 2 sampling campaigns were performed.

In terms of dominance, five species were always present among the group of species that comprised at least 90% of the fish community abundance: *Pomatoschistus microps*, *P. minutus*, *Dicentrarchus labrax*, *Solea solea*, and *Platichthys flesus* (Fig. 4).

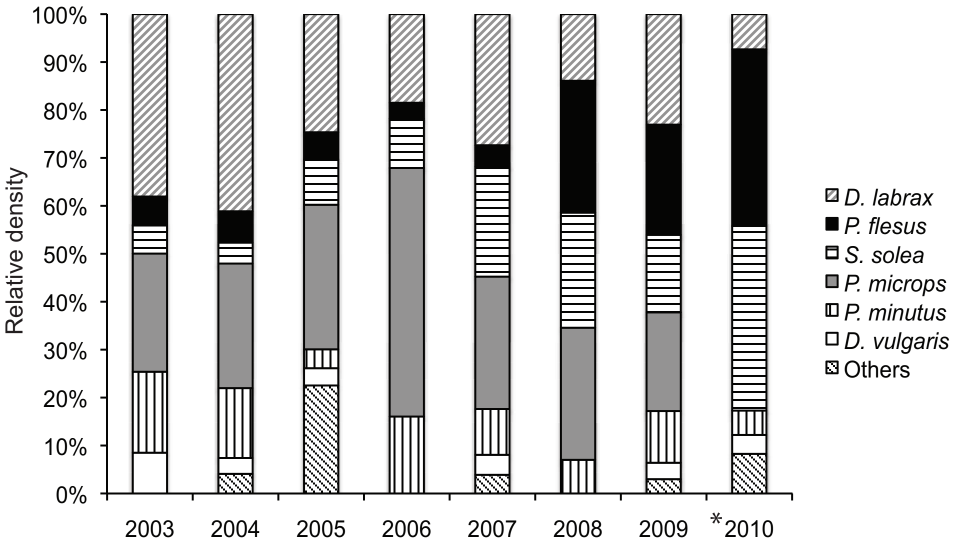

Figure 4 Relative abundance of the dominant species that comprised at least 90% of the fish assemblage for each year. The ‘Others’ category includes the following species: *A. anguilla*, *A. boyeri*, *C. lucernus*, *C. lyra*, *L. ramada*, *S. acus*, *S. pilchardus*, *T. luscus*; * only 2 sampling campaigns were performed.

Diplodus vulgaris had also high abundance values and was among the most abundant species throughout the study period (except in 2006 and 2008). In 2005, the number of dominant species almost doubled, compared with the other years. Besides the five most abundant species described above, *Atherina boyeri*, *Anguilla anguilla*, *Sardina pilchardus*, *Trisopterus luscus*, *Syngnathus acus*, and *Callionymus lyra* occurred with high relative abundance in this year (Fig. 4). In the other years, other species occurred among the dominant species: *A. anguilla* (2004), *Chelidonichthys lucerna* (2007), and *Callionymus lyra* (2009).

During the study period, the species abundance rank changed with year: *D. labrax* had the highest total and relative abundance in 2003, 2004, and 2009, while *P. microps* was the most abundant species in 2005, 2006, 2007, and 2008. Considering only the two sampling occasions in 2010 (January and March), *Solea solea* was the most abundant species (Fig. 4).

Significant differences (ANOSIM) regarding seasonal abundance data per year were only detected in the autumn (global R: 0.489, $p < 0.05$). For this season, the k-dominance plots confirmed the tendency that the structure and composition of the fish assemblage in 2005 was significantly different from the remaining years, with higher species richness and lower species relative abundance (dominance) in the fish assemblage (Fig. 5).

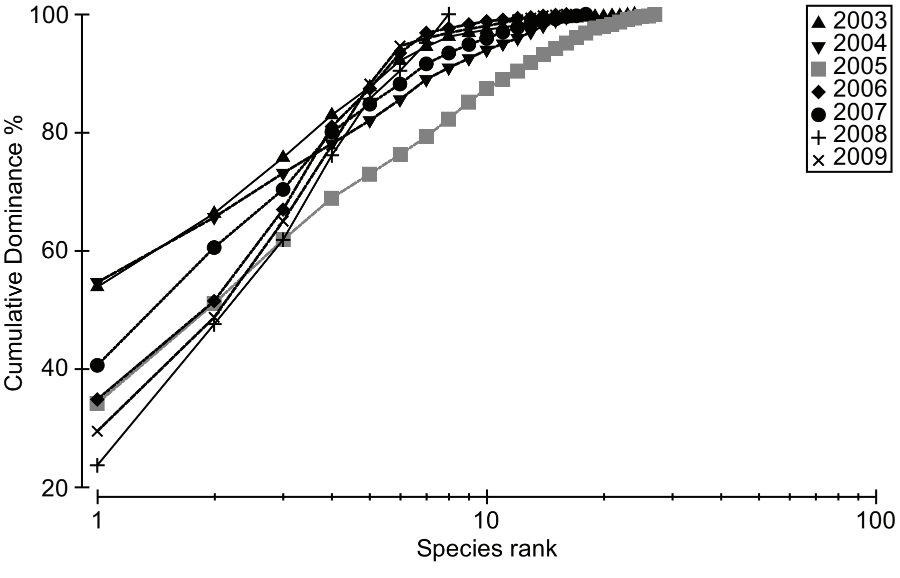

Figure 5 *k*-dominance curves for the fish assemblage sampled in autumn (aut) of each year.

Functional composition of the fish assemblage

The most abundant ecological guilds were the estuarine residents (ER, represented mainly by *P. microps* and *P. minutus*), the marine estuarine-dependents (MMD, represented mainly by *D. labrax*, *P. flesus*, and *S. solea*) and, with lower percentage, the marine estuarine-opportunists (MMO, represented mainly by *D. vulgaris*) (Fig. 6).

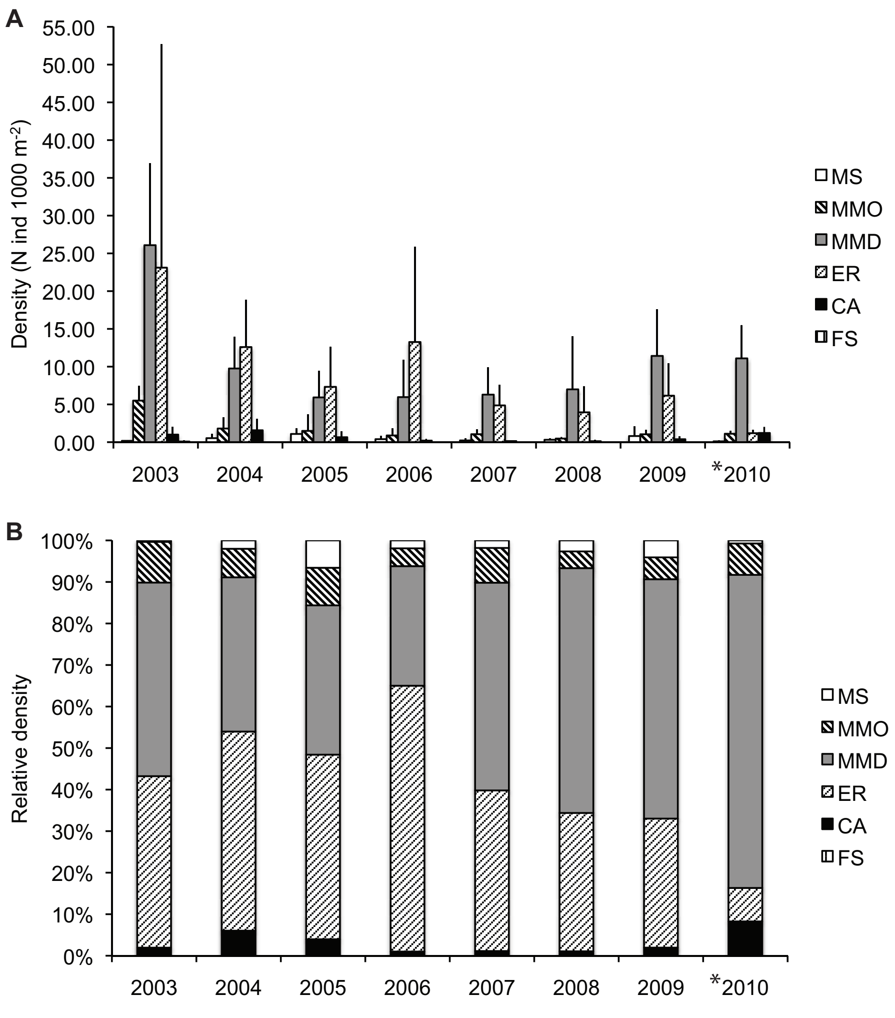

Figure 6 Annual variation of the ecological functional guilds for: (**a**) total density, with indication of standard deviation; and (**b**) relative density. *MS* marine straggler species, *MMO* marine estuarine-opportunist species, *MMD* estuarine-dependent species, *ER* estuarine resident species, *CA* catadromous species, *FS* freshwater stragglers; * only 2 sampling campaigns were performed.

These three ecological guilds had the highest densities in 2003, taking into account the whole study period (Fig. 6a). In 2003, MMD dominated the fish assemblage, and then from 2004 to 2006, most of the fish assemblage was dominated by ER species.

From 2007 onwards, the opposite pattern occurred, with MMD species showing considerably higher total and relative abundance values (Fig. 6). The abundance of MMO decreased throughout the study period. Migrant catadromous species (CA) were more abundant in 2004 and in the winter of 2009/2010 (Fig. 6a), when the fish assemblage was highly dominated by the MMD, mostly due to *S. solea* (Fig. 6b). During the extreme drought of 2005 (Fig. 2a), marine straggler (MS) species increased in abundance, while freshwater stragglers (FS) were only captured until the winter of 2004.

Concerning the feeding guilds, the most abundant and dominant group was the invertebrate and fish feeders (IF) (Fig. 7).

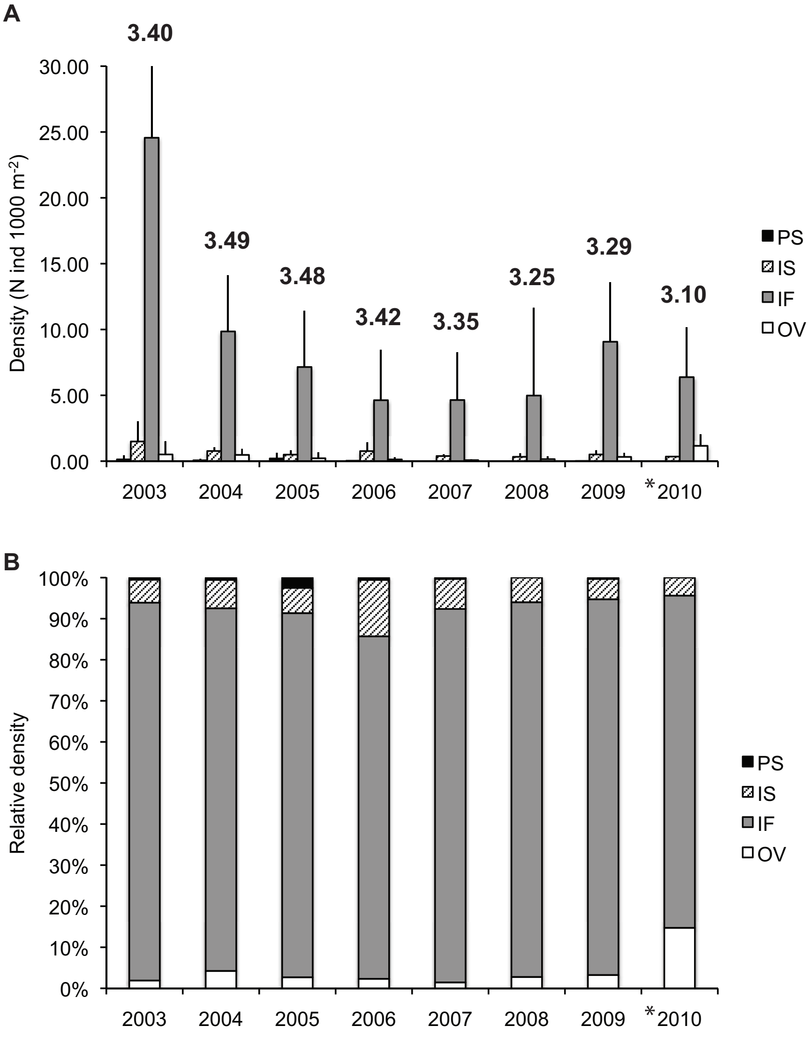

Figure 7 Annual variation of the feeding guilds for: (**a**) total density with indication of the standard deviation and the mean trophic level (*TLm*) above the *bars*; and (**b**) relative density. *PS* planktivorous species, *IS* invertebrate feeders, *IF* invertebrate and fish feeders, *OV* omnivorous species; * only 2 sampling campaigns were performed.

In 2005, a small increase of planktivorous species (PS) was observed, as well as of invertebrate feeders (IS) in 2006 (Fig. 7). A gradual decrease in the relative importance of omnivorous (OV) species along the study period up to 2010 was also observed (Fig. 7B).

The mean trophic level (*TLm*) of the fish assemblage was the highest in the drought years of 2004 and 2005 (3.49 and 3.48, respectively). Afterward, it gradually decreased, with the lowest values in 2008 and in the 3-month period of 2010 (January to March) (Fig. 7a).

Among the six most abundant species, the density and biomass values of *D. labrax*, *D. vulgaris*, *P. microps*, and *P. minutus* were the highest at the beginning of the study period, and then it gradually decreased onwards (Fig. 8a, b, c, d).

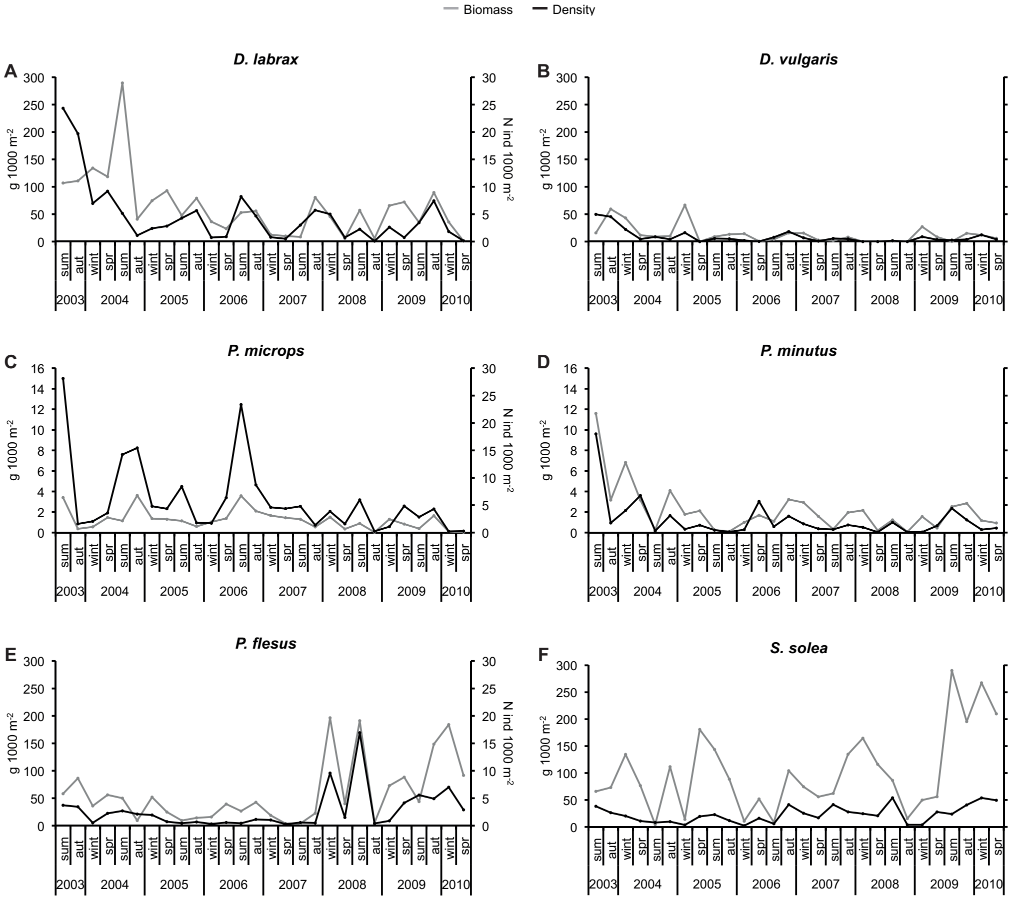

Figure 8 Seasonal variation of mean density (*thick line*) and mean biomass (*thin line*) of the six most abundant species in the Mondego estuary: (**a**) *Dicentrarchus labrax*, (**b**) *Diplodus vulgaris*, (**c**) *Pomatoschistus microps*, (**d**) *Pomatoschistus minutus*, (**e**) *Platichthys flesus*, (**f**) *Solea solea*.

D. labrax, *P. microps*, and *P. minutus* showed summer and autumn abundance peaks (Fig. 8a, c, d), while *D. vulgaris* was more abundant in autumn and winter periods (Fig. 8b). The mean densities of *P. flesus* and *S. solea* were relatively constant, while their mean biomass values showed an increasing trend along the study period (Fig. 8e, f). *P. flesus* exhibited the highest mean density and mean biomass values in the winter and summer of 2008 and appeared in very high biomass in the winter of 2009/2010 (Fig. 8e). *S. solea* had the highest biomass values at the end of the study period, in the summer of 2009, and in the winter of 2009/2010 (Fig. 8f).

Discussion

Fish assemblage composition and dominance patterns

In central Portugal, climate patterns changed considerably from 2003 to 2010, especially regarding precipitation and freshwater runoff, which led to the definition of drought and non-drought years. The observed differences in river runoff caused changes in the salinity gradient, a determinant factor influencing the structure of estuarine fish assemblages (Marshall and Elliott, 1998; Meynecke et al. 2006; Dolbeth et al. 2010).

In this long-term study, 43 species were observed throughout the estuary, which reveals a decrease in almost 20 species when compared with historical data available for the Mondego estuary (1988–1992) (Leitão et al. 2007). The previous authors pointed out that changes in river habitat due to anthropogenic interventions led to an increase in salinity in the middle and upper reaches, which resulted in the gradual disappearance of freshwater species from the estuary over the last 20 years.

Species richness declined toward the end of the study period, while evenness was relatively constant. Both species number and Shannon-Wiener index were the highest in 2005, the extreme dry year, driven mainly by the presence of several new marine species inside the estuary, such as *A. laterna*, *B. luteum*, *P. lascaris*, and *T. luscus*. During the drought period in 2005, the higher incursion of seawater created a more suitable environment for these marine species, resulting in the observed higher abundances. In agreement, Nicolas et al. (2010) also referred this pattern for several European estuaries, in which higher seawater penetration generally leads to the migration and higher densities of marine species in estuaries. In fact, the number of species that comprised 90% of the fish assemblage almost doubled in this year and evenness became higher, providing an indication of lower dominance by single taxa. In addition, the subtropical flatfish species *A. laterna*, *B. luteum*, and *P. lascaris* also increased in the estuary, which might have been induced by the increase in water temperature inside the estuary in 2005 (Martinho et al. 2010), given a temporary optimization of their thermal niche.

A small decrease in evenness was observed in 2006, which may be attributed to the weather conditions in the autumn and winter, since precipitation was considerably higher than in the previous years, causing a decrease in salinity throughout the estuary. This salinity decrease forced the marine occasional species back into the adjacent coastal areas, and only a few dominant species were present in the estuary. Previous studies in South African (Whitfield and Harrison, 2003) and European estuaries (Costa et al. 2007; França et al. 2010) have shown similar results, as species richness in fish assemblages tends to decrease as river flow increases considerably.

Although the occasional changes in fish composition attributed to differences in hydrology, the fish assemblage of the Mondego estuary was dominated by relatively few species, similarly to several other estuaries worldwide (e.g. Jackson and Jones, 1999; Akin et al. 2003; Hagan and Able, 2003; Ecoutin et al. 2005). In addition, the dominant species remained relatively unchanged along the study period, and the major changes were among the species that occurred with lower frequencies in the estuary. Similar results were found by Henderson et al. (2011) regarding the fish and crustacean community of the Bristol Channel, where the most abundant species showed long-term stability, while lower abundance species were dynamically less stable and highly responsive to environmental change.

Distinct rank abundance patterns were observed between *D. labrax* and *P. microps* throughout the study period that can be explained by changes in precipitation and runoff: in the years with higher precipitation and river runoff, *D. labrax* was the most abundant (except 2004), while during the drought years, *P. microps* occurred with the highest abundance (except 2006). In 2010 (only January and March), *S. solea* and *P. flesus* appeared with the highest relative abundance, probably due to the high winter precipitation and freshwater flows.

Estuarine habitat use functional groups

The guild approach to categorizing estuarine fish assemblages presents an opportunity to compare and contrast estuarine and other transitional habitats worldwide (Elliott et al. 2007). In addition, the presence of species of either marine or freshwater affinity may indicate the influence of natural and anthropogenic stressors external to the estuary, such as changes in freshwater flow (Chícharo et al. 2006; Costa et al. 2007; Martinho et al. 2007b). In the Mondego estuary, the most representative ecological guilds were the estuarine residents (ER), the marine estuarine-dependents (MMD), and the marine estuarine-opportunists (MMO), as observed in many European estuarine systems (e.g. Franco et al. 2006; Selleslagh and Amara, 2008). Estuarine residents (ER) dominated the fish assemblage in terms of numbers, but their species number was relatively low, also a common pattern in European estuaries (Selleslagh et al. 2009; França et al. 2010) and coastal lagoons (Franco et al. 2006; Ribeiro et al. 2006).

An alteration in dominance was observed along the study period between estuarine residents (ER) and marine estuarine-dependent species (MMD), which can be attributed to the changes in precipitation and river runoff conditions. In particular, river runoff is a determinant factor for the recruitment success of marine estuarine-dependent species (MMD) (Martinho et al. 2008), as their larvae depend on the extent of river plumes in coastal areas for finding estuaries (e.g. Marshall and Elliott, 1998; Vinagre et al. 2008; Martinho et al. 2008).

In contrast, marine stragglers (MS) densities increased in 2005, matching with the period of lower precipitation and river runoff (i.e. drought) and a higher salinity incursion into the estuary, creating a more suitable environment for these species (see Baptista et al. 2010 for further details). Generally, marine stragglers (MS) occur inside the estuary in lower densities, probably due to the relatively small area and small opening of the estuary compared with other systems, which may limit the entrance of these species (Martinho et al. 2007b; Dolbeth et al. 2010).

Migrant catadromous species (CA) were more abundant during the winter of 2009/2010, probably as a response to high precipitation values (highest precipitation since 1970), which translated into an increase in river flow and consequent decrease in salinities. Freshwater stragglers (FW) only appeared in the estuary until the winter of 2004, as a consequence of an upstream displacement of the estuarine salinity gradient. In addition, salinity levels have increased over the last years, mainly due to continuous dredging activities to deepen the main shipping channel (Leitão et al. 2007). A similar pattern was found in the Guadiana estuary (Portugal), where the Alqueva dam reduced river flow, leading to a reduction in freshwater fish species (Chícharo et al. 2006). In agreement, anthropogenic transformations have been pointed out as the main reason for the loss of freshwater species in estuaries (Smith et al. 2008).

Based on our results, the joint action of ongoing climate change and extreme weather events that were verified throughout the study period had important effects on the fish assemblage, particularly on the marine estuarine-dependent species that use the estuaries as a nursery area (MMD). Lower freshwater influx was responsible for a reduced extension of the river plume in the adjacent coastal area, which is a determinant factor for the recruitment success of these species. In this sense, the increasing frequency and intensity of extreme weather events, combined with continuous changes in the environment, may in the future lead to functional changes of estuarine systems, particularly in their role as a nursery area.

Feeding mode functional groups and mean trophic level

Feeding guilds are good indicators of the main types of food exploited by fish within estuarine environments and the estuarine compartments where these resources are taken from (e.g. pelagic, benthic) (Franco et al. 2008). Most of the fish assemblage was dominated by invertebrate and fish feeders (IF), similarly to some other small-sized Portuguese estuaries (Cardoso et al. 2011) and European tidal marshes (Mathieson et al. 2000). The highest abundance of the invertebrate and fish feeders

(IF) was observed in 2003, then gradually decreasing that relies on the species composition, as the most abundant species of this feeding guild were *D. labrax* and *P. flesus*, which occurred in lower densities during the dry years. In 2004 and 2005, there was also a slight change in the species composition of this group and some marine predator species (e.g. *A. laterna*, *Ciliata mustela*, *T. luscus*) occurred with higher densities than the remaining years. In 2005, there was an increase of the planktivorous species (PS), mainly marine species that generally occur in the estuary in lower numbers, and whose higher densities in this year were attributed to the higher salinity inside the estuary. An opposite pattern was found in the Forth estuary (UK) by Mathieson et al. (2000), where in general, the planktivorous species (PS) dominated the fish assemblages.

Invertebrate feeders (IS) comprised a major part of the fish assemblage. In general, this feeding guild occurs in high densities in European estuaries (e.g. Franco et al. 2006; França et al. 2009), taking advantage of the high productivity that characterizes estuarine systems. An increase in the relative proportion of omnivorous species (OV) was observed in 2010, probably as a response to higher precipitation and river runoff, despite that this year refers only to the winter period. These species feed mainly on algae, macrophytes, periphyton, epifauna, and infauna (Elliott et al. 2007). During the rainy winter period, probably food availability increased due to higher freshwater input, while the opposite could have been verified in lower freshwater flow regimes.

The mean trophic level of the fish assemblage was the highest during dry years (2004 and 2005), which can be explained by the increase in marine predator species number and biomass. From this point onwards, the mean trophic level gradually decreased: between the beginning and the end of the study period, the mean trophic level of the fish assemblage decreased a total of 0.11 values, which according to Pauly et al. (2001) and Laurans et al. (2004) can be considered significant. This decrease suggests a gradual replacement of top predators with invertebrate feeders. Similar results have been found in the Sine Saloum estuary (Senegal) facing environmental degradation and overfishing, where the mean trophic level of the fish assemblage decreased also by 0.11 over a 10-year period (Ecoutin et al. 2010).

Although both legal and illegal catches do occur in the Mondego estuary, the environmental changes seemed to be the most important stressors for the reduction in the mean trophic level of the fish assemblage.

Dominant species: abundance and distribution

A gradual decrease could be observed in the mean densities and mean biomass of *D. labrax*, *D. vulgaris*, *P. microps*, and *P. minutus* during the study period, probably due to the changes in the estuarine environment, such as increasing salinity over the past years (Leitão et al. 2007). *D. labrax*, *P. microps*, and *P. minutus* were more abundant during summer and autumn periods. The same pattern was observed in the Severn Estuary (UK), where the high autumn abundance of these species was explained by the arrival of juveniles that were born earlier in the same year (Henderson and Bird, 2010). *D. vulgaris* appeared with the highest densities in autumn and winter, which can probably be explained by several factors: according to Vasconcelos et al. (2010), the occurrence of this species within estuarine systems along the Portuguese coast can be a function of salinity, depth, percentage of mud in the sediment, importance of intertidal areas, distance to the estuary mouth, and macrozoobenthos density. *P. flesus* appeared with the highest mean biomass and densities in the winter and summer of 2008, which could be attributed to the recruitment success of this species. Both *P. flesus* and *S. solea* had the highest biomass values at the end of the study period, showing an increasing trend in mean length of these species along the study period.

Conclusions

In this work, it was possible to assess changes in the structure and composition of the Mondego estuary fish assemblage over a 7-year period. Although some changes in species richness, distribution, and abundance were observed, the main core of the fish community remained relatively unchanged, with some alterations in the order of dominance. This indicates that the major changes occurred in species that were less abundant in the estuary, which were probably less resilient to environmental disturbances. The combined approach of using indicators such as dominance, composition, estuarine habitat use, and feeding guilds applied to a monitoring program provided support for analyzing trends over time. Variations in salinity, runoff, and precipitation regimes seemed the most determinant factors for the changes in the fish assemblage, mainly by the displacement of estuarine habitats and by influencing the migration and recruitment of several species. The changes in the ecological and feeding guilds along the study period provided important information on the functional changes of estuarine systems facing climatic changes.

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CHAPTER

III

Effects of local and large-scale climate patterns on estuarine resident fishes: The example of *Pomatoschistus microps* and *Pomatoschistus minutus*

Abstract

Large-scale and local climate patterns are known to influence several aspects of the life cycle of marine fish. In this paper, we used a 9-year database (2003-2011) to analyse the populations of two estuarine resident fishes, *Pomatoschistus microps* and *Pomatoschistus minutus*, in order to determine their relationships with varying environmental stressors operating over local and large scales. This study was performed in the Mondego estuary, Portugal. Firstly, the variations in abundance, growth, population structure and secondary production were evaluated. These species appeared in high densities in the beginning of the study period, with subsequent occasional high annual density peaks, while their secondary production was lower in dry years. The relationships between yearly fish abundance and the environmental variables were evaluated separately for both species using Spearman correlation analysis, considering the yearly abundance peaks for the whole population, juveniles and adults. Among the local climate patterns, precipitation, river runoff, salinity and temperature were used in the analyses, and North Atlantic Oscillation (NAO) index and sea surface temperature (SST) were tested as large-scale factors. For *P. microps*, precipitation and NAO were the significant factors explaining abundance of the whole population, the adults and the juveniles as well. Regarding *P. minutus*, for the whole population, juveniles and adults river runoff was the significant predictor. The results for both species suggest a differential influence of climate patterns on the various life cycle stages, confirming also the importance of estuarine resident fishes as indicators of changes in local and large-scale climate patterns, related to global climate change.

Keywords

Pomatoschistus microps; *Pomatoschistus minutus*; Mondego estuary; environmental variables; river runoff; NAO

Introduction

Climate change has significant impacts on marine and estuarine ecosystems (Harley et al. 2006; Montoya and Raffaelli, 2010). These impacts can be induced by the alterations in local climate patterns such as temperature, freshwater flow, wind, tidal circulation and currents (e.g. Henderson and Seaby, 2005; Martinho et al. 2009), or by changes in large-scale factors such as North Atlantic Oscillation (NAO) and sea surface temperature (SST) (Attrill and Power, 2002; Vinagre et al. 2009). In addition, local stochastic events such as weather extremes (e.g. droughts, floods, heat/cold waves) can induce fluctuations in the conditioning factors, influencing the biological processes and ecosystem development of estuaries (Kantoussan et al. 2012; Pasquaud et al. 2012). As transitional areas, estuaries are among the most productive ecosystems of the world, supporting important ecological links with other environments (McLusky and Elliott, 2004; Able, 2005). These areas support high abundance of different biological communities, of which fish are a very important component (Whitfield, 1999). In particular, estuaries provide nursery and reproduction grounds for several species, offering a favourable habitat for resident species, juveniles of marine species and migratory routes for catadromous and anadromous species (Elliott and McLusky, 2002; Martinho et al. 2007). Nevertheless, the functioning of these transitional systems is strongly affected by environmental pressures linked to eutrophication, industrial pollution, overfishing and climate change (Martinho et al. 2008; Dolbeth et al. 2010).

Estuarine fish populations are highly dynamic and characterized by changing levels of recruitment and migration (Costa et al. 2002), being highly affected by hydrological parameters and climate (Costa et al. 2007; Martinho et al. 2009). In particular, fish dynamics, growth and production of estuarine ecosystems seem to be strongly affected by altered hydrology patterns, which can be regulated by floods and drought events (Whitfield, 2005; Dolbeth et al. 2008a, 2010; Baptista et al. 2010). Fish recruitment, growth and production can also be influenced directly by changes in physico-chemical parameters, such as salinity, turbidity and dissolved oxygen (Pampoulie et al. 2001; Selleslagh and Amara, 2008) or indirectly, through changes in food availability (Whitfield, 2005). Temperature has also important effects on fish reproduction, growth and migration patterns (Attrill and Power, 2002; Vinagre et al. 2009). Therefore fishes are widely used as indicators of environmental changes (e.g. Martinho et al. 2008), as they provide the possibility to evaluate the condition of the environment without having to capture the full complexity of the system (Whitfield and Elliott, 2002).

In most European estuaries, the common goby *Pomatoschistus microps* and the sand goby *Pomatoschistus minutus* are ubiquitous and abundant species (Bouchereau and Guelorget, 1998; Leitão et al. 2006). In general, these species spend their entire life cycle within estuaries, showing relatively short life spans and distinct behavioural characteristics (Bouchereau and Guelorget, 1998; Leitão et al. 2006; Dolbeth et al. 2007). In addition, gobies are also important for the estuarine foodweb as intermediate predators (Dolbeth et al. 2008b). Taking into account these characteristics, it is important to better understand their life cycle and to evaluate their responses to different environmental scenarios.

Based on the mentioned above, the overall aims of the present study were to evaluate the effects of climatic variations on two estuarine resident fish species, and to highlight the important role of estuarine residents fishes as indicators of environmental changes. More specifically, the main objectives were (1) to evaluate the abundance, growth and production patterns of *Pomatoschistus microps* and *Pomatoschistus minutus* over a nine year period (June 2003 to June 2011); (2) to estimate the responses of the two species to different environmental variables, including local and large-scale climate patterns; (3) to assess the distinct response patterns to the environmental variables by the different life stages of each species.

Materials and methods

Study site

The Mondego estuary is a small intertidal estuary of 8.6 km^2, located on the Atlantic coast of Portugal (40º 08'N, 8º 50'W). The estuary is divided in two distinct arms (north and south) in the terminal part at about 7 km from the shore that join again near the mouth (Fig. 1).

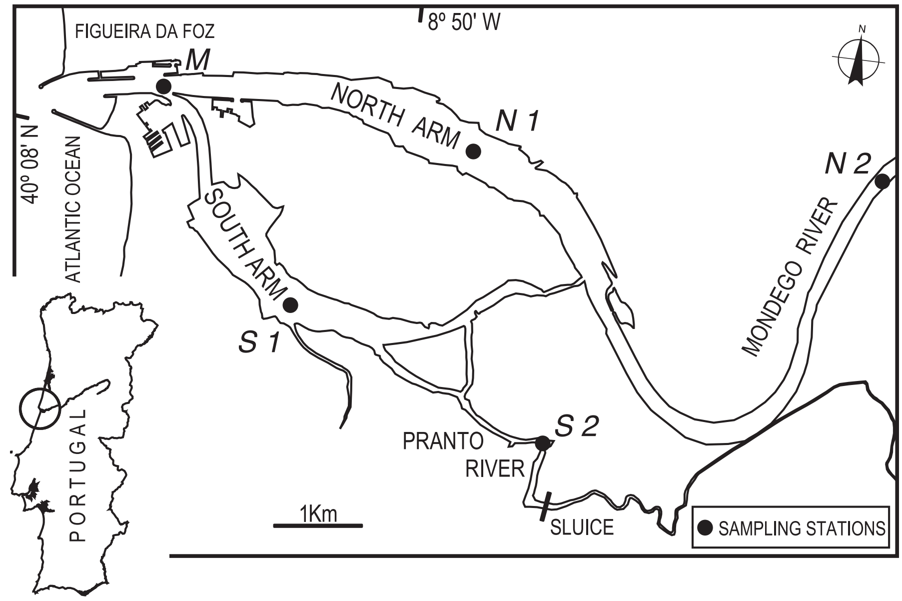

Figure 1 The Mondego estuary with the location of the five sampling stations.

The north arm is deeper, with 5-10 m depth at high tide, with a tidal range of 2-3 m, while the south arm is shallower, with 2-4 m during high tide, and a tidal range of 1-3 m. The north arm constitutes the main navigation channel and the location of the Figueira da Foz commercial harbour. The constant dredging and shipping that occur in this area causes physical disturbance of the bottom. The south arm is characterized by large areas of intertidal mudflats that comprise about 75% of the total area. Freshwater flows mainly through the north arm, as the south arm is almost silted up in the upstream areas. The water circulation on the south arm is mainly dependent on the tides and on the small freshwater input from the Pranto River, which is a small tributary system, regulated by a sluice according to the water needs in the surrounding rice fields. In 2006, the connection between the two arms was enlarged, allowing a higher water circulation through the south arm.

Sampling and laboratory procedures

Sampling was conducted monthly from June 2003 until January 2007, and then bimonthly until June 2011 (except in July, September, October and December 2004, October and November 2008, September and November 2010 and March 2011, owing to technical constraints or bad weather conditions). Fishing took place during the night at five sampling stations (Fig. 1), at high water of spring tides, using a 2-m beam trawl with one tickler chain and 5-mm stretched mesh size in the cod end. At each sampling station, three hauls were towed at the speed of two knots for an average of 3 min each, covering at least an area of 500 m^2. Samples were transported in iceboxes to the lab, where fish were sorted, and all *Pomatoschistus microps* and *Pomatoschistus minutus* present in the samples were measured (total length to nearest 1 mm) and weighted (wet weight, 0.01 g precision). Bottom water was analysed for temperature and salinity at each sampling station during the fishing campaigns.

Acquisition of environmental data

Freshwater runoff was acquired from the Portuguese Water Institute (INAG; http://snirh.inag.pt; 12.03.2012) station Açude Ponte Coimbra 12G/01A, near the city of Coimbra, located 40 km upstream of the estuary. Monthly precipitation was obtained from the Soure 13F/01G station (INAG), and the long-term average precipitation (1971-2000) was obtained in http://www.meteo.pt from Coimbra station (IM).

The North Atlantic Oscillation (NAO) index (defined as the pressure difference between Lisbon, Portugal, and Reykjavik, Iceland) data were supplied by NOAA/National Weather Service – Climate Prediction Centre (http://www.cdc.noaa.gov, 21.03.2012). Sea surface temperature (SST) data concerning the 1º Lat x 1º Long square in the Portuguese coast nearest to the Mondego estuary were obtained from the International Comprehensive Ocean – Atmosphere Data Set (ICOADS) online database (http://dss.ucar.edu/pub/coads, Slutz et al. 1985; 09.03.2012).

Data analysis

For both species, monthly density data (individuals per 1000 m^2) were calculated by averaging the total number of individuals in relation to the five sampling stations. Mean annual densities were calculated by averaging the monthly data from each year (from January to December).

The population structure of both species was determined by tracking recognizable cohorts from the consecutive sampling dates. Each spatial sample was aggregated and analysed using the size-frequency distribution of the consecutive sampling dates. Cohorts were determined using the FAO – ICLARM Stock Assessment Tools software (FISAT II, http://www.fao.org/fi/statist/fisoft/fisat/index.htm). Bhattacharya's method was used at first to identify the location of the modes, and then the estimated mean length for each age group was refined with the NORMSEP procedure, which separates normally distributed components of the size-frequency samples (Gayanilo et al. 2005). This analysis provides the mean length, standard deviation, population sizes and the separation indices for the identified age groups.

After identification of the cohorts, annual production was calculated using the cohort increment summation method (Winberg, 1971), according to:

$$P_{cn} = \sum_{t=0}^{T-1} \left(\frac{N_t + N_{t+1}}{2} \right) \times \left(\overline{w}_{t+1} - \overline{w}_t \right)$$

where P_{cn} is the growth production (g ww 1000 m^{-2} year $^{-1}$) of cohort *n*; *N* is the density (ind 1000 m^{-2}), *w* is the mean individual weight (g ww), and *t* and *t*+1, consecutive sampling dates. Population production estimates correspond to the sum of each cohort production (P_{cn}). Negative production values were not included in the overall estimates and were considered as zero production. Annual production was calculated for each year, from June to May.

The mean annual biomass (*B*) was estimated according to:

$$\overline{B} = \left(\frac{1}{T} \right) \times \sum_{n=1}^{N_c} \left(\overline{B}_{cn} t_{cn} \right)$$

where *T* is the period of study, which is always 365 days (yearly cycles) as the mean annual biomass is being computed; N_c is the number of cohorts found in the study period; B_{cn} is the mean biomass (g ww 1000 m^{-2}) of cohort *n*; t_{cn} is the time period of the cohort *n* (days), from the first appearance of individuals until they disappeared.

For each cohort, absolute growth rates (AGR, cm day^{-1}) were calculated, according to:

$$AGR = \frac{L_{t+1} - L_t}{t + 1 - t}$$

where L_{t+1} and L_t are the total length at time $t + 1$ and t respectively.

The relationships between the fish densities and environmental variables were analysed with Spearman correlations using R software (R Development Core Team, 2008). These analyses were performed separately for the two species, considering the population as a whole, and then adults and juveniles separately. The separation between adults and juveniles was determined taking into account the minimum length of the last maturation stage found for each species (for *Pomatoschistus microps*, 2.5 cm; for *Pomatoschistus minutus*, 3.9 cm), previously defined by Dolbeth et al. (2007). The explanatory variables for these analyses included precipitation, freshwater runoff, mean estuarine salinity, mean estuarine temperature, the North Atlantic Oscillation (NAO) index and sea surface temperature (SST) in the coastal area near the estuary. For each year, the sampling date with the highest density value of each species was used in the analyses (whole population, adults and juveniles separately) (Table 1), and compared against the monthly average values of each environmental variable of the corresponding date. We also tested a time-lag of one and two months to detect small time scale patterns, and six and twelve months in order to detect larger time scale patterns, since it has been recognized that the environmental background may influence fish spawning and larval immigration, and hence, fish recruitment over a wider time frame (e.g. Vinagre et al. 2009; Martinho et al. 2012). For the mean estuarine salinity and temperature, only the monthly average values of the corresponding date with the highest density values were used in the models. A significance level of 0.05 was considered in all test procedures.

Table 1 Annual density peaks of *Pomatoschistus microps* and *Pomatoschistus minutus* (total population, juveniles and adults) and the respective sampling date they were recorded.

	P. microps **total population**		***P. microps*** **juveniles**		***P. microps*** **adults**	
Year	Highest density	Date	Highest density	Date	Highest density	Date
2003	48.69	29-Jun	29.80	29-Jun	18.89	29-Jun
2004	20.73	06-Jul	16.53	23-Apr	13.20	07-Dec
2005	14.39	21-Jul	12.62	24-Mar	6.77	22-Aug
2006	41.73	26-Jun	19.29	29-May	22.60	26-Jun
2007	7.30	22-Mar	0.99	29-Oct	6.31	22-Mar
2008	5.97	04-Aug	2.10	04-Aug	5.97	04-Aug
2009	8.08	23-Sep	1.77	23-Sep	5.93	28-May
2010	5.90	15-Jul	0.65	15-Jul	5.48	15-Jul
2011	40.64	20-Apr	24.57	03-Jun	16.07	20-Apr
	P. minutus **total population**		***P. minutus*** **juveniles**		***P. minutus*** **adults**	
Year	Highest density	Date	Highest density	Date	Highest density	Date
2003	28.34	29-Jun	16.51	29-Jun	11.83	29-Jun
2004	10.72	23-Apr	9.83	23-Apr	4.52	19-Feb
2005	2.44	24-Mar	0.92	26-May	2.44	24-Mar
2006	13.58	29-May	11.34	29-May	6.23	26-Sep
2007	2.83	29-Oct	0.36	28-Nov	2.74	29-Oct
2008	1.85	04-Aug	0.65	04-Aug	1.69	04-Aug
2009	5.00	23-Sep	2.14	28-May	5.00	23-Sep
2010	42.44	15-Jul	24.50	15-Jul	18.27	15-Jul
2011	2.35	03-Jun	1.59	03-Jun	0.92	03-Jun

Results

Environmental characterization

Both precipitation and freshwater runoff showed clear seasonal fluctuations, characteristic of temperate regions, along the 9-year study period (Fig. 2A). In the periods with higher precipitation, freshwater runoff increased and consequently salinity decreased inside the estuary, while during periods of low precipitation an opposite pattern could be observed. In general, 2003, 2006 and 2009 were considered as regular hydrologic years regarding precipitation, 2004, 2005, 2007, 2008 and 2011 were dry years, and 2010 was considered as rainy year, by comparing against the mean precipitation regime for central Portugal during the period of 1971-2000 (INAG; http://snirh.inag.pt). The harshest drought occurred in 2005, when precipitation values were far below the long-term average, considered the worst drought since 1931 in the Portuguese territory (Fig. 2A). The highest precipitation values were observed in the autumns of 2003 and 2006 and in the winter of 2009/2010 (Fig. 2A), when some of the highest levels occurred since 1970, inducing an abrupt increase in river runoff (Fig. 2A) and consequent decrease in salinities (Fig. 2B).

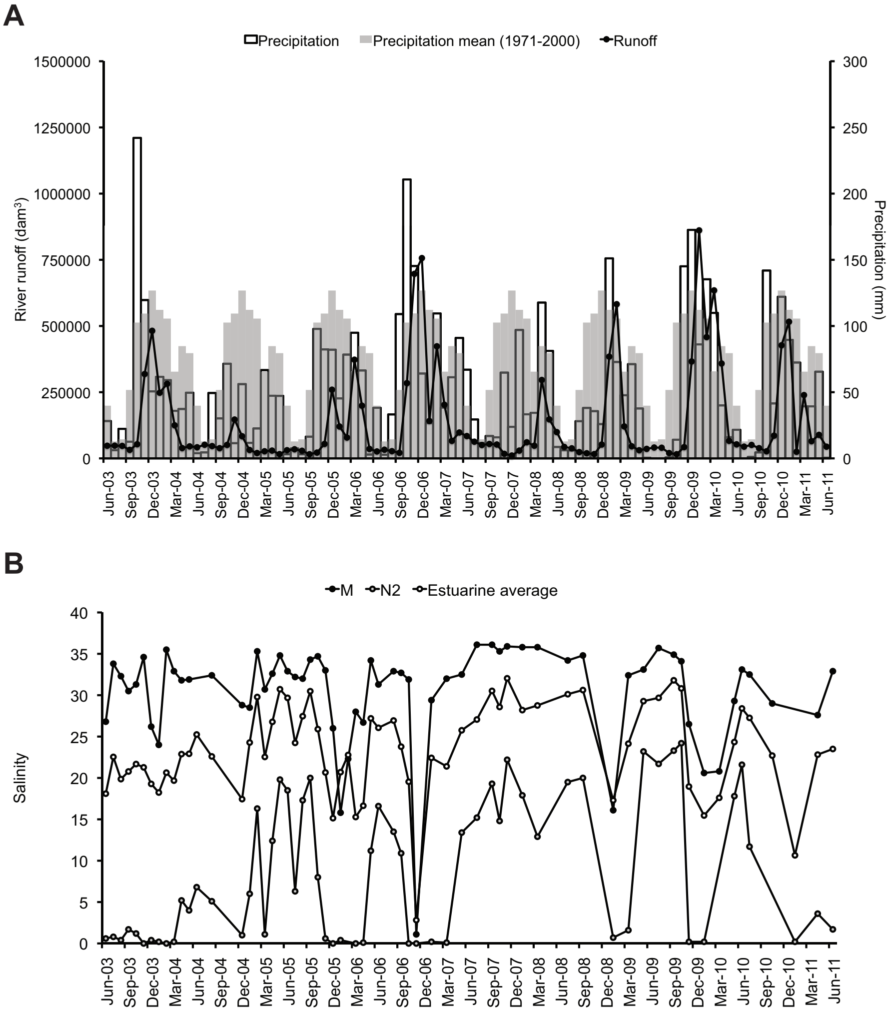

Figure 2 Monthly variation of (**a**) precipitation and river runoff (cubic decameter, dam^3) during the study period and average precipitation values during the period of 1971-2000 in the Mondego river basin; (**b**) salinity at stations M (farthest downstream station), N2 (furthest upstream station) and estuarine average salinity values.

The mean estuarine water temperature was in general lower than the SST in the adjacent coastal area (varying between 9 ºC and 23 ºC, compared to 11 ºC and 29 ºC, respectively) (Fig. 3A). Estuarine water temperature was the lowest in January 2005, while the highest values were observed in July 2005, with 2005 characterized by the highest variation in temperature along the year (Fig. 3A). SST was the lowest in the winter of 2004 and 2005, while the highest values occurred in the summer of 2003 (Fig. 3A). The highest annual range variation of SST was observed in 2009 (13 ºC in January and 28 ºC in August) (Fig. 3A).

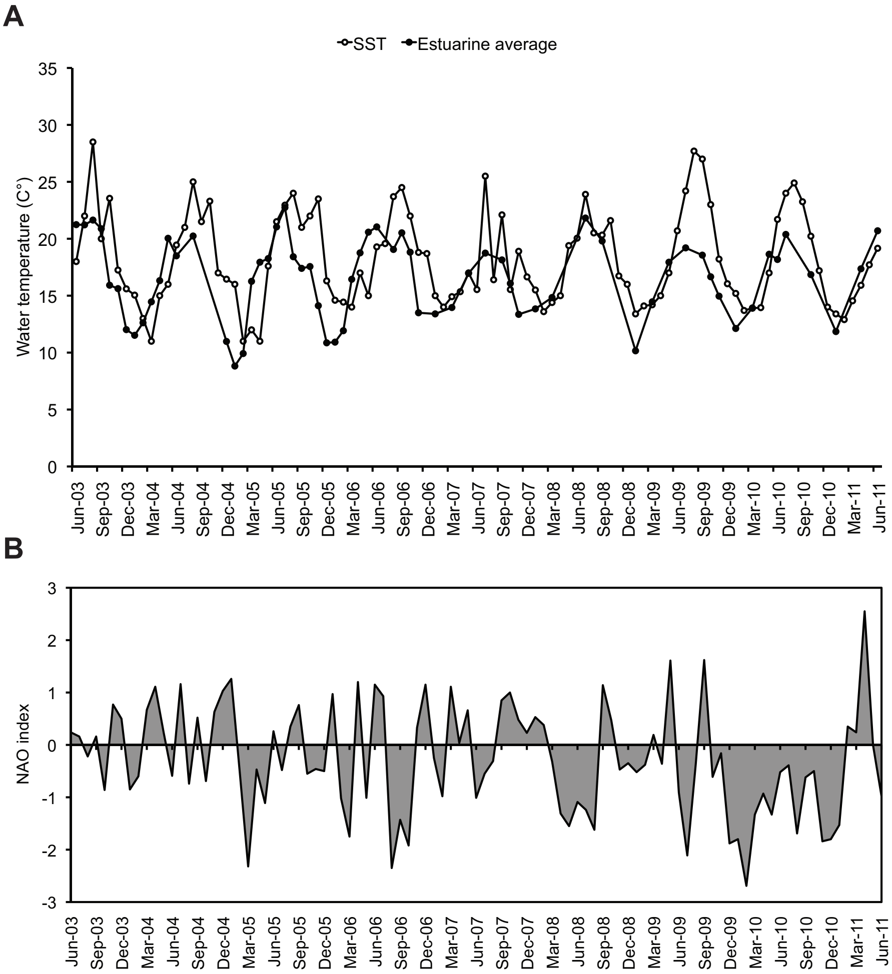

Figure 3 Estuarine average temperature in the Mondego estuary and sea surface temperature (SST) in the adjacent coastal area (**a**); monthly variation of the North Atlantic Oscillation index (NAO index) (**b**) during the study period.

The NAO index ranged from –2.69 to 2.55 and showed a general decreasing tendency towards the end of the study period, denoting a transition from a positive to a negative phase (Fig. 3B).

Abundance, population structure and absolute growth rates

In general, *Pomatoschistus microps* was more abundant than *Pomatoschistus minutus* (Fig. 4A). Both species had high densities in 2003, after which they decreased and showed constant values. Apart from this, *P. microps* was also more abundant in 2004, 2006 and in 2011, showing the highest values along the study period (Fig. 4A).

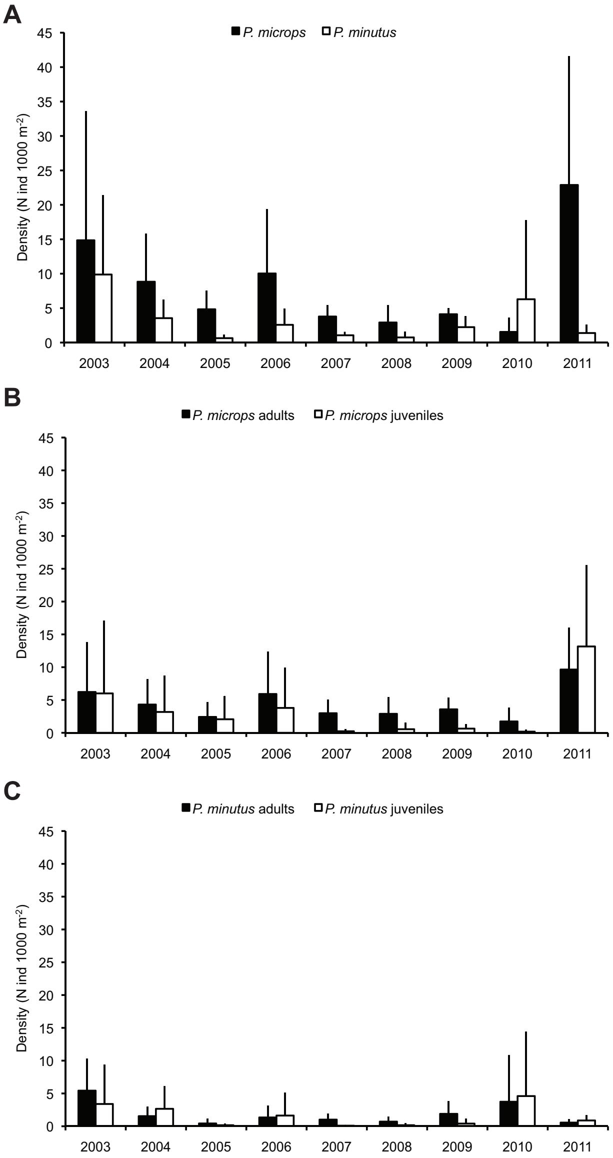

Figure 4 Total annual density (±standard deviation) of (**a**) *Pomatoschistus microps* and *P. minutus*; (**b**) *P. microps* adults and juveniles and (**c**) *P. minutus* adults and juveniles from 2003 to 2011.

Both adults and juveniles of *P. microps* showed similar abundances, but between 2007 and 2010, juveniles occurred in lower densities (Fig. 4B). In 2011, juveniles occurred in higher densities than the adults (Fig. 4B). *Pomatoschistus minutus* was more abundant in 2003, 2004, 2006 and in 2010 (Fig. 4A), mainly due to juveniles (Fig. 4C). In 2003, 2005 and between 2007 and 2009, *P. minutus* juveniles were less abundant than the adults (Fig. 4C).

Pomatoschistus microps had three recruitment periods per year (January, April and June/July), while for *Pomatoschistus minutus* only two recruitment periods were observed (April and November) (Fig. 5A and B).

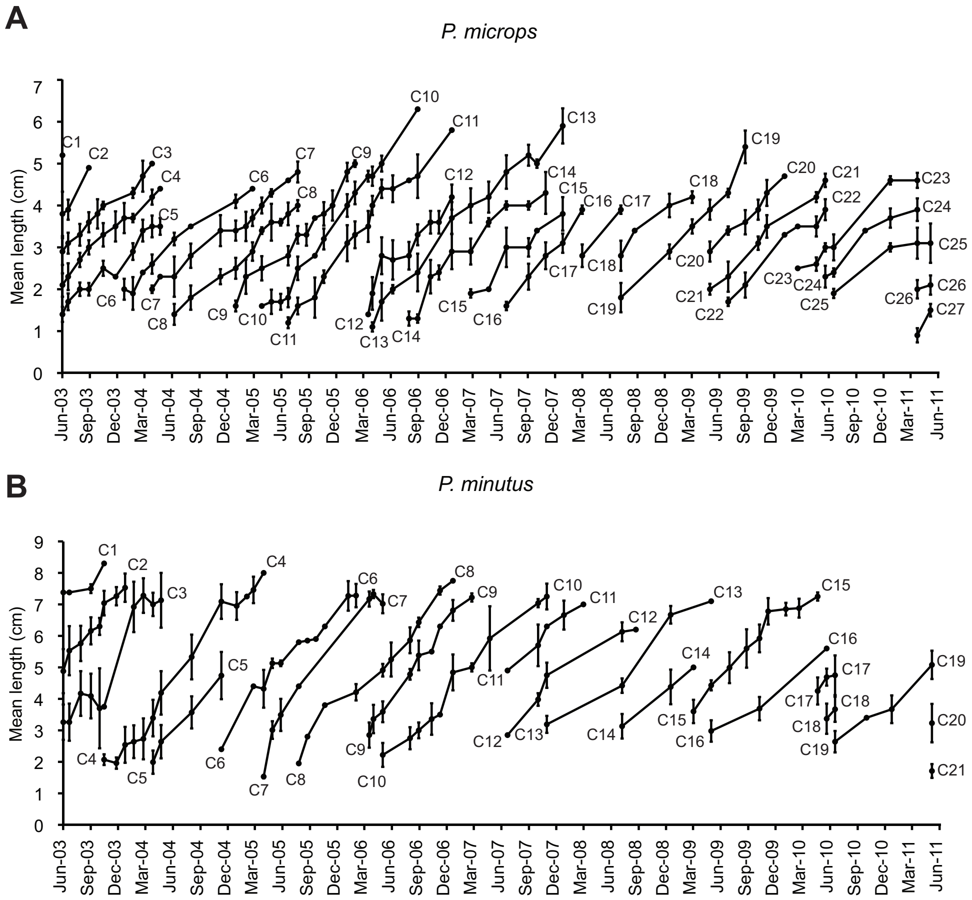

Figure 5 Mean cohort length of *Pomatoschistus microps* (**a**) and *P. minutus* (**b**) (±standard deviation) with indication of the cohorts (C).

Smaller juveniles of both species were not detected between 2007 and 2009 (Fig. 5A and B). Larger individuals were observed in 2006, 2007 and 2009 for both species, as well as in 2003 for *P. minutus* (Fig. 5A and B).

For *Pomatoschistus microps*, the mean growth rate of January recruits was similar to those of the April and the June/July recruits (0.007, 0.008 and 0.008 cm day^{-1}, respectively). Regarding *Pomatoschistus minutus*, the April and November recruits showed also similar growth rates (0.010 and 0.011 cm day^{-1}, respectively).

Production dynamics

The mean annual production and biomass of *Pomatoschistus microps* were highly variable along the study period, with the maximum values observed in 2006/07 (Table 2). High production values were observed in 2003/04, 2004/05, 2005/06 and 2006/07, while the lowest values were obtained in 2008/09 (Table 2). Mean biomass was highest in 2003/04, 2006/07 and at the end of the study period in 2010/11 (Table 2). The lowest mean annual biomass values were obtained in 2008/09. P/B ratios were higher in 2005/06 and 2009/10, while the lowest values were observed in 2008/09 and 2010/11 (Table 2).

Table 2 Production values (g ww 1000 m^{-2} year^{-1}), mean biomass (g ww 1000 m^{-2}) and P/B ratios for *Pomatoschistus microps* and *Pomatoschistus minutus* for each year (June-May) during the study period.

	2003/04	**2004/05**	**2005/06**	**2006/07**	**2007/08**	**2008/09**	**2009/10**	**2010/11**
Production (g ww 1000 m^{-2} year^{-1})								
P. microps	3.61	3.04	3.09	5.00	1.47	0.91	2.60	2.43
P. minutus	16.41	4.26	1.58	5.69	2.31	2.52	4.14	2.55
Biomass (g ww 1000 m^{-2})								
P. microps	1.39	1.28	0.96	2.00	0.77	0.62	0.80	1.74
P. minutus	5.04	1.84	1.20	2.05	0.86	0.75	1.73	0.91
P/B (year^{-1})								
P. microps	2.6	2.4	3.2	2.5	1.9	1.5	3.2	1.4
P. minutus	3.3	2.3	1.3	2.8	2.7	3.4	2.4	2.8

For *Pomatoschistus minutus*, both mean annual production and biomass were the highest at the beginning of the study period (2003/04), and then presented constant values (Table 2). Nevertheless, annual production was the lowest in 2005/06, while mean biomass had the lowest values in 2008/09. The highest P/B ratios were observed in 2003/04 and in 2008/09, and the lowest in 2005/06 (Table 2). In general, both production and mean biomass values were higher for *P. minutus* than for *Pomatoschistus microps* (Table 2).

Relation between environmental parameters and fish abundance

According to the Spearman correlation analysis, the different life stages of *Pomatoschistus microps* and *Pomatoschistus minutus* showed different response patterns to the environmental variables (Table 3).

Table 3 Spearman correlations fitted to the abundance data of *Pomatoschistus microps* and *Pomatoschistus minutus* (total population, juveniles and adults).

Species	Parameters	Spearman correlations
P. microps total population	NAO (time-lag 2 months)	0.70*
	Precipitation (time-lag 2 months)	0.90***
P. microps adults	NAO (time-lag 12 months)	0.72*
	Precipitation (time-lag 2 months)	0.81**
P. microps juveniles	NAO (time-lag 1 month)	0.74*
	Precipitation (time-lag 2 months)	0.87**
P. minutus total population	River runoff (time-lag 6 months)	0.85**
P. minutus adults	River runoff (time-lag 6 months)	0.81**
P. minutus juveniles	River runoff (time-lag 6 months)	0.75*

* – Significance codes: 0 ‘***’, 0.001 ‘**’, 0.01 ‘*’.

Regarding the total *P. microps* population, both precipitation and NAO two months prior to the peak abundance were significant factors explaining year-to-year variations in abundance (Table 3): in years of high precipitation and positive NAO values, higher abundance of *P. microps* was observed.

For the *Pomatoschistus microps* juveniles, precipitation two months prior and NAO one month prior to the highest abundance peak were significant factors explaining abundance (Table 3). For the adult *P. microps* individuals, the NAO index with a time-lag of twelve months and precipitation two months prior to the highest abundance peak were significant predictors (Table 3). In particular, higher abundances were observed during positive NAO values and higher precipitation.

Concerning the whole population of *Pomatoschistus minutus*, river runoff with a time-lag of six months to the highest abundance peak was the only significant predictor (Table 3). Likewise, for the *P. minutus* adults and juveniles, the same parameter was also significant. In general, higher abundance of *P. minutus* was observed in periods with higher river runoff (Table 3).

Discussion

Abundance, growth and production

Estuarine resident fish species, such as those in the present study, are highly abundant across European estuaries (e.g. França et al. 2010; Henderson et al. 2011). Similarly also to most European estuaries, *Pomatoschistus microps* was more abundant than *Pomatoschistus minutus* in the Mondego estuary, which can be related to the particular physiological characteristics of the two species: *P. microps* tolerates a wider range of temperature and salinity variations, which seems an advantage towards *P. minutus* (Dolbeth et al. 2007, 2010).

The abundance peaks observed for both species occurred in years with higher precipitation and river runoff levels (2003, 2004, 2006, 2010 and 2011). Although 2004 and 2011 were considered as dry years, high precipitation and river runoff were observed in summer and autumn, which could have influenced the abundance patterns of the two species by reducing the overall salinity within the estuary for *Pomatoschistus microps*, or increasing food availability from allochthonous sources. Salinity plays an important role on the egg development of Gobidae fish, whose survival is lower at higher salinities for *P. microps* (Fonds and Van Buurt, 1974). In fact, the juveniles of both species appeared with lower densities in the years with higher salinity levels within the estuary, mainly in the driest years (e.g. 2005, 2007, 2009 and 2010). According to Maes et al. (1998), besides the adults, the juveniles of *Pomatoschistus minutus* also undertake migrations to the coastal area to avoid predation and to find food, which could also explain the lower abundance of juveniles during this period. However, predation pressure might also have been higher during the droughts (as hypothesised for the extreme drought of 2005 by Dolbeth et al. 2007), which could have contributed to the eventual migration or mortality of juveniles in those years.

For both species, higher annual productions were observed in years when precipitation was higher and salinities consequently decreased inside the estuary. This pattern confirms the important role of freshwater flow that has both direct and indirect effects on fish abundance (Costa et al. 2007; Martinho et al. 2007) and production (Dolbeth et al. 2008a,b, 2010). For *Pomatoschistus microps*, mean annual production and biomass were relatively constant along the study period, confirming the higher resilience of this species to temperature and salinity variations (Riley, 2003; Dolbeth et al. 2007). Both annual production and mean biomass of *Pomatoschistus minutus* were the highest at the beginning of the study period, and then remained constant. Contrary to *P. microps*, this species is less tolerant to the interrelated environmental variations that were relatively strong during the study period, mainly precipitation, freshwater flow and salinity. On the other hand, the lowest production values in 2005/06 could be attributed to predation, as during this extreme drought period some piscivorous species appeared inside the estuary, exploiting the temporarily available suitable habitat created by a higher salinity incursion, which might have caused a higher predation pressure on the resident species (Dolbeth et al. 2007; Martinho et al. 2010).

Relationship between environmental variables and fish abundance: the role of local and large-scale climate patterns

Both species provided different responses to the selected environmental variables, suggesting different tolerance thresholds and adaptation strategies to the surrounding environment. For the whole population, juveniles and adult individuals of *Pomatoschistus microps*, the NAO with a time-lag of two, one and twelve months respectively, and precipitation with a time-lag of two months prior to the year-to-year abundance peak were significant factors explaining interannual variability in abundance. The significant effects of NAO on the abundance patterns of *P. microps* show that large-scale factors can influence fish species over a prolonged time frame (Ottersen et al. 2001; Vinagre et al. 2009), and also suggest its influence on local climate patterns: in the central Atlantic region, the NAO is responsible for changes in sea surface temperature (SST) and also for wind and current patterns (Stenseth et al. 2002; Henriques et al. 2007). In addition, recent studies highlighted the indirect effects of NAO on the abundance and productivity of fish communities (Attrill and Power, 2002; Henriques et al. 2007) and also on the recruitment and migration patterns of species (Sims et al. 2004; Henderson and Seaby, 2005). Moreover, the relationship between the NAO and water temperature (Ottersen et al. 2001; Attrill and Power, 2002) might also contribute for the interannual variability of *P. microps*, as previous studies in the Mondego estuary described that abundance patterns of this species were positively correlated with water temperature (Dolbeth et al. 2007).

Our results also confirm the important regulating effects of local environmental processes on fish abundance, such as precipitation and consequently freshwater inflow (e.g. Costa et al. 2007; Gillson et al. 2009; Martinho et al. 2009), with consequent repercussion on the production levels (Dolbeth et al. 2007, 2010), as observed by the increased production of *Pomatoschistus microps* in the years with higher precipitation levels. In general, higher river flow is responsible for an increased transport of organic matter towards estuaries, inducing an increase in primary and secondary production that provides higher food availability for fishes (Costa et al. 2007; Baptista et al. 2010; Dolbeth et al. 2010).

For the whole population, juveniles and adults of *Pomatoschistus minutus*, river runoff with a time-lag of six months prior to the yearly abundance peak was the only significant factor explaining abundance. Higher abundances of *P. minutus* adults were observed during periods with higher river runoff, confirming the important effects of freshwater flow on fish abundance, as sources of primary and secondary production available for fish consumption (Costa et al. 2007; Gillson et al. 2009). In addition, the winter reproductive migrations of this species could also have been influenced by river runoff, as during higher river runoff food availability might increase, which may have induced the migration of larvae and young individuals towards the estuary.

Surprisingly, there were no significant relationships between fish abundance and temperature, although previous studies found positive relation between fish production and temperature (e.g. Dolbeth et al. 2007). As ectotherms, metabolic processes in fish are dependent on temperature (Fry, 1947; Neill et al. 1994), which include growth and reproduction. In addition, temperature has also been determined to be an important regulatory factor for egg size and developmental rates (Fox et al. 2003). However, the influence of temperature on the abundance patterns of both *Pomatoschistus* species might not be easily isolated, since they are relatively resilient to temperature fluctuations (Riley, 2003, 2007), and the rate of regime shift is much slower when compared to the rapid changes in salinity or freshwater flow.

The present study showed that *Pomatoschistus* populations might be highly affected by climatic variability (associated with changes in precipitation, river runoff and large-scale patterns such as the NAO), through changes in abundance patterns, growth and production potential. To a further extent, global climatic changes might induce notable alterations in estuarine fish assemblages, which could have significant effects on the structure and functioning of coastal marine ecosystems (e.g. Philippart et al. 2011; Rose and Allen, 2013).

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CHAPTER

IV

Efficacy of single and multi-metric fish-based indices in tracking anthropogenic pressures in estuaries: an 8-year case study

Abstract

Facing a generalized increase in water degradation, several programmes have been implemented for protecting and enhancing the water quality and associated wildlife, which rely on ecological indicators to assess the degree of deviation from a pristine state. Here, single (species number, Shannon-Wiener H’, Pielou J’) and multi-metric (Estuarine Fish Assessment Index, EFAI) community based ecological quality measures were evaluated in a temperate estuary over an 8-year period (2005-2012), and established their relationships with an anthropogenic pressure index (API). Single metric indices were highly variable and neither concordant amongst themselves nor with the EFAI. The EFAI was the only index significantly correlated with the API, indicating that higher ecological quality was associated with lower anthropogenic pressure. Pressure scenarios were related with specific fish community composition, as a result of distinct food web complexity and nursery functioning of the estuary. Results were discussed in the scope of the implementation of water protection programmes.

Keywords

Water Framework Directive; Anthropogenic pressures; Marine fish; EFAI; Estuaries; Ecological quality assessment

Introduction

Historical and relentless human disturbance have altered the stability, diversity and ecological integrity of Earth's ecosystems (Kennish, 2002; MacDougall et al. 2013), inducing the loss of several attributes such as taxonomic, phylogenetic, genetic and functional diversity (Solan et al. 2004; Ives and Carpenter, 2007; Cardinale et al. 2012; Naeem et al. 2012; Dolbeth et al. 2013). In particular, marine and estuarine waters have been affected by man's activities worldwide (Borja and Dauer, 2008), resulting in generalized signs of habitat degradation, reduced water quality and loss of aquatic biota (Pérez-Domínguez et al. 2012), threatening the long-term health and sustainability of these important ecosystems (Goldberg, 1995; Costa et al. 2002; Kennish, 2002; Vasconcelos et al. 2007).

Considering that transitional ecosystems provide a wide range of goods and services that benefit human populations (Costanza et al. 2007), the equilibrium between socio-economic growth and environmental protection has become a central topic in marine environmental management (Borja and Dauer, 2008; Elliott and Whitfield, 2011). Over the last decades, a worldwide effort has been dedicated to developing and implementing policy and tools for the protection and/or restoration of aquatic systems, ensuring a sustainable use of water resources, such as the USA and Canada Clean Water Act, Australia's Oceans Policy, the National Water Act in South Africa and the EU Water Framework and Marine Strategy Framework Directives (see details in (Cabral et al. 2012)). For European countries, the Water Framework Directive (EU WFD, 2000/60/EC) has set the main agenda for community action in the domain of water policy, whose main objective is the protection and enhancement of groundwater, inland surface waters, transitional and coastal waters.

In transitional areas, assessment of ecological quality faces one important challenge, given the need to disentangle between changes due to natural variability and to man's activities, a concept usually termed as the "Estuarine Quality Paradox" (Dauvin, 2007; Elliott and Quintino, 2007). Hence, an index used to measure ecological integrity should be sensitive to anthropogenic stressors, but sufficiently robust against natural variability at different spatial and temporal scales (Rice and

Cooper, 2003; Dauvin, 2007; Elliott and Quintino, 2007; Nõges et al. 2009). Consequently, the inclusion of measures of community structure, such as species diversity and composition, and function, such as nursery function and trophic integrity, is determinant for a proper ecological assessment in transitional waters (Elliott and Hemingway, 2002; Harrison and Whitfield, 2004; Elliott and Quintino, 2007; Fonseca et al. 2013). This approach has been followed in several multimetric indices developed for the USA, South Africa and European countries (see Martinho et al. 2008; Pérez-Domínguez et al. 2012).

Apart from multi-metric tools, traditional diversity measures can also be used to infer changes in the health status of an ecosystem, mainly due to their ease of estimation. For instance, a fish community facing significant environmental disturbances, such as habitat loss, will be characterized by a more simplified structure, with lower diversity and complexity (Kennish, 2002; Fonseca et al. 2013). Although several studies delved into relating ecological quality based on estuarine fish assemblages with anthropogenic pressures (e.g. Delpech et al. 2010; Cabral et al. 2012; Drouineau et al. 2012; Fonseca et al. 2013; Pasquaud et al. 2013), one aspect that is clearly lacking is the inclusion of a large and continuous timeframe. The use of large datasets allows a thorough assessment of how climate may shape the patterns of marine biodiversity (Cheung et al. 2009), providing a background of climate-species relationships than can be related with changes due to increasing human pressure.

In this context, the main objective of this work was to evaluate the changes in community-based measures of ecological quality in the Mondego estuary (Portugal) over an eight-year period, and to infer the interrelationship between them and the trends in anthropogenic pressures in the same time frame. This objective was achieved by evaluating a) changes in anthropogenic pressure over time; b) changes in several fish-based diversity measures; and by c) establishing relationships between diversity measures and anthropogenic pressures over time, based on the principle that higher stressed environments will result in lower diversity and ecological quality status.

Materials and Methods

Fish sampling surveys and laboratory procedures

Fish were collected in the Mondego estuary, located on the Atlantic coast of Portugal (40°08’N, 8°50’W) (see detailed description of the Mondego estuary in (Martinho et al. 2007). Sampling was performed between 2005 and 2012 at five stations (Fig. 1) during the night, starting at high water of spring tides, using a 2m beam trawl with one tickler chain and a stretched mesh size of 5mm in the cod end.

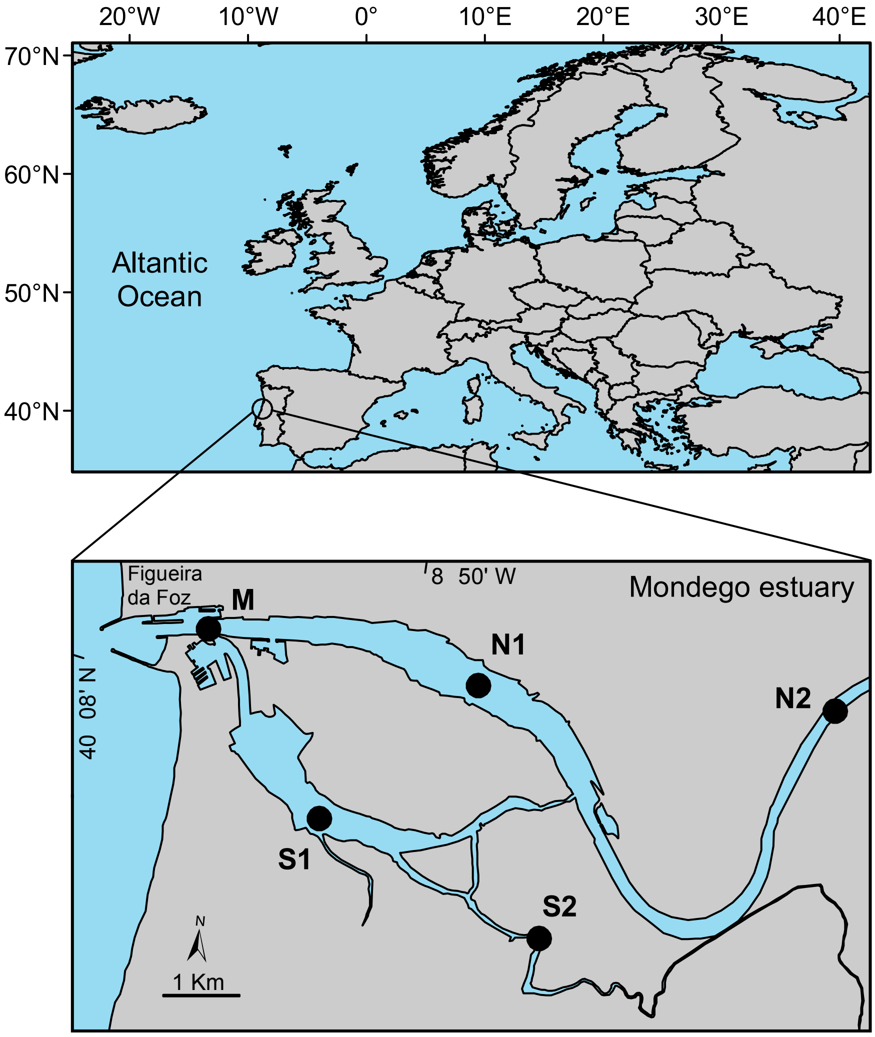

Figure 1 Location of the Mondego estuary in the Portuguese Atlantic coast, highlighting the sampling stations (black circles).

The selected sampling sites covered the majority of the estuarine area, representing several habitats and salinity ranges, providing a good overview of the whole ecosystem. At each sampling station, 3 hauls were towed at an average speed of two knots, covering at least an area of $500m^2$. Samples were transported in iceboxes to the laboratory, where fish were sorted, identified, measured (total length – TL, to the nearest 1 mm) and weighted (wet weight – WW, with 0.01 g precision).

Considering the recommendations for evaluating the ecological quality of Portuguese transitional waters for the EU WFD, that fish sampling should be performed in the spring due to higher species richness and abundance (Cabral et al. 2012), sampling was performed in two months in each year during spring, and data from all five stations in the two months every year were averaged, in order to provide a suitable record of the structure and composition of the fish assemblages. Due to technical constraints, data was only available for one month in 2008. A total of 203 hauls were considered in this study.

Data acquisition and analysis

The assessment of anthropogenic pressures was initially based on 12 descriptors according to (Fonseca et al. 2013), taking into account the availability and quality of data. These pressures reported in Table 1 were chosen since they were considered to produce a significant negative effect on fishes, and included bank regulation, dredging, interference in the hydrographic regime, river flow and dams, industry, population density, shellfish quality, agriculture production, aquaculture production, intensity of port/marina developments, commercial and recreational fishing. Data was acquired from public and governmental sources (obtained in online datasets or by specific request), which provide suitable data required for these types of studies (Vasconcelos et al. 2007; Fonseca et al. 2013), and merged into a single data frame for the lower Mondego area (see Table 1). Given the time frame of 8 consecutive years, data on sediment metals and PAHs was not included since there were no available records that covered this whole period, and overall values were particularly low (e.g. Coelho et al. 2006; Nunes et al. 2011; Baptista et al. 2013). Data on shellfish quality, aquaculture production, bank regulation and interference on the hydrographic regime within the estuary were also not considered further, since the available data consisted of equal values in all years.

Table 1 Pressure indicators used to evaluate the total anthropogenic pressure in the Mondego estuary, Portugal, between 2003 and 2012, including the types and sources of data. (Abbreviations: GE – Google Earth; INAG – National Institute of Water; INE – National Institute of Statistics; DRAP – General Direction of Agriculture and Fisheries; Aquamondego – Aquaculture Producers Association of Figueira da Foz; [1]Dataset provided upon request; [2]Online dataset).

Pressure indicators	Description	Source
Bank regulation	Percentage of regulated estuarine bank length	GE
Dredging	Mean volume and intensity	Port Authority [1]
Interference in hydrographic regime	Percentage of area occupied by structures interfering with the hydrographic regime	GE
River flow and dams	Flow (dam^3) and number of large dams	INAG [2]
Industry	Number of industries in the watershed	INE [2]
Population	Population density of watershed surrounding areas	INE [2]
Shellfish quality	Categories according to national standards	IPMA [2]
Agriculture	Used agricultural surface area	DRAP [1]
Aquaculture	Number and area occupied	AQUAMONDEGO [1]
Intensity of port/marina developments	Number of berths in marinas/port areas	Port Authority [1]
Commercial fishing	Number of licensed boats	Port Authority [1]
Recreational fishing	Number of recreational licensed fishermen	INE [2]
Anthropogenic Pressure Index - API	Sum of all standardized indicators	

Global anthropogenic pressure was assessed through a multi-metric pressure index (Anthropogenic Pressure Index, API), based on the final set of eight descriptors, and calculated as the sum of the standardized values of each pressure descriptor (Table 1). Standardization of data was required since most descriptors were obtained in different units, and was performed according to (Vasconcelos et al. 2007) and (Fonseca et al. 2013):

$$P_{S,i} = (P_{A,i} - P_{\min,i})/(P_{\max,i} - P_{\min,i})$$

where P_S represents the standardized pressure value for each descriptor for each year *i*; P_A the original pressure value, and P_{min} and P_{max} the minimum and maximum pressure values for each indicator, respectively. The overall score for each descriptor ranges between 0 and 1, representing the lowest and highest pressure values, respectively.

Fish catch data was converted to densities (Ind. $1000m^{-2}$) (Table 2), and each species was assigned to several functional guilds according to (Franco et al. 2008) (see Table 1 in Nyitrai et al. 2012 for the complete classification).

Table 2 Average yearly densities (Ind. $1000m^{-2}$) of each fish species for the time period between 2005 and 2012 in the Mondego estuary, including also the total species number per year.

Species	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**
Ammodytes tobianus	0.06	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Anguilla anguilla	1.61	0.19	0.12	0.50	0.74	0.04	0.64	0.05
Aphia minuta	0.00	0.08	0.00	0.00	0.10	0.16	0.13	0.00
Atherina boyeri	0.06	0.00	0.00	0.00	0.00	0.00	0.17	0.00
Atherina presbyter	0.06	0.00	0.00	0.08	0.44	0.00	0.04	0.00
Carassius auratus	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.00
Challionymus lyra	0.55	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Chelidonichthys lucerna	0.05	0.00	0.49	0.17	0.19	0.38	0.13	1.00
Chelon labrosus	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00
Ciliata mustela	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Conger conger	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.11
Dicentrarchus labrax	2.28	0.39	0.52	0.83	1.75	0.67	1.10	1.57
Diplodus vulgaris	0.00	0.00	0.08	0.00	0.35	0.41	0.17	0.17
Engraulis encrasicolus	0.09	0.04	0.00	0.00	0.05	0.00	0.04	0.00
Gobius niger	0.10	0.04	0.17	0.25	0.24	0.26	0.21	0.69
Hippocampus hippocampus	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.15
Liza aurata	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Liza ramada	0.00	0.08	0.00	0.08	0.13	1.29	0.21	0.06
Mullus surmuletus	0.06	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Platichthys flesus	0.67	0.27	0.27	2.33	6.07	1.76	3.05	9.96
Pomatoschistus microps	3.49	3.03	4.37	1.58	4.84	0.90	32.67	4.13
Pomatoschistus minutus	1.82	7.58	0.69	0.08	1.24	0.97	1.17	1.58
Sardina pilchardus	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Solea senegalensis	0.00	0.00	0.00	0.08	0.14	0.07	0.00	0.00
Solea solea	1.01	0.97	1.70	2.08	2.78	8.13	4.82	3.05
Sparus aurata	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00
Spondyliosoma cantharus	0.05	0.00	0.00	0.00	0.18	0.00	0.04	0.00
Symphodus bailloni	0.09	0.04	0.00	0.17	0.00	0.00	0.00	0.00
Syngnathus abaster	0.05	0.04	0.55	0.00	0.00	0.00	0.59	0.05
Syngnathus acus	0.24	0.08	0.09	0.00	0.09	0.23	0.08	0.12
Trisopterus luscus	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.10
Species number	20	18	13	13	17	13	18	15

Data was analysed for the whole estuary (following Gonçalves et al. 2012), considering also the inexistence of spatially explicit data on anthropogenic pressures available for a more detailed analysis at a water body level.

In order to assess changes in diversity and ecological quality, a set of traditional descriptors that included species number, Shannon-Wiener diversity index (H') and Pielou Evenness index (J'), and a multi-metric index the Estuarine Fish Assessment Index (EFAI - Cabral et al. 2012) were determined. These analyses were performed in order to determine whether or not single- or multi-metric indices could signal the changes in anthropogenic pressures. The traditional indices were computed using the "vegan: Community Ecology Package" library (Oksanen et al. 2013) in R software (R Development Core Team, 2008). The Ecological Quality Ratios (EQR; 0-1) and corresponding Ecological Quality Status (EQS; Bad, Poor, Moderate, Good, High) were calculated based on the EFAI (Cabral et al. 2012). In sum, the EFAI is composed of the following metrics: species richness, percentage of individuals of marine migrants, number of estuarine resident species, percentage of individuals of estuarine resident species, number of piscivorous species, percentage of individuals of piscivorous species, diadromous, introduced and disturbance sensitive species, and a score of 1, 3 or 5 is attributed to each metric, depending on the established reference conditions. The EFAI is calculated by summing the scores of each individual metric, and then divided by the maximum possible score (35) in order to obtain the respective EQR (Cabral et al. 2012).

Relationships between pressure indicators (considering the API and the individual pressure data) and the measures of diversity and ecological quality, and between the diversity and ecological quality measures were determined by Pearson correlation coefficients, performed in R software (R Development Core Team, 2008). After recognition of the significant correlations between diversity and ecological quality measures with the API, a non-metric Multidimensional Scaling (MDS) analysis based on a Bray-Curtis similarity matrix was performed on standardized individual anthropogenic pressure data, in order to define periods of contrasting anthropogenic influence in the estuary. In order to validate the interpretation of the MDS, an analysis of similarities (ANOSIM), built on a simple nonparametric permutation procedure, was applied to the similarity matrix underlying the ordination of the samples (treatments) (Clarke and Warwick, 2001). Subsequent MDS and ANOSIM procedures were also performed on the individual metrics composing the EFAI, and also on square root transformed species densities data, in order to identify possible changes in community structure in the fish assemblage through time. The SIMPER procedure provided the percentage contributions of each species and metrics to the average dissimilarity between groups. All these analyses were performed in PRIMER software package (version 5.0) (Clarke and Warwick, 2001), considering a significance level of 0.05 in all test procedures.

Results

Anthropogenic pressures

In general, anthropogenic pressures showed a decreasing tendency towards the end of the study period, as seen in Figure 2. This decrease was mainly observed in dredging activities, recreational fishing, population density, agriculture occupied area and number of industries in the watershed. In contrast, fish landings in the Figueira da Foz harbour and river runoff were higher near the end of the study period (Fig. 2). In particular, higher river runoff matched the periods with higher rainfall in the river basin (see Martinho et al. 2007; Nyitrai et al. 2012, for further information). This decreasing tendency in global anthropogenic pressure was evident in the continuous reduction of the API scores from 5.73 in 2005 to 2.88 in 2012 (Fig. 2).

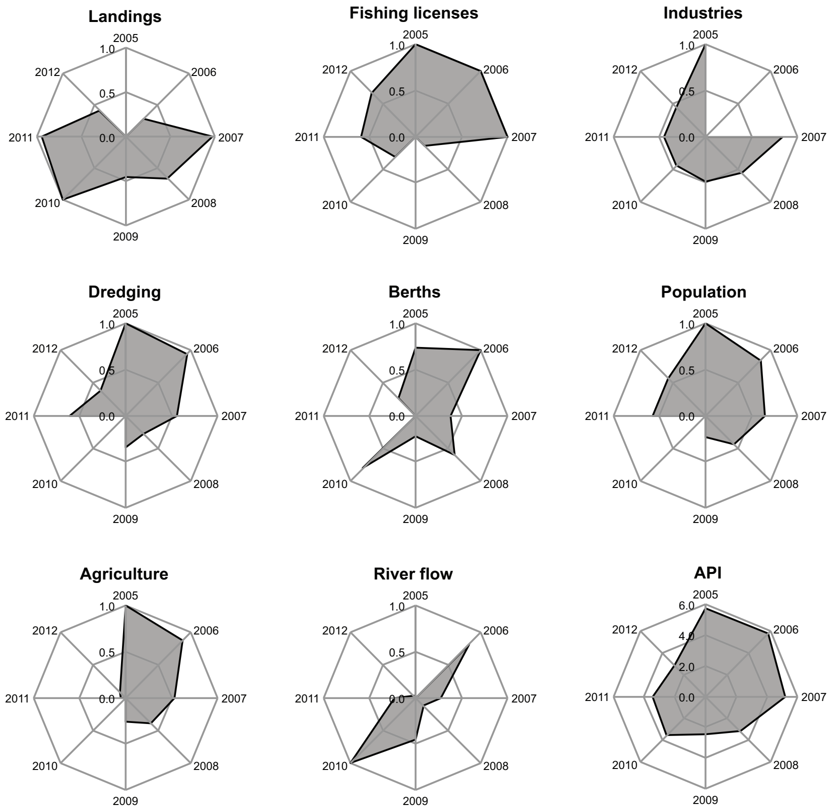

Figure 2 Standardized pressure indicators used to evaluate the total anthropogenic pressure in the Mondego estuary between 2005 and 2012, and the Anthropogenic Pressure Index (API, last chart), determined as the sum of all standardized pressure indicators.

Diversity and ecological quality descriptors

A total of 31 fish species were found in the estuary between 2005 and 2012 (Table 2). Higher densities were observed for the marine juvenile migrants *Dicentrarchus labrax*, *Platichthys flesus* and *Solea solea*, and the estuarine residents *Pomatoschistus microps* and *Pomatoschistus minutus*. Higher species number was observed in 2005 (20 species), followed by a decreasing trend until 2008 (13 species) (Fig. 3a). Between 2009 and 2012, an oscillatory pattern was observed in terms of species number, as seen in Figure 3a. Shannon-Wiener’s diversity (H’) was higher in 2005 and 2009, and lower in 2006 and 2011, a trend also observed for Pielou’s Evenness (J’) (Fig. 3a). Overall, 2006 and 2011 came out as the lower ranking years in terms of diversity measures (H’ and J’), but not in species number.

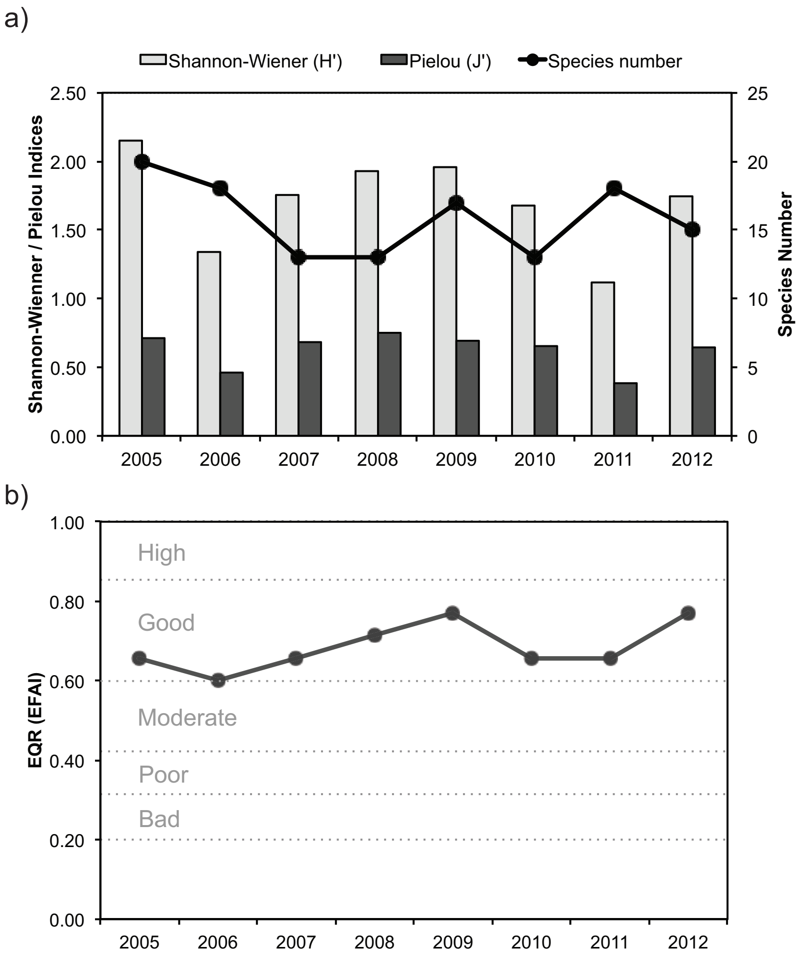

Figure 3 Yearly variation of single- (**a**) and multi-metric (**b**) indices between 2005 and 2012, based on fish data.

According to the EFAI, the global ecological status of the Mondego estuary increased slightly between 2005 and 2009, and between 2010 and 2012. All years were classified as in Good status, with EQR values ranging from 0.60 in 2006 to 0.77 in 2009 and 2012 (Fig. 3b).

Relationships between diversity, ecological quality and anthropogenic pressures

A comprehensive analysis of the relationship between diversity and ecological quality descriptors with individual anthropogenic pressures and the API was performed using Pearson correlation coefficients (Table 3).

Table 3 Pearson correlation coefficients between the standardized pressure indicators, the Anthropogenic Pressure Index and the several ecological quality indices for the period between 2005 and 2012. Significant values (p<0.05) are highlighted with *.

	EQR (EFAI)	Shannon-Wiener (H')	Pielou (J')	Species number
Landings	-0.09	-0.43	-0.17	-0.69
Fishing licenses	-0.62	-0.20	-0.30	0.35
Industries	0.15	0.66	0.61	-0.01
Dredging	-0.50	-0.10	-0.34	0.78
Berths	-0.56	0.18	0.17	0.00
Population	-0.40	-0.01	-0.20	0.61
Agriculture	-0.49	0.29	0.11	0.51
River flow	-0.43	-0.36	-0.28	-0.18
API	-0.81*	-0.03	-0.14	0.36

A significant correlation was obtained between the EQR (EFAI) scores and the API, denoting a strong negative linear relationship (r=-0.81; p<0.05). No other correlations were obtained between pressure indicators and diversity and ecological quality measures, or either between the any of diversity indices and the ecological quality (EFAI) measures.

Responses of fish assemblages to distinct pressure scenarios

Two distinct groups of standardized pressure data were observed in the MDS plot (2005/06 and 2007/12, figure not shown, stress value=0.05), validated by the ANOSIM procedure (Global R=0.66; p<0.05). In terms of pressure scenarios, the period of 2005/06 was characterized by higher anthropogenic pressures considering nearly all indicators (related with habitat disturbance and indirect sources of pollution), with the exception of fisheries landings in the Figueira da Foz harbour and river flow (Fig. 4a). In contrast, a lower anthropogenic pressure characterized the period of 2007/12, with the exception of fisheries landings, which increased towards the end of the study period (Fig. 4b; see also Fig. 2).

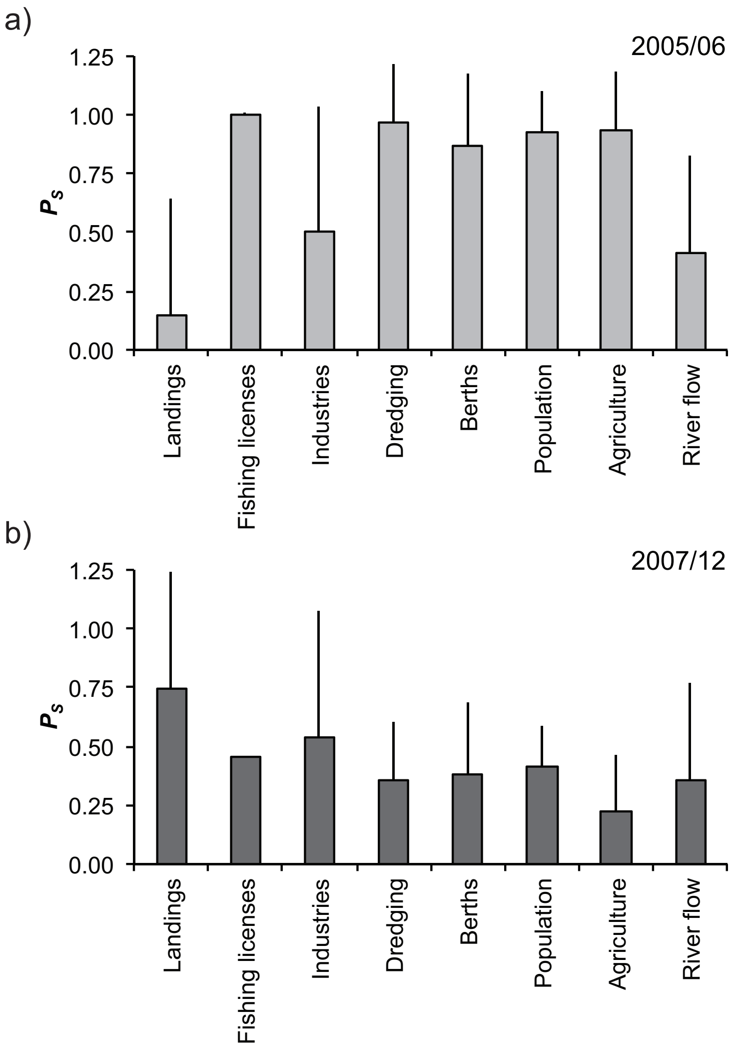

Figure 4 Average values of the standardized pressure indicators that compose the Anthropogenic Pressure Index (API) considering the two periods determined by the MDS analysis: (**a**) 2005/06 (light grey) and (**b**) 2007/12 (dark grey). Vertical lines represent the standard deviation of the average values.

A SIMPER analysis performed on the Bray-Curtis similarity matrix for the individual metrics of the EFAI determined that the percentage of marine migrants, estuarine residents, piscivorous and diadromous species contributed with 94.1% for the dissimilarity between anthropogenic pressure scenarios of 2005/06 and 2007/12, explaining 32.7% of the total variation. In particular, the percentage of marine migrants and piscivorous species was higher in the period of 2007/12, while the percentage of estuarine residents and diadromous was higher in 2005/06 (Fig. 5).

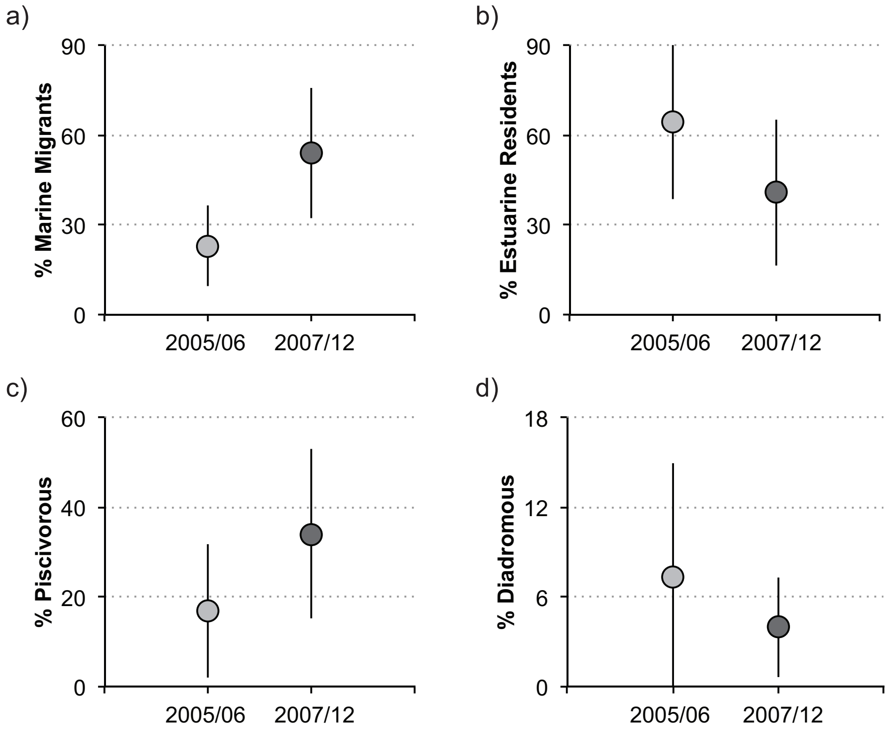

Figure 5 Average values (circles) and respective standard deviation (vertical lines) for the relative contribution of each metric that contributed up to 94% of the dissimilarity between the year periods of 2005/06 (light grey) and 2007/12 (dark grey), according to the SIMPER analysis (33%).

A more in-depth analysis on the fish community composition at the species level also revealed two distinct groups in 2005/06 and 2007/12 (ANOSIM Global R=0.82; p<0.05) (Fig. 6).

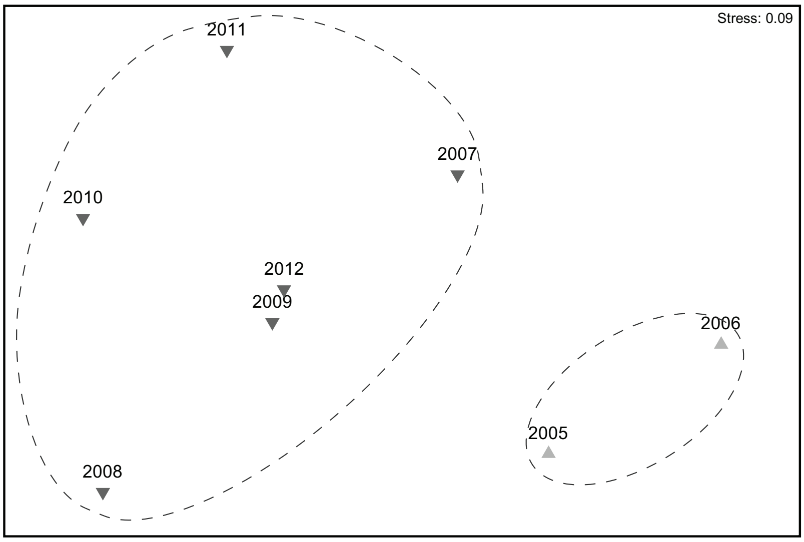

Figure 6 Two-dimensional MDS plot of the species composition in each year, separated by the year periods previously defined: 2005/06 (light grey) and 2007/12 (dark grey). The dashed line represents 60% similarity between year periods.

In total, six species were responsible for >50% of the dissimilarity between anthropogenic pressure scenarios: *P. minutus*, *P. flesus*, *P. microps*, *S. solea*, *A. anguilla* and *C. lucerna*, while 15 other species contributed with nearly 40% to the dissimilarity between scenarios (SIMPER, 43.7% of total variation explained) (Table 4).

Table 4 List of fish species and respective cumulative percentage that contributed up to 90% of inter-group dissimilarity between the periods of 2005/06 and 2007/12 (SIMPER, 43.7%).

Species	2005/06 Average Abundance	2007/12 Average Abundance	Cumulative Percentage
Pomatoschistus minutus	2.05	0.93	12.04
Platichthys flesus	0.67	1.79	23.25
Pomatoschistus microps	1.80	2.37	32.68
Solea solea	1.00	1.87	41.19
Anguilla anguilla	0.85	0.52	46.13
Chelidonichthys lucerna	0.11	0.59	51.05
Challionymus lyra	0.47	0.00	55.60
Dicentrarchus labrax	1.07	1.01	59.98
Diplodus vulgaris	0.00	0.39	63.73
Liza ramada	0.14	0.42	66.97
Syngnathus abaster	0.20	0.29	69.88
Gobius niger	0.26	0.53	72.62
Trisopterus luscus	0.26	0.05	75.14
Symphodus bailloni	0.25	0.07	77.46
Mullus surmuletus	0.22	0.00	79.64
Ammodytes tobianus	0.21	0.00	81.78
Atherina presbyter	0.12	0.19	83.72
Engraulis encrasicolus	0.25	0.07	85.62
Aphia minuta	0.14	0.18	87.41
Solea senegalensis	0.00	0.15	88.99
Syngnathus acus	0.38	0.29	90.57

Of these species, *P. flesus* and *S. solea* were more abundant in the period characterized by a lower anthropogenic pressure.

Discussion

This work contributed to a more thorough understanding of the usefulness of estuarine fish assemblages as indicators of anthropogenic pressures, particularly considering a time frame of eight consecutive years. Estuarine fish assemblages have been used as indicators of change over the last several years, in the scope of several programmes implemented for the protection of water resources worldwide (Borja and Dauer, 2008; Cabral et al. 2012; Pérez-Domínguez et al. 2012; for a review). The use of fish as indicators relies mainly on their ubiquitous presence in aquatic ecosystems, easy collection, handling and identification, presence in several trophic and ecological levels, sensitivity to habitat loss, and on their wide range of likely responses to stress (Elliott and Hemingway, 2002; Harrison and Whitfield, 2004; Pérez-Domínguez et al. 2012).

Anthropogenic pressures have dramatically increased around transitional areas, leading to changes in their physical, chemical and biological compartments (Marchand et al. 2002; Kennish, 2002), inducing an overall lower trophic integrity in highly impacted ecosystems (Fonseca et al. 2013). The assessment of anthropogenic pressure status between 2005 and 2012 comprised a thorough assessment of human pressure gradients, constrained by local conditions and data availability. As in (Borja et al. 2011), pressure data was not weighted regarding the expected impact on fish assemblages, also due to the lack of adequate spatially and temporally explicit data. Most of the obtained data belonged to the category of important pressures such as port and other industrial activities, resource exploitation (e.g. fisheries and agriculture), and population density, a characteristic of many estuaries worldwide (e.g. Marchand et al. 2002; Aubry and Elliott, 2006; Vasconcelos et al. 2007). Despite that chemical pollution (by metals and/or organic compounds) has been used as a proxy of anthropogenic stress (e.g. Delpech et al. 2010; Drouineau et al. 2012; Fonseca et al. 2013) mainly due to its disruptive potential of the life cycle of fishes (Kennish, 2002; Marchand et al. 2002; Shahidul Islam and Tanaka, 2004), data that covered the spatial and temporal scales of this study were not available, and thus were not included in the pressure assessment. However, previous studies reported that the levels of metals (Coelho et al. 2006), PCBs (Baptista et al. 2013), PCDD/Fs and dioxin-like PCBs (Nunes et al. 2011) in the Mondego estuary's sediments are particularly low. Hence, and despite that the availability of such data would certainly have improved the evaluation of the global pressure status, the existing chemical load most certainly did not influence the structure and composition of the fish assemblage in a significant way.

In terms of anthropogenic stress, the Mondego estuary is a moderate-to-low impacted estuary, particularly due to low chemical loads and industrialization in the catchment area (as also observed by Vasconcelos et al. 2007). The system has also been recovering from a particularly harsh eutrophication scenario in the early 1990's, showing significant improvements in water quality and faunal composition (see (Dolbeth et al. 2013). In agreement, a decreasing trend in the total anthropogenic pressure measured by the API was observed between 2005 and 2012, reflecting mainly a decline in dredging activities, total number of ship berths, population density, agriculture occupied area and number of industries in the watershed over the last decade.

Considering estuarine habitat use, the fish assemblage was composed mainly of estuarine resident and marine migrant species, in parallel with many estuarine ecosystems worldwide. Lower values of the traditional diversity descriptors Shannon-Wiener (H') and Pielou (J') were observed in 2006 and 2011. In contrast, lower species number was observed in 2007, 2008 and 2010, providing an inconsistent trend when comparing with the previous indices. According to the EFAI, the global ecological status of the Mondego estuary was stable during the study period, with all years classified as in Good status; however, EQR values increased from 0.60 in 2006 to 0.77 in 2012. The increase in EQR was mainly attributed to higher contributions of marine migrants and piscivorous species, as observed in similar studies (e.g. Gonçalves et al. 2012; Fonseca et al. 2013). Lower abundances of these functional groups have been particularly related with higher anthropogenic stress like chemical pollution, habitat loss or overfishing (e.g. Kennish, 2002; Marchand et al. 2002; Coates et al. 2007; Courrat et al. 2009; Uriarte and Borja, 2009; Fonseca et al. 2013; Pasquaud et al. 2013), implying a significant reduction in the nursery role of an estuary. In this case, and despite a reduction in species number from 2005 to 2012, there seemed to be an increase in the nursery function of the estuary (as also observed by (Nyitrai et al. 2012) synchronized with the increase in ecological status.

However, there was no concordance between the diversity measures and the EFAI, implying that management actions based on both approaches can, and will, provide different outcomes regarding the evaluation of an ecosystem's health status. In addition, the EFAI was the only index that was significantly correlated with the API (r=-0.81), confirming the initial paradigm that an estuary with higher anthropogenic disturbance has an overall lower ecological quality, and vice versa. This delivers a prominent argument in favour of multi-metric tools in ecological quality assessment when comparing to single-metric ones, as postulated by several other authors (e.g. Hughes et al. 1998; Weisberg et al. 1997; Dauvin, 2007; Borja et al. 2011), particularly those that include a combination of structural and functional compartments of fish communities, as well as disturbance-sensitive taxa (Cabral et al. 2012; Pérez-Domínguez et al. 2012; Harrison and Kelly, 2013).

These results also demonstrate that the EFAI was developed robustly enough, being able to detect changes in fish assemblages related to anthropogenic pressures, against a background of natural variability, in agreement with several authors (e.g. Cabral et al. 2012; Gonçalves et al. 2012; Fonseca et al. 2013). This is, on its own, quite a challenging task, considering that environmentally dynamic areas such as estuaries can dampen the effects of anthropogenic stress, turning even more difficult the detection of anthropogenic signals against a background of environmental noise (i.e. the "Estuarine Quality Paradox", Elliott and Quintino, 2007). In addition, most stressors usually act simultaneously with additive, synergistic or antagonistic effects that depend on the biological organizational level and on the stressor's characteristics (Brown et al. 2002; Crain et al. 2008), increasing the overall uncertainty levels in ecological quality assessments. This is particularly important in this case study, where despite the high influence of climatic variability on the local fish fauna over the last decade (e.g. Martinho et al. 2007; Baptista et al. 2010; Nyitrai et al. 2012), it was still possible to ascertain changes in the fish assemblage attributable to anthropogenic stress.

In the present study, two distinct periods of generalized higher (2005/06) and lower (2007/12) anthropogenic pressure were observed, allowing relating the composition and functioning of fish assemblages with both pressure scenarios. In more detail, the first period (2005/06) was characterized by a higher pressure intensity, namely regarding the number of fishing licenses, dredging activities, ship berths, population density and agriculture activities, when comparing with the second period of 2007/12. All these pressures can directly impact on habitat usage by fish and on their overall abundance patterns within an estuary (Kennish, 2002; Marchand et al. 2002), and were reflected in the overall lower ecological quality of the estuary. Despite that the detection signal between pressure scenarios was not very strong, differences in anthropogenic pressure were still matched in terms of the fish community structure, considering the individual metrics that compose the EFAI. In fact, the main groups that contributed for the dissimilarity between periods were the marine migrants and piscivorous species, which presented higher relative abundance during the period of lower pressure, and the estuarine residents and diadromous, which presented a contrasting trend.

A higher abundance of marine migrant species was characteristic of a higher ecological quality, in agreement with several other studies (e.g. Uriarte and Borja, 2009; Fonseca et al. 2013; Pasquaud et al. 2013), reinforcing the role of estuaries as nursery grounds. The higher abundance of piscivorous species in the lower pressure period provided a clear signal of higher trophic integrity and complexity of estuarine food webs, given the presence of higher trophic levels (Cabral et al. 2012). On the other hand, the reduction in relative proportion of estuarine residents was contrary to what would be expected in a higher ecological status, as estuarine resident species are a structuring component of estuarine fish assemblages worldwide. However, this might be related to an effective decrease in estuarine resident species number from 2005 to 2012 and to the decrease in relative abundance of the sand goby *P. minutus*, the species that most contributed to the dissimilarity between both periods in terms of species composition. Regarding the diadromous species, there was a slight decrease in relative abundance between 2005/06 and 2007/12, which was mainly due to a reduction in the abundance of *A. anguilla*. These species are good indicators of sea-estuary-river connectivity, as they are strongly impacted by man-made barriers such as dams (Cabral et al. 2012), and their abundance trends might be linked to the high inter-annual variability in river runoff. In terms of community composition, a similar trend was observed regarding the determination of year periods, reinforcing the concept that different anthropogenic pressure scenarios would have specific effects on distinct compartments of the estuarine fish fauna.

The use of anthropogenic pressure indices in transitional waters has gained attention over the last years, mainly in the scope of water protection programmes such as the EU WFD (e.g. Aubry and Elliott, 2006; Vasconcelos et al. 2007; Courrat et al. 2009; Delpech et al. 2010; Borja et al. 2011; Cabral et al. 2012; Fonseca et al. 2013). On one side, they have been quite effective in delivering valuable tools for assessing total anthropogenic pressure within a system, identifying similarities and/or differences between estuaries at wide spatial scales, and relating particular changes with the responsible source (Vasconcelos et al. 2007; Borja et al. 2011). However, they have also some shortcomings, mainly concerning their application in the already variable transitional areas (Borja et al. 2011). In fact, the current methods for disentangling between climate- and anthropogenic-induced pressures are mostly effective when the anthropogenic stressor is particularly harsh (Elliott and Quintino, 2007). However, if the overall level and intensity of anthropogenic pressure is relatively low and/or stable in time, as in the present case, it can be more difficult to pinpoint the most probable causes of change within a fish assemblage. Another concern for management purposes is the high variability in the sensitiveness of the detection of change when relating ecological quality measures with pressure indices, as required by the EU WFD (Borja et al. 2011; Pasquaud et al. 2013). An improvement in these methodologies would be the development of specific pressure impact models (Drouineau et al. 2012), increasing the overall availability and reliability of the data used for evaluating pressure gradients (Brown et al. 2002; Delpech et al. 2010; Pérez-Domínguez et al. 2012), and improving the definition and quantification of the responses of transitional ecosystems to human activities (Elliott and Quintino, 2007; Borja et al. 2011).

Assessing pressure-response patterns in an estuary should take into account the time frame between pressure and response from the biological assemblages, as well as the response time of fish-based indicators to pressure, which is not always observed (e.g. Pasquaud et al. 2013). In fact, while some pressures have an immediate effect on fish populations, others may act on a longer time scale (Elliott and Quintino, 2007). In this sense, one aspect that improves from the previous studies is the inclusion of a large time frame, which provides a more detailed overview when analysing ecological responses to anthropogenic stress. While some studies have considered a larger time frame (>10 years) for the evaluation of ecological quality, which have nonetheless allowed for a better characterization of changes (South Africa - Harrison and Whitfield, 2004), (UK - Coates et al. 2007), none of them related fish data with anthropogenic pressure gradients as in the present work, a critical aspect in ecological quality assessment.

Despite that this study was performed during a period of particular climatic instability, related with highly variable precipitation regimes (e.g. Leitão et al. 2007; Martinho et al. 2007; 2009; Baptista et al. 2010; Dolbeth et al. 2010; Nyitrai et al. 2012), the multi-metric index was still able to recognize changes in the fish assemblage significantly correlated with the trends in anthropogenic pressure over time. This work also highlighted the possible difficulties that managers may face when assessing variability in anthropogenic pressure in a continuous time frame, particularly considering that, with few exceptions, existing pressures will change in a continuous manner, making it harder to evaluate pressure-response relationships. Prolonging existing long-term datasets and testing these methodologies in other estuarine ecosystems with different degrees of anthropogenic stress will certainly improve our capability of disentangling the role of climate and anthropogenic forcing in estuarine fish assemblages and the implementation of water protection programmes worldwide.

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CHAPTER

V

General Discussion and Conclusions

Estuarine fish assemblages: structure, composition and functionality

The first main objective of the present thesis was to assess the influences of distinct environmental conditions on the structure and composition of an estuarine fish assemblage. Although weather conditions were highly variable in the Portuguese territory over the last decade including droughts, heat waves and heavy rainy periods, the main core of the Mondego estuary fish assemblage remained relatively unchanged during this period. The most significant changes due to climate occurred among the species that were usually less abundant inside the estuary. This pattern has been observed in several other studies as well, when the major changes due to environmental variations occurred among less frequent species, while the dominant species remained relatively stable (e.g. James et al. 2008; Selleslagh and Amara, 2008; Henderson et al. 2011).

The most affected groups were the marine and freshwater stragglers, which were strongly influenced by the variations in freshwater runoff, salinity and water temperature. A general trend could be observed in the Mondego estuary regarding marine stragglers, as during the dry years its species number and abundance increased due to the higher salinity intrusion inside the estuary, while in rainy periods they appeared in lower numbers. This pattern has been observed in many other estuarine systems as well (Ecoutin et al. 2005; Selleslagh and Amara, 2008; Pasquaud et al. 2012). The increased number of marine stragglers during dry years suggests its influence on estuarine food webs (Dolbeth et al. 2008) and consequent impacts on local fish and invertebrate assemblages. A prolonged appearance of marine stragglers in estuaries might cause considerable changes in estuarine food webs and in the ecosystem functioning.

Freshwater stragglers only appeared in the estuary in the beginning and in the end of the study period, in very low numbers. Besides climatic changes, anthropogenic interventions such as continuous dredging activities also contributed to an upstream displacement of the estuarine salinity gradient (Leitão et al. 2007), leading to a gradual decrease of freshwater species over the last years. In general, variations of species of marine or freshwater affinity may suggest the influence of natural or anthropogenic stressors (Chícharo et al. 2006; Martinho et al. 2007; Vasconcelos et al. 2012).

Regarding dominant species, estuarine resident and marine-estuarine species composed the majority of the fish assemblage among the estuarine habitat use functional groups, a common pattern in European estuaries (e.g. Selleslagh and Amara, 2008; Nicolas et al. 2010; Vasconcelos et al. 2012). Hence, the Mondego estuary fish assemblage was similar in structure with the typical European Atlantic seaboard estuarine fish assemblage, as described by Elliott and Dewailly (1995). However, there was a decrease in abundance and production of estuarine resident and marine-estuarine dependent species during the dry periods and an increase in rainy periods. The decrease in abundance of marine-estuarine dependent species during dry periods could be attributed to the reduction in extension of the river plumes in the adjacent coastal area, a determinant factor for the recruitment success of these species (Martinho et al. 2009, 2012; Vasconcelos et al. 2012). In fact, recruitment strength of these species is determined over the pelagic larval stage (van der Veer et al. 2000), and the larvae of these species depend on river plumes in coastal areas for finding estuaries (Vinagre et al. 2007; Martinho et al. 2012). In this process, several cues play an important role, including acoustic, chemical, rheotactic, magnetic or thermal features for orienting fish larvae towards estuarine nursery grounds (Arvedlund and Takemura, 2006). In rainy periods, an opposite pattern was observed, which was most probably related with an increase in the extension of river plumes, increasing the probability of fish larvae detecting the specific cues that orient them towards the estuaries.

The estuarine resident species also decreased in abundance during the dry years, while in rainy periods no considerable changes were observed in these species. High salinities can be detrimental for the survival of the eggs of these species (Fonds and van Buurt, 1974) that could have been the reason for their decrease during dry periods. The larvae and juveniles of this group remain most of their life cycle within estuaries (Whitfield, 1999) and in addition, these species produce benthic eggs (Pampoulie, 2001), which reduces the risk of being flushed out. Probably therefore the abundance of these species remained relatively stable in rainy periods.

The marine-estuarine dependent species *Dicentrarchus labrax*, *Platichthys flesus* and *Solea solea*, and the estuarine residents *Pomatoschistus microps* and *Pomatoschistus minutus* were the most abundant species in the Mondego estuary. This is a common pattern in most European estuaries (e.g. Selleslagh and Amara, 2008; Nicolas et al. 2010; Vasconcelos et al. 2010). *D. labrax*, *P. flesus* and *S. solea* are commercially important species: their adults inhabit the coast and continental shelf and are highly valuable marine fishes captured in Portuguese fisheries, mainly in multi-species fisheries such as trammel nets and longlines (Vasconcelos et al. 2010). *P. microps* and *P. minutus* are important inhabitants of temperate estuaries, lagoons and coastal areas (Arruda et al. 1993; Leitão et al. 2006; Dolbeth et al. 2007), also acting as prey for larger demersal fish and predators of small benthic and epibenthic organisms (Freitas et al. 2011). These are small-sized species with a short life span, and can be used as ecological indicators of environmental changes in small and larger-scales as well (Dolbeth et al. 2007, 2010; Nyitrai et al. 2013). The generally high abundance of marine migrant species demonstrates the importance of the Mondego estuary as a nursery area.

The invertebrate and fish feeders were the most abundant feeding mode, which has also been observed in estuaries (Cardoso et al. 2011), coastal lagoons (Malavasi et al. 2004), tidal marshes (Mathieson et al. 2000) and coastal areas worldwide (Unsworth et al. 2007). In the Mondego estuary, the most abundant species of this group were *D. labrax* and *P. flesus*, which generally occurred in high densities within the estuary. During the dry years, some marine predator species belonging to this group appeared with higher densities, probably due to the higher salinity intrusion inside the estuary, demonstrating that during the dry period the estuarine conditions were more similar to those of the adjacent coastal area. Invertebrate feeders also occurred with high densities in the estuary, a common pattern in European estuaries (e.g. Malavasi et al. 2004; Ramos et al. 2015). Given that estuaries are highly productive ecosystems in terms of macrobenthic invertebrates, the high food availability that characterizes these habitats allows for a high abundance and prevalence of invertebrate feeders. The most abundant species of this group were *P. microps* and *P. minutus*, the two estuarine resident species that predominantly feed on benthic invertebrates (Leitão et al. 2006) and *S. solea*, a marine-estuarine dependent species that feeds mainly on invertebrates (Dolbeth et al. 2008).

The guild approach used in the present study provides a suitable basis to determine changes across systems and geographical areas (Elliott et al. 2007; Franco et al. 2008). Categorizing fish into functional categories supports the evaluation of the functioning of estuarine ecosystems (Elliott et al. 2007). The estuarine habitat use and feeding mode functional groups demonstrate that there are similarities between estuarine fish assemblages on the functional level, indicating a shared functional role of transitional waters not detected by the taxonomical composition analysis (Franco et al. 2008).

Impacts of local and large-scale climatic events on estuarine fish fauna

Local and large-scale climatic events have considerable influences on marine and estuarine fish assemblages worldwide (e.g. Nicolas et al. 2010; James et al. 2013; Pasquaud et al. 2015). Marine fish are influenced by climate directly through physiology, and also indirectly through affecting interactions with predators, prey and competitors, in addition to regulating suitable habitat (Ottersen et al. 2004).

Moreover, the effects of climate change on marine organisms can be detected at various levels, such as growth, swimming speed and activity rates, reproduction, recruitment, phenology, distribution or mortality as well (Rijnsdorp et al. 2009; Drinkwater et al. 2010). In particular, climate change may have strong influence on fish distribution and abundance through changes in growth, survival, reproduction, or responses to changes at other trophic levels (Perry et al. 2005). Also as a consequence of global warming, several marine species in the Northern hemisphere have been observed to migrate deepward and/or northward to remain within their suitable thermal limits (Rose, 2005; Parmesan, 2006; ter Hofstede et al. 2010).

Estuarine fish assemblages are also strongly affected by climatic variability, by influencing estuarine production through changes in either growth or abundance (Attrill and Power, 2002). Furthermore, due to the highly variable and complex environment, fish assemblages in estuaries tend to vary considerably in both composition and abundance between years and seasons (McLusky and Elliott, 2006). Changes in environmental conditions within an estuary may be predictable, or they may be induced by short- and/or long-term climatic fluctuations that have large effects on the abundance and distribution of estuarine fish species (Kupschus and Tremain, 2001; Desmond et al. 2002).

Among local climate patterns, the effects of precipitation, river runoff, salinity and temperature on the Mondego estuary fish assemblage were tested in the present study. Changes in precipitation and consequently in river runoff had considerable effects on the Mondego estuary fish assemblages, as observed elsewhere (e.g. Gillson et al. 2009; Taylor et al. 2010; James et al. 2013). Increased precipitation induces elevated freshwater runoff, causing a higher extension of river plumes in the adjacent coastal area, which is a crucial factor for the juveniles of numerous marine species in finding estuaries (e.g. Le Pape et al. 2003; Baptista et al. 2010; Martinho et al. 2012). A decrease in river runoff induced higher salinity intrusion inside the estuary, which in turn favoured an increase in the proportion of marine species that are adapted to higher salinity levels (Martinho et al. 2007; Nyitrai et al. 2012; Pasquaud et al. 2012). In general, prolonged drought periods that induce higher salinity incursion in the downstream areas of estuaries could result in an upward displacement of suitable habitats, which may cause the disappearance of freshwater species from the whole estuary; this situation has occurred in the Mondego estuary during the last two decades (Leitão et al. 2007; Martinho et al. 2007).

Another important factor that is influenced by the changes in river runoff is the nutrient availability, as during periods of high river runoff nutrient input increases, causing a consequent increase in primary and secondary production (Gillanders and Kingsford, 2002; Baptista et al. 2010). During dry periods, an opposite pattern could be observed. Besides the higher nutrient inputs, elevated river runoff also prevents hypersaline conditions that could cause a substantial decline in species richness, diversity and abundance (Simier et al. 2004; Cyrus and Vivier, 2006). For instance, in the St Lucia estuarine system in South Africa, at major declines in freshwater, salinities increased considerably and a decrease in estuarine and marine species diversity and abundance were observed that decreased onwards, concurrently with a rise in salinities (Vivier et al. 2010). In the Mondego estuary an increase in marine species abundance could be observed during the dry period when there was a higher salinity incursion inside the estuary and species diversity became higher during this period (Martinho et al. 2007, 2010; Nyitrai et al. 2012).

Besides this, another relevant factor that influences the structure and composition of estuarine fish assemblages is air and water temperature. Fish species have their specific temperature range (Coutant, 1977; Scott, 1982) within their physiological functioning and reproduction is optimal. For instance, in the Atlantic coast of Portugal a northward distributional shift could be observed among subtropical flatfish species due to the increase in seawater temperature (Martinho et al. 2010). This general trend has also been observed in other areas, such as in the Bay of Biscay (France) (Hermant et al. 2010) and in the North Sea (Perry et al. 2005). Estuarine organisms are generally more adapted to environmental changes, including changes in temperature, than organisms that live in more stable aquatic environments (Elliott and Whitfield, 2011). Still, temperature is responsible for several factors structuring estuarine fish assemblages (Attrill and Power, 2002; Henderson, 2007) such as spawning migration (Sims et al. 2004), egg mortality (von Westernhagen, 1970), growth (Amara et al. 2009) or small-scale distributions as well (Vinagre et al. 2009).

In addition, the temperature differential between estuarine and marine waters permits fish species to optionally exploit suitable thermal habitats (Attrill and Power, 2004).

As large-scale climate patterns, the influences of the North Atlantic Oscillation (NAO) and sea surface temperature (SST) on the Mondego estuary fish assemblage were measured. The effects of both local and large-scale climate patterns were evaluated with a shorter and longer time lag as well, as climate factors are known to influence fish species over a prolonged time frame (Ottersen et al. 2001; Vinagre et al. 2009). The North Atlantic Oscillation (NAO) is a hemispheric meridional oscillation in atmospheric mass with centers of action near Iceland and the Azores (Trigo et al. 2002; Hurrell and Deser, 2009). The NAO has a dominant influence on storms and precipitation, SST and also controls fluctuations in temperature and salinity, vertical mixing, circulation patterns and ice formation of the North Atlantic Ocean, which affects marine biology through both direct and indirect pathways (Drinkwater et al. 2003; Hurrell and Deser, 2009). In the central Atlantic, the NAO is mainly responsible for changes in SST, and wind and current patterns as well (Stenseth et al. 2002; Henriques et al. 2007). In addition, the NAO is highly correlated with precipitation regimes in the Iberian Peninsula, as it interferes with the trajectory of depressions in the North Atlantic (Trigo et al. 2004; Gallego et al. 2005).

For the estuarine resident species of the Mondego estuary, the NAO with time lag of one, two and twelve months prior to the highest abundance peaks were significant predictors, demonstrating that large-scale climate patterns can act in a prolonged time frame (Vinagre et al. 2009; Martinho et al. 2012). Moreover, the NAO had significant effects on the fish species simultaneously with precipitation, showing that large-scale patterns influence local climatic events as well (Drinkwater et al. 2003; Trigo et al. 2004). During periods of positive NAO values and high precipitation, higher abundance of *P. microps* was observed. Several studies have emphasized the distinct effects of the positive and negative NAO phases in local climate patterns and in ecosystems as well (e.g. Ottersen et al. 2001; Henriques et al. 2007; López-Moreno et al. 2011). For instance, a positive NAO phase is associated with strong wind circulation in the North Atlantic and also with high atmospheric and sea temperatures in western Europe (Mann and Lazier, 1991). Moreover, the prevailing westerly winds are strengthened, which causes increased precipitation and temperatures over northern Europe and southeastern USA (Ottersen et al. 2001). During a negative NAO phase the westerly winds are weaker, and temperatures decrease in northern Europe (Ottersen et al. 2001). Similarly to our results, Ottersen and Stenseth (2001) found that positive NAO index anomalies created favourable conditions for Arcto-Norwegian cod. In addition, in the central Baltic in the late 1980s, a dominant positive NAO led to increased water temperatures and consequently to higher food availability, which in turn resulted in increases in sprat populations (Alheit et al. 2005). In the Portuguese coast, the surface waters are dominated by the Canary current that brings a regular supply of cold water from the north (Bischof et al. 2003), and probably brings eggs and young fish (Henriques et al. 2007). During periods when the NAO is continuously positive, this current usually prevails in all seasons (Henriques et al. 2007). When the negative NAO is dominant, this current may be replaced by a poleward flow (Frouin et al. 1990). This pattern also demonstrates the indirect effects of the NAO to fish species.

The NAO has also significant effects on the variations of sea surface temperature (SST, Hurrell and Deser, 2009): in years when the NAO is extremely positive, in the British Isles and adjacent areas the southwest winds become dominant, winters are mild and rainy and SST is relatively higher, while in southwest Europe and in the Mediterranean, winters are sunny, dry and cold, and northern winds are the dominant, SST is relatively lower (Henriques et al. 2007). In years of negative NAO, an opposite pattern can be observed. Sea surface temperature (SST) has been described to exert considerable influences on numerous marine and estuarine fishes (e.g. Genner et al. 2004; Henriques et al. 2007; Vinagre et al. 2009). In the Northeast Atlantic, SST data series demonstrate the existence of a long-term warming since the mid-1970s (Gómez-Gesteira et al. 2008; Michel et al. 2009). In particular, a significant increase in SST has been detected in the Central Atlantic from 2000 to 2010, as well as on the Bay of Biscay (France) since 1987 (Hermant et al. 2010). As a probable consequence, boreal fish species became scarcer at the southern edge of their distributional ranges, while temperate and southern fish species are tending to expand their distributions (ter Hofstede et al. 2010; van Hal et al. 2010). Moreover, in the North Atlantic SST is predicted to increase by 0.5-4 ºC over the next century (Hulme et al. 2002). This projected increase along with a reduction in the Atlantic thermohaline circulation, and an increase in stratification probably will change the geographical distribution of fish species within this area (Chust et al. 2011). However, SST did not have significant influences on the two observed estuarine resident fish species of the Mondego estuary. According to Attrill and Power (2002), estuaries may act as thermal buffers against more severe open-sea conditions, and therefore are not affected directly by marine conditions. This could have been the reason why SST did not have significant impacts on the two observed estuarine resident fish species, since they spend most of their life-cycle within estuarine areas.

Importance of fish-based indices in determining anthropogenic pressures on transitional waters

During the last decades, several fish-based indices have been developed in order to assess the ecological quality of transitional waters related to anthropogenic pressures (e.g. Ramm, 1988; Quinn et al. 1999; Harrison and Whitfield, 2004; Coates et al. 2007; Delpech et al. 2010; Cabral et al. 2012; Pérez-Domínguez et al. 2012; Pasquaud et al. 2013). These indices have been proposed worldwide, e.g. the Estuarine Fish Community Index (EFCI) in South Africa (Harrison and Whitfield, 2004), Index of Biotic Integrity (IBI) in USA (Meng et al. 2002), and several indices in Europe, such as the Estuarine and Lagoon Fish Index (ELFI) in France (Delpech et al. 2010), the Zone-specific Fish-based Estuarine Biotic Index (Z-EBI) in Belgium (Breine et al. 2010), the Transitional fish classification index (TFCI) in the UK (Coates et al. 2007) and the Estuarine Fish Assessment Index (EFAI) in Portugal (Cabral et al. 2012), due to the implementation of the EU Water Framework Directive (WFD; EU, 2000). The WFD has set up a framework for the protection of groundwater, inland surface waters, estuarine and coastal waters, comprising a new view for the management of water resources in Europe (Borja, 2005). According to this directive, its main objective is to obtain a “Good” Ecological Status in all European water bodies by 2015. The European fish-based indices were developed to reflect the ecological quality of a water body over a period of three years, in accordance with the WFD. Generally, these indices are a combination of several metrics, in order to allow a better sensitivity and robustness than any of the individual metrics that they are comprised of (Deegan et al. 1997; Hughes et al. 1998), and to provide also integrative and more holistic perspectives (Roset et al. 2007). The first steps in the development of multimetric indices are the selection of the constituent metrics (Hering et al. 2006), to guarantee that the diversity of ecological functions of a community are represented and that these biological measures are sensitive sufficiently to respond to human stressors that are distinguishable from natural variation (EPA, 2000).

In the present work, changes in single such as species number, Shannon-Wiener (H') and Pielou (J') indices, and also a multi-metric community-based measure of ecological quality - the Estuarine Fish Assessment Index (EFAI), were evaluated in the Mondego estuary, and determined their relationships with an anthropogenic pressure index (API). A particularly important aspect was the evaluation of ecological quality over an eight-year period, since long-term approaches for evaluating ecological quality are rare in literature. A previous study evaluated the similarities between fish-based indices used in the context of the WFD in the North-East Atlantic Geographical Intercalibration Group (NEAGIG), and grouped the indices of Belgium (Breine et al. 2010), Portugal (Cabral et al. 2012) and Spain (Basque country) (Borja et al. 2004) together (Cabral et al. 2012). This group was related to several metrics based on the relative abundance of functional guilds, and metrics relative to sensitive and introduced species as well (Cabral et al. 2012).

Single metric indices have been widely used to assess changes in diversity though time in marine ecosystems (e.g. Selleslagh and Amara, 2008; Borja et al. 2011; González-Ortegón et al. 2012; Kantoussan et al. 2012). However, distinct trends could be observed for diversity and evenness indices, as single metric indices were neither concordant amongst themselves nor with the EFAI. In turn, the EFAI indicated an increasing tendency in ecological quality, and also significantly correlated with the API, showing that a higher ecological quality was associated with lower anthropogenic pressure. These results emphasize the usefulness of this index in the evaluation of the ecological quality of Portuguese estuaries. Moreover, all the studied years were classified as in Good status by the EFAI, similar to the results by Cabral et al. (2012), where 13 estuaries were studied along the Portuguese coast and all of them presented a Good water quality status, except the Douro estuary in a particular year.

Two distinct periods could be observed in terms of pressure scenarios (2005/06 and 2007/12), from which the first period (2005/06) was characterized by higher anthropogenic pressures, when comparing with the second period (2007/12). Lower abundances of marine migrant species was observed during the period of higher pressure, demonstrating that anthropogenic pressures may have deteriorating influences on the functioning of estuarine nursery grounds. Moreover, many of the marine migrants are commercially important species; therefore, their reduction in abundances may have considerable impacts on human populations as well (Vasconcelos et al. 2010, 2012; Martinho et al. 2012).

One of the major difficulties in ecological quality assessment is to distinguish anthropogenic pressures from natural variability, which is known as the “Estuarine Quality Paradox” (Elliott and Quintino, 2007). Previous studies recommended to develop methods that use functional and structural symptoms as well, as functional properties may be more robust than structural ones (Hooper et al. 2005; de Jonge et al. 2006). Indeed, the EFAI was also developed including functional and structural metrics, enabling a higher capability for distinguishing between anthropogenic pressures and natural variability. Hence, and despite that the anthropogenic pressure scenarios were not particularly harsh, it was still possible to distinguish between two distinct periods, and to correlate the EFAI with the API. The obtained results indicate the robustness and sensibility of the EFAI, which proved to be a valuable tool in assessing the ecological quality of Portuguese estuaries in the highlight of the EU WFD.

Conclusions

The present work demonstrated the effects of natural variability including local and large-scale climate patterns, as well as anthropogenic pressures, on a temperate European estuarine fish assemblage. A long-term database was analysed considering functional and structural features, in order to evaluate the influences of environmental changes and human disturbance on the estuarine fish assemblage. As a general trend, among local climate patterns precipitation and consequently river runoff had considerable influences on the fish assemblage, mostly by the displacement of suitable habitats and by influencing the recruitment and migration of numerous species, as well as by alterations in the trophic structure of the fish assemblage. Among large-scale climate patterns, the North Atlantic Oscillation (NAO) exerted significant influence on the observed estuarine resident fish species that spend their entire life cycle inside the estuary, indicating the broad-scale spatial and temporal effects of this climatic phenomenon. The Estuarine Fish Assessment Index (EFAI) proved to be a useful tool in assessing ecological quality of Portuguese estuaries, as with the relatively low human disturbance of the Mondego estuary it was still possible to detect changes in the fish assemblage attributable to anthropogenic pressures. The predicted increase in natural extreme events such as droughts, floods and heat waves may have even harsher impacts on estuarine fish assemblages mainly through the displacement or degradation of suitable habitats and through reduced water quality. As these ecosystems provide highly valuable goods and services, these impacts may have influences on the human populations that live in the surrounding areas of these ecosystems. Furthermore, the present work reinforced the fact that estuarine fish assemblages can be used as indicators of environmental changes and anthropogenic pressures.

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