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Estuarine fish assemblages as indicators of environmental changes: *the Mondego estuary case study*

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CONTENTS

Abstract

The main objective of this thesis was to assess the influence of an extreme weather event (drought) in the functioning of the Mondego estuary (Portugal) fish assemblage, based on field surveys conducted from 2003 to 2007. The occurrence of an extreme drought event during this time frame, provided an important case study on the effects of a significant reduction in precipitation and freshwater flow in estuarine fish communities, namely at two different scales: (a) structure and composition of the functional guilds composing the estuarine fish assemblage, as well as the classification of the Ecological Quality Status, and (b) interannual variability in abundance and recruitment levels in the marine species that use the estuary as a nursery area.

The response of the Mondego estuary fish assemblage to the drought was firstly evaluated regarding the variations in structure and composition of the habitat use guilds that compose the assemblage: estuarine residents, marine juveniles, marine stragglers, freshwater, catadromous and marine species that use the estuary as a nursery area. The fish assemblage was composed mainly of estuarine residents and marine species that use the estuary as a nursery area, in conformity of most of the European Atlantic estuaries. During the drought period, depletion in freshwater species was observed, while marine stragglers increased in abundance and species number, as a response to a higher saline intrusion inside the estuary. In addition, a reduction in abundance of the estuarine residents was also observed. Among the environmental variables analyzed, precipitation was the one that best explained the variability in the fish assemblage.

The flatfish community was analyzed in more detail, being possible to define three groups of species: one group composed of species present throughout the study period and in high densities - *Platichthys flesus* and *Solea solea*; one group composed of less abundant species but regularly present - *Scophthalmus rhombus* and *Solea senegalensis*; and another group, composed of occasional species, present only during the drought period - *Arnoglossus laterna, Buglossidium luteum, Dicologlossa hexophthalma* and *Pegusa lascaris*. Abundance data was complemented by multivariate analysis, with river runoff, salinity and dissolved oxygen being the environmental variables that significantly influenced the distribution of flatfish species. In addition, the species

most affected by the drought event, in which a significant reduction in abundance was observed, were those whose southern limit of distribution occurs at higher latitudes. In opposition, the flatfish assemblage of the Mondego estuary was composed of a higher species number during the drought, as a consequence of an increase in marine straggler species, typical of southern latitudes. Early summer average salinity and precipitation values were appointed as good proxies for estimating abundance levels of *P. flesus* and *S. solea*, respectively, emphasizing the importance of hydrodynamics for the recruitment and abundance of these commercially important species.

The interannual variability in abundance of 0-group fish of the marine species that use the Mondego estuary as a nursery area was evaluated, and related to a set of environmental variables. For *Dicentrarchus labrax* and *P. flesus*, a decreasing tendency in abundance of 0-group fish was observed, with river runoff, precipitation and the east-west wind component having been identified as variables with significant influence for both species. The north-south wind component was also significant for *P. flesus*. The abundance patterns of *S. solea* juveniles were only related with river runoff from the Mondego River, with the lowest densities found during the drought. However, after the drought, *S. solea* 0-group fish densities increased to similar levels of 2003. Taking into account the recent climate change projections for the Iberian Peninsula, namely a reduction in precipitation levels, this study led to the conclusion that marine species whose recruitment occurs in early spring (*D. labrax* and *P. flesus*) are expected to be more affected by a lesser extent of river plumes to coastal areas.

To evaluate the influence of sampling gear in the determination of the structure and composition of the Mondego estuary fish assemblage, two commonly used fishing gears for scientific purposes were compared - beam and otter trawl. Despite both sampling gears provided the same species richness (20 espécies), the structure and composition of type-specific fish assemblages was to some extent divergent, with a higher relative abundance of estuarine residents in beam trawl samples driving the main differences. Regarding the commercially important and most abundant species in both gears, *D. labrax*, *P. flesus* and *S. solea*, differences

in median total length and length-frequency distributions were observed, with beam trawl samples providing individuals that belong to smaller length-classes, a determining factor when targeting 0-group fish in recruitment studies. Abundance estimates for these species were also different in both sampling gears, which can be related to differences in spatial occupation of different size-classes along the estuary, as larger fish tend to move to deeper estuarine areas. Regarding the compliance with the Water Framework Directive (WFD), different structures of estuarine fish assemblages may provide with different classifications of the Ecological Quality Status (EQS).

Also in the context of the WFD, the classification of the EQS of the Mondego estuary by five selected multimetric fish-based indices was compared, as well as the indices' responses to an extreme drought. The selected indices were the EBI (Deegan et al., 1997), EDI (Borja et al., 2004), EFCI (Harrison and Whitfield, 2004), EBI (Breine et al., 2007) and TFCI (Coates et al., 2007). Based on these indices, the EQS of the Mondego estuary ranged between "Poor" and "High" status, evidencing the high level of mismatch among methodologies. The EBI (Breine et al., 2007) was the only index that evidenced clear interannual and seasonal variations, while others were seasonally constant. The EDI (Borja et al., 2004) and TFCI (Coates et al., 2007) classified regularly the Mondego estuary with the highest status, being proposed as the most suitable indices for application in this estuary. The results are examined in the scope of the EU WFD, regarding monitoring strategies, application of indices and EQS assessment.

Resumo

A presente tese teve como objectivo principal avaliar a influência de um evento climático extremo (seca) no funcionamento da comunidade piscícola do estuário do Rio Mondego (Portugal), tendo como base campanhas de amostragem realizadas de 2003 a 2007. A ocorrência de uma seca extrema durante o período de amostragem, permitiu um caso de estudo muito importante em que os efeitos de uma redução significativa na precipitação e no caudal do Rio Mondego foram avaliados na comunidade de peixes estuarinos. A resposta destas comunidades à seca extrema foi avaliada em escalas distintas: (a) estrutura e composição dos grupos funcionais que constituem a comunidade, bem como a classificação do Estado de Qualidade Ecológica (EQE), e (b) variabilidade interanual na abundância e níveis de recrutamento nas espécies marinhas que utilizam o estuário como zona de viveiro.

A resposta da comunidade piscícola do estuário do Mondego à seca foi inicialmente avaliada tendo em conta a variação na estrutura e composição dos grupos ecológicos relacionados com o uso do habitat: residentes estuarinos, marinhos juvenis, marinhos ocasionais, espécies dulçaquícolas, migradores catádromos e espécies marinhas que utilizam o estuário como zona de viveiro. Os grupos mais abundantes na comunidade foram os residentes estuarinos e as espécies marinhas que utilizam o estuário como zona de viveiro, em conformidade com a maioria dos estuários da costa Atlântica europeia. Durante o período de seca, foi observado o desaparecimento das espécies dulçaquícolas, enquanto que as espécies marinhas ocasionais aumentaram em abundância e em riqueza específica, em resposta a uma maior extensão da cunha salina no interior do estuário. No mesmo período, foi observada também uma redução na abundância dos residentes estuarinos. Entre as variáveis ambientais consideradas, a precipitação foi a que melhor explicou a variabilidade na comunidade de peixes.

A comunidade de Pleuronectiformes foi analisada com particular detalhe, tendo sido possível definir três grupos: um grupo composto por espécies presentes durante todo o período de estudo e em densidades elevadas – *Platichthys flesus* e *Solea solea*; um grupo composto por espécies menos abundantes mas também com presença regular – *Scophthalmus rhombus* e

Solea senegalensis; e finalmente, um outro grupo composto por espécies ocasionais, presentes apenas durante o período de seca – *Arnoglossus laterna, Buglossidium luteum, Dicologlossa hexophthalma* e *Pegusa lascaris*. Os dados relativos à abundância das espécies foram complementados com uma análise multivariada, através da qual foram determinadas as variáveis ambientais que influenciaram significativamente a distribuição destas espécies no estuário do Mondego: escoamento, salinidade e oxigénio dissolvido. As espécies mais afectadas pela seca, para as quais foi observada uma redução significativa na abundância, foram as espécies cujo limite Sul da distribuição ocorre a latitudes mais elevadas. Durante o período de seca, a comunidade de Pleuronectiformes apresentou uma maior riqueza específica devido a um aumento de espécies marinhas ocasionais, típica de zonas de menor latitude. Os valores de salinidade média e precipitação do início do Verão foram apontados como bons predictores para estimar os níveis de abundância de *P. flesus* e *S. solea*, respectivamente, realçando a importância da hidrodinâmica para o recrutamento e abundância destas espéceis com interesse comercial.

A variabilidade interanual nos níveis de abundância de juvenis das espécies marinhas que utilizam o estuário como zona de viveiro foram avaliadas e relacionadas com um conjunto de variáveis ambientais. Para *Dicentrarchus labrax* e *P. flesus*, posteriormente ao período de seca, foi observada uma tendência decrescente na abundância dos juvenis, tendo sido identificado o escoamento, a precipitação e a componente este-oeste do vento como variáveis explicativas com influência significativa para ambas as espécies. A componente norte-sul do vento também foi significativa para *P. flesus*. Os padrões de abundância dos juvenis de *S. solea* foram apenas relacionados com o escoamento do Rio Mondego, tendo sido observadas as densidades mais baixas durante o início da seca. Contudo, posteriormente a este período, a abundância dos juvenis de *S. solea* recuperou para valores semelhantes aos de 2003. Tendo em conta as mais recentes projecções para a Península Ibérica relacionadas com as alterações climáticas, nomeadamente a redução dos valores de precipitação, este trabalho permitiu inferir que as espécies cujo período de recrutamento ocorre no início da primavera (*D. labrax* e *P. flesus*) serão as mais afectadas pela redução na extensão da pluma dos rios nas zonas costeiras.

De modo a avaliar a infuência do método de amostragem na determinação da estrutura e composição da comunidade piscícola do estuário do Rio Mondego, duas artes de pesca usualmente utilizadas em campanhas de pesca para fins científicos foram comparadas - arrasto de vara e arrasto de portas. Apesar de ambas as técnicas terem capturado o mesmo número de espécies (20 espécies), a estrutura e composição das comunidades associadas a cada técnica foram até certo ponto divergentes, com o arasto de vara a capturar uma maior abundância relativa de residentes estuarinos. No que diz respeito às espécies de interesse comercial e mais abundantes em ambas as técnicas de amostragem, *D. labrax*, *P. flesus* e *S. solea*, foram obtidas diferenças na mediana do comprimento total e também na distribuição das frequências de comprimentos. As amostras provenientes do arrasto de vara foram compostas por indivíduos pertencentes às classes de menores comprimentos, um factor determinante no estudo de padrões de recrutamento de juvenis. As estimativas de abundância para estas espécies também foram distintas para as duas artes de amostragem. De acordo com os resultados previamente obtidos, é provável que diferentes determinações da estrutura das comunidades piscícolas (relacionado com a arte de amostragem) possa influenciar a classificação do Estado de Qualidade Ecológica (EQE) para a implementação da Directiva Quadro da Água (DQA).

Ainda no contexto da DQA, foi avaliada a classificação do EQE por cinco índices multimétricos para peixes, tendo sido avaliada também a sua resposta ao período de seca. Os índices seleccionados foram os seguintes: EBI (Deegan et al., 1997), EDI (Borja et al., 2004), EFCI (Harrison and Whitfield, 2004), EBI (Breine et al., 2007) e TFCI (Coates et al., 2007). Tendo por base estes índices, o EQE do estuário do Mondego variou entre “Pobre” e “Excelente”, evidenciando a elevada falta de correspondências entre índices. O EBI (Breine et al., 2007) foi o único índice cujos resultados apresentaram uma clara variação sazonal e interanual, contrariamente aos restantes. O EDI (Borja et al., 2004) e o TFCI (Coates et al., 2007) foram os índices que classificaram regularmente o estuário do Rio Mondego com o estado ecológico mais elevado, tendo sido propostos como os mais indicados para utilização neste sistema. Os

resultados foram também avaliados no âmbito da DQA, tendo em conta estratégias de monitorização, aplicação de índices e determinação do EQE.

General Introduction

Estuaries: drivers, pressures and responses

Estuaries are among the most productive ecosystems of the world (Kennish, 2002; McLusky and Elliott, 2004; Dolbeth et al., 2007b), supporting an important range of flora and fauna. As transition areas, estuaries are arguably more complex than other ecosystems, with highly interrelated processes between their physical, chemical and biological components (Molles, 1999; Elliott et al., 2002). Allied to their high productivity, the availability of different habitats provides optimal settlement conditions for invertebrates, fish and birds, either for estuarine residency, as nursery areas or as migration routes, including tidal flats, oyster beds, saltmarshes and seagrass beds (Beck et al., 2001; Pihl et al., 2002; Vasconcelos et al., 2007). One of the main attributes of estuarine areas is the salinity gradient, from the brackish waters of the upper reaches to the euhaline downstream areas. The salinity regime, coupled with the typical hydrodynamic fluctuations of freshwater flow and seawater intrusion, creates the one of the main gradients that is responsible for the distribution of organisms within estuarine waters (Thiel et al., 1995; Whitfield, 1999; Kimmerer, 2002; Leitão et al., 2007). Moreover, water circulation due to tidal and freshwater currents is responsible for the transport of organisms, nutrient and oxygen cycling (Molles, 1999; Gibson et al., 2003; Lillebø et al., 2004). As a consequence, changes in hydrological regimes, due to droughts, floods or water retention in dams, can significantly impact the estuarine species, mainly by the displacement of suitable habitats (Kimmerer, 2002).

The wide range of goods and services provided by estuarine areas, namely tourism, recreational areas, protection against floods, replenishment of coastal fisheries stocks, sediment and nutrient cycling, among others, led Costanza et al. (2007) to classify them among the most valuable ecosystems on Earth. However, due to their privileged location, estuarine and coastal areas have long been subjected to intense human activities, leading to an over-exploitation of natural resources and to dramatic changes in land-use, contributing to the deterioration of the natural environment (de Jonge et al., 2002; Dauvin and Ruellet, 2009) (Fig. 1). These include habitat destruction by bank reclamation, water quality impoverishment by domestic, agricultural and industrial effluent discharges and nutrient enrichment (Cloern, 2001), and stock depletion by overfishing. In fact, direct disturbance on the sediment-water interface (such as the one created by activities referred before) has the potential to impact estuarine communities through the many functional links between them (Austen et al., 2002). Ultimately, increasing stress levels can change the way the estuarine system functions for local fish, invertebrate and bird communities, with strong implications for the supply of goods and services.

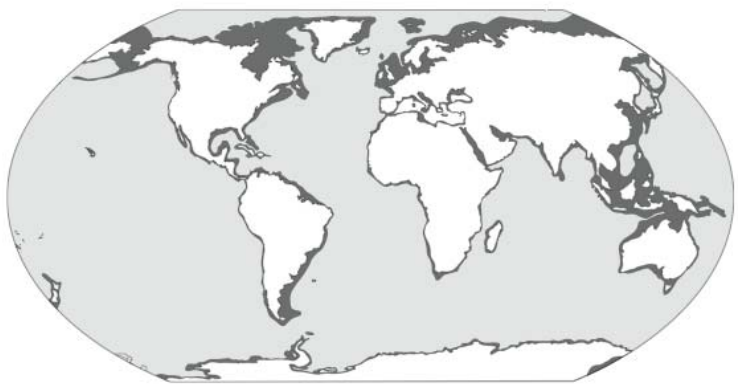

Figure 1. Continental shelves of the World (dark shade), defining the extension of coastal waters (From Holligan and Reiners, 1992).

Nutrient enrichment, and consequently eutrophication, is seen as one of the major threats for the functioning of the estuarine ecosystem. Recently, Flemer and Champ (2006) pointed out that the mobilization of nitrogen and phosphorus through land clearing, application of fertilizers, discharge of human wastes, animal production and combustion of fossil fuels is the main source of eutrophication in estuaries. Likewise, and especially in densely populated areas, several effects of eutrophication have been described, including the growth of opportunistic macroalgae and consequent reduction in seagrass beds, development of anoxia and hypoxia events, and as a worst case scenario, mass mortality of fish and invertebrate fauna (e.g. Raffaelli et al., 1998;

Cloern, 2001; Pardal et al., 2004). In summary, structural changes in local communities have been observed due to eutrophication, mainly by a reduction in species abundance and diversity (Dolbeth et al., 2003; Cardoso et al., 2004, 2008; Pardal et al., 2004).

Recently, the DPSIR approach (e.g. Elliott, 2002; Borja and Dauer, 2008) has been introduced for managing estuarine and coastal ecosystems, in which *Drivers* (human demands on the systems) and *Pressures* (the precise activities leading to change) result in *State Changes* (in the natural features) and *Impacts* on the socio-economic uses of the systems; the latter in turn require a *Response* in order to reduce, mitigate and/or compensate any adverse effects (McLusky and Elliott, 2004). This conceptual framework is intended to identify a causal relationship within the *DPS* component and most importantly to determine and evaluate human and ecological impacts (Borja and Dauer, 2008). This approach, in combination with recently developed worldwide legislation for the protection of water resources, such as the EU Water Framework Directive (WFD, 2000/60/EC), the US Clean Water Act (USEPA, 2002), the EU Marine Strategy Framework Directive (MSD, 2008/56/EC), the National Land and Water Resources Audit in Australia (Heap et al., 2001) and the Water Act in South Africa (Adams et al., 2002), will imply a more rational use of water resources, but will also provide a framework for an integrated management of the highly variable and valuable estuarine ecosystems.

Estuarine fish fauna

Estuaries have long been regarded as important areas for fish, acting mainly as nursery areas, migration routes, feeding and shelter areas, providing an alternative habitat to the marine environment that supports large numbers of fish (Haedrich, 1983; Beck et al., 2001; McLusky and Elliott, 2004). Over the last years, several studies have been conducted regarding estuarine fish assemblages, trying to relate the occurrence of fish species in relation with the estuarine habitat. When considering estuarine habitat use by fish assemblages, alternative views of the assemblage are increasingly being explored, based on their functional rather than taxonomic aspects (Elliott and Dewailly, 1995; Mathieson et al., 2000; Franco et al., 2008). The allocation of all taxa to a number of functional guilds allows a description of fish assemblages in terms of vertical zonation, habitat preferences, including the substratum preference of benthic/demersal species, and dietary preferences (Mathieson et al., 2000). Elliot and Dewailly (1995) first introduced the concept of classifying fish species in functional guilds, as a result of a comparison of the structure and composition of European estuarine fish assemblages. As a result, the previous authors defined the typical European Atlantic seaboard estuarine fish assemblage, showing that there were common patterns in estuarine usage by fihses, in spite of the differences in specific composition. More recently, in order to standardize their concepts and applications, Elliott et al. (2007) reviewed the guild approach in categorizing estuarine fish species, since several functional guilds had been either created or adapted to other geographical areas, leading to overlap and confusion between concepts. Moreover, an understanding of the ecosystem processes (i.e. the functioning) of these transitional environments is necessary to enable their protection and the sustainable management required by legislation, such as the EU Habitats (92/43/EEC) and Water Framework Directive (2000/60/EC) (Franco et al., 2008). Concurrently, the functional guild approach has been extensively used worldwide (e.g. Mathieson et al., 2000; Malavasi et al., 2004; Costa et al., 2007; Jovanovic et al., 2007; Martinho et al., 2007; Selleslagh and Amara, 2008; James et al., 2008), providing a good opportunity for comparing the functioning of transitional waters for fish along different geographical areas.

Among estuarine fish assemblages, the resident species are one of the most abundant groups, mainly the common and sand gobies, as described elsewhere (e.g. Laffaille et al., 2000; Dolbeth et al., 2007a; Jovanovich et al., 2007; Martinho et al., 2007; França et al., 2008; Selleslagh and Amara, 2008). These species are of utmost importance in estuarine trophic webs as intermediate predators, being consumers of plankton, meio- and macrobenthos (Doornbos and Twisk, 1987; Salgado et al., 2004; Leitão et al., 2006), but also consumed by macrobenthos (Baeta et al., 2006), several larger fishes (Arruda et al., 1993; Martinho et al., 2008) and birds (Doornbos, 1984). The plasticity and adaptability of these species towards environmental changes endows them with the capacity to successfully occupy different biotopes, from brackish waters to euhaline areas (Bouchereau and Guelorget, 1998; Leitão et al., 2006).

Estuaries also play an important role as nursery area for marine fish (Beck et al., 2001; Cabral et al., 2007; Martinho et al., 2007a). The nursery concept was firstly introduced for fish that have complex life cycles, in which larvae are transported to estuaries, where they metamorphose, grow to subadult stages, and then move to adult habitats offshore (Beck et al., 2001). This ontogenic niche separation generates a great ecological distinction between life-stages, which prevents population size at one stage from being regulated in accordance with the carrying capacity of the next ontogenic habitat (Costa et al., 2002). Among these species that use estuaries as nursery habitats (marine-estuarine dependent species), competition seems to be diminished by abundant food resources (e.g. Dolbeth et al., 2007b) or by resource partitioning produced by the likely competitors (Amara et al., 2001; Costa et al., 2002), namely temporal and spatial segregation within nursery areas (Martinho et al., 2007b). In this context, numerous factors have been catalogued as determinant for the distribution of these species within estuarine waters, most of them related to substrate type, hydrological features and the presence of rooted vegetation, creating a complex mosaic of specific habitats (Cabral and Costa, 1999; Pihl et al., 2002; França et al., 2009). To a great extent, these species are of high commercial value, requiring efficient protection measures, preferably based on the best available knowledge on habitat descriptors, physiological tolerances and ecological requirements.

Recruitment variability of marine species

Marine species that use estuaries as nursery areas are one of the abundant ecological groups in estuarine fish assemblages (e.g. Elliott and Dewailly, 1995; Malavasi et al., 2004; Cabral et al., 2007; Martinho et al., 2007a,b). Particularly, the European sea bass (*Dicentrarchus labrax*), flounder (*Platichthys flesus*) and the common sole (*Solea solea*) are among the most abundant in Portuguese estuaries (Cabral et al., 2007; Martinho et al., 2007a,b; Ribeiro et al., 2008). Accordingly, these species have high commercial value mainly in adult stages, so understanding the dynamics of recruitment variability is a crucial step for an accurate fisheries management strategy. In these species, spawning takes place offshore, implying the migration of larvae from the continental shelf towards coastal areas and estuaries (e.g. Norcross and Shaw, 1984; Koutsikopoulos and Lacroix, 1992). Larval dispersion has several advantages, such as the possibility of colonization of new settlement habitats, gene flow and the minimization of intra-specific competition. Nevertheless, these advantages can be diminished by the high mortality and predation rates that young larvae are subjected to, and also due to the difficulties in finding the access to suitable estuarine habitats (Bailey et al., 2005). Moreover, slight variations in daily mortality rates operating over the egg and larval stages are capable of generating variations in recruitment orders of magnitude greater than those actually observed (Heath, 1992). For these species, connectivity between estuarine and coastal areas is of great importance (Dame and Allen, 1996; Ray, 2005), being not only influenced by natural conditions (e.g. river flow), but also by the anthropogenic factors that are conspicuous in most European estuaries (Able et al., 1999; Le Pape et al., 2007, Vasconcelos et al., 2007).

Recruitment variability has been attributed as one of the main causes of fluctuating stock numbers, either a consequence of density-dependent factors acting mainly inside estuarine waters, such as predation, mortality, competition for food and shelter (van der Veer et al., 2000), or density-independent factors acting essentially during the estuarine colonization phase (van der Veer et al., 2000), such as hydrological features, coastal wind speed and direction, currents, salinity, turbidity, water temperature precipitation and large ocean-atmosphere patterns such as the North Atlantic Oscillation (NAO) (e.g. Marchand, 1991; Amara et al., 2000; van der Veer et al., 2000; Attrill and Power, 2002; Bailey et al., 2005; Vinagre et al., 2007; Wilson et al., 2008; Martinho et al., 2009).

For the Portuguese coast, spawning data is unavailable for most marine fish species, being impossible to relate abundance patterns and recruitment variability with spawning stock biomass. However, some attempts have been made to relate variations in hydrodynamics and in the NAO with the recruitment levels of marine-estuarine dependent species (Vinagre et al 2007; Martinho et al., 2009), marine-estuarine opportunists (Santos et al., 2001, 2004) and with the structure and composition of coastal marine fish assemblages (Henriques et al., 2007). Hydrodynamics, mainly related with river runoff patterns and the extent of river plumes to coastal areas, has been identified as critical for recruitment, acting as proximity indicators of nursery areas for fish larvae (e.g. Marchand, 1991; Amara et al., 2000; Metcalfe et al., 2006; Vinagre et al., 2007; Martinho et al., 2009). Since most of these species use, at some part of their larval cycle, selective tidal stream transport (STST) from the continental shelf to estuarine nurseries (van der Veer et al., 1991; Jennings and Pawson, 1992; Amara et al., 2000), other aspects such as tidal currents, local topography and upwelling events also contribute, mainly locally, for variations in water circulation, retention and transport patterns, which may influence the amount of potential settlers and ultimately recruitment levels (Rijnsdorp et al., 1985; Lancaster et al., 1998; Metcalfe et al., 2006). Moreover, and given that river runoff is presently influenced by water retention in dams and to extreme weather events such as droughts or floods, it is expected that high recruitment variability in these species will occur in a nearby future.

On the other hand, it has been stressed the important effect of temperature on recruitment variability. As an example, Sims et al. (2004) noted that the reproductive migration phenology of a marine-estuarine dependent species (*P. flesus*) appears to be driven to a large extent to short-term climate-induced changes in thermal resources of their overwintering habitat. Moreover, it has been suggested its eggs are highly sensitive to water temperatures higher than 12°C in the winter, inducing high mortality levels (Von Westernhagen, 1970). The reduction in abundance of this species in the southern Atlantic coast of Portugal has been related with this fact, possibly as a consequence of climate change-induced increase in sea temperature (Cabral et al., 2007). For other marine-estuarine dependent species, temperature has been also reported as a determining factor in recruitment, such as *D. labrax* and *S. solea* (e.g. van der Veer et al., 2001; Le Pape et al., 2003a; Picket et al., 2004). Ultimately, Attrill and Power (2004) stated that the thermal resources within estuarine nursery areas have a temporal (rather than spatial) dimension, which may be responsible for fish migration patterns, and to a large extent, fish use estuarine habitats to exploit optimal thermal habitats rather than food supply.

Large-scale oceanographic patterns have also been considered to influence recruitment variability in marine fishes. In fact, the NAO has been correlated with a range of long-term ecological measures, including certain fish stocks. Such environmental influences are most likely to affect susceptible juveniles during estuarine residency, as estuaries are critical juvenile nursery or over-wintering habitats (Attrill and Power, 2002). In general, a positive phase of the NAO induces dry weather in southern European and wet weather in northern Europe, while a negative phase induces an opposite pattern. On the Portuguese coast, the NAO has been determined to influence not only the sea surface temperature (SST), but also the wind and current patterns (Henriques et al., 2007), which can have a synergistic effect on the factors that interact directly with recruitment variability.

Recently, a first attempt to determine the nursery of origin of several marine fish species was performed by Vasconcelos et al. (2008), assessing the contribution of each estuary to the coastal stocks by means of otolith elemental fingerprints. As noted elsewhere, this technique has proven to be suitable for discriminating nursery origins and connectivity between juvenile and adult habitats, being crucial for the management and conservation of estuarine and coastal stocks.

Climate forcing and systems' changes

Since the industrial revolution, an important milestone for the development of Humankind, CO_2 and other greenhouse gases (GHG) have been increasing in the atmosphere, mainly due to human activities. Particularly, GHG emissions increased in about 80% between 1970 and 2004, as a primary outcome of the use fossil fuels, with land-use change providing also a small contribution (IPCC, 2007). As a result, global warming, and consequently climate change and its main expressions, pose an important challenge for both scientists and stakeholders for the management of terrestrial and marine ecosystems, mainly in what concerns the developing of adaptation and mitigation strategies. Accordingly, one of the major challenges in the 21st century will be the access of the global population to freshwater resources (Gleick, 2002; IPCC, 2007). The frequency and magnitude of extreme weather events are expected to increase in the future due to climate change (Mirza, 2003), inducing changes in the Earth's global oceanic and atmospheric circulation patterns. Moreover, ocean acidification, sea level rise, reduction in thickness and extent of glaciers, ice sheets and sea ice, and increase in ocean and air temperature are all severe consequences of global warming (IPCC, 2007), with strong implications for coastal ecosystems. In estuarine areas, the main impacts of climate change are thought to be associated with river flow, either by flood or drought events. In the particular case of droughts, several authors have pointed out the effects of reductions in river flow at several latitudes, mainly for fish and invertebrates (e.g. Attrill and Power, 2000a; Le Pape et al., 2003b; Harrison and Whitfield, 2006; Costa et al., 2007; Marques et al., 2007; Martinho et al., 2007a) and for the water quality in general (Attrill and power, 2000b; Elsdon et al., 2009). Moreover, Erzini et al. (2005) and Meynecke et al. (2006) also reported on the reduction in landings of commercial species as a consequence of a reduced extent of river plumes to coastal areas.

For the Iberian Peninsula, a reduction in precipitation amounts (concentrated in the winter months) and consequent droughts, combined with the expected increase in air temperature are among the main projected regional impacts of global warming (Miranda et al., 2006; IPCC, 2007). In fact, six of the last ten years have been classified by the Portuguese Weather Institute (*http://www.meteo.pt*) among the driest and/or warmest since 1931. When looking at both precipitation and river runoff values for the last twenty years for the Mondego River basin area, it is notable the rapid shift between floods and droughts in consecutive years (Fig. 2). In addition, in 2005 a severe drought was recorded, with significant reductions in precipitation and river runoff, when compared to the long-term average values. As a consequence, a change in the structure and composition of estuarine planktonic and fish communities was observed, as a response to the upward displacement of brackish habitats (Marques et al., 2007; Martinho et al., 2007a), as well as a reduction in abundance and production of the estuarine residents and marine-estuarine dependent species (Martinho et al., 2007a, 2009; Dolbeth et al., 2008).

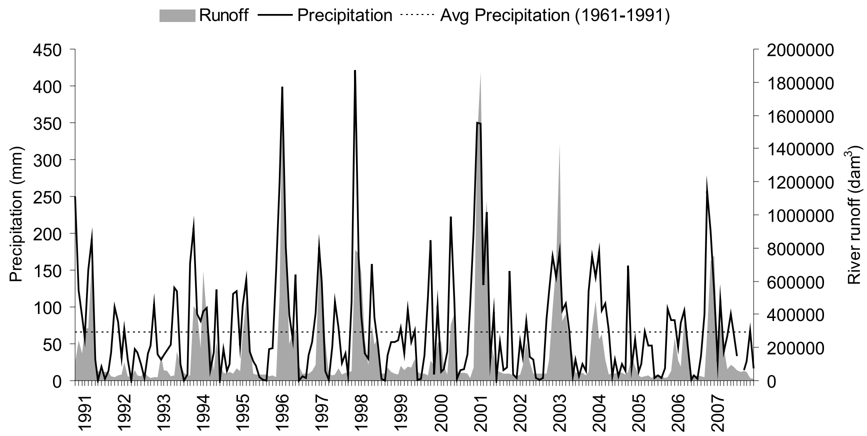

Figure 2. Long-term variation of precipitation and river runoff in the Mondego River basin.

Thus, since stochastic phenomena like the ones previously reported are expected to increase in frequency over the next years, it is essential to understand their influence on estuarine ecosystems, since the goods and services provided by them might be dangerously threatened. Moreover, changes in abundance of key species in the estuarine system, such as fish or decapods, will have serious implications for the structure and dynamics of estuarine food webs (Attrill and Power, 2000a), and ultimately for the functioning of whole ecosystem.

Assessing ecological status of transitional waters

The Water Framework Directive (WFD; 2000/60/EC) is a milestone document concerning the protection and enhancement of European rivers, lakes, groundwater, transitional and coastal waters. The main objectives of this directive are: (a) to prevent further deterioration, to protect and to enhance the status of water resources; (b) to promote sustainable water use; (c) to enhance protection and improvement of the aquatic environment, through specific measures for the progressive reduction of discharges; (d) to ensure the progressive reduction of pollution of groundwater and prevent its further pollution; and (e) to contribute to mitigating the effects of floods and droughts. Ultimately, EU member states are required by the WFD to achieve a Good Ecological Status in all water bodies by 2015. Recently, the Marine Strategy Framework Directive (MSFD; 2008/56/EC) was adopted by the EU, in order to extend this level of protection for coastal waters, providing an opportunity for a comprehensive policy for a sustainable use of Europe's environmentally degraded seas (Mee et al., 2008).

The concept of ecological status developed in the WFD is defined in terms of the biological community quality, as well as the systems' hydrological and chemical characteristics (Teixeira et al., 2008). Moreover, the application of the WFD requires methods capable of distinguishing between different levels of ecological quality to classify surface waters by comparing present data with a reference situation (Borja, 2005). However, one aspect is of particular relevance: since estuarine waters constitute naturally stressed, highly variable ecosystems that are also exposed to high levels of anthropogenic stress (Dauvin, 2007; Elliott and Quintino, 2007), is it possible to distinguish between natural and anthropogenic-induced stress? This question has been identified as the *Estuarine Quality Paradox* (e.g. Dauvin, 2007; Elliot and Quintino, 2007), and is of particular relevance when using ecological indicators to determine the Ecological Quality Status for transitional waters. In fact, since estuaries are naturally high variable habitats (i.e. in terms of salinity, temperature, oxygen and sediment dynamics), an over-reliance on ecosystem structural features, such as diversity, in quality indicators makes it more difficult to detect anthropogenic stress (Elliot and Quintino, 2007).

In estuaries, the biological elements considered for determining the Ecological Quality are algae, benthic invertebrates and fish. The use of fish as indicators of environmental change has recently gained attention, mainly due to the advantages in using these organisms, when compared to other groups such as the benthic invertebrates. According to Whitfield and Elliott (2002), the main advantages of using fish as indicators are: (1) they are typically present in all aquatic systems, with the exception of highly polluted waters; (2) there is extensive life-history and environmental response information available for most species; (3) in comparison to many invertebrates, fishes are relatively easy to identify and most samples can be processed in the field, with the fishes being returned to the water (non-destructive sampling); (4) fish communities usually include a range of species that represent a variety of trophic levels and include foods of both aquatic and terrestrial origin; (5) fishes are comparatively long-lived and therefore provide a long-term record of environmental stress; (6) they contain many life forms and functional guilds and thus are likely to cover all components of aquatic ecosystems affected by anthropogenic disturbance; (7) they are both sedentary and mobile and thus will reflect stressors within one area as well as providing groups to give a broader assessment of effects; (8) acute toxicity and stress effects can be evaluated in the laboratory using selected species, some of which may be missing from the study system; (9) they have a high public awareness value such that the general public are more likely to relate to information about the condition of the fish community than data on invertebrates or aquatic plants; (10) societal costs of environmental degradation, including cost-benefit analyses, are more readily evaluated because of the economic, aesthetic and conservation values attached to fishes. Recently, several ecological indicators have been developed worldwide for fish assemblages, based on multimetric approaches (e.g. Deegan et al., 1997; Borja et al., 2004; Harrison and Whitfield, 2004; Breine et al., 2007; Coates et al., 2007). As required by the WFD, fish-based indicators for transitional waters must evaluate the composition and abundance of fish species and/or functional groups in comparison with a reference value, determined either by a physical comparison with a control area (which is difficult to locate), hindcasting (which requires good previous data), predictive modelling (which requires adequate empirical or stochastic models) or expert judgement (subjective and difficult to quantify) (Whitfield and Elliott, 2002). Moreover, and according to Muxika et al. (2007) one of the most challenging aspects of the WFD is to determine reference conditions for each typology, which should be a developing topic for future research.

Study site – the Mondego estuary

The present study was carried out in the Mondego estuary (40º08'N; 8º50'W), located in the western Atlantic coast of Portugal, a warm temperate region, with a continental temperate climate. The source of the Mondego river is located at Serra da Estrela and extends along 227 Km, draining a hydrological basin of approximately 6670 Km^2, the largest entirely comprised in the Portuguese territory (Lourenço, 1986; Marques et al., 2002) (Fig. 3A). In its terminal part, the Mondego River forms a vast alluvial plain, the Lower Mondego Region, which consists of 15000 ha of agricultural land (Marques et al., 2002), where the main harvested crop is rice. Over half of a million people live in the basin, mainly in the medium and lower areas.

Near the coast, the estuary is divided in two arms, north and south, separated by an alluvium-formed island – the Murraceira Island (Fig. 3B). The two arms have different hydrological features: the north arm is deeper, with 4-10m during high tide and tidal range of 1-3m, while the south arm is shallower, with 2-4m deep at high tide and tidal range of 1-3m and was largely silted up in its upstream areas until 2006. The main freshwater circulation is carried out trough the north arm, while in the south arm water circulation in mainly caused by tidal influence and the freshwater input from the Pranto River, a small tributary, which is controlled by a sluice, according to the water needs of the surrounding rice fields. The estuary occupies an area of 8.6Km^2, where 75% corresponds to intertidal mudflats in the south arm, and less than 10% in the north arm. The downstream areas of the south arm exhibit *Scirpus maritimus* and *Spartina maritima* marshes and *Zostera noltii* beds (Lillebø et al., 2004). The estuary has been classified as highly productive, particularly the intertidal macrobenthic assemblages (Dolbeth et al., 2003, 2007b), with higher values in the seagrass *Z. nolttii* beds. Regarding special protection legislation, the estuary has been classified as an Important Bird Area (IBA; PT039) and as a RAMSAR site (site no. 1617).

In this system, the anthropogenic activities coupled to favourable physical characteristics (high water residence time and shallowness) and climate conditions (low precipitation) have imposed a high environmental pressure resulting in an eutrophication process (Martins et al., 2001;

Dolbeth et al., 2003; Marques et al., 2003; Cardoso et al., 2004; Pardal et al., 2004; Leston et al., 2008).

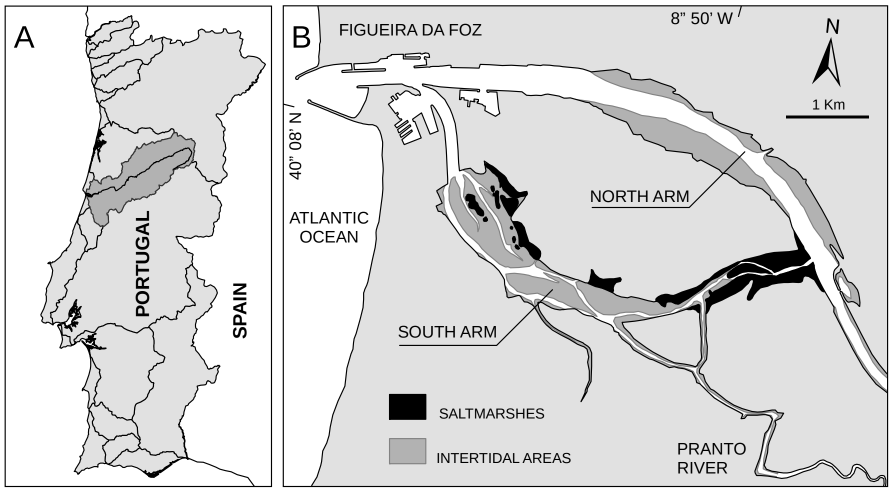

Figure 3. Geographical location of the Mondego River basin (shaded area) (A) and detailed scheme of the estuary (B), showing the two arms, intertidal and saltmarsh areas.

As an outcome, and mainly due to the increase in occurrence of green macroalgae *Ulva* spp blooms, the *Z. noltii* beds of the south arm declined from 15 ha in 1986 to 0.02 ha in 1997. From 1998 onwards, a restoration programme was implemented, in order to protect and enhance the *Z. noltii* beds, mainly by physically protecting the existing areas, and by diverting the organic-enriched freshwater discharges from the Pranto River into the north arm of the estuary (Martins et al., 2005). From that moment on, the area covered by *Z. noltii* has been increasing, reaching 4.7 ha in 2006. In 2006 was started another physical intervention in the estuary, by enlarging and keeping permanently open the upstream communication between both arms, which allowed to reduce the water residence time in the south arm through a higher water circulation.

Among the main anthropogenic pressures, organic pollution, bank reclamation and shipping activities are the most important. Since 1984, several interventions were conducted in the lower Mondego valley in order to improve irrigation efficiency for the surrounding agricultural fields, which included the construction of channels, regularization of margins to reduce floods and the construction of sluices to control the water level in the rice fields (Cardoso et al., 2007). On the other hand, the city of Figueira da Foz has over 60.000 inhabitants, whose wastewater (partially untreated) in still discharged in the north arm of the estuary. In addition, most of the traditional salt-farms in the Murraceira Island have been converted into semi-intensive aquacultures, whose wastewater is also discharged in both arms. Additionally, the port of Figueira da Foz, which in 2006 moved 1.1 million tons of total cargo, is responsible for the main industrial pressure in the estuarine area. At the moment the Port Authority is expanding the port's facilities, with an expected increase in yearly total cargo of 1.8 to 2 million tons. Consequently, constant dredging for maintaining the man navigation areas constitutes one of the major threats for benthic organisms, such as fish and invertebrates. Resource exploitation from fishing activities targeting mainly seasonal species, such as sea lamprey *Petromizon marinus* and shad *Alosa alosa*, coupled with illegal catches of European eel *Anguilla anguilla*, sea bass *D. labrax*, flounder *P. flesus*, and sole *S. solea* (Jorge et al., 2002; Leitão et al., 2007), as well as the capture of cockles and clams (Cardoso et al., 2007) increase the anthropogenic pressure that the estuary is subjected to.

Over the last 15 years, applied research has been conducted in the Mondego estuary, providing a comprehensive dataset on several areas, such as sediment and nutrient dynamics (e.g. Coelho et al., 2004), primary and secondary production (e.g. Cardoso et al., 2002; Dolbeth et al., 2003; 2007b), intertidal and subtidal benthic invertebrates (e.g Cardoso et al., 2004; 2007; Verdelhos et al., 2005; Viegas et al., 2007), plankton (e.g. Marques et al., 2007), fish (Dolbeth et al., 2007a; 2008; Leitão et al., 2007; Martinho et al., 2007a,b,; 2008) and bird species (e.g Lopes et al., 2006). Regarding the fish component, the Mondego estuary has been described as an active nursery area for marine fish (Leitão et al., 2007; Martinho et al., 2007a,b; 2008; Dolbeth et al., 2008), being responsible for important stocks off the central Portuguese coast (Vasconcelos et al., 2008).

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General Aim and Thesis Outline

Estuarine fish assemblages are among the most important faunal groups in transitional waters, mainly due to their commercial, social and aesthetic values. However, and despite their ecological aspects are relatively well known, there is still much to learn about their relationships with global climate change, as some vital features such as recruitment, productivity and ecological functions might be threatened. In this thesis, it is presented a conceptual and integrative approach on using estuarine fish assemblages as indicators on environmental changes. The primary aim was to assess the influence of an extreme weather event (drought) in the functioning of the Mondego estuary fish assemblage at two levels: fish assemblage (structure, composition, ecological quality status) and particular functional groups (abundance, recruitment levels, interannual variability). More precisely, the influence of the drought event on the fish assemblage was assessed according to the following questions:

- Is the structure and composition of estuarine fish assemblages influenced by droughts?
- Is recruitment variability in marine-estuarine dependent fish species related with environmental forcing?
- Do distinct sampling methods provide different estimates of the structure and composition of estuarine fish assemblages?
- Is the assessment of the ecological quality status in transitional waters conditioned by climate instability?

These main questions are addressed in the five chapters that constitute the focal point of this thesis. In the end, a general discussion and overview of the results is presented, integrating the response to the previous questions, main conclusions and future research areas.

Chapter I

In this thesis, the premise that estuarine fish assemblages are good indicators of environmental changes was tested and implemented. Hence, the first chapter deals with the response of the Mondego estuary fish assemblage to a severe drought that occurred during the study period. In *The influence of an extreme drought event in the fish community of a southern Europe temperate estuary* (Estuar. Coast. Shelf Sci., 2007, 75: 537-546), a comprehensive approach on the variations in the structure and dynamics of the fish assemblage is conducted by means of the estuarine use guild approach, based on the changes in the fish community as a response to the upward displacement of brackish habitats, due to a lower freshwater flow into the estuary, coupled with a higher seawater intrusion. This chapter provides the first long-term approach on the study of fish assemblages in the Mondego estuary, being used as a basis for future work.

Chapter II

The second chapter brings a new approach, by focusing only the estuarine flatfish community. The main objectives of *Does the flatfish community of the Mondego estuary (Portugal) reflect environmental changes?* were to study the distribution and abundance patterns of the flatfish species in relation with the drought event, and ultimately, to determine if the flatfish community could be used as an effective early indicator of state changes, such as droughts. To complement the information on seasonal abundance data of flatfish species, multivariate analysis were used to determine the relationships between species and significant environmental parameters. In addition, two different steps were taken, by analyzing the response of flatfish species with different latitudinal distributions to variations in freshwater runoff and temperature regimes, and also to determine if early summer environmental parameters could be used as a proxy for abundance values of flatfish species.

Chapter III

Recruitment variability is one of the main causes of interannual variations in abundance of marine fishes. In *Environmental effects on the recruitment variability of nursery species* (Estuar. Coast. Shelf Sci., 2009, *in press*), several environmental factors such as river runoff, precipitation, sea surface temperature, salinity, the NAO index and wind speed and direction were tested, in order to determine which were most significant in explaining interannual variability in recruitment levels in three marine fish that use the estuary as a nursery area: *D. labrax*, *P. flesus* and *S. solea*. For achieving this purpose, a gamma-based Generalized Linear Model was used to relate the yearly abundance levels of 0-group fish with the environmental data. The results were compared with recent projections for climate change scenarios for the Iberian Peninsula, mainly an increase in extreme weather events such as droughts.

Chapter IV

Efficient monitoring and management plans need to be based on cost-effective sampling methodologies. Thus, chapter four focuses on assessing the effect of sampling gear used in the determination of the structure and composition of estuarine fish communities. In *Sampling estuarine fish assemblages with beam and otter trawls: differences in structure, composition and diversity*, the gear-specific fish assemblages were analyzed and compared based on estuarine use and feeding guilds. Multivariate analyses were used to detect differences regarding species composition and relative abundance of gear-specific assemblages.

Regarding the most abundant and commercially important species in both gears, *D. labrax*, *P. flesus* and *S. solea*, differences in median total length and length-frequency distributions were analyzed, as well as abundance trends over time. Results are discussed regarding the design of more efficient field surveys. Concerning the WFD, distinct results in the structure and composition of estuarine fish assemblages (either in species or functional groups) obtained by different sampling gears can significantly influence the determination of the Ecological Quality Status.

Chapter V

The WFD requires that fish assemblages should be sampled every three years for the determination of the Ecological Quality Status (EQS) in transitional waters. However, since different indices for determining the EQS can provide distinct results, it is necessary to analyze and assess differences and similarities between them, to insure that in the intercalibration process, European Union (EU) member states standardize their approaches. In the last chapter, *Assessing estuarine environmental quality using fish-based indices: performance evaluation under climatic instability* (Mar. Poll. Bull., 2008, 56: 1834-1843), the variation of five selected multimetric indices for the determination of the EQS of transitional waters was evaluated, as well as the indices' responses to an extreme drought. The selected indices were the EBI (Deegan et al., 1997), EDI (Borja et al., 2004), EFCI (Harrison and Whitfield, 2004), EBI (Breine et al., 2007) and TFCI (Coates et al., 2007). The consistency between indices was analyzed by the percentage of mismatch in the total sampling period (i.e. the percentage of the total number of times in which one index classifies as "Good" or "High" status, while another classifies as "Medium", "Poor" or "Bad" status). Since the indices were developed for other regions such as the United States, United Kingdom, South Africa, Belgium and Spain, their applicability in Portuguese estuaries was discussed. The results are examined in the scope of the EU WFD, regarding monitoring strategies, application of indices and EQS assessment.

Chapter I

The influence of an extreme drought event in the fish community of a southern Europe temperate estuary

Abstract

Between 2003 and 2006, a severe drought occurred throughout the Mondego River catchment's area, inducing lower freshwater flows into the estuary. As a consequence, both 2004 and 2005 were considered as extreme drought events. From June 2003 to June 2006, the fish assemblage of the Mondego estuary was sampled monthly in five stations during the night, using a 2-m beam trawl. Fish abundance was standardized as the number of individuals per 1000 m^2 per season and the assemblage was analysed based on ecological guilds: estuarine residents, marine juveniles, marine adventitious, freshwater, catadromous and marine species that use the estuary as a nursery area. A total of 42 species belonging to 23 families were identified, with estuarine residents and nursery species dominating the fish community. Variations in the fish community were assessed using non-metric MDS, being defined three distinct periods: summer and autumn 2003, 2004/2005 and winter and summer 2006. The main drought-induced effects detected were the depletion of freshwater species and an increase in marine adventitious in 2004/05, due to an extended intrusion of seawater inside the estuary and a significant reduction in abundance during the driest period of estuarine resident species. Nevertheless, from the management point of view, it could be stated that although some variations occurred due to environmental stress, the main core of the Mondego estuary fish community remained relatively unchanged.

Keywords

Fish assemblages :: Ecological guilds :: Climatic changes :: Drought events :: Temperate estuary :: Mondego

Introduction

The increasing rate of global climate change seen in the last century and the predictions to accelerate into the present one, will significantly impact the Earth's oceans and coastal waters (Short and Neckles, 1999; Mirza, 2003). Stochastic events such as weather extremes can cause fluctuations in the conditioning factors but often affect the state directly, for example, by eliminating parts of populations (Scheffer et al., 2001). Extreme environmental events (e.g. extreme droughts/floods) can seriously change the amount of water flowing within river systems (Tallaksen et al., 1997). In estuaries, these effects have only been addressed in a few studies concerning invertebrates (Attrill et al., 1996; Attrill and Power, 2000) and fishes (e.g. Garcia et al., 2001; Ecoutin et al., 2005). Such environmental instability, combined with anthropogenic derived pressures such as dredging activities, waste water and general organic pollution, will have significant impacts on estuarine fish communities. Hence, understanding the effects of changes in freshwater flow on estuarine systems is crucial to comprehend more about the dynamics of these key ecosystems, since river discharge into many estuaries is substantially altered due to human socio-economic development and may be in fact sensitive to climate change (Gleick, 2003).

Estuaries have long been regarded as important sites for fish (e.g. Haedrich, 1983; Mclusky and Elliot, 2004), and are characterized by a relatively low diversity but high abundance of individual taxa, most of which exhibit wide tolerance limits to the fluctuating conditions found within these systems (Whitfield, 1999). In order to simplify information and to allow a better comparison among different systems, Elliott and Dewailly (1995) defined the structure of the typical Atlantic Seaboard estuarine fish community, in terms of ecological guilds: estuarine residents, marine adventitious visitors, diadromous (catadromous or anadromous) migrant species, marine seasonal migrants, marine juvenile migrants which use estuaries as nursery grounds and freshwater adventitious species. Therefore, the knowledge of the effects and changes caused by environmental extreme variations in the above ecological groups should yield insights on the present status and to foresee future changes in fish communities.

Previous works regarding the Mondego estuary fish community focused mainly on the structure according to the ecological guilds previously referenced and also on the importance of the estuary as a nursery area for commercial marine species (for further details see Jorge et al., 2002; Leitão et al., 2006; 2007; Martinho et al., 2007). As a general trend, in the last 15 years it was noticed a major decline in species number from 1989-1992 to 2003/04, mostly freshwater species (Leitão et al., 2007). According to the same authors, this decline could be partially attributed to a change in the estuarine environment, as salinity increased over the past years due to continuous dredging activities to deepen the main shipping channel, within the estuary. Also, the decrease in the environmental quality of the estuary due to eutrophication pulses and consequent macroalgae blooms (Cardoso et al., 2004; Dolbeth et al., 2007a) could have been responsible for the loss of less resilient species.

In Portugal, several weather extremes have been recorded in the past years. According to the Portuguese Weather Institute (*http://web.meteo.pt/pt/clima/clima.jsp*), in 2003 was registered the second hottest summer since 1931, with the longest heat wave since 1941. In 2004, an extremely dry year, precipitation levels were far below average. June 2004 was the hottest month since 1931. In the year 2005, which was also an extremely dry year, the lowest precipitation values since 1931 were registered. June and August 2005 were the second and third hottest months since 1931. This year was considered of severe drought until September throughout the Portuguese territory, and was the harshest of the past 60 years. Until June 2006, 96% of the Portuguese territory remained under weak drought conditions (Portuguese Weather Institute, *http://web.meteo.pt/pt/clima/clima.jsp*). This series of consecutive changes allowed a direct comparison between years of different hydrological regimes.

Within this framework of events, the objectives of this work were a) to identify the changes that occurred in the fish community of the Mondego estuary along a three year period, from June 2003 to June 2006 and b) to assess if drought conditions influenced the distribution and composition of the fish community.

Materials and Methods

Study site

The Mondego River estuary (40º08'N, 8º50'W) is a small intertidal system, located in the western coast of Portugal (Fig. 1). It comprises two arms (north and south) that separate at about 7 km from the shore and join again near the mouth. The north arm is deeper, with 5 to 10 m depth at high tide and tidal range of 2 to 3 m, while the south arm is shallower, with 2 to 4 m depth at high tide and tidal range of 2 to 3 m. The south arm is almost silted up in the upstream areas, which causes the freshwater to flow mainly by the north arm. The water circulation in the south arm is mainly dependent on the tides and on a small freshwater input, carried out through the Pranto River, a small tributary system, which is regulated by a sluice according to the water needs in the surrounding rice fields. In the south arm, about 75% of the total area consists of intertidal mudflats, while in the north arm they stand for less than 10%.

The main point sources of pressure and disturbance in the Mondego are: a) dredging and shipping in the north arm and b) raw sewage disposal and high nutrient inputs from agricultural and fish farms in the upstream areas of the south arm. Combined with a high water residence time, this led to an eutrophication process, resulting in occasional spring macroalgae blooms of *Enteromorpha* spp. over the past two decades (e.g. Pardal et al., 2004; Dolbeth et al., 2007). As a result the ancient large meadows of *Zostera noltii* (Horneman, 1832) are restricted to a small patch in the downstream area (Cardoso et al., 2004).

Sampling procedures and hydrological data

The fish community was sampled monthly at five stations (Fig. 1), from June 2003 to June 2006 (except in July, September, October and December 2004, due to technical constraints). Fishing took place during the night, at high water of spring tides, using a 2m beam trawl with one tickler chain and 5 mm mesh size in the cod end. At each station, three trawls were towed for an average of 5 minutes each, covering at least an area of 500 m^2. All fish caught were identified and counted. Bottom water was analyzed for temperature, salinity, dissolved oxygen and pH while fishing took place.

Hydrological data was obtained from INAG – Portuguese Water Institute (*http://snirh.inag.pt*). Both monthly precipitation (from January 2002 to June 2006) and long-term monthly average precipitation (from 1933 to 2006) were obtained from the Soure 13F/01G station. Freshwater runoff was acquired from INAG station Açude Ponte Coimbra 12G/01A, near the city of Coimbra (located 40 km upstream).

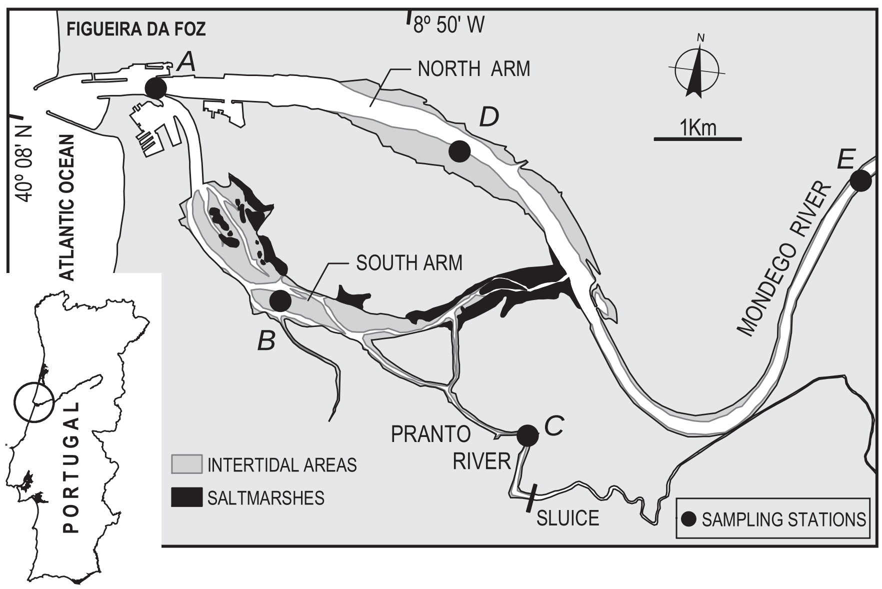

Figure 1. The Mondego River estuary – location of the 5 sampling stations.

Data analysis

The community structure was analyzed based on six ecological guilds established from habitat pattern usage (adapted from Elliott and Dewailly, 1995): marine adventitious species (MA), marine juvenile migrant species (MJ) (occurring usually in low densities in estuaries as an alternative habitat), marine species that use the estuary as nursery grounds (NU) (occurring in clear seasonal patterns, higher densities and remaining longer periods in estuaries), estuarine resident species (ER), catadromous adventitious species (CA) (no anadromous species were found) and freshwater adventitious species (FW). Monthly fish abundance data were expressed as the number of individuals per 1000 m^2. For a straightforward synthesis and analysis of the obtained results, monthly data were averaged by season, as follows: summer – from June to August; autumn – from September to November; winter – from December to February; spring – from March to May. Whenever one (or more months) was absent, seasonal values were calculated with the available data. Spatial variability was not taken into account due to the small size of the estuary.

Multivariate statistics were used to investigate variations in the structure of the fish community throughout the study period. A Bray-Curtis similarity matrix was computed using seasonal abundances of fishes of each guild (square root transformed), from which the non-metric multidimensional scaling (MDS) ordination plot was generated using PRIMER software package (version 5.0) (Clarke and Warwick, 2001). To validate the interpretation of the MDS, it was performed the ANOSIM test (analysis of similarities), built on a simple nonparametric permutation procedure, applied to the similarity matrix underlying the ordination of the samples (treatments) (Clarke and Warwick, 2001). The BIOENV procedure (PRIMER software package, version 5.0) (Clarke and Warwick, 2001) (all permutations of trial variables, Spearman rank correlation) was used to find the best combination of environmental variables explaining the variations in the fish community.

Salinity anomalies were calculated as the average monthly salinity subtracted to the corresponding average throughout the study period. Differences between years were tested using an ANOVA. Tukey-type *a posteriori* tests were used, whenever the null hypothesis was rejected. Spearman rank correlations between fish abundance (per ecological guild) and environmental parameters were calculated. A significance level of 0.05 was considered in all test procedures.

Results

Environmental characterization

Considerable variations in precipitation and freshwater runoff were observed during the surveys. While in 2003/04 precipitation values were higher than the 1931-2006 average, 2004 and 2005 were considered of extreme drought, with below average precipitation and low freshwater discharge (Fig. 2). Freshwater flow evidenced a severe reduction, with the lowest value in 2005 almost ten fold lower than the highest in 2003.

Salinity was highly variable at all stations, particularly at the most upstream ones ($F=34.1$, $p<0.05$). In 2005, salinity values were higher in all stations than in the remaining years. This fact was particularly marked in station E (reaching 20 in September 2005). Significant differences were found between salinity values of 2003/04 and 2004/05 ($q=0.006$, $p<0.05$, Tukey-type a posteriori test). Negative anomalies occurred mainly in 2003/04, while positive anomalies occurred mostly in 2005, indicating higher than average salinity values (Fig. 3). For further detailed information on salinity variations in the sampling stations see Marques et al. (2007). Temperature showed a typical variation for the temperate regions, varying from 8.82 ± 1.69 ºC to 22.74 ± 2.55 ºC (Table 1).

Fish assemblage

Throughout the study period, a total of 10697 individuals, belonging to 42 species and 23 families composed the Mondego estuary fish assemblage (Table 2). The most abundant species were the estuarine residents (ER) *Pomatoschistus microps* and *Pomatoschistus minutus*, the species that use the estuary as nursery grounds (NU) *Dicentrarchus labrax*, *Solea solea* and *Platichthys flesus*, and the marine juvenile migrants (MJ) *Diplodus vulgaris*. Juvenile stages composed most of the estuarine community (unpublished results).

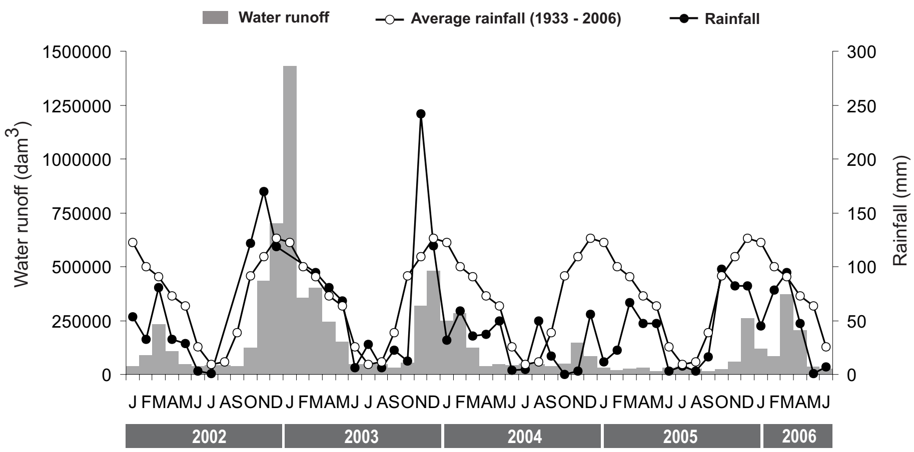

Figure 2. Monthly precipitation and river runoff values from January 2002 to June 2006, plotted against the average precipitation values for 1933-2006.

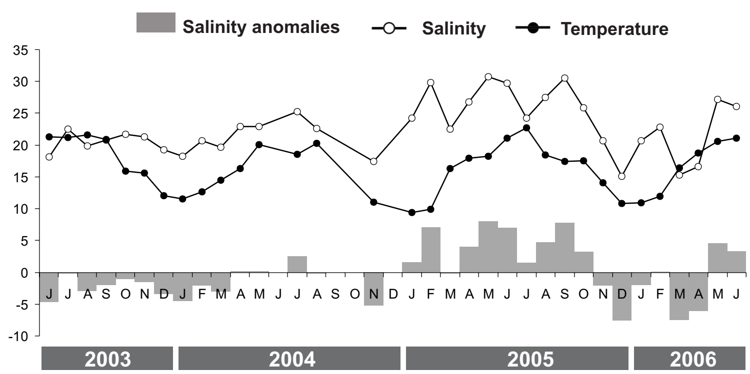

Figure 3. Average monthly variation of bottom salinity, temperature (ºC) and salinity anomalies for the study period, in the Mondego estuary.

Table 1. Monthly variation (mean values ± standard deviation) of bottom water salinity, temperature (ºC), dissolved oxygen (mg/l) and pH.

Month	Salinity	Temperature (ºC)	O_2 (mg/l)	pH	Month	Salinity	Temperature (ºC)	O_2 (mg/l)	pH
Jun-03	18.10 ± 11.52	21.24 ± 1.11	5.53 ± 0.72	8.05 ± 0.20	**Mar-05**	22.54 ± 12.97	16.26 ± 0.75	9.57 ± 1.37	8.03 ± 0.23
Jul-03	22.54 ± 13.45	21.22 ± 3.61	8.24 ± 1.76	8.04 ± 0.26	**Apr-05**	26.78 ± 8.78	17.94 ± 1.78	9.07 ± 1.43	8.13 ± 0.21
Aug-03	19.86 ± 12.22	21.64 ± 2.28	8.09 ± 1.71	8.03 ± 0.26	**May-05**	30.72 ± 6.28	18.26 ± 2.60	9.08 ± 1.18	8.05 ± 0.18
Sep-03	20.78 ± 14.14	20.88 ± 1.26	7.51 ± 0.97	7.95 ± 0.16	**Jun-05**	29.68 ± 6.39	21.04 ± 2.91	8.73 ± 1.32	7.81 ± 0.16
Oct-03	21.68 ± 13.34	15.92 ± 0.42	9.29 ± 1.07	7.85 ± 0.27	**Jul-05**	24.24 ± 11.67	22.74 ± 2.55	7.62 ± 1.30	7.91 ± 0.13
Nov-03	21.28 ± 14.06	15.62 ± 0.28	8.93 ± 1.06	7.86 ± 0.44	**Aug-05**	27.46 ± 6.29	18.42 ± 2.29	7.99 ± 0.75	8.06 ± 0.09
Dec-03	19.28 ± 11.88	12.02 ± 0.97	10.76 ± 1.13	8.00 ± 0.28	**Sep-05**	30.48 ± 6.63	17.40 ± 1.78	8.63 ± 0.92	7.99 ± 0.12
Jan-04	18.24 ± 11.23	11.52 ± 1.52	10.46 ± 0.35	7.89 ± 0.27	**Oct-05**	25.90 ± 11.35	17.56 ± 0.67	8.84 ± 1.28	7.92 ± 0.10
Feb-04	20.64 ± 13.01	12.62 ± 1.17	9.77 ± 1.39	8.28 ± 0.11	**Nov-05**	20.66 ± 13.73	14.12 ± 0.72	8.24 ± 0.21	-
Mar-04	19.68 ± 12.53	14.46 ± 1.48	10.13 ± 0.45	8.31 ± 0.22	**Dec-05**	15.14 ± 10.91	10.86 ± 0.94	-	-
Apr-04	22.88 ± 10.82	16.32 ± 1.26	9.98 ± 1.02	8.00 ± 0.19	**Jan-06**	20.70 ± 13.14	10.92 ± 1.36	10.06 ± 0.59	7.72 ± 0.07
May-04	22.92 ± 11.31	20.04 ± 2.71	7.89 ± 1.76	8.10 ± 0.19	**Feb-06**	22.78 ± 8.17	11.93 ± 0.88	10.59 ± 0.53	7.78 ± 0.13
Jun-04	25.25 ± 12.49	18.50 ± 3.05	7.53 ± 1.19	8.04 ± 0.14	**Mar-06**	15.28 ± 14.26	16.44 ± 1.28	9.25 ± 0.42	8.37 ± 0.14
Aug-04	22.60 ± 12.74	20.24 ± 2.73	8.45 ± 1.04	7.99 ± 0.46	**Apr-06**	16.64 ± 14.82	18.76 ± 2.27	8.41 ± 1.20	8.08 ± 0.36
Nov-04	17.44 ± 10.22	10.98 ± 1.06	9.52 ± 0.48	-	**May-06**	27.20 ± 9.86	20.58 ± 3.37	8.00 ± 1.04	8.02 ± 0.12
Jan-05	24.28 ± 10.58	8.82 ± 1.69	-	-	**Jun-06**	26.06 ± 6.02	21.04 ± 2.36	8.02 ± 0.85	7.99 ± 0.16
Feb-05	29.78 ± 7.99	9.92 ± 0.38	11.99 ± 0.79	8.28 ± 0.04					

Freshwater species (FW) (*Barbus bocagei*, *Carassius auratus* and *Gambusia holbrooki*) were only occasionally collected until the winter of 2004. Marine adventitious species (MA) such as *Arnoglossus laterna*, *Buglossidium luteum*, *Gaidropsarus mediterraneus*, *Solea lascaris* and *Symphodus bailloni* only appeared after the summer of 2004. In fact, *A. laterna*, *B. luteum*, *S. lascaris* and *Trisopterus luscus* were only captured inside the estuary throughout 2005.

The number of species per month was constant over the study period, with an average number of 15 (±3). However, differences in the total number of species per year were observed, with the highest values recorded in 2005/06 – 35 species, then 2003/04 – 34, and finally 2004/05 – 29 species.

Regarding the number of species per ecological guild (Fig. 4A), there was an increase in marine adventitious species (MA) since the spring of 2005, composing almost 25% of the species number. On the opposite, there was a decrease in marine juvenile migrant species (MJ). Estuarine residents (ER) and nursery species (NU) comprised about 50% of the species number. In terms of densities (Fig. 4B), the nursery species (NU) dominated the assemblage in 2003/04, while in 2004/05 and 2005/06 the community was dominated by the estuarine residents (ER), particularly *Pomatoschistus* species, comprising about 75% of the community in the autumn 2004 and spring and summer 2006. In 2005, the relative abundance of marine adventitious species (MA) also increased.

The most significant changes in the fish assemblage were detected in three guilds: estuarine residents (ER), freshwater adventitious (FW) and marine adventitious (MA) (Fig. 5). Considering marine adventitious species (MA), there was an increase in densities mainly in 2005, during the period of extremely low precipitation and freshwater flow. In 2006, abundance values were similar to those found in 2003. An opposite pattern was found for estuarine residents (ER), with low densities in the driest periods (2004 and 2005). These species exhibited summer abundance peaks, but in higher magnitude in 2003 and 2006.

Table 2. Mondego estuary fish community: distribution of species according to Family and Ecological Guild (CA – catadromous; ER – estuarine resident; MA – marine adventitious; FW – freshwater; MJ – marine juvenile; NU – nursery) and average number of individuals per 1000 m^2 for all the study period.

Species	Family	Ecological Guild	Average N ind.1000 m^{-2}	Species	Family	Ecological Guild	Average N ind.1000 m^{-2}
Ammodytes tobianus	Ammodytidae	MA	0.119 ± 0.34	***Liza aurata***	Mugilidae	MJ	0.014 ± 0.04
Anguilla anguilla	Anguillidae	CA	0.633 ± 0.94	***Liza ramada***	Mugilidae	CA	0.250 ± 0.58
Aphia minuta	Gobiidae	MA	0.062 ± 0.22	***Mugil cephalus***	Mugilidae	MJ	0.005 ± 0.02
Arnoglossus laterna	Scophthalmidae	MA	0.016 ± 0.06	***Mullus surmuletus***	Mullidae	MJ	0.104 ± 0.17
Atherina boyeri	Atherinidae	ER	0.789 ± 1.29	***Nerophis lumbriciformis***	Syngnathidae	ER	0.008 ± 0.05
Atherina presbyter	Atherinidae	ER	0.098 ± 0.21	***Parablennius gattorugine***	Blenniidae	MA	0.003 ± 0.02
Barbus bocagei	Cyprinidae	FW	0.007 ± 0.04	***Platichthys flesus***	Pleuronectidae	NU	1.501 ± 1.64
Buglossidium luteum	Soleidae	MA	0.003 ± 0.02	***Pomatoschistus microps***	Gobiidae	ER	8.155 ± 11.58
Callionymus lyra	Callionymidae	MA	0.148 ± 0.27	***Pomatoschistus minutus***	Gobiidae	ER	3.685 ± 6.04
Carassius auratus	Cyprinidae	FW	0.002 ± 0.01	***Sardina pilchardus***	Clupeidae	MJ	0.275 ± 1.10
Chelon labrosus	Mugilidae	MJ	0.009 ± 0.04	***Scophthalmus rhombus***	Scophthalmidae	MJ	0.050 ± 0.07
Ciliata mustela	Gadidae	MJ	0.129 ± 0.21	***Solea lascaris***	Soleidae	MA	0.025 ± 0.07
Conger conger	Congridae	MA	0.018 ± 0.04	***Solea senegalensis***	Soleidae	MJ	0.099 ± 0.15
Dicentrarchus labrax	Moronidae	NU	7.507 ± 7.93	***Solea solea***	Soleidae	NU	1.663 ± 1.42
Dicologlossa hexophthalma	Soleidae	MJ	0.002 ± 0.01	***Sparus aurata***	Sparidae	MJ	0.020 ± 0.05
Diplodus vulgaris	Sparidae	MJ	1.422 ± 1.84	***Spondyliosoma cantharus***	Sparidae	MA	0.018 ± 0.07
Echiichthys vipera	Trachinidae	MA	0.027 ± 0,07	***Symphodus bailloni***	Labridae	MA	0.047 ± 0.11
Engraulis encrasicolus	Engraulidae	MA	0.052 ± 0.17	***Syngnathus abaster***	Syngnathidae	ER	0.165 ± 0.25
Gaidropsarus mediterraneus	Gadidae	MA	0.002 ± 0.01	***Syngnathus acus***	Syngnathidae	ER	0.257 ± 0.52
Gambusia holbrooki	Poeciliidae	FW	0.011 ± 0.06	***Trigla lucerna***	Triglidae	MJ	0.120 ± 0.26
Gobius niger	Gobiidae	ER	0.124 ± 0.14	***Trisopterus luscus***	Gadidae	MA	0.102 ± 0.24

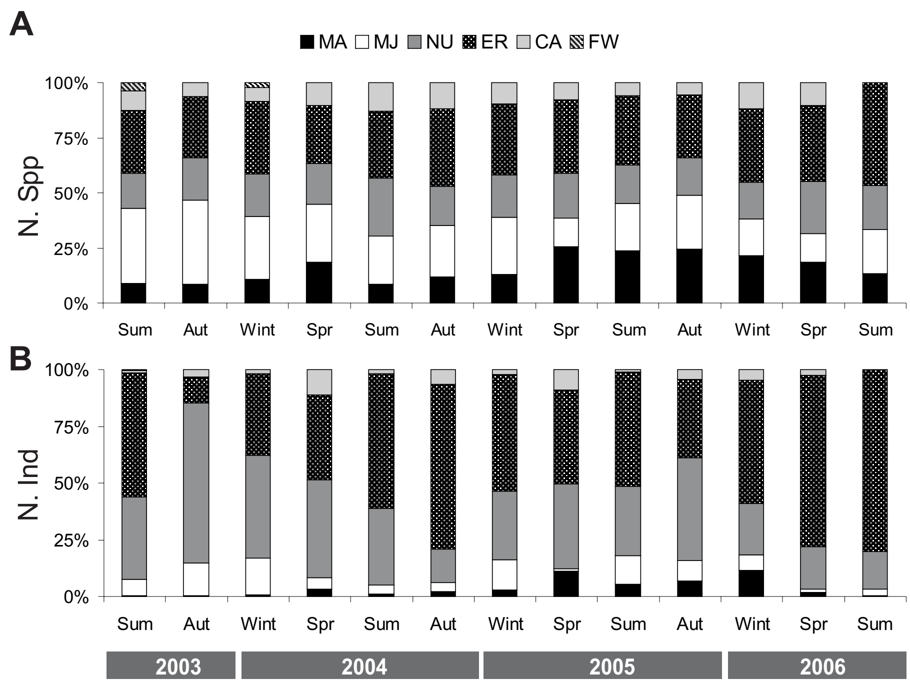

Figure 4. Seasonal variation of the relative composition of each Ecological Guild, according to the number of species (A) and number of individuals per 1000 m^2 (B). CA – Catadromous; ER – Estuarine residents; MA – Marine adventitious; FW – Freshwater; MJ – Marine juvenile migrants; NU – Nursery. Sum – summer; Aut – autumn; Wint – winter; Spr – spring.

Freshwater species (FW) were last recorded in estuarine waters in the winter of 2004 and in very low densities. Taking into account marine juvenile migrant (MJ) and nursery (NU) species, a decrease in densities was also observed along the study period.

Statistical analysis of the fish assemblage

The fish community (based on the ecological guilds) was seasonally separated in the MDS ordination plot, particularly the years of 2003 and 2006 (Fig. 6). Differences between seasons and years were identified, mainly in 2003 and 2006, which were farther separated from the remaining years. Furthermore, 2006 exhibited high variability among seasons, showing the greatest scatter within the same year. Both summers of 2003 and 2006 were closely linked in the ordination plot. All seasons of 2004 and 2005 were closer in the diagram, along with the spring of 2006. An opposition between the autumn of 2003 and 2004 was observed.

Significant differences were detected with ANOSIM between years at the 5% level: 2005 was significantly different from 2006 (R=0.426; p=0.029). Although not significantly, differences were also found between 2003 and 2004 (R=0.857; p=0.067) and between 2003 and 2005 (R=1; p=0.067). The BIOENV procedure revealed that dissolved oxygen, pH and precipitation were responsible for explaining 34% of the variability within the fish community.

Spearman rank correlations of the abundance of each ecological guild with the environmental conditions showed a positive correlation of the nursery and residents guilds with temperature (r_S=0.56; $p<0.05$ and r_S=0.64; $p<0.05$, respectively) (Table 3). Negative correlations were found between the estuarine residents and precipitation (r_S=-0.54; $p<0.05$) and between marine adventitious and water runoff (r_S=-0.81; $p<0.05$).

Discussion

Fish community

The Mondego estuary fish assemblage was similar to the typical European Atlantic seaboard estuarine community defined by Elliott and Dewailly (1995), Pihl et al. (2002) and by Mathieson et al. (2000) for tidal marshes, with a dominance of estuarine residents (ER) and species that use estuaries as nursery areas (NU). The present results corroborate the findings of Malavasi et al. (2004), in which the estuarine residents comprise about 50% of the fish community of the Venice lagoon. Unlike larger estuaries, marine juvenile migrants (MJ) were less important in both number of species and total densities. Pihl et al. (2002) found that within a group of selected estuarine systems, great regional variance exists in the composition and abundance of the ecological guilds making up the estuarine fish assemblages, mainly due to particular characteristics of each estuarine system. This seems to agree with our results, as the differences found can be attributed to the small size of the Mondego estuary and to its small opening, which can limit the entrance of marine species.

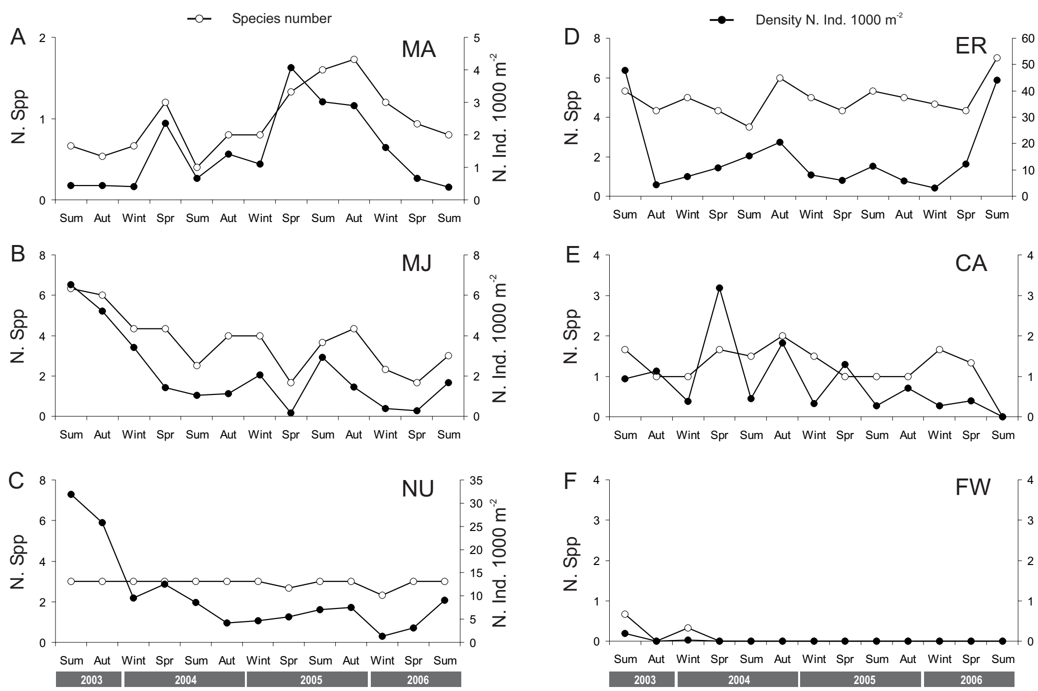

Figure 5. Seasonal variation of the species number and number of individuals per 1000 m^2 of each Ecological Guild, A – Marine adventitious (MA); B – Marine juvenile migrants (MJ); C – Nursery (NU); D - Estuarine residents (ER); E – Catadromous (CA); F – Freshwater (FW). Sum – summer; Aut – autumn; Wint – winter; Spr – spring.

The most important families of the Mondego estuary fish assemblage were Gobiidae, Moronidae, Soleidae, Pleuronectidae, Sparidae and Atherinidae. When comparing with other European estuarine systems (e.g. Laffaille et al., 2000; Cabral et al., 2001; Gordo and Cabral, 2001; Potter et al., 2001; Costa et al., 2002; Hampel et al., 2003; Malavasi et al., 2004), the main difference that can be found is the absence of Mugilidae as one of the most important families.

Beam trawling is often considered a suitable method for sampling benthic species, but tends to underestimate pelagic species (Thiel et al., 2003) such as mugilids.

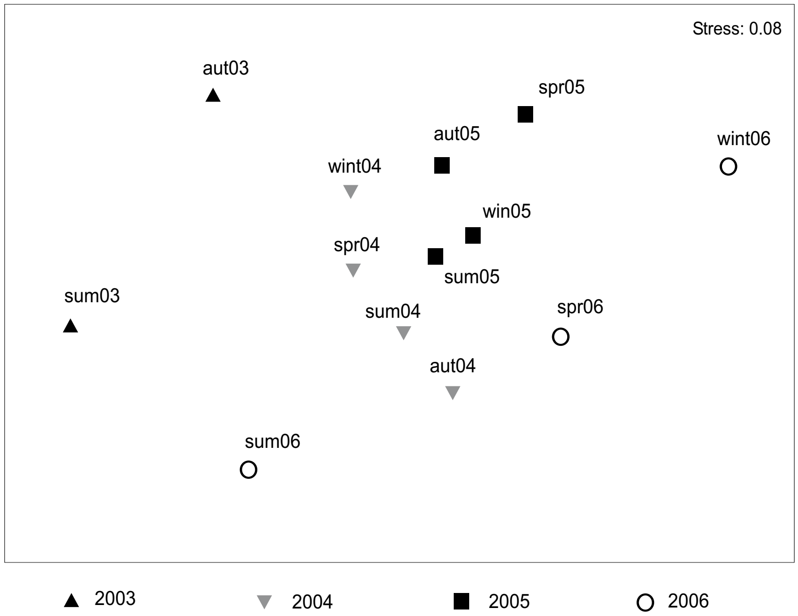

Figure 6. Two dimensional MDS ordination plot of the fish community according to season. Sum – summer; Aut – autumn; Wint – winter; Spr – spring.

The nursery species (NU) *D. labrax*, *P. flesus* and *S. solea* comprised a significant part of the assemblage, in conformity with other European estuaries (e.g. Elliott and Dewaily, 1995; Laffaille et al., 2000; Gordo and Cabral, 2001; Malavasi et al., 2004), reinforcing the importance of small estuaries as nursery areas and their role for the maintenance of coastal stocks. As outlined by Martinho et al. (2007), seasonal abundance peaks for these species were recorded, and directly linked to the seasonal patterns of recruitment and migration between enclosed and coastal waters. In terms of numbers, the fish community was dominated by *P. microps*. This estuarine resident (ER) was the most abundant since the autumn 2004, and in agreement with Leitão et al.

(2006), tends to occupy inner areas of the estuary, thus reducing competition with other Gobiidae species such as *P. minutus*, which is mainly concentrated at the mouth of the estuary (Leitão et al., 2006; Dolbeth et al., 2007b).

One significant aspect of this work was the reappearance of *S. bailloni*, a marine species that lives in association with seagrass beds. This species had been captured in the previous study performed by Jorge et al. (2002) in the early nineties, but was absent until the summer of 2004. The relevance of this finding can be related to the success of the eutrophication mitigation measures implemented in the estuary since 1998 (for further details see Lillebø et al., 2005), which led to a recovery of the area covered by *Z. noltii* (15 ha in 1986; 0.02 ha in 1997; 4.7 ha in 2006) in the south arm of the estuary, increasing the available habitat for this species. Currently, a management program is taking place, in order to restore the communication of the north and south arms, to improve the water circulation and to reduce the residence time, aiming at the restoration of the seagrass meadows.

Extreme drought events and their impacts in fish assemblages

Having in mind that the measurement of any ecological response by the fish community, or individual species within a community, must take cognisance on the key role played by the physico-chemical environment in influencing the structure and functioning of that estuary (Whitfield and Elliott, 2002), this work tried to assess the influence of extreme drought events in the fish community.

Due to the occurrence of two consecutive extreme dry years, several changes occurred in the Mondego river basin. In fact, according to the Portuguese Weather Institute (*http://web.meteo.pt/pt/clima/clima.jsp*), in these last years severe differences in climate have been recorded when compared to the general climate patterns for the period of 1961-1990. With decreasing precipitation levels and in an extreme drought situation, freshwater was stored in dams located upstream, which led to a decrease in water runoff to the estuary, from 2004 to 2005. According to Whitfield (1999), variations in the river flow in estuaries influence not only the salinity but also the biochemical properties of the water body. Furthermore, decreasing freshwater flow results in the incursion of saline waters into reaches of the estuary previously dominated by freshwater (Attrill et al., 1996), and in agreement, strong positive salinity anomalies were observed mainly in 2005, indicating that higher than average salinity values occurred throughout the estuary.

Table 3. Spearman rank correlations between the average abundance of fishes of each Ecological Guild and some environmental parameters. Sal – salinity; Temp – temperature; O_2 – dissolved oxygen; pH – pH; Avg Precip – average precipitation; Avg Runoff – average river runoff. * are significant values for *p*<0.05.

Guild	Sal	Temp (ºC)	O_2 (mg l^{-1})	pH	Avg Precip (mm)	Avg Runoff (dam^3)
MA	0.49	0.00	0.26	0.23	0.24	-0.81 *
MJ	0.24	0.34	0.27	-0.01	0.05	0.05
NU	0.24	0.56 *	0.06	0.18	0.17	0.05
ER	0.18	0.64 *	-0.36	0.51	-0.54 *	-0.16
CA	0.01	0.11	0.29	0.48	0.29	-0.06
FW	0.01	0.43	0.17	0.38	0.17	-0.05

Typical marine adventitious species (MA) like *A. laterna*, *B. luteum*, *S. lascaris* and *T. luscus* were only captured in the estuary during the period where the highest drought effects were experienced – 2005, benefiting from a higher extent of the incursion of seawater into the estuary (due to low freshwater runoff). In fact, none of these species had ever been described in the Mondego estuary, according to the baseline study performed by Jorge et al. (2002) in the early nineties. This points out an increase in salinity over the past decade inside the estuary, related to the effects of low freshwater flow combined with continuous dredging activities in the north arm (the location of the commercial harbour) which increases the salinity incursion inside the estuary (e.g. Marchand et al., 2002; Leitão et al., 2007).

According to the present data, it was possible to define three distinct periods in terms of environmental conditions: a) 2004 and 2005, when the harshest drought induced effects were felt; b) 2003, when precipitation levels were considered normal and c) 2006, when precipitation levels were slightly lower than normal/regular years. These periods were identified in the MDS plot, showing the seasons of 2004 and 2005 grouped together, and although high dissimilarity between 2003 and 2006 was detected, summer values of both years look similar.

This indicates that the changes in the fish community due to the effects of low precipitation and freshwater flow were more significant in 2004/05. Accordingly, the main changes in the fish community were the depletion of freshwater species (FW) and an increase in marine adventitious species (MA), supported by the strong negative correlation between marine adventitious species (MA) and freshwater runoff, and a decrease in abundance of the estuarine residents (ER) during the driest period. The increase in marine adventitious species (MA) had already been reported in 1992 by Jorge et al. (2002), as a consequence of a hydrological dry year. The opposition found between the autumn 2003 and autumn 2004 seems to be caused by a shift in the relative proportions of estuarine residents (ER) and nursery species (NU). In addition, the ordination of samples in the MDS plot suggests that the fish community showed more seasonal changes in wet years than in dry years, as previously observed by Potter et al. (1986) and Cuesta et al. (2006) in other temperate estuaries. As outlined in the BIOENV analysis, precipitation was one of environmental parameters best explaining the variability in the fish community.

References of the impacts of drought events on temperate estuarine fish communities are scarce in literature, with more available information on planktonic (e.g. Marques et al., 2007) and invertebrate benthic communities (e.g. Attrill, 1996; Attrill and Power, 2000; Cuesta et al., 2006). The previous authors concluded that in years of low freshwater flow, and mainly in the upper reaches of estuarine areas, freshwater communities were gradually replaced by others tolerant to higher salinities due to a higher saline incursion. This seems to corroborate the present results, even if comparing different trophic levels. Furthermore, Attrill and Power (2000) pointed out that fluctuations in key benthic species such as *Carcinus maenas* or *Crangon crangon* in response to climatic changes can induce variations in the whole estuarine community. Drake et al. (2002) suggested that short-term salinity fluctuations during warmer periods may negatively affect species that complete their lifecycle within the estuary. Likewise, the estuarine residents (ER) presented lower densities during the dry period and according to Fonds and Van Buurt (1974), the mortality of *P. microps* and *P. minutus'* eggs (the most abundant resident species) is highly dependent on salinity, conditioning the recruitment's strength (maximum egg survival at 5-35 with maximum larval size at 5-15 for *P. microps* and, 15-35 for maximum egg survival with maximum larval size at 35 for *P. minutus*). Also, predation pressure on these species could have increased due to the higher abundance of marine adventitious (MA) species, particularly at the mouth of the estuary (for further discussion on this topic see Dolbeth et al., 2007b). According to Garcia et al. (2001), surveys in a warm-temperate south-western Atlantic intermittently closed lagoon (South Brazil) led to the conclusion that during *La Niña* episodes (cold and dry events) there was an increase in marine species inside the lagoon, as a consequence of low freshwater input and a higher intrusion of sea water into the upper reaches. The same pattern was observed by Ecoutin et al. (2005) in a coastal lagoon in the Ivory Coast.

However, and despite the changes described above, the majority of the fish assemblage remained rather unchanged. In the three years of sampling, the ten most abundant species always comprised more than 90% of the number of individuals of the fish community, composing the main core of the community (Table 4) and indicating that the major changes occurred in species with lower frequencies. Similar results were obtained by Costa et al. (2007) for the Tagus estuary, when comparing various sampling periods from 1978 to 2002. The previous authors reported that the structure of the fish community remained relatively unchanged during subsequent dry and wet years, although higher densities were recorded in dry years. To assess whether climatic extremes have long term and more significant impacts on estuarine fish communities will require even longer and geographically wider data bases, due to the slow response time to disturbance that characterizes fish species (Cabral et al., 2001).

Table 4. Species abundance ranking (% of number of individuals per 1000 m^2) for the periods of 2003/04, 2004/05 and 2005/06. Only the 20 most abundant species are shown.

2003/04		2004/05		2005/06	
Species	**%**	**Species**	**%**	**Species**	**%**
D. labrax	34.58	*P. microps*	42.85	*P. microps*	40.43
P. microps	20.31	*D. labrax*	15.89	*D. labrax*	17.79
P. minutus	17.55	*P. flesus*	8.70	*P. minutus*	8.79
D. vulgaris	7.00	*S. solea*	5.92	*S. solea*	7.05
P. flesus	5.69	*P. minutus*	5.79	*A. boyeri*	6.82
S. solea	5.52	*D. vulgaris*	3.46	*P. flesus*	2.64
A. anguilla	2.46	*A. boyeri*	3.38	*S. pilchardus*	2.39
S. acus	0.89	*A. anguilla*	3.34	*D. vulgaris*	2.09
A. boyeri	0.86	*A. tobianus*	1.47	*S. acus*	1.20
L. ramada	0.78	*L. ramada*	1.33	*A. anguilla*	1.19
S. pilchardus	0.62	*C. lyra*	1.22	*T. luscus*	1.11
T. lucerna	0.60	*S. abaster*	1.13	*C. lyra*	1.04
C. mustela	0.56	*S. acus*	0.64	*M. surmuletus*	0.90
S. abaster	0.44	*C. arautus*	0.61	*L. ramada*	0.89
G. niger	0.39	*A. presbyter*	0.55	*A. presbyter*	0.86
A. minuta	0.28	*E. encrasicolus*	0.55	*S. senegalensis*	0.74
S. rhombus	0.25	*G. niger*	0.50	*S. abaster*	0.58
S. senegalensis	0.21	*M. surmuletus*	0.48	*G. niger*	0.55
A. tobianus	0.17	*S. bailloni*	0.45	*C. mustela*	0.43
E. vipera	0.12	*T. luscus*	0.41	*E. encrasicolus*	0.36

In conclusion, and despite interanual variations in recruitment and mortality rates, it was possible to assess some effects of extreme drought events on a temperate estuarine fish community. This influence seems to be increased in the case of the Mondego due to its small area (3.4 km^2), with lower thresholds to fast environmental variations. Moreover, and in conformity with Attrill et al. (1996), the influence of severe drought conditions, which are predicted to increase over the subsequent years, should be strongly considered when undertaking management plans for estuaries and river catchment areas. Also, prolonging ongoing monitoring programmes should be encouraged, in order to understand the long term effects of climatic changes in key systems such as the estuarine environment. The use of ecological guilds proved to be a strong tool in assessing changes in the estuarine system by reducing variability and allowing direct comparisons between estuaries in the future.

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Chapter II

Does the flatfish community of the Mondego estuary (Portugal) reflect environmental changes?

Abstract

The temporal and spatial patterns of abundance of the flatfish community of the Mondego estuary were investigated from 2003 to 2007, based on monthly beam trawl samples. During the study period, a severe drought occurred, with consequent reductions in river runoff. A total of eight flatfish species occurred within the estuary, with seasonal specific richness and abundance varying considerably, being possible to identify three main groups: one group composed of species present throughout the study period and in high densities: *Platichthys flesus* and *Solea solea*; one group composed of less abundant species but regularly present: *Scophthalmus rhombus* and *Solea senegalensis*; and another group, composed of occasional species, that occured mainly during the drought period: *Arnoglossus laterna, Buglossidium luteum, Dicologlossa hexophthalma* and *Pegusa lascaris*. Flatfish distribution patterns varied according to the estuarine use guild: marine-estuarine dependent fish occurred mainly in the upper reaches, while the marine stragglers and marine-estuarine opportunists occurred mostly in the downstream areas. Species with more northern latitudinal affinities were the most affected by the drought related with a lesser extent of river plumes to the coastal area, resulting in a reduction in abundance levels. On the other hand, flatfish species with more southern affinities increased in abundance during the drought, benefiting also from an increase in estuarine water temperature. Early summer salinity and precipitation values were good proxies for estimating abundance levels of *P. flesus* and *S. solea*, respectively, emphasizing the importance of hydrodynamics for the recruitment and abundance of these commercially important species.

Keywords

Flatfish :: Drought :: River runoff :: Mondego estuary :: *Solea solea* :: *Platichthys flesus*

Introduction

Flatfish species are one of the main components of estuarine fish assemblages (e.g. Mathieson et al., 2000; Cabral et al., 2007; Franco et al., 2008), using estuaries mainly as nursery areas or temporary habitats as an alternative to coastal areas (Beck et al., 2001). In estuaries, the distribution and abundance of flatfishes has been related to both biotic (prey abundance, predation) and abiotic factors, such as temperature, salinity, sediment type and freshwater runoff (e.g. Rogers, 1994; Amara et al., 2000; Werner et al., 1997; Power et al., 2000; Sims et al., 2004). In flatfish species, reproduction strategies usually include batch spawning in offshore waters, and larvae finding estuarine and coastal waters, where the chances of survival and maximizing growth are higher. However, given the natural variability in current speed and direction, the potential for drift and migration to nursery environments, themselves of varying quality, might be expected to result in high recruitment variability (Bailey et al., 2005), with consequent high mortality in juvenile stages. In agreement, recruitment variability in flatfish seems to be mainly regulated by density independent (i.e. environmental) factors, related with transport and retention mechanisms, wind, tidal currents and estuarine circulation (see van der Veer et al., 2000; Bailey et al., 2005 for reviews), being the availability of high quality nursery areas also essential for a successful recruitment (Florin et al., 2009). Differences in ontogenic state (i.e. juveniles and adults) interact with seasonal fluctuations in abiotic and biotic factors to produce differential distribution and habitat use at a variety of spatial and temporal scales (Gibson et al., 1996).

The Atlantic coast of Portugal provides a natural case study for the latitudinal distribution of flatfish species, since it is the southern range limit for northern species, and the northern limit for southern species, representing the transition between northeastern Atlantic warm-temperate and cold-temperate regions (Ekman, 1953; Briggs, 1974). From the more than twenty-five species that occur in the Portuguese coast (Albuquerque, 1956; Cabral, 2002), eight have been observed in the Mondego estuary (Leitão et al., 2007; Martinho et al., 2007a). Most of these species have high commercial value, providing important local offshore fisheries (Power et al., 2000; Vasconcelos et al., 2008). Hence, understanding the dynamics of flatfish abundance and recruitment patterns in estuaries can offer an insight on the success of coastal fisheries in a nearby future. Recently, Cabral et al. (2007) reported on the relative importance of Portuguese estuarine nurseries for flatfish species, stating that their occurrence was not only dependent on the latitudinal gradient, but also on specific habitat preferences. Flatfish species are known to be impacted by environmental degradation of anthropogenic origin (such as dredging, bank reclamation, chemical and organic pollution), which can affect their distribution within nurseries by changes in habitat suitability and availability, food abundance or sediment contamination (Vinagre et al., 2005; Pérez-Rusafa et al., 2006; Schlacher et al., 2007; Vasconcelos et al., 2007; Courrat et al., 2009). Additionally, it is known also that channel morphology and habitat niche requirements influence fish and demersal assemblages (Elliott and Hemingway, 2002).

Due to their location, transitional waters are expected to be influenced by climate change and its main expressions, such as an increase in frequency of extreme weather events and sea level rise. According to the Portuguese Weather Institute (*http://www.meteo.pt*), six of the last twelve years have ranked among the hottest and driest of the past one hundred years. In 2004 and 2005, a severe drought was observed in the Portuguese territory, with several implications for the estuarine communities, such as a decline in secondary production of the intertidal benthic invertebrate and fish communities (Dolbeth et al., 2007, 2008), a displacement of planktonic and fish assemblages to upstream areas (Marques et al., 2007; Martinho et al., 2007a) and a reduction in abundance and recruitment levels of several marine-estuarine dependent species (Dolbeth et al., 2008; Martinho et al., 2009). For this reason, understanding the effects of weather extremes, and the hydrological features associated with them, on estuarine and coastal ecosystems is an added value for the establishment of suitable management plans as well as for the implementation of climate change mitigation measures. The objectives of this work were to study the distribution and abundance patterns of flatfish species in the Mondego estuary, their relation with the drought event that took place during the study period, and to determine if estuarine flatfish communities can be used as effective early indicators of climatic events, such as droughts.

Materials and Methods

Study site

This study was conducted in the Mondego estuary, located in the Atlantic coast of Portugal (40º08'N, 8º50'W). In the terminal part, the estuary is divided in two arms – north and south (Fig. 1) that join again near the mouth. The two arms have different hydrological features: the north arm is deeper, with 5 to 10 m depth at high tide and tidal range of 2 to 3 m, while the south arm is shallower, with 2 to 4 m depth at high tide and tidal range of 1 to 3 m. In the north arm is located the commercial harbour, being subjected to constant dredging to deepen the main shipping channel. The south arm is silted up to some extent in the upstream areas, which causes the freshwater to flow mainly by the north arm. In the south arm, about 75% of the total area consists of intertidal mudflats, while in the north arm they account for less than 10%. The water circulation in the south arm is mainly dependent on tides and on a small freshwater input, carried out through the Pranto River, a small tributary river which is regulated by a sluice according to the water needs in the surrounding rice fields.

Flatfish sampling and data acquisition

Flatfish abundance data was obtained from monthly night samples, from June 2003 to July 2007. Sampling was conducted at five stations (M, N1, N2, S1, S2; Fig. 1), using a 2m beam trawl with one tickler chain and 5mm mesh size in the cod end. At each station, three tows of about five minutes at the speed of one knot were carried out, covering at least an area of 500 m^2. All fish caught were immediately frozen, and taken to the lab for posterior identification and counting. After fishing, temperature, salinity, dissolved oxygen and pH were measured in one sample of bottom water.

Hydrological data were obtained from the Portuguese Water Institute (INAG, *http://snirh.inag.pt*): monthly precipitation (from 2003 to 2007) and long-term average precipitation (1961-1990) were obtained from the Soure 13F/01G station (near the estuary), and freshwater runoff was acquired from INAG station Açude Ponte Coimbra 12G/01A, near the city of Coimbra (located 40 km upstream). Sea surface temperature (SST) for the 1º Lat x 1º Long square nearest to the Mondego estuary was obtained from the International Comprehensive Ocean-Atmosphere Data Set (ICOADS) online database (*http://dss.ucar.edu/pub/coads*, Slutz et al., 1985).

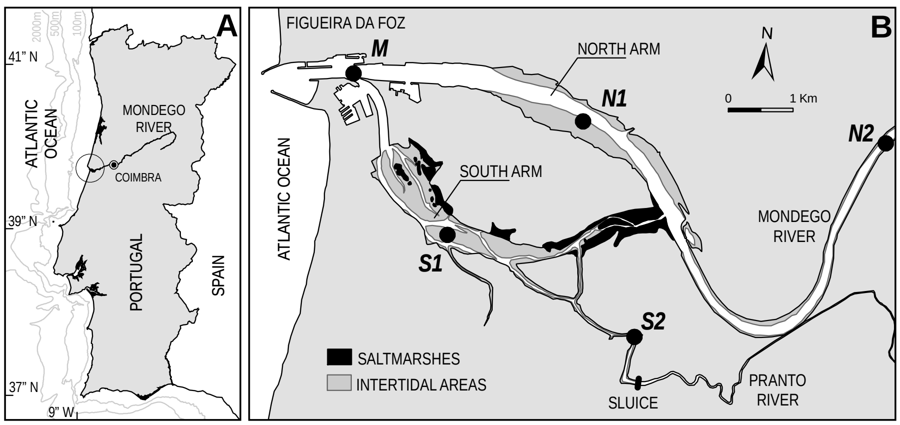

Figure 1. Location of the Mondego estuary on the Portuguese coast (A) and the sampling stations within the estuary (B).

Data analysis

Monthly flatfish abundance data (individuals per 1000 m^2) were obtained from averaging the total number of individuals according to the surveyed area. Seasonal abundance estimates were determined by averaging monthly data, according to the following routine: winter (December, January, February) and spring (March, April, May), summer (June, July, August) and autumn (September, October, November). The environmental variables were also averaged by season, to provide an easier approach on the five-year data set. Spearman rank correlations were used to determine if significant relationships existed between the seasonal abundance estimated for each species and the environmental parameters. In addition, the abundance values in June, considered as a proxy for early summer abundance levels and corresponding to the end of the estuarine colonization period for both species, were compared with the environmental variables measured in the same month using linear regression analyses. Prior to the analysis, the assumptions (normality, homogeneity and independence of the variance) of the linear regression were verified.

To evaluate the relationship between flatfish species abundance and environmental variables, a redundancy analysis (RDA) was performed. For the RDA it was used the seasonal abundance data for each species. All environmental variables were used in a first analysis and their significance was tested with a forward selection procedure. Afterwards, a second db-RDA was performed using only the significant environmental variables. The RDA was chosen after detecting the linear response of the flatfish abundance data with the Detrended Correspondence Analysis (DCA). These analyses were performed with CANOCO software (version 4.5) (Ter Braak, 1995). A significance level of 0.05 was considered in all test procedures.

Results

Environmental background

During the study period a severe drought occurred, with a significant reduction in precipitation and freshwater runoff to the estuary (Fig. 2). The hydrological years of 2003 and 2006 were considered as regular, while 2004, 2005 and 2007 were extremely dry years, with the harshest effects observed from the summer 2004 to the end of 2005 (classified as the worst drought since 1931 by the Portuguese Weather Institute). Significant differences were obtained for yearly runoff values during the study period ($H=16.923$; $p<0.001$). Regarding precipitation, during the drought period nearly all values were below the 1961-1990 long-term average levels (Fig. 2). In the autumn 2006, heavy rainfall was recorded (Table 1, Fig. 2), inducing the lowest salinity values throughout the study period. This variation in precipitation and runoff regimes influenced considerably the salinity patterns inside the estuary, with higher average salinity values in 2005 (Fig. 3A).

In the summers of 2003 and 2005, heat waves were also recorded (Portuguese Weather Institute), which translated into the highest average water temperature measured inside the estuary, especially in 2005, and also into the highest summer difference between estuarine water temperature and SST in the adjacent coastal area (3.24ºC both in 2003 and 2005) (Fig. 3B). Moreover, 2005 was the year with the highest range between the lowest and highest average water temperature (8.8ºC in January and 22.7ºC in July, respectively) (Fig. 3B). An overview of the environmental conditions (seasonal average ± standard deviation) is presented in Table 1.

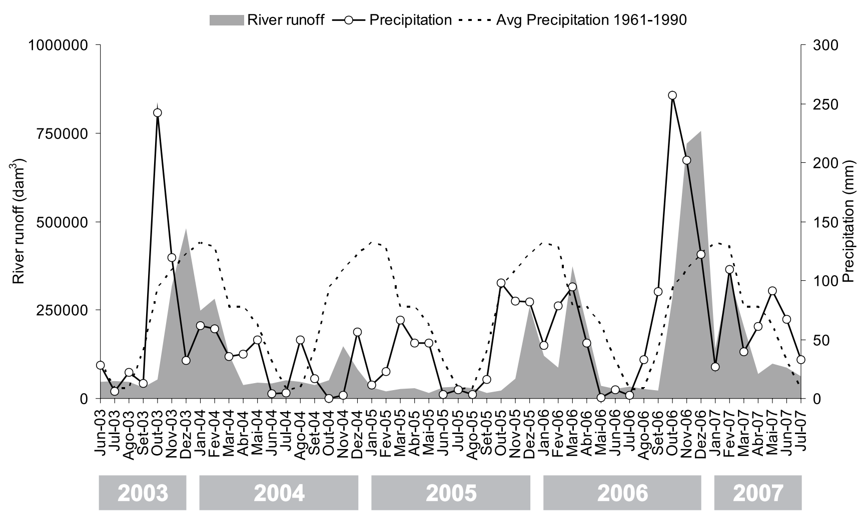

Figure 2. Monthly variation of precipitation (mm), long-term precipitation average (mm) (1961-1990) and river runoff (dam^3) in the Mondego river basin.

Table 1. Seasonal variation of average values (standard deviation between brackets) of the environmental parameters measured from 2003 to 2007.

Year	Season	Salinity	Temperature (ºC)	O_2 (mg l^{-1})	pH	Runoff (dam^3)	Precipitation (mm)	SST (ºC)
2003	Summer	20.17 (2.24)	21.37 (0.24)	9.15 (2.27)	8.26 (0.05)	47718.67 (455.68)	18.87 (11.44)	19.20 (1.11)
	Autumn	21.25 (0.45)	17.47 (2.95)	9.57 (1.17)	8.11 (0.12)	134833.00 (159587.73)	124.77 (114.84)	18.35 (2.05)
2004	Winter	19.39 (1.20)	12.05 (0.55)	9.77 (2.29)	8.07 (0.17)	337229.67 (126423.41)	50.93 (16.36)	14.55 (0.40)
	Spring	21.83 (1.86)	16.94 (2.84)	10.72 (0.46)	8.25 (0.08)	69822.67 (47941.03)	40.93 (7.54)	14.68 (0.60)
	Summer	23.93 (1.87)	19.37 (1.23)	9.52 (0.00)	7.62 (0.00)	46705.00 (4710.65)	19.27 (26.10)	20.10 (0.85)
	Autumn	17.44 (0.00)	10.98 (0.00)	9.40 (0.00)	-	78794.00 (59163.03)	6.60 (8.96)	16.65 (0.00)
2005	Winter	27.03 (3.89)	9.37 (0.78)	13.22 (0.00)	8.16 (0.21)	45317.00 (33791.99)	30.13 (23.14)	14.30 (0.42)
	Spring	26.68 (4.09)	17.49 (1.07)	10.89 (0.23)	8.25 (0.04)	24203.67 (7096.59)	53.73 (11.23)	15.33 (1.53)
	Summer	27.13 (2.74)	20.73 (2.18)	9.33 (0.53)	8.00 (0.14)	31398.00 82842.42)	4.57 (2.54)	19.45 (0.78)
	Autumn	25.68 (4.91)	16.36 (1.94)	9.73 (1.27)	8.11 (0.06)	31399.67 (21905.51)	65.50 (43.22)	17.97 (0.91)
2006	Winter	19.54 (3.95)	11.24 (0.60)	10.21 (1.24)	7.74 (0.01)	155682.67 (91389.55)	68.60 (20.26)	15.03 (0.87)
	Spring	19.71 (6.52)	18.59 (2.08)	9.21 (0.60)	8.16 (0.19)	204779.33 (168483.29)	47.57 (47.00)	15.57 (1.91)
	Summer	26.50 (0.62)	18.32 (3.85)	9.36 (0.29)	7.99 (0.23)	29084.00 (3266.24)	14.33 (16.49)	20.05 (0.49)
	Autumn	15.38 (11.38)	17.30 (3.40)	9.40 (0.00)	7.63 (0.20)	342115.67 (352776.52)	183.13 (84.89)	19.47 (0.42)
2007	Winter	22.43 (0.00)	14.40 (0.00)	10.65 (0.21)	7.87 (0.39)	414494.33 (313290.91)	86.07 (51.79)	15.18 (0.46)
	Spring	23.58 (3.08)	14.25 (0.78)	8.20 (0.00)	7.55 (0.00)	122979.33 (70556.38)	63.97 (25.80)	16.00 (0.00)
	Summer	27.06 (3.89)	14.90 (0.00)	8.50 (0.00)	7.53 (0.00)	75159.50 (17084.41)	50.00 (0.00)	19.50 (0.00)

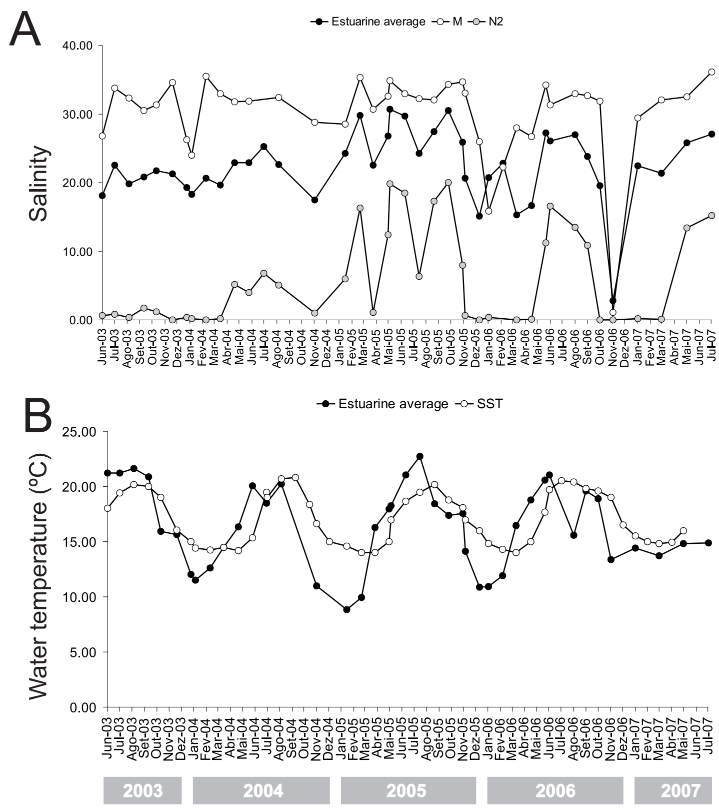

Figure 3. Monthly variation of salinity at stations M (most downstream station), N2 (most upstream station) and estuarine average salinity values (A), and estuarine average temperature and sea surface temperature (SST) for the 1º Lat x 1º Long nearest to the Mondego estuary (B).

Flatfish distribution and abundance patterns

During the study period eight flatfish species were captured in the Mondego estuary: *Arnoglossus laterna* (scaldfish), *Buglossidium luteum* (solenette), *Dicologlossa hexophthalma* (ocellated wedge sole), *Pegusa lascaris* (sand sole), *Platichthys flesus* (flounder), *Scophthalmus rhombus* (brill), *Solea senegalensis* (Senegalese sole) and *Solea solea* (common sole). *P. flesus* and *S. solea* were the most ubiquitous and abundant species (maximum density of 3.71±2.74 and 4.14±0.00 Ind 1000m^{-2}, respectively), showing seasonal summer abundance peaks (Fig. 4A, B). From 2005 onwards, the abundance peaks of *P. flesus* were of lower magnitude than in the previous years. Regarding *S. solea*, summer abundance peaks were of similar magnitude during the study period, with lower values in 2004. In 2006, the abundance peak was displaced to the autumn (Fig. 4B), coinciding with an increase in precipitation and river runoff. *S. rhombus* and *S. senegalensis* were less abundant, but were present in most of the sampling events during the study period (maximum densities of 0.15±0.12 and 0.25±0.27 ind. 1000m^{-2}, respectively). Seasonal abundance peaks were observed for *S. rhombus* in the summer months (Fig. 5A), while for *S. senegalensis* abundance peaks were observed in the autumn (Fig. 5B). Abundance peaks for these species were in the same order of magnitude between species and seasons, but *S. rhombus* was absent in the summers of 2004 and 2007. As for the remaining species, abundance patterns varied between seasons, but were all captured inside the estuary during the dry period (Fig. 6). *A. laterna* was captured in the autumn 2004, when the highest densities were found (0.28±0.00 Ind 1000m^{-2}), and in the summer and autumn 2005 (Fig. 6A). *B. luteum* and *D. hexophthalma* were only caught in one occasion (autumn 2005 and winter 2006, respectively), with maximum densities of 0.03±0.03 and 0.02±0.04 ind. 1000m^{-2} (Fig. 6B, C). *P. lascaris* was only observed in the estuary during 2005, from spring to autumn, being the maximum densities recorded in this last season (0.15±0.14 Ind 1000m^{-2}) (Fig. 6D).

The ordination diagram obtained by the DCA (Fig. 7) revealed a clear salinity gradient from the mouth of the estuary to the upstream areas, with the flatfish species distributing accordingly: the marine stragglers *A. laterna, B. luteum, D. hexophthalma*, *P. lascaris* and the marine-estuarine opportunists *S. rhombus* and *S. senegalensis* occurring in more saline downstream areas (M, N1 and S1), with higher oxygen levels, while the abundant marine–estuarine dependent species *S. solea* and *P. flesus* distributed essentially in the upstream areas (N2 and S2). Particularly, *S. solea* was influenced by higher runoff values (mostly in the winters of 2003, 2006 and 2007, Fig. 2).

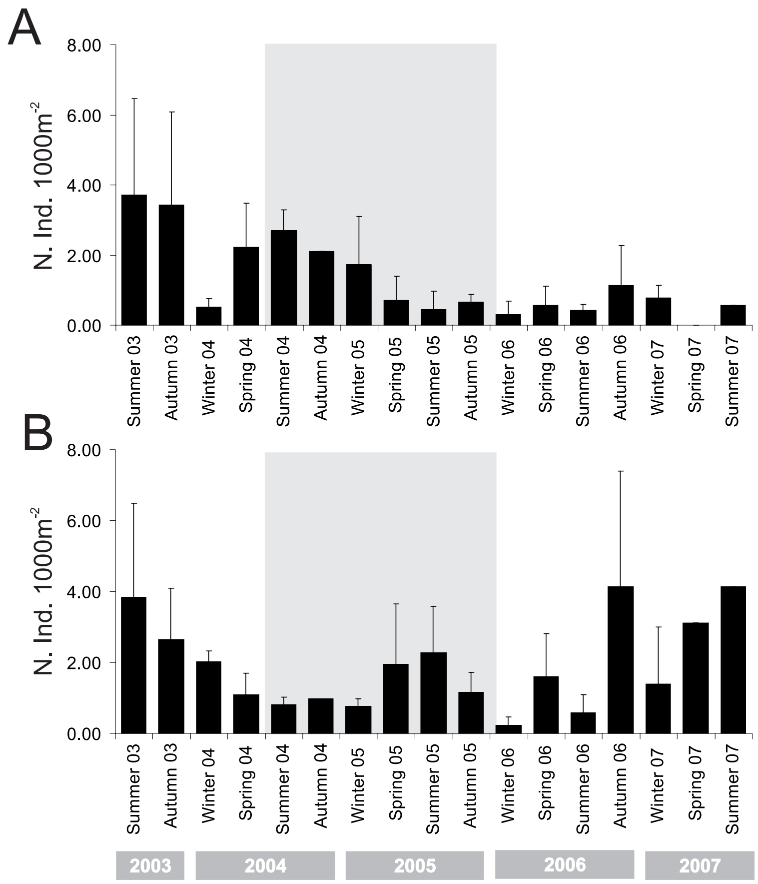

Figure 4. Seasonal abundance patterns (N. Ind 1000m^{-2}) of *P. flesus* (A) and *S. solea* (B) from June 2003 to July 2007. Bars represent the standard deviation. Shaded area represents the drought period.

Significant correlations (Spearman rank correlation) between the average seasonal abundance values and the corresponding environmental parameters were obtained for *P. lascaris* and estuarine average salinity (r_S=0.494; p<0.05), for *P. lascaris* and river runoff (r_S=-0.584; p<0.05) and for *S. solea* and dissolved oxygen (r_S=-0.498; p<0.05).

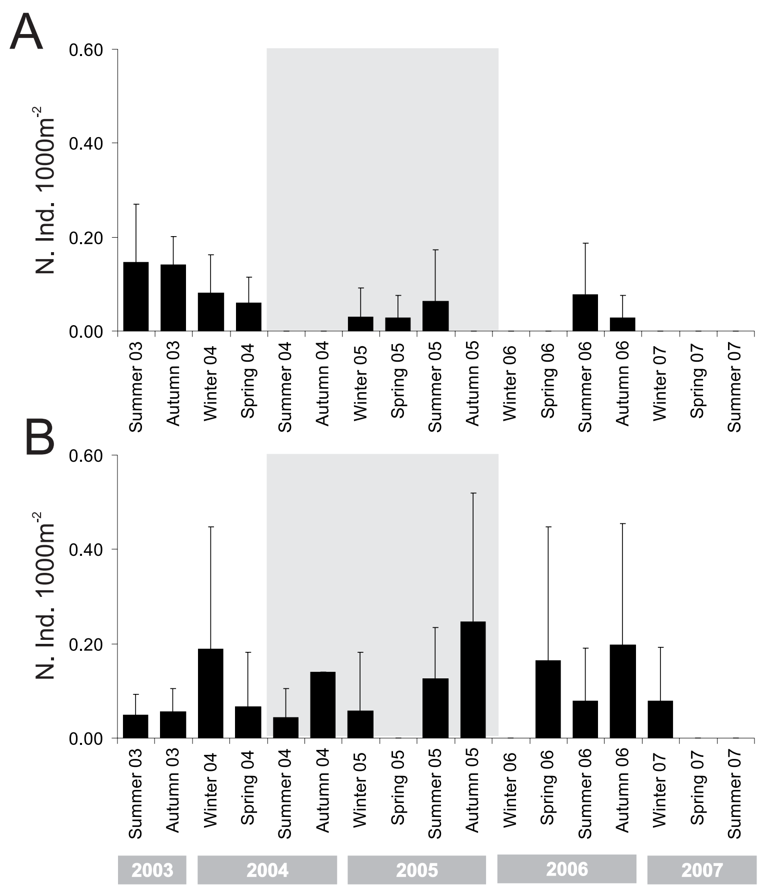

Figure 5. Seasonal abundance patterns (N. Ind 1000m^{-2}) of *S. rhombus* (A) and *S. senegalensis* (B) from June 2003 to July 2007. Bars represent the standard deviation. Shaded area represents the drought period.

These results are in accordance with the DCA analysis. For evaluating the relationship between early summer abundance of the dominant species and the environmental parameters, a regression analysis was used to model the densities in June for each species. Significant relationships were obtained for *P. flesus* and estuarine average salinity (R^2=0.896; t=-5.059; p<0.05), and for *S. solea* and precipitation (R^2=0.765; t=3.125; p<0.05) (Fig. 8).

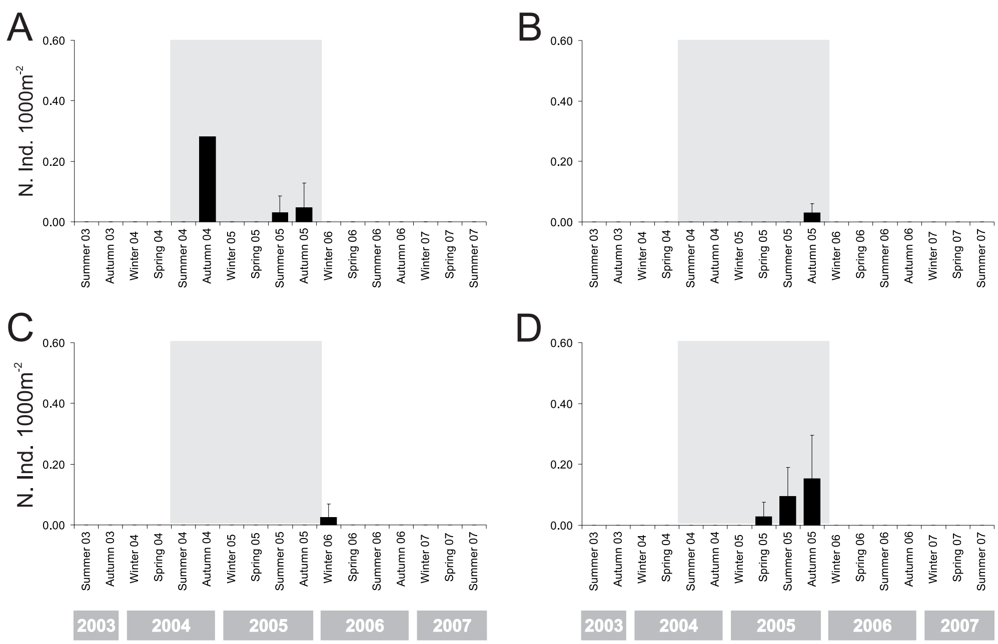

Figure 6. Seasonal abundance patterns (N. Ind 1000m^{-2}) of *A. laterna* (A), *B. luteum* (B), *D. hexophthalma* (C) and *P. lascaris* (D) from June 2003 to July 2007. Bars represent the standard deviation. Shaded area represents the drought period.

Discussion

The flatfish community of the Mondego estuary

Among the twenty-five species that have been reported for the Portuguese Atlantic coast

(Albuquerque, 1956; Cabral, 2002), eight were caught inside the Mondego estuary. Among these species, several estuarine habitat use guilds were represented, being possible to detect different responses regarding the weather events that took place. Accordingly, the life strategies of estuarine organisms create the structure of the estuarine ecosystem, reflect its functioning and can be used to determine the spatial and temporal utilization of the available resources of space and food (Franco et al., 2008). Over the study period, flatfish specific richness and abundance varied considerably, being possible to identify three main groups: one group composed of species present throughout the study period and in high densities (*P. flesus*, *S. solea*), one group composed of less abundant species but regularly present (*S. rhombus, S. senegalensis*) and another group, composed of occasional species, present mainly during the drought period (*A. laterna, B. luteum, D. hexophthalma, P. lascaris*). In fact, the two most abundant flatfish species in this study, *P. flesus* and *S. solea*, are among the most abundant species of the estuarine fish assemblage, as previously reported by Martinho et al. (2007a). At a wider scale, Pleuronectidae and Soleidae are one of the most abundant families in European estuarine fish assemblages (e.g. Laffaille et al., 2000; Mathieson et al., 2000; Potter et al., 2001; Courrat et al., 2009). Accordingly, these species use the estuary mainly as a nursery area, in which juveniles stay for a period of about two years, migrating afterwards to the adjacent coastal area (Dolbeth et al., 2008; Martinho et al., 2008). Both species presented summer abundance peaks reflecting mainly the estuarine colonization period by 0-group fish, although *P. flesus* showed a decreasing tendency in abundance, mainly after the drought period.

S. rhombus and *S. senegalensis*, although common species in the flatfish assemblage, were present in much lower densities, with summer and autumn abundance peaks, respectively. Both species belong to the marine-estuarine opportunists ecological guild, who often enter estuarine waters, particularly as juveniles, but use to different degrees nearshore marine waters as alternative habitats (Elliott et al., 2007). The seasonal differences in abundance peaks between both species reflected their different spawning periods: from March to August for *S. rhombus* (Nielsen, 1986c) and from May to August for *S. senegalensis* (Quéro et al., 1986).

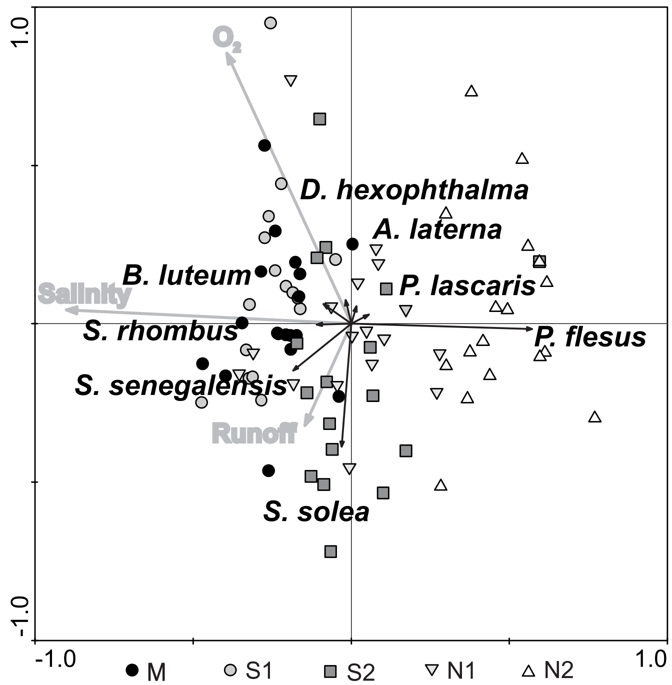

Figure 7. RDA analysis, showing the relationship between flatfish community densities throughout the estuary and significant environmental variables (after forward selection). Symbols, position of the sampling stations within the ordination space; black vector lines, flatfish species densities; grey vector lines, relationship of environmental variables to the ordination axis, whose length is proportional to their relative significance. Total variation explained by the diagram: 25%.

The remaining species, *A. laterna, B. luteum* and *D. hexophthalma*, marine stragglers, and *P. lascaris*, a marine-estuarine opportunist species, were only captured inside the estuary during the dry period, occurring mainly in the lower reaches of the estuary, where the marine influence is still high. Generally, these species appear in estuarine waters in small numbers, being associated with coastal marine waters (Elliott et al., 2007). Given the latitudinal location of the estuary, the flatfish community was composed of species with both subtropical and temperate affinities, since many flatfish species have their southern and northern distribution limits along the Portuguese coast (Cabral, 2002) (Table 2). Moreover, this area represents two distinct coastal climatic divisions, being the transition between northeastern Atlantic warm-temperate and cold-temperate regions (Ekman, 1953; Briggs, 1974).

Influence of environmental changes in estuarine flatfish communities

According to Cabral et al. (2007), the occurrence of flatfish species in Portuguese estuaries seems to be dependent mainly on the latitudinal gradient and on specific habitat preferences. In the Mondego estuary, flatfish species were distributed in different estuarine areas, with the marine-estuarine dependent species *P. flesus* and *S. solea* occurring in the innermost areas, while the other species, all marine stragglers and marine-estuarine opportunists, occurred mainly in the lower estuarine reaches. In fact, distribution of flatfish in temperate estuarine areas has been related with sediment preferences, food availability and salinity tolerances (e.g. Power et al., 2000; Vinagre et al., 2005; Martinho et al., 2007b). Regarding latitudinal distributions, the flatfish species present in the estuary belong to different biogeographic ranges, either showing more northern (such as *P. flesus* and *S. rhombus*) or southern affinities (such as *D. hexophthalma* and *P. lascaris*).

Concurrently, patterns related with latitudinal distribution and habitat preferences can be influenced by climate change, either by a northward displacement of suitable habitats due to the increase in seawater temperature, or by local weather events induced by global changes, such as droughts, floods and heat waves. In fact, several authors have reported a consistent effect of environmental changes on the recruitment and abundance of flatfish species in temperate estuaries, mainly hydrodynamics and freshwater flow (e.g. Legget and Frank, 1997; van der Veer et al., 2000; Vinagre et al., 2007; Wilson et al., 2008; Martinho et al., 2009). In the present work, several changes in the flatfish community were mainly induced by the reduction in freshwater flow, essentially at two levels: a decrease in abundance of the marine-estuarine dependent *P. flesus* in the years that succeeded the drought period, and an increase in marine straggler

Table 2. Life history traits of the eight flatfish species observed in the Mondego estuary from 2003 to 2007, ranked according to the distribution range. Data sources: [1] Nielsen, 1986a; [2] Nielsen, 1986b; [3] Nielsen, 1986c; [4] Quéro et al., 1986; [5] Froese and Pauly, 2009.

Species	Distribution range	Biogeographical area	Habitat	Spawning period
P. flesus	72°N - 30°N [5] Temperate	Eastern Atlantic, from the White Sea to Mediterranean and Black Sea [2]	Continental shelf, brackish waters (juveniles), soft bottoms [2]	February-June [2]
S. rhombus	64°N - 30°N [5] Temperate	Eastern Atlantic, Mediterranean and Black Sea [3]	Continental shelf (shallower areas), sandy bottoms [3]	March-August [3]
S. solea	67°N - 17°N [5] Subtropical	Eastern Atlantic, Mediterranean and southward to Senegal [4]	Continental shelf, estuaries and lagoons, mainly between 0-200m, sand and mud [4]	January-April (Mediterranean), December-May (Bay of Biscay), April-June (North Sea) [4]
B. luteum	64°N - 3°S [5] Subtropical	Eastern Atlantic and Mediterranean [4]	Continental shelf, mainly between 10-40m, sandy bottoms [4]	February (Mediterranean), March-June (Bay of Biscay), July-August (English Channel, North Sea) [4]
A. laterna	62°N - 17°S [5] Subtropical	Eastern Atlantic, Mediterranean and southward to Cape Blanc [1]	Continental shelf up to 200m, mixed muddy bottoms [1]	April-August [1]
P. lascaris	57°N - 32°S [5] Subtropical	Eastern Atlantic, western Mediterranean and southward to South Africa [4]	Continental shelf, mainly between 20-50m, gravel, sand and muddy sand [4]	May-September, peak in June-July (Iberian Peninsula, Bay of Biscay, west English Channel) [4]
S. senegalensis	47°N - 14°N [5] Subtropical	Eastern Atlantic, Mediterranean (rare) and southward to Senegal [4]	Continental shelf, estuaries and lagoons, mainly between 0-100m, sand and mud [4]	May-August (peak in June) (Iberian Peninsula, Bay of Biscay) [4]
D. hexophthalma	34°N - 17°S [5] Subtropical	Eastern Atlantic, Mediterranean and southward to Angola [4]	Demersal, shallow waters [4]	No data available

flatfishes during the driest period, as a consequence of higher salinity intrusion in the lower reaches (Martinho et al., 2007a).

For *P. flesus* and *S. solea*, recruitment variability, and consequently estuarine abundance, has been described as mostly depending on density independent factors, such as precipitation, freshwater runoff, SST, the North Atlantic Oscillation (NAO), tidal and wind circulation (e.g. Marchand, 1991; Amara et al., 2000; Attrill and Power, 2002; Sims et al., 2004; Henderson and Seaby, 2005; Bailey et al., 2008). In addition, for Portuguese estuaries, river runoff was identified as one of the main sources of variation inducing the abundance for *P. flesus* and *S. solea* (Vinagre et al., 2007; Martinho et al., 2009). Moreover, two different responses were obtained for these species regarding the impacts of the drought period: *P. flesus*, which is the only flatfish species in Europe that can be found in freshwater, was mostly affected by the reduction in river flow, as lower densities were found in the estuary after this period (in accordance with the significant negative relationship between *P. flesus* early summer abundance and salinity). This could be related to its early spring spawning period (when river runoff was more reduced), comparing to the early winter spawning period of *S. solea*, when river runoff is at its maximum extension. On the other hand, for *S. solea* no significant impact on abundance levels was observed during the drought period, except for the displacement of the abundance peak in 2006 to the autumn, which matched an increase in precipitation and river runoff. The role played by land-based runoff in increasing coastal fishery production has been recognized for the common sole (Salen-Picard et al., 2002), and in addition, the significant relationship between early summer *S. solea* abundance and precipitation reinforced this statement.

Regarding *S. senegalensis*, studies in the Portuguese coast are mainly restricted to the Tagus estuary. In this context, Vinagre et al. (2007) noted that the abundance of this species in estuarine areas was also positively correlated with river runoff, mainly through a higher extension of river plumes to coastal areas that act as proximity indicators of the nursery areas for fish larvae. As for *P. lascaris*, studies are scarce and mainly related to fisheries management (e.g. Pinheiro et al., 2005; Marques et al., 2006). In agreement with the present results, Cabral et al.

(2000) found that in the Sado estuary (southern Portugal) this species was only present occasionally. Furthermore, the significant correlations with river runoff and salinity (negative and positive, respectively) reinforce the idea that the abundance of this species in the estuary was mainly driven by the drought-induced changes.

The species with more northern temperate affinities*, P. flesus* and *S. rhombus*, seemed the most affected by the drought period, either by the reduction in freshwater runoff, but also by temperature, which exerts its influence in all environments and directly affects organism physiology (Attrill and Power, 2004). In fact, during the drought period, and as a consequence of a heat wave, the estuarine temperature was higher than in the other years. Since both species are at the southern limit of their distribution range (Nielsen, 1986b, c; Froese and Pauly, 2009), an increase in estuarine temperature could be a limitation for those species. *P. flesus*, which occurs almost exclusively in the northern estuaries of the Portuguese coast above 39ºN (Cabral et al., 2007; Vasconcelos et al., 2007), may be subjected to a more significant impact of extreme climatic events such as the ones here described, since it has been suggested that *P. flesus* eggs are highly sensitive to water temperatures higher than 12°C in the winter, inducing high mortality levels (Von Westernhagen, 1970).

Likewise, Sims et al. (2004) pointed out that the migration phenology of *P. flesus* in the western English Channel appears to be driven to a large extent by short-term, climate-induced changes in the thermal resources of their overwintering habitat. This suggests that climate fluctuations may have significant effects on the timing of the peak abundance of fish populations (Sims et al., 2004). Moreover, and in agreement with Martinho et al. (2009), since this species spawns in early spring (Nielsen, 1986b), further reductions in river flow and the increase in SST that are expected to take place in the Iberian Peninsula (Miranda et al., 2006) will significantly influence future recruitment levels, as well as the occurrence of this species in this estuary. On the other hand, the increase in estuarine temperature can potentially benefit the flatfish species with more southern affinities (Table 2).

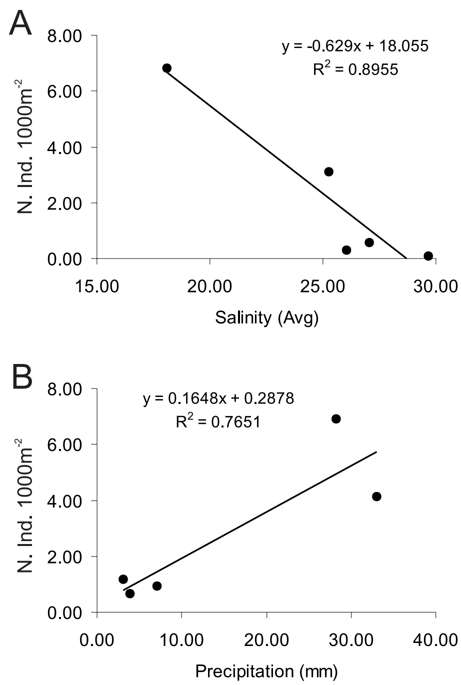

Figure 8. Significant linear regressions (p<0.05) between the abundance estimates in June for each year of the two most abundant species, *Platichthys flesus* (A), *Solea solea* (B) and the correspondent environmental parameters.

For the Bristol Channel (United Kingdom), Henderson and Seaby (2005) identified that an increase in abundance levels of *S. solea* was highly positively correlated with an increase in water temperature in the early growing season. In addition, this species spawns mainly in the early winter (Quéro et al., 1986; Koutsikopoulos and Lacroix, 1992) when reductions in freshwater runoff were not so evident, which may have contributed to a constancy of recruitment and abundance levels (in accordance with Vinagre et al., 2007; Martinho et al., 2009). For the less abundant species *A. laterna, B. luteum, D. hexophthalma* and *P. lascaris*, all with southern affinities, an increase in SST could induce higher abundance values inside the estuarine area.

Although no significant relationships of flatfish abundance with SST were determined, which can be a limitation induced by the five-year database. Attrill and Power (2002) demonstrated that long-term juvenile fish abundance is best explained by temperature differentials between estuarine and coastal waters. Thus, further investigations in relating the abundance of key species with water temperature should be conducted, mainly in what concerns recruitment patterns and the timing of migrations between estuarine and coastal waters.

Conclusions

This work was provided a new insight of using flatfish assemblages as indicators of environmental changes. Evidence that this particular group is a good indicator of environmental status in estuarine waters relies on the recent inclusion in multi-metric indices for evaluating both the ecological quality status of transitional waters (Uriarte and Borja, 2009) and the anthropogenic pressures in the main Portuguese estuaries (Vasconcelos et al., 2007). Ultimately, the species richness and abundance of fishes in estuaries comes down to a matter of function, in accordance with Elliott et al. (2007) and Franco et al. (2008): if flatfish assemblages change, will the function of the ecosystem remain or will it change? In this case, evidence was presented that the function of the Mondego estuary as a nursery area for flatfish species can be reduced, in light of the present climate change scenarios, where salinity and temperature seem to be having synergistic effects, acting over the abundance and distribution ranges of flatfish species. Finally, the use of early summer environmental parameters, namely salinity for *P. flesus* and precipitation for *S. solea*, demonstrated to be a good surrogate for expected abundance levels, so further research on this topic should be addressed, in order to provide prompt and reliable indicators of estuarine abundance of these commercially important flatfish species.

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Chapter III

Environmental effects on the recruitment variability of nursery species

Abstract

The recruitment variability of the marine fish species *Dicentrarchus labrax*, *Platichthys flesus* and *Solea solea* was evaluated in the Mondego estuary (Portugal) from 2003 to 2007. The relationships between sea surface temperature, NAO index, coastal wind speed and direction, precipitation and river runoff prior to the estuarine colonization and the densities of 0-group fish were evaluated using gamma-based Generalized Linear Models. *D. labrax* and *P. flesus* 0-group decreased in densities towards the end of the study period, while *S. solea*, despite low densities in 2004, increased densities in 2007. For *D. labrax*, river runoff, precipitation and east-west wind were significant; for *P. flesus*, precipitation, river runoff and both north-south and east-west wind components were significant parameters, while for *S. solea* only river runoff was important. Results were compared with recent projections for climate change scenarios, to evaluate their effects on future recruitment levels.

Keywords

Recruitment variability :: Flatfish :: Sea Bass :: River runoff :: Hydrodynamics :: NAO

Introduction

Estuaries are regarded as highly important sites for fish, particularly as nursery areas for marine species (Beck et al., 2001; McLusky and Elliott, 2004). In general, spawning takes place offshore, implying the migration of larvae from the continental shelf towards coastal areas and estuaries (e.g. Norcross and Shaw, 1984; Koutsikopoulos and Lacroix, 1992). Among other factors, hydrodynamics is a key factor for the recruitment success of marine fishes (van der Veer et al., 2000; Wilson et al., 2008); given the natural variability in current speeds and direction, the potential for drift into nursery environments of varying quality might be expected to result in high recruitment variability (Bailey et al., 2005).

Larval dispersion in early stages has several advantages, such as the potential for dispersion and colonization of new habitats, gene flow and minimization of intra-specific competition. Nonetheless, there are eminent risks in dispersion towards estuarine waters, one of which is the high variability in recruitment strength. This problem is acute for marine fishes whose planktonic stages have high mortality rates (commonly measured at 5–40% per day) and whose larval stages may metamorphose into quite different juvenile forms (Bailey et al., 2008). Among other species, many flatfish have their major spawning period in winter-early spring, when strong storm-related winds predominate, so there could be adaptations to wind-induced circulation (Marchand, 1991; Bailey et al., 2005). In the case of many estuarine-dependent species whose juvenile nursery habitats are spatially distinct from spawning locations, physical processes affecting the transport during the pelagic stages are of great importance to year-class strength (Amara et al., 2000; Bailey et al., 2005). In fact, recruitment success in marine species seems to be regulated mostly by density-independent factors (van der Veer et al., 2000) related with the surrounding environment and climate, but also by density-dependent mechanisms such as predation, feeding and mortality. The strongest evidence of density-dependent regulation of recruitment in temperate flatfishes, both coastal and offshore, is the strong and direct relationship between the size of the nursery area and the output of juveniles (level of recruitment) (Rijnsdorp et al., 1992; van der Veer et al., 2000).

It is generally agreed that river plumes may have a crucial role as indicators of the proximity of nursery areas for fish larvae (e.g. Marchand, 1991; Amara et al., 2000). This means that in years of high river drainage these plumes extend over a greater area, increasing the probability of being detected by fish larvae spawned in the coast that will then direct their movement towards the nursery grounds (Vinagre et al., 2007). In agreement, Martinho et al. (2007a) reported higher densities of estuarine residents and marine species that use estuaries as nursery grounds in years with higher freshwater flow. Changes in precipitation and river runoff regimes (due to climate change) will significantly impact on coastal ecosystems, and particularly on marine fish species whose larval stages depend on finding estuarine waters. In addition, large oceanic patterns such as the North Atlantic Oscillation (NAO) have been correlated with a range of long-term ecological measures, including the abundance of certain fish. Such environmental influences are most likely to affect susceptible juveniles during estuarine residence (Attrill and Power, 2002), thus having a potentially influential role in determining stock recruitment levels. Accordingly, variations in the NAO index were linked to different precipitation scenarios over the Portuguese territory (Zhang et al., 1997), being a potential indicator of changes in climate patterns over the years.

Previous work conducted in the Mondego estuary (Portugal, Southern Europe) (Leitão et al., 2007; Martinho et al., 2007a, b; Dolbeth et al., 2008; Martinho et al., 2008) reported that this estuary acts as an important nursery ground for marine species such as the European seabass *Dicentrarchus labrax*, flounder *Platichthys flesus* and sole *Solea solea*. In fact, these are among the most abundant species of the fish community (Martinho et al., 2007a). Thus, understanding the combined effects of both large-scale oceanic and hydrological patterns with local specific pressures is crucial to the estimation of recruitment levels and ultimately, to the stock assessment for coastal fisheries. Accordingly, the objectives of this work were to assess the influence of the NAO, coastal wind speed and direction, sea surface temperature (SST), precipitation and river runoff on the recruitment variability of three marine species that use estuaries as nursery areas: *D. labrax*, *P. flesus* and *S. solea* and to evaluate the impact of future climate change scenarios on the recruitment of these species.

Materials and Methods

Study site

The Mondego River estuary is a small intertidal system, located in the western Atlantic coast of Portugal (40º08'N, 8º50'W) (Fig. 1). Its terminal part is divided in two arms (north and south) that join again near the mouth. The north arm is deeper, with 5 to 10 m depth at high tide and tidal range of 2 to 3 m, while the south arm is shallower, with 2 to 4 m depth at high tide and tidal range of 1 to 3 m. The south arm is quite silted up in the upstream areas, which causes the freshwater to flow mainly by the north arm. In the south arm, about 75% of the total area consists of intertidal mudflats, while in the north arm they account for less than 10%. The water circulation in the south arm is mainly dependent on the tides and on a small freshwater input, carried out through the Pranto River, a small tributary system, which is regulated by a sluice according to the water needs in the surrounding rice fields.

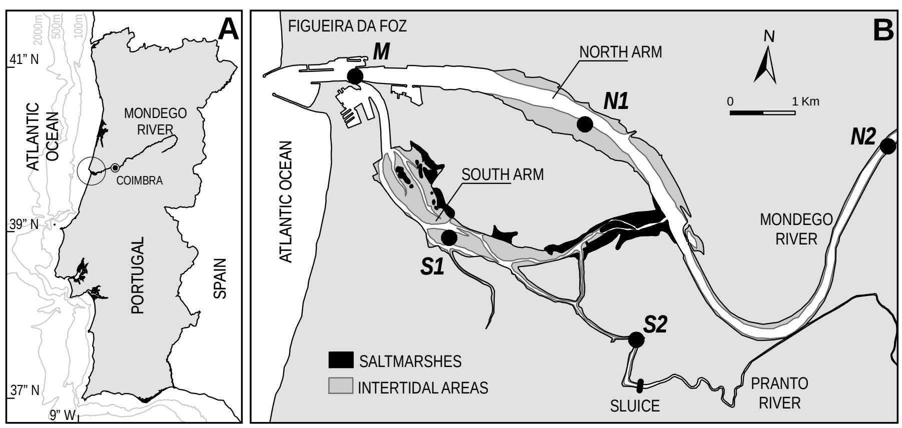

Figure 1. Geographical location of the Mondego estuary in the Portuguese coast (A) and of the five sampling stations within the estuary (B).

The Atlantic coast of the Iberian Peninsula is dominated in the summer months by equator-ward winds that generally start in late May or early June and persist through until late September or early October (Smyth et al., 2001). These winds cause an equator-ward mean surface flow to form above the predominantly pole-ward slope current and hence to generate coastal upwelling, inducing Ekman transport of the surface water away from the coast (Huthnance, 1995; Smyth et al., 2001; Mason et al., 2005). During the winter, winds change between northerly and southerly components, favourable of both upwelling and downwelling events, respectively (e.g. Santos et al., 2004). Local features of the northwest coast of Portugal include the Western Iberia Buoyant Plume (WIBP), a low salinity surface water fed by the winter-intensified runoff of several rivers (Santos et al., 2004), and the Iberian Poleward Current (IPC), a weak undercurrent that often extends to the surface in the winter (Peliz et al., 2003).

Sampling strategies and data acquisition

Fish were collected from June 2003 to July 2007, in the following regime: from June 2003 to November 2006 on a monthly basis, and from January 2007 to July 2007, every two months. Sampling was carried out at five selected stations (M, N1, N2, S1 and S2) (Fig. 1B) at night, using a 2 m beam trawl, with one tickler chain and 5mm mesh-size in the cod end. At each station, three tows of about five minutes were carried out, covering at least an area of 500 m^2. All fish caught were immediately frozen, and in the lab were identified and counted.

Hydrological data was obtained from the INAG – Instituto da Água (*http://snirh.inag.pt*): monthly precipitation (from 2003 to 2007) was obtained from the Soure 13F/01G station (near the estuary), and freshwater runoff was acquired from INAG station Açude Ponte Coimbra 12G/01A, near the city of Coimbra (located 40 km upstream) (See Fig. 1A). North Atlantic Oscillation (NAO) Index (given by the pressure differences between Lisbon (Portugal) and Reykjavik (Iceland)) data was supplied by NOAA/National Weather Service – Climate Prediction Center (*http://www.cdc.noaa.gov*). Sea surface temperature (SST), wind data, both north-south and east-west components, were acquired from the International Comprehensive Ocean-

Atmosphere Data Set (ICOADS) online database (*http://dss.ucar.edu/pub/coads*, Slutz et al., 1985) concerning the 1º Lat x 1º Long square nearest to the Mondego estuary.

Data analysis

Data on abundance of 0-group juveniles was plotted from the original trawl data matrix according to Dolbeth et al. (2008) and Martinho et al. (2008) who determined that only one cohort is produced each year. Fish densities (individuals per 1000 m^2) were determined as the number of 0-group fish caught in the total sampled area. Monthly data was calculated as the average of all densities from the five sampling stations for each species. From each yearly cohort, the densities of the first three months when estuarine catches start were chosen, since they represented the onset of the 0-group settlement period; at this time, fish are approximately three months old. In general, *S. solea*'s recruitment to estuarine waters starts in the winter, whereas for *P. flesus* and *D. labrax* the recruitment usually starts in early and late spring, respectively (Dolbeth et al., 2008; Martinho et al., 2008). The environmental parameters were obtained on the third month prior to the first estuarine catch, based on their expected influence on the colonization process.

The inter-annual relationship between the densities of the three species and the environmental patterns (predictors) were analyzed with a Generalized Linear Model (GLM) in R software (R Development Core Team, 2008), where the number of fish is related to other measured variables through distributional assumptions (Stefánsson, 1996). A gamma distribution (only positive densities) was used to model the non-zero catches (Myers and Pepin, 1990; Stefánsson, 1996). The GLM was built with an additive methodology: predictors were tested independently for significance and subsequently, significant predictors were added to determine the residual deviance, the percentage explained by each predictor and the total percentage of the deviance explained by the model. The final model was fitted only with the significant variables. Given that the considered species have different characteristics in terms of spawning, larval development and recruitment patterns, GLM analyses were performed separately for each species. A significance level of 0.05 was considered in all test procedures.

Results

Environmental background

Throughout the study period, both precipitation and river runoff had clear seasonal and yearly variations (Fig. 2). During 2004 and 2005 an extreme drought was recorded, with precipitation and river runoff values much lower than the 1961-1990 average (Fig. 2). The harshest effects of the drought were experienced in 2005. In 2003, 2006 and 2007, precipitation levels were comparable among years and to the 1961-1990 average (Fig. 2), being considered regular years. River runoff levels over the third month prior to the estuarine colonization by 0-group juveniles presented high values in 2003 and 2004 for *D. labrax*, in 2003, 2004 and 2006 for *P. flesus* and in 2003, 2006 and 2007 for *S. solea* (Table 1). Over the same period, precipitation levels ranged from 35.90mm to 80.90mm for *D. labrax*, from 45.30mm to 80.90mm for *P. flesus* and from 0.00mm to 257.00mm for *S. solea*.

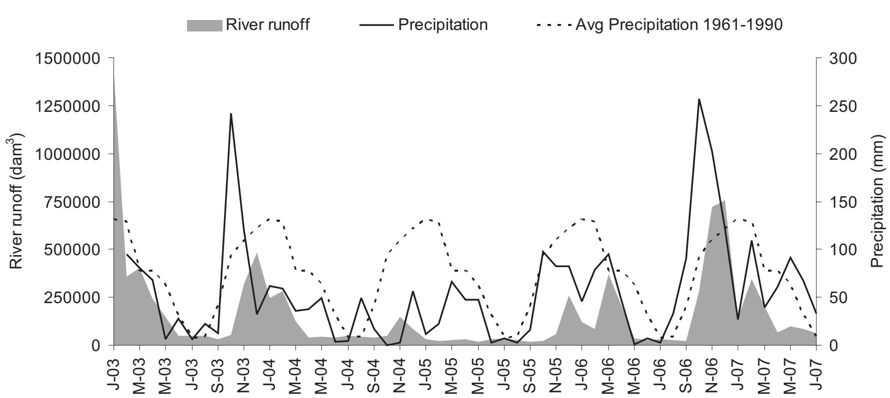

Figure 2. Monthly variation of precipitation (mm), long-term average precipitation (1961-1990) (mm) and river runoff (dam^3) from 2003 to 2007 in the Mondego River basin.

Sea surface temperature (SST) ranged from 13.74 ºC to 15.57 ºC for *D. labrax*, with the lowest values in 2005 and 2006 (Table 1). For *P. flesus*, SST varied from 14.15 ºC to 15.57 ºC, with the lowest values in 2003, and for *S. solea* higher SST values were recorded, ranging from 14.15 ºC to 19.59 ºC. As a general trend, SST over the third month prior to the estuarine colonization increased towards the end of the study period. NAO index in the third month prior to 0-group first appearance in the estuary (Table 1) ranged from -1.83 to 1.02 for *D. labrax*, being negative in 2005 and 2006, and positive in the remaining years. For *P. flesus*, the NAO index had negative values in 2004 and 2005, and positive values in 2003, 2006 and 2007, ranging from -0.30 to 1.26. For *S. solea*, the NAO index was positive in 2003 and 2006, being negative in 2004, 2005 and 2007, varying from -2.24 to 1.26.

Table 1. Values of the environmental variables used in the gamma-based GLM analysis by year and species.

Species	Year	SST (ºC)	River Runoff (dam^3)	Precipitation (mm)	NAO Index	Wind N-S ($m\ s^{-1}$)	Wind E-W ($m\ s^{-1}$)
D. labrax	2003	14.15	403410.00	80.90	0.32	-1.55	0.00
	2004	14.28	125055.00	35.90	1.02	-3.50	0.89
	2005	13.91	26931.00	66.70	-1.83	2.95	0.00
	2006	13.74	87139.00	78.40	-0.51	-1.65	4.65
	2007	15.57	68402.00	61.30	0.17	-6.00	1.22
P. flesus	2003	14.15	403410.00	80.90	0.32	-1.55	0.00
	2004	14.46	248020.00	61.60	-0.29	-2.67	0.00
	2005	14.68	29532.00	47.30	-0.30	-3.06	2.50
	2006	14.52	120468.00	45.30	1.26	-5.36	-0.94
	2007	15.57	68402.00	61.30	0.17	-6.00	1.22
S. solea	2003	14.15	403410.00	80.90	0.32	-1.55	0.00
	2004	18.64	53594.00	242.10	-1.26	0.00	0.00
	2005	18.17	51028.00	0.00	-1.10	-2.76	0.68
	2006	14.52	120468.00	45.30	1.26	-5.36	-0.94
	2007	19.59	284262.00	257.00	-2.24	3.51	2.90

Concerning the upwelling favourable winds (North-South component; negative values for northerly winds) (Table 1), for *P. flesus* there was a clear increase in intensity along the study period from -1.55 m s^{-1} to -6.00 m s^{-1}. For *D. labrax*, positive winds were only registered in 2005, varying from -6.00 m s^{-1} to 2.95 m s^{-1}, and for *S. solea*, southerly winds only occurred in 2007, ranging from -5.36 m s^{-1} to 3.51 m s^{-1}. For all species, there was an increase in north-south wind intensity towards the end of the study period. East-West wind component (Table 1) (negative values for easterly winds) had in general lower and positive values for all species, evidencing an increase in intensity towards the end of the study period. The only negative values were recorded in 2006 for *P. flesus* and *S. solea*.

Yearly variations in 0-group fish densities

Densities of 0-group fish where highly variable between 2003 and 2007, with the highest densities for the three species being reported in 2003 (Fig. 3). *D. labrax* was the species whose highest average yearly densities were recorded (19.1 Ind. $1000m^{-2}$ in 2003) (Fig. 3A). From 2004 onwards, densities decreased considerably, with a minimum of 1.0 Ind. $1000m^{-2}$ in 2007. A similar tendency was reported for *P. flesus*, with the highest average yearly densities in 2003 (2.8 Ind. $1000m^{-2}$) and the lowest in 2006 (0.3 Ind. $1000m^{-2}$) (Fig. 3B). *S. solea* 0-group fish evidenced a distinct pattern: average yearly densities in 2003 where higher (2.8 Ind. $1000m^{-2}$), but the lowest values where achieved in 2004 (0.6 Ind. $1000m^{-2}$), and from 2005 to 2007 there was a tendency to increase almost to 2003 levels (Fig. 3C).

Peak densities for the three species were compared to the ones found in the most important Portuguese estuarine systems, according to the available literature, mainly from beam trawl surveys (Table 2). For *D. labrax*, peak densities in the Mondego estuary were higher than in other estuaries, and for *P. flesus* and *S. solea* densities were comparable across the range of selected estuarine systems, with the exception of the Minho estuary, where densities were clearly higher.

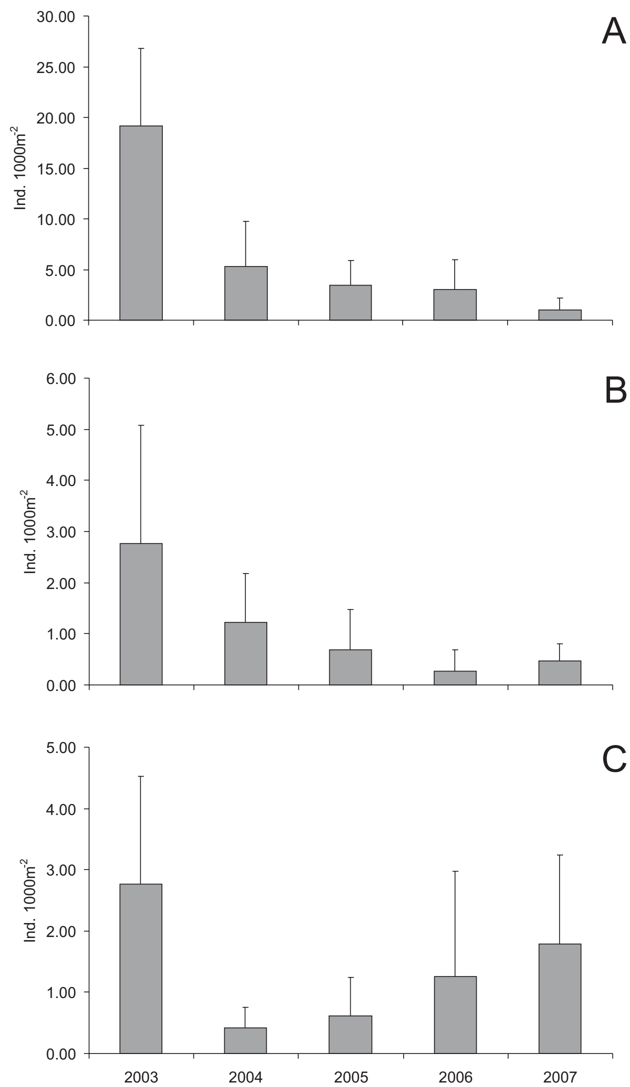

Figure 3. Mean densities (± standard deviation) of *D. labrax* (A), *P. flesus* (B) and *S. solea* (C) by year, from 2003 to 2007.

Table 2. Peak densities reported for *D. labrax*, *P. flesus* and *S. solea* along the main estuarine systems of the Portuguese coast.

Species	Location (Estuary)	Max Density (Individuals $1000m^{-2}$)	Sampling gear	Reference
D. labrax	Ria de Aveiro	145.3	Beach seine	Pombo et al. (2007)
	Mondego	320.0	Beam trawl	Martinho et al. (2007b)
	Tejo	81.8	Beam trawl	Cabral and Costa (2001)
	Guadiana	10.0	Otter trawl	Chícharo et al. (2006)
P. flesus	Minho	236.0	Beam trawl	Cabral et al. (unpublished data)
	Douro	9.1	Beam trawl	Vinagre et al. (2005)
	Ria de Aveiro	23.2	Beach seine	Pombo et al. (2007)
	Mondego	30.0	Beam trawl	Martinho et al. (2007b)
S. solea	Minho	67.0	Beam trawl	Cabral et al. (unpublished data)
	Douro	8.0	Beam trawl	Vinagre et al. (2005)
	Ria de Aveiro	0.6	Beach seine	Pombo et al. (2007)
	Mondego	23.1	Beam trawl	Martinho et al. (2007b)
	Tejo	25.9	Beam trawl	Cabral and Costa (1999)
	Sado	24.5	Beam trawl	Cabral (2000)
	Mira	15.7	Beam trawl	Cabral et al. (unpublished data)

Relation between environmental parameters and 0-group abundance

The final results of the Generalized Linear Models (GLM) are reported in Table 3. The analysis of deviance for *D. labrax* showed that river runoff, precipitation and the east-west wind component where significant predictors explaining the 0-group densities ($p<0.05$). Besides the main effects, the first-order interaction between river runoff and precipitation also was significant. The model explained 78.6% of the deviance, being river runoff mostly responsible for this (44.9%) (see Table 3). The predictors precipitation and east-west wind component contributed similarly to the deviance (15.3% and 17.6%, respectively), and the interaction between river runoff and precipitation contributed with 0.7%. In general, there was a tendency for higher densities of 0-group *D. labrax* during the period of estuarine settlement in years of high river runoff and precipitation (Fig. 4A, B), in accordance with the previous analysis. On the contrary, years with higher intensity of westerly winds were associated with lower densities (Fig. 4C).

Analysis of deviance for *P. flesus* indicated that river runoff, precipitation and both north-south and east-west wind components were significant predictors (Table 3). The model explained 74.9% of the deviance, with river runoff also being responsible for the majority of the deviance (73.7%). The remaining predictors: precipitation, north-south and east west wind components accounted for 1.2% of the deviance (0.89%, 0.003% and 0.27%, respectively). No significant first-order interactions were found between the main effects. Similar to *D. labrax*, *P. flesus* 0-group abundance was higher in years with higher river runoff and precipitation (Fig. 5A, B). Likewise, lower densities were where associated with higher intensity of westerly winds, and lower densities where also associated with higher intensity of northerly winds (Fig. 5C, D). Regarding *S. solea*, the model accounted for 46.1% of the deviance (Table 3).

From the set of chosen predictors, only river runoff was significant in the GLM analysis, being responsible for the whole deviance. Similarly to *P. flesus*, no first-order interactions between the main effects where found. In accordance with the previous species, higher densities of *S. solea* 0-group fish were found in years with higher river runoff (Fig. 6).

Discussion

Recruitment variability: role of environmental stressors

The present work focuses on the influence of environmental aspects on the recruitment variability of marine fish species that use estuaries as nursery areas: *D. labrax*, *P. flesus* and *S. solea*. These species are among the most abundant marine fishes in Portuguese estuaries (e.g. Ribeiro et al., 2006; Cabral et al., 2007; Martinho et al., 2007a), with typical seasonal abundance peaks reflecting estuarine colonization by the young-of-the-year. Since the present database concerns only five consecutive years, it was not possible to determine trends regarding recruitment variability and strength, since these species are known to have highly variability in recruitment rates in consecutive years (Cabral and Costa, 2001). Climate and its main features such as rain, SST, the NAO, wind speed and direction, as well as tidal movements and ocean currents, have been recognized as key issues in the estuarine colonization and settlement processes of both marine fish and invertebrate larvae (e.g. Marchand, 1991; Amara et al., 2000; Attrill and Power, 2002; Almeida and Queiroga, 2003; Bailey et al., 2008).

One of the most significant aspects of this work was the identification of the importance of river runoff in this process for the three species in study.

In fact, this was the only parameter that was significant for all species. Previous studies in the Tagus estuary (Portugal) revealed that river drainage is a determining factor in the estuarine colonization process undertaken by marine species, particularly for soles (Vinagre et al., 2007). In the Bay of Vilaine (France), Amara et al. (2000) pointed out that the initiation of sole estuarine colonization is regulated by a combination of river flow, wind direction and intensity and tidal cycle.

During the study period a severe drought occurred, leading to a significant decrease in precipitation and river runoff values. According to the present results, higher runoff values were associated with higher densities of *D. labrax*, *P. flesu*s and *S. solea*, as well as precipitation for *D. labrax* and *P. flesu*s. River runoff not only affects the extent of river plumes into coastal areas, but can also be responsible for salinity changes within estuarine waters (one of the main factors responsible for the structuring of estuarine fish communities (e.g. Marshall and Elliott, 1998; Drake et al., 2002; Costa et al., 2007; Leitão et al., 2007), protracting or diminishing the available habitats for fish and suitable nursery areas. Effectively, Marques et al. (2007) and Martinho et al. (2007a) described a higher proportion of marine species in plankton and fish communities during low runoff periods, respectively, due to a higher extent of the salinity incursion inside estuarine waters.

Table 3. Analysis of deviance table for the gamma-based GLM fitted to the densities data of the species considered in study. (Res. Dev. – Residual deviance; % Expl. – Percentage of the deviance explained by the model).

Species	Parameters	*p*-value	Res. Dev.	Deviance	% Expl.
D. labrax	Null		31.772		
	Main effects				
	Runoff	0.0163	17.478	14.294	44.989
	Precipitation	0.0003	12.628	19.144	15.265
	Wind E-W	0.0109	7.035	24.737	17.604
	Interactions				
	Runoff : Precipitation	0.0034	6.799	24.973	0.742
	Total explained				78.600
P. flesus	Null		11.207		
	Main effects				
	Runoff	0.0006	2.944	8.263	73.729
	Precipitation	0.0024	2.845	8.362	0.885
	Wind N-S	0.046	2.845	8.362	0.003
	Wind E-W	0.0166	2.815	8.392	0.266
	Total explained				74.883
S. solea	Null		24.055		
	Main effects				
	Runoff	0.0093	12.977	11.078	46.053
	Total explained				46.053

The importance of river plumes for the estuarine colonization of fish and invertebrate larvae has been related to the existence of chemical cues that orient larvae towards estuarine areas and settlement habitats, such as odor, temperature, salinity and turbidity (e.g. Miller, 1988; Gibson, 1997; Arvelund and Takemura, 2006; Krimsky and Epifanio, 2008) and to potential higher primary production (Costa et al., 2007). Also, in accordance with Vinagre et al. (2007), a wider extent of river plumes during high freshwater flow will enhance the chance of larvae detecting and moving towards estuarine waters, which is in agreement with the present results.

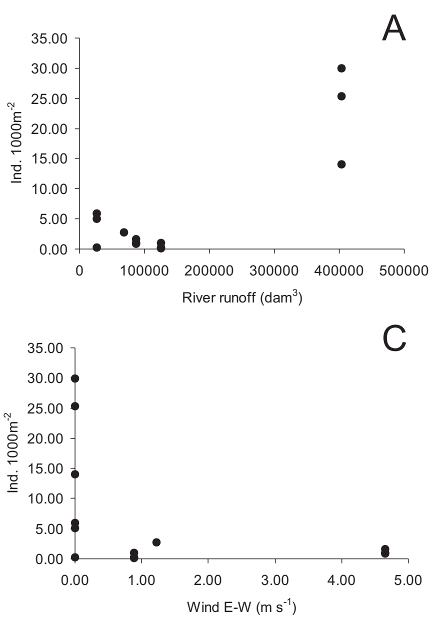

Figure 4. Densities of 0-group *D. labrax* (Ind. 1000m^{-2}) (first three months) in relation with river runoff (dam^{3}) (A), precipitation (mm) (B) and wind E-W component (m s^{-1}) (C) concerning the third month prior to the period of estuarine colonization.

Regarding fisheries, Salen-Picard et al. (2002) identified a positive relationship between high runoff values and *S. solea* landings after a 5-year time lag in the Golf of Lions (France, Mediterranean coast). The same authors pointed out that flooding events were responsible for an increase in benthic food resources for juvenile fish. Sentchev and Korotenko (2004) based on three dimension particle-tracking transport models, evidenced that tides and freshwater discharge play a critical role in influencing flounder larval transport over the eastern English Channel, from spawning areas to nursery grounds. For the three studied species, the spawning season in temperate latitudes and estuarine colonization processes seem to have evolved to match the season when river plumes have their maximum extent, thus increasing the chance for recruitment success.

One important regulatory aspect in the estuarine colonization of larvae and young stages seems to be transportation from the continental shelf to estuarine nurseries, with several authors stressing the importance of wind-driven and tidal-driven circulation (e.g. Jennings and Pawson, 1992; Amara et al., 2000; Sentchev and Korotenko, 2004). In the present study, wind was determined as a significant predictor for *D. labrax* (E-W component) and for *P. flesus* (N-S and E-W components). Nevertheless, for both wind components, the weakest intensities were associated with the highest densities of 0-group fish, possibly indicating that lower wind intensity induces either weaker upwelling events or turbulence. The present results are in accordance with prior results obtained by Amara et al. (2000), who pointed out that strong offshore winds can be unfavourable to the estuarine dispersion. For *D. labrax*, Lancaster et al. (1998) concluded that differences in recruitment levels could be attributed to variations in coastal wind direction and strength.

For several species, selective tidal stream transport (STST) has been proposed as the main process for larvae entering estuaries. For *P. flesus*, passive transport occurs in their early life, followed by active STST (van der Veer et al., 1991), in which fishes make use of strong tidal currents over one part of the tidal cycle for transportation (Forward and Tankersley, 2001). In the Bay of Biscay (France), Lagardère et al. (1999) observed that *S. solea* larvae were mainly located in the lower part of the water column before migration to estuaries. Vinagre et al. (2007) pointed out that soles undertake vertical movements to escape the top layer of water masses that are most susceptible to wind and offshore advection, important in upwelling systems such as the Portuguese coast. In opposition, field studies conducted in South Wales indicated that *D. labrax* larvae tended to occur in the upper part of the water column, and the arrival of post-larvae into sheltered bays and estuaries usually takes place during spring tides (Jennings and Pawson, 1992). These differences could be attributed to the different vertical guilds of *D. labrax*, a demersal and more active swimming species, and *P. flesus* and *S. solea*, both benthic species.

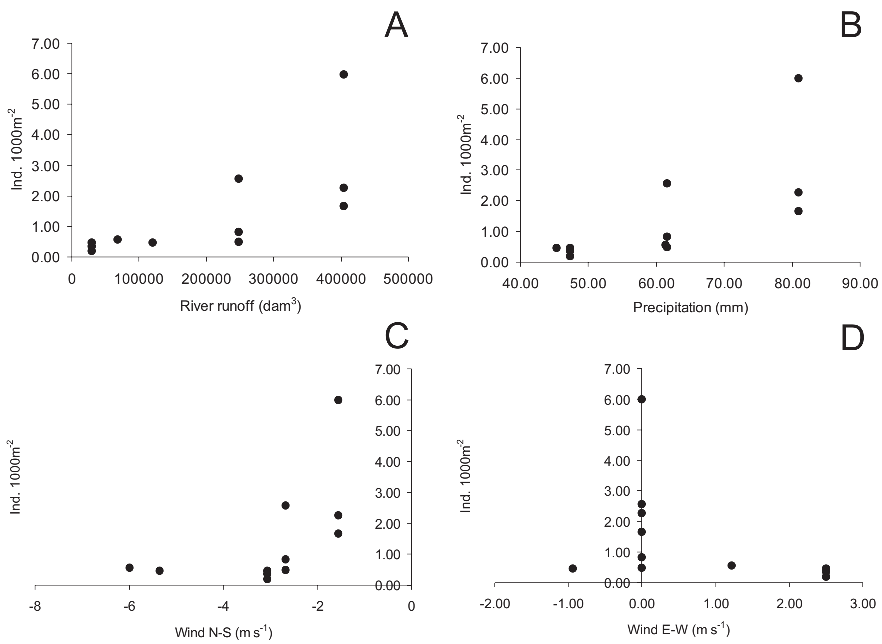

Figure 5. Densities of 0-group *P. flesus* (Ind. $1000m^{-2}$) (first three months) in relation with river runoff (dam^3) (A), precipitation (mm) (B), wind N-S component ($m\ s^{-1}$) (C), and wind E-W component ($m\ s^{-1}$) (D) concerning the third month prior to the period of estuarine colonization.

The dominance of density-independent factors operating at a local scale on the egg and larval stages stresses the importance of hydrodynamic circulation as a key factor in determining year-class strength (Legget and Frank, 1997). Also, recruitment variability has been shown to depend on the pelagic stage duration (van der Veer et al. 2000), either before metamorphosis or during the juvenile period (Lagardère et al., 1999; Amara et al., 2000). Despite fish larvae having been described as active swimmers, Gibson (1997) stressed that during the estuarine colonization processes they are still under the influence of hydrodynamic forces and successful recruitment during this stage is mostly dependent upon favorable hydrographic conditions. Although density-independent factors have been shown to influence recruitment variability in the marine environment prior to estuarine colonization (e.g. Rijnsdorp et al., 1992), density dependent factors may also have a high preponderance within nursery grounds, inducing high levels of juvenile mortality (Gibson, 1994; Rogers, 1994).

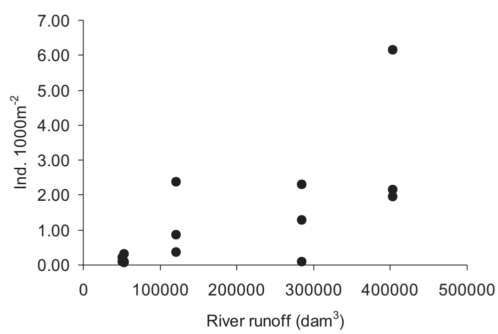

Figure 6. Densities of 0-group *S. solea* (Ind. $1000m^{-2}$) (first three months) in relation with river runoff (dam^3) concerning the third month prior to the period of estuarine colonization.

For the species in study there was no relationship between early juvenile densities and sea surface temperature. However, according to Rijnsdorp et al. (1992), recruitment is likely to be mostly influenced by water temperature in the marine environment prior to or during the immigration to nursery areas. For *S. solea*, Le Pape et al. (2003) found a positive relationship between SST and the growth of young recruits. The same authors also pointed out that warmer temperatures are an important factor for the attraction to estuarine nurseries. In opposition, for several flatfish species (dab and plaice), a negative relationship between year-class strength and water temperature has been determined (van der Veer et al., 2000). Regarding *D. labrax*, Jennings and Pawson (1992) pointed out that, in UK waters, post-larvae migrate to inshore waters when temperatures are higher than those of the surrounding sea.

The North Atlantic Oscillation (NAO) has been correlated with a range of long-term ecological measures, including fish stocks. Such environmental influences are most likely to affect susceptible juveniles during estuarine residency, as estuaries are critical juvenile nursery or over-wintering habitats (Attrill and Power, 2002). Nonetheless, none of the selected species revealed to have a significant influence by the NAO, which as a large-scale oceanographic process, influences not only the SST, but also the wind and current patterns along the Portuguese coast (Henriques et al., 2007). The main effects of the NAO on the recruitment and estuarine colonization of marine fish can be most likely observed at a broader scale, either in time or space, than the ones used in this study. Nevertheless, the isolation of single factors that can influence recruitment patterns can be somewhat difficult, since the movement from offshore waters to inshore protected areas is a complex and intricate process. In fact, it is likely that local- and meso-scale singularities in hydrography, bathymetry or landscape structure may also contribute to regional differences in transport processes (Bailey et al., 2008). In addition, and according to Mestres et al. (2007), the extension of river plumes, derived from the only predictor that was considered significant in the GLM analysis for the three species (river runoff), is clearly influenced by the main local driving mechanisms, namely the prevailing wind and the freshwater discharge rate, and even within a species, there may be local adaptations reflecting quite different strategies (Bailey et al., 2008).

D. labrax, *P. flesus* and *S. solea* in Portuguese estuaries

Regarding *D. labrax*, the highest densities in Portuguese estuaries were recorded in the Mondego estuary. In a recent work by Vasconcelos et al. (2008), the authors identified by otolith elemental fingerprints that about 40% of the coastal stocks of this species were originated in the Mondego nurseries, thus reinforcing the role of the Mondego estuary for coastal stocks. For *P. flesus*, a decrease in densities towards south is consistent with Cabral et al. (2001), who reported the decrease in densities near its southern limit of distribution, located along the center of the Portuguese Atlantic coast. The highest densities were recorded in the Minho estuary (North Portuguese coast), and in agreement, Vasconcelos et al. (2008) indicated that at least half of this species' coastal stocks were originated in a northern estuary (Douro estuary). As for *S. solea*, nursery origins were determined to be mostly from the Mondego and Tagus estuaries, although with less reliability (Vasconcelos et al. 2008). This species seems to be the most ubiquitous in the Portuguese coast, with similar densities found over the selected estuaries. However, the highest densities were recorded at the Minho estuary, similarly to *P. flesus*. As a general trend, higher densities were found in northern estuaries.

Climate change scenarios: future perspectives in recruitment levels

Recent climate change projections for the Iberian Peninsula point out that both temperature and drought periods are expected to increase, with a concentration of rainfall in the winter months (Miranda et al., 2006). More precisely, and based on the Intergovernmental Panel on Climate Change – Special Report on Emissions Scenarios (IPCC-SRES, 2001) models, winter precipitation by the end of the XXI century is predicted to decrease an average 15% (Miranda et al., 2006). According to the previous authors, reductions in precipitation amounts throughout the year are expected to be higher: -20% to -50%. Considering this, the decrease in precipitation and consequently river runoff will lead to a reduction of the extent of river plumes to coastal waters, which has been determined as an important factor for the recruitment success of marine fish that use estuaries as nursery areas. As a significant outcome, runoff regime shifts can induce changes in coastal fisheries: Meynecke et al. (2006) based on a data series from 1988 to 2004, concluded that dry years were often associated with lower catches, leading to important economic and social losses. Due to the concentration of precipitation in time, restricted to the winter months, it is possible that species that migrate to estuaries in spring, such as *D. labrax* and *P. flesus*, can be significantly impacted due to the decrease in runoff and to the lesser extent of river plumes, in agreement with the present results and with Vinagre et al. (2007). In fact, in the present study, these species showed a decrease in densities during and after the drought, in opposition to *S. solea*. In addition, previous work by Dolbeth et al. (2008) indicated a breakdown of secondary production in nursery species during the drought, which can be an indicator that continued decreases in river runoff can in fact impact on these species. Regarding this scenario, it is possible to infer that *D. labrax* and *P. flesus* will be more affected by drought events in the future, thus requiring efficient management measures.

Further improvements on this approach would be the integration of plankton and reproductive biology data in order to obtain a more accurate scenario of the estuarine colonization processes. In addition, protracting this time-series would allow a better definition of the physical and environmental conditions that contribute to the recruitment variability in the studied species and that operate over larger time and spatial scales.

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Chapter IV

Sampling estuarine fish assemblages with beam and otter trawls: differences in structure, composition and diversity

Abstract

The effect of sampling gear used in the determination of estuarine fish communities' structure and composition were assessed during four seasonal field surveys in the Mondego estuary, Portugal, conducted in 2003 and 2004. Sampling with beam and otter trawl was performed at night, at two different locations in the north arm of the estuary. In general, seasonal otter trawl samples were composed with a higher species number, but underestimated the estuarine residents in comparison with beam trawl samples. The relative abundance of zoobenthivores was similar in both gears, while for the piscivores, their relative abundance was about two times higher in otter trawl samples in 2003, and opposite in 2004. Multivariate analysis detected significant differences in the structure and composition of estuarine fish assemblages captured by the two techniques, with higher within-gear variability in beam trawl samples. Significant differences were detected in median total length and length-frequency distributions of the most abundant and commercially important species *Dicentrarchus labrax*, *Platichthys flesus* and *Solea solea*, with the smallest fish captured by beam trawl. Results are discussed regarding the design of field surveys, and compared with other fishing gears and several other factors, such as diel period, tow duration and tidal conditions. Concerning the European Water Framework Directive, distinct results in the structure and composition of estuarine fish assemblages (either in species or functional groups) obtained by different sampling gears can significantly influence the determination of the Ecological Quality Status.

Keywords

Sampling design :: Fish assemblages :: Ecological guilds :: Water Framework Directive :: Mondego

Introduction

Estuaries are among the most productive systems throughout the world and at the same time among the most threatened from a variety of conflicting human activities, which impair their ecological functions (McLusky and Elliott, 2004; Dolbeth et al., 2007a; Vasconcelos et al., 2007; Hewitt et al., 2008). Increasing pressures can result in the impairment of estuaries' main functions, goods and services, with severe consequences for the environment and the population. Within estuarine (=transitional waters) communities, fish have been used mainly for the assessment of (a) changes in composition and structure through time (e.g. Araújo et al., 2000; Hemingway and Elliott, 2002), (b) spatial distribution (e.g. Martinho et al., 2007a), (c) direct impacts of pollution sources (e.g. Lancaster et al., 1998), (d) recruitment variability of commercially important species and nursery areas (e.g. van der Veer et al., 2001, Cabral et al., 2007, Martinho et al., 2007a) and (e) production of individual species and/or communities (e.g. Pombo et al., 2007, Dolbeth et al., 2008a).

One common aspect of using fish as indicators of state changes is the need for efficient, cost-effective and representative sampling methods, in order to retain the most information in the least number of samples possible, since they usually involve destructive, expensive and time-consuming techniques. In fact, one of the most important decisions in developing a sampling program is gear selection (Rozas and Minello, 1997). Accordingly, several methodologies have been used for sampling estuarine fish communities (some derived from commercial fisheries) in order to capture elements from the pelagic, demersal, benthic, marginal and cryptic communities: beam trawl (e.g. Marshall and Elliott, 1998; Cabral et al., 2007; Martinho et al., 2007b), otter trawl (e.g. Thiel et al., 1995; Rotherham et al., 2008), gillnets (e.g. Harrison and Whitfield, 2004), beach seine and fish traps (e.g. Gell and Whittington, 2002), fyke nets (e.g. Mathieson et al., 2000) power plant coolers (e.g. Araújo et al., 2000; Attrill and Power, 2002; Greenwood, 2008), drop samplers (e.g. Almeida et al., 2008) and visual census (mostly for rocky substrates and caves) (e.g. Henriques et al., 2007; Bussotti and Guidetti, 2009) (See Hemingway and Elliott, 2002 for a full review). Recent work has noted that the structure and composition of fish samples can be affected by the choice of gear type (Greenwood, 2008) (due to different catch efficiencies and area sampled (Hemingway and Elliott, 2002), diel period (Johnson et al., 2008), tow duration (Godø et al., 1990; Rotherham et al., 2008) and speed (Weinberg et al., 2002). Although some of the factors here described refer to fisheries in coastal and deeper marine areas, the same concepts can be applied to estuarine sampling. Given the above-cited diversity of sampling gears and specifications, before implementing large-scale and long-term monitoring surveys, an important first step is to test the effects of different configurations of gear and sampling practices on retained samples (Gunderson, 1993; Rotherham et al., 2007). According to Hemingway and Elliott (2002), the choice of sampling methodologies should at least take into account the target organisms, the substratum, hydrodynamic regime, habitat types, spatial coverage and also logistic and safety requirements. For studies that aim to combine or compare the results of previous studies, developing spatial comparisons, or use the relative proportion of species within assemblages, sampling biases related with gear choice may seriously mislead ecological interpretations (Guest et al., 2003).

The European Water Framework Directive (WFD; 2000/60/EC) requires for transitional waters that fish communities should be sampled every three years for the determination of the Ecological Quality Status (EQS). In this particular case, it is very important that the sampling methodologies used in different estuaries and countries produce comparable results, given the possibility of similar assemblages being classified as different or in opposition, different assemblages being classified as equal. In fact, several authors have used different sampling methods for the assessment of the EQS: otter trawl - Deegan et al. (1997), trawl - Borja et al. (2004), gillnets - Harrison and Whitfield (2004), fyke-nets – Breine et al. (2007) or multi-method sampling (seine nets, beam and otter trawls) - Coates et al. (2007), whose different results have considerable implications in establishing reference conditions against which present and future results are to be compared. Recently, Martinho et al. (2008b) evaluated the results obtained by the above authors, applying the proposed ecological indices in a small intertidal estuary (Mondego estuary, Portugal, SW Europe), reinforcing the suggestion that similar sampling methodologies should be adopted by EU member states. Hence, the objectives of this study were to assess the differences between two fish sampling methods: beam and otter trawl, considering specific richness, structure and composition of estuarine fish communities, and to determine differences in abundance trends over time, median total length and length frequency distributions of the most abundant species common to both techniques.

Material and Methods

Study site

The Mondego estuary is a small estuarine system, located on the Atlantic coast of Portugal (40º08'N, 8º50'W) (Fig. 1). Its terminal part is divided in two arms (north and south) that join again near the mouth. The north arm is deeper, with 5 to 10 m depth at high tide, while the south arm is shallower, with 2 to 4 m depth at high tide, being the tidal range of 2 to 4 m. The south arm is silted up to some extent in the upstream areas, which causes the freshwater to flow mainly by the north arm. In the south arm, about 75% of the total area consists of intertidal mudflats, while in the north arm they account for less than 10%. The water circulation in the south arm is mainly dependent on the tides and on a small freshwater input from the Pranto River, a small tributary system. A commercial harbour is located in the north arm, with the estuarine sediments being subjected to constant dredging in the main navigation areas.

Sampling design

In this experiment, two sampling sites were chosen in the Mondego estuary, separated by approximately 3km (Fig. 1): A – with typical marine influence, located 1.5 km from the estuary's mouth, 8.7 $\pm$ 1.2 m depth; B - with regular freshwater flow, 5.5 $\pm$ 0.5 m depth, located 4.5 km from the estuary's mouth. The restriction to use the otter trawl in the deeper waters of the north arm of the estuary determined the choice of the sampling sites. Sampling was carried out at night, since previous surveys indicated that daytime sampling was inefficient in this estuary, and fishing started at high water of spring tides. Fieldwork was conducted along one year (June 2003 to May 2004), with seasonal periodicity (one sampling occasion per season) and in similar tide conditions but in different days, to insure the independence of data (see Underwood, 1997). At each station, three replicates were collected with a 2m beam trawl with one tickler chain and 5mm stretched mesh size in the cod end, and with a 10m headline otter trawl with 10mm stretched mesh size in the cod end. The two sampling gears were chosen based on the fact that they are among the most extensively used fishing gears for studying estuarine fish assemblages (see Hemingway and Elliott, 2002). At each station, three hauls along the current were performed with each gear, covering at least an area of 500m^2. Distance travelled by each haul was measured with a GPS device, and tow speed was approximately 1 knot for both gears. A total of 48 tows were performed. All fish caught were immediately frozen, and subsequently identified, counted and measured for total length (TL) to the nearest 1 mm.

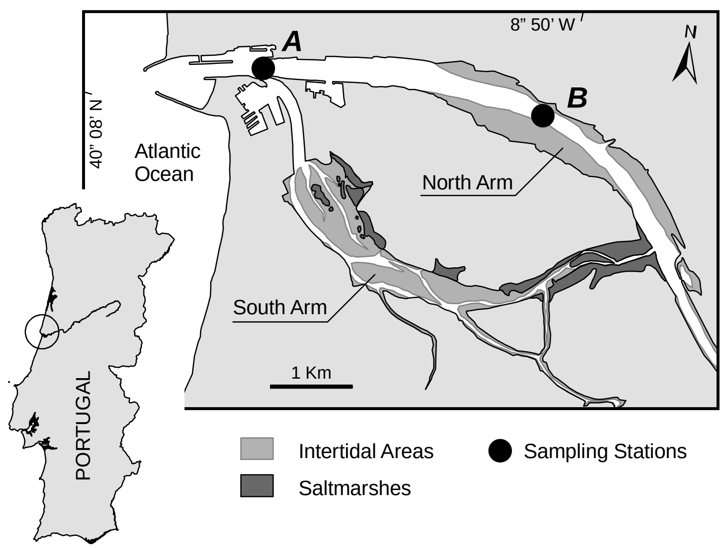

Figure 1 Location of the Mondego estuary and of the selected sampling stations in the north arm of the estuary (A and B).

Data analysis

To eliminate the variability inherent to different mesh sizes, all fish with less than 50mm TL were removed from the analysis, since it was the smallest TL captured by the 10mm mesh size otter trawl. Fish data was standardized as the number of individuals per $1000m^2$, and were analyzed according to the sampling regime: beam and otter trawl. Monthly data were calculated as the average of the three hauls for each gear, and species abundance data were standardized as the percentage of total catches, to enable direct comparison between sampling methods. In order to assess the fish community structure, fish species were classified in ecological guilds, according to the recent review by Elliott et al. (2007): marine stragglers (MS), marine-estuarine opportunists (MMO), marine-estuarine dependents (MMD), estuarine residents (ER), catadromous (CA); and in the two most representative feeding guilds in estuarine fish assemblages: piscivores (PV) and zoobenthivores (ZB).

Differences in catch composition (species number per ecological guild, ecological and feeding guild relative abundance) were assessed with Kruskal-Wallis non-parametric ANOVA. Data were explored using ANOSIM to test for differences between sampling gears, and a non-metric multidimensional scaling (nMDS) (Bray-Curtis similarity matrix) was used to determine trends in structure and composition of fish samples. The SIMPER procedure was performed to determine which species contributed most for both the similarity in sampling gears and for the dissimilarity between beam and otter trawl. Prior to these analyses, abundance data were standardized using a square root transformation. Results were compared to determine if there were differences in the standardized proportions of fish species in the assemblages, and presence/absence data to detect different species composition between sampling gears. These analyses were performed using PRIMER software (Clarke and Warwick, 2001).

For the most abundant species caught in both gears, *Dicentrarchus labrax*, *Platichthys flesus* and *Solea solea*, differences regarding the median total length were assessed with Kruskal-Wallis non-parametric ANOVA. For this purpose, the dataset used concerned the total catches of the four sampling dates for both beam and otter trawl. Differences in length-frequency distributions were assessed with Kolmogorov-Smirnov two-sample tests, using the same dataset and methodology. Regarding the seasonal abundance trends for each species, results between gears were compared with Spearman rank correlations. A significance level of 0.05 was considered in all test procedures.

Results

Structure and composition of fish communities

A total of 2800 fish (26 species) were collected during the study, 1134 from beam trawl and 1666 from otter trawl surveys, corresponding to 20 species in both gears (Table 1). Otter trawl samples captured a higher species number and individuals when analysed seasonally (with the exception of the summer 2003) (Fig. 2), particularly for marine-estuarine opportunists (MMO), marine stragglers (MS) and catadromous (CA) groups. In opposition, the beam trawl samples were composed of a higher number of estuarine resident species (ER) (Fig. 2A, C, E, G). Marine estuarine dependent (MMD) species, composed by some of the most abundant species in the estuary, were equally represented in both gears in terms of species number (Fig. 2). For both gears, samples collected at station A (located at the estuary's mouth) had generally higher species number of all ecological guilds. Significant differences between sampling gears were obtained for the species number of all ecological guilds: MS, H=6.610, $p<0.05$; MMO, H=7.580, $p<0.05$; MMD, H=5.974, $p<0.05$; ER, H=0.773, $p<0.05$; CA, H=4.651, $p<0.05$. When comparing the densities (transformed in relative abundance for a better comparison between sampling gears), considerable differences were found: beam trawl samples were mainly composed by marine-estuarine dependents (MMD) and estuarine residents (ER), while otter trawl samples were mainly composed by marine-estuarine dependents (MMD) and marine-estuarine opportunists (MMO) (Fig. 3).

Table 1. Species list of the Mondego estuary fish assemblage collected during the study period, with respective Family, Ecological Guild, Feeding Guild, average density estimates (N. Ind $1000m^{-2}$ ± standard deviation) and total species number for beam and otter trawl samples; CA – catadromous, ER – estuarine resident, MS – marine stragglers, MMO – marine estuarine opportunists, MMD – marine estuarine dependents, and: ZP – zooplanktivores, ZB – zoobenthivores, PV – piscivores, OV – omnivores, DV - detritivores.

Species	Family	Ecological Guild	Feeding Guild	Beam trawl	Otter trawl
Anguilla anguilla	Anguillidae	CA	ZB/OV	0.29 ± 0.39	0.10 ± 0.08
Aphia minuta	Gobiidae	MS	PV	0.26 ± 0.64	-
Atherina boyeri	Atherinidae	ER	PV/OV	0.07 ± 0.14	-
Atherina presbyter	Atherinidae	ER	ZB/OV	0.04 ± 0.10	0.02 ± 0.04
Callionymus lyra	Callionymidae	MS	ZB/OV	0.12 ± 0.35	0.01 ± 0.02
Chelidonichthys lucerna	Triglidae	MMO	PV	0.16 ± 0.35	0.20 ± 0.20
Ciliata mustela	Gadidae	MMO	ZB	0.12 ± 0.24	0.04 ± 0.10
Conger conger	Congridae	MS	PV	-	0.05 ± 0.10
Dicentrarchus labrax	Moronidae	MMD	PV	3.34 ± 6.50	1.56 ± 2.28
Diplodus vulgaris	Sparidae	MMO	ZB/OV	1.54 ± 2.24	2.59 ± 4.78
Echiichthys vipera	Trachinidae	MS	ZB/OV	-	0.06 ± 0.06
Gobius niger	Gobiidae	ER	ZB	0.08 ± 0.22	0.03 ± 0.06
Liza aurata	Mugilidae	MMO	DV/OV	0.03 ± 0.09	0.01 ± 0.02
Liza ramada	Mugilidae	CA	DV/OV	-	0.01 ± 0.02
Mugil cephalus	Mugilidae	MMO	DV/OV	-	0.03 ± 0.06
Mullus surmuletus	Mullidae	MMO	ZB	0.08 ± 0.23	0.04 ± 0.08
Platichthys flesus	Pleuronectidae	MMD	ZB	1.41 ± 2.00	1.31 ± 1.62
Pomatoschistus microps	Gobiidae	ER	ZB	0.21 ± 0.28	-
Pomatoschistus minutus	Gobiidae	ER	ZB	3.84 ± 5.91	-
Sardina pilchardus	Clupeidae	MMO	PV	0.04 ± 0.12	-
Scophthalmus rhombus	Scophthalmidae	MMO	PV	0.11 ± 0.24	0.20 ± 0.14
Solea senegalensis	Soleidae	MMO	ZB	-	0.11 ± 0.23
Solea solea	Soleidae	MMD	ZB	4.14 ± 4.62	4.19 ± 5.49
Sparus aurata	Sparidae	MMO	ZB/OV	0.03 ± 0.09	-
Syngnathus acus	Syngnathidae	ER	ZB	1.24 ± 3.41	0.05 ± 0.08
Trisopterus luscus	Gadidae	MS	ZB/OV	-	0.04 ± 0.10
Total species				20	20

The high relative abundance of ER in beam trawl samples was related to the high abundance of *Pomatoschistus microps* and *P. minutus*. Also, in the beam trawl samples, a dominance of MMD and ER was observed in all sampling events, with the exception of the Spring 2004 (Fig. 3G), when a high equitability between guilds was observed. In the otter trawl samples, MMD and MMO comprised the majority of the fish assemblage, as observed in all sampling events (Fig. 3B, D, F, H). Significant differences between beam and otter trawl were only obtained for the estuarine residents (ER) (H=16.877, p<0.05).

In general, the zoobenthivores (ZB) comprised a significant higher proportion of the beam and otter trawl samples (Fig. 4B, D, F, H). The exception was the winter 2004, when the piscivores (PV) were more abundant (Fig. 4A, C, E, G). This pattern was observed for both beam and otter trawl samples. However, no significant differences were found between the catch composition of the feeding guilds (PV, H=0.480, p>0.05; ZB, H=2.083, p>0.05).

The ANOSIM procedure revealed significant differences between assemblages captured by beam and otter trawl regarding the structure (standardized relative abundance, R=0.496, p=0.001) and species composition (presence/absence, R=0.587, p=0.001). Average similarity values for beam trawl samples where 44.2% and 55.0% (standardized relative abundance and presence/absence data, respectively), while for otter trawl samples, average similarity values were 56.7% and 67.6% (standardized relative abundance and presence/absence data, respectively). These results were also supported by the MDS analysis (Fig. 5), and in both cases, higher within-gear variability was registered for beam trawl samples. In agreement with the previous guild approach, the species that most contributed for the similarity (Bray-Curtis similarity) in beam trawl surveys were the ER *P. microps* and *P. minutus*, the MMD *D. labrax*, *S. solea* and *P. flesus*, and the MMO *Diplodus vulgaris* (SIMPER) (Table 2). In otter trawl samples, *S. solea*, *D. labrax*, *P. flesus* (all MMD), *D. vulgaris*, *Scophthalmus rhombus* and *Ciliata mustela* (all MMO), and *Anguilla anguilla* (CA) contributed for more than 90% of the similarity between samples, according to the SIMPER procedure (Table 2).

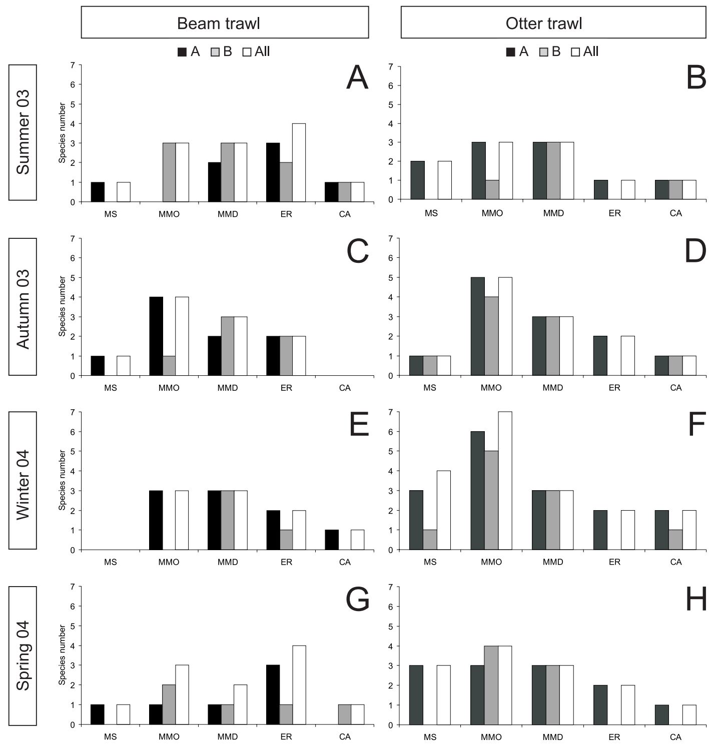

Figure 2. Species number for each ecological guild captured with beam and otter trawl at sampling stations A, B and in all samples; MS - marine stragglers, MMO - marine-estuarine opportunists, MMD - marine-estuarine dependents, ER - estuarine residents, CA – catadromous.

Differences in typifying species were driven by a higher species number in the presence/absence analysis, which included also *Chelidonichthys lucerna* (MMO) and *A. anguilla* (CA) in beam trawl samples, and *Echiichthys vipera* (MS) in otter trawl samples (SIMPER) (Table 2). A large number of species contributed for the dissimilarity between sampling methods (Bray-Curtis dissimilarity) (Table 3), corresponding to an average value of 64.4% (standardized relative abundance data) and 53.5% (presence/absence data) (ANOSIM).

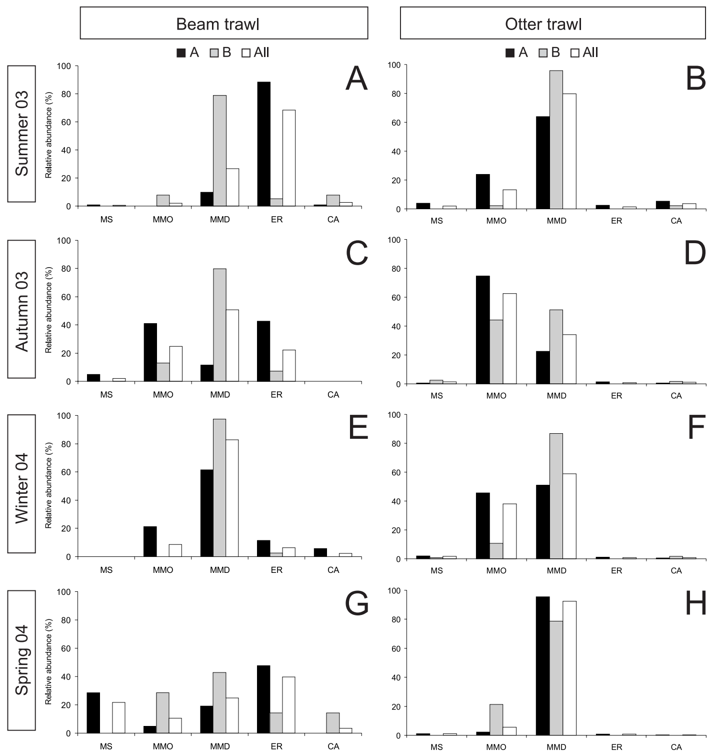

Figure 3. Relative abundance of each ecological guild captured by beam and otter trawl, at sampling stations A, B and in all samples; MS - marine stragglers, MMO - marine-estuarine opportunists, MMD - marine-estuarine dependents, ER - estuarine residents, CA – catadromous.

Variations in abundance, total length and length-frequency distributions

For the most abundant and commercially important species in both gears, *D. labrax*, *P. flesus* and *S. solea* (all MMD) (see Fig. 6), significant differences where found in median total length of fish caught by beam and otter trawl (*D. labrax*: H=119.683, p<0.001; *P. flesus*: H=19.194, p<0.001; *S. solea*: H=112.772, p<0.001) (Fig. 6).

Table 2. Species percent contribution for the similarity in each sampling gear comprising more than 90% of the similarity. Results from standardized relative abundance data and presence/absence data are compared.

	Species	Beam trawl	Otter trawl
Standardized relative abundance	*P. microps*	27.60	
	P. minutus	25.72	
	S. solea	15.24	31.85
	D. vulgaris	10.08	11.66
	D. labrax	9.48	15.82
	P. flesus	4.94	15.64
	S. rhombus		8.56
	C. lucerna		6.00
	A. anguilla		4.76
	Average similarity	44.15	56.66
Presence/Absence	*P. microps*	21.38	
	P. minutus	21.38	
	S. solea	16.15	15.26
	D. labrax	11.65	15.26
	P. flesus	7.87	15.26
	D. vulgaris	7.04	7.63
	C. lucerna	4.11	10.47
	A. anguilla	3.82	10.90
	S. rhombus		12.31
	E. vipera		5.01
	Average similarity	55.01	67.63

Significant differences in length-frequency distributions were also obtained with Kolmogorov-Smirnov tests for the three species considered (*D. labrax*: KS=5.162, $p<0.001$; *P. flesus*: KS=2.776, $p<0.001$; *S. solea*: KS=5.658, $p<0.001$).

Table 3. Species percent contribution for the dissimilarity between sampling gears comprising more than 90% of the dissimilarity. Results from standardized relative abundance data and presence/absence data are compared.

Species	Standardized relative abundance	Presence/Absence
P. minutus	14.53	10.36
P. microps	13.79	10.36
S. solea	10.53	
D. vulgaris	9.53	4.70
D. labrax	9.52	2.51
P. flesus	9.08	3.77
S. rhombus	4.81	6.49
C. lucerna	4.39	5.33
A. anguilla	3.31	5.49
E. vipera	2.71	5.67
S. acus	1.99	4.15
M. surmuletus	1.67	3.13
S. senegalensis	1.66	4.87
S. pilchardus	1.56	2.39
A. minuta	1.56	2.51
G. niger		3.58
C. mustela		3.50
C. conger		3.36
A. presbyter		2.98
M. cephalus		2.22
A. boyeri		2.21
T. luscus		2.09
Average dissimilarity	64.36	53.47

Larger fish captured with the otter trawl and also the small length-classes captured by the beam trawl mainly drove these differences (Fig. 6). Comparing the seasonal trends in densities of the selected species along the sampling period, *S. solea* was the most abundant of the three species, both in beam and otter trawl samples (Fig. 7C). Regarding *D. labrax*, an abundance peak was observed in the winter samples (in both sampling gears) (Fig. 7A), while as for *P. flesus*, lower densities were observed (Fig. 7B). However, no significant correlations (Spearman rank correlation) were found for the abundance values in beam and otter trawl samples for each species.

Discussion

Gear type-specific fish assemblages

An underlying assumption in estuaries management is that modified habitats contain altered biological communities, and that species richness is, in general, inversely proportional to degree of deterioration (Araújo et al., 2000). In this context, fish assemblages have been used because they are known to change in composition as their habitats are modified (Araújo et al., 2000). Thus, this study aimed at identifying differences in estuarine fish assemblages sampled by different fishing gears, in order to establish future monitoring programmes either at a local or a broader scale. Effectively, the two fishing gears here tested gave significantly different catch compositions, regarding the structure and composition of the Mondego estuary fish assemblage. The main difference between both gears was driven by the absence of the estuarine resident gobies *P. microps* and *P. minutus* in the otter trawl samples. In fact, these are among the most abundant species in the estuary (Dolbeth et al., 2007b; Martinho et al., 2007b), being clearly underestimated by the otter trawl.

Although the species richness was equal in both gears, seasonal otter trawl samples had higher species number, with the exception of the summer 2003. In addition, the main difference between sampling gears was a higher species number of the estuarine residents (ER) in the beam trawl and a higher number of marine-estuarine opportunists (MMO) and marine stragglers (MS) in the otter trawl. Regarding the relative abundance of the ecological guilds, the beam trawl samples were more homogeneous, although with a domination of marine-estuarine dependent (MMD), marine-estuarine opportunists (MMO) and estuarine residents (ER).

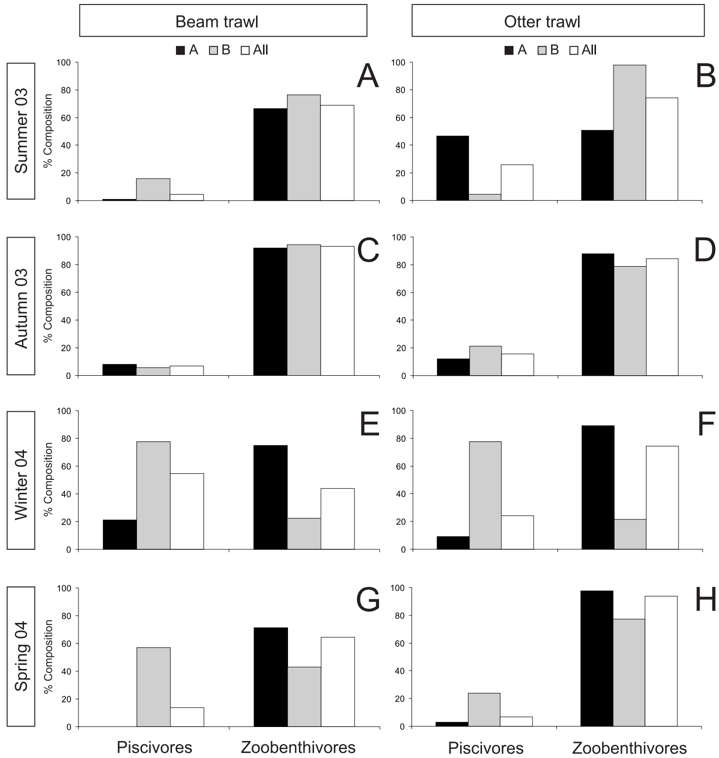

Figure 4. Relative composition of the most abundant feeding guilds (piscivores and zoobenthivores) captured by beam and otter trawl.

In otter trawl samples, a clear domination by the marine estuarine dependent (MMD) and marine estuarine opportunists (MMO) was always evident. Such divergence between samples can be attributed to the lack of estuarine residents in the otter trawl samples, as referred previously. In agreement, and according to Olin and Malinen (2003), estimates of fish density, species composition and size distributions can differ notably depending on the sampling method and time.

In the present study, the effect of diel period was not taken into account, since previous surveys reported that the catch efficiency of the beam trawl was noticeably poor during daytime, and did not serve the purpose for sampling the estuary's fish fauna. In agreement, several authors have stated that nocturnal samples provide higher species number and abundance of fish species (e.g. Gray et al., 1998; Guest et al., 2003; Unsworth et al., 2007; Johnson et al., 2008; Rotherham et al., 2008), as the efficiency of trawl nets increases with decreased visibility (van de Broek, 1979). Moreover, it has been noted that more organisms and species are caught in estuarine seagrass beds at night (e.g. Gray et al. 1998; Guest et al., 2003; Unsworth et al., 2007), probably as a consequence of increased availability of invertebrate food resources (e.g. Sogard and Able, 1994; Guest et al., 2003; Unsworth et al., 2007).

Significant differences in median total length (TL) and length-frequency distributions were found for the most abundant and economically important species: *D. labrax*, *P. flesus* and *S. solea* (all MMD), as beam trawl samples collected mainly the smaller length-classes of these species. In opposition, otter trawl samples were composed of larger fishes, as well as a larger minimum TL caught. A similar pattern was reported by Guest et al. (2003), who observed differences in TL frequency distributions between beam trawl and seine net samples, being the smaller size-classes captured by the beam trawl. Similar results were also reported by Greenwood (2008). As for the seasonal trends in abundance for these species, no significant correlations could be attained between beam and otter trawl samples. Regarding *D. labrax*, a similar trend in abundance could be observed for both beam and otter trawl: the highest densities in winter samples, can be explained by a concentration of fish in the most downstream areas, near the mouth of the estuary. As for the two flatfishes, *P. flesus* and *S. solea*, two different communities could be detected: summer and autumn samples, mainly dominated by 0-group fish (Martinho et al., 2007a), being less abundant in otter trawl samples (due to a lesser cacthability by this gear), and winter and spring samples, composed of older fish (namely 1- and 2-group fish) that concentrate in the most downstream areas (Martinho et al., 2007a).

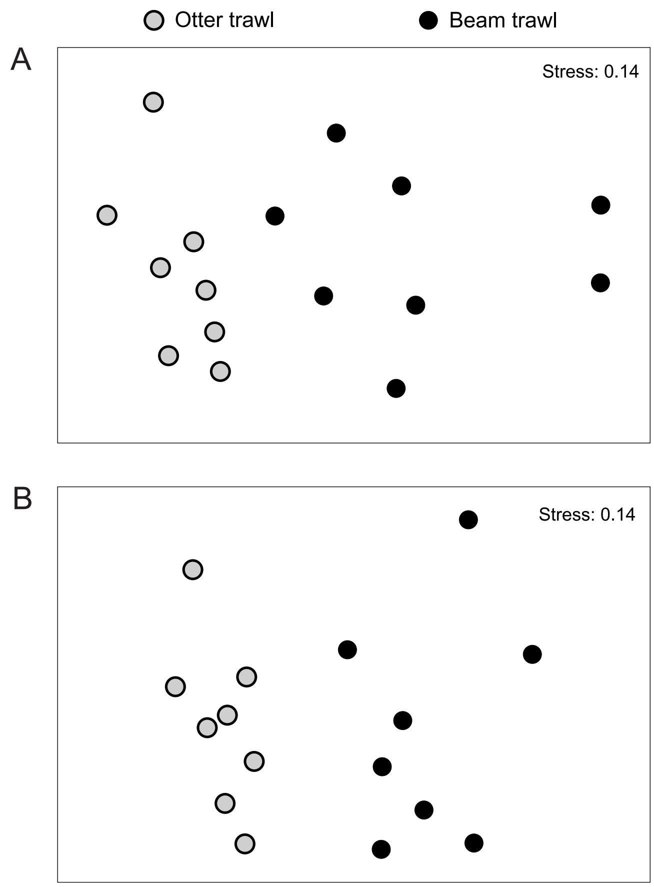

Figure 5. Multidimensional Scaling (MDS) ordination plots of standardized relative abundance (A) and presence/absence data (B) of the estuarine fish communities sampled with beam and otter trawl. Samples include all sampling stations and dates.

Thus, the differences between species (demersal vs. benthic) can be related to differences in spatial occupation of different size-classes along the estuary (namely in the sampled areas), as larger fish tend to move to deeper estuarine areas (e.g. Leitão et al., 2006, Martinho et al., 2007a). On the contrary, recent investigations by Greenwood (2008) pointed out that strong positive correlations existed in several species' abundance trends collected at both power plant cooling-water intake screens and two trawling methods (pelagic and Agassiz trawls).

Implications for the design of field surveys

When designing a sampling programme, the unique characteristics of shallow estuarine habitats must be considered, and the choice of a technique depends on several factors including the overall objectives of the survey, the target species and the sampling area (Hemingway and Elliott, 2002; Guest et al., 2003). In addition, the sampling design should incorporate as few samples as possible (Rozas and Minello (1997), thus reducing the number of fish killed and the need for sub-sampling (Godø et al., 1990). Recently, some work has been conducted to compare fish sampling methods, in an attempt to design more efficient field surveys, introducing the variables of gear type, diel period, tow duration and net height (e.g. Wieland and Storr-Paulsen, 2006; Greenwood, 2008; Johnson et al., 2008; Rotherham et al., 2008).

According to Morrison et al. (2002), the choice of tide for fish sampling is a determinant factor, as high tide sampling in mudflats can be substantially less informative. To reduce bias related with tides, in this work all sampling events were carried out at spring tides, starting at ebbing water. A further improvement of this approach would be to test differences related to spring/neap tides (i.e. lunar cycles), as some fish species (e.g. soles) have been demonstrated to perform tidal- and lunar-related migrations (see Vinagre et al., 2006). Regarding tow duration, several authors have pointed out that short tows (between 5 and 15 minutes) capture more species diversity (Godø et al., 1990; Rotherham et al., 2008) and abundance (Somerton et al., 2003) relatively to longer tows. Smaller tows also allow for increasing replication and precision in surveys, without large increases in costs (Pennington and Vølstad, 1991; Rotherham et al., 2008). Regarding the diel period in which sampling should preferably be carried out, most studies point to night sampling, with considerable increases in species number, diversity and abundance (Gray et al., 1998; Guest et al., 2003; Unsworth et al., 2007; Rotherham et al., 2008).

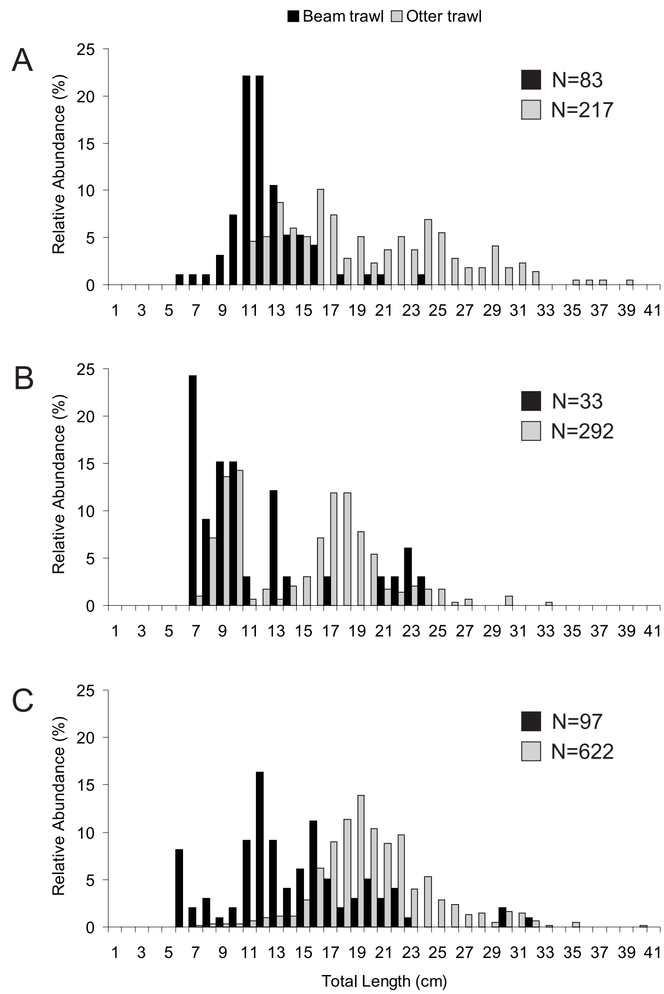

Figure 6. Comparison of total length frequency distributions for the most abundant and commercially important species *D. labrax* (A), *P. flesus* (B) and *S. solea* (C) in beam and otter trawl samples.

Consequently, future monitoring plans should include night sampling (as the present work), despite the technical constraints and difficulties associated. Towing speed is another factor that should be considered, as the catch of benthic species such as flatfish can be more affected by the loss of footrope contact with the bottom (Weinberg et al., 2002).

As an alternative to a single method, multi-method sampling programmes can be used for estuarine fish assemblages. Unlike sampling for other components such as the benthos or plankton, the fish assemblage comprises many different groups representing different niches, and thus requires many different – though complementary – methods (Hemingway and Elliott, 2002). Such an approach has been implemented in the United Kingdom since 2002, given that it was considered a more effective way of assessing the ecological status of transitional waters (Coates et al., 2007). The sampling gears used are seine nets, otter trawls and beam trawls, targeting both pelagic and benthic fish species, deployed according to site-specific characteristics (see Coates et al., 2007). Despite the advantages of such practice are evident, for many countries its feasibility can be questioned, due to the considerable human resources and costs involved. Ultimately, the choice of sampling gear shall fall on one single method. As with all sampling gears, it is important to acknowledge that not all members of the fish assemblage will be collected and those that are will be caught with differing efficiencies (Greenwood, 2008), so the choice of sampling gear must be a compromise between catch characteristics, ease of use and the objectives of the study.

One limitation of this work was the inability of sampling the whole estuarine area, imposed by the restriction of using the otter trawl only in the deeper areas. This can be seen as a constraint in using this technique in shallow estuaries, as the case of the Mondego, since it is not possible to have a representative sample of the whole estuary. Apart from this exercise, a sampling programme was started in June 2003 (see Leitão et al., 2006; Dolbeth et al., 2007b, 2008a; Martinho et al., 2007a,b, 2008a for further details), using a 2m beam trawl (5mm mesh size), providing a wider picture of the changes in structure and composition of the fish assemblages. Accordingly, and since the species richness was equal in both gears (although not the same species composition), the beam trawl seems a more suitable method to use in shallow estuaries.

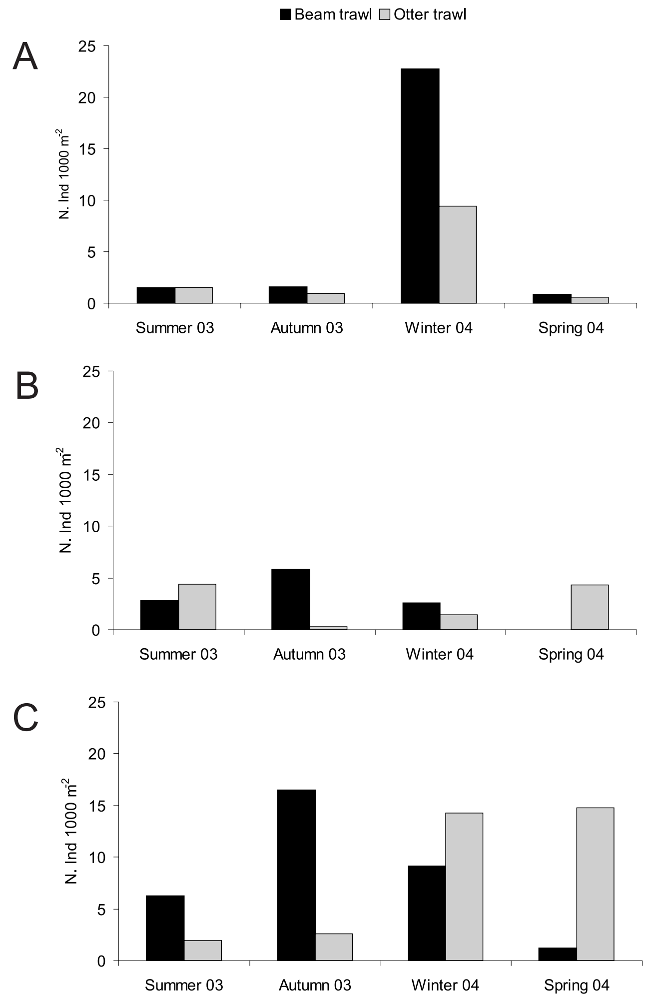

Figure 7. Seasonal variation of density estimates for *D. labrax* (A), *P. flesus* (B) and *S. solea* (C) based on beam and otter trawl samples.

Other advantages rely on the fact that it is possible to estimate the sampled area (in opposition to seine nets) and the ease of use (Hemingway and Elliott, 2002). Effectively, the beam trawl is one of the most extensively used methods for scientific sampling (Hemingway and Elliott, 2002), but if needed, the otter trawl could be used as a complement for capturing larger fish and pelagic species (as in Martinho et al., 2008a).

Compliance with WFD requirements

The WFD requires that for the fish component of transitional waters, sampling is conducted every three years, and that the evaluation of the Ecological Quality Status (EQS) is based on species richness and abundance of species/functional groups. As a consequence, differences in the EQS may occur due to bias introduced by different sampling methodologies. Within this framework, Coates et al. (2007) compared the EQS obtained for the Thames estuary (UK) by beam trawl, otter trawl and seine net samples, and concluded that the beam trawl consistently provided the lowest estimates of species richness and EQS, since this gear targets mainly benthic fish communities (Hemingway and Elliott, 2002). This fact was not so evident in the present work, since the beam trawl captured the same species number as the otter trawl, and also showed more equitability in the distribution of the species number trough the estuarine use guilds. In addition, the otter trawl tended to underestimate the estuarine residents either in species number or relative abundance, as is the case of the beach seine, which can underestimate gobies and blennies (Gell and Whittington, 2002). The resident species are a particularly important element of estuarine food webs, as determined by stomach contents analysis (Leitão et al., 2007; Dolbeth et al., 2008b) and stable isotope approaches (Baeta et al., 2008), and are also contemplated in several multimetric indices used to determine the EQS: EBI (species number) – Deegan et al. (1997), EDI (species number and abundance) – Borja et al. (2004), EFCI (species number and abundance) – Harrison and Whitfield (2004), TFCI (species number and functional guilds number) – Coates et al. (2007) (See Martinho et al. (2008b) for the comparison of indices). On the other hand, the otter trawl was more effective in capturing marine-estuarine opportunist species (MMO) in most of the sampling occasions.

Regarding the feeding guilds, no significant differences between sampling gears could be attained during the study period. However, in the particular case of the summer 2003 samples, the variability in the relative abundance of the piscivores was very high between fishing gears. Community indicators such as the proportion of feeding guilds (among others) were also used by Trenkel et al. (2004) to identify differences in catch composition for UK and French trawl surveys in the Celtic Sea. Like pointed out before, differences in EQS obtained by beam and otter trawl samples may occur, considering that the feeding guilds are also contemplated in several indices used: EDI (relative abundance) – Borja et al. (2004), EFCI (species number and relative abundance) – Harrison and Whitfield (2004), EBI (relative abundance) – Breine et al. (2007), TFCI (species number of functional guilds) – Coates et al. (2007). As noted by Coates et al. (2007), the sampling regime will significantly affect the determination of the EQS, mainly due to gear selectivity.

Conclusions

This study showed that, in low budget and human resources situations, both sampling gears could give effective quantification of estuarine fish assemblages (with their inherent limitations and selectivity). However, a better coverage of the total estuarine area would imply the use of the beam trawl, which can be operated in shallower areas such as small channels and near salt marshes. In addition, the beam trawl captured significantly smaller length-classes, a determining issue when targeting in more detail recruitment patterns and smaller species, such as the estuarine resident gobies. Efficient management plans, as required by the European Water Framework Directive, need to be based on cost-effective and both quantitative and qualitative sampling, so further development on comparing other aspects (such as tow duration, tidal cycle and mesh size) should be conducted in order to optimise resources and improve the data required for both researchers and stakeholders.

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Chapter V

Assessing estuarine environmental quality using fish-based indices: performance evaluation under climatic instability

Abstract

The seasonal variation of five selected multimetric indices for the determination of the Ecological Quality Status (EQS) of transitional waters was evaluated, as well as the indices' responses to an extreme drought event that occurred in 2005. The database used regards the Mondego river estuary, which was sampled from June 2003 to August 2006 on a monthly basis. Among the selected indices (EBI - Deegan et al. (1997), EDI - Borja et al. (2004), EFCI - Harrison and Whitfield (2004), EBI - Breine et al. (2007) and TFCI - Coates et al. (2007), the EBI by Breine et al. (2007) was the only that evidenced clear interanual and seasonal variations. The EQS by the several indices ranged from "Poor" to "High", depending on the index considered, evidencing the high level of mismatch between indices. The results are discussed in the scope of the EU Water Framework Directive, regarding monitoring strategies, application of indices and EQS assessment.

Keywords

Water Framework Directive :: Fish-based indices :: Ecological Quality Status :: Ecological indicators :: Fish community :: Mondego Estuary

Introduction

The Water Framework Directive (WFD, 2000/60/EC) has set a new approach to the management and monitoring of water resources, aiming to achieve a "Good" ecological status by 2015 in all European water bodies (i.e.: The values of the biological quality elements for the surface water body type should show low levels of distortion resulting from human activity, deviating only slightly from those normally associated with undisturbed conditions (Vincent et al., 2002). This directive establishes a framework for the protection of groundwater, inland surface waters, estuarine (= transitional) and coastal waters (Borja et al., 2005), whose main objectives are: (a) to prevent further deterioration, to protect and to enhance the status of water resources; (b) to promote sustainable water use; (c) to enhance protection and improvement of the aquatic environment, through specific measures for the progressive reduction of discharges; (d) to ensure the progressive reduction of pollution of groundwater and prevent its further pollution; and (e) to contribute to mitigating the effects of floods and droughts. Accordingly, all EU member states are required to assess the Ecological Quality Status (EQS) of water bodies, and in transitional waters (=estuaries) the measurement of biological integrity will be emphasized on phytoplankton, macroalgae, benthos and fishes.

Transitional waters are of great importance for the fish fauna, playing a vital role by providing nursery habitats, reproduction grounds, refuge from predators and migratory routes (Haedrich, 1983; Elliott and McLusky, 2002; Cabral et al., 2007; Martinho et al., 2007a,b). Nevertheless, these systems are being subjected to high environmental pressure due to anthropogenic forcing, such as eutrophication, overfishing, bank reclamation and general environmental degradation.

The use of fishes as indicators of environmental change has recently gained attention (Whitfield and Elliott, 2002), with several authors developing multimetric tools in order to assess the estuarine ecosystem status for the fish component at various latitudes (ex: Deegan et al., 1997; Borja et al., 2004; Harrison and Whitfield, 2004; Breine et al, 2007; Coates et al., 2007). In addition, studies of population dynamics, food-web organization and structure of communities have been more successful than single species bioassays at predicting the effects of multiple stresses on biological systems (Schindler, 1987; Plafkin, 1989; Dolbeth et al., 2007a). The use of indicators provides the possibility to evaluate the fundamental condition of the environment without having to capture the full complexity of the system (Whitfield and Elliott, 2002), and according to the WFD guidance, the evaluation methods for the fish component should take in account both aspects of composition and abundance of fish species.

Extreme climatic events, such as floods or droughts are increasing in frequency worldwide (Mirza, 2003), and as a consequence, river discharge into many estuaries may be affected (Gleick, 2003). In the Mondego River basin, a severe drought occurred in 2005, and was classified by the Portuguese Weather Institute (*http://web.meteo.pt/clima/clima.jsp*) as the worst drought of the past 60 years. As a result, the decreasing precipitation and runoff induced changes in the estuary's planktonic and fish communities, with an increase in typical marine species during the drought (Marques et al., 2007; Martinho et al., 2007b). Since the implementation of the WFD by EU member states will be a continued process in time, to cope the various methodologies with climate instability is a key issue for the success of such an ambitious and promising directive. Within this framework, the objectives of the present work were to compare the results obtained by the methodologies developed by Deegan et al. (1997), Borja et al. (2004), Harrison and Whitfield (2004), Breine et al. (2007) and Coates et al. (2007) for determining the Ecological Quality Status of transitional waters using fish data, and to evaluate their responses in different climatic scenarios, namely in the presence of an extreme event (severe drought).

Materials and Methods

Study site

The Mondego estuary is a small intertidal system, located on the Atlantic coast of Portugal (40º 08' N, 8º 50' W) (Fig. 1), where approximately 1072 ha correspond to wetland habitats. In its terminal part, it comprises two arms that join near the mouth, separated by an alluvium-formed island (Murraceira Island). The northern arm is deeper (average 10 m during high tide) and is the main navigation channel and the location of the commercial harbour. The southern arm is shallower (2–4 m during high tide) and water circulation is mostly dependent on the tides and on the freshwater input from the Pranto River, a small tributary system. For further detailed information on the Mondego estuary's characteristics see Teixeira et al. (2008).

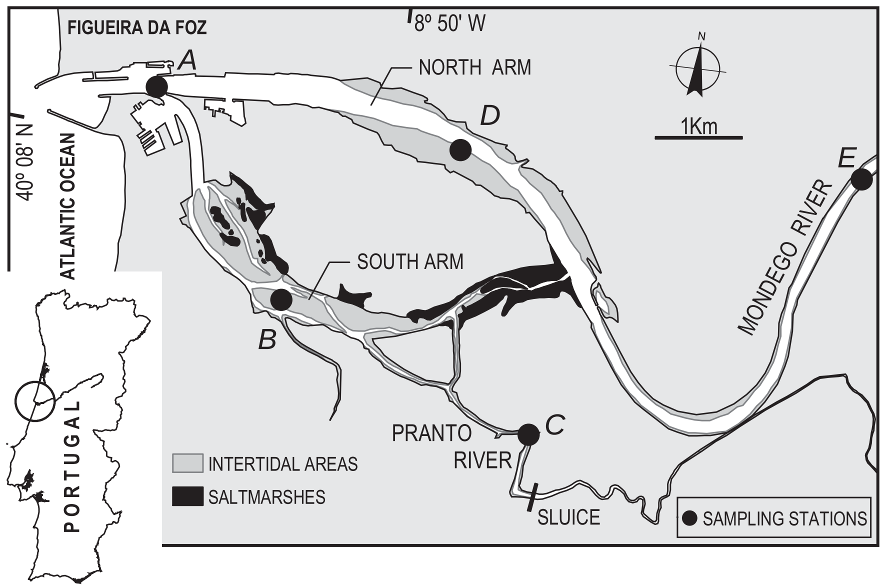

Figure 1. Geographical location of the Mondego estuary and the five sampling stations (A-E).

Sampling procedures and data acquisition

Fish sampling was performed monthly from June 2003 to August 2006 (except in July, September, October, December 2004 and July 2006, due to technical constraints or bad weather conditions), using a 2 x 0.5 m beam trawl with one tickler chain and 5 mm mesh size in the cod end. Samples were collected during the night, at high water of spring tides and in 5 stations throughout the estuary (Fig. 1). At each station, three tows were carried out, covering at least an area of 500 m^2 each. All fish caught were identified, counted, measured (total length) and weighted (wet weight). Bottom water salinity and temperature were measured after fishing took place.

Hydrological data was obtained from INAG – Portuguese Water Institute (*http://snirh.inag.pt*). Monthly precipitation (from January 2002 to June 2006) and long-term monthly average precipitation (from 1933 to 2006) were obtained from the Soure 13F/01G station (located near the estuary). Freshwater runoff was acquired from INAG station Açude Ponte Coimbra 12G/01A, near the city of Coimbra (located 40 km upstream).

Description of the multimetric indices

A variety of indices have been used to assess the Ecological Quality Status of estuarine systems. In the present study, the results of five multimetric indices and their responses to an extreme drought event were evaluated: Estuarine Biotic Integrity Index (EBI) (Deegan et al., 1997), Estuarine Demersal Indicators (EDI) (Borja et al., 2004), Estuarine Fish Community Index (EFCI) (Harrison and Whitfield, 2004), Fish-based Estuarine Biotic Index (EBI) (Breine et al., 2007) and the Transitional Fish Classification Index (TFCI) (Coates et al., 2007). All the methodologies and respective metrics are based on presence/absence, relative proportions and number of taxa of different species and functional groups. Unlike in larger estuaries (e.g. Breine et al., 2007; Coates et al., 2007), the Mondego estuary was not divided in sub areas, due to its relatively small size.

Estuarine Biotic Integrity Index (EBI) (Deegan et al., 1997)

The Estuarine Biotic Integrity Index (EBI) (Table 1) was developed using data from Waquoit Bay and validated using data from Buttermilk Bay, southern Massachusetts, USA. Each metric has an associated score of 0 or 5, and the EBI ranges from 0 to 40, being calculated as the sum of scores for each metric. Due to the inexistence of reference data, the authors only considered two Ecological Quality Status (EQS): Medium (EBI≥25); Low (EBI<25). Although the EBI can be used with either density or biomass data, in the present case only densities were used. Sampling was carried out using a 4.8m otter trawl (0.3 cm mesh size cod end).

Estuarine Demersal Indicators (EDI) (Borja et al., 2004)

The Estuarine Demersal Indicators (Table 2) were developed for the Basque Country, using fish and crustaceans data due to the small size of the Basque estuaries (in the present study, only fish were considered). Each indicator/metric has an associated score of 1, 3 or 5. The sum of all scores provides the final classification for the fish community, being then converted into the Ecological Quality Ratio (EQR), which ranges from 0 to 1 with five equal thresholds, each corresponding to an Ecological Quality Status (EQS): Bad (<0.2), Poor (0.2-0.4), Moderate (0.4-0.6), Good, (0.6-0.8) and High (0.8-1.0). The sampling method was trawling.

Estuarine Fish Community Index (EFCI) (Harrison and Whitfield, 2004)

The Estuarine Fish Community Index (EFCI) (Table 3) was developed for South African estuaries. Each metric has an associated score of 0, 3 or 5, and the EFCI is calculated as the sum of the scores. The EFCI ranges from 0 to 70 with five thresholds: Very Poor (EFCI<20), Poor (22≥EFCI≤38), Moderate (40≥EFCI≤44), Good (46≥EFCI≤62) and Very Good (62≥EFCI≤70). Fishes were sampled using a 30m x 1.7m seine (15mm bar mesh size) and a fleet of 10 x 1.7m gillnets (45, 75 and 100mm stretched mesh panels).

Fish-based Estuarine Biotic Index (EBI) (Breine et al., 2007)

The Fish-based Estuarine Biotic Index (EBI) (Table 4) was developed for the brackish section of the Schelde River estuary. Each metric has an associated score of 0, 0.25, 0.5, 0.75 and 1, and the EBI is calculated as the scores' average value. Due to the absence of reference data, the authors only defined the thresholds until Moderate status: Bad (EBI≤0.15), Poor (0.15>EBI≤0.30), Moderate (EBI>0.30). However, and since the EBI ranges from 0 to 1, it was decided to define the remaining thresholds, in order to better compare the results with the other methodologies, as follows: Moderate (0.30>EBI≤0.55), Good (0.55>EBI≤0.80), High (EBI>0.80). Sampling was performed using a pair of two fyke-nets (type 120/80), deployed at low tide and emptied in the following day.

Table 1. Description of the Estuarine Biotic Integrity Index (EBI), after Deegan et al. (1997).

No.	Metric	Scores	
		0	5
1	Number of species (N)	< 6	≥ 6
2	Dominance	< 3	≥ 3
3	Fish abundance	< 3.8	≥ 3.8
4	Nursery species (N)	< 3	≥ 3
5	Estuarine spawners (N)	< 3	≥ 3
6	Resident species (N)	< 4	≥ 4
7	Proportion benthic fishes (%)	< 0.70	≥ 0.70
8	Proportion abnormal (%)	< 0.01	≥ 0.01

Table 2. Description of the Estuarine Demersal Indicators (EDI), after Borja et al. (2004).

No.	Metric	Scores		
		1	3	5
1	Number of species (N)	< 3	4 - 9	> 9
2	Pollution indicator species	Presence		Absence
3	Introduced species	Presence		Absence
4	Fish health (damage) (% affection)	> 50	5 - 49	< 5
5	Flatfish presence (%)	< 5	5 - 10 or < 60	10 - 60
6	Abundance of omnivorous species (%)	< 1 or > 80	1 - 2.5 or 20 - 80	2.5 - 20
7	Abundance of piscivorous species (%)	< 5 or > 80	5 - 10 or 50 - 80	10 - 50
8	Estuarine resident species (N)	< 2	2 - 5	> 5
9	Abundance of resident species (%)	< 5 or > 50	5 - 10 or 40 - 50	10 - 40

Table 3. Description of the Estuarine Fish Community Index (EFCI), after Harrison and Whitfield (2004).

No.	Metric	Scores		
		1	3	5
1	Number of species (N)	≥ 22	< 22 and ≥ 12	<12
2	Rare or threatened species	Presence	Absence	
3	Exotic or introduced species		Absence	Presence
4	Species composition (% similarity)	≥ 80	< 80 and ≥ 50	< 50
5	Species relative abundance (% similarity)	≥ 60	< 60 and ≥ 40	< 40
6	Species that make up 90% of the abundance (N)	≥ 8	< 8 and ≥ 4	< 4
7	Estuarine resident species (N)	≥ 5	< 5 and ≥ 3	< 3
8	Estuarine-dependent marine species (N)	≥ 14	< 14 and ≥ 8	< 8
9	Abundance of estuarine resident species (%)	25 – 75	≥ 10 and < 25 or > 75 and ≤ 90	< 10 or > 90
10	Abundance of estuarine-dependent marine species (%)	25 – 75	≥ 10 and < 25 or > 75 and ≤ 90	<10 or >90
11	Benthic invertebrate feeding species (N)	≥ 7	< 7 and ≥ 4	< 4
12	Piscivorous species (N)	≥ 3	< 3 and ≥ 2	< 2
13	Abundance of benthic invertebrate feeding species (%)	≥ 10	< 10 and ≥ 5	< 5
14	Abundance of piscivorous species (%)	≥ 1	< 1 and ≥ 0.5	< 0.5

Table 4. Description of the Fish-based Estuarine Biotic Index (EBI), after Breine et al. (2007).

No.	Metric	Scores				
		0	0.25	0.5	0.75	1
1	Number of species (N)	≤ 7	> 7	> 9	> 10	> 11
2	*Osmerus eperlanus* individuals (%)	≤ 0.33		> 0.33	> 1.12	> 2.68
3	Marine juvenile migrating individuals (%)	≤ 33.0	> 33.0	> 54.2	> 73.1	> 82.0
4	Omnivorous species (%)	≥ 16.44	< 16.44	< 7.90	< 3.37	< 1.17
5	Piscivorous species (%)	≤ 12.84	> 12.84	> 19.44	> 27.23	> 41.19

Transitional Fish Classification Index (TFCI) (Coates et al., 2007)

The Transitional Fish Classification Index (TFCI) (Table 5) was developed for the Thames River estuary, and compared to a reference estuarine fish community, derived from a number of estuaries of the same typology as the Thames. Each metric has an associated score of 1, 2, 3, 4, or 5, and the TFCI is calculated as the total score for each sampling date divided by the maximum possible score. The EQS thresholds are the same as in the methodology by Borja et al. (2004). Sampling was carried out based on a multi-method approach: in the upper to mid estuary a 45 x 3.5 m seine net with a 5 mm knotless mesh centre and 20 mm wings was deployed from the shore; a 1.52 m wide beam trawl with a 20 mm knotless outer mesh and 5 mm knotless cod end was trawled for 250 m parallel to the seining site; in the mid and lower estuary were also used paired 8 m wide otter trawls with a 40 mm outer mesh with a 5 mm knotless 'cod end' mesh (Coates et al., 2007). According to the authors, and for the TFCI only, the mean number of taxa within the upper quintile (top 20%) was determined and used as the boundary value between RS4 and RS5. Percentages of this value were used to calculate the boundaries for each metric (see Table 5).

Reference data

An ideal reference community is derived from the same site at the same time of year using the same methods, during a period when the environment is pristine and no anthropogenic changes have occurred (Coates et al., 2007). Since reference data was not available for the Mondego estuary (or any other Portuguese A2 type estuary - Mesotidal well-mixed estuaries with irregular river discharge; Bettencourt et al., 2004) to use in the Estuarine Fish Community Index (EFCI) (Harrison and Whitfield, 2004) and in the Transitional Fish Classification Index (TFCI) (Coates et al., 2007), it was determined that the average densities from June 2003 to May 2004 would be used as reference, since it was the period when environmental conditions (namely precipitation and freshwater runoff) were within regular values. This decision was taken in accordance with Martinho et al. (2007b), who outlined that the fish community is sensitive (to some degree) to variations in precipitation and freshwater runoff regimes.

Table 5. Description of the Transitional Fish Classification Index (TFCI), after Coates et al. (2007) (Metrics 4, 5, 6, 8 and 9 scores defined according to the Mondego estuary fish community).

No.	**Metric**	**Scores**				
		1	**2**	**3**	**4**	**5**
1	Species composition (% similarity)	< 19.9	20 - 39.9	40 - 59.9	6 - 79.9	80 - 100
2	Presence of Indicator Species	Presence				
3	Species relative abundance (% similarity)	< 19.9	20 - 39.9	40 - 59.9	6 - 79.9	80 - 100
4	Taxa that make up 90% of the abundance (N)	< 0.6	0.6 - 1.19	1.2 - 1.79	1.8 - 2.39	≥ 2.4
5	Estuarine resident species (N)	< 0.6	0.6 - 1.19	1.2 - 1.79	1.8 - 2.39	≥ 2.4
6	Estuarine-dependent marine species (N)	< 0.6	0.6 - 1.19	1.2 - 1.79	1.8 - 2.39	≥ 2.4
7	Functional guild composition (N)	0 - 1	2	3		
8	Benthic invertebrate feeding species (N)	< 0.6	0.6 - 1.19	1.2 - 1.79	1.8 - 2.39	≥ 2.4
9	Piscivorous species (N)	< 0.6	0.6 - 1.19	1.2 - 1.79	1.8 - 2.39	≥ 2.4
10	Feeding Guild Composition (N)	0	1	2	3	4

Data analysis

The structure of the fish community was analyzed based on Ecological Guilds, derived from habitat usage patterns (adapted from Elliott and Dewailly, 1995): marine adventitious species (MA), marine juvenile migrant species (MJ; occurring usually in low densities in estuaries as an alternative habitat), marine species that use the estuary as nursery grounds (NU; occurring in clear seasonal patterns, higher densities and remaining longer periods in estuaries), estuarine resident species (ER), catadromous adventitious species (CA) (no anadromous species were found) and freshwater adventitious species (FW), and Feeding Guilds: planktivorous (PLANK), benthic invertebrate feeders (INVV), piscivorous (PISV), omnivorous (OMN) (adapted from Elliott and Dewailly, 1995, Breine et al., 2007 and Coates et al., 2007). Fish densities were estimated as the number of individuals per 1000 m^2. Whenever needed, data was transformed according to the procedures proposed by each index.

Results from the indices were compared based on Kendall's coefficient of correlation. This correlation coefficient varies between -1 (total disagreement) and 1 (perfect agreement), and if the correlation equals zero, the rankings are completely independent. Differences between seasonal results were tested with an ANOVA and Tukey-type a posteriori tests were used whenever the null hypotheses were rejected. A significance level of 0.05 was considered in all test procedures.

Results

Environmental background

Within the study period, a severe drought occurred in 2004/2005, with associated reduction in precipitation and freshwater runoff (Fig. 2A). In fact, only in 2003/2004 was recorded precipitation values above the 1931-2006 average, and freshwater runoff to the estuary was reduced almost 10-fold from the highest value in 2003 to the lowest value in 2005. According to the Portuguese Weather Institute (*http://web.meteo.pt/pt/clima/clima.jsp*), the 2005 drought was the harshest since 1931.

Temperature showed a typical pattern for temperate latitudes, ranging from 8.8 ± 1.7 to 22.7 ± 2.6 ºC, while salinity showed a clear increase during the drought (Fig. 2B). For further detailed information on the drought conditions and its main effects on estuarine planktonic and fish communities see Dolbeth et al. (2007b), Marques et al. (2007) and Martinho et al. (2007b).

Estuarine fish community

The Mondego estuary fish community was studied from June 2003 to August 2006 on a monthly basis, being so far identified 42 species, belonging to 23 Families (Leitão et al., 2007; Martinho et al., 2007a,b) (Table 6). As a general pattern, the fish community was dominated by the estuarine residents (ER) *Pomatoschistus microps* and *Pomatoschistus minutus*, the marine species that use the estuary as nursery area (NU) *Dicentrarchus labrax*, *Solea solea* and *Platichthys flesus*, and the marine juvenile (MJ) *Diplodus vulgaris*. Freshwater adventitious species (FW) (*Barbus bocagei*, *Carassius auratus* and *Gambusia holbrooki*) were only occasionally caught until the winter of 2004, and marine adventitious species (MA) such as *Arnoglossus laterna*, *Buglossidium luteum*, *Gaidropsarus mediterraneus*, *Solea lascaris* and *Symphodus bailloni* only appeared after the summer of 2004. In fact, *A. laterna*, *B. luteum*, *S. lascaris* and *Trisopterus luscus* were only captured inside the estuary in 2005.

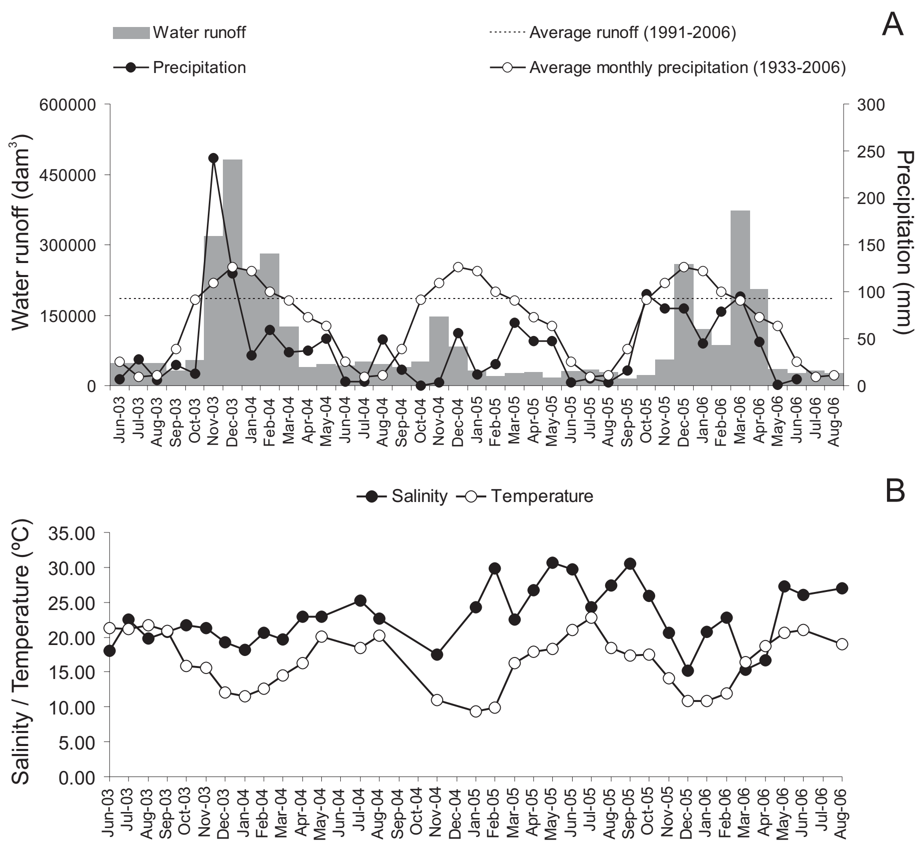

Figure 2. Monthly variation of precipitation (mm) and freshwater runoff (dam^3) (A) and average temperature (ºC) and salinity (B) from June 2003 to August 2006. Dashed line indicates the average value of freshwater runoff for the period 1933-2006.

Regarding the total number of species (Fig 3A), throughout the study period were captured an average of 15 ($\pm$3) per month; the highest species number was collected in January 2005 (22 spp). Total fish densities (Fig. 3B) were higher in the beginning of the study (~140 ind. 1000 m^{-2}), with an average value of 27.4$\pm$25.1 ind. 1000 m^{-2} throughout the study period. No clear seasonal patterns were identified both for the number of species and total densities.

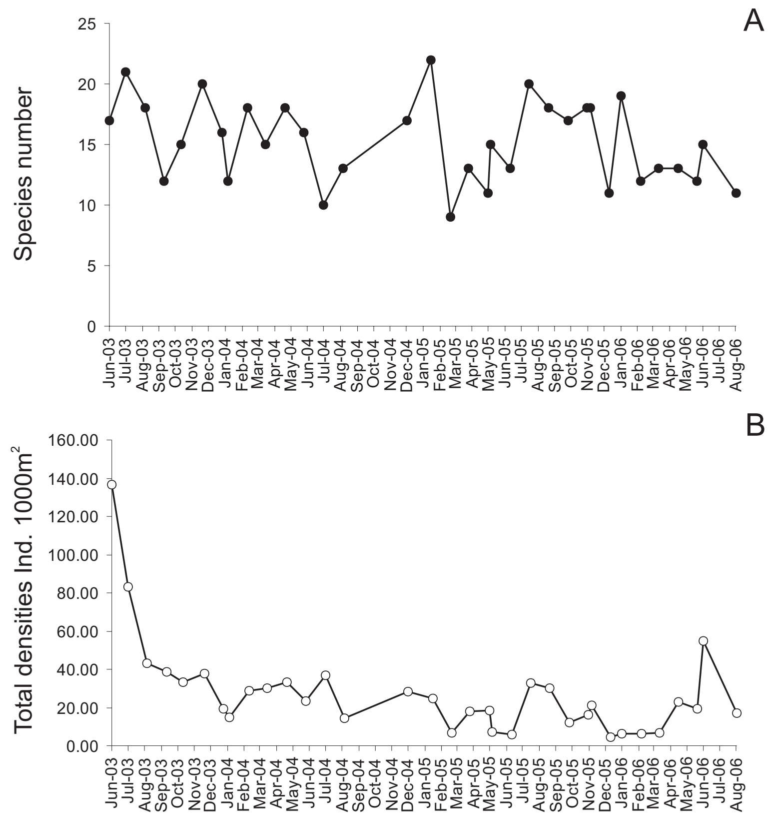

Figure 3. Monthly variation of the number of species (A) and total densities (N. Ind.1000m^{-2}) (B) of the fish community of the Mondego estuary during the study period.

Ecological quality: metrics results

The monthly evaluation by the Estuarine Biotic Integrity Index (EBI) is shown in Fig. 4A. The index exhibited a constant value over the study period, corresponding to a Medium Ecological Quality Status (EQS). The exception was December 2005, in which the index presented a Low EQS. The Estuarine Demersal Indicators (EDI) proposed by Borja et al. (2004) (Fig. 4B) classified in general as Good status, with the highest amplitude of values during the drought period: in May 2005, the EDI classified as Moderate status and in August 2005 as High status. In May 2006, the lowest EQR was obtained (0.44 – Moderate) and in the winter of 2004 this index classified as High status.

Although quite constant, the Estuarine Fish Community Index (EFCI) (Harrison and Whitfield, 2004) (Fig. 4C) showed a slight decreasing tendency regarding the EQS of the Mondego estuary. As a general pattern, this index classified as Good status from 2003 to 2005 (with few exceptions), while all 2006 was classified as Moderate status. The Fish-based Estuarine Biotic Index (EBI) (Breine et al., 2007) was the only index that evidenced a clear decrease in the EQS (Fig. 4D), particularly during the drought period (from mid-2004 to 2005). As a result, during 2003 a Good status was obtained, while in 2004/2005 the values decreased and the estuary was classified in Moderate and Poor status. In 2006, the index values increased, and a Good status was obtained in the end of the study period. Figure 4E reports the classification of the EQS according to the Transitional Fish Classification Index (TFCI) (Coates et al., 2007). This index evidenced the highest and more constant results, classifying as High status almost all the sampling situations, with the exception of August 2004, December 2005 and June 2006.

Comparison of Indices

Table 7 shows the results of the Kendall tau rank correlation coefficient between the selected indices ($p<0.05$). Significant positive correlations were found between Borja et al. (2004) and Breine et al. (2007) (T=0.41), Coates et al. (2007) and Breine et al. (2007) (T=0.31), Harrison and Whitfield (2004) and Coates et al. (2007) (T=0.64); the only significant negative correlation was found between Deegan et al. (1997) and Borja et al. (2004) (T=-0.30).

Table 6. Species list of the Mondego Estuary fish community, with respective Family, Ecological Guild, Feeding Fuild, Indicator status and average density (number of individuals per 1000 m^2) trhoughout the study period; CA – catadromous; ER – estuarine resident; MA – marine adventitious; FW – freshwater; MJ – marine juvenile; NU – nursery; PLANK – planktivorous; INVV – benthic invertebrate feeder; PISV – piscivorous; OMN – omnivorous.

Species	Family	Ecological Guild	Feeding Guild	Indicator Species	Average N ind.1000 m^{-2}
Ammodytes tobianus	Ammodytidae	MA	PLANK	N	0.115 ± 0.33
Anguilla anguilla	Anguillidae	CA	INVV/OMN	Y	0.614 ± 0.93
Aphia minuta	Gobiidae	MA	PLANK	N	0.060 ± 0.22
Arnoglossus laterna	Scophthalmidae	MA	PISV	N	0.015 ± 0.05
Atherina boyeri	Atherinidae	ER	PLANK/OMN	N	0.768 ± 1.27
Atherina presbyter	Atherinidae	ER	INVV/OMN	N	0.096 ± 0.21
Barbus bocagei	Cyprinidae	FW	INVV/OMN	N	0.007 ± 0.04
Buglossidium luteum	Soleidae	MA	INVV	N	0.003 ± 0.02
Callionymus lyra	Callionymidae	MA	INVV/OMN	N	0.143 ± 0.26
Carassius auratus	Cyprinidae	FW	INVV/OMN	Y	0.002 ± 0.01
Chelon labrosus	Mugilidae	MJ	DETR/OMN	N	0.009 ± 0.04
Ciliata mustela	Gadidae	MJ	INVV	N	0.126 ± 0.21
Conger conger	Congridae	MA	PISV	N	0.018 ± 0.04
Dicentrarchus labrax	Moronidae	NU	PISV	N	7.540 ± 7.82
Dicologlossa hexophthalma	Soleidae	MJ	INVV/OMN	N	0.002 ± 0.01
Diplodus vulgaris	Sparidae	MJ	INVV/OMN	N	1.394 ± 1.81
Echiichthys vipera	Trachinidae	MA	INVV/OMN	N	0.026 ± 0,07
Engraulis encrasicolus	Engraulidae	MA	PLANK/OMN	N	0.050 ± 0.16
Gaidropsarus mediterraneus	Gadidae	MA	INVV	N	0.002 ± 0.01
Gambusia holbrooki	Poeciliidae	FW	INVV/OMN	N	0.011 ± 0.06
Gobius niger	Gobiidae	ER	INVV	N	0.121 ± 0.14
Liza aurata	Mugilidae	MJ	DETR/OMN	N	0.014 ± 0.04

Table 6 (cont.). Species list of the Mondego Estuary fish community, with respective Family, Ecological Guild, Feeding Fuild, Indicator status and average density (number of individuals per 1000 m^2) trhoughout the study period; CA – catadromous; ER – estuarine resident; MA – marine adventitious; FW – freshwater; MJ – marine juvenile; NU – nursery; PLANK – planktivorous; INVV – benthic invertebrate feeder; PISV – piscivorous; OMN – omnivorous.

Species	Family	Ecological Guild	Feeding Guild	Indicator Species	Average N ind.1000 m^{-2}
Liza ramada	Mugilidae	CA	DETR/OMN	N	0.242 ± 0.57
Mugil cephalus	Mugilidae	MJ	DETR/OMN	N	0.005 ± 0.02
Mullus surmuletus	Mullidae	MJ	INVV	N	0.106 ± 0.17
Nerophis lumbriciformis	Syngnathidae	ER	INVV	N	0.008 ± 0.05
Parablennius gattorugine	Blenniidae	MA	INVV	N	0.003 ± 0.02
Platichthys flesus	Pleuronectidae	NU	INVV	N	1.473 ± 1.63
Pomatoschistus microps	Gobiidae	ER	INVV	N	8.061 ± 11.41
Pomatoschistus minutus	Gobiidae	ER	INVV	N	3.623 ± 5.96
Sardina pilchardus	Clupeidae	MJ	PLANK	N	0.267 ± 1.08
Scophthalmus rhombus	Scophthalmidae	MJ	PISV	N	0.053 ± 0.08
Solea lascaris	Soleidae	MA	INVV	N	0.024 ± 0.07
Solea senegalensis	Soleidae	MJ	INVV	N	0.096 ± 0.15
Solea solea	Soleidae	NU	INVV	N	1.621 ± 1.42
Sparus aurata	Sparidae	MJ	INVV/OMN	N	0.019 ± 0.04
Spondyliosoma cantharus	Sparidae	MA	INVV/OMN	N	0.018 ± 0.06
Symphodus bailloni	Labridae	MA	INVV/OMN	N	0.053 ± 0.12
Syngnathus abaster	Syngnathidae	ER	INVV/OMN	N	0.161 ± 0.25
Syngnathus acus	Syngnathidae	ER	INVV	N	0.251 ± 0.52
Trigla lucerna	Triglidae	MJ	PISV	N	0.117 ± 0.25
Trisopterus luscus	Gadidae	MA	INVV/OMN	N	0.099 ± 0.24

The conformity between the methods tested was in general low (Table 7), as the relative number of cases when one index classified a sampling occasion as “High” or “Good” and the other as “Moderate”, “Poor” or “Bad” (mismatch) was high. The lowest mismatch value was obtained between the indices by Borja et al. (2004) and Coates et al. (2007) (6%). Concerning the seasonal variation of the ecological status of the system, only the EBI (Breine et al. 2007) found significant seasonal differences (F=0.982; $p<0.05$), and in particular, between the Autumn 2003 and Autumn 2004 ($q=0.04$; $p<0.05$) and between the Winter 2004 and Winter 2005 ($q=0.048$; $p<0.05$). For the other indices, no significant seasonal variations were found.

Discussion

Assessing the EQS and its relation with drought events

An ecologically parsimonious approach dictates that investigators should place greater emphasis on evaluating the suitability of indices that already exist prior to developing new ones (Diaz et al., 2004). In agreement, this work aimed at evaluating the performance of five selected multimetric indices to assess the Ecological Quality Status of transitional waters using fish data and their response to an extreme drought event that occurred in 2005. Testing of indices is an exercise aiming not only to select the best appropriate index for each case, but also to assure that results are comparable among two or more indices (Simboura and Reizopulou, 2008). One of the major concerns when undertaking this exercise was the lack of publications in this subject, in opposition with the benthic component of transitional waters, which has generated a large debate and accordingly, several multimetric indices are being tested by European member states (e.g. AMBI - Borja et al., 2000; BENTIX - Simboura and Zenetos, 2002; BQI - Rosenberg et al., 2004).

In general, all tested methodologies gave constant results throughout the study period, particularly the indices proposed by Harrison and Whitfield (2004) and Coates et al. (2007) (the metrics with the highest correlation values between them). This would be expected, since the metric by Coates et al. (2007) is an adaptation of the South African index developed by Harrison and Whitfield (2004) to European transitional waters, particularly to the Thames River.

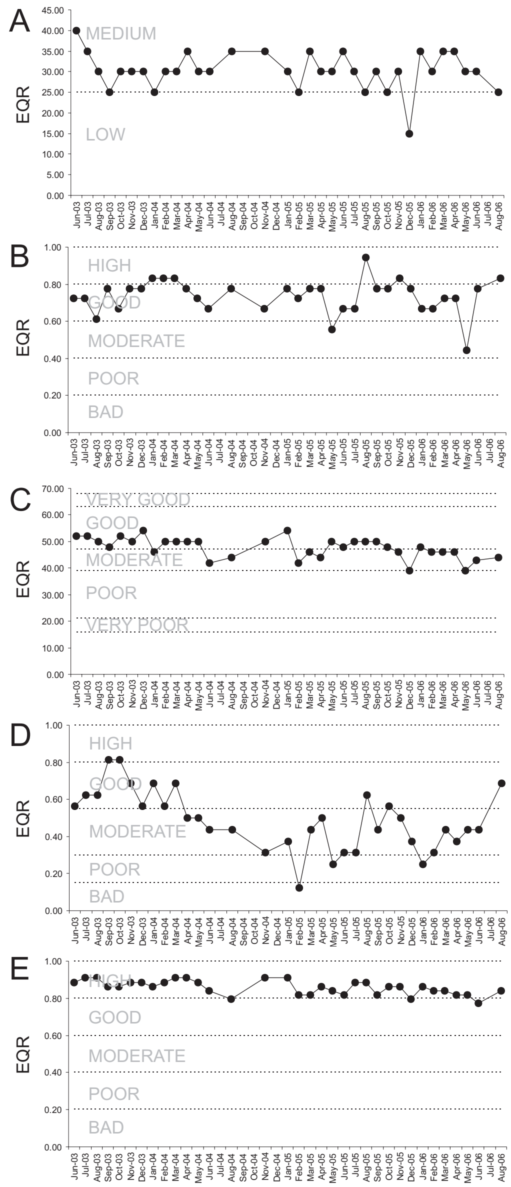

Figure 4. Classification results of the selected indices (EQR) and correspondent Ecological quality status (EQS): (A) EBI - Deegan et al. (1997), (B) EDI – Borja et al. (2004), (C) EFCI – Harrison and Whitfield (2004), (D) EBI – Breine et al. (2007), (E) TFCI – Coates et al. (2007).

In the particular case of the work due to Coates et al. (2007), the system was almost always classified as High status, possibly due to the type of data used as reference condition (the first year of the study). This will be a common issue in implementing the WFD, since quality reference data will not be available for most estuaries, thus becoming an important source of bias when determining the EQS. In terms of status classification, the indices proposed by Borja et al. (2004) and Coates et al. (2007) classified the estuary with the highest status, evidencing the lowest level of mismatch among all the indices tested (Table 7).

Table 7. Kendall tau rank correlation coefficient between the tested indices and conformity between the different methods, given by the percentage of mismatch (relative number of cases in which one of the methods classified a sampling occasion as "High" or "Good" and the other as "Moderate", "Poor" or "Bad" (after Borja et al., 2007). * are significant values for $p< 0.05$.

	EBI Deegan et al. (1997)	**EDI Borja et al. (2004)**	**EFCI Harrison and Whitfield (2004)**	**EBI Breine et al. (2007)**	**TFCI Coates et al. (2007)**
EBI Deegan et al. (1997)	-	91.18 %	73.53 %	41.18 %	97.06 %
EDI Borja et al. (2004)	-0.30 *	-	23.53 %	55.88 %	5.88 %
EFCI Harrison and Whitfield (2004)	0.21	-0.01	-	44.12 %	23.53 %
EBI Breine et al. (2007)	-0.23	0.41 *	0.22	-	61.76 %
TFCI Coates et al. (2007)	0.09	0.06	0.64 *	0.31 *	-

The only index that presented clear interannual and seasonal variations between year fluctuations was the EBI by Breine et al. (2007), which could be the result of two aspects: a) the elimination of the metric correspondent to the abundance of *O. eperlanus* (due to the inexistence of this species in Portuguese and southern European waters, since the southern limit of distribution is the Gironde estuary, France (Rochard and Elie, 1994) and b) this is the index that has the lowest number of metrics (4) within the indices compared in this study. Thus, the use of a larger number of metrics seems more adequate for the fish component of transitional waters (acting as a buffer) and also the use of metrics based on single species should be discouraged, since there is a small number of species that could be considered as indicator and present in all European estuaries. The disadvantage of relying on one single species had already been stated by Breine et al. (2007).

When comparing the classification status of all indices for the same period, considerable variations existed (Fig. 4). The diverse or not consistent responses of the different indices may lead to doubt in managers' minds regarding the value of the methods (Quintino et al., 2006). According to the same authors, the outcome of the use of the indicators has a financial dimension, such that areas misclassified as being in ''Poor status'' will then require expensive remediation measures. This was quite evident when analyzing the level of mismatch between the indices tested, induced by the different background of sampling methods, geographical areas, seasons, pressures and determination of metrics and thresholds for the EQS ranges. In particular, the index by Deegan et al. (1997) gave consistently the lowest results, possibly due to the determination of only the thresholds between "Low" and "Medium" status. It would also have been the case of the EBI by Breine et al. (2007), since reference conditions could not be attained and the boundaries between the highest statuses could not be defined. However, the use of quintile methods and the EQS scale from 0 to 1 allowed defining the thresholds between "Moderate" and "Good" and between "Good" and "High" statuses.

One of the aspects highlighted by this work was the seasonal constancy of the indices (except in the EBI), evidencing that the changes induced by the drought in the fish community, namely the increase in marine adventitious species and a decrease of the estuarine residents, mainly *P. minutus* (Dolbeth et al., 2007b; Martinho et al., 2007b) were not reflected at other guild levels, which are the main components of the indices tested. A characteristic of a good ecological indicator is that it should reflect changes in the ecosystem, while taking into account the natural variability inherent of natural processes, in agreement with the Estuarine Quality Paradox (Elliott and Quintino, 2007), which was verified for all indices except for the EBI (Breine et al., 2007). Thus, it is recommended that this last index should be used with cautiously, considering the Mondego estuary fish database.

Sampling methodologies

One of the main problems in applying and comparing the selected indices is the discrepancy in sampling methodologies, which included gillnetting, beam and otter trawling, deployment of fyke and seine nets, all with different efficiencies, catch rates and sampling efforts. An ideal approach for the implementation of the WFD would be a multi-method sampling regime, in agreement with Coates et al. (2007), since the particular limitations of one sampling gear would be surpassed by other. This, however, would have high costs implicated for the EU Member States, in terms of sampling gears and facilities, human and time resources.

In the specific case of the Mondego estuary, Leitão et al. (2007) found that otter trawl samples did not collect as many species as beam trawl samples, due to restrictions in operating the otter trawl imposed by the lower depths of the upstream areas. Also, the beam trawl is one of the most extensively methods used for scientific sampling of estuarine fish assemblages (Hemingway and Elliott, 2002), being possible to estimate the area covered by each trawl, in opposition to seine nets. However, and according to Coates et al. (2007), the beam trawl is likely to produce samples with lower relative scores than the seine net and otter trawl because it targets benthic fish communities, since it is a much more discriminative technique than the other methods, capturing lower species diversity. In spite of the sampling method used (or a combination of more), it should be standardized the units in which data is converted (e.g. catch per unit effort

(CPUE), number of individuals per unit area), enabling to test and compare different methods in the future.

Definition of guilds

An important component of fish-based indices are the functional guild analysis and classifications. Nonetheless, and due to different classification schemes, some variation occurred between indices, with some species being differently classified in the various approaches and others not assigned to particular guilds. This could be corrected by building an European database of fish species allocated to respective functional guilds (ecological, feeding, vertical preferences, among others), using the recently reviewed and generally accepted concepts of the guild approach for categorizing fish assemblages by Elliott et al. (2007).

Also, it is known that some species can have ontogenic variations at different latitudes, thus being included in different guilds, which should be taken into account. As an example, flounder (*P. flesus*) is classified as a resident species in the UK (Elliott and Dewailly, 1995), while in southern Europe is classified as species that uses estuaries as nursery areas (Leitão et al., 2007; Martinho et al., 2007a). Thus, it is recommended that a standardized guild approach should take place, reducing the variability between indices and according to Elliott et al. (2007), presenting an opportunity to compare and contrast estuarine and other transitional habitats worldwide.

Monitoring

According to the WFD guidance, the minimal monitoring frequency for the fish component of transitional waters should be once every three years, which may have little biological relevance, being probably inconsistent in terms of natural spatial and temporal variability, management actions or decision making (de Jonge et al., 2006). In agreement, and despite that the majority of the selected methodologies showed a good tolerance to the changes induced by an extreme drought event, such a long time period will probably miss important events that can take place in the highly variable estuarine environment (such as sudden pollution and eutrophication, disease outbreaks or even a synergistic effect of extreme climatic episodes). For the fish component, and since no significant variations were found between seasons, the minimal monitoring frequency should be reduced to once every year. The challenging aspect of the WFD is its holistic approach (de Jonge et al., 2006), by assessing the river basin as a whole (ecosystem-based management); thus, the biological and chemical elements that are being used to assess the Ecological Quality Status of water bodies should ideally have shorter minimal monitoring frequencies (which would certainly also imply a higher effort by the EU member states in terms of budget, time and human resources).

Conclusions

As a main conclusion it can be stated that despite some variation, all the indices gave consistent results throughout the study period. However, the ones that seem more adequate for an immediate application and assessment of the EQS are the indices by Borja et al. (2004) and by Coates et al. (2007), given the available dataset for the Mondego estuary. Since there is no reference data available, the index by Borja et al. (2004), with a few modifications, adjusting it to a larger size of estuaries, since it was built for small sized estuaries (Borja et al., 2004), is the one that can could be readily used and validated. One of the modifications that would enable this index to be used in a broader scale would be changing the number of species in the metric concerning the total number of species to a percentage of the maximum number of species ever caught in a given estuary, since the number of species in the Basque estuaries (fish + crustaceans or fish only) is quite low, when compared to other transitional waters.

Nevertheless, the high level of mismatch between the selected indices indicates that there is still a great amount of work to be done in the intercalibration process, and concurrently, further comparisons of different indices for the fish component of transitional waters throughout European member states should be encouraged, in order to test their responses in different water body typologies, time series, sampling methods and designs. Furthermore, the determination of the EQS in transitional waters using fish data will be a challenging task, due to the high mobility of fish species, coupled with the unstable environment that characterizes

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General Discussion

In this section, an integrative approach on the various topics dealt with in the previous chapters will be addressed, by summarizing and discussing the use of estuarine fish assemblages as indicators of environmental changes, as well as the major implications of extreme weather events, such as droughts. During the study period, an extreme drought occurred throughout the Portuguese territory, providing a natural case study to ascertain in more detail the processes and relationships between fish and the surrounding estuarine environment. According to Whitfield and Elliott (2002), the measurement of any ecological response by a fish community, or individual species within a community, must take cognisance on the key role played by the physico-chemical environment in influencing the structure and functioning of that estuary. Thus, the study of the estuarine fish assemblage was performed at two levels: assemblage, by focusing on variations in structure and composition of functional guilds and in the ecological quality status, and specific functional guilds, namely the marine species that use the estuary as a nursery area, by concentrating on aspects related with recruitment levels, interannual variability and abundance patterns.

Estuarine fish assemblages: structure and composition

The first objective of this thesis was to determine if the structure and composition of estuarine fish assemblages was influenced by drought events. In fact, several differences were found in the estuarine use guilds composing the Mondego estuary fish assemblage during the dry period. Particularly, the fish belonging to the species that use marginal estuarine areas, such as the marine and freshwater stragglers, experienced one of the most significant changes, by an increase and decrease, respectively, of the number of species and abundance. Changes in freshwater regimes due to droughts, and consequent higher salinity intrusion in the upper reaches of estuarine waters, lead to an upstream displacement of suitable habitats (Kimmerer, 2002, Fernández-Delgado et al., 2007), having a potential impact on the distribution of fish and planktonic species (e.g. Attrill and Power, 2000; Marques et al., 2007; Martinho et al., 2007). As for the estuarine resident and marine-estuarine dependent species, which composed the majority of the fish community, a decrease in abundance and production was also observed during the dry period (Dolbeth et al., 2008). Seasonal abundance peaks, mainly due to the recruitment patterns of the species belonging to the previous ecological guilds were generally observed, as noted elsewhere (McLusky and Elliott, 2004; Selleslagh and Amara, 2008).

Trends in species composition related with variations in river flow have been reported for estuarine systems worldwide (e.g. Garcia et al., 2001; Ecoutin et al., 2005; Chícharo et al., 2006; Costa et al., 2007; Henderson, 2007; Selleslagh and Amara, 2008), with the common pattern of an increase in marine species during dry periods. Accordingly, a similar response was also observed for the flatfish community, with the main changes in the structure and composition being induced by different river flow regimes. In fact, one of the main impacts of drought events is the reduction in seasonality, as observed by Potter et al. (1986) and Cuesta et al. (2006), essentially in what precipitation and river flow is concerned. The pattern of occurrence of higher species number and abundance of marine stragglers during dry years, as reported elsewhere, can point to a change in estuarine food webs and predator-prey interactions (Attrill and Power, 2000; Dolbeth et al., 2008), with significant impacts for local fish and invertebrate assemblages and for the ecosystem functioning.

However, given the difficulty in distinguishing the effects of river flow from those of other environmental variables (Costa et al., 2007), it is possible that the changes reported can be masked by other factors, such as temperature. In fact, temperature is another important factor structuring estuarine fish assemblages (Attrill and Power, 2002; Henderson, 2007), being also responsible for specific patterns, such as spawning migration (Sims et al., 2004), egg mortality (von Westernhagen, 1970), growth (Amara et al., 2009) or small-scale distributions (Vinagre et al., 2009). Accordingly, temperature may have had an important contribution: during the drought, the flatfish species whose distribution range include higher latitudes were the ones with a more distinct decrease in abundance, which can be related with an increase in water temperature and also to a higher summer temperature differential between estuarine waters and the surrounding coastal area. A similar hypothesis has also been raised by Attrill and Power (2004), stating that the temperature differential between estuarine and marine waters allows fish to optionally exploit optimal thermal habitats. Because most fish species tend to prefer a specific temperature range (Coutant 1977; Scott 1982), long-term changes in temperature may lead to expansion or contraction of their distribution range. These changes are generally most evident near the northern or southern boundaries of the species range; warming results in a geographic shift northward and cooling draws species southward (Ottersen et al., 2004).

The best evidence of the effects of climate change in marine ecosystems comes, in fact, from distributional shifts in marine organisms, more evident near the northern or southern boundaries of their distribution ranges (Drinkwater et al., 2009). Moreover, Henderson (2007) pointed out that a change to warm water communities occurred due to an increase in SST in the Bristol Channel (UK). This may also have occurred in the Mondego estuary during the drought period, with an increase in flatfish species whose distribution range includes subtropical areas, such as *A. laterna*, *B. luteum*, *D. hexophthalma* and *P. lascaris.* Since the Portuguese coast represents the transition between northeastern Atlantic warm-temperate and cold-temperate regions (Ekman, 1953; Briggs, 1974), the presence of several species in sympatry within this latitudinal area constitutes an interesting and unique ecological context (Cabral et al., 2007), with particular relevance for the study of species shifts in habitat use due to global changes.

To identify changes across systems and geographical areas, the guild approach provides a suitable basis (Elliott and Dewailly, 1995; Elliott et al., 2007; Franco et al., 2008). In fact, the importance of the evaluation of the functioning of estuarine ecosystems by categorizing fish into functional categories (such as habitat use, feeding and reproduction), relies on the ability to separate fish strategies in the use of transitional water systems (hence functional aspects) from “accidental behaviors”, e.g., connected to species identities and their distribution along geographical ranges (Elliott et al., 2007). The estuarine habitat use approach here used supports the principle of the previous auhtors, that similarities between estuarine fish assemblages exist on the functional level (rather than on the species composition), suggesting a shared functional role of transitional waters not detected by the taxonomical composition analysis (Franco et al., 2008). Accordingly, the Mondego estuary fish assemblage was similar in structure with the typical European Atlantic seaboard estuarine fish community, as described by Elliot and Dewailly (1995), with a dominance of estuarine resident species and marine-estuarine species. The main variations across estuarine systems, mostly in species composition and relative abundance of functional guilds, have been attributed to particular conditions at each estuary (Pihl et al., 2002), which include hydrodynamic regimes, local topography, anthropogenic pressures and habitat suitability (Schlacher et al., 2007; Vasconcelos et al., 2007).

Linking climate patterns to recruitment variability in fish species

In recent decades there has been growing interest in climate variability and its effects on fisheries and marine ecosystems (Parsons and Lear, 2001). Given the high commercial importance of the marine fish species that use estuaries as nursery areas (i.e. marine-estuarine dependent species), it was essential to evaluate the magnitude in which environmental and climatic variables, both at local and global scales, influence the recruitment and abundance processes in estuarine nurseries. In fact, climate influences marine fish directly through physiology, as well as indirectly through affecting interactions with predators, prey, and competitors in addition to regulating suitable habitat (Ottersen et al., 2004). Thus, the influence of several local environmental variables such as precipitation, river runoff, salinity and estuarine water temperature, as well as large-scale patterns including the NAO index, sea surface temperature, and coastal wind speed and direction was measured, in order to describe the interannual variability in recruitment strength of three key species in the Mondego estuary.

For the three marine species that use the Mondego estuary as a nursery area, *D. labrax*, *P. flesus* and *S. solea*, river runoff was significant in explaining yearly 0-group abundance, with lower densities found during the drought period. These results emphasize the importance of maintaining ecological freshwater flows, since river runoff is interrelated with the extension of river plumes to coastal areas, an important cue for orienting fish larvae towards estuarine nurseries (Amara et al., 2000; Forward and Tankersley, 2001; Vinagre et al., 2007). Wind speed and direction were also determined as significant factors for the yearly abundance of 0-group *D. labrax* and *P. flesus*. Although in lower magnitude, wind is responsible for several circulation patterns, such as coastal upwelling, surface currents and turbulence, which combined with local topography, tidal and ocean currents can influence transport and retention mechanisms, essential for fish larvae. In particular, flatfish larvae tend to concentrate in the lower water masses (e.g Lagardère et al., 1999), mainly for escaping to the top layer masses that are more susceptible to wind and offshore advection, typical of upwelling systems such as the Portuguese coast (Vinagre et al., 2007).

All these reported influences of environmental factors in the recruitment variability of marine fish lead to one question: how will marine species cope with the environmental instability that is prone to take place in the forthcoming years? One of the major patterns of atmospheric variability in the Northern Hemisphere is the North Atlantic Oscillation (NAO), a hemispheric meridional oscillation in atmospheric mass with centers of action near Iceland and over the subtropical Atlantic (Visbeck et al., 2001; Trigo et al., 2002). This large-scale pattern is highly correlated with precipitation regimes in the Iberian Peninsula, as it interferes with the trajectory of depressions in the North Atlantic (Zhang et al., 1997; Trigo et al., 2002). Additionally, the NAO has also been correlated with changes in sea surface temperature (SST), as well as wind and current patterns off the Portuguese coast (Henriques et al., 2007). In this work, the effects of the NAO could not be ascertained, probably due to the small time frame of field sampling, as its effects are mainly experienced in the long-term. Nevertheless, recent studies have related the NAO with abundance and productivity of fish communities (Parsons and Lear, 2001; Attrill and Power, 2002; Henriques et al., 2007), as well as on the recruitment and migration patterns of particular species (Sims et al., 2004; Henderson and Seaby, 2005; Santojanni et al., 2006). Although the NAO is a natural mode of atmospheric variability, surface (ocean and land), stratospheric, or even anthropogenic processes may influence its phase and amplitude (Visbeck et al., 2001). Hence, a different combination of the NAO, high temperature and salinity may, in the future, produce a considerable less favourable combination resulting in recruitment collapse for many species (Henderson, 2007). In addition, since fish species are often found in, or seek, a limited range of hydrographic conditions, large-scale shifts in water mass boundaries can also lead to distributional changes (Drinkwater et al., 2009), due to changes in water stratification, turbulence and advection mechanisms, essential for the dispersion of eggs and larvae to estuarine nurseries (Sentchev and Korotenko, 2004).

Local patterns also play an important role in determining recruitment strength, which include precipitation, river runoff and salinity regimes. Recent projections for the Iberian Peninsula indicate that a reduction in precipitation amounts is expected to take place, concentrated in the winter months (IPCC, 2001, 2007; Miranda et al., 2006), as well as an increase in extreme weather events, such as droughts or floods (Mirza, 2003; Oki and Kanae, 2006). Thus, since river runoff has been appointed as one determining factor for recruitment of marine fishes worldwide, it is expected that species that recruit later in spring will be more affected by the reduction in the extension of river plumes into the continental shelf, where spawning takes place. Precipitation has also been determined as an important factor influencing estuarine fish production worldwide. As an example, Meynecke et al. (2006) determined that the catches of several Australian estuarine fish species were correlated with rainfall, indicating that fisheries will be sensitive to the effects of climate change. In agreement, similar patterns have been described in southern Portugal, regarding river flow and local offshore fisheries (Erzini et al., 2005). In the present work, both these factors were determinant either in structuring the estuarine fish assemblage, or in the recruitment variability of the selected species, pointing out the importance of such density-independent mechanisms. Moreover, and since Dolbeth et al. (2008) reported on a significant reduction in estuarine production of these species during the drought, it is expected that the function of the estuary as a nursery area for these species may be impaired in the future.

The effects of climate change on marine organisms can be felt at various levels, such as growth, swimming speed and activity rates, reproduction, phenology, recruitment, distribution or mortality (Drinkwater et al., 2009). Of the previous set of environmental factors, temperature seems to be another important variable by controlling several physiological functions, such as growth and reproduction. For the English Channel, Sims et al. (2004) observed that flounder (*P. flesus*) migrated earlier from estuarine nurseries to coastal spawning grounds when the largest differences in temperatures between near-estuary and offshore environments occurred, which were in turn, related significantly to cold, negative phases of the NAO. For the same species, seawater temperature over 12ºC has a deleterious effect on the eggs' survival (von Westernhagen, 1970), which has been pointed out as the main cause for the reduction in abundance of this species in southern Portuguese estuaries (Cabral et al., 2007). Moreover, Pörtner et al. (2001) demonstrated a clear relationship between climate induced temperature fluctuations and the recruitment, growth performance and fecundity of cod stocks, as a consequence of maintaining internal energy budgets. Given these early indicators of change in phenology and habitat use patterns, it is probable that a significant reduction in the abundance of these species in Portuguese estuaries may take place, mainly for those species whose distribution range includes higher latitudes.

The use of statistical methods such as GLM for determining which environmental variables influence significantly the recruitment variability showed to be a powerful tool. This technique has been extensively used worldwide, mainly for determining habitat suitability, species occurrence and analysing fisheries catch data (e.g. Stefánsson, 1996; Le Pape et al., 2007; Teixeira and Cabral, 2009; Vinagre et al., 2009). Hence, this approach combined with other models such as Generalized Additive Models (GAM) and Habitat Suitability Indices (HSI), can improve the assessment of optimal habitats, as well as the occurrence of species given a set of environmental characteristics. Moreover, descriptors of habitat suitability provide valuable information, as required for implementing effective ecosystem-based management plans (e.g. River Basin Plans, Integrated Coastal Zone Management Plans), either for fisheries, marine protected areas, estuarine and coastal ecosystems.

Management of transitional waters

The EU Water Framework Directive (WFD) has established 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. For the first time, water management is: (a) based mainly upon biological and ecological elements, with ecosystems being at the centre of the management decisions; (b) applied to European water bodies, as a whole; and (c) based upon the whole river basin, including also the adjacent coastal area (Borja, 2005). The main objective of this directive is to obtain a "Good" Ecological Status in all European water bodies by 2015. In this context, the requirement for the use of comparable methodologies among different countries relies on the fact that, during the intercalibration process for the implementation of the WFD, EU member states need to be in a broad consensus in the meaning of the different concepts included in the directive, mainly in the operative aspects (Borja, 2005), such as sampling methodologies and processes for the ecological quality assessment. Thus, the need for establishing the Ecological Quality Status (EQS) in transitional waters based on the fish component (in addition with algae and benthic invertebrates), led to the evaluation of the previous aspects: sampling methodologies and multimetric indices.

Regarding the first approach, two widely used fishing gears for sampling estuarine fish assemblages were compared in terms of structure and composition of the fish assemblage, since the effects of gear type have considerable implications for the accuracy and precision of samples obtained from ecological and fishery-independent surveys (Greenwood, 2008; Rotherham et al., 2008). Effectively, and despite the same species richness was observed in both beam and otter trawl samples, different type-specific assemblages were determined. In fact, a higher relative proportion of estuarine resident species in the beam trawl drove the main differences between beam and otter trawl samples. However, seasonal otter trawl samples were composed of a higher species number, as a result of a wider catchability of pelagic species in comparison with the beam trawl, which targets mainly benthic and demersal species (Hemingway and Elliott, 2002). Similar results when comparing different sampling methods have been obtained by several authors, attributing such variability to gear selectivity (e.g. Olin and Malinen, 2003; Greenwood, 2008).

Other factors such as diel period, tow duration, sampling gear, tidal phase and sediment type have been determined to exert an effect on the structure and composition of estuarine fish samples. As an example, night samples consistently provide higher abundance and species richness estimates (e.g. Gray et al., 1998; Guest et al., 2003; Unsworth et al., 2007; Johnson et al., 2008; Rotherham et al., 2008), mainly due to an increased catch efficiency of sampling gears, and to the increase in food availability, mainly in seagrass beds (e.g. Sogard and Able, 1994; Guest et al., 2003; Unsworth et al., 2007). Despite the difficulties and constraints of sampling at night (Heminway and Elliott, 2002), forthcoming monitoring plans should be based on night samples. In addition, habitat heterogeneity should also be considered, as it has been demonstrated that different fish assemblages are associated with habitat complex mosaics, such as mudflats, bare sand, saltmarshes, oyster and seagrass beds (Nagelkerken and van der Velde, 2004; Ribeiro et al., 2006; França et al., 2009). For the Mondego estuary, the beam trawl was determined as the most suitable sampling gear for fish assemblages, given the impossibility of operating the otter trawl in the shallower upper reaches of the estuary. Beam trawls are among the most extensively used sampling gears for scientific purposes throughout Europe (Hemingway and Elliott, 2002), thus providing a suitable method for further comparisons among estuarine areas.

The reported differences in the functional guilds of the Mondego estuary fish assemblage due to different sampling methods, mainly regarding the estuarine use and feeding guilds, is of great importance for the determination of the EQS, leading to the another crucial issue that is the choice and harmonization of sampling methodologies. Notwithstanding the fact that in the present case both beam and otter trawl captured the same species number, their composition in ecological and feeding guilds was to some extent different, which might provide with different qualification if the EQS. In agreement, Coates et al. (2007) stated that different sampling gears contributed to differences in the EQS, as a result of gear selectivity. As with all sampling gears, it is important to acknowledge that not all members of the fish assemblage will be collected and those that are, will be caught with differing efficiencies (Greenwood, 2008), so as a result, most fish-based indices will be method-specific regarding guild composition, metric scoring thresholds and reference conditions.

The use of estuarine fish assemblages as indicators of environmental changes was further analyzed, by comparing several multimetric indices developed worldwide in terms of their evaluation of the Ecological Quality Status (EQS) of the Mondego estuary. Moreover, it was also assessed their response to the extreme drought event that took place during the sampling period. The use of environmental indicators not only helps monitor changes in an ecosystem, but also aid in communication by summarizing complex information about the environment (Harrison and Whitfield, 2004). In addition, these biologically criteria-based tools have the advantage of providing a reliable and flexible evaluation, allowing at the same time an easier communication of results to managers, stakeholders and policymakers (Henriques et al., 2008).

For this work, several multimetric indices were selected: Estuarine Biotic Integrity Index (EBI) (Deegan et al., 1997), Estuarine Demersal Indicators (EDI) (Borja et al., 2004), Estuarine Fish Community Index (EFCI) (Harrison and Whitfield, 2004), Fish-based Estuarine Biotic Index (EBI) (Breine et al., 2007) and the Transitional Fish Classification Index (TFCI) (Coates et al., 2007). In general, the EQS determined for the Mondego estuary based on the fish component ranged between “Bad” and “High”, with the EDI by Borja et al. (2004) and the TFCI by Coates et al. (2007) giving constantly the higher results. These were also the indices that obtained the lowest mismatch values, providing consistent evaluations of the EQS among them. Thus, it is recommended that, if no specific index is developed in a near future, one of these two indices should be used (or adapted) for Portuguese transitional waters. The EBI by Breine et al. (2007) was the only that detected changes in the structure and composition of the fish assemblage during the drought period. In fact, testing of indices aims not only to select the best appropriate index for each case, but also assures that results are comparable among two or more indices (Simboura and Reizopoulou, 2008), which is a determinant process for the successful implementation of the WFD.

Moreover, fulfilling the requirements for the evaluation of the EQS raises a few questions: (a) How to disentangle the "signal" of anthropogenic effects against the "noise" of natural variability? and also, (b) how to obtain suitable reference conditions, to whom compare the degree of similarity between fish assemblages? In fact, one major difficulty in evaluating the EQS is to distinguish between the multiple impacts that aquatic ecosystems are subjected to, turning it more intricate to study the response of estuarine fish assemblages to these pressures (Vasconcelos et al., 2007; Uriarte and Borja, 2009). In addition, since transitional waters consist of highly dynamic and naturally stressed systems, separating the causes of change in the structure and composition of fish assemblages can be a demanding task (Estuarine Quality Paradox – see General Introduction), mainly if the available classification tools rely only on ecosystem structural features, such as diversity, instead of functional ones (Elliott and Quintino, 2007). According to the previous authors, failing to do so creates the risk that any indices based on those features and used to plan environmental improvements are flawed. Hence, the use of functional attributes in multimetric indices is highly advisable, as they will enable a better understanding of the changes in the ecosystem functioning, a determinant feature within the WFD. A suitable approach for reducing the influence of natural variability is to adjust the metric's criteria to seasons and sampling gears, as stated by Breine et al. (2007).

The second question was related to the existence of reference data. In fact, one challenging aspect of the WFD is to determine reference conditions, either based on historical data or expert judgement (Muxika et al., 2007). Since little quality historical data is available for Portuguese estuaries, reference conditions need to be derived from either expert judgement, predictive modelling, or as the present case, to rely on recent sampling data. This has the possible disadvantage of biasing the determination of the EQS, since at the present, aquatic ecosystems and the associated fauna have already a degree of deviation regarding what could be determined as a reference condition. However, this method has been successfully implemented by several authors worldwide (e.g. Harris and Silveira, 1999; USEPA, 2000; Harrison and Whitfield, 2004), in which was used the best values observed to define the expectations (i.e. reference conditions) for each biotic attribute or metric given the available dataset. Furthermore, the definition of reference conditions should account for the great natural variability that characterizes estuaries, namely regarding the different habitats available for fish.

Conclusions

This work presented a comprehensive approach on the use of estuarine fish assemblages as indicators of environmental changes. As a major outcome, the components included in the previous chapters, namely the fish assemblages, the marine species that use the estuary as a nursery area as well as the assessment of the Ecological Quality Status, were all influenced in critical aspects by climate and in one of its main expressions: precipitation regimes. Given the expected increase in extreme weather events, such as droughts and floods, resource use patterns by estuarine fish fauna will face new challenges, due to changes in nursery functioning, habitat suitability and reduced water quality. In view of this, the impacts of global changes may be far reaching than the estuarine borders, due to the highly valuable goods and services that these ecosystems provide, including numerous economic activities directly or indirectly connected to them. Ultimately, this work also strengthened the viewpoint that the use of biological criteria, particularly estuarine fish assemblages, is a practical and suitable approach to provide information that supports management decisions, as required by recently developed legislation for the protection of water resources.

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Future Perspectives

One usual pattern of scientific work is that when one objective is fulfilled, several other questions arise, most of which interrelated with the previous work, posing new challenges and defining new research directions. In this thesis, this was not an exception. Consequently, in this section are presented new research areas drawn from the present work, stressing the importance of further investigation on the processes relating estuarine fish assemblages and the surrounding estuarine and marine environments.

Long-term studies and climate change

The importance of long-term studies has been widely recognized, being the basis of reference efforts in determining causal relationships between biota and the environment. A good example is the Continuous Plankton Recorder survey, in which the ecology and biogeography of marine plankton of the North Atlantic and North Sea has been studied since 1931. One important feature in studying the highly dynamic estuarine ecosystems is cross-referencing data at different levels, which reinforces the establishment of long-term monitoring programmes. It also known that global patterns such as the North Atlantic Oscillation (NAO) operate over a large time-scale, being responsible for some of the most important aspects in marine ecosystems such as wind and water circulation, sea water temperature and precipitation regimes. In this work, a relatively small time frame was analyzed, but it was possible to detect some trends as a response to the occurrence of extreme weather events. Prolonging the current monitoring programme will allow evaluating in what extension these extreme weather events impacted on the estuarine communities, as well as determining the effects of different NAO phases on the distribution and abundance of estuarine fauna. In addition, long-term data can also be the fulcrum for a holistic approach in understanding the effects of stochastic and/or small-scale events in estuarine communities, such as pollution outbreaks and toxic algal blooms.

Connectivity analysis

The issue of connectivity between the estuarine and coastal areas is determinant for the fish species whose complex life cycle includes migration between these two environments. This analysis has an invaluable importance, mainly due to the high commercial value of these species. Thus, it would be worthy to analyze the contribution of the Mondego estuary to the coastal stocks of the most important fish species that use the estuary as a nursery area: *D. labrax, P. flesus and S. solea*. Further developments on the use of otolith microchemistry structure can provide more detailed information on the export rates, and also to which degree these rates are influenced by environmental fluctuations and extreme weather events. The analysis of specific patterns related with habitat heterogeneity can also provide suitable information regarding the importance of estuaries as nursery areas for marine fish, being possible to estimate the function and value of particular habitats for each fish species. This can be performed by comparing growth rates, RNA:DNA ratios and condition factors, or even by means of Dynamic Energy Budget models, in order to assess the quality and performance of estuarine nurseries. By combining this information with the anthropogenic pressures in the estuary, valuable insights can be provided for a more adequate management of the estuarine ecosystem.

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