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Abundance, structure and distributional patterns of core secondary producers in a temperate estuary: Driving factors and climate change scenario simulations

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This Thesis includes two scientific papers published in international journals and six submitted articles originated from part of the results obtained in the experimental work referenced as:

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Souza AT, Dias E, Marques JC, Antunes C, Martins I (submitted) Population structure, production and feeding habit of the sand goby *Pomatoschistus minutus* (Actinopterygii: Gobiidae) in a estuary dominated by the sympatric species *P. microps.*

Souza AT, Dias E, Nogueira A, Campos J, Marques JC, Martins I (2013) Population ecology and habitat preferences of juvenile flounder *Platichthys flesus* (Actinopterygii: Pleuronectidae) in a temperate estuary. Journal of Sea Research 79: 60–69.

Souza AT, Freitas F, Campos J, Marques JC, Martins I (submitted) Top-abundant demersal species in a temperate salt-wedge estuary: Assemblages structure and dominance patterns.

Souza AT, Ilarri MI, Campos J, Marques JC, Martins I (2011) Differences in the neighborhood: Structural variations in the carapace of shore crabs *Carcinus maenas* (Decapoda: Portunidae). Estuarine, Coastal and Shelf Science 95: 424–430.

Souza AT, Marques JC, Martins I (submitted) Modeling the effects of temperature and salinity variations on the shore crab *Carcinus maenas*.

Souza AT, Marques JC, Martins I (submitted) Modeling the effects of climate change on a remarkably abundant population of the common goby *Pomatoschistus microps*.

One should not pursue goals that are easily achieved. One must develop an instinct for what one can just barely achieve through one's greatest efforts.

Albert Einstein

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I would like to thank everyone who one way or another has helped me during the course of my PhD, namely my laboratory (LMCEE) and PhD colleagues (PROMAR), my supervisors, my family and friends. I surely could not complete this task without the help of each one of them.

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Summary

Population dynamics is a field of ecology which studies how a population of a given species varies through time according to intrinsic and extrinsic driven forces. This ecology domain is responsible for describing the dynamic response of species to a continuously changing environment. Estuaries are one of the most ecological-rich and dynamic systems on Earth, once they are located in the threshold of marine and freshwater systems, accumulating nutrients from both systems, and hence functioning as nutrient traps. Therefore, estuaries are submitted to a continuous chemical and physical water mixing processes, which makes them complex systems. For this reason, estuaries are characterized by a few number of species, which are often highly abundant and geographically widespread. These species are known as core species, and they present a remarkably interesting opportunity to study the effects of environmental driven forces in populations, since most of them are able to tolerate a wide range of environmental pressures, but often respond differently to stressors. In this context, the present work aimed at assessing the population dynamics of four core species from European estuaries, namely the shore crab *Carcinus maenas*, the common and the sand gobies *Pomatoschistus microps* and *P. minutus* and the European flounder *Platichthys flesus* and to construct ecological models, in order to predict how these species will cope with the effects of rising water temperature and salinity variations promoted by global climate change. To achieve this, epibenthic organisms were sampled during 18 consecutive months at Minho estuary (NW Iberian Peninsula). Overall, the population dynamics studies revealed that the four core species were more abundant during autumn and reproduce either continuously (*C. maenas*), once (*P. minutus* and *P. flesus*) or twice a year (*P. microps*) in Minho estuary. Three of the four studied species (*P. microps*, *P. minutus* and *P. flesus*) presented density values higher than in other European systems. However, the production values recorded in the present work were not significantly higher than in other estuaries, since the core species populations were dominated by juveniles. One exception was the gobies' populations, which were dominated by adults and also presented higher production compared to nearby systems. The present work also highlighted that each estuarine section has its own community structure and dynamics and hence were dominated by different species throughout the year cycle. In areas in the vicinity of the sea, the crustaceans seemed to flourish, while near the threshold of marine water

intrusion, estuarine fishes appeared to outnumber crustaceans. Notwithstanding, we were able to demonstrate that different estuarine sections within an estuary can sustain morphologically different individuals of a given species, which was the case of the shore crab, that presented structural differences in their carapace thickness between close areas within Minho estuary. Finally, the modeling approach suggests how two of the most important species in the system will respond to changes in water temperature and salinity. The *C. maenas* population probably will increase in the near future due to rising water temperature, while changes in salinity will cause minor effects on the population, due to the wide salinity tolerance and the high osmoregulatory skills of the species. On the other hand, the common goby population probably will decrease as a direct response to temperature increase in the next decades, whereas changes in salinity will have important consequences for the *P. microps* population, due to the positive response towards an increased freshwater input and a negative response triggered by a salinity increase.

Resumo

A dinâmica populacional é um campo da Ecologia que estuda como uma população de determinada espécie varia ao longo do tempo de acordo com pressões ambientais intínsecas e extrínsecas. Este ramo de Ecologia é responsável por descrever as variações ao longo do tempo de populações animais e vegetais num ambiente em constante transformação. Os estuários são um dos sistemas mais produtivos e dinâmicos da Terra, uma vez que eles estão localizados no limite dos sistemas marinhos e duceaquícolas, acumulando nutrientes oriundos de ambos os sistemas, funcionando como acumuladores naturais de nutrientes. Devido às variações diárias e sazonais a que estão sujeitos, os estuários são sistemas complexos, onde apenas algumas espécies subsistem. Na realidade, os estuários abrigam um pequeno número de espécies, que frequentemente são muito abundantes e de ampla distribuição geográfica. Estas espécies são conhecidas como espécies centrais, e constituem uma oportunidade extremamente interessante para o estudo dos efeitos de variáveis ambientais sobre as suas populações, uma vez que a maioria destas espécies são capazes de tolerar uma ampla gama de gradientes e pressões ambientais, respondendo, contudo, de maneira diferente à cada uma destas pressões. Neste contexto, o presente trabalho teve como objetivo avaliar a dinâmica populacional de quatro espécies centrais de estuários europeus, nomeadamente o caranguejo verde *Carcinus maenas*, o caboz comum e da areia, respectivamente, *Pomatoschistus microps* e *P. minutus* e a solha das pedras *Platichthys flesus*, bem como desenvolver modelos ecológicos capazes de prever a variação destas espécies sob condições de aumento de temperatura da água e variação da salinidade, promovidas pelas alterações climáticas globais. Para isso, os organismos epibentónicos foram amostrados durante 18 meses consecutivos no estuário do Minho (NO da Península Ibérica). Em geral, os estudos de dinâmica populacional revelaram que as quatro espécies estudadas foram mais abundantes durante o outono e reproduziram continuamente (*C. maenas*), uma vez (*P. minutus* e *P. flesus*) ou duas vezes por ano (*P. microps*). Três das quatro espécies estudadas (*P. microps*, *P. minutus* e *P. flesus*) apresentaram valores de densidade mais elevados do que em outros sistemas europeus. No entanto, os valores de produção registados no presente trabalho não foram significativamente maiores do que em outros estuários, uma vez que as populações destas espécies no estuário do Minho foram maioritariamente compostas por jovens. Exceto a

população de cabozes, que foi predominantemente composta por adultos, apresentando também valores de produção secundária superiores quando comparados com sistemas próximos. Os presentes resultados sugerem ainda que cada seção estuarina possui uma estrutura e dinâmica de comunidade próprias, sendo dominadas por diferentes espécies ao longo do ano. Em áreas próximas ao mar, os crustáceos pareceram ser dominantes, ao passo que próximo ao limite de intrusão de água marinha, os peixes estuarinos foram mais bem-sucedidos do que os crustáceos. Além disto, os presentes resultados também indicaram que diferentes seções estuarinas podem sustentar formas morfologicamente diferentes de uma determinada espécie, como no caso do caranguejo-verde, que apresentou diferenças estruturais na espessura da carapaça entre áreas próximas dentro do estuário do Minho. Finalmente, os modelos ecológicos simularam cenários possíveis da variação de duas das espécies mais importantes do sistema como resposta às mudanças de temperatura e salinidade da água. A população de *C. maenas* provavelmente aumentará num futuro próximo, devido ao aumento da temperatura da água, enquanto as variações na salinidade causarão efeitos menores sobre a população, devido à ampla tolerância às variações de salinidade e às capacidades osmorregulatórias desta espécie. Por outro lado, a população do caboz-comum provavelmente irá diminuir em resposta direta ao aumento da temperatura nas próximas décadas, enquanto as alterações na salinidade terão consequências importantes para a população *P. microps*, devido à resposta positiva em relação a um aumento da entrada de água doce no sistema e também a uma resposta negativa provocada pelo um aumento da salinidade.

CHAPTER 1

GENERAL INTRODUCTION

1.1. Estuaries and climate change

Estuaries are defined as a semi-enclosed coastal body of water with one or more rivers or streams flowing into it, and with a free connection to the open sea (Pritchard, 1967). These systems are among the most complexes, productive and valuables ecosystems on Earth (Costanza et al. 1997; McLusky and Elliot 2004). A number of aquatic species, belonging to different taxonomic groups, inhabit estuarine areas for different periods of their life cycle. Some species are residents, remaining inside the estuary during their entire life cycle (Elliott et al. 2007); while others are migratory or transitory, using the estuary during shorter periods of time, on their way to the spawning grounds, where reproduction and/or juveniles' recruitment take place (Claridge et al. 1986; Potter et al. 1997). Actually, several species use estuaries as nursery grounds (Beck et al. 2001; Peterson 2003), since they provide abundant prey resources and low predation risk for juveniles (Joseph 1973). Therefore, sexually immature individuals can enhance their feeding and growth rates (Houde 1989; Cabral et al. 2007; Martinho et al. 2007a) inside estuaries.

In addition to their high ecological value, estuarine systems have a significant socio-economical importance, since in its waters and/or adjacent areas, fishery, aquaculture, salt mining, tourism, among other economic activities take place (Kennish 2002; Svensson et al. 2007; Vasconcelos et al. 2007). Despite its high ecological and socio-economical value, estuaries are currently subjected to stress caused by a wide variety of anthropogenic activities, including nutrient over-enrichment (Pardal et al. 2000; Nixon 2009), habitat loss (Nicholls et al. 1999; Castro and Freitas 2006), over exploitation of natural resources (Blaber et al. 2000; Vasconcelos et al. 2007; Ferguson et al. in press), introduction of invasive species (Levin and Crooks 2011), altered freshwater inflow (Whitfield and Bruton 1989; Hallim 1991; Ferguson et al. in press), pollution (Matthiessen and Law 2002) and climate change (Henderson et al. 2006; Thorne et al. 2012).

Among all threats posed by anthropogenic activities over estuaries, climate change is certainly one of the most difficult to predict and, thus, to manage, since it occurs in a global scale, affecting uniquely different geographic regions (see IPCC 2007). This phenomenon is considered a direct result of varied human activities and currently is one of the most controversial topics in environmental sciences, ecology, and governance (Halpern et al. 2007; 2008). Climate change will affect oceanic and coastal systems in various ways (Kjerfve et al. 1994): through changes in temperature, sea level, ocean circulation, storminess and hence vertical mixing and wave regimes. Therefore, climate change will significantly influence and modify aquatic ecosystems. In this context, one can expect changes in nutrient availability, biological productivity, phenology (the timing of biological events such as spawning), population biogeography, community structure, and predator-prey relationships from the bottom to the top of the food web (Philippart et al. 2007).

1.2. Climate change signals in Southern Europe and their potential impacts on estuaries

Climate change is already affecting several parts of Europe, contributing to a wide range of impacts on society and in the environment (EEA 2012). The recent climate change predictions made by the Intergovernmental Panel on Climate Change (IPCC) suggest that the air temperature across the continent will increase at a rate of 0.1 to 0.4ºC per decade, with the warming being stronger at southern Europe and northeast Europe (IPCC 2007). Also, it is predicted that winters currently classified as cold will become rarer, while hot summers will occur in a much higher frequency (IPCC 2007). It is also expected that the precipitation will change in southern Europe causing drier summers (5% drier per decade) and wetter winters (1 to 4% wetter per decade) (IPCC 2007). Additionally, it is expected that the sea level will rise (13-68 cm by the 2050s) and heat waves will increase in frequency and length throughout Europe (IPCC 2007).

If the IPCC predictions about temperature increase prove correct, southern European estuaries will warm in the near future, and these climatic changes may trigger a cascade of events within estuaries that ultimately will affect the entire biological community. For instance, changes in water temperature can influence the oxygen solubility (Henry's law), water stratification and mixing (Stefan et al. 1998). These processes can cause oxygen depletion on the estuarine bottom, which may to lead to massive die-offs of aquatic organisms (Diaz and Rosenberg, 1995). Moreover, the low oxygen can also contribute to changes in the water quality, altering the levels of ammonia, nitrate and phosphorus in the water column (see Whitehead et al. 2009). Also, the mix of the nutrient-rich bottom waters with the surface water could lead to algal blooms (see Whitehead et al. 2009).

Changes in precipitation probably will affect the river discharge of southern European estuaries. The wetter winters and drier summers probably will cause a higher number of floods and droughts in estuaries (IPCC 2007). A reduction in river discharge will also produce a decrease in the nutrient supply for estuarine organisms, since the river flow provides and transports both dissolved and particulate organic matter (Meybeck 1982). Also, the decrease in precipitation probably will change the seawater intrusion within estuaries, with seawater reaching more often the upper portions of estuaries and increasing its influence near the river mouth. Conversely, an increase in precipitation probably would cause higher river run-offs, which in turn ultimately may cause changes in water salinity, stratification, turbidity, nutrient availability and sediment erosion.

All abovementioned changes in physical and chemical characteristics of estuaries will certainly affect the aquatic species inhabiting these systems. Thus, it should be expected that significant changes in estuarine communities will occur in response to climate changes.

1.3. Relationships of climate change, population dynamics and ecological modeling

Estuaries are one of the most studied ecosystems (e.g. Gaston and Nasci 1988; Day et al. 1989; McLusky 1999); however, most studies focused on the impacts of anthropogenic disturbances while only a few attempted to investigate population dynamics of estuarine species (e.g. Baeta et al. 2005; Sant'anna et al. 2009) and the effects of climate change (e.g. Henderson et al. 2006). In this context, it is important to know the dynamics and productivity of estuarine species, in order to make predictions regarding the imminent impacts of climate change on European estuarine systems.

Some estuarine species are highly abundant along a wide geographic area in Europe, which makes them core-species in their habitats and hence suitable as target-species for such an approach. Yet only few studies took place near the edges of the geographic distribution, and those probably are the areas where species are most vulnerable to climate change. Although traditional methods of field research are the only way of describing population dynamics, generalizations are limited and only reliable within the range of observations. Moreover, models are a more reliable tool to link population dynamics to environmental conditions and to make long-term predictions. Therefore, modeling approaches have gained notability by enabling extrapolations (Clark 2005).

Population dynamics models simulate the species dynamics over time, taking as state variables the abundance or biomass of individuals or species, addressing aspects of age and/or population groups (juveniles, males and females), growth and recruitment. Populations vary over time according to different parameters such as age class distribution, rates of birth, mortality, dispersal, and growth. In addition, environmental factors (e.g. temperature, salinity, availability of resources and food) are included in the models, influencing population dynamics, through their effects on population processes. Finally, whenever validated, ecological models allow formulating predictions under different environmental scenarios including climate change, extreme weather events, etc.

1.4. Case study: Minho estuary (NW Iberian Peninsula)

The field campaigns for the present work were performed in Minho estuary (41º52'N, 8º52'W), which covers about 23 km^2 in the area of the northern Portuguese-Spanish border (Fig. 1.1). The estuary extends from 70 km upstreams, with tidal influence reaching the first 40 km, while the uppermost 30 km being classified as a tidal freshwater wetland (Souza et al. 2013).

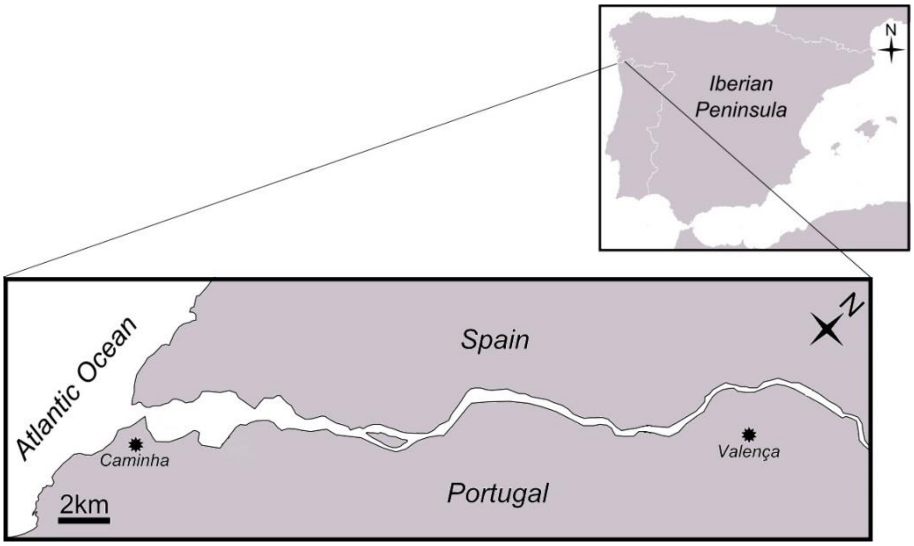


Fig. 1.1 Location of the Minho estuary in the northern Portuguese-Spanish border (NW Iberian Peninsula).

The aquatic resources of Minho estuary have long been explored (see Sousa et al. 2008a and the references therein), however only during the last decade; several studies have focused the ecology of aquatic communities in the Minho estuary. During this period, the aquatic fauna have been investigated, with particular focus on macroinvertebrates (e.g. Sousa et al. 2005; 2007; 2008b; Ilarri et al. 2012), crustaceans (e.g. Freitas 2011; Souza et al. 2011; Sousa et al. in press) fishes (e.g. Freitas et al. 2009; Morais et al. 2011; Sousa et al. 2012a; Souza et al. 2013) and birds (Dias et al. 2012). Several studies reported that, in the Minho estuary, different species present higher density and/or production compared to nearby estuaries (e.g. Sousa et al. 2005; 2007; 2008b; Freitas et al. 2009; Souza et al. 2013), suggesting that the Minho estuary has a significant ecological value in Southern Europe, probably due to higher habitat preservation when compared to other systems (Sousa et al. 2008a).

Nevertheless, Minho estuary has been under different anthropogenic threats in the recent years, such as biological invasions (e.g. Sousa et al. 2007; Ilarri et al. 2012), floods (e.g. Sousa et al. 2012b) droughts (e.g. Dolbeth et al. 2010) and heatwaves (e.g. Ilarri et al. 2011; Sousa et al. 2011), which have caused noteworthy alterations in the ecosystem. Actually, there are records of abnormal climatic events (floods and droughts) since 1953, with 8 droughts (3 moderate, 3 severe and 2 extreme) and 5 floods (1 moderate, 2 severe and 2 extreme) being recorded from 1953 to 2010 (Fig. 1.2). Interestingly, the last two droughts in Minho estuary (2004 and 2008) were extreme and appear to have lasted longer than previous events.

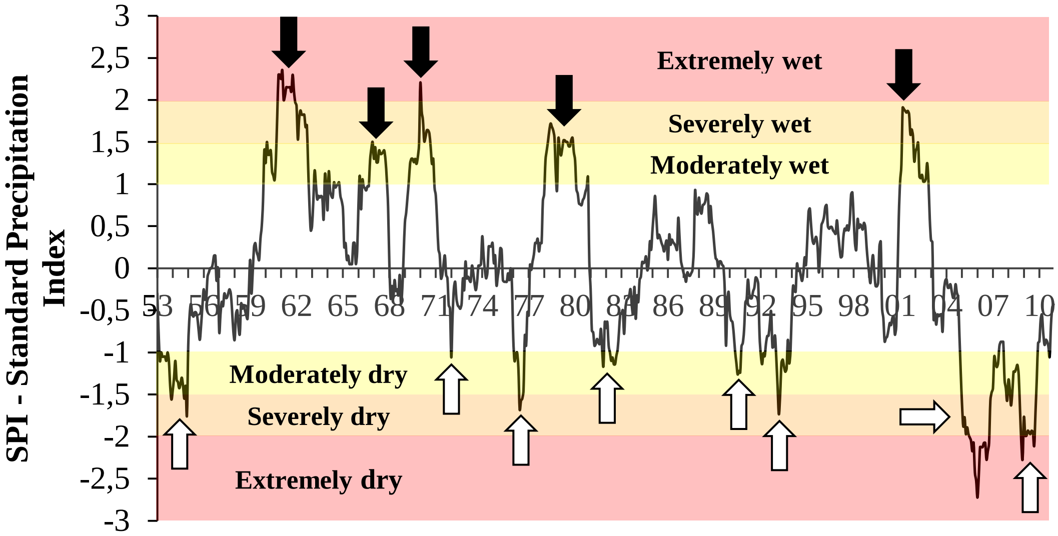


Fig. 1.2 Monthly variation of the Standard Precipitation Index (SPI) in Minho estuary (NW Iberian Peninsula) from 1953 to 2010. Arrows indicate abnormal climatic events (white arrows = droughts; black arrows = floods). Source: Integrated Climate Data Center (http://icdc.zmaw.de).

Nevertheless, river discharge did not follow, exactly, the same pattern of floods and droughts as reported by the SPI. For instance, in 2002 the river discharge was low, but the SPI indicated that this year had precipitation values above the average (Fig. 1.3). The mismatch between SPI and the river discharge may be probably caused by the presence of five dams along the river extension (Peares, Belesar, Velle, Castrelo and Frieira), which manage the water discharge according to needs for electrical power production.

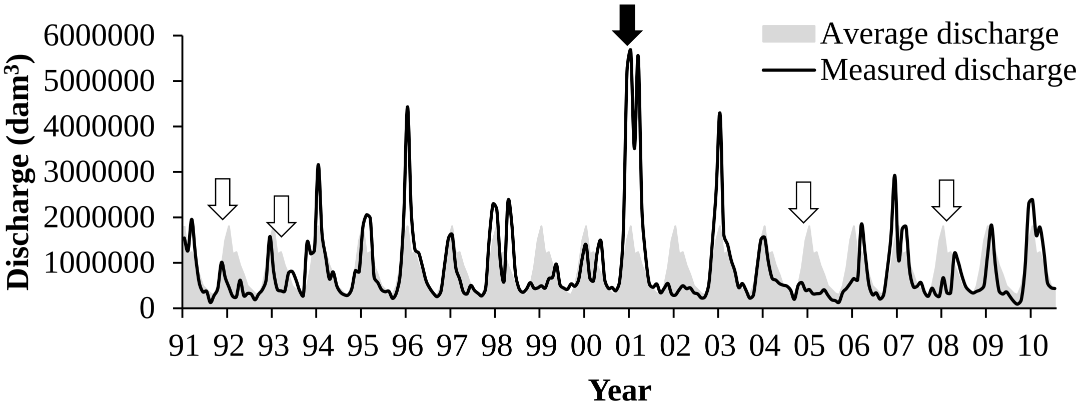


Fig 1.3. Monthly values of river discharge (dam³) from 1991 to 2010, with indication of the average value for the period of 1990 to 2010. Arrows indicate abnormal climatic events (white arrows = droughts; black arrows = floods). Source: Sistema Nacional de Informação de Recursos Hídricos - SNIRH (www.snirh.pt).

Also, since the 80's, that the air temperature in Minho estuary seem to have increased, with the 90's and the years after 2000 being 0.58ºC and 0.66ºC warmer than the average, respectively (Fig. 1.4).

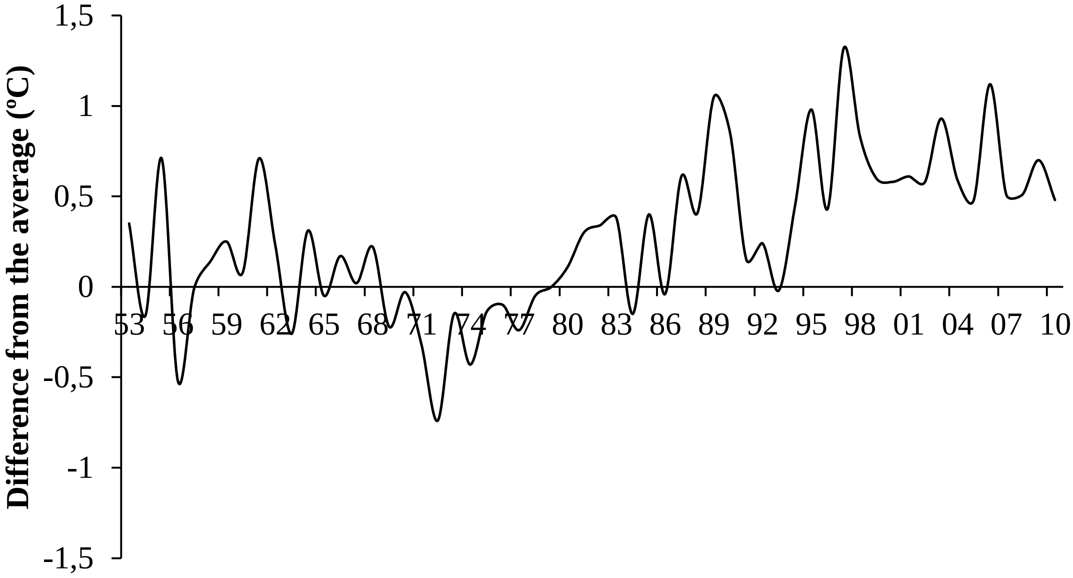


Fig. 1.4 Annual surface temperature difference from the 1951-1980 average in Minho estuary (NW Iberian Peninsula). Source: NASA Goddard Institute for Space Studies - Surface Temperature Analysis (www.giss.nasa.gov).

In this context, it is safe to suggest that climatic changes are already occurring in this system, and it is likely that aquatic organisms are already responding to these changes. In spite some evidence of the occurrence of changes in temperature and precipitation in this system, only few studies dealing with climate change were carried out in this area (e.g. Dolbeth et al. 2010; Sousa et al. 2011; 2012b; Ilarri et al. 2011). Interestingly, all these studies reported the consequences of extreme climatic events on a certain group of species. Clearly, there is a lack of studies that attempt to understand how climate changes will affect the population dynamics of aquatic species in estuarine ecosystems.

CHAPTER 2

STUDIED SPECIES

Carcinus maenas Shore crab / Green crab

Identification: The shore or green crab is a common component of the estuarine and coastal fauna in Europe. This crab can reach up to 60mm of carapace breadth (CB) and 90mm of carapace width (CW). Their dorsum is usually greenish to brownish, while the thoracic sternum in the abdomen can be green or red.

Habitat: This species can be found in different habitat types including the marine sublittoral (up to 60 m depth), estuaries, coastal lagoons and rocky shores. Usually, it lives near the bottom associated with almost all substrates types (muddy, sandy and rocky). Shore crabs are very resistant to environmental variability, being able to tolerate salinities ranging from 4 to 52 and to survive in temperatures varying from 0 to 30ºC (Cohen et al. 1995).

Distribution: Being indigenous from European and Northern Africa coasts and estuaries, (spanning a latitudinal gradient of *ca*. 49º) (Roman and Palumbi 2004), the invasion of *C. maenas* has been reported for several systems in Argentina, Australia, Japan, South Africa and both coasts of North America (Brenchley 1982; Lafferty and Kuris 1996; Behrens Yamada 2001; Hidalgo et al. 2005) (Fig. 2.1).

Fig. 2.1 Area of occurrence of the shore crab *Carcinus maenas*. Blue = indigenous area of distribution; Red = Invaded areas; Green = potential to invasion. Black = occasional records. Source: Wikimedia.

Knowledge in Portugal: There are 55 published papers dealing with the shore crab in Portugal. The species started to be studied in Portuguese waters in the 80's,

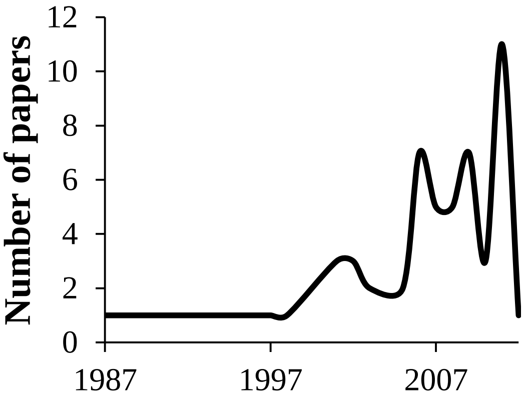


Fig. 2.2 Published papers focusing on the shore crab *Carcinus maenas* in Portugal.

but the scientific interest increased after 2006, when 71% of the studies were published (Fig. 2.2). Studies focusing on ecotoxicology (n = 15) and distribution and habitat preferences (n = 10) accounted for 45% of the total, with other scientific fields being investigated in a smaller rate (Fig. 2.3). The systems where the species was most studied in Portugal were Ria de Aveiro (33% of the published papers), coastal waters (15% of the published papers), Mira estuary (14% of the published papers) and Minho estuary (10% of the published papers), with other systems representing less than 30% of the studies.

Knowledge in Minho estuary: There are 6 published papers dealing with *C. maenas* in Minho estuary, 5 dealing with ecotoxicology (Elumalai et al. 2002; 2005; 2007; Mesquita et al. 2011; Rodrigues et al. 2012) and one with morphology (Souza et al. 2011). Despite of being abundant, until now, nothing is known about the population structure of the species in the Minho estuary.

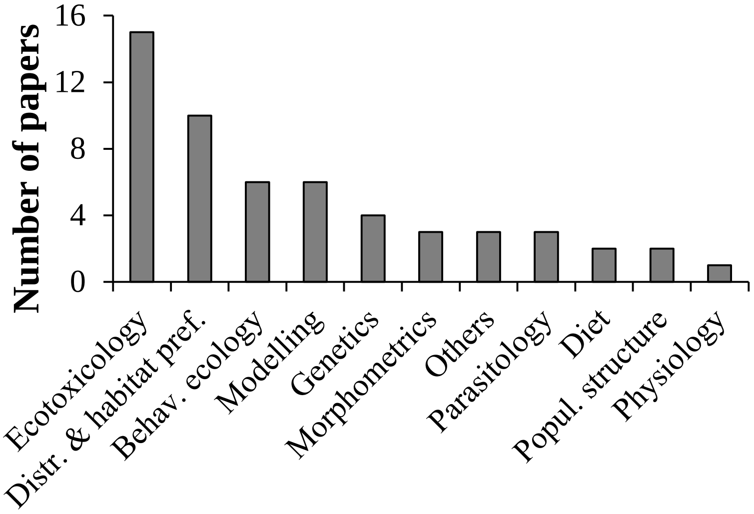
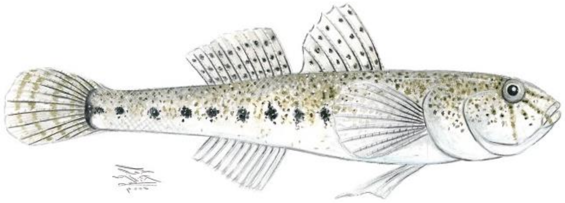


Fig. 2.3 Papers dealing with the shore crab *Carcinus maenas* in Portugal grouped according to their research field.

Pomatoschistus microps Common goby



Identification: The common goby is a small-bodied (maximum T*L* = 90 mm) demersal euryhaline species. This goby is often grey or sandy brown in color, often with darker blotches across the back and faint marks along the side of the body. Males often displays a conspicuous darker spot on the first dorsal fin, and during breeding period, males' color darkens and the fins become more colored.

Habitat: This species usually inhabit estuaries, tidal pools, lagoons, but can also be found in coastal waters. The common goby is often associated to shallow waters, with the depth range varying between 0 and 12 m. Usually, *P. microps* lives associated with muddy and sandy bottoms.

Distribution: The common goby is distributed from Norway to Mauritania, including the Canary Islands, western Mediterranean and Baltic Sea, spanning ca. 44° of latitudinal range (Froese and Pauly 2010).

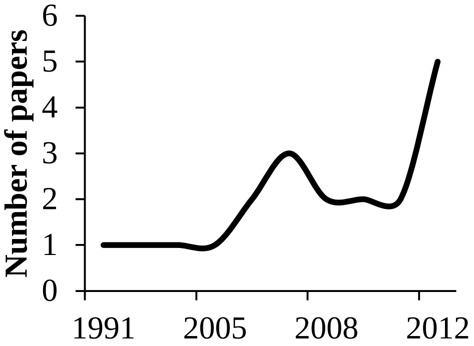


Fig. 2.4 Published papers focusing on the common goby *Pomatoschistus microps* in Portugal.

Knowledge in Portugal: There are 20 published papers dealing with the common goby in Portugal. The species started to be studied in Portuguese waters in the 90's, but the scientific interest increased after 2006, when 80% of the studies were published (Fig. 2.4). Studies focusing on ecotoxicology represented 55% of the total, followed by population structure (20%) and parasitology studies (10%) respectively (Fig. 2.5). The systems where the species was most studied in Portugal were Tagus estuary and Minho estuary (22% of the published papers each), followed by Ria de Aveiro lagoon (19% of the published papers) and Guadiana estuary (8% of the published papers), with other systems accounting for 28% of the studies.

Knowledge in Minho estuary: There are 8 published papers dealing with *P. microps* in Minho estuary, all of them dealing with ecotoxicology (Monteiro et al. 2005; 2006, 2007; Quintaneiro et al. 2008; Vieira et al. 2008; 2009; Guimarães et al. 2012; Oliveira et al. 2012). Despite of being abundant, until now, nothing is known about the population structure, the distribution and habitat preferences of the species in the Minho estuary.

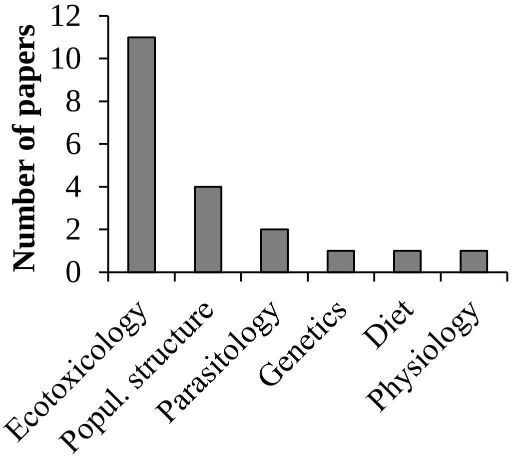
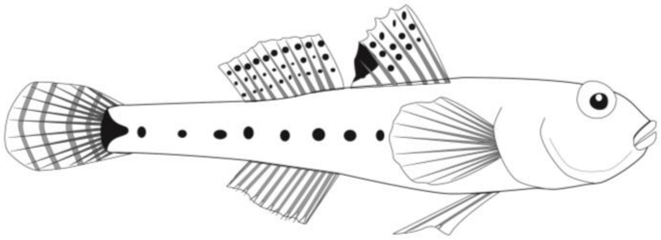


Fig. 2.5 Papers dealing with the common goby *Pomatoschistus microps* in Portugal grouped according to their research field.

Pomatoschistus minutus Sand goby



Identification: The sand goby is a small-bodied (maximum T*L* = 110 mm) demersal species. This goby is often light sandy brown in color, with brown and black spots on the head, a dark reticulation on the back and a dark triangular mark on the base of the caudal fin. Males often displays a conspicuous darker spot on the first dorsal fin, and during breeding period, males' color darkens and the fins become more colored.

Habitat: This species usually inhabit coastal areas, juveniles often can be found in estuaries, tidal pools and lagoons. Usually, *P. minutus* lives in sandy or muddy areas from the low shore to a depth of approximately 20m.

Distribution: The sand goby is widely distributed and commonly observed in the North-eastern Atlantic from Norway to the south of Spain, in some zones of the western Mediterranean (Gulfs of Lions and Genoa), in Adriatic (Venice Gulf) and along the western Black Sea coast. The precise geographical distribution of the species is difficult to define because it has been often mistaken with other goby species (e.g. Doornbos & Twisk, 1987; Henderson 1989).

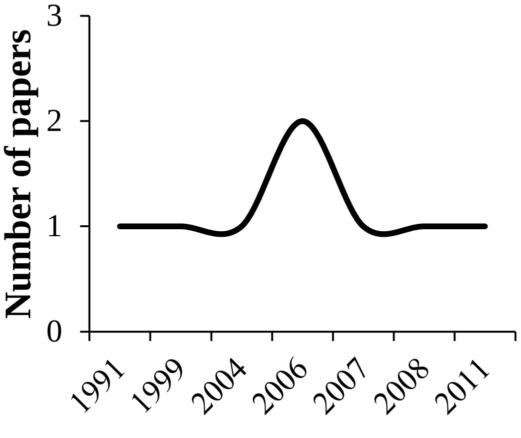


Fig. 2.6 Published papers focusing on the sand goby *Pomatoschistus minutus* in Portugal.

Knowledge in Portugal: There are only 8 published papers dealing with the sand goby in Portugal. The species started to be studied in Portuguese waters in the early 90's, but the scientific interest on *P. minutus* seems to be unimportant, once the species was sporadically investigated in the last two decades (Fig. 2.6). Studies focusing on population structure represented 50% of the total, with ecotoxicology, behavioral ecology, diet and morphometrics contributing with 12.5% of the total number of papers each (Fig. 2.7). The systems where the species was most studied in Portugal were the Tagus and Mondego estuaries (37% of the published papers each), followed by Minho estuary (18% of the published papers) and Lima estuary (9% of the published papers).

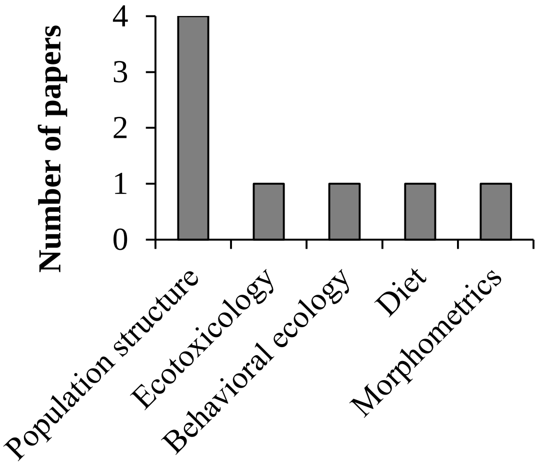
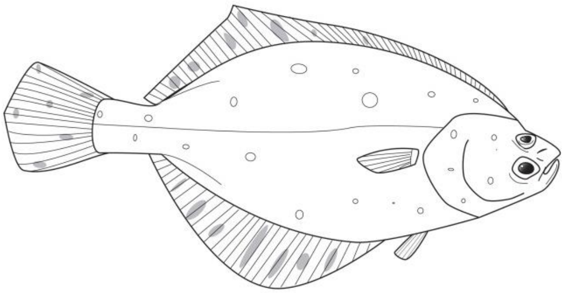


Fig. 2.7 Papers dealing with the sand goby *Pomatoschistus minutus* in Portugal grouped according to their research field.

Knowledge in Minho estuary: There are 2 published papers dealing with *P. minutus* in Minho estuary, one dealing with ecotoxicology (Rodrigues et al. 2006) and one dealing with behavioral ecology (Freitas et al. 2011). Despite of being abundant, until now, nothing is known about the population structure, the distribution and habitat preferences of the species in the Minho estuary.

Platichthys flesus European flounder



Identification: The European flounder is a demersal fish that can grow up to 60 cm of T*L* and weight up to 60 kg. It has a roughly oval shape, with both eyes on the right side of its head, a small mouth and pointed snout. The topside color is variable from dull brown to greyish-green, often with small orange spots, while the underside is white.

Habitat: This species usually inhabit coastal and brackish waters being often classified as a catadromous species (Riede 2004). The European flounder *P. flesus* lives associated with muddy bottoms in depths varying between 1 to 100 m (Muss and Nielsen 1999).

Distribution: This flounder is a widely distributed species naturally occurring in the Black Sea, the Mediterranean Sea, the European Atlantic Coast (including the British Isles and Ireland), the North, the Baltic, the Barents and the White Sea (Nielsen 1986; Rochard and Elie 1994). The described distribution of *P. flesus* in the Atlantic waters ranges from Norway to Morocco (Nielsen 1986), but more recently, other studies pointed out that the northern and central coastal areas of Portugal are more likely to be the current southern limit for its distribution (Cabral et al. 2007; Dolbeth et al. 2008).

Knowledge in Portugal: There are 38 published papers dealing with *P. flesus* in Portugal. In Portuguese waters, the species started to be studied in the 2000, but the scientific interest on *P. flesus* increased after 2007, when 79% of the studies were published (Fig. 2.8). Studies focusing on distribution and habitat preferences represented 45% of the total, while ecotoxicology (16%), parasitology (16%) and physiology (11%) were also investigated in high frequency in Portugal (Fig. 2.9). The systems where the species was most investigated were the Douro estuary (21% of the published papers), Mondego

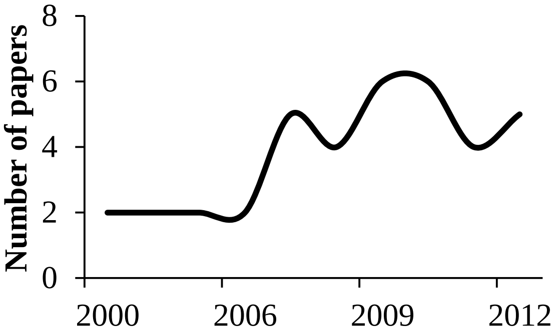


Fig. 2.8 Published papers focusing on the European flounder *Platichthys flesus* in Portugal.

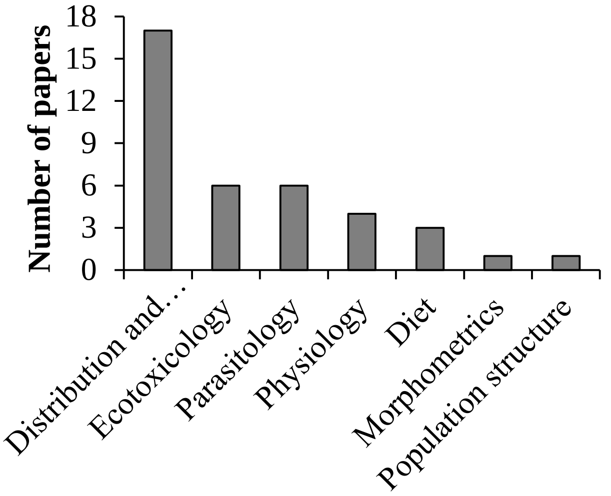


Fig. 2.9 Papers dealing with the European flounder *Platichthys flesus* in Portugal grouped according to their research field.

estuary (16% of the published papers), the coast (11% of the published papers) and the Tagus estuary (10% of the published papers), with the other 6 estuaries accounting for less than half of the studies.

Knowledge in Minho estuary: There are 6 published papers dealing with *P. flesus* in Minho estuary, 5 dealing with distribution and habitat preferences (Cabral et al. 2007; Freitas et al. 2009; Vasconcelos et al. 2010; Morais et al. 2011; Daverat et al. 2012) and one dealing with *P. flesus* physiology (Vasconcelos et al. 2010). The interest in the species in Minho estuary is recent, and this probably is related to the high density of juveniles found in the system. Despite of other papers have investigated the distribution and habitat preferences of the species in the system, none is known about the population structure of the European flounder in Minho estuary.

PART I

ABUNDANCE, STRUCTURE AND DISTRIBUTIONAL PATTERNS

CHAPTER 3

DISTRIBUTIONAL PATTERNS OF THE SHORE CRAB *CARCINUS MAENAS* IN A SALT-WEDGE ESTUARY: SEXUAL AND ONTOGENETIC SEGREGATION

Distributional patterns of the shore crab *Carcinus maenas* in a salt-wedge estuary: sexual and ontogenetic segregation

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Souza AT, Ribas FO, Dias E, Campos J, Marques JC, Martins I (submitted) Distributional patterns of the shore crab *Carcinus maenas* in a salt-wedge estuary: sexual and ontogenetic segregation.

Abstract

The shore crab *Carcinus maenas* is a worldwide distributed species. Due to its wide geographical distribution, biological and behavioral traits, the shore crab is considered a key-species in many coastal systems. This species is highly tolerant to environmental constraints; however, our results indicated that salinity, river discharge and depth probably were responsible for a spatial and temporal segregation between males, females and juveniles. Half of *C. maenas* population in Minho estuary (NW Iberian Peninsula) is constituted of crabs smaller than 35 mm of CW, while females and small-bodied juveniles were more frequently found near the river mouth; males were more abundant inside a saltmarsh area. The high river discharge and low salinity of the Minho estuary probably leaves this estuary voided of berried females, and makes the reproduction occurs on coastal areas. Shore crab recruitment was continuous and the population is up to 4 times denser than in other estuaries from southern Europe. Our results suggest that in salt-wedged estuaries, shore crab populations might be sexually and ontogenetic segregated, and these estuaries may serve primarily for growth and developmental areas for shore crab juveniles.

Keywords: green crab / life cycle / Minho estuary / Portugal / secondary production

3.1. Introduction

In estuaries, the habitat heterogeneity is particularly high, with marked dissimilarities in space and time of the abiotic parameters such as salinity, temperature, flow and tidal energy; and therefore for the associated flora and fauna (Hoffman et al. 2008). Estuaries also contain diverse aquatic habitat types, including seagrass, kelp and shellfish beds, hard-bottom, soft bottom communities with mud and sand, rocky inter-tidal zones, fringing mangrove forests and vegetated marshes/wetlands (Nelson and Monaco 2000). These systems are also classified according to their salinity distribution and flow characteristics, being categorized into one of the three following groups: highly stratified or salt-wedge, partially mixed, and vertical homogeneous or well mixed (Dyer 1996). Salt-wedge estuaries are the most heterogeneous type and are characterized to present abrupt changes on environmental conditions. Thus, the heterogeneity of this ecosystem is ideal for developing generalities about population ecology, structure and habitat preferences of different population groups.

In estuarine communities, only a few species of decapods are present, but usually they are very abundant (Raffaelli et al. 1989; Lazzari 2002; Neves et al. 2007). The most abundant crab inhabiting European estuaries is the shore crab *Carcinus maenas* (Queiroga 1998). This species presents a complex life cycle, which includes both planktonic and benthic phases (Anger 2006). During the larval phase of its development, the shore crab lives offshore in the pelagic domain (Cohen et al. 1995). After the metamorphosis, the young crab moves towards the intertidal zones and sinks to the bottom (Cohen et al. 1995); in the adulthood, *C. maenas* stays in the bottom, living over almost all types of substrata of the estuarine and coastal systems (Crothers 1967; Cohen et al. 1995).

The shore crab is highly tolerant to environmental constraints, being able to tolerate salinities ranging from 4 to 52 and to survive in temperatures varying from 0 to 30ºC (Cohen et al. 1995). *C. maenas* is an opportunistic feeder, preying on a large variety of species from different taxonomic groups, which includes crustaceans, insects, molluscs, fishes and others (Cohen et al. 1995; Baeta et al. 2006). The voracious predatory behavior of the species is an essential feature in structuring marine and estuarine benthic communities (Raffaelli et al. 1989); therefore, *C. maenas* is currently considered a key-species in European estuaries.

Due to the ecological relevance of the species, the shore crab has been the focus of several studies, which assessed different aspects, such as its biology, physiology and reproduction (McVean 1976; Reid and Aldrich 1989; Reid et al. 1994), diet (Cohen et al. 1995; Baeta et al. 2006), agonistic behavior (Sneddon et al. 1997; Sneddon et al. 2000; Fletcher and Hardege, 2009), biological invasion (Roman and Palumbi 2004; Hidalgo et al. 2005; Yamada et al. 2005), pollution effects (Elumalai et al. 2007; Stewart et al. 2010) and ecology (Warman et al. 1993). Despite of being highly studied, there are few studies concerning its population ecology (Baeta et al. 2005; Bessa et al. 2010) and the effects of environmental driven forces on population structure and distribution (Yamada and Kosro 2010). Therefore, this study aimed at understanding how *C. maenas* population is structured in a heterogeneous salt-wedge estuarine system.

3.2. Materials and Methods

3.2.1. Study area and sampling campaign

This study was conducted in the Minho Estuary (NW Iberian Peninsula), which ranges up to 40 km (considering the upstream limit of spring tides), covering a total area of 23 km2. The Minho estuary is a very shallow system (Moreno et al. 2005), with a mean depth of 2.6 m (Freitas et al. 2009) and characterized as a mesotidal and partially mixed system, although during periods of high river discharge, it tends towards a salt wedge estuary (Sousa et al. 2005). The present study was carried out at the lower estuary (first 8 km from the estuary mouth), which corresponds to the area where the shore crab is regularly found in this estuary (personal observation).

Carcinus maenas individuals were collected during 18 consecutive months, from February/09 to July/10 in three nearby subtidal areas in the lower Minho estuary (Fig. 3.1). The three sites were chosen in order to assess possible dissimilarities on the population structure related to differences in the habitat type and also to environmental features.

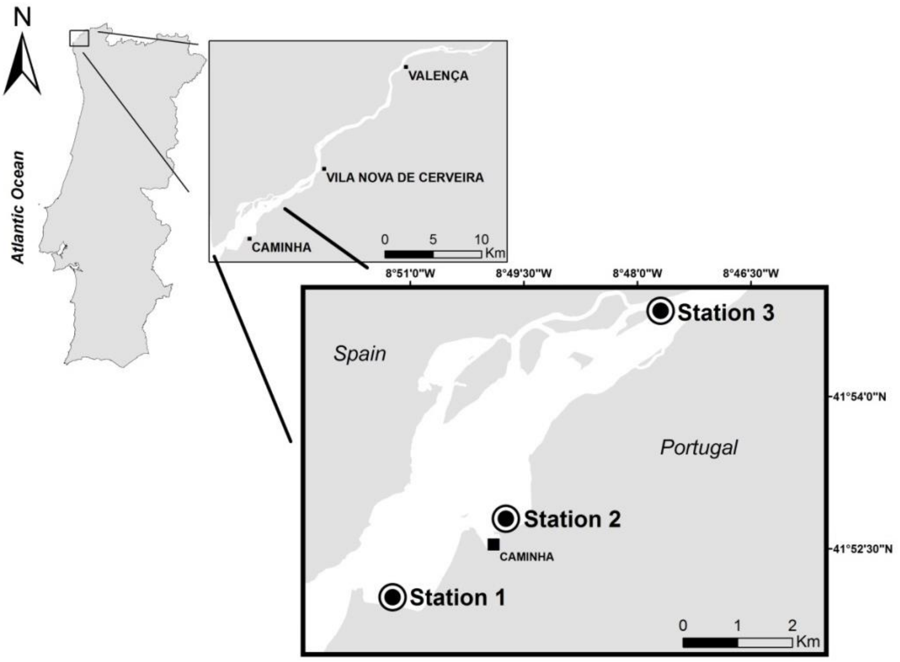


Fig. 3.1 Map of the study site showing the three sampling stations at the lower Minho Estuary, NW Iberian Peninsula.

Station 1 is closer to the river mouth (ca. 1.5km), and is characterized by muddy soft bottoms often densely covered by debris, such as drifting seaweeds, dead leaves and empty mollusks' shells (Souza et al. 2011; 2013). Station 2 is located inside the Coura salt marsh (ca. 3.5 km apart from the river Minho mouth), which is a relatively small sub-system characterized by the presence of narrow channels, typically bordered by the seagrass Spartina maritima. The channels' soft bottom is often sparsely covered by debris and empty peppery furrow shells *Scrobicularia plana* (Souza et al. 2011; 2013). Station 3 is located ca. 8 km upstream from the mouth and is characterized by presenting high densities of the Asian clam *Corbicula fluminea*, with its soft bottoms often sparsely covered by debris and submerged vegetation (Sousa et al. 2008b,c; Souza et al. 2013) In each station, three replicates per month were collected during the day at high tide of spring tides using a 1m beam trawl (5 mm mesh size) towed at constant speed (2 km.h^{-1}) for 2 to 3 minutes. The density of individuals was calculated by averaging densities from individual tows for each site of each month. Additionally, environmental variables (water temperature, salinity, pH and oxidation reduction potential (ORP)) were measured with a multiparameter probe YSI 6820 deployed to 20 cm off the bottom (two replicates).

The monthly river discharge data measured at Foz do Mouro hydrometric station between February/09 and July/10 was obtained from the INAG – Instituto da Água, I.P. (http://snirh.inag.pt).

3.2.2. Laboratory procedures

All *C. maenas* individuals were counted, measured to the nearest 0.01 mm (carapace width - CW) and sexed (based on the observation of abdomen morphology). The reproductive condition (berried females) and color morphotype were recorded based on the predominant color of the thoracic sternum (McGaw and Naylor 1992). Crabs smaller than 15 mm of CW were classified as juveniles (corresponding size of the smallest crab that it sex could be determined at naked eye). Afterwards, all crabs were classified into one of the 7 size classes: A (< 15 mm), B (16-25 mm), C (26-35 mm), D (36-45 mm); E (46-55 mm), F (56-65 mm) and G (> 65 mm).

3.2.3. Data analysis

Prior to the analyses of abiotic data, all variables were normalized. Posteriorly, a resemblance matrix based on the Euclidean distance was calculated. The Principal Components Analysis (PCA) was performed in order to detect habitat differences among sampling stations based on environmental data.

A two-way SIMPER routine was applied to determine the contribution of each abiotic variable for the dissimilarities among groups of stations and seasons.

Afterwards, a Permutational Multivariate Analysis of Variance (PERMANOVA) (type-III) was employed in a two-way crossed design, with station as a fixed factor (3 levels: station 1, station 2 and station 3) and season as a fixed factor (4 levels: winter, spring, summer and autumn), in order to test the statistical significant differences among sampling stations and seasons using all abiotic variables (multivariate approach) and comparing each variable separately (univariate approach). The PERMANOVA analysis is capable of handling unbalanced statistical designs, such as in this study (due to unequal number of samples collected in each season), and calculates an identical F statistic that would be produced using traditional ANOVA and it is not affected by non-normal distribution of data (Anderson 2001).

A similar analysis procedure was adopted to investigate the shore crab data. Prior to the shore crab data analysis, all variables were normalized, and resemblance matrices based on Euclidean distance were calculated. The overall density, the density of each sex (male, female and juvenile), the density of the two color morphotypes (green and red), the density of the seven size classes, the overall size of shore crabs and the size of males and females shore crabs were statistically tested using a two-way PERMANOVA (type-III) in a two-way crossed design, with station as a fixed factor (3 levels: station 1, station 2 and station 3) and season as a fixed factor (4 levels: winter, spring, summer and autumn), using all both multivariate and univariate approaches (comparing each variable separately). Additionally, a PERMANOVA (type-III) in a two-way crossed design, with sexes as a fixed factor (3 levels: male, female and juvenile) and color morphotype as fixed factor (2 levels: green and red) was performed to verify if the shore crab sized varied according to these two factors.

In PERMANOVA, the statistical significance of variance components was tested using 9999 permutations of residuals under a reduced model. When the possible number of permutations was lower than 150, the Monte Carlo-p value was considered. Subsequently, significant terms and interactions were investigated using a posteriori pair-wise comparisons to determine which pairs of stations and seasons were significantly different.

Also, a two-way SIMPER routine was applied to determine the contribution of each size class for the dissimilarities among groups of stations and seasons.

The PCA, the SIMPER and the PERMANOVA analyses were performed using PRIMER v 6.1.11® (Clarke and Gorley 2006) with PERMANOVA+1.0.1. add-on package (Anderson et al. 2008).

Canonical Correspondence Analysis (CCA) was performed to determine the relationship between the density of each size class and groups (genders and color morphotypes) of *C. maenas* and the measured environmental variables using CANOCO 5 software (Ter Braak and Verdonschot 1995). The Monte-Carlo randomization test (499 permutations under the reduced model) was performed to determine the statistical significance of the correlations (Ter Braak 1986).

Finally, the secondary production was computed using the size-frequency method (Hynes method). This method calculates production by summing the biomass lost between size classes (Krueger and Martin 1980). In order to determine the annual production of *C. maenas* in the lower Minho estuary, the Krueger and Martin (1980) equation was applied:

(1) $$P = \sum_{j=1}^{a-1} 0.5\left[\left(Y_{i,j} + Y_{i+1,j}\right) - \left(Y_{i,j+1} + Y_{i+1,j+1}\right)\right].\left(W_j.W_{j+1}\right).(t)^{-1}$$

where *P* is the annual production of the species, $Y_{i,j}$ is the mean density (ind.m-^{2}) of the size class j in the date i, $Y_{i+1,j}$ is the mean density of the size class j in the date i+1, $Y_{i,j+1}$ is the mean density of the size class j+1 in the date i, $Y_{i+1,j+1}$ is the mean density of the size class j+1 in the date i+1, W_j is the biomass (g.m^{-2}) of the size class j, W_{j+1} is the biomass of the size class j+1 and t is the time between i and i+1. Population production estimates correspond to the sum of each size class production (*P*). Negative production values were not included in the overall estimates and were regarded as zero production.

3.3. Results

3.3.1. Abiotic data

Overall, values of depth, pH and salinity were higher in station 1, while temperature and ORP were higher in station 3, with station 2 presenting intermediare values (Supplementary material 3.1).

The two-way PERMANOVA results indicated that there were no differences among sampling stations and seasons (Table 3.1).

Table 3.1 Two-way PERMANOVA results on the effects of sampling stations and seasons and their interaction term on the abiotic variables in lower Minho estuary, NW Iberian Peninsula. * = $p < 0.01$; ** = $p < 0.001$; ^{ns} = non-significant.

Source	df	SS	MS	Pseudo-*F*
All abiotic variables				
Station	2	55.73	27.87	8.83**
Season	3	64.44	21.48	6.81**
Station x Season	6	10.00	1.67	0.53^{ns}
Depth				
Station	2	33.06	16.53	57.78**
Season	3	5.90	1.97	6.87**
Station x Season	6	1.67	0.28	0.97^{ns}
Salinity				
Station	2	21.62	10.81	22.83**
Season	3	8.20	2.73	5.77*
Station x Season	6	1.56	0.26	0.55^{ns}

Temperature				
Station	2	0.24	0.12	0.27^{ns}
Season	3	28.44	9.48	21.63**
Station x Season	6	5.70	0.95	2.17^{ns}
pH				
Station	2	0.59	0.29	0.39^{ns}
Season	3	19.84	6.61	8.77**
Station x Season	6	0.90	0.15	0.20^{ns}
ORP				
Station	2	0.22	0.11	0.09^{ns}
Season	3	2.06	0.69	0.57^{ns}
Station x Season	6	0.17	0.03	0.02^{ns}

Nevertheless, the PCA routine indicated that the first two axis of the PCA explained 62.3% of the variance among samples (PC1 = 34.2% and PC2 = 28.1%) and revealed slight, but relevant differences between sampling stations, with station 1 differing from the other stations, mainly due to its lower values of temperature and pH and higher values of depth and salinity. The water temperature (eigenvalue = -0.674) and the pH (eigenvalue=-0.597) presented the strongest correlations in PC1, while salinity (eigenvalue=0.709) and depth (eigenvalue=0.698) were the most important variables in explaining the PC2 (Fig 3.2).

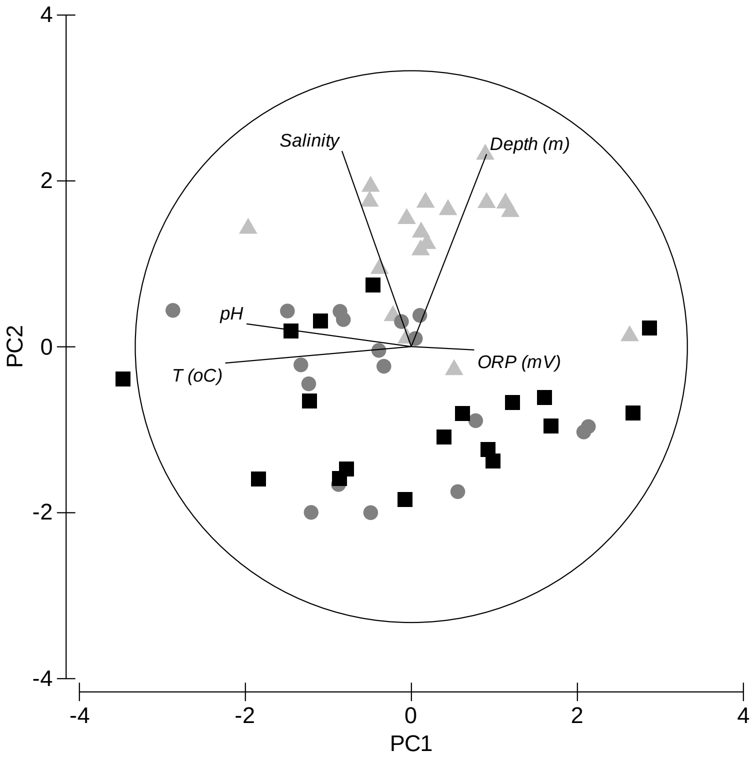


Fig. 3.2 PCA showing the differences of the environmental variables among sampling stations at the lower Minho Estuary, NW Iberian Peninsula. ▲= station 1; ● = station 2 and ■ = station 3. T(oC) = water temperature; ORP(mV) = potential redox.

Moreover, the two-way SIMPER results revealed that 60.7% of the dissimilarity between stations 1 and 2 was explained by depth (41.7%) and ORP values (19.3%); while the dissimilarity between stations 1 and 3 was mostly explained by salinity (31.2%) and depth (22.1%); on the other hand, dissimilarity between stations 2 and 3 was mainly explained by differences in ORP (30.5%) and salinity (25.7%).

The two-way SIMPER also revealed that temperature was the factor which most contributed to differences between the winter and other seasons (with dissimilarities ranging from 32.4 to 37.6%), while the dissimilarities between spring and summer was mostly explained by pH (40.4%) and ORP values (21.9%); similarly, spring and autumn also differed mostly due to ORP (22.7%) and pH values (22.5%); as well as summer and autumn, which also presented their dissimilarities explained mostly by pH (42.3%) and ORP values (19.4%).

3.3.2. Shore crab data

A total of 3358 crabs were collected (station 2 = 52.9%, station 1 = 42.6% and station 3 = 4.5%). Of these, 31.1% were collected during autumn, 28.0% in summer, 24.0% in spring and 16.9% during the winter. Males were more abundant (46.0% of the total), compared with females (38.1%) and juveniles (15.9%). The green morphotype was far more frequent than the red morphotype, corresponding to 90.5% and 9.5% of the individuals, respectively.

3.3.2.1. Density patterns

The mean density of *C. maenas* in Minho estuary, considering the entire sampling period and the 3 stations, was 26.1 ± 2.8 ind.100m^{-2} (mean ± SE), with the highest density being recorded in October/09 (51.3 ± 17.7 ind.100m^{-2}) and the lowest in February/09 (4.0 ± 3.2 ind.100m^{-2}) (Fig. 3.3).

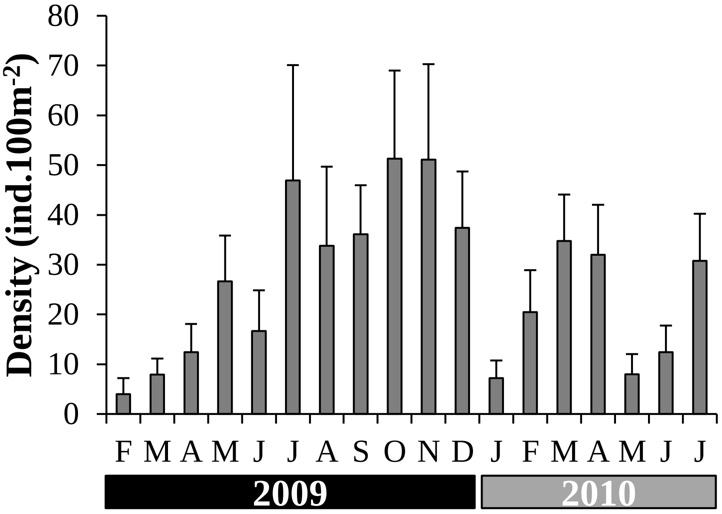


Fig. 3.3 Mean density (± SE) of the Shore crab *Carcinus maenas* during 18-months study period in the lower Minho estuary, NW Iberian Peninsula.

The Two-way PERMANOVA for stations and seasons of the *C. maenas* density was highly significant for both main effects (stations: pseudo-*F* = 46.23, p < 0.001; seasons: pseudo-*F* = 13.01, p < 0.001) and for the *stations x seasons* interaction (pseudo-*F* = 7.92, p < 0.001). Therefore, each sampling station presented different spatiotemporal distributional patterns. In station 1, the density peak was recorded during autumn (67.6 ± 17.0 ind.100m^{-2}), while in station 2 the shore crab was denser during summer (86.8 ± 13.0 ind.100m^{-2})

and autumn (67.7 ± 14.3 ind.100m^{-2}). In station 3, *C. maenas* was absent during spring, and barely found during winter, with the highest density in this sampling station being recorded during summer (11.3 ± 4.6 ind.100m^{-2}) (Fig. 3.4).

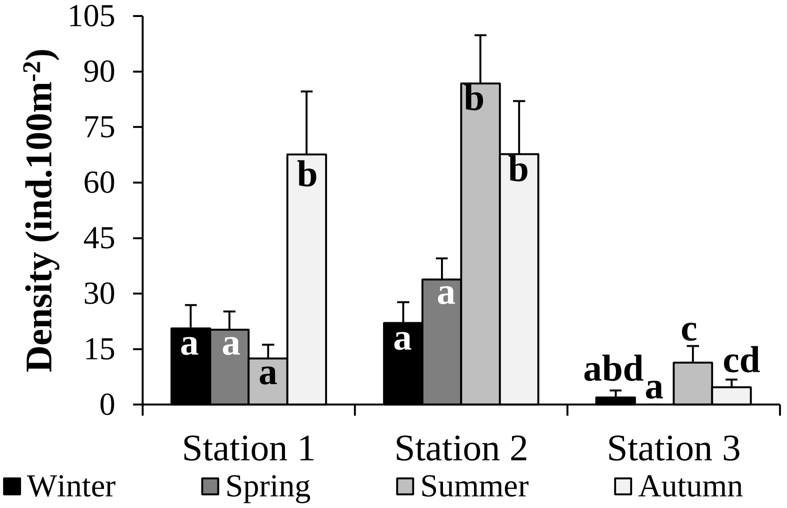


Fig. 3.4 Comparisons on the densities (mean ± SE) of the shore crab *Carcinus maenas* between seasons in each sampling station of the lower Minho estuary, NW Iberian Peninsula. Different letters (a, b, c or d) indicate significant differences between them (Two-way PERMANOVA pairwise tests: $p<0.05$).

3.3.2.2. Sexes

The two-way PERMANOVA indicated that the densities of males, females and juveniles were significantly different for both main effects (stations: pseudo-$F = 31.16$, $p < 0.001$; seasons: pseudo-$F = 6.59$, $p < 0.001$) and for the *stations x seasons* interaction (pseudo-$F = 4.86$, $p < 0.001$).

Differences were also detected when analyzing the density of males, females and juveniles independently. Overall, males were denser in station 2 in all seasons; with the density of males being higher during autumn in station 1, while in stations 2 and 3, male density was higher during summer. Females followed a different pattern, with higher densities being found in station 1 during winter and autumn, while during the spring and summer, density was higher in station 2. Juveniles, on the other hand, were denser in station 1 in all seasons (Table 3.2).

Table 3.2 Comparisons on the density (mean ± SE) of males, females and juveniles of the shore crab *Carcinus maenas* among three sampling stations and four seasons in the lower Minho estuary, NW Iberian Peninsula. ^{ns} = non-significant; * = $p < 0.001$. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test: $p < 0.05$).

	Seasons				Two-way PERMANOVA		
	Winter	Spring	Summer	Autumn	Stations	Seasons	Interaction
Male							
Station 1	$8.3\pm2.7^{ac(A)}$	$3.2\pm0.8^{ab(A)}$	$1.5\pm0.5^{b(A)}$	$17.0\pm3.9^{c(A)}$	Pseudo-*F* = 87.16*	Pseudo-*F* = 12.75*	Pseudo-*F* = 8.60*
Station 2	$14.5\pm4.0^{a(A)}$	$21.4\pm3.5^{a(B)}$	$50.6\pm7.8^{b(B)}$	$44.3\pm7.0^{b(B)}$			
Station 3	$1.2\pm1.2^{ab(B)}$	$0.0\pm0.0^{a(C)}$	$5.9\pm2.5^{b(A)}$	$3.3\pm1.7^{b(AC)}$			
Female							
Station 1	$6.7\pm2.0^{a(A)}$	$7.6\pm1.5^{a(A)}$	$5.8\pm1.6^{a(A)}$	$38.7\pm11.7^{b(A)}$	Pseudo-*F* = 22.44*	Pseudo-*F* = 10.67*	Pseudo-*F* = 8.48*
Station 2	$6.0\pm1.9^{a(A)}$	$10.8\pm2.3^{a(A)}$	$33.3\pm5.5^{b(B)}$	$17.6\pm8.7^{ab(A)}$			
Station 3	$0.3\pm0.3^{ad(B)}$	$0.0\pm0.0^{a(B)}$	$4.1\pm2.1^{bc(A)}$	$1.1\pm0.7^{cd(B)}$			
Juvenile							
Station 1	$5.6\pm2.5^{(A)}$	$9.5\pm4.5^{(A)}$	5.2±2.2	$11.9\pm4.0^{(A)}$	Pseudo-*F* = 11.40*	Pseudo-*F* = 1.05^{ns}	Pseudo-*F* = 0.73^{ns}
Station 2	$1.3\pm0.6^{a(AB)}$	$1.4\pm0.6^{a(A)}$	2.7 ± 1.2^{ab}	$5.4\pm1.7^{b(A)}$			
Station 3	$0.4\pm0.4^{ab(B)}$	$0.0\pm0.0^{a(B)}$	1.1 ± 0.4^{b}	$0.1\pm0.1^{a(B)}$			

3.3.2.3. Color morphotypes

The two-way PERMANOVA indicated that the density of the two color morphotypes, was significantly different for both main effects (stations: pseudo-*F* = 42.87, $p < 0.001$; seasons: pseudo-*F* = 7.51, $p < 0.001$) and for the *stations x seasons* interaction (pseudo-*F* = 5.21, $p < 0.001$).

Differences were also detected when analyzing the density of males and females of the green and red morphotypes. The two-way PERMANOVA indicated that the density of males of the green morphotype was significantly different for both main effects (stations: pseudo-*F* = 94.40, $p < 0.001$; seasons: pseudo-*F* = 12.61, $p < 0.001$) and for the *stations x seasons* interaction (pseudo-*F* = 8.65, $p < 0.001$). A similar result was obtained when comparing the density of red males (stations: pseudo-*F* = 15.66, $p < 0.001$; seasons: pseudo-*F* = 6.71, $p = 0.001$; *station x seasons*: pseudo-*F* = 7.15, $p < 0.001$) and green females (stations: pseudo-*F* = 12.80, $p < 0.001$; seasons: pseudo-*F* =

6.74, p < 0.001; *station x seasons*: pseudo-F = 5.31, p < 0.001), but red females were only different among sampling stations (stations: pseudo-*F* = 27.23, p < 0.001; seasons: pseudo-*F* = 0.95, p = 0.42; *station x seasons*: pseudo-*F* = 0.99, p = 0.44) (Table 3.3).

Table 3.3 Comparisons on the density (mean ± SE) of males and females of the green and red morphotype of the shore crab *Carcinus maenas* among sampling stations and seasons in lower Minho estuary, NW Iberian Peninsula. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test: p < 0.05).

	Green			Red		
	Station 1	Station 2	Station 3	Station 1	Station 2	Station 3
Male						
Winter	6.7±2.2$^{abd(A)}$	14.3±4.1$^{a(A)}$	1.2±1.2$^{ab(B)}$	1.5±0.6$^{a(A)}$	0.4±0.2$^{a(AB)}$	0.0±0.0$^{(B)}$
Spring	2.8±0.7$^{bc(A)}$	20.4±3.1$^{a(B)}$	0.0±0.0$^{a(C)}$	0.3±0.2$^{a(AB)}$	1.2±0.5$^{ab(A)}$	0.0±0.0$^{(B)}$
Summer	1.3±0.5$^{c(A)}$	48.9±7.6$^{b(B)}$	6.2±2.6$^{b(A)}$	0.2±0.2$^{a(A)}$	2.1±0.7$^{b(B)}$	0.0±0.0$^{(A)}$
Autumn	12.3±2.7$^{d(A)}$	43.3±6.6$^{b(B)}$	3.3±1.7$^{b(C)}$	4.7±1.3$^{b(A)}$	1.6±0.5$^{b(B)}$	0.0±0.0$^{(C)}$
Female						
Winter	4.4±1.4$^{a(A)}$	6.1±1.9$^{a(A)}$	0.3±0.3$^{ac(B)}$	2.3±0.7$^{(A)}$	0.2±0.2$^{(B)}$	0.0±0.0$^{(B)}$
Spring	3.6±0.9$^{a(A)}$	10.6±2.2$^{a(B)}$	0.0±0.0$^{a(C)}$	4.0±0.8$^{(A)}$	0.5±0.3$^{(B)}$	0.0±0.0$^{(B)}$
Summer	2.5±0.6$^{a(A)}$	33.3±5.4$^{b(B)}$	4.1±2.1$^{b(A)}$	3.3±1.4$^{(A)}$	0.6±0.3$^{(AB)}$	0.0±0.0$^{(B)}$
Autumn	33.7±10.5$^{b(A)}$	17.7±8.7$^{ab(A)}$	1.1±0.7$^{bc(B)}$	4.9±1.4$^{(A)}$	0.0±0.0$^{(B)}$	0.0±0.0$^{(B)}$

3.3.2.4. Size, recruitment patterns and production estimates

The range of CW varied between 1.3 and 82.0 mm, with an average value of 32.3 ± 0.3 mm (mean ± SE).

The mean size of shore crabs was significantly different among sampling stations (stations: pseudo-*F* = 27.20, p < 0.001) and seasons (seasons: pseudo-*F* = 54.00, p < 0.001) and for the *stations x seasons* interaction (pseudo-*F* = 164.16, p < 0.001).

Differences were also detected when analyzing the CW of males and females independently. Both males and females were larger in stations closer to the river mouth, except during the winter. Larger males were found during autumn in stations 1 and 2, whilst during winter larger males were found in station 3.

Females showed a different pattern, with larger individuals being caught during summer, autumn and winter in stations 1, 2 and 3 respectively (Table 3.4).

Table 3.4 Comparisons of the size (mean ± SE) of males and females of the shore crab *Carcinus maenas* among sampling stations and seasons in lower Minho estuary, NW Iberian Peninsula. ^{ns} = non-significant; * = $p < 0.001$. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test: $p<0.05$).

	Seasons				Two-way PERMANOVA		
	Winter	Spring	Summer	Autumn	Stations	Seasons	Interaction
Male							
Station 1	$38.1\pm1.4^{a(A)}$	$42.6\pm2.6^{a(A)}$	$42.2\pm5.1^{ab(A)}$	$48.0\pm0.8^{b(A)}$	Pseudo-*F* = 102.27*	Pseudo-*F* = 33.96*	Pseudo-*F* = 53.89*
Station 2	$30.7\pm0.7^{a(B)}$	$32.5\pm0.5^{a(B)}$	$37.7\pm0.3^{b(B)}$	$38.5\pm0.5^{b(B)}$			
Station 3	$43.3\pm3.9^{a(A)}$	$0.0\pm0.0^{b(C)}$	$18.1\pm1.3^{c(C)}$	$29.2\pm2.2^{d(C)}$			
Female							
Station 1	$40.5\pm1.1^{a(A)}$	$41.0\pm0.8^{a(A)}$	$46.8\pm0.6^{b(A)}$	$44.6\pm0.4^{b(A)}$	Pseudo-*F* = 219.84*	Pseudo-*F* = 53.29*	Pseudo-*F* = 104.33^{ns}
Station 2	$26.4\pm0.8^{a(B)}$	$29.1\pm0.5^{b(B)}$	$34.8\pm0.3^{c(B)}$	$38.0\pm0.6^{d(B)}$			
Station 3	$41.4\pm5.3^{a(A)}$	$0.0\pm0.0^{b(C)}$	$18.9\pm1.3^{c(C)}$	$24.3\pm2.0^{c(C)}$			

The two-way PERMANOVA indicated that the density of the seven size classes of the shore crab was significantly different for both main effects (stations: pseudo-$F = 24.23$, $p < 0.001$; seasons: pseudo-$F = 7.39$, $p < 0.001$) and for the *stations x seasons* interaction (pseudo-$F = 6.13$, $p < 0.001$). In station 1, the size classes A and E accounted for 60.3% of all individuals sampled, whilst in station 2 size classes C and D summed 81% of C. maenas individuals, whether in station 3 crabs belonging to the size classes A and B accounted for 67.3% of the total (Fig. 3.5).

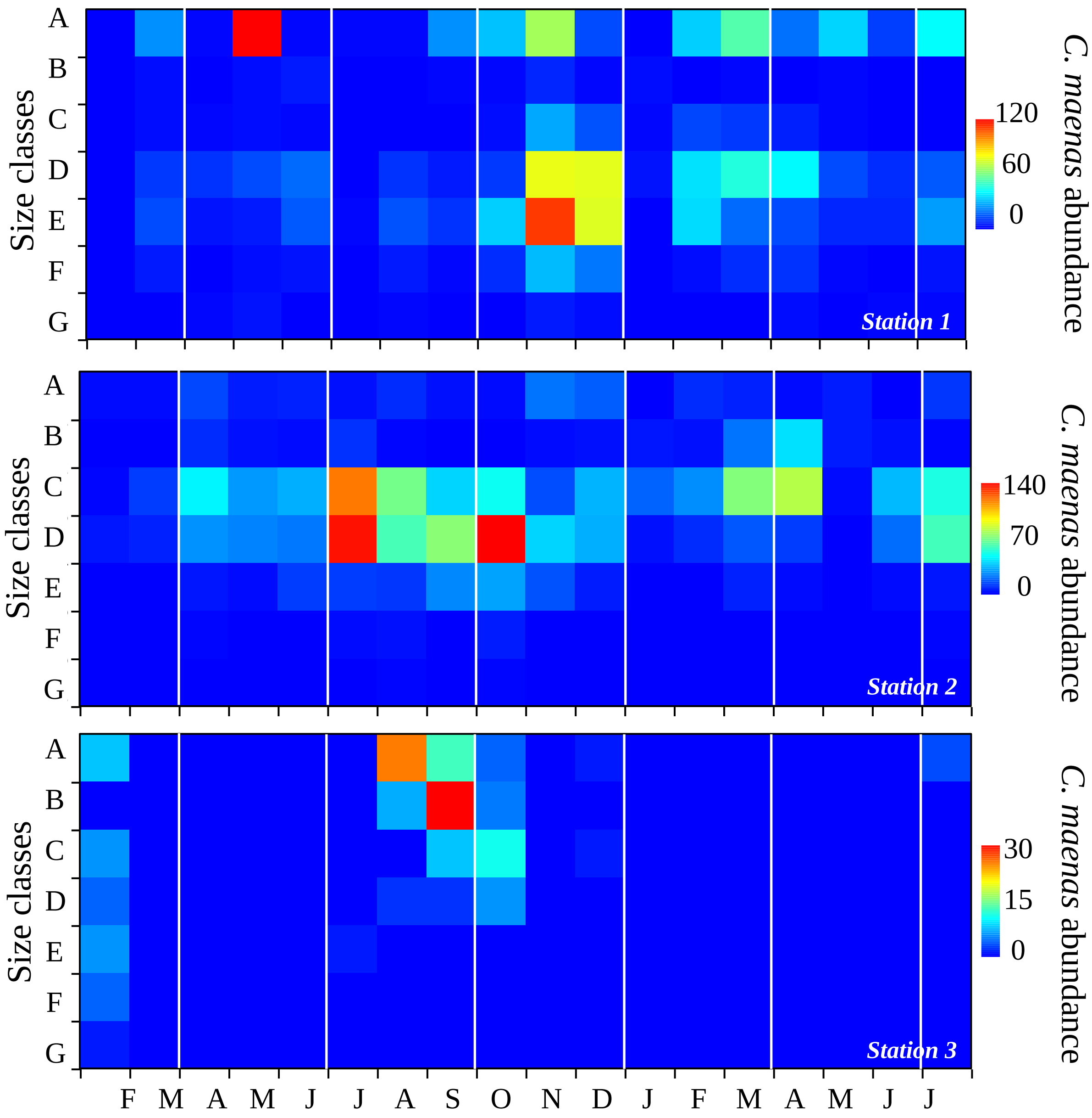
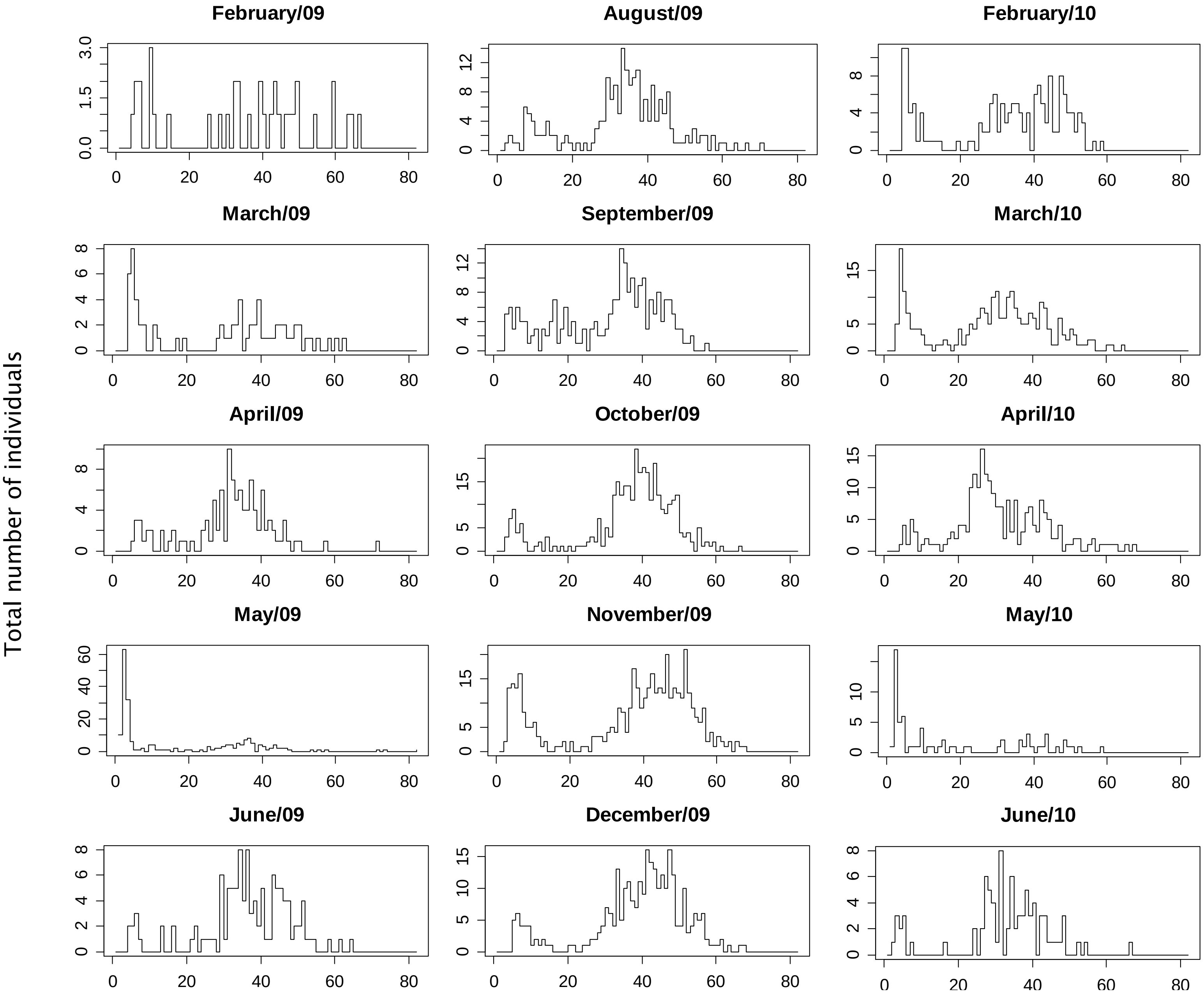


Fig. 3.5 Abundance of different size classes of the shore crab *Carcinus maenas* throughout the 18 months sampled in Minho estuary, NW Iberian Peninsula. Vertical white bars separate different seasons. Size classes: A (< 15 mm), B (16-25 mm), C (26-35 mm), D (36-45 mm); E (46-55 mm), F (56-65 mm) and G (> 65 mm).

The two-way SIMPER results revealed that the spatiotemporal dissimilarities were mainly related to the abundance of size classes A, C and D (Supplementary material 3.2).

The size frequency distribution of the shore crab revealed that the population from Minho estuary has a continuous recruitment, since individuals smaller than 15 mm of CW were found during the entire sampling period (Fig. 3.6).

However, it was possible to detect two reproduction peaks (twice more individuals >15mm of CW than on the other months) during the 18 months of sampling, namely in May/09 and November/09. Also, it is worth mentioning that small crabs (>15mm of CW) were almost absent in early summer of 2009 (June and July) and 2010 (June) and were hardly found in winter of 2009 (February/09) and 2010 (January/10). Additionally, sexually mature individuals (> 30 mm of CW) were found in high densities during almost the entire sampling period, except in January/10, where only 30% of the individuals were larger than 30 mm of CW.



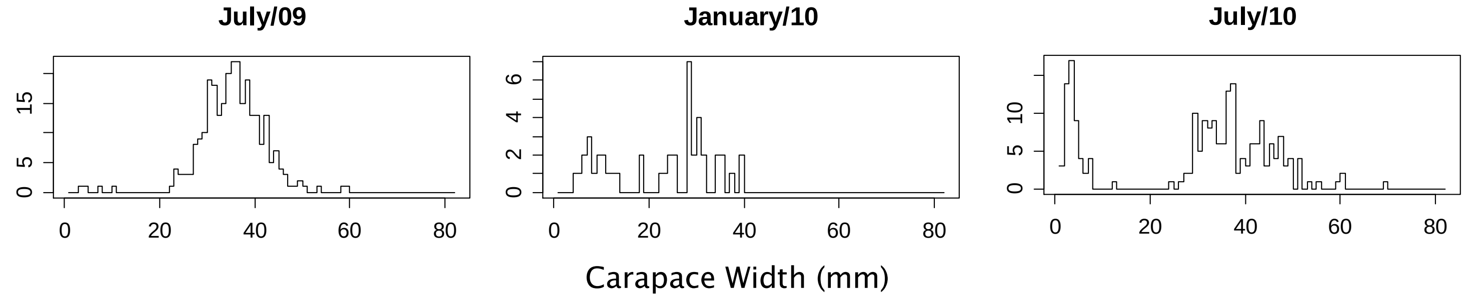


Fig. 3.6 Size frequency distribution of the shore crab *Carcinus maenas* in the lower Minho estuary, NW Iberian Peninsula.

Only four berried females were caught during the entire sampling campaign, with two individuals in May/09, one in April/10 and other in May/10. The average carapace width of berried females was 52.2 ± 4.9 mm, with the largest one being 58.5 mm and the smallest one 47.0 mm. Additionally, the four berried females presented the red color morphotype, and were all recorded in station 1.

The overall secondary production of the subtidal population of *C. maenas* in the lower Minho estuary was estimated at 266.4 $gWWm^{-2}$; with the annual production being estimated at 189.5 $gWWm^{-2}year^{-1}$.

3.3.2.5. Crab-habitat associations

Overall, different groups of *C. maenas* (sexes and color morphotypes) associated differentially with environmental variables. Males of the green morphotype were associated mostly with shallow upstream zones of the lower estuary, while red males associated mainly with high salinities. Females of the green morphotype showed weak association with environmental variables, while red females associated preferably with deeper waters near the river mouth; a similar pattern was recorded for juveniles (Fig. 3.7a). The Monte-Carlo test was significant (test of significance of all canonical axes: trace = 0.29; F-ratio = 4.97; $p < 0.01$) and cumulatively, axes 1 and 2 accounted for 95.9% of the total variance, with correlations between the density of *C. maenas* groups (sexes and color morphotypes) and environmental variables of 0.830 (axis 1) and 0.643 (axis 2).

Significant associations were also found between the density of different size classes of the shore crab and environmental variables. Size classes B and C associated mostly with shallow upstream zones of the lower estuary, while the size class D showed weak associations with the environmental variables. On the other hand, size classes A and G associated with deeper waters near the river mouth, a similar pattern was recorded for the size classes E and F, but the later groups associated also with high salinities (Fig. 3.7b). The Monte-Carlo test was significant (test of significance of all canonical axes: trace = 0.44; F-ratio = 5.08; $p < 0.01$) and cumulatively, axes 1 and 2 accounted for 83.5% of the total variance, with correlations between *C. maenas* size classes densities end environmental variables of 0.854 (axis 1) and 0.603 (axis 2).

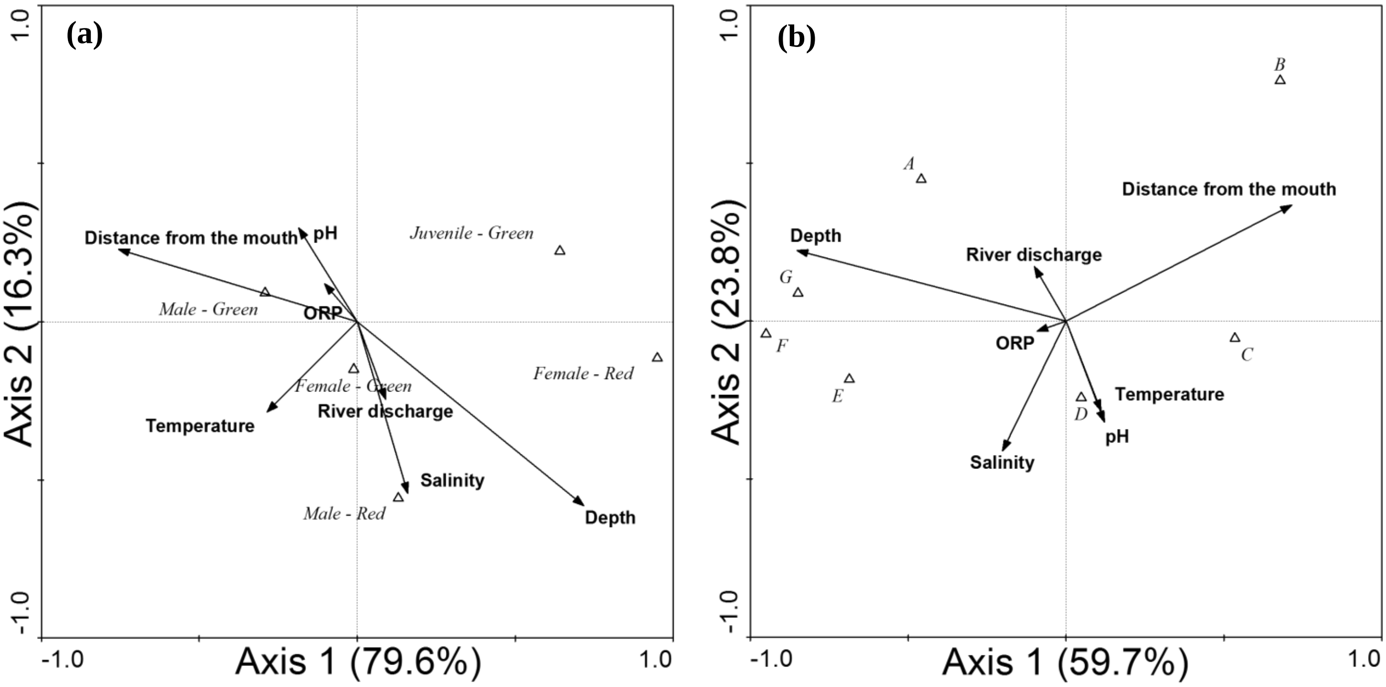


Fig. 3.7 Canonical plotting of environmental characteristics (arrows) and different groups of individuals – sexes and color morphotypes (a) and size classes (b) – of the shore crab *Carcinus maenas* in the lower Minho estuary, NW Iberian Peninsula. ORP = Redox potential. Size classes: A (< 15 mm), B (16-25 mm), C (26-35 mm), D (36-45 mm); E (46-55 mm), F (56-65 mm) and G (> 66 mm).

3.4. Discussion

Our results highlighted that shore crabs of different sizes, sexes and color morphotypes are unevenly distributed, both spatially and temporally in the Minho estuary, suggesting that *C. maenas* population is spatially and temporally structured within this estuary. This may be related to the observed variations in salinity, temperature and depth among sampling stations, which are considered as important predictors of the shore crab distribution (Baeta et al. 2005; Bessa et al. 2010). The different combination of these factors at each sampling station may have significantly contributed to the observed differences in the population structure of *C. maenas*.

The high density of *C. maenas* adults found in station 2 (saltmarsh), suggests that this is the preferred habitat for the species in Minho estuary. This preference seems to be related with the high abundance of seagrasses (e.g. *Spartina* sp.), once shore crabs usually associates with seagrasses (Cohen et al. 1995). Also this site is located inside a narrow channel, with high water flow. The channel becomes almost dry during ebb tides and quickly reaches approximately 2 meters depth during high tide (personal observation). Since shore crab densities are higher in high flow areas (Leonard et al. 1998), this water flow regime may also contribute to the high densities found in station 2. Moreover, common preys of the shore crabs (Baeta et al. 2006) are also found in high densities in this area, namely the brown shrimp *Crangon crangon* and the common goby *Pomatoschistus microps* (Campos 2009; Souza et al. accepted), which may further influence the density of the *C. maenas* in this sampling station. In fact, when caught, most of the shore crabs in this station were feeding on *P. microps*, while this behavior was less frequently observed in other sampling stations (personal observation).

In station 1, the size range of shore crabs was wide, with very small (< 15 mm of CW) and large crabs (> 45 mm of CW) being especially abundant in this area. Small-bodied crabs are often associated with areas containing high algae biomass and turbid waters (Baeta et al. 2005), which were conditions observed in this station. Additionally, due to high river discharge (Ferreira et al. 2003), this station continuously houses a high amount of debris and drifting algae (personal observation), contributing to increase the habitat structural complexity, which may benefit small-bodied individuals due the protection provided from these habitats (Thiel and Dernedde 1994). Moreover, the reproduction of shore crabs usually occurs in coastal zones, and since larger individuals migrate to downstream areas to reproduce, it is more likely that younger individuals are more often found in estuarine zones closer to the sea (Baeta et al. 2005).

Conversely, in station 3, the density of shore crabs was fairly low, with just a few crabs being recorded, especially during summer and autumn. This is probably related to the low salinity values recorded in this station. Constantly, during the months when salinity was remarkable low (0.4 ± 0.7) shore crabs were not recorded at this station. Previous studies indicate that shore crabs prefer mesohaline to polyhaline salinities (10 to 30) (Broekhuysen 1936; Dawirs 1985), and despite of tolerating salinities as low as 4 (Crothers 1967), shore crabs cannot persist in areas with lower values of salinity. Such extremely low salinity values were recorded in station 3 during 13 of the 18 months sampled, which seems to explain the absence of *C. maenas* in this station during most of the sampling period.

The density of shore crabs recorded in Minho estuary was higher than in other estuarine systems. In fact, in other Portuguese estuaries, the density of *C. maenas* was much lower than in Minho estuary (3.7 times lower in Tagus and 2.2 times lower in Mondego) (Table 3.5).

Table 3.5 Mean density of *Carcinus maenas* in different locations in Atlantic estuaries.

Location	Mean density (100 ind.m^{-2})	Period of sampling	Habitat type	Reference
Minho estuary, Portugal	26.1	Diurnal	Whole estuary	This study
Mondego estuary, Portugal	11.6	Nocturnal	Whole estuary	Baeta et al. 2005
Canche estuary, France	10.9	Diurnal	Intertidal sand beach	Selleslagh and Amara 2008
Tagus estuary, Portugal	7.0	Diurnal and Nocturnal	Subtidal (upper estuary)	França et al. 2008

Despite of Minho presented higher density of individuals than Mondego; the secondary production of the shore crab in the former estuary is 2.8 to 8 times lower than the later estuary (Bessa et al. 2010). This difference is probably related to different sizes of shore crabs between sites, with Minho estuary being dominated by small-sized crabs densely distributed, while Mondego sustains a subtidal population with a higher contribution of larger crabs sparsely distributed within the estuary (Baeta et al. 2005; Bessa et al. 2010).

Other studies also reported extremely high densities of estuarine species in Minho, for instance, the Asian clam *Corbicula fluminea* (Sousa et al. 2008d), the flatfish *Platichthys flesus* (Cabral et al. 2007; Freitas et al. 2009; Souza et al. 2013) , the common goby *Pomatoschistus microps* (Souza et al. accepted) and the sand goby *P. minutus* (Souza et al. unpublished data); indicating that high density of individuals may be the pattern for various benthic species of both epifauna and infauna in Minho estuary. Compared to other systems, Minho estuary is considered as low-environmentally impacted (see Sousa et al. 2008a), which may be related to the high abundances and biodiversity values found in found in this estuary. However, this system is facing several kinds of environmental threats, which have severely altered the ecosystem structure in the recent years (Sousa et al. 2011). The establishment and increase in populations of invasive species may have significantly changed the ecosystem pattern of functioning, and may even cause the local extinction of species (Sousa et al. 2011). Additionally, the construction of dams along the river basin, the fishing pressure and climate change might also influence the ecosystem stability. Under environmental stresses, only some species can persist, often reaching high densities. According to recent studies, the aquatic fauna in Minho has been suffering important declines in populations of mollusks (e.g. *Anodonta cygnea*, *A. anatina*, *Margaritifera margaritifera*, *Psilunio littoralis* and *Unio pictorum*) and fishes (e.g. *Acipenser sturio* and *Lampetra fluviatilis*) (Antunes and Weber 1996; Sousa et al. 2008a). The present results related to high densities of *C. maenas* in Minho estuary may be somehow related to environmental pressures. In fact, the decline in fish populations, especially invertebrate feeders and piscivores species, may favor the population of its potential preys, such as the shore crab. However, the lack of historical data regarding the shore crab population in Minho does not allow us to confirm or even refute this hypothesis.

The shore crab is sexually mature when larger than 30 mm of CW (d'Udekem d'Acoz 1993; Behrens Yamada et al. 2005), and individuals with this sizes were present throughout the entire sampling period; with smaller crabs (size class A) being found during the 18 sampled months, indicating that recruitment in Minho is continuous. The presence of size A individuals was especially high in station 1, reaching up to 83% of individuals in May/09 and being higher than 40% in 9 of the 18 sampled months. As mentioned before, the proximity to the sea and the high amount of debris and seaweeds found in station 1 may favor the density of smaller crabs. However, the percentage of smaller crabs in Minho population was higher during spring, indicating that this season may be the preferred for the recruitment of the species.

The scarce occurrence of berried females inside Minho estuary (only 4 individuals) may indicate that this system is not a spawning ground for *C. maenas*. Additionally, berried females can avoid remaining within the estuary due to the low salinity values found in Minho, since the shore crabs' eggs cannot develop normally in salinities below 26 (Crothers 1967). On the other hand, the small number of ovigerous females might also be related with a selective advantage for these females to remain hidden and seek deeper more saline waters, thus being harder to be caught (Baeta et al. 2005). When comparing our results with other Southern European estuaries, we have noticed that the Minho population differs from adjacent estuarine systems, wherein ovigerous females were found in higher densities and during the entire year (Baeta et al. 2005; Queiroga 1993). These differences might be related to the lower salinity profile of Minho estuary compared to other estuarine systems in nearby areas (Ferreira et al. 2003).

In Minho estuary, shore crab males' and females' were unevenly distributed through sampling stations, with a remarkable higher density of males over females in stations 2 and 3. Similarly with the results of Baeta et al. (2005) , the number of females was proportionally higher in stations closer to the sea, especially during spring, indicating that females probably migrate to downstream areas, when they are near to reach maturity or ready to mate (Baeta et al. 2005). The dominance of males was also recorded in Mondego estuary (Baeta et al. 2005), but in Lima estuary, a system located near Minho (ca. 20 km southward), the density of females is much higher than males' (Aurélie Rodrigues, personal communication). The Lima estuary usually presents higher values of salinities (Ferreira et al. 2003), and since the density of females is higher in saltier waters (Baeta et al. 2005), this difference in sex-ratio might also be associated with differences in salinity levels between estuaries.

Regarding color morphotypes, our results are consistent with the ecophysiological differences reported in the literature between the two color morphotypes. The green morphotype is known to withstand a greater environmental stress than red crabs (McGaw and Naylor 1992; Reid et al. 1997). This characteristic is essential in the relative distribution of the two color morphotypes, with green crabs being found more often in estuarine and intertidal habitats than red individuals, which are most frequently found in open shore and subtidal habitats. Moreover, at estuarine areas, red females are more abundant than red males (Crothers 1967; Reid et al. 1997).

Our results highlight that shore crab distributes unevenly in the estuary throughout the year; being the differences probably related to the complex life cycle of the species, in which they constantly migrate to different areas in coastal and estuarine systems, with each ecosystem being important to its autecology. Due to the ecological relevance of this species to estuarine ecosystems, it is important to preserve and/or protect the systems that they inhabit, especially those that are used as nursery grounds, such as estuaries and salt marshes.

3.5. Acknowledgements

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CHAPTER 4

STRUCTURE, GROWTH AND PRODUCTION OF A REMARKABLY ABUNDANT POPULATION OF THE COMMON GOBY *POMATOSCHISTUS MICROPS* (ACTINOPTERYGII: GOBIIDAE)

Structure, growth and production of a remarkably abundant population of the common goby *Pomatoschistus microps* (Actinopterygii: Gobiidae)

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Souza AT, Dias E, Campos J, Marques JC, Martins I (accepted) Structure, growth and production of a remarkably abundant population of the common goby *Pomatoschistus microps* (Actinopterygii: Gobiidae). Environmental Biology of Fishes.

Abstract

The common goby *Pomatoschistus microps* is a relevant species from estuarine food webs, playing important roles as predator of polychaetes and crustaceans and as prey for larger fishes and crustaceans. The Minho estuary (NW Portugal) is a relatively well preserved and productive system. To assess the population structure and production of *P. microps* in this estuary, monthly samples were undertaken in three different areas along an estuarine gradient in the lower estuary. The density of *P. microps* varied considerably among seasons and sampling stations; with higher densities occurring in summer and autumn. The lowest abundances were found closer to the sea. In general, the density of females was higher than the density of males in all sampling stations, while juveniles were more abundant within a salt marsh area. Compared with other European estuaries, our data showed a remarkable higher density and production values of *P. microps*. This may be related to the high freshwater input and the low salinities found in this estuary. In addition, we hypothesize that the lower density of the sympatric species *P. minutus* and the high availability of bivalve shells observed in Minho estuary may have also contributed to the present results, once *P. minutus* and *P. microps* often display a diet overlap and the bivalve shells are crucial for the common goby reproduction.

Keywords: *Corbicula fluminea* / life cycle / Minho / *Pomatoschistus minutus* / secondary production

4.1. Introduction

Estuaries contain diverse aquatic habitat types, including seagrass, kelp and shellfish beds, muddy and sandy environments, among others (Nelson and Monaco 2000). These are highly biologically productive ecosystems that provide important forage, spawning, refuge, and nursery habitat for several fish species at various stages in their development (Chambers 1992; Nelson and Monaco 2000; Able 2005).

The Minho estuary (NW Iberian Peninsula) is a relatively well-preserved system where several aquatic species present higher values of density and/or secondary production compared with other systems (see Sousa et al. 2008d; Freitas et al. 2009; Dolbeth et al. 2010; Freitas 2011; Souza et al. 2013). In this estuary, five species of Gobiidae are present, namely, the common goby *Pomatoschistus microps*, the sand goby *P. minutus*, the painted goby *P. pictus*, the transparent goby *Aphia minuta* and the black goby *Gobius niger*, with *P. microps* being largely more abundant than other Gobiidae species (Antunes pers. comm.; Antunes and Rodrigues 2004; Costa-Dias et al. 2010).

The common goby is a small-bodied demersal euryhaline species that usually occurs in high densities, from Norway to Mauritania, including the Canary Islands, western Mediterranean and Baltic Sea, spanning ca. 44º of latitudinal range (Froese and Pauly 2010). This species is of great importance in marine and estuarine ecosystems as it links low and high levels of the food-web, by acting as a mesopredator, thus connecting microbenthos to larger predators such as birds and fishes (Doornbos 1984; Moreira et al. 1992; Cabral 2000).

The ecology of *P. microps* has been the focus of several studies, which include population structure (Arruda et al. 1993; Pampoulie 2001; Leitão et al. 2006; Dolbeth et al. 2007b), breeding behavior (Magnhagen 1998; Pampoulie et al. 2000; Pampoulie 2001) and diet (Mehner 1992; Leitão et al. 2006; Jackson and Rundle 2008). However, few studies have focused on *P. microps* populations from the Atlantic coast of Southern Europe (but see Arruda et al. 1993; Leitão et al. 2006; Dolbeth et al. 2007b). Besides, in this area, nearly 55% of published studies regarding the species focused on ecotoxicology (e.g. Monteiro et al. 2007; Vieira et al. 2008; 2009; Guimarães et al. 2012; Oliveira et al. 2012).

The common goby is often reported as one of the most abundant fish in estuaries (Martinho et al. 2007b; Dolbeth et al. 2010), and it is frequently found in areas where the sympatic species *P. minutus* co-occurs. Both species are often found in equal or slightly different densities (e.g. Arruda et al. 1993; Leitão et al. 2006; Dolbeth et al. 2007b; Martinho et al. 2007b). However, in Minho estuary the common goby seems to be much more abundant than the sand goby (pers. observ.; Costa-Dias et al. 2010).

In this context, the present study aims at assessing the population structure, density, production and growth of *P. microps* in Minho estuary. By focusing on the distributional patterns of *P. microps* within a system where the species seems to be especially abundant, we expect to increase the understanding of the ecology of this core species in several estuarine systems across Europe.

4.2. Materials and Methods

4.2.1. Study area and sampling procedures

This study was conducted in the Minho Estuary (NW of the Iberian Peninsula – 41º53'N 8º50'0), which ranges up to 40 km (considering the upstream limit of spring tides), covering a total area of 23 km^2. The Minho estuary is a very shallow system (Moreno et al. 2005), with a mean depth of 2.6 m (Freitas et al. 2009); it is characterized as a mesotidal and partially mixed system, although it tends towards a salt wedge estuary during periods of high river flow (Sousa et al. 2005).

This study was carried out during 18 months, from February/09 to July/10, with months being grouped into four seasons, namely winter (January, February and March), spring (April, May and June), summer (July, August and September) and autumn (October, November and December). Sampling was carried out in 3 nearby stations within the first 8 km of the Minho estuary, considering a gradient of distance to the river mouth (Fig. 4.1).

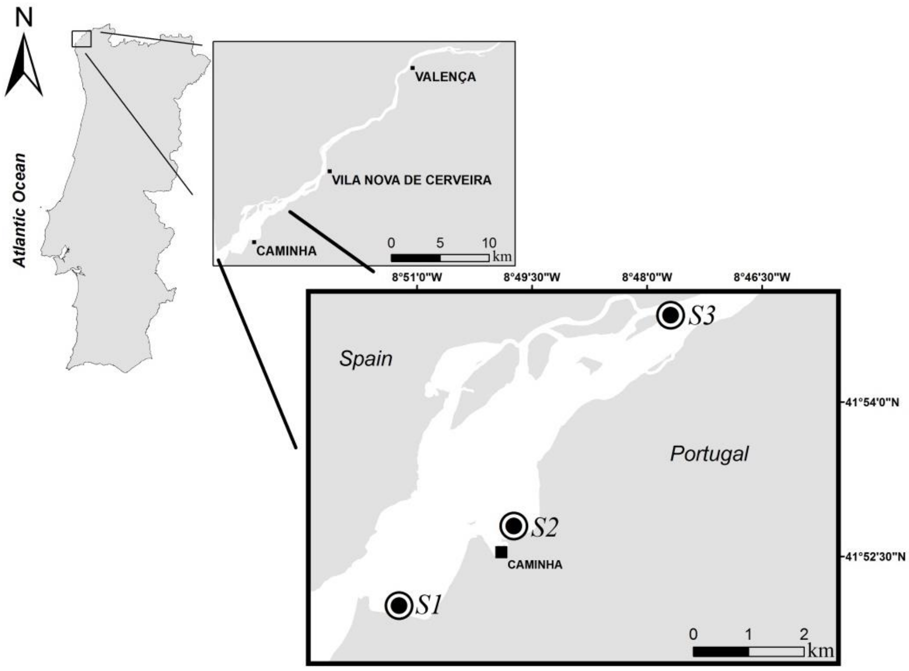


Fig. 4.1 Sampling stations on the lower Minho Estuary (NW Iberian Peninsula).

The 3 stations (S1, S2 and S3) were chosen in order to assess possible dissimilarities on the population dynamics related to differences in habitat type and environmental parameters. S1 was closer to the river mouth (*ca.* 1.5 km), and characterized by the presence of soft bottoms, often densely covered by debris such as drifting seaweeds, dead leaves and empty mollusks' shells (Souza et al. 2011; 2013). S2 was located inside a salt marsh (*ca.* 3.5 km upwards from the river Minho mouth), which is a relatively small system characterized by the presence of narrow channels, typically bordered by the seagrass *Spartina maritima*. The channel's soft bottoms are often sparsely covered by debris and empty peppery furrow shells *Scrobicularia plana* (Souza et al. 2011; 2013). S3 was located *ca.* 5 km upstream from S2, and characterized by high densities of the Asian clam *Corbicula fluminea*, with the soft bottoms often sparsely covered by debris and submerged vegetation (Sousa et al. 2008b,c; Souza et al. 2013). In each site, with monthly periodicity, three replicates were collected using a 1 m beam trawl (5 mm mesh size) towed at constant speed (2 km.h^{-1}) for two to three minutes, during daylight at the high tide of spring tides. Previous studies in Minho estuary indicated that the sampling area covered by a three minutes tow, at constant speed, was equivalent to 100 ± 4 m^2 on average (Freitas et al. 2009). Density data was standardized to the same scale (ind.100 m^2) prior to the statistical analyses. Additionally, at each site, water temperature, salinity, pH and oxidation reduction potential (ORP) were measured at 20 cm above the bottom with a multiparameter probe YSI 6820 (two replicates).

The monthly river discharge data measured at Foz do Mouro hydrometric station between February/09 and July/10 was obtained from the INAG – Instituto da Água, I.P. (http://snirh.inag.pt).

4.2.2. Laboratory procedures

At each sampling date, the density of *P. microps* was determined by counting all sampled individuals. The size (total length - T*L*) of each fish was measured (0.01 mm precision) using a digital caliper, and subsequently assigned into one of the following seven size classes: A (< 15.00 mm), B (15.01 to 20.00 mm), C (20.01 to 25.00 mm), D (25.01 to 30 mm); E (30.01 to 35.00 mm), F (35.01 to 40.00 mm) and G (> 40.01 mm).

Fifty randomly selected individuals of each sample were observed under a magnifying glass for sex distinction based upon dimorphic features on their morphology and gonads (Whitehead et al. 1986). Individuals smaller than 26 mm of T*L* showed no clear morphological signs of their sex, and thus were considered sexually immature and classified as juveniles (see Bouchereau et al. 1989). Females with advanced stage of gonad development (i.e. presence of eggs) were assigned as ovigerous females. The total density of juveniles, males and females was estimated based on the percentage of contribution of each group within the fifty randomly selected fishes at each sample.

Additionally, 291 randomly selected fish from the three sampling stations were wet weighted in a precision scale to the nearest 0.001 g in order to determine the length-weight relationship. The condition factor of *P. microps* was calculated using the allometric equation:

$$(1) \qquad W = a.L^b$$

Where W is the wet weight of the fish (in grams), *L* is the total length of the fish (in centimeters) and *a* and *b* refer to the allometric coefficients obtained through the length-weight relationship (Reiss 1989).

4.2.3. Data analysis

Prior to the analyses of abiotic data, all variables were normalized. Posteriorly, a resemblance matrix based on the Euclidean distance was calculated. Principal

Components Analysis (PCA) was performed in order to detect habitat differences among sampling stations based on environmental data.

A two-way SIMPER routine was applied to determine the contribution of each abiotic variable for the dissimilarities among groups of stations and seasons.

Afterwards, a Permutational Multivariate Analysis of Variance (PERMANOVA) (type-III) was employed in a two-way crossed design, with station as a fixed factor (three levels: S1, S2 and S3) and season as a fixed factor (four levels: winter, spring, summer and autumn), in order to test the statistical significant differences among sampling stations and seasons using all abiotic variables (multivariate approach) and comparing each variable separately (univariate approach). The PERMANOVA analysis is capable of handling unbalanced statistical designs, such as in this study (due to unequal number of samples collected in each season), and calculates an identical *F* statistic that would be produced using traditional ANOVA and it is not affected by non-normal distribution of data (Anderson 2001).

A similar analysis procedure was adopted to investigate the common goby data. Prior to the analyses, all variables were normalized, and resemblance matrices based on Euclidean distance were calculated. The overall density, the density of each gender (juvenile, male and female), the density of ovigerous females, the density of *P. microps* size classes, the size of juveniles, males and females and the size of ovigerous females were statistically tested using a two-way PERMANOVA (type-III) in a two-way crossed design, with station as a fixed factor (three levels: S1, S2 and S3) and season as a fixed factor (four levels: winter, spring, summer and autumn), using both multivariate and univariate approaches (comparing each variable separately).

In PERMANOVA, the statistical significance of variance components was tested using 9999 permutations of residuals under a reduced model. When the possible number of permutations was lower than 150, the Monte Carlo-p value was considered. Subsequently, significant terms and interactions were investigated using a posteriori pair-wise comparisons to determine which pairs of stations and seasons were significantly different.

The PCA, the SIMPER and the PERMANOVA analyses were performed using PRIMER v 6.1.11® (Clarke and Gorley 2006) with PERMANOVA+1.0.1. add-on package (Anderson et al. 2008).

Canonical Correspondence Analysis (CCA) was performed to determine the relationship between the density of *P. microps* juveniles, males and females and the measured environmental variables using CANOCO 5 software (ter Braak and Verdonschot 1995). The data used for the CCA were organized by the total abundance of each group (juveniles, males and females) in each sampling station by month. The Monte-Carlo randomization test (499 permutations under the reduced model) was performed to determine the statistical significance of the correlations (see ter Braak 1986). The population structure of *P. microps* was defined by tracking recognizable cohorts from the successive sampling dates. Spatial samples were pooled together and analyzed using the size frequency distribution of successive dates. The mixture analysis method was used to identify cohorts within size frequency distributions constructed from population samples in each month using the PAST® software. The mixture analysis is a maximum-likelihood method for estimating the parameters (mean, standard deviation and proportion), of univariate normal distributions, based on a pooled univariate sample. This software uses the EM algorithm described by Dempster et al. (1977) and considers a histogram of frequency as a mixture of probability density functions. The number of modes in each month was determined by visual analysis of the histogram.

Absolute growth rates (AGR, mm day^{-1}) were determined for each cohort according to:

$$(2) \qquad AGR = \frac{L_{t2} - L_{t1}}{t_2 - t_1}$$

where L_{t2} and L_{t1} are the total length at time t_2 and t_1 respectively.

Finally, the secondary production was computed using the size-frequency method (Hynes method). This method calculates production by summing the biomass lost between size classes (Krueger and Martin 1980). In order to determine the production of *P. microps* in the lower Minho estuary, the Krueger and Martin (1980) equation was applied:

$$(3) \qquad P = \sum_{j=1}^{a-1} 0.5\left[\left(Y_{i,j} + Y_{i+1,j}\right) - \left(Y_{i,j+1} + Y_{i+1,j+1}\right)\right].\left(W_j.W_{j+1}\right).(t)^{-1}$$

where *P* is the annual production of the species, $Y_{i,j}$ is the mean density (ind.m^{-2}) of the size class *j* in the date *i*, $Y_{i+1,j}$ is the mean density of the size class *j* in the date *i+1*, $Y_{i,,j+1}$ is the mean density of the size class *j+1* in the date *i*, $Y_{i+1,j+1}$ is the mean density of the size class *j+1* in the date *i+1*, W_j is the biomass (g.m^{-2})

of the size class *j*, W_{j+1} is the biomass of the size class *j+1* and t is the time between *i* and *i+1*. Population production estimates correspond to the sum of each size class production (*P*). Negative production values were not included in the overall estimates and were regarded as zero production.

Annual production was calculated by a cross-multiplication, based on the values obtained for the overall production (18 months).

4.3. Results

4.3.1. Abiotic data

The Two-way PERMANOVA for stations and seasons of all abiotic variables was significant for both main effects (stations: pseudo-*F* = 8.83, $p < 0.001$; seasons: pseudo-*F* = 6.81, $p < 0.001$), but was not significant for the *stations x seasons* interaction (pseudo-*F* = 0.53, p = 0.97).

Depth was significantly higher in S1 during all seasons. On the other hand, pH was significantly higher during summer compared to winter in S2 and S3. Salinity was higher in S1 during all seasons; and in S3, salinity was significantly higher during summer compared with other seasons. Temperature varied seasonally, with significant lower values being recorded during winter in all sampling stations The ORP values did not vary among sampling stations and seasons (Table 4.1).

Table 4.1 Values of the five environmental variables (mean ± SE) measured in the three sampling stations throughout the seasons in the lower Minho estuary, NW Iberian Peninsula and the Two-way PERMANOVA results on the effects of sampling stations and seasons and their interaction term. * = $p < 0.01$; ** = $p < 0.001$; ^{ns} = non-significant. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test: $p < 0.05$).

	Seasons				Two-way PERMANOVA		
	Winter	Spring	Summer	Autumn	Stations	Seasons	Interaction
Depth							
S1	$4.2\pm0.3^{ac(A)}$	$3.1\pm0.3^{b(A)}$	$3.7\pm0.2^{ab(A)}$	$4.3\pm0.1^{c(A)}$	Pseudo-F = 57.78**	Pseudo-F = 6.87**	Pseudo-F = 0.97^{ns}
S2	$1.9\pm0.2^{a(B)}$	$1.3\pm0.2^{a(B)}$	$1.8\pm0.1^{a(B)}$	$1.8\pm0.1^{a(B)}$			
S3	$2.7\pm0.4^{a(B)}$	$1.9\pm0.3^{a(B)}$	$2.8\pm0.3^{a(C)}$	$2.1\pm0.1^{a(B)}$			

ORP							
S1	180.8±37.6	156.1±28.9	144.4±36.3	169.2±27.0	Pseudo-F = 0.09^{ns}	Pseudo-F = 0.57^{ns}	Pseudo-F = 0.02^{ns}
S2	189.1±45.6	153.6±32.0	159.5±41.5	173.4±19.0			
S3	201.2±52.2	159.9 ± 31.5	167.2±43.7	170.6±26.7			
pH							
S1	7.8±0.2^{a}	7.8±0.3^{a}	8.5±0.3^{a}	8.0±0.2^{a}	Pseudo-F = 0.39^{ns}	Pseudo-F = 8.77**	Pseudo-F = 0.20^{ns}
S2	7.8±0.2^{a}	8.0±0.2^{ab}	8.8±0.3^{b}	8.1±0.3^{ab}			
S3	7.7±0.1^{a}	8.1±0.2^{ab}	8.9±0.3^{b}	8.1±0.2^{ab}			
Salinity							
S1	25.8±6.5$^{a(A)}$	26.0±2.9$^{a(A)}$	32.0±1.0$^{a(A)}$	31.8±1.9$^{a(A)}$	Pseudo-F = 22.83**	Pseudo-F = 5.77*	Pseudo-F = 0.55^{ns}
S2	12.8±7.8$^{a(AB)}$	15.9±6.2$^{a(A)}$	30.8±1.9$^{a(A)}$	23.0±6.9$^{a(AB)}$			
S3	0.4±0.2$^{a(B)}$	0.4±0.2$^{a(B)}$	19.1±4.2$^{b(B)}$	4.3±4.2$^{ab(B)}$			
Temperature							
S1	11.9±0.9$^{a(A)}$	14.6±0.5$^{b(A)}$	15.0±0.6$^{b(A)}$	15.8±0.4$^{b(A)}$	Pseudo-F = 0.27^{ns}	Pseudo-F = 21.63**	Pseudo-F = 2.17^{ns}
S2	11.7±0.8$^{a(A)}$	16.4±0.9$^{b(A)}$	15.9±0.5$^{b(AB)}$	15.1±1.5$^{b(A)}$			
S3	9.6±0.8$^{a(A)}$	16.4±1.2$^{b(A)}$	18.3±1.2$^{b(B)}$	14.7±1.8$^{b(A)}$			

The two-way SIMPER results revealed that 60% of the dissimilarity between S1 and S2 was explained by depth and ORP values; while the dissimilarity between S1 and S3 was mostly explained by salinity and depth. On the other hand, dissimilarity between S2 and S3 was mainly related to differences in ORP and salinity. This analysis also revealed that temperature was the abiotic parameter that most varied among seasons (Supplementary material 4.1).

Additionally, the PCA routine indicated that the first two axis explained 62.3% of the variance among samples (PC1: eigenvalue = 1.71, explain 34.2% of variation; PC2: eigenvalue = 1.40, explain 28.1%) and revealed marked differences between sampling stations, with S1 differing from the other stations, mainly due to its lower values of temperature and pH and higher values of depth and salinity (Fig. 4.2).

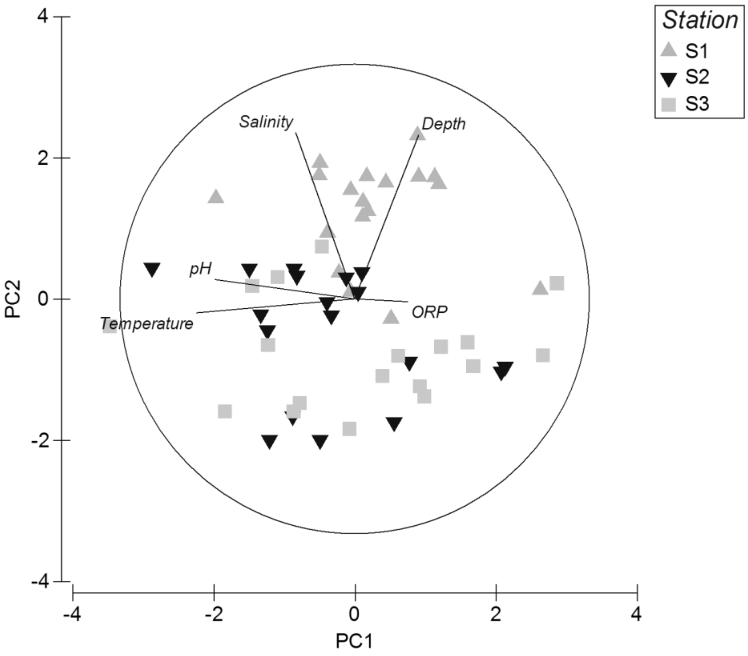


Fig. 4.2 PCA showing the differences of the environmental variables among sampling stations in the lower Minho Estuary, NW Iberian Peninsula.

4.3.2. Common goby data

A total of 15001 fishes were sampled (S2 = 59.9%, S1 = 21.4% and S3 = 18.7%), with 36.6% of these being caught during autumn, 33.0% in winter, 18.3%, in spring and 12.1% in summer.

4.3.2.1. Density patterns

The mean density of *P. microps* in Minho estuary, considering the entire sampling period and the three stations, was 118.3 ± 14.7 ind.100m^{-2} (mean ± SE). The density of *P. microps* varied throughout the sampling period, with higher values being found during the autumn (429.0 ± 140.0 ind.100m^{-2} in December/09) (Fig. 4.3A).

The density of *P. microps* varied significantly through space and time (stations: pseudo-$F = 40.14$, $p < 0.001$; seasons: pseudo-$F = 18.89$, $p < 0.001$; *stations x seasons* interaction pseudo-$F = 10.11$, $p < 0.001$), with each sampling station presenting a different pattern. In S1, the density of *P. microps* was lower during the summer (17.8 ± 4.8 ind.100m^{-2}) compared with other seasons (pairwise test: $p < 0.05$). In S2 the density of individuals was higher during the autumn (591.0 ± 102.0 ind.100m^{-2}) and winter (302.9 ± 78.3 ind.100m^{-2}) compared to spring and summer (pairwise test: $p < 0.05$); whereas in S3, the common goby was more abundant during autumn and summer (pairwise test:

p < 0.001). Overall, the density of *P. microps* was higher in S2 during all seasons, except during summer, when S3 presented the highest density (102.0 ± 18.4 ind.100m^{-2}) (Fig. 4.3B).

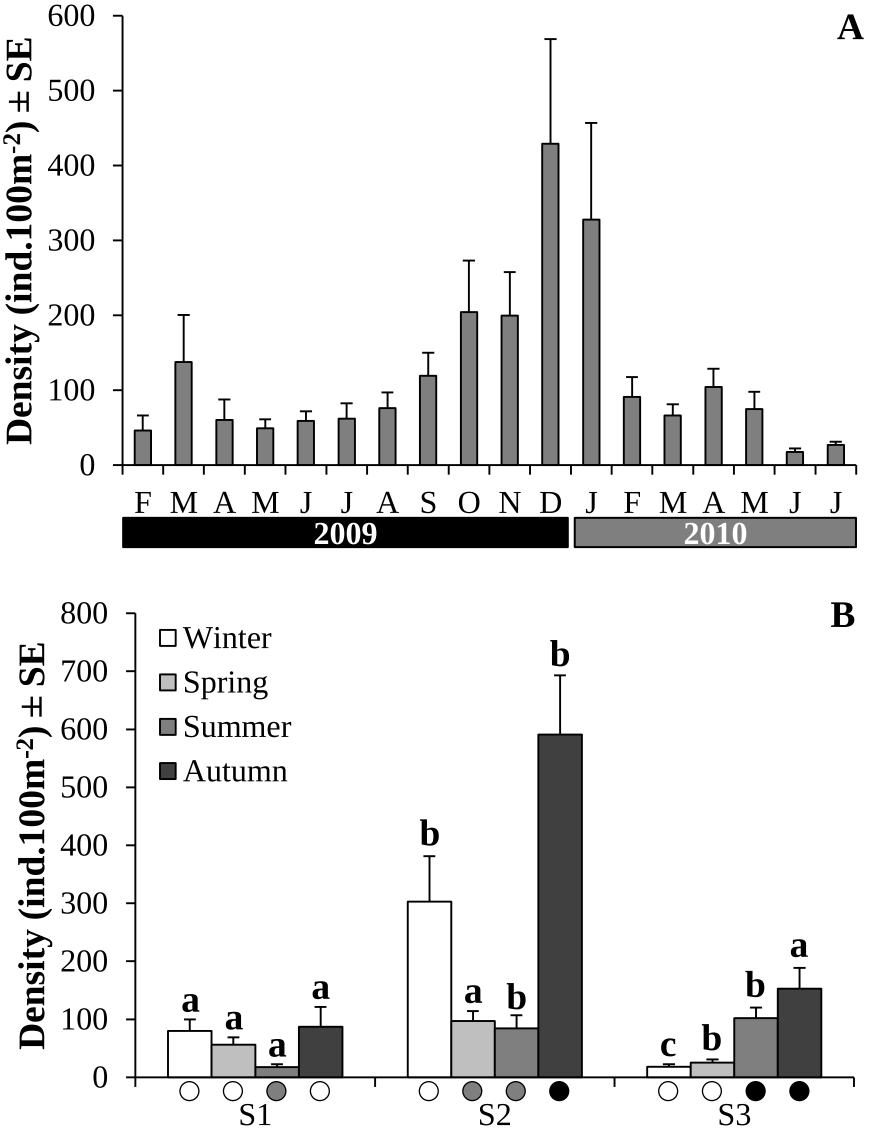


Fig. 4.3 (A) Mean density (± SE) of the common goby *Pomatoschistus microps* during 18-months study period in Minho estuary. (B) Comparisons on the densities (mean ± SE) of *P. microps* between sampling stations and seasons. Different letters (a, b or c) indicates post-hoc differences among sampling stations within the same season. Post-hoc differences among seasons within each sampling station are indicated by circles of different colors (white, grey or black) (Two-way PERMANOVA pairwise tests: p < 0.05).

The estimated densities of juveniles, males and females varied significantly through space and time (stations: pseudo-*F* = 25.08, p < 0.001; seasons: pseudo-*F* = 11.79, p < 0.001; *stations x seasons* interaction (pseudo-*F* = 7.03, p < 0.001).) Males and females were more abundant in S2 during all seasons, except in summer, when density was higher in S3. Juveniles, on the other hand, were denser in S2 during all seasons but spring, when the higher density was recorded in S1; with the lowest density value being recorded during summer in S1, spring in S2 and winter in S3 (Table 4.2).

Table 4.2 Comparisons on the estimated density (mean ± SE) of males, females and juveniles of the common goby *Pomatoschistus microps* among three sampling stations and four seasons in Minho estuary, NW Iberian Peninsula. * = p < 0.001. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test: p < 0.05).

	Seasons				Two-way PERMANOVA		
	Winter	Spring	Summer	Autumn	Stations	Seasons	Interaction
Male							
S1	$20.5\pm 4.6^{a(A)}$	$19.4\pm4.1^{a(A)}$	$6.0\pm1.5^{b(A)}$	$15.6\pm6.0^{ab(A)}$	Pseudo-*F* = 22.04*	Pseudo-*F* = 7.13*	Pseudo-*F* = 5.38*
S2	$51.0\pm11.6^{a(B)}$	$47.4\pm7.9^{a(B)}$	$6.2\pm1.4^{b(A)}$	$61.3\pm10.1^{a(B)}$			
S3	$4.7\pm1.5^{a(C)}$	$8.3\pm2.5^{a(C)}$	$19.1\pm4.0^{b(B)}$	$32.1\pm7.4^{b(A)}$			
Female							
S1	$46.6\pm12.2^{a(A)}$	$29.9\pm7.9^{a(A)}$	$7.4\pm2.0^{b(A)}$	$57.7\pm25.0^{a(A)}$	Pseudo-*F* = 37.42*	Pseudo-*F* = 22.18*	Pseudo-*F* = 11.64*
S2	$156.1\pm42.4^{a(B)}$	$43.3\pm8.9^{b(A)}$	$43.5\pm18.0^{ab(B)}$	$370.0\pm63.8^{c(B)}$			
S3	$12.1\pm3.0^{a(C)}$	$12.0\pm3.3^{a(B)}$	$59.4\pm13.3^{b(B)}$	$80.7\pm21.1^{b(A)}$			
Juvenile							
S1	$12.7\pm4.2^{ab(A)}$	$7.1\pm2.2^{ab(A)}$	$4.1\pm1.8^{a(A)}$	$14.1\pm4.3^{b(A)}$	Pseudo-*F* = 19.31*	Pseudo-*F* = 8.88*	Pseudo-*F* = 5.36*
S2	$95.9\pm30.6^{ac(B)}$	$6.3\pm2.5^{b(A)}$	$29.9\pm9.1^{a(B)}$	$160.2\pm53.4^{c(B)}$			
S3	$1.5\pm0.9^{a(C)}$	$4.8\pm2.0^{a(A)}$	$23.6\pm4.8^{b(B)}$	$40.4\pm24.5^{b(A)}$			

The sampled population was dominated by females (53.0%), with males and juveniles contributing with a smaller portion of individuals (26.3% and 20.7% respectively). The sex ratio (M/F) was not significantly different for stations (pseudo-*F* = 0.62, p = 0.55), but was significant for seasons (pseudo-*F* = 10.34, p < 0.001) and for the *stations x seasons* interaction (pseudo-*F* = 2.49, p <

0.05). The highest (spring: 1.7 ± 0.4) and the lowest (autumn: 0.2 ± 0.1) values of sex ratio were recorded in S2 (Supplementary material 4.2).

A total of 349 ovigerous females were caught in 18 months, with 20 individuals in autumn (5.7%), 41 in winter (11.8%), 190 in spring (54.4%) and 98 in summer (28.1%) with an average density of 2.2 ± 0.3 ind.100m^{-2} (mean ± SE). The density of ovigerous females also varied through space and time (stations: pseudo-*F* = 2.14, p = 0.12; pseudo-*F* = 5.91, p < 0.01; *stations x seasons* interaction (pseudo-*F* = 2.71, p < 0.05), with higher densities being recorded during spring (S1 and S2) and summer (S3) (Fig. 4.4).

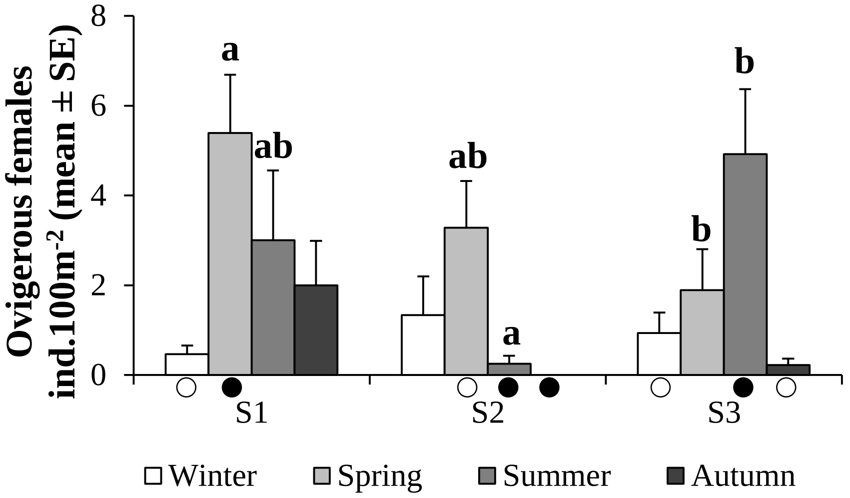


Fig. 4.4 Comparisons on the density (mean ± SE) of ovigerous females of the common goby *Pomatoschistus microps* among three sampling stations and seasons in lower Minho estuary, NW Iberian Peninsula. Different letters (a or b) indicates post-hoc differences among sampling stations within the same season. Post-hoc differences among seasons within each sampling station are indicated by circles of different colors (white or black) (Two-way PERMANOVA pairwise tests: p < 0.05).

4.3.2.2. Size and recruitment patterns

The range of T*L* varied between 9.3 and 71.4 mm, with an average value of 30.4 ± 0.1 mm (mean ± SE). The size of males did not vary significantly among sampling stations, but larger males were found during winter and autumn. On the other hand, the size of females varied significantly among sampling stations and seasons, with larger females being recorded in S3 during all seasons, except in autumn, when larger females were recorded in S1. Juveniles also presented significant differences among sampling stations and seasons, with smaller fish being recorded in S3 during all seasons, except in autumn, when smaller gobies were found in S1 (Table 4.3).

Table 4.3 Comparisons on the total length (mean ± SE) of males and females of the common goby *Pomatoschistus microps* among sampling stations and seasons in Minho estuary, NW Iberian Peninsula. ^{ns} = non-significant; * = $p < 0.001$. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test: $p < 0.05$).

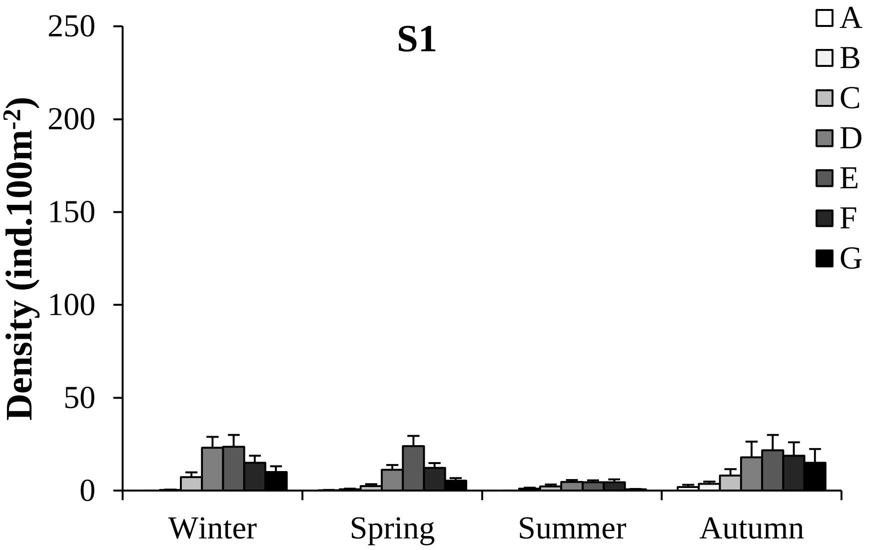
	Total length (mm)				Two-way PERMANOVA		
	Winter	Spring	Summer	Autumn	Station	Season	Interaction
Male							
S1	$36.7 \pm 0.5^{a(A)}$	$34.0 \pm 0.3^{b(A)}$	$33.1 \pm 0.5^{b(A)}$	$37.3 \pm 1.0^{a(A)}$	Pseudo-F = 1.46^{ns}	Pseudo-F = 40.51*	Pseudo-F = 3.84*
S2	$37.4 \pm 0.4^{a(AB)}$	$34.5 \pm 0.3^{b(A)}$	$34.3 \pm 0.6^{b(A)}$	$36.8 \pm 0.8^{a(A)}$			
S3	$38.6 \pm 0.50^{a(B)}$	$36.1 \pm 0.4^{b(B)}$	$31.8 \pm 0.3^{c(B)}$	$36.8 \pm 0.6^{b(A)}$			
Female							
S1	$33.1 \pm 0.3^{a(A)}$	$34.7 \pm 0.3^{b(A)}$	$32.5 \pm 0.6^{a(A)}$	$34.6 \pm 0.4^{b(A)}$	Pseudo-F = 86.87*	Pseudo-F = 30.47*	Pseudo-F = 7.06*
S2	$32.4 \pm 0.2^{a(A)}$	$32.7 \pm 0.3^{a(B)}$	$30.1 \pm 0.3^{b(B)}$	$32.3 \pm 0.3^{a(B)}$			
S3	$35.5 \pm 0.4^{a(B)}$	$36.4 \pm 0.3^{b(C)}$	$33.1 \pm 0.3^{c(A)}$	$33.4 \pm 0.3^{c(C)}$			
Juvenile							
S1	$25.7 \pm 0.4^{a(A)}$	$24.6 \pm 0.6^{a(A)}$	$21.8 \pm 0.4^{b(A)}$	$22.3 \pm 0.8^{b(A)}$	Pseudo-F = 21.06*	Pseudo-F = 26.87*	Pseudo-F = 49.04*
S2	$24.2 \pm 0.3^{a(B)}$	$24.5 \pm 0.6^{a(A)}$	$23.5 \pm 0.3^{a(B)}$	$23.8 \pm 0.3^{a(A)}$			
S3	$23.6 \pm 0.5^{a(B)}$	$14.8 \pm 0.4^{b(B)}$	$21.3 \pm 0.4^{a(A)}$	$26.8 \pm 0.5^{c(B)}$			

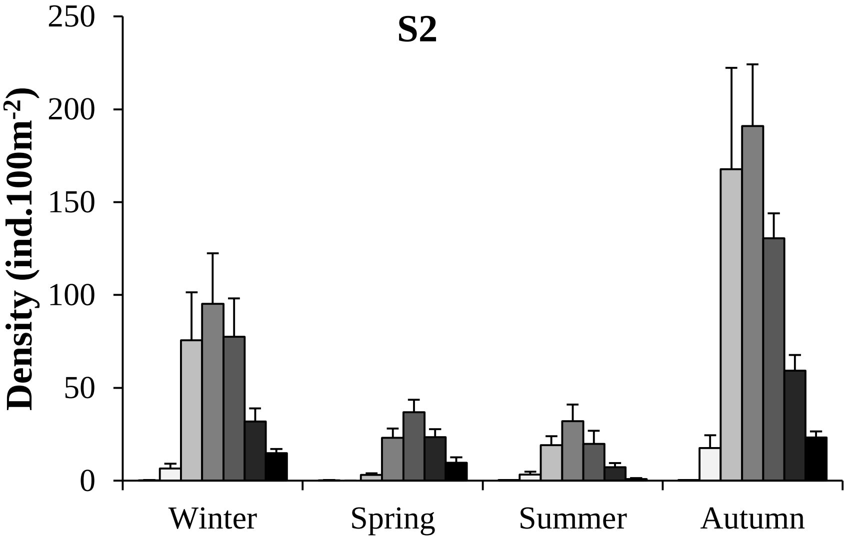
The T*L* of the ovigerous females varied between 26.8 and 46.9 mm, with an average value of 35.9 ± 0.2 mm. The Two-way PERMANOVA indicated that size of ovigerous females varied significantly among seasons (pseudo-F = 6.72, $p < 0.001$), but not among stations (pseudo-F = 0.11, $p = 0.73$), the *stations x seasons* interaction was also non-significant (pseudo-F = 1.02, $p = 0.41$) (Table 4.4).

Table 4.4 Comparisons on the total length (mean ± SE) of ovigerous females of the common goby *Pomatoschistus microps* among sampling stations and seasons in Minho estuary, NW Iberian Peninsula. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test: $p < 0.05$).

Station\Season	Winter	Spring	Summer	Autumn
S1	40.6±1.5$^{a(A)}$	35.9±0.4$^{b(AB)}$	34.9±0.7$^{b(A)}$	38.5±0.9$^{a(A)}$
S2	35.7±0.9$^{a(B)}$	34.9±0.5$^{a(A)}$	32.3±1.8$^{b(A)}$	-
S3	37.9±1.2$^{a(AB)}$	37.1±0.8$^{a(B)}$	35.3±0.5$^{b(A)}$	38.3±2.8$^{ab(B)}$

The density of the different size classes of *P. microps* varied through space and time (stations: pseudo-*F* = 17.61, p < 0.001; seasons: pseudo-*F* = 11.47, p < 0.001; *stations x seasons* interaction: pseudo-*F* = 5.06, p < 0.001). Results indicated that most of the size classes were more abundant in S2, except size class A, which was more abundant in S3. In S1 and S3, the most abundant size classes were E, D and F respectively, while classes D, E and C were the most abundant in S2 (Fig. 4.5).





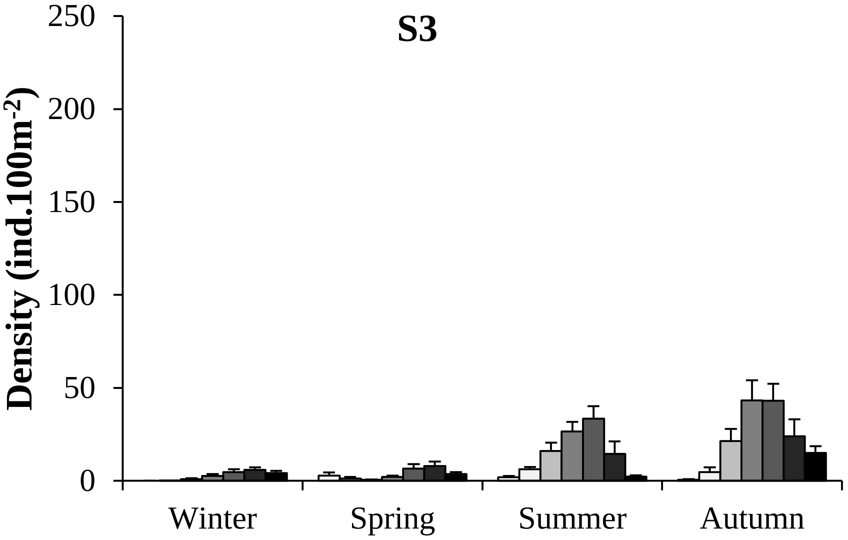


Fig. 4.5 Mean density (± SE) of the seven size classes of the common goby *Pomatoschistus microps* among seasons in each sampling station of the lower Minho estuary, NW Iberian Peninsula. Size classes: A (< 15.00 mm), B (15.01 to 20.00 mm), C (20.01 to 25.00 mm), D (25.01 to 30 mm); E (30.01 to 35.00 mm), F (35.01 to 40.00 mm) and G (> 40.01 mm).

The mixture analysis indicated that the recruitment of *P. microps* occurred three times during the sampled period, twice in 2009 (May and October) and once in 2010 (June) (Fig. 4.6).

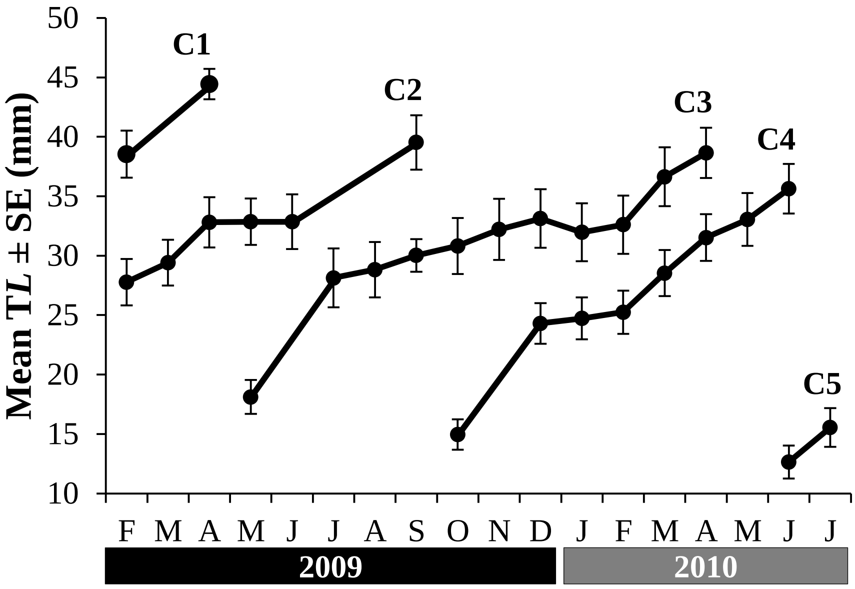


Fig. 4.6 Mean cohort length of the common goby *Pomatoschistus microps* in lower Minho estuary, with indication of the five cohorts identified by the mixture analysis (C1, C2, C3, C4 and C5).

4.3.2.3. Fish-habitat associations

Juveniles, males and females were associated differentially with environmental variables. The Monte-Carlo test was significant (test of significance of all canonical axes: trace = 0.05; F-ratio = 2.53; p < 0.01) and cumulatively, axes 1 and 2 accounted for 26.1% of the total variance. Correlation between fish and abiotic variables in axis 1 was higher (0.57) than in axis 2 (0.37) (Fig. 4.7).

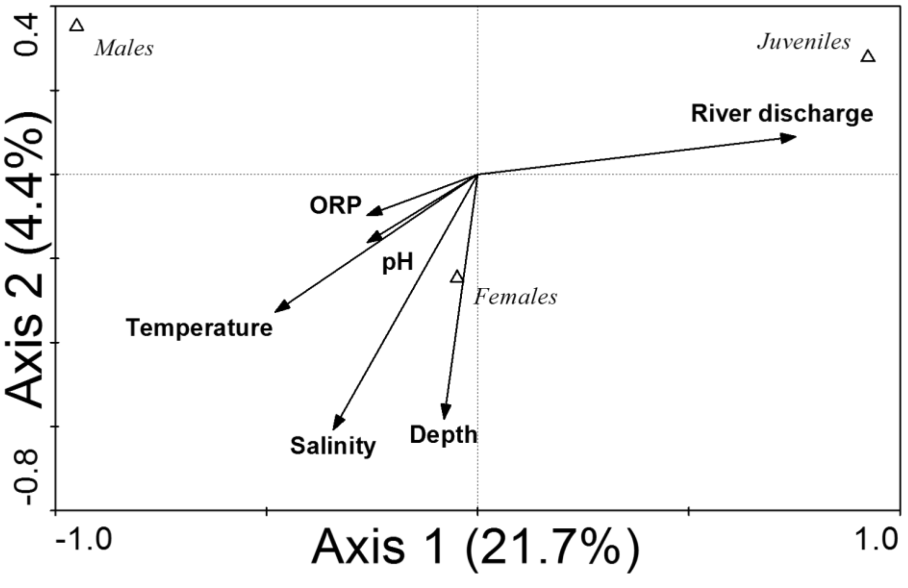


Fig. 4.7 Canonical plotting of environmental characteristics (arrows) and males, females and juveniles of the common goby *Pomatoschistus microps* in Minho estuary, NW Iberian Peninsula. ORP = Redox potential.

4.3.2.4. Length-weight relationship, growth and secondary production

The calculated length and wet weight relationship of the *P. microps* presented a high allometric b value (b = 3.6489) and low allometric a value (a = 0.000001).

Growth of the C2 and C3 cohorts was similar, while individuals from the C4 cohort grew faster than others cohorts (Table 4.5).

Table 4.5 Absolute growth rates (AGR, mm.day^{-1}) for the common goby *Pomatoschistus microps* cohorts. The AGR and the 95% confidence interval (CI) were computed only when the cohort was recorded for more than 3 months.

Cohort	Growth±CI
C2	0.045±0.002
C3	0.046±0.001
C4	0.069±0.001

The estimated overall secondary production of *P. microps* was 5.38 g.WWm^{-2}, with an annual production of 3.83 g.WWm^{-2}.year^{-1}.

4.4. Discussion

The common goby has a great capacity of adaptation, which allows it to successfully occupy habitats with different environmental characteristics (Bouchereau and Guelorget 1998). However, it seems that in Minho estuary, the species preferentially occupied the salt marsh area (S2), since the density of *P. microps* at this station is constantly higher compared with its density at the river mouth (S1) and the upper station (S3) (approximately 4 times). In the salt marsh area, most of the fishes were small-bodied, with high proportion of individuals between 20 and 30mm of T*L*, suggesting that this area may serve as a refuge or shelter site for juveniles and subadults. Actually as reported in other studies, the high complexity and productivity of salt marshes' biological communities might provide an ideal environment for feeding, sheltering and growing for the common goby (Koutsogiannopoulou and Wilson 2007). In contrast, at the river mouth (S1) most of the fishes were larger, with length ranging between 30 and 40 mm of T*L*, with *ca.* 70% of the individuals being sexually mature throughout the year. In the upper station (S3), the density of gobies varied considerably throughout seasons, with lower values being recorded during winter and spring, compared with summer and autumn. At this station, most of the fishes were young adults (between 25 and 35 mm of T*L*). Such variations on *P. microps* density suggest that the upper station presented important seasonal oscillations in abiotic variables and might be located near the upstream limit of distribution of the species in winter. Our results suggest that the area occupied by the common goby in the estuary might vary throughout the year due to different degrees of saline intrusion. During summer, the saline plume often reaches upstream areas of the estuary, noticeably influencing the distribution of the species. The saline intrusion in Minho estuary fluctuates between 10km during winter and 20 km during summer, exceptionally ranging up to 30 km in extreme conditions (Antunes pers. comm.). In fact, during summer this species can be found in a much wider spectrum within the estuary, being recorded *ca.* 13 km upstream from the river mouth; whilst during winter, *P. microps* is usually restricted to the lowest areas of the estuary (ca. 9 km) (pers. observ.).

Our results indicated that juveniles, males and females might have distinct habitat preferences. Females were largely dominant within the estuary and showed a weak correlation with the measured abiotic parameters. The numerical dominance of females over males is relatively common in *Pomatoschistus* spp. populations (e.g. Bouchereau et al. 1993; Fouda et al. 1993; Koutrakis and Tsikliras 2009) and may be related to different behaviors exhibited by sexes. According to Miller (1984), during the breeding season, there is a marked reduction in the proportion of males due to their nest guarding behavior. *P. microps* males guard eggs under shells or stones, consequently males are less easily caught. In fact, males were less abundant during summer at the river mouth and at the salt marsh.

Also, our results indicated that juveniles may be associated with shallow and polyhaline waters, as well as with months of high river discharge. Differently from Leitão et al. (2006), which noted that 0+ group associated with high temperatures, in our study, juveniles associated with colder waters, indicating that juveniles recruited in different months in Mondego and in Minho, two estuaries from the Atlantic coast of Portugal. According to the present study, recruitment occurred twice per year (in late spring/early summer and in the autumn), while in Mondego, *P. microps* recruited three times per year (winter, spring and summer) (Leitão et al. 2006). Notwithstanding, a third pattern was reported for Minho and Mondego estuaries, with recruitment occurring once per year in Minho (in early summer), and twice (spring and summer) or three times (winter, spring and summer) per year in Mondego (Dolbeth et al. 2010). Actually, the recruitment of fishes in Minho estuary often takes place later and growth rates are lower compared with the Mondego estuary, and those differences probably are related to differences in temperature between estuaries (Dolbeth et al. 2010). Also, in the present study, we found different growth rates among *P. microps* cohorts, with the autumn recruits (C4) growing faster than the spring ones (C3). Different growth rates between fish cohorts in a given population are common and often related to environmental conditions, but their ecological causes and implications are still uncertain (e.g. Fuiman et al. 2005 and the references therein). However fast growing is often related to low temperatures (e.g. Dolbeth et al. 2010), which could explain the faster growth of autumn recruits compared with spring ones; since in spring water was slightly warmer than in autumn.

The observed differences in the density of ovigerous females may also indicate that breeding grounds varied seasonally within the estuary, probably due to differences in the temperature and in the availability of substrate for egg attachment, once each sampling station presented different substrate characteristics. The presence of bivalve shells is important for the species, since *P. microps* use it during the reproduction (Nyman 1953), and also for shelter. Thus, the high density of empty bivalve shells found in S3, can be related with the high density of ovigerous females found in this station during the summer, when most of the Asian clams *C. fluminea* dies due to salinity increase (see Ilarri et al. 2011). In a small spatial scale experiment, Ilarri (2012) found that the densities of *P. microps* and *C. fluminea* were positively correlated, suggesting that the common goby prefer to inhabit areas with higher availability of bivalve shells in Minho estuary.

P. microps is an iteroparous species (Miller 1984), being able to spawn several times along the reproductive cycle, normally spawning when water temperature is between 15ºC and 20ºC (Wiederholm 1987). In Minho estuary, the water temperature in the salt marsh and the upper station varied within the optimal levels for *P. microps* reproduction from May to November, whereas in the river mouth, the optimum range of temperature occurred for a shorter period of time (from September to December). These results suggest that the common goby reproduction may occur in different periods of time in each estuarine section. It is also possible that the optimal temperature range for *P. microps* reproduction differs among different latitudes, once this species has a great plasticity on reproductive traits (Pampoulie et al. 2000; Dolbeth et al. 2010); however, due to the lack of data, this hypothesis cannot be confirmed or contradicted, and hence further studies are necessary to investigate if the optimal values suggested by Wiederholm (1987), based on individuals from the Bothnian Sea, are valid for *P. microps* populations in southern Europe.

Differences in recruitment between this and other studies may also be related to breeding season duration. In higher latitudes, breeding season is typically shorter than in lower latitudes, but in certain cases, breeding season can last longer in lower latitudes (see Bouchereau and Guelorget 1998). This seems to be the case at the Minho estuary, once it presented a longer breeding duration (6 months) than estuaries located in higher latitudes (e.g. Miller 1975; Rogers 1988). Nevertheless, breeding season in Minho estuary was also longer than in systems located in lower latitudes (e.g. Arruda et al. 1993; Leitão et al. 2006). A longer breeding season might have an important effect on the *P. microps* population, due to the improvement of its reproductive success (Bouchereau and Guelorget 1998), which would also help to explain the high densities recorded in Minho estuary. In fact, the densities recorded in this study are much higher than those reported in southern (e.g. Leitão et al. 2006; Dolbeth et al. 2007b; Martinho et al. 2007b; Almeida et al. 2008; França et al. 2008) and in northern European estuaries (e.g. Norte-Campos and Temming 1994; Selleslagh and Amara 2008) (Table 4.6). Latitudinal comparisons in *P. microps* density are difficult to be made, once the sampling strategies, techniques and periodicity are often different in each study. However, considering only studies that have adopted similar sampling methods, latitudinal trends become clearer. For instance, in studies which used encircling nets or traps (throw and drop), densities appear to be higher in northern compared with southern systems. Similarly, in studies that used beam trawls, fish density seems to be higher in higher latitudes. On the other hand, the densities recorded in Minho estuary were much higher than elsewhere, suggesting that in this system, *P. microps* might find a combination of factors which helps the species to achieve higher densities than would be expected, given the latitude of this estuary.

Table 4.6 Mean density of the common goby *Pomatoschistus microps* in different Atlantic estuaries.

Location	Mean density.100m^{-2}	Study period	Seasons	Day period	Sampling gear	Reference
Sylt-Rømø Bight, Germany	230.0 – 1070.0 (seagrass); 30.0 – 200.0 (sand)	2003	Summer	Diurnal	Portable drop trap	Polte et al. 2005
Wadden Sea, Germany	6.0	1991/92	Summer	Diurnal and nocturnal	Beam trawl	Norte-Campos and Temming 1994
Canche estuary, France	8.2	2006/07	All seasons	Diurnal	Beam trawl	Selleslagh and Amara 2008
Minho estuary, Portugal	118.3	2009/10	All seasons	Diurnal	Beam trawl	This study
Minho estuary, Portugal	60.1	2006	Summer	Diurnal	Beam trawl	Costa-Dias et al. 2010
Mondego estuary, Portugal	0.8	2003/06	All seasons	Nocturnal	Beam trawl	Martinho et al. 2007
Mondego estuary, Portugal	0.8	2003/04	All seasons	Nocturnal	Beam trawl	Dolbeth et al. 2007
Mondego estuary, Portugal	0.8 0.5 0.8	2004/05 2005/06 2003/04	All seasons All seasons All seasons	Nocturnal Nocturnal Nocturnal	Beam trawl Beam trawl Beam trawl	Leitão et al. 2006
Tagus estuary, Portugal	60.0 (upper intertidal); 117.1 (lower intertidal); 89.9 (subtidal)	2005	All seasons	Diurnal and nocturnal	Encircling nets	França et al. 2008
Ria Formosa, Portugal	115.0	2001/02	All seasons	-	Throw trap	Almeida et al. 2008

The common goby is sympatric with the sand goby *P. minutus* in several estuaries, and presents some degree of habitat and diet overlap (Leitão et al. 2006; Złoch and Sapota 2010). Nevertheless, the sand goby is less tolerant to salinity and temperature fluctuations than the common goby, thus *P. minutus* prefers to inhabit estuarine zones with higher salinities or the sea, whereas *P. microps* is preferentially found in brackish waters (Fonds and Van Buurt 1974; Pampoulie et al. 1999). In Minho estuary, during winter, salinity can reach extremely low values even near the river mouth, for instance, in January/10, salinity at the bottom reached 0.12 in S1. The occurrence of such low salinities within the estuary may benefit *P. microps* by potentially expanding its favorable habitat and also by reducing the detrimental effects of competition with its congener *P. minutus*, which is much less abundant in areas with low salinities in this estuary (Souza et al. unpublished data).

Also, the sampling strategy adopted in the present study may have contributed to the observed differences between population density in Minho and other estuaries, since we performed diurnal 1-m beam trawl catches, while other studies performed night catches using 2-m trawls. Changes in trawling time can result in differences on fish density (Rotherhan et al. 2008) or not (Ribeiro et al. 2006); and as far as we know, there are no published studies that compare the efficiency of 1-m and 2-m beam trawls for *P. microps*.

Other studies also reported remarkable high densities of estuarine species in Minho, for instance, the Asian clam *Corbicula fluminea* (Sousa et al. 2008d), the European flounder *Platichthys flesus* (Cabral et al. 2007; Freitas et al. 2009, Souza et al. 2013) and the shore crab *Carcinus maenas* (Souza et al. unpublished data). These high densities may be linked to the particular abiotic profile of Minho estuary, namely the high river discharge and possibly the high availability of nutrients and organic matter. These characteristics may lead to a scenario of dominance of a few species, highly tolerant to freshwater in detriment of species with higher affinity with salt water, suggesting that in stressful environments certain species can be benefited.

Secondary production can be used to infer the population fitness (Rigler and Downing, 1984) and therefore, it may represent an interesting proxy with regard to the functional responses of populations to environmental stressors (Dolbeth et al. 2012). The annual production of *P. microps* in Minho estuary was notably higher than in other systems (see Dolbeth et al. 2010 and the references therein), suggesting that this system provide advantageous environmental conditions for this particular species to thrive. Nonetheless, in a previous study, Dolbeth et al. (2010) recorded production values four times lower for *P. microps* in the same estuary, suggesting that the common goby production might have increased over the years, probably due to the fluctuations on the abiotic conditions caused by drought events that occurred in this estuary recently (see Dolbeth et al. 2010; Ilarri et al. 2011). Alternatively, differences in production values obtained for *P. microps* in Minho estuary can be the result of different sampling strategies and production estimation method adopted in each study.

4.5. Conclusions

In summary, our results highlights that the observed high density and production of *P. microps* are probably explained by a combination of factors that favored growth and reproduction of the common goby in the system. The tolerance for low salinities (Rigal et al. 2008), the long breeding season of *P. microps* and the low density of the competing species *P. minutus* (Costa-Dias et al. 2010; Souza et al. unpublished data) probably are the responsible for the remarkable abundance of *P. microps* in Minho estuary. Also, it is possible that the high availability of bivalve shells of the non-indigenous clam *C. fluminea* (Sousa et al. 2008c) could be exerting a significant influence on *P. microps* population (Ilarri 2012). In fact, the common goby might be using the shells as a refuge and shelter site, as well as for reproduction (Bouchereau et al. 1991). In conclusion, the common goby may found in Minho estuary, an area with a combination of factors that created optimal conditions for the species to thrive.

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CHAPTER 5

POPULATION STRUCTURE, PRODUCTION AND FEEDING HABIT OF THE SAND GOBY *POMATOSCHISTUS MINUTUS* (ACTINOPTERYGII: GOBIIDAE) IN A ESTUARY DOMINATED BY THE SYMPATRIC SPECIES *P. MICROPS*

Population structure, production and feeding habit of the sand goby *Pomatoschistus minutus* (Actinopterygii: Gobiidae) in a estuary dominated by the sympatric species *P. microps*

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Souza AT, Dias E, Marques JC, Antunes C, Martins I (submitted) Population structure, production and feeding habit of the sand goby *Pomatoschistus minutus* (Actinopterygii: Gobiidae) in a estuary dominated by the sympatric species *P. microps*.

Abstract

The density of the sand goby *Pomatoschistus minutus* varied significantly among estuarine areas and seasons (Two-way PERMANOVA: $p < 0.001$), with most of the individuals being caught near the river mouth during the autumn (38.4% of the total). Males, females and juveniles were spatially segregated (Monte-Carlo test: $p < 0.01$), as well as fishes belonging to different size classes (Monte-Carlo test: $p < 0.01$). The sand goby fed mostly on crustaceans (Frequency of Occurrence - FO) = 35%; Relative Abundance - RA = 40%), detritus (FO = 27%; RA = 23%) and annelids (FO = 15%; RA = 14%), with no obvious difference between the diet of males and females (One-way PERMANOVA: $p = 0.75$). Despite of a remarkable abundance of the sympatric species *P. microps* in Minho estuary, the density (up to 20 times) and the secondary production (up to 16 times) of the sand goby was higher than in other southern European estuaries.

Keywords: competition / diet / fish / Minho / population ecology / Portugal

5.1. Introduction

Estuaries are highly productive ecosystems that provide important forage, spawning, refuge, and nursery habitat for several fish species during one or more of their life history stages (Chambers 1992; Nelson and Monaco 2000). These systems are known to present marked differences in the abiotic characteristics among different estuarine sections, with significant seasonal variations, especially due to the instability on the salt and freshwater equilibrium over time (Pritchard 1967). The spatial and seasonal variations of abiotic features within estuaries provide an excellent opportunity to study the distribution and structure patterns, as well as the habitat preferences of aquatic populations.

The sand goby *Pomatoschistus minutus* is a small fish (up to 110 mm of T*L*) that lives in the shore and inside estuaries, and is one of the most abundant fish species along the Atlantic European coasts and its estuaries (Healey 1971; Bouchereau and Guelorget 1998). This species is widely distributed and commonly observed in the North-eastern Atlantic from Norway to the south of Spain, in some zones of the western Mediterranean (Gulfs of Lions and Genoa), in Adriatic (Venice Gulf) and along the western Black Sea coast. The precise geographical distribution of the species is difficult to define because it has been often mistaken with other goby species (e.g. Doornbos and Twisk 1987; Henderson 1989).

The sand goby also form an important ecological link between benthic invertebrates and larger predatory fish (Jaquet and Raffaelli 1989; Maes et al. 2003; Salgado et al. 2004). This species does not generally remain on a permanent basis in the lagoons or estuaries, but performs seaward migration to reproduce (Fonds 1973). Also, in northern regions, *P. minutus* carries out thermal migration towards deeper water when the temperature drops below 4-5ºC (Fonds 1973). In a recent study, Guelinckx et al. (2008) found that *P. minutus* can perform repeated migration between the upper and the lower estuary, suggesting that the species can actively select habitats according to favourable environmental conditions.

Due to its ecological relevance and abundance in several systems, the population structure of this species has been previously studied, but interestingly, most of the studies conducted in southern Europe simultaneously investigated the sympatric species *P. microps* (Krøyer 1838) (e.g. Leitão et al. 2006; Dolbeth et al. 2007b). The density of both species usually is even in estuaries, however marked differences in habitat preferences was previously acknowledged (Leitão et al. 2006; Dolbeth et al. 2007b). However, in Minho estuary, the density of the common goby *P. microps* is remarkably higher than other estuarine systems (Souza et al. in revision).

In this context, the present study aimed at assessing the habitat use of the sand goby in an estuary where the sympatric species shows remarkable high values of density (Souza et al. unpublished data). Our goals were threefold: (a) to investigate the habitat use and population density of *P. minutus*; (b) to investigate the distributional patterns of *P. minutus* along a salinity gradient and (c) to investigate ontogenetic shifts in the spatial and temporal preferences of *P. minutus*. By focusing on the distributional patterns of *P. minutus* within a system where the species seems to be outnumbered by the sympatric species *P. microps*, we aim to contribute to increase the knowledge and understanding about the ecology of *P. minutus*.

5.2. Material and methods

5.2.1. Study area and sampling campaign

This study was conducted in the Minho estuary (NW Iberian Peninsula), which ranges up to 40 km (considering the upstream limit of spring tides), covering a total area of 23 km^2. The Minho estuary is a very shallow system (Moreno et al. 2005), with a mean depth of 2.6 m (Freitas et al. 2009) and characterized as a mesotidal and partially mixed system, although during periods of high river discharge, it tends towards a salt wedge estuary (Sousa et al. 2005). The present study was carried out at the lower estuary (first 8 km from the estuary mouth).

Pomatoschistus minutus individuals were collected during 18 consecutive months, from February 2009 to July 2010 in three nearby subtidal areas in the lower Minho estuary (Fig. 5.1). The three sites were chosen in order to assess possible dissimilarities on the population dynamics related to differences in the habitat type and also to environmental features.

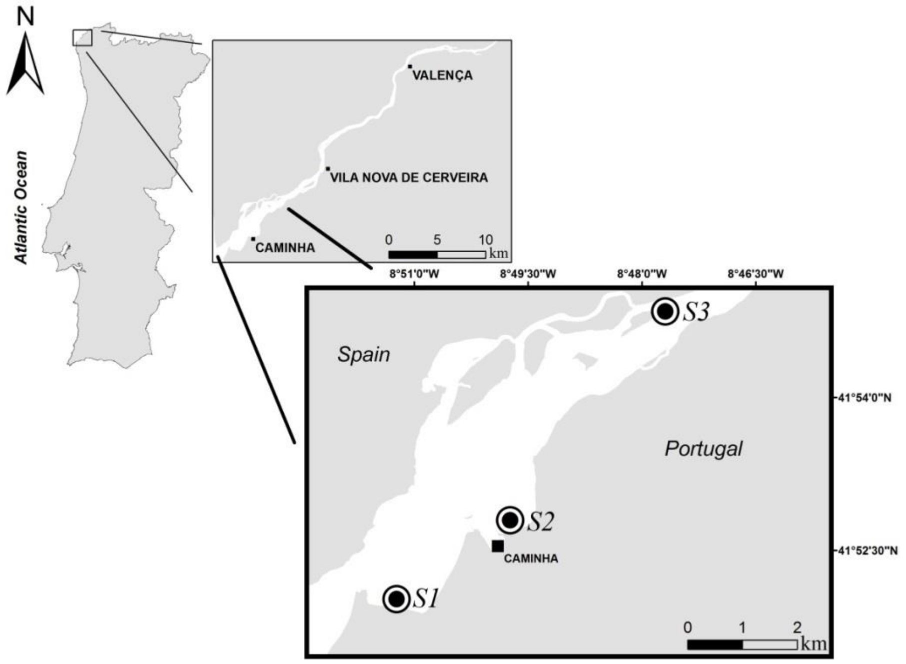


Fig. 5.1 Map of the study site showing the three sampling stations at the lower Minho Estuary, NW Iberian Peninsula.

Station 1 (S1) is closer to the river mouth (ca. 1.5 km), and is characterized by muddy soft bottoms often densely covered by debris, such as drifting seaweeds, dead leaves and empty molluscs' shells (Souza et al. 2011). Station 2 (S2) is located inside the Coura salt marsh (ca. 3.5 km apart from the river Minho mouth), which is a relatively small sub-system characterized by the presence of narrow channels, typically bordered by the seagrass *Spartina maritima*. The channels' soft bottom is often sparsely covered by debris and empty peppery furrow shells *Scrobicularia plana* (Souza et al. 2011). Station 3 (S3) is located ca. 8 km upstream from the mouth and is characterized by presenting high densities of the Asian clam *Corbicula fluminea*, with its soft bottoms often sparsely covered by debris and submerged vegetation (personal observation; Sousa et al. 2008b,c). In each station, three replicates per month were collected during the day at high tide of spring tides using a 1m beam trawl (5 mm mesh size) towed at constant speed (2 km.h^{-1}) for 2 to 3 minutes. The density of individuals was calculated by averaging densities from individual tows for each site of each month. Additionally, environmental variables (water temperature, salinity, pH and oxidation reduction potential (ORP)) were measured with a multiparameter probe YSI 6820 deployed to 20 cm off the bottom (two replicates).

The monthly river discharge data measured at Foz do Mouro hydrometric station between February 2009 and July 2010 was obtained from the INAG – Instituto da Água, I.P. (http://snirh.inag.pt).

5.2.2. Laboratory procedures

All *P. minutus* individuals were counted, measured to the nearest 0.01 mm (total length – T*L*), wet weighted to the nearest 0.001 g in a precision scale and observed under a stereomicroscope for sex distinction based upon dimorphic features on their morphology and gonads (Whitehead et al. 1986). Individuals smaller than 26 mm of T*L* did not show clear morphological signs of its gender and thus were considered sexually immature and classified as juveniles. Afterwards, all sand gobies were classified into one of the six size classes: C1 (< 29.99 mm), C2 (30.00 to 39.99 mm), C3 (40.00 to 49.99 mm), C4 (50.00 to 59.99 mm), C5 (60.00 to 69.99 mm) and C6 (> 70.00 mm).

The stomach contents of 138 sand gobies were analysed using a stereomicroscope. The food items found in the *P. minutus* stomachs were counted and identified to the lowest taxonomic level possible with the aid of identification keys for aquatic invertebrates (Tachet et al. 2003) and fishes (Iglésias 2012). Afterwards, food items were grouped into one of the seven functional groups (Annelids, Crustaceans, Detritus, Insects, Molluscs, N.I. (non-identified material) and Others (sum of arachnids and fishes)).

Feeding activity was evaluated by the vacuity index defined as the percentage of empty stomachs (Hyslop 1980). The frequency of occurrence (FO%) for each prey category was defined according to the percentage of samples in which that particular item occurred. The relative abundance (RA%) of the different prey items was expressed as the percentage of a given item in all samples in relation to the numerical sum of the all prey items recorded.

5.2.3. Data analysis

Prior to the analyses of abiotic data, all variables were normalized. Posteriorly, a resemblance matrix based on the Euclidean distance was calculated. The Principal Components Analysis (PCA) was performed in order to detect habitat differences among sampling stations based on environmental data. A two-way SIMPER routine was applied to determine the contribution of each abiotic variable for the dissimilarities among groups of stations and seasons.

Afterwards, a Permutational Multivariate Analysis of Variance (PERMANOVA) (type-III) was employed in a two-way crossed design, with station as a fixed factor (3 levels: S1, S2 and S3) and season as a fixed factor (4 levels: winter, spring, summer and autumn), in order to test the statistical significant differences among sampling stations and seasons using all abiotic variables (multivariate approach) and comparing each variable separately (univariate approach). The PERMANOVA analysis is capable of handling unbalanced statistical designs, such as in this study (due to unequal number of samples collected in each season), and calculates an identical *F* statistic that would be produced using traditional ANOVA and it is not affected by non-normal distribution of data (Anderson 2001).

A similar analysis procedure was adopted to investigate the sand goby data. Prior to the sand goby data analysis, all variables were normalized, and resemblance matrices based on Euclidean distance were calculated. The overall density, the density of each gender (male, female and juvenile), the density of *P. minutus* size classes and the size of the sand goby were statistically tested using a two-way PERMANOVA (type-III) in a two-way crossed design, with station as a fixed factor (3 levels: S1, S2 and S3) and season as a fixed factor (4 levels: winter, spring, summer and autumn). The feeding habit of the sand goby was statistically compared among genders (2 levels: males and females) and seasons (4 levels: winter, spring, summer and autumn) using one-way PERMANOVA (type-III) test. A one-way SIMPER routine was employed to verify the contribution of each food item for the dissimilarities among males and females. Also, a similarity index based on Euclidean distance was employed to group seasons according to the RA% of the food items consumed by the sand goby.

In PERMANOVA, the statistical significance of variance components was tested using 9999 permutations of residuals under a reduced model. When the possible number of permutations was lower than 150, the Monte Carlo-p value was considered. Subsequently, significant terms and interactions were investigated using a posteriori pair-wise comparisons to determine which pairs of stations and seasons were significantly different.

The PCA, SIMPER and PERMANOVA analyses were performed using PRIMER v 6.1.11® (Clarke and Gorley, 2006) with PERMANOVA+1.0.1. add-on package (Anderson et al. 2008).

Canonical Correspondence Analysis (CCA) was performed to determine the relationship between the density of *P. minutus* size classes and the density of males, females and juveniles with the measured environmental variables using CANOCO 5 software (ter Braak and Verdonschot 1995). The Monte-Carlo randomization test (499 permutations under the reduced model) was performed to determine the statistical significance of the correlations (see ter Braak 1986).

The population structure of *P. minutus* was defined by tracking recognizable cohorts from the successive sampling dates. Spatial samples were pooled together and analysed using the size frequency distribution of successive dates. The mixture analysis method was used to identify cohorts within size frequency distributions constructed from population samples in each month using the PAST® software. The mixture analysis is a maximum-likelihood method for estimating the parameters (mean, standard deviation and proportion), of univariate normal distributions, based on a pooled univariate sample. This software uses the EM algorithm described by Dempster et al. (1977) and considers a histogram of frequency as a mixture of probability density functions. The number of modes in each month was determined by visual analysis of the histogram.

Finally, the secondary production was computed using the size-frequency method (Hynes method). This method calculates production by summing the biomass lost between size classes (Krueger & Martin, 1980). In order to determine the annual production of *P. minutus* in the lower Minho estuary, the Krueger and Martin (1980) equation was applied:

$$(2) \qquad P = \sum_{j=1}^{a-1} 0.5\left[\left(Y_{i,j} + Y_{i+1,j}\right) - \left(Y_{i,j+1} + Y_{i+1,j+1}\right)\right].\left(W_j.W_{j+1}\right).(t)^{-1}$$

where *P* is the annual production of the species, $Y_{i,j}$ is the mean density (ind.m^{-2}) of the size class *j* in the date *i*, $Y_{i+1,j}$ is the mean density of the size class *j* in the date *i+1*, $Y_{i,,j+1}$ is the mean density of the size class *j+1* in the date *i*, $Y_{i+1,j+1}$ is the mean density of the size class *j+1* in the date *i+1*, W_j is the biomass (g.m^{-2}) of the size class *j*, W_{j+1} is the biomass of the size class *j+1* and t is the time between *i* and *i+1*. Population production estimates correspond to the sum of each size class production (*P*). Negative production values were not included in the overall estimates and were regarded as zero production.

5.3. Results

5.3.1. Abiotic data

The Two-way PERMANOVA for stations and seasons of all abiotic variables was significant for both main effects (stations: pseudo-*F* = 8.83, $p < 0.001$; seasons: pseudo-*F* = 6.81, $p < 0.001$), but was not significant for the *stations x seasons* interaction (pseudo-*F* = 0.53, p = 0.97).

Depth was significantly higher in S1 during all seasons, with significant lower depth being recorded during spring in this station. On the other hand, pH did not vary among sampling stations, but was significantly higher during summer compared to winter in S2 and S3. Salinity was higher in S1 during all seasons, and in S3 salinity significantly varied among seasons, due to the higher values observed in summer. Sampling stations presented similar values of temperature in all seasons except during summer, which presented warmer waters in S3; while during the winter, significantly colder waters were registered in all sampling stations. The ORP values did not vary among sampling stations and seasons (Table 5.1).

Table 5.1 Values of the five environmental variables (mean ± SE) measured in the three sampling stations throughout the seasons in the lower Minho estuary, NW Iberian Peninsula and the Two-way PERMANOVA results on the effects of sampling stations and seasons and their interaction term. * = $p < 0.01$; ** = $p < 0.001$; ^{ns} = non-significant.

	Seasons				Two-way PERMANOVA		
	Winter	Spring	Summer	Autumn	Stations	Seasons	Interaction
Depth							
S1	$4.2 \pm 0.3^{ac(A)}$	$3.1 \pm 0.3^{b(A)}$	$3.7 \pm 0.2^{ab(A)}$	$4.3 \pm 0.1^{c(A)}$	Pseudo-F = 57.78**	Pseudo-F = 6.87**	Pseudo-F = 0.97^{ns}
S2	$1.9 \pm 0.2^{a(B)}$	$1.3 \pm 0.2^{a(B)}$	$1.8 \pm 0.1^{a(B)}$	$1.8 \pm 0.1^{a(B)}$			
S3	$2.7 \pm 0.4^{a(B)}$	$1.9 \pm 0.3^{a(B)}$	$2.8 \pm 0.3^{a(C)}$	$2.1 \pm 0.1^{a(B)}$			
ORP							
S1	180.8±37.6	156.1±28.9	144.4±36.3	169.2±27.0	Pseudo-F = 0.09^{ns}	Pseudo-F = 0.57^{ns}	Pseudo-F = 0.02^{ns}
S2	189.1±45.6	153.6±32.0	159.5±41.5	173.4±19.0			
S3	201.2±52.2	159.9±31.5	167.2 ± 43.7	170.6±26.7			
pH							
S1	7.8 ± 0.2^{a}	7.8 ± 0.3^{a}	8.5 ± 0.3^{a}	8.0 ± 0.2^{a}	Pseudo-F = 0.39^{ns}	Pseudo-F = 8.77**	Pseudo-F = 0.20^{ns}
S2	7.8 ± 0.2^{a}	8.0 ± 0.2^{ab}	8.8 ± 0.3^{b}	8.1 ± 0.3^{ab}			

S3	7.7±0.1^{a}	8.1±0.2^{ab}	8.9±0.3^{b}	8.1±0.2^{ab}			
Salinity							
S1	25.8±6.5$^{a(A)}$	26.0±2.9$^{a(A)}$	32.0±1.0$^{a(A)}$	31.8±1.9$^{a(A)}$	Pseudo-F = 22.83**	Pseudo-F = 5.77*	Pseudo-F = 0.55^{ns}
S2	12.8±7.8$^{a(AB)}$	15.9±6.2$^{a(A)}$	30.8±1.9$^{a(A)}$	23.0±6.9$^{a(AB)}$			
S3	0.4±0.2$^{a(B)}$	0.4±0.2$^{a(B)}$	19.1±4.2$^{b(B)}$	4.3±4.2$^{ab(B)}$			
Temperature							
S1	11.9±0.9$^{a(A)}$	14.6±0.5$^{b(A)}$	15.0±0.6$^{b(A)}$	15.8±0.4$^{b(A)}$	Pseudo-F = 0.27^{ns}	Pseudo-F = 21.63**	Pseudo-F = 2.17^{ns}
S2	11.7±0.8$^{a(A)}$	16.4±0.9$^{b(A)}$	15.9±0.5$^{b(AB)}$	15.1±1.5$^{b(A)}$			
S3	9.6±0.8$^{a(A)}$	16.4±1.2$^{b(A)}$	18.3±1.2$^{b(B)}$	14.7±1.8$^{b(A)}$			

Moreover, the two-way SIMPER results revealed that near 60% of the dissimilarity between S1 and S2 was explained by depth and ORP values; while the dissimilarity between S1 and S3 was mostly explained by salinity and depth. On the other hand, dissimilarity between S2 and S3 was mainly explained by differences in ORP and salinity. The two-way SIMPER also revealed that temperature was the factor which most contributed to differences between the winter and other seasons (with dissimilarities ranging from 32.4 to 37.6%), while pH and ORP were the abiotic factors which contributed the most for the observed differences among the remaining seasons (Supplementary material 5.1).

The PCA routine indicated that the first two axis explained 62.3% of the variance among samples (PC1 = 34.2% and PC2 = 28.1%) and revealed marked differences between sampling stations, with station 1 differing from the other stations, mainly due to its lower values of temperature and pH and higher values of depth and salinity. The water temperature (eigenvalue = - 0.67) and the pH (eigenvalue = - 0.60) presented the strongest correlations in PC1, while salinity (eigenvalue = 0.71) and depth (eigenvalue = 0.70) were the most important variables in explaining the PC2 (Fig. 5.2).

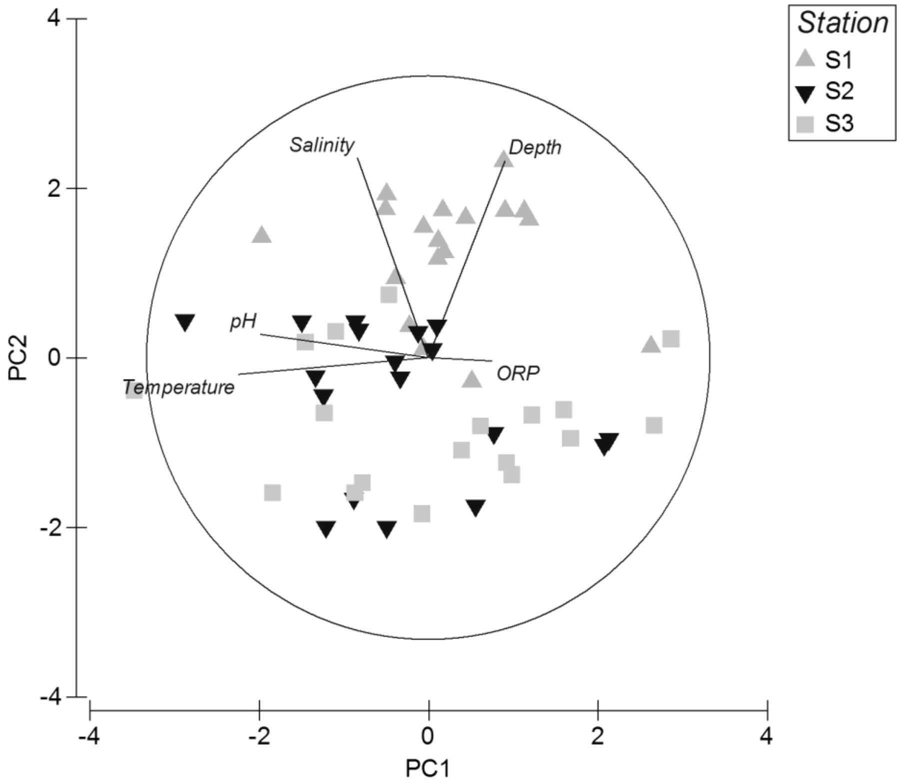


Fig. 5.2 PCA showing the differences of the environmental variables among sampling stations in the lower Minho Estuary, NW Iberian Peninsula.

5.3.2. Population structure

A total of 320 fishes were collected (station 1 = 88.7%, station 2 = 9.1% and station 3 = 2.2%). Of these, 42.2% were sampled during autumn, 27.5% in spring, 24.4%, in summer and 5.9% during winter. Overall, 282 fishes were sexed, with a proportion of 80.9% of males, 14.5% of females and 4.6% of juveniles.

5.3.2.1. Density patterns

The mean density of *P. minutus* in Minho estuary, considering the entire sampling period and the 3 stations, was 1.98 ± 0.45 ind.100m^{-2} (mean ± SE), with the highest density being recorded in December/09 (21.83 ± 5.80 ind.100m^{-2}) and the lowest in April/09 and July/09, when no sand goby was captured (Fig. 5.3a).

The Two-way PERMANOVA for stations and seasons of *P. minutus* density differences was significant for both main effects (stations: pseudo-F = 24.71, $p < 0.001$; seasons: pseudo-F = 5.21, $p < 0.01$) and for the *stations x seasons* interaction (pseudo-F = 4.66, $p < 0.001$). Overall, densities in S1 were higher than in S3 in all seasons except during spring (Two-way PERMANOVA, pairwise test: $p < 0.05$); also, the density in S1 was significantly higher than S2 during the summer and autumn (Two-way PERMANOVA, pairwise test: $p < 0.05$). When comparing the density of *P. minutus* among seasons within each sampling stations, S2 and S3 presented an even pattern, while in S1the significantly higher number of individuals were caught during the autumn (Two-way PERMANOVA, pairwise test: $p < 0.05$), also the *P. minutus* density was significantly higher during summer compared with winter (Two-way PERMANOVA, pairwise test: $p < 0.001$) (Fig. 5.3b).

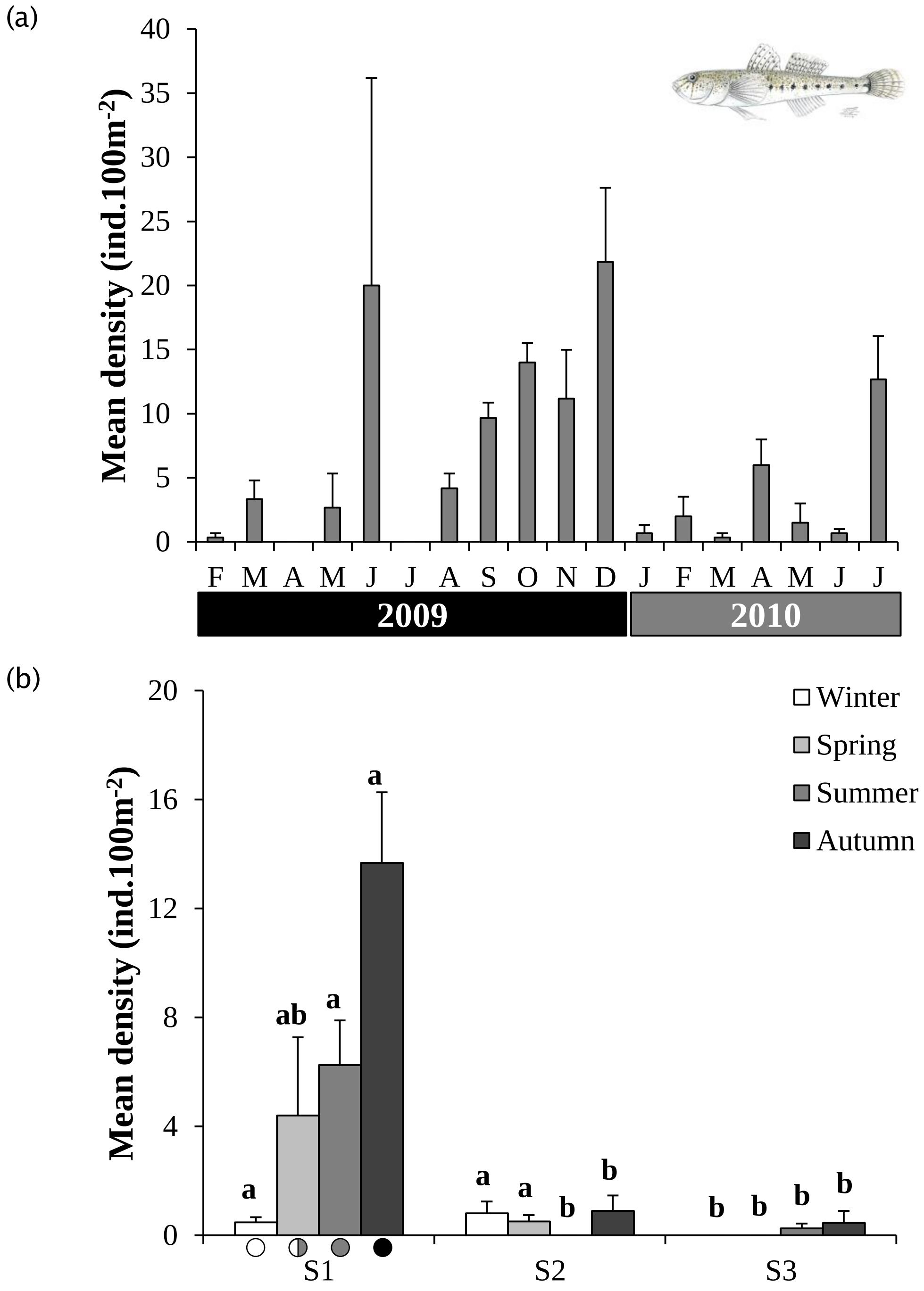


Fig. 5.3 (a) Mean density (± SE) of the sand goby *Pomatoschistus minutus* throughout 18 consecutive months in the lower Minho estuary, NW Iberian Peninsula. (b) Comparisons on the densities (mean ± SE) of *P. minutus* between sampling stations and seasons. Different letters (a or b) indicates post-hoc differences among sampling stations within the same season. Post-hoc differences among seasons within each sampling station are indicated by circles of different colours (white, grey or black) (Two-way PERMANOVA pairwise tests: $p < 0.05$).

The two-way PERMANOVA for the density of males, females and juveniles were significantly different for both the main effects (stations: pseudo-$F = 13.01$, $p < 0.001$; seasons: pseudo-$F = 3.40$, $p < 0.01$), and for the *stations x seasons* interaction (pseudo-$F = 2.44$, $p < 0.01$). Overall, males were denser in S1 and seasonal differences were only detected in S1, wherein significant higher values were recorded during autumn. On the other hand, females presented significant higher densities in S1 only in summer and autumn. Juveniles presented a somewhat even distribution among sampling stations and seasons, with significant differences being recorded only in S1 when comparing values of winter and autumn (Table 5.2).

Table 5.2 Comparisons on the density (mean ± SE) of males, females and juveniles of the sand goby *Pomatoschistus minutus* among three sampling stations and four seasons in Minho estuary, NW Iberian Peninsula. ^{ns} = non-significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test: $p < 0.05$).

	Seasons				Two-way PERMANOVA		
	Winter	Spring	Summer	Autumn	Stations	Seasons	Interaction
Male							
S1	$0.3\pm0.2^{a(A)}$	$3.3\pm2.4^{ab(A)}$	$4.3\pm1.3^{b(A)}$	$11.4\pm2.5^{c(A)}$	Pseudo-F = 22.89^{***}	Pseudo-F = 4.64^{**}	Pseudo-F = 4.98^{***}
S2	$0.7\pm0.4^{a(AB)}$	$0.1\pm0.1^{a(A)}$	$0.0\pm0.0^{a(B)}$	$0.2\pm0.2^{a(B)}$			
S3	$0.0\pm0.0^{a(B)}$	$0.0\pm0.0^{a(A)}$	$0.0\pm0.0^{a(B)}$	$0.1\pm0.1^{a(B)}$			
Female							

S1	0.1±0.1$^{a(A)}$	0.5±0.3$^{ab(A)}$	1.3±0.6$^{b(A)}$	1.1±0.4$^{b(A)}$	Pseudo-F = 14.35***	Pseudo-F = 3.07*	Pseudo-F = 2.31*
S2	0.0±0.0$^{a(A)}$	0.1±0.1$^{a(AB)}$	0.0±0.0$^{a(B)}$	0.1±0.1$^{a(B)}$			
S3	0.0±0.0$^{a(A)}$	0.0±0.0$^{a(B)}$	0.2±0.1$^{a(AB)}$	0.0±0.0$^{a(B)}$			
Juvenile							
S1	0.1±0.1$^{a(A)}$	0. 6±0.4$^{ab(A)}$	0.7±0.4$^{ab(A)}$	1.1±0.4$^{b(A)}$	Pseudo-F = 4.46*	Pseudo-F = 2.74*	Pseudo-F = 0.66^{ns}
S2	0.1±0.1$^{a(A)}$	0.3±0.1$^{a(A)}$	0.0±0.0$^{a(A)}$	0.6±0.6$^{a(A)}$			
S3	0.0±0.0$^{a(A)}$	0.0±0.0$^{a(A)}$	0.1±0.1$^{a(A)}$	0.3±0.3$^{a(A)}$			

5.3.2.2. Size and recruitment patterns

The range of T*L* varied between 20.76 and 86.20 mm, with an average value of 48.90 mm. Overall, fish size significantly varied among genders (One-way PERMANOVA: pseudo-*F* = 23.63, $p < 0.001$), with males (52.5 ± 1.1 mm), females (39.4 ± 1.8 mm) and juveniles (24.8 ± 0.6 mm) being different among each other.

The two-way PERMANOVA for differences in *P. minutus* T*L* revealed that fish size significant varied among sampling stations and seasons (*stations x seasons* interaction: pseudo-*F* = 4.49, $p < 0.01$). Individual comparisons among seasons and stations could not be performed due to the absence of fishes in S2 during summer and during winter and spring in S3. Nevertheless, pairwise comparisons revealed that fish size varied among seasons within S1 and S2, with larger sand gobies being captured during winter and autumn. Also, fish size decreased according to the distance from the river mouth, with larger individuals being recorded in S1 and smaller in S3 (Fig. 5.4).

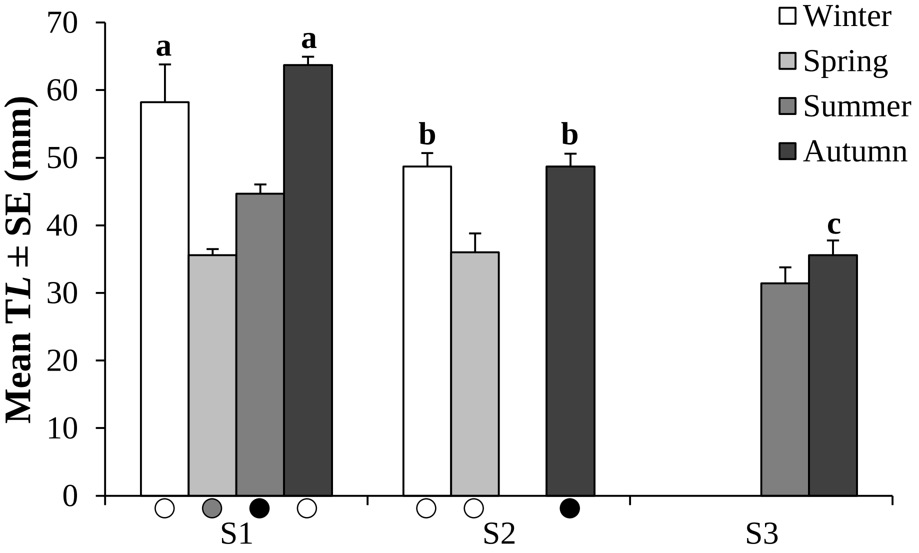
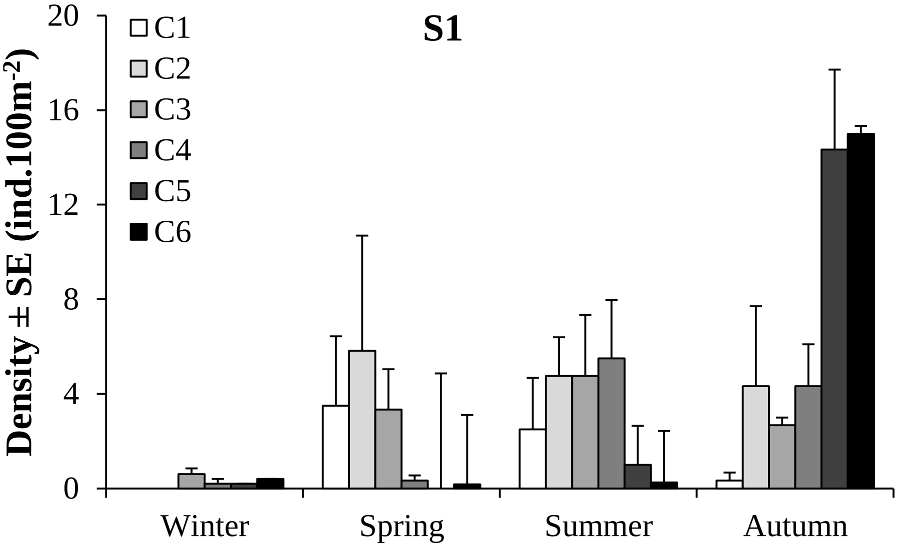
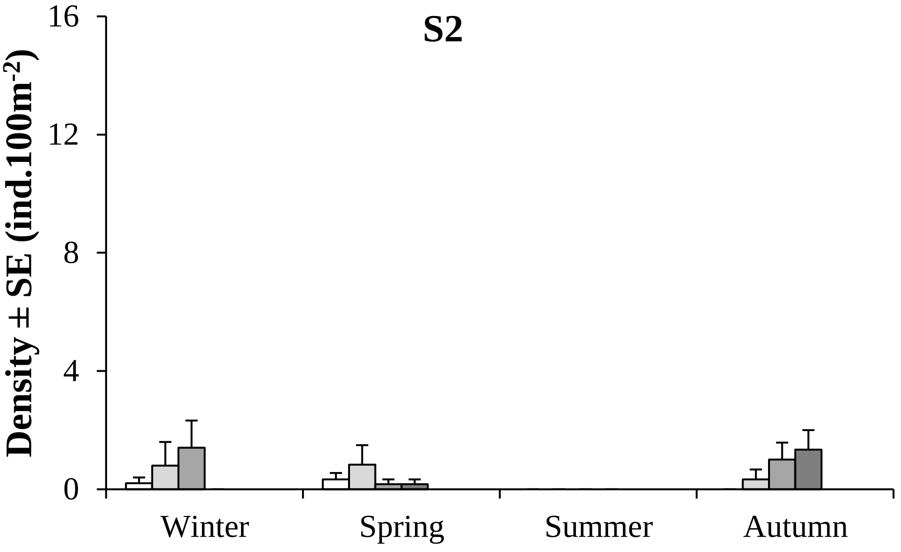


Fig. 5.4 Comparisons on the total length (mean ± SE) of *P. minutus* between sampling stations and seasons. Different letters (a, b or c) indicates post-hoc differences among sampling stations within the same season. Post-hoc differences among seasons within each sampling station are indicated by circles of different colours (white, grey or black) (Two-way PERMANOVA pairwise tests: $p < 0.05$).

The two-way PERMANOVA for the size classes density of *P. minutus* were significantly different for both the main effects (stations: pseudo-$F = 7.21$, $p < 0.001$; seasons: pseudo-$F = 2.88$, $p < 0.01$), and for the *stations x seasons* interaction (pseudo-$F = 2.96$, $p < 0.001$). Overall, size classes C5 and C6 were only recorded in S1, wherein they reached higher values during autumn. On the other hand, S2 was dominated by smaller fish, belonging to C1, C2, C3 and C4 size classes; whereas in S3 only C1 and C2 size classes were present (Fig. 5.5).





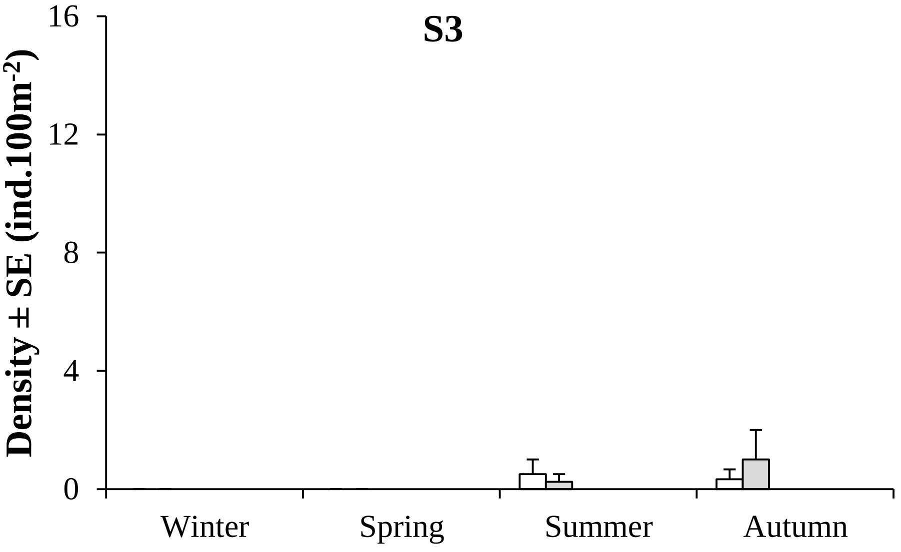


Fig. 5.5 Mean density ± SE of the six size classes of the sand goby *Pomatoschistus minutus* among seasons in each sampling station of the lower Minho estuary, NW Iberian Peninsula. Size classes: C1 (< 29.99 mm), C2 (30.00 to 39.99 mm), C3 (40.00 to 49.99 mm), C4 (50.00 to 59.99 mm), C5 (60.00 to 69.99 mm) and C6 (> 70.00 mm).

The density of small fishes (C1) was high during spring of 2009, with a second peak of abundance being recorded in the spring of 2010. On the other hand, larger fishes (C6) were mainly found during autumn. Overall, fishes larger than 50 mm of T*L* (C4, C5 and C6) were captured exclusively during summer and autumn of 2009, while smaller fishes were found throughout almost the entire sampling period (Fig. 5.6a). The mixture analysis revealed that *P. minutus* recruited once a year in Minho estuary, with recruitment occurring in different seasons in 2009 and 2010: new recruits were detected during spring 2009 (May), while in 2010, recruits were detected in winter (February) (Fig. 5.6b).

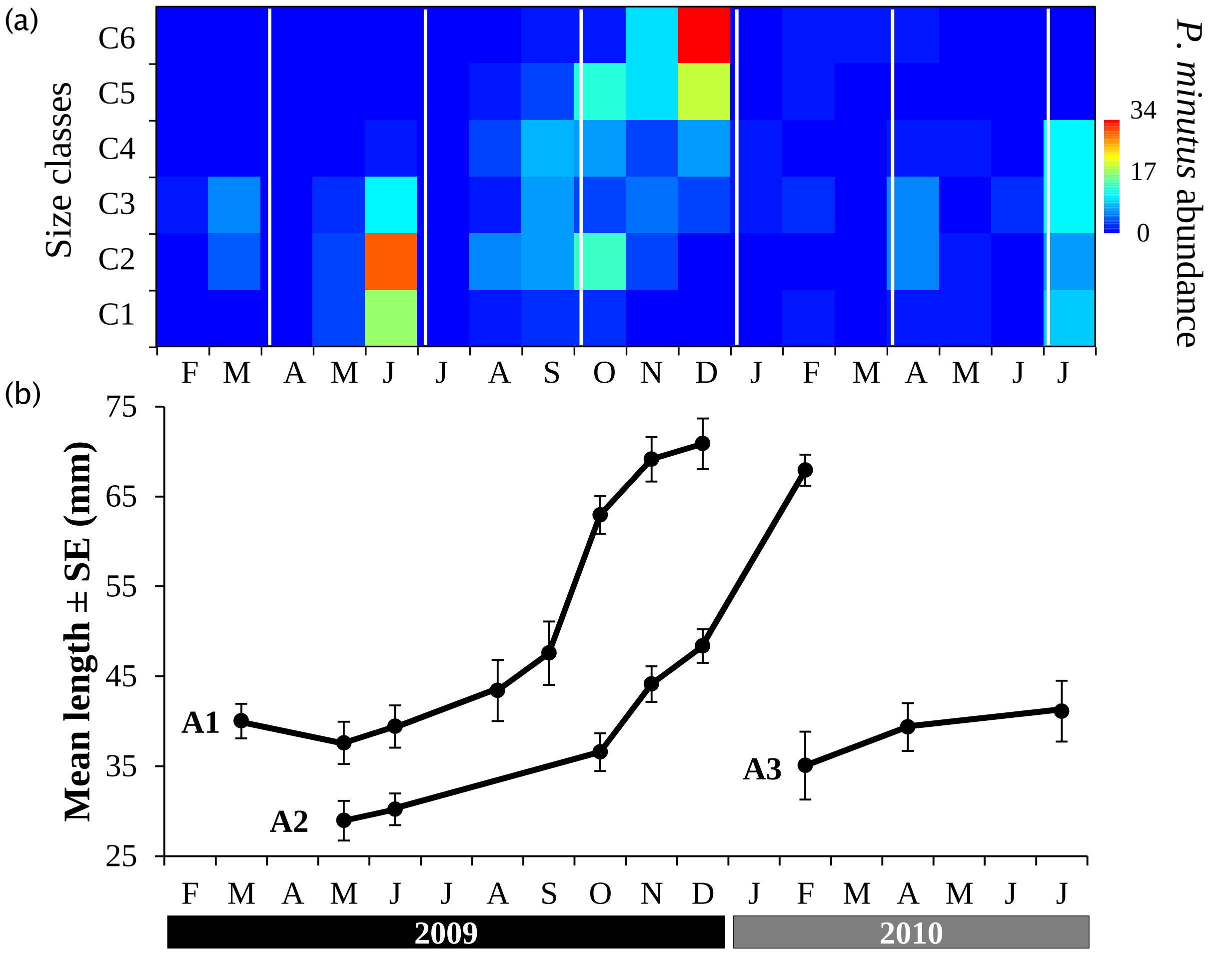


Fig. 5.6 (a) Density of different size classes of the sand goby *Pomatoschistus minutus* throughout the 18 months sampled in Minho estuary, NW Iberian Peninsula. Vertical white bars separate different seasons. Size classes: C1 (< 29.99 mm), C2 (30.00 to 39.99 mm), C3 (40.00 to 49.99 mm), C4 (50.00 to 59.99 mm), C5 (60.00 to 69.99 mm) and C6 (> 70.00 mm). (b) Mean cohort length of *P. minutus* in Minho estuary, with indication of the three cohorts identified by the mixture analysis (A1, A2 and A3).

Only 6 ovigerous females were recorded during the entire sampling period (1.9% of the individuals caught), being all found in S1. The T*L* of ovigerous females varied between 39.1 and 82.6 mm, with an average value of 50.9 ± 6.9 mm (mean ± SE).

5.3.2.3. Fish-habitat association and secondary production

Overall, *P. minutus* genders associated differentially with environmental variables indicating that each group has dissimilar habitat preferences. Juveniles showed a marked preference for warmer, shallower and less saline waters located farther from the river mouth; whereas females tended to be associated with waters presenting higher values of pH and lower values of ORP and river discharge. On the other hand, males tended to be associated with deeper, colder and saltier waters, preferentially located near the river mouth (Fig. 5.7a). The Monte-Carlo test was significant (test of significance of all canonical axes: trace = 0.233; F-ratio = 2.669; $p < 0.01$) and cumulatively, axes 1 and 2 accounted for 53.9% of the total variance, with correlations between *P. minutus* genders and environmental variables of 0.790 (axis 1) and 0.583 (axis 2). Also, a partitioning pattern between large and small fishes was recorded, with size classes associating differently with environmental variables. The larger fishes (C5 and C6) tended to be associated with higher depths and river discharges, whereas smaller fishes (C1, C2, C3 and C4) tended to be associated with high values of pH, lower depth and upstream areas of the lower estuary (Fig. 5.7b). The Monte-Carlo test was significant (test of significance of all canonical axes: trace = 0.781; F-ratio = 4.705; $p < 0.01$) and cumulatively, axes 1 and 2 accounted for 60.3% of the total variance, with correlations between *P. minutus* size classes and environmental variables of 0.963 (axis 1) and 0.745 (axis 2).

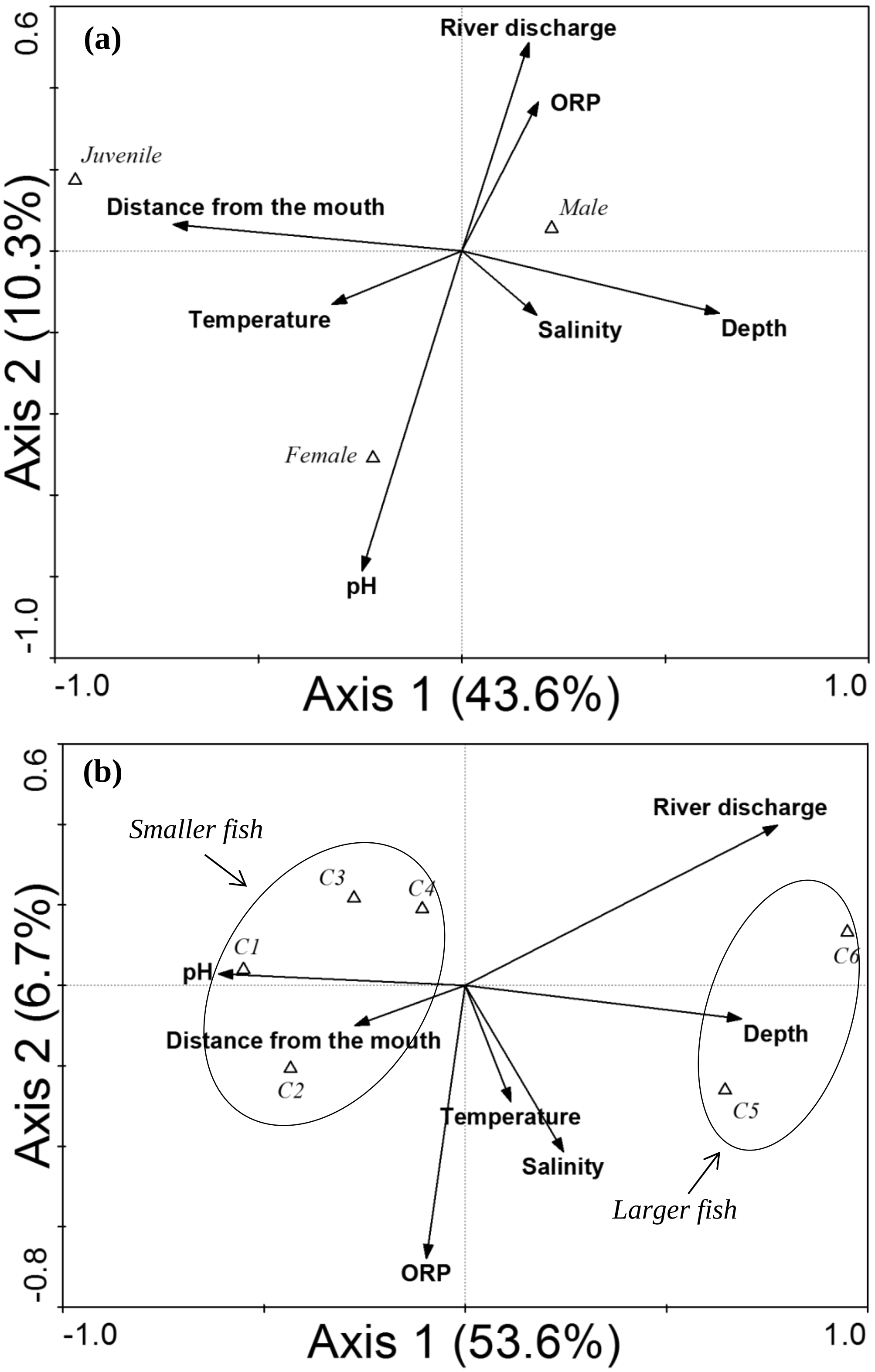


Fig. 5.7 Canonical plotting of environmental characteristics (arrows) and males, females and juveniles (a) and different size classes (b) of the sand goby *Pomatoschistus minutus* in the lower Minho estuary, NW Iberian Peninsula. ORP = Redox potential. Size classes: C1 (< 29.99 mm), C2 (30.00 to 39.99 mm), C3

(40.00 to 49.99 mm), C4 (50.00 to 59.99 mm), C5 (60.00 to 69.99 mm) and C6 (> 70.00 mm).

The overall secondary production of *P. minutus* in Minho estuary was estimated in 0.231 g.WWm^{-2}, with an annual production of 0.164 g.WWm^{-2}.year^{-1}.

5.3.3. Feeding habits

A total of 138 *P. minutus* individuals (88.4% males and 11.6% females) had their stomach contents analysed. 32 fishes had their stomachs empty (vacuity index = 23.2%). Gut content analyses revealed that the sand goby fed on 23 different food items, with males feeding on 22 items and females feeding only on 7 different items (Table 5.3).

Table 5.3 Frequency of occurrence (FO%) and relative abundance (RA%) of the 23 food items consumed by males and females of the sand goby *Pomatoschistus minutus* in Minho estuary, NW Iberian Peninsula. NI = Non-identified.

	Male		Female	
Food item	FO%	RA%	FO%	RA%
Annelids				
Annelidae NI	9.1	7.9	-	-
Polychaeta	6.6	6.4	6.7	5.3
Molluscs				
Bivalve NI	0.1	0.7	-	-
Gastropoda	1.7	1.4	-	-
Mytilus sp.	-	-	6.7	5.3
Arachnids				
Aracnidae NI	0.8	0.7	-	-
Crustaceans				
Amphipoda NI	5.8	5.7	-	-
Cyathura carinata	1.7	1.4	-	-
Crangon crangon	6.6	5.7	-	-
Carcinus maenas	0.8	1.4	-	-
Copepoda	4.1	7.9	-	-

Corophium multisetosum	1.7	1.4	-	-
Crustacea NI	12.4	11.4	13.3	10.5
Gammaridae	0.8	0.7	-	-
Gnathiidae	0.8	0.7	-	-
Idoitea	0.8	0.7	-	-
Isopoda NI	1.7	1.4	-	-
Ostracoda	2.5	3.6	6.7	5.3
Insects				
Insect larvae	0.8	0.7	20.0	21.1
Heterometabole	0.8	2.1	-	-
Fishes				
Teleostei	0.8	0.7	-	-
Detritus	28.1	24.3	20.0	15.8
NI	14.9	12.9	46.7	36.8

The frequency of occurrence of food items belonging to different functional groups were different between genders, with crustaceans and detritus representing the highest percentages for males, while NI (non-identified) represented almost the 50% of the females' diet. Males and females presented similar diets (one-way PERMANOVA: Pseudo-*F* = 0.46, p = 0.75). Nevertheless, genders presented slight differences in food item consumption (SIMPER test: Overall average dissimilarity of 87.8%), with 6 food items (NI, Detritus, Insect larvae, Crustacea NI, Polychaeta and Ostracoda) being responsible for more than 75% of the observed dissimilarities found. Considering the functional groups, the SIMPER routine revealed a dissimilarity of 80.4%, with crustaceans being the food item which most contributed to the differences observed between males and females (Table 5.4).

Table 5.4 SIMPER percentages of the food items that most contributed to the differences of the diet between males and females of the sand goby *Pomatoschistus minutus* in the lower Minho estuary (NW Iberian Peninsula).

Food item	Dissimilarity (%)	Cumulative dissimilarity (%)	Male – RA%	Female – RA%
Crustaceans	34.0	34.0	42.1	15.8
NI	21.5	55.4	12.9	36.8

Detritus	20.8	76.2	24.3	15.8
Annelids	9.9	86.2	14.3	5.3
Insects	9.7	95.9	2.9	21.1
Molluscs	3.6	99.5	2.1	5.3
Others	0.5	100	1.4	0.0

Since males and females did not differ in their feeding habits, data was pooled to compare the diet of *P. minutus* among seasons. The one-way PERMANOVA indicated that the sand goby presented an even diet through seasons (Pseudo-*F* = 1.48; p = 0.12).

Despite of no significant differences being detected, the cluster analysis indicated that the diet of the sand goby during summer and autumn were similar, consisting in at least 40% of NI and detritus, whereas during spring these items accounted for 20%, and annelids summed 30% of the total (Fig. 5.8).

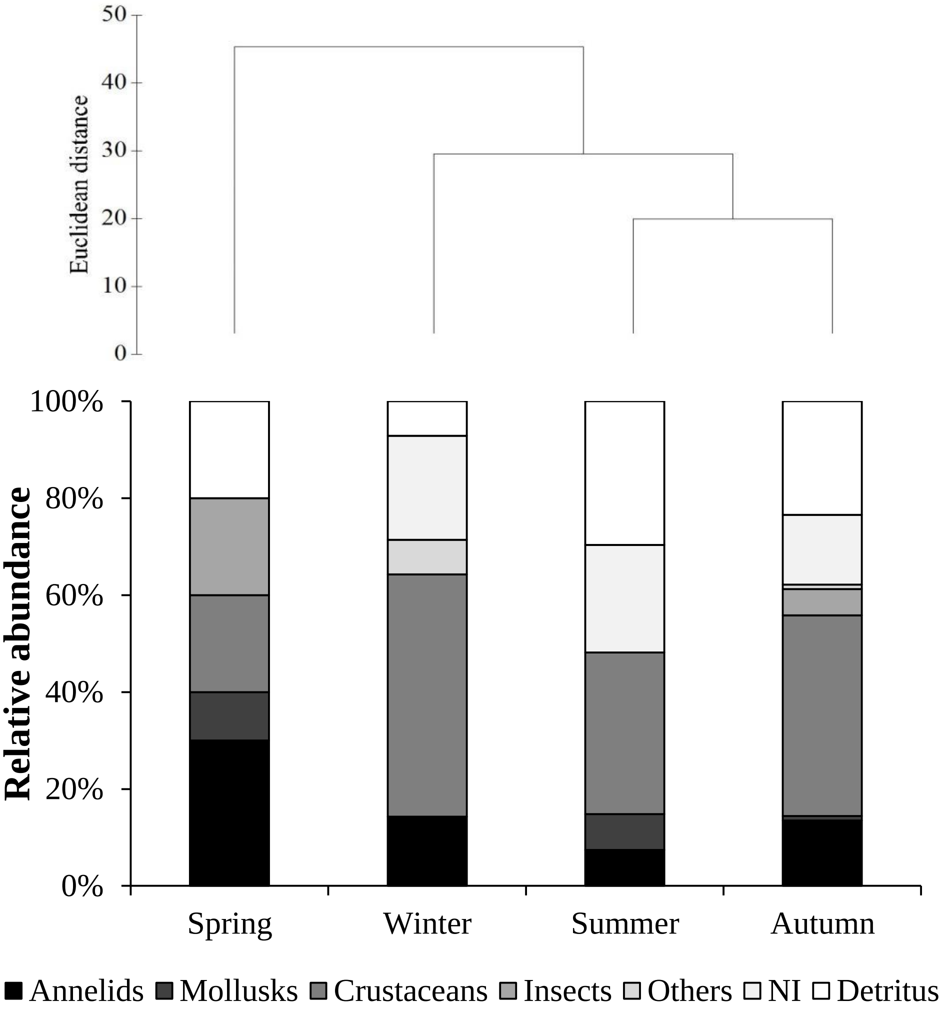


Fig. 5.8 Relative abundance of the food items (functional groups) consumed by the sand goby *Pomatoschistus minutus* in the lower Minho estuary, NW Iberian Peninsula. Others = Sum of fishes and arachnids. The upper portion of the figure refers to the similarity index between seasons.

5.4. Discussion

The sand goby population presented spatial and temporal differences in its distribution in Minho estuary. Spatially, *P. minutus* showed a clear preference for S1, which is near the river mouth, with density values being 7 and 27 times higher than in S2 and S3 respectively. The higher density of *P. minutus* in S1 probably is related with high salinity and depth values found at this station, corroborating with previous studies which report a preference for saltier and deeper waters within estuaries (Leitão et al. 2006; Dolbeth et al. 2007b). Moreover, in S1 there is a high amount of debris and drifting seaweeds, which possibly can function as a refuge or shelter for the sand goby.

The density pattern of *P. minutus* can also be directly related to the presence of the sympatric species *P. microps*, which showed an opposite pattern of distribution, with lower densities in S1 and higher densities in S2 and S3 (Souza et al. in revision). The competition theory states that similar sympatric species compete for similar resources and, therefore, tend to avoid each other by fine-scale niche partitioning (Dobzhansky 1950; Colwell 1973). In fact, these species are known to present some degree of niche and diet overlap (Leitão et al. 2006; Złoch and Sapota 2010). Therefore, the two species might distribute in different estuarine zones, thus avoiding the negative impacts of competition for space and/or food. Moreover, the sand goby is a less tolerant species to salinity and temperature fluctuations when compared to the common goby, thus *P. minutus* prefers to inhabit estuarine zones with higher levels of salinity or the sea, whereas *P. microps* is preferentially found in brackish waters (Fonds and Van Buurt 1974; Pampoulie et al. 1999). Leitão et al. (2006) studying the population structure of *P. microps* and *P. minutus* in Mondego estuary (located 190 km southward from Minho estuary) found an almost even density of both species, and a similar spatial distribution within the Mondego estuary, although *P. minutus* was mostly found near the river mouth, while *P. microps* was more abundant in upper areas of the estuary. According to our data (Souza et al. unpublished data), in Minho estuary, the density of *P. minutus* is 60 times lower than the density of *P. microps*. This fact might be related to some abiotic characteristics of Minho estuary, namely, the occurrence of high values of river discharge and low values of salinity, especially during winter (see Ferreira et al. 2003). Since the sand goby is less tolerant to low salinity values than the common goby, the former species may be restricted to a small portion of the estuary, while the later species take advantage of the low densities of the sympatric species in the upper portion of the lower estuary and reaches remarkable high densities (Souza et al. unpublished data). Nevertheless, the density of *P. minutus* recorded in this study is higher than those reported in other studies conducted in nearby estuarine systems, but much lower when comparing to Northern European systems (Table 5.5).

Table 5.5 Mean density of the sand goby *Pomatoschistus minutus* in different locations in Atlantic estuaries.

Location	Mean density (ind.100m^{-2})	Study period	Day period	Sampling gear	Reference
Wadden Sea, Germany	40	1991/92	Diurnal and Nocturnal	Beam trawl	Norte-Campos and Temming 1994
Tagus estuary, Portugal	11.8	2005	Diurnal and Nocturnal	Encircling nets	França et al. 2008
Minho estuary, Portugal	1.98	2009/10	Diurnal	Beam trawl	This study
Mondego estuary, Portugal	0.59	2003/04	Nocturnal	Beam trawl	Leitão et al. 2006
Mondego estuary, Portugal	0.37	2003/06	Nocturnal	Beam trawl	Martinho et al. 2007b
Minho estuary	0.37	2003/07	Diurnal	Beam trawl	Dolbeth et al. 2010

The peak of density of *P. minutus* in Minho occurred during autumn, similarly to other locations (Healey 1971; Maes et al. 2005). Additionally, other species in Minho estuary also present density peaks during autumn, namely the shore crab *Carcinus maenas*, the common goby *P. microps* and the European flounder *Platichthys flesus* (Souza et al. unpublished data), with a remarkable drop being recorded during the 2010th winter. This dramatic decline in the density occurred immediately after an unusual raining event in January/10, which was also related to important die-off of other aquatic species indeed (Sousa et al. 2012b).

In Minho estuary, the recruitment of *P. minutus* occurred once per year, while in other Portuguese coastal and estuarine systems (Ria de Aveiro lagoon and Mondego estuary), the recruitment of this species occurs twice per year (Arruda et al. 1993; Leitão et al. 2006; Dolbeth et al. 2010). Differences in recruitment of estuarine fishes between Minho and Mondego estuaries were previously recorded, and usually recruitment seasons tend to occur later and growth rates are lower in Minho compared to Mondego estuary, probably due to temperature differences between estuaries (see Dolbeth et al. 2010). However, recruitment differences may also be related to salinity dissimilarities between estuaries, once *P. minutus* reproduction occurs when salinity is between 15 and 35, while their eggs dies in salinities near 5 (Fonds and Van Buurt 1974). Actually, in Minho estuary, the optimal temperature (between 8 and 15ºC) and salinity (between 15 and 35) levels for the sand goby reproduction (Fonds and Van Buurt 1974) is limited to one short time interval per year, occurring between February and August (2009) or between February and May (2010) in S1, and was limited to April in 2009 and spanned from February to April in 2010 in S2. Consequently, it is more likely that recruitment occurs once per year in Minho estuary between late winter and spring. Also, the scarcity of ovigerous inside the estuary might indicate that reproduction occurs preferably in the sea, probably during late autumn and winter.

Differently from Dolbeth et al. (2007), which did not found spatial segregation among males, females and juveniles in Mondego estuary, in the present study, a marked difference in habitat preference of these groups were found. Juveniles were more often found in upper portions of the lower estuary, which could be associated with the shelter and protection against larger predators often present in low densities in these areas (e.g. Hamerlynck 1990).

Regarding males and females, different patterns were also recorded, indicating that genders may have different habitat preferences, which probably is related to differences in behaviour and ecophysiological requirements. The sand goby males display a marked parental care, vigorously guarding the eggs which are laid on empty bivalve shells (Pampoulie et al. 2004). Therefore, it should be more likely to find males in areas where bivalve shells are more abundant, which is the case of S1, where a high amount of debris occur possibly due to high river discharge (Ferreira et al. 2003). On contrary, females were more abundant at shallow and warmer waters of the estuary.

As well as the density, the secondary production of *P. minutus* was higher than in other nearby estuaries. In fact, production in Minho estuary was 16 times higher than in Mondego (see Dolbeth et al. 2010). Interestingly, the average size of sand gobies in Minho was smaller than in Mondego estuary (Leitão et al. 2006; Dolbeth et al. 2007b). These results suggest that Minho estuary may serve as an important nursery area for the sand goby. Actually, Minho estuary was previously recognized as a significant nursery in southern Europe for the shore crab *C. maenas* (Souza et al. unpublished data), indicating that this estuary is in fact an important nursery for euryhaline species.

The diet of *P. minutus* in Minho estuary was similar to what was reported in other studies, where crustaceans were the main food item consumed by the sand goby (Hamerlynck and Cattrijsse 1994; Salgado et al. 2004; Leitão et al. 2006). Also, the differential food consumption of the sand goby during the spring may be related to the reproduction. Actually, Salgado et al. (2004) noted that the feeding habits of *P. minutus* varied during reproduction, because males exhibit parental care of the eggs. Since the majority of the individuals analysed in the present study were males, our results are in line with those reported by Salgado et al. (2004).

5.5. Acknowledgements

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CHAPTER 6

POPULATION ECOLOGY AND HABITAT PREFERENCES OF JUVENILE FLOUNDER *PLATICHTHYS FLESUS* (ACTINOPTERYGII: PLEURONECTIDAE) IN A TEMPERATE ESTUARY

Population ecology and habitat preferences of juvenile flounder Platichthys flesus (Actinopterygii: Pleuronectidae) in a temperate estuary

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Abstract

The European flounder *Platichthys flesus* is a widely distributed epibenthic species and an important component of demersal fish assemblages in the European Atlantic coastal waters. In Portuguese estuaries, this species reaches high densities, especially in Minho estuary (NW Iberian Peninsula, Europe), potentially playing an important role in the system's ecology. In this context, the population structure, production and the habitat use of juvenile *P. flesus* were investigated. Sampling took place monthly, from February 2009 until July 2010 along the entire estuarine gradient (5 sampling stations distributed in the first 29 km from the river mouth, with S1 located near the river mouth, S2 inside a salt marsh, S3 in a salinity transition zone, while S4 and S5 were located in the upper estuary). Flounder's density varied significantly among sampling stations and seasons (Two-way PERMANOVA: $p < 0.001$), with the majority of the individuals being found during the spring (30.1%) and in S3 and S4 (72.6%). Males and females presented an even distribution, with a higher proportion of males observed during summer. Fish length also differed among sampling stations and seasons (Two-way PERMANOVA: $p < 0.001$), with larger fishes being found in S1 during the autumn (168.50 ± 59.50 mm) and the smallest in S4 during the spring (33.80 ± 3.12 mm). Size classes associated differently with environmental variables, with larger juveniles being more abundant in the downstream areas of the estuary, whereas smaller juveniles were related to higher water temperatures, suggesting a habitat segregation of *P. flesus* of different sizes. The fish condition of *P. flesus* in Minho estuary was higher than in other systems, probably due to the dominance of juveniles on the population. Also, the densities found in this estuary were up to 32 times higher than in other locations, suggesting that Minho estuary is an important nursery area for the species. The estimated secondary production of *P. flesus* was lower than previous studies acknowledged in the system ($0.037\ g.WWm^{-2}.year^{-1}$), indicating that the production of this species in estuaries can vary considerably depending of several factors such as the sampling year and strategy, population and fish size.

Keywords: flatfish / migration / nursery ground / secondary production / Portugal

6.1. Introduction

The spatial distribution of organisms is generally not homogeneous in space and time as a result of habitat heterogeneity and complex mechanisms (Chesson, 1998). Environmental-driven habitat selection is directly related to individual-level responses to different biotic and abiotic features that unequally are distributed in space across habitats. This results in how different proportions of a population use different habitats (Křivan and Sirot 2002). Habitat features such as depth, current, substratum type (e.g. Schlosser 1982; Bain et al. 1988; Ramos et al. 2009), presence of shelter or habitat diversity (Gorman and Karr 1978; Mérigoux et al. 1998) may play a major role in shaping fish population in space; whereas precipitation, river discharge, water temperature and salinity (e.g. Matthews 1998; Martinho et al. 2009) are the main physical parameters structuring fish population in time.

In estuaries, the habitat heterogeneity is particularly high, with marked dissimilarities in space and time of the abiotic parameters such as salinity, temperature, flow and tidal energy; and therefore for the associated flora and fauna (Hoffman et al. 2008). Additionally, estuaries contain diverse aquatic habitat types, including seagrass, kelp and shellfish beds, hard-bottom, soft bottom communities with mud and sand, rocky inter-tidal zones, fringing mangrove forests and vegetated marshes/wetlands (Nelson and Monaco 2000). Thus, the heterogeneity of this ecosystem is ideal for developing generalities about population ecology and habitat preferences, especially for those euryhaline species which can inhabit the saline, brackish and the freshwater portions of the estuary.

Estuaries are also highly productive environments that provide important forage, spawning, refuge, and nursery habitat for commercial, recreational, and forage fish species during one or more of their life history stages (Chambers 1992; Nelson and Monaco 2000; Able 2005). Thus, estuaries are considered an essential habitat for many fish species at various stages in their development (Able 2005).

Interestingly, several fish species also change their habitat preferences during growth, with habitat relationships associated with particular life history stages reflecting the changes in morphology, physiology and trophic guild membership that occur throughout ontogeny (Swain et al. 1998; Garrison and Link 2000).

Flatfishes of the Pleuronectidae family are a worldwide spread group of fishes, occurring in fresh, brackish and marine waters. Pleuronectidae includes 104 species distributed in 41 genera (Froese and Pauly 2012), and it is among the most ecological relevant fish family in European estuaries. Therefore, its ecology has received much attention in several estuarine systems across the continent (e.g. Becker 1988; Van der Veer et al. 1991; Modin and Pihl 1996; Cabral et al. 2007; Ramos et al. 2009, 2010).

One of the most important flatfish species from European waters is the European flounder *Platichthys flesus*. This flounder is a widely distributed species in coastal and brackish waters, naturally occurring in the Black Sea, the Mediterranean Sea, the European Atlantic Coast (including the British Isles and Ireland), the North, the Baltic, the Barents and the White Sea (Nielsen 1986; Rochard and Elie 1994). The described distribution of *P. flesus* in the Atlantic waters ranges from Norway to Morocco (Nielsen 1986), but more recently, other studies pointed out that the northern and central coastal areas of Portugal are more likely to be the current southern limit for its distribution (Cabral et al. 2007; Dolbeth et al. 2008). Some of these estuaries were already identified as important nursery grounds for flounder, particularly Mondego, Ria de Aveiro, Douro, Lima and Minho (Cabral et al. 2007; Vasconcelos et al. 2008, 2010; Freitas et al. 2009; Ramos et al. 2010). From these, Minho estuary has the highest density values, 2.93 ind.100m^{-2} (Freitas et al. 2009), which are up to 11 times higher than the maximum values found in other Portuguese estuaries (e.g. Vinagre et al. 2005; Pombo et al. 2007; Martinho et al. 2007b; Ramos et al. 2010; Vasconcelos et al. 2010; França et al. 2011).

Freitas et al. (2009) found that *P. flesus* population in Minho estuary is largely dominated by juveniles of 0- and 1-year group individuals, with upstream zones (freshwater) of the estuary being the preferred by the species. Moreover, in a recent study on flounder migration patterns, it is discussed the possibility of Minho estuary to be also a spawning area for this species, rather than just a nursery and feeding ground, as previously described (Morais et al. 2011). The European flounder have been receiving an increasing attention by scientists in the past years (e.g. Freitas et al. 2009; Morais et al. 2011). Nevertheless, there are still lacks of information in the patterns of population function and structure in Minho estuary, particularly related to fish-habitat association, recruitment and secondary production. Due to the economic and ecological relevance of *P. flesus*, the continuous monitoring of the different populations across the entire range of distribution is recommended by the IUCN to determine stock status of the species (Munroe 2010). In this context, the present field-based study aimed to assess the habitat use of the European flounder in an estuary where the species reaches remarkable high values of density (Freitas et al. 2009). Our goals were twofold: (a) to investigate the population structure, the recruitment pattern, the secondary production and the distributional patterns of *P. flesus* juveniles along the entire salinity gradient (saline, brackish and freshwater) and (b) to investigate the spatio-temporal habitat preferences of the European flounder juveniles of different sizes. By focusing on the distributional patterns of *P. flesus* within a system where the species seems to be especially abundant, we expect to increase the understanding on the ecology of a key-species in European estuaries.

6.2. Material and Methods

6.2.1. Study area and sampling campaign

The Minho estuary is located in the NW-Iberian Peninsula (SW Europe) and covers a total area of 23 km^2. The limit of tidal influence is about 40 km inland, and the uppermost 30 km are a tidal freshwater wetland. The estuary is mesotidal, with tides ranging between 0.7 m and 3.7 m (Alves 1996). The mean depth of the estuary is 2.6 m and the maximum depth is about 26 m (Antunes et al. 2011), and the average annual freshwater run-off is about 300 m^3.s^{-1} (Ferreira et al. 2003).

The present study was carried out in five stations along the entire salinity gradient. Sampling was conducted during 18 consecutive months, from February/09 to July/10 in four subtidal areas (S1, S2, S3 and S4) and during 12 consecutive months (from February/09 to January/10) in the most upstream sampling station (S5) (Fig. 6.1). The five stations were selected in order to assess possible dissimilarities on the population structure related to differences in the habitat type and environmental characteristics.

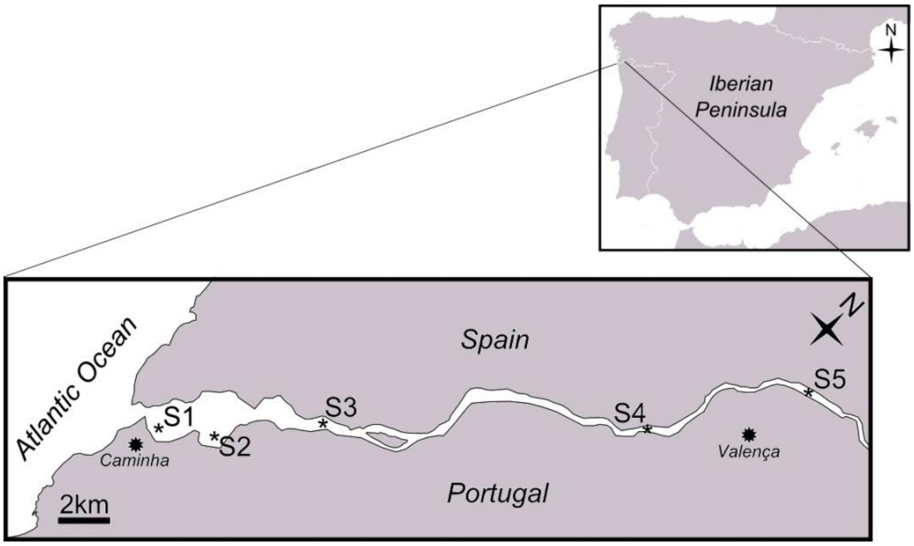

Fig. 6.1 Map of the study site showing the five sampling stations in Minho estuary, NW Iberian Peninsula.

S1 is located near the river mouth (ca. 1.5km), and characterized by muddy soft bottoms often densely covered by debris, such as drifting seaweeds, dead leaves and empty mollusks' shells (Souza et al. 2011). S2 is located inside the Coura salt marsh (ca. 3.5km apart from the river Minho mouth), which is a relatively small sub-system characterized by the presence of narrow channels, typically bordered by the seagrass *Spartina maritima*. The channels' soft bottom is sparsely covered by debris and empty peppery furrow shells *Scrobicularia plana* (Souza et al. 2011). S3 is located ca. 8 km upstream from the river mouth and characterized by presenting high densities of the Asian clam *Corbicula fluminea* and by the soft bottom sparsely covered by debris and submerged vegetation (Sousa et al. 2008b,c; Ilarri et al. 2012). S4 is located in the freshwater portion of the estuary (ca. 21 km upstream from the river mouth) and characterized by presenting high densities of submerged vegetation, specially water crowfoots (*Ranunculus* sp.) and high densities of the Asian clam *C. fluminea*. S5 is located ca. 29 km upstream from the river mouth and ca. 1 km upstream from the Louro river (tributary to the Minho river). This station is characterized by presenting freshwater (salinity < 0.05) during almost the entire year, the bottom is also covered by Asian clams and submerged vegetation.

In each station, three replicates per month were collected during the day at high tide of spring tides using a 1m beam trawl (5 mm mesh size) towed at constant speed (2 km.h^{-1}). Duration of the tows varied between 2 to 3 minutes depending on the sediment type, in order to avoid net clogging by detritus or obstruction of the gear. The sampling area covered by a 3 min tow corresponds to 100 ± 4 meters (Freitas et al. 2009). Additionally, environmental variables (water temperature, salinity, pH and oxidation reduction potential (ORP)) were measured with a multiparameter probe YSI 6820 deployed to 20 cm off the bottom.

The monthly river discharge data measured at Foz do Mouro hydrometric station between February 2009 and July 2010 was obtained from the INAG – Instituto da Água, I.P. (http://snirh.inag.pt).

6.2.2. Laboratory procedures

The density of *P. flesus* juveniles was determined by counting all individuals caught in each sampling station. Size (standard length – S*L* and total length – T*L*) of individual fish was measured (0.5mm precision) using a ruler and subsequently, all individuals were assigned into six different size classes based on S*L*: F1 (< 25.0 mm), F2 (25.0 to 49.9 mm), F3 (50.0 to 74.9 mm), F4 (75.0 to 99.9 mm); F5 (100.0 to 125.0 mm) and F6 (> 125.0 mm). Individuals larger than 185 mm of T*L* were considered adults (Kosior et al. 1996).

All individuals had their sex identified based upon dimorphic features on their morphology and gonads, in order to compare the spatio-temporal distribution and habitat use of juveniles of different sexes. Additionally, all *P. flesus* individuals were wet weighted in order to determine the total biomass captured in Minho estuary.

The condition factor of *P. flesus* was calculated using the allometric equation:

$$(3) \qquad W = a.L^b$$

Where W is the wet weight of the fish (in grams), *L* is the total length of the fish (in centimeters), a is the coefficient related to body form and b is an exponent indicating isometric growth when equals to 3 (Reiss 1989; Beverton and Holt 1996).

6.2.3. Data analysis

Prior to the analyses of abiotic data, all variables were normalized. Posteriorly, a resemblance matrix based on the Euclidean distance was calculated.

The Permutational Multivariate Analysis of Variance (PERMANOVA) (type-III) was employed in a two-way crossed design, with station as a fixed factor (5 levels: S1, S2, S3, S4 and S5) and season as a fixed factor (4 levels: winter, spring, summer and autumn), in order to test the statistical significant differences among sampling stations and seasons using all abiotic variables (multivariate approach) and comparing each variable separately (univariate approach). The PERMANOVA analysis is capable of handling unbalanced statistical designs, such as in this study (due to unequal number of samples collected in each season and in each station), and calculates a pseudo-*F* (based on permutations) which is identical to the *F* statistic that would be produced using traditional ANOVA and it is not affected by non-normal distribution of data (Anderson 2001).

The Principal Components Analysis (PCA) was performed in order to detect habitat differences among sampling stations based on environmental data.

Similarly, the flounder data were also normalized, and resemblance matrices based on Euclidean distance were calculated. The overall density, the density of each sex (male and female), the density of the six size classes and the size of males and females *P. flesus* individuals were statistically tested using a two-way PERMANOVA (type-III) in a two-way crossed design, with station as a fixed factor (5 levels: S1, S2, S3, S4 and S5) and season as a fixed factor (4 levels: winter, spring, summer and autumn), using all both multivariate and univariate approaches (comparing each variable separately).

In PERMANOVA, the statistical significance of variance components was tested using 9999 permutations of residuals under a reduced model. When the possible number of permutations was lower than 150, the Monte Carlo-p value was considered. Subsequently, significant terms and interactions were investigated using a posteriori pair-wise comparisons to determine which pairs of stations and seasons were significantly different.

The PCA and PERMANOVA analyses were performed using PRIMER v 6.1.11® (Clarke and Gorley 2006) with PERMANOVA+1.0.1. add-on package (Anderson et al. 2008).

Canonical Correspondence Analysis (CCA) was performed to determine the relationship between the density of each size class of *P. flesus* and abiotic data using CANOCO 5 software (ter Braak and Verdonschot 1995). The Monte-Carlo randomization test (499 permutations under the reduced model) was performed to determine the statistical significance of the correlations (see ter Braak 1986).

The population structure of *P. flesus* was defined by tracking recognizable cohorts from the successive sampling dates. Spatial samples were pooled together and analyzed using the size frequency distribution of successive dates. The mixture analysis method was used to identify cohorts within size frequency distributions constructed from population samples in each month using the PAST® software. The mixture analysis is a maximum-likelihood method for estimating the parameters (mean, standard deviation and proportion), of univariate normal distributions, based on a pooled univariate sample. This software uses the EM algorithm described by Dempster et al. (1977) and considers a histogram of frequency as a mixture of probability density functions. The number of modes in each month was determined by visual analysis of the histogram.

The secondary production was computed using the size-frequency method (Hynes method), which calculates production by summing the biomass lost between size classes (Krueger and Martin, 1980). The annual production of *P. flesus* in Minho estuary was calculated according to the Krueger and Martin (1980) equation:

$$(2) \qquad P = \sum_{j=1}^{a-1} 0.5\left[\left(Y_{i,j} + Y_{i+1,j}\right) - \left(Y_{i,j+1} + Y_{i+1,j+1}\right)\right].\left(W_j.W_{j+1}\right).(t)^{-1}$$

where *P* is the annual production of the species, $Y_{i,j}$ is the mean density (ind.m^{-2}) of the size class *j* in the date *i*, $Y_{i+1,j}$ is the mean density of the size class *j* in the date *i+1*, $Y_{i,,j+1}$ is the mean density of the size class *j+1* in the date *i*, $Y_{i+1,j+1}$ is the mean density of the size class *j+1* in the date *i+1*, W_j is the biomass (wet weight) (g.m^{-2}) of the size class *j*, W_{j+1} is the biomass (wet weight) of the size class *j+1* and t is the time between *i* and *i+1*. Population production estimates correspond to the sum of each size class production (*P*). Negative production values were not included in the overall estimates and were regarded as zero production.

6.3. Results

6.3.1. Abiotic data

The two-way PERMANOVA for stations and seasons of the abiotic variables differences was highly significant for both main effects (stations: pseudo-*F* = 8.30, $p < 0.001$; seasons: pseudo-*F* = 9.12, $p < 0.001$) but not for the *stations x seasons* interaction (pseudo-*F* = 1.01, p = 0.45). When analyzed independently, each abiotic variable displayed a different pattern. The variables that varied the most among sampling stations and seasons were depth, salinity and temperature, while pH varied slightly, and ORP was not different among sampling stations and seasons. Overall, the pairwise tests of the two-way PERMANOVA indicated that the depth was lower in S2 and S3 compared with other stations. Salinity varied among sampling stations, with S1 presenting higher values than other stations; the only station where significant differences in salinity were detected among seasons was

S3, which presented significantly saltier waters during summer. Temperature varied seasonally, with winter differing from other seasons; moreover, water temperature was significantly higher in S4 and S5 during summer compared to other stations (Table 6.1).

Table 6.1 Two-way PERMANOVA results on the effects of sampling stations and seasons and their interaction term on the abiotic variables in Minho estuary, NW Iberian Peninsula. ^{ns} = non-significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test for the interaction effect: $p < 0.05$).

	Seasons				Two-way PERMANOVA		
	Winter	Spring	Summer	Autumn	Stations	Seasons	Interaction
Depth							
S1	4.2±0.3$^{ad(A)}$	3.1±0.3$^{b(A)}$	3.7±0.2$^{bd(A)}$	4.3±0.1$^{ac(A)}$	Pseudo-F = 22.20***	Pseudo-F = 3.81*	Pseudo-F = 2.53**
S2	1.9±0.2$^{(B)}$	1.3±0.2$^{(B)}$	1.8±0.1$^{(B)}$	1.8±0.1$^{(B)}$			
S3	2.7±0.4$^{(BC)}$	1.9±0.3$^{(B)}$	2.8±0.3$^{(C)}$	2.1±0.1$^{(B)}$			
S4	2.4±0.4$^{a(BC)}$	4.3±0.6$^{ab(AC)}$	5.9±1.2$^{b(A)}$	3.9±0.4$^{ab(A)}$			
S5	4.3±0.4$^{(AC)}$	3.3±0.6$^{(AC)}$	4.3±0.2$^{(A)}$	4.0±0.6$^{(A)}$			
ORP							
S1	180.8±37.6	156.1±28.9	144.4±36.3	169.2±27.0	Pseudo-F = 0.68^{ns}	Pseudo-F = 0.95^{ns}	Pseudo-F = 0.21^{ns}
S2	189.1±45.6	153.6±32.0	159.5±41.5	173.4±19.0			
S3	201.2±52.2	159.9±31.5	167.2±43.7	170.6±26.7			
S4	235.8±59.6	190.3±41.2	165.7±23.70	189.4±21.9			
S5	264.1±46.7	247.0±0.5	157.0±18.2	206.2±38.8			
pH							
S1	7.8±0.2	7.8±0.3	8.5±0.3	8.0±0.2	Pseudo-F = 1.99^{ns}	Pseudo-F = 8.75***	Pseudo-F = 0.35^{ns}
S2	7.8±0.2^{a}	8.0±0.2^{ab}	8.8±0.3^{b}	8.1±0.3^{ab}			
S3	7.7±0.1^{a}	8.0±0.2^{ab}	8.9±0.3^{b}	8.1±0.2^{a}			
S4	7.6±0.2	7.7±0.3	8.1±0.3	8.0±0.4			
S5	7.8±0.1	7.7±0.1	8.1±0.4	7.9±0.3			
Salinity							
S1	25.8±6.5$^{(A)}$	26.0±2.9$^{(A)}$	32.0±1.0$^{(A)}$	31.8±1.9$^{(A)}$	Pseudo-F = 41.47***	Pseudo-F = 4.85**	Pseudo-F = 1.21^{ns}
S2	12.8±7.8$^{(AB)}$	15.9±6.2$^{(AD)}$	30.8±1.9$^{(A)}$	23.0±6.9$^{(AB)}$			

S3	$0.4\pm0.2^{a(B)}$	$0.4\pm0.2^{a(BC)}$	$19.1\pm4.2^{b(B)}$	$4.3\pm4.2^{ab(BC)}$			
S4	$0.0\pm0.0^{(B)}$	$0.1\pm0.0^{(BC)}$	$0.1\pm0.0^{(C)}$	$0.0\pm0.0^{(C)}$			
S5	$0.1\pm0.0^{(B)}$	$0.1\pm0.0^{(BCD)}$	$0.1\pm0.0^{(C)}$	$0.1\pm0.0^{(C)}$			
Temperature					Pseudo-*F* = 1.12^{ns}	Pseudo-*F* = 48.50***	Pseudo-*F* = 3.29***
S1	11.9 ± 0.9^{a}	14.6 ± 0.5^{b}	$15.0\pm0.6^{b(A)}$	15.8 ± 0.4^{b}			
S2	11.7 ± 0.8^{a}	16.37 ± 0.9^{b}	$15.9\pm0.5^{b(AB)}$	15.1 ± 1.5^{ab}			
S3	9.6 ± 0.8^{a}	16.4 ± 1.2^{b}	$18.3\pm1.2^{b(B)}$	14.7 ± 1.8^{b}			
S4	9.8 ± 0.5^{a}	15.6 ± 1.4^{b}	$22.5\pm0.3^{c(C)}$	15.1 ± 2.2^{b}			
S5	9.9 ± 1.1^{a}	15.6 ± 2.2^{b}	$21.9\pm0.1^{c(C)}$	14.9 ± 2.6^{b}			

The PCA routine indicated that the first two axis of the PCA explained 55.8% of the variance among samples and revealed differences between sampling stations, with S1 and S2 clearly differing from S4 and S5, while S3 was associated to both main groups (Fig. 2). The pH (eigenvalue = -0.628) and the water temperature (eigenvalue = -0.566) presented the strongest correlations with PC1, while depth (eigenvalue = 0.749) was the most important variable in explaining PC2 (Fig. 6.2).

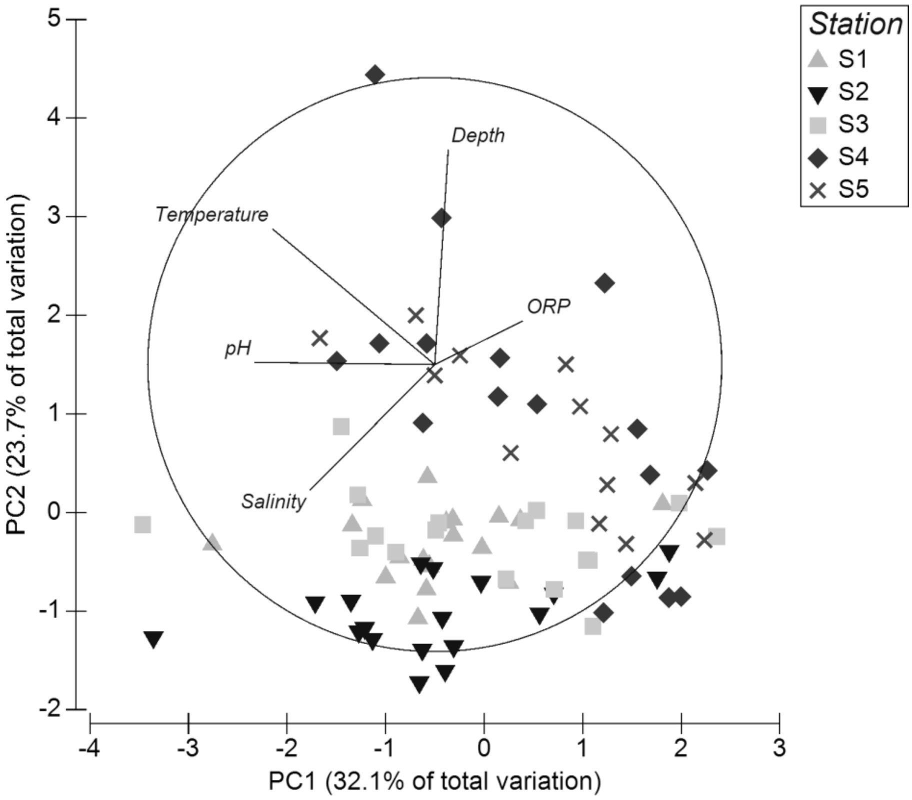

Fig. 6.2 Principal Components Analysis (PCA) biplot for the abiotic data collected from February 2009 to July 2010 in five sampling stations (S1, S2, S3, S4 and S5) in Minho estuary, NW Iberian Peninsula.

6.3.2. Flounder data

A total of 528 fishes were collected (S3 = 43.6%, S4 = 29.0%, S2 = 11.9%, S5 = 9.3% and S1 = 6.2%). Of these, 30.1% were sampled during spring, 24.8% in summer, 24.5% in autumn and 20.6% during winter. Males and females were evenly found in Minho estuary (46.6% and 46.8% of the total respectively), while undefined individuals accounted only for 6.6% of the total number of individuals sampled.

6.3.2.1. Density patterns

The mean density of *P. flesus* in Minho estuary, considering the entire sampling period and the 5 stations, was 2.8 ± 0.3 ind.100m^{-2} (mean ± SE). The density of *P. flesus* varied throughout the sampling period, with higher values being found during the spring (4.5 ± 2.3 ind.100m^{-2} in May/09) and summer months (4.6 ± 1.2 ind.100m^{-2} in August/09) (Fig. 6.3A).

The two-way PERMANOVA for stations and seasons of *P. flesus* density differences was significant for the *stations x seasons* interaction (pseudo-$F = 2.28$, $p < 0.05$). The density of *P. flesus* was even throughout seasons in S1, S3 and S5, while in S2, a significant higher number of individuals were captured during the summer (2.8 ± 1.0 ind.100m^{-2}) in comparison with autumn (0.4 ± 0.3 ind.100m^{-2}) (Two-way PERMANOVA, pairwise test: $t = 2.33$, $p < 0.05$), and in S4 the density was higher during the autumn (8.4 ± 1.5 ind.100m^{-2}) in comparison with the other three seasons (Two-way PERMANOVA, pairwise tests – *Winter x Autumn*: $t = 4.53$, $p < 0.001$; *Spring x Autumn*: $t = 2.12$, $p < 0.05$; *Summer x Autumn*: $t = 2.37$, $p < 0.05$). Overall, the density of *P. flesus* was higher in S3 during all seasons, except during autumn, when S4 presented the highest density (Fig. 6.3B).

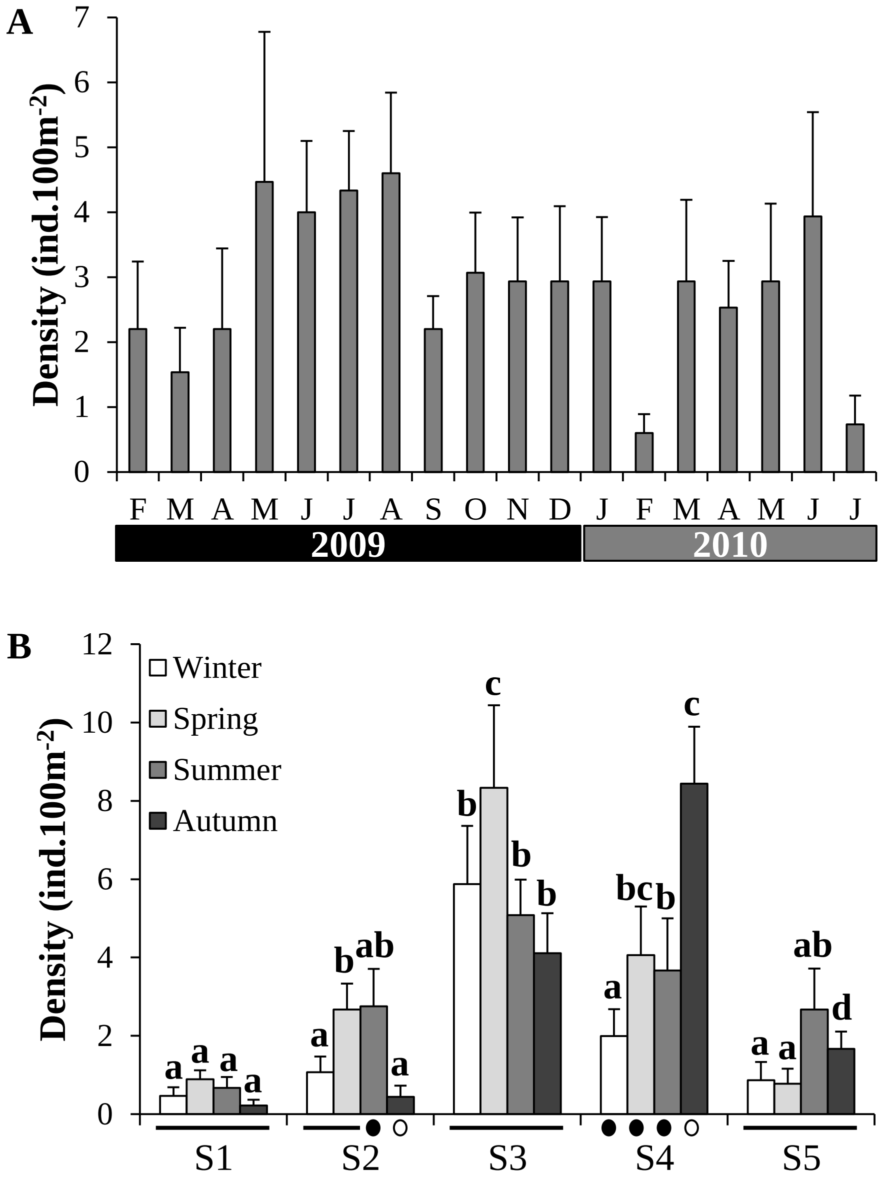

Fig. 6.3 (A) Mean density (± SE) of the European flounder *Platichthys flesus* during 18-months study period in Minho estuary. (B) Comparisons on the densities (mean ± SE) of *P. flesus* between sampling stations and seasons. Different letters (a, b, c or d) indicates post-hoc differences among sampling stations within the same season. Post-hoc differences among seasons within each sampling station are indicated by circles of different colors (black or white), while continuous line indicates that there are no significant differences among seasons crossed by it (Two-way PERMANOVA pairwise tests: $p < 0.05$).

During winter, spring and summer, most of the *P. flesus* individuals (71.4%, 70.3% and 58.3% respectively) were captured in the three stations located in the lower and in the intermediary estuary (S1, S2 and S3); while during the autumn, the pattern was different with 69.0% of the individuals being caught in the sampling stations located in the upper estuary (S4 and S5).

Overall, males and females presented an even distribution along the five sampling stations and the four seasons, with a higher sex ratio (Male/Female) being found during the summer (1.3), followed by winter (1.0), spring (0.8) and autumn (0.7) respectively. The density of males was slightly higher than females' in stations S1 and S3, whereas the females' density was slightly higher in stations S2, S4 and S5. Additionally, the interaction effect of stations and seasons for the density males was significant, with higher density values being recorded in S3 and S4 during spring and autumn respectively. Females also showed a similar pattern, with higher densities being recorded in S4 and S3 during autumn and spring respectively (Table 6.2).

Table 6.2 Comparisons on the density (mean ± SE) of males and females of the European flounder *Platichthys flesus* among five sampling stations and four seasons in Minho estuary, NW Iberian Peninsula. ^{ns} = non-significant; * = $p < 0.05$; ** = $p < 0.001$. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test for the interaction effect: $p < 0.05$).

	Seasons				Two-way PERMANOVA		
	Winter	Spring	Summer	Autumn	Stations	Seasons	Interaction
Male							
S1	$0.2\pm0.2^{(A)}$	$0.4\pm0.2^{(A)}$	$0.5\pm0.3^{(A)}$	$0.22\pm0.15^{(A)}$	Pseudo-*F* = 16.43**	Pseudo-*F* = 1.04^{ns}	Pseudo-*F* = 2.44**
S2	$0.5\pm0.2^{(A)}$	$1.2\pm0.4^{(A)}$	$1.0\pm0.5^{(A)}$	$0.22\pm0.22^{(A)}$			
S3	$2.9\pm0.8^{(B)}$	$4.6\pm1.2^{(B)}$	$3.2\pm0.6^{(B)}$	$2.33\pm0.60^{(B)}$			
S4	$0.9\pm0.4^{a(A)}$	$1.4\pm0.6^{a(A)}$	$1.6\pm0.7^{a(AB)}$	$4.33\pm1.13^{b(B)}$			
S5	$0.5\pm0.2^{a(A)}$	$0.0\pm0.0^{b(C)}$	$1.1\pm0.5^{a(A)}$	$0.22\pm0.22^{ab(A)}$			
Female							
S1	$0.27\pm0.12^{(A)}$	$0.44\pm0.17^{(A)}$	$0.2\pm0.1^{(A)}$	$0.0\pm0.0^{(A)}$	Pseudo-*F* = 11.20**	Pseudo-*F* = 0.93^{ns}	Pseudo-*F* = 1.87*
S2	$0.60\pm0.25^{(A)}$	$1.50\pm0.41^{(BC)}$	$1.7\pm0.6^{(B)}$	$0.2\pm0.2^{(A)}$			
S3	$3.07\pm0.87^{(B)}$	$3.39\pm0.98^{(C)}$	$2.3\pm0.5^{(B)}$	$1.9\pm0.7^{(BC)}$			
S4	$0.87\pm0.32^{a(A)}$	$1.67\pm0.40^{a(BC)}$	$1.8\pm0.6^{a(B)}$	$3.9\pm0.7^{b(B)}$			
S5	$0.33\pm0.23^{a(A)}$	$0.78\pm0.38^{ab(AB)}$	$1.6\pm0.7^{ab(AB)}$	$1.4\pm0.5^{b(C)}$			

6.3.2.2. Size and recruitment patterns

Almost the entire population was composed by juveniles (only one adult individual was captured in S1), and the range of S*L* varied between 7.5 and 184.0 mm, with an average value of 54.9 ± 1.4 mm (mean ± SE). The two-way PERMANOVA for stations and seasons of *P. flesus* size differences was significant for both main effects (stations: pseudo-*F* = 54.22, $p < 0.001$; seasons: pseudo-*F* = 22.75, $p < 0.001$), and for the *stations x seasons* interaction (pseudo-*F* = 2.63, $p < 0.001$).

When analyzing fish size of each sex separately, both males and females were frequently larger in S1 and smaller in the stations located in the intermediate (S3) and the upper zone (S4) of the estuary. Additionally, *P. flesus* individuals were often smaller during the spring and larger during the autumn (Table 6.3).

Table 6.3 Comparisons on the standard length (mean ± SE) of males and females of the European flounder *Platichthys flesus* among sampling stations and seasons in Minho estuary, NW Iberian Peninsula. ^{ns}= non-significant; *= $p < 0.05$; ** = $p < 0.01$ ***= $p < 0.001$. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test for the interaction effect: $p < 0.05$).

	Standard length (mm)				Two-way PERMANOVA		
	Winter	Spring	Summer	Autumn	Station	Season	Interaction
Male							
S1	$92.0\pm11.6^{(A)}$	$112.4\pm6.5^{(A)}$	$133.3\pm16.1^{(A)}$	$168.5\pm59.1^{(A)}$	Pseudo-*F* = 48.67***	Pseudo-*F* = 7.90***	Pseudo-*F* = 3.39**
S2	$65.7\pm5.1^{a(B)}$	$64.0\pm7.9^{a(C)}$	$62.9\pm12.2^{a(B)}$	$208.5\pm0.0^{b(A)}$			
S3	$63.4\pm3.5^{a(B)}$	$38.2\pm2.9^{b(B)}$	$55.4\pm7.3^{a(B)}$	$65.1\pm6.4^{a(B)}$			
S4	$47.8\pm3.4^{a(C)}$	$35.0\pm3.7^{b(B)}$	$39.5\pm11.0^{ab(B)}$	$53.6\pm4.3^{a(B)}$			
S5	$66.0\pm10.6^{(ABC)}$	-	$66.6\pm13.7^{(B)}$	$89.5\pm0.0^{(AB)}$			
Female							
S1	$76.6\pm8.1^{(A)}$	$107.6\pm11.5^{(A)}$	$102.0\pm14.0^{(A)}$	-	Pseudo-*F* = 4.48*	Pseudo-*F* = 0.36^{ns}	Pseudo-*F* = 3.54**
S2	$64.8\pm6.2^{(AB)}$	$50.4\pm5.1^{(B)}$	$63.3\pm5.7^{(B)}$	$81.0\pm0.0^{(A)}$			
S3	$60.0\pm2.5^{a(B)}$	$39.2\pm3.1^{b(B)}$	$60.8\pm6.8^{a(AB)}$	$76.8\pm15.0^{a(A)}$			
S4	$52.0\pm5.3^{ab(B)}$	$52.1\pm5.2^{ab(B)}$	$39.1\pm3.8^{a(C)}$	$49.6\pm2.6^{b(B)}$			
S5	$47.4\pm3.9^{a(B)}$	$54.6\pm9.5^{a(B)}$	$56.5\pm6.7^{a(B)}$	$91.6\pm7.5^{b(A)}$			

The two-way PERMANOVA for stations and seasons of the European flounder size classes densities was significant for both main effects (stations: pseudo-*F* = 5.11, p < 0.001; seasons: pseudo-*F* = 2.21, p < 0.01) and for the *stations x seasons* interaction (pseudo-*F* = 1.81, p < 0.001).

Overall the Minho estuary population is dominated by small-bodied individuals, with the smaller size classes (F1, F2 and F3) representing at least 52% of the population throughout the entire study period, and reaching up to 94% of the population in April/09 (Fig. 6.4A).

The mixture analysis indicated that the recruitment of *P. flesus* occurred once a year (March/09 and May/10) in in Minho estuary (Fig. 6.4B).

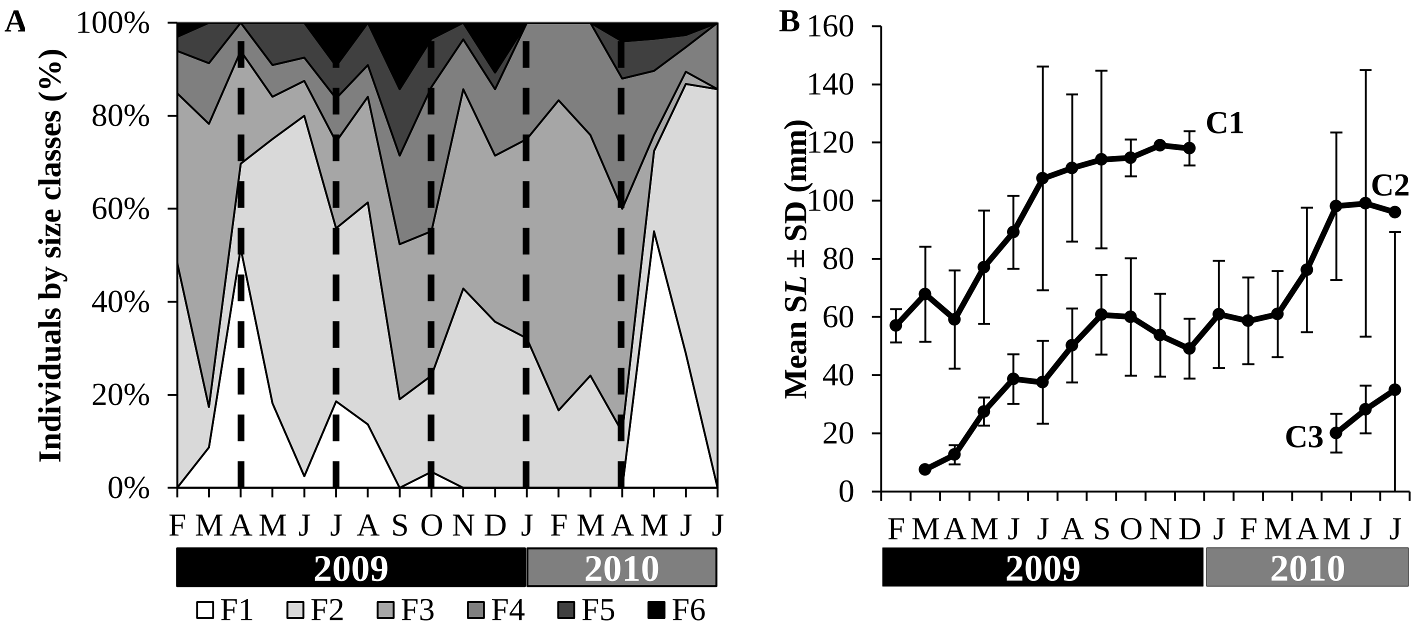

Fig. 6.4 (A) Size classes proportions of the European flounder *Platichtys flesus* in Minho estuary throughout time. Size classes: F1 (< 25 mm), F2 (25 to 49.9 mm), F3 (50 to 74.9 mm), F4 (75 to 99.9 mm), F5 (100 to 124.9 mm) and F6 (> 125 mm). Vertical dashed lines separate different seasons. (B) Mean cohort length of *P. flesus* in Minho estuary, with indication of the three cohorts identified by the mixture analysis (C1, C2 and C3).

6.3.2.3. Fish-habitat associations

Overall, size classes associated differentially with environmental variables. The smallest fishes (F1) tended to be associated with high water temperature and low river discharge and salinity, whereas larger fishes (F5 and F6) were associated with high values of salinity and depth. On the other hand, intermediate size fishes

(F2, F3 and F4) showed weaker associations with environmental variables (Fig. 6.5). The Monte-Carlo test was significant (test of significance of all canonical axes: trace = 0.39; F-ratio = 2.92; $p < 0.01$) and cumulatively, axes 1 and 2 accounted for 86.3% of the total variance, with correlations between *P. flesus* size classes densities and environmental variables of 0.574 (axis 1) and 0.536 (axis 2).

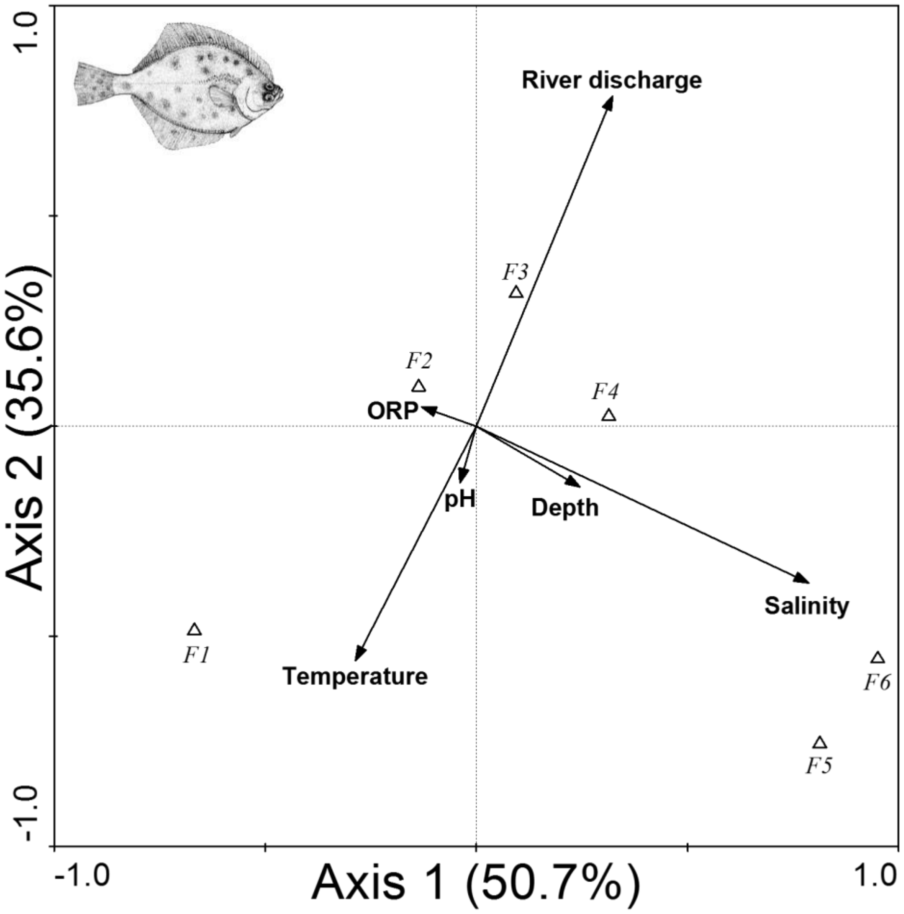

Fig. 6.5 Canonical plotting of environmental characteristics (arrows) and different size classes of the European flounder *Platichthys flesus* in Minho estuary, NW Iberian Peninsula. ORP = Redox potential. Size classes: F1 (< 25 mm), F2 (25 to 49.9 mm), F3 (50 to 74.9 mm), F4 (75 to 99.9 mm), F5 (100 to 124.9 mm) and F6 (> 125 mm).

No significant associations were found between the density of males and females and the environmental variables (test of significance of all canonical axes: trace = 0.10; F-ratio = 0.81; $p = 0.56$), indicating that males and females have similar habitat preferences in Minho estuary.

6.3.2.4. Length-weight relationship and secondary production

During the study period, a total of 2739 g (wet weight) of *P. flesus* was collected in Minho estuary. Males presented a ratio of T*L*/wet weight slightly higher than females. When comparing the results of the length and weight relationship of the *P. flesus*, the values found in Minho estuary was different from others, since the allometric coefficient a values were lower and the allometric coefficient b values were higher than other locations (Table 6.4).

Table 6.4 Comparison of the values obtained by length and weight relationship of the European flounder *Platichthys flesus* in different locations.

Country	Locality	allometric coefficient a	allometric coefficient b	Sex	T*L* range (cm)	Reference
Germany	Western Baltic	0.0220	2.830	Male	15.0 to 42.0	Froese and Freiß 1992
Germany	Western Baltic	0.0158	2.956	Female	15.0 to 42.0	Froese and Freiß 1992
France	East and West Channel	0.0116	2.963	Unsexed	5.0 to 48.0	Dorel 1986
England	-	0.0125	2.968	Both	-	Bedford et al. 1986
France	Bay of Biscay	0.0093	3.066	Unsexed	-	Dorel 1986
Scotland	Moray Firth and Buchan	0.0087	3.098	Unsexed	12.0 to 38.0	Coull et al. 1989
France	Gulf of Lyon	0.0063	3.100	Both	5.0 to 43.0	Vianet et al. 1989
Croatia	Mirna estuary	0.0070	3.110	Unsexed	11.0 to 43.0	Dulcic and Glamuzina 2006
Portugal	Minho estuary	0.0045	3.310	Both	1.3 to 27.5	Present study
Portugal	Minho estuary	0.0048	3.267	Male	1.3 to 27.5	Present study
Portugal	Minho estuary	0.0041	3.363	Female	1.7 to 22.5	Present study

The overall the secondary production of *P. flesus* in Minho estuary was of 0.052 g.WWm^{-2}, with the annual production of 0.037 g.WWm^{-2}.year^{-1}.

6.4. Discussion

6.4.1. Distribution patterns and habitat use

European flounder juveniles presented spatial and temporal differences in its distribution in Minho estuary. Spatially, *P. flesus* seemed to prefer the intermediate (S3) and upper (S4) zones of the estuary. Interestingly, these two stations are about 14 km apart from each other and present marked differences in their environmental characteristics, mainly related to depth and salinity. While in S3 the salinity values varied between 0.03 (February/09) and 27 (August/09), the S4 presented salinity values constantly lower than 0.05; additionally, S4 (4.1 ± 1.4 m) is much deeper than S3 (2.3 ± 0.9 m) and presented important differences in the benthic habitat characteristics, once in S4, the submerged vegetation is copious, whereas in S3 the presence of the Asian clam *C. fluminea* is high. Despite the abovementioned differences in habitat characteristics, both sampling stations sustained high densities of *P. flesus* throughout the whole study period, indicating that other factors not measured in this study may be also important to *P. flesus*. The European flounder is able to tolerate a wide range of salinity (Nielsen 1986) and is also known to exhibit great plasticity in of their life history patterns (Daverat et al. 2012), thus residing in zones where the food availability and environmental conditions are optimal. In fact, this species can be spatially distributed in patches (Modin and Pihl 1996).

The density of the European flounder juveniles in S1 (close to the river mouth) was the lowest found in the estuary, indicating that the Minho estuary population may prefer zones with lower values of salinity. Similar patterns of preference for low salinity were previously acknowledged in the literature (e.g. Kerstan 1991; Van der Veer et al. 1991; Thiel et al. 2003; Martinho et al. 2007a; Freitas et al. 2009).

Sexual segregation is a relatively common pattern in flatfish's distribution (see Becker 1988); but this issue have be somehow neglected; one exception was Bartolino et al. (2011), which investigated the ontogenetic and sex-specific differences in habitat selection of the yellowfin sole *Limanda aspera* finding that sexual segregation is more evident in adult stages, with juveniles did not differing much in habitat preferences according to its sex (Bartolino et al. 2011), as recorded in Minho estuary for *P. flesus* juveniles. Interestingly, previous studies involving the *P. flesus* population patterns in Portuguese estuaries did not measured possible sexual segregation (e.g. Cabral et al. 2007; Martinho et al. 2007a; Freitas et al. 2009).

Similarly with Freitas et al. (2009), higher densities of *P. flesus* were recorded during summer in Minho estuary; however, in the present study, high densities were also found during the spring. Differences were also recorded in the months where the density peaks between two consecutive years, indicating that abundance peaks may change over the years according to changes in environmental conditions. Actually, interannual variations in fish abundance in estuaries are common, and can result from differences in recruitment success among years or periods (Potter et al. 2001; Maes et al. 2005). Furthermore, density peaks can also occur in different seasons, for instance in the Baltic Sea, the peak seem to occur in late summer and the autumn (see Aarnio 2000).

High *P. flesus* densities were recorded in upstream stations during the autumn, while during the remaining seasons, density values in upstream stations decreased for less than 30% of the total, suggesting that perhaps juveniles may move from the polyhaline portion of the estuary into the tidal freshwater (TFW) area during autumn. The movement towards the TFW area of the estuary could be related with increased growth rates at intermediate salinities compared with extreme salinities (Gutt 1985). In fact, Bos and Thiel (2006) found that 0-group flounder selected the lower salinity conditions in laboratory migration experiment. Nonetheless, the salinity found in S4 and S5 is always near 0.05, thus it should be expected that juveniles avoid to remain in an area with such low values of salinity, and other factors may be driven the upward movements of *P. flesus* juveniles during the autumn.

The ecological interaction among other co-occurring key-species, such as the common goby *Pomatoschistus microps*, the sand goby *P. minutus*, the shore crab *Carcinus maenas* and the Great cormorant *Phalacrocorax carbo* might also be related with the different patterns of abundance of *P. flesus* juveniles among sampling stations. During autumn, the mean density of the common goby *Pomatoschistus microps* (288.4 ind.100m^{-2}), the sand goby *P. minutus* (5.2 ind.100m^{-2}) and the shore crab *Carcinus maenas* (57.2 ind.100m^{-2}) were high in the lower (S1 and S2) and in the intermediary (S3) estuary compared with the remaining seasons (Souza et al., unpublished results); with flounder presenting low values in these three sampling stations (1.6 ± 0.5 ind.100m^{-2}); but reaching densities three fold higher (5.1 ± 1.1 ind.100m^{-2}) in the upstream stations (S4 and S5). The high densities of these species may favor the upstream dislocation of juvenile flounder, since they can possibly be competing for space and/or food. In fact, Złoch and Sapota (2010) found a niche overlap of 40% between *P. flesus* and *P. microps* and *P. minutus*, suggesting that competition between these species can also be occurring in Minho estuary. Additionally, it is possible that *P. flesus* might be preyed by the Shore crab and the Great cormorant in the lower and the intermediary estuary. Inside estuaries, fishes represent an important fraction of the *C. maenas* diet (see Baeta et al. 2006), while *P. flesus* represent *c.a.* 30% of total number of preys consumed by the Great cormorant, being the most representative food item of the species in Minho estuary (Dias et al. 2012). Of the four abovementioned species, only the Great cormorant co-occurs with flounder in the upstream stations, but the occurrence of *P. carbo* near S4 and S5 is noticeably lower than in the lower estuary (personal observation), indicating that in the upstream stations, *P. flesus* may find less competition and predators, thus being a favorable location for juveniles, particularly during the autumn.

The seasonal differences in the density of the European flounder among sampling stations can also be related with changes in the abundance of its preys, since *P. flesus* can actively move towards areas where its preys are more abundant (Modin and Pihl 1996). Nevertheless, due to the lack of published information regarding the spatio-temporal density of the subtidal macrozoobenthic assemblages in Minho estuary, we cannot confirm that the availability of *P. flesus* preys is greater during autumn in upstream areas of the estuary or not. Further studies involving *P. flesus* and subtidal macrozoobenthos density in Minho estuary are necessary in order to confirm this possible correlation.

Despites of the spatio-temporal differences in *P. flesus* density patterns found in this study, we cannot conclusively say that the European flounder can move from one estuarine area to another, since we did not use appropriated techniques to measure fish dislocations. Therefore, further studies are necessary to describe *P. flesus* movements within the estuary.

The absence of larger *P. flesus* in all studies conducted in Minho estuary so far (e.g. Freitas et al. 2009; Dolbeth et al. 2010; our study) can be related with the sampling strategy adopted in all of them (1m beam trawl tows), which is a selective sampling gear for smaller size *P. flesus*. On the other hand, studies that used a different sampling gear (2m beam trawl) found much lower density values of *P. flesus* in Minho estuary than the present study (e.g. Vasconcelos et al. 2010; França et al. 2011), indicating that Minho's population may be primarily constituted by juveniles indeed. Still, larger flounders were occasionally recorded in upper Minho estuary (ca. 70 km upstream) (C. Antunes unpublished results).

Recruitment occurred during winter (March) in 2009 and during the spring in 2010 (May). This result is in accordance with Dolbeth et al. (2010), which identified the recruitment of a single cohort of *P. flesus* per year (during spring) in Minho and Mondego estuaries between 2004 and 2007. Differences in the months when *P. flesus* recruitment occurs may perhaps be related with differences in environmental conditions between years (Philippart et al. 1996).

Flounder distribution in coastal and estuarine systems is strongly influenced by a number of abiotic and biotic factors (Able et al. 2005). For flatfishes many studies indicate depth, temperature, salinity and substratum type as the best environmental predictors of habitat use within a study area (Able et al. 2005). In our study we found that these abiotic features were also good predictors of *P. flesus* distribution. However, fishes from different size classes, showed marked differences in habitat preference in Minho, indicating that fishes from different sizes might be spatially segregated. It is important to highlight that the smallest fish (class F1) showed a strong correlation with low values of salinity and high values of temperature, indicating that these fish preferred to inhabit areas with low salinities (< 1), namely S3 (during winter and spring), S4 and S5. On the other hand, larger juveniles, tended to be associated with higher values of salinity, temperature and depth, indicating that these fishes (classes F5 and F6) tended to remain in the lower (S1 and S2) and the intermediary (S3) estuary. Otherwise, the intermediate size classes (F2, F3 and F4) showed weaker association with the environmental variables measured in this study thus can be associated either with the downstream or upstream estuarine zones.

Salinity is known to greatly influence the distribution of *P. flesus*, in fact, within estuaries, densities of juvenile *P. flesus* significantly increased with decreasing salinity (Kerstan 1991; Van der Veer et al. 1991; Thiel et al. 2003; Martinho et al 2007; Freitas et al. 2009), suggesting that there is an active choice of juveniles for low salinity waters (Bos and Thiel 2006). Our results, however, showed that different size classes of *P. flesus* have different association with salinity, emphasizing that even during the juvenile phase, preferences can be different.

6.4.2. Flounder condition and production

In allometric equations for fish length and weight relationship, high allometric coefficient b values may indicate that fish present good condition, and the results obtained in Minho estuary were higher than those recorded in other places (see Table 4). This could be an indicative that in Minho estuary, *P. flesus* may find optimal conditions for growth, thus helping to explain the high flounder density observed in this estuary compared with other locations. Additionally, the second highest b value was also found in an estuarine system (Mirna estuary in Croatia) (Dulcic and Glamuzina 2006). Inside estuaries, fishes find favorable conditions for rapid growth due to the higher availability of food and shelter (Haedrich 1983), and not arbitrarily, these locations function as nursery grounds (Beck et al. 2001). On the other hand, the high fish condition found in this study might be related with the small range in fish size observed in Minho estuary compared with other systems (see Table 4). Fish condition can vary ontogenetically (Azuma et al. 1998), with smaller juveniles presenting higher condition than larger juveniles and subadults (Costa and Araújo 2003). Therefore, the higher condition of *P. flesus* in Minho estuary could be related with the dominance of small-bodied individuals in the population.

The high fish condition measured for *P. flesus* in Minho estuary, associated with the noticeably high densities of several aquatic species, such as the Asian clam *C. fluminea* (Sousa et al. 2008d), the common goby *P. microps* (Souza et al. in revision), the sand goby *P. minutus* (Souza et al. unpublished results) the shore crab *C. maenas* (Souza et al., unpublished results) and also the European flounder *P. flesus* (Cabral et al. 2007; Freitas et al. 2009, our study) found in Minho estuary, can be an indicative that this estuary presents good condition for this and other aquatic species (Gibson 1994; Amara et al. 2009). This fact may perhaps be related to the higher habitat preservation of Minho estuary compared to other estuaries (see Sousa et al. 2008a).

The secondary production of *P. flesus* was lower than previously reported for Minho (39 times lower) and Mondego (2.7 times lower) estuaries (see Dolbeth et al. 2010). Similarly with Dolbeth et al. (2010), we performed diurnal tows using the same sampling gear (1 m beam trawl with 5 mm mesh size), but our sampling strategy was highly different, since we performed replicates in each of the five sampling stations (from the first 29 km of the estuary), while they used data only from 6 sampling stations located in the first 12 km of the estuary with no replicates within each sampling station. In our study, most of the individuals captured were very small in size, and interestingly smaller fishes were sampled in the areas not sampled by Dolbeth et al. (2010). Also, the mean density value found in our study (2.8 ± 0.3 ind.100m^{-2}) was lower than previous studies conducted in Minho (see Freitas et al. 2009; Dolbeth et al. 2010), and since production is directly related with fish size and density, it should be expected that our production estimate would be lower than theirs' and most of the dissimilarities obtained by both studies might possibly be related to differences in the experimental design.

6.5. Conclusions

The juvenile flounder population in Minho estuary was spatially and temporally structured, with marked differences in habitat preferences among size classes. Flounders also seem to perform upstream dislocations during autumn, probably due to better environmental conditions for the species found in this portion of the estuary during this season compared with the lower and the intermediary estuary. Flounders from Minho estuary had higher densities (see Vinagre et al. 2005; Pombo et al. 2007; Martinho et al. 2007a; Ramos et al. 2010; Vasconcelos et al. 2010; França et al. 2011) and condition (see Bedford et al. 1986; Dorel 1986; Coull et al. 1989; Vianet et al. 1989; Froese and Freiß 1992; Dulcic and Glamuzina 2006) when compared with other areas, indicating that this estuary might present better conditions for the growth of *P. flesus* juveniles. Nonetheless, the secondary production obtained in our study was lower than previous studies acknowledged.

6.6. Acknowledgements

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CHAPTER 7

TOP-ABUNDANT DEMERSAL SPECIES IN A TEMPERATE SALT-WEDGE ESTUARY: ASSEMBLAGES STRUCTURE AND DOMINANCE PATTERNS

Top-abundant demersal species in a temperate salt-wedge estuary: Assemblages structure and dominance patterns

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Abstract

Estuaries are characterized to present a lower diversity of species, but a high density of individuals. In general, species of temperate estuarine systems present a wide geographical distribution. Despite of several estuaries sustain similar epibenthic fauna in Europe, patterns of species dominance can vary geographically due to differences in abiotic characteristics of systems, as well as due to differential patterns of ecological interactions. Actually, different patterns of dominance can also be found inside the same system. In order to investigate the spatio-temporal changes in the dominance patterns of epibenthic species, a 16 month sampling campaign was performed in three different estuarine areas (river mouth – S1, salt marsh – S2 and upper estuary – S3) in Minho estuary (Northern Portugal). Five species were numerically dominant, namely the shore crab *Carcinus maenas*, the brown shrimp *Crangon crangon*, the European flounder *Platichthys flesus*, the common (*Pomatoschistus microps*) and the sand goby (*P. minutus*), altogether these species accounted for 104981 individuals (weighting 49679.8 g). Considering the density of individuals, the brown shrimp was the dominant species of the estuary; but, in terms of biomass, the shore crab dominated the system. These two crustaceans were especially dominant in the stations closer to the river mouth (S1 and S2), while in the upper station (S3), the common goby displayed a marked dominance, both in terms of density and biomass. Overall, Minho estuary presents different patterns of dominance among estuarine areas, with a predominance of crustaceans in areas with lower variation in abiotic characteristics (mainly salinity), while fishes were dominant in the area with higher abiotic variation throughout the year. Our data also shows that, in general, the epibenthic assemblages of Minho estuary are dominated by juveniles. However marked differences among sampling stations were observed, with the sampling stations closer to the river mouth (S1 and S2) presenting a dominance of juveniles, especially during spring and summer months, while adults were dominant in S3 during all seasons. Our results highlight that dominance patterns of species in estuaries is highly dependent of the spatial and seasonal variability, and also that *P. microps* can take advantage of its high physiological adaptability to thrive harsh environmental conditions and became dominant in stressful estuarine areas.

Keywords: dominant species / salinity / crustacean / Minho / secondary production

7.1. Introduction

Estuaries are one of the most complexes, productive and valuables ecosystems in earth (Costanza et al. 1997; McLusky and Elliot 2004). A number of aquatic species, belonging to different taxonomic groups, inhabit these systems for different periods of their life cycle. Some species are residents, remaining inside the estuary during their entire life cycle (Elliott et al. 2007); while others are migratory or transitory, using the estuary during shorter periods of time, on their way to the spawning grounds, where reproduction and/or juveniles' recruitment take place (Claridge et al. 1986; Potter et al. 1997). Actually, several species uses estuaries as nursery grounds, since they provide abundant prey resources and low predation risk for juveniles (Joseph 1973). Therefore, sexually immature individuals can enhance their feeding and growth rates (Houde 1989; Cabral et al. 2007; Martinho et al. 2007a).

Estuaries are also characterized to present a lower diversity of species, but a high density of individuals (Whitfield 1999). This pattern is derived from the high variability of the abiotic characteristics of these systems (see Pritchard 1967), that makes estuaries rigorous and stressful habitats for most of the aquatic organisms.

In general, species of temperate estuarine systems present a wide geographical distribution, for instance, in European estuaries, several fishes and crustaceans can span up to 49º of a latitudinal range (e.g. Miller 1986; Nielsen 1986; Rochard and Elie 1994; Roman and Palumbi 2004; Campos et al. 2009). Also, the dominant estuarine species presents a long-term stability in abundance, being less affected by environmental changes than lower abundant species (Henderson et al. 2011; Nyitrai et al. 2012). Nevertheless, community structure is highly influenced by interactions among species in a given location (Toft et al. 1982). Despite of several estuaries sustain similar epibenthic fauna in Europe, patterns of species dominance can vary geographically due to differences in abiotic characteristics of systems, as well as due to differential patterns of ecological interactions (Nicolas et al. 2010). Actually, different dominance patterns can also be found inside the same system, once spatial distribution of organisms is normally not homogeneous in space and time (Chesson 1998).

Two taxonomic groups stand out in terms of abundance and biomass within estuaries, namely crustaceans and fishes (De Ben et al. 1990). Due to their representativeness, these two groups have been focus of several studies dealing with the population ecology (e.g. Baeta et al. 2005; Leitão et al. 2006; Viegas et al. 2007; Dolbeth et al. 2007b; Martinho et al. 2010) and with assemblages structure (e.g. Amara and Paul 2003; Martinho et al. 2009; Dolbeth et al. 2010; Henderson and Bird 2010; Nyitrai et al. 2012). However, few studies attempted to the dominance patterns of top-abundant species focusing in different estuarine sections through time. In this context, the present study aimed to assess the spatio-temporal distribution and the dominance patterns of two crustaceans species (*Carcinus maenas* and *Crangon crangon*) and three fishes species (*Platichthys flesus*, *Pomatoschistus microps* and *P. minutus*), that are widely distributed and abundant in several estuaries in Europe.

7.2. Material and methods

7.2.1. Study area, sampling and laboratory procedures

This study was conducted in Minho estuary (NW of the Iberian Peninsula – 41º53'N 8º50'0), which covers a total area of 23 km^2. This estuary is a shallow system, with a mean depth of 4 m according to the hydrographic zero, and has a maximum depth of 23 m (Reis et al. 2009). In the first 13 km of the estuary there are several sand banks that become exposed during low tides, forming small river islands (Reis et al. 2009). Minho estuary is also characterized by several bathymetric constraints like strangling and abrupt variations in depth (Reis et al. 2009). The limit of salt water intrusion in Minho estuary is 35 km from the mouth, and this estuary is classified as a mesotidal system in which vertical stratification occurs during periods of high freshwater discharge (Ferreira et al. 2003). The mean annual river discharge is 300 m^{-3}s^{-1}, but it can reach 1000 m^{-3}s^{-1} during winter (Ferreira et al. 2003).

A 16 month's sampling program was established, from April/09 to July/10 in three nearby sites within the first 8 km of the Minho estuary (Fig. 7.1). The three sites (S1, S2 and S3) were chosen in order to assess possible dissimilarities on the crustaceans and fishes populations structures related to differences in the habitat type and environmental characteristics.

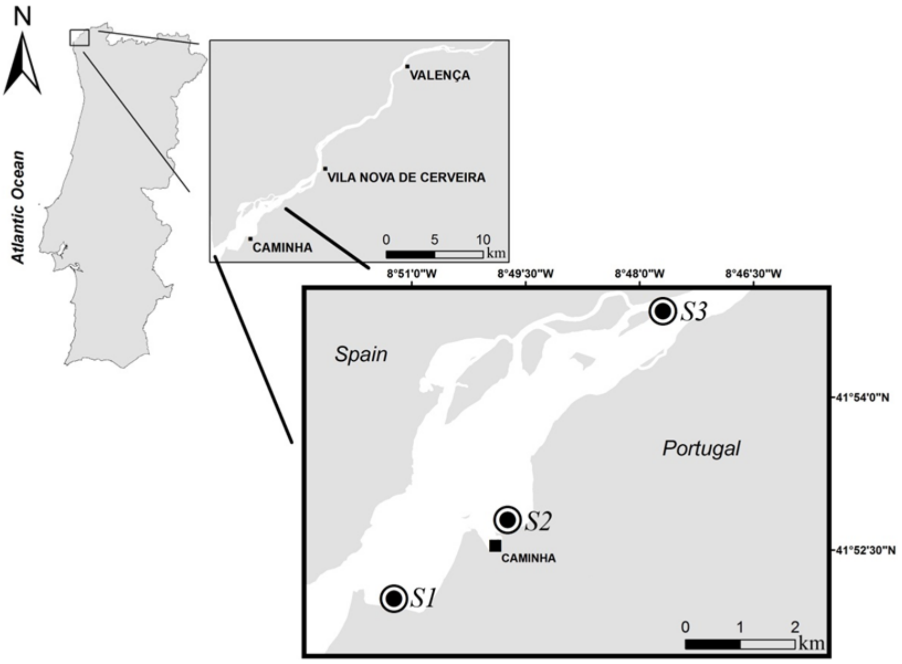


Fig. 7.1 Map of the study site showing the three sampling stations at the lower Minho Estuary, NW Iberian Peninsula.

S1 was closer to the river mouth (ca. 1.5 km), and characterized by the presence of a soft bottom often densely covered by debris, such as drifting seaweeds, dead leaves and empty mollusks' shells (Souza et al. 2011). S2 was located in the Coura salt marsh (ca. 3.5 km upwards from the river Minho mouth), which is a relatively small system characterized by the presence of narrow channels, typically bordered by the seagrass *Spartina maritima*. The channel's soft bottoms are often sparsely covered by debris and empty peppery furrow shells *Scrobicularia plana* (Souza et al. 2011). S3 was located ca. 5 km upstream from S2, and characterized by high densities of the Asian clam *Corbicula fluminea*, with the soft bottoms often sparsely covered by debris and submerged vegetation (Sousa et al. 2008b,c). In each site, with monthly periodicity, three replicates were collected using a 1 m beam trawl (5 mm mesh size) towed at constant speed (2 km.h^{-1}) for 2 to 3 minutes, during daylight at the high tide of spring tides. Previous studies in Minho estuary indicated that the sampling area covered by a 3 minutes tow, at constant speed, was equivalent to 100 ± 4 m^2 on an average (Freitas et al. 2009). Density data was standardized to the same scale (ind.100 m^2) prior to the statistical analyses. Additionally, at each site, water temperature, salinity, pH and oxidation reduction potential (ORP) were measured at 20 cm above the bottom with a multiparameter probe YSI 6820 (two replicates).

The monthly river discharge data measured at Foz do Mouro hydrometric station between February/09 and July/10 was obtained from the INAG – Instituto da Água, I.P. (http://snirh.inag.pt).

At each sampling date, the abundance of the five numerically dominant epibenthic species was determined by counting all individuals caught, while size (total length – T*L* or carapace width – CW) were measured (0.01 mm precision) using a digital caliper. Due to the enormous density of the brown shrimp *Crangon crangon*, subsamples were applied to estimate the total abundance of the species in each sample.

Randomly selected individuals (up to 500 individuals) of each species were wet weighted in a precision scale to the nearest 0.001 g. The wet weight and size relationship was calculated using the allometric equation:

$$\text{(1)} \qquad \mathrm{W} = \mathrm{a}.L^{\mathrm{b}}$$

Where W is the wet weight of the individual (in grams), L is the total length of the individual (in centimeters) and a and b refers to the allometric coefficients obtained through the length-weight relationship (Reiss 1989).

Each individual caught was classified as juvenile or adults based on its size (Table 7.1).

Table 7.1 Size of maturity of the five numerically dominant demersal species in the lower Minho estuary, NW Iberian Peninsula.

Species	Common name	Taxonomic group	Adults	Reference
Carcinus maenas	Shore crab	Crustacean	> 35 mm (CW)	Baeta et al. 2005
Crangon crangon	Brown shrimp	Crustacean	> 22 mm (T*L*)	Campos and van der Veer 2008
Platichthys flesus	European flounder	Fish	> 185 mm (T*L*)	Kosior et al. 1996
Pomatoschistus microps	Common goby	Fish	> 26 mm (T*L*)	Bouchereau et al. 1989
Pomatoschistus minutus	Sand goby	Fish	> 25 mm (T*L*)	Rochard and Elie 1994

7.2.2. Data analysis

Prior to the analysis of the abiotic data, all variables were normalized. The Principal Components Analysis (PCA) was performed in order to detect habitat differences among sampling stations based on environmental data. Posteriorly, a resemblance matrix based on the Euclidean distance was calculated.

The Permutational Multivariate Analysis of Variance (PERMANOVA) (type-III) was employed in a two-way crossed design, with station as a fixed factor (3 levels: S1, S2 and S3) and season as a fixed factor (4 levels: spring, summer, autumn and winter), in order to test the statistical significant differences among sampling stations and seasons using all abiotic variables (multivariate approach) and to compare each variable separately (univariate approach). The PERMANOVA analyses is capable of handling unbalanced statistical designs, such as in this study (due to unequal number of samples collected in each season), and calculates an identical F statistic that would be produced using traditional ANOVA and it is not affected by non-normal distribution of data (Anderson 2001).

Similarly, the biotic data were also normalized, and resemblance matrices based on Euclidean distance were calculated. The overall density, the overall biomass, the density of each species, and the biomass each species were statistically tested using a three-way PERMANOVA (type-III) in a three-way crossed design, with station as a fixed factor (3 levels: S1, S2 and S3), season as a fixed factor (4 levels: spring, summer, autumn and winter) and life stage as a fixed factor (2 levels: juvenile and adult) using all both multivariate and univariate approaches (comparing each species separately).

Additionally, the density and biomass of the top dominant species were tested using a four-way PERMANOVA (type-III) in a four-way crossed design, with station as a fixed factor (3 levels: S1, S2 and S3), season as a fixed factor (4 levels: spring, summer, autumn and winter), life stage as a fixed factor (2 levels: juvenile and adult) and taxonomic groups as a fixed factor (2 levels: crustacean and fish).

In PERMANOVA, the statistical significance of variance components was tested using 9999 permutations of residuals under a reduced model. When the possible number of permutations was lower than 150, the Monte Carlo-p value was considered. Subsequently, significant terms and interactions were investigated using a posteriori pair-wise comparisons to determine which pairs of stations and seasons were significantly different.

To determine which one of the five species was numerically dominant in each sampling station, radar plots were constructed using the mean density (ind.100m^{-2}) of each species during each season. Density data were log transformed to avoid bias due to different scales of variations in density among species. A similar procedure were used to plot the biomass dominance per station among seasons, with the mean density value of each species being multiplied by the weight (wet weight in grams) correspondent to the average size of a particular species in each season. Weights were obtained using the allometric equations of the length-weight relationships.

The ecological indexes of Pielou's evenness and the Berger-Parker dominance was calculated for each trawl using the PRIMER v 6.1.11® (Clarke and Gorley 2006) and the PAST® software respectively.

Canonical Correspondence Analysis (CCA) was performed to determine the relationship between the density of each life stage of the five dominant species and abiotic data using CANOCO 5 software (ter Braak and Verdonschot 1995). The Monte-Carlo randomization test (499 permutations under the reduced model) was performed to determine the statistical significance of the correlations (see ter Braak 1986).

Distance-based linear modeling (DistLM) using distance-based redundancy analysis (dbRDA) was carried out to assess the relative contribution of abiotic data in the density of the five dominant demersal species in Minho estuary.

The PERMANOVA, PCA, DistLM and dbRDA analyses were performed using PRIMER v 6.1.11® (Clarke and Gorley 2006) with PERMANOVA+1.0.1. add-on package (Anderson et al. 2008).

7.3. Results

7.3.1. Abiotic data

The Two-way PERMANOVA for stations and seasons of all abiotic variables was significant for both main effects (stations: pseudo-F = 7.99, $p < 0.001$; seasons: pseudo-F = 6.41, $p < 0.001$), but was not significant for the *stations x seasons* interaction (pseudo-F = 0.53, $p = 0.97$) (Supplementary material 7.1).

Depth was significantly higher in S1 during all seasons. On the other hand, pH did not varied among sampling stations, but was significantly higher during summer compared to winter in all stations. Salinity was higher in S1 during all seasons, and in S3 salinity significantly varied among seasons, due to the higher values observed in summer. Sampling stations presented similar values of temperature in all seasons but the summer, which presented warmer waters in S3; while during the winter, significantly colder waters compared to spring and summer were observed in S2 and S3. The ORP values did not varied among sampling stations and seasons (Table 7.2).

Table 7.2 Values of the five environmental variables (mean ± SE) measured in the three sampling stations throughout the seasons in the lower Minho estuary, NW Iberian Peninsula and the Two-way PERMANOVA results on the effects of sampling stations and seasons and their interaction term. * = $p < 0.01$; ** = $p < 0.001$; ^{ns} = non-significant. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test: $p < 0.05$).

	Seasons				Two-way PERMANOVA		
	Spring	Summer	Autumn	Winter	Stations	Seasons	Interaction
Depth							
S1	$3.1\pm0.3^{b(A)}$	$3.7\pm0.2^{ab(A)}$	$4.3\pm0.2^{c(A)}$	$4.4\pm0.3^{ac(A)}$	Pseudo-F = 59.04^{**}	Pseudo-F = 9.51^{**}	Pseudo-F = 1.20^{ns}
S2	$1.3\pm0.2^{a(B)}$	$1.8\pm0.1^{ab(B)}$	$1.8\pm0.1^{ab(B)}$	$2.0\pm0.2^{b(B)}$			
S3	$1.9\pm0.3^{a(B)}$	$2.8\pm0.3^{a(C)}$	$2.1\pm0.1^{a(B)}$	$3.1\pm0.6^{a(AB)}$			
ORP							
S1	156.1±28.9	144.4±36.3	169.2±27.0	123.7±16.6	Pseudo-F = 0.03^{ns}	Pseudo-F = 0.96^{ns}	Pseudo-F = 0.03^{ns}
S2	153.6±32.0	159.5±41.5	173.4±19.0	117.9±22.1			
S3	159.9±31.5	167.2±43.7	170.6±26.7	119.3±17.7			
pH							
S1	7.8 ± 0.3^{ab}	8.5 ± 0.3^{a}	8.0 ± 0.2^{ab}	7.5 ± 0.1^{b}	Pseudo-F = 0.75^{ns}	Pseudo-F = 9.90^{**}	Pseudo-F = 0.03^{ns}
S2	8.0 ± 0.2^{ab}	8.8 ± 0.3^{a}	8.0 ± 0.2^{ab}	7.6 ± 0.2^{b}			
S3	8.1 ± 0.2^{ab}	8.9 ± 0.3^{a}	8.1 ± 0.2^{ab}	7.6 ± 0.2^{b}			
Salinity							
S1	$26.0\pm2.9^{a(A)}$	$32.0\pm1.0^{a(A)}$	$31.8\pm1.9^{a(A)}$	$22.4\pm11.2^{a(A)}$	Pseudo-F = 19.91^{**}	Pseudo-F = 4.85^{*}	Pseudo-F = 0.54^{ns}
S2	$15.9\pm6.2^{a(A)}$	$30.8\pm1.9^{a(A)}$	$23.0\pm6.9^{a(AB)}$	$21.2\pm10.6^{a(A)}$			
S3	$0.4\pm0.2^{a(B)}$	$19.1\pm4.2^{b(B)}$	$4.3\pm4.2^{ab(B)}$	$0.4\pm0.4^{a(A)}$			
Temperature							
S1	$14.6\pm0.5^{a(A)}$	$15.0\pm0.6^{a(A)}$	$15.8\pm0.4^{a(A)}$	$11.9\pm1.5^{a(A)}$	Pseudo-F = 0.25^{ns}	Pseudo-F = 14.60^{**}	Pseudo-F = 2.06^{ns}
S2	$16.4\pm0.9^{a(A)}$	$15.9\pm0.5^{a(AB)}$	$15.1\pm1.5^{ab(A)}$	$12.0\pm1.2^{b(A)}$			
S3	$16.4\pm1.2^{a(A)}$	$18.3\pm1.2^{a(B)}$	$14.7\pm1.8^{a(A)}$	$8.8\pm0.6^{b(A)}$			

Additionally, the PCA routine indicated that the first two axis explained 62.4% of the variance among samples (PC1 = 34.3% and PC2 = 28.1%) and revealed marked differences between sampling stations, with station 1 differing from the other stations, mainly due to its lower values of temperature and pH and higher values of depth and salinity. The water temperature (eigenvalue = - 0.64) and the pH (eigenvalue = - 0.63) presented the strongest correlations in PC1, while salinity (eigenvalue = 0.72) and depth (eigenvalue = 0.57) were the most important variables in explaining the PC2 (Fig 7.2).

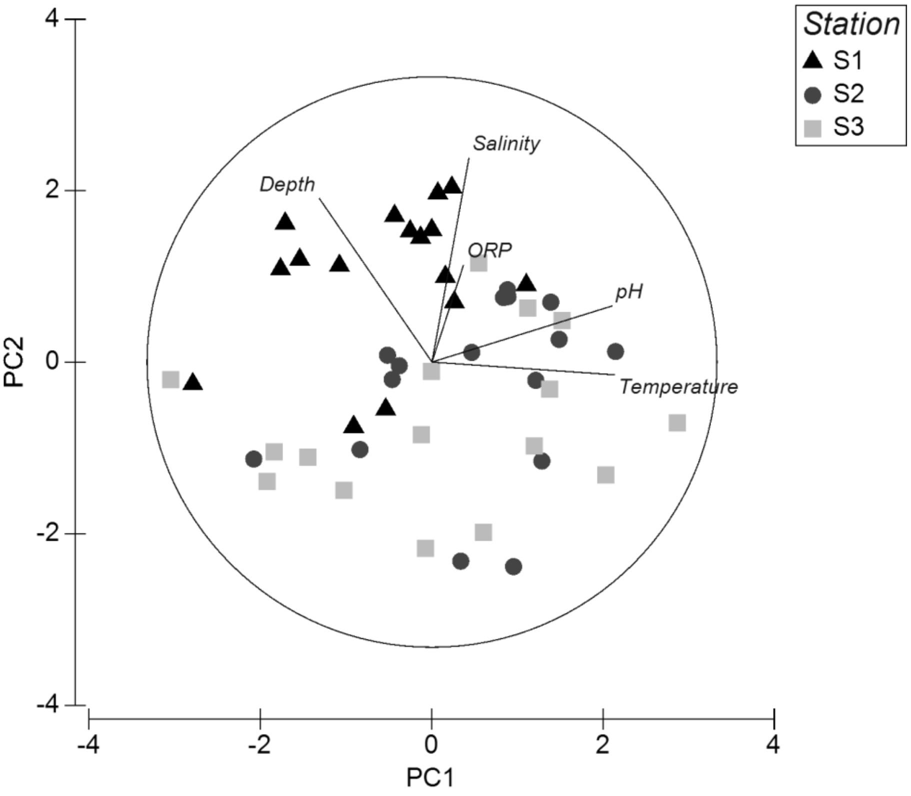


Fig. 7.2 PCA showing the differences of the environmental variables among sampling stations in the lower Minho Estuary, NW Iberian Peninsula.

7.3.2. Biotic data

7.3.2.1. Density patterns

A total of 104981 individuals were caught in all sampling stations during the studied period, with the brown shrimp *C. crangon* being the most abundant species (78.7%), followed by the common goby *P. microps* (16.7%), the shore crab *C. maenas* (4.0%), the European flounder *P. flesus* (0.3%) and the sand goby *P. minutus* (0.3%) respectively.

In general, Minho estuary is numerically dominated by juveniles (53.1%), with the population of *C. maenas*, *C. crangon* and *P. flesus* being dominated by juveniles (Fig. 7.3).

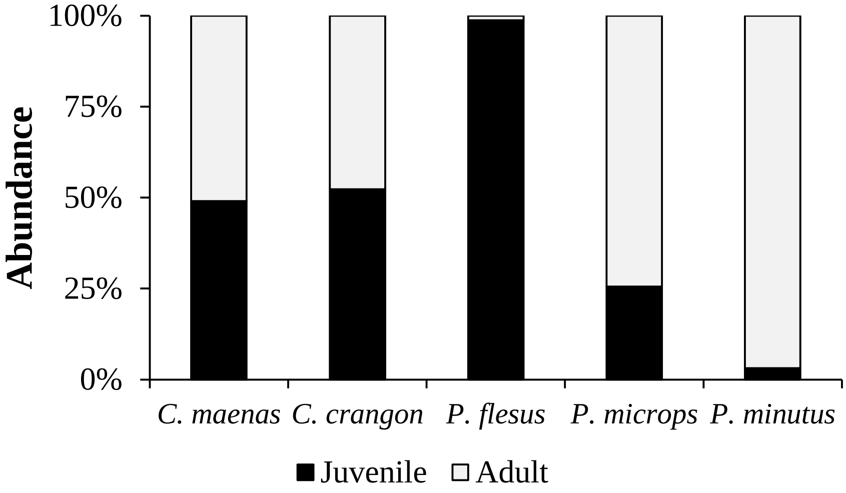


Fig. 7.3 Percentage of contribution of juveniles and adults for the population of the five most abundant demersal species in Minho lower estuary, NW Iberian Peninsula.

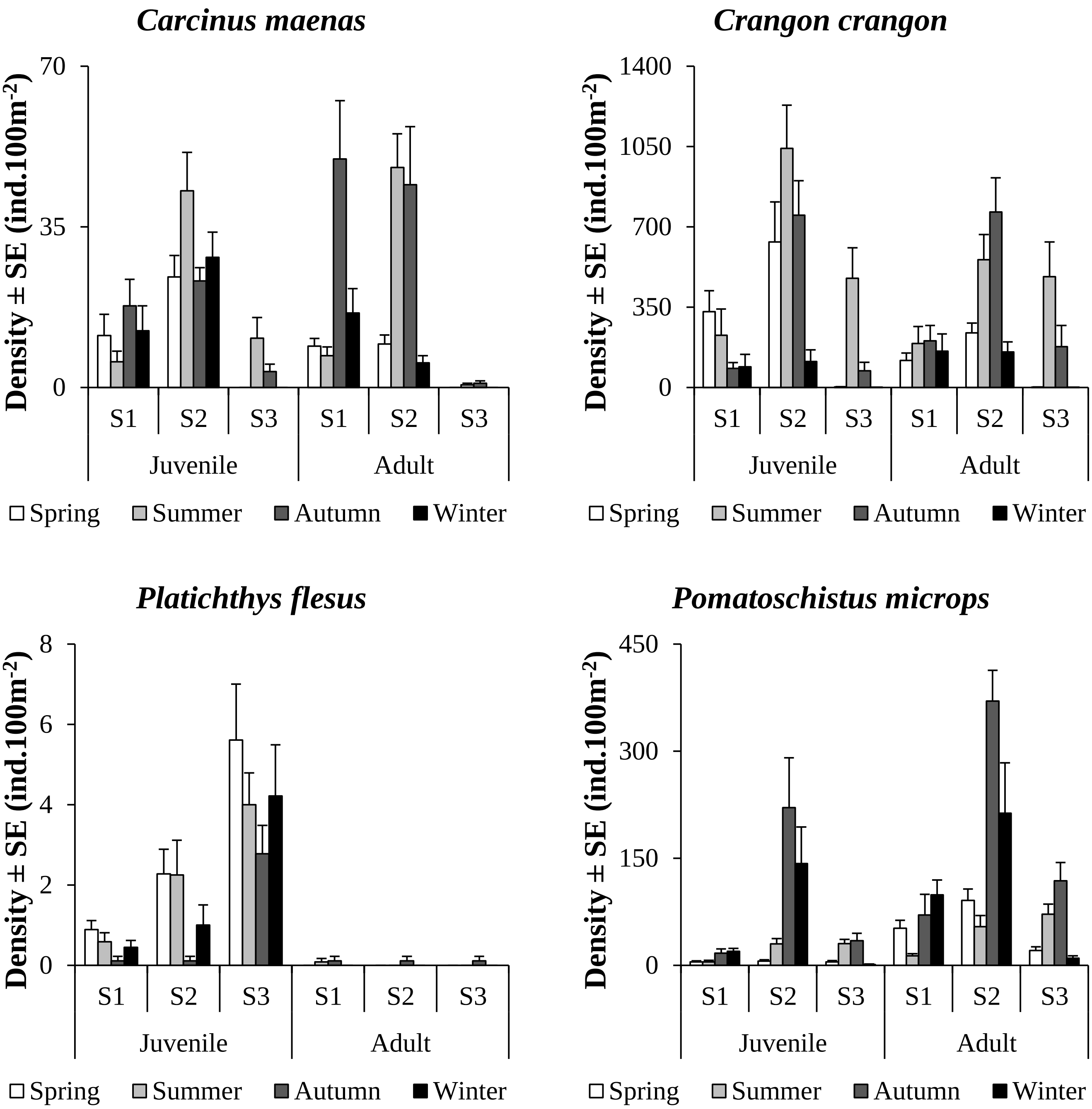
The three-way PERMANOVA for stations, seasons and life stages of the five dominant demersal species was significant for all effects and interactions (Table 7.2). However, each sampling station presented a distinct pattern, for instance in S1, the density of juveniles were significantly higher than adults' during spring and summer, while the opposite pattern was recorded during autumn and winter. A somewhat similar pattern was recorded in S2, which also presented a higher density of juveniles during spring and summer, but showed an even density of juveniles and adults during autumn and winter. On the other hand, the density of adults was continuously higher than juveniles' in S3 (Table 7.3).

Table 7.3 Three-way PERMANOVA results comparing the density of life stages (juveniles and adults) of the five most abundant demersal species (*Carcinus maenas*, *Crangon crangon*, *Pomatoschistus microps*, *P. minutus* and *Platichthys flesus*) in Minho estuary (NW Iberian Peninsula) among sampling stations (S1, S2 and S3) and seasons (Spring, Summer, Autumn, Winter). ns = non-significant p value. * Pairwise tests among life stages using the t-statistic on Pseudo-*F* values for detecting significantly differences in density of juveniles and adults.

Source	df	SS	MS	Pseudo-*F*	p
Station	2	218.64	109.32	37.20	< 0.001
Season	3	112.56	37.52	12.77	< 0.001
Life stage	1	79.68	79.68	27.12	< 0.001
Station x Season	6	114.18	19.03	6.48	< 0.001
Station x Life stage	2	59.10	29.55	10.06	< 0.001
Season x Life stage	3	36.87	12.29	4.18	< 0.001
Station x Season x Life stage	6	32.45	5.41	1.84	< 0.01
Residual	264	775.73	2.94		
Total	287	1435			
Pairwise comparison		Juvenile (ind.100m^{-2})	Adult (ind.100m^{-2})	t	p
S1 Spring		347.3±92.5	182.0±39.9	1.63	< 0.001
Summer		238.0±118.0	217.6±77.9	2.67	< 0.001
Autumn		117.8±33.5	336.9±97.7	3.79	< 0.001
Winter		121.4±56.3	273.2±82.8	1.77	< 0.05
S2 Spring		666.0±172.0	339.1±40.1	3.01	< 0.001
Summer		1118.0±195.0	659.0±115.0	1.85	< 0.05
Autumn		996.0±212.0	1180.0±194.0	1.56	ns
Winter		285.9±90.6	374.1±86.0	1.47	ns
S3 Spring		12.5±3.39	21.8±5.93	4.03	< 0.001
Summer		521.0±135.0	556.0±160.0	2.78	< 0.001
Autumn		113.0±43.7	298.0±105.0	2.80	< 0.001
Winter		5.4±1.32	10.1±3.61	3.33	< 0.001

Each species presented a different pattern of spatio-temporal distribution of their life stages. For instance, adults of *C. maenas* were significantly denser than juveniles in S1 during autumn, and juveniles were denser than adults during spring and winter in S2, and during summer in S3. On the other hand, *C. crangon* juveniles were denser than adults during spring (S1 and S2) and summer (S2), with adults never outnumbering the density of juveniles. As well as the brown shrimp, the density of *P. flesus* juveniles was frequently higher than adults', with significant differences being recorded in S1 (spring and winter), S2 (spring, summer and winter) and S3 (all seasons). Conversely, the density of adult both gobies species were frequently higher than juveniles: *P.*

microps (S1: summer, autumn and winter; S2: spring; S3: all seasons) and *P. minutus* (S1: summer, autumn and winter; S2: spring) (Fig. 7.4; Supplementary material 7.2).



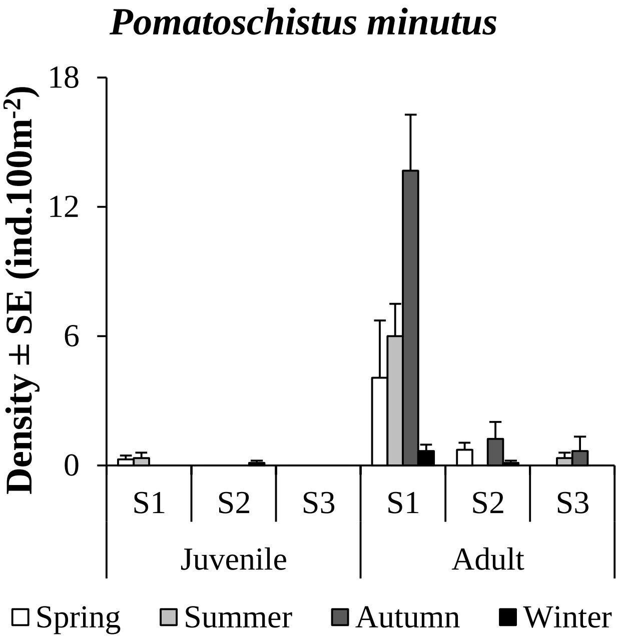


Fig. 7.4 Comparisons on the density of juveniles and adults of the five top-abundant demersal species in Minho estuary (NW Iberian Peninsula) among sampling stations and seasons.

The four-way PERMANOVA for stations, seasons, life stages and taxonomic groups was significant for all the effects and interactions, indicating that density of juveniles and adults of crustaceans and fishes was uneven among sampling stations and seasons (Supplementary material 7.3).

7.3.2.2. Biomass patterns

A total of 49679.8 g were caught in all sampling stations during the studied period, with the shore crab *C. maenas* the species with the highest biomass (75.2%), followed by the brown shrimp *C. crangon* (11.6%), the common goby *P. microps* (6.7%), the European flounder *P. flesus* (5.4%) and the sand goby *P. minutus* (1.0%) respectively.

In general, the biomass of adults (81.4%) was higher than juveniles' (18.6%), with most of the biomass of *C. maenas*, *C. crangon*, *P. microps* and *P. minutus* populations being represented by adults' biomass (Fig. 7.5).

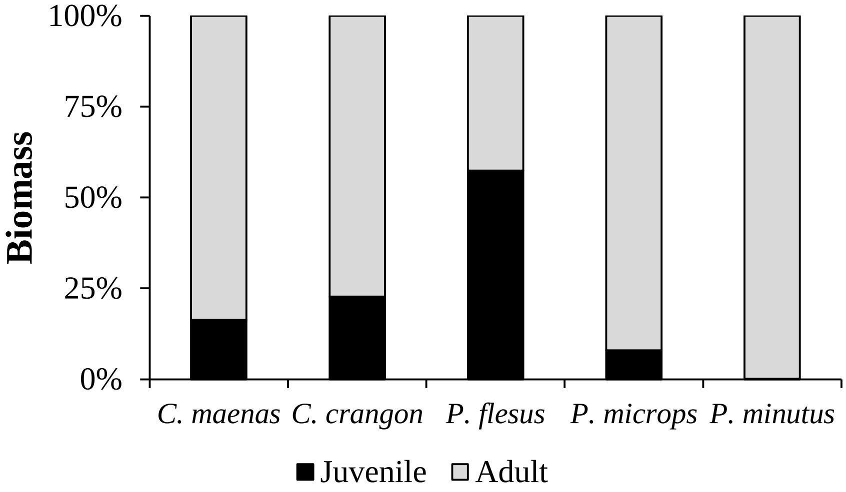


Fig. 7.5 Percentage of contribution of juveniles and adults biomasses of the five dominant demersal species in Minho lower estuary, NW Iberian Peninsula.

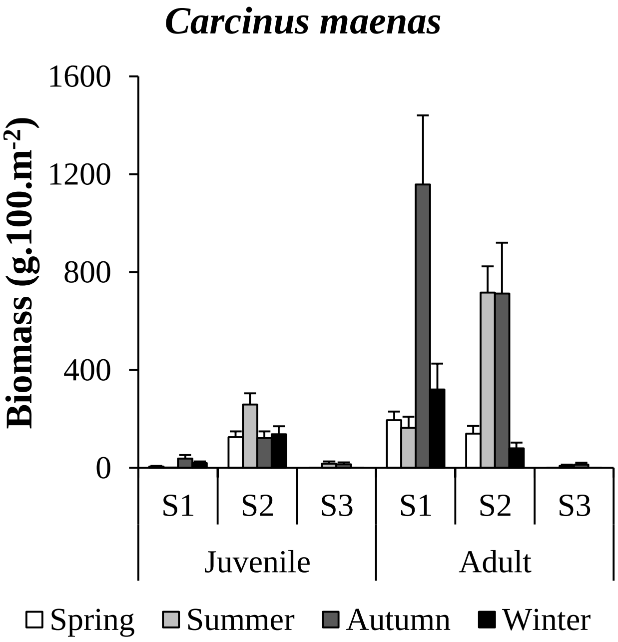
The three-way PERMANOVA for stations, seasons and life stages of the five dominant demersal species was significant for all effects and interactions. However, each sampling station presented a distinct pattern, for instance, the biomass of adults were higher than juveniles' in S1 and S2 during all seasons, whereas in S3, the biomass of juveniles were greater than adults' in spring and winter (Table 7.4).

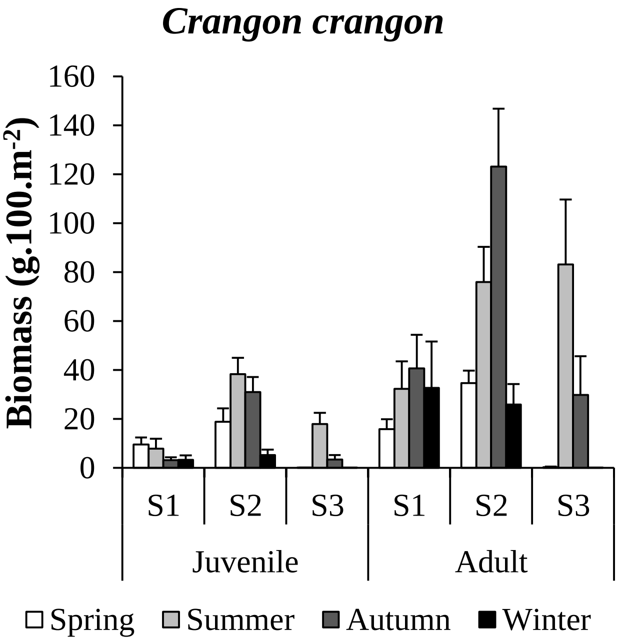
Table 7.4 Three-way PERMANOVA results comparing the biomass of life stages (juveniles and adults) of the five most abundant demersal species (*Carcinus maenas*, *Crangon crangon*, *Pomatoschistus microps*, *P. minutus* and *Platichthys flesus*) in Minho estuary (NW Iberian Peninsula) among sampling stations (S1, S2 and S3) and seasons (Spring, Summer, Autumn, Winter). ns = non-significant p value. * Pairwise tests among life stages using the t-statistic on Pseudo-*F* values for detecting significantly differences in biomass of juveniles and adults.

Source	df	SS	MS	Pseudo-*F*	p
Station	2	118.82	59.41	20.66	< 0.001
Season	3	118.25	39.42	13.71	< 0.001
Life stage	1	149.18	149.18	51.88	< 0.001
Station x Season	6	115.14	19.19	6.67	< 0.001
Station x Life stage	2	63.50	31.75	11.04	< 0.001

Season x Life stage	3	87.17	29.06	10.10	< 0.001
Station x Season x Life stage	6	79.94	13.32	4.63	< 0.01
Residual	264	759.19	2.88		
Total	287	1435			
Pairwise comparison		Juvenile (g.100m^{-2})	Adult (g.100m^{-2})	t	p
S1 Spring		35.2±8.2	238.2±41.4	2.58	< 0.001
Summer		22.0±7.4	225.9±48.5	1.89	< 0.01
Autumn		45.3±14.3	1296.0±287.0	3.21	< 0.001
Winter		28.9±7.1	389.0±100.0	2.66	< 0.001
S2 Spring		156.6±25.6	206.1±33.0	3.83	< 0.001
Summer		313.8±53.0	805.0±109.0	2.92	< 0.001
Autumn		171.1±31.4	966.0±218.0	3.37	< 0.001
Winter		160.4±30.5	166.9±25.6	2.84	< 0.01
S3 Spring		9.78±2.56	8.83±2.24	3.99	< 0.001
Summer		60.0±17.6	119.3±30.9	2.43	< 0.01
Autumn		37.2±11.4	122.7±61.6	1.35	< 0.05
Winter		19.1±6.2	4.6±2.0	2.93	< 0.001

Overall, the biomass of adults was significantly higher than juveniles' for all species but *P. flesus*, which presented a significant higher biomass of juveniles compared to adults (Fig. 7.6; Supplementary material 7.4).





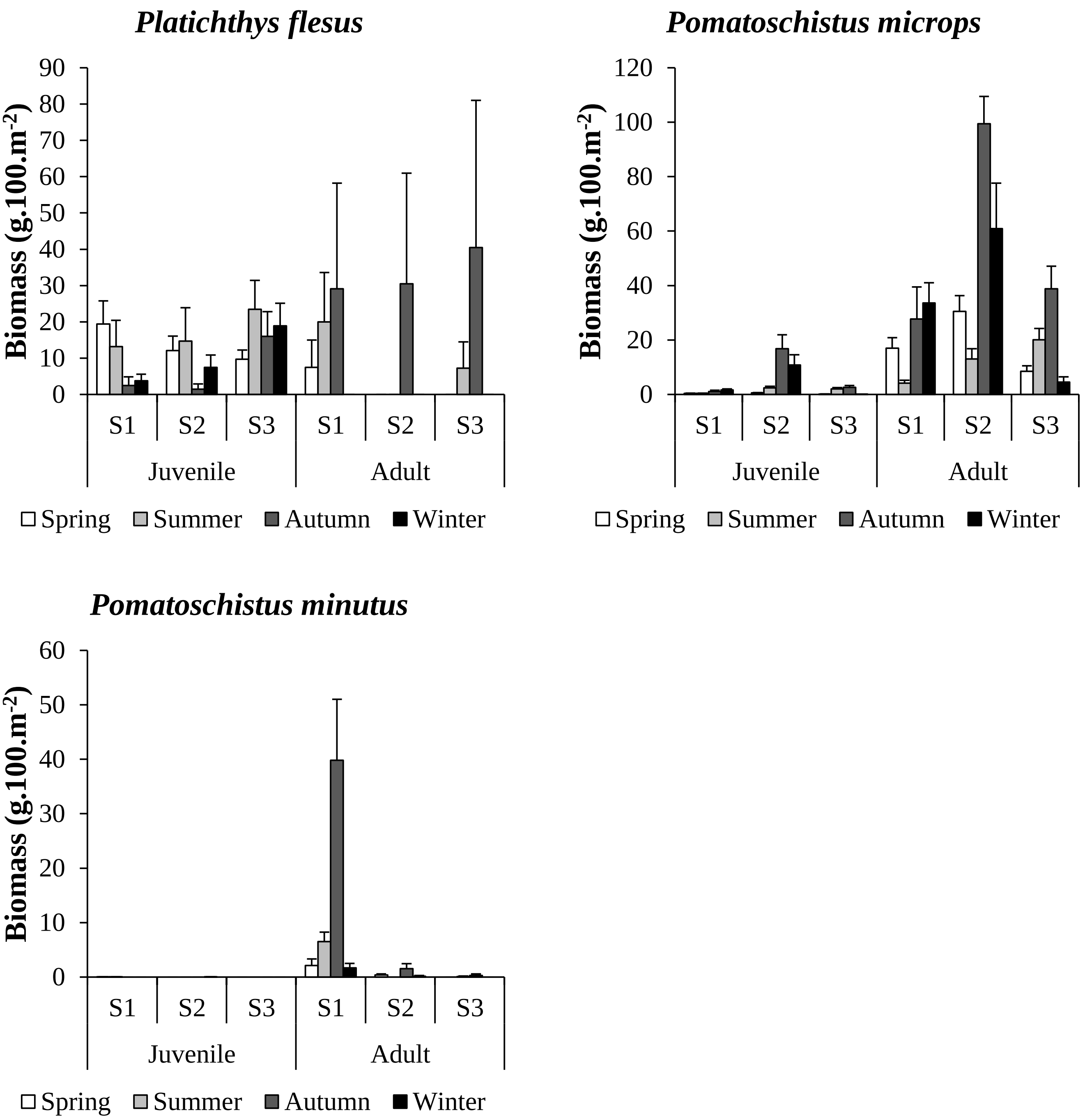


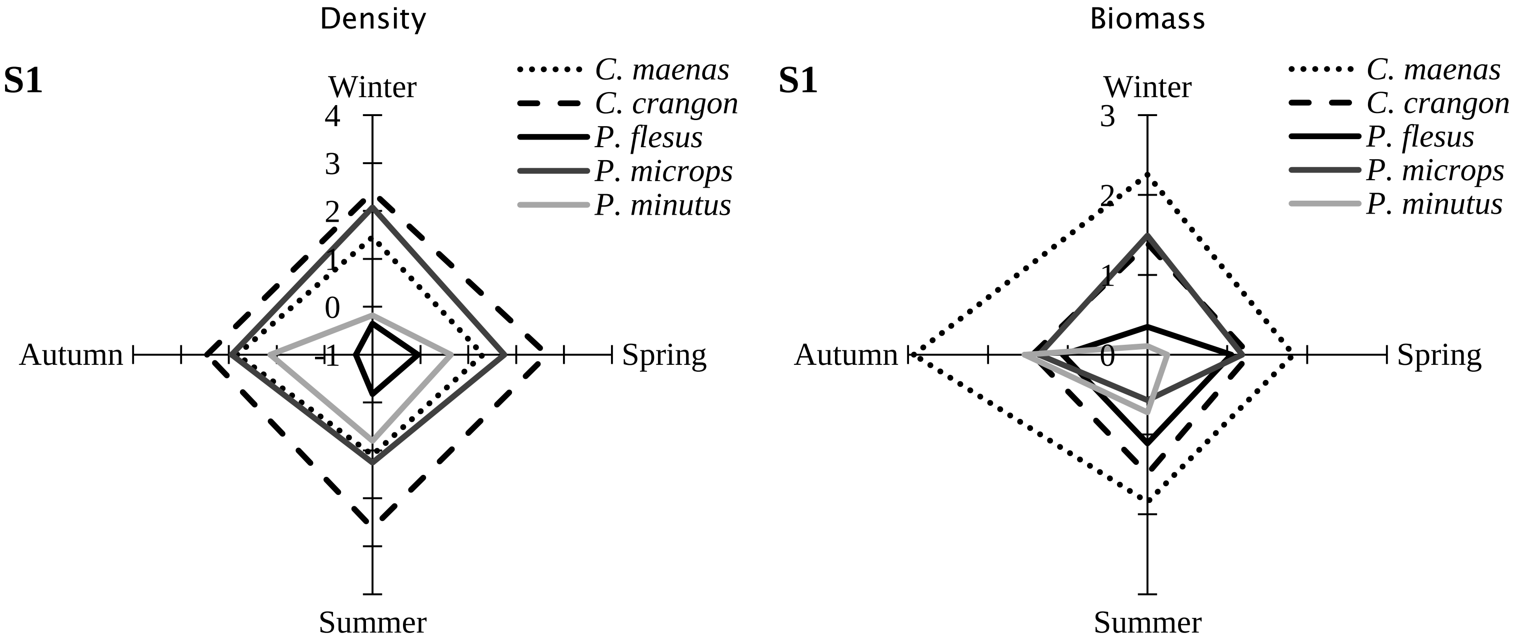
Fig. 7.6 Comparisons on the biomass of juveniles and adults of the top five abundant demersal species in Minho estuary (NW Iberian Peninsula) among sampling stations and seasons.

The four-way PERMANOVA for stations, seasons, life stages and taxonomic groups was significant for all the effects and interactions, indicating that biomass (Supplementary material 7.5) of juveniles and adults of crustaceans and fishes was uneven among sampling stations and seasons.

7.3.2.3. Dominance patterns

When considering the density of individuals, the brown shrimp *C. crangon* and the common goby were the dominant species, with the European flounder *P. flesus* the most discrete species in S1. In S2, a similar pattern was detected, with the dominance of *C. crangon* and *P. microps*; however the sand goby *P. minutus* was the most discrete species in this station. On the other hand, the pattern observed in S3 highly differed from others stations, with a numerical dominance of *P. microps*. The density of *C. crangon* greatly varied in S3. The brown shrimp was the dominant species during the summer, but showed a discrete pattern during winter and spring (Fig. 7.7).

A different pattern was recorded when considering the biomass of the five most abundant species. In S1 and S2 the dominant species was the shore crab *C. maenas*, with the brown shrimp *C. crangon* and the common goby *P. microps* being equally important. On the other hand, in S3, *P. flesus* and *P. microps* were the most important species during all seasons, except during summer, where *C. crangon* was the dominant species in this sampling station (Fig. 7.7).



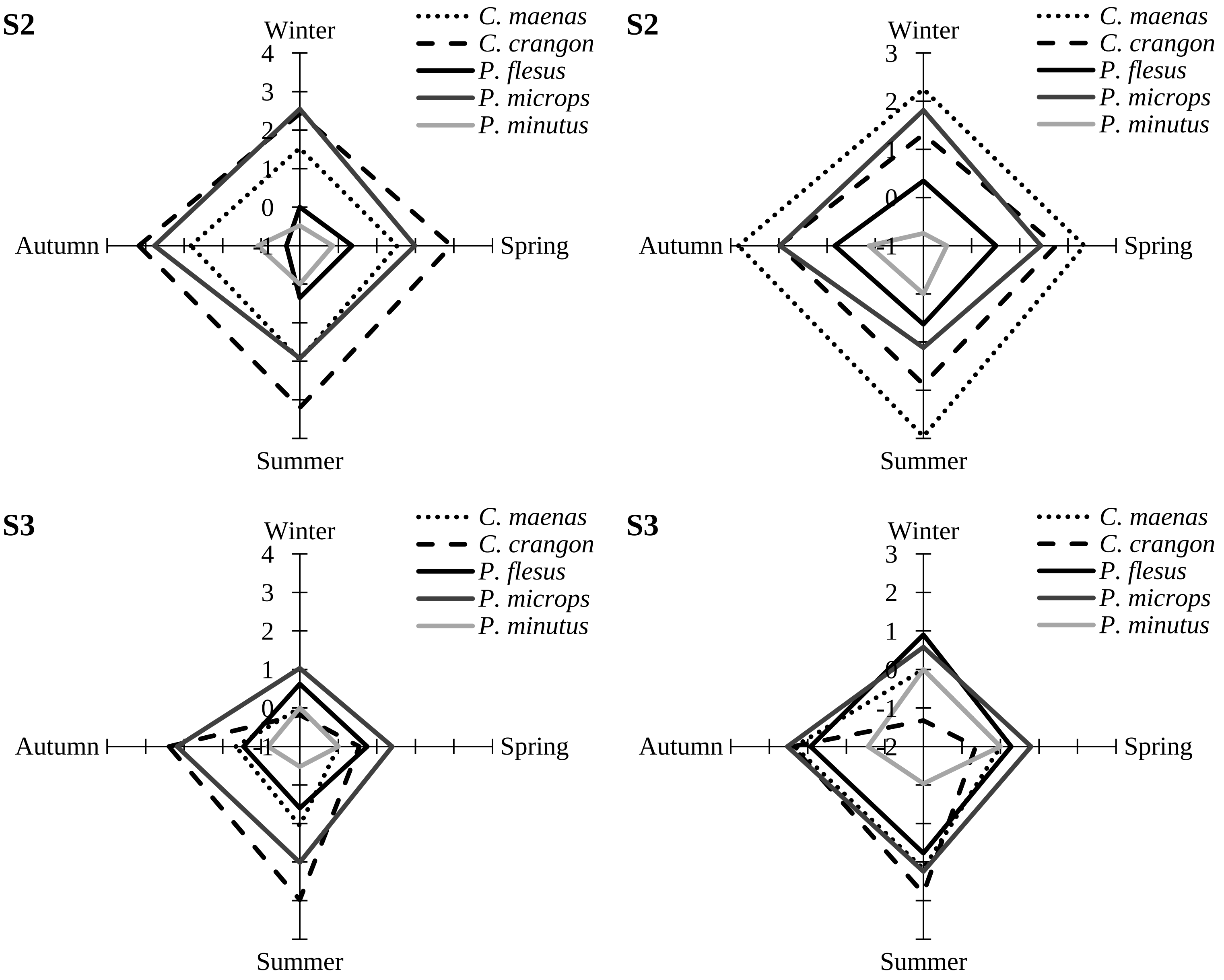


Fig. 7.7 Dominance patterns: Comparisons on the density (left column) and biomass (right column) of the top-abundant demersal species in Minho lower estuary, NW Iberian Peninsula. Density (ind.100m^{-2}) data refers to the mean values (log transformed) of each species in every sampling station and season. Biomass data refers to a value (log transformed) calculated by the simple multiplication of the mean density (ind.100m^{-2}) by the average wet weight (g) of each species during the respective season.

The two-way PERMANOVA for stations and seasons of the Pielou's evenness index was not significant for the station effect (pseudo-F = 0.88, p = 0.42), but was significant for the season effect (pseudo-F = 8.49, p < 0.001) and the *stations x seasons* interaction (pseudo-*F* = 4.69, p < 0.001). The equilibrium among the top-abundant species was higher during the autumn when compared to spring in S1. In S2 the top-abundant species was less evenly distributed during the spring and summer. In S3 the evenness was significantly higher during spring and winter compared to summer and autumn. During spring, the evenness was significantly higher in S3 when compared to S1 and S2. On the other hand, evenness was significantly lower in S3 during autumn compared to S1 and S2. No significant differences among sampling stations were detected during the summer and winter (Fig. 7.8A).

The Two-way PERMANOVA for stations and seasons of the Berger-Parker dominance index was significant for both of the main effects (stations: pseudo-*F* = 8.38, p < 0.001; seasons: pseudo-*F* = 3.98, p < 0.01), and for the *stations x seasons* interaction (pseudo-*F* = 2.80, p < 0.05). The dominance in S1 was significantly higher during spring when compared to autumn and winter. In S2, the dominance was higher during summer compared to autumn and winter; also, the dominance was significantly lower in autumn compared to spring and summer. On the other hand, the dominance was higher during autumn compared to spring in S3. During autumn, the Berger-Parker index was significantly lower in S1 when compared to S2 and S3; whereas during winter, dominance was significantly higher in S3 compared to S1 and S2. On the other hand, no significant differences among sampling stations were observed during spring and summer (Fig. 7.8B).

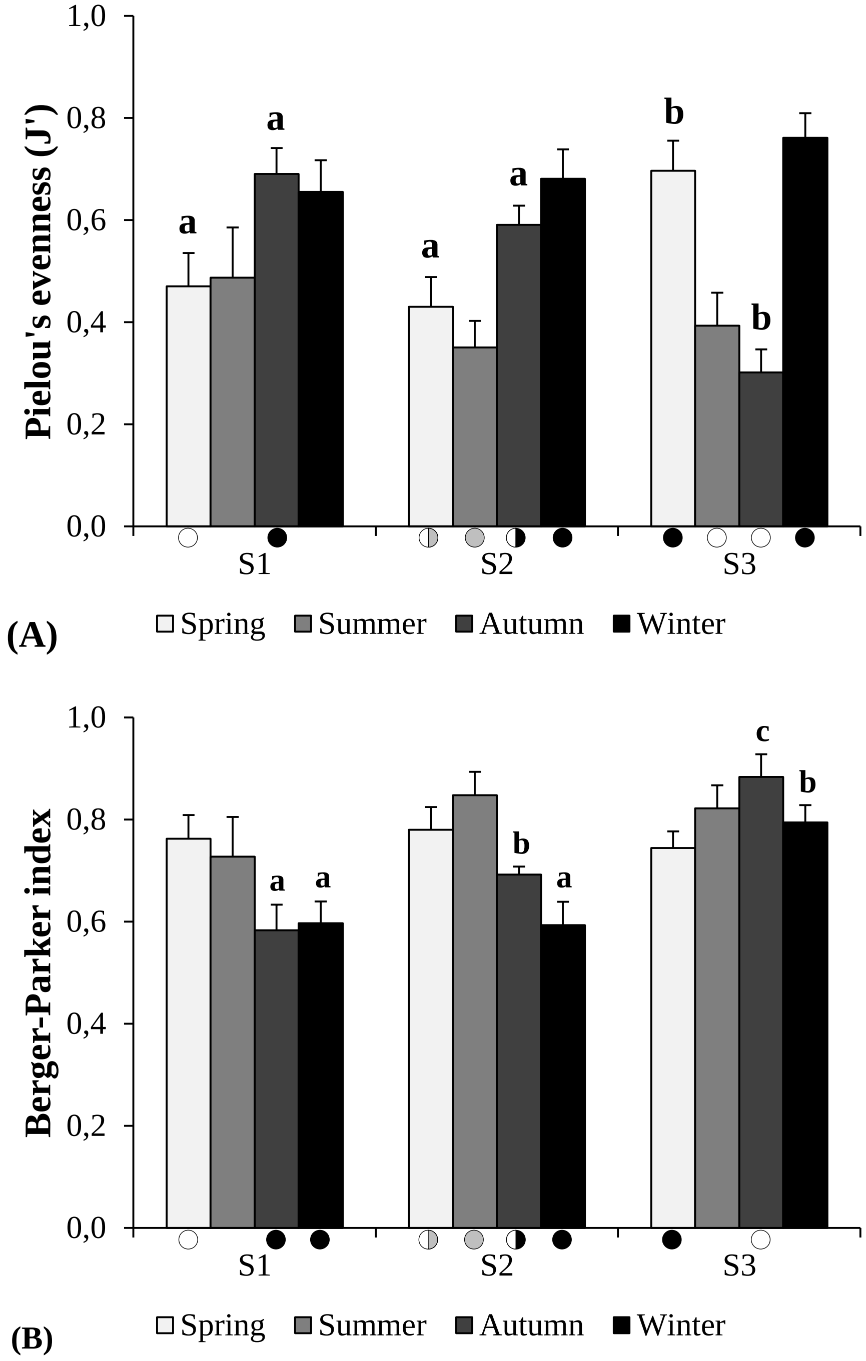
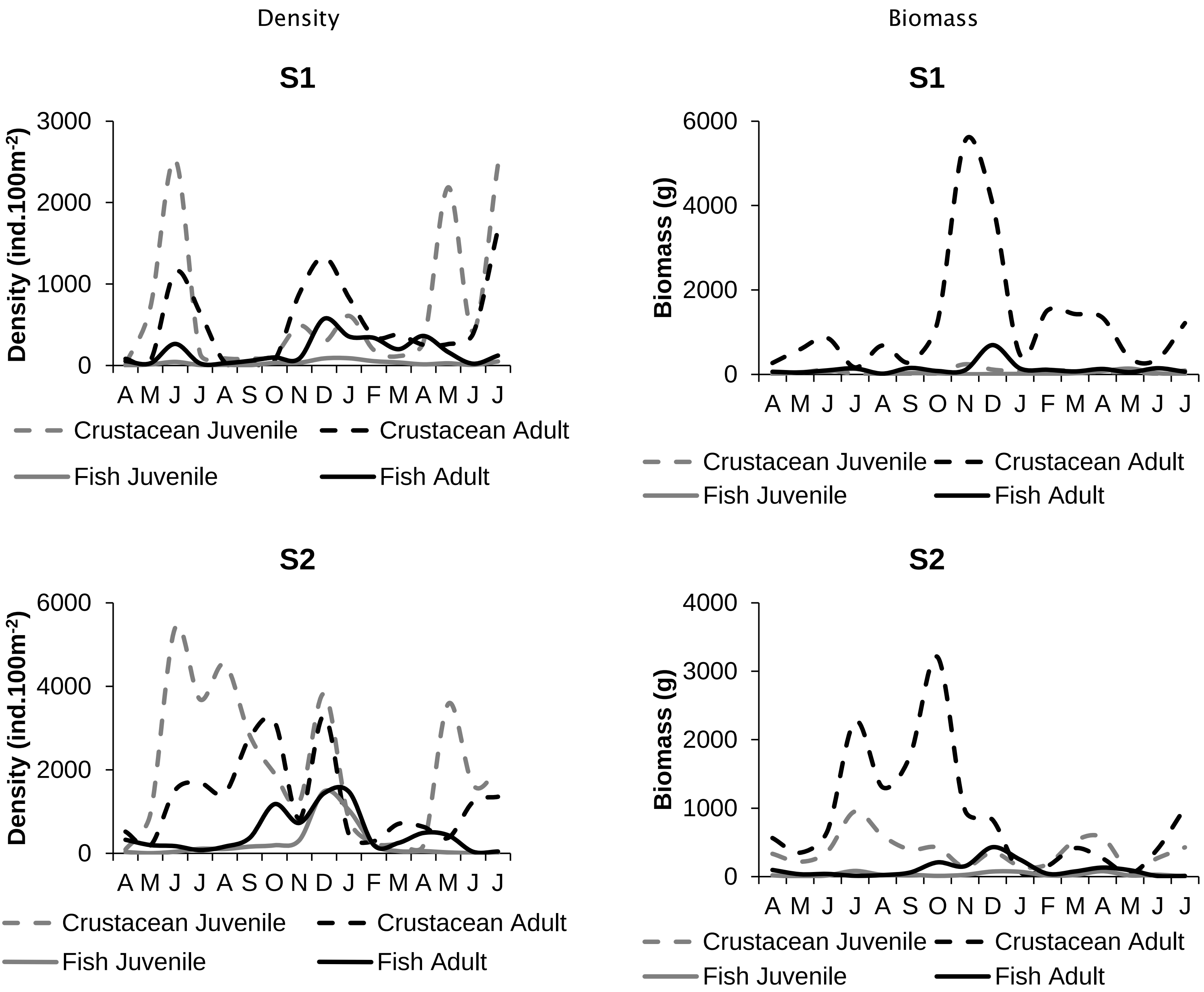


Fig. 7.8 Comparisons on the Pielou's evenness index (A) and the Berger-Parker dominance index (B) values for the top five dominant demersal species in Minho estuary (NW Iberian Peninsula) between sampling stations and seasons. Different letters (a or b) indicates post-hoc differences among sampling stations within the same season. Post-hoc differences among seasons within each sampling station are indicated by circles of different colours (white, grey or black) (Two-way PERMANOVA pairwise tests: $p < 0.05$).

Overall, crustaceans were dominant in S1 and S2 both in terms of density and biomass, while fishes were dominant in S3 during most of the sampling period, with the exception of summer months (Fig. 7.9).



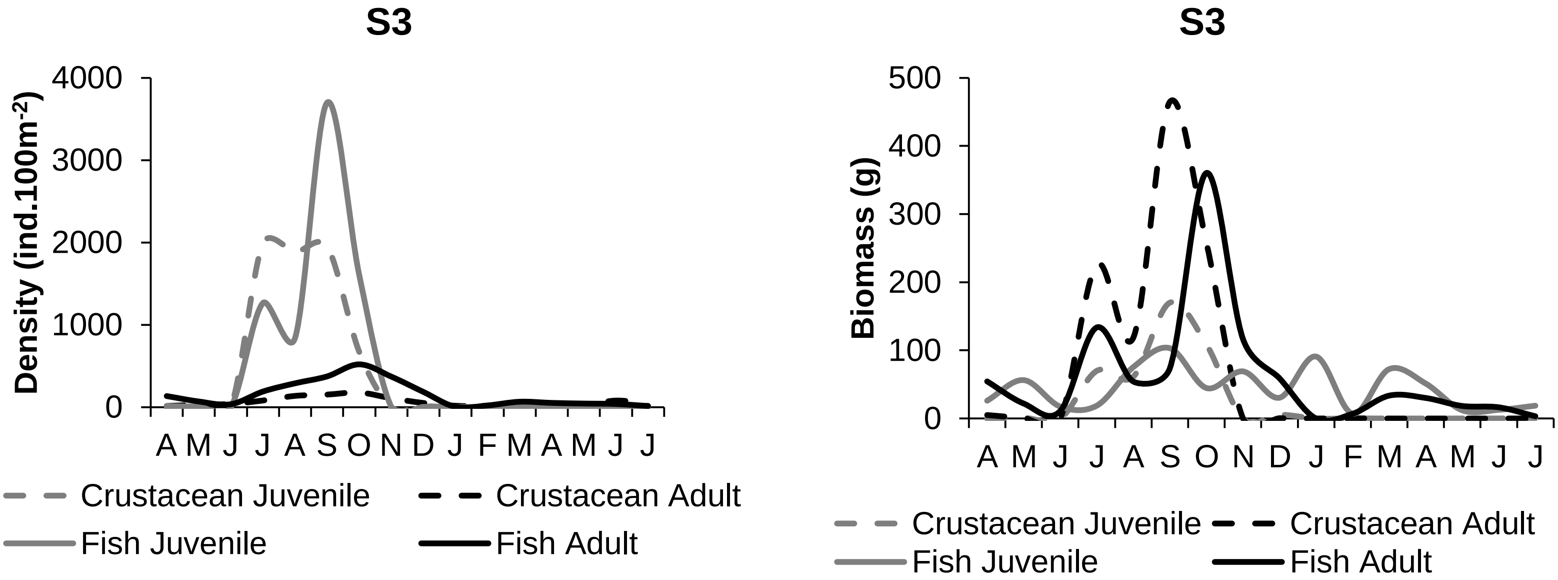


Fig. 7.9 Density and biomass of juveniles and adults of the two dominant taxonomic groups (crustaceans and fishes) in the three sampling stations of the lower Minho estuary (NW Iberian Peninsula) throughout 16 consecutive months (April/09 to July/10).

7.3.2.4. Species-habitat association

Overall, juveniles and adults of the five most abundant species in the lower Minho estuary associated differentially with environmental variables indicating that each group within each species has dissimilar habitat preferences. The common goby presented a weak differentiation between juveniles and adults, with both life stages associating with low values of temperature, salinity and pH and with high values of river discharge. On the other hand, the sand goby showed a marked differentiation in habitat preferences between life stages, with juveniles associating with saltier and warmer waters, while adults associated mostly with deeper waters. The European flounder showed an even stronger dissociation of habitat preferences between life stages, with juveniles being associated to low salinity and depth values, whit adults presenting an opposite preference. The shore crab juveniles showed a weak association with abiotic factors, while adults associated with high values of salinity and depth. The brown shrimp adults also presented weak association to the measured abiotic variables, whereas juveniles prefer to inhabit shallow estuarine zones (Fig. 7.10). The Monte-Carlo test was significant (test of significance of all canonical axes: trace = 0.243; F-ratio = 3.710; $p < 0.01$) and cumulatively, axes

1 and 2 accounted for 83.9% of the total variance, with correlations between species and environmental variables of 0.755 (axis 1) and 0.781 (axis 2).

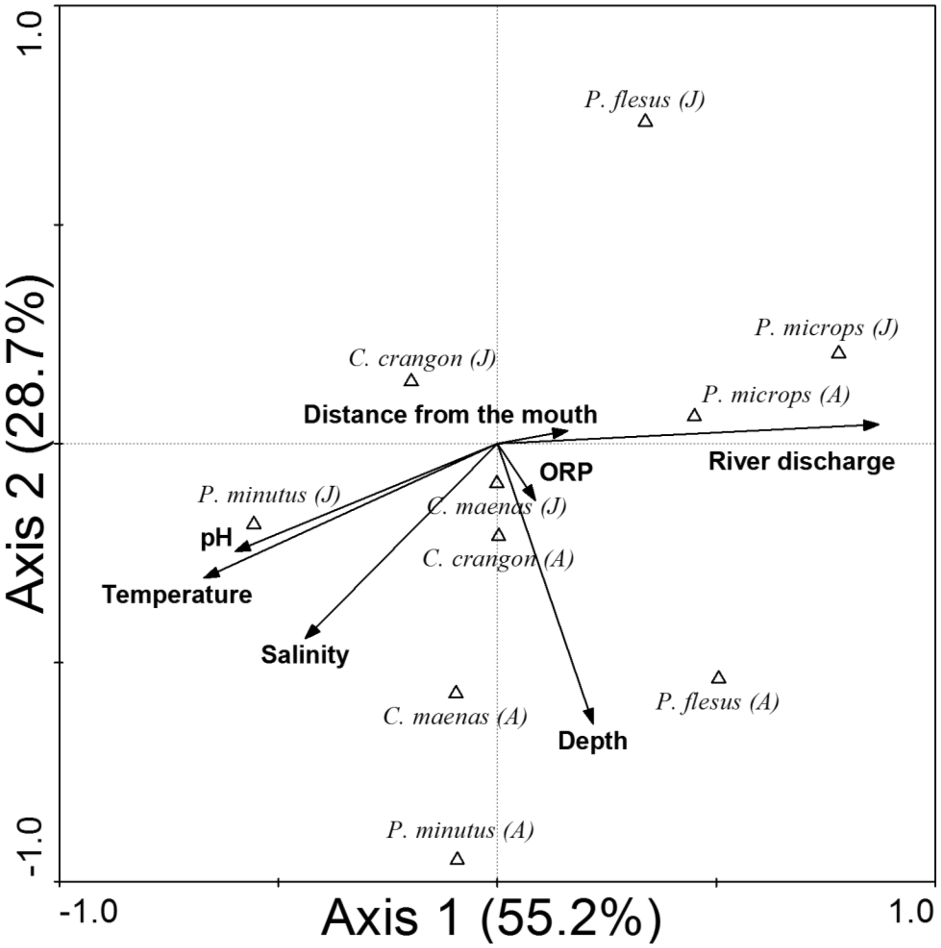


Fig. 7.10 Canonical plotting of abiotic characteristics (arrows) and juveniles *(J)* and adults *(A)* of the top five dominant demersal species in the lower Minho estuary, NW Iberian Peninsula. ORP = Redox potential.

Additionally, the DistLM analysis showed that of the five abiotic variables measured, only depth (AIC = 107.84, SS trace = 26.09, Pseudo-F = 2.93, $p < 0.01$) and salinity (AIC = 108.87, SS trace = 43.33, Pseudo-F = 4.67, $p < 0.001$) significantly influenced the distribution of the top five dominant species in Minho estuary.

7.4. Discussion

Our data shows that, in general, the epibenthic assemblages of Minho estuary are dominated by juveniles. However marked differences among sampling stations were observed, with the sampling stations closer to the river mouth

(S1 and S2) presenting a dominance of juveniles, especially during spring and summer months, while adults were dominant in S3 during all seasons. The numerical dominance of juveniles is relatively common in estuaries (e.g. Garcia et al. 2001), and it is derived from the presence of physical structures (mainly vegetation and debris), shallow depth, which provides refuge from predators (Lubbers et al. 1990; Ruiz et al. 1993) and high prey accessibility (Meador and Kelso 1990). The sampling stations S1 and S2 present a high availability of refuges, thus is more likely that juveniles preferred these areas. Despite of the high availability of bivalve shells in S3 (Ilarri et al. 2012), adults were dominant in this sampling station, and this is probably related to the abiotic characteristics of this particular estuarine area, which is located in the transition zone between the mesohaline and oligohaline portions of the estuary (Dias et al. unpublished data). Usually, adults exhibit a higher tolerance to harsh environments than juveniles (Pattillo et al. 1995), and then in a stressful area such as S3, adults can stick at, while juveniles may find difficulties to thrive in harsh environmental conditions such as the high variability in hydrodynamics, salinity and turbidity. In such stressful area, only few species are physiologically able to colonize, inducing a low biological competition and high abundances (McLusky and Elliot 2004). In fact, in S3, the common goby *P. microps* presented high density and biomass, being the dominant species in this area. The common goby *P. microps* is classified as an estuarine resident fish (Elliot et al. 2007), and is known to present a great capacity of adaptation, which allows them to successfully occupy habitats with different environmental characteristics (Bouchereau and Guelorget 1998). On the other hand, the brown shrimp *C. crangon* and the shore crab *C. maenas*, which were the dominant species in terms of density and biomass respectively in S1 and S2, presented low densities in S3, with peaks of abundance being recorded in summer. Despite of these crustaceans present a broad tolerance to variations in environmental characteristics (e.g. Crothers 1967; Kuipers and Dapper 1984), they cannot thrive in areas with such low salinity values as in S3 (down to 0.04) (see Crothers 1967; Bodekke 1975). Due to the capacity of *P. microps* to inhabit this area, and since *C. maenas*, *C. crangon* and *P. microps* prey on similar food items (see Mendonça et al. 2009), the lower density of the crustaceans might have favored the common goby, which become dominant under these conditions, corroborating with Souza et al. (in revision), which reported that a stressful environment can be favorable to *P. microps*.

Moreover, the dominance of *P. microps* in S3 may also be related to the presence of the invasive clam *Corbicula fluminea*. Actually, in a recent study, Ilarri et al. (unpublished data) found that in an area in Minho estuary near S3, *C. fluminea* significantly influence the epibenthic assemblages. The shells of *C. fluminea* seemed to favor small-bodied animals; particularly the common goby *P. microps,* that may find refuge from predators within the shell reef, also using the shells in its reproduction (Ilarri et al. unpublished data).

Our results also showed that the demersal compartment of Minho estuary is dominated by crustaceans, with the brown shrimp being the most abundant species, and the shore crab the species with the highest biomass in the estuary. The dominance of crustaceans is relatively common in temperate estuaries (e.g. Maes et al. 1998; Beyst et al. 2002; Drake et al. 2002; Amara and Paul 2003). And in Minho estuary, this dominance might be related to differences in their life cycle, since *C. crangon* (Freitas et al. 2011) and *C. maenas* (Souza et al. unpublished data) present a continuous recruitment in this system, while *P. microps*, *P. minutus* and *P. flesus* present recruitment peaks (Table 7.5). The continuous recruitment of crustaceans, allows them to uninterruptedly input juveniles into the system, while fishes' input is concentrated in specific periods of time (mainly during spring). Therefore, the gross number of crustaceans' recruits in Minho estuary in a year cycle probably is greater than fishes', allowing crustaceans to dominate the system. Moreover, the dominance pattern of crustaceans in Minho estuary may also be related with differences in the reproduction strategies between these fishes and crustaceans' species. In recent studies, the population ecology of the dominant demersal species in Minho estuary was investigated, and 3 of the 5 studied species reproduces in the sea, namely *C. crangon* (Freitas 2011), *C. maenas* (Souza et al. unpublished data) and *P. minutus* (Souza et al., unpublished data), while *P. microps* (Souza et al. in revision) and *P. flesus* might reproduce inside the estuary (Morais et al. 2011). The variations in the abiotic parameters in the sea is much less pronounced than in estuaries (Pritchard 1967), consequently species which reproduces in the sea can more often find optimal conditions for reproduction, while the reproduction of estuarine species may be restricted to specific periods of time. Actually, crustaceans were dominant in the two sampling stations closer to the sea (S1 and S2), while fishes dominated in the upper area (S3). Moreover, juveniles were more abundant in S1 and S2, suggesting that the reproduction strategy and the proximity to the sea may play an important role in the structure of estuarine assemblages indeed.

Table 7.5 Summary of the information regarding the five most abundant demersal species in Minho estuary, NW Iberian Peninsula.

Species	*C. maenas*	*C. crangon*	*P. flesus*	*P. microps*	*P. minutus*
Mean density (ind.100m^{-2})	29.0±6.1	573.7±27.8	2.3±1.8	121.7±13.9	2.2±2.5
Secondary production (g.WWm^{-2}.year^{-1})	189.5	0.264 (AFDW)*	0.04	3.8	0.16
Recruitment pattern	Continuous	Continuous*	Once a year	Twice a year	Once a year
Recruitment season	All year	All year*	Late winter or early spring	Late spring or early summer and autumn	Late winter or early spring
Preferred area	Salt marsh	Salt marsh	Mid-estuary	Salt marsh	River mouth
Ovigerous females	Rare	Rare	Absent	Common	Rare
Gender dominance	Male	Female	Even	Female	Male
Life stage dominance	Juvenile	Juvenile	Juvenile	Adult	Adult
Size range (mm)	1.3 to 82.0	6.5 to 58.0	10.0 to 228.0	9.5 to 71.4	20.8 to 86.2
Mean size (mm)	32.3±3.9	21.9±2.7	59.5±5.8	30.4±2.5	49.4±4.1
Reference	Souza et al. (unpublished data)	Freitas (2011)	Souza et al. (in press)	Souza et al. (in revision)	Souza et al. (unpublished data)

Despite of *P. minutus* and *P. flesus* present higher densities in Minho estuary when compared to other estuaries in Southern Europe (see Souza et al. 2013), the epibenthic assemblage in Minho estuary is dominated by *C. maenas*, *C. crangon* and *P. microps*. These three species also reach high densities when compared to other estuaries (Freitas 2011; Souza et al. in revision; Souza et al. unpublished data), and therefore can be considered the most important species of the demersal compartment in the lower estuary. Apart from the fact of the larger size of *C. maenas* compared to *C. crangon* and *P. microps*, the dominance in terms biomass of the shore crab can be related to the fact that *C. maenas* preys on the brown shrimp and the common goby (Baeta et al. 2006). The remarkable high densities of these two species in Minho estuary may increase the odds of the shore crab obtains food, consequently, the shore crab may find optimal conditions to increase its biomass, reaching high rates of secondary production in the system (Souza et al. unpublished data). The remarkable abundance of *C. crangon* found in Minho estuary is in line with other studies, which also recorded high densities of brown shrimps and considered this species as one of the most ecological important species in European estuaries (Amara and Paul 2003; Viegas et al. 2007). Contrary, despite of being an important species in European estuaries (e.g. Wiederholm 1987; Leitão et al. 2006; Dolbeth et al. 2007b), the common goby exhibits remarkable higher densities in Minho estuary compared to other estuaries in Europe (Souza et al. in revision), fact that probably is directly related to the high river discharge and low salinity found in Minho (Souza et al. in revision). The dominance of *P. microps* in S3 further confirms the hypothesis that this species is benefited in areas with high fluctuations in salinity.

Overall, the five dominant species of the epibenthic community of Minho estuary present higher densities and production than nearby systems (Freitas, 2011; Souza et al. unpublished data). The high density of individuals and secondary production recorded in Minho estuary might be related to the high river discharge in the system, compared to other Portuguese estuaries (see Ferreira et al. 2003). Once, a high river discharge can positively affect the secondary production of fishes and crustaceans within estuaries (Loneragan and Bunn 1999). Also, salinity seems to play a crucial role in structuring fish and crustacean dominance patterns in Minho estuary. In the CCA analysis species were clearly divided into two groups, those with higher affinity with the sea, namely *C. maenas*, *C. crangon* and *P. minutus*, and those with higher correlation with oligohaline and/or freshwater, namely *P. flesus* and *P. microps*. Interestingly, the first group was dominant in S1 and S2, while the later dominate in S3.

Despite of the five species present high densities and biomass compared to other systems, the common goby and the European flounder seems to be highly benefited by the low salinity and high river discharge, since they reach remarkable abundances and production compared to other nearby estuaries (Souza et al. in revision; Souza et al. 2013), therefore Minho estuary can be considered one of the most important nursery areas for *P. microps* and *P. flesus* in southern Europe.

The ecological indexes (Pielou's evenness and Berger-Parker dominance index) indicated that dominance is higher in S3, especially during autumn and winter, and lower in S1, particularly during autumn and winter. The proximity with the sea makes S1 an estuarine area with less noticeable fluctuations in abiotic parameters, allowing species with different degrees of environmental tolerance to coexists, which explains the higher evenness and the lower dominance patters in this sampling station. On the other hand, the high dominance and the low evenness observed in S3 probably is related to the high environmental variability found in this station, which seems to favor mainly species with high tolerance to freshwater input, in this case, the common goby *P. microps*. Additionally, in S3, fishes are the dominant group throughout most of the sampling period, with crustaceans dominating only during summer months. This pattern suggests that this crustacean species may be more susceptible to fluctuations in environmental parameters than fishes.

7.5. Conclusions

Both crustacean species were dominant in terms of density (*C. crangon*) and biomass (*C. maenas*), indicating that this group is the dominant in Minho estuary. On the other hand, the common goby dominates in terms of density and biomass in S3, which is the most stressful estuarine portion of the lower estuary, indicating that this species can persist in this estuarine section throughout the entire year, even when environmental conditions are harsh for most species.

7.6. Acknowledgments

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CHAPTER 8

DIFFERENCES IN THE NEIGHBORHOOD: STRUCTURAL VARIATIONS IN THE CARAPACE OF SHORE CRABS *CARCINUS MAENAS* (DECAPODA: PORTUNIDAE)

Differences in the neighborhood: Structural variations in the carapace of shore crabs *Carcinus maenas* (Decapoda: Portunidae)

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Abstract

The present study compared the carapace structure of *Carcinus maenas* in two nearby sites (2 km apart) within Minho estuary, submitted to different physicochemical and ecological conditions (water temperature, pH, crabs' density and sex-ratio). Regarding the carapace structure of the carapace and chelae, crabs presented significant differences between sampling sites (t-test; $p < 0.01$). The SIMPER analysis revealed that the Weight/CW and Thickness/CW ratios explained all the dissimilarities found among sites. Overall, males' carapace was proportionally thicker in station 2 (t-test; $p < 0.01$), while females' carapace was proportionally thicker in station 1 (t-test; $p < 0.001$). A thicker carapace can be advantageous when competing for food or sexual partner. We hypothetized that, since in station 2, the density of individuals was twice higher than in station 1, it is likely that agonistic encounters are more frequent, thus favouring a thicker carapace.

Keywords: competition / cuticles / exoskeleton / green crab / agonistic behavior / Minho

8.1. Introduction

One unmistakable feature of crustaceans is their hard, calcified exoskeleton. The exoskeleton has multiple functions: body support, resistance to mechanical loads, environmental protection and resistance to desiccation (Vincent 1991; 2002; Vincent and Wegst 2004; Sanchez et al. 2005). However, the rigidity of the carapace restricts growth, once any increase in size requires the shedding of the exoskeleton. Therefore, crustaceans present a discontinuous growth pattern, characterized by successive ecdysis (periodic shedding of the exoskeleton) over time (Hartnoll 2001).

Molting is a complex process, affected by a range of environmental cues and regulated by a cascade of hormonal signals involving changes in gene expression, cellular commitment, mitotic and secretory activity, endocrinology, behavior and cell death (Loeb 1993). Several environmental and ecological factors may be involved in the molting process, such as water temperature, salinity, photoperiod, tidal cycle, food availability and population clusters (Chang 1995; Hartnoll 2001). Also, several hormones are involved in the molting process control (Lachaise et al. 1993; Hartnoll 2001), thus molting results from complex interactions between endogenous regulation and environmental inputs and involves hormonal, neurohormonal and enzymatic control (Styrishave et al. 2004).

The green or shore crab *Carcinus maenas* is a common and easily found inhabitant of intertidal systems worldwide. It is indigenous from European and Northern Africa coasts and estuaries, spanning a latitudinal gradient of ca. 49º (from Norway to Mauritania) (Roman and Palumbi 2004). Recently, this species has also colonized several systems in Argentina, Australia, Japan, South Africa and both coasts of North America (Brenchley 1982; Lafferty and Kuris 1996; Behrens Yamada 2001; Hidalgo et al. 2005) and has, therefore, been considered one of the worst invasive species in the world (Lowe et al. 2000).

During its life cycle, *C. maenas* performs about 18 moltings, four of them on the first developmental stages (zoea and megalopa) and fourteen after larval stage. Shore crabs grow up and molt until the terminal ecdysis (cessation of growth), which occurs when they reach 86 mm of carapace width (CW) (Crothers 1967). Moreover, the shore crab takes about 10 molts to reach 20 mm carapace width (CW) in its first year, if conditions are favorable (Crothers 1967). *Carcinus maenas* may molt more than once per year after the first year if conditions are good but molting rate slows once maturity is reached and is probably about once per year post maturity (Crothers 1967).

Considerable variability in morphology, physiology and behavior is commonly found in *C. maenas* in relation to sex, size and carapace coloration (e.g. McGaw and Naylor 1992a; Reid et al. 1994; Ledesma et al. 2010). This intraspecific variability reflects the phenotypic adaptive responses of individual crabs in relation to their ability to withstand environmental unevenness (McGaw and Naylor 1992a; Warman et al. 1993; Abelló et al. 1997). Differences in morphology, physiology and/or behavior are often displayed when organisms are submitted to different environmental and ecological conditions (e.g. Reid et al. 1997). However, the mechanisms that lead to these differences are seldom investigated in the field. While the effect of environmental variables can be easily replicated in laboratory, ecological processes are hard to reproduce. In this context, this study aimed to compare the carapace structure of the shore crab sexes (males and females) and color morphotypes (green and red) among different sampling sites. The comparisons were made in two nearby areas within the same ecosystem, and with distinct habitat utilization by crabs, reflected in differential densities, sex ratio and environmental conditions (ATS personal observation).

8.2. Materials and Methods

8.2.1. Sampling and laboratory analyses

This study was conducted in the Minho Estuary (NW of the Iberian Peninsula), which ranges up to 40 km (considering the upstream limit of spring tides), covering a total area of 23 km^2. The Minho estuary is a very shallow system (Moreno et al. 2005), with a mean depth of 2.6 m (Freitas et al. 2009) and characterized as a mesotidal and partially mixed system, although during periods of high river flow, it tends towards a salt wedge estuary (Sousa et al. 2005). The present study was carried out at the lower estuary (first 8 km from the estuary mouth).

C. maenas individuals were collected in April 2010 in two nearby sites (approximately 2 km apart) in the lower Minho estuary (Fig. 8.1). The two sites were chosen in order to assess possible dissimilarities on carapace structure related to *C. maenas* density, sex's ratio and environmental variables. Both sampling stations are located in the subtidal zone. However, during low water, in station 2 the water column is very shallow (ca. 20 cm), while in station 1 water level reaches c.a. 1 m of minimum depth. Moreover, station 1 is closer to the river mouth, with the soft bottom often sparsely covered by debris, such as drifting seaweeds and empty mollusks' shells, while station 2 is located inside the Coura salt marsh, dominated by the seagrass *Spartina maritima* and its soft bottom houses an especially abundant but poorly diverse fauna represented mainly by crabs *C. maenas*, gobies *Pomatoschistus* spp., common shrimps *Crangon crangon* and the peppery furrow shells *Scrobicularia plana* (ATS personal observation). In each site, three samples were taken using a 1m beam trawl (5 mm mesh size) towed at constant speed (2 km.h^{-1}) for 2 to 3 minutes, during high water. Additionally, at each site, environmental variables (water temperature, salinity, conductivity, pH and oxidation reduction potential (ORP)) were taken through a multiparameter probe YSI 6820 on the surface (two replicates) and 20 cm above the bottom (two replicates). For statistical analysis, four measurements of each site (2 x surface and 2 x bottom) were used to assess differences between sampling stations.

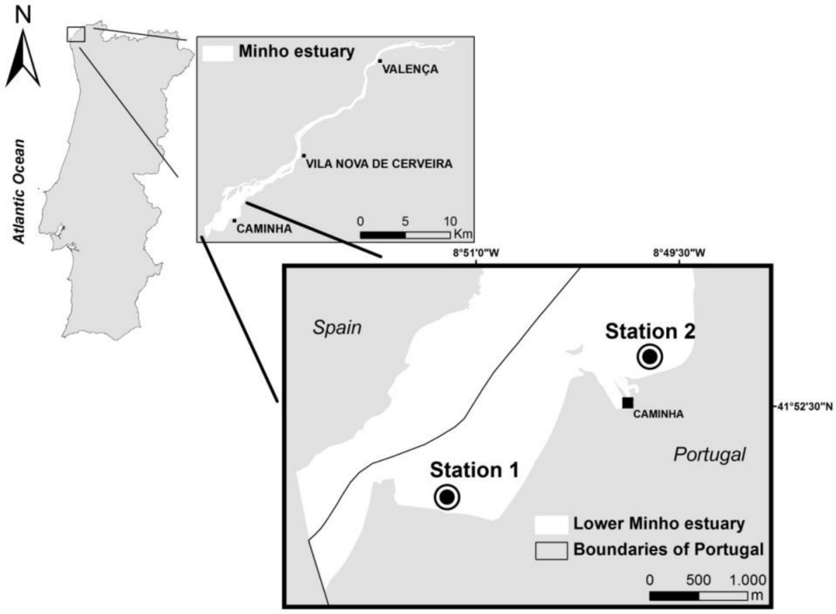

Fig. 8.1 Map of the study site showing the two sampling stations on the lower Minho Estuary.

All collected *C. maenas* individuals were counted in order to assess its density on the two sampling sites. Intermolt crabs were sexed (males or females) according to their abdomen's anatomy (see Crothers 1967) and classified into color morphotypes (green or red), based on the predominant color of the thoracic sternum (McGaw and Naylor 1992a). Afterwards, the carapace width (CW) and propodus length (PL) of right and left claw were measured to the nearest 0.01 mm using a digital caliper. Carapaces were then air-dried for 4 days and afterwards weighted to the nearest 0.0001 g using a digital scale. A small segment of the carapace (approximately 1 cm^2) was removed from each tested crab, along the fracture line, and its thickness was measured to the nearest 0.01 mm with a digital caliper. A similar procedure was used to assess the claws' thickness, which was also air-dried for 4 days, and had a small segment (approximately 0.5 cm^2) removed from the area corresponding to the maximum propodus height on the frontal part of the chelae (Fig. 8.2). For thickness samples, the average of three separate measurements was recorded, and its mean value used in statistical analysis.

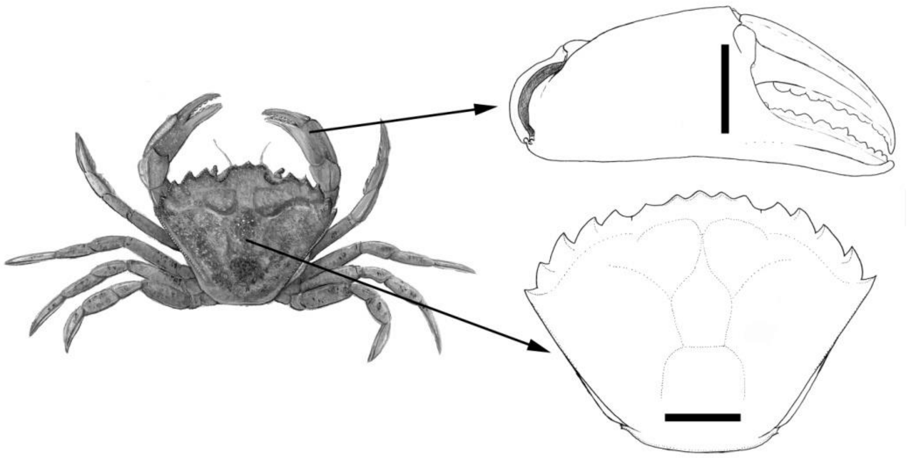

Fig. 8.2 *Carcinus maenas* areas measured for carapace and claw thickness (black bars).

For the comparisons in the carapace structure between mature versus immature crabs, we considered *C. maenas* individuals as sexually mature when they were larger than approximately 30 mm of CW (d'Udekem d'Acoz 1993; Behrens Yamada et al. 2005).

For all variables, ratios were calculated in order to avoid bias due to increasing carapace thickness with individual growth (i.e. Thickness/CW, for this ratio was assumed that the higher the ratio, thicker is the individual; Weight/CW, for this ratio was assumed that the higher the ratio, heavier is the individual). The same procedure was used with right (RC) and left chelae (LC) (i.e. right and left propodus length) and chelae thickness (i.e. RC thickness/RC, for this ratio was assumed that the higher the ratio, thicker is the RC of the individual; LC thickness/LC, for this ratio was assumed that the higher the ratio, the thicker is the LC of the individual). Since most of the ratio values presented two or more non-significant decimal digits, a simple multiplication (times 1000) was applied when needed, in order to highlight the numerical differences present. Nevertheless, this process did not affect the calculation, since the same procedure was applied in all cases.

8.2.2. Data analysis

Data were analyzed with the PRIMER v6® package (Clarke and Warwick 2001). A one way ANOSIM (analysis of similarity) and a nMDS (non-metric multi-dimensional scaling) were used to independently infer the putative differences among sampling stations, regarding environmental descriptors and *C. maenas* carapace (proportions of CW with thickness and weight, as well as the proportion of chelae thickness with propodus length). All data were normalized, without prior data transformation, in order to avoid bias due to differences in variability range of each variable (Clarke and Warwick 2001). Due to the character of the data (morphometrics), the similarity matrices were based upon Euclidian distances as recommended by Clarke and Warwick (2001). In case of significance, we used the SIMPER routine to determine the percent contribution of each variable to the observed dissimilarities between sampling stations. Additionally, to examine differences between sampling stations regarding environmental variables and carapace variables between, sex and color morphotype, pairwise comparisons were performed using Student’s t-test (unpaired). Also, the Pearson’s correlation test was used to determine whether a correlation existed between CW with carapace thickness and weight; these analyses were carried out with Statistica 5 software.

8.3. Results

8.3.1. Ecological data

Overall, the environmental descriptors were similar in the two sampling stations (ANOSIM; R= 0.26; S.L. > 0.05). However, when compared individually, there were significant differences in pH (t-test, $p<0.05$) and temperature of the water (t-test, $p<0.05$), with both variables presenting higher values in station 2. The SIMPER procedure also suggested that those variables were the most important, accounting for near the half of the dissimilarities observed among sampling sites (44.90%).

Crabs' density in station 1 was 33.5 ± 4.95 ind.m^{-2} (mean ± SD), being 58.2 % females, 29.9 % males and 11.9% juveniles (CW < 10 mm), while in station 2, density nearly doubled (72.0 ± 6.36 ind.m^{-2}), with a predominance of males (56.3 %) over females (43.8 %), while juveniles were absent. On a year basis, station 1 is dominated by juveniles and females, whereas at station 2, males are dominant (ATS personal observation).

8.3.2. Carapace structure: range of observations

A total of 99 individuals (green morphotype = 68.7%; red morphotype = 31.3%) were analyzed, being 43 males (green morphotype = 81.4%; red morphotype = 18.6%) and 56 females (green morphotype = 58.9%; red morphotype = 41.1%).

The range of CW size varied between 14.2 and 65.2 mm, with an average value of 35.6 mm. The carapace thickness varied from 0.07 to 0.39 mm, with an average value of 0.22 mm. Although, in general, individuals showed the right chelae larger than the left one (81.8% of the individuals), this difference was not significant (paired t-test; p > 0.05).

Significant differences were detected between males and females (both color morphotypes) in four carapace variables, namely, carapace thickness, carapace thickness/CW, RC thickness/RC, LC thickness/LC, indicating that females were proportionally thicker than males (Table 8.1).

Table 8.1 Carapace variables and ratios (mean ± SD) of *Carcinus maenas* color morphotypes and sexes in Minho estuary. S.L. = Significance level; CW = carapace width; PL = propodus length; RC = right chelae; LC = left chelae. NS = non-significant; * = S.L. < 0.05; ** = S.L. < 0.01; *** = S.L. < 0.001; ^{a} = ratio times 1000.

Variables	Green			Red		
	Male	Female	S.L.	Male	Female	S.L.
CW (mm)	38.6±10.6	34.8±10.0	NS	37.6±10.2	34.1±10.3	NS
Carapace weight (g)	0.8±0.5	0.6±0.5	NS	0.8±0.5	0.6±0.5	NS
Carapace thickness (mm)	0.2±0.1	0.2±0.1	*	0.2±0.1	0.2±0.1	*
PL - right chelae (mm)	19.2±6.3	17.2±5.7	NS	18.6±5.9	16.8±5.8	NS
RC thickness (mm)	0.4±0.1	0.3±0.1	NS	0.3±0.1	0.3±0.1	NS

PL - left chelae (mm)	18.6±6.4	16.7±5.7	NS	18.0±6.1	16.4±5.9	NS
LC thickness (mm)	0.3±0.1	0.3±0.1	NS	0.3±0.1	0.3±0.1	NS
Weight/CW	0.02±0.01	0.02±0.01	NS	0.02±0.01	0.02±0.01	NS
Carapace thickness/CW [a]	5.3±1.1	6.2±1.2	**	5.7±0.8	7.2±1.0	***
RC Thickness/RC [a]	15.6±5.0	17.5±3.8	*	18.9±3.8	19.7±1.9	NS
LC Thickness/LC [a]	16.6±4.0	17.8±3.7	*	17.5±1.9	20.1±1.6	***

Significant differences between crabs with the red and green morphotypes were detected when regarding the thickness/CW and weigth/CW ratios, with red crabs being proportionally thicker (t-test; $p < 0.001$) and heavier (t-test; $p < 0.001$) than individuals of the green morphotype.

The Pearson's correlation between carapace thickness and CW was significant either for males ($r^2 = 0.55$; $p < 0.001$) and females ($r^2 = 0.86$; $p < 0.001$). A similar result was obtained when regarding weight and CW, with the Pearson's correlation being significant for males ($r^2 = 0.90$; $p < 0.001$) and females ($r^2 = 0.92$; $p < 0.001$), with the carapace of females being heavier than males'.

8.3.3. Carapace structure: mature versus immature crabs

Significant differences between males (t-test; $p < 0.01$) and females (t-test; $p < 0.001$) were detected on the thickness/CW ratio when splitted in mature or immature individuals, with immature males presenting a relatively thicker carapace than immature females, whereas when mature this patterns shifts, with females becoming proportionally thicker than males (Fig. 8.3). Mature males also presented a larger ratio of chelae size (right and left) with CW than immature males, whilst females did not differ. Regarding the ratio of chelae size with its thickness, males only differed in the left chelae, which was thicker when immature. In contrast, females presented thicker chelae (right and left) when mature. Table 8.2 sums up the differences detected in the chelae, as well as the cases when the differences were significant.

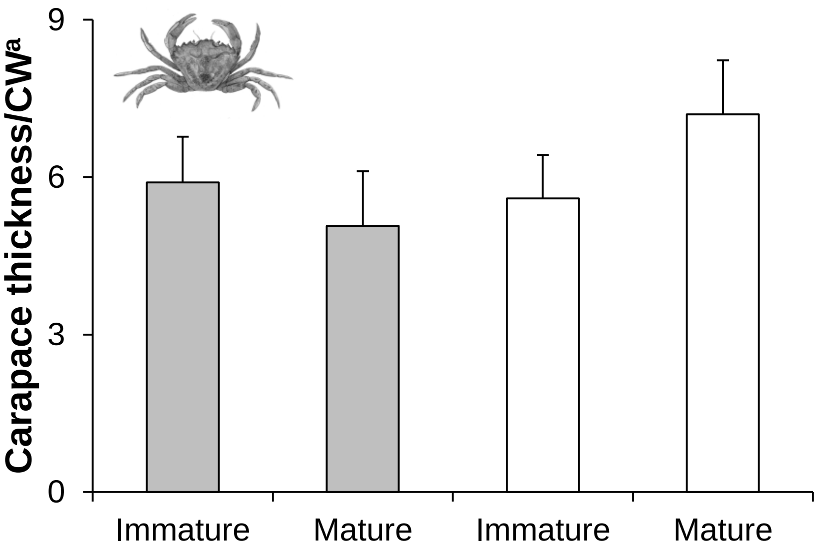

Fig. 8.3 Differences in the ratio of carapace width (CW) and thickness (mean ± SD) of *Carcinus maenas* immature (CW < 30 mm) and mature (CW > 30 mm) individuals in Minho estuary. Grey bars = males; white bars = females; [a] = ratio times 1000.

Table 8.2 Differences in chelae (right and left) ratios (mean ± SD) of *Carcinus maenas* males and females between immature (CW < 30 mm) and mature (CW > 30 mm) individuals from the lower Minho estuary. S.L. = Significance level; CW = carapace width; RC = right chelae; LC = left chelae; NS = non-significant; * = S.L. < 0.05; ** = S.L. < 0.01; *** = S.L. < 0.001; [a] = times 1000.

Variable	Male			Female		
	Immature	Mature	S.L.	Immature	Mature	S.L.
CW/RC	2.0±0.1	1.9±0.1	**	2.1±0.1	2.0±0.1	NS
CW/LC	2.1±0.1	2.0±0.1	***	2.1±0.1	2.1±0.2	NS
RC thickness/RC[a]	17.9±2.9	16.3±3.6	NS	18.0±2.1	19.2±2.2	*
LC thickness/LC[a]	18.3±2.2	16.4±2.7	**	18.1±1.1	19.6±2.1	**

8.3.4. Carapace structure: differences between sites

Regarding the ratios of the carapace width with thickness and weight, as well as the ratios of chelae thickness with propodus length, *C. maenas* individuals of both sexes and color morphotypes differed significantly between sampling sites (ANOSIM; R = 0.49; S.L. < 0.01). The nMDS ordination also suggested a strong dissimilarity between individuals of different sampling sites (2D Stress = 0.01) (Fig. 8.4). The SIMPER analysis further confirmed a high dissimilarity between sampling sites which was 100 % explained by two variables: ratio thickness/CW (53.86 %) and ratio Weight/CW (46.14 %).

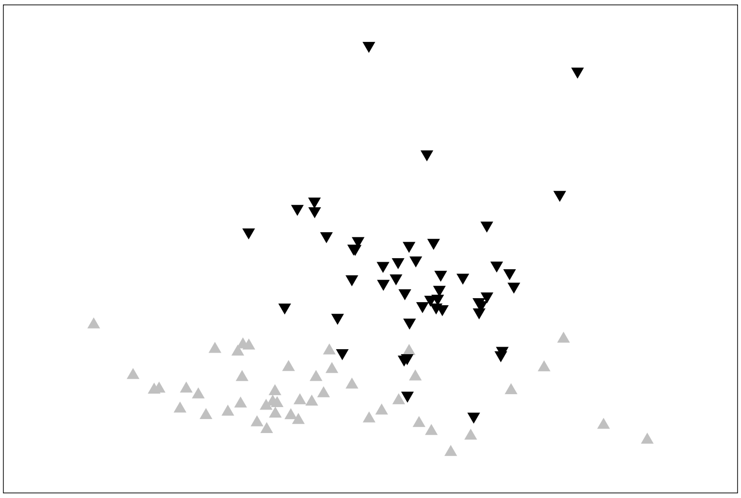

Fig. 8.4 Two-dimensional nMDS ordination of *Carcinus maenas* carapace (ratios of carapace thickness and weight with width) and chelae variables (ratios of chelae thickness with length) in two sampling sites in Minho estuary. Station 1 = grey triangles; Station 2 = black triangles.

Male and female crabs significantly differed between sites in respect to the ratio of carapace weight and width (t-test; $p < 0.001$). Moreover, the ratio of carapace thickness and width of males (t-test; $p < 0.01$) and females (t-test; $p < 0.001$) were significanty different between sampling sites (Fig. 8.5).

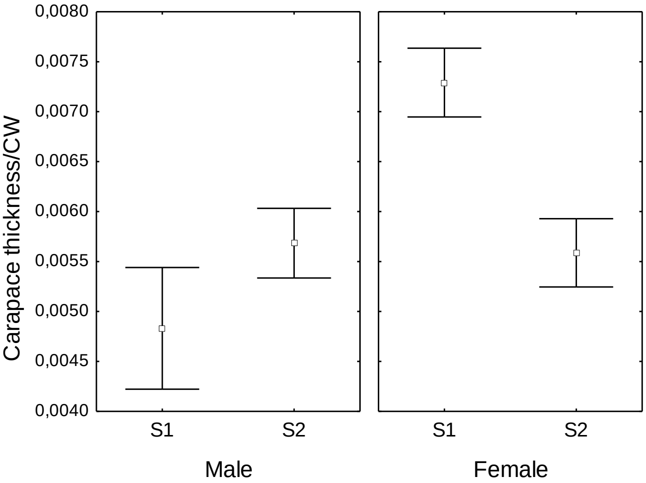

Fig. 8.5 Ratios (mean ± SD) of width and thickness of the *Carcinus maenas* carapace at the two sampling stations in lower Minho estuary. CW = Carapace width; S1 = Station 1; S2 = Station 2.

Table 8.3 shows the differences and its significance levels between sampling sites for both males and females crabs. Mature males of station 1 presented a smaller ratio (i.e. proportionally larger chelae) of chelae size (right and left) with CW than males from station 2, whilst females did not differ. Regarding the ratio of chelae size with its thickness, males only differed in the left chelae, presenting thicker left chelae in station 2. Conversely, females presented thicker chelae both right and left in station 1.

Table 8.3 Differences in chelae (right and left) ratios (mean ± SD) of mature (CW > 30 mm) *Carcinus maenas* (males and females) between sampling stations in the lower Minho estuary. S.L. = Significance level; CW = carapace width; RC = right chelae; LC = left chelae; NS = non-significant; *= S.L. < 0.05; **= S.L. < 0.01; [a] = times 1000.

Variable	Male			Female		
	Station 1	Station 2	S.L.	Station 1	Station 2	S.L.
CW/RC	1.9±0.1	2.0±0.1	**	2.0±0.1	2.0±0.1	NS
CW/LC	1.9±0.1	2.0±0.1	**	2.1±0.2	2.1± 0.0	NS
RC thickness/RC[a]	16.1±4.3	16.6±2.7	NS	19.4±2.0	17.2±3.7	*
LC thickness/LC[a]	15.5±3.1	17.5±1.7	*	19.8±1.9	16.9±2.4	**

8.4. Discussion

Remarkable variability on the carapace structure of *C. maenas* was revealed in this study between functional groups i.e. sexes, color morphotypes and life stages, as well as among sampling stations.

8.4.1. Differences among *C. maenas* functional groups

C. maenas males and females presented a notably different pattern in their carapace structure, both in their cephalothoraxes and chelipeds. Overall, females were proportionally thicker than males, whereas males presented proportionally larger chelae.

Differences in carapace thickness may possibly be related to different life strategies between sexes. Since males might preferentially invest their energy in rapid growth, and hence shedding their exoskeleton faster than females, which preferentially allocate their energy in reproduction, leading them to longer intermolt stages that may ultimately result in a thicker carapace due to the higher accumulation of minerals. An injury in the crab's carapace and/or appendages may directly influence molting, with the regeneration process being concluded after the complete shedding of the damaged carapace (McVean 1976). Since males crabs are more often engaged in disputes for food and mates than females (Sneddon et al. 2003), they are more susceptible to damages in their carapace and hence they may be forced to molt more often than females. Indeed, the frequency of the ecdysis in males is slightly higher than in females especially when the carapace width is larger than 20mm (see Crothers 1967).

Additionally, sexual dimorphism in carapace may be a common trend in crabs (e.g. Lewis 1977; González-Gurriarán 1985; Rufino et al. 2004; Ledesma et al. 2010). Usually, males and females differ in carapace shape, with females presenting larger abdomen that ultimately is beneficial to reproduction (Hartnoll 1982; Haefner 2000), while males often present broader carapaces and larger chelae that are advantageous in agonistic encounters (Reid et al. 1994). Thus it is possible that differences in carapace shape may also be reflected in carapace structure, with female carapace being relatively thicker than male carapace.

In this study we also detected significant differences in carapace and cheliped structure between immature and mature individuals. However, there were no differences between males and females during the immature stage. Present observations suggest that, during growth, the carapace of males becomes proportionaly thinner, whereas females' becomes thicker. Furthermore, males increase desproportionaly the size of their cheliped, thus, presenting a larger chelae (right and left) when adults, while females did not present differences between the immature and the mature stages. In fact, frequently dominant individuals have larger or stronger chelipeds as shown by other studies (Barki et al. 1997; Sneddon et al. 1997; 2000). Furthermore, allometric growth and sexual dimorphism in cheliped size is common in decapods, with males having larger chelipeds than females (Hartnoll 1974).

Overall, the red morphotype individuals from all functional groups (i.e. sexes and life stages) and from both sampling stations presented a thicker and heavier carapace, whilst green individuals were lighter and thinner. Several aspects, both physiological and behavioral, differ considerably between color morphotypes including osmoregulation (McGaw and Naylor 1992a), tolerance to hypoxia (Reid and Aldrich 1989), molting rate (Reid et al. 1997; Wolf 1998), growth, mating, muscular and carapace composition and aggressiveness (Reid et al. 1997). Then, it could be expected that green and red morphotypes also differ in carapace structure as was confirmed by our data. Reid et al. (1997) claimed that green and red morphs differ due to differential use of resources, with green individuals allocating most of their energy in growth, while red individuals allocate it to reproduction. Moreover, usually red crabs are dominant over green morphs in aggressive disputes for prey (Keiser et al. 1990). Consequently, the stronger carapace of red crabs reflects its competitive advantage.

8.4.2. Differences between sites: ecological and behavioral implications

Dissimilarities in carapace variables between sites were observed. In general, females presented a thicker carapace than males, for both color morphtypes. However, in the salt marsh this pattern was somewhat different, with males presenting a slightly thicker carapace than females. It is hard to state the specific reasons that led to this fact; however several explanations can be drawn.

The carapace of aquatic crabs is complex in its composition, being constituted by chitin-protein fibers stacked in numerous lamellae that are deeply calcified by minerals such as calcite or amorphous calcium carbonate (Roer and Dillaman 1984; Giraud-Guille et al. 1994). Previous studies have acknowledged that the amount of calcium carbonate available in the environment can directly influence the carapace composition of crustaceans (Greenaway 1983; Cameron 1985). Also, the water temperature may further influence on the carapace’s hardening time, being up to 4 times faster in warmer than in colder waters (see Crothers 1967). Since the water temperature in the salt marsh was higher, it could be expected that the crabs’ carapace probably should be thicker. However, this trend was recorded for males but not for females, suggesting that other factors might be influencing the observed pattern.

The pH value, as well, differed between sampling stations, also being higher in the salt marsh. A higher value of the pH might favor the deposition of minerals in the crabs’ calcified carapace, since acidic waters usually reduce the calcification rates of calcified marine organisms (Fabry et al. 2008). Thus, it also could be expected that in this sampling station, the carapace of crabs should be thicker than in areas with lower values of pH. On the other hand, since there is more available calcium carbonate in saltier waters (Neufeld and Cameron 1992), it would be expected that crabs inhabiting areas closer to the sea would present higher amounts of calcium carbonate in its carapace and, thus, a thicker carapace. So, the thicker carapace of females in station 1 could be related to environmental dissimilarities between sampling stations, however, males presented an opposite trend, indicating that other factors might be driving males to become thicker in station 2.

After an ecdysis, crustaceans suffer significant calcium lost that needs to be replaced in order to preserve the carapace functions. This calcium uptake is obtained primarily from the environment after molting, and/or by the use of calcium reservoirs formed before the molt (Greenaway 1985). The environmental uptake of calcium can occur by the active absorption or transfer from the surrounded water, or by feeding. However, it is believed that feeding may play a less noticeable role in aquatic crabs. In fact, Neufeld and Cameron (1992) studying the calcium uptake of the blue crab *Callinectes sapidus*, stated that the diet is not the primary source of calcium carbonate for the species, being the uptake from the surrounded water the major source of its carapace mineralization. However, they claimed that the mineral re-absorption before the ecdysis and the feeding of exuviae may perhaps play an important role in the calcium carbonate uptake under natural conditions for other crab species.

However, despite the apparently minor importance of the diet in the carapace structure of crabs, its importance cannot be totally discharged (Neufeld and Cameron 1992). Differences in food availability of both sampling sites may perhaps further influence the development of a differential carapace structure pattern, because the carapace composition in decapods is related to the individual's diet (Kennish 1996). Moreover, the diet of *C. maenas* can greatly differ in nearby areas owing mainly to its opportunistic feeding behavior (see Baeta et al. 2006). It may be possible that feeding also contributes to the observed dissimilarities in carapace structure recorded in the present study. On the other hand, *C. maenas* did not present an obvious discrepancy in their diet according to sex and/or size (Baeta et al. 2006), and since males and females presented a distinct trends in their carapace structure, it seems unlike that diet may significantly contributed for the observed dissimilarities recorded. Still, additional studies need to be performed in order to elucidate the relative importance of diet in the carapace structure of *C. maenas*.

A well constituted and thicker carapace certainly can be advantageous in competitive disputes for food or sexual partner. A stronger carapace certainly provides a better protection, and also, it further enables a stronger and more effective attack tool against conspecifics during feeding and mating interactions. Predator abundance can modulate the expression of defensive morphologies, inducing changes that can reflect within-generation developmental responses (Trussell and Smith 2000). In this case, it can be expected that when agonistic encounters are often, a stronger carapace will be much more common, and that in crowded areas the agonistic encounters will occur more frequently, contributing to a higher proportion of thicker crabs than is expected.

In the salt marsh, the crabs' density was twice higher than near the river mouth. Therefore, it is likely that agonistic encounters are more frequent, and thus favouring a thicker carapace, which provides a better protection during disputes. This is specially true for males, since they usually fight over resources (food, individual space, females), with the larger males being dominant (Berrill and Arsenault 1982). Fights between males are significantly more intense in the presence of females (Sneddon et al. 2003) than of food. These fights are costly with 40% of fighting crabs incurring an injury (Fletcher and Hardege 2009). Therefore in a highly densely occupied space, like in the Coura salt marsh, it is reasonable to expect that males with a thicker and stronger carapace will have more easily access to food and also to females. Intraspecific competition could then be leading to thicker males as a pattern.

In decapod crustaceans, the first pereiopod generally bears a chelae (claw) formed by a modification of the dactylus and propodus which is known as a cheliped. This appendage is typically used in feeding, as well as a defense tool against predators and during agonistic behavior, and hence has also been considered a multifunctional organ (Brown et al. 1979; Lee 1995) which can also present a large variability. Mature males presented significantly larger cheliped than immature males, whereas this was not registered in females. Males further presented a larger ratio of CW with chelae size (right and left) near the river mouth, while females did not differ. These results can be due to intraspecific competition for food in the case of immature individuals. In respect to mature males, the stronger chelae might be determinant in the competition for mating, since during agonistic encounters males with larger chelipeds often become dominant over smaller individuals (Sneddon et al. 1997; 2000). In fact, in Minho estuary, ovigerous females are exclusively recorded in areas near the river mouth of this estuary (ATS personal observation).

This study further highlighted that even in nearby areas within an ecosystem, significant morphological differences occurs. These differences probably were related to ecological and behavioral dissimilarities among microhabitats, thus it is important to conduct further researches that address a wide ecological and behavioral spectrum, aiming to assess the importance of each feature in the ecomorphology of the species.

8.5. Acknowledgements

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PART II

CLIMATE CHANGE SCENARIO SIMULATIONS

CHAPTER 9

MODELING THE EFFECTS OF TEMPERATURE AND SALINITY VARIATIONS ON THE SHORE CRAB *CARCINUS MAENAS*

Modeling the effects of temperature and salinity variations on the shore crab *Carcinus maenas*

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Souza AT, Marques JC, Martins I (submitted) Modeling the effects of temperature and salinity variations on the shore crab *Carcinus maenas.*

Abstract

Despite of the IPCC predictions about the temperature rising in the near future and the global awareness about this issue, there are only few studies that use these predictions to simulate the effects of climate change on the population dynamics of estuarine crustaceans. In this context, the present study aimed at assessing the effects of temperature and salinity variations on the population dynamics of the shore crab *Carcinus maenas* in an important nursery area for the species within its indigenous range of occurrence. This aim was achieved by modeling the dynamics of *C. maenas* population in Minho estuary (Portugal). The model focused only on subadults and adults inhabiting the estuary, but it also accounted for the two stages of *C. maenas* development, namely, the planktonic and benthic phases, that occurs at the sea and within the estuary respectively. Linear regressions between the observed and the predicted density of subadults (ANOVA: $F= 10.66$; $p < 0.01$), females (ANOVA: $F = 17.41$; $p < 0.01$), males (ANOVA: $F = 17.38$; $p < 0.01$) and the total estuarine population (ANOVA: $F = 8.77$; $p < 0.05$) were significant, which accounted for the accuracy of the model to describe *C. maenas* population dynamics, and, thus, used to simulate the effects of climatic changes on the crab population. Results suggest that *C. maenas* is more sensitive to oscillations on temperature than on salinity. Temperature rises of up to 2ºC causes increases on the population density of *C. maenas* from Minho estuary, whilst further temperature increase leads to a noticeable decrease on shore crab density (up to 60%). Assuming a temperature rise of 2ºC in the next decades, simulations indicate that *C. maenas* density will continuously increase. Due to the voracious and generalist feeding behavior of the species, a *C. maenas* population increase will probably have significant effects on the estuarine community in the studied area.

Keywords: Ecological modeling / climate change / global warming / Minho / Portugal

9.1. Introduction

The most abundant crab inhabiting European estuaries is the shore crab *Carcinus maenas* (Queiroga 1998). In Minho estuary (NW Iberian Peninsula), the shore crab is one of most abundant species; dominating the epibenthic assemblage in terms of biomass (Souza et al. unpublished data). In this shallow mesotidal and partially mixed estuary (Moreno et al. 2005; Sousa et al. 2005), shore crabs make up a highly dense population dominated by small-bodied individuals (Souza et al. unpublished data), which is reflected on a low secondary production, but on a high density of individuals compared to other estuaries in southern Europe (e.g. Baeta et al. 2005; Bessa et al. 2010).

This mid-size swimming crab can reach up to 90 mm of carapace width (CW) (Klassen and Locke 2007) and can be found in different habitat types, including the marine sublittoral, estuaries, coastal lagoons and rocky shores (Grosholz and Ruiz 1996). Usually, it lives near the bottom associated with almost all substrates types (muddy, sandy and rocky) (Crothers 1968). This species presents a complex life cycle, which includes both planktonic and benthic phases (Anger 2006). During the larval phase of its development, the shore crab lives offshore in the pelagic domain (Cohen et al. 1995). After the metamorphosis, the young crab moves towards the intertidal zones and sinks to the bottom (Cohen et al. 1995); in the adulthood, *C. maenas* stays in the bottom, living over almost all types of substrata of the estuarine and coastal systems (Crothers 1968; Cohen et al. 1995).

The shore crab is highly tolerant to environmental constraints, being able to tolerate salinities ranging from 4 to 52 and to survive in temperatures varying from 0 to 30ºC (Cohen et al. 1995). However, despite of being able to thrive even in harsh environmental conditions, several biological and ecological traits of *C. maenas*, such as growth, sexual maturation, reproduction, migration and mortality are highly dependent upon temperature and salinity conditions (Broekhuysen 1936; Crothers 1967; 1968; Klassen and Locke 2007). Given that, it is expected that these two parameters will change in a near future due to changes in global climate (IPCC 2007), the present study aimed at predicting the effects of changes on temperature and salinity on *C. maenas* population in Minho estuary, a system used as a nursery area for the species within its indigenous area of occurrence.

9.2. Data set

9.2.1. Crab data

The model was developed and calibrated with data of *C. maenas* obtained at Minho estuary, which ranges up to 40 km (considering the upstream limit of spring tides), covering a total area of 23 km^2 (Ferreira et al. 2003).

Shore crabs were monthly collected, from February 2009 to January 2010, in two nearby sites (about 2 km apart) in the lower Minho estuary (Fig. 9.1). Detailed descriptions of the two sampling sites and the main abiotic characteristics are available in previous studies (Souza et al. 2011; 2013). Every month, at each site, three samples were taken using a 1m beam trawl (5 mm mesh size) towed at constant speed (2 km.h^{-1}) for 2 to 3 minutes, during daylight at high tide of spring tides. Previous studies in Minho estuary indicated that the sampling area covered by a three minutes tow, at constant speed, was equivalent to 100 ± 4 m^2 on average (Freitas et al. 2009). Density data was standardized to the same scale (ind.100 m^2).

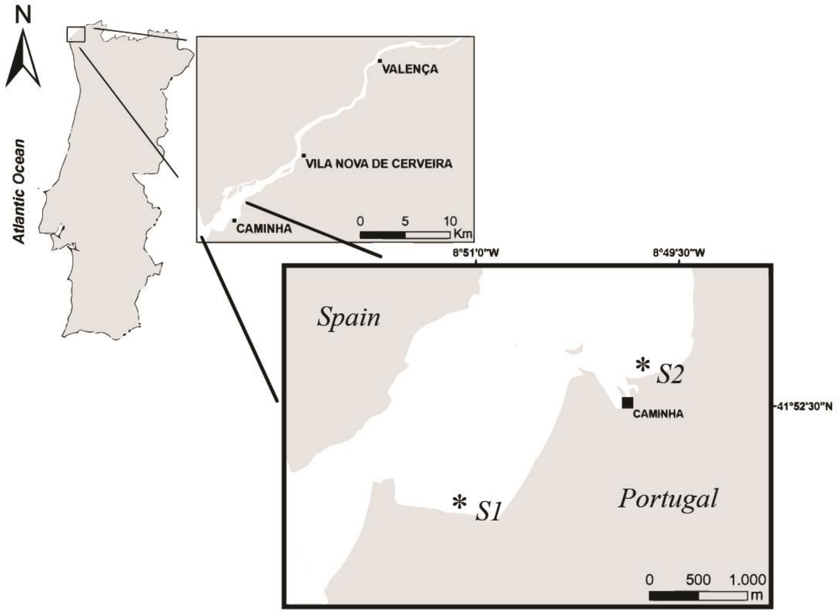


Fig. 9.1 Map of the study site showing the two sampling stations (S1 and S2) at the lower Minho estuary, NW Iberian Peninsula.

In the lab, all *C.maenas* individuals were counted, measured to the nearest 0.01 mm (carapace width - CW) and sexed (based on the observation of abdomen morphology). Afterwards, all crabs were assigned to one of the four considered groups based upon the sexual development and size (Crothers

1968): newly-settled juveniles (NSJ) (crabs < 15 mm of CW); subadults (males < 27.5 mm and females < 23.0 mm of CW), females (females > 23 mm of CW) and males (males > 27.<5 mm of CW).

9.2.2. Abiotic data

The water temperature and salinity at the bottom were measured each month in the two sampling stations using a multiparameter probe YSI 6820 (Fig. 9.2). Because the larval phase of *C. maenas* occurs predominately in the sea (Crothers 1967; Roff et al. 1986; Lindley 1987), mean monthly values of the SST (Sea Surface Temperature) at the vicinity of the Minho estuary mouth during the study period were used to describe the temperature oscillation (Fig. 9.2). The high resolution SST data were obtained using an optimum interpolation (OI) version 2 (Reynolds et al. 2007) from the National Oceanic and Atmospheric Administration's (NOAA) web site (www.esrl.noaa.gov/psd/data/gridded/data.noaa.oisst.v2.html).

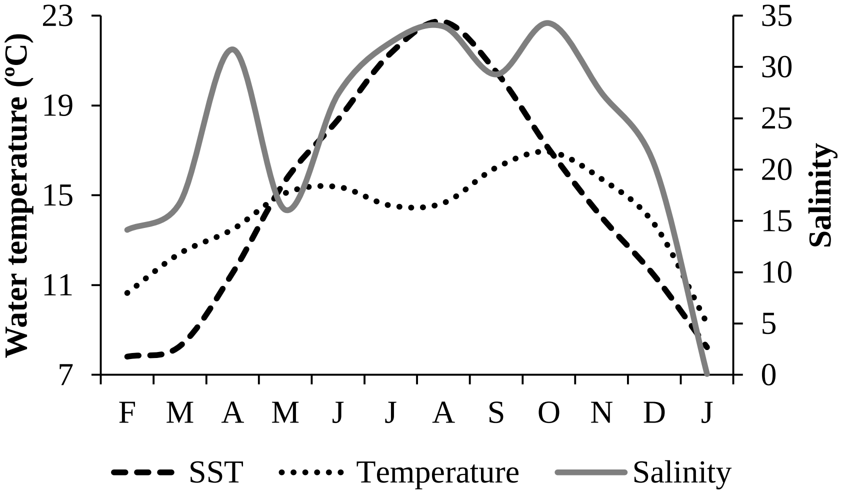


Fig. 9.2 Sea Surface Temperature (SST) at the sea nearby the mouth of the Minho estuary, and the temperature and the salinity variation at the lower Minho estuary during the study period.

Daylight duration data (Fig. 9.3) were also obtained from the NOAA website (www.esrl.noaa.gov/gmd/grad/solcalc/sunrise.html). The mean daylight duration in minutes at each month was used as a proxy for the variation on the day length at the study site.

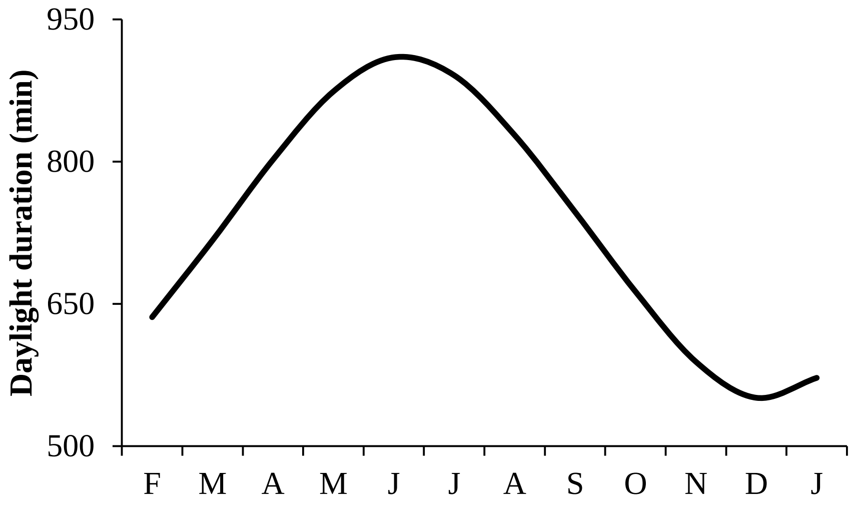


Fig. 9.3 Monthly variation in daylight duration at the Minho estuary, NW Iberian Peninsula.

9.3. Model formulation and conceptualization

Size structured models had proven to be advantageous because they take into account different kinetics and parameters that regulate the dynamics and physiology of different life stages of a given species (e.g. larvae, recruits, subadults, males and females) (Batchelder and Miller 1989; Labat 1991).

In the present model, the population of *C. maenas* was divided into four groups: newly settled juveniles (NSJ), subadults, females and males, which constitute the state variables of the model. The flows between state variables are individuals per unit of time, while the units of the state variables are individuals 100m^{-2}. The processes that regulate the number of individuals in each group over time are: growth, which determines the number of individuals transferred from one group to another; death, which describes the number of individuals subtracted to each group by mortality; migration, that describes the number of individuals subtracted to females and males by the overwintering migration (Klassen and Locke 2007); and finally reproduction/recruitment, which describes the input of NSJ.

The model forcing functions are daylight duration, salinity and water temperature (at the sea and within the estuary), which affect reproduction/recruitment, growth, mortality and migration. The model was written in STELLA 5.0 software and uses a time step of one month for 12 months. The time step was chosen to allow a direct comparison with the data obtained in the field (Souza et al. unpublished results). A simplified STELLA diagram of the model is shown in Fig. 9.4.

Long-term simulations were run for 48 months, to test the stability of the model.

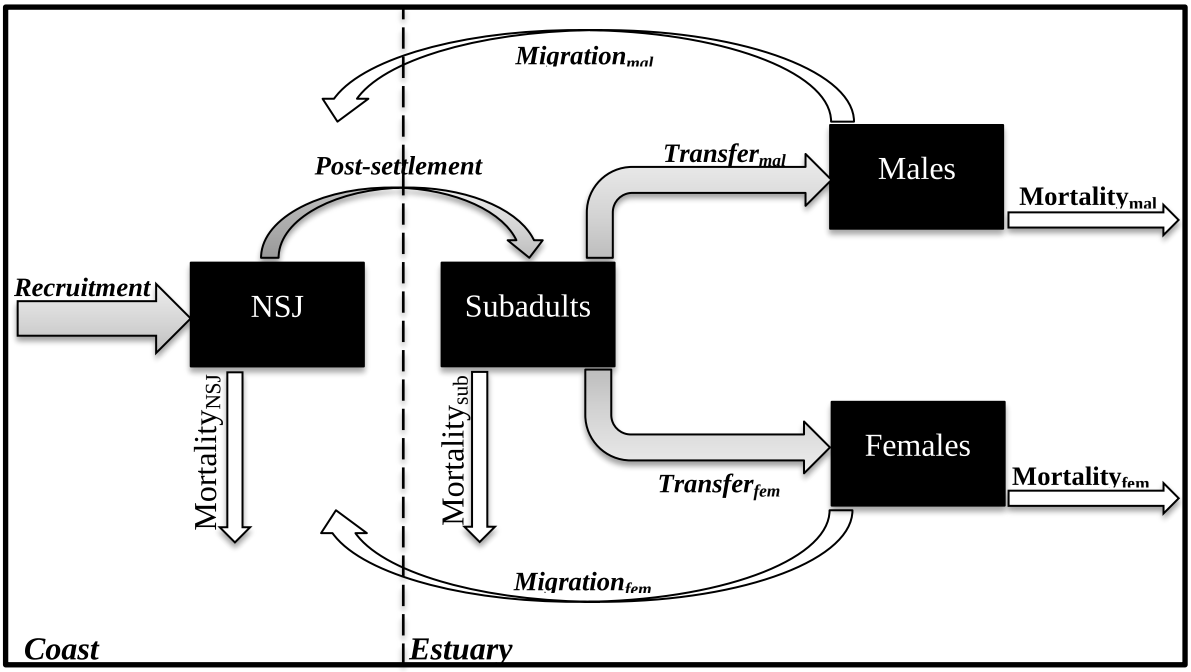


Fig. 9.4 Simplified STELLA diagram of the population dynamics model of the shore crab *Carcinus maenas*. Grey arrows indicate the input or the transference of individuals between population groups. White arrows indicate the outputs of the state variables. All state variables and fluxes indicate the number of individuals in 100m^{-2} per month. NSJ = Newly settled juveniles.

9.3.1. Larval phase, recruitment and post-settlement

Carcinus maenas life cycle is complex and requires both a planktonic (four zoeal and one megalopal stage) and a benthic phase (juveniles, subadults and adults) (Crothers 1967; Rice and Ingle 1975; Queiroga and Blanton 2004). The larval phase occurs predominantly offshore, with larvae settling in coastal areas after being transported to near-shore environment by oceanic currents (Roff et al. 1986; Lindley 1987; Queiroga 1996); therefore, the input of recruits into an estuary is predominantly allochthonous.

In our study we focused only on the post-settled crabs inside Minho estuary, thus *C. maenas* larvae were not sampled. Additionally, the shore crab reproduction does not seem to occur inside Minho estuary (Souza et al.

unpublished data). Taking this into consideration, we assumed that the input of NSJ in the model was not directly related with the number of adult individuals inside the estuary. Therefore, recruitment was calculated based on an estimated number of berried females, which was obtained by calibration, while the fecundity per female (Cohen et al. 1995), the percentage of eggs hatched (Van Engel 1958; Hill et al 1989) and the survival in the larval phase (Moloney et al. 1994) were obtained in the literature and posteriorly calibrated, once they referred to other crab species. Despite of the high number of eggs produced by each berried female (Cohen et al. 1995), the egg hatch success rate is low in portunid crabs (Hill et al. 1989), for instance, the egg hatch success rate for the blue crab *Callinectes sapidus* is about one in one million (Van Engel 1958). There is no available information about the egg hatch success rate for *C. maenas* in the field; therefore, the value used in the model was obtained by calibration.

Dawirs (1985) claimed that *C. maenas* larval development in the field is mainly controlled by temperature dynamics, whereas the effects of salinity during the larval phase may be minor, since salinity is relatively stable in the sea.

In the model, we assumed that *C. maenas* recruitment occurs throughout the year, once the shore crab population seems to present a continuous recruitment pattern in Minho estuary, showing a peak of abundance during spring (Souza et al. unpublished data). All values and equations used to describe the shore crab recruitment are presented in tables 9.1 and 9.2 respectively.

Table 9.1 Elements of the model and their respective values, symbols, description, units and their estimation method and initial values used on the model, when applied. NSJ = newly settled juveniles.

Type	Symbol	Description	Units	Used value
Equation	F_{sal}	Equation for the variation on salinity	Non-dimensional	Variable
Equation	F_{SST}	Equation for the variation on SST (Sea Surface Temperature)	ºC	Variable
Equation	F_{temp}	Equation for the variation on	ºC	Variable

		temperature		
Equation	$Migra_{fem}$	Number of migrating females	Per $100m^{-2}\cdot month^{-1}$	Variable
Equation	$Migra_{mal}$	Number of migrating males	Per $100m^{-2}\cdot month^{-1}$	Variable
Equation	$Mortality_{fem}$	Number of dead females	Per $100m^{-2}\cdot month^{-1}$	Variable
Equation	$Mortality_{mal}$	Number of dead males	Per $100m^{-2}\cdot month^{-1}$	Variable
Equation	$Mortality_{sub}$	Number of dead subadults	Per $100m^{-2}\cdot month^{-1}$	Variable
Equation	$Mortality_{NSJ}$	Number of dead NSJ	Per $100m^{-2}\cdot month^{-1}$	Variable
Equation	Post-settlement	Number of post-settled subadults	Per $100m^{-2}\cdot month^{-1}$	Variable
Equation	Recruitment	Number of NSJ entering in the system	Per $100m^{-2}\cdot month^{-1}$	Variable
Equation	Total abundance	Sum of the number of subadults, females and males	Per $100m^{-2}\cdot month^{-1}$	Variable
Equation	$Transfer_{fem}$	Transference from subadults to females	Per $100m^{-2}\cdot month^{-1}$	Variable
Equation	$Transfer_{mal}$	Transference from subadults to males	Per $100m^{-2}\cdot month^{-1}$	Variable
Forcing function	Daylight duration	Monthly variation of the daylight duration	Minutes	Variable/experimental
Forcing function	Sal	Monthly variation of the salinity inside the estuary	Non-dimensional	Variable/experimental
Forcing function	SST	Monthly variation of the surface sea temperature	ºC	Variable/experimental
Forcing function	Temp	Monthly variation of the water temperature inside the estuary	ºC	Variable/experimental
Parameter	Eggs	Number of eggs generated by reproductive females	Per $100m^{-2}\cdot month^{-1}$	Variable

Parameter	Fecundity	Number of eggs generated per reproductive female	Per 100m^{-2}·month^{-1}	15416/literature
Parameter	Hatched eggs	Rate of successful hatched eggs	Per 100m^{-2}·month^{-1}	0.01/literature, calibration
Parameter	MigraRate$_{fem}$	Rate of migrating females	Per 100m^{-2}·month^{-1}	0.85/calibration
Parameter	MigraRate$_{mal}$	Rate of migrating females	Per 100m^{-2}·month^{-1}	0.85/calibration
Parameter	MortRate$_{fem}$	Rate of female mortality	Per 100m^{-2}·month^{-1}	0.10/calibration
Parameter	MortRate$_{mal}$	Rate of male mortality	Per 100m^{-2}·month^{-1}	0.10/calibration
Parameter	MortRate$_{NSJ}$	Rate of NSJ mortality	Per 100m^{-2}·month^{-1}	0.25/calibration
Parameter	MortRate$_{sub}$	Rate of subadult mortality	Per 100m^{-2}·month^{-1}	0.15/calibration
Parameter	Reproductive females	Estimated number of reproductive females contributing to the estuarine population	Per 100m^{-2}·month^{-1}	10/calibration
Parameter	S_{max}	Maximum salinity for *C. maenas* growth	Non-dimensional	52/literature
Parameter	S_{min}	Minimum salinity for *C. maenas* growth	Non-dimensional	4/literature
Parameter	S_{opt}	Optimum salinity for *C. maenas* growth	Non-dimensional	34/literature, calibration
Parameter	Survival in larval phase	Rate of larvae surviving the planktonic stage	Per 100m^{-2}·month^{-1}	0.5/literature, calibration
Parameter	TMax$_{benthic}$	Maximum temperature for *C. maenas* growth	ºC	30/literature
Parameter	TMax$_{planktonic}$	Maximum temperature for *C. maenas* larvae development	ºC	22.5/literature
Parameter	TMin$_{benthic}$	Minimum temperature for *C. maenas* growth	ºC	10/literature
Parameter	TMin$_{planktonic}$	Minimum temperature	ºC	9/literature

		for *C. maenas* larvae development		
Parameter	$TOpt_{benthic}$	Optimum temperature for *C. maenas* growth	ºC	14/calibration
Parameter	$TOpt_{planktonic}$	Optimum temperature for *C. maenas* larvae development	ºC	14/literature, calibration
Parameter	Tx_{fem}	Transfer rate from subadults to females	Per $100m^{-2}\cdot month^{-1}$	0.6/experimental
Parameter	Tx_{mal}	Transfer rate from subadults to males	Per $100m^{-2}\cdot month^{-1}$	0.71/experimental
State variable	Females	Initial density of females	Per $100m^{-2}\cdot month^{-1}$	0.5/experimental
State variable	Males	Initial density of males	Per $100m^{-2}\cdot month^{-1}$	2/experimental
State variable	NSJ	Initial density of newly settled juveniles	Per $100m^{-2}\cdot month^{-1}$	12.5/experimental
State variable	Subadults	Initial density of subadults	Per $100m^{-2}\cdot month^{-1}$	1.5/experimental

Table 9.2 Equations used in the model of the population dynamics of the shore crab *Carcinus maenas* in Minho estuary, NW Iberian Peninsula.

NSJ density NSJ(t) = NSJ(t-∂t) + (Recruitment) * ∂t *Inflow*: ○ Recruitment = IF (SST < 10) THEN ((((Reproductive females * Fecundity) * Hatched eggs) * Survival in larval phase) * F_{SST}) ELSE ((((Reproductive females * Fecundity) * Hatched eggs) * Survival in larval phase) * F_{SST} * 0.01) *Outflows*: ○ $Mortality_{NSJ}$ = NSJ * $MortRate_{NSJ}$ ○ Post-settlement = NSJ * F_{temp} * F_{sal}	Variation in NSJ density per unit of time	(1)
Subadult density Subadult(t) = Subadult(t-∂t) + (Post-settlement – $Transfer_{mal}$ – $Transfer_{fem}$ – $Mortality_{sub}$) * ∂t	Variation in subadult density per unit	(2)

Inflows: ○ Post-settlement = NSJ * F_{temp} * F_{sal} *Outflows*: ○ $Transfer_{mal}$ = Subadult * Tx_{mal} * F_{temp} * F_{sal} ○ $Transfer_{fem}$ = Subadult * Tx_{fem} * F_{temp} * F_{sal} ○ $Mortality_{sub}$ = Subadult * $MortRate_{sub}$	of time	
Female density Female(t) = Female(t-∂t) + ($Transfer_{fem}$ – $Mortality_{fem}$ – $Migra_{fem}$) * ∂t *Inflows*: ○ $Transfer_{fem}$ = Subadult * Tx_{fem} * F_{temp} * F_{sal} *Outflows*: ○ $Mortality_{fem}$ = Female * $MortRate_{fem}$ ○ $Migra_{fem}$ = IF (Daylight duration < 575) OR (Temperature < 10) THEN ($MigraRate_{fem}$ * Female) ELSE (0)	Variation in female density per unit of time	(3)
Male density Male(t) = Male(t-∂t) + ($Transfer_{mal}$ – $Mortality_{mal}$ – $Migra_{mal}$) * ∂t *Inflows*: ○ $Transfer_{mal}$ = Subadult * Tx_{mal} * F_{temp} * F_{sal} *Outflows*: ○ $Mortality_{mal}$ = Male * $MortRate_{mal}$ ○ $Migra_{mal}$ = IF (Daylight duration < 575) OR (Temperature < 10) THEN (Male * $MigraRate_{mal}$) ELSE (0)	Variation in male density per unit of time	(4)
SST (Sea Surface Temperature) F_{SST} = IF (SST ≤ $TOpt_{planktonic}$) THEN (EXP(-2.3*((SST - $TOpt_{planktonic}$) / ($TMin_{planktonic}$ - $TOpt_{planktonic}$))^2)) ELSE (EXP-2.3*((SST - $TOpt_{planktonic}$) / ($TMax_{planktonic}$ - $TOpt_{planktonic}$))^2))	Equation for the limit factor of SST	(5)
Temperature F_{temp} = IF (Temperature ≤ $TOpt_{benthic}$) THEN (EXP(-2.3*((Temperature - $TOpt_{benthic}$) / ($TMin_{benthic}$ - $TOpt_{benthic}$))^2)) ELSE (EXP-2.3*((Temperature - $TOpt_{benthic}$) / ($TMax_{benthic}$ - $TOpt_{benthic}$))^2))	Equation for the limit factor of temperature	(6)
Salinity F_{sal} = IF (Salinity ≤ S_{Opt}) THEN (EXP(-2.3*((Salinity - S_{Opt}) / (S_{Min} - S_{Opt}))^2)) ELSE (EXP-2.3*((Salinity - S_{Opt}) / (S_{Max} - S_{Opt}))^2))	Equation for the limit factor of temperature	(7)

9.3.2. Effect of temperature on *C. maenas* growth

Since crustaceans are not able to control their body temperature to a significant degree, the typical response is that their metabolic rate varies directly with environmental temperature (Vernberg 1983). Empirical and experimental evidence suggests that *C. maenas* has different temperature ranges during the planktonic and benthic phases of its development, with the tolerance of larvae ranging from 9 to 22.5ºC (Dawirs et al. 1986; deRivera et al. 2007), while subadults and adults can grow at temperatures ranging from 10 to 30 ºC (Berrill 1982; Cohen et al. 1995; Behrens Yamada et al. 2005). This kind of variation is indicative of an optimum-type curve (Martins et al. 2008), which can be described by:

(1) $$f(T) = e^{-2.3\left(\frac{(T-T_{Opt})}{(T_{Min}-T_{Opt})}\right)^2} \text{ for } T \leq T_{\text{Opt}}$$

and

(2) $$f(T) = e^{-2.3\left(\frac{(T-T_{Opt})}{(T_{Max}-T_{Opt})}\right)^2} \text{ for } T > T_{\text{Opt}}$$

Where T = STT (for the planktonic phase) or temperature (for the benthic phase); T_{Opt} = optimum temperature for development (planktonic phase) and growth (benthic phase); T_{Min} = minimum temperature development (planktonic phase) and growth (benthic phase); T_{Max} = maximum temperature for development (planktonic phase) and growth (benthic phase).

The temperature tolerance for the shore crab larvae range from 9 to 22.5ºC (Dawirs et al. 1986; deRivera et al. 2007), but survivorship is higher at intermediate values and decreases with temperature increase (deRivera et al. 2007). Thus, the $\text{TOpt}_{\text{planktonic}}$ was obtained in the literature and calibrated. On the other hand, the $\text{TOpt}_{\text{benthic}}$ value was estimated from field data and calibration (see Table 9.1).

9.3.3. Effect of salinity on *C. maenas* growth

Salinity is an important environmental variable for several physiological, behavioral and ecological traits of estuarine crustaceans (e.g. Dorgelo 1976). Such as in the case of temperature, the metabolic rate of crustaceans also varies directly with salinity (e.g. Taylor et al. 1977; Anger et al. 1998). To cope with this, the model uses an optimum curve to describe the effect of salinity on

C. maenas growth. This optimum curve is mathematically identical with the one used for describing the effect of temperature on *C. maenas* development and growth (see equations 1 and 2).

Where S = salinity; S_{Opt} = optimum salinity for growth; S_{Min} = minimum salinity for growth; S_{Max} = maximum salinity for growth.

The SOpt value was estimated from field data and calibration (see Table 9.1).

9.3.4. Mortality

The number of *C. maenas* individuals subtracted to each population group was defined by:

(3) $$Mortality_i = MortRate_i \times D_i$$

Where $Mortality_i$ = mortality of the population group *i*; $MortRate_i$ = mortality rate of the population group *i*; D_i = density of the population group *i*.

The mortality rates of each population group were calibrated due to the absence of this information on the literature (Table 9.1). The mortality equations for each population group are shown in Table 9.2.

9.3.5. Migration

Migration is the movement of animals from one place to another in response to seasonal changes. Usually a sharp drop in temperature serves as an environmental trigger for migration in crustaceans (e.g. Kanciruk and Herrnkind 1978). The shore crab has long been recognized as an intertidal migrant both on a tidal (Crothers 1968; Hunter and Naylor 1993; Warman et al. 1993) and on a seasonal basis (Crothers 1968; Atkinson and Parsons 1973). Shore crab migration towards offshore is likely to be temperature controlled, with *C. maenas* migrating seawards when temperature level starts to drop in late autumn or early winter (Naylor 1963). An offshore overwintering migration is typical of most estuarine populations (Klassen and Locke 2007). Shore crabs usually move out of estuaries to deeper, warmer, coastal waters in winter (Broekhuysen 1936). Temperature seems to be the responsible for the overwintering in shore crabs, but it can vary according to each location, for instance, in the Netherlands, the overwintering temperature of *C. maenas* is 8.5ºC (Broekhuysen 1936), whilst in Ria de Aveiro lagoon (Portugal) is 10ºC (Gomes 1991).

Furthermore, the daylight duration can also be an important stimulus for migration and/or reproduction of crustaceans (e.g. Shuster 1982; Barlow et al. 1986; Sutcliffe 1993). Despite of this relationship has not been reported for *C. maenas* yet, our field observations suggest that this parameter is also associated to *C. maenas* overwintering migration (personal observation). Notwithstanding, shore crab locomotor activity patterns have been shown to vary according to daylight and incident light (McGaw and Naylor 1992b, Warman et al. 1993). Therefore, this parameter was also included in the equation that describes *C. maenas* migration. The number of migrating *C. maenas* individuals in each population group was defined by:

(4) $$Migra_i = MigraRate_i \times D_i$$

Where $Migra_i$ = migration of the population group *i*; $MigraRate_i$ = migration rate of the population group *i*; D_i = density of the population group *i*.

The migration rates of each population group were obtained by calibration due to the absence of this information on the literature. The values of the migration rate and the equations for males and females migration are shown in Tables 1 and 2 respectively.

9.3.6. Sensitivity analysis

Sensitivity analysis was calculated for variations of ± 10% on each parameter:

(5) $$Y'_{X_i} = \frac{\partial Y}{\partial X_i}$$

Where *Y'* is the sensitivity of the model outputs to variations on parameters (X_i). The variation of each parameter and, the resulting response of the model, were calculated with all other parameters fixed according to OAT (on-at-a-time) approach.

9.3.7. IPCC predictions

The IPPC (Intergovernmental Panel on Climate Change) Fourth Assessment Report (AR4) predicted that the surface air warming in the 21st century will range from 1.1 to 6.4ºC (IPCC 2007). It has also been predicted that the annual temperature in Europe warms at a rate of between 0.1 and 0.4 Cº per decade, and that the warming will be greatest over southern Europe and northeast Europe (Christensen et al. 2007).

The IPCC (2007) also predicted that the annual precipitation will decrease across southern Europe (maximum 1% per decade), producing drier summers and wetter winters (Christensen et al. 2007). These changes probably will cause changes on the salinity levels of estuarine systems, since it is most likely that droughts and floods events will be triggered in these systems, as recently reported (e.g. Cardoso et al. 2008; Dolbeth et al. 2010; Santos et al. 2010; Ilarri et al. 2011).

In this context, we have simulated several scenarios of temperature and salinity variations in Minho estuary. Simulations included six levels of water temperature increase (+1, +2, +3, +4, +5 and +6ºC), three different scenarios of salinity variation (-5 psu, +5 psu and oscilattory (-5 psu from November to April and +5 psu from May to October)). Additionally, we have also simulated the combined effects of temperature and salinity variations.

Finally, we have also simulated the most likely scenarios of climate change in the 21st century (temperature increasing and oscillatory variation pattern on salinity) on the shore crab population in Minho estuary. Notwithstanding, two different rates of water temperature increase were simulated: slow (increase of +0.1ºC per decade) and rapid (increase of +0.4ºC per decade).

9.4. Results

9.4.1. Model results

The predicted density of *C. maenas* subadults followed the same pattern of the observed variation, with a peak of density being recorded in May (Fig. 9.5). The predicted densities of females, males and total density also followed similar patterns of the observed results, with the density continuously increasing after spring, remaining high until late autumn and then dropping during winter (Fig. 9.5).

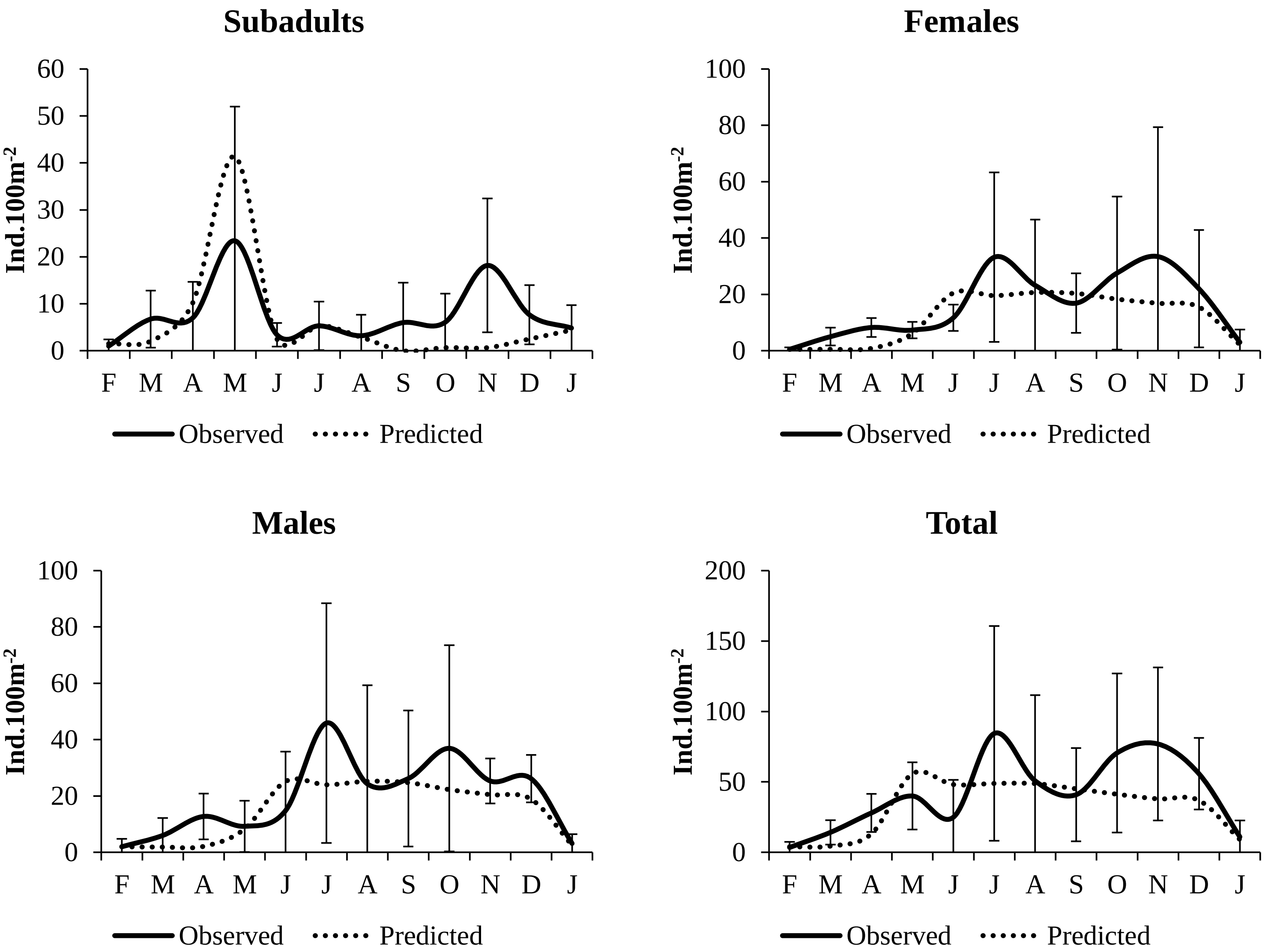


Fig. 9.5 Observed ± SD and predicted variation of subadults, females, males and the total density (ind.100m^{-2}) of the shore crab *Carcinus maenas*.

9.4.2. Model stability and sensitivity analysis

The model showed stability for long term simulations (48 months) (Fig. 9.6), thus supporting its internal logic (Jørgensen 1994).

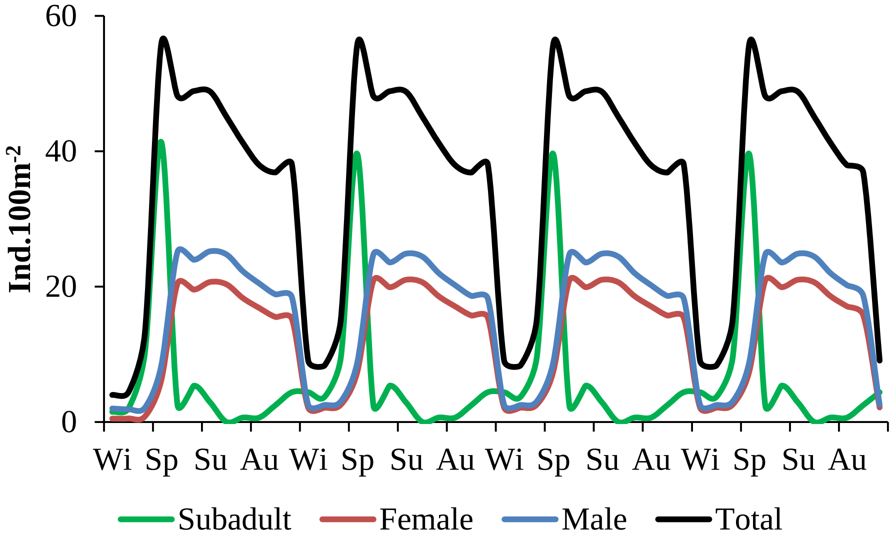


Fig. 9.6 Long term simulation (48 months) for the shore crab *C. maenas* population in Minho estuary (NW Iberian Peninsula), showing longtime stability of the model, with every population group presenting consistent stability through time. Wi = winter; Sp = spring; Su =summer; Au = autumn.

Sensitivity analysis showed that $TMin_{planktonic}$ and $TOpt_{planktonic}$ were the most sensitive parameters (Table 9.3).

Table 9.3 Sensitivity (*sensu* Jørgensen 1994) of the population density to ± 10% variations of the parameters used on the population dynamics model of the shore crab *Carcinus maenas* in Minho estuary, NW Iberian Peninsula.

Parameter	-10%	Base simulation	+10%	Sensitivity
Fecundity	13874.4	15416	16957.6	0.83
Hatched eggs	0.009	0.01	0.011	0.83
$MigraRate_{fem}$	0.765	0.85	0.935	-0.03
$MigraRate_{mal}$	0.765	0.85	0.935	-0.04
$MortRate_{fem}$	0.09	0.10	0.11	-0.11
$MortRate_{mal}$	0.09	0.10	0.11	-0.14
$MortRate_{NSJ}$	0.225	0.25	0.275	-0.16
$MortRate_{sub}$	0.135	0.15	0.165	-0.13
Reproductive females	9	10	11	0.83
$SMax_{benthic}$	46.8	52	57.2	0.00
$SMin_{benthic}$	3.6	4	4.4	-0.02

$SOpt_{benthic}$	30.6	34	37.4	-0.65
Survival in larval phase	0.45	0.50	0.55	0.83
$TMax_{benthic}$	27	30	33	0.00
$TMax_{planktonic}$	20.25	22.5	24.75	0.33
$TMin_{benthic}$	9	10	11	-0.36
$TMin_{planktonic}$	8.1	9	9.9	-7.58
$TOpt_{benthic}$	12.6	14	15.4	-0.77
$TOpt_{planktonic}$	12.6	14	15.4	2.04
Tx_{fem}	0.54	0.60	0.66	0.01
Tx_{mal}	0.639	0.71	0.781	0.01

9.4.3. Climatic change simulations

Once the correlation between the model outputs and real data was shown to be satisfactory (Table 9.4), the model was considered suitable to simulate the effects of the upcoming climatic changes on the shore crab population.

Table 9.4 Linear regressions between the observed and the predicted values for the *C. maenas* density inside Minho estuary, NW Iberian Peninsula.

Group	Df	SS	MS	F	P
Subadults	1	238.0	238.0	10.66	0.008*
Females	1	554.5	554.5	17.41	0.002*
Males	1	1314.8	1314.8	17.38	0.002*
Total	1	3631.8	3631.8	8.77	0.014*

9.4.3.1. Temperature variations

Simulations showed that *C. maenas* population increases when the temperature increases in 1ºC (about 126%) or 2ºC (about 96%), with all other scenarios of temperature increase leading to a significant reduction (decrease of nearly 60% in scenarios with a temperature increase > 2ºC) in *C. maenas* density within the estuary. Additionally, a temperature increase of 1ºC seems to promote an earlier occurrence of the spring peak of density of subadults, females and males, which in this scenario, occurred one (+1ºC) or two (+2ºC) months earlier compared to the basic run (Fig. 9.7).

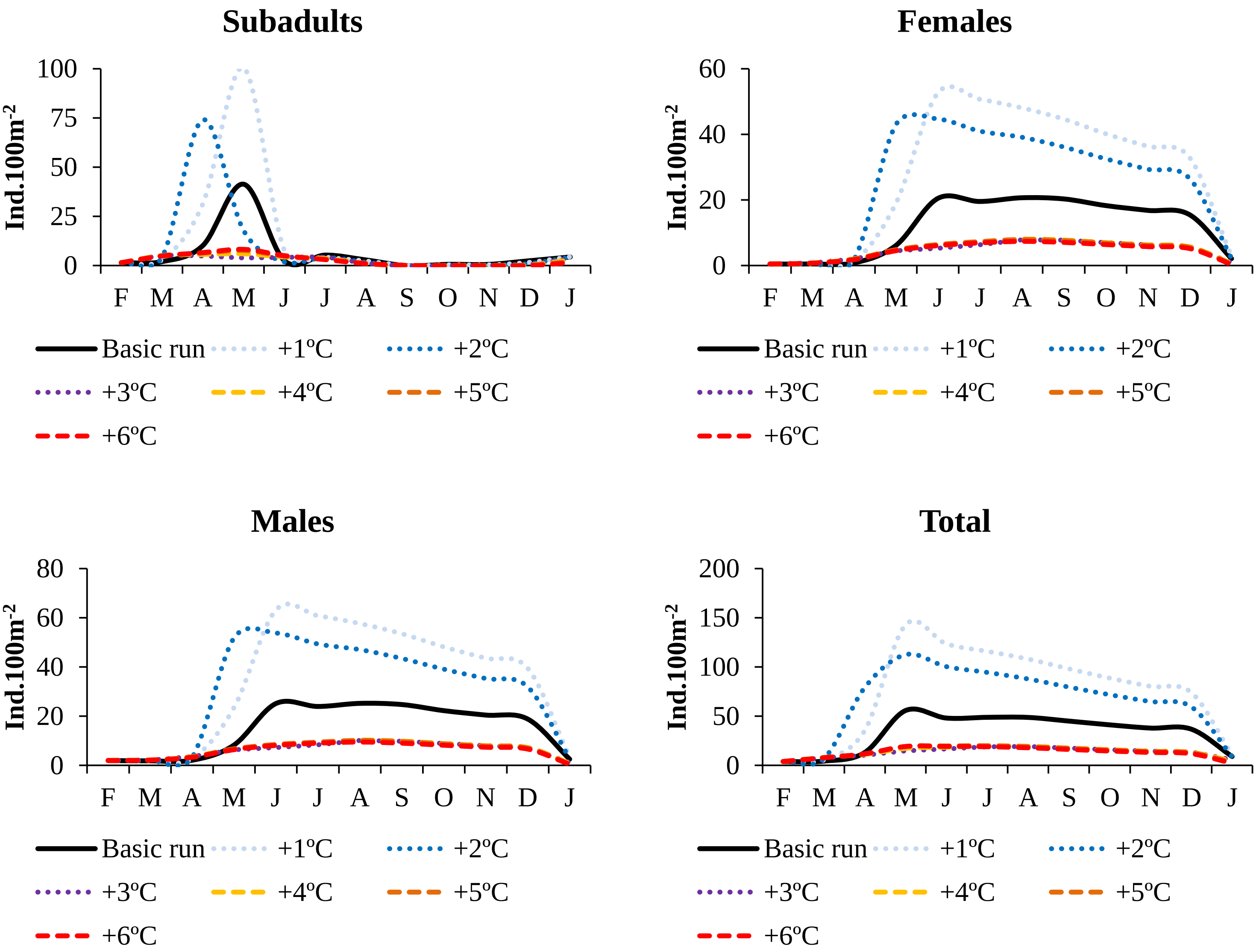


Fig. 9.7 One year simulations of subadults, females, males and total density of the shore crab *Carcinus maenas* in different scenarios of temperature increase according to the IPCC projections for the future. Dotted lines refer to low scenarios of climate change and dashed lines refer to high scenarios of climate change.

9.4.3.2. Salinity variations

Simulations with salinity decreases, increases and oscillations suggest that *C. maenas* population would barely change, with a slight increasing in density (13%) being recorded with a salinity increase, and a slight decrease when salinity is oscillatory (8%) or decreased (11%) (Fig. 9.8).

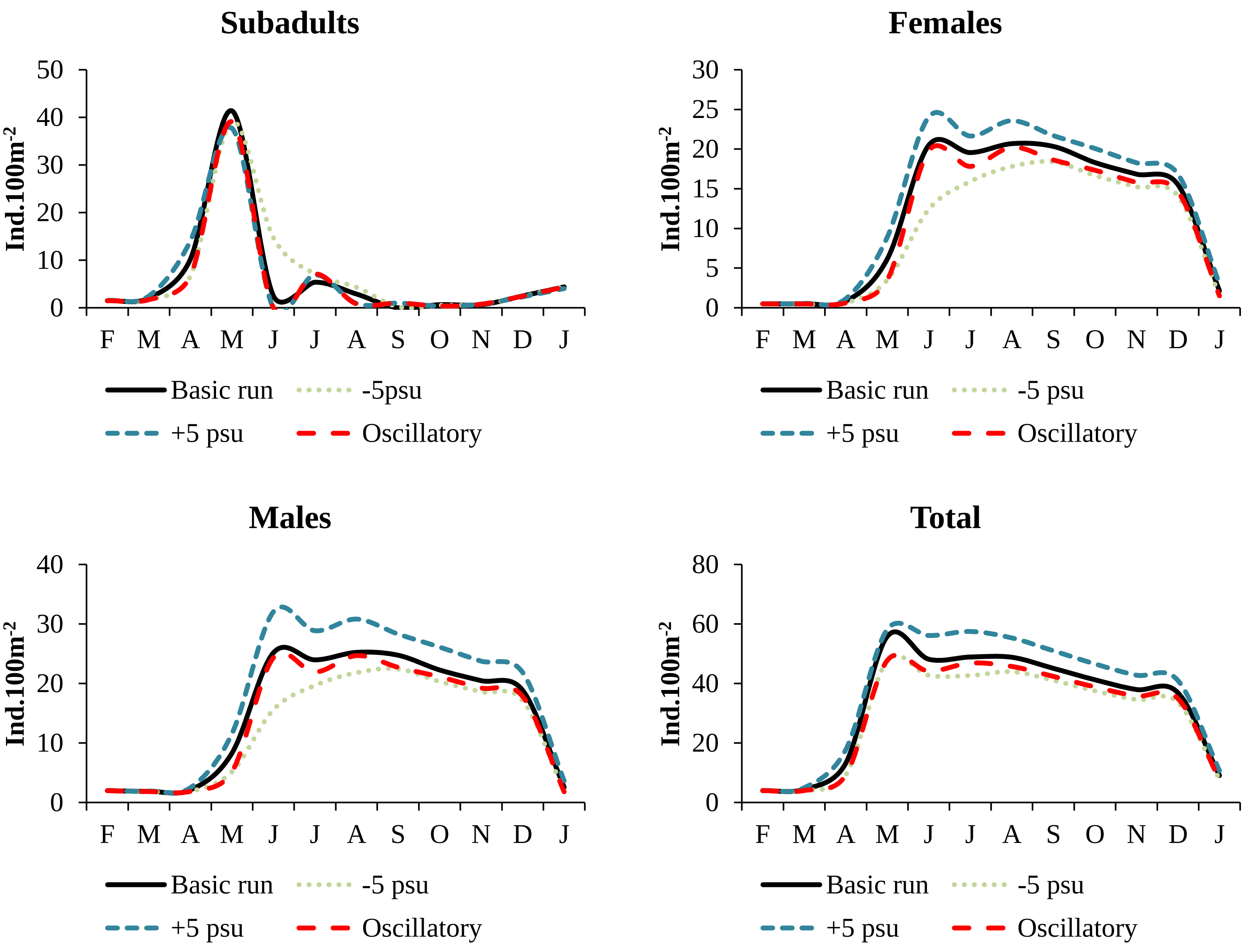
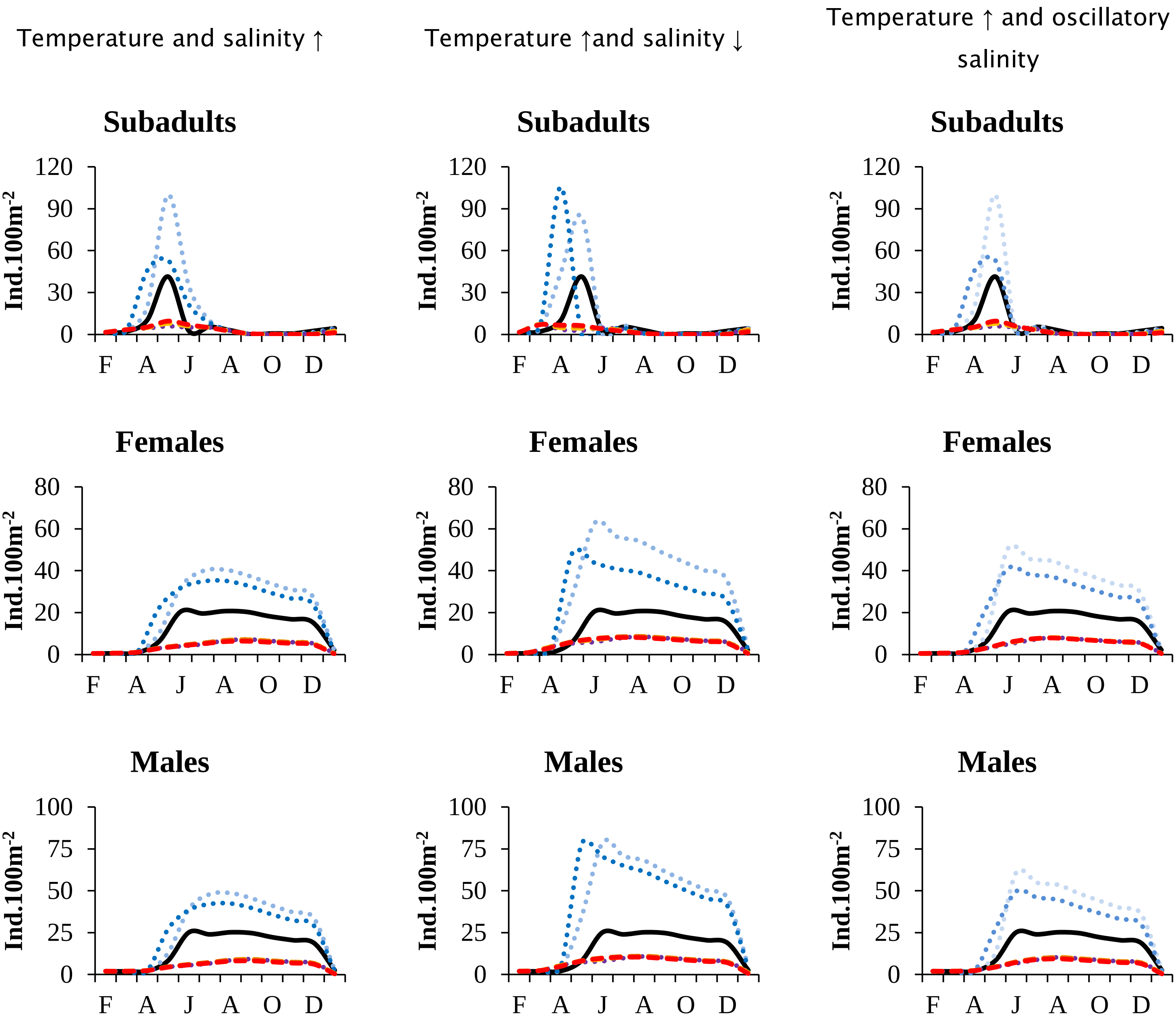


Fig. 9.8 One year simulations of subadults, females, males and total density of the shore crab *Carcinus maenas* in scenarios of salinity decreasing (-5 psu) and increasing (+5 psu) and oscillatory pattern (-5 psu from November to April, and +5 psu from May to October).

9.4.3.3. Combined effects of temperature and salinity variations

The combined effect of temperature and salinity variations on the shore crab population would be similar to those recorded for temperature rising, with *C. maenas* population increasing only with +1ºC and +2ºC of temperature increase. In the scenarios of temperature increase and salinity decrease, *C. maenas* population responded with a less noticeable variation of density (about 12% less) compared to a scenario of temperature increase alone. On the other hand, in scenarios of temperature and salinity increase, the shore crab population would respond with a more noticeable increase of abundance. Temperature increase combined with an oscillatory pattern of salinity variation would cause a less marked increase of *C. maenas* density at +1ºC (11% less) and +2ºC (10% less) compared to the temperature increase alone (Fig. 9.9).



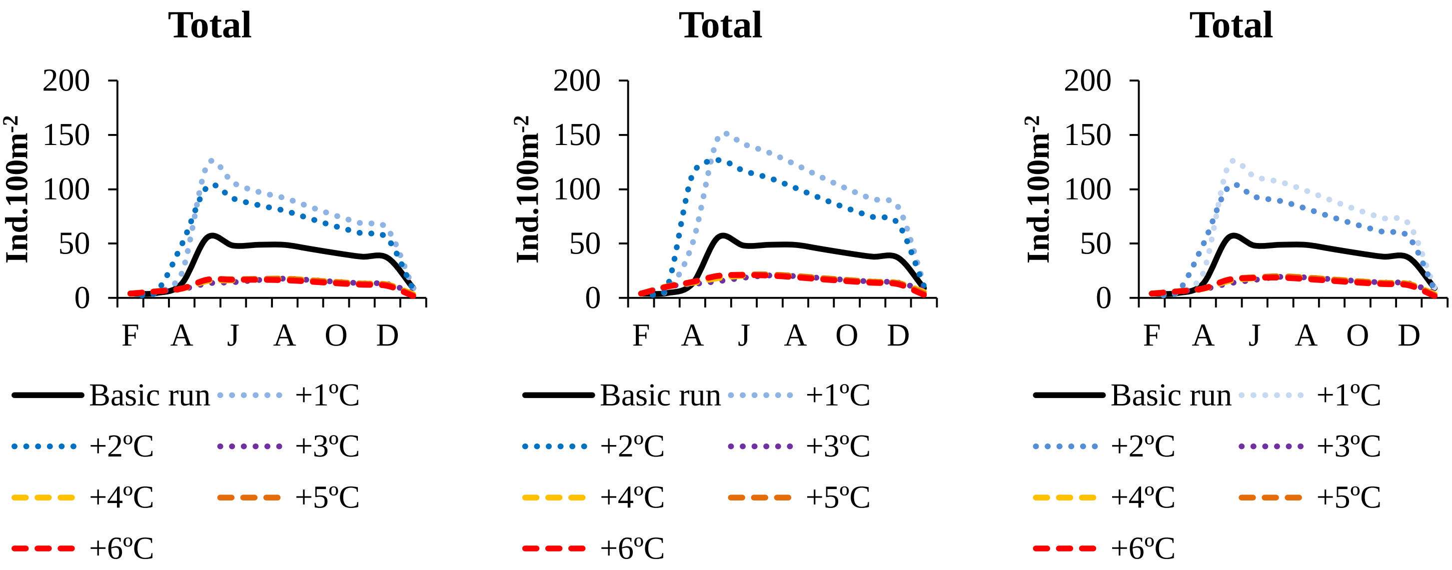


Fig. 9.9 One year simulations of subadults, females, males and the total density of the shore crab *Carcinus maenas* in different climatic change scenarios of temperature and salinity increase (left column), temperature increase and salinity decrease (middle column) and temperature increase and oscillatory salinity (-5 psu from November to April, and +5 psu from May to October).

9.4.3.4. Long-term effects of temperature and salinity variations

The combined effect of temperature increase and an oscillatory salinity pattern through an extended period of time suggests that *C. maenas* population in Minho estuary may increase for the next 30 years before it starts to decline in a scenario of a rapid increase of temperature. On the other hand, in a scenario of a slow increasing of temperature, the *C. maenas* population will continuously increase until the end of the 21st century (Fig. 9.10).

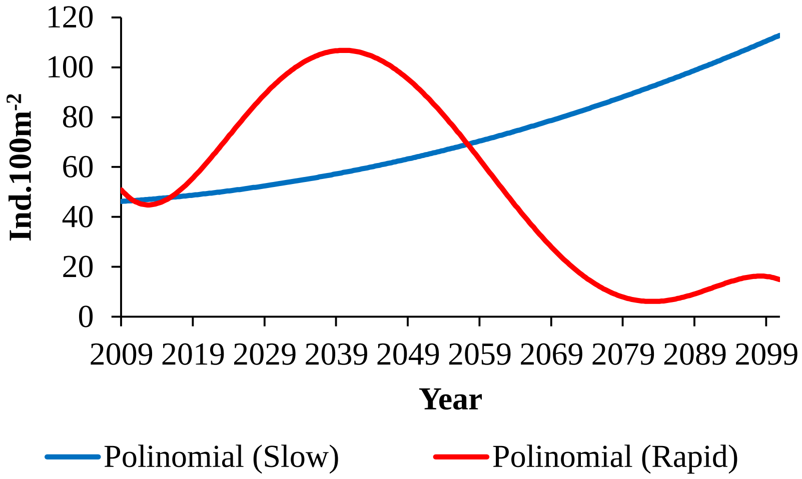


Fig. 9.10 Long-term effects of temperature increasing and an oscillatory pattern of salinity in *C. maenas* population in Minho estuary, NW Iberian Peninsula.

9.5. Discussion and conclusions

The model was capable to satisfactory predict the variation of *C. maenas* density in the Minho estuary. Model results also suggest that *C. maenas* is more sensitive to changes in temperature than in salinity. This result is in accordance with Styrishave et al. (1999), which found that the water temperature was the abiotic variable that exerted the largest influence on the physiological traits of *C. maenas*.

A lower sensitivity to salinity compared to temperature is particularly appropriate for shore crabs inhabiting estuaries because, within these systems, salinity presents drastic changes on a daily basis, while significant changes in water temperature usually occur within months. Thus, one should expect that shore crabs could cope with salinity oscillations much better than with changes in temperature (see Styrishave et al. 1999). Actually, *C. maenas* is classified as an efficient hyperosmoregulator (Rankin and Davenport 1981), which can even withstand short-term exposure to freshwater (McGaw and Naylor 1992b). On the other hand, even slight changes in temperature can affect several traits in *C. maenas* life, such as the locomotor rhythm (Naylor 1963); the heart rate and respiratory frequency (Taylor et al. 1973); the immunity response against antigens (Brockton and Smith 2008); the feeding rate (Wallace 1973; Sanchez-Salazar et al. 1987; Murray and Seed 2010); and the agonistic behavior (Souza et al. unpublished data). In this context, rises in water temperature would have much more consequences on the *C. maenas* populations than increases or decreases in salinity, as evidenced by the model.

Because *C. maenas* is not able to control its body temperature, and this abiotic variable plays a major role in the physiology of the species, shore crabs developed behavioral strategies to cope with temperature fluctuations. During cold months, *C. maenas* performs overwintering migrations to cope with the low temperatures in estuaries (Klassen and Locke 2007). Also, when exposed to high temperatures (> 25ºC), shore crabs can migrate into air to lower their body temperature (see Taylor and Wheatly 1979). In scenarios of temperature increase, the migration into the air may perhaps increase mortality rate of *C. maenas*, once it might increase the odds of being preyed by birds, which are one of the most important predators for the species (Moreira 1999; Klassen and Locke 2007).

Kelley et al. (2011) demonstrated that the range in *C. maenas* thermotolerance varied between 0.7 to 3ºC in 12º of latitude, suggesting that the thermotolerance of shore crabs differs according to latitude, reflecting their native thermal environment. Therefore, the thermal tolerance of the shore crab population from Minho estuary may perhaps be different from those reported in the literature (e.g. Berrill 1982; Cohen et al. 1995; Behrens Yamada et al. 2005). Ideally, the values of temperature tolerance used in the model should be site specific; however, due to the lack of those data for Minho estuary, we have use values reported in the literature. As far as we know, there is no published study comparing the *C. maenas* temperature tolerance across its native range of distribution. Thus, further studies are necessary to ascertain the range of variation in temperature tolerance of *C. maenas* populations from different locations, in order to improve the accuracy of population dynamics models. Knowing that populations from higher latitudes have a shorter upper limit of temperature tolerance (Kelley et al. 2011), and that several values used in the model were obtained from studies conducted with populations located at higher latitudes, it is possible that the maximum temperature tolerance of the considered *C. maenas* population is higher, but further studies are necessary to confirm this.

According to the model, the planktonic stage is more sensitive to temperature variations than the benthic stage, which is corroborated by experimental data, where subadults and adults show a higher range of temperature tolerance compared with larvae (Berrill 1982; Dawirs et al. 1986; Cohen et al. 1995;

Behrens Yamada et al. 2005; deRivera et al. 2007). The narrow range of temperature tolerance of *C. maenas* larvae is actually responsible for the predicted sharp decrease in *C. maenas* abundance with a temperature increase larger than 2ºC, because temperature regulates the input of individuals into the system. Therefore, the effects of climate change will be firstly noticeable at *C. maenas* larval stages. In this context, it would be important to establish monitoring programs of the *C. maenas* larvae in the future, in order to detect the early signs of a potential population collapse.

The shore crab can significantly impact community structure of the systems where it inhabits. This species can exert influence on the population level of various taxonomic groups, including algae, plants, mollusks, crustaceans and fishes (see Klassen and Locke 2007). A number of studies have demonstrated the negative effects of *C. maenas* introduction in non-indigenous systems (e.g. Grosholz and Ruiz 1995; deRivera et al. 2011; Gregory and Quijón 2011), but shore crabs have had similar ecological impacts in their native range, even though their habitat use may vary between areas (Klassen and Locke 2007). Therefore, it should be expected that, in scenarios of temperature and/or salinity change in Minho estuary, the shore crab may cause significant impacts on the ecosystem functioning and the benthic community structure. Actually, the model suggests that in scenarios of temperature and/or salinity increase in Minho estuary, the shore crab population would respond with a noticeable (up to 126% in a year) and long lasting (minimum of 30 years) increase in the population size. Thus, due to the voracious and generalist behavior of the species (Raffaelli et al. 1989), an increase in *C. maenas* density probably would have important consequences to the estuarine community. Also, the feeding rate of shore crabs is substantially higher at higher temperatures (Wallace 1973). Therefore, it should be expected a significant increase in the food uptake of *C. maenas*, which could significantly alter the community structure and existent ecological interactions within the estuary (Grosholz and Ruiz 1995; 1996).

Since the Minho estuary is characterized by abundant and productive populations of several aquatic species and is considered an important nursery area for estuarine species in SW Europe (e.g. Sousa et al. 2008a; Freitas et al. 2009; Dolbeth et al. 2010; Freitas 2011; Souza et al. 2013; Souza et al. unpublished data), the possible impacts of variations on the abundance of *C. maenas* may have significant consequences on the ecosystem, which in turn may have socioeconomical implications, given that the system sustains an active community of artisanal fishermen (see Sousa et al. 2008a). In this context, our study presents a forecasting approach on how a core and worldwide distributed species will cope with climatic change in the near future. This type of approach represents a useful tool for future planning and management of estuarine systems, once results predict how *C. maenas* will vary with global effects of climate change.

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CHAPTER 10

MODELING THE EFFECTS OF CLIMATE CHANGE ON A REMARKABLY ABUNDANT POPULATION OF THE COMMON GOBY *POMATOSCHISTUS MICROPS*

Modeling the effects of climate change on a remarkably abundant population of the common goby *Pomatoschistus microps*

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Souza AT, Marques JC, Martins I (submitted) Modeling the effects of climate change on a remarkably abundant population of the common goby *Pomatoschistus microps*.

Abstract

Despite of the IPCC predictions about the temperature rising in the near future and the global awareness about this issue, there are only few studies that use these predictions to simulate the effects of climate change on the population dynamics of estuarine fishes. In this context, the present study aimed at assessing the effects of temperature and salinity variations on the population dynamics of the common goby *Pomatoschistus microps* in a system where the species is remarkably abundant. This aim was achieved by modeling the dynamics of *P. microps* population in Minho estuary (Portugal). The state variables used in the model were juveniles, females and males. The linear regressions between the observed and the predicted density of males (ANOVA: $F = 3.06$; $p = 0.11$) was not significant, but regressions for observed versus predicted density of juveniles (ANOVA: $F = 5.39$; $p < 0.05$), females (ANOVA: $F= 25.89$; $p < 0.001$) and the total estuarine population (ANOVA: $F = 44.57$; $p < 0.001$) were significant. Therefore, the model was considered able to satisfactory describe the *P. microps* population dynamics, and it was used to simulate the effects of climatic changes on the fish population. The common goby responded toward changes in both temperature and salinity. In all scenarios of temperature increase the common goby responded with a population decrease. Similarly, increased salinities also caused decreases in *P. microps* density; whilst scenarios of decreased salinity caused marked increases in the abundance of the common goby. Long-term simulations indicated that population will continuously decrease in the next decades and, it is likely that, common gobies may experience sharp population shrinkage if the IPCC predictions prove correct. Given the trophic position and abundance of the species in Minho estuary, this may have significant impacts on the estuarine biological communities.

Keywords: Ecological modeling / climate change / global warming / Minho / Portugal

10.1. Introduction

The common goby *Pomatoschistus microps* is a widely distributed estuarine species spanning ca. 44º in latitudinal range, occurring from Norway to Mauritania, including the Canary Islands, western Mediterranean and Baltic Sea (Froese and Pauly 2010). This species is of great importance in marine and estuarine ecosystems, as it links low and high levels of the food-web, by acting as a mesopredator, thus connecting microbenthos to larger predators such as birds and fishes (Doornbos 1984; Moreira et al. 1992; Cabral 2000).

The common goby is often reported as one of the most abundant fish in northern Atlantic estuaries (Martinho et al. 2007b; Dolbeth et al. 2010). This species is frequently found in areas where the sympatric species *P. minutus* co-occurs and both species tend to present similar densities (e.g. Arruda et al. 1993; Leitão et al. 2006; Dolbeth et al. 2007b; Martinho et al. 2007b). However, in Minho estuary (NW Iberian Peninsula) the common goby seems to be much more abundant than the sand goby, presenting remarkably dense and productive population, which is attributed to site-specific favorable conditions to thrive (Souza et al. in revision).

The common goby is highly tolerant to environmental constraints, being able to tolerate wide ranges of temperature and salinity (Fonds 1973; Moreira et al. 1992; Rigal et al. 2008). However, despite of being able to thrive even in harsh environmental conditions, several biological and ecological traits of *P. microps*, such as growth, reproduction, migration and mortality are highly dependent upon temperature and salinity conditions (Jones and Miller 1966; Fonds 1973; Claridge et al. 1985; Wiederholm 1987; Moreira et al. 1992; Rigal et al. 2008). Since, it is expected that these two parameters will change in a near future due to changes in global climate (IPCC 2007), the present study aimed at assessing the possible effects of changes on temperature and salinity on *P. microps* population in a system where the species is highly dense and productive.

10.2. Study area

This study was conducted in Minho estuary (NW Iberian Peninsula – 41º53'N 8º50'0), which ranges up to 40 km (considering the upstream limit of spring tides), covering a total area of 23 km^2. This estuary is a shallow system (Moreno et al. 2005), with a mean depth of 2.6 m (Freitas et al. 2009); and is characterized as a mesotidal and partially mixed system, although it tends towards a salt wedge estuary during periods of high river flow (Sousa et al. 2005).

This study was carried out during 12 months, from February/09 to January/10. Samples were collected at 3 nearby stations (S1, S2 and S3) within the first 8 km of the Minho estuary, considering a gradient of distance to the river mouth (Fig. 10.1). S1 is located closer to the river mouth (ca. 1.5 km), and characterized by the presence of soft bottoms, often densely covered by debris such as drifting seaweeds, dead leaves and empty mollusks' shells (Souza et al. 2011; in press). S2 is located inside a salt marsh area (ca. 3.5 km upwards from the river Minho mouth), which is a relatively small sub-system characterized by the presence of narrow channels, typically bordered by the seagrass *Spartina maritima*. The channel's soft bottoms are often sparsely covered by debris and empty peppery furrow shells *Scrobicularia plana* (Souza et al. 2011; in press). S3 is located ca. 5 km upstream from S2, and characterized by high densities of the Asian clam *Corbicula fluminea*, with the soft bottoms often sparsely covered by debris and submerged vegetation (Sousa et al. 2008b,c; Souza et al. 2013).

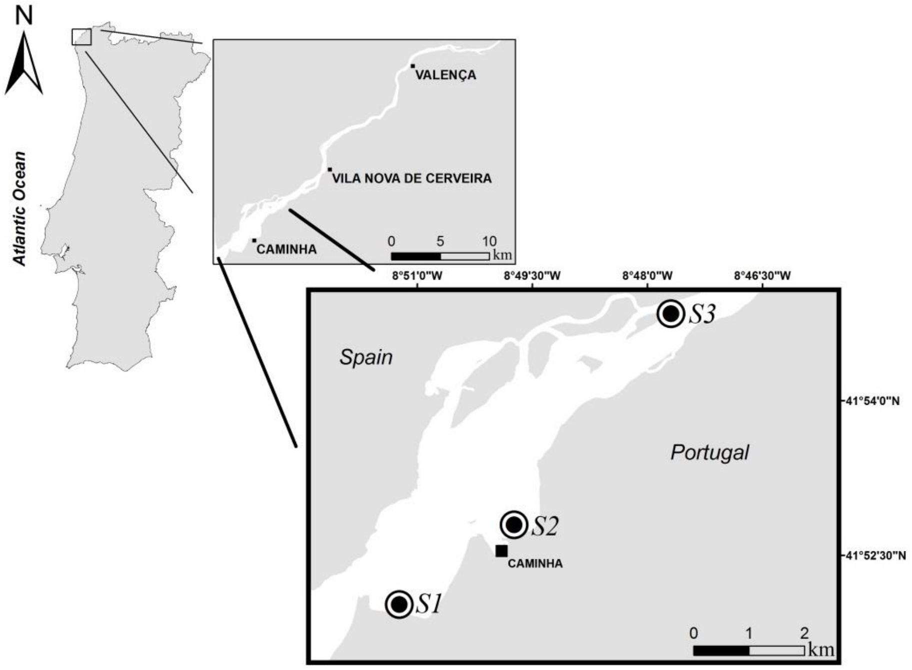


Fig. 10.1 Map of the study site showing the three sampling stations (S1, S2 and S3) at the lower Minho estuary, NW Iberian Peninsula.

10.3. Data set

10.3.1. Fish data

The model was developed and calibrated with data of *P. microps* sampled, from February 2009 to January 2010, in the three nearby stations at the lower Minho estuary (Fig. 10.1). In each site, three replicates per month were sampled using a 1m beam trawl (5 mm mesh size) towed at constant speed (2 km.h^{-1}) for 2 to 3 minutes, during daylight at high tide of spring tides. Previous studies in Minho estuary indicated that the sampling area covered by a 3 minutes tow, at constant speed, was equivalent to an average area of 100 ± 4 m^2 (Freitas et al. 2009).

10.3.2. Abiotic data

The water temperature and salinity at the bottom were measured each month in the 3 sampling stations using a multiparameter probe YSI 6820 (Fig. 10.2).

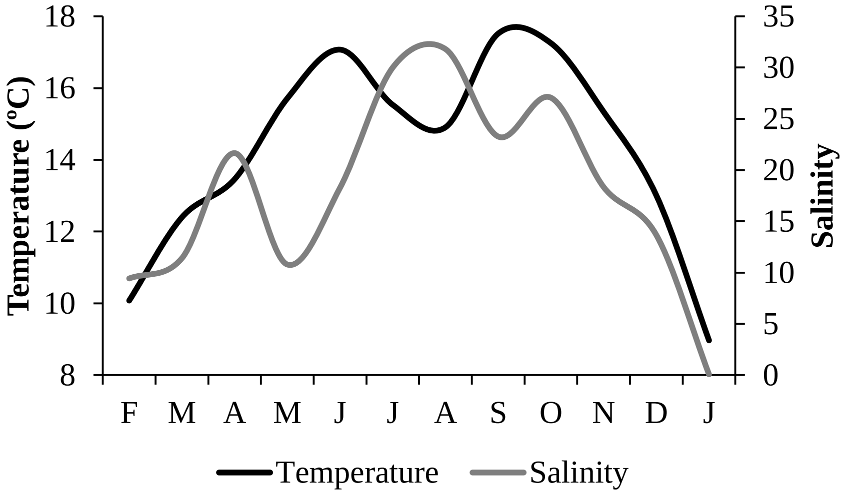


Fig. 10.2 Water temperature and the salinity variation at the lower Minho estuary during the study period.

The daylight duration data (Fig. 10.3) was obtained from NOAA website (www.esrl.noaa.gov/gmd/grad/solcalc/sunrise.html). Monthly mean daylight duration (minutes) was used as a proxy for the variation on the day length at the study site.

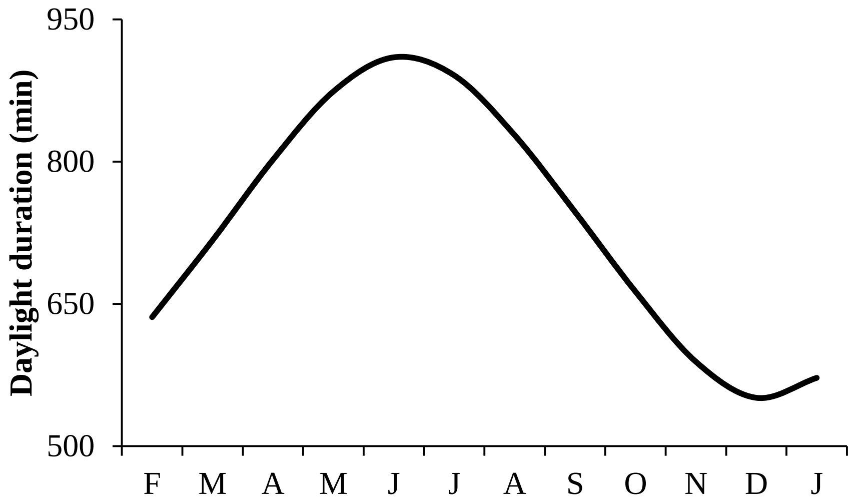


Fig. 10.3 Monthly variation in daylight duration at the Minho estuary, NW Iberian Peninsula.

10.4. Model formulation and conceptualization

Size structured models had proven to be advantageous because they take into account different kinetics and parameters that regulate the dynamics and physiology of different life stages of a given species (e.g. juveniles, males and females) (Batchelder and Miller 1989; Labat 1991).

In the present model, the *P. microps* population was divided into three groups: juveniles, females and males, which constitute the state variables of the model. The flows between state variables are individuals per unit of time, while the units of the state variables are individuals.100m^{-2}. The processes that regulate the number of individuals in each group over time are: growth, which determines the number of individuals transferred from one group to the next; death, which describes the number of individuals subtracted to each group by mortality; migration, that describes the number of individuals subtracted to females and males by the overwintering migration (Jones and Miller 1966); and finally recruitment, which describes the input of juveniles on the population.

The model forcing functions are daylight duration, salinity and water temperature, which affect recruitment, growth, mortality and migration. The model was written in STELLA 5.0 software and uses a time step of one month for 12 months. The time step was chosen to allow a direct comparison with the data obtained on the field (Souza et al. in revision). A simplified STELLA diagram of the model is shown in Fig. 10.4. Long-term simulations run for 48 months, to test the stability of the model.

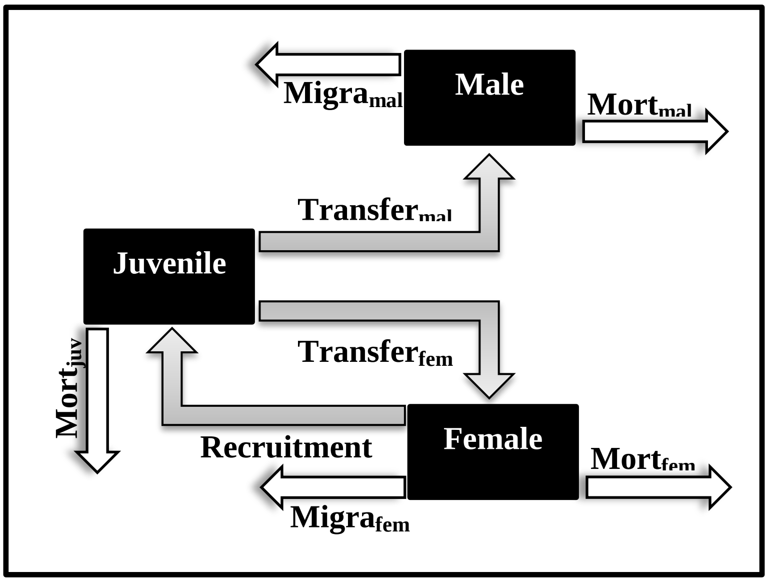


Fig. 10.4 Simplified STELLA diagram of the population dynamics model of the common goby *Pomatoschistus microps*. Grey arrows indicate the input or the transference of individuals between population groups. White arrows indicate the outputs of the state variables. All state variables and fluxes indicate the number of individuals in $100m^{-2}$ per month.

10.4.1 Reproduction and recruitment

The reproductive behavior of *P. microps* is relatively well known, with spawning usually occurring when water temperature is between 15 and 20ºC (Wiederholm 1987). During reproduction season, mature females lay their eggs on the nests built by males using empty bivalve shells (Nyman 1953; Jones and Reynolds 1999; Pampoulie et al. 2001). Afterwards, males fertilize the eggs, fan and guard them until hatching (Svensson et al. 1998; Jones and Reynolds 1999; Pampoulie et al. 2001). During nest guarding behavior, males often prey on their own brood (Magnhagen 1992) removing about 30% of the egg mass on a clutch (Forsgren et al. 1996).

Common gobies are known to have a high individual fecundity (Bouchereau and Guelorget 1998), with each mature female being able to generate from 460 to 3400 eggs (Miller 1986; Bouchereau et al. 1989; Bouchereau and Guelorget 1998). Despite of the high fecundity, the mortality rate during the early stages of fish development is very high (Leis 2007). Actually, the survival rate of marine and diadromous fish larvae varies between $6.7x10^{-5}$ and 0.1% according to Dahlberg (1979). Given that there is any information regarding the mortality rate of *P. microps* larvae, we have to calibrate this rate based on the values of other marine and estuarine fish species.

The percentage of mature females on the population during spawning season was estimated as the ratio between the amount of females in advanced stages of gonadal development and the total number of females.

Parameters values and the equations describing the common goby recruitment are described in tables 10.1 and 10.2 respectively.

Table 10.1 Elements of the model and their respective values, symbols, description, units and their estimation method and initial values used on the model, when applied.

Type	Symbol	Description	Units	Used value
Equation	F_{sal}	Equation for the variation on salinity	Non-dimensional	Variable
Equation	F_{temp}	Equation for the variation on temperature	ºC	Variable
Equation	$Migra_{fem}$	Number of migrating females	Per month	Variable
Equation	$Migra_{mal}$	Number of migrating males	Per month	Variable
Equation	$Mort_{fem}$	Mortality rate of females	Per month	Variable
Equation	$Mort_{juv}$	Mortality rate of juveniles	Per month	Variable
Equation	$Mort_{mal}$	Mortality rate of males	Per month	Variable
Equation	Recruitment	Number of juveniles entering in the system	Individuals.100m^{-2}	Variable
Equation	Total abundance	Sum of the number of juveniles, females and males	Individuals.100m^{-2}	Variable
Equation	$Transfer_{fem}$	Transfer rate from juveniles to females	Per month	Variable
Equation	$Transfer_{mal}$	Transfer rate from juveniles to males	Per month	Variable
Forcing	Daylight	Monthly variation of the	Minutes	Variable/experimental

function		daylight duration		
Forcing function	Sal	Monthly variation of the salinity inside the estuary	Non-dimensional	Variable/experimental
Forcing function	Temp	Monthly variation of the water temperature inside the estuary	ºC	Variable/experimental
Parameter	Egg loss	Rate of eggs not hatched in nests	Per month	0.3/literature
Parameter	Fecundity	Mean number of eggs generated per mature female	Per month	2000/literature; calibration
Parameter	Larval dispersal and mortality	Rate of larvae that fail to recruit	Per month	0.020338/literature; calibration
Parameter	Mature females	Rate of reproductive females	Per month	0.13/experimental
Parameter	$\text{MigraRate}_{\text{fem}}$	Rate of migrating females	Per month	0.65/calibration
Parameter	$\text{MigraRate}_{\text{mal}}$	Rate of migrating females	Per month	0.65/calibration
Parameter	$\text{MortRate}_{\text{fem}}$	Mortality rate of females	Per month	0.15/calibration
Parameter	$\text{MortRate}_{\text{juv}}$	Mortality rate of males	Per month	0.175/calibration
Parameter	$\text{MortRate}_{\text{mal}}$	Mortality rate of juveniles	Per month	0.2/calibration
Parameter	S_{max}	Maximum salinity for *P. microps* growth	Non-dimensional	51/literature
Parameter	S_{min}	Minimum salinity for *P. microps* growth	Non-dimensional	0.3/literature
Parameter	S_{opt}	Optimum salinity for *P. microps* growth	Non-dimensional	10/literature; calibration
Parameter	T_{max}	Maximum temperature for *P. microps* growth	ºC	24/literature
Parameter	T_{min}	Minimum temperature for *P. microps* growth	ºC	-1/literature

Parameter	T_{opt}	Optimum temperature for *P. microps* growth	ºC	16/calibration
Parameter	TransfRate$_{fem}$	Rate of juveniles maturing into females	Individuals.100m^{-2}	0.6/experimental
Parameter	TransfRate$_{mal}$	Rate of juveniles maturing into males	Individuals.100m^{-2}	0.2/experimental
State variable	Female	Initial density of females	Individuals.100m^{-2}	23/experimental
State variable	Juvenile	Initial density of juveniles	Individuals.100m^{-2}	10/experimental
State variable	Male	Initial density of males	Individuals.100m^{-2}	13/experimental

Table 10.2 Equations used in the model of the population dynamics of the common goby *Pomatoschistus microps* in Minho estuary, NW Iberian Peninsula.

Juvenile density Juvenile(t) = Juvenile(t-∂t) + (Recruitment – Transfer$_{mal}$ – Transfer$_{fem}$ – Mort$_{juv}$) * ∂t *Inflows*: ○ Recruitment = IF(Temp>15)AND(Temp<20)THEN(Female * Mature females * Fecundity * Egg loss * Larval dispersal and mortality * F$_{temp}$*F$_{sal}$)ELSE(0) Outflows: ○ Transfer$_{mal}$ = Juvenile * TransfRate$_{mal}$ * F$_{temp}$ * F$_{sal}$ ○ Transfer$_{fem}$ = Juvenile * TransfRate$_{fem}$ * F$_{temp}$ * F$_{sal}$ ○ Mort$_{juv}$ = Juvenile * MortRate$_{juv}$ * F$_{temp}$ * F$_{sal}$	Variation in juvenile density per unit of time	(1)
Male density Male(t) = Male(t-∂t) + (Transfer$_{mal}$ – Mort$_{mal}$ – Migra$_{mal}$) * ∂t *Inflows*: ○ Transfer$_{mal}$ = Juvenile * TransfRate$_{mal}$ * F$_{temp}$ * F$_{sal}$ *Outflows*: ○ Mort$_{mal}$ = IF (Temp < 10) THEN (Male * MortRate$_{mal}$ * 1.3) ELSE (Male * MortRate$_{mal}$) ○ MigraMal = IF(Temp<10)OR(Daylight<575)THEN(Male*MigraRate_Mal)EL	Variation in male density per unit of time	(2)

SE(0) ○ $Migra_{mal}$ = IF (Temp < 10) OR (Daylight < 575) THEN (Male * $MigraRate_{mal}$) ELSE (0)		
Female density $Female(t) = Female(t-\partial t) + (Transfer_{fem} - Mort_{fem} - Migra_{fem}) * \partial t$ *Inflows*: ○ $Transfer_{fem}$ = Juvenile * $TransfRate_{fem}$ * F_{temp} * F_{sal} *Outflows*: ○ $Mort_{fem}$ = IF (Temp < 10) THEN (Female * $MortRate_{fem}$ * 1.3) ELSE (Female * $MortRate_{fem}$) ○ $Migra_{fem}$ = IF (Temp < 10) OR (Daylight < 575) THEN (Female * $MigraRate_{fem}$) ELSE (0)	Variation in female density per unit of time	(3)
Temperature F_{temp} = IF (Temperature ≤ T_{opt}) THEN (EXP(–2.3 * ((Temperature – T_{opt}) / $(T_{min}-T_{opt}))^2$)) ELSE (EXP(–2.3 * ((Temperature – T_{opt}) / $(T_{max} - T_{opt}))^2$))	Equation for the limit factor of temperature	(4)
Salinity F_{sal} = IF (Salinity ≤ S_{opt}) THEN (EXP(–2.3 * ((Salinity – S_{opt}) / $(S_{min} - S_{opt}))^2$)) ELSE (EXP(–2.3 * ((Salinity – S_{Opt}) / $(S_{max} - S_{opt}))^2$))	Equation for the limit factor of temperature	(5)

10.4.2. Mortality

One of the most important shortcomings in the knowledge of estuarine fishes is the lack of estimates of the source of mortality for any life history stage (Houde 2008). Even where mortality estimates have been made for estuarine species, the influence of confounding factors (i.e. gear avoidance, inaccessible habitats, etc.) makes it difficult to determine mortality rate (Able and Fahay 2010). For the common goby, as far as we know, there is any published paper addressing the mortality rate of the species in nature, therefore the mortality rate used in the model was obtained by calibration.

Notwithstanding, mortality rate may vary throughout the year in temperate estuarine fishes (Able and Fahay 2010). In fact, during winter, small and relatively immobile fish experience an increase in their mortality rates, due net energy deficits caused by low temperatures and food scarcity (Sogard 1997; Hurst et al. 2000; Hales and Able 2001; Hurst 2007), which leads to an increase in mortality of about 33% during winter in estuarine fishes (Able and Fahay 2010). The seasonal variation in *P. microps* mortality was also taken into account in the model, with an increment of 30% in mortality rate of juveniles, females and males when the water temperature was lower than 10ºC.

The number of *P. microps* individuals subtracted to each population group was defined by:

$$(6) \qquad Mortality_i = MortRate_i \times D_i$$

Where Mortality$_i$ = mortality of the population group *i*; MortRate$_i$ = mortality rate of the population group *i*; D$_i$ = density of the population group *i*.

The parameters values and the equations of each population groups are presented in Tables 10.1 and 10.2, respectively.

10.4.3. Migration

The typical life cycle of the common goby lasts for one year, with adults migrating to warmer waters during winter (Jones and Miller 1966; Muus 1967). Given that the common goby presents a dynamic and plastic behavior in several of its life traits (e.g. Reynolds and Jones 1999; Pampoulie et al. 2000 Heubel et al. 2008), one should expect that the temperature level which triggers seasonal migration in estuarine populations should also be different across the geographical range of the species (Jones and Miller 1966). For instance, Jones and Miller (1966) reported that migration is triggered when temperature is lower than 7ºC, while Claridge et al. (1985) mentioned that at 5ºC migration is triggered. For other estuarine overwintering migrating species in nearby systems, it is argued that 10ºC is responsible for triggering seasonal migration (Gomes 1991). Given the scarcity of information regarding the temperature level that trigger overwintering migration of common gobies in Southern European estuaries, we assumed that temperatures lower than 10ºC are capable to induce *P. microps* migration in Minho estuary.

Migration can be triggered by a number of environmental cues, such as the temperature, precipitation, drought, water discharge and photoperiod (Bauer et al. 2011). In a recent study, McNamara et al. (2011) ascertained that the photoperiod is probably the most prominent and universal variable, entraining organisms to time of the year. Also, photoperiod is a reliable indicator of time of the year and thus can be a useful predictor for the phenology of resources (Bauer et al. 2011). In this context, the photoperiod was taken into account in the migration equation. The number of migrating *P. microps* individuals in each population group was defined by:

(7) $$Migra_i = MigraRate_i \times D_i$$

Where $Migra_i$ = migration of the population group *i*; $MigraRate_i$ = migration rate of the population group *i*; D_i = density of the population group *i*.

The migration rates of each population group were obtained by calibration due to the absence of this information on the literature. The values of the migration rate and the equations for females and males migration are shown in Tables 10.1 and 10.2, respectively.

10.4.4. Effect of temperature on growth

Since *P. microps* is not able to control its body temperature to a significant degree, the typical response is that its metabolic rate varies directly with ambient temperature (von Oertzen 1983). The common goby presents a relatively wide tolerance range for temperature variation, being able to withstand temperatures ranging from -1ºC to 24ºC (Fonds 1973; Moreira et al. 1992). Freitas et al. (2010) assumed that the optimal temperature for *P. microps* growth is 20ºC, but since this value could not be validated due to the lack of experimental data, the value used in the model was obtained by calibration, using the optimal temperature reported by Freitas et al. (2010) as a proxy. The effect of temperature on *P. microps* growth was described as an optimum-type curve (Martins et al. 2008), which can be described by:

(8) $$f(i) = e^{-2.3\left(\frac{(i-i_{opt})}{(i_{min}-i_{opt})}\right)^2} \text{ for } i \leq i_{opt}$$

and

(9) $$f(i) = e^{-2.3\left(\frac{(i-i_{opt})}{(i_{max}-i_{opt})}\right)^2} \text{ for } i > i_{opt}$$

Where i = temperature; i_{opt} = optimum temperature for growth; i_{min} = minimum temperature for growth; i_{max} = maximum temperature for growth.

10.4.5. Effect of salinity on growth

Salinity is one of the most important environmental factors affecting the growth and survival of aquatic organisms since it can influence both physiological and ecological processes (Poizat et al. 2004; Nordlie 2006). Moreover, many authors have demonstrated the influence of external salinity on growth capacities in fish (see Boeuf and Payan 2001). The metabolic rate of *P. microps* varies directly with salinity (Rigal et al. 2008), and the species has a relatively wide tolerance range for salinity variation, being able to withstand salinities ranging from 0 to 51 (Rigal et al. 2008), with better physiological performances recorded at low salinities (Pampoulie et al. 2000; Rigal et al. 2008). To cope with this, the model uses an optimum curve to describe the effect of salinity on *P. microps* growth. This optimum curve is mathematically identical to the one used for describing the effect of temperature on *P. microps* development and growth (see equations 1 and 2).

The optimum salinity value for the species (SOpt) was obtained in the literature and followed by calibration (see Table 10.1)

10.4.6. Sensitivity analysis

Sensitivity analysis was calculated for variations of ± 10% on each parameter:

$$(10) \qquad Y'_{X_i} = \frac{\partial Y}{\partial X_i}$$

Where *Y'* is the sensitivity of the model outputs to variations on parameters (X_i). The variation of each parameter and, the resulting response of the model, were calculated with all other parameters fixed according to OAT (on-at-a-time) approach (see Table 10.3).

10.4.7. IPCC predictions

The IPPC (Intergovernmental Panel on Climate Change) Fourth Assessment Report (AR4) predicted that, the surface air warming in the 21st century will range from 1.1 to 6.4ºC (IPCC 2007). Also, the annual temperature over Europe will warm at a rate of 0.1 and 0.4 Cº per decade, and warming will be greatest over southern Europe and northeast Europe (Christensen et al. 2007).

The IPCC (2007) also predicted that the annual precipitation will decrease across southern Europe (maximum 1% per decade), producing drier summers and wetter winters (Christensen et al. 2007). These changes will probably cause changes on the salinity levels of estuarine systems, since it is most likely that droughts and floods events will be more frequently triggered in these systems, as recently reported (e.g. Cardoso et al. 2008; Dolbeth et al. 2010; Santos et al. 2010; Ilarri et al. 2011).

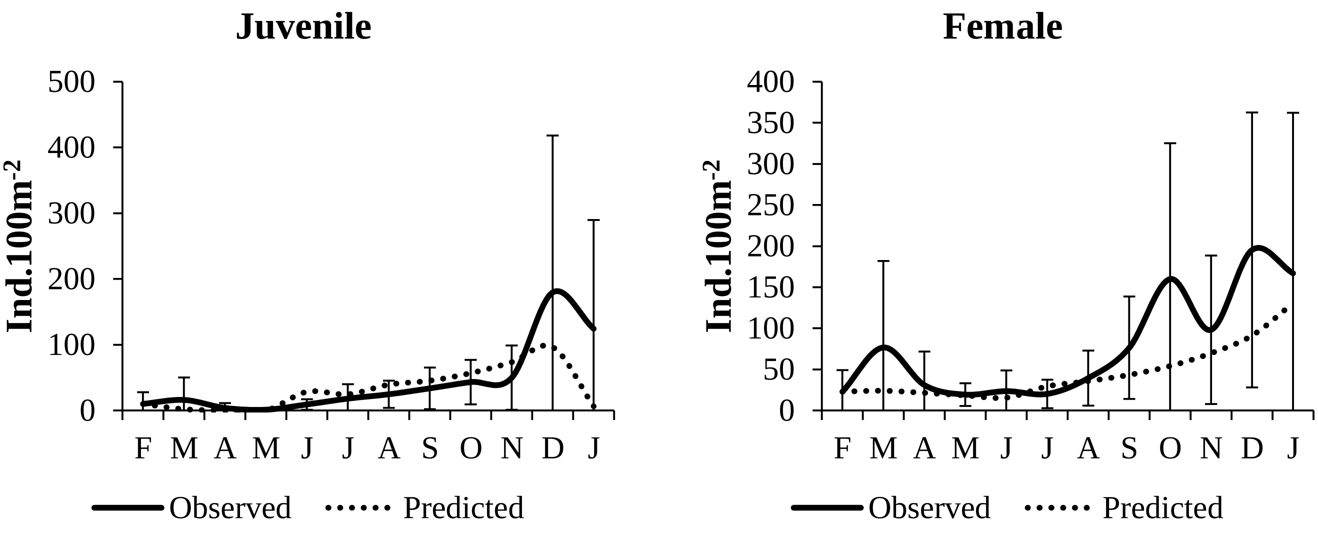
In this context, several scenarios of temperature and salinity variations in Minho estuary were simulated. Six levels of water temperature increasing (+1, +2, +3, +4, +5 and +6ºC), three different scenarios of salinity variation (-5 psu, +5 psu and oscilattory (-5 psu from November to April and +5 psu from May to October)) were simulated. Additionally, we have simulated the combined effects of temperature and salinity variations.

Finally, we performed long run simulations (20 years) to assess the long-term effects of climatic change on the common goby population in Minho estuary. Two different rates of temperature increase were simulated: slow (+0.01ºC per year) and rapid (+0.04ºC per year) accompanied by different scenarios of salinity (normal, -5 psu, +5 psu and oscillatory).

10.5. Results

10.5.1. Model results

The predicted density of *P. microps* juveniles followed the same pattern of the observed variation, with a marked peak of abundance being recorded in December (Fig. 10.5). The predicted density of females, males and total population also followed similar patterns to the observed data, with density continuously increasing after spring and reaching a peak in December or January (Fig. 10.5).



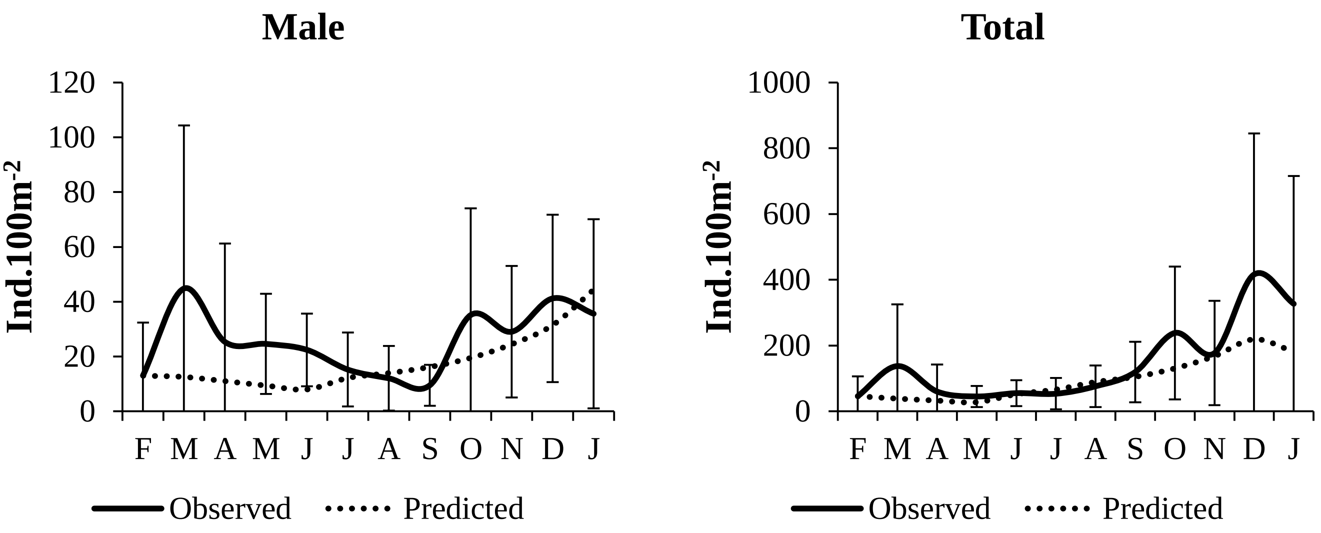


Fig. 10.5 Observed ± SD and predicted variation of juveniles, females, males and the total density (ind.100m^{-2}) of the common goby *Pomatoschistus microps*.

10.5.2. Model stability and sensitivity analysis

The model showed long-term stability (48 months) (Fig. 10.6), which supports the internal logic of the model (Jørgensen 1994).

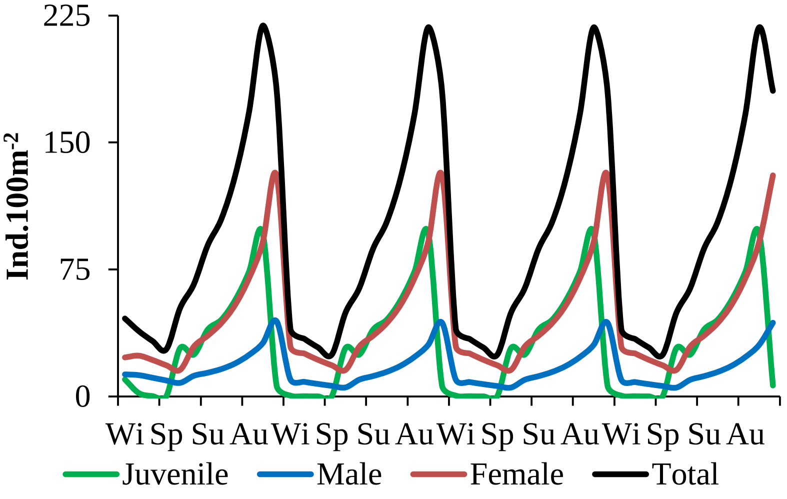


Fig. 10.6 Long term simulation for the common goby *P. microps* population in Minho estuary (NW Iberian Peninsula), showing longtime stability of the model, with every population group presenting consistent stability through time.

The sensitivity analysis showed that the parameters related to reproduction (egg loss; fecundity; larval dispersal and mortality; mature females) were the most sensitive ones (Table 10.3).

Table 10.3 Sensitivity (*sensu* Jørgensen 1994) of the population density to ± 10% variations of the parameters used on the population dynamics model of the common goby *Pomatoschistus microps* in Minho estuary, NW Iberian Peninsula.

Parameter	-10%	Base	+10%	Sensitivity
Egg loss	0.27	0.30	0.33	1.33
Fecundity	1800	2000	2200	1.33
Larval dispersal and mortality	0.0183042	0.020338	0.0223718	1.33
Mature females	0.117	0.13	0.143	1.33
$MigraRate_{fem}$	0.585	0.65	0.715	0.00
$MigraRate_{mal}$	0.585	0.65	0.715	0.00
$MortRate_{fem}$	0.135	0.15	0.165	-0.76
$MortRate_{juv}$	0.18	0.20	0.22	-0.27
$MortRate_{mal}$	0.135	0.15	0.165	-0.07
S_{max}	45.9	51	56.1	1.03
S_{min}	0.27	0.30	0.33	0.00
S_{opt}	9	10	11	0.34
T_{max}	21.6	24	26.4	0.15
T_{min}	-0.9	-1	-1.1	0.00
T_{opt}	14.4	16	17.6	0.48
$TransfRate_{fem}$	0.54	0.60	0.66	0.48
$TransfRate_{mal}$	0.18	0.20	0.22	-0.08

10.5.3. Climatic change simulations

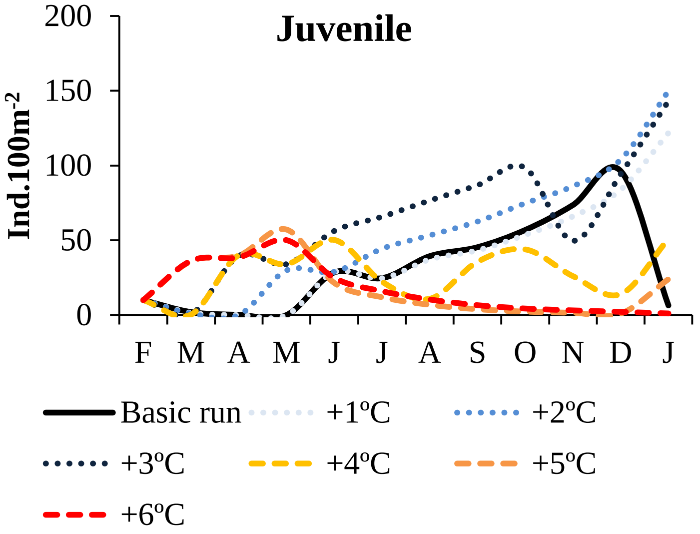
Once the correlation between the model outputs and real data was shown to be satisfactory (Table 10.4), the model was considered suitable to simulate the effects of the upcoming climatic changes on the common goby population during a year cycle (12 months).

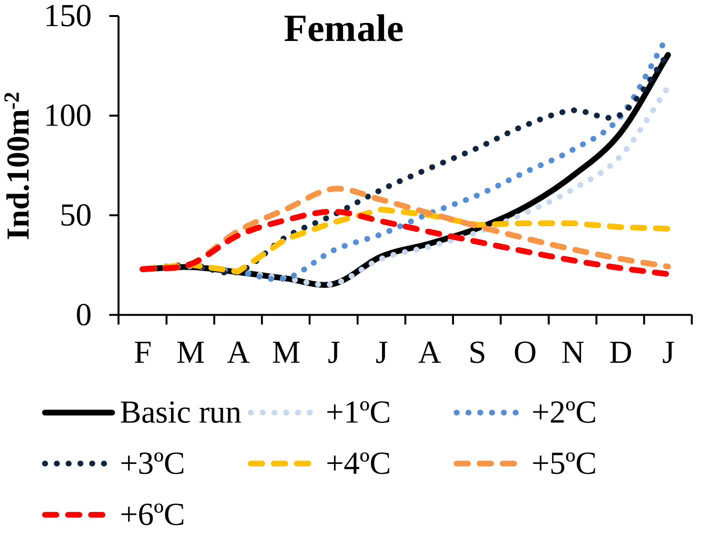
Table 10.4 Linear regressions between the observed and the predicted values for the common goby *P. microps* density inside Minho estuary, NW Iberian Peninsula.

Group	SS	MS	F	P
Juvenile	11435	11435	5.39	0.04*
Female	32642	32642	25.89	0.000*
Male	359.2	359.2	3.06	0.111^{ns}
Total	134817	134817	44.57	0.000*

10.5.3.1. Temperature variations

Simulations suggest that *P. microps* population will be greatly affected by water temperature warming in. For the low IPCC scenarios (+1ºC, +2ºC and +3ºC), the overall density of *P. microps* would increase (3%, 35% and 61% respectively) in one year simulation, while for the high IPCC scenarios (+4ºC, +5ºC and 6ºC) the common goby population would decline on a year basis (12%, 23% and 32% respectively) (Fig. 7). Also, the timing of the density peaks change for the increasing water temperature scenarios, with the juveniles recruiting earlier in the year in all scenarios except in +1ºC. In high IPCC scenarios, the density peak of juveniles will change from December to May, while females and males would peak in June instead of January (Fig. 10.7).





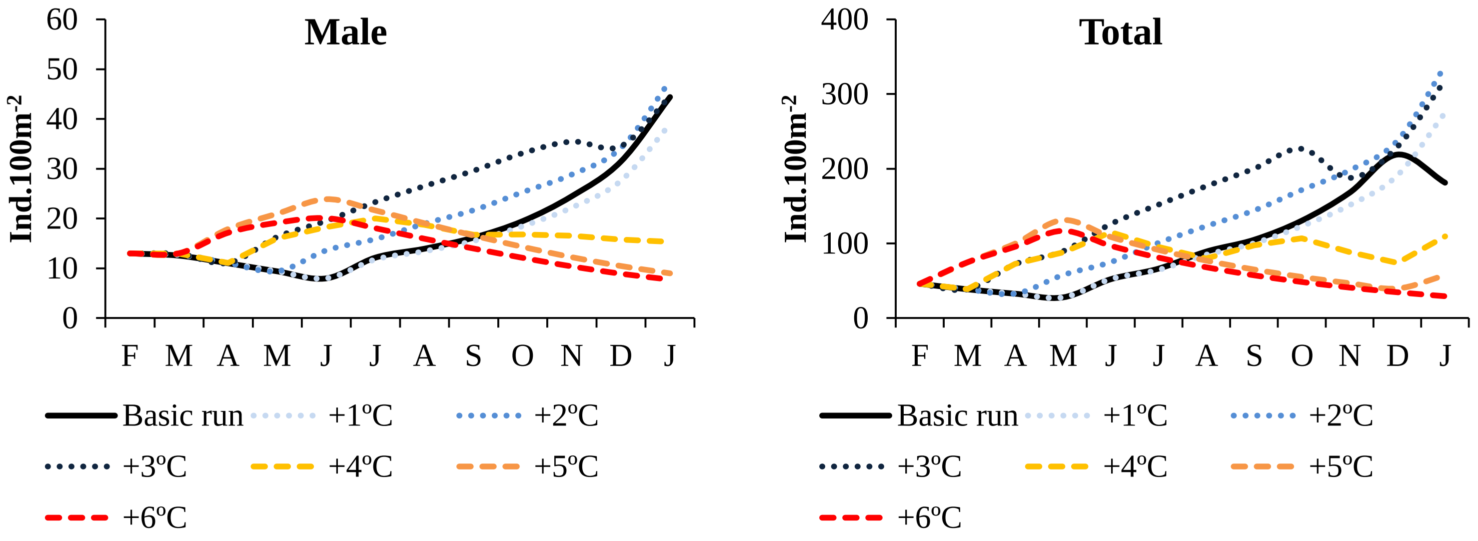
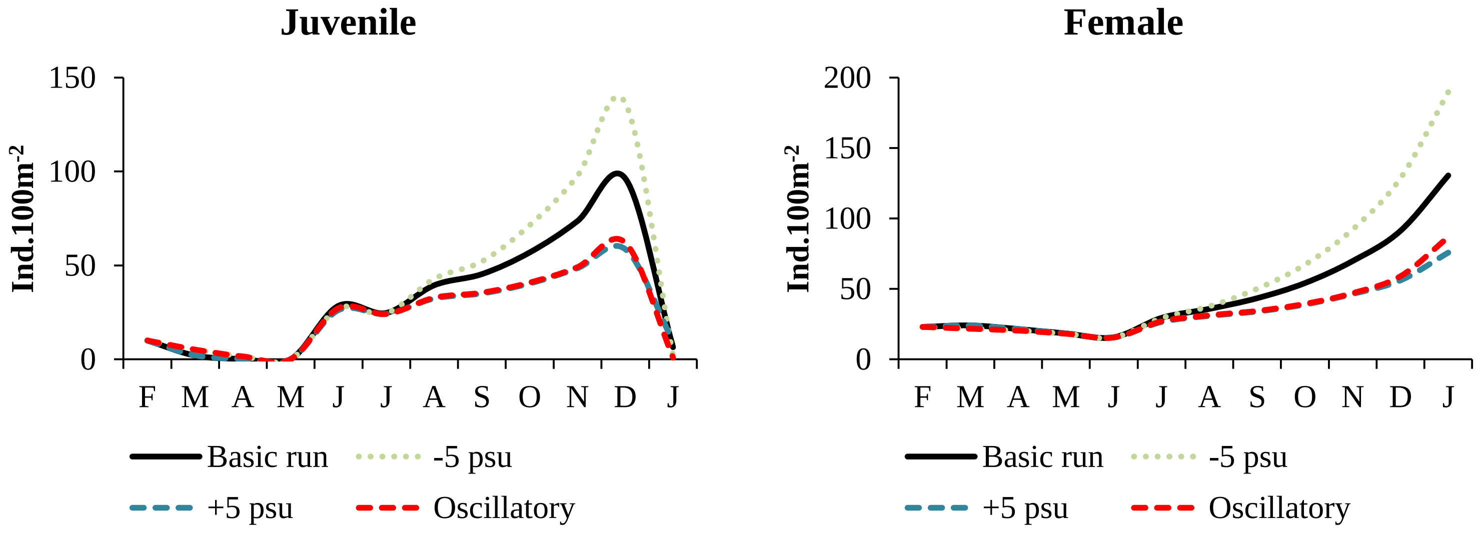


Fig. 10.7 One year simulations of juveniles, females, males and total density (ind.100m^{-2}) of the common goby *Pomatoschistus microps* in different scenarios of temperature increase according to the IPCC projections for the future. Dotted lines refer to low scenarios of climate change and dashed lines refer to high scenarios of climate change.

10.5.3.2. Salinity variations

Simulations with salinity variations suggest that *P. microps* population would be benefited by a decrease in salinity (23% increase of the total density in one year), while an increase or an oscillatory pattern in salinity would cause a decrease in *P. microps* population (25% and 24% respectively) (Fig. 10.8).



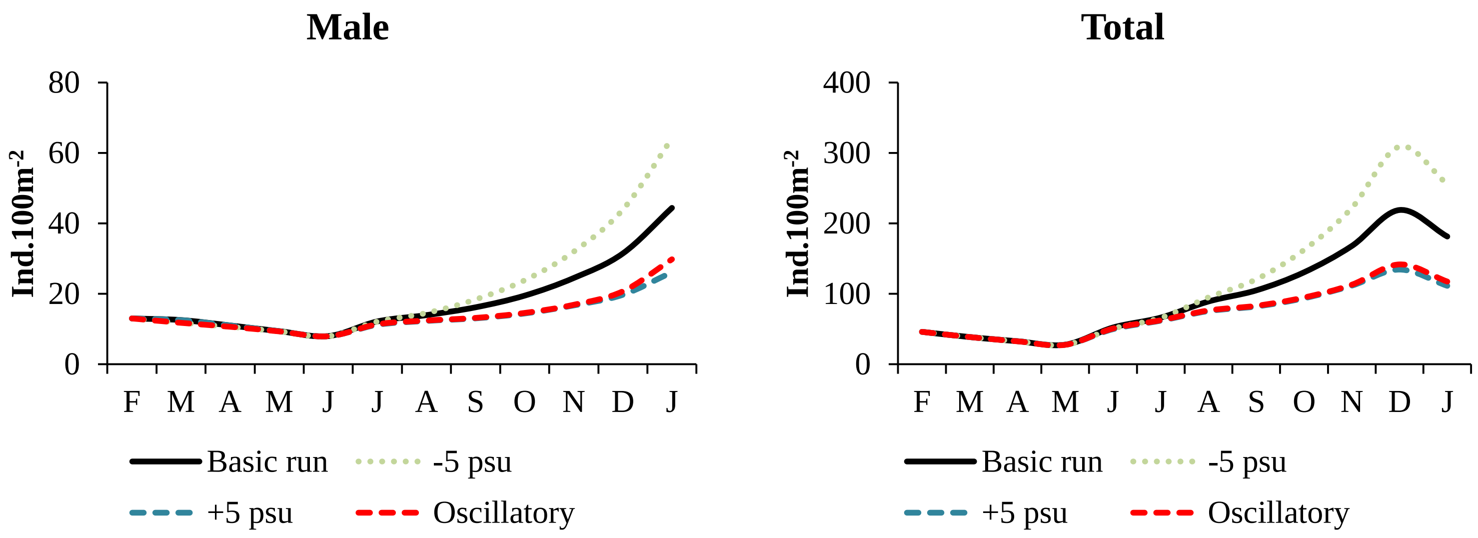
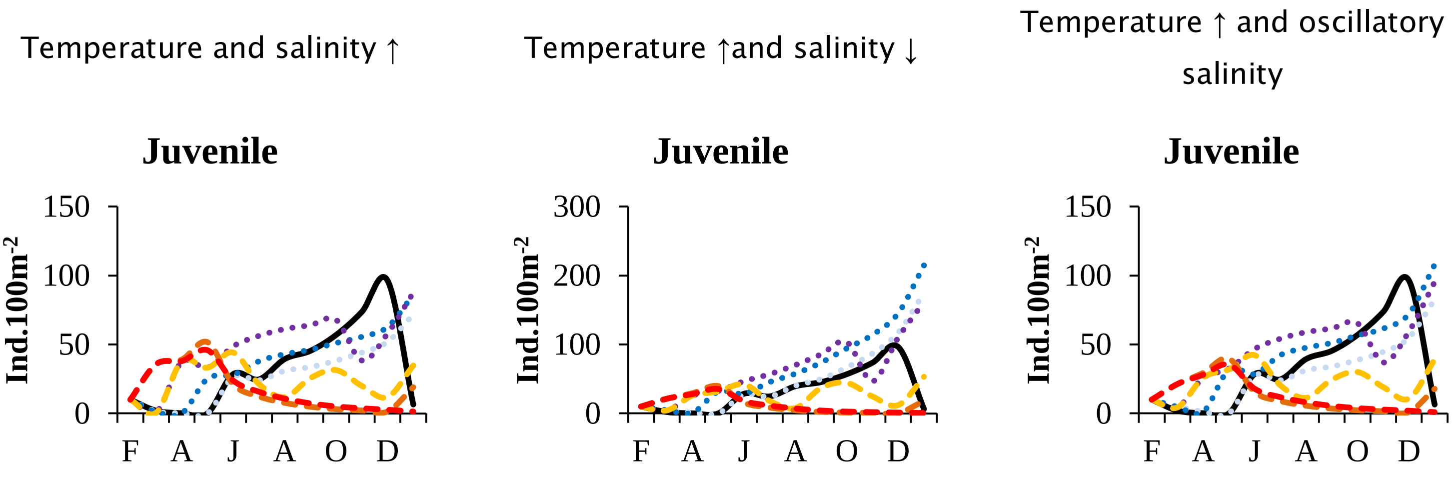


Fig. 10.8 One year simulations of juveniles, females, males and total density of the common goby *Pomatoschistus microps* in different scenarios of salinity decreasing (-5 psu) and increasing (+5 psu) and oscillatory pattern (-5 psu from November to April, and +5 psu from May to October).

10.5.3.3. Combined effects of temperature and salinity variations

Overall, the combined effects of temperature and salinity increase would lead to a decrease in *P. microps* population in all IPPC scenarios. Similarly, when salinity pattern is oscillatory, the population would respond in the same way, but with slighter less marked decrease in *P. microps* density in all IPCC scenarios. Conversely, a temperature increase combined with a salinity decrease would cause an accentuated increase in population levels of *P. microps* in low IPCC scenarios (ranging from 28% to 68%) (Fig. 10.9).



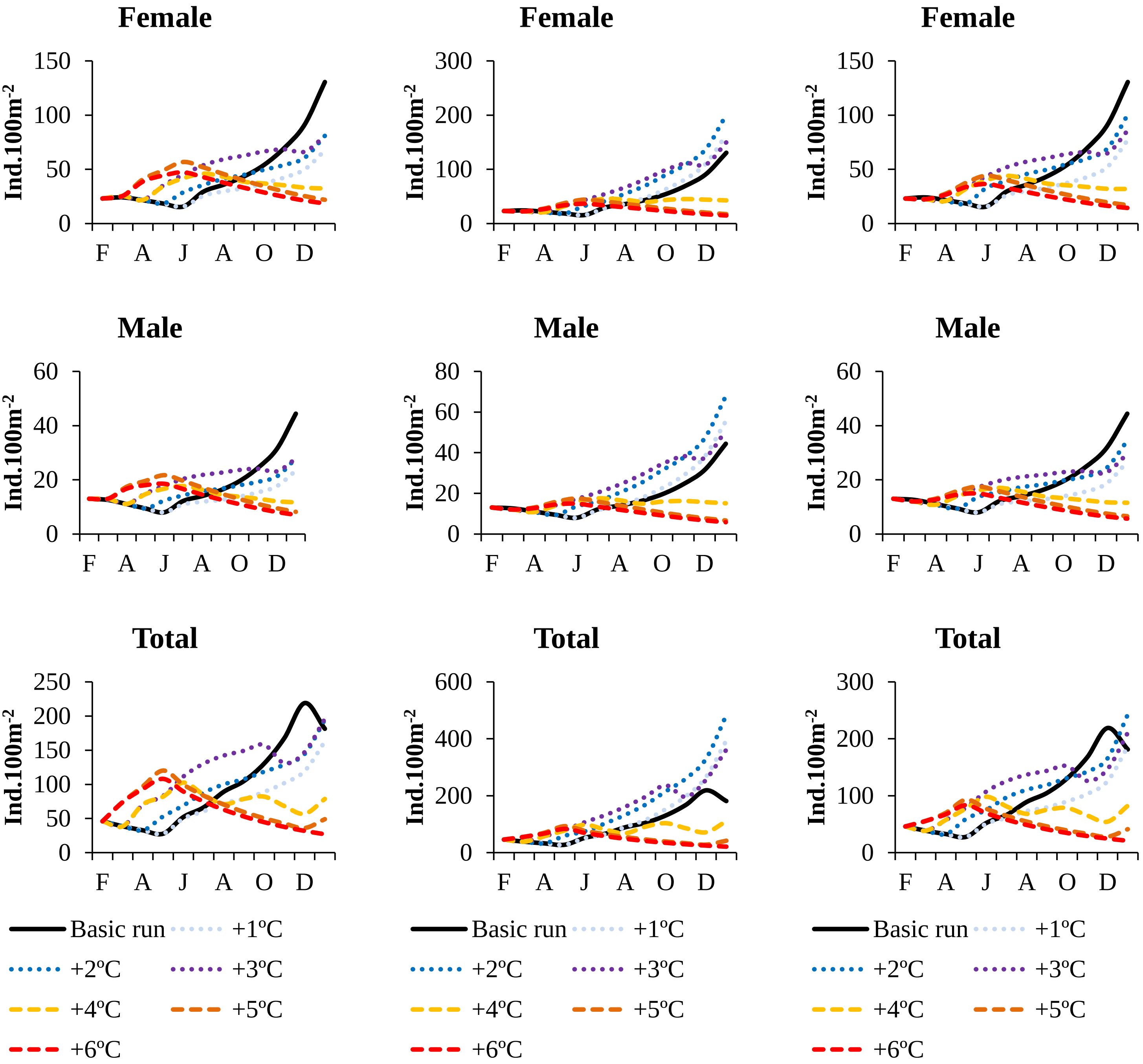


Fig. 10.9 One year simulations of juveniles, females, males and the total density of the common goby *Pomatoschistus microps* in different climatic change scenarios of temperature and salinity increase (left column), temperature increase and salinity decrease (middle column) and temperature increase and oscillatory salinity (-5 psu from November to April, and +5 psu from May to October).

10.5.3.4. Long-term effects

Long-term effects of temperature increasing would have significant consequences for *P. microps* population in Minho estuary, with a continuous decreasing in population density being predicted for the next 20 years both in low and high IPCC scenarios (Fig. 10.10).

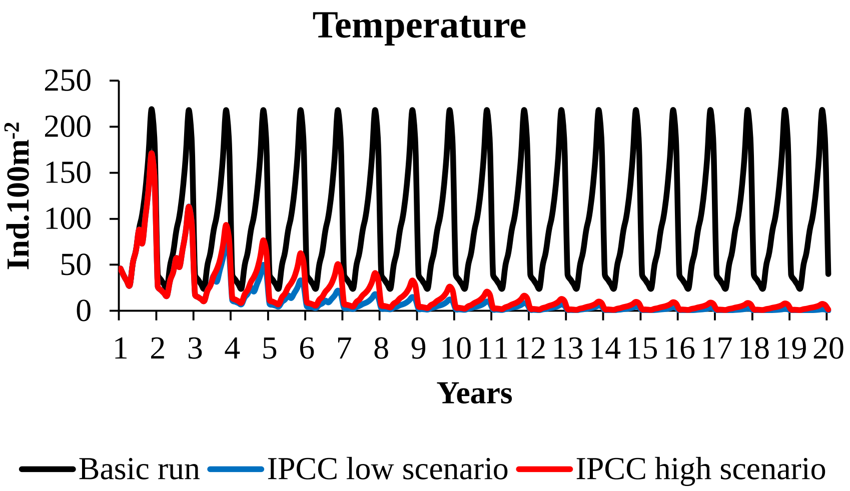


Fig. 10.10 Long-term effects of temperature increasing on *P. microps* population in Minho estuary, NW Iberian Peninsula. IPPC low scenario = +0.01°C.y^{-1} and IPPC high scenario = +0.04°C.y^{-1}.

Similarly an oscillatory pattern of salinity or a salinity increase would lead to a marked decrease in *P. microps* population, while a salinity decrease would have the opposite trend (Fig. 10.11).

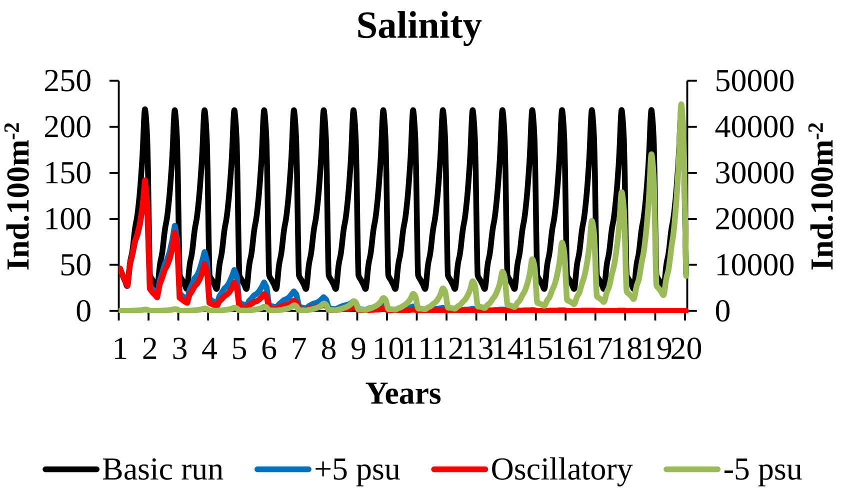


Fig. 10.11 Long-term effects of salinity variation on *P. microps* population in Minho estuary, NW Iberian Peninsula. Oscillatory = -5 psu from November to April, and +5 psu from May to October. The -5 psu scenario is plotted on the secondary vertical axis.

The combined effects of temperature increase and salinity variation would cause an even faster decrease in *P. microps* population levels in all scenarios with an oscillatory salinity pattern and with salinity increase. Whereas with a salinity decrease, the common goby population would initially decrease, recovering after 20 years on the low IPCC scenario. Conversely, on the high IPCC scenario, the population would immediately increase, reaching density values 5 times higher compared with the present scenario (no increase in temperature and salinity) (Fig. 10.12).

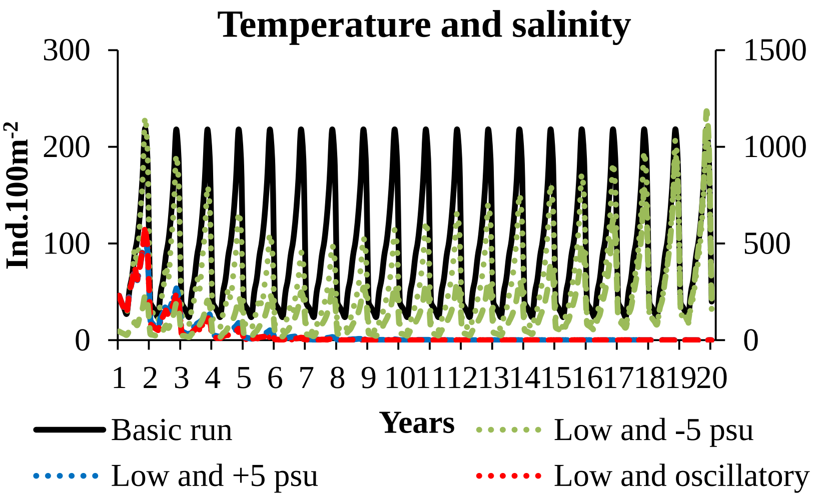


Fig. 10.12 Long-term effects of temperature increase combined with salinity variation on *P. microps* population in Minho estuary, NW Iberian Peninsula. Oscillatory = -5 psu from November to April, and +5 psu from May to October. Low scenario = +0.01°C.y^{-1} and High scenario = +0.04°C.y^{-1}. The High and -5 psu scenario is plotted on the secondary vertical axis.

10.6. Discussion and conclusions

The model was capable of satisfactory predicting the variation of *P. microps* density within the Minho estuary. Long-term simulations indicate that *P. microps* population will be highly sensitive to changes in both temperature and salinity. According to predictions, rises in water temperature will cause long-term detrimental effects on *P. microps* population, with the high IPCC scenarios affecting the species more severely than low IPCC scenarios.

Predictions also indicated that the spawning season will be modified by increasing water temperature. In low IPPC scenarios, the changes in spawning season would cause an extension in the recruitment season, with common gobies spawning earlier in the year. However, in high IPCC scenarios, the spawning season would be greatly altered, with juveniles starting to recruit in winter but with a marked shortage in the duration of the recruitment season. The duration of spawning seasons have a major effect on *P. microps* populations (Bouchereau and Guelorget 1998), and it may be one of the reasons behind the high density of the species in Minho estuary, once in this system, the reproduction season appear to be longer than in other estuaries (Souza et al. in revision).

Freitas et al (2010) assumed that the optimal temperature for *P. microps* growth is 20ºC. Nevertheless, previous empirical observations (Dolbeth et al. 2010) and the results from the present model indicate that the species is more abundant and productive at lower temperatures. However, unless specific experimental studies are conducted to determine the optimum temperature value for the growth of *P. microps*, all other values are assumed and may be subject to revision. Hence, further studies are needed to obtain the correct value of this parameter.

The common goby population also responds negatively towards salinity increases, indicating that droughts may cause a reduction in *P. microps* population, which agrees with the results reported by Dolbeth et al. (2010), who noted a decrease in *P. microps* production after drought events in Minho estuary. On the other hand, model outputs suggest that the common goby population will be largely benefited by flood events, due to the decrease in salinity within the estuary. This result agrees with those reported by Pampoulie et al. (2000), who described an increased reproductive investment by *P. microps* after a high freshwater inflow in a coastal lagoon. Also, the common goby seemed to be further benefited by the reduction of competitors such as, the sand goby *P. minutus*, within the lagoon (Pampoulie et al. 2000). Notwithstanding, the model showed that the effect of a reduced salinity is voided when accompanied by an increased salinity during summer, suggesting that *P. microps* populations would decrease in the next years, if the IPCC predictions of wetter winters and drier summers (see Christensen et al. 2007) proves correct.

Also, it is important to refer that the subsequent effects of climatic extremes may have opposite trends. For instance, resulting from a massive die-off of bivalves caused after droughts (Ilarri et al. 2011) and floods (Sousa et al. 2012b), may lead to a significant increase on the quantity of empty shells that might be used for *P. microps* reproduction in the next breeding season, which may cause an increase in the population density after one generation. Actually, the reproduction of common gobies seems to be limited by the presence of nest substrates (Nyman 1953; Magnhagen 1998) and their abundance and availability can directly influence the amount of breeding males (Breitburg 1987; Lindström 1988).

The model was able to predict correctly the dynamics of juveniles and females, whilst the predicted male dynamics differed more from real data. This may be related to the nest guarding behavior of males that makes them difficult to be caught within estuaries (Miller 1984), which also is one of the reasons for the dominance of females in estuarine populations of *Pomatoschistus* spp. (e.g. Bouchereau et al. 1993; Fouda et al. 1993; Koutrakis and Tsikliras 2009). In fact, most of the dissimilarities between the observed and the predicted density of males occurred during the breeding season, suggesting that guarding behavior may have influenced the results indeed.

The model was most sensitive to variations in the parameters related to *P. microps* reproduction. This result was somehow expected given that the common gobies present high plasticity on their reproductive traits (e.g. Reynolds and Jones 1999; Pampoulie et al. 2000 Heubel et al. 2008), suggesting that the species can respond to environmental constrains and rapidly adapt to new environmental conditions.

Despite of the IPCC predictions referring to temperature increase in the air, it should be expected that the water temperature will also increase due to global changes in climate (IPCC 2008). Nevertheless, it is unlikely that water temperature will increase at the same ratio as the air's, given the differences on the thermal properties between the two fluids; and hence, temperature increase in water probably would be slighter than in air. There are uncertainties in projected changes in hydrological systems since it often depends on a number of variables such as precipitation, evapotranspiration, soil moisture and runoff (IPCC 2008). In this context, we opted to use the IPCC projections for air temperature increase despite of knowing that the temperature increase in water would be slighter. Notwithstanding, it is unlikely that the water temperature would increase as much as the most extreme high IPCC scenarios, therefore, the projections on the *P. microps* population dynamics at +5ºC and +6ºC should be carefully evaluated.

The model predicted that, in any scenario of temperature increase, the *P. microps* population would experience a gradual decrease in long-term simulations. Also, the most likely scenario of salinity change (oscillatory pattern) would lead to a sharp decrease in *P. microps* density. In this context, it is likely that common gobies experience population shrinkage, and given the trophic position and abundance of the species, this could cause important consequences for estuarine biological communities, especially in a system where the species is remarkably abundant such as in Minho estuary (Souza et al. in revision). Considered a mesopredator, the common goby connects low and high levels of the food-web fishes (Doornbos 1984; Moreira et al. 1992; Cabral 2000); therefore, changes in *P. microps* population would affect both higher and lower trophic levels. Nevertheless, the real ecological impact of the *P. microps* population reduction is hard to predict, since the sympatric species *P. minutus* may play a similar ecological role (Salgado et al. 2004). Actually, both species are morphologically and ecologically similar, differing mostly on salinity preferences, with *P. minutus* preferring to inhabit saltier waters compared to *P. microps* (Leitão et al. 2006; Dolbeth et al. 2007b). Also, both species can often compete for food and space (Złoch and Sapota 2010) and hence, it is reasonable to assume that *P. minutus* may perhaps be benefited by a decrease in *P. microps* population, and fulfill the ecological gaps left by the common goby. Notwithstanding, given the uncertainty about the ecological cascade of effects that a decrease in P. microps density would trigger, it would be interesting to perform further studies on the *P. microps* and *P. minutus* interaction at different conditions of temperature, salinity and density.

Given that the *P. microps* geographical range of occurrence is wide, and our study was conducted in a system located nearer to the southern than the northern edge of the species distribution (Froese and Pauly 2010), the populations inhabiting systems submitted to colder temperatures could experience a milder effects of climate change, while populations located further south may suffer more serious consequences. Still, given the plasticity of *P. microps*, each population may respond differently to environmental changes, and hence, each system should be treated as a unique case of study, despite of the trend presented in this study of a decrease in density caused by warming waters.

Finally, our study presents a forecasting approach on how a core species will cope with climatic change in the near future. This type of approach represents a useful tool for future planning and management of estuarine systems, once results predict how *P. microps* will vary with global effects of climate change.

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CHAPTER 11

CONCLUSIONS AND FUTURE DIRECTIONS

Conclusions and future directions

Despite the noticeable increase on the knowledge about the ecology of species inhabiting the Minho estuary during the last decade, there was still a gap of knowledge related to the population dynamics of core species from this estuary, which was somehow fulfilled with the present work.

The present results have contributed to increase the understanding of the Minho estuary function, as well as, they have contributed to define and identify research issues that may help to achieve a further development and knowledge on the ecology of the Minho estuary.

The present thesis reported, for the first time, the population dynamics of four of the most abundant and widely distributed species of secondary producers in the Minho estuary and in several other European estuaries. The obtained results highlighted the fact that the Minho estuary is an important nursery area for the shore crab *Carcinus maenas* and the for the European flounder *Platichthys flesus*, with populations being mainly constituted by juveniles and reaching density values significantly higher than other nearby estuarine systems. Moreover, in the Minho estuary, the common *Pomatoschistus microps* and the sand goby *P. minutus* reached densities and production values remarkably higher than at other estuaries across Europe. Thus, it seems safe to ascertain that the Minho estuary is a relevant and important nursery area for estuarine core species. The present results have also highlighted that, within the Minho estuary, the downwards areas, closer to the estuarine mouth, hold a substantially higher density of estuarine crustaceans compared to estuarine fishes, while in areas with marked variations in salinity throughout the year, estuarine fishes seemed to be favored over estuarine crustaceans. We have also shown that, the shore crab presents significant differences on the carapace structure between nearby areas of the estuary, which is potentially related with differences in the composition of communities and function of different sections within the estuary.

The present results corroborate the idea that, the abiotic characteristics of Minho estuary are essential to determine the high production of this estuarine system. Namely, the high river run-off and freshwater input seem to be important for the remarkable high density of common gobies, and probably, it is also the reason behind the high density of juvenile flounders in Minho estuary. Notwithstanding, other factors also contribute to the high density and production of core species in the system, such as, habitat preservation and low to moderate anthropogenic pressures (e.g. relatively low domestic sewage and agricultural runoffs), although the presence of the invasive clam *Corbicula fluminea* may be changing the ecosystem structure due to its bioengineering abilities. In this context, further studies related to the role of *C. fluminea* on the epibenthic community should be conducted; it would also be interesting to investigate the role of the *C. fluminea* shells on the *P. microps* and *P. minutus* reproduction.

Moreover, the present thesis presents a forecasting approach on how core species will cope with climatic changes. This type of approach represents a useful tool for future planning and management of estuarine systems because results aim to predict how species will vary with global effects of climate change.

Simulations indicated that the population of *C. maenas* will increase and the population of *P. microps* will decrease if the IPCC predictions prove correct. Since both species are important constituents of estuarine communities, one should expect significant changes in the ecosystem functioning and structure in the upcoming years. Therefore, both populations should be continuously monitored in order to detect changes in population levels, as well as their potential consequences for the ecosystem, especially related to biodiversity and production.

Since most of the estuarine species spend, at least one stage of their development in the sea, studies integrating both coastal and estuarine waters should be performed, in order to better understand the population dynamics of the species inhabiting the estuary. Special attention should be given to the planktonic phase of fishes and crustaceans and the migration patterns of adult individuals, since this information is important to improve our knowledge about the phenology of estuarine species.

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Appendix

Supplementary material 3.1 Values of the five environmental variables (mean ± SE) measured in the three sampling stations throughout the seasons in the lower Minho estuary, NW Iberian Peninsula.

	Station 1	Station 2	Station 3
Winter			
Depth	4.2 ± 0.3	1.9 ± 0.2	2.7 ± 0.4
ORP	180.8 ± 37.6	189.1 ± 45.6	201.2 ± 52.2
pH	7.8 ± 0.2	7.8 ± 0.2	7.7 ± 0.1
Salinity	25.8 ± 6.5	12.8 ± 7.8	0.4 ± 0.2
Temperature	11.9 ± 0.9	11.69 ± 0.76	9.6 ± 0.8
Spring			
Depth	3.1 ± 0.3	1.3 ± 0.2	1.9 ± 0.3
ORP	156.1 ± 28.9	153.6 ± 32.0	159.9 ± 31.5
pH	7.8 ± 0.3	8.0 ± 0.2	8.1 ± 0.2
Salinity	26.0 ± 2.9	15.9 ± 6.2	0.4 ± 0.2
Temperature	14.6 ± 0.5	16.4 ± 0.9	16.4 ± 1.2
Summer			
Depth	3.7 ± 0.2	1.8 ± 0.1	2.8 ± 0.3
ORP	144.4 ± 36.3	159.5 ± 41.5	167.2 ± 43.7
pH	8.5 ± 0.3	8.81 ± 0.32	8.9 ± 0.3
Salinity	32.0 ± 1.0	30.8 ± 1.9	19.1 ± 4.2
Temperature	15.0 ± 0.6	15.9 ± 0.5	18.3 ± 1.2
Autumn			
Depth	4.3 ± 0.1	1.8 ± 0.1	2.1 ± 0.1
ORP	169.2 ± 27.0	173.4 ± 19.0	170.6 ± 26.7
pH	8.0 ± 0.2	8.1 ± 0.3	8.1 ± 0.2
Salinity	31.8 ± 1.9	23.0 ± 6.9	4.3 ± 4.2
Temperature	15.8 ± 0.4	15.1 ± 1.5	14.7 ± 1.8

Supplementary material 3.2 Two-way SIMPER results of the shore crab *Carcinus maenas* size classes' abundance in the lower Minho estuary, NW Iberian Peninsula throughout 18-months study period (February/09 to July/10).

	Dissimilarity (%)

	Size class						
	A	B	C	D	E	F	G
Stations							
S1 x S2	18.5	7.4	34.8	26.6	9.5	2.7	0.5
S1 x S3	30.8	6.0	6.1	29.6	21.3	5.2	1.0
S2 x S3	13.9	10.8	41.5	27.6	5.4	0.7	0.1
Seasons							
Winter x Spring	25.1	8.7	24.5	26.2	11.6	3.1	0.8
Winter x Summer	31.0	9.0	15.3	25.5	15.9	2.9	0.4
Winter x Autumn	18.8	5.3	21.2	30.7	19.1	4.4	0.5
Spring x Summer	35.6	10.1	12.4	24.7	14.0	2.5	0.7
Spring x Autumn	20.0	6.6	21.6	28.5	18.6	4.2	0.5
Summer x Autumn	27.3	9.1	17.5	23.1	18.2	4.3	0.5

Supplementary material 4.1 Two-way SIMPER results of the five abiotic variables measured in the lower Minho estuary, NW Iberian Peninsula throughout 18-months study period (February/09 to July/10).

	Dissimilarity (%)				
	Depth	ORP	pH	Salinity	Temperature
Stations					
S1 x S2	41.7	19.3	14.9	17.1	7.1
S1 x S3	22.1	19.7	13.9	31.2	13.1
S2 x S3	12.8	30.5	15.2	25.7	15.9
Seasons					
Winter x Spring	13.4	26.3	13.4	10.7	36.2
Winter x Summer	3.9	19.4	30.0	14.2	32.4
Winter x Autumn	7.4	25.3	13.8	15.9	37.6
Spring x Summer	9.7	21.9	40.4	18.1	9.9
Spring x Autumn	17.3	22.7	22.5	15.4	22.1
Summer x Autumn	6.1	19.4	42.3	12.9	19.3

Supplementary material 4.2 Comparisons on the sex ratio (mean ± SE) of the common goby *Pomatoschistus microps* among three sampling stations and four seasons in Minho estuary, NW Iberian Peninsula. * = $p < 0.001$. Different letters indicates significant differences among them. Lower case letters refers to comparisons among seasons, while upper case letters in brackets refers to comparisons among sampling stations (Two-way PERMANOVA, pairwise test: $p < 0.05$).

Station\Season	Winter	Spring	Summer	Autumn
S1	$0.8\pm0.3^{a(A)}$	$1.0\pm0.2^{a(AB)}$	$1.1\pm0.3^{b(A)}$	$0.3\pm0.1^{a(A)}$
S2	$0.5\pm0.1^{a(B)}$	$1.7\pm0.4^{b(A)}$	$0.3\pm0.1^{c(B)}$	$0.2\pm0.0^{d(B)}$
S3	$0.5\pm0.1^{a(C)}$	$1.3\pm0.4^{ab(B)}$	$0.4\pm0.1^{b(B)}$	$0.4\pm0.0^{c(A)}$

Supplementary material 5.1 Two-way SIMPER results of the five abiotic variables measured in the lower Minho estuary, NW Iberian Peninsula throughout 18-months study period (February/09 to July/10).

	Dissimilarity (%)				
	Depth (m)	ORP (mV)	pH	Salinity	Temperature (ºC)
Stations					
S1 x S2	41.7	19.3	14.9	17.1	7.1
S1 x S3	22.1	19.7	13.9	31.2	13.1
S2 x S3	12.8	30.5	15.2	25.7	15.9
Seasons					
Winter x Spring	13.4	26.3	13.4	10.7	36.2
Winter x Summer	3.9	19.4	30.0	14.2	32.4
Winter x Autumn	7.4	25.3	13.8	15.9	37.6
Spring x Summer	9.7	21.9	40.4	18.1	9.9
Spring x Autumn	17.3	22.7	22.5	15.4	22.1
Summer x Autumn	6.1	19.4	42.3	12.9	19.3

Supplementary material 7.1 Two-way PERMANOVA results of all abiotic variables (depth, ORP, pH, salinity and temperature) in the lower Minho estuary, NW Iberian Peninsula.

Source	df	SS	MS	Pseudo-*F*	p
Stations	2	55.03	25.02	7.99	< 0.001
Seasons	3	60.23	20.08	6.41	< 0.001
Stations x Seasons	6	9.93	1.66	0.53	ns
Residual	36	112.73	3.13		
Total	47	235			
Pairwise comparison for the *Seasons* factor				t	p
S1 Spring x Summer				1.23	ns

	Spring x Autumn	1.14	ns
	Spring x Winter	1.37	ns
	Summer x Autumn	1.16	ns
	Summer x Winter	1.82	ns
	Autumn x Winter	1.56	ns
S2	Spring x Summer	1.38	ns
	Spring x Autumn	0.66	ns
	Spring x Winter	1.38	ns
	Summer x Autumn	1.09	ns
	Summer x Winter	1.92	< 0.05
	Autumn x Winter	1.19	ns
S3	Spring x Summer	1.66	< 0.05
	Spring x Autumn	0.55	ns
	Spring x Winter	2.45	< 0.05
	Summer x Autumn	1.46	ns
	Summer x Winter	3.02	< 0.01
	Autumn x Winter	2.24	< 0.05

Supplementary material 7.2 Three-way PERMANOVA results comparing the density of life stages (juveniles and adults) of the top five dominant species *Carcinus maenas*, *Crangon crangon*, *Pomatoschistus microps*, *P. minutus* and *Platichthys flesus* in Minho estuary (NW Iberian Peninsula) among sampling stations (S1, S2 and S3) and seasons (Spring, Summer, Autumn, Winter). ns = non-significant p value. * Pairwise tests among life stages using the t-statistic on Pseudo-*F* values for detecting significantly differences in density of juveniles and adults.

C. maenas	Source	df	SS	MS	Pseudo-*F*	p
	Station	2	65.38	32.69	59.48	< 0.001
	Season	3	20.96	6.99	12.71	< 0.001
	Life stage	1	0.10	0.10	0.19	ns
	Station x Season	6	29.95	4.99	9.08	< 0.001
	Station x Life stage	2	4.36	2.18	3.96	< 0.05
	Season x Life stage	3	11.10	3.70	6.73	< 0.001
	Station x Season x Life	6	10.54	1.76	3.20	< 0.01

	stage					
	Residual	264	145.09	0.55		
	Total	287	287			
	Pairwise comparison		Juvenile (ind.100m^{-2})	Adult (ind.100m^{-2})	t	p
S1	Spring		11.3±4.7	9.0±1.7	0.46	ns
	Summer		5.6±2.3	6.9±1.9	0.44	ns
	Autumn		17.8±5.8	49.8±12.7	2.30	< 0.05
	Winter		12.3±5.4	16.2±5.3	0.51	ns
S2	Spring		24.6±4.7	9.4±2.0	2.86	< 0.01
	Summer		42.8±8.4	47.9±7.4	0.46	ns
	Autumn		23.2±2.9	44.2±12.6	1.62	ns
	Winter		28.3±5.5	5.3±1.6	4.06	< 0.001
S3	Spring		-	-	-	-
	Summer		10.8±4.5	0.6±0.3	2.29	< 0.05
	Autumn		3.4±1.7	0.9±0.5	1.42	ns
	Winter		-	-	-	-
C. crangon	Source	df	SS	MS	Pseudo-*F*	p
	Station	2	42.9	21.5	33.21	< 0.001
	Season	3	31.9	10.6	16.42	< 0.001
	Life stage	1	1.5	1.5	2.28	ns
	Station x Season	6	20.4	3.4	5.26	< 0.001
	Station x Life stage	2	3.7	1.8	2.85	ns
	Season x Life stage	3	5.7	1.9	2.93	< 0.05
	Station x Season x Life stage	6	3.3	0.6	0.86	ns
	Residual	264	170.6	0.7		
	Total	287	287			
	Pairwise comparison		Juvenile (ind.100m^{-2})	Adult (ind.100m^{-2})	t	p
S1	Spring		330.1±90.8	117.3±33.1	2.20	< 0.05
	Summer		227.0±115.0	191.3±74.2	0.26	ns
	Autumn		83.0±25.7	202.9±66.7	1.68	ns
	Winter		89.2±55.1	157.6±74.9	0.73	ns

S2	Spring		634.0±174.0	237.9±42.9	2.21	< 0.05
	Summer		1042.0±188.0	556.0±110.0	2.23	< 0.05
	Autumn		751.0±150.0	764.0±150.0	0.06	ns
	Winter		112.8±51.4	155.0±43.1	0.63	ns
S3	Spring		2.2±1.5	1.3±0.6	0.51	ns
	Summer		476.0±133.0	483.0±151.0	0.04	ns
	Autumn		72.2±36.9	177.9±92.3	1.06	ns
	Winter		0.3±0.3	0.3±0. 3	0.00	ns
P. flesus	Source	df	SS	MS	Pseudo-*F*	p
	Station	2	23.50	11.75	18.95	< 0.001
	Season	3	4.67	1.56	2.51	ns
	Life stage	1	41.19	41.19	66.43	< 0.001
	Station x Season	6	1.59	0.26	0.43	ns
	Station x Life stage	2	23.65	11.82	19.07	< 0.001
	Season x Life stage	3	6.10	2.03	3.28	< 0.05
	Station x Season x Life stage	6	1.74	0.29	0.47	ns
	Residual	264	163.7	0.62		
	Total	287	287			
	Pairwise comparison		Juvenile (ind.100m^{-2})	Adult (ind.100m^{-2})	t	p
S1	Spring		0.9±0.2	0.0±0.0	3.92	< 0.001
	Summer		0.6±0.2	0.1±0.1	2.05	ns
	Autumn		0.1±0.1	0.1±0.1	0.00	ns
	Winter		0.4±0.2	0.0±0.0	2.53	< 0.05
S2	Spring		2.3±0.6	0.0±0.0	3.90	< 0.001
	Summer		2.3±0.9	0.0±0.0	2.74	< 0.05
	Autumn		0.1±0.1	0.1±0.1	0.00	ns
	Winter		1.0±0.5	0.0±0.0	2.14	< 0.05
S3	Spring		5.6±1.4	0.0±0.0	4.04	< 0.001
	Summer		4.0±0.8	0.0±0.0	5.22	< 0.001
	Autumn		2.8±0.7	0.1±0.1	3.77	< 0.01
	Winter		4.2±1.3	0.0±0.0	3.33	< 0.001
P. microps	Source	df	SS	MS	Pseudo-*F*	p
	Station	2	59.10	29.55	62.53	< 0.001

	Season	3	45.29	15.10	31.94	< 0.001
	Life stage	1	18.43	18.43	39.00	< 0.001
	Station x Season	6	48.56	8.09	17.12	< 0.001
	Station x Life stage	2	2.18	1.09	2.31	ns
	Season x Life stage	3	3.54	1.18	2.49	ns
	Station x Season x Life stage	6	2.94	0.49	1.04	ns
	Residual	264	124.78	0.47		
	Total	287	287			
	Pairwise comparison		Juvenile (ind.100m^{-2})	Adult (ind.100m^{-2})	t	p
S1	Spring		4.7±1.5	51.7±11.5	4.06	< 0.001
	Summer		4.8±2.0	13.3±3.1	2.31	< 0.05
	Autumn		16.9±6.0	70.4±28.9	1.81	ns
	Winter		19.4±4.4	98.8±20.6	3.78	< 0.001
S2	Spring		6.0±1.7	90.8±16.2	5.20	< 0.001
	Summer		30.0±7.4	54.0±15.7	1.37	ns
	Autumn		220.9±69.7	370.0±43.4	1.82	ns
	Winter		142.6±51.0	213.1±51.0	0.81	ns
S3	Spring		4.7±2.0	20.5±5.4	2.76	< 0.01
	Summer		30.2±6.3	71.7±13.8	2.74	< 0.05
	Autumn		34.3±10.6	118.4±25.7	3.02	< 0.01
	Winter		0.9±0.8	9.8±3.6	2.41	< 0.01
P. minutus	Source	df	SS	MS	Pseudo-*F*	p
	Station	2	27.72	13.86	21.33	< 0.001
	Season	3	9.80	3.27	5.03	< 0.01
	Life stage	1	18.47	18.47	28.44	< 0.001
	Station x Season	6	13.69	2.28	3.51	< 0.01
	Station x Life stage	2	25.24	12.62	19.42	< 0.001
	Season x Life stage	3	10.44	3.48	5.36	< 0.001
	Station x Season x Life stage	6	13.90	2.32	3.57	< 0.001
	Residual	264	171.52	0.65		
	Total	287	287			

	Pairwise comparison	Juvenile (ind.100m^{-2})	Adult (ind.100m^{-2})	t	p
S1	Spring	0.3±0.2	4.1±2.7	1.42	ns
	Summer	0.3±0.3	6.0±1.5	3.73	< 0.001
	Autumn	0.0±0.0	13.7±2.6	5.25	< 0.001
	Winter	0.0±0.0	0.7±0.3	2.31	< 0.05
S2	Spring	0.0±0.0	0.7±0.3	2.15	< 0.05
	Summer	-	-	-	-
	Autumn	0.0±0.0	1.2±0.8	1.58	ns
	Winter	0.1±0.1	0.1±0.1	0.00	ns
S3	Spring	-	-	-	-
	Summer	0.0±0.0	0.3±0.3	1.39	ns
	Autumn	0.0±0.0	0.7±0.7	1.00	ns
	Winter	-	-	-	-

Supplementary material 7.3 Four-way PERMANOVA results comparing the density of individuals (ind.100m^{-2}) of the different taxonomic groups (fishes and crustaceans), life stages (juveniles and adults), sampling stations (S1, S2 and S3) and seasons (Spring, Summer, Autumn, Winter) in the lower Minho estuary (NW Iberian Peninsula). ns = non-significant p value. * Pairwise tests among taxonomic groups using the t-statistic on Pseudo-*F* values for detecting significantly differences in density of crustaceans and fishes.

Source	df	SS	MS	Pseudo-*F*	p
Station	2	35.38	17.69	45.87	< 0.001
Season	3	32.26	10.75	27.88	< 0.001
Life stage	1	40.54	40.54	105.13	< 0.001
Taxonomic group	1	57.49	57.49	149.07	< 0.001
Station x Season	6	23.64	3.94	10.22	< 0.001
Station x Life stage	2	18.30	9.15	23.73	< 0.001
Station x Taxonomic group	2	30.28	15.14	39.25	< 0.001
Season x Life stage	3	27.72	9.24	23.96	< 0.001
Season x Taxonomic group	3	20.37	6.79	17.60	< 0.001
Life stage x Taxonomic group	1	25.38	25.38	65.81	< 0.001
Station x Season x Life stage	6	16.51	2.75	7.13	< 0.001

Station x Season x Taxonomic group	6	26.96	4.49	11.65	< 0.001
Station x Life stage x Taxonomic group	2	14.37	7.18	18.63	< 0.001
Season x Life stage x Taxonomic group	3	15.79	5.26	13.65	< 0.001
Station x Season x Life stage x Taxonomic group	6	17.05	2.84	7.37	< 0.001
Residual	528	203.62	0.39		
Total	575	575			

Density (ind.100m^{-2}) ± SE			Crustacean	Fish	t	p
S1	Spring	Juvenile	15.4±3.2	19.8±6.4	0.61	ns
		Adult	211.5±36.7	26.6±8.1	4.91	< 0.001
	Summer	Juvenile	8.5±4.1	13.6±7.2	0.62	ns
		Adult	195.5±49.3	30.6±13.5	3.22	< 0.01
	Autumn	Juvenile	41.7±14.9	3.5±2.5	2.52	< 0.01
		Adult	1199.0±287.0	96.6±41.4	3.80	< 0.001
	Winter	Juvenile	23.5±6.0	5.4±1.8	2.88	< 0.01
		Adult	354.0±101.0	35.3±7.2	3.16	< 0.01
S2	Spring	Juvenile	144.0±23.1	12.6±4.1	5.61	< 0.001
		Adult	175.2±33.5	30.9±5.9	4.25	< 0.001
	Summer	Juvenile	296.6±48.9	17.1±9.5	5.61	< 0.001
		Adult	792.0±109.0	13.0±3.8	7.15	< 0.001
	Autumn	Juvenile	152.8±30.7	18.2±5.0	4.33	< 0.001
		Adult	835.0±219.0	131.5±35.7	3.17	< 0.001
	Winter	Juvenile	142.2±32.6	18.2±5.4	3.75	< 0.001
		Adult	106.1±28.8	61.0±16.6	1.35	ns
S3	Spring	Juvenile	0.1±0.1	9.8±2.5	3.86	< 0.001
		Adult	0.4±0.2	8.5±2.0	3.97	< 0.001
	Summer	Juvenile	34.8±11.4	25.5±8.2	0.66	ns
		Adult	91.8±27.7	27.4±7.6	2.24	< 0.05
	Autumn	Juvenile	18.7±9.1	18.5±6.7	0.18	ns
		Adult	43.1±22.8	79.6±41.4	0.77	ns
	Winter	Juvenile	0.0±0.0	19.0±6.2	3.05	< 0.001
		Adult	0.0±0.0	4.6±1.9	2.35	< 0.01

Supplementary material 7.4 Three-way PERMANOVA results comparing the biomass of life stages (juveniles and adults) of the top five dominant species *Carcinus maenas*, *Crangon crangon*, *Pomatoschistus microps*, *P. minutus* and *Platichthys flesus* in Minho estuary (NW Iberian Peninsula) among sampling stations (S1, S2 and S3) and seasons (Spring, Summer, Autumn, Winter). ns = non-significant p value. * Pairwise tests among life stages using the t-statistic on Pseudo-*F* values for detecting significantly differences in biomass of juveniles and adults.

C. maenas	Source	df	SS	MS	Pseudo-*F*	p
	Station	2	35.47	17.74	40.82	< 0.001
	Season	3	25.67	8.56	19.70	< 0.001
	Life stage	1	31.72	31.72	73.03	< 0.001
	Station x Season	6	30.20	5.03	11.59	< 0.001
	Station x Life stage	2	19.87	9.94	22.87	< 0.001
	Season x Life stage	3	22.38	7.46	17.18	< 0.001
	Station x Season x Life stage	6	20.43	3.40	7.84	< 0.001
	Residual	264	114.69	0.43		
	Total	287	287			
	Pairwise comparison		Juvenile (g.100m^{-2})	Adult (g.100m^{-2})	t	p
S1	Spring		5.8±1.9	195.6±35.1	5.40	< 0.001
	Summer		0.6±0.3	163.2±45.8	3.55	< 0.001
	Autumn		38.5±14.5	1158.0±282.0	3.97	< 0.001
	Winter		20.2±6.4	321.0±105.0	2.86	< 0.05
S2	Spring		125.2±24.2	140.5±30.9	0.39	ns
	Summer		258.4±46.8	716.0±108.0	3.90	< 0.001
	Autumn		121.8±26.9	712.0±208.0	2.81	< 0.001
	Winter		136.9 ± 33.1	80.2 ± 23.7	1.39	ns
S3	Spring		-	-	-	-
	Summer		16.9±9.6	8.6±4.6	0.79	ns
	Autumn		15.2±7.5	13.3±7.4	0.19	ns
	Winter		-	-	-	-
C. crangon	Source	df	SS	MS	Pseudo-*F*	p
	Station	2	23.79	11.89	19.81	< 0.001
	Season	3	32.60	10.87	18.10	< 0.001
	Life stage	1	33.20	33.20	55.31	< 0.001
	Station x Season	6	19.69	3.28	5.47	< 0.001

	Station x Life stage	2	2.70	1.35	2.25	ns
	Season x Life stage	3	13.88	4.63	7.71	< 0.001
	Station x Season x Life stage	6	8.97	1.50	2.49	< 0.05
	Residual	264	158.45	0.60		
	Total	287	287			
	Pairwise comparison		Juvenile (g.100m^{-2})	Adult (g.100m^{-2})	t	p
S1	Spring		9.6±2.8	15.8±4.0	1.28	ns
	Summer		7.9±4.0	32.3±11.3	2.02	< 0.05
	Autumn		3.2±1.1	40.7±13.7	2.73	< 0.01
	Winter		3.3±1.8	32.7±19.0	1.54	< 0.001
S2	Spring		18.8±5.5	34.7±5.0	2.14	< 0.05
	Summer		38.3±6.7	75.9±14.4	2.37	< 0.05
	Autumn		31.0±6.1	123.2±23.6	3.78	< 0.01
	Winter		5.2±2.2	25.9±8.3	2.41	< 0.01
S3	Spring		0.1±0.1	0.4±0.2	1.35	ns
	Summer		17.9±4.6	83.2±26.5	2.43	< 0.01
	Autumn		3.5±1.8	29.8±15.9	1.64	ns
	Winter		0.0±0.0	0.0±0.0	0.40	ns
P. flesus	Source	df	SS	MS	Pseudo-*F*	p
	Station	2	1.30	0.65	0.65	ns
	Season	3	5.69	1.90	1.90	ns
	Life stage	1	0.02	0.02	0.02	ns
	Station x Season	6	2.79	0.47	0.47	ns
	Station x Life stage	2	0.77	0.38	0.38	ns
	Season x Life stage	3	11.29	3.76	3.77	< 0.01
	Station x Season x Life stage	6	1.36	0.23	0.23	ns
	Residual	264	263.58	1.00		
	Total	287	287			
	Pairwise comparison		Juvenile (g.100m^{-2})	Adult (g.100m^{-2})	t	p
S1	Spring		19.4±6.3	7.5±7.5	1.22	ns
	Summer		13.2±7.2	20.0±13.6	0.44	ns
	Autumn		2.4±2.4	29.1±29.1	0.91	ns
	Winter		3.8±1.8	0.0±0.0	2.07	ns

S2	Spring		12.1±4.0	0.0±0.0	3.01	< 0.001
	Summer		14.7±9.2	0.0±0.0	1.58	ns
	Autumn		1.4±1.4	30.5±30.5	0.95	ns
	Winter		7.4±3.5	0.0±0.0	2.14	ns
S3	Spring		9.7±2.5	0.0±0.0	3.84	< 0.001
	Summer		23.5±8.0	7.2±7.2	1.51	ns
	Autumn		16.0±6.9	40.5±40.5	0.60	ns
	Winter		18.9±6.2	0.0±0.0	3.03	< 0.01
P. microps	Source	df	SS	MS	Pseudo-*F*	p
	Station	2	31.75	15.88	40.74	< 0.001
	Season	3	32.92	10.97	28.16	< 0.001
	Life stage	1	69.00	69.00	177.05	< 0.001
	Station x Season	6	24.61	4.10	10.52	< 0.001
	Station x Life stage	2	13.71	6.86	17.60	< 0.001
	Season x Life stage	3	18.20	6.07	15.56	< 0.001
	Station x Season x Life stage	6	11.28	1.88	4.82	< 0.001
	Residual	264	102.88	0.39		
	Total	287	287			
	Pairwise comparison		Juvenile (g.100m^{-2})	Adult (g.100m^{-2})	t	p
S1	Spring		0.3±0.1	17.0±3.8	4.33	< 0.001
	Summer		0.4±0.1	4.2±1.1	3.54	< 0.001
	Autumn		1.1±0.5	27.7±11.8	2.26	< 0.01
	Winter		1.7±0.4	33.6±7.5	4.26	< 0.001
S2	Spring		0.6±0.2	30.5±5.8	5.16	< 0.001
	Summer		2.4±0.6	13.0±3.8	2.76	< 0.001
	Autumn		16.8±5.1	99.4±10.1	7.28	< 0.001
	Winter		10.8±3.9	60.9±16.7	2.93	< 0.01
S3	Spring		0.1±0.1	8.5±2.0	4.11	< 0.001
	Summer		2.0±0.5	20.1±4.2	4.30	< 0.001
	Autumn		2.6±0.7	38.8±8.3	4.35	< 0.001
	Winter		0.1±0.1	4.6±1.9	2.33	< 0.01
P. minutus	Source	df	SS	MS	Pseudo-*F*	p
	Station	2	26.51	13.25	29.26	< 0.001

	Season	3	21.38	7.13	15.73	< 0.001
	Life stage	1	15.24	15.24	33.65	< 0.001
	Station x Season	6	37.85	6.31	13.93	< 0.001
	Station x Life stage	2	26.44	13.22	29.19	< 0.001
	Season x Life stage	3	21.42	7.14	15.76	< 0.001
	Station x Season x Life stage	6	37.90	6.32	13.94	< 0.001
	Residual	264	119.58	0.45		
	Total	287	287			
	Pairwise comparison		Juvenile (g.100m^{-2})	Adult (g.100m^{-2})	t	p
S1	Spring		0.0±0.0	2.1±1.2	1.70	< 0.01
	Summer		0.0±0.0	6.5±1.8	3.72	< 0.001
	Autumn		0.0±0.0	39.8±11.2	3.56	< 0.001
	Winter		0.0±0.0	1.7±0.8	2.01	ns
S2	Spring		0.0±0.0	0.4±0.2	1.86	ns
	Summer		-	-	0.00	ns
	Autumn		0.0±0.0	1.5±0.9	1.67	ns
	Winter		0.0±0.0	0.1±0.1	0.91	ns
S3	Spring		-	-	0.00	ns
	Summer		0.0±0.0	0.1±0.1	1.48	ns
	Autumn		0.0±0.0	0.3±0.3	1.00	ns
	Winter		-	-	0.00	ns

Supplementary material 7.5 Four-way PERMANOVA results comparing the biomass of individuals (g.100m^{-2}) of the different taxonomic groups (fishes and crustaceans), life stages (juveniles and adults), sampling stations (S1, S2 and S3) and seasons (Spring, Summer, Autumn, Winter) in the lower Minho estuary (NW Iberian Peninsula). ns = non-significant p value. *Pairwise tests among taxonomic groups using the t-statistic on Pseudo-*F* values for detecting significantly differences in density of crustaceans and fishes.

Source	df	SS	MS	Pseudo-*F*	p
Station	2	35.38	17.69	45.87	< 0.001
Season	3	32.26	10.75	27.88	< 0.001
Life stage	1	40.54	40.54	105.13	< 0.001

Taxonomic group	1	57.49	57.49	149.07	< 0.001
Station x Season	6	23.64	3.94	10.22	< 0.001
Station x Life stage	2	18.30	9.15	23.73	< 0.001
Station x Taxonomic group	2	30.28	15.14	39.25	< 0.001
Season x Life stage	3	27.72	9.24	23.96	< 0.001
Season x Taxonomic group	3	20.37	6.79	17.60	< 0.001
Life stage x Taxonomic group	1	25.38	25.38	65.81	< 0.001
Station x Season x Life stage	6	16.51	2.75	7.13	< 0.001
Station x Season x Taxonomic group	6	26.96	4.49	11.65	< 0.001
Station x Life stage x Taxonomic group	2	14.37	7.18	18.63	< 0.001
Season x Life stage x Taxonomic group	3	15.79	5.26	13.65	< 0.001
Station x Season x Life stage x Taxonomic group	6	17.05	2.84	7.37	< 0.001
Residual	528	203.62	0.39		
Total	575	575			

Biomass (g.100m^{-2}) ± SE			Crustacean	Fish	t	p
S1	Spring	Juvenile	15.4±3.2	19.8±6.4	0.61	ns
		Adult	211.5±36.7	26.6±8.1	4.91	< 0.001
	Summer	Juvenile	8.5±4.1	13.6±7.2	0.62	ns
		Adult	195.5±49.3	30.6±13.5	3.22	< 0.001
	Autumn	Juvenile	41.7±14.9	3.5±2.5	2.52	< 0.01
		Adult	1199.0±287.0	96.6±41.4	3.80	< 0.001
	Winter	Juvenile	23.5±6.0	5.4±1.8	2.88	< 0.01
		Adult	354.0±101.0	35.3±7.2	3.16	< 0.01
S2	Spring	Juvenile	144.0±23.1	12.6±4.1	5.61	< 0.001
		Adult	175.2±33.5	30.9±5.9	4.25	< 0.001
	Summer	Juvenile	296.6±48.9	17.1±9.5	5.61	< 0.001
		Adult	792.0±109.0	13.0±3.8	7.15	< 0.001
	Autumn	Juvenile	152.8±30.7	18.2±5.0	4.33	< 0.001
		Adult	835.0±219.0	131.5±35.7	3.17	< 0.001
	Winter	Juvenile	142.2±32.6	18.2±5.4	3.75	< 0.001
		Adult	106.1±28.8	61.0±16.6	1.35	ns
S3	Spring	Juvenile	0.1±0.1	9.8±2.5	3.86	< 0.001
		Adult	0.4±0.2	8.5±2.0	3.97	< 0.001
	Summer	Juvenile	34.8±11.4	25.5±8.2	0.66	ns

	Adult	0.0±0.0	4.6±1.9	2.24	< 0.05
Autumn	Juvenile	18.7±9.1	18.5±6.7	0.18	ns
	Adult	43.1±22.8	79.6±41.4	0.77	ns
Winter	Juvenile	0.0±0.0	19.0±6.2	3.05	< 0.001
	Adult	0.0±0.0	4.6±1.9	2.35	< 0.001