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Long-term predation pressure exerted by juvenile flatfish on macrobenthic communities in an estuarine area

Susana Rita Paulo Pinheiro

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica do Professor Doutor Miguel Pardal (Universidade de Coimbra) e do Doutor Filipe Martinho (Universidade de Coimbra)

Susana Rita Paulo Pinheiro

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Index

Resumo

A pressão de predação exercida por dois abundantes pleuronectiformes, o linguado (*Solea solea*) e a solha (*Platichthys flesus*), foi analisada no estuário do Mondego, Portugal, durante 10 anos (2003-2013). A amostragem foi realizada com colheitas mensais e bimensais, usando arrastos de vara em 5 estações. A pressão de predação anual em comunidades macrobênticas, foi estimada para os três grupos de idade grupo 0, I e II de cada espécie, determinando os Requisitos para Manutenção Diária (DMR) e os Requisitos para Crescimento Diário (DGR), que somados consistem no Consumo Diário de Alimento (DFI). As abundancias revelaram uma elevada variabilidade interanual na solha, e em menor escala no linguado, que tem vindo a aumentar desde 2004. No geral, o linguado mostrou uma maior abundancia e pressão de predação anual, principalmente nos grupos 0 e I, enquanto que a solha não mostrou nenhum padrão particular. Esta variabilidade pode estar relacionado com fatores específicos intrínsecos e tolerâncias a factores ambientais, que são conhecidos por regular a variabilidade de recrutamento. A pressão de predação anual exercida por ambas as espécies aumentaram de 0.175 g AFDM m^{-2} (2004) para o valor máximo de 0.654 g AFDM m^{-2} (2009), diminuindo depois para to 0.174 g AFDM m^{-2} em 2011 e depois aumentando no ano seguinte para 0.254 g AFDM m^{-2}, mantendo-se estável até 2013. Baseado em análises da dieta, anfípodes, poliquetas, decápodes e crustáceos foram as presas mais consumidas, refletindo a variabilidade na pressão de predação da solha e linguado. Considerando a baixa ordem de magnitude de presas consumidas, o controlo "top-down" dos pleuronetiformes no macrobentos parece não ser relevante, particularmente quando comparado com outras áreas de viveiro destas espécies.

Palavras-chave: Pressão de predação, solha, linguado, controlo top-down, interações bentos-peixe

Abstract

The predation pressure exerted by two abundant flatfishes, sole (*Solea solea*) and flounder (*Platichthys flesus*), was evaluated in the Mondego estuary, Portugal, over a period of 10 years (2003-2013). Sampling was performed with monthly and bimonthly surveys using beam trawls, in five selected stations. Annual predation pressure on macrobenthic communities by each species was estimated for three age classes: 0, I and II-group, by determining the Daily Maintenance Requirements (DMR) and the Daily Growth Requirements (DGR), which added consist of the Daily Food Intake (DFI). Abundances showed a high inter-annual variability in flounder, and to a lesser extent in sole, which has been increasing in numbers since 2004. Overall, sole showed a higher abundance and annual pressure, mainly in 0 and I-group, while flounder did not show any particular pattern. Such variability may be related with species-specific intrinsic factors and tolerance to environmental factors, which are known to regulate recruitment variability. The total annual predation pressure carried out by both species increased from 0.175 g AFDM m^{-2} (2004), to a maximum value of 0.654 g AFDM m^{-2} (2009), decreasing afterwards to 0.174 g AFDM m^{-2} in 2011 and then increase in the next year to 0.254 g AFDM m^{-2}, maintaining stable until 2013. Based on diet analysis, amphipods, polychaetes, decapods and crustaceans were the most consumed preys, reflecting the variability in predation pressure by flounder and sole. Considering the low order of magnitude of consumed prey, top-down control of flatfish on macrobenthos seemed to be negligible, particularly when compared to other nursery areas for these species.

Key-words: Predation pressure, flounder, sole, top-down control, benthos-fish interaction

Chapter 1. Introduction

1.1 Importance of estuaries and coastal areas for marine fauna

Habitat quality represents one popular ecological issue, including the combined effects of abiotic and biotic environmental conditions and the available resources to the existing infauna. Dynamic systems such as estuaries are unique since they share both sea and river features, being recognized as transition areas. Countless studies on estuarine dynamics have described the myriad of specific abiotic factors like salinity, temperature, water currents, dissolved oxygen, sediment type substrate and vegetation, high turbidity and sheltered areas (Rogers, 1994; Werner et al., 1997; Amara et al., 2000), creating a high diversity of habitats that provides favorable conditions for invertebrates, birds and fish (Bergman et al., 1989; Piersma, 1994; Beck et al., 2001). However, in general, fish assemblages in estuaries have low diversity, but high abundances of individual taxa (Whitfield, 1999), usually concentrated in certain areas (Elliott et al., 2002), creating a very specific ecosystem with solid biological interactions. França et al. (2009) reported a variation of habitats regarding their availability and diversity of resources within the Portuguese coast, presenting differences in the fish community structure. Also, some fish communities are particularly associated with specific habitat types, representing the diversity of interactions. Besides, there is a certain codependence between all of the different habitats inside an estuary, where one can't exist without the others (Pihl et al., 2002).

Estuaries and coastal areas systems are known to have a high ecological value, mainly due to their high productivity (Costanza et al., 1997). In fact, the combination of specific biotic and abiotic factors leads to high abundance of primary producers and intertidal macrobenthic assemblages (McLusky, 1989), which play an important basal role in habitat balance, since they represent an abundant resource for many animals that use estuaries as feeding grounds, mainly birds and fish (Bergman et al., 1988; Piersma, 1994; Pihl et al., 2002; Dolbeth et al., 2003; Pedro, 2006). Indeed, through complex trophic chains, they provide a foundation to the development of the fish communities. In agreement, estuaries provide an additional set of important functions for fishes, such as migration routes, reproduction sites and feeding areas (Haedrich, 1983; Beck et al., 2001; Elliot et al., 2007). They are also often referred to as nursery grounds for marine fish, i.e. places that produce/generate more adult recruits per area unit than other juvenile habitats used by a particular species (Beck et al., 2001), being of invaluable importance for the restocking of marine migrant coastal populations.

For fishes, the occupancy of estuarine areas can vary from permanent (estuarine species), transitional (anadromous and catadromous), occasional (freshwater and marine stragglers) or for certain life-history periods, such as the marine species that use estuaries as nursery grounds (Elliott et al., 2007; Franco et al., 2008). The life cycle of these last species depends highly on migrations and connectivity between habitats, as it rotates between the sea and estuary. There is adult reproduction and spawning in the sea (offshore, in deeper waters), followed by migration of pelagic larvae and benthic juveniles into the estuary, drifting with the currents. There, the juveniles grow into sub-adult stages, where they migrate back to offshore areas (recruitment process), where they will reproduce and spawn, restarting the life cycle again (Miller et al., 1985; Zijlstra, 1972; Bergman et al., 1988; Koutsikopoulos et al., 1989; Rijnsdorp et al., 1995). These life stages follow a certain amount of density fluctuations, matching transitions in the life-history of fishes.

1.2 Recruitment variability to adult fish populations

From fish early life stages to adult phases there is a general abundance decrease that results from controlling factors occurring in transition phases, named bottlenecks. These can act on the transition between distinct life history stages, mainly caused by changes in the habitat conditions (Rijnsdorp et al., 1995). Within these stages there are some conditioning factors besides environmental fluctuations, such as some biological conditions and risks, such as predation. This latter is considered to be the main source of mortality for smaller fish (Harter and Heck, 2006), and is most likely to act on transitional phases such as the arrival of recruits to the estuary (MacGregor and Houde, 1994) affecting the population dynamics (e.g. survival rates) and so the recruitment strength and variability.

When compared to other fish, flatfishes are known to have smaller inter-annual variability in recruitment (Beverton, 1995), due to their life-history characteristics. These differences are mainly due to the metamorphosis that occurs just before the settlement, allowing them to obtain a demersal life style (van der Veer and Leggett, 2005). In flatfishes, there are two distinct bottlenecks that lead to recruitment variability: 1) migration of larvae to the estuary (pelagic stage) and 2) the development stage inside the estuary, followed by emigration to deeper waters offshore (adult stages). Presently, there is some debate as for which processes are responsible for most of the recruitment variability: a) density-independent processes, that affect mainly the pelagic phase (eggs and larvae), consisting mainly on abiotic factors, such as temperature, nutrients, hydrodynamics, large-scale factors; and b) density-dependent processes that influence the nursery stage (within the estuaries), made up by biotic factors such as competition for food, space or shelter, prey availability and predation (van der Veer et al., 2000), affecting the growth and survival of the juveniles and hence, the recruitment to adult populations. Nevertheless, the pelagic stage is considered to be the main source of recruitment strength, affected by environmental factors, such water temperature, salinity, tidal transport, fluvial discharge and wind regimes (van der Veer et al., 2000). On the other hand, Le Pape et al., (2003a) reported that nursery ground occupation can also result in a bottleneck in which the recently settled juveniles will suffer from density-dependent mortality factors (e.g. food limitation and diseases), due to the increasing of densities of larvae, limitation of estuary's carrying capacity and habitat quality. These limitations will most likely generate reduced survival of the less fit, since they will probably be targeted by predators.

It is thought that this juvenile mortality dampens the inter-annual variability of metamorphosed individuals that enter the estuary and thus to produce a lower annual variability of adult recruited individuals (Beverton and Iles, 1992). This variation in individuals that join the adult stocks results from a complex combination of processes that interact and can differ through time (Brunel and Boucher, 2006), generated in the pelagic stage and additionally regulated during the nursery residency period.

1.3 Predation as a regulatory process within estuarine nurseries

Juvenile habitat quality is driven mostly by water temperature, food availability and predation (Gibson, 1994; van der Veer et al., 2000), possibly affecting the recruitment processes. In fact, prey availability is known to be some of the main factors that lead to a sustainable habitat during this stage (Gibson, 1994), being considered a limiting factor for growth and survival for flatfish juveniles. On the other hand, predation is another main factor responsible for the variability in juveniles mortality and thus in the recruitment (van der Veer et al., 2000; Harter and Heck, 2006; Freitas et al., 2008), mainly in transition areas, which can lead to bottlenecks (van der Veer and Bergman, 1987; Rijnsdorp et al., 1995), and thus variations in juvenile population densities.

The post-settlement stage, characterized by an increase of densities inside the estuaries (Gibson, 1994), represents the more vulnerable phase to predation due to a reduced ability to feed, which can lead to lower growth rates and survival rates (Nunn et al., 2012). During this stage there have been reports of predation on flatfish juveniles, especially from brown shrimps and crabs (van der Veer and Bergman, 1987; van der Veer et al., 1990; Ansell et al., 1999), in which the duration of early-life stages influences the predation risk (Shepherd and Cushing, 1980; Hovenkamp and Witte, 1991; Sogard, 1997). These predator-prey relationships in intertidal systems are extremely complex (Freitas et al., 2008), depend on predator and prey sizes (Gibson et al., 1995), are highly size-selective (Sogard, 1997), and lead to high inter-annual variability (up to 40%, as reported in the Wadden Sea by van der Veer (1986) and Bergman et al. (1988)). Ultimately, predator-prey relationships dampen the inter-annual variability in year-class strength.

Within estuarine nurseries, prey availability is one important factor for determining habitat quality for juvenile fish (Gibson, 1994; 1997; Le Pape et al., 2003a; Vinagre et al., 2006). For instance, differences in juvenile *Solea solea* maximal growth rates have been attributed to distinct food availability and composition in several areas of an extensive intertidal area (Rijnsdorp and van Beek, 1991; van der Veer et al., 2001). The impact that one species has on their prey community is an example of what can be addressed as predation pressure (de Vlas, 1979), which is a major biotic factor that regulates the prey recruitment process (e.g. van der Veer et al., 1998a) and community structure. This is a complex mechanism that can depend on biotic conditions such as density/abundance, growth rates (Koot, 2009), predators preying strategy and prey detection methods, and alternate prey availability (Miller et al., 1991). The spatial variability and habitat complexity, such as sediment type and distance to gullies/channels (Koot, 2009), existence of refuges, and several abiotic factors controlling encounter rate (McGurk, 1989) such as turbidity (Miller et al., 1991) can also lead to a change in the feeding success.

Since fish are poikilothermic animals, temperature also plays a crucial role in their development, and it is believed that it can have a significant effect on predation, since it can influence encounter rates, prey fleeing ability, and chasing success (Persson, 1986; Englund et al., 2011). However, there is not enough information to conclude how the temperature affects the predators' behavior, and thus predation pressure on benthos. Since predation pressure of fish on their prey results from an intricate interaction between biotic and abiotic factors, they need to be considered when assessing predation pressure due to its complexity and variability under various scenarios. Then, it will be possible to determine the energy flows through estuarine trophic webs, which have a great effect on community species productivity, stability and dynamics (Ricklefs, 2008).

In biological terms, trophic webs are mainly controlled and regulated in two possible ways: bottom-up control, which consists in primary and secondary production variability that affects the upper levels of the trophic web; and top-down control when there is a reflection of the variability of higher trophic levels on lower productivity levels

(Ricklefs, 2008). In this specific case, predation is one of the critical top-down processes in structuring the composition and size distribution of macrobenthic communities, being highly dependent on the predator preferences for certain prey species (Kuipers, 1977). In flatfishes there is a known preference for macrobenthic prey, having a relatively wide range of alternative prey for both juveniles and adults (e.g. Cabral, 2000; Amara et al., 2001; Martinho et al., 2008). Therefore, each time there is a migration of juveniles into an estuary and the densities increase, predation pressure increases, which can regulate the recruitment of their macrobenthic prey (van der Veer et al., 1998a).

In temperate estuaries, macrobenthic prey populations seem to be most often controlled by bottom-up processes (primary production) (Herman et al., 1999), while top-down control by predators act on the regulation of prey species composition (van Der Veer et al., 2011). Even though it is thought that it's not the only factor that regulates macrobenthic prey communities, flatfish diet certainly have an impact on its production (Evans, 1983; Gee, 1987), as it is almost restricted to these prey items, such as polychaetes, molluscs and amphipods (e.g. Dolbeth et al., 2008; Martinho et al., 2008), exerting a strong impact on these assemblages.

Assessment and quantification of flatfish predation pressure in temperate estuaries has not been widely explored in literature. The few existing studies have focused on plaice *Pleuronectes platessa* and flounder *Platichthys flesus* in the Dutch Wadden Sea, using the methodologies developed by de Vlas (1979), and adapted recently by Koot (2009) and van de Veer et al. (2011). These studies assessed the amount of food that the species need in order to grow, and evaluated whether or not the system was providing the necessary resources for the development of the fish populations. Effectively, both studies showed a decline in the predation pressure along time, indicating a decline in the use of this area as a nursery ground. Hence, using the same methodologies in other datasets on estuarine nurseries worldwide will provide a comparable assessment of the variation in nursery habitat use by flatfishes, and on their value as feeding grounds.

1.4 Study species

The model species for this study are the common sole (*Solea solea* (Linnaeus, 1758)) and the European flounder (*Platichthys flesus* (Linnaeus, 1758)). Both of them are North Atlantic species, with commercial importance (e.g. Costa and Cabral, 1999; Vasconcelos et al., 2010). These are marine estuarine-dependent species that can live up to 2 years inside the estuary, with different colonization timings and area occupation (Martinho et al., 2007; 2008; Primo et al., 2013). As for intrinsic factors, such as the diet, these species have a specialized and opportunistic feeding strategy, depending mainly on macrobenthic assemblages (Summers, 1980; Amara et al., 2001; Dolbeth et al., 2008; Martinho et al., 2008), belonging to a complex trophic chain that is mediated mainly by this benthic pathway (França et al., 2011). Also, both species have different tolerance to some environmental factors and changes, such as temperature (Freitas et al., 2010), dissolved oxygen (Tallqvist et al., 1999), and salinity (Fonds, 1975) which can be reflected in their geographic range and distribution within estuaries (Rogers, 1992; Hermant et al., 2010).

1.5 Objectives

The main goal of this work was to determine the changes in predation pressure by two juvenile flatfishes (flounder and sole) in an estuarine nursery area for 10-year period. The specific aims were to: a) determine the abundance patterns of both flatfishes; b) assess the predation pressure by flatfish on macrobenthic assemblages, using the methodology of de Vlas (1979) on the Mondego estuary data, over the last 10 years (2003-2013), and c) determine possible “top-down” effects of juvenile flatfish on these communities.

Chapter 2. Materials and Methods

2.1 Study Site

Sampling was performed in the Mondego estuary, on the west coast of Portugal, located near Figueira da Foz. The estuary has a total area of 8.6 km^2 and splits into a southern and a northern arm, 7 km from the coast, unifying again near the river mouth. Both arms have different features: the northern arm is deeper, with 5–10m during high tide and its tidal range reaches up to 2 to 3 m. About less than 10% of the total area of the northern arm consists of intertidal mudflats. In order to keep it as the main navigation channel, depth maintenance through dredging is necessary and done frequently. On the other hand, the south arm is shallower, reaching 2 to 4 m deep during high tide and has a tidal range of 1–3m. The small input of freshwater into the southern arm is coming out through the Pranto River, which comes from the surrounding rice fields, and is controlled by a sluice that regulates the respective water needs. This arm stands out due to the intertidal mudflat areas, which correspond to about 75% of the total estuary area, being highly influenced by tidal cycles.

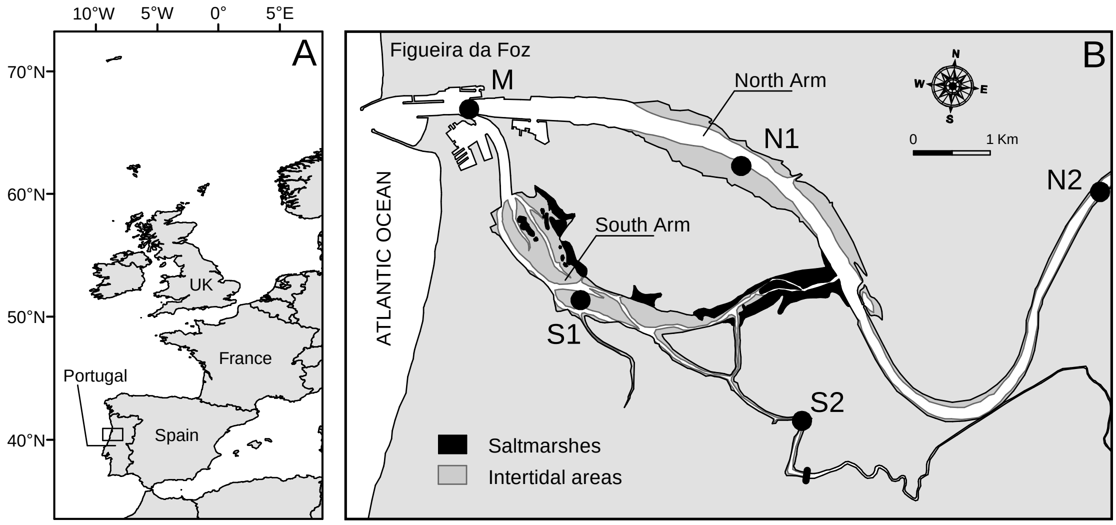

Figure 1 – Geographical location of the Mondego river estuary (A) and of the five sampling stations (black circles) (B).

2.2 Sampling

Fishing took place from 2003 to 2013 at five stations (M, N1, N2, S1, S2, see Fig.1) in the Mondego estuary, Portugal, with a monthly (from June 2003 until November 2006) and bimonthly frequency (since January 2007 until the end of the study period). Fishing was performed at night with a 2-m beam trawl with one tickler chain and 5 mm mesh-size in the cod end. Three trawls were hauled for 3 min per sampling station, covering a minimum area of 500 m^2 each, at a constant speed of about two knots. During fishing campaigns, referred to as cruises in this methodology, the hydrological records of bottom water temperature, salinity and dissolved oxygen were measured. In the field, fish were separated from crabs and shrimps to avoid interspecific predation, and the samples were stored in plastic bags, and transported in iceboxes to the laboratory.

All fish were identified, counted, measured (total length to nearest 1 mm) and weighed (wet weight, with 0.01 g precision). Since this work dealt only with the flatfishes flounder *Platichthys flesus* and sole *Solea solea*, data on these species were pooled from the original dataset. Fish length was converted into 0.5 cm size classes, and densities were calculated as individuals per $10^3 m^2$ (Ind. $[10^3 m^2]^{-1}$), being previously corrected for size-selective mesh size (Dapper, 1978), catch efficiency (Kuipers 1975), as well as for differences in sampling effort. All samples from one cruise were clustered into one single value per 0.5 cm size class and species. Afterwards, catches were split into age classes (0, I and II-group) according to length, based on previous work on these species in the estuary (Martinho et al., 2008; Dolbeth et al., 2010). Instead of performing a specified analysis per station, the arithmetic mean of all stations sampled during each survey was calculated, considered to be the total mean density at the Mondego estuary. The length-weight relationship was assessed using measurements for each species, modeled, and subsequently used in all calculations.

2.3 Estimation of predation pressure

The estimation of predation pressure by 0, I and II group of each species, which corresponds to the assessment of food intake, was based on the method of annual food intake calculation described by De Vlas (1979) and adapted by van der Veer et al. (2011). Daily food intake, which is the total amount of food consumed on a daily basis (DFI_j, g AFDM m^{-2} d^{-1}) consists of two distinct components: a) contribution for metabolism (daily maintenance requirements - DMR), and b) contribution for observed growth (daily growth requirements - DGR), which, summed annually, results in a total annual food intake. The energy spent on minor tasks, like swimming, are not included in the calculations, since it was assumed to already be considered in the estimate of the metabolism (De Vlas, 1979).

All calculations were performed in the open source R software (R Core Team 2014), using several packages listed at the end of this thesis: reshape2 (Wickham, 2007), plyr (Wickham, 2011), lubridate (Grolemund and Wickham, 2011), MASS (Venables and

Ripley, 2002), polynom (Venables et al., 2014), gridExtra (Auguie, 2012), ggplot2 (Wickham, 2009) and stringr (Wickham, 2012)

2.3. a) Daily maintenance requirements (DMR)

The estimation of the daily maintenance requirements (DMR) was based on the relationships of daily maintenance with metabolic fish mass ($W^{0.8}$) and temperature (Fonds et al. 1992). In the original method, the fish wet weight was unknown and estimated using the species-specific weight-length relationship:

$$\boldsymbol{W = (a \times L)^{b}} \qquad \text{Equation 1}$$

where L is total length in cm; W is wet mass in g, and "a" is the shape coefficient ($g^{1/3}\ l^{-1}$). "a" and "b" are species-specific parameters. For accuracy purposes, and because in our case this information was obtained, the wet weight data was used, creating a model that represented this species-specific length-weight relationship, and used in the following calculations. The fish wet mass (W_i) was estimated for each 0.5 cm length-based size class (L_i) taking the mean length of each size class.

Then, W_i was converted into metabolic mass ($W_i^{0.8}$) and multiplied by the number of individuals of each size class (n_i) found per 1000 m^2. Then, the $W_i^{0.8}$ were summed for all size classes in order to obtain the total metabolic wet mass of the population per 1000 m^2 ($\sum \boldsymbol{i(n_i W_i^{0.8}}$)). Lastly, the daily food intake per m^2 needed to meet daily maintenance requirements (*DMR*; g AFDM $m^2 d^{-1}$) was calculated using the same formula by De Vlas (1979) (Equation 2):

$$\boldsymbol{DMR = WA \times Q_{10}^{0.1 \times T} \times Main \sum i(n_i W_i^{0.8}))} \qquad \text{Equation 2}$$

where *T* is temperature in °C, Q_{10} is the Q_{10} (the increase in rate with a temperature increase of 10 °C), *Main* is the maintenance coefficient ($WW^{-0.8}d^{-1}$) and *WA* is the conversion factor from wet mass to ash-free dry mass (De Vlas 1979). The maintenance requirements were estimated for each sampling day separately, following the methodology by De Vlas (1979) and van der Veer (2011). The Q_{10} was calculated using the temperature sensitivity T_A (K) and optimum temperature T_{opt} ºC (K) values described in Table 2 in Freitas et al. (2010). The Q_{10}, *WA* and *Main* parameters were used according to the species (Table 1).

Table 1 – Coefficients used for estimating the daily maintenance requirements for sole and flounder.

Species	Q10	Wet Weight-AFDM conversion	Maintenance Coefficient	Assimilation Efficiency
S. solea	2.787	0.2	0.02	2
P. flesus	2.326	0.17	0.02	2

2.3. b) Daily growth requirements (DGR)

The daily food intake needed to achieve an observed growth (daily growth requirements, DGR) was assessed following the methods by van der Veer et al. (2011). Firstly, the average length per cruise was estimated for 0, I and II age group of sole and flounder separately. Then, using the species-specific weight-length relationship assessed before (Equation 1), estimates of mean wet mass were used to determine the instantaneous growth rate (G; d^{-1}) between 2 successive cruises:

$$\boldsymbol{G_{j\to j+1} = \frac{\ln W_{j+1} - \ln W_j}{t}} \qquad \text{Equation 3}$$

where W_j and W_{j+1} are the mean wet mass at cruise j and the following cruise $j+1$, respectively, and t is the time difference in days between the two cruises, being G the growth rate at the midpoint between two consecutive cruises.

In order to determine the daily growth requirements (DGR), the growth rates (G) were estimated over time allowing the fitting of an exponential curve of G as a function of time, for each age group and species. This relationship of G over time was used to smooth some of the sampling points where the surveys didn't occur or where are outliers/uncertainties. Therefore, an estimate of G at the sampling dates was obtained for each age group of both species. It was assumed that all of the individuals of all size-classes within each age group have the same growth rate, on a specific sampling date. This relationship was then applied to determine daily growth in mass for each size class (ΔW_{ij}).

Then, daily population growth (DPG; g wet mass d^{-1}) on sampling date j was calculated as the sum, for all size classes i, of the product of daily growth multiplied by the number of individuals per m^2 (n_i):

$$\boldsymbol{DPG_j} = \sum\nolimits_{\text{all}\,\boldsymbol{i}} \boldsymbol{n_i} \Delta \boldsymbol{W_{ij}} \qquad \text{Equation 4}$$

The amount of food needed for growth was calculated as 2 times the increase in wet mass (De Vlas 1979). Therefore, total daily food requirements for growth (DGR) were achieved by multiplying the $\mathrm{DPG_j}$ by an assimilation efficiency factor (FAC, in this case 2), and by AW - conversion to AFDM (De Vlas, 1979), resulting in an estimate on sampling date j (DGR_j; g AFDM m^{-2} d^{-1}):

$$\boldsymbol{DGR_j} = \boldsymbol{AW} \times \boldsymbol{FAC} \times \boldsymbol{DPG_j} \qquad \text{Equation 5}$$

Accordingly to this methodology, the fraction of food intake invested into maintenance, and so, the food intake invested into growth, is highly related with temperature, hence the need to explore this relationship by plotting these two variables.

2.3. c) Total food intake:

Daily food intake on sampling date *j* (DFI_j, g AFDM m^{-2} d^{-1}) for flounder and sole was calculated summing the daily maintenance requirements (DMR_j) and daily growth requirements (DGR_j) of each age group:

$$\boldsymbol{DFI_j} = \boldsymbol{DMR_j} + \boldsymbol{DGR_j} \qquad \text{Equation 6}$$

In order to determine annual food intake, a polynomial was fitted through the *DFI*, and the function was solved by integrating over the whole year. 2003 and 2008 are not represented in the results due to the lack of a minimum of 5 points/surveys needed for the growth determination. Also, there are some years where there are only 5 or 6 cruises, because the sampling frequency/schedule was monthly and bimonthly.

Finally, the diet composition was estimated based on the percentage of each prey item on the total food intake per species based on diet studies in 2003/2004 on sole and flounder (see Martinho et al., 2008). This was then used to estimate the amount of each prey item that was ingested in each year, assuming that the diet didn’t change through time and ontogeny.

Chapter 3. Results

3.1 Environmental characterization

The environmental parameters within the study period (2003-2013) showed a typical seasonal variation of temperate latitudes (Fig. 2). For instance, temperature showed a relatively constant pattern, varying between 8 and 22ºC, and with the minimum values seemingly increasing since 2003. Higher water temperatures were observed in 2005 (22.74 ºC), 2008 (21.82 ºC) and 2013 (22.28 ºC). Similarly, dissolved oxygen exhibited a seasonal variation with higher values in the winter and lower in the summer months, ranging from 5.5 to 12.0 mg/L. Dissolved oxygen showed an opposite trend to temperature, as higher dissolved oxygen values corresponded to lower temperatures, and vice versa.

Salinity showed a high variability inside the estuary (ranging between 2 and 32), reflecting the precipitation and river runoff variations (data not shown). Nonetheless, some anomalies were observed, such as extreme low salinity levels in the winter of 2006 and beginning of 2013. The highest salinity values occurred in 2005, 2007, 2008 and 2009 (mainly during the summer), representing dry years. Among these years, 2005 was considered as an extreme drought, regarded as the worst drought since 1931 (Portuguese Weather Institute—IM). The salinity values in 2008, 2009 and 2010 were low relatively to normal years, indicating rainy winters. Also, in the winter of 2011 the salinity levels were high taken into account the pattern of a regular winter.

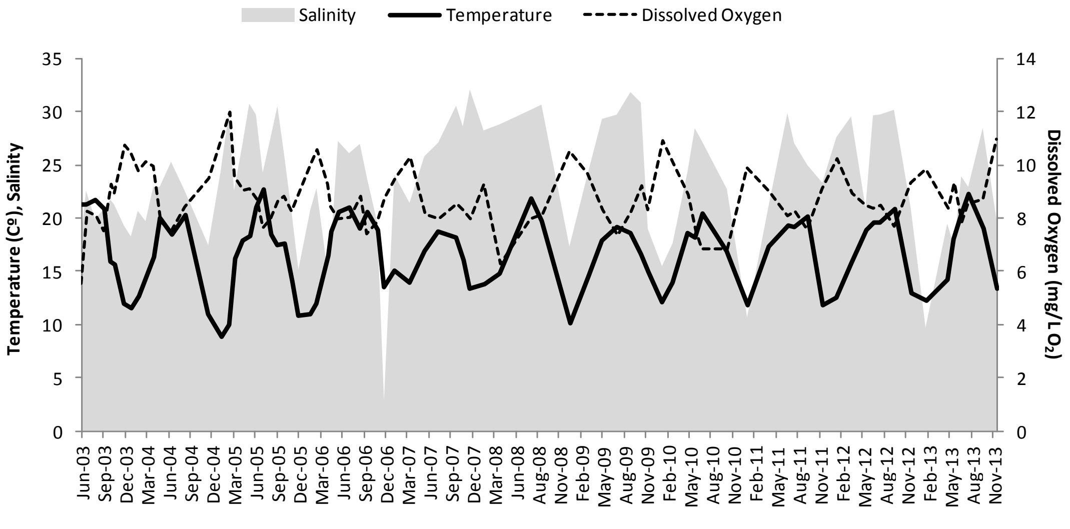

Figure 2: Monthly average salinity, temperature (ºC) and dissolved oxygen (mg/L) in the Mondego estuary surveys during between 2003 and 2013.

3.2 Flatfish abundance in nursery grounds

Flounder and sole showed higher density values for 0 and I-group juveniles (Fig. 3). Abundance of flounder 0- and I-group was particularly variable during the study period, with higher values in 2009, 2010 and 2012 (0-group) and in 2008, 2009 and 2010 (I-group). Mean densities of II-group flounder were low when comparing with younger age groups, and were higher in 2010. For sole there was a higher and more stable abundance, and so a lower inter-annual variability, with continuous density increase of juveniles (0-group) since 2004, being less noticeable in the I-group. The older juveniles (II-group) were more abundant in flounder than in sole.

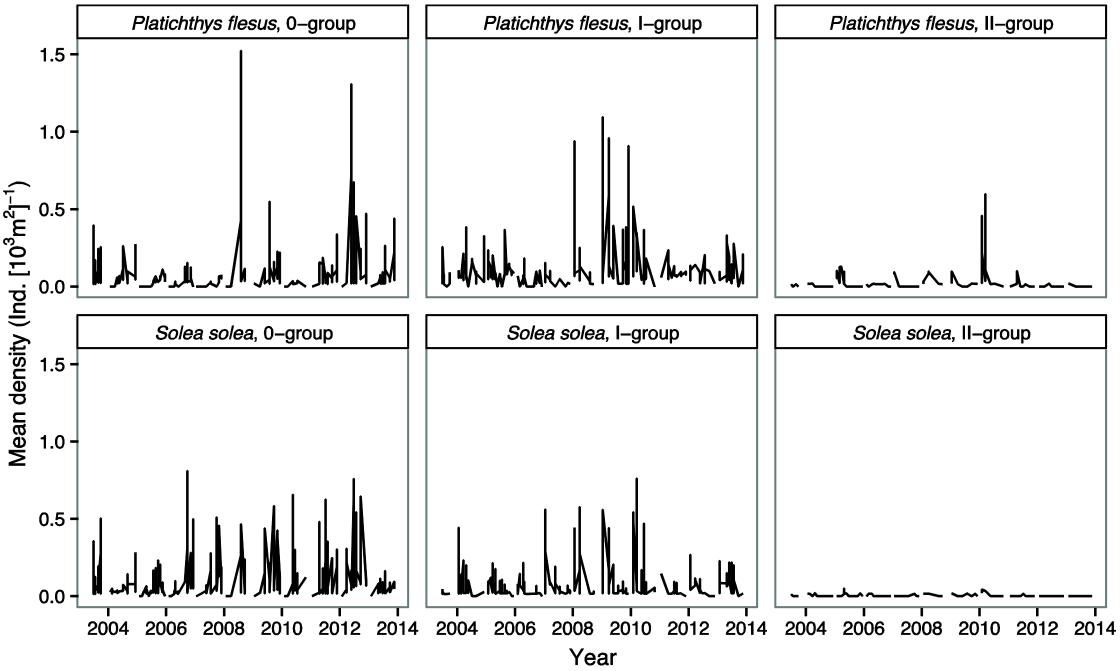

Figure 3 – Mean density (ind. $[10^3 m^2]^{-1}$) of 0, I- and II-group flounder and sole between 2003 and 2013 in the Mondego estuary.

In order to obtain a species-specific weight-length relationship, a non-linear model was fitted through the wet weight and total length values. Significant relationships between total length (mm) and weight (g) were obtained for both species (Fig. 4; $p<0.05$), resulting in the following species-specific exponential models:

P. flesus: Wet Weight = $(0.215 \times \text{Total Length}^{3.002})$

S. solea: Wet Weight = $(0.182 \times \text{Total Length}^{3.269})$

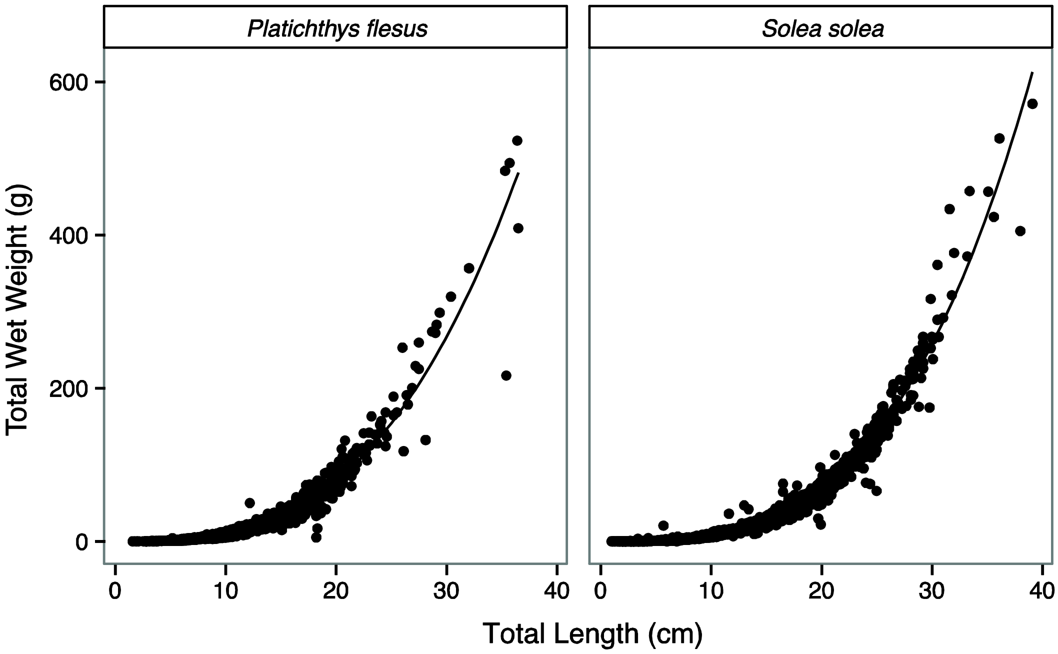

Figure 4 - Relationship weight-length for flounder and sole.

The maximum wet weight for *P. flesus* was 523.1 g and for *S. solea* was 571.3 g, having the respective total length of 36.40 cm and 39.10 cm. The minimum wet weight for *P. flesus* was 0.001 g and for *S. solea* was 0.001 g, having the respective total length of 1.60 cm and 1.00 cm.

3.3 Estimation of predation pressure on benthos

The estimate of G at each cruise was obtained for each age group, where an exponential decrease of instantaneous growth rate was observed for both sole and flounder, starting at approximately 0.09 mm per day (d^{-1}) for 0-group sole and flounder (Fig. 5), reaching nearly 0 in II-group. Compared with flounder, sole showed higher growth rates until approximately 200mm in length, more evident in the smaller size categories (TL<100mm). There were significant differences in flounder (p(a)=0.000; p(b)=0.037) and sole (p(a)=0.000; p(b)=0.000) G between years by fitting an exponential regression model through both species instantaneous growth:

Flounder: $G= 0.021\ e^{(-0.005*\mathrm{Length})}$; $r^2=0.057$

Sole: $G= 0.048\ e^{(-0.008*\mathrm{Length})}$; $r^2=0.284$

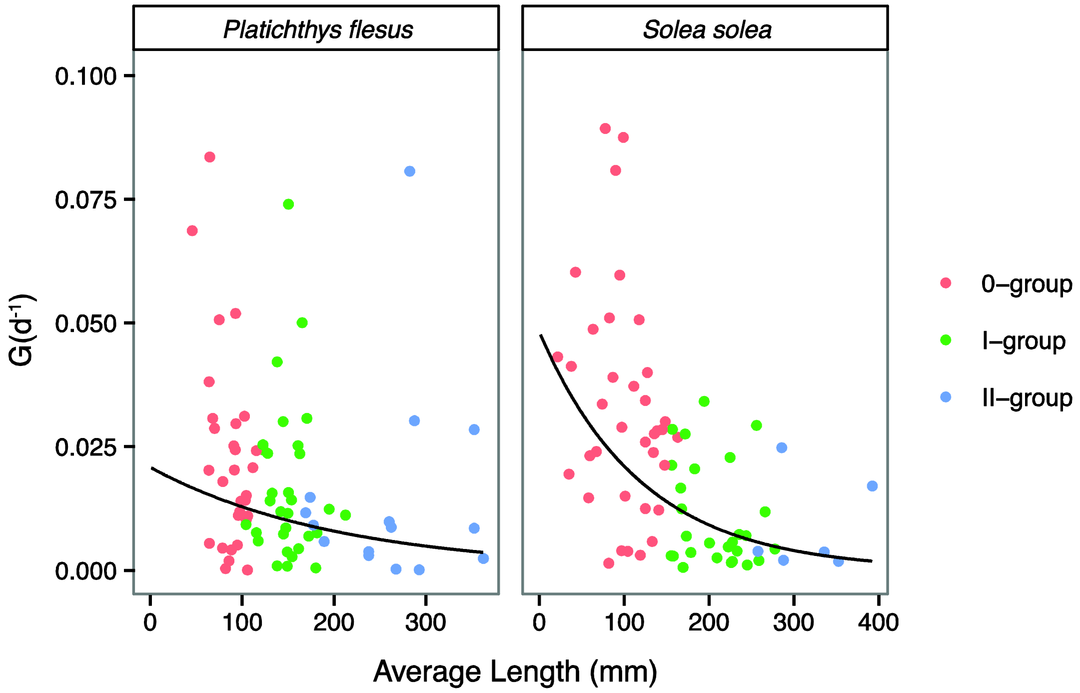

Figure 5 – Instantaneous growth rates (G; d^{-1}) for each age group sole and flounder, in relation to size (mm). All years combined.

Taking into account the metabolic and growth requirements, both species shared a similar pattern in the fraction of energy redirected for growth (Fig. 6), where the peak of food intake invested in growth occurs during colder seasons, and the lower values in warm months (Fig. 6a, b). Indeed, a higher investment in growth was observed during winter and consequently, a lower investment in maintenance (low maintenance requirements). Regardless of this tendency, flounder and sole showed opposite trends throughout time and according with body size. Flounder showed a decrease on the food invested into growth (energy flux into growth) reaching almost zero in II-group individuals (Fig. 6a, b). Contrarily, sole displayed an increasing investment in growth from the first to the third growing season, indicating a high growth potential for this species, mainly for the II-group.

The fraction of food intake invested into growth was also related with water temperature, especially concerning I and II-groups, since the investment into growth of 0-group did not seem to differ between these two species (Fig. 6c). At a given temperature of 12ºC, for instance, sole (I-group) invested 50% in growth (r^2=0.45; “a”= 0.709; “b”= -0.020), and flounder (I-group) invested around 40% (r^2=0.35; “a”= 0.541; “b”= -0.016). As for the II-group, sole invested 60% of the ingested food into growth (r^2=0.91; “a”= 0.831; “b”= -0.021), whereas flounder II-group invested approximately only 25% (r^2=0.75; “a”= 0.477; “b”= -0.019).

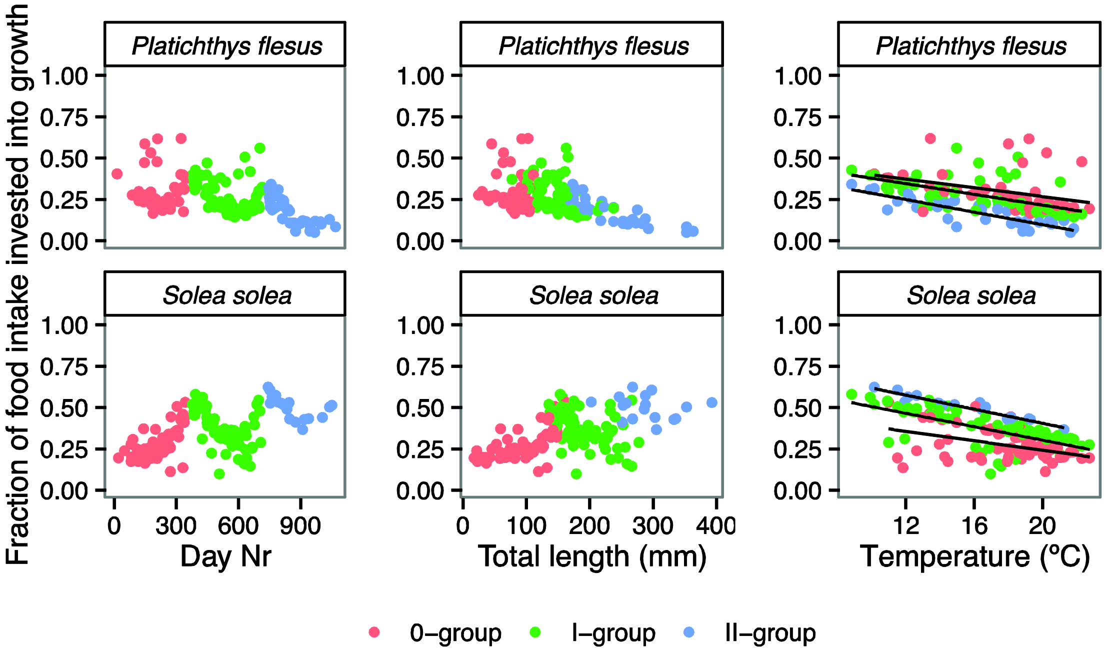

Figure 6 – Fraction of food intake invested into growth for juvenile flounder and sole, considering each age group, plotted against day number, total length (mm) and temperature (ºC). All years combined.

In order to determine the predation pressure by sole and flounder on the benthos, the daily food intake (mg AFDM m^{-2} d^{-1}) was estimated (Fig. 7, 8), which comprises daily maintenance requirements and daily growth requirements. Overall, diary predation pressure exerted by sole was higher than by flounder. During the 10 years, flounder I-group has a higher daily food intake, followed by 0 and II-group (Fig. 7), and sole 0-group shows higher food intake, followed by I- and II-groups (Fig. 8). Sole (0 and I-group) had the highest food intake in 2007; flounder and sole (0 and I-group) were the main responsible for the highest values of food intake in 2009; in 2010, the highest values belonged to flounder (I and II-group) and sole (I-group); in 2012 the higher values were of flounder and sole (0 and I-group), which can also be observed in Table 2.

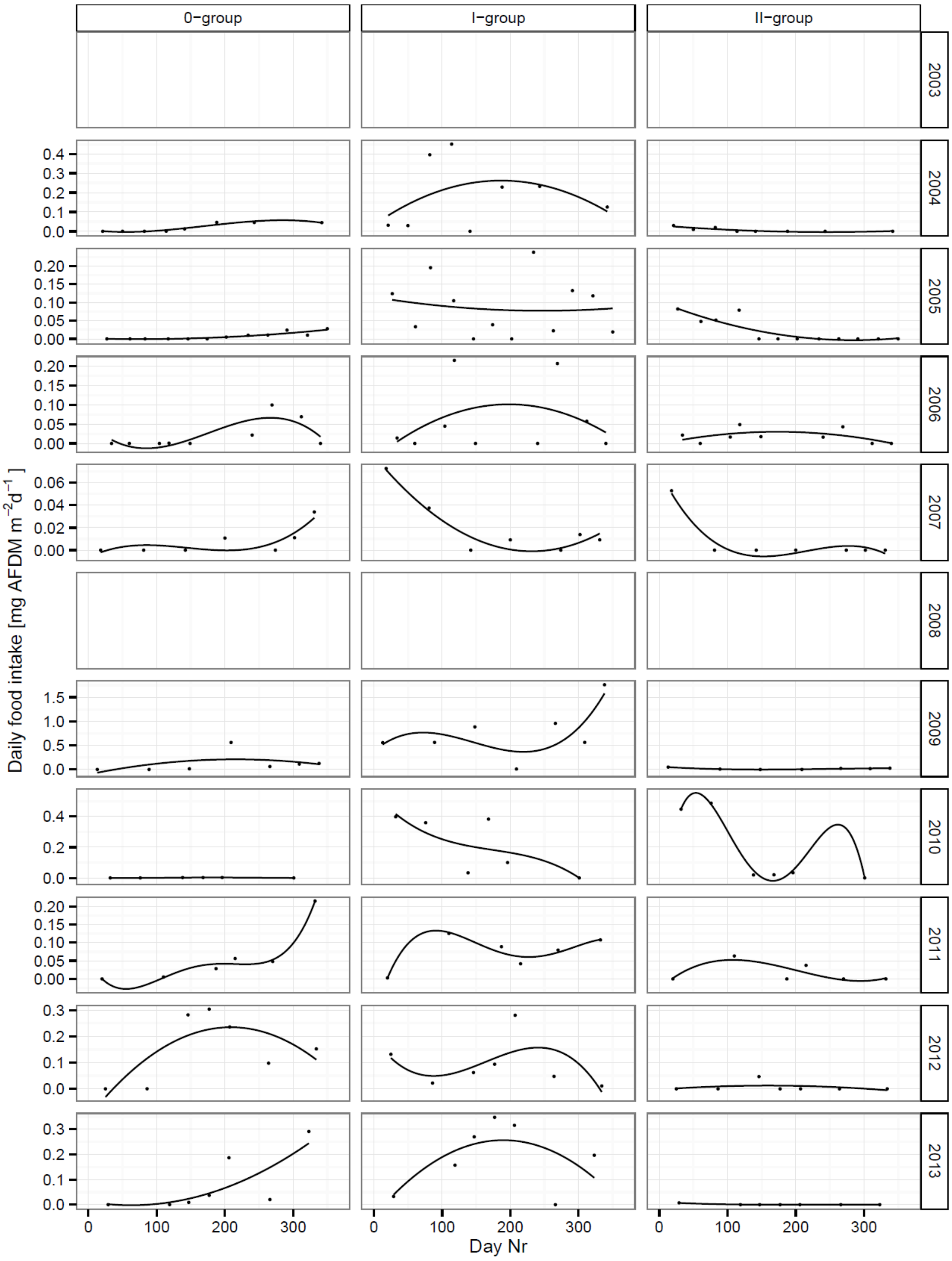

Figure 7 – Daily food intake (mg AFDM m^{-2} d^{-1}) in function of day number, by *Platichthys flesus* in the Mondego estuary over the period 2003-2013, based on food intake estimates.

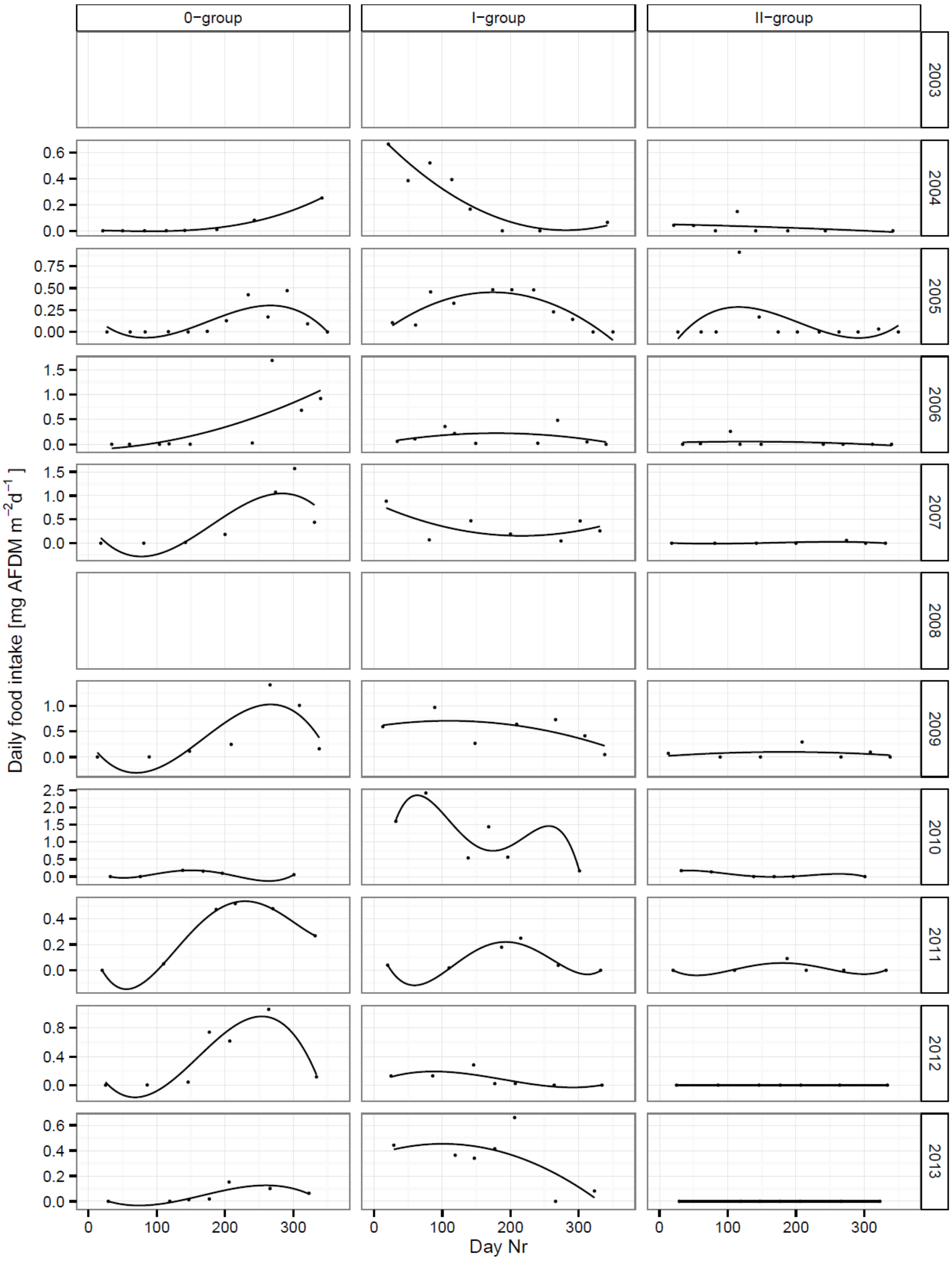

Figure 8 – Daily food intake (mg AFDM m^{-2} d^{-1}) in function of day number, by *Solea solea* in the Mondego estuary over the period 2003-2013, based on food intake estimates.

The annual food intake was determined by fitting fourth-order polynomials (1st to 4th order polynomials were used) through this daily food intake data for each cohort (Fig. 7) and calculating the surface area bellow the season curve, from the 1st January until the 31th December. In general, sole had a higher annual food intake than flounder (Table 2). Comparing the three age groups of both species, the annual predation pressure of I-group was higher, but there was no clear increasing or decreasing trend over time. Within the 10 years, the annual predation pressure carried out by sole and flounder, increased from

0.175 g AFDM m^{-2}, in 2004, to peak in 2009, with 0.654 g AFDM m^{-2}. In 2010 there was a decrease to 0.563 g AFDM m^{-2}, declining abruptly to 0.174 g AFDM m^{-2} in 2011 and then increase in the next year to 0.254 g AFDM m^{-2}, maintaining stable at the same order of magnitude until 2013.

Table 2 - Annual predation pressure (g AFDM m^{-2} y^{-1}) by juvenile *Platichthys flesus* and *Solea solea* in the Mondego estuary over the period 2003-2013, based on food intake estimates. ((-) insufficient data for yearly food intake estimation).

Year	*Platichthys flesus*			*Solea solea*			Total
	0-group	I-group	II-group	0-group	I-group	II-group	
2003	-	-	-	-	-	-	-
2004	0.010	0.068	0.002	0.021	0.066	0.008	0.175
2005	0.002	0.029	0.008	0.041	0.097	0.037	0.214
2006	0.009	0.022	0.007	0.125	0.053	0.011	0.227
2007	0.002	0.006	0.002	0.144	0.107	0.004	0.265
2008	-	-	-	-	-	-	-
2009	0.048	0.238	0.005	0.144	0.194	0.025	0.654
2010	0.000	0.058	0.070	0.018	0.398	0.019	0.563
2011	0.016	0.030	0.008	0.091	0.024	0.005	0.174
2012	0.054	0.032	0.002	0.137	0.029	0.000	0.254
2013	0.027	0.062	0.000	0.018	0.109	0.000	0.216

Considering the annual food intake for each species, a diet analysis was performed in order to determine the differences in the amount of ingested food through time, based on stomach content analysis on flounder and sole juveniles (0-group), performed in 2003/2004 by Martinho et al. (2008). In this case, and since this was the only source of data for both species' diet, it was assumed that diet composition did not change with time or age. The diet of the sole consisted mostly on polychaeta (65%) and decapoda (21%), while flounder fed mainly on amphipoda (72%) and polychaeta (17%) (Table 3). Overall, sole had a more diverse diet composition compared to flounder, which fed mostly on amphipods. Considering the differential prey consumption by both flatfishes, polychaetes and amphipods were the most heavily preyed group by sole and flounder, respectively. This was particularly evident in 2009 and 2010, matching the periods of higher densities of juvenile flounder and sole (Fig. 8).

Table 3: Proportions of ingested prey items (% in wet weight) for 0-group *Platichthys flesus* and *Solea solea*, determined by stomach content analysis (data obtained from Martinho et al., 2008).

	Percentage (%)	
Prey group	*P. flesus*	*S. solea*
Polychaeta	17.25	65.33
Mollusca	2.15	4.16
Amphipoda	72.14	3.34
Decapoda	2.87	20.51
Teleostei	0.13	1.16
Crustacea	1.18	1.82
Plant material	0.00	0.20
Echinodermata	0.00	0.40
Non-identified	5.27	3.06

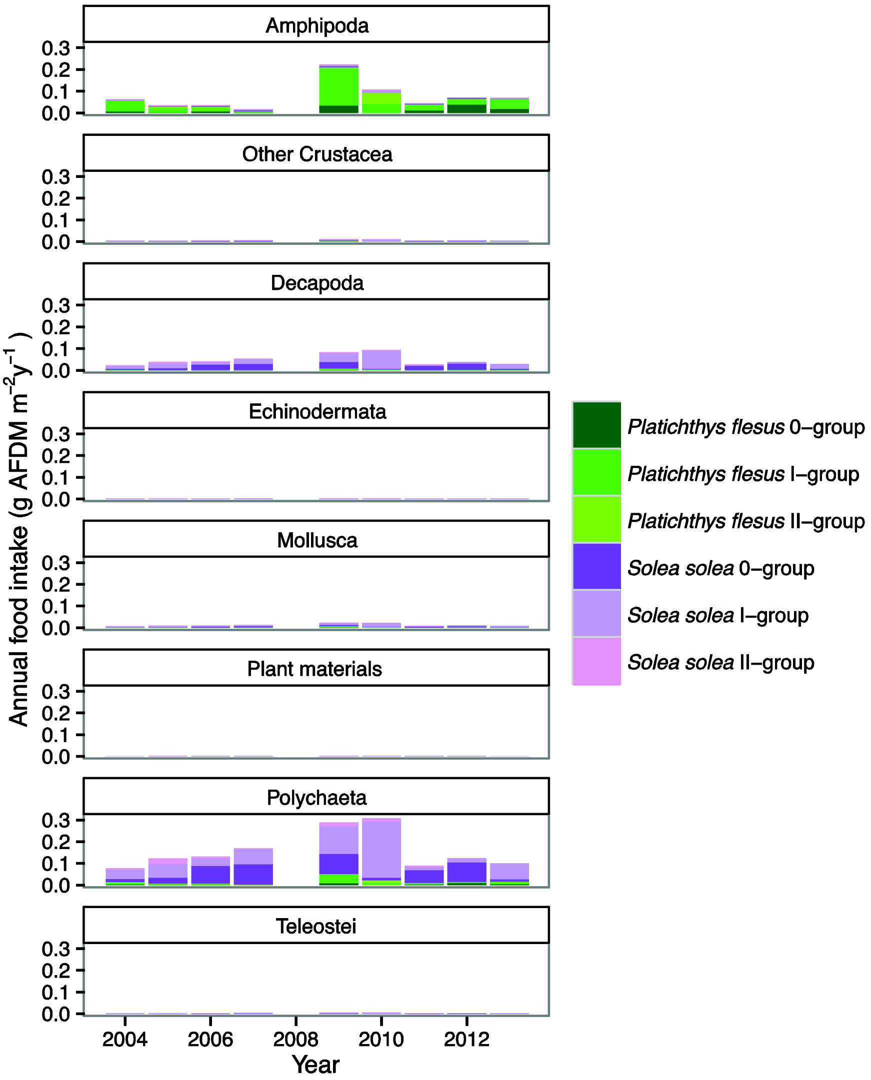

Figure 8 – Proportions of potentially ingested prey items by each age class of *Platichthys flesus* and *Solea solea* in the Mondego estuary between 2003 and 2013.

Chapter 4. Discussion

Long-term studies allow the detection of developmental patterns through time, preventing errors and downscaling possible factors that can influence the outcomes of marine ecological research. This study is based on a 10-year database, allowing the tracking of the density variations of sole and flounder populations within this period. The density variability through time was a required base in order to assess the predation pressure using the methodology developed by de Vlas (1979) and adapted by van der Veer et al. (2011). Previous work regarding estuaries demonstrated the importance of these areas as nursery grounds for sole and flounder, some of the most abundant species (e.g. Le Pape et al., 2003a; Andersen et al., 2005; Cabral et al., 2007; Martinho et al., 2007, 2008; Vasconcelos et al., 2010; Primo et al., 2013), reinforcing their role for the replenishment of marine coastal stocks. In addition, the Portuguese coast has a particular ecological relevance since it represents the transition between northeastern Atlantic warm-temperate and cold-temperate regions (Ekman, 1953), being an important source of adult recruits (Vasconcelos et al., 2008; 2011). When it comes to flatfishes, its latitudinal location includes the limits of both subtropical and temperate species distribution, such as flounder (Cabral, 2002).

4.1 Long-term flatfish abundance

A typical characteristic of estuarine nursery grounds worldwide is the presence of juvenile stages of fish, where they live for some years prior to their ontogenic migrations towards coastal areas. In our dataset, three age groups of both flounder and sole are represented, despite that the II-group was much less present than the other age groups. In fact, these species have been reported to stay within the estuary for a period of about 2 years, entering the estuary afterwards only occasionally (Martinho et al., 2007; 2008; Primo et al., 2013). Still, it was opted to include this age group for a more consistent assessment of predation pressure that integrated most individuals caught in the estuary, even with very low densities. Less inter-annual variability was observed in sole juveniles (0-group), when compared with flounder. Still, flounder showed higher peak densities in all age-groups. Such highly dynamic variability in juvenile densities has been reported in several studies, which is generated during the pelagic stage (van der Veer et al., 2000), mainly by variations in density-independent factors, such as hydrodynamic circulation and in the mortality rates of eggs and larvae (van der Veer et al., 1998b; van der Veer et al., 2000; Vinagre et al., 2008a; Martinho et al., 2009).

For instance, spawning success seems to be controlled by prevailing water temperatures: for flounder, spawning success decreases above 12ºC (Von Westernhagen, 1970), while for sole, increasing temperatures have been related with higher spawning success (Henderson and Seaby, 2005). In addition, other hydrological aspects have also been related with recruitment variability in marine fish, mainly the dispersion of river plumes into coastal areas, which play a pivotal role as proximity indicators of nursery areas for fish larvae (e.g. Marchand, 1991; Amara et al., 2000; Le Pape et al., 2003b), given the presence of chemical cues that direct larvae towards the estuarine grounds. However, density-dependent factors acting in shallow nurseries are also hypothesized to be responsible for regulating inter-annual variability (e.g. Zijlstra, 1972; Bergman et al., 1988), as a consequence of a high concentration of individuals in these areas. These include, for instance, density-dependent mortality and changes in predator-prey relationships (see review in Freitas et al. (2008)). In agreement, the main goal of this study was to tackle the juvenile stages within the estuary, and the processes that affect their growth and development.

4.2 Energy partition – Maintenance vs Growth

Fish are poikilothermic animals, adapting their body mechanisms to environmental conditions, mainly in response to temperature variations. Compared with other, more active fish, flatfishes convert relatively more of their food intake into growth than into metabolism (Fonds et al., 1992), considering that the total energy intake is divided between maintenance and growth (assimilation), and assuming that there are no losses in the process (De Vlas, 1979, van der Veer et al., 2011). However, this partition may not be constant maybe because of seasonal temperature variations, being the most probable cause for the energy flux patterns between growth and maintenance. In this case, both flounder and sole showed lower investment in growth with increasing temperatures in the warmer seasons, implying the existence of higher maintenance costs, partially coherent with the higher investment in flounder growth in the beginning of the growing seasons (winter) reported in van der Veer et al. (2011). In an unlimited food availability situation, temperature is the most important factor that can control fish growth and development (Gibson, 1994; Neill et al., 1994), namely at the level of metabolic processes (Fry, 1947). Consequently, metabolic rates increase with water temperature, causing an increase in energy demands for maintenance (Sumner and Lanham, 1942), corroborating the results obtained for both species and respective age-groups. In fact, the influence of higher water temperature on fish metabolism and energy partition has been observed elsewhere. Brett (1979) suggested that growth rates can vary accordingly to the food-metabolism-temperature relationship, in a way that high metabolic rates, caused by high water temperatures, results in an increased energy demand to maintain a constant body weight, which may surpass the energy obtained by feeding, thus reducing the scope available for growth. A similar pattern, linked also with poor foraging or diet, has been observed for the sockeye salmon (*Oncorhynchus nerka*) (Hinch et al., 1995). It has also been discussed in Freitas et al. (2012) that the stress resulting from high temperature can interfere with growth rates if it exceeds metabolic optimum temperatures (Vinagre et al., 2008b).

Also, high metabolic rates due to higher water temperatures lead to a decrease in the energy available for other tasks, such as swimming (Fry and Hart, 1948), which might be a significant issue for flatfish, given their lower swimming activity (Fonds et al., 1992). Ultimately, stochastic episodes such as disease and parasite outbreaks can't be discarded, since they are often triggered by higher temperatures, and can affect fish swimming ability, growth, weight gain and survival (Moller and Anders, 1986; Wagner et al., 2005; Elliott and Hemingway, 2008), and lead to indirect density-dependent mortality, as it results from predation on weaker fish individuals (van der Veer et al., 1994).

Dissolved oxygen is another abiotic factor that can limit individual fish growth. Dissolved oxygen showed higher values during the cold seasons, corresponding to lower temperatures. In this case, warmer seasons were marked by a lower investment in growth, which can be partly explained by this decrease in dissolved oxygen since, like temperature, it can lead to a decrease in metabolic rates and food intake, increasing ventilation costs (maintenance), potentially decreasing individual growth (Brett, 1979; Kramer, 1987; Yamashita et al., 2001). In fact, Sepúlveda (1994) estimated that dissolved oxygen concentrations inferior to 4.5 mg L^{-1} caused growth reduction in smelt (*Osmerus eperlanus)* larvae. Sole showed reduced activity below 40% of air saturation (Van den Thillart et al., 1994) and flounder exhibited the same at less than 30% oxygen (Tallqvist et al., 1999). However, and since the values obtained in the field never went below 5.5 mg L^{-1} it is assumed that DO didn't represent a direct limitation for the growth of juvenile flounder and sole.

Even though this was a similar pattern in both species, flounder described a decrease in investment in growth through time, whilst sole presented the opposite. The decreasing flounder investment into growth along time was expected as the older individuals (I and II-group) grow less, having less growth requirements, also observed in van der Veer et al. (2011). The fact that it decreased to nearly zero in II-group flounder is an indication that the larger individuals don't take full advantage of the resources provided by the estuary as a nursery ground, when compared to 0 and I-group. This might also be related with the carrying capacity of the estuary, given its relatively small size, which limits the potential use by larger and older fish. Still, it might also be related with the small number of II-group fish used in the analyses, which can lead to an underestimation of the food intake.

Sole investment into growth increased with age and size, contrary to flounder, which may represent the slight differences in growth rates observed, presenting maximum values similar to flounder growth in the Wadden Sea plaice (van der Veer et al., 2011). This difference in energy use by both species can be explained by species-specific features, such as latitudinal position and distribution, tolerance to environmental factors such as day duration, temperature, salinity, food availability, prey preferences and energy content, and feeding strategies, and competition, as follows.

Each species has a temperature tolerance range that will influence and establish their geographic distribution limits, in a way that populations can grow under optimal conditions. Flatfish are known to have a warm-water limit of distribution, which is the point at which the metabolic costs exceed the energy uptake (van der Veer and Leggett, 2005). Flounder is a temperate species, whose southern limit of distribution is presently the Mondego estuary (Nielsen, 1986; Cabral et al., 2001; 2007), while sole is a subtropical species, with a wider distribution, from the eastern Atlantic from Norway southward to Senegal, occurring also in the Mediterranean and the south-western Black Sea (Fonds, 1977; Henderson and Seaby, 2005). In this sense, sole can easily tolerate higher water temperatures than flounder, whose Mondego populations show some signs of thermal stress (La Roche, personal observation), typical of populations at the limits of their distribution range (Miller et al., 1991).

Within estuaries, several abiotic conditions influence the distribution and abundance of flatfishes like sole and flounder, which include temperature, salinity and sediment type (e.g. Riley et al., 1981; Dorel et al., 1991; Dorel and Desaunay, 1991; Kerstan, 1991; Bos, 1999; Amezcua and Nash, 2001; Vasconcelos et al., 2010). Due to the high dependence of fish maintenance on water temperature, it is likely that this factor is one of the main drivers for species occurrence. The observed temperature range within the estuary showed a slight increase since 2008, particularly during the cold season, in parallel with observations for the Northeast Atlantic (Perry et al., 2005). This can become a test to species tolerance ranges, especially for flounder, which is already at its limits of tolerance. In the methodology used, the assumed Q_{10} values (calculated using values from Freitas et al. (2010)) are slightly higher for sole (Q_{10}=2.787) than for flounder (Q_{10}=2.326), meaning that if the temperature increases 10ºC, sole will have a slightly higher metabolic rate, being capable of keeping up with temperature variations, i.e. its energy input into maintenance may be lower. Also, food intake invested into growth showed that, for the same temperature, sole I and II-group invested more into growth than flounder of the same age-groups, suggesting a higher tolerance to temperature increases and higher survival chances facing climate change.

Another factor that can be responsible for a divergence in growth responses by these two species is salinity, which plays an important role in maintenance metabolism (Morgan and Iwama, 1991), affecting indirectly the energy available for growth, in a way that higher growth rates should occur at salinity levels where the metabolic cost for osmoregulation is lowest (Yamashita et al., 2001); i.e., salinity variations may represent metabolic losses and lower growth rates. However, salinity variations do not seem to be determinant in this case, since both species' juveniles are usually located in lower salinity areas, specifically in upstream areas (e.g. Cabral et al., 2007; Martinho et al., 2007; Primo et al., 2013), and are typically exposed to daily salinity fluctuations due to tidal action.

Growth rates can act as indicators of the quantity and quality of the ingested food (Le Pape and Bonhommeau, 2013), and hence, of habitat quality for marine fish. Sole and flounder diet consists of macrobenthos, being considered opportunistic species (De Vlas, 1979; Miller et al., 1985; Dolbeth et al., 2008) which differ in terms of prey selection: sole has a wider range of prey items besides the main group, polychaeta, such as decapoda, mollusca, amphipoda, and other crustaceans (Beyst et al., 1999; Cabral, 2000; Amara, 2001; Martinho et al., 2008), while flounder's diet is almost restricted to amphipoda and polychaeta (Moore and Moore, 1976; De Vlas, 1979; Summers, 1980; Aarnio and Mattila, 2000). Besides feeding on whole individuals, flatfishes also consume regenerative parts like bivalve siphons, as well as polychaete tails and clam siphon tips reported for flounder and plaice (de Vlas, 1979, van der Veer et al., 2011), which can represent about 80% of these fishes diet (Ansell and Gibson, 1990). In the Mondego estuary intertidal areas, food limiting conditions have never been reported due to its high productivity (Dolbeth et al., 2003), although during critical conditions, such as droughts or floods, macrobenthic species can be affected, changing food availability. Grilo et al. (2011) reported that heat waves and floods were responsible for the more negative effects on the macrobenthic communities, when compared with droughts, causing overall lower species richness and abundances of prey.

An adequate feeding strategy is also a major advantage in order to have a successful development and recruitment within estuarine grounds. While sole is an olfactory feeder (De Groot, 1969) with poor vision ability and small mouth (Piet et al., 1998), which may explain why its preference for polychaetes, flounder is a visual feeder, showing a preference for more mobile prey (De Groot, 1971; Martinho et al., 2008), such as amphipods. Such differences in diet composition can also reflect the distinct habitat use patterns by both species: while flounder is mostly restricted to the upper reaches of the estuary, sole juveniles can occur throughout the estuary, including the most turbid areas of the south arm (Martinho et al., 2007; Dolbeth et al., 2008), where visual acuity is not so required. In addition, sole does not depend on daylight to forage, in contrast with flounder (Lagardère, 1987). Polychaetes are also among the most caloric prey found in intertidal mudflats (24,33 ± 0,54 KJ/g), when compared to other macrobenthic species (Pedro, 2006). Overall, feeding strategies, coupled with distinct habitat requirements and use patterns, reflect an advantage for sole juveniles, as higher growth rates lead to higher resistance under starvation conditions, providing also a size advantage in accessing a wider variety of prey and to avoid being predated (Nunn et al., 2012; Le Pape and Bonhommeau, 2013).

Competition can also be a source of divergent investment growth. Nevertheless, and despite that some spatial niche overlap exists between sole and flounder, competition seems to be diminished by the high food availability (Cabral et al., 2007; Dolbeth et al., 2008), the exploitation of different microhabitats, the different timings of estuarine colonization (Macpherson, 1981; Martinho et al., 2007): sole – mid winter, and flounder – mid-spring (Martinho et al., 2007; Primo et al., 2013), and by the low diet overlap observed between both species in previous studies (Martinho et al., 2008).

4.3 Long-term changes in predation pressure

Daily and annual food intake was assessed for each species and respective age groups, and sole clearly presented higher food intake than flounder. In addition, 0-group sole showed a higher food intake than I and II-groups, which is to be expected due the higher metabolic rate of smaller size compared with bigger sized individuals, due to the different body surface proportions (Schaeperclaus, 1933). However, I-group flounder exhibited higher daily food intake, followed by 0-group, and then by II-group, contrary to what was expected. This can be due to despite the fewer individuals in I group, they have higher food requirements in order to maintain and enhance their body biomass. These results are also all consistent with the density patterns, matching the periods of higher and lower fish numbers in the estuary. Analysis of this 10 year dataset showed a higher predation pressure exerted by sole, increasing since 2003 to its peak in 2009 (0.654 g AFDM m^{-2} y^{-1}). Since 2010, this pressure decreased to the initial lower values (2004). In addition, and also in conformity with density data, these results confirm that the estuary is an important nursery for 0 and I-group fish, but with limited value for older II-group fish.

Studies on predation pressure exerted by marine fish on macrobenthos are scarce in literature. Compared with a similar approach by van der Veer et al (2011) in the Wadden Sea, predation pressure by juvenile flatfish plaice (*Pleuronectes platessa*) and flounder were several orders of magnitude superior to the Mondego populations, particularly in the 1970's and 80's. However, and due to a dramatic reduction in flatfish abundance in this area in the last years, predation pressure values between both areas are actually comparable, particularly for flounder. The lack of a clear trend in flatfish abundance in the Mondego estuary prevented the inference of whether or not there has been a reduction in its nursery role over the last decade. Some methodological constraints were also encountered while estimating annual predation pressure, especially the limitation of a minimum of 5 surveys per year, which led to discarding most data for 2003 and 2008. In addition, the use of a bimonthly sampling design did not produce a desirably continuous dataset, and hence, annual predation pressure can be considered as underestimates for both species.

Predation pressure peaks were highly related to fish densities inside the estuaries, but may also be linked with environmental conditions, such as water temperature, which can control prey-predator relationships, for example, by inducing a shift in the distributions of predators/prey (e.g. Ciannelli and Bailey, 2005), affecting predator behavior by changing searching, handling time, and movement velocity (Barbeau and Scheibling, 1994; Freitas et al., 2008), reduction of feeding activity in the presence of a predator (e.g. plaice, Burrows and Gibson, 1995), encounter rates (see review in Hunsicker et al. (2011)) and even appetite changes (Brett, 1979), and acting on the sediment oxygen levels, which may affect prey burrowing ability and its visibility to predators (Freitas et al., 2008). The high precipitation levels of 2008-2010 can also be responsible for an increase in food availability, since increased river flow allows for higher primary and secondary productivity. In fact, the predation pressure peak in 2009 is supported by the high juvenile densities of both species, which may be related to a significant increase in seagrass Z*ostera noltii* beds in the Mondego estuary since 2008, followed by an increase of the gastropod *Hydrobia ulvae* and accompanying macrofauna (see Grilo et al., 2012). This seems to confirm that, according to the present estimates, flounder and sole populations are regulated by "bottom-up" control, an ecological process known to occur in temperate estuaries (Herman, 1999).

4.4 “Top-Down” control - trophic chain cascades:

The main basis for estuarine production lays on the terrestrial organic matter transported by rivers, followed by detritus food chains (detritivores), as well as on the high availability of phytoplankton, benthic microalgae, macroalgae and marsh plants, which presents a seasonal and space variability (e.g. Deegan and Garritt, 1997; Whitfield, 1999; Dolbeth et al., 2003; Elliott et al., 2002; Svensson et al., 2007). Variations in one of these components will be reflected in the next trophic levels, which includes benthos, zooplankton, and fishes (Whitfield, 1999). The feeding strategies and habits of fish, as well as their position within the food web vary substantially with time and space, especially in opportunistic feeders as sole and flounder, being considered rather complex and dynamic (Deudero et al., 2004; França et al., 2011). These food webs can be controlled by “top-down” processes, which consist in the impact of flatfishes on their prey through predation dictating their abundance levels. However, from these results (predation pressure peak of 0.654 g AFDM m^{-2}) and when compared to predation pressure peak of 5 g AFDM m^{-2} elsewhere (van der Veer et al., 2011), it’s not likely to be enough to exert direct top-down control. On the other hand, prevailing environmental conditions seem to be reflected in the trophic chains, through changes in productivity, abundance of prey, and growth and survival of predators, and in this case, exerting a bottom-up control on the abundance of juvenile flatfish.

4.5 Conclusion

In conclusion, estimating predation pressure allowed for a complementary approach on more traditional evaluation of changes in fish densities, with the added value of the possibility of estimating the existence of possible top-down control. Using a long-term dataset allowed the identification of constancy in juvenile flounder and sole densities over time, as well as the primordial nursery function for 0 and I-group fish. This methodology also allowed determining the potential prey biomass removed from the system by both species, and also inferring on the non-existence of a top-down control exerted by juvenile flatfish. Still, the availability of data on macrobenthos would have allowed for a direct comparison between prey and predator density data, and fine-tuning the interpretation of the interdependency between predator and prey in an estuarine trophic web.

Chapter 5. References

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