

**FEEDING ECOLOGY OF EUROPEAN FLOUNDER,
PLATICHTHYS FLESUS, IN THE LIMA ESTUARY (NW
PORTUGAL)**

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Dissertação de Mestrado em Ciências do Mar – Recursos Marinhos

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Resumo

A função viveiro é uma das funções mais relevantes providenciada pelos estuários para as espécies de piscícolas. Os estados iniciais de desenvolvimento de muitas espécies de peixes marinhos tomam partido dos factores abióticos e bióticos favoráveis dos habitats estuarinos. Estes ecossistemas podem fornecer uma elevada disponibilidade de presas e refúgio contra a predação que maximizam o crescimento e sobrevivência dos estados iniciais de desenvolvimento. Os peixes chatos, incluindo a solha, *Platichthys flesus*, são utilizadores comuns dos estuários como zonas viveiro. Na realidade, *P. flesus* é uma das espécies de peixes chatos que utiliza o estuário do Lima como local de viveiro para os estados iniciais de desenvolvimento. Assim, este estudo pretende abordar a ecologia alimentar dos juvenis de *P. flesus* na área viveiro do estuário do Lima, bem como investigar as relações predador-presa que afectam os juvenis desta espécie. Com esse fim, foram realizadas quatro campanhas de amostragem em 2010 para recolher solhas juvenis, obter parâmetros abióticos e bióticos associados à ecologia alimentar na água e sedimentos. As comunidades de macroinvertebrados e crustáceos (*Crangon crangon* e *Carcinus maenas*), considerados as principais presas e predadores dos juvenis de peixes chatos, respectivamente, foram igualmente estudadas. Os padrões alimentares das solhas juvenis foram estimados através da análise de conteúdos estomacais, tendo sido identificadas as principais presas relativas às diferentes classes de tamanho. Os índices numérico, de ocorrência e gravimétrico, bem como os índices de importância relativa e de preponderância foram estimados para quatro classes de tamanho dos juvenis: classe 1: 0-49 mm TL; classe 2: 50-99 mm TL; classe 3: 100-149 mm TL, e classe 4: 150-199 mm TL. Adicionalmente, a selecção de presas, expressa pelo índice de selectividade de Strauss, foi investigada, com base em dados derivados da caracterização da comunidade de macroinvertebrados do estuário do Lima. A amplitude do nicho trófico (índices Shannon-Wiener a Levins) e a sobreposição da dieta entre classes de tamanho foram também determinadas. Para avaliar a pressão predatória pelo *C. crangon* e *C. maenas*, as suas densidades foram comparadas com as densidades das solhas e com a sua condição, expressa pelo índice de Fulton. Relativamente à comunidade de macroinvertebrados, os *Oligochaeta* ni, *Hediste diversicolor* e *Corophium* spp. foram os principais taxa encontrados. A abundância total da comunidade não apresentou nenhum padrão sazonal ou espacial evidente. Contudo, no estuário inferior, a macrofauna foi mais diversa e apresentou um maior número de espécies. A dieta dos juvenis incluiu macroinvertebrados, peixes, detritos vegetais e areia. De acordo com os índices

alimentares utilizados, *Corophium* spp. e os Chironomidae ni foram as principais presas das solhas juvenis. A dieta tornou-se gradualmente mais generalista à medida que os juvenis cresceram, incluindo presas de maiores dimensões. Contudo, não foram detectadas diferenças importantes entre a dieta das diferentes classes de tamanho. Por outro lado, a dieta das solhas apresentou alguma sazonalidade, associada a flutuações das presas macrobênticas no estuário do Lima. Apenas ocorreu sobreposição da dieta entre as classes 2 e 4, ambas apresentando *Corophium* spp. como uma das principais presas. A baixa sobreposição da dieta observada entre as diferentes classes de tamanho poderá ser indicativa de uma estratégia de particionamento de recursos que minimiza a competição intraspecífica. Assim, os presentes resultados parecem indicar que as alterações sazonais da dieta foram mais relevantes do que as variações entre classes de tamanho das solhas. De facto, essas alterações coincidiram com eventuais flutuações sazonais das presas macrobentónicas no estuário. A localização restrita das classes de menores dimensões na secção superior do estuário do Rio Lima é um indicador da função viveiro que esta zona desempenha. Adicionalmente, a escolha desta zona como viveiro poderá estar relacionada com a presença única de determinadas presas, nomeadamente os Chironomidae ni e *Corophium* spp., principais itens alimentares das classes de menores dimensões. Por outro lado, os resultados também demonstraram uma relação inversa entre as abundâncias de juvenis de solha com *C. maenas*, o que pode indicar uma possível pressão predatória. No entanto, a presença de *C. maenas* não afectou a condição dos juvenis, pelo que não ocorreram alterações aparentes no comportamento alimentar das solhas.

Abstract

The nursery function is one of the most relevant role that estuaries provide to fish species. Early life stages of many marine fish species make use of the favorable abiotic and biotic factors of the estuarine habitats. These ecosystems comprise high prey availability and refuge from predation that maximize growth and survival of the initial development stages of fishes. Flatfishes, including the flounder *Platichthys flesus*, are common users of estuaries as nursery grounds. In fact, *P. flesus* is one of the flatfish species that uses the Lima estuary as a nursery ground for early life stages. Thus, this study aimed the study of the feeding ecology of *P. flesus* juveniles in the Lima estuary nursery area and also to investigate the predator and prey relationships affecting juveniles of this species. For that purpose, four seasonal surveys were conducted in 2010 in order to collect flounder juveniles, as well as several abiotic and biotic parameters associated to the feeding ecology. Environmental parameters of the water column and sediments were analyzed, as well as the macroinvertebrates community and crustaceans (*Crangon crangon* and *Carcinus maenas*) considered as the main prey and predators of flatfish juveniles, respectively. The feeding patterns of the flounder juveniles were ascertained from the analysis of stomach contents, including the identification of the main prey items for the different size classes. Numerical, occurrence and gravimetric indices, as well as the relative importance and preponderance indices were estimated for four size classes of juveniles: class 1: 0-49 mm TL; class 2: 50-99 mm TL; class 3: 100-149 mm TL, and class 4: 150-199 mm TL. Furthermore, prey selection expressed by the Strauss elective index was also investigated, based on data derived from the characterization of the macroinvertebrates community of the Lima estuary. Niche breadth (Shannon-Wiener and Levins indices) and diet overlap between size classes were also determined. In order to assess potential predatory pressure, the influence of *C. crangon* and *C. maenas* on the juveniles flounder abundance, and on their condition, expressed by the Fulton's index, were determined. Regarding the macroinvertebrates community, Oligochaeta ni, *Hediste diversicolor* and *Corophium* spp. were the main taxa found. Overall macrofauna abundance did not present any important seasonal or spatial trend. However, in the lower estuary, the macrofauna was more diverse and comprised a higher number of species. The flounder juveniles diet included macroinvertebrates, fishes, plant debris and sand. According to the feeding indexes used, *Corophium* spp. and Chironomidae ni were the main prey items of flounder juveniles. The diet gradually became more generalist as juveniles grew, including prey with greater dimensions. However, no relevant differences

between the diet of the different size classes were detected. On the contrary, flounder diet showed some seasonality, what was associated with seasonal fluctuations of the macrobenthic prey in the Lima estuary. Diet overlap only occurred between classes 2 and 4, when *Corophium* spp. emerged as a major prey item. The reduced dietary overlap observed between different size classes may be indicative of resource partitioning strategy that minimizes intraspecific competition. Thus, the present results showed that seasonal changes in the macroinvertebrate prey availability might be more relevant in defining the diet of the juveniles than the size class of flounder. The restricted location of smaller classes in the upper estuarine section was an indicator of the nursery role of that area of the estuary. Moreover, the choice of this zone as nursery could be due to the presence of unique prey, namely Chironomidae and *Corophium* spp. main prey items of the smaller classes. On the other hand, results also showed an inverse relationship between the abundance of flounder juveniles and *C. maenas*, indicating a possible predatory pressure. However, the presence of *C. maenas* did not affect the juveniles condition, so no apparent changes in the feeding behavior emerged.

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1. Introduction

1.1 Estuarine environments

Estuaries have been classified as the most productive and valuable aquatic ecosystems on earth (Costanza et al. 1997), with high biological importance (Elliott and McLusky 2002; Yáñez-Arancibia and Day 2004). Several definitions have been proposed to these systems. Odum (1959) presented one of the earliest, stating that an estuary is “a river mouth where tidal action brings about a mixing of freshwater and saltwater”. Later, Pritchard (1967) defined an estuary as “semi-enclosed body of water which has a free connection with the open sea and within which sea water is measurably diluted with fresh water derived from land drainage”. This concept, however, did not consider the tidal influence. Thus, more recently, Dyer (1997), developed the concept proposed by Pritchard, taking into account the tidal influence: “an estuary is a semi-enclosed coastal body of water which has a free connection to the open sea, extending into the river as far as the limit of the tidal influence, and within which sea water is measurably diluted with fresh water derived from land drainage”.

As transition areas between freshwater and salt water, extreme gradients are often observed within estuarine chemical and physical variables, namely salinity, temperature, pH, dissolved oxygen, nutrients and quantity and quality of particles. These environmental gradients favor the recruitment of a variety of species with diverse physical and trophic structures (Harris et al. 2001). Freshwater inputs support high primary productivity by the existent phytoplankton, benthic algae and emergent vegetation (Odum 1959; Day et al. 1989), whose decomposition is essential to maintain the complex estuarine food webs. Indeed, the high estuarine productivity, combined with high food and refuge availability, supports high abundances of organisms, such as fishes, crustaceans and also macroinvertebrates. However, diversity is generally low in these habitats because few species have adapted to the physiological stress induced in organisms by the estuarine environmental oscillations (McLusky and Elliott 2004).

1.2 Estuarine communities

Despite the transitional and unstable nature of estuaries, these are the temporary or permanent habitat for several animals and plants (McLusky and Elliott 2004). Macroinvertebrates are one of the most relevant groups of the estuarine communities, including freshwater and marine species (Edgar and Shaw 1995). These organisms represent an important link in the energy flow to higher trophic levels, recycling organic matter in marine and estuarine ecosystems (DeLancey 1989; Edgar and Shaw 1995). Moreover, they also constitute important food sources for several demersal fish and invertebrate species.

The estuarine fish fauna includes both resident and transient species at different life stages (Able and Fahay 1998) and with different life history patterns (Haedrich 1983). However, fish diversity in these ecosystems is low, compared to the adjacent continental shelf because few species are adapted to the constant environmental oscillations (McLusky and Elliott 2004). In consequence, a reduced number of species, most of them small in size, tends to dominate the ichthyofauna, not only in numbers but also in biomass (Elliott et al. 1990; Whitfield 1994b). Estuarine fish communities have been extensively studied worldwide, and there have been several attempts to define common features of these communities in order to apply these criteria to the different types of estuaries (e.g. Elliott and Dewailly 1995; Mathieson et al. 2000; Elliott and Hemingway 2002; Able 2005). In this context, fishes are often classified into different guilds, which are defined as groups of species that exploit the same class of environmental resources in a similar way (Root 1967). The functional guild approach assigns fishes of estuarine assemblages into different functional guilds, according to their estuarine use, mode of feeding and reproductive strategy (Franco et al. 2008). According to the ecologic guilds proposed by Elliot et al. (2007), fish can be classified into the following functional groups:

- Marine stragglers - species that spawn at sea and typically enter estuaries only in low numbers and occur most frequently in the lower reaches where salinities are approximately 35 psu. These species are often stenohaline and associated with coastal marine waters;
- Marine migrants - species that spawn at sea and often enter estuaries in large numbers and particularly as juveniles. Some of these species are highly euryhaline and move throughout the full length of the estuary. This group is divided into marine estuarine-opportunist species and marine estuarine

dependent species;

- Estuarine species – this category is divided in two groups: estuarine residents, species capable of completing their entire life cycle within the estuarine environment, and estuarine migrants, species that have larval stages of their life cycle completed outside the estuary or are also represented by discrete marine or freshwater populations;
- Anadromous - species that undergo their greatest growth at sea and which, prior to the attainment of maturity, migrate into rivers where spawning subsequently occur;
- Semi-anadromous - species whose spawning run from the sea extends only as far as the upper estuary rather than going into freshwater;
- Catadromous - species that spend all of their trophic life in freshwater and which subsequently migrate out to sea to spawn;
- Semicatadromous - species whose spawning run extends only to estuarine areas rather than the marine environment;
- Amphidromous - species which migrate between the sea and freshwater and in which the migration in neither direction is related to reproduction;
- Freshwater migrants - freshwater species found regularly and in moderate numbers in estuaries and whose distribution can extend beyond the oligohaline sections of these systems;
- Freshwater stragglers - freshwater species found in low numbers in estuaries and whose distribution is usually limited to the low salinity, upper reaches of estuaries.

Estuaries provide a diversity of roles for many fish species, both resident and transient, with marine species visiting these habitats for feeding, reproduction, growth and protection (Able and Fahay 1998). One of the most relevant roles is the nursery function, provided to transient species, such as migratory anadromous and catadromous species, as well as marine species, whose larvae and juveniles inhabit the estuaries temporarily. A nursery habitat may be described as a restricted area where initial development stages of a species spend a limited period of their life cycle, during which they are spatially and temporally separated from the adults (although some spatial overlap may occur). In these areas, the survival of initial development stages is enhanced through optimal conditions for feeding, growth, and/or predation refuge (Beck et al. 2001; Pihl et al. 2002; Beck et al. 2003). Recently, Beck et al. 2001 proposed that a habitat only functions as a nursery

when its contribution with new recruits to the adult populations per unit area is greater, on average, than other juveniles habitats. However, larger habitats with less contribution per unit area might as well be essential fish habitats (Dahlgren et al. 2006).

1.3 Estuarine nursery use by flatfish species

Flatfish are among the fish that use estuaries as nursery areas. Generally, nurseries grounds are reached by the early life stages, either before or after the larvae undergo metamorphosis and settlement, two processes closely associated. The metamorphosis involves a series of morphological (e.g. eye migration, completion of squamation and full pigmentation), anatomical and physiological transformations (Able and Fahay 1998) that enable the shift from the symmetric pelagic larva to a benthic juvenile form during settlement. The settlement process can be either direct, when pelagical larvae enter the estuarine nurseries where they settle after metamorphosis; or indirect when it occurs in the coastal areas and then the newly settled juveniles migrate to nursery areas (Gibson 1973; Lockwood 1974). Settlement should occur in areas with high prey abundance and low predatory risk, in order to maximize growth and survival of the initial development stages (Lenanton and Potter 1987; Bergman et al. 1988; Gibson 1999; Beck et al. 2001). In fact, recruitment to a suitable nursery area is crucial for the survivorship of young flatfishes and, ultimately to the species recruitment success (van der Veer et al. 2001). It is thought that settlement, as well as the habitat and behavioral changes associated, rather than metamorphosis per se, may have a greater impact on successful recruitment of the flatfishes (Geffen et al. 2007).

Habitat selection in the nursery areas results from a compromise between different environmental factors, including biotic and abiotic (Burrows 1994; Hugie and Dill 1994). The influence of each factor varies throughout the ontogenetic development (Phelan et al. 2001) and also at a variety of temporal and spatial scales (Gibson et al. 1996). For instance, temperature and salinity may exhibit gradients at a variety of temporal and spatial scales (Gibson 2005), therefore determining the distribution of individuals within a nursery area, although they may exert no effect in nursery areas where they show none or little variations. Also, as diet and main predators change throughout ontogeny, juveniles may reorganize their distribution in function of these factors (Burke 1995; Modin and Pihl 1996; Castillo-Rivera et al. 2000) explain this, is not clear. Furthermore, both differences

in ontogenetic state and seasonal fluctuations in the abiotic and biotic factors act together to produce characteristic distribution patterns and differential habitat use at different spatial and temporal scales (Gibson et al. 1996). Small scale differences in habitat characteristics might influence distribution, creating patchy distribution patterns (Modin and Pihl 1996).

Several abiotic factors, namely salinity (Vinagre et al. 2006; Andersen et al. 2005; Ramos et al. 2009), temperature (Power et al. 2000), depth (Vinagre et al. 2006; Cabral et al. 2007; Vasconcelos et al. 2010), dissolved oxygen (Power et al. 2000; Maes et al. 2007), turbidity and sediment composition (Gibson 1994; Stoner et al. 2001; Zuccheta et al. 2010) have influence on the habitat selection within the nursery areas. The correlation between the abiotic factors and abundance of juveniles does not imply that these factors have a direct effect on the distribution patterns. Instead, abiotic variables may be proxies for biological attributes of the habitat, such as reduced risk of predation or high food availability (Gibson 2005). For instance, sediment type is hypothesized to act indirectly by influencing prey distribution and abundance (Gibson 1994; McConnaughey and Smith 2000; Amezcua and Nash 2001) and also controlling the fish ability to dig (Gibson and Robb 1992), in order to escape predation. Thus, abiotic factors can be used by flatfishes to locate areas with favorable biotic conditions. Studies showing that physical variables were not enough to explain variability in flatfish juveniles distribution (Le Pape et al. 2007) and that biotic factors such as predation pressure and prey availability affected the habitat selection by juveniles (Adams et al. 2004; Le Pape et al. 2007), seem to corroborate this theory.

Macroinvertebrates are one of the main prey items of flatfish juveniles as evidenced by diet studies (e.g. Aarnio et al. 1996; Cabral et al. 2002; Link et al. 2002). Besides providing a quantitative description of the diet of the target fish, diet studies may also give valuable information about the spatial and temporal variations and the degree of specialization of their diet, thus assessing the habitat use and ecological niche they occupy, as well as similarities and possible competition for resources between populations and different species (Marshall and Elliott 1997). Therefore, the study of the diet throughout different life stages in a given habitat provides information about the ecological niches and interaction between cohabiting sizes (Knight and Ross 1994; Haroon and Pittman 1998; Darnaude et al. 2001; Cabral et al. 2002; Vinagre et al. 2005). Generally, ontogenetic shifts in the diet are responsible for a decrease in intraspecific niche overlap

between flatfish size classes (Darnaude et al. 2001). Many studies have also compared macroinvertebrates communities of a nursery habitat with the juvenile flatfish stomach contents, in order to evaluate prey selection and relate macroinvertebrates with juvenile distribution patterns. These studies have concluded that the often patchy distribution of macroinvertebrates, presenting variable densities, is an important factor affecting juveniles' distribution (Andersen et al. 2005; Vinagre et al. 2005; Vinagre and Cabral 2008).

Several factors affect prey selection by fish, namely prey availability, prey and predator characteristics and predator ability to detect the prey. For a prey to be incorporated in the diet of a fish, must be available and accessible, considering the constraints imposed by the morphology and sensority capacities of the fish. Prey characteristics, such as size, contrast with the background and movement, and predator characteristics, such as visual acuity, body form and locomotion of the predator that determine their ability to successfully capture the prey, must be taken into account (Wootton 1998). Diel changes in the diet often occur and probably reflect changes in prey activity, hence, prey vulnerability. Seasonal changes may also occur and are related with variations in the habitats availability for foraging, changes resulting from the life history patterns of prey organisms and changes caused by the feeding activities of the fish themselves (Wootton 1998).

During the early pos-settlement period, flatfishes are most vulnerable to predation, responsible for the higher mortality rates observed compared to other life stages (Van der Veer 1986; Beverton and Iles 1992; Sogard 1997). Indeed, predation is thought to be the main responsible for 0-group juveniles mortality in nursery sites (Steele and Edwards 1970; Van der Veer and Bergman 1987; Van der Veer 1991), causing a rapid depletion of juveniles after their arrival to the estuarine nurseries (Van der Veer 1991; Beverton and Iles 1992). Several studies have demonstrated predation as a density-dependent mortality cause (Lockwood 1980; Van der Veer 1991; Nash and Geffen 2000).

Size is an important factor affecting an individual vulnerability to predation (Van der Veer and Bergman 1987; Witting and Able 1993; Wennhage 2000), and smaller individuals of early life stages are generally more vulnerable, being consumed by a broader taxonomic variety and range size of predators (Ellis and Gibson 1995). The "bigger is better" hypothesis predicts that there is a proportional relationship between size and vulnerability

to predation (Litvak and Leggett 1992; Leggett and Deblois 1994). Therefore, the faster the growth, the less is the fish vulnerability and, consequently, lower is the predation impact on juvenile flatfishes. However, the smallest flatfish individuals (8-12 mm TL) may experience less predatory encounter rates, thus being less vulnerable than the intermediate size ones (13-17 mm TL) (Taylor 2003). Besides the direct effects on mortality of the juveniles, the presence of predators also drives changes in feeding activity, affecting growth of fishes (Jones and Paszkowski 1997; Maia et al. 2009), as well as in the settlement behavior and thus influencing the habitat selection (Wennhage and Gibson 1998). In fact, changes in predation risk may be responsible for ontogenic habitat shifts in juvenile flatfish (Werner and Gilliam 1984; Halpin 2000; Byström et al. 2003). Hence, the magnitude of predation is determined by the juvenile growth rates, the timing and location of settlement, habitat choice and therefore the degree of overlap in size distribution of juvenile flatfish and their predators (Ellis and Gibson 1995).

Although major predators of juvenile flatfish differ among nurseries (Van der Veer et al. 1990), crustaceans have been recognized as important predators across different nursery areas (Wennhage and Gibson 1998; Ansell et al. 1999). Several studies identified the shrimp *Crangon crangon* and the shore crab *Carcinus maenas* as important predators (Ansell et al. 1999), causing a significant density dependent mortality in flatfish populations (Van der Veer and Bergman 1987). Actually, these crustaceans may be responsible for the regulation of many flatfish populations (Van der Veer 1986; Van der Veer and Bergman 1987; Van der Veer et al. 1990), minimizing interannual variations in year class strength that result from the pelagic phase (Van der Veer and Bergman 1987). According to Van der Veer and Bergman (1987), newly settled flatfish are vulnerable to shrimp until attaining a refuge size of 30 mm for shrimp predation and 50 mm for *Carcinus* spp.. Only *C. crangon* and *C. maenas* of a minimum size of 30 mm length and 26 mm carapace width, respectively, can prey upon the juvenile flatfish (Van der Veer and Bergman 1987; Ansell et al. 1999; Van der Veer et al. 2000).

1.4 The flounder, *Platichthys flesus*

The flounder (*Platichthys flesus* Linnaeus, 1758) is a ray-finned (Class Actinopterygii) flatfish (Order Pleuronectiformes), right eye flounder (Family Pleuronectidae) species (Figure 1.1), reaching up to 60 cm and 2.5 Kg (Munk and Nielsen 2005). The flounder has an ellipsoid body form and it usually has the eyes on the right side, but in some areas up to 30 % flounders are left-eyed. There are small, body knobs especially along the lateral line, a rough scale at the basis of each dorsal and anal fin ray and the body often presents red spots.

The flounder geographical distribution ranges from the White Sea in the North to the Mediterranean and the Black Sea (Ré and Meneses 2009), and the Portuguese coasts have been pointed as the Southern distribution limit (Cabral et al. 2007). *P. flesus* is a common species around the coasts of northern Europe and the Mediterranean, where it is an important component of demersal fish assemblages economically exploited (Maes et al. 1998; Thiel and Potter 2001; Ramos et al. 2010). According to FAO (2011), there was an increase of the flounder reported global landings between 1950 and 2009. In fact, the minimum of 7,407 tonnes reported in 1970, peaked to the maximum of 24,461 tonnes registered in 2005 (FAO, 2011). The countries with the largest catches in 2006 were Poland (42.1%), Netherlands (18.0%) and Denmark (15.1%) accounting for 75.2% of the total catches (22,739 tonnes) (FAO, 2011). Portugal accounted only for 0.06. % of the global catches (FAO, 2011). However, *P. flesus* is one of the dominant flatfish species and an important commercial species in the Portuguese estuaries, where their nursery grounds are mainly located in low salinity areas (Cabral 2000; Vinagre et al. 2005; Martinho et al. 2007; Cabral et al. 2007; Vasconcelos et al. 2009; Freitas et al. 2009; Ramos et al. 2010).

Flounder spends most of its lifecycle in estuaries. This species occurs on fine sandy and muddy bottoms from shallow water down to 50 m, typical of sheltered and low saline areas (Riley et al. 1981), spending most of the day buried into the sediment. *P. flesus* is a euryhaline species, tolerating salinities from 0 to 35 and it also demonstrates a great tolerance to temperature (5 -25 °C) (Baensch and Riehl 1997) and oxygen (Muus 1967; Kerstens 1979). Sexual maturity is attained at 2-4 years age. This species is oviparous and spawning takes place from January to July (Munk and Nielsen 2005). The pelagic eggs present 0.82-1.13 mm in diameter (Munk and Nielsen 2005) and larvae, also

pelagic, hatch after 5-7 days (Russel 1976) with 10-12 mm body length (Ré e Meneses 2009). The young flounders leave the plankton towards the bottom (settlement) at a length of about 10 mm, when the left eye has reached the dorsal ridge, when metamorphosing is complete. While the adult flounders migrate offshore from the estuaries to spawn, the post larvae generally occur nearer the shore than other pleuronectids (Russel 1976).

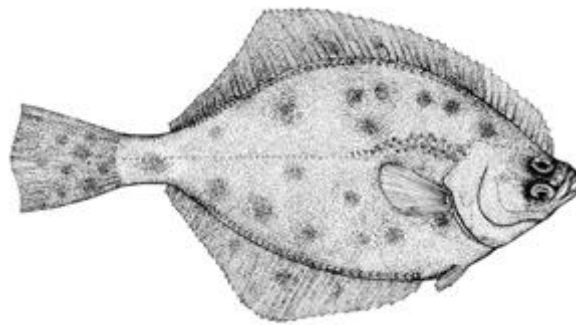


Figure 1.1 – The flounder, *Platichthys flesus*.

Considering the life cycle of *P. flesus*, it is not clear to which ecological functional guild should flounder be assigned, according to their estuarine use. For example, flounder may be viewed as a catadromous species (McDowall 1988) (Figure 1.2a), although there is no obligate freshwater phase in their lifecycles (Elliot et al. 2007). Some may also consider it as a semi-catadromous species (Figure 1.2b) because rivers are not their first choice at any life stage, although these habitats are often occupied (Elliot et al. 2007). However, recent evidence of the use of estuaries as spawning grounds (Morais 2011) discards this species as exclusively catadromous. It is also classified as an estuarine resident (Figure 1.2c), despite the spawning emigration to the sea, with their larvae using selective tidal stream to immigrate to the estuaries (Elliot et al. 2007). *P. flesus* may also be regarded as a marine estuarine-opportunist, as spending most of their life in the estuaries, but also using nearshore marine waters as an alternative habitat, such as what occurred in the Bristol Channel, a marine embayment located outside the Severn estuary (Claridge et al. 1986). At last, it is also sometimes classified as an estuarine migrant (Figure 1.2d), because it migrates between marine and estuarine environments throughout its lifecycle, although spending most of it in estuarine areas (Elliot et al. 2007).

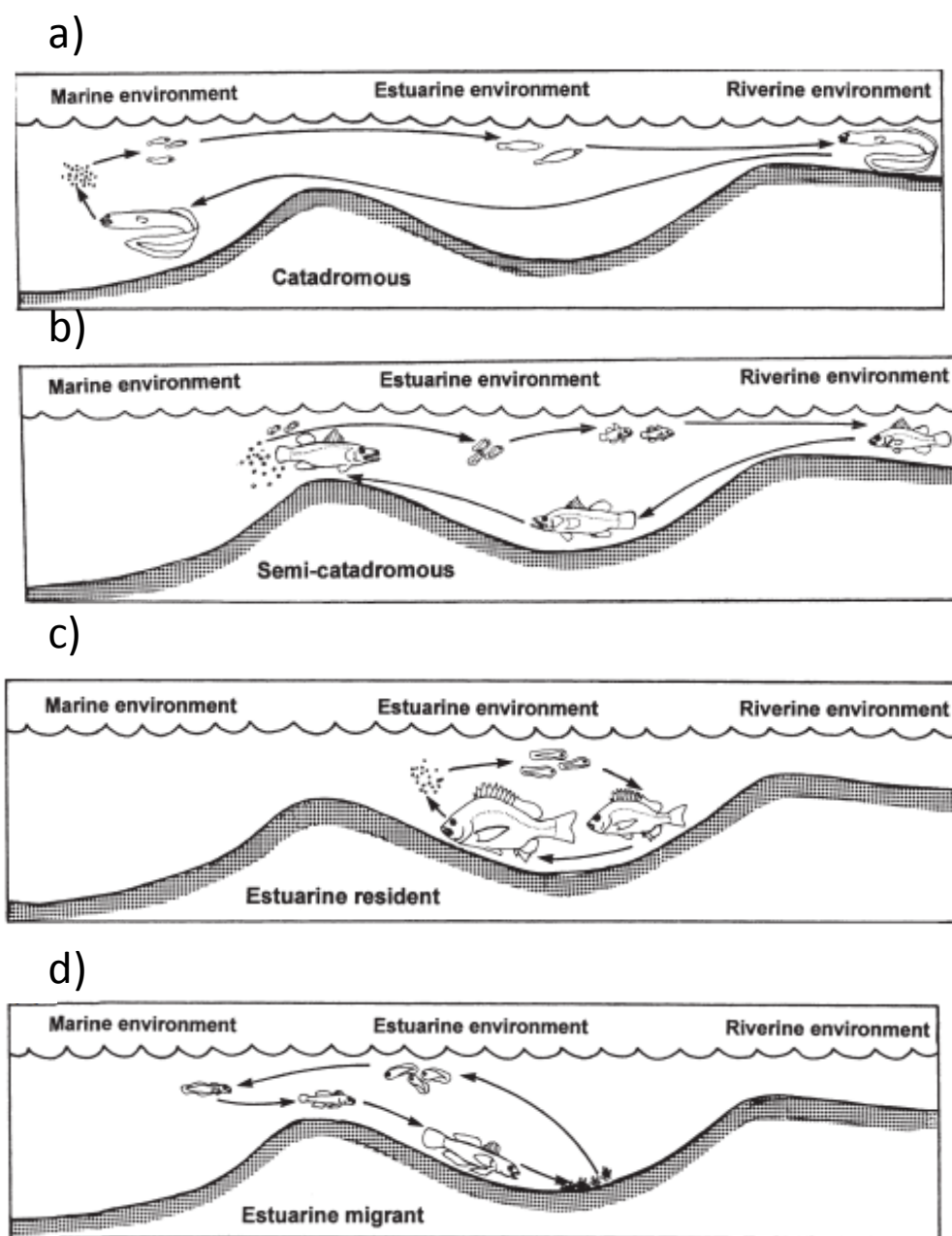


Figure 1.2 – Different life cycle categories proposed for *Platichthys flesus*: a) Catadromous b) Semi-catadromous c) Estuarine resident d) Estuarine migrant (adapted from Elliot et al. 2007).

Estuarine and other shallow water areas are usually used as feeding and nursery grounds (e.g. Summers 1979, Van der Veer et al. 1991; Cabral et al. 2007; Ramos et al. 2010). Feeding grounds are mainly intertidal mudflats, estuarine and coastal areas. It is widely accepted that flounder is a day-feeder (De Groot 1971; Matilla and Bonsdorff 1998), with feeding peak activities at dawn and dusk (De Groot 1971; Muus 1967). As a visual predator, it usually feeds upon active mobile prey, such as amphipods (De Groot 1971),

presenting an opportunistic behavior (De Groot 1971), feeding on the most available macroinvertebrate prey. The diet of *P. flesus* has been broadly studied across European nursery grounds, along northwestern Europe (e.g. Jager et al. 1995; Aarnio et al. 1996) and in the Black Sea (e.g. Banaru and Harmelin-Vivien 2009), and also along the Portuguese coast (Teixeira et al. 2010) and estuaries (Costa and Bruxelles 1989; Martinho et al. 2008; Vinagre et al. 2005). Juveniles main prey items include crustaceans (Teixeira et al. 2010), specially amphipods (Vinagre et al. 2005), polychaetes (Vinagre et al. 2005), oligochaetes, chironomids (Florin and Lavados 2010), bivalves (Pihl 1982) and mysids (Mariani et al. 2011). The amphipod *Corophium* spp. and the polychaete *Hediste diversicolor* were shown as important prey items in the Danish east coast (Andersen et al. 2005) and in several estuaries across different geographical locations, namely in the Schelde estuary (Hampel et al. 2005; Stevens 2006), and Tejo (Costa and Bruxelles 1989) and Douro Portuguese estuaries (Vinagre et al. 2005). Environmental factors, such as wave exposure and vegetation, and also prey related factors like size, burrowing ability, mobility and diel activity pattern can have an effect on the flounder diet (Florin and Lavados 2010). Seasonal variations of prey availability may reflect in seasonal variations in the type of prey consumed (Aarnio et al. 1996). The diet also varies along ontogeny and between different juvenile size classes (Ustups et al. 2003). Moreover, Aarnio et al. (1996) also reported a transition from meio- to macrofauna preys, when juveniles reach 45 mm total length. As juveniles develop, the diet tends to become more diverse, registering a gradual shift from smaller prey such as amphipods to larger prey as polychaetes and bivalves (Vinagre et al. 2008). Nevertheless, small prey still continues to be consumed by all flounder size classes (Vinagre et al. 2008).

In the nursery grounds, the juvenile flounder environmental control seems to be related to abiotic factors, such as depth (Cabral et al. 2007; Vasconcelos et al. 2009), salinity (Vinagre et al. 2005; Ramos et al. 2009; Zuccheta et al. 2010), temperature (Power et al. 2000), dissolved oxygen (Power et al. 2000; Maes et al. 2007), sediment type (Amezcuca and Nash 2001; Vinagre et al. 2005; Zuccheta et al. 2010) and turbidity (Zuccheta et al. 2010). Although flounder is known to be an euryhaline species (Power et al. 2000), 0-group juveniles are usually concentrated in low-salinity areas with mesohaline or polyhaline waters (Jager 1998; Vinagre et al. 2005; Van der Veer et al. 1991; Anderson et al. 2005; Ramos et al. 2009). Several authors reported temperature as a strong predictor of juveniles flounder distribution (Freitas et al. 2009; Marshall and Elliott 1998; Power et al. 2000; Vasconcelos et al. 2009), although Martinho et al. (2009) found no relationship.

Besides affecting physiological tolerances and preferences (Power et al. 2000) and also interactions with other physico-chemical variables such as dissolved oxygen (Marchand 1993), temperature also affects growth (Yamashita et al. 2001, Stevens et al. 2006) and processes such as spawning time (Sims et al. 2004), migration (Stevens 2006), and recruitment patterns (Marshall and Elliot 1998), thus indirectly affecting flounder juvenile distribution within estuarine habitats. Dissolved oxygen is also a known factor affecting flatfish distribution (Pomfret et al. 1991; Marchand 1993). In the Lima estuary, juvenile flounder was associated to areas with high oxygen saturation values (Ramos et al. 2009). Regarding the sediments, flounder seems to have a preference for fine sandy and muddy bottoms (Riley et al. 1981; Greenwood and Hill 2003), typical of more sheltered and less saline areas (Gibson 1994), which may be related to prey availability (Gibson 1994; Amezcua and Nash 2001). In the Lima estuary, it was suggested that juvenile flounder spatial distribution could have been related to sediment composition, possibly through effects on prey availability (Ramos et al. 2009). A preference for turbid waters is also known, since these areas may present large food resources (Power et al. 2000; Zuccheta et al. 2010).

In addition to the vast list of abiotic parameters, biotic factors such as prey and predator availability (Gibson 1994; Power et al. 2000; Cabral et al. 2007) also influence the juvenile flounder distribution within the estuarine nursery grounds. On the contrary to the abiotic parameters, few studies had approached the effects of prey-predator interactions influence on flounder nursery habitats. However, these factors are thought to have great relevance in flatfish distribution patterns, including *P. flesus*. For example, flounder densities are generally positively correlated with macrozoobenthos densities, their main prey. In fact, macroinvertebrates density has been included in the fish distribution models, in order to enhance the predictability of the high flounder density areas (Nicolas et al. 2007; Vinagre et al. 2009; Vasconcelos et al. 2010).

Flounder juveniles may present an ontogenetic differential distribution along a depth gradient, with smaller individuals occurring in shallower water (Martinsson and Nissling 2011). As the diet varies along ontogenetic development, differences in the diet may be responsible for this distribution pattern (Burke 1995, Modin and Pihl 1996, Castillo-Rivera et al. 2000). It is also hypothesized that changes in predation risk may be responsible for these ontogenetic habitat shifts (Werner and Gilliam 1984; Byström et al. 2003; Manderson et al. 2006). In this context, smaller individuals usually concentrate in more

shallow areas where they may escape larger predators (Gibson et al. 2002) and the distribution becomes broader as juveniles develop and attain a size refuge from different types of predators. The crangonids shore crab *Carcinus* and shrimp *Crangon* are important predators of juvenile flounder (Ansell et al. 1999, Van der Veer et al. 1991) whose vulnerability is highest during larval immigration and at 8 mm size (Van der Veer et al. 1991). There is a lack of studies relating predation pressure by crustaceans on flounder abundances. Henderson and Seaby (1994) and Power (2000) found no relationship between the predator shrimp *Crangon* and flounder abundances, although Power et al. (2000) highlighted that most of the fish sampled were outside the predation range (> 30 mm; Van der Veer and Bergman 1987) of that predator. Modin and Pihl (1996), however, found evidence of negative influence of the brown shrimp on the small-scale distribution of young juvenile flounder.

1.5 Objectives

As a common user of estuarine and other shallow coastal areas as nurseries and attending to the economical importance of the species, flounder juveniles diet has been widely studied throughout European nurseries, as mentioned above. Besides providing a quantitative description of the diet of the target fish, feeding ecology studies may also give valuable information about the spatial and temporal variations of fish abundance. Moreover, these studies also allow to estimate the degree of specialization of fish diet and assess the habitat use and ecological niche they occupy, as well as similarities and possible competition for resources between populations and different species (Marshall and Elliot 1997). Therefore, the study of the diet throughout different life stages in a given habitat provides information about the ecological niches and interactions between cohabiting sizes (Cabral et al. 2002, Vinagre et al. 2005). Usually, ontogenetic shifts in the diet are responsible for a decrease in intraspecific niche overlap between size classes (Keast 1977, Pen et al. 1993, Darnaude et al. 2001).



Figure 1.3 – The Lima estuary at Viana do Castelo, Portugal.

The Lima estuary, NW Portugal (Figure 1.3), has been identified as an important nursery area for several flatfish species, including the larval and juvenile stages of flounder, *P. flesus*, (Ramos et al. 2010). Thus, the present study aims to:

- study of the feeding ecology of the flounder juveniles in the Lima estuary;
- evaluate prey selection by the flounder juveniles;
- investigate the potential predatory impact of crustaceans predators, such as the shore crab (*Carcinus maenas*) and the shrimp (*Crangon crangon*).

Such studies were never performed in the Lima estuary nursery area, thus the results will give *valuable insights* for the feeding patterns of *P. flesus*, and also on the prey-predator relationships affecting their distribution. Also, given the need to identifying and conserving essential habitat and considering the economical importance of flounder, understanding how the biotic factors affect the distribution dynamics of flounder during their development is crucial in order to take appropriate management decisions.

2. Material and Methods

2.1 Study Area

The Lima River is an international water body, with a water basin located in the northern region of the Iberian Peninsula, covering approximately 2,480 km² of which 1,177 km² (47%) are located in the Portuguese territory. It has two large hydroelectric dams (Alto do Lindoso and Touvedo) in operation since 1992. The Lima estuary is a small open estuary with a semidiurnal and mesotidal regime (3.7 m). Salt intrusion can extend up to 20 km upstream, with an average flushing rate of 0.4 m s⁻¹ and a residence time of 9 days (Ramos et al. 2006). From 1967 to the present, the estuary suffered heavy modifications for commercial navigation and fisheries purposes. Nowadays, the river mouth is partially obstructed by a 2 km long jetty, causing a deflection of the river flow to the south.

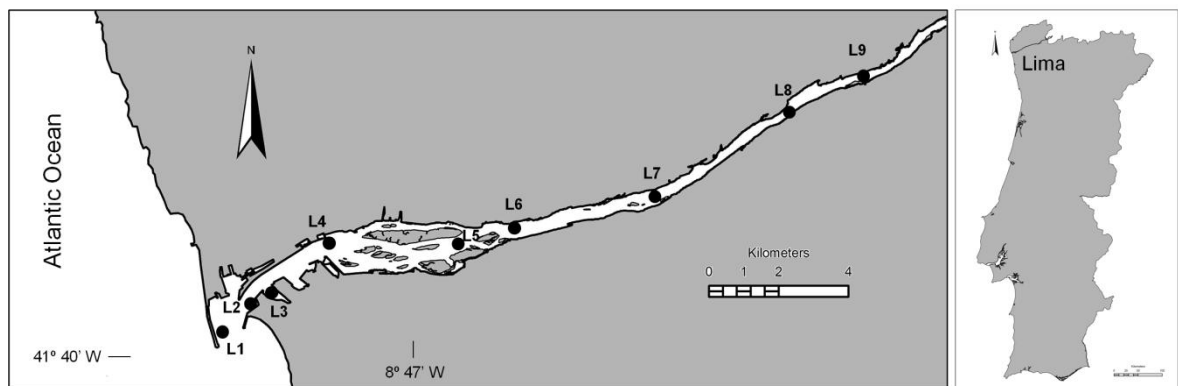


Figure 2.1 – Lima estuary with the location of the nine sampling stations (L1-L9).

For this study, nine sampling stations covering the lower, middle and upper estuary were chosen. The lower estuary (stations L1-L3), located in the initial 2.5 km, is a narrow, deep navigational channel, highly industrialized, with walled banks. It includes a large shipyard, a commercial seaport, and a fishing harbour. The average depth of 10 m is maintained by constant dredging. The middle estuary (stations L4-L6) comprises a broad shallow intertidal saltmarsh zone, mainly colonized by the common rush (*Juncus* spp.), with a large longitudinal sandy island (Cavalar Island). During high tide, mean depth is 4 m, but this zone is almost completely drained during low tide. This saltmarsh area is an important wetland that provides food and shelter to vertebrates such as mammals, birds, reptiles, amphibians and fishes (PBHL 2002). The upper estuary (Stations L7-L9) is a narrow

shallow channel, less disturbed, with natural banks and few presenting intertidal banks and sand islands.

2.2 Data Collection

In order to study the feeding ecology of *P. flesus* juveniles in the Lima estuary, environmental and biological data were collected in 2010, in the lower, middle and upper estuarine sections. Seasonal surveys, including winter (February), spring (April), summer (July) and autumn (October) were performed during the nightly ebb tides. In addition to the collection of *P. flesus*, sampling also contemplated the macroinvertebrate community, considered to be the flounder main prey items (Andersen et al. 2005; Hampel et al. 2005; Martinho et al. 2008) as well as their crustacean predators *C. maenas* and *C. crangon* (Ansell et al. 1999; Van der Veer et al. 1991).

2.2.1 Environmental parameters

This component included the collection of physical parameters of the water column as well as sediment samples for grain characterization and organic matter content estimation. At each sampling station, vertical profiles of temperature and salinity were obtained by means of a YSI 6820 CTD. Similarly, at each sampling station, triplicate sediment samples were taken using a Petit Ponar grab with an area of 0.023 m². Samples were stored at 4 °C in plastic bags for further laboratory procedures.

2.2.2 Macroinvertebrates

Three replicates per sampling station were collected with a Petit Ponar grab with an area of 0.023 m². Samples were fixed in 5 % buffered formalin stained with Rose Bengal and stored for further laboratory analysis.

2.2.3 Fish and crustaceans

Flounder juveniles, as well as their crustaceans predators, the shore crab *C. maenas* and the shrimp *C. crangon*, were collected with a 2 m beam trawl, with a mesh size of 5 mm in the cod end and a tickler chain. Trawls were made at a constant speed and lasted 10 min. Samples were refrigerated in boxes with ice and transported to the laboratory where they were frozen until sorting. Geographic location of the sampling stations and distance traveled during each tow was measured by a Magellan 315 GPS.

2.3 Laboratory Procedures

2.3.1 Sediment characterization

Unfixed sediments were treated in order to determine the percentage of organic matter, by drying the samples at 105 °C (24 h) and then by loss on ignition at 500 °C (4 h; APHA, 1992). Sediments were previously dried at 100 °C and grain size analysis was performed by wet (fraction < 0.063 mm) and dry (other fractions) sieving (CISA Sieve Shaker Mod. RP.08) of samples. Sediments were divided into four fractions: silt and clay (<0.063 mm), fine sand (0.063–0.250 mm), sand (0.250–1.000 mm) and gravel (>1.000 mm). Each fraction was weighed and expressed as a percentage of the total weight.

2.3.2 Macroinvertebrates

Sediment samples were sieved on a 0.5 mm mesh size and the macroinvertebrates were kept in 70 % alcohol until sorting. Macroinvertebrates were then counted and identified to the species level whenever possible, using a binocular magnifier (Leica MZ12-5). Whenever individuals were fragmented, only the heads were considered for counting purposes.

2.3.3 Fish

Flounder specimens were sorted from the beam trawl samples. Fishes were measured in terms of total (TL) and standard length (SL) (1 mm precision), and weighed (wet weight, 0.01 mg precision). Considering that the length at first maturation is 200 mm TL (Diniz 1986), fishes presenting less than 200 mm TL were considered juveniles. The maximum mouth gape width (mm) of the juveniles was measured.

Stomachs were excised, contents removed and preserved in alcohol 70 %, for further prey identification. Each prey item was identified to the lowest taxonomic level possible, using a binocular magnifier (Leica MZ12-5), counted and weighed (wet weight to 0.001 g). Whenever individuals were fragmented, only the heads were considered for counting purposes. In addition, the minimum and maximum prey lengths (mm) of each stomach were determined.

2.3.4 Crustaceans

Similarly to *P. flesus*, *C. maenas* and *C. crangon* were also sorted from the beam trawl samples. The body measurements considered were the total length for the shrimps and carapace width for the crabs (1 mm precision).

2.4 Data Analysis

2.4.1 Macroinvertebrates community

Macroinvertebrates abundance data was standardized as the number of individuals per m² of sediment. Frequency of occurrence was determined for each taxon. Diversity of macrobenthos was expressed by the Shannon-Winner index (H') (Shannon and Weaver, 1949):

$$H' = \sum_{i=1}^s P_i \cdot \ln P_i,$$

Where P_i is the numerical proportion of the i th macroinvertebrate species in the environment and s is the total number of different macroinvertebrate taxonomic groups in the environment. Equitability was also measured by the Pielou's evenness index (J') (Pielou, 1966):

$$\frac{H'}{H'_{max}} = \frac{\sum_{i=1}^s P_i \cdot \ln P_i}{\ln s}$$

Two-way ANOVA was performed to assess spatial and temporal differences on the macrofauna abundance, diversity (H') and equitability (J'), with estuarine sections and seasons as fixed factors. Abundance data was log transformed ($\log(x + 1)$). Furthermore, in the event of significance, a posteriori Fisher was used to determine which means were significantly different at a 0.05 level of probability (Zar, 1996). These analyzes were performed with Statistica software (version 10.0, Statsoft Inc., Tulsa, OK, USA). Two-way crossed ANOSIM was performed to investigate seasonal and spatial variations of the macrofauna species structure. The similarities percentage procedure (SIMPER) was used to assess which species contributed more to the dissimilarities observed. These analyzes were performed with the PRIMER statistical package (Plymouth Marine Laboratory, PRIMER v6).

2.4.2 Flounder diet

Trawl opening (2 m) and distance travelled (determined by GPS) were used to estimate the sampled area and densities were standardized as the number of individuals per 1000 m² swept. Fishes were divided into four size classes according to their total length: class 1 (0-49 mm), class 2 (50-99 mm), class 3 (100-149 mm) and class 4 (150-199 mm). Fish condition was assessed by the Fulton's condition factor, K , determined from morphometric data with the formula:

$$K = 100 \cdot \frac{W_t}{L_t^3}$$

where W_t is total wet weight (mg) and L_t total length (mm) (Ricker, 1975).

Feeding activity was evaluated by the vacuity index (I_v), defined as the percent of empty stomachs (Hyslop 1980). Several dietary indices were used to quantitatively describe the fish diet and also to assess the relative contribution of the different prey taxa, such as:

- numerical index (NI) – percentage of the number of individuals of a prey item over the total number of individuals of all prey;
- occurrence index (OI) – percentage of non-empty stomachs in which a prey occurred over the total number of occurrences;
- gravimetric index (GI) – percentage in weight of a prey item over the total weight of all prey (Hyslop, 1980).

Thus, the relative importance of each prey item in the *P. flesus* diet was evaluated by these three indices. Accordingly to Hyslop (1980), none of these indices should be used individually, given that each one can over- and underestimate a given group of prey. For example, the numerical index overestimates small prey that are generally present in the stomach in higher numbers, contrarily to the gravimetric index which tends to overestimates bigger prey, present in smaller numbers, but with greater weight. Thus, the information provided by each of these indices should be looked in a complementary way. Therefore, compound indices, based on the combination of two or more of the simple indices are also frequently used since they provide a more balanced view of the dietary importance of each prey item (Pinkas et al. 1971, Liao et al. 2001). In the present study, the relative importance index (RI) and the preponderance index (I_P) were used. The RI uses the sum of the three simple indices, while the I_P integrates the product of the GI and OI. The sum and product of simple indices are the two most common processes used for the compound indices determination, thus justifying their use. The relative importance index (RI) (George and Hadley 1979) was determined by first summing the NI, OI and GI of each prey item, thus generating the index of absolute importance (AI) for each prey item, where:

$$AI_j = NI_j + OI_j + GI_j$$

Then, a sum of all AI values was used to calculate the RI for each prey item:

$$RI_j = 100 \cdot AI_j / \sum_{i=1}^n AI_i$$

where n is the number of prey items. The index of preponderance (I_P) (Natarajan and Jhingran 1961) ranks each prey item i based on their occurrence and weight and is expressed as:

$$I_{P_i} = (GI.OI) / (\sum GI.OI) .100$$

Diet variation throughout different juvenile size classes was assessed through the calculation of the diet indices for each of the size classes. The graphical method of Costello (1990) was also used, providing a scatter plot of weight values in the y axis and occurrence values in the x axis. Points located near 100 % of occurrence and 1 % of weight, demonstrate that predator consumed different preys in low quantity, indicating that is a generalist species. On the other hand, points located near 1% of occurrence and 100 % weight show that the fish diet is specialized on a given prey. Dominant preys are represented by points near 100 % occurrence and 100 % weight, while rare prey items are represented by points near the axis origin.

Dietary differences between flounder size classes and seasons were investigated using multivariate data analysis, available in the PRIMER statistical package (Plymouth Marine Laboratory, PRIMER v6). Hierarchical agglomerative clustering with complete linkage was used to investigate differences between the diet of the four size classes, using the five dietary indices (NI, OI, GI, RI and PI). Tests were based on the Bray–Curtis similarity measure (Bray and Curtis 1957) applied to $\log(x+1)$ transformed data. SIMPROF test was applied to assess the significance of the clusters produced.

Seasonal variations on the diet of each size class were assessed by one-way analysis of similarity (ANOSIM) based on RI and performed on $\log(x+1)$ transformed data. Only RI was chosen for this analysis because it was considered the most representative index of the diet, integrating information provided by the simple indices used. SIMPER (Similarity of percentages) analysis was used to identify which prey items were responsible for the differences found. In addition, non-metric multidimensional scaling (MDS), based on Bray–Curtis similarity matrix (Bray and Curtis, 1957) was carried out using $\log(x+1)$ transformed RI data.

2.4.3 Prey-predator interactions

Interactions between flounder juveniles and their macroinvertebrate prey were investigated based on the stomach content data.

Prey selection by flounder juveniles was quantified by comparing the contributions of different prey categories present in the diet with the relative proportions of those prey species in the environment (Lima macroinvertebrate community), using the Strauss elective index (Strauss, 1979). The expression

$$S_i = fd_i - fe_i$$

was used to estimate electivity (S_i), where fd_i is the relative frequency of the item i in the diet and fe_i is the relative frequency of the item i in the environment.

Niche breadth measures the degree of specialization relatively to the use of a certain resource. Niche breadth of the juvenile flounders was determined by the Levins index (B) and also by the Shannon-Wiener diversity index (H'). The Levins index down-weights the rarer prey items, making it more suitable for interspecific comparisons (Marshall and Elliot 1997) or in this case for the comparison between the different size classes. On the other hand, Shannon-Wiener index presents a greater sensitivity to the rarer items, presenting a better indication of the overall niche breadth (Marshall and Elliot 1997). The Levins index was determined by the following formula:

$$Bi = \frac{1}{\sum p_{ij}^2} \text{ (Levins, 1968),}$$

where p_{ij} is the proportion of the diet of predator i comprising prey species j and n is the number of prey categories. The index has a minimum of 1.0 when only one prey type is found in the diet and a maximum at n , where n is the total number of prey categories, each representing an equal proportion of the diet. The Shannon-Wiener diversity index H' (Shannon and Weaver, 1949) was determined by:

$$H' = - \sum_{i=1}^s P_i \ln P_i,$$

where P_i is the numerical proportion of the i th prey category in the diet and s is the total number of different prey categories consumed by the juveniles.

The potential diet overlap between the four size classes was measured by the Schoener index (SI) (Schoener et al. 1970):

$$SI = 1 - 0.5 \left(\sum_{i=1}^n |P_{iA} - P_{iB}| \right),$$

where p_{iA} and p_{iB} are the numerical frequencies of the item i in the size class A and B, respectively. Values of the diet overlap vary between 0, when no food is shared, and 1, when there is the same proportional use of all food resources. Values higher than 0.6 are considered to demonstrate significant overlap (Wallace and Ramsey 1983).

In order to study the influence of prey size on the flounder diet, Pearson correlations were used. First, the relationships between flounder total length and maximum mouth gap width were determined and after, Pearson correlations between fishes total length and minimum, maximum and mean prey length for the overall individuals and for each size class were determined, using the GraphPad Prism version 5.0 software (GraphPad Software).

The potential predatory action of *C. crangon* and *C. maenas* on juvenile flounder abundance and also condition was investigated. Taking into consideration that the predatory capability is size dependent, only *C. crangon* over 30 mm and *C. maenas* with a carapace width over 26 mm are considered as potential predators of small flounders (*P. flesus* TL<50 mm) (Van der Veer and Bergman 1987). Thus, only crustaceans following those requisites were considered for the present study. Densities of *C. crangon* and *C. maenas* were expressed by the number of individuals per 1000 m². Linear regression was used to assess the potential effect of predators on juvenile flounder abundances and condition, using the GraphPad Prism version 5.0 software (GraphPad Software).

3. Results

3.1 Environmental parameters

During the study period, the water column temperature followed the usual seasonal pattern. There was a general trend for a winter temperature decrease and summer-autumn increase, in the three estuarine zones (Table 3.1). However, this temporal pattern was more evident in the upper estuary, where the minimum (7.4 °C) and maximum (25.0 °C) temperature values were observed. The typical estuarine horizontal salinity gradient was always present, with salinity decreasing upstream. In average, the lower estuarine zone was in the euryhaline range (27.6), as well as the middle estuary (23.7), while the upper section was in the oligohaline range (3.3). Contrarily to temperature, seasonal salinity variations were more evident in the lower and middle sections of the Lima estuary (Table 3.1).

Table 3.1 – Mean temperature (T) and salinity (S) of water column, and sediment organic matter content (OM) of the lower, middle and upper sections of the Lima estuary.

		T(°C)	S	OM(mg g ⁻¹)
Lower	Winter	11.8	27.4	37.5
	Spring	15.2	18.8	33.6
	Summer	14.9	33.2	37.1
	Autumn	16.4	33.4	39.5
Middle	Winter	10.7	16.3	19.0
	Spring	14.0	14.9	12.5
	Summer	16.7	30.1	39.9
	Autumn	16.4	32.3	38.9
Upper	Winter	9.2	0.0	4.0
	Spring	13.1	0.3	10.0
	Summer	23.3	7.7	5.5
	Autumn	16.0	8.5	11.9

Sediments composition varied across the estuary (Figure 3.1). The lower estuary was mainly composed by sand and fine sand, while in the upper estuarine section gravel was the predominant fraction of the sediments. The middle estuary presented the most equitable distribution of different types of sediment. There was a trend for an upstream

increase of the gravel and a decrease of the silt and clay fractions of the Lima estuarine sediments. In fact, silt and clay reached 15% in the lower estuary and less than 1% in the upper estuarine sediments. Similarly, the organic matter content showed a general trend to decrease from the lower (36.9 mg g^{-1}), to the middle (27.6 mg g^{-1}) and upper estuarine sections (7.8 mg g^{-1}) (Table 3.1). In the lower estuary, it maintained a stable level throughout the year. On the other hand, in the middle estuary, organic matter content was higher during summer (39.9 mg g^{-1}) and autumn (38.9 mg g^{-1}). In the upper estuary, organic matter content was very low, increasing during spring (10.0 mg g^{-1}) and autumn (11.9 mg g^{-1}).

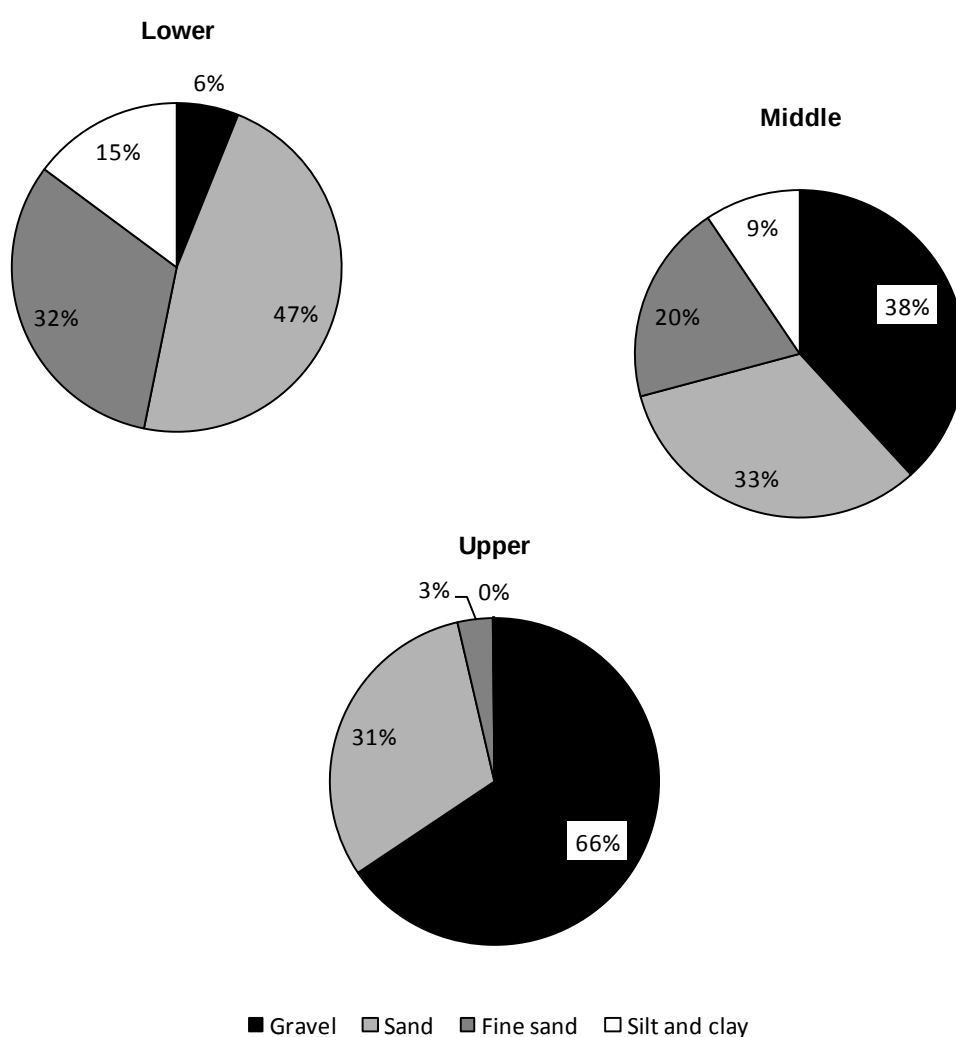


Figure 3.1 –Sediment composition of the lower, middle and upper estuarine sections of the Lima estuary.

3.2 Macroinvertebrates community

A total of 3,601 individuals were identified, belonging to 63 taxa, distributed by six Phyla (Table 3.4). Annelida was the most abundant phyla, representing 68.6 % of the total macroinvertebrates, followed by the Arthropoda (25.9 %), Nemertea (2.7 %), Mollusca (2.6 %), Nematoda (0.1 %) and Cnidaria (0.02 %). *Oligochaeta* ni, *Corophium* spp. and *Hediste diversicolor* were the most abundant taxa, corresponding to 29.6 %, 21.3 % and 10.3 % of the total macrofauna, respectively.

The abundance of the Lima estuarine macrofauna was in average 1788 ± 2597 individuals m^{-2} , ranging from a minimum of 0 individuals m^{-2} and the maximum of 16826 individuals m^{-2} , both situations observed in the upper estuary. Despite the lack of significant seasonal ($F=2.8$, $p \geq 0.06$) or spatial ($F=2.1$, $p \geq 0.15$) variations, macrofauna abundance exhibited different seasonal trends in each section of the Lima estuary. In the lower and middle sections, the highest values of total abundance were recorded during the winter and autumn. On the other hand, in the upper estuary, macroinvertebrates were more abundant during the autumn (Figure 3.2).

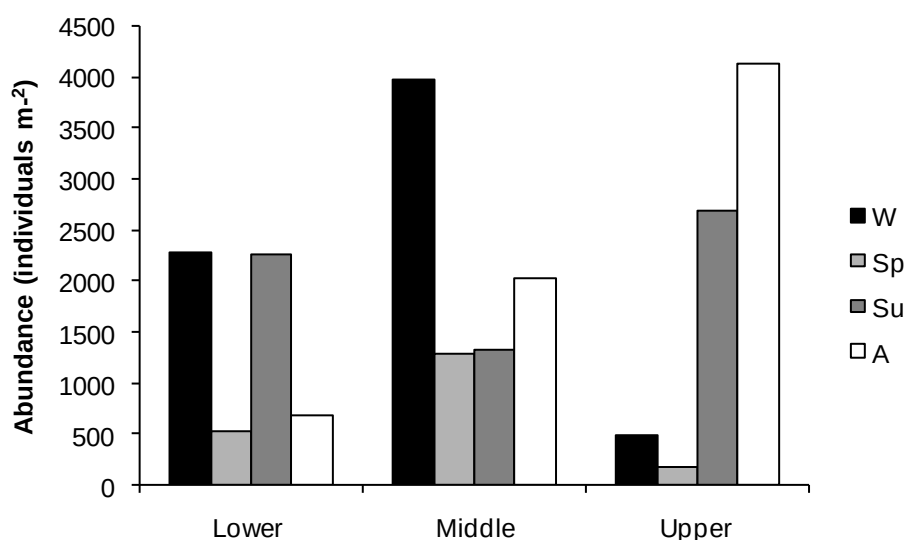


Figure 3.2 – Seasonal mean abundance of macroinvertebrates in the lower, middle and upper estuarine sections (W, winter; Sp, spring; Su, summer, A, autumn).

The number of species varied between 5 and 26 (Table 3.2). In general, the lower estuary tended to comprise more species, with an average of 17 species, followed by

the middle (average of 13) and upper estuarine sections (average of 8). An exception occurred during the winter, when the highest values (9 species) occurred in the middle estuary. In the lower and middle sections of the estuary, the number of species followed a common seasonal pattern, increasing from winter to spring, reaching a peak during the summer and decreasing in the following autumn (Figure 3.3). In the upper estuary, a similar pattern also occurred, although a decrease in number of species occurred from winter to spring (Figure 3.3).

Table 3.2 – Average number of species (S), Shannon and Wiener index (H') and equitability (J') of the macroinvertebrates community of the lower, middle and upper sections of the Lima estuary.

		S	H'	J'
Lower	Winter	6	1.5	0.8
	Spring	16	2.0	0.7
	Summer	26	1.9	0.6
	Autumn	18	2.0	0.7
Middle	Winter	9	0.4	0.2
	Spring	14	2.2	0.8
	Summer	18	2.1	0.7
	Autumn	9	1.4	0.7
Upper	Winter	7	1.5	0.8
	Spring	5	1.2	0.8
	Summer	11	1.6	0.7
	Autumn	7	0.8	0.4

The Shannon-Wiener index (H') presented a significant spatial variation ($F= 3.9$, $p < 0.05$), but did not varied seasonally ($F=2.5$, $p \geq 0.08$). Similarly to the number of species, diversity was generally higher in the lower and middle estuarine sections (Table 3.2). In the lower estuary, there was a winter decrease of the community diversity, and slight variations throughout the rest of the year (Figure 3.3). The same pattern occurred in the middle estuary, but the winter decrease was more evident ($H'=0.4$), despite the high number of species present in this season (Figure 3.3). During this period, the community of macroinvertebrates in the middle estuary was dominated by *Oligochaeta ni* (Table 3.4) that represented 93 % of the total abundance, probably explaining the low diversity values. In the upper estuary, diversity remained relatively stable throughout the year, decreasing only in autumn in association with a decrease in

the number of species (7) (Figure 3.3). The Pielou evenness index (J') did not vary significantly between the estuarine sections ($F=0.6$, $p \geq 0.54$) or seasons ($F=0.8$, $p \geq 0.51$). In the lower estuary, equitability did not vary considerably throughout the year (Figure 3.3). Similarly to the H' pattern, a sharp increase was observed from winter to spring in the middle estuary (Figure 3.3). In the upper estuary, J' remained stable throughout the year, with only a greater decrease occurring from summer to autumn (Figure 3.3).

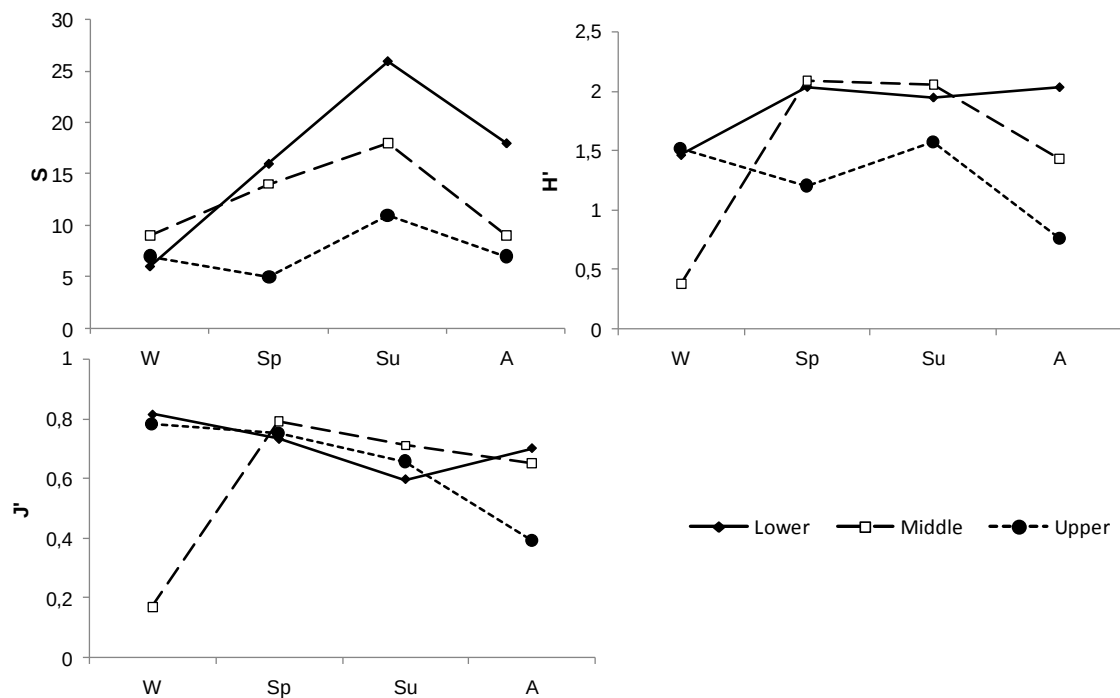


Figure 3.3 - Seasonal variation of the average number of species (S), Shannon–Wiener index (H') and equitability (J') (W, winter; Sp, spring; Su, summer, A, autumn).

According to ANOSIM results, the structure of the macroinvertebrates community varied significantly between the estuarine sections ($R=0.6$, $p < 0.05$), but no significant differences were found between seasons ($R = -0.0$, $p \geq 0.51$). In fact, the macroinvertebrates community present in the upper estuary was significantly different from that observed in the lower ($R=0.7$, $p < 0.05$) and middle ($R=0.7$, $p < 0.05$) sections of the Lima estuary (Table 3.3). SIMPER results identified *Oligochaeta* ni, *Corophium* spp. and Chironomidae ni as responsible for 43% of the average dissimilarity observed between the macrobenthic community of the lower and of the upper sections of the estuary (Table 3.3). In fact, *Oligochaeta* ni was more abundant in the lower estuary,

while *Corophium* spp. was considerably more abundant in the upper estuary and Chironomidae ni was only present in this estuarine section (Table 3.4). Regarding the differences between the middle and upper estuary, *Hediste diversicolor*, which was more abundant in the upper estuary, was responsible for 15% of the total dissimilarity, while *Capitella* spp. and Oligochaeta ni, more abundant in the middle estuary, contributed together with 28% of the total dissimilarity (Table 3.3).

Table 3.3 - Results of ANOSIM (R values and significance levels) and SIMPER analyses on abundance of macroinvertebrate taxa (SIMPER results for the three most important taxa contributing to dissimilarities are shown).

Groups	ANOSIM		Average dissimilarity (%)	SIMPER Discriminating taxa	Cumulative contribution (%)
	R	p			
Lower vs. Middle	0.5	0.06	69.8	<i>Hediste diversicolor</i>	15.3
				Oligochaeta ni	30.4
				Nemertea ni	30.9
Lower vs. Upper	0.7	0.03*	83.4	Oligochaeta ni	15.1
				<i>Corophium</i> spp.	30.1
				Chironomidae ni	43.3
Middle vs. Upper	0.7	0.03*	78.4	<i>Hediste diversicolor</i>	14.9
				<i>Capitella</i> spp.	29.3
				Oligochaeta ni	43.4

* significant values

Table 3.4 –Abundance (mean $\pm$ standard deviation, individuals m^{-2}) and frequency of occurrence (%) of the macroinvertebrate community of the Lima estuary in the lower, middle and upper sections during winter, spring, summer and autumn of 2010.

Phylum	Taxa	Total abundance	Frequency (%)	Lower estuary	Middle estuary	Upper estuary
Cnidaria	<i>Edwardsia clapedii</i>	0.5 $\pm$ 4.6	0.02	1.4 $\pm$ 7.9	0.0	0.0
Nemertea	Nemertea ni	56.0 $\pm$ 201.6	2.70	10.1 $\pm$ 40.7	136.2 $\pm$ 331.7	21.7 $\pm$ 54.5
Nematoda	Nematoda ni	2.9 $\pm$ 14.3	0.13	0.0	8.7 $\pm$ 24.0	0.0
Annelidae	Oligochaeta ni	357.5 $\pm$ 1216.7	29.57	207.2 $\pm$ 387.8	740.6 $\pm$ 2021.8	124.6 $\pm$ 272.1
	<i>Capitella</i> spp.	113.04 $\pm$ 270.84	6.63	123.2 $\pm$ 287.1	136.2 $\pm$ 221.6	79.7 $\pm$ 303.0
	<i>Mediomastus fragilis</i>	53.1 $\pm$ 494.9	2.43	2.9 $\pm$ 15.9	156.5 $\pm$ 857.3	0.0
	<i>Tharyx marioni</i>	129.9 $\pm$ 587.3	7.76	389.9 $\pm$ 976.6	0.0	0.0
	<i>Glycera convoluta</i>	1.0 $\pm$ 6.4	0.04	2.9 $\pm$ 11.0	0.0	0.0
	<i>Glycera</i> spp.	1.0 $\pm$ 6.44	0.04	1.4 $\pm$ 7.9	1.4 $\pm$ 7.9	0.0
	<i>Micronephtys</i> spp.	1.0 $\pm$ 9.2	0.04	2.9 $\pm$ 15.9	0.0	0.0
	<i>Nephtys cirrosa</i>	4.3 $\pm$ 24.4	0.20	13.0 $\pm$ 41.4	0.0	0.0
	<i>Nephtys convergi</i>	1.0 $\pm$ 9.2	0.04	0.0	2.9 $\pm$ 15.9	0.0
	<i>Nephtys incisa</i>	0.5 $\pm$ 4.6	0.02	1.4 $\pm$ 7.9	0.0	0.0
	<i>Nephtys</i> spp.	2.4 $\pm$ 10.0	0.11	1.4 $\pm$ 7.9	5.8 $\pm$ 15.0	0.0
	<i>Hediste diversicolor</i>	210.6 $\pm$ 503.5	10.25	10.1 $\pm$ 35.5	266.7 $\pm$ 410.7	355.1 $\pm$ 735.9
	<i>Scoloplos armiger</i>	1.5 $\pm$ 7.9	0.07	4.3 $\pm$ 13.3	0.0	0.0
	<i>Eteone barbata</i>	1.9 $\pm$ 18.3	0.09	0.0	5.8 $\pm$ 31.8	0.0

	<i>Eteone flava</i>	0.5 ± 4.6	0.02	0.0	1.4 ± 7.9	0.0
	<i>Eumyidae bahusiensis</i>	31.9 ± 213.6	1.46	81.2 ± 364.8	14.5 ± 55.2	0.0
	<i>Mysta picta</i>	0.5 ± 4.6	0.07	0.0	1.4 ± 7.9	0.0
	<i>Phyllodoce maculata</i>	0.5 ± 4.6	0.02	1.4 ± 7.9	0.0	0.0
	<i>Pisione remota</i>	6.3 ± 59.6	0.29	0.0	18.8 ± 103.2	0.0
	Phyllodocidae ni	10.1 ± 38.6	0.46	0.0	30.4 ± 62.7	0.0
	<i>Cossura</i> spp.	19.8 ± 97.6	0.99	59.4 ± 163.7	0.0	0.0
	<i>Prionospio delta</i>	1.0 ± 6.4	0.04	2.9 ± 11.0	0.0	0.0
	<i>Prionospio</i> spp.	0.5 ± 4.6	0.02	1.4 ± 7.9	0.0	0.0
	<i>Pygospio elegans</i>	1.0 ± 9.2	0.04	0.0	2.9 ± 15.9	0.0
	<i>Scolecopsis squamata</i>	1.0 ± 9.2	0.04	0.0	0.0	0.0
	<i>Spio martinensis</i>	2.4 ± 15.1	0.15	2.9 ± 15.9	1.4 ± 7.9	0.0
	<i>Streblospio shrubsolii</i>	70.5 ± 198.9	3.27	5.8 ± 24.8	189.9 ± 302.4	21.7 ± 87.5
	Spionidae ni	44.4 ± 220.3	3.93	0.0	2.9 ± 15.9	27.5 ± 150.8
	<i>Amage adspersa</i>	8.2 ± 45.6	0.38	102.9 ± 347.0	24.6 ± 77.2	0.0
	<i>Lanice conchilega</i>	0.5 ± 4.6	0.02	0.0	1.4 ± 7.9	0.0
	Terebeliidae ni	0.5 ± 4.6	0.02	0.0	0.0	0.0
	Polychaeta ni	0.5 ± 4.6	0.07	1.4 ± 7.9	0.0	1.4 ± 7.9
Mollusca	<i>Cerastoderma edule</i>	2.4 ± 16.4	0.11	0.0	7.2 ± 28.2	0.0
	<i>Laevicardium crassum</i>	0.48 ± 4.58	0.02	0.0	0.0	1.4 ± 7.9
	<i>Donax vittatus</i>	0.5 ± 4.6	0.02	1.4 ± 7.9	0.0	0.0
	<i>Lutraria lutraria</i>	1.0 ± 9.2	0.04	0.0 ± 0.0	2.9 ± 15.9	0.0
	<i>Tellimya ferruginosa</i>	1.5 ± 13.7	0.07	4.3 ± 23.8	0.0	0.0
	<i>Abra alba</i>	46.4 ± 417.4	2.12	139.1 ± 722.1	0.0	0.0

	<i>Scrobicularia plana</i>	1.0 ± 6.4	0.04	1.4 ± 7.9	1.4 ± 7.9	0.0
	<i>Spisulida spuncata</i>	0.5 ± 4.6	0.02	1.4 ± 7.9	0.0	0.0
	<i>Tellina fabula</i>	0.5 ± 4.6	0.02	1.4 ± 7.9	0.0	0.0
	<i>Telinna temys</i>	0.5 ± 4.6	0.02	1.4 ± 7.9	0.0	0.0
	<i>Venerupis senegalensis</i>	1.0 ± 6.4	0.04	2.9 ± 11.0	0.0	0.0
	Bivalvia ni	1.0 ± 6.4	0.09	1.4 ± 7.9	0.0	1.4 ± 7.9
Arthropoda	<i>Ciathura</i> spp.	1.0 ± 9.2	0.04	0.0 ± 0.0	0.0	2.9 ± 15.9
	<i>Saduriella losadai</i>	0.5 ± 4.6	0.02	0.0 ± 0.0	0.0	1.4 ± 7.9
	<i>Sphaeroma serratum</i>	2.9 ± 15.7	0.13	0.0 ± 0.0	2.9 ± 11.0	5.8 ± 24.8
	Tanaidacea ni	0.5 ± 4.6	0.02	1.4 ± 7.9	0.0	0.0
	Isopoda ni	8.2 ± 39.6	0.60	4.3 ± 13.3	20.3 ± 66.3	0.0
	<i>Corophium</i> spp.	461.8 ± 1808.6	21.30	49.3 ± 206.7	1.4 ± 7.9	1334.8 ± 2968.6
	<i>Gammarus</i> spp.	7.3 ± 25.5	0.33	2.9 ± 15.9	13.0 ± 36.4	5.8 ± 18.9
	Amphipoda ni	1.0 ± 6.4	0.04	1.4 ± 7.9	0.0	1.4 ± 7.9
	<i>Gastrosaccus spinifer</i>	0.5 ± 4.6	0.02	1.4 ± 7.9	0.0	0.0
	<i>Siriella</i> spp.	0.5 ± 4.6	0.02	1.4 ± 7.9	0.0	0.0
	Mysidae ni	0.5 ± 4.6	0.07	0.0	1.4 ± 7.9	0.0
	<i>Carcinus maenas</i>	1.5 ± 7.9	0.07	2.9 ± 11.0	1.4 ± 7.9	0.0
	<i>Crangon crangon</i>	1.0 ± 9.2	0.04	2.9 ± 15.9	0.0	0.0
	Chironomidae ni	53.6 ± 198.5	3.12	0.0	0.0	160.9 ± 321.0
	Diptera ni	0.5 ± 4.6	0.02	0.0	0.0	1.4 ± 7.9
	Non identified	1.0 ± 6.4	0.04	1.4 ± 7.9	1.4 ± 7.9	0.0
	TOTAL			1256.5	1804.3	2149.3

3.3 Diet of *P. flesus* juveniles

During the study period, a total of 102 flounder juveniles were collected, with the total length ranging between 19 and 175 mm, and total weight varying between 0.1 and 61.9 g (Table 3.5).

Table 3.5 - Number of *P. flesus* juveniles sampled per size class, mean total length (mm) and mean total weight (g).

Size class	Number of fish sampled	Total length (mm)	Total weight (g)
1	49	28.9 ± 8.1	0.3 ± 0.3
2	37	64.4 ± 8.9	3.0 ± 1.3
3	10	121.2 ± 14.1	17.8 ± 5.6
4	6	166.2 ± 8.2	47.3 ± 10.0

Class 1 was the most abundant, presenting the highest abundances, although these individuals were restricted to the upper estuary, and only occurred during spring and also in summer, but in considerable lower numbers (Table 3.6). Class 2 juveniles also tended to spread mostly in the upper estuary during summer, although, during the spring, their presence was also recorded in the lower and middle estuary (Table 3.6). Class 3 juveniles, were frequently observed not only in upper, but also in the middle estuary, despite of the higher abundances still occurring in the upper estuary. Older juveniles, as those belonging to class 4, were absent in the upper estuary, but were frequently observed in the lower and middle estuarine sections of the Lima estuary (Table 3.6).

Table 3.6 – Mean abundance (individuals m⁻²) (mean ± sd) of *P. flesus* juveniles of the low, middle and upper sections of the Lima estuary.

		Class 1	Class 2	Class 3	Class 4
Lower	Winter	0.0	0.0	0.00	1.3 ± 2.2
	Spring	0.0	0.8 ± 1.3	0.0	0.0
	Summer	0.0	0.0	0.0	0.0
	Autumn	0.0	0.0	0.0	0.5 ± 0.8
Middle	Winter	0.0	0.0	0.7 ± 1.2	3.9 ± 6.7
	Spring	0.0	1.2 ± 2.1	0.9 ± 1.5	0.0
	Summer	0.0	0.0	0.7 ± 1.1	0.7 ± 1.1
	Autumn	0.0	0.0	0.0	0.0
Upper	Winter	0.0	1.6 ± 2.8	1.3 ± 1.1	0.0
	Spring	111.6 ± 25.0	0.0	1.3 ± 2.2	0.0
	Summer	2.2 ± 3.8	16.8 ± 17.4	0.0	0.0
	Autumn	0.0	1.3 ± 1.1	0.6 ± 1.1	0.0

The condition of the flounder juveniles, expressed by Fulton's *k* factor, varied between 0.3 (class 2) and 2.9 (class 1) and, in average presented (Table 3.7) similar values for all the size classes.

Table 3.7 – Fulton's *k* condition factor (mean ± standard deviation) for each *P. flesus* size classes.

Class	1	2	3	4
<i>k</i>	1.0 ± 0.3	1.1 ± 0.2	1.0 ± 0.1	1.0 ± 0.1

From the 102 stomachs analyzed, 16 stomachs were empty, leading to a vacuity index of 15.7 %. The percentage of empty stomachs was considerable higher during the winter (42%), comparatively to spring (12%), summer (13%) and autumn (10%). The vacuity index increased along the size classes, with class 1 presenting the lowest value (8.2 %), followed by classes 2 (16.2 %), 3 (30.0 %) and 4 (50.0 %). Percentages of 100% empty stomachs were observed for classes 2 and 4 (Table 3.8).

Table 3.8 – Vacuity index for each size class throughout the year of 2010 (W, Winter; Sp, Spring; Su, Summer, A, Autumn; values in brackets represent number of empty stomachs).

Class	Season			
	W	Sp	Su	A
1	-	9% (4)	0% (0)	0% (0)
2	33% (1)	100% (2)	12% (3)	0% (0)
3	60% (3)	0% (0)	0% (0)	-
4	25% (1)	-	100% (1)	100% (1)

The diet composition of *P. flesus* included sixteen different taxa, including macroinvertebrates, fishes, plant debris and sand (Table 3.9). The flounder juveniles' diet was mainly composed by Chironomidae ni, *Corophium* spp. and to a much lesser extent Elmidae ni, (Table 3.9). Considering the gravimetric index, *Corophium* spp., *C. crangon* and Chironomidae ni were the main prey items. Bivalvia and the gastropod *Ecreobia truncata* were also important items, according to the gravimetric and occurrence indices, although presenting values widely below the mentioned main items. These results were corroborated by the Costello graphical method (Figure 3.4) that identified *Corophium* spp., Chironomidae ni, *C. crangon* and Elmidae ni as the main prey items of the flounder diet. This method also showed that other prey items, such as gastropods and polychaetes were rare in this species diet, thus appearing near the axis origin.

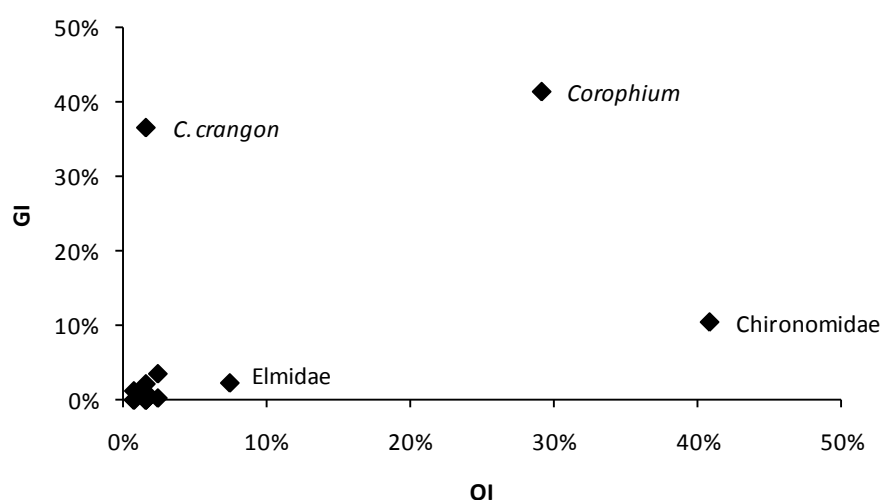


Figure 3.4 – Costello graphical method applied to the diet of *P. flesus* juveniles.

Table 3.9 – Numerical (NI), occurrence (OI), gravimetric (GI), relative importance (RI) and preponderance (PI) indices values of prey found in stomachs of 86 *P. flesus* juveniles.

Food items	NI	OI	GI	RI	PI
Phylum Annelidae					
Class Polychaeta					
Family Spionidae	0.1	1.7	<0.1	0.6	<0.1
Polychaeta n.i.	0.1	0.8	<0.1	0.3	<0.1
Phylum Mollusca					
Class Bivalvia	0.3	2.5	0.3	1.0	0.0
Class Gastropoda					
<i>Ecrobia truncata</i>	0.7	2.5	3.6	2.3	0.5
<i>Potamopyrgus jenkinsi</i>	0.1	0.8	1.3	0.7	1.3
Phylum Arthropoda					
Class Crustacea					
Order Isopoda	0.2	1.7	<0.1	0.6	<0.1
Order Amphipoda					
<i>Corophium</i> spp.	10.6	29.2	41.5	27.0	69.7
Order Decapoda					
<i>Crangon crangon</i>	0.1	1.7	36.6	12.8	3.5
Crustacea n.i.	0.1	1.7	0.2	0.7	<0.1
Class Insecta					
Order Coleoptera					
Family Elmidae	7.4	7.5	2.3	5.7	1.0
Order Diptera					
Family Chironomidae	79.4	40.8	10.5	43.7	24.7
Family Simuliidae	0.1	1.7	<0.1	0.6	<0.1
Diptera n.i.	0.3	1.7	0.1	0.7	<0.1
Order Ephemeroptera					
Family Caenidae	<0.1	0.8	0.1	0.3	<0.1
Ephemeroptera n.i.	0.1	1.7	0.2	1.0	<0.1
Phylum Chordata					
Infraclass Teleostei	0.1	1.7	2.2	1.3	2.2
Non identified	0.1	1.7	1.1	0.9	0.1

Regarding the diet of Class 1 juveniles, a total of 1437 items were found. According to all indices used, Chironomidae ni, *Corophium* spp. and Elmidae ni (Figure 3.5) were the most important items, especially for the preponderance index. Indeed, this item was present in large numbers and with a high representativity among all stomachs, despite the reduced weight of this prey. *Corophium* spp. assumed a greater importance in the occurrence, gravimetric and relative importance indices. Although present in relatively low numbers, this species occurred in most of the stomachs and their weight was higher comparatively to other items like Elmidae ni. The latter only appeared in some of the stomachs, but with an important contribution to the total contents in terms of number and weight. Consequently, it was a major item of the diet, according to the numerical and gravimetric indices.

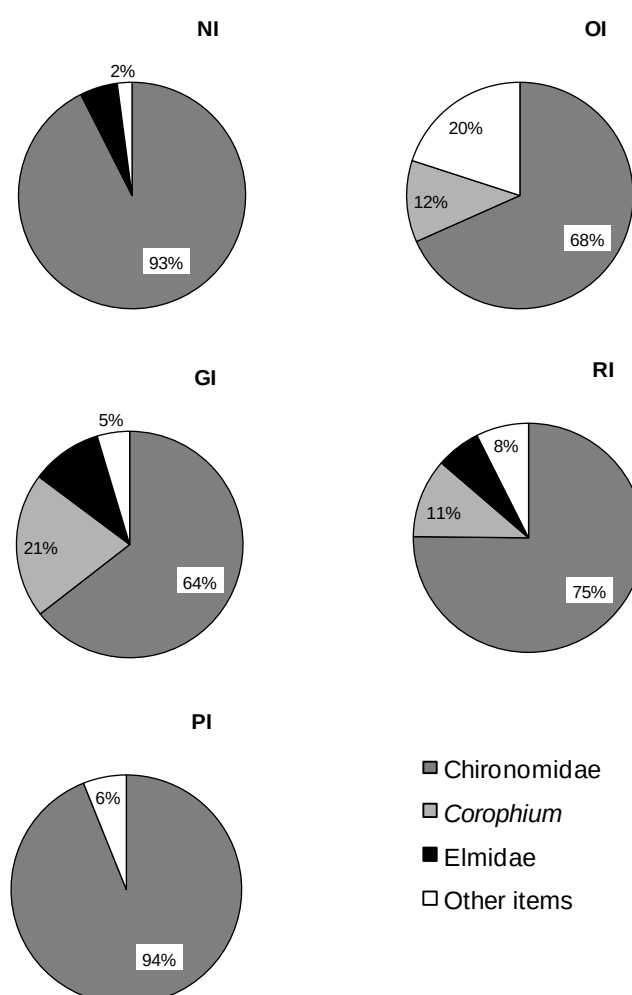


Figure 3.5 – Numerical index (NI), occurrence index (OI), gravimetrical index (GI), relative importance index (RI) and preponderance index (PI) of the prey items of class 1 *P. flesus* juveniles (other items: prey items with a contribution < 5 %).

The diet of class 2 juveniles was more diversified, and included 230 different prey items. *Corophium* spp. was the main prey item, according to all dietary indices applied (Figure 3.6). Their presence in most of the stomachs in high numbers and, also their relatively great body dimension, contributed greatly to the total weight of the stomach contents, justifying these results. Chironomidae ni was also a major item according to the NI, OI and RI indices. The low weight of these organisms was responsible for the lower importance accordingly with the gravimetric index. The gastropod *E. truncata* was also an important item, according to the OI and RI indices.

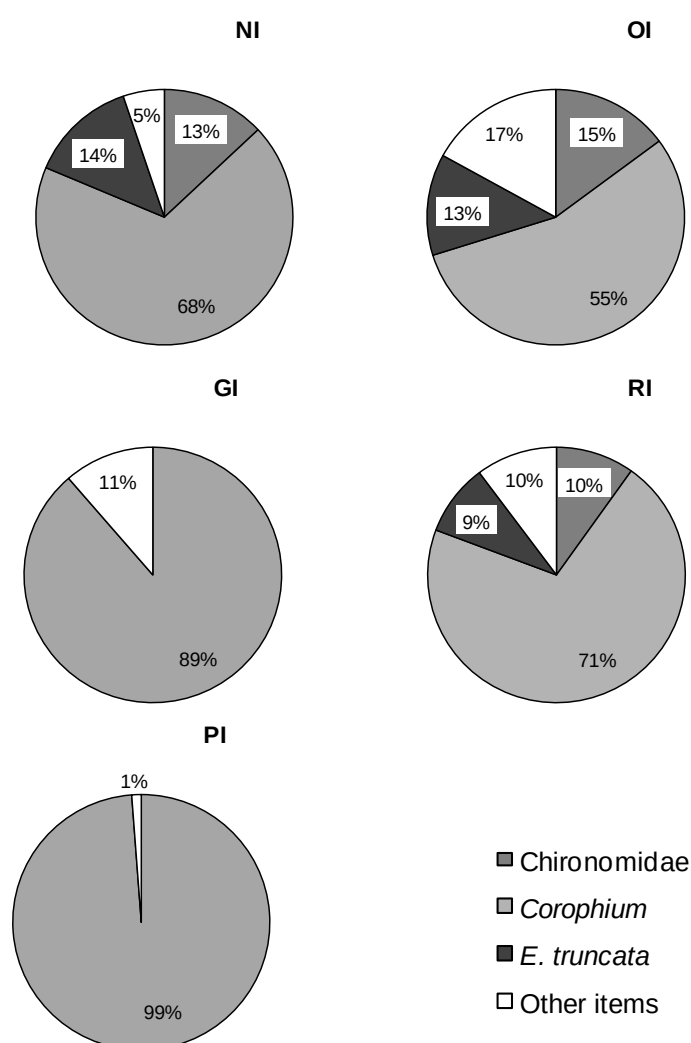


Figure 3.6 – Numerical index (NI), occurrence index (OI), gravimetrical index (GI), relative importance index (RI) and preponderance index (PI) of the prey items of class 2 *P. flesus* juveniles (other items: prey items with a contribution < 5 %).

Class 3 juveniles presented the most diverse diet, including 33 prey items. Similarly to class 2, *Corophium* spp. emerged as an important item according to all of the dietary indices, along with *E. truncata* (Figure 3.7). The importance of these items was due to their presence both in terms of number, occurrence and weight. Another gastropod, *Potamopyrgus jenkinsi*, was also a representative item in several stomachs, leading to the importance attributed by the NI, OI and RI indices.

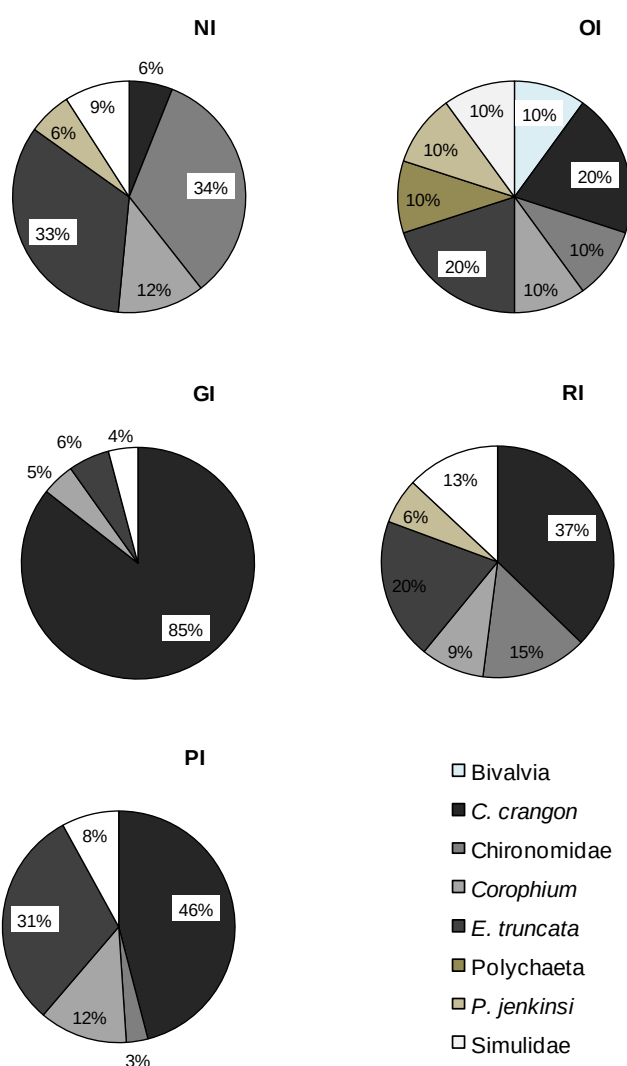


Figure 3.7 – Numerical index (NI), occurrence index (OI), gravimetric index (GI), relative importance index (RI) and preponderance index (PI) of the prey items of class 3 *P. flesus* juveniles (other items: prey items with a contribution < 5 %).

Chironomidae ni was also an important prey according to the NI and PI indices, since it occurred in high numbers. The presence of *C. crangon* in the diet was unique of this class. This item was important in the diet, when considering the OI, GI and RI indices, due to its presence in a considerable number of stomachs and its contribute to the total weight of the stomach contents, due to their high body weight. Bivalve ni and Polychaeta ni were minor items, although contributing with 10% each in the occurrence index. Moreover, Simuliidae ni was only considered important according to the compound indices RI and PI.

The diet of older juveniles, Class 4, only included two prey items, *Corophium* spp. and Teleostei ni (Figure 3.8). Thus, these individuals revealed a preference for prey items of a greater size, comparatively to other classes. However, these results may not be representative, because from the 6 fishes only three presented stomach contents. Therefore, the diet may include more prey items.

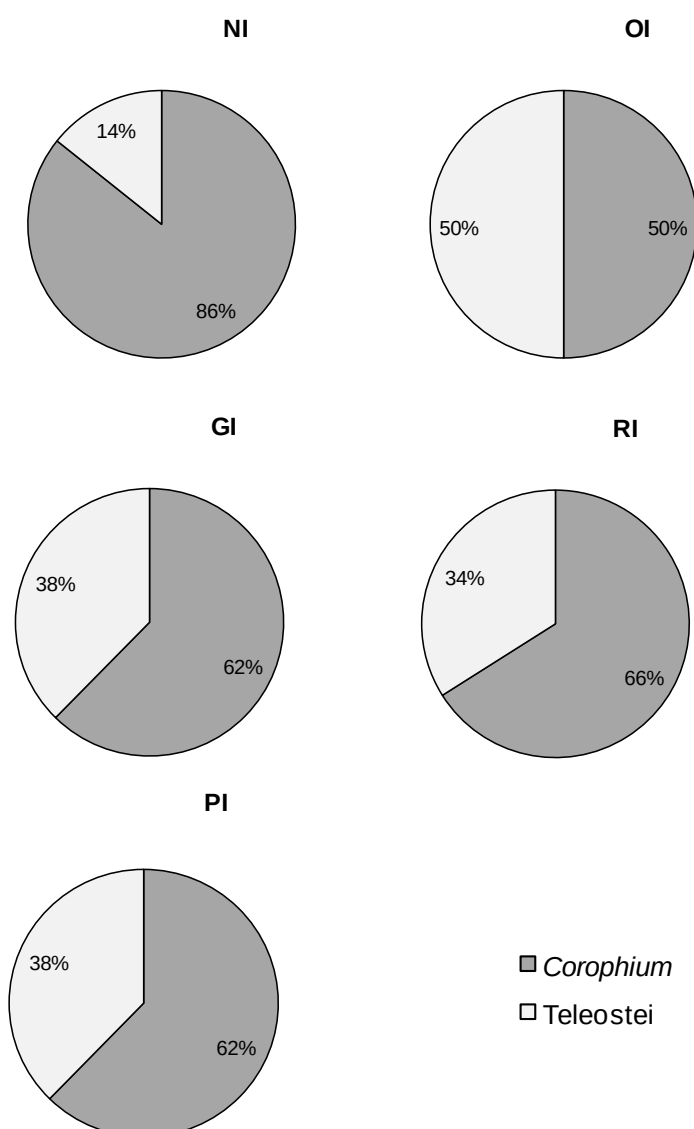


Figure 3.8 – Numerical index (NI), occurrence index (OI), gravimetric index (GI), relative importance index (RI) and preponderance index (PI) of the prey items of class 4 *P. flesus* juveniles (other items: prey items with a contribution < 5 %).

Cluster analysis based on NI, OI and RI showed that classes 1 and 2 clustered at 50% of similarity, while the remaining classes appeared as separate clusters (Figure 3.9). However, according to SIMPROF analysis, the diet of the four classes was not significantly different ($p > 0.05$) for all the indices studied. The high similarity between class 1 and 2 can probably be explained by the main prey items shared by these classes, namely Chironomidae ni and *Corophium* spp. (Figures 3.5 and 3.6). These items were

also present in the class 3 diet, which, however, was more diversified (Figure 3.7), leading to the lower level of similarity with classes 1 and 2. Cluster analyses based on GI and PI, showed that classes 2 and 4 exhibited approximately 60% and 75% of similarity, respectively (Figure 3.9). The diet of classes 2 and 4 was mainly composed by *Corophium* spp. in terms of weight (Figures 3.6 and 3.8), thus explaining the high similarity between those classes, based on GI and consequently on PI index. Moreover, classes 1 and 3 also showed 40% of similarity when considering PI index, what might be a reflex of the common presence of Chironomidae ni as one of the most important prey items of those classes.

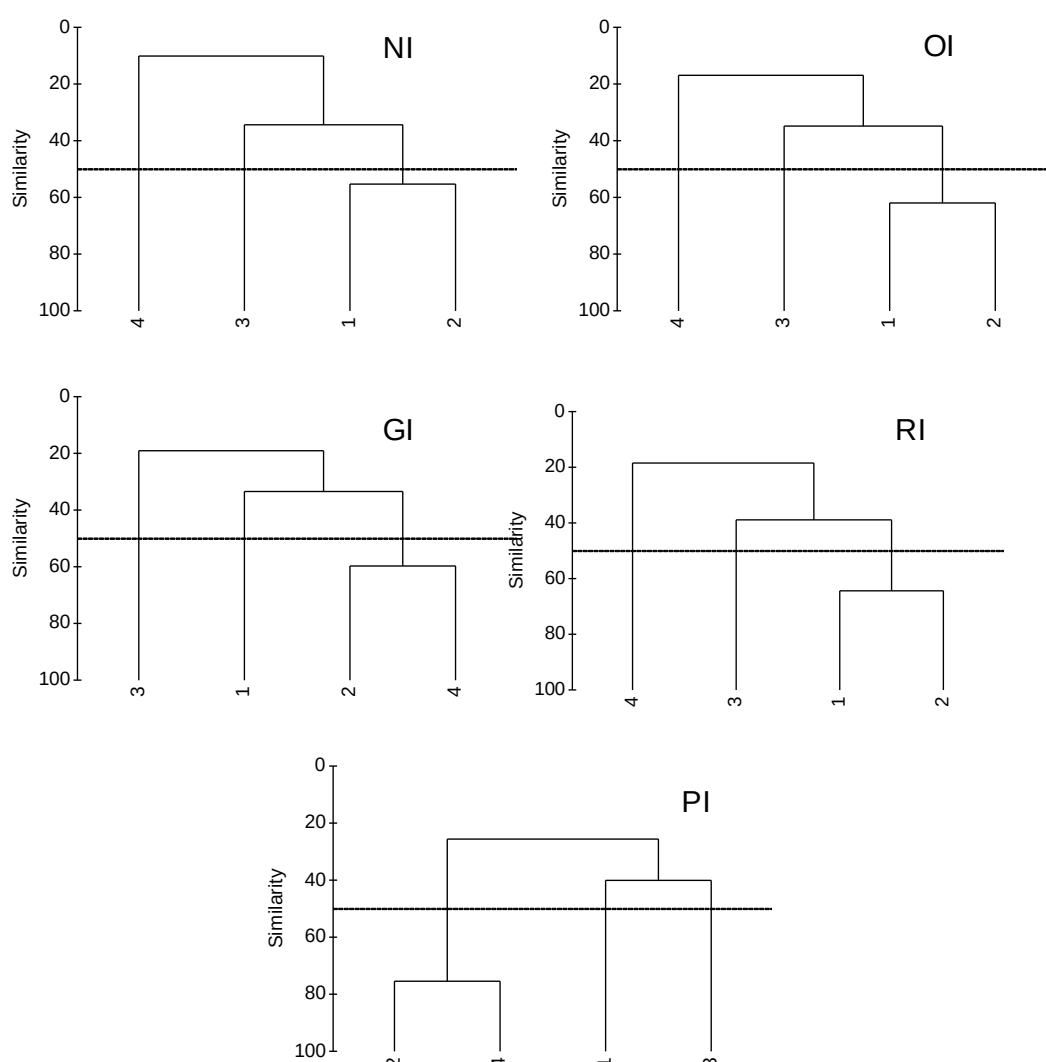


Figure 3.9– Cluster analysis of the four *P. flesus* size classes, based on numerical index (NI), occurrence index (OI), gravimetric index (GI), relative importance index (RI) and preponderance index (PI).

ANOSIM analysis performed on RI index revealed no significant differences between the diet of samples of different seasons ($R = 0.2$, $p \geq 0.27$) or size classes ($R = -0.4$, $p \geq 0.93$). However, the MDS analysis based on RI revealed that samples tended to cluster according to the sampling season, and two main groups were isolated: one, in the right part of the MDS plot and containing the winter samples of class 2 and 3; and another group with spring and summer samples of classes 1 and 2 that clustered in the middle of the plot (Figure 3.10). Autumn samples of classes 1 and 2 also clustered and formed a third group of samples, isolated in the left part of the plot. Winter diet of class 4, as well as the spring diet of class 3 were separated from the three main groups (Figure 3.10).

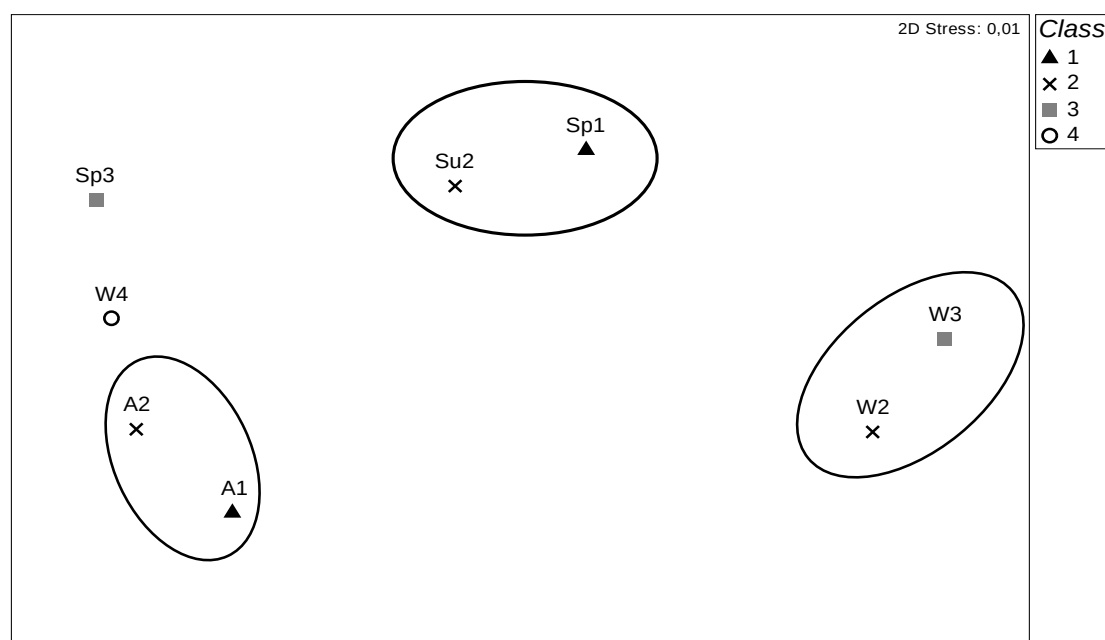


Figure 3.10 – MDS plot of the RI prey items of *P. flesus* juveniles diet per size classes (1, 2, 3 and 4) and season (W - Winter, Sp – Spring, Su – Summer and A- Autumn).

Regarding the SIMPER results, seasonal variations of the flounder diet were mainly related to seasonal variations of three prey items: Elmidæ ni, *C. crangon* and *E. truncata* (Table 3.10). Elmidæ ni was the important item for the diet differences between the autumn and the other seasons, despite the flounder size class. In fact, this prey was only available for juvenile flounder during the autumn. *C. crangon*, only occurring during the summer, was responsible for 17% and 22% of the average dissimilarity between summer and winter and spring diets, respectively. Finally, *E. truncata* was identified as responsible

for 15% of the average dissimilarity between winter and spring diets, since this prey was only present in the flounder diet during the winter.

Table 3.10—SIMPER results for differences of the diet between seasons: average dissimilarity and contribution percentage (%) of discriminating taxa to the differences observed (W- winter; Sp – Spring; Su – Summer; A- Autumn).

Groups	Average dissimilarity (%)	Discriminating taxa	Contribution (%)
A vs. W	83.8	Elmidae	27.4
Sp vs. W	78.9	<i>E. truncata</i>	15.1
W vs. Su	73.2	<i>C. crangon</i>	16.7
Sp vs A	70.1	Elmidae	30.8
A vs. Su	66.8	Elmidae	26.2
Sp vs. Su	54.3	<i>C. crangon</i>	21.6

3.4 Prey-predator relationships

3.4.1 Prey selection

Niche breadth, expressed by the Levins index, revealed some degree of specialization of the flounder diet, showed by the low values obtained for all the size classes (Figure 3.11). There was an increase in the niche breadth throughout the flounder growth until 150 mm TL (i.e. until reaching class 4), in a ratio of approximately 1:2. Diet diversity, measured by the Shannon-Wiener index (H') presented rather low values for classes 1 and 4. Similarly, the diet diversity increased with *P. flesus* size, until reaching class 4 (Figure 3.12), in agreement with results obtained for the Levins index. However, caution is needed when interpreting the results of class 4, since the reduced variety of prey found in the stomachs of this class and consequently, the results indicating high degree of specialization of the diet may be due to the reduced number of fish sampled. Overall, results indicate that the diet of *P. flesus* juveniles became more diverse with increasing size.

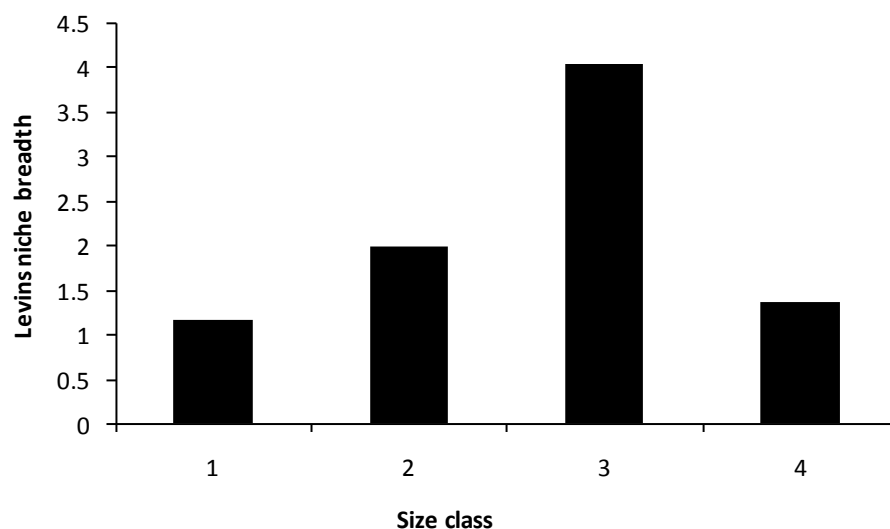


Figure 3.11 – Levins niche breadth for each *P. flesus* size classes (1-4).

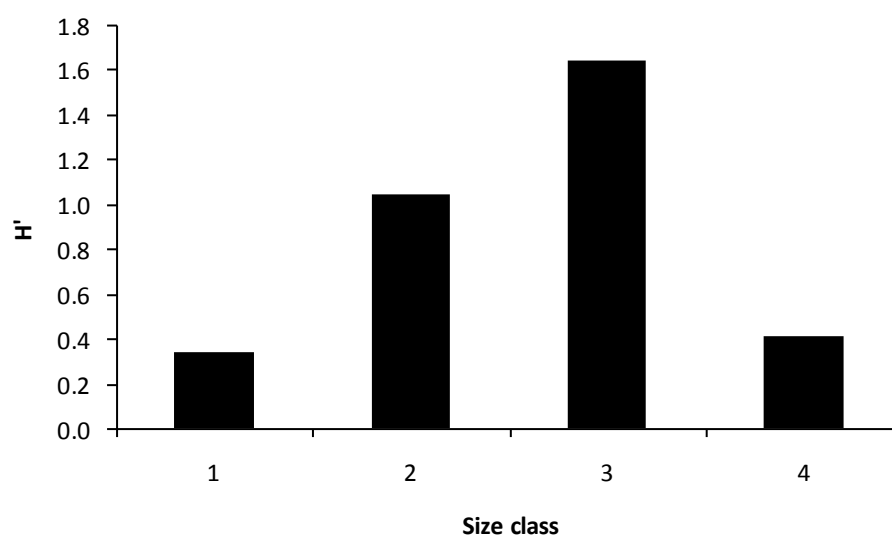


Figure 3.12 – Prey diversity estimated by the Shannon-Wiener diversity index, H' , for each *P. flesus* size classes (1-4).

When comparing the seasonal variation of the flounder main prey items with the Lima estuarine macrobenthic community, *Corophium* spp. showed a pronounced seasonal variation, peaking during the autumn (Figure 3.13). At the same time, this taxon was also

among the main prey items of all flounder size classes throughout the year. Chironomidae ni was also present in the environment from winter to summer, as well as in the stomach contents of juveniles of classes 1, 2 and 3. This item was the dominant prey of class 1 juveniles during the spring. During the autumn, when no Chironomidae ni was found in the macroinvertebrate community, the diet of class 1 juveniles was mainly composed of Elmidae ni and *Corophium* spp.. The class 2 diet also varied between seasons, being dominated by Chironomidae ni and *E. truncata* during the winter, while during summer *Corophium* spp. was the dominant prey. The decrease in consumption of Chironomidae ni from winter to summer was probably a reflex of the decrease observed in the macrobenthic community. During autumn, the item Elmidae ni dominated the diet, together with *Corophium* spp. Class 3 juveniles presented different prey items throughout the year. *E. truncate* and Chironomidae ni were the main prey items during winter, coinciding with the period of great abundance of Chironomidae ni in the Lima estuary. The spring diet was mainly constituted of *Corophium* spp., polychaetes, and bivalves. On the other hand, items found in the stomach contents of this class during summer included only *C. crangon*. Both the items Elmidae and *E. truncata*, although important prey of the juveniles, were absent in the macroinvertebrate community samples. Data regarding the diet of class 4 is only available for the winter. During this season, *Corophium* spp. as the only macrobenthic prey present in the diet, although coinciding with the period when this prey was less abundant in the environment.

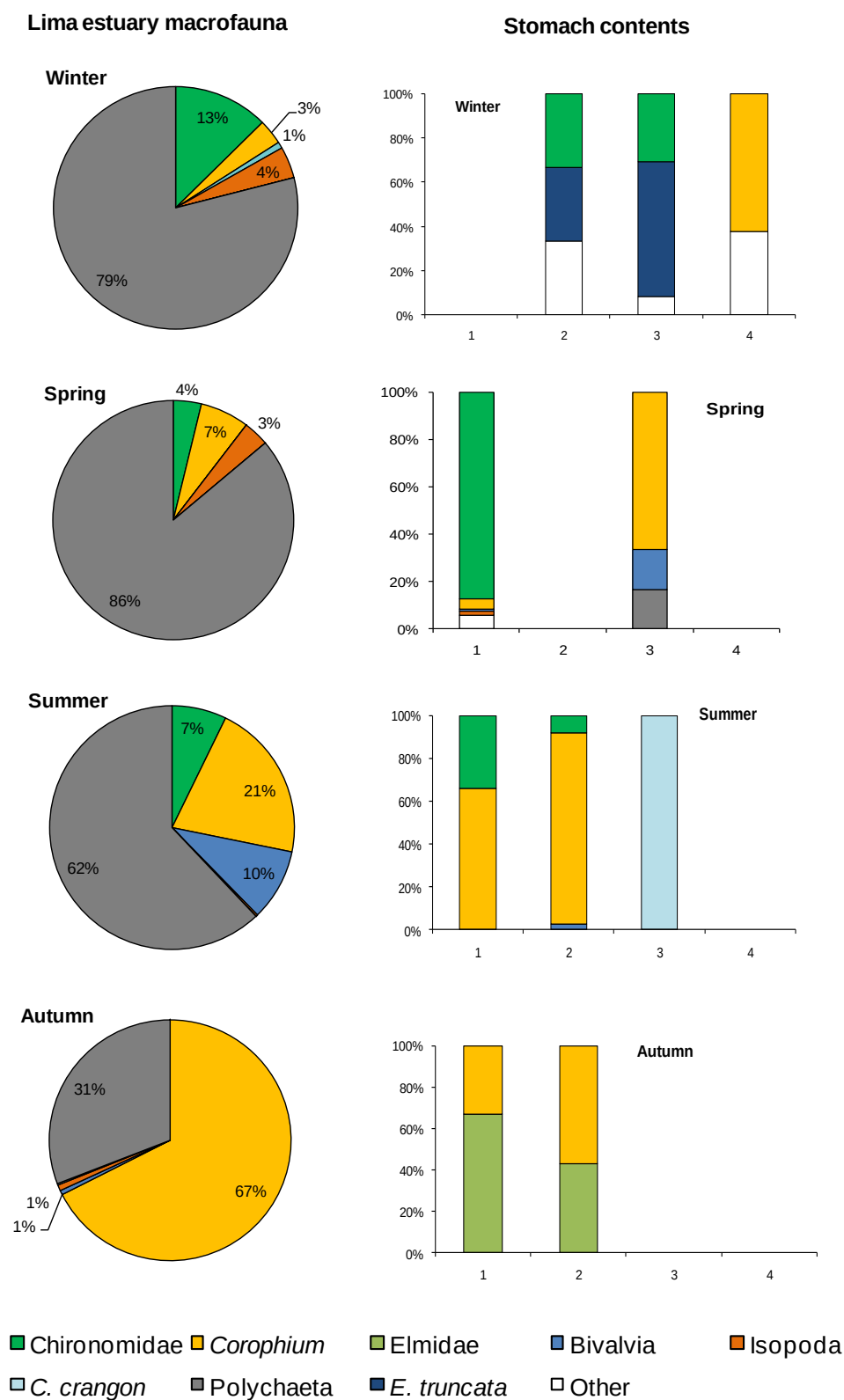


Figure 3.13 – Seasonal abundance of macrobenthos prey in the Lima estuary and seasonal variation of RI diet of the different *P. flesus* size classes (other items: prey items with a contribution < 6 %).

Regarding the Strauss electivity index, some prey items, namely Elmidae ni, *E. truncata* and *P. jenkinsi*, were not considered due to their absence among the macrobenthos samples. According to this index, class 1 flounders presented a high positive selection for Chironomidae ni during spring and summer, indicated by the positive values obtained for the Strauss index (Figure 3.14). Negative values occurred in autumn as expected, since Chironomidae ni was absent from the Lima estuarine community (Figure 3.14). *Corophium* spp. was only positively selected as prey during the summer, when it was the dominant prey item of class 1. Although this item was the second most important in the class 1 diet during autumn, its proportion in the diet was below that found in the macroinvertebrate community (Figure 3.13). Thus, negative selection of *Corophium* spp. occurred during spring and in a greater extent in autumn (Figure 3.14). Bivalvia ni, Diptera ni, Isopoda ni and Spionidae ni presented values near zero, indicating random feeding on these items. Class 2 individuals highly selected Chironomidae ni during the winter and in a lesser extent during the summer, accompanying the decrease of its abundance in the Lima estuarine community. Negative values were observed in autumn due to the absence of Chironomidae ni in the environment. Regarding *Corophium* spp., negative values were registered during winter and positive values during summer and autumn, when its abundance was higher in the macroinvertebrates community (Figure 3.14). Class 3 individuals presented a positive selection for Chironomidae ni and a negative selection for *Corophium* spp. during the winter. The inverse pattern was observed during spring. In the environment, abundance of *Corophium* spp. increased from winter to spring, while there was a decrease of Chironomidae abundance. Thus, once more, the selection pattern coincided with seasonal variations of the items in the environment (Figures 3.13 and 3.14). No preference was shown for Bivalvia ni, *C. crangon*, Diptera ni or Spionidae ni, which presented electivity index values close to zero (Figure 3.14). However, the item Polychaeta ni had a different behavior, since it was negatively selected. In fact, Polychaeta were practically absent from the flounder juveniles diet, despite being one of the dominant groups of the Lima macrobenthic fauna (Figure 3.13). Class 4 individuals only selected *Corophium* spp. as a prey item (Figure 3.14).

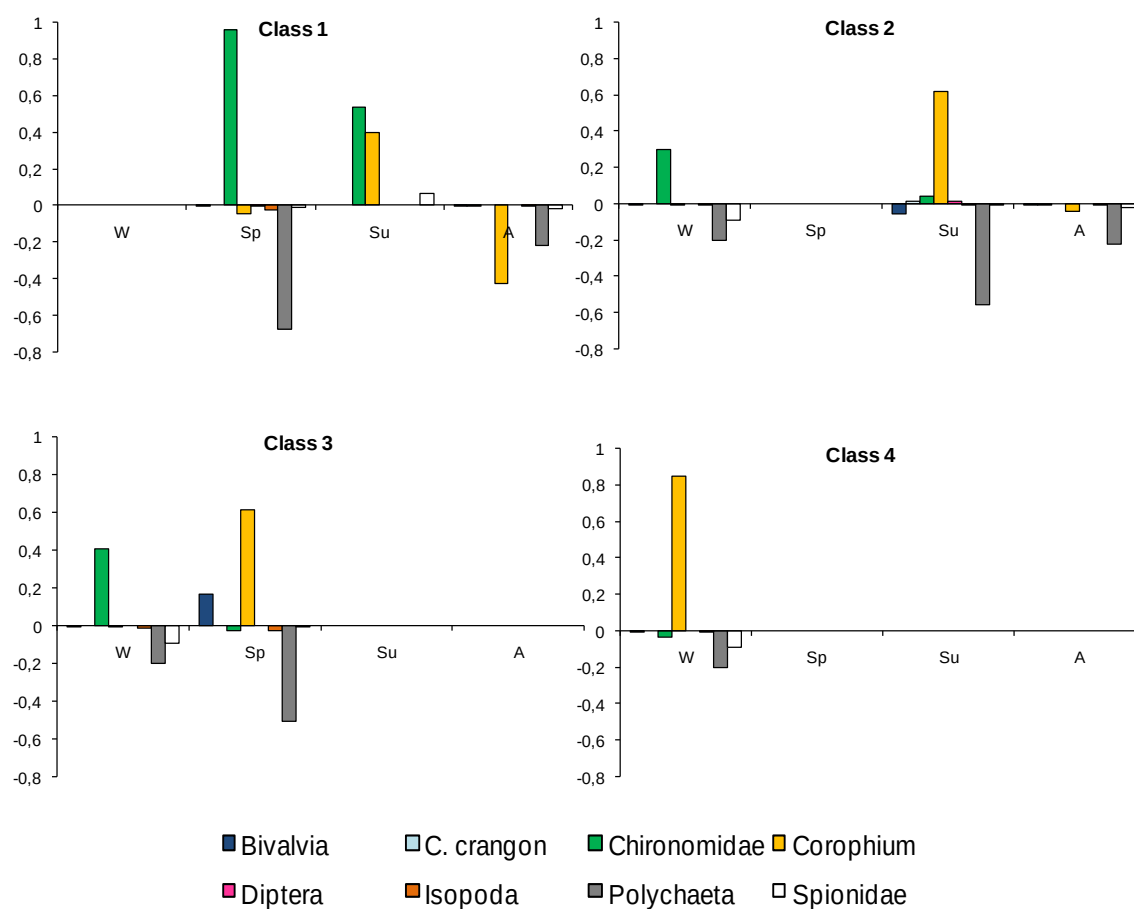


Figure 3.14 - Electivity values for the main prey items of *P. flesus* size classes (W – winter; Sp – spring, Su – summer, A- autumn).

Concerning the niche overlap between flounder size classes, values higher than 0.6 (suggesting overlap), only occurred between class 2 and 4 (Table 3.11). All other values were not indicative of niche overlap, since Schoener index values ranged 0.1 - 0.3 regarding NI index and 0.0 - 0.4 when considering GI dietary index values.

Table 3.11 – Schoener index values of trophic niche overlap between the different *P. flesus* size classes, based on NI (numbers in *italic*) and GI.

Class	1	2	3	4
1	-	<i>0.2</i>	<i>0.4</i>	<i>0.0</i>
2	0.3	-	<i>0.3</i>	<i>0.7</i>
3	0.1	0.1	-	<i>0.1</i>
4	0.2	0.7	0.1	-

Flounder juveniles showed a high positive correlation between total length and mouth gape length ($r^2 = 0.9$, Figure 3.15), with bigger fish presenting wider mouth gape size as expected.

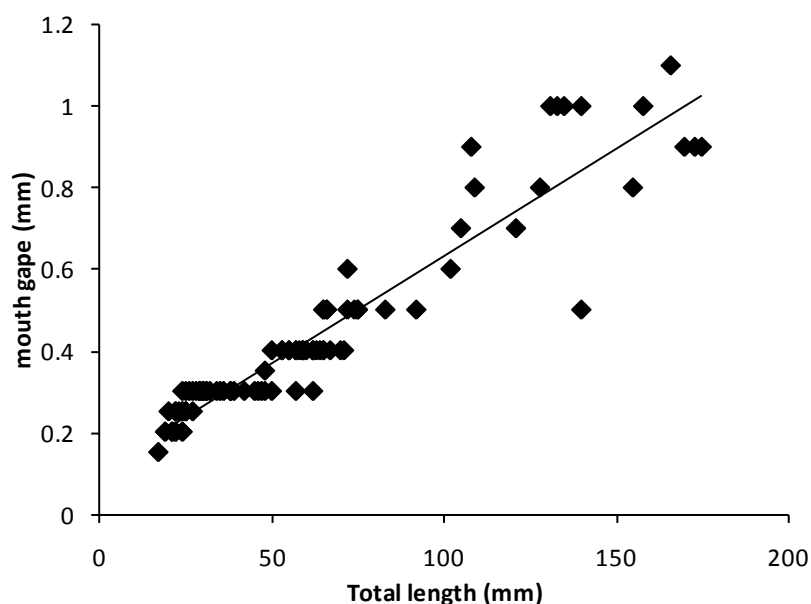


Figure 3.15— *P. flesus* total length (mm) and mouth gape length (mm) relationship.

When considering individuals of all size classes, a significant positive correlation between fishes total length and prey length was found ($R^2 = 0.5$, $p < 0.0001$), with prey length increasing with fish total length (Figure 3.16).

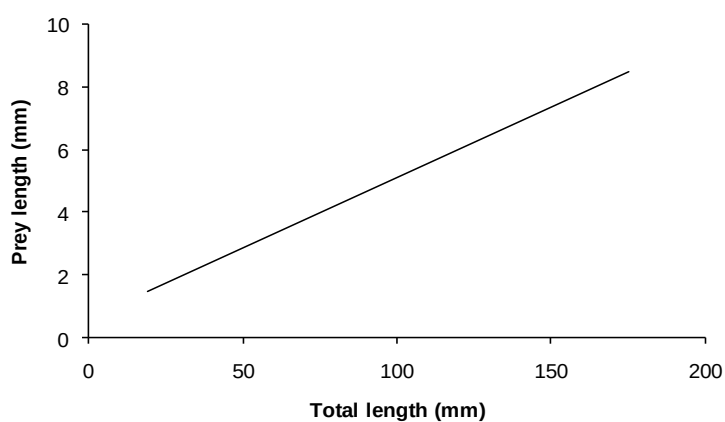


Figure 3.16 - Mean prey length relationship with total length (mm) of *P. flesus* juveniles.

For class 1, there was a significant increase of the minimum ($R^2 = 0.2$, $p < 0.05$) and mean ($R^2 = 0.1$, $p < 0.05$) size of the prey consumed with the body length (Figure 3.17) and, ultimately with the mouth gape size. Thus, the smallest prey gradually ceased to be consumed as the class 1 fishes grew. However, no significant relation was found between minimum ($R^2 = 0.016$, $p > 0.05$), mean ($R^2 = 0.014$, $p > 0.05$) and maximum ($R^2 = 0.011$, $p > 0.05$) prey length and fishes total length for flounder belonging to class 2, despite the general trend for a decrease of prey items with increase of juveniles body length (Figure 3.17). Similarly, in class 3 no significant relationships between minimum ($R^2 = 0.7$; $p > 0.05$), mean ($R^2 = 0.6$; $p > 0.05$) and maximum ($R^2 = 0.6$; $p > 0.05$) prey length and fishes total length were found (Figure 3.17). However, for class 3 individuals, values of minimum and maximum prey length tended to converge. Therefore, the range of prey sizes consumed by class 3 juveniles tended to be narrower as fish grew, restricting to prey of about 20 mm. Regarding class 4, there were not enough samples to analyze the relationship between the prey size and body size of class 4 individuals, so data was not presented here.

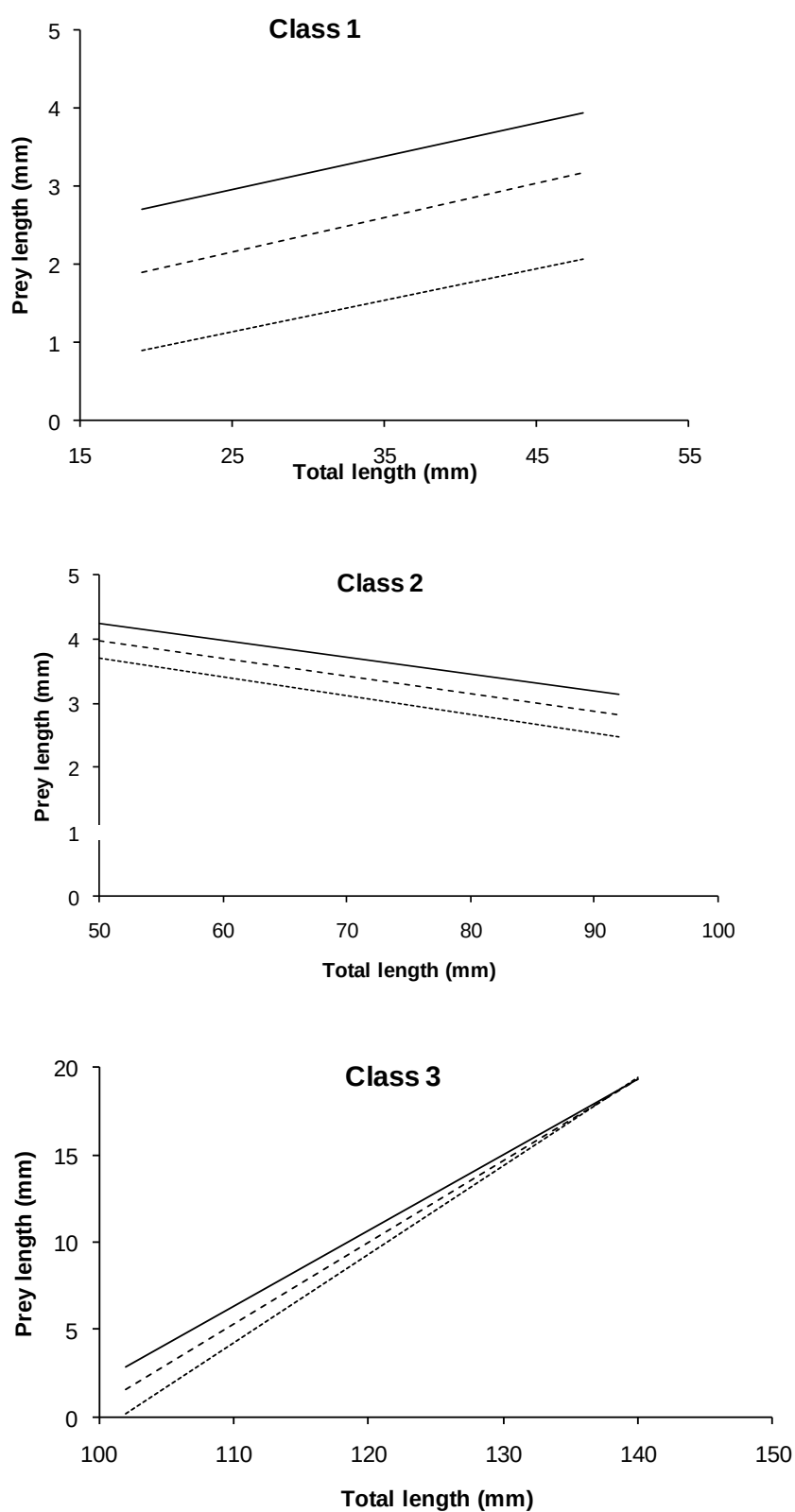


Figure 3.17 - Minimum, mean and maximum prey length relationships with total length (mm) of *P. flesus* of different size classes.

3.4.2 Predatory pressure

The potential predation effect of *C. crangon* on *P. flesus* juveniles, with dimensions greater than 30 mm TL only occurred during spring in the upper estuary, and coincided with the appearance of the smallest flounder individuals, within the predation range of this predator (Table 3.12). Thus, during this period, flounder with less than 21 mm (TL) and with 21-30 mm (TL) co-occurred with their potential predator *C. crangon* (>30 mm and >40 mm, respectively). Because this co-occurrence was limited to the spring season, there was not enough data to establish relationships between the occurrence of *C. crangon* and *P. flesus* densities.

Table 3.12 – Condition (Fulton condition factor, k) and abundance (individuals 1000 m⁻²) of *P. flesus* and their predators *C. maenas* and *C. crangon* (dimensions: *C. maenas* – carapace width (mm); *C. crangon* and *P. flesus* – total length (mm); density – individuals 1000 m⁻²)

Season	<i>P. flesus</i> size (mm)	Predator	Predator density	<i>P. flesus</i> density	k
Spring	< 21	<i>C. crangon</i> >30 mm	7.49 ± 7.77	2.30 ± 3.99	1.45 ± 0.97
Spring	21-30	<i>C. crangon</i> >40 mm	2.18 ± 1.77	23.21 ± 15.44	0.95 ± 0.13
Spring	< 50	<i>C. maenas</i> > 26 mm	35.72 ± 51.36	38.23 ± 35.51	1.02 ± 0.33
Summer	< 50	<i>C. maenas</i> > 26 mm	49.90 ± 55.61	2.89 ± 6.15	1.08 ± 0.04
Autumn	< 50	<i>C. maenas</i> > 26 mm	50.20 ± 67.29	1.33 ± 2.83	1.20 ± 0.17

The predator *C. maenas* (>26 mm carapace width) co-occurred with *P. flesus* (< 50 mm) during spring, summer and autumn, in the upper estuary. From spring to summer, the increase in *P. flesus* density coincided with a decrease in the *P. flesus* density (Table 3.12). From summer to autumn, *C. maenas* and *P. flesus* maintained their densities. Again, data was not enough robust to analyze the predator-prey relationships between this predator and flounder densities. Regarding the influence of predator on flounder condition, no significant correlation was found between *C. maenas* density and the condition of juveniles (Pearson correlation, $R = 0.1$, $p = 0.43$, Table 3.12).

4. Discussion

4.1 The macroinvertebrates community

The macroinvertebrates community of the Lima estuary was previously characterized (Sousa et al. 2006) and results were different from those obtained in the present study. *Oligochaeta* ni, *Hediste diversicolor* and *Corophium* spp. were the most dominant taxa found in this study, while Sousa et al. (2006) and Sousa (2007) found that *H. diversicolor* and the bivalve *Abra alba* presented the highest densities. Indeed, lower abundances of bivalves in general, and *A. alba* in particular, were found in the current investigation. This could be due to the lower area (0.023 m^2) of the grab used, comparatively to Sousa et al. (2006, 2007) (0.5 m^2). Moreover, oligochaetes presented very high densities compared to Sousa et al. (2006) results. A lower mesh size (0.5 mm) was used when sieving the samples, when compared to Sousa et al. (2006) (1mm; larger macroinvertebrates), possibly allowing a greater capture of these small organisms. Oligochaetes are opportunist and commonly found in organic enrichment associated with anoxic conditions (Ysebaert et al. 1998). These organisms were observed in very high densities, which could be an indicative of site contamination. Nevertheless, there was not a decrease in number of species and diversity of the macrobenthic community, typical of contamination situations. Actually, both minimum and maximum number of species ($S_{\min} = 5$; $S_{\max} = 26$) and Shannon-Wiener ($H'_{\min} = 0.37$; $H'_{\max} = 2.09$) values were within the range of the previous studies performed in the Lima estuary ($S_{\min} = 6$, $S_{\max} = 30$; $H'_{\min} = 0.00$; $H'_{\max} = 1.96$, Sousa et al. 2006; $S_{\min} = 1$, $S_{\max} = 20$; $H'_{\min} = 0.22$; $H'_{\max} = 2.80$, Sousa et al. 2007).

The average abundance of macroinvertebrates ($1,788 \text{ individuals m}^{-2}$) in the Lima estuary was slightly higher than the observed by Sousa et al. (2006) ($1,581 \text{ individuals m}^{-2}$) and Sousa et al. (2007) ($1,219 \text{ individuals m}^{-2}$). There were some seasonal oscillations in the abundance (Figure 3.2) which, according to Sousa et al. (2007), could be due to movements of species from the marine adjacent area. The number of species, Shannon-Wiener and Pielou indices were lower during the winter and maintained relatively stable throughout the rest of the year (Figure 3.3), in agreement with prior results obtained for the Lima estuary (Sousa et al. 2007). This seasonal stability is common in several estuaries (Marques et al. 1993; Mucha et al. 2005). Typically, the number of species (Ysebaert et al. 1998; Ysebaert et al. 2003) and diversity (Michaelis, 1983; Mannino and

Montagna, 1997; Ysebaert et al. 2003) tend to decrease upstream. Thus, in the Lima estuary, the number of species was in average higher in the lower stretches. The diversity was generally higher in the lower and middle sections, where a variety of species typical of marine environments, especially polychaetes were observed. However, the abundance of the dominant taxa, *Oligochaeta* ni, *Hediste diversicolor* increased from the lower to the upper estuary, while *Oligochaeta* ni presented the highest abundances in the middle estuary. The same pattern regarding these taxa has been reported in other estuaries (Ysebaert et al. 1993; Seys et al. 1999; Ysebaert et al. 2003). The species structure varied across the estuary. In particular, the structure of the upper stretch differed the most, with Chironomidae ni and *Corophium* spp. being characteristic of this section. These observations are concomitant with the results of Sousa et al. (2006), who observed that Insecta and *Corophium* spp. were restricted to the upper estuary.

4.2 Distribution of *P. flesus* juveniles

Flounder densities observed in the Lima estuary (Table 3.6) were within the range of values observed by Ramos et al. (2009) and for other Portuguese estuaries, namely the Douro (Vinagre et al. 2005), Mondego (Martinho et al. 2007) and Tejo (Cabral et al. 2007). Highest abundances of flounder juveniles were recorded during spring, when new settled individuals arrived to the estuary, thus explaining the predominance of class 1 individuals. Individuals from class 2 observed in summer were probably young of the year who arrived during spring and then grew until attain the class 2 size. These results reflect the spring spawning season of *P. flesus*, with larvae entering the Lima estuary during spring period (Ramos et al. 2010). Indeed, colonization of the estuary by the new settled individuals occurred during late spring, slightly earlier than the observed in the Lima estuary by Ramos et al. (2010) and in the Douro (Vinagre et al. 2005) and Mondego (Martinho et al. 2007) estuaries, where colonization occurred during early summer (June- July).

Flounder juveniles tend to show an active preference for low salinity waters (Bos and Thiel 2006). Indeed, class 1 and the great majority of 2 juveniles were restricted to the upper estuary, coinciding with Ramos et al. (2010) results showing that new settled juveniles appeared at this section of the Lima estuary. Vinagre et al. (2008) also obtained similar results in the Douro estuary, as well as Martinho et al. (2007) in the Mondego estuary, although in the later lower densities were observed (maximum density: 15 fishes 1000 m⁻³

²) when compared to Lima during the current investigation (maximum density: 41.3 fishes 1000 m⁻²). Results may indicate that the distribution of flounder juveniles became wider as they grew, a pattern commonly observed in other estuarine habitats (Kerstan 1991). Thus, class 3 juveniles presented a broader distribution, namely at the middle estuarine section, despite higher abundances still occurred in the upper estuary. Additionally, older juveniles of class 4, probably from cohorts of the previous year, were only found at the lower and middle estuarine sections. As the nursery concept implies some degree of spatial segregation from the older individuals (Beck et al. 2001), the restricted and unique location of the smaller juveniles in the upper estuary confirms its role as a nursery area in the Lima estuary.

Juveniles condition was within the range of the results obtained for other European estuaries (Amara et al. 2009), and higher than in other Portuguese estuaries, such as Minho, Douro and Mondego (Vasconcelos et al. 2009). The condition remained stable between the different size classes, indicating that juveniles maintained a good nutritional state throughout their ontogenetic development, so food availability was not limited.

4.3 Diet of *P. flesus* and prey selection

The main prey items of the flounder juveniles included *Corophium* spp. and Chironomidae ni. These items were highly abundant in the upper estuary, where most of the juveniles concentrated, thus explaining their relevance in the diet. *Corophium* spp. has been pointed as one of the main prey items of flounder diet in several studies (Summers 1980; Hampel et al. 2005; Stevens 2006), including in the Portuguese estuaries Tejo (Costa and Bruxelas 1989) and Douro (Vinagre et al. 2005). In the Lima estuary, it was a major item across all size classes of juveniles. Chironomidae ani are commonly present in the flounder diet, particularly of the smaller juveniles (Aarnio et al. 1996, Weatherley 1989, Nissling et al. 2007, Florin and Lavados 2010). This was also observed in the Lima estuary, since Chironomidae ni dominated the diet of class 1 juveniles and was a major item of the class 2. On the other hand, polychaetes, specially the species *H. diversicolor*, often dominated the diet, along with *Corophium* spp. (Hampel et al. 2005; Vinagre et al. 2005). Although polychaetes dominated the macroinvertebrate community, they were only present as minor prey items of class 3 flounders. Particularly, the species *H. diversicolor*, the most abundant polychaete of the macroinvertebrate community, was absent from the diet of the juveniles. Vinagre et al. (2008) suggested that reduced mouth gape might represent a

challenge for smaller flounder juveniles (<170 mm) to ingest polychaetes. This implies that only individuals of class 3 and 4 would be able to consume this type of prey. In agreement with these observations, results indicate that polychaetes were absent from the diet of the smaller classes, only representing a minor importance in the class 3 diet. Gastropods *E. truncata* and *P. jenkinsi*, although common items in the diet, were absent in the Lima estuarine macroinvertebrate community. However, these species were frequently captured with the beam trawls. Intriguingly, some Diptera species that occurred in the stomachs were not found in the macroinvertebrate samples. These species are typical of freshwater environments. That could imply that the juveniles could have been feeding in other locations, namely further upstream of the sampling sites. On the other hand, a methodological problem must also be considered as a possible cause for the absence of Diptera from the macroinvertebrate samples. Considering that each sampling location can include several types of sediment and vegetation, and consequently, distinct a macroinvertebrate community, the sampling method used showed some limitations for the recovery of all macroinvertebrate species.

Prey length increased with the flounder size. This increase was observed when taking all fishes in consideration, but not within the range of each size class. Thus, fishes gradually consumed prey of increasing sizes along their ontogenetic development, a trend commonly reported in several studies (Keast and Webb 1966; Juanes 1994; Dorner and Wagner 2003). For class 3, minimum and maximum prey length tended to converge, meaning that there was a decrease in the length range of prey consumed. Possibly, not only fishes consume larger prey as they grow, but also smaller preys stop to integrate their diet. These results are contradictory to those obtained by Vinagre et al. (2008) who reported that smaller prey never ceased to be consumed by the larger individuals, despite their ability to capture larger prey.

Diversity of prey items also increased with the size class, larger individuals presenting a higher number of prey taxa in their diet. Concomitantly with these results, niche breadth determined both as Shannon-Wiener index and Levins index showed an increasingly generalist diet along the flounder juvenile development. An exception was observed for class 4, whose diet consisted only of *Corophium* spp. and Teleostei ni. However, the reduced number of full stomachs could explain this lack of prey variability. Therefore, the results here presented may not be representative of the diet of this size class, due to the reduced number of stomachs analyzed. The Shannon-Wiener index values were within

the range of those obtained in other studies of the diet of flounder juveniles (Aarnio et al. 1996, Andersen et al. 2005, Hampel et al. 2005). Moreover, diet diversity of class 3 was higher than the obtained in such studies.

According to SIMPROF results, no significant differences occurred between the diet of the size classes. However, class 1 and 2 exhibited higher diet similarity than the other classes. These smaller classes included Chironomidae ni and *Corophium* spp. as the main prey items. In fact, *Corophium* spp. was the only item present in the diet of all classes, varying in terms of importance. Despite the similarity of the diet between all the four size classes, significant diet overlap only occurred for class 2 and 4. Attending to the fact that both classes consumed *Corophium* spp. (Figures 3.6 and 3.8), the niche overlap observed could have been a consequence of sharing the same prey item. The absence of diet overlap could indicate resource partitioning between the size classes, possibly minimizing intraspecific competition. In the nursery grounds, where high densities of flatfishes juveniles of different species occur, both inter- and intraspecific competition may arise (Martinsson and Nissling 2011). However, species have evolved strategies to avoid this competition. Regarding intraspecific competition, ontogenetic shifts in the diet have been reported (Andersen et al. 2005; Florin and Lavados 2009), enabling resource partitioning between different life stages.

Diet similarities between samples of the same season were greater than similarities within each size class. In fact, the diet of *P. flesus* appeared to be more sensitive to temporal variations, than to ontogenetic development, expressed by the lack of diet variations between the four size classes. The seasonal variations were probably related to seasonal fluctuations in the prey items availability, and to the fact that the presence of some prey taxa may be restricted to some seasons. For example, variations of the main prey items *Corophium* spp. and Chironomidae ni in the Lima estuarine sediments were generally accompanied by variations in the proportions of these taxa in the flounder diet. On the other hand, when Chironomidae ni, the main prey item of the smaller juveniles, was not present in the macrobenthic community, other items were included in the diet of these juveniles, namely Elmidae ni. Thus, these results highlight the opportunistic feeding behaviour of *P. flesus* juveniles. This finding does not exclude that some degree of prey selection may occur, as evidenced by the absence of highly abundant macroinvertebrates in the diet. Particularly, oligochaetes are frequent prey items in the diet of *P. flesus* juveniles in other locations, such as in the River Dee, North Wales (Weatherley 1989) and

in the Danish east coast (Andersen et al. 2005), but were absent in the diet of juveniles of the Lima estuary.

Interestingly, the diet of classes 1 and 2 was based on Chironomidae ni, an item only present in the upper estuary, and *Corophium* spp., which was also far more abundant in the upper estuary. Thus, the location of prey may be the main cause for the restricted location of these smaller juveniles in the upper section of the Lima estuary. Moreover, Ramos et al. (2010) showed that, in the Lima estuary, the sediment composition was related to *P. flesus* juveniles spatial distribution, possibly through its effect on prey abundance. In fact, environmental variables such as sediment composition and physico-chemical parameters such as salinity may only act indirectly (Gibson 2005), by influencing the distribution of the macroinvertebrate prey (Gibson 1994; McConnaughey and Smith 2000; Amezcua and Nash 2001) and, consequently, the location of juveniles.

The vacuity index increased over the size classes. This result was not expected owing to the fact that as the diet became more diverse along the growth of juveniles, a larger choice of prey was available, and consequently less empty stomachs should be found. The bulk of class 2 individuals was caught in the upper estuary and did not present empty stomachs. Interestingly, all the individuals of this class occurring in the lower and middle stretches presented empty stomachs. This may reinforce the concept of the upper estuary role as a nursery, since high food availability is one of the characteristics of the nursery areas that make them attractive to the juveniles (Beck et al. 2001).

In resume, flounder juveniles diet was dominated by organisms present in high abundances. Furthermore, *P. flesus* showed a trend to consume larger and more diverse preys as they grew, varying their diet accordingly to the type of prey present in the environment. The unique prey present in the upper estuary, namely Chironomidae ni and *Corophium* spp. may be responsible for the choice of this estuarine section as nursery by the flounder juveniles.

4.4 Predatory pressure

The species *C. crangon* and *C. maenas* are important predators of flatfishes juveniles (Ansell et al. 1999; Van der Veer and Bergman 1987). Their predation capacity is size dependent. Both these species occurred in the upper estuary, where the juveniles

vulnerable to their predation were concentrated. *C. crangon* (TL > 30 mm) and *P. flesus* juveniles (TL < 30 mm) only co-occurred in the upper estuary during spring. During the remaining seasons, there were no flounder juveniles vulnerable to predation by *C. crangon*. This co-occurrence may imply predatory pressure exert on *P. flesus* in the Lima estuary. Nevertheless, more data would be necessary to evaluate the real impact of *C. crangon* predation on flounder juveniles.

The density of *P. flesus* (TL < 50 mm) vulnerable to predation by *C. maenas* decreased over time, from spring to autumn. This decrease was possibly related to the growth of new settled juveniles until they attain a size refuge from predation. However, during this timeline there was also an increase of the density of *C. maenas* in the upper estuary. Thus, there was the possibility that predation impact by *C. maenas* was also contributing to the decrease of the density of juveniles. Besides direct effects on mortality, the presence of predators often induces changes in feeding behavior (Jones and Paszkowski 1997; Maia et al. 2009), thus affecting the vacuity index and the condition of fishes. However, the reduced number of empty stomachs of class 1 juveniles was not indicative of such changes in the feeding behaviour. Moreover, the potential predatory effect of *C. maenas* did not induced a decrease in the condition of young flounders.

5. General considerations and future directions

The present study revealed that *Corophium* spp. and Chironomidae ni were the main prey items of *P. flesus* juveniles in the Lima estuary. The diet of the juveniles gradually became more diverse as they grew, including prey with greater dimensions. *P. flesus* presented an opportunistic feeding behavior, with the juveniles feeding on abundant prey and changes in the diet reflecting seasonal fluctuations of the macrobenthic prey, namely *Corophium* spp. and Chironomidae ni, in the Lima estuarine macrofauna. However, some degree of prey selection occurred since the diet composition did not reflect entirely the macroinvertebrate community composition and highly abundant organisms such as oligochaetes and polychaetes were absent or low represented in the diet. The low dietary overlap observed between different size classes possibly reflected a resource partitioning strategy, in order to minimize intraspecific competition.

Smaller *P. flesus* were restricted to the upper section of the Lima estuary and separated from the older juveniles, evidencing the nursery role of this area. The unique macroinvertebrates community of the upper section of the Lima estuary, presenting taxa actively chosen as prey by *P. flesus* juveniles, may be responsible for this distribution pattern.

The co-occurrence of potential predators *C. crangon* and *C. maenas* with flounder juveniles within a vulnerable range size was indicative of a possible impact on flounder mortality and densities. Besides direct effects on mortality, the presence of predators often drives changes in feeding behavior and, consequently, in the juveniles condition. That did not seem to be the case in the Lima estuary regarding the predator *C. maenas*, since no effects on juvenile condition were observed.

Further investigations with an extended study period and a large number of fish sampled would enable a deeper understanding of diet variations throughout ontogenetic development allowing a better assessment of the degree of specialization of the diet of different size classes. It would also be interesting to analyze the diet of other flatfish species that use the Lima estuary as nursery area, namely *Solea solea* and *S. senegalensis*, in order to establish comparisons and investigate interspecific relationships, but also additional insights on the predation impact of *C. crangon* and *C. maenas* on the densities of juveniles.

6. References

- Aarnio, K., Bonsdorff, E., Rosenback, N. 1996. Food and feeding habits of juvenile flounder *Platichthys flesus* (L.), and turbot *Scophthalmus maximus* L. in the Åland archipelago, northern Baltic Sea. *Sea Res* 36 (3-4): 311-320.
- Able, K. 2005. A re-examination of fish estuarine dependence: Evidence for connectivity between estuarine and ocean habitats. *Est Coast Shelf* 64(1):5-17.
- Able, K. W. and Fahay, M. P. 1998. *The first year in the life of estuarine fishes in the Middle Atlantic Bight*. Rutgers University Press, New Brunswick, NJ.
- Adams, A.J., Locascio, J.V., Robbins, B.D. 2004. Microhabitat use by a post-settlement stage estuarine fish: evidence from relative abundance and predation among habitats. *J Exp Mar Bio Ecol* 299: 17– 33
- Amara, R., Selleslagh, J., Billon, G., Minier, C. 2009. Growth and condition of 0-group European flounder, *Platichthys flesus* as an indicator of estuarine habitat quality. *Hydrobiologia* 627: 87–98.
- Amezcu, F. and Nash, R.D.M. 2001. Distribution of the order Pleuronectiformes in relation to sediment type in the North Irish Sea. *J Sea Res.* 45: 293–301.
- Andersen, B.S., Carl, J.D., Grønkjær, P., Støttrup, J.C. 2005. Feeding ecology and growth of age 0 year *Platichthys flesus* (L.) in a vegetated and a bare sand habitat in a nutrient rich fjord. *J Fish Biol.* 66: 531–552.
- Ansell, A. D., Comely, C. A., Robb, L. 1999. Distribution, movements and diet of macrocrustaceans on a Scottish sandy beach with particular reference to predation on juvenile fishes. *Mar Ecol Prog Ser* 176: 115-130.
- Baensch, HA, Riehl, R. 1997. *Aquarien Atlas*, Band 5. Mergus Verlag, Melle, Germany.
- Bănar, D., Harmelin-Vivien, M. 2009. Feeding behaviour of Black Sea bottom fishes: Did it change over time? *Acta Oecol* 35(6): 769-777.
- Beck, M. W., Heck, K. L. J., Able, K. W., Childers, D. L., Eggleston, D. B., Gillanders, B. M., Halpern, B., Hays, C. G., Hoshino, K., Minello, T. J., Orth, R. J., Sheridan, P. F. and

- Weinstein, M. P. 2001. The identification, conservation, and management of estuarine and marine nurseries for fish and invertebrates. *Bioscience* 51(8):633-641.
- Beck, M. W., Heck, K. L. J., Able, K. W., Childers, D. L., Eggleston, D. B., Gillanders, B. M., Halpern, B., Hays, C. G., Hoshino, K., Minello, T. J., Orth, R. J., Sheridan, P. F. and Weinstein, M. P. 2003. The role of nearshore ecosystems as fish and shellfish nurseries. *Issues Ecol* 11:1–12.
- Bergman, M.J.N., Van der Veer, H.W., Zijlstra, J.J. 1988. Plaice nurseries: effects on recruitment. *J Fish Biol* 33 (Suppl A):201-218.
- Beverton, R.J.H., Iles, T.C. 1992. Mortality rates of 0-group plaice (*Pleuronectes platessa* L.), dab (*Limanda limanda* L.) and turbot (*Scophthalmus maximus*) in European waters. II. Comparison of mortality rates and construction of life table for 0-group plaice. *Neth J Sea Res* 29:49-59.
- Byström, P., L. Persson, E. Wahlström and E. Westman 2003. Size- and density-dependent habitat use in predators: consequences for habitat shifts in young fish. *J Anim Ecol* 72(1): 156-168.
- Bos, A.R., Thiel, R. 2006. Influence of salinity on the migration of postlarval and juvenile flounder *Pleuronectes flesus* L. in a gradient experiment. *J Fish Biol.* 68: 1411–1420.
- Bray, J.R., Curtis, J.T. 1957. An ordination of the upland forest communities of southern Wisconsin. *Ecol Monogr* 27:325–34.
- Burke, J.S. 1995. Role of feeding and prey distribution of summer and southern flounder in selection of estuarine nursery habitats. *J Fish Biol* 47:355–366.
- Burrows, M.T. 1994. An optimal foraging and migration model for juvenile plaice. *Evol Ecol* 8(2): 125-149.
- Byström, P., Persson, L., Wahlström, E., Westman, E. 2003. Size- and density-dependent habitat use in predators: consequences for habitat shifts in young fish. *J Anim Ecol* 72: 156-168.
- Cabral, H.N., 2000. Distribution and abundance patterns of flatfishes in the Sado estuary, Portugal. *Estuaries* 53: 351–358.

- Cabral, H.N., Lopes, M., Loeper, R. 2002. Trophic niche overlap between flatfishes in a nursery area on the Portuguese coast. *Sci Mar* 66(3): 293-300.
- Cabral, H.N., Vasconcelos, R., Vinagre, C., França, S., Fonseca, V., Maia, A., Reis-Santos, P., Lopes, M., Ruano, M., Campos, V., Santos, P.T., Costa, M.J. 2007. Relative importance of estuarine flatfish nurseries along the Portuguese coast. *J Sea Res* 57:209–217.
- Castillo-Rivera, B.M., Kobelkowsky, A. et al. 2000. Feeding biology of the flatfish *Citharichthys spilopterus* (Bothidae) in a tropical estuary of Mexico. *J Appl Ichthyol* 16(2): 73-78.
- Claridge, P.N., Potter, I.C., Hardisty, M.W. 1986. Seasonal changes in movements, abundance, size composition and diversity of the fish fauna of the Severn estuary. *J Mar Biol Ass UK* 66: 229– 258.
- Costanza, R., d'Arge, R., de Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R., Paruelo, J., Raskin, R., Sutton, P., van den Belt, M. 1997. The value of the world's ecosystem services and natural capital. *Nature* 387:253-260.
- Costa, M.J., Bruxelas, A., 1989. The structure of fish communities in the Tagus estuary, Portugal, and its role as a nursery for commercial fish species. *Sci Mar* 53: 561–566.
- Costello, M.J. 1990. Predator feeding strategy and prey importance: a new graphical analysis. *J Fish Biol* 36:261-263.
- Dahlgren, C.P., Kellison, G.T., Adams, A.J., Gillanders, B.M., Kendall, M.S., Layman, C.A., Ley, J.A., Nagelkerken, I., Serafy, J.E.. 2006. Marine nurseries and effective juvenile habitats: concepts and applications. *Mar Ecol Prog Ser* 312: 291–295.
- Darnaude AM, Harmelin-Vivien ML, Salen-Picard C. 2001. Food partitioning among flatfish (Pisces: Pleuronectiforms) juveniles in a Mediterranean coastal shallow sandy area. *J Mar Biol Ass UK* 81: 119-127.
- Day, J.W.J., Hall, C.A.S., Kemp, W. M., Yáñez-Arancibia, A. 1989. *Estuarine ecology*. John Wiley and Sons, New York.
- De Groot, S.J. 1971. On the interrelationships between morphology of the alimentary tract, food and feeding behaviour in flatfishes (Pisces: Pleuronectiformes). *Neth J Sea Res* 5:121-196.

- DeLancey, L.B. 1989. Trophic relationship in the surf zone during the summer at Folly Beach, South Carolina. *J Coast Res* 5:477-488.
- Dorner, H., Wagner, A. 2003. Size-dependent predator-prey relationships between perch and their fish prey. *J Fish Biol* 62: 1021–1032.
- Dyer, K.R. 1997. *Estuaries. A physical introduction*. 2nd edition. John Wiley and Sons, Chichester.
- Edgar, G.J., Shaw, C. 1995. The production and trophic ecology of shallow-water fish assemblages in southern Australia. II. Diets of fishes and trophic relationships between fishes and benthos at Western Port, Victoria. *J Exp Mar Biol Ecol* 194:83-106.
- Elliot, M., O'Reilly, M.G., Taylor, C.J.L. 1990. The Forth estuary a nursery and overwintering area for North sea fishes. *Hydrobiologia* 195: 89-103.
- Elliott, M. and Dewailly, F. 1995. The structure and components of European fish assemblages. *Neth J Aquat Ecol* 29(3-4):397-417.
- Elliott, M. and Hemingway, K. L. 2002. *Fishes in Estuaries*. Blackwell, Oxford.
- Elliott, M., McLusky, D.S. 2002. The need for definitions in understanding estuaries. *Estuar Coast Shelf Sci* 55:815-827.
- Elliott, M., Whitfield, A.K., Potter, I.C., Blaber, S.J.M., Cyrus, D.P., Nordlie, F., Harrison, T.D. 2007. The guild approach to categorizing estuarine fish assemblages: a global review. *Fish Fish* 8(3): 241-268.
- Ellis, T., Gibson, R. 1995. Size-selective predation of 0-group flatfishes on a Scottish coastal nursery ground. *Mar Ecol Prog Ser* 127: 27-37.
- FAO - <http://www.fao.org/fishery/species/2550/en>
- Florin, A.B., Lavados, G. 2010. Feeding habits of juvenile flatfish in relation to habitat characteristics in the Baltic Sea. *Estuar Coast Shelf Sci* 86(4): 607-612.
- Franco, A., Elliot, M., Franzoi, P., Torricelli, P. 2008. Life strategies of fishes in European estuaries: the functional guild approach. *Mar Ecol Prog Ser* 354: 219-228.

- Freitas, V., Costa-Dias, S., Campos, J., Bio, A., Santos, P., Antunes, C., 2009. Patterns in abundance and distribution of juvenile flounder *Platichthys flesus*, in Minho estuary (NW Iberian Peninsula). *Aquatic Ecology* 43 (4): 1143–1153
- Geffen, A.J., Van der Veer, H.W., Nash, R.D.M. 2007. The cost of metamorphosis in flatfish. *J Sea Res* 58: 35– 45.
- George, E.L., Hadley, W.F. 1979. Food and habitat partitioning between rock bass (*Ambloplites rupestris*) and smallmouth bass (*Micropterus dolomieu*) young of the year. *T Am Fish Soc* 108: 253–261.
- Gibson, R. N. 1973. The intertidal movements and distribution of young fish on a sandy beach with special reference to the plaice (*Pleuronectes platessa* L.). *J Exp Mar Biol Ecol* 12:79-102.
- Gibson, R.N. 1994. Impact of habitat quality and quantity on the recruitment of juvenile flatfishes. *Neth J Sea Res* 32(2): 191-206.
- Gibson, R. N. 1999. The ecology of the early life stages of the plaice, *Pleuronectes platessa* L. A review. *Bulletin of Tohoku Regional Fisheries Research Laboratory* 62:17-50.
- Gibson, R.N. 2005. *Fish and Aquatic Resources Series*, vol. 9 , in: R.N. Gibson, Editor, *Flatfishes: Biology and Exploitation*, Blackwell Publishing, Oxford.
- Gibson, R.N., Robb, L., 1992. The relationship between body size, sediment grain size and the burying ability of juvenile plaice, *Pleuronectes platessa*. *J Fish Biol* 40: 771–778.
- Gibson, R.N., Robb, L., Burrows, M.T., Ansell, A.D. 1996. Tidal, diel and longer term changes in the distribution of fishes on a Scottish sandy beach. *Mar Ecol Prog Ser* 130: 1–17.
- Gibson, R.N., Rob, L., Wennhage, H., Burrows, M.T. 2002. Ontogenetic changes in depth distribution of juvenile flatfishes in relation to predation risk and temperature on a shallow-water nursery ground. *Mar Ecol Prog Ser* 229: 233–244.
- Greenwood, M.F.S., Hill, A.S. 2003. Temporal, spatial and tidal influences on benthic and demersal fish abundance in the Forth estuary. *Estuar Coast Shelf Sci* 58: 211–225

-
- Haedrich, R. L. 1983. Estuarine Fishes, in B. H. Ketchum, editor. *Estuaries and Enclosed Seas. Ecosystems of the World*. Elsevier Scientific, New York.
- Halpin, P.M., 2000. Habitat use by an intertidal salt-marsh fish: trade-offs between predation and growth. *Mar Ecol Prog Ser* 198: 203– 214.
- Hampel, H.A., Cattrijse, Elliot, M. 2005. Feeding habits of young predatory fishes in marsh creeks situated along the salinity gradient of the Schelde estuary, Belgium and The Netherlands. *Helgoland Mar Res* 59(2): 151-162.
- Haroon, Y. A. K., Pittman, K.A. 1998. Intraspecific dietary breadth, overlap indices and feeding strategies of *Puntius gonionotus* Bleeker and *Oreochromis* spp. in a shallow pond from Bangladesh. *Asian Fish Sci* 10: 303-316.
- Harris, S.A., Cyrusa, D.P., Beckleyb, L.E. 2001. Horizontal Trends in Larval Fish Diversity and Abundance Along an Ocean-Estuarine Gradient on the Northern KwaZulu-Natal Coast, South Africa. *Estuar Coast Shelf Sci* 53:221–235.
- Henderson, P.A., Seaby, R.M.H. 1994. On the factors influencing juvenile flatfish abundance in the lower Severn Estuary, England. *Neth J Sea Res* 32(3-4): 321-330.
- Hugie, D.M. and Dill, L.M. 1994. Fish and game: a game theoretic approach to habitat selection by predators and prey. *J Fish Biol* 45: 151-169.
- Hyslop, E.J. 1980. Stomach content analysis - a review of methods and their application. *J Fish Biol* 17:411-429.
- Jager, Z. 1998. Accumulation of flounder larvae (*Platichthys flesus* L.) in the Dollard (Ems estuary, Wadden Sea). *J Sea Res* 40:43–57.
- Jager, Z., Kleef H.L., et al. 1995. Mortality and growth of 0-group flatfish in the brackish dollard (Ems Estuary, Wadden Sea). *Neth J Sea Res* 34(1-3): 119-129
- Jones, H.M. and Paszkowski1 C.A.1997. Effects of exposure to predatory cues on territorial behaviour of male fathead minnows. *Environ Biol Fishes* 49: 97–109.
- Juanes, F. 1994. What determines prey size selectivity in piscivorous fishes?, D.J. Stouder, K.L. Fresh, R.J. Feller, Editors , *Theory and Application in Fish Feeding Ecology*, University of South Carolina Press, Columbia, S.C.

- Keast, A. 1977. Diet overlaps and feeding relationships between the year classes in the yellow perch (*Perca flavescens*). *Environ Biol Fishes* 2(1): 53-70.
- Keast, A., Webb, D., 1966. Mouth and body form relative to feeding ecology in fish fauna of a small lake, Lake Opinicon, Ontario. *J Fish Res Board Can* 23: 1845–1874.
- Kerstan, M. 1991. The importance of rivers as nursery grounds for 0- and I-group flounder (*Platichthys flesus* L.) in comparison to the Wadden Sea. *Neth J Sea Res* 27: 353-366.
- Kerstens, A., Lomoholt, J.P., Johansen, K. 1979. The ventilation, extraction and uptake of oxygen in undisturbed flounders, *Platichthys flesus*: responses to hypoxia acclimatation. *J Exp Biol* 83: 169–179.
- Knight, J. G., Ross, S.T. 1994. Feeding habits of the Bayou darter. *Trans Amer Fish Soc* 123: 794-802.
- Le Pape, O., L. Baulier, Cloarec, A., Martin, J., Le Loc'h, F., Désaunay, Y. 2007. Habitat suitability for juvenile common sole (*Solea solea*, L.) in the Bay of Biscay (France): A quantitative description using indicators based on epibenthic fauna. *J Sea Res* 57(2-3): 126-136.
- Leggett, W.C., DeBlois E. 1994. Recruitment in marine fishes: Is it regulated by starvation and predation in the egg and larval stages? *Neth J Sea Res* 32:119–134.
- Lenanton, R.C.J., Potter, I.C. 1987. Contribution of estuaries to commercial fisheries in temperate Western Australia and the concept of estuarine dependence. *Estuaries* 10(1): 28-35.
- Liao, H., Pierce, C.L., Larscheid, J.G. 2001. Empirical assessment of indices of prey importance in the diets of predacious fish. *Trans Amer Fish Soc* 130: 583-591.
- Link, J.S., Bolles, K., Milliken, C.G. 2002. The feeding ecology of flatfish in the Northwest Atlantic. *J. Northwest Atl. Fish. Sci.* 30: 1–17.
- Litvak, M.K., Leggett, W.C. 1992. Age and size-selective predation on larval fishes - the bigger-is-better hypothesis revisited *Mar Ecol Prog Ser* 81:13-24.
- Lockwood, S. J. 1974. The settlement, distribution and movements of 0-group plaice *Pleuronectes platessa* (L.) in Filey Bay, Yorkshire. *Journal of Fish Biology* 6:465-577.

- Lockwood, S.J. 1980. Density-dependent mortality in 0-group plaice (*Pleuronectes platessa* L.) populations. *Journal du Conseil* 39(2): 148-153
- Maes, J., Taillieu, A., Van Damme, P.A., Cottenie, K., Ollevier, F. 1998. Seasonal patterns in the fish and crustacean community of a turbid temperate estuary (Zeeschelde Estuary, Belgium). *Estuar Coast Shelf Sci* 47:143-151.
- Maes, J., Stevens, M., Breine, J., 2007. Modelling the migration opportunities of diadromous fish species along a gradient of dissolved oxygen concentration in a European tidal watershed. *Estuar Coast Shelf Sci* 75: 151–162.
- Maia, A., Vinagre, C., Cabral, H.N. 2009. Impact of predator in the foraging behavior of *Solea senegalensis*. *J Mar Biol Ass UK* 89(3): 645–649.
- Manderson, J.P., Pessutti, J., Shaheen, J., Juanes, F. 2006. Dynamics of early juvenile winter flounder predation risk on a North West Atlantic nursery ground. *Mar Ecol Prog Ser* 328: 249–265.
- Mannino, A., Montagna, P.A. 1997. Small-scale spatial variation in macrobenthic community structure. *Estuaries* 20: 159–173.
- Marchand, J., 1993. The influence of seasonal salinity and turbidity maximum variations on the nursery function of the Loire estuary (France). *Neth J Aquat Ecol* 27: 427–436.
- Mariani, S., Boggan, C., Balata, D. 2011. Food resource use in sympatric juvenile plaice and flounder in estuarine habitats. *Mar Ecol* 32: 96-101.
- Marques, J.C., Maranhão, P., Pardal, M.A. 1993. Human impact assessment on the subtidal macrobenthic community structure in the Mondego estuary (Western Portugal). *Estuar Coast Shelf Sci* 37: 403-419.
- Marshall, S., Elliott, M. 1997. Environmental influences on the fish assemblage of the Humber estuary, UK. *Estuar Coast Shelf Sci* 46: 175–184.
- Marshall, S., Elliott, M., 1998. Environmental influences on the fish assemblages of the Humber Estuary, U.K. *Estuar Coast Shelf Sci* 46: 175-184
- Martinho, F., Leitão, R., Neto, J.M., Cabral, H.N., Marques, J.C., Pardal, M.A. 2007. Then use of nursery areas by juvenile fish in a temperate estuary, Portugal. *Hydrobiologia* 587: 281–290.

- Martinho, F., Leitão, R., Neto, J.M., Cabral, H., Lagardère, F., Pardal, M.A. 2008. Estuarine colonization, population structure and nursery functioning for 0-group sea bass (*Dicentrarchus labrax*), flounder (*Platichthys flesus*) and sole (*Solea solea*) in a mesotidal temperate estuary. *J Appl Ichthyol* 24(3): 229-237.
- Martinho, F., Dolbeth, M., Viegas, I., Teixeira, C.M., Cabral, H.N., Pardal, M.A. 2009. Environmental effects on the recruitment variability of nursery species. *Estuar Coast Shelf* 83: 460–468.
- Martinsson, J., Nissling, A. 2011. Nursery area utilization by turbot (*Psetta maxima*) and flounder (*Platichthys flesus*) at Gotland, central Baltic Sea. *Boreal Env Res* 16:60-70.
- Mathieson, S., Cattrijsse, A., Costa, M. J., Drake, P., Elliott, M., Gardner, J. and Marchand, J. 2000. Fish assemblages of European tidal marshes: a comparison based on species, families and functional guilds. *Mar Ecol Prog Ser* 204:225-242.
- Mattila, J., Bonsdorff, E. 1998. Predation by juvenile flounder (*Platichthys flesus* L.): a test of prey vulnerability, predator preference, switching behaviour and functional response. *J Exp Mar Biol Ecol* 227(2): 221-236.
- McConnaughey, R.A., Smith, K.R., 2000. Associations between flatfish abundance and surficial sediments in the eastern Bering Sea. *Can J Fish Aquat Sci* 57: 2410–2419.
- McDowall, R.M. 1988. *Diadromy in Fishes*. Croom-Helm, London
- McLusky, D.S., Elliott, M. 2004. *The estuarine ecosystem*. John Wiley and Sons, New York.
- Michaelis, H. 1983. Intertidal benthic animal communities of the estuaries of the rivers Ems and Weser. In W. J. Wolff (Ed.), *Ecology of the Wadden Sea Vol. I* (pp. 4/158–4/188). Balkema, Rotterdam.
- Modin, J., Pihl, L. 1996. Small-scale dispersal of 0-group plaice (*Pleuronectes platessa* L.) and flounder (*Platichthys flesus* L.) in a shallow Swedish bay. *J Fish Biol* 49:1070-1085.
- Morais, P., Dias, E., Babaluk, J., Antunes, C. 2011. The migration patterns of the European flounder *Platichthys flesus* (Linnaeus, 1758) (Pleuronectidae, Pisces) at the southern limit of its distribution range: Ecological implications and fishery management. *J Sea Res* 65:235-246.

- Mucha, A.P. Vasconcelos, M.T.S.D. Bordalo, A.A. 2003. Macrobenthic community in the Douro Estuary: relations with trace metals and natural sediment characteristics. *Environ Pollut* 121: 169–180.
- Munk, P., Nielsen, G.N. 2005. *Eggs and larvae of North Sea fishes*. Biofolia Frederiksberg Denmark.
- Muus, B.J. 1967. The fauna of Danish estuaries and lagoons. Distribution and ecology of dominating species in the shallow reaches of the mesohaline zone. *Meddr Danm Fisk.-og Havunders.* 5: 7–316.
- Nash, R.D.M., Geffen, A.J. 2000. The influence of nursery ground processes in the determination of year-class strength in juvenile plaice *Pleuronectes platessa* L. in Port Erin Bay, Irish Sea. *J Sea Res* 44(1-2): 101-110.
- Natarajan, A.V., Jhingran, A.G. 1961. Index of preponderance-a method of grading the food elements in the stomach analysis of fishes. *Ind J Fish* 8: 54-59.
- Nicolas, D., Le Loc'h, F., Désaunay, Y., Hamon, D., Blanchet, A., Le Pape, O. 2007. Relationships between benthic macrofauna and habitat suitability for juvenile common sole (*Solea solea*, L.) in the Vilaine estuary (Bay of Biscay, France) nursery ground. *Estuar Coast Shelf Sci* 73 (3-4): 639-650.
- Nissling, A., Jacobsson, M., Hallberg, M. 2007. Feeding ecology of juvenile turbot *Scophthalmus maximus* and flounder *Pleuronectes flesus* at Gotland, Central Baltic Sea. *J Fish Biol* 70 (6): 1877–1897.
- Odum, E. P. 1959. *Fundamentals of Ecology.*, second ed. edition. Saunders, Philadelphia.
- Pen, L., Potter, I., Calver, M.C. 1993. Comparisons of the food niches of three native and two introduced fish species in an Australian river. *Environ Biol Fish* 36(2): 167-182.
- Phelan, B.A., Manderson, J.P., Stoner, A.W., Bejda, A.J. 2001. Size-related shifts in the habitat associations of young-of-the-year winter flounder (*Pseudopleuronectes americanus*): field observations and laboratory experiments with sediments and prey. *J Exp Mar Biol Ecol* 257: 297–315.
- Pielou, E.C. 1966. Shannon's formula as a mean of specific diversity: its use and misuse. *Am Nat* 100: 463–465.

- Pihl, L. 1982. Food intake of young cod (*Gadus morhua*) and flounder (*Platichthys flesus*) in a shallow bay on the Swedish West Coast. *Neth J Sea Res.* 15: 419–432.
- Pihl, L., Cattrijsse, A., Codling, I., Mathieson, D.S., McLusky, D.S., Roberts, C., 2002. Habitat use by fishes in estuaries and other brackish areas. In: Elliott, M., Hemingway, K. (Eds.), *Fishes in Estuaries*. Blackwell Science, Oxford.
- Pinkas, L., Oliphant, M.S. & Iverson, I.L.K. 1971. Food habits of albacore, bluefin tuna, and bonito in California waters. California Fish and Game, *Fish Bulletin* 152:1- 105.
- Pomfret, J.R., Elliott, M., O'Reilly, M.G., Phillips, S. 1991. Spatial and temporal patterns in the fish communities in two U.K. North Sea estuaries. In: Elliott, M., Ducrotoy, J.-P. (Eds.). *Estuaries and Coasts: Spatial and Temporal Intercomparisons*. Olsen and Olsen, Fredensborg.
- Power, M., Attrill, M.J. and Thomas, R.M. 2000. Temporal abundance patterns and growth of juvenile herring and sprat from the Thames estuary 1977-1992. *J Fish Biol* 56: 1408-1426.
- Pritchard, D. W. 1967. What is an estuary: a physical viewpoint. *Am Assoc Adv Sci Pub* 83:3-5.
- Ramos, S., Cowen, R.K., Paris, C., Ré, P., Bordalo, A.A., 2006. Environmental forcing and larval fish assemblage dynamics in the Lima River estuary (northwest Portugal). *J Plankton Res* 28 (3): 275–286.
- Ramos, S., Ré, P., Bordalo, A. 2009. Environmental control on early life stages of flatfishes in the Lima estuary (NW Portugal). *Estuar Coast Shelf S* 83: 252-264.
- Ramos, S., Ré, P., Bordalo, A. 2010. Recruitment of flatfish species to an estuarine nursery habitat (Lima estuary, NW Iberian Peninsula). *J Sea Res* 64(4): 473-486.
- Ré, P., Meneses, I. 2009. Early stages of marine fishes occurring in the Iberian Peninsula.
- Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. *Bulletin of the Fisheries Research Board of Canada* 191:1-382.
- Riley, J.D., Symonds, D.J., Woolner, L. 1981. On the factors influencing the distribution of 0-group demersal fish in coastal waters. *Rapp P-v Reun Cons int Explor Mer* 178: 223–228.

- Root, R.B. 1967. The niche exploitation pattern of the blue-grey gnatcatcher. *Ecol Monograph* 37: 317–350.
- Russel, F.S. 1976. *The Eggs and Planktonic Stages of British marine Fishes*. Academic Press, New York.
- Schoener, T.W. 1970. Non-synchronom spatial overlap of lizards in patchy habitats. *Ecology* 51: 408-418.
- Seys, J., Vincx, M., Meire, P. 1999. Spatial distribution of Oligochaeta (Clitellata) in the tidal freshwater and brackish parts of the Schelde estuary (Belgium). *Hydrobiologia* 406: 119–132.
- Shannon CE and Weaver W. 1949. *The mathematical theory of communication*. Urbana, IL: University of Illinois Press.
- Sims, D.W., Wearmouth, V.J., Genner, M.J., Southward, A.J., Hawkins, S.J., 2004. Low-temperature-driven early spawning migration of a temperate marine fish. *J Anim Ecol* 73: 333–341.
- Sogard, S.M., 1997. Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bull Mar Sci* 60 (3): 1129– 1157.
- Sousa, R., Dias, S., Antunes, C. 2006. Spatial subtidal macrobenthic distribution in relation to abiotic conditions in the Lima estuary, NW of Portugal. *Hydrobiologia* 559:135-148.
- Sousa, R., Dias, S., Antunes, C. 2007. Subtidal macrobenthic structure in the lower Lima estuary, NW of Iberian Peninsula. *Annales Zoologici Fennici* 44: 303 - 313.
- Steele, J.H. and Edwards R.R.C. 1970. The ecology of 0-group plaice and common dabs in loch ewe. IV. Dynamics of the plaice and dab populations. *J Exp Mar Biol Ecol* 4(2): 174-187.
- Stevens, M., 2006. Intertidal and basin-wide habitat use of fishes in the Scheldt estuary. PhD Thesis. Katholieke Universiteit Leuven, Laboratorium voor Aquatische Ecologie: Heverlee, Leuven, (Belgium).
- Stevens, M., Maes, J., Ollevier, F., 2006. A bioenergetics model for juvenile flounder *Platichthys flesus*. *J Appl Ichthyol* 22: 79–84.

- Stoner, A.W., Manderson, J.P., Pessutti J. 2001. Spatially-explicit analysis of estuarine habitat for juvenile winter flounder (*Pseudopleuronectes americanus*): combining generalized additive models and geographic information systems. *Mar Ecol Prog Ser* 213: 253–271.
- Strauss, R.E. 1979. Reliability estimates for Ivlev's electivity index, the forage ratio, and a proposed linear index of food selection. *Trans Am Fish Soc* 108: 344-35
- Summers, R.W. 1979. Life cycle and population ecology of the flounder *Platichthys flesus* (L) in the Ythan estuary, Scotland. *J Nat Hist* 13:703–723.
- Taylor, D.L. 2003. Size-dependent predation on post-settlement winter flounder *Pseudopleuronectes americanus* by sand shrimp *Crangon septemspinosa*. *Mar Ecol Prog Ser* 263: 197-215.
- Teixeira, C.M., Batista, M.I., Cabral, H.N. 2010. Diet, growth and reproduction of four flatfishes along the Portuguese coast. *Sci Mar* 74(2): 223-233
- Thiel, R., Potter, I.C. 2001. The ichthyofaunal composition of the Elbe estuary: an analysis in space and time. *Mar Biol* 138:603–616.
- Ustups D, Urtans E, Minde A., Uzars D. 2003. The structure and dynamics of fish communities in the Latvian coastal zone (Pape – Pērkone), Baltic Sea. *Acta Universitatis Latviensis* 662: 33-44.
- Van der Veer, H. W. 1986. Immigration, settlement and density-dependent mortality of a larval and early post-larval 0-group plaice (*Pleuronectes platessa* L.) population in the western Wadden Sea. *Mar Ecol Prog Ser* 29:223–236.
- Van der Veer, H.W. Bergman, M. 1987. Predation by crustaceans on newly settled 0-group plaice *Pleuronectes platessa* populations in the western Wadden Sea. *Mar Ecol Prog Ser* 35:203–215.
- Van der Veer, H.W., Pihl, L., Bergman, M.J.N. 1990. Recruitment mechanisms in North Sea plaice *Pleuronectes platessa*. *Mar Ecol Prog Ser* 64: 1-12.
- Van der Veer, H.W., Bergman, M.J.N., Dapper, R., Witte, J.I.J. 1991. Population dynamics of an intertidal 0-group flounder *Platichthys flesus* population in the western Dutch Wadden Sea. *Mar Ecol Prog Ser* 73: 141-148.

- Van der Veer, H.W., Berghahn, R., Miller, J.M., Rijnsdorp, A.D. 2000. Recruitment in flatfish, with special emphasis on North Atlantic species: progress made by the Flatfish Symposia. *J Mar Sci* 57: 202–215.
- Van der Veer, H. W., Dapper, R. and Whitte, J. I. 2001. The nursery function of the intertidal areas in the western Wadden Sea for 0-group sole *Solea solea* (L.). *J Sea Res* 45:271-279.
- Vasconcelos, R. P., P. Reis-Santos, Fonseca, V., Ruano, M., Tanner, S., Costa, M.J., Cabral, H.N. 2009. Juvenile fish condition in estuarine nurseries along the Portuguese coast. *Estuar Coast Shelf Sci* 82(1): 128-138.
- Vasconcelos, R. P., P. Reis-Santos, A. Maia, V. Fonseca, S. França, N. Wouters, M. J. Costa and H. N. Cabral 2010. Nursery use patterns of commercially important marine fish species in estuarine systems along the Portuguese coast. *Estuar Coast Shelf Sci* 86(4): 613-624.
- Vinagre, C., França, S., Costa, M.J., Cabral, H.N., 2005. Niche overlap between two flatfishes, *Platichthys flesus* (Linnaeus, 1758) and *Solea solea* (Linnaeus, 1758), in a southern European estuary and adjacent coastal waters. *J Appl Ichthyol* 21: 1–7.
- Vinagre, C., Fonseca, V., Cabral, H., Costa, M. 2006. Habitat suitability index models for juvenile soles, *Solea solea* and *S. senegalensis*, in the Tagus estuary: defining variables for species management. *Fish Res* 82:140-149.
- Vinagre, C., Cabral, H., Costa, M.J. 2008. Prey selection by flounder, *Platichthys flesus*, in the Douro estuary, Portugal. *J Appl Ichthyol* 24: 238-243.
- Weatherley, N.S. 1989. The diet and growth of 0-group flounder, *Platichthys flesus* (L), in the river Dee, North Wales. *Hydrobiologia* 178:193–198.
- Wallace, H., Ramsey, J.S. 1983. Reliability in measuring diet overlap. *Can J Fish Aquat. Sci* 40: 347-351.
- Wennhage, H. 2000. Vulnerability of settling plaice *Pleuronectes platessa* to predation: effects of developmental stage and alternative prey. *Mar Ecol Prog Ser* 203: 289–299
- Wennhage, H., Gibson, R.N. 1998. Influence of food supply and a potential predator (*Crangon crangon*) on settling behaviour of plaice (*Pleuronectes platessa*). *J Sea Res* 39(1-2): 103-112.

- Werner, E.E., Gilliam, J.F. 1984. The ontogenetic niche and species interactions in size-structured populations. *Ann Rev Ecol Syst* 15: 393– 425.
- Whitfield, A. K. 1994b. Fish species diversity in southern African estuarine systems: an evolutionary perspective. *Environmental Biology of Fishes* 40(1):37-48.
- Witting, D.A., Able, K.W. 1993. Effects of body size on probability of predation for juvenile summer and winter flounder based on laboratory experiments. *Fish Bull* 91:577–581.
- Wootton, R.J. 1998. *The ecology of teleost fishes*. 2nd edn. Fish & Fisheries Series, no. 24. Dordrecht: Kluwer.
- Yamashita, Y., Tanaka, M., Miller, J.M. 2001. Ecophysiology of Juvenile Flatfish in Nursery Grounds. *J Sea Res* 45: 205-218.
- Yáñez-Arancibia, A., Day, J. W. 2004. The Gulf of Mexico: towards an integration of coastal management with large marine ecosystem management. *Ocean Coast Manage* 47:537-563.
- Ysebaert, T., Meire, P., Maes, D. and Buijs, J. 1993. The benthic macrofauna along the estuarine gradient of the Schelde estuary. *Neth J Aquat Ecol* 27: 327–341.
- Ysebaert, T., Meire, P., Coosen, J., Essink, K. 1998. Zonation of intertidal macrobenthos in the estuaries of Schelde and Ems. *Aquat Ecol* 32: 53–71.
- Ysebaert, T., Herman, P.M.J., Meire, P., Craeymeersch, J., Verbeek, H., Heip, C.H.R. 2003. Large-scale spatial patterns in estuaries: estuarine macrobenthic communities in the Schelde estuary, NW Europe. *Est Coast Shelf S* 57: 335-355.
- Zar, J. H. 1996. *Biostatistical analysis*. Third editions Prentice-Hall International Editions, New Jersey.
- Zuccheta, M., Franco, A., Torricelli, P., Franzoi, P. 2010. Habitat distribution model for European flounder juveniles in the Venice lagoon. *J Sea Res* 64: 133–144.