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Intertidal and basin-wide habitat use of fishes in the Scheldt estuary

Getij- en bekkengebonden habitatgebruik door vissen in het Schelde-estuarium

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CHAPTER 1

Introduction

1

In this introductory chapter, we first briefly review the factors that influence the selection of habitats by fishes. Next, the nature and the importance of these factors for juvenile fishes in estuarine nurseries are discussed. Also a description is given of our study area (the Scheldt estuary), its main physicochemical characteristics and the different habitats present. Finally, the objectives and outline of this thesis are presented.

1. Habitat selection

Living organisms generally select those habitats that optimize their fitness. Individuals occupying habitats that maximize their lifetime reproductive success produce on average the highest number of progeny. Therefore natural selection favors individuals that are able to distinguish between high- and low-quality habitats, based on the habitats' potential to confer fitness on their inhabitants. According to Morris (2003), a habitat is defined as a spatially-bounded area, with a subset of physical and biotic conditions, within which the density of interacting individuals, and at least one of the parameters of population growth, is different from adjacent subsets. As our study deals with mobile organisms (fishes), habitat should not be regarded as fixed in relation to their ontogeny. Fishes are indeed free to move from one habitat to another and their different life history stages may occupy different habitats (e.g. feeding habitats, spawning habitats, nursery habitats).

When animals choose habitats in which they forage, find shelter or reproduce, their preference will be affected by both habitat quality and conspecifics. There is a general agreement that habitats with high intrinsic quality are preferred for settlement, i.e. those areas that offer high resource densities, protection from predators, or other features that enhance growth, survivorship or offspring production (Frettwell and Lucas, 1970; Morin, 1999; Morris, 2003). As also predators are free to move from one habitat to another, the distribution of most species between different habitats may be determined by a trade-off between predation risk and site-specific resources (e.g. foraging) (Burrows, 1994; Hugie and Dill, 1994; Morin, 1999). As a result of maximizing energy intake and minimizing mortality, organisms can be restricted to so-called less favourable habitats (Werner and Gilliam, 1984).

Given the scope of this thesis, we focus on the factors that influence the habitat quality for fishes. Their habitats vary temporally and spatially; so in order to optimize their survival, growth and reproduction, fishes need to select the optimum available combination of biotic and abiotic environmental factors. The effect of these factors on their growth and survival and how they contribute to habitat quality is briefly discussed hereafter.

1.1. Abiotic factors

The distribution of fishes is more affected by the abiotic environment, than it is the case for endothermic vertebrates. For fishes temperature, oxygen, salinity and water movement are the dominant abiotic factors (Wootton, 1992). Other factors, such as pH, may only play a differentiating role in rather extreme environments. Fish can only survive within a limited range of each specific abiotic variable; outside this range the fish dies and the variable acts as a lethal factor (Fry, 1971; Wootton, 1992). Temperature, oxygen and salinity can therefore act as lethal factors. It may be clear that the actual values of these factors are interdependent and that the effect of their combinations may be different from that of a single factor. When fishes are free to migrate between habitats, some of these factors can rather act as directive factors, which induce movements from areas with unfavorable conditions to areas that enhance growth and survival (Fry, 1971; Yamashita, 2001). Some species are tolerant to a wide range of a factor (eurytopic species), whereas others only tolerate a narrow range (stenotopic species). The fundamental niche of a species is defined by both the diet of a species and the range of abiotic factors that allows the species to successfully maintain itself by natural recruitment. Interactions with other organisms (inter- or intraspecific) restrict the species to the smaller realized niche (Wootton, 1992). Furthermore, the tolerance of a species for a specific abiotic variable may change during its ontogeny. Larvae and juveniles, for example, often have a higher optimum temperature for growth than their larger conspecifics (Fonds *et al.,* 1992; Jobling, 1994).

1.1.1. Temperature

Temperature controls the maximum rate of metabolic processes in ectotherms. As such, it has an impact on the rate of feeding, growth and even reproduction. Fish can detect a temperature gradient in their environment, which allows them to exert some behavioral control over their body temperature by selecting a range of suitable temperatures (Wootton, 1992). If food is not limiting, then temperature is likely to be the most important factor controlling growth. Rapid growth enables juvenile fish to reduce their vulnerability to size-selective predation. 0-group flatfish, for example, are vulnerable to predation by crangonid shrimps until they reach a size refuge at a length of 25 mm (van der Veer *et al*., 2000b; Taylor and Collie, 2003). However, temperature may also have an indirect effect on the survival of juveniles, as extreme low winter temperatures may reduce the predator population (Pihl, 1990). Fast growth also allows fishes to exploit a wider range of prey items and to mature at an earlier age. Fish that reach the age of recruitment faster are likely to make a more substantial contribution to the overall reproductive output (Gibson, 1994). Fish may therefore use temperature as a resource in a manner consistent with their use of other ecological resources (Magnuson *et al.,* 1979; Attrill and Power, 2004). Temperatures of many habitats will be optimal for several species and therefore these habitats might be the subject of potential competition.

1.1.2. Salinity

Remark: salinity is reported using the Practical Salinity Scale. In the Practical Salinity Scale salinity is defined as a pure ratio, and has no dimensions or units.

Fishes that migrate between habitats differing in salinity probably have to invest additionally in osmotic capacity. An increased energetic cost for osmoregulation may reduce the scope for growth. Consequently, growth should be enhanced at salinities close to the fish plasma concentration, at least for species that are physiologically adapted to it (Gutt, 1985; Bœuf and Payan, 2001). However, salinity seems to have only limited effects on growth of euryhaline species (Wuenschel *et al.*, 2004) and mainly controls the distribution and movement of fishes (Malloy and Target, 1991; Gibson, 1994; 1998; Bœuf and Payan, 2001). As such, salinity is considered a dominant and persistent factor affecting the structure of fish assemblages in rivers and estuaries (Cyrus and Blaber, 1992; Higgins and Wilde, 2005).

The capacity to osmoregulate may vary during ontogeny (See review by Varsamos *et al.*, 2005). Generally, the ability to osmoregulate increases with size, according to a timing which is closely related to the ecology of the species. The larvae of many diadromous fishes are stenohaline during the first weeks or months after hatching and acquire the ability to osmoregulate just before the onset of migration from fresh to salt water or *vice versa* (e.g. downstream migration of salmonids). In many marine species (e.g. *Clupea harengus* and *Pleuronectes platessa*) the larvae and juveniles are transported to coastal areas where they are exposed to salinity variations. In these species the osmoregulatory capacity already develops during the early larval phase which allows them to migrate into the coastal nursery areas where food and temperature are more favourable.

1.1.3. Oxygen

The gills allow fishes effective functioning when the oxygen saturation of the water is high enough. When the oxygen level drops too far below saturation, a critical oxygen concentration is reached below which the metabolism is limited by restricted oxygen supply. The solubility of oxygen decreases with increasing temperature (and salinity), while the metabolic rate of fishes increases with increasing temperature. As a result, the risk of reaching a lethal oxygen level is generally higher in warm than in cold water. Below the critical oxygen concentration, dissolved oxygen acts as a limiting factor by constraining food intake, conversion efficiency and metabolic rate (Fry, 1971; Wootton, 1992; Jobling, 1994). However, most fishes show avoidance responses to hypoxic conditions and consequently do not experience direct reduced growth rates due to low oxygen concentrations in open systems like rivers and seas. It is, however, possible that this avoidance response forces fish to temporally retreat in habitats with lower food density or higher predation risk, which, in turn, negatively affects growth and survival (Gibson, 1994). A study by Eby *et al.* (2005) in a river in North-Carolina (USA) showed that intermittent hypoxic conditions reduce the habitat quality for demersal fishes through three pathways: (1) hypoxia restricts fishes to shallow, oxygenated areas, where fewer prey resources are available. (2) The concentration of fish into smaller areas may result in density-dependent reduction of growth rates. (3) Mortality of sessile infauna in areas exposed to intermittent hypoxia decreases prey densities.

1.1.4. Hydrodynamics

Water movement influences directly and indirectly the habitat use of fishes. By the high momentum of moving water, fishes may experience difficulties in maintaining their position in habitats with much turbulence (Wootton, 1992; Booker *et al.*, 2004). Currents and wave action may indirectly influence habitat use by decreasing food intake, compared with the possibilities in calm water (Gibson, 1994; Metcalfe *et al.*, 1997). Particularly planktivorous fishes encounter problems with prey capture at higher current velocities (McFarland and Levin, 2002). In addition, increased turbulence suspends sediment thereby reducing the visual field and hence prey capture. However, at intermediate levels, turbidity may have a positive effect on the attack rate of larval fishes because of an increased encounter rate and a shortened reaction distance due to light scattering and absorption (Utne-Palm, 2004). Finally, hydrographical conditions are important for the transport of fish larvae to and their retention in shallow coastal areas and as such may determine the amount of potential settlers (Costa *et al.*, 2002). Pihl (1990) for example showed that onshore winds during spring favour good recruitment of plaice on the Swedish west coast. The wind influences the distribution of surface water in which eggs and larvae drift passively.

1.2. Biotic factors

While the abiotic environment defines the absolute limits for survival, growth and reproduction of an organism, its occurrence in a habitat may also be precluded by the presence and action of other organisms (Begon *et al.,* 1996). The biotic interactions experienced by an individual may be towards members of its own species (conspecific interactions) or from other species (heterospecific interactions) (Wootton, 1992). These organisms may either act as prey, competitors or predators. The role of the interacting species may change seasonally, locally and also during their ontogeny.

1.2.1. Food availability and competition

The availability of suitable food items is probably one of the most important factors determining habitat quality. The range of available potential food items is largely influenced by the physical environment. The range utilized by a species depends on its ability to detect, acquire and process the food. The morphology and size of both predator and prey influence the success by which preys are detected and consumed. The accessibility of prey species and the maximum prey size a fish can ingest are determined by the gape size and the structure of the feeding apparatus of the fish (De Groot, 1971; Wootton, 1992). As most fishes are visual feeders, high levels of turbidity reduce the visual field of the predator and decreases the capture success. Prey densities vary spatially and temporally and are generally highest during the warmer (more productive) periods of the year. This may translate in a seasonal migration of fishes between habitats according to the relative availability of food resources (Wootton, 1992; Madsen and Shine, 1996). In addition, ontogenetic shifts in the diet of fishes may trigger shifts in habitat use and may be one of the reasons why juvenile and adult habitats are spatially separated (Beck *et al.,* 2001).

The concentration of fish in habitats where prey is generally more abundant can nevertheless lead to exploitative competition and density-dependent growth (Davey *et al*., 2006). In most cases, however, direct competition between species is avoided by the partitioning of resources in space and time, which is of course already the result of competition in the past (Morris, 2003). High predator densities may locally reduce the prey population and ultimately prey may become limiting. Such habitats are thought to have reached their maximum carrying capacity. The carrying capacity is defined as the population density of a habitat at which the *per capita* population growth rate is zero. When the carrying capacity of a habitat is reached, population growth declines and may even become negative if the capacity is exceeded (Goss-Custard *et al.,* 2001).

1.2.2. Predation

Predation can have direct and indirect effects on the quality of habitats. Predators directly influence the survival of a prey fish by killing it or indirectly by affecting growth, forcing its prey in habitats which are safer, but contain less food (Burrows, 1994; Hugie and Dill, 1994; Creel et al., 2005). The probability of encountering a predator may be reduced by moving into habitats where predators are visually restricted (turbidity: Snickars *et al*., 2005; Van de Meutter *et al*., 2005) or where they are hindered by the physical structure of the habitat (vegetation: Snickars *et al*., 2005 / depth: Gibson *et al.,* 2002). Predation may also alter the use of time (temporal niche) of prey species as was shown in a study by Fraser *et al.* (2004). This study showed that predation threat can induce a facultative shift in the temporal niche and vital rates of a prey species. The authors tested the effect of the presence of a nocturnal predator (*Hoplias malabaricus*) on the behaviour of guppies (*Poecilia reticulate*), which are viewed as a diurnal species. Their study demonstrated that guppies expand their foraging into the nocturnal period in the absence of severe piscivorous predation, resulting in higher growth rates and increased courtship intensity during daytime. Apparently, daytime feeding energetically frees the guppies to increase reproductive activity during the day.

The vulnerability of a fish to predation is dependent on its size and the opportunities to decrease the encounters with predators. As gape size sets the limits of the prey size a predator can ingest, juvenile fishes that are able to maximize their growth rate get a benefit from reaching earlier a refuge in size. They can do so by selecting habitats in which the abiotic environmental factors optimize growth and the food densities are highest. The effect of prey growth on predation mortality was examined in a pond experiment where the growth rate of spot (*Leiostomus xanthurus*) was controlled by manipulating the food availability in the presence of a predator. The experiment showed that slow-growing spot experience higher predation mortality than those growing rapidly (Craig *et al*., 2006).

2. Use of the estuarine habitat

Estuaries are located at the boundaries of oceans and seas. As such, they are the main transition zones between fresh water from the land and salt water from the oceans. Rivers import high loads of organic matter from upstream reaches and wetlands into the estuarine system. Many estuaries are highly urbanized and lie in densely populated areas, from which they receive high nutrient loads and pollutants. These increased nutrient loadings lead to an increased primary production and affect secondary production. In turbid systems, however, primary production is often light-limited and only a fraction of the nutrients can be used for the growth of algae (Heip *et al.,* 1995). A large part of the imported organic matter is trapped in the estuary and fuels the higher trophic levels. Generally, two food webs can be distinguished which are tightly coupled: a bed-dominated detrital food chain, fueled by the input of allochthonous material, and a phytoplankton-based food chain, supported by autochthonous production of suspended benthic diatoms (microphytobenthos). The generally well-mixed nature of the estuarine water column intensifies the exchange of energy between both food webs (Day *et al.,* 1989; Heip *et al.,* 1995; Elliott *et al.,* 2002). The continuous arrival of allochthonous organic material supports a very high biomass per unit of area, which places estuaries amongst the most productive ecosystems on earth. Their productivity is of the same level as coral reefs and mangroves and much higher than that of the open ocean or the continental shelf (Day *et al.,* 1989; Begon *et al.,* 1996; McLusky and Elliott, 2004).

Estuaries supply many vital ecological services in coastal waters. Most notably, these ecosystems provide food and refuge that support a great abundance and diversity of ecologically and commercially important fish, as well as shrimp and other invertebrates. Because of the increased productivity and survival, nearshore estuarine and marine ecosystems are often considered “nurseries” for juvenile fish and macroinvertebrates. In the following paragraphs, we briefly describe the importance of estuarine habitats for juvenile fishes. Moreover, we discuss the function of estuaries as essential fish habitats and describe the environmental abiotic and biotic factors that contribute to this. We focus our discussion on a particular life history stage, the juvenile stage, because this stage is directly affected by the quality of nursery habitats.

2.1. Estuaries as nurseries

The importance of shallow coastal zones and estuaries in particular, for juvenile fishes, has been established in the nursery-role concept. Beck *et al.* (2001) define nurseries as a subset of juvenile habitats that contribute disproportionately to the production of individuals that recruit to adult populations. In this definition, any habitat that is used by juvenile fishes is considered a juvenile habitat. Dahlgren *et al.* (2006) extended the definition of nursery habitat to Effective Juvenile Habitat (EJH). Here, the authors consider the *overall* contribution of individuals from juvenile habitats to the adult population, rather than the *per-unit-area* contribution in the nursery definition. Generally, a habitat is called a nursery if juveniles of a fish or invertebrate species occur at higher density, avoid predation more successfully, or grow faster there than in a different habitat (Able *et* *al.,* 2005). Implicit to the definition of a nursery is the ability of individuals to migrate between nurseries and the adult habitat (Gillanders *et al.,* 2003).

Generally, the spawning and nursery grounds of fishes are geographically separated, which implies that larvae have to be transported from the spawning areas to nursery grounds. The transport to and retention in nurseries of fish larvae is determined by hydrographical, physical and morphological variables (Pihl, 1990; Forward and Tankersley, 2001). After spawning, newly hatched larvae are transported passively by ocean and tidal currents to nearshore areas. Once they arrive in the coastal areas, transport to the nursery areas includes an active component by using selective tidal stream transport (STST; Jager *et al.,* 1999; Forward and Tankersley, 2001; Gibson, 2003). Larvae using STST are supposed to ascend actively in the water column during flood and return to the bottom when the tide turns, thus preventing being flushed back during ebb (Jager *et al.,* 1999).

Inside the estuary, the larvae migrate further into specific nursery habitats. Formerly, the entire estuary was considered to be the nursery, nowadays however, the focus has shifted to specific areas within estuaries as nurseries. Some juvenile habitats make a smaller than average contribution to the recruitment of adults and should not be considered a nursery habitat. Pihl *et al.* (2002) describe nine habitats in European estuarine waters important to fishes (Table 1.1). The same authors recognize four different functions of these estuarine habitats: feeding ground, nursery area, spawning area and migration pathway. We will focus on the nursery function of estuarine habitats. As foraging is essential for growth and survival, it will be discussed as a part of the nursery function.

The factors determining the attraction of juveniles to nurseries are roughly the same as the factors mentioned above (habitat selection). We will confine our discussion to those biotic and abiotic factors that are widely recognized as being responsible for most of the site-specific variations in nursery value (Gibson, 1994; Beck *et al.,* 2001; Costa *et al.,* 2002). The interactions between these abiotic and biotic factors and the components of the nursery-concept are presented in figure 1.1.

Table 1.1*. Extent of the nine fish habitats in the Scheldt estuary (Figure 1.2) and the number of species found in each of these habitats. If a habitat is used by fishes for one or more of the four functions, it is indicated in the last column (S = spawning, N = nursery, F = feeding, D = diadromy (migration)). The habitat use that is applied by most fishes is underlined. There are no data available about the use of reed beds in the Scheldt estuary. After Pihl et al., 2002.*

Habitat type	Surface area (km^2)	# species	Habitat utilization
1. Tidal freshwater	30	18	S, N, **F**, D
2. Reed beds	5.2	?	?
3. Saltmarsh	31.7	14	S, **N**, F, D
4. Intertidal soft substratum	112.4	24	S, **N**, F, D
5. Intertidal hard substratum	0	-	-
6. Subtidal soft substratum	176	43	S, N, **F**, D
7. Subtidal hard substratum	0	-	-
8. Subtidal seagrass beds	0	-	-
9. Biogenic reefs	0	-	-
Total	**355.3**	56	

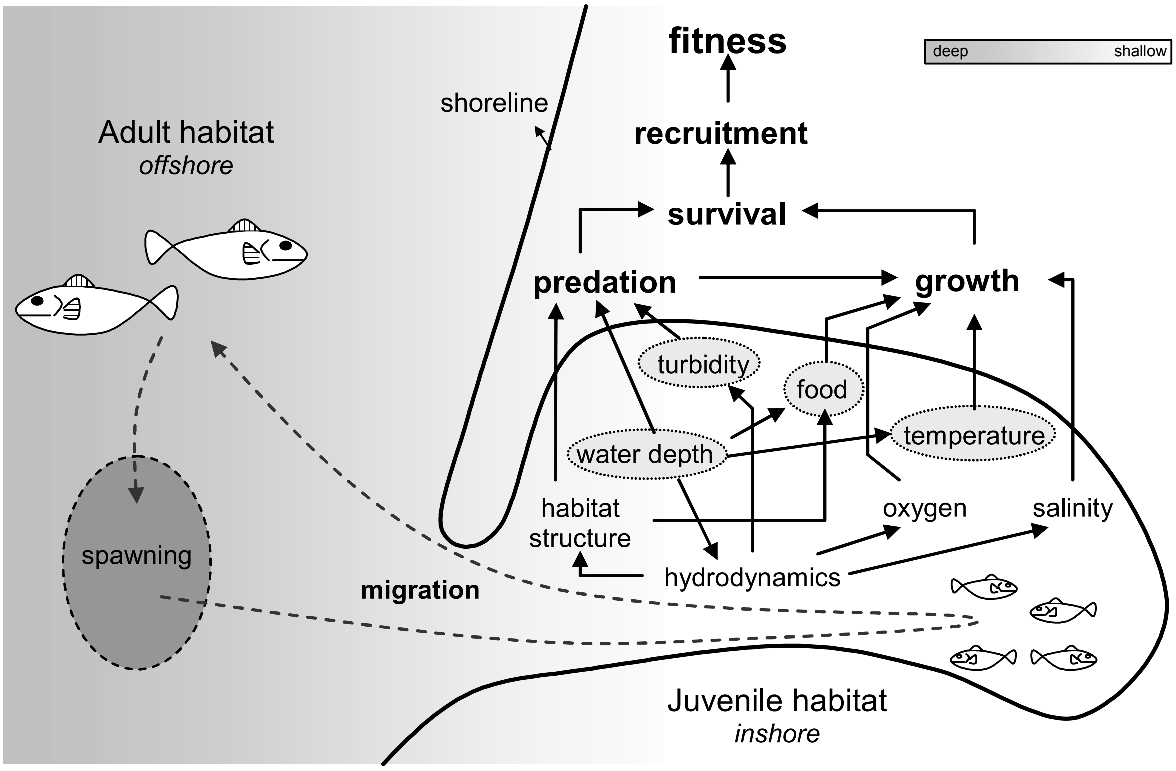

***Figure 1.1**. Various factors affecting the habitat quality of estuarine juvenile habitats. Adapted from Gibson, 1994.*

2.1.1. Abiotic environmental factors

Estuaries are by definition the transition zone between the freshwater of the land and marine salt water. They are highly dynamic systems and are characterized by a large spatial and temporal variation of the environmental abiotic factors. These spatial and temporal variations are mainly caused by tidal mixing and river runoff (Day *et al.,* 1989). While the tides daily impose the marine influence on the estuarine ecosystem, the runoff of inland water mainly determines the seasonal variation of the water quality. This environmental variability represents a problem for the organisms living and migrating in estuaries. They have to cope with very different conditions amonst others of salinity, temperature and oxygen. Contrary to less mobile species, which have to tolerate the highly variable environment, fishes are able to avoid unfavorable conditions by migrating in and out the estuarine habitats, as a kind of behavioral enviroregulation (Blaber and Blaber, 1980; Wootton, 1992; Gibson, 1994; Gibson, 1997).

The view of estuaries as stressful environments conflicts with their role as important nursery grounds for juvenile marine fish (Blaber and Blaber, 1980; Elliott and Hemingway, 2002). The overriding view on which organisms are present in estuaries is that their distribution is related to their ability to endure fluctuating environmental conditions, which limit their ability to exploit the large food resources present in estuaries (Elliott and Hemingway, 2002; Attrill and Power, 2004). Due to the interaction between these abiotic factors, it is difficult to separate each of their effects on growth and survival of fishes. The interaction between temperature and oxygen concentration, for example, is well understood. Also salinity and water temperature are often correlated in estuaries, as higher summer temperatures are generally found in the upper (freshwater) reaches of the estuary. Furthermore, some flatfishes have a preference for muddy bottoms, which are typically found in more sheltered and less saline areas (Gibson, 1994).

a) Temperature

Temperature has been reported as one of the main environmental factors affecting the distribution and abundance of fishes in estuaries (Blaber and Blaber, 1980; Henderson, 1989; Power *et al.,* 2000). In temperate, well-mixed estuaries, summer temperatures are generally higher in the upstream reaches of the estuary. In winter, water temperature at sea is higher compared to the estuary. The temperature differential between the sea and estuarine waters might act as a cue for the migration of fishes from and to estuaries (Leggett, 1977; Gibson, 1997; White and Knights, 1997). Attrill and Power (2002) demonstrated that long-term juvenile fish abundance is best explained by temperature differentials between estuarine and coastal waters. Higher numbers of young fish were present in the Thames estuary when winter estuarine water was warmer than the North Sea because juvenile fish in estuaries grow faster at higher temperatures (Attrill and Power, 2002). Temperature may be viewed as a resource, which is used by fishes to enhance growth and hence fitness (see predation below). Direct lethal effects of extreme temperatures in estuaries have only rarely been recorded, because fish gradually adjust their distribution in response to temperature change. Only under high temperatures in combination with low oxygen concentrations, fish populations might suffer a reduction in numbers (Pomfret *et al.,* 1991; Costa *et al.,* 2002). In addition, Attrill and Power (2004) suggested that the temporal migration of fish in estuaries could be interpreted as a response to resource separation along the spatial temperature axis, which limits potential competition between functionally similar species.

b) Salinity

Salinity fluctuates daily and seasonally and mainly depends on the tidal regime and the freshwater discharge (Day *et al.,* 1989). The energetic costs of osmoregulation can be quite substantial for estuarine fishes and consequently may reduce growth. However, the effect of salinity on growth in euryhaline species seems to be much less important than that of temperature (Wuenschel *et al.,* 2004). Salinity-induced mortality of fishes is observed in intermittent open estuaries (tropical regions). When disconnected from the sea, salt water intrusion in these estuaries is prevented and oligohaline or freshwater conditions develop, which increases osmoregulatory stress in stenohaline species (Griffiths, 2001). In most cases, however, salinity seems to act as a controlling factor for the distribution of fishes in estuaries (Blaber and Blaber, 1980; Henderson, 1989; Cyrus and Blaber, 1992; Maes *et al.,* 1998b). The distribution is mainly determined by the salinity variation, rather than the absolute tolerance for salinity (Attrill, 2002). Salinity may also act as a cue for the attraction of post-larvae and juveniles towards nursery areas with lower salinity (Gibson, 1997; Costa *et al.,* 2002; Whitfield, 2005).

c) Dissolved oxygen

The enormous import of nutrients and organic matter into the estuary supports a high bacterial activity and results in a high biological oxygen demand (Day *et al.,* 1989; Soetaert *et al.,* 2006). In warmer periods, bacterial activity intensifies, which often leads to hypoxic or even anoxic conditions in some parts of the estuary. The effect of oxygen on the abundance of estuarine fish operates through interaction with temperature. Most fishes show avoidance behaviour when the oxygen concentration drops below a critical level (Phelan *et al.,* 2000). If fishes are able to leave the areas of low oxygen concentration, they form distributions along the oxygen gradient according to their tolerance to oxygen depletion (Maes *et al.,* 1998b; Costa *et al.,* 2002). The oxygen levels may also drop dramatically in vegetated bays at night and fish may experience oxygen depletion (Costa *et al.,* 2002).

The annual formation of hypoxic conditions in certain areas of the estuary during summer may hinder the migration of diadromous fish (Pomfret *et al.,* 1991; Solomon and Sambrook, 2004, Maes Stevens and Breine, in press). Anadromous species like smelt (*Osmerus eperlanus*) spawn in freshwater and the occurrence of hypoxic conditions in their spawning area may cause high larval mortality (Thiel *et al.,* 1995).

2.1.2. Piscivorous predation

One of the key factors of the nursery-concept is the increased survival of juveniles in those areas. In this respect, it is argued that estuaries provide a refuge from predation. The refuge hypothesis is based on the assumption that the effectiveness of visual predation is reduced due to turbid conditions in many estuaries (Blaber and Blaber, 1980; Maes *et al.,* 1998a) and/or that estuaries provide habitats where there are relatively few predators (Ruiz *et al.,* 1993, Ellis and Bell, 2004; Verweij *et al.,* 2006). De Robertis *et al.* (2003) showed that planktivorous fishes are less vulnerable to predation by piscivores under turbid conditions, while their ability to capture zooplankton prey was hardly affected. It is suggested that the structural complexity of certain estuarine habitats reduces the predation efficiency on fishes and shrimps (Gibson, 1994). In this respect, it has been shown that seagrass beds (Verweij *et al.,* 2006) and salt marshes (Kneib, 1997; Halpin, 2000; Salgado *et al.,* 2004) have an important predator refuge value for juvenile fishes. Also water depth *in se* is thought to act as a cue to avoid predators. Several studies report on the segregation of nekton by body size along a water depth gradient (Paterson and Whitfield, 2000; Gibson *et al.,* 2002), which might reduce the encounter rate of juvenile fishes with large piscivores (Ruiz et al, 1993; Gibson, 1994). However, a recent review by Sheaves (2001) shows that small piscivores can be numerous in shallow tropical waters and have the potential to be a major structuring force on the estuarine fish community.

Piscivores in estuaries range from invertebrates such as coelenterates and crustaceans to vertebrates, including teleosts, birds and mammals (Elliott *et al.*, 2002). The impact of the different predators changes as prey fish grow, consequently becoming prey to a smaller range of predators. The main fish predators in temperate coastal zones and estuaries are considered Gadoidae (Pihl, 1982; Ellis and Gibson, 1995; Gibson and Robb, 1996). A process of top-down control was shown for the Baltic ecosystem, where cod predation on clupeids and clupeid predation on cod eggs could produce either a cod-dominated or a clupeid-dominated system (Rudstam *et al.*, 1994). In tropical estuaries, the piscivore community is clearly more diverse. Baker and Sheaves (2005) reviewed the piscivore assemblage of shallow tropical estuaries and identified members of the Sphyraenidae, Scomberidae, Carangidae and Platycephalidae as the main fish predators.

Several pelagic coelenterates such as medusae, siphonophores and ctenophores are known to include ichthyoplankton (eggs and larvae) in their diet and their effects on the fish larvae can be substantial (see review by Purcell and Arai, 2001). van der Veer (1985) showed that the coelenterates *Pleurobrachia pileus* and *Aurelia aurita* are important predators of flounder (*Platichthys flesus*) and plaice (*Pleuronectes platessa*) larvae in the Wadden Sea and that they might be responsible for an abrupt termination of larval flatfish immigration. Their overall impact on flatfish recruitment however, is thought to be small since most larvae have already completed their immigration into the nurseries when the coelenterate outburst starts.

Because of their demersal life style, newly settled flatfishes are vulnerable to shrimp (Crangonidae) predation (van der Veer *et al.,* 1991; van der Veer *et al.*, 2000b). Predation by shrimps is highly size-dependent and only important in the early life (larval) stages of the flatfishes, as they reach a size refuge for crustacean predation at a length of 25 mm (Taylor and Collie, 2003). Juvenile (flat)fishes should therefore select habitats that enhance their growth. The higher temperatures in shallow estuarine waters may therefore contribute indirectly to the survival of juvenile fishes (Gibson, 1994; Costa *et al.,* 2002).

For the larger juvenile size classes, bird predation may become important in shallow nursery areas. Leopold *et al.* (1998) show that cormorant (*Phalacrocorax carbo*) predation can be an important source of mortality for 0-group flatfish in the Wadden Sea. However, the effect of bird predation on the fish community in turbid estuarine waters is probably negligible (Gibson, 1994).

2.1.3. Food availability

As mentioned earlier, estuaries are highly productive areas, in which both autochthonous and allochthonous detrital material is trapped. Most of the estuarine food webs seem to be fueled by detritus-based pathways. Detritus provides abundant food resources for filter- and deposit-feeding invertebrates (Elliott *et al.,* 2002; Riera *et al.,* 2004). In addition to detritus, microphytobentos (benthic microalgae) is an important food source for the invertebrates inhabiting the estuarine mudflats and may account for a large fraction of plankton-based food webs as it becomes resuspended in the water column during flood (Herman *et al.,* 2000; Riera *et al.,* 2004). The highest productivity in estuaries is generally found in the intertidal zones (Day *et al.,* 1989; Elliott and Taylor, 1989; Ysebaert *et al.,* 2003), where benthic diatoms and sedimentary organic matter are an important food source for (surface) deposit feeders (Herman *et al.,* 2000). Of this group, the small epibenthic and hyperbenthic crustaceans form the main link between the detritus and fish (Hostens and Mees, 1999; Elliott *et al.,* 2002).

The high secondary production in intertidal areas provides abundant food resources to estuarine fish and macrocrustaceans when the flats are covered by the tide. At low tide, they are important foraging grounds for waterfowl (Day *et al.,* 1989; Raffaelli and Hawkins, 1996; McLusky and Elliott, 2004). Although food may be abundant in estuaries, it is not inexhaustible. Several studies indicate that secondary consumers (e.g. crustaceans, fishes and birds) can have significant effects on the prey population (Baird and Milne, 1981; Gee *et al.,* 1985; Thiel, 1996). A study of the impact of predator consumption on the production of *Corophium volutator* in a Swedish estuary has shown that up to 98% of the annual production is consumed by shrimps, crabs and fish (Pihl, 1985). However, experimental exclusion of epibenthic predators on intertidal sediments, has generally not led to dramatic changes in the prey community structure (Raffaelli and Milne, 1987; Raffaelli *et al.,* 1989; Hall *et al.,* 1990b). In these studies, only relatively small changes in the densities of one or a few species were observed (but see Virnstein, 1977; Gee *et al.,* 1985; Reise, 1985). On sheltered mudflats shorebirds are often the most conspicuous predators; they are capable of seriously depleting the density of particular prey (Daborn *et al.,* 1993; Raffaelli and Hawkins, 1996; Goss-Custard *et al.,* 2001). The effects of bird predation on benthic prey can cascade through the system and influence sediment stability (Daborn *et al.,* 1993). Predation effects are most likely to be observed in late autumn and winter, when shorebird densities are highest and prey production is lowest (McLusky and Elliott, 2004). The outcome of predation studies in sediment communities seems to vary from study to study, probably reflecting differences between locations and the timing of arrival of migrating predators (Raffaelli and Hawkins, 1996).

In contrast to the situation in sediment communities, predation seems to be much more important as a structuring force in rocky intertidal areas (Raffaelli and Hawkins, 1996; Horn and Martin, 1999). The observed differences between rocky and soft sediment systems might be the result of the three-dimensional nature of sediment habitats, compared to the two-dimensional nature of rocky shores. In soft sediments, benthic prey are able to burry in the sediment, thereby reducing their vulnerability to predation (Raffaelli and Hawkins, 1996).

The impact of pelagic predators on the estuarine zooplankton community is less well documented, but also here predation may be a structuring force (Mehner and Thiel, 1999; Maes *et al.,* 2005c). Thiel (1996) calculated that consumption by planktivorous fish larvae and 0+ juveniles accounted for about 85% of total zooplankton consumption. A modelling study on the predation impact of *Clupea harengus* and *Sprattus sprattus* on zooplankton in the Scheldt estuary suggested that the zooplankton community might be top-down controlled (Maes *et al.,* 2005c). But similar to the benthic situation, the effects of pelagic predation on the zooplankton community are highly variable in space and time.

2.1.4. Competition

The increased food concentration in estuaries attracts large numbers of predators, which may lead to competition if food resources are limited. As mentioned earlier, exploitative competition may lead to density-dependent regulation of the growth and mortality. Limited availability of resources suggests that fish populations in estuarine nurseries may reach their carrying capacity. A carrying capacity effect is thought to dampen the recruitment variability of species that tend to concentrate spatially on nursery grounds during their early life (e.g. flatfishes) (Beverton, 1995; Iles and Beverton, 2000). However, the evidence for density-dependent regulation of estuarine fish populations is equivocal (see review Cowan *et al.,* 2000) and saturation of nursery grounds seems to be rare or non-existent (Gibson, 1994; van der Veer *et al.,* 2000a). The general trend seems to be that direct competition between fishes in estuaries is avoided by resource partitioning on a trophic, temporal and spatial scale (Gibson, 1994; Thiel *et al.,* 1996; Elliott *et al.,* 2002). It is further suggested that resources are not limiting, due to the superabundance of available prey items. As a result, fish populations remain below the carrying capacity of the system.

2.2. The Scheldt estuary, habitats in an urbanized system

The Scheldt estuary is one of the last genuine estuaries in Western Europe, with a complete salinity gradient ranging from Vlissingen near the mouth to Gent, where the influence of the tides is stopped by sluices (Figure 1.2). The River Scheldt is divided in three zones: the Westerschelde ranging from Vlissingen to the Dutch-Belgian border, followed by the Zeeschelde segment to Gent and the Upper Scheldt, upstream the sluices in Gent. The Zeeschelde is sometimes subdivided in the Lower Zeeschelde between the Dutch-Belgian border and Antwerp and the Upper Zeeschelde between Antwerp and Gent.

The tidal Scheldt is a shallow, well-mixed and relatively turbid macrotidal estuary. Due to the funnel shape of the estuary, the mean tidal height is maximal in the freshwater reaches. The maximum range (5.3 m) is observed near the mouth of the River Rupel. The vertical tidal range is 3.8 m in Vlissingen and 1.9 m in Gent (Meire *et al.,* 2005). The location of the maximum turbidity zone depends on the freshwater discharge and is situated at about 110 km from the mouth during dry periods and at about 50 km during wet periods (Meire *et al.,* 2005). Two maximum turbidity zones might be observed: one at the freshwater/seawater interface and a second resulting from tidal asymmetry (Baeyens *et al.,* 1998; Herman and Heip, 1999). The average freshwater discharge is about 100 m^3 s^{-1} and varies seasonally between 60 m^3 s^{-1} (exceptionally 20 m^3 s^{-1}) during summer and 180 m^3 s^{-1} (exceptionally 600 m^3 s^{-1}) during winter (Baeyens *et al.,* 1998). In summer, at low discharge, most of the water of the Upper Scheldt is deviated by the Gent-Terneuzen canal to the Westerschelde, to maintain a minimum water level for shipping in the canal. As a result the Rupel discharge can amount twice the discharge of the Upper Scheldt (Meire *et al.,* 2005). The salinity gradient in the Scheldt estuary is mainly determined by the freshwater discharge and shows a clear seasonal trend. The polyhaline zone (salinity 18-30) ranges from the mouth of the estuary to approximately 40 km upstream. The mesohaline (brackish; salinity 5-18) zone is highly variable in space and time, stretching from km 37 to km 55 in March 1998, and from km 48 to km 73 in October 2003. The freshwater zone refers to the oligohaline (salinity 0.5-5) and limnetic zone and is situated roughly upstream Antwerp.

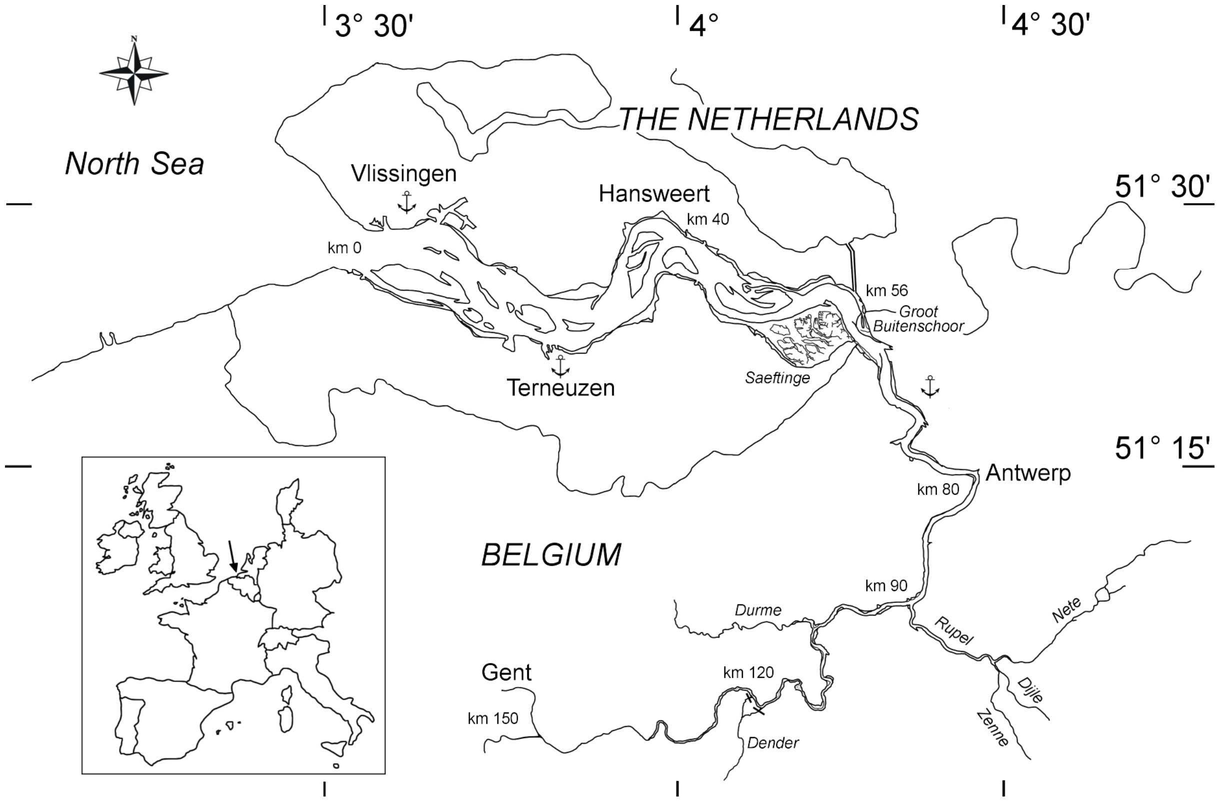

Figure 1.2*. Map of the Scheldt estuary. The kilometers represent the distance to the mouth of the estuary. The River Dender is a major tributary of the Scheldt, but is disconnected from the Zeeschelde by sluices. The samples for the first part of this thesis (chapter 2-4) were taken on a mudflat near the Dutch-Belgian border, a few hundred meters north of the Groot Buitenschoor.*

The catchment of the Scheldt is highly urbanised. Between 1970 and 1980, intense bacterial activity, due to the high nutrient loads from untreated industrial and domestic sewage effluents, caused temporal anoxic conditions in the freshwater part of the estuary. Because of increasing wastewater treatment, oxygen concentrations gradually improved during the eighties and the nineties (Soetaert *et al.,* 2006). However, low oxygen concentrations still persist around the mouth of the Rupel. Due to the turbid conditions, phytoplankton production is often light limited and growth is low. Because of the high import of allochthonous organic matter, the system respiration is greater than the autotrophic production and the estuary can be considered as a heterotrophic system (Heip *et al.,* 1995).

The Westerschelde is characterized by multiple channels, surrounding large intertidal sand flats and bordered by mudflats and salt marshes. These intertidal areas are important feeding and resting places for fish, crustaceans and birds (Hostens and Mees, 1999; Cattrijsse et al, 1994; Hampel *et al.,* 2005; Van den Bergh *et al.,* 2005). Pihl *et al.,* 2002 reviewed the quantity of estuarine fish habitats in European estuaries. They found six habitats for the Westerschelde (Table 1.1), of which the interand subtidal soft substratum covers the largest surface. The marsh of Saeftinghe in the mesohaline zone is one of the largest brackish marshes in NW-Europe. The geomorphological history of the estuary is characterized by the loss of intertidal habitat because of embanking, dredging and dike building (Meire *et al.,* 2005).

The fish communities of the various compartments in the Scheldt estuary are relatively well documented. The research of Maes and coworkers mainly focused on the fish community in the Zeeschelde. Field studies were done on the composition and structure of the fish community (Maes *et al.,* 1998a,b; 2005b) and the diet of the dominant species (Maes *et al*., 2003). In addition, mathematical models were constructed to predict the functioning of the estuarine nursery (Maes *et al*., 2005a) and the predation impact of clupeids on estuarine zooplankton (Maes *et al*., 2005c). In the Westerschelde on the other hand, the spatial and temporal patterns and the diet of the demersal fish community were studied by Hostens (2000) and Hostens and Mees (1999). A description of the postlarval fish community of the Westerschelde is given in Beyst *et al.* (1999b) and the different aspects of the nekton communities in marshes is given in Cattrijsse *et al.* (1994 and Hampel *et al.* (2004, 2005). However, so far no consistent monitoring program exists for the fish community in the entire estuary.

3. Objectives and outline

This thesis describes the habitat use of estuarine fishes in the Scheldt estuary. In the **first part** (chapter 2 to chapter 4) we examine the importance of intertidal migration for fishes and discuss the different factors that affect the intertidal habitat quality. The **second part** (chapter 5 and 6) describes the effect of abiotic factors on the use of the Scheldt estuary by European flounder *Platichthys flesus* (L.). We approach these questions by combining monitoring, field experiments and a modelling study.

Despite the importance of estuarine intertidal areas for fishes, comparatively little research has been done on the role and processes of short-term movements in and out unvegetated mudflats (Morrison *et al.,* 2002). ***Chapter 2*** describes the seasonal structure of the fish community on a brackish water mudflat. The specific aims were to quantify the structure of the fish assemblage on the higher, middle and lower shore and to relate the migration patterns of fishes on the mudflat with the use of the intertidal habitat. The migration and zonation of the most common fishes are discussed in terms of the prevailing (tidal) currents and the biotic and abiotic factors that affect their distribution.

Food availability is thought to be one of the most important factors determining habitat quality and one of the main triggers for intertidal migration (Gibson, 1994). The available food resources in nurseries like the Scheldt estuary can affect both fish recruitment and survival. In ***chapter 3,*** we examine the trophic relations within the fish community on an intertidal mudflat. Food availability in the field is compared with the diet of the most common species. The two main questions that were addressed are: firstly, which species utilize the mudflat as a feeding ground and to what extent do they depend on this zone for their food supply? Secondly, how does trophic niche sharing affect the composition and functioning of the feeding guilds on the mudflat? We also address the question as to whether competition is a structuring force for the intertidal fish community and whether resources are partitioned on a spatial and/or temporal scale.

Given the intense use of the intertidal resources, the question raises whether benthivorous predators are able to deplete the benthic prey populations. Besides fishes, also shorebirds forage intensively on the uncovered mudflats and can have significant effects on the abundance of their prey (Goss-Custard *et al.,* 2001). The objective of ***chapter 4*** is to estimate the impact of predation on the intertidal macrobenthic community and to differentiate herein between the effects by birds and fishes. This is tested with both field surveys and exclosure experiments.

In the second part, we take a closer look at how the abiotic environmental factors influence habitat suitability in the entire estuary. We first construct a bioenergetics model, which describes the growth of European flounder as a function of the ambient water temperature and body weight (***Chapter 5***). Hereto the field data of a flounder population in the Ythan estuary, Scotland are used (Summers, 1974). In ***chapter 6***, this bioenergetics model is further extended with an oxygen and salinity dependent function. The resulting growth model is used to develop a spatially explicit model of flounder growth in the Scheldt estuary. As growth of juvenile fish can be used to estimate the quality of nursery habitats (Gibson, 1994; Tylor and Brandt, 2001), the model results are used as an indicator of habitat suitability in the Scheldt estuary.

Finally, we define some key factors that influence the use and the quality of intertidal estuarine nurseries. We discuss how these factors limit or favor the use of estuarine habitats and formulate further research questions.

CHAPTER 2

Zonation and tidal stream migration of fishes on an estuarine mudflat

2

Maarten Stevens, Joachim Maes, Bart Van Asten and Frans Ollevier

Abstract

Intertidal migration is relatively well-studied in marine fish biology. Most studies focused on marine sandy beaches and rocky shores. Comparatively little work, however, has been done on the distribution and migration patterns of fishes on estuarine mudflats. The seasonal composition of an intertidal estuarine fish community was studied with directional fyke nets at different heights on the mudflat. These fyke nets each sampled an opposite direction and as such could be used to analyze the migration patterns of fishes on the mudflat. The fish assemblage was dominated by juveniles of *Clupea harengus*, *Dicentrarchus labrax*, *Platichthys flesus* and *Solea solea*. The composition of the intertidal fish community reflected the cyclicity of seasonal recruitment of species into the estuary. Analysis of the directional data of the fyke nets showed that the flatfish species, flounder and sole, migrated actively onto the mudflat. In contrast, the distribution of herring and seabass in the fyke nets suggests that they were transported passively by the tidal currents. These (semi-)pelagic species can be considered opportunistic vagrants onto the mudflat. They might follow their migrating hyperbenthic and pelagic prey and may find a valuable supplement to their diet in the infauna that disperses into the water column. Because of the low predator abundance and high turbidity in this part of the Scheldt estuary, predation is probably not an important trigger for intertidal migration. Species didn't show a clear zonation on the mudflat and, if observed, this was mainly the result of species-specific differences in mobility. The zonation of flatfishes tended to be affected by density-dependent processes. When fish densities were higher, relatively more flounder were caught on the upper shore. By doing so, they avoid competition for food or space with sole, which stays on the middle and lower reaches of the mudflat.

Key words: *Circular statistics; Intertidal fishes; Scheldt estuary; Tidal migration; Zonation*

Unpublished manuscript

1. Introduction

Intertidal areas make up a significant proportion of the estuarine habitat available to fish. A review of fish habitats in European estuaries revealed that intertidal soft substratum accounted for almost 30% of the total surface of the estuarine systems in the Boreal/Atlantic region (Pihl *et al.,* 2002). Intertidal areas are highly productive compartments in the estuarine environment. They contain a high density and large biomass of macrobenthos, which provides abundant food for estuarine fish and macrocrustaceans when the flats are covered by the tide. At low tide, they may be important for waders and waterfowl (McLusky and Elliott, 2004). Intertidal shallow waters may also act as nurseries for juvenile fishes: there where favourable temperatures optimize growth and where they find a refuge against predators (Gibson, 1994; Gibson *et al.,* 2002; Able *et al.,* 2005).

Many fish species undertake short movements synchronously with the tide to take advantage of the intertidal zone (Gibson, 2003; Able *et al.,* 2005). These tidally-synchronised migrations have been attributed to increased feeding potential (Wirjoatmodjo and Pitcher, 1984) and predator avoidance (Gibson, 1973; Ellis and Bell, 2004; Franco *et al.*, 2006). Predation pressure and feeding may be spatially and temporally unevenly distributed in the intertidal zone. This translates in both seasonal and depth-related differences in the distribution of fishes in the intertidal zone (Gibson, 1972; 1973; Hernández *et al.,* 2002; Griffiths *et al.,* 2003). Where the primary function of upshore movements is feeding, zonation has been linked to the behavior of both predator and prey, the avoidance of competition and a heterogeneous distribution of prey species (Gibson, 1973; Miltner *et al.,* 1995). Several studies reported on the segregation of fishes by body size along a gradient of water depth and demonstrated that the density of larger piscivores is lower in the upper reaches of the intertidal zone (although predation can be important in shallow water; see Baker and Sheaves, 2005). As a result, smaller fishes migrate higher on the mudflat to avoid predation (Blaber and Blaber, 1980; Burrows, 1994; Patterson and Whitfield, 1996; Gibson *et al.,* 2002). Furthermore, physical factors like temperature often vary with water depth and may influence fish's intertidal distribution as growth is mainly regulated by ambient temperature (Van der Veer and Bergman, 1986; Gibson *et al.,* 2002; Chapter 5).

Despite its potential importance for fishes, comparatively little work has been done on the species diversity that utilizes intertidal estuarine mudflats. The vast majority of studies on intertidal habitat use is restricted to marine sandy beaches (Gibson, 1973; Pihl, 1982; Burrows, 2001) and vegetated habitats such as mangroves and marsh creeks (Cattrijsse *et al.,* 1994; Ellis and Bell, 2004; Hindell and Jenkins, 2004; Hampel *et al.,* 2005). However, studies on the role and process of short term movements in and out unvegetated mudflats are relatively scarce (Morrison *et al.,* 2002).

The transitional position of the intertidal zone, between the marine and terrestrial environments, makes it vulnerable to human interference in the estuarine system and climate induced sea level rise. In the Scheldt estuary more than 50% of the intertidal habitat was lost by dike building, dredging and embankment over the past century (Meire *et al.,* 2005). As the pressure on the estuary will probably increase, a further decrease of the low dynamic area (mudflats and shallow water) is expected. The role of the Scheldt estuary as a nursery for fish has been emphasized by several authors (Cattrijsse, 1994; Beyst *et al.,* 1999b; Hostens, 2003; Hampel, 2003; Maes *et al.,* 2005b), yet no studies focused on how and which fish species utilize the intertidal mudflats.

The present study was designed to assess the importance of intertidal migration for estuarine fishes. The specific aims were to quantify the structure of the fish assemblage on the higher, middle and lower shore and to relate the migration patterns of fishes on the mudflat with the use of the intertidal habitat. It is hypothesized that the distribution of fishes and the direction in which they migrate on the mudflat, provides information about their intertidal dependence. Fishes that move in a directed way on the mudflat are assumed to actively take advantage of the intertidal area. We predicted that their distribution would change according to the shore level in response to changing physical and/or biotic factors. Contrary, fishes that are transported passively by the ebb and flood currents on the mudflat are considered opportunistic vagrants and their distribution will be mainly determined by the area made available by the tide.

2. Materials and methods

2.1. Study area

The fish community was sampled on a mudflat in the brackish water part of the Scheldt estuary near the Dutch-Belgian border (Figure 2.1). A detailed description of the Scheldt estuary can be found in Meire *et al.* (2005). The mudflat is on average 450 m wide, has a gentle slope and is bound in the north by the outlet of a drainage sluice and in the south by the Groot Buitenschoor, a large brackish water intertidal area (Figure 2.1).

Currents on the mudflat are altered by a dam, which was constructed to maintain open access to the sluices of the harbor docks. The main flood currents pass by a flood channel (Appelzak) near the right border, while the ebb currents follow the main channel near the west bank. Mean flood currents range from 0.45 $m \cdot s^{-1}$ on the mudflat to 1.3 $m \cdot s^{-1}$ in the main channel. The ebb currents range on average from 0.2 $m \cdot s^{-1}$ on the mudflat to 0.8 $m \cdot s^{-1}$ in the main channel (IMDC 1997). The sediment on the mudflat is sandy towards the lower edge with a mean grain size of 170 µm. The largest part of the mudflat however is very muddy with a mean grain size of 50 µm. The upper shoreline is bordered by small patches of reed (*Phragmites australi*s). The tidal range is up to 6.1 m during spring tide and 4.1 m during neap tide. Turbidity in this part of the estuary is generally high and is positively related to the freshwater discharge (Fettweis *et al.,* l998).

The study area is situated in the mesohaline part of the estuary. The salinity near the Dutch-Belgian border is mainly determined by the discharge from upstream rivers and harbor docks and reaches a maximum of 16 in summer and a minimum of 3 in winter (Fettweis *et al.,* l998). Salinity is locally and temporally influenced by the drainage of fresh water from two fresh water lakes at a flow capacity of 100 $m^3 \cdot s^{-1}$. On a daily basis 8.5 million $m^3 \cdot s^{-1}$ of fresh water may be discharged into the

Westerschelde (Data obtained from the Ministry of Transport, Public Works and Water Management of The Netherlands - ZEGE).

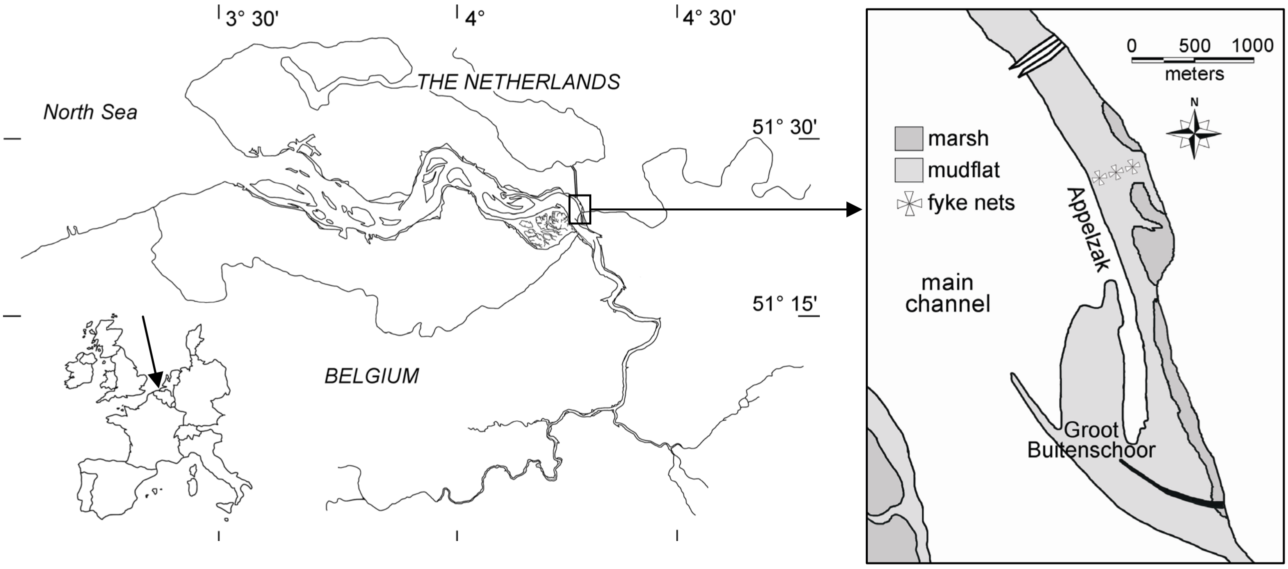

***Figure 2.1**. Map of the Scheldt estuary and detail of the study area (inset), situated on the right bank of the river.*

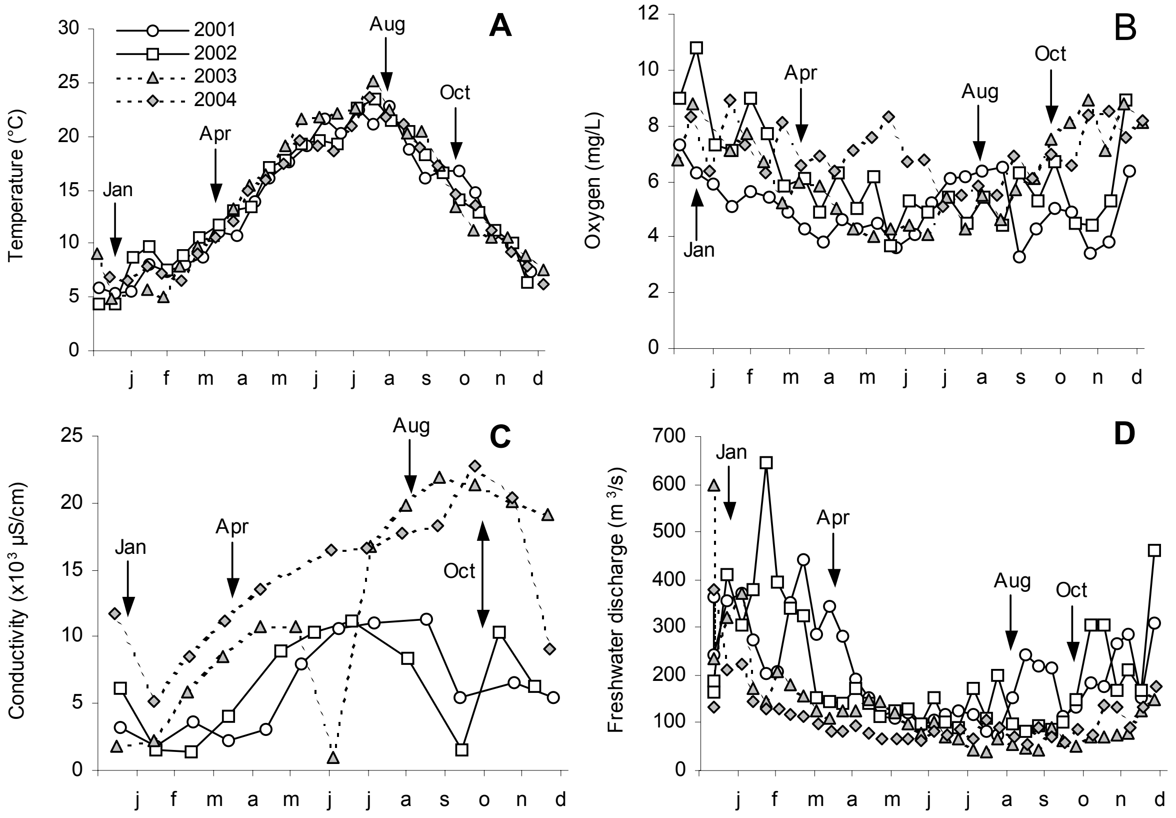

***Figure 2.2**. Seasonal changes in (A) water temperature, (B) oxygen concentration, (C) conductivity near the Dutch-Belgian border and (D) combined freshwater discharge of the Zeeschelde and the harbor docks during the four sampling years. Months refer to the sampling scheme. Data were obtained from http://www.awz.be, http://www.vmm.be and http://www.waterbase.nl.*

2.2. Sampling

Samples were taken on a seasonal basis in August and October 2001 and 2002, in January 2003 and 2004 and in April 2003. Each month four consecutive tidal cycles were sampled, with the exception of October 2001, when only three cycles were sampled.

Fish and macrocrustaceans were captured with directional fyke nets. Four nets were placed such that each fyke sampled an opposite direction. Each fyke net consisted of two side panels in front of a chamber, leading the fish in the actual fyke net (Figure 2.3A). The stretched mesh size (knot to knot) of the lead nets, the chamber, as well as the pot measured 2 cm. The last hoop of the pot, where the fish is collected, measured 1.5 cm. In order to explore the zonation of fishes on the mudflat, the fyke nets were deployed at three zones on the mudflat (Figure 2.3B).

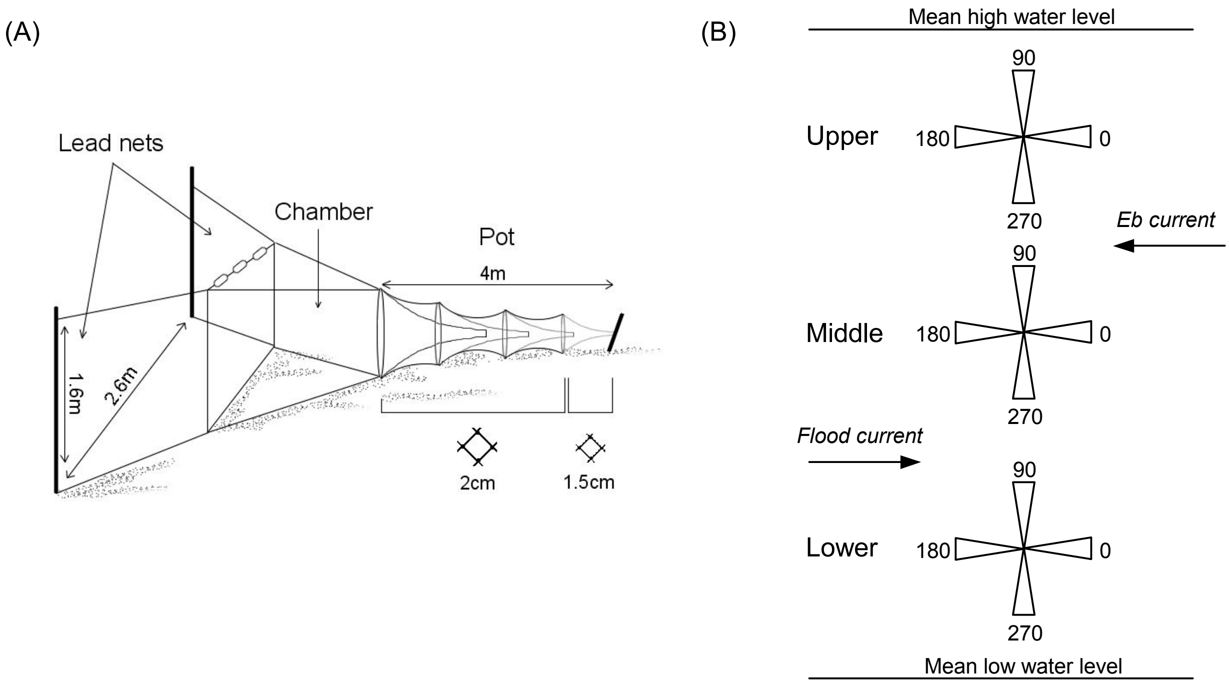

Figure 2.3. (A) Dimensions of the fyke nets used and (B) positioning of the fyke nets on the mudflat. On three zones on the mudflat four fykes were mounted in a star-shape, each sampling an opposite direction. The numbers next to the fyke nets represent the direction (degrees) of the fyke mouth; e.g. the fyke nets facing the upstream zone of the mudflat are represented by zero degrees.

The inundation time of the upper, middle and lower cluster of fykes was on average 3 h 20 min, 4 h 30 min and 5 h 40 min respectively. All fishes were anaesthetized with benzocaine and fixed in 7% formaline for further analysis. In the lab they were counted, measured (1 mm) and weighted (0.1 g). Species were identified according to Nijssen and De Groot (1987).

During sampling water temperature (°C), oxygen concentration ($mg \cdot L^{-1}$) and salinity were measured. Data about the freshwater discharges of the Zeeschelde and tributaries were made available by the Flemish Administration of Waterways and Maritime Affairs (http://www.awz.be). Monthly abiotic water parameters were obtained from the Flemish Environmental Agency (http://www.vmm.be) and from the Ministry of Transport, Public Works and Water Management of The Netherlands (http://www.waterbase.nl) (Figure 2.2).

2.3. Data analysis

For each sampling month, the total monthly abundance (N) and biomass (W; g), the Shannon-Wiener diversity index (H') and the equitability (J) were calculated. Datasets were compiled according to the level of analysis. The dataset for the temporal analysis (differences between years and months) consisted of the added numbers over one tidal cycle, resulting in a set of 27 samples (tides). For the spatial analysis the samples were grouped per cluster (height) and standardized by dividing the number of fishes in each fyke by the inundation time. To analyze the directional data, each individual fish was replaced by the direction of the fyke in which it was caught (e.g. 10 flounders in the fyke facing the high water level correspond to 10 times 90°, Figure 2.3B).

Multivariate ordination techniques (based on log(x+1) transformed abundance data) were used to analyze the community structure and its relation with measured environmental variables. Based on the results (lengths of gradients in standard deviation) of a detrended correspondence analysis (DCA) a linear response model (PCA) was applied (Ter Braak and Smilauer, 1998). Relationships between environmental variables and the first two axes of the PCA were analyzed with a Spearman rank correlation test.

The monthly abundance of species over different years (e.g. 2001 vs. 2002) was analysed by one-way analyses of variance (ANOVAs) of Year. Differences in species abundance between mudflat zones in the same year were tested with one-way ANOVAs (factor Height). Possible interaction effects between the main effects Year and Height were analyzed with two-way ANOVAs. All analyses were carried out on log-transformed abundance data (plus one to correct for zero catches) and all factors in the model were fixed. Homogeneity of variances was tested using Levene's test. A post-hoc Tukey test was used to determine the significant differences between group means.

Directional data were analyzed using circular statistics. The circular scale is a special type of interval scale where there is no true zero and the designation of high and low values is arbitrary (Batschelet, 1981; Zar, 1998). A common example of circular measurements is compass direction, where the circle is divided into 360 equal intervals (degrees) and for which the zero point is arbitrary. Circular distributions can not be analyzed using the common statistical methods and other techniques should be used. Since our data can be considered as grouped (group size 45°), the randomness of fish distribution in the fykes was evaluated with a χ^2-test. This is a non-parametric test which checks the uniformity of angles (Batschelet, 1981; Zar, 1998). Only the commonly occurring species and the freshwater species as a group were included in the analysis. Differences in angular dispersion between species were explored with a pairwise-multisample χ^2-test. Analysis of uniformity and differences between species was done with Oriana package 2.02.

Table 2.1. *Species list and total monthly catch. Species were assigned to a life cycle category according to Thiel and Potter (2001).*

Scientific name	**Common name**	**JAN** 2003	**JAN** 2004	**APR** 2003	**AUG** 2001	**AUG** 2002	**OCT** 2001	**OCT** 2002
Anadromous species								
Gasterosteus aculeatus	Three-spined stickleback	7	2			1		
Osmerus eperlanus	European smelt	146	7	11	6	1	3	7
Catadromous species								
Anguilla anguilla	European eel				2	8	2	2
Freshwater species								
Abramis brama	Carp bream	48	4	16		1		
Blicca bjoerkna	White bream			2			6	
Gymnocephalus cernuus	Ruffe	18		54	1			
Perca fluviatilis	European perch				15	4	5	
Rhodeus sericeus	Amur bitterling				2			
Rutilus rutilus	Roach	5	1		9	4	10	1
Sander lucioperca	Zander	4	1	1	20	9	10	3
Scardinius erythrophthalmus	Rudd				1			
Marine estuarine opportunists								
Atherina presbyter	Sand smelt	1						1
Ciliata mustela	Fivebeard rockling							1
Clupea harengus	Atlantic herring	526	163	60	179	23	500	117
Dicentrarchus labrax	European seabass	14	116	37	1	15	5	73
Gadus morhua	Atlantic cod							1
Liza ramado	Thinlip mullet					1		2
*Micropogonias undulatus**	Atlantic croaker						1	
Platichthys flesus	Flounder	20	28	72	157	49	134	74
Pleuronectes platessa	European plaice		4					
Pomatoschistus minutus	Sand goby						119	9
Solea solea	Common sole			2	984	251	139	55
Sprattus sprattus	European sprat	6					1	
Trisopterus luscus	Pouting			1				
Estaurine residents								
Pomatoschistus microps	Common goby				6			3
	Number of species	11	9	10	13	12	13	14
	Diversity (H’)	1.15	1.18	1.73	0.98	1.17	1.38	1.69
	Equitability (J)	0.48	0.53	0.75	0.38	0.47	0.53	0.64

* *Non-indigenous species (Stevens et al., 2004)*

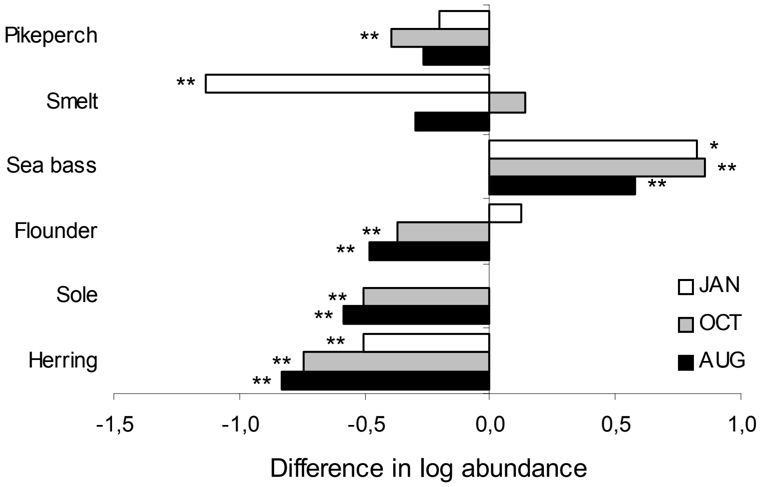

Figure 2.4. *Inter-annual variation in log abundance. August and October: 2001-2002, January: 2003-2004. A positive difference means that there were more fishes in 2001 or 2003 than in 2002 or 2004. Significant differences are marked with an asterisk (** P *< 0.05, *** P *< 0.01). For the one-way ANOVA, the F-test had 1 and 6 degrees of freedom.*

3. Results

3.1. Species composition, abundance and biomass

A total of 25 species was recorded, half of which (13 species) were categorized as marine estuarine opportunists (Table 2.1). Most species occurred sporadically and showed a clear seasonal trend in abundance. The small-sized sand goby *Pomatoschistus minutus* was only recorded in October. Freshwater species (n = 8) were always present in reasonable numbers, but didn't contribute much to the total catch. The group of estuarine residents was only represented in very low numbers by *Pomatoschistus microps*. One catadromous (*Anguilla anguilla*) and two anadromous species were caught, of which *Osmerus eperlanus* ranked fifth in the total catch.

August and October were the species richest months but diversity was highest in April. The lower diversity in August and January may be explained by the dominance of *Solea solea* and *Clupea harengus*, respectively (Table 2.1). Several species (12) were recorded occasionally and were not included in the further analysis. *Micropogonias undulates*, a species occurring in North American estuaries, was recorded for the first time in Europe (Stevens *et al.,* 2004).

Species were ranked by contribution to the cumulative total abundance and biomass. Five species (herring, sole, flounder, seabass and smelt) made up 90% of the total fish abundance, while seven species made up 90% of the total biomass (the five most abundant species together with pikeperch and eel). The five numerically most important species were selected for further analysis. The role of *Sander lucioperca* as a top predator in the ecosystem justifies its selection in the analysis.

For most species there were significant differences in abundance between years (one-way ANOVA). Log-transformed species abundances of the same months of different years (e.g. August 2001 and 2002) were subtracted to display the inter-annual variation (Figure 2.4). The highest species abundances were recorded in August-October 2001 and January 2003; seabass showed the opposite pattern with its highest numbers in 2002 and 2004 (Figure 2.4).

The vast majority of most species consisted of juveniles with a mean length between 5 and 15 cm (Figure 2.5). The length distribution of *C. harengus*, (mean length 8.1 cm ± 1.1 cm SD) was highly calibrated, indicating that the population consists of a single year class. For flounder and pikeperch we also caught some adults. The larger proportion of juvenile flounder in January compared to October suggests that larger individuals leave the estuary in winter and return in spring. There were some significant differences in size composition of the populations between the years. In 2001 there were much more juvenile fishes than in 2002, probably reflecting differences in interannual recruitment strength. This pattern was particularly clear for flounder and sole. Both taxa had quite similar lengths in August 2001, while flounder was significantly larger than sole in August 2002. Again, the pattern was reversed (more juveniles in 2002) for seabass. Only larger adults of *A. anguilla* were caught (minimum length: 29 cm).

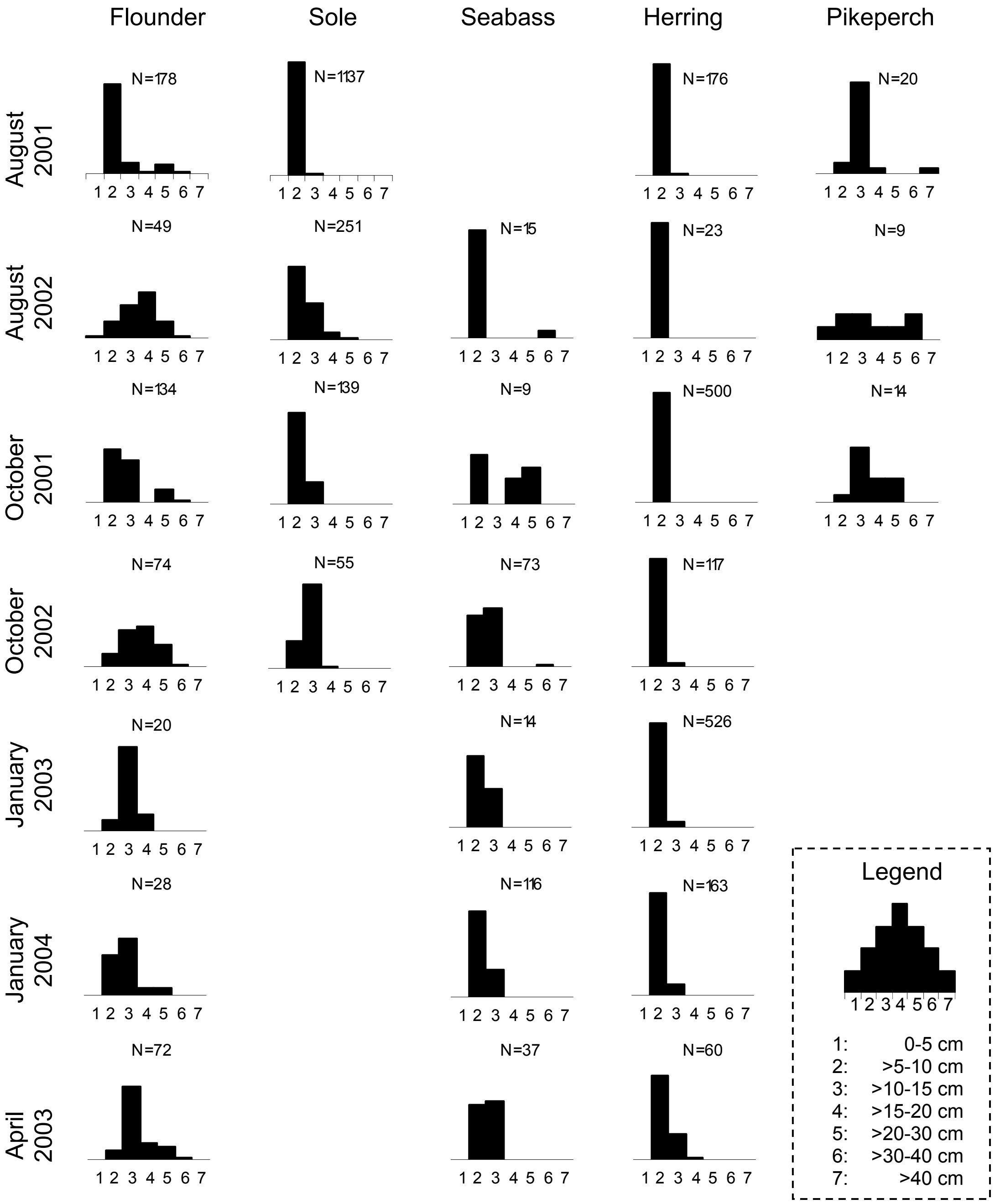

Figure 2.5. *Length-frequency (cm) of the most common species over the different sampling months (N = number caught). The numbers below each x-axis refer to the length class.*

3.2. Seasonal structure of the fish assemblage

Densities of the more common species were ordinated to explore the seasonal community structure. Figure 2.6 shows the projection of species (A) and sampling events (B) on the first two axes of the principal component analysis. Relevant abiotic variables were plotted as supplementary variables. The first two axes explained 56% of the total variance in the dataset. The first axis sorted samples according to water temperature and freshwater discharge. Temperature correlated negatively and discharge and oxygen positively with axis 1 (Table 2.2). The second axis was positively correlated with salinity.

The six species in the analysis showed a clear seasonal trend in abundance (Figure 2.6, Table 2.1). Herring and seabass reached their maximal abundance in October and January, smelt peaked in January. Sole and pikeperch on the other hand were mainly present in August. Flounder occurred throughout the year but dipped in January. The freshwater species *A. brama* and *G. cernuus* reached their highest density in January and April, while *P. fluviatilis* and *R. rutilus* were caught mainly in August and October (Table 2.1).

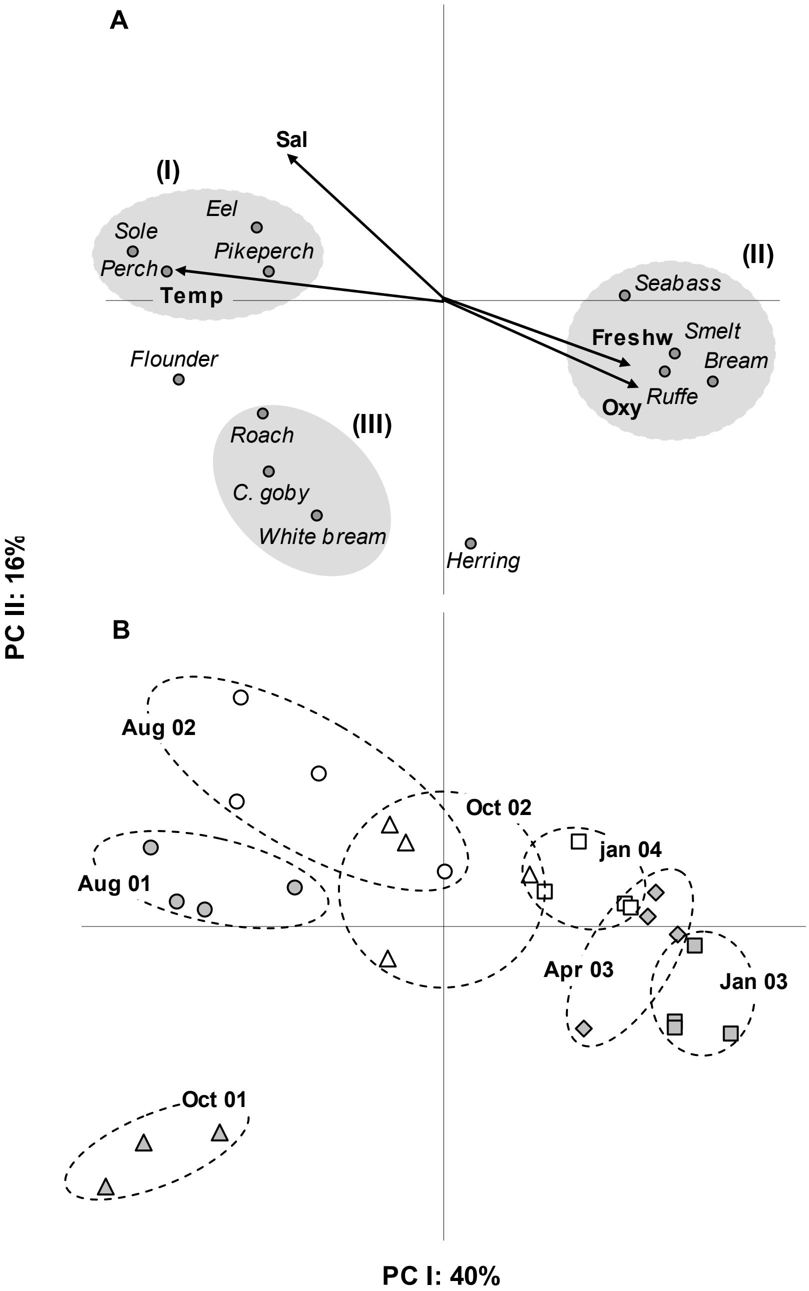

Figure 2.6. *Principal Component Analysis (PCA) biplots based on the analysis of abundance. The top figure (A) shows the vectors of the species and the environmental variables as points. Only the most important species are shown. The bottom figure (B) shows the sample scores. Sample scores were grouped per month.*

In order to clarify the patterns in the graph, the species and sampling events were grouped and three clusters of species could be distinguished. A first group represented by sole, pikeperch, perch and eel was associated with warmer and more saline water in the August samples. The samples from January and April are found opposite the first group and were characterised by a high freshwater discharge and more oxygenated water. Species that reached their maximal abundance during January and April were seabass, smelt, bream and ruffe. A third group that consisted of roach, common goby and white bream took an intermediate position on the biplot and was associated with samples from October 2001. Flounder and herring were placed in an intermediate position on the biplot according to their seasonal occurrence.

Table 2.2. *Spearman rank correlation between the environmental variables and the first two principal components of figure 2.6. Significant correlations at* $P < 0.05$ *are marked (*).*

	PC 1	**PC 2**
Temperature	-0.66*	0.29
Salinity	-0.26	0.72*
Oxygen	0.56*	-0.25
Discharge	0.56*	-0.22

3.3. Zonation of fishes on the mudflat

For the analysis of the vertical distribution of fishes over the mudflat, fish densities were standardized by dividing the total catch per level by the mean inundation time of each cluster of fykes ($\text{number}\cdot h^{-1}$). A one-way (height) or two-way (height-year) ANOVA was used to examine any zonation of species on the mudflat within a single month. The results have been summarized in figure 2.7.

No consistent trend was found in the distribution of species on the mudflat. Generally the density of species was highest on the middle and lower shore and the number of fish caught in the upper fyke cluster near the HW level was significantly lower than in the other clusters (Figure 2.7). Seabass in April 2004 and particularly smelt in January 2003 showed the opposite pattern and were found mainly in the fykes on the upper mudflat. Except for differences in total abundance, the vertical distribution patterns across years were quite similar (no significant interaction effect).

In 2001, a year with high fish density (Figure 2.4), we couldn't find a statistically significant difference in the density of flounder between the three zones. In the low-density year 2002 however, the abundance of flounder in October was significantly lower on the upper part on the mudflat (two-way ANOVA interaction term: $F_{(2, 15)} = 5.62$, $P = 0.015$; Figure 2.7d). A similar trend was observed for flounder in August, but here the interaction term was not significant ($P = 0.15$). The interaction term Height*Year was also significant for flounder in January ($F_{(2, 18)} = 7.53$, $P = 0.0045$; Figure 2.7h).

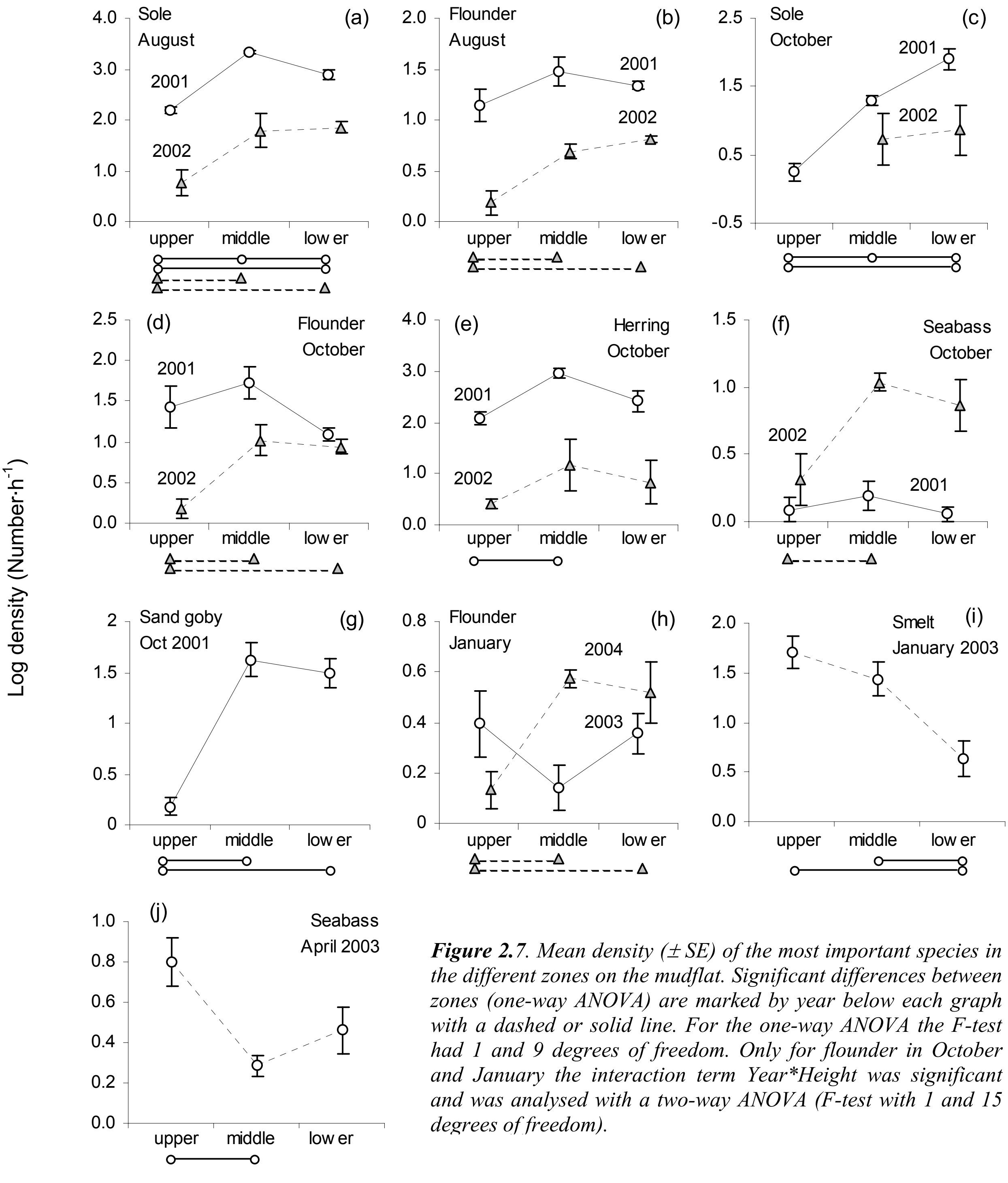

***Figure 2.7**. Mean density (± SE) of the most important species in the different zones on the mudflat. Significant differences between zones (one-way ANOVA) are marked by year below each graph with a dashed or solid line. For the one-way ANOVA the F-test had 1 and 9 degrees of freedom. Only for flounder in October and January the interaction term Year*Height was significant and was analysed with a two-way ANOVA (F-test with 1 and 15 degrees of freedom).*

3.4. Dispersion patterns

Directional data were analysed using circular statistics for the calculation of the mean vector. Species and samples were excluded from the analysis when there weren't enough observations available for a particular test. For the same reason the analysis was limited to differences between species and months. Testing the differences in species dispersion between zones was not possible because of sample size limitations for the χ^2-test.

The dispersion pattern of species was first tested for uniformity using a χ^2-test (Batschelet, 1981). Non-uniformity indicates that the species under analysis is not distributed randomly over the directional fyke nets and suggests that it has a 'preferred' direction in which it moves on the mudflat. Table 2.3 shows that none of the examined species, except pikeperch, has a uniform distribution.

***Table 2.3**: Distribution of the most common species in the fyke nets. Results of the χ^2-test of uniformity of angles. Significant* P*-values (bold) indicate non-uniform distributions. The freshwater species group encloses all species mentioned in table 2.1 except pikeperch.*

	N	**μ**	**r**	**Circ. Stdev**	**X²**	***P***
P. flesus	534	83°	0.42	75°	247.65	**<0.01**
S. solea	1431	87°	0.14	113°	228.35	**<0.01**
C. harengus	1567	107°	0.15	111°	553.14	**<0.01**
D. labrax	260	119°	0.19	103°	95.78	**<0.01**
S. lucioperca	47	106°	0.17	107°	3.98	0.264
Freshw. sp.	133	46°	0.28	92°	16.68	**<0.01**

N = number of observations, *μ* = mean vector (angle), *r* = length of mean vector

The mean vectors of all species are situated within the 90° ± 45° interval. However, the mean vector may be rather misleading if compared to figure 2.10, where the density distribution of some species matches with the axis perpendicular to the calculated mean vector (Figure 2.8). The unreliability of their mean vector is also suggested by their small lengths (r). Species such as herring and seabass have a diametrically bimodal distribution, having data with two modes lying opposite to each other on the diameter of the circle. As a result, the calculated mean angle may be far from the diameter along which the bulk of the observations lie. This problem is solved by doubling of the angles (Zar, 1998).

As can be seen from table 2.4 the modes from the distributions of flounder and sole lie along 90° (perpendicular to the longitudinal axis of the mudflat); the modes for herring and seabass lie along 0° (parallel to the longitudinal axis of the mudflat). The vector lengths (r) of pikeperch and the freshwater species are quite low and the angular deviations (s') high, which confirms their deviating distribution.

***Table 2.4**. Results of the doubling of angles procedure. Orientation (α) of the diameter along which the bimodal distribution lies;* r *is the vector length and* s' *the angular deviation.*

	α	**r**	**s'**
Flounder	90°	0.42	0.44
Sole	90°	0.36	0.46
Herring	0°	0.56	0.38
Seabass	0°	0.55	0.38
Pikeperch	0°	0.19	0.51
Freshwater sp.	90°	0.02	0.56

Table 2.5 summarizes the χ^2-tests for differences in dispersion between species. The test was only not significant for the difference between seabass and herring, which means that they had a similar distribution in the fykes. Based on the above results and figure 2.8 we would have expected that

flounder and sole also had equal distributions. However, if we compare the test statistics, the χ^2-value for flounder-sole was much smaller than for the other significant differences. Because of the large sample size, the χ^2-test detects already small differences and the null hypothesis is rejected. We therefore believe that the migration patterns of sole and flounder are largely similar as was already suggested in table 2.4.

Table 2.5: *χ^2 scores (lower half) and probabilities (upper half) for between-species differences in distribution. Species with the same distribution ($p > 0.05$) are indicated in bold.*

	P. flesus	***S. solea***	***D. labrax***	***C. harengus***
P. flesus		< 0.01	< 0.01	< 0.01
S. solea	35.2		< 0.01	< 0.01
D. labrax	144.1	126.9		**0.616**
C. harengus	390.8	457.5	1.8	

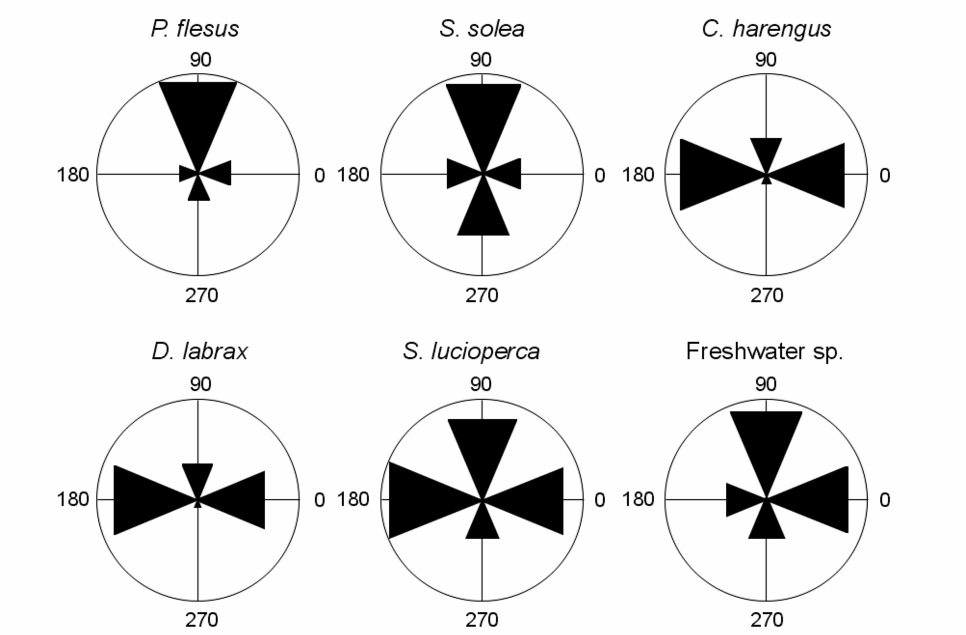

Figure 2.8. *Rose histograms of the distribution of the most important species in the fyke nets. Species were grouped per month. The group of the freshwater species includes all species mentioned in table 2.1, except pikeperch (presented separately).*

According to their distribution in the fyke nets, two groups of species were distinguished: the first group consists of flounder and sole, both benthic species that were mainly caught in the fykes facing the high water level. It suggests that they move independently of the currents on the mudflat. The distribution of herring and seabass on the other hand is perpendicular to that of the previous group and lies in the main direction of the ebb and flood currents. The freshwater species have an intermediate distribution with the majority of fishes caught in the fykes opposite the high water level and the ebb current. According to the χ^2-test *S. lucioperca* has a random distribution pattern with no directional preference. Just like the other species, pikeperch seems to avoid the fykes facing the low water level.

If the analysis was repeated for each month separately, the observed patterns persisted but for most species, the freshwater component (ebb current) was stronger in January and April, while in summer (higher salinity) the marine component (flood current) was more pronounced. This was most obvious for the 'current-dependent' species herring and seabass.

4. Discussion

4.1. Species composition

The intertidal fish community in the mesohaline zone of the Scheldt estuary (Southern North Sea) was sampled on a seasonal basis over four years (2001-2004). Because of logistic reasons our study could not be replicated on different mudflats. Consequently, one should be careful in extrapolating the results of our study on this brackish water mudflat to other systems. We were able to catch 25 species during seven sampling campaigns (campaign = four consecutive tides). A maximum of 14 species (October 2002) and a minimum of 9 species (January 2004) was caught during one campaign. In a synthesis of 10 years data collection from the cooling water intake of the nuclear power plant at Doel, Maes *et al.* (2005b) report a total of 62 species in the brackish zone of the estuary. All but two species of the present study also occurred in the cooling water at Doel. The only species not occurring in the cooling water were *Scardinius erythrophthalmus* and *Micropogonias undulates*. This last one is a non-indigenous species that was new for European coastal waters (Stevens *et al.,* 2004). Of both species only one specimen was caught.

Four species, all marine estuarine opportunists (Thiel and Potter, 2001), numerically dominated the catches: two flatfish species *P. flesus* and *S. solea* and *C. harengus* and *D. labrax*. These species also ranked in the 10 most common species in the cooling water at Doel. Of the other top-ten species however, gobiids, three-spined stickleback and sprat were underrepresented or absent (*Syngnathus rostellatus*) in our samples. *S. rostellatus* probably escaped through the meshes of the fyke because of its needle-like shape. Possibly also the gobiids and three-spined sticklebacks are too small to be retained efficiently. Although herring and sprat co-occur in mixed schools in the estuary (Maes and Ollevier, 2002), sprat was virtually absent in the fykes. Our data suggest that sprat unlike herring doesn't migrate onto the mudflat and stays in the subtidal zone during high water. The reason for this behaviour is unclear. In a study about the environmental influences on the fish assemblage of the Humber estuary, the authors found a negative correlation between abundance and depth for herring but not for sprat (Marshall and Elliott, 1998). As the estuarine status of sprat is poorly known (Costello *et al.,* 2002) further research on habitat use of *C. harengus* and *S. sprattus* in the estuary is needed for species-specific management.

Other flatfish species such as *Limanda limanda* (dab) and *Pleuronectes platessa* (plaice) were lacking in our samples, although they were found in substantial numbers in other studies in the mesohaline part of the estuary (Hostens, 2003). According to Elliott and Dewailly (1995), dab prefers sandy bottoms, while the mudflat in our study can be classified as soft, muddy bottom. Furthermore dab do usually not migrate tidally and continue to feed in the subtidal areas (McLusky and Elliott, 2004). Another explanation which also holds for plaice could be that our study site is near the lower salinity range of distribution for these species. In the Elbe estuary plaice only occurred in very low numbers at the site were salinity fluctuated around 7 and was not found in sites with lower salinities (Thiel and Potter, 2001).

Freshwater species were always present in reasonable numbers, but didn't contribute much to the total catch. Only in April they made up to 30% of the catch. It is not known to what extent the freshwater discharge from the channel in Bath contributed to the presence of freshwater species. Hostens (2003) studied the subtidal fish community in the mesohaline part of the Westerschelde and couldn't find any freshwater species, suggesting that the fishes in our samples were vagrants from nearby freshwater discharge points.

The present study was designed to examine the spatial structure on the mudflat on a seasonal basis, for which quarterly samples should be adequate (Hemmingway and Elliott, 2002). Because of the significant interannual variation in the abundance (Figure 2.4) and because not all seasons could be sampled in every year, any conclusions about the seasonality of the fish assemblage are tentative. The seasonal structure of the fish assemblage is very similar to the cyclic structure described in other studies (Power *et al.,* 2000; Thiel and Potter, 2001; Maes *et al.,* 2005b). The largest amount of temporal variability in the data was determined by differences in annual recruitment and then by predictable cyclical patterns of species abundance (Maes *et al.,* 2005b). Environmental variables as temperature and salinity may act more as a final trigger for migration in and out the estuary. The same conclusions were also found for a study of the intertidal fish and macrocrustacean community on a sandy beach, where it was suggested that annual cycles mainly result from recruitment rather than being a response to physical factors (Gibson *et al.,* 1993).

The intertidal fish community was dominated by juvenile fishes, confirming the nursery function of the intertidal zone. Adult specimens were only recorded sporadically and large piscivorous predators were virtually absent. Flounder was the sole species for which elder individuals were recorded during the whole year, confirming its status of an estuarine resident species (Elliott and Dewailly, 1995).

In other studies which specifically looked at the fish assemblage of intertidal mudflats, the mudflat community was dominated by Gobiidae, Pleuronectidae, Mugilidae (Morrison *et al.,* 2002; Hindell and Jenkins, 2004; Salgado *et al.,* 2004) and Engraulidae (Morrison *et al.,* 2002). Although it is difficult to compare our results with studies from other zoogeographic regions with a different faunistic composition, the absence of gobiids and mugilids in our study is striking. Based on the seasonal patterns of gobies in the Scheldt estuary (Hostens, 2003; Maes *et al.,* 2005b) we know that gobies peek in the Scheldt estuary between June and December. The selectivity of the sampling gear could have been a reason for an underestimation of the numbers of gobies. As with dab, substrate preference may also be part of the explanation as *Pomatoschistus sp.* prefer sandy substratum (Elliott and Dewailly, 1995). The *Liza ramado* population in the mesohaline part of the

Scheldt estuary is mainly composed of small juveniles (4 – 4.5 cm) (J. Maes, pers. communication and Hostens, 2003). Fishes of this length were probably not retained in the net.

A study in a nearby saltmarsh by Hampel *et al.* (2004) between April and October 2000 yielded only five species (of which four species only occurred in the fykes) and was dominated by *Platichthys flesus*, *Dicentrarchus labrax* and *Pomatoschistus microps*. Also a comparison between the fish assemblages of saltmarsh creeks and adjacent mudflats in the Tejo estuary in Portugal (Salgado *et al.,* 2004) showed that the species richness in the saltmarshes was lower than on the mudflat. The structural complexity of saltmarshes might not only function as a predator refuge but also as a hindrance for the invasion of more pelagic and semi-pelagic species. Mudflats on the other hand are easily accessible and contain huge amounts of benthic prey (Chapter 3), making it to a pantry for the fish visiting the mudflat.

4.2. Intertidal dispersion

Most of the studies on intertidal migration deal with post-larval or early juvenile flatfish species on sandy coastal beaches and relate migration patterns to predator avoidance and foraging (Gibson *et al.*, 1998; Gibson *et al.,* 1993; Burrows, 1994). Little information, however, is available on movements of juvenile fishes on estuarine intertidal mudflats. Intertidal migration can be influenced by the day-night cycle or tidal rhythm. The former seems mainly to occur in places where the tidal amplitude is negligible (Able *et al.,* 2002; Gibson, 1997).

The specific design of the present study using directional fyke nets at different levels on the shore should allow us to obtain an idea of the extent and direction of intertidal migration. In the introduction we hypothesized that the zonation of fishes on the mudflat should be species-specific and should be caused by biotic and environmental factors. However, most species in our study use the mudflat to its full extent as suggested by their presence in fykes over all shore levels (Figure 2.7). Only the upper shore was relatively less used by most species. The distance a species covers during one tide depends on species-specific traits as mobility and site-specific properties such as currents. Flounders are known to move several hundred meters onto tidal flats (Wirjoatmodjo and Pitcher, 1984; Able *et al.,* 2002). Our data also suggest that flounder is one of the species moving to the upper part of the tidal flat. Sole on the other hand is known to feed in the subtidal zone while in our study sole also migrated onto the mudflat (McLusky and Elliott, 2004). The cost of migration can be significant for less mobile species which therefore might be restricted to the lower shore. When present, *P. minutus*, one of the less mobile species, was almost exclusively caught in the fykes on the low and middle shore (Figure 2.7g).

The directional fyke nets allowed us to sample the intertidal fish assemblage from different directions. We hypothesized that the distribution patterns of fishes in the fykes tell us something about the way they migrate on the mudflat. Fishes which are transported more passively by the currents on the mudflat will be captured primarily in the fykes that filter the largest part of the ebb and flood currents. Contrary, fishes that move actively over the mudflat will be distributed in the fyke nets either randomly or independently of the ebb and flood currents.

According to their distribution over the fyke nets, we found two groups of fish species. A first group included the flatfish species flounder and sole which were mainly caught in the fykes perpendicular to the axis of the ebb and flood currents. The majority of these species occurred in the fykes facing the high water level (Figure 2.8), indicating that they probably migrate down the shore, following the edge of the retreating water at ebb. The second group included herring and seabass which occurred primarily in the fykes parallel to the main currents on the mudflat (Figure 2.3B). When the distribution patterns were analyzed for each month separately, the patterns described above persisted, but for most species and particularly those of the second group, the marine (Figure 2.3B – 180°) or the freshwater (Figure 2.3B – 0°) component of the distribution became more important respectively in October and April. Based on these findings we argue that both groups of species use the mudflat differently. Flatfishes move actively on the mudflat as demonstrated in the past by telemetry (Wirjoatmodjo and pitcher, 1984) and direct observations (Gibson, 1980). (Semi-)pelagic species like herring and demersal species like seabass seem to use the tidal flow as a vector for intertidal migration. For clupeids it has already been shown that they move with tidal flow in the Forth estuary as a kind of behavioural enviroregulation (Welsby *et al.,* 1964; Gibson, 1997).

Of the factors explaining intertidal migration, **foraging** seems the most important (Chapter 3). This is obvious for flatfishes, for which numerous studies already demonstrated the importance of intertidal areas as feeding grounds (Summers, 1980; Cabral *et al.,* 2002). Semi-pelagic and demersal species such as herring and seabass on the other hand, can be considered as rather opportunistic migrants taking advantage of the benefits of intertidal migration. They possibly follow their hyperbenthic and pelagic prey species (e.g. mysids and copepods), which are passively transported by the currents on the mudflat (Speirs *et al.,* 2002).

Intertidal migration might also be related to **predator avoidance** as suggested by the low abundance of large piscivores in estuarine habitats and shallow waters in particular (Blaber and Blaber, 1980; Burrows, 1994; Patterson and Whitfield, 1996; but, see Baker and Sheaves, 2005). The main fish predators in coastal zones are thought to be gadoids (Pihl, 1982; Ellis and Gibson, 1995), but they do not seem to penetrate very far into estuaries (Maes *et al.,* 1997; Greenwood and Hill, 2003). This is in agreement with our study where one cod and one pouting was caught. Other possible piscivores (pikeperch) were only present in very low numbers. In addition, a modelling study by Maes *et al.* (2005) in which the fitness of individual herring in the Scheldt estuary and adjacent coastal areas was modelled as a function of foraging efficiency and predation risk, showed that mortality decreases considerably at turbidities >75 NTU. The mean turbidity on the mudflat fluctuates around 60 NTU, which might be suboptimal for visual predation. This supports the idea that predation in turbid areas like the brackish part of the Scheldt estuary, is probably insignificant and not a strong cue for intertidal migration.

Several studies indicate that **higher intertidal temperatures** during summer are likely to result in higher growth rates of juvenile flatfishes (Van der Veer and Berman, 1986; Gibson, 1994; Gibson *et al.,* 2002; Stevens *et al.,* 2006). It remains however a question to what extent the temperature-related growth benefit during small scale intertidal movements contributes to the overall growth compared to larger scale movements on extensive intertidal flats as the Wadden Sea. The Scheldt estuary is well mixed, which means that vertical temperature gradients are small or negligible. We couldn't find a correlation between daily water or air temperature and the distribution of species on the mudflat. In relative small intertidal areas like our study area, the temperature effect is probably negligible compared to other advantages from intertidal migration such as the availability of high prey densities.

A final stimulus for intertidal migration, which received relatively little attention in literature is **competition**. Some studies suggest that interspecific competition can regulate the vertical distribution of fishes, especially when food is limiting. For example, plaice and dab occupy a similar ecological niche and differ mainly in their distribution during the first few months of their life. While dab settles in deeper water, plaice uses the intertidal water in the earliest developmental stages, when densities are highest and competition with dab is likely to be greatest (Gibson, 1973; Gibson *et al.,* 2002). Also in the Wadden Sea plaice has been observed to move onshore in the presence of competition for food (Berghahn, 1987). However, direct evidence for exploitative competition is scarce and the general consensus seems to be that direct competition is rare and prevented by abundant food resources or separation in time or space (Gibson, 1994; Cabral *et al.,* 2002).

In the present study, flounder was observed to migrate relatively higher onto the shore in 2001 when the abundance of most species was significantly higher than in 2002 (Figure 2.4). Competition with sole may force flounder to move up further on the shore to exploit the available food sources. Further evidence for enhanced competition on the lower and middle shore can be obtained from the density pattern of *Corophium volutator*, the most important intertidal prey item, in 2001 and 2002 (Chapter 3). In August 2001 and 2002, the density of *C. volutator* on the high and middle shore is about the same. In October 2001 however, numbers dropped dramatically on the middle shore whilst staying high on the upper shore, whereas in 2002 they stay high at both levels. This might suggest that food limitation can occur in years with high fish density, resulting in spatial segregation of fishes on the mudflat.

From our results, we conclude that the fish assemblage on this mudflat in the Scheldt estuary wasn't clearly zonated as expected from the hypothesis of the nursery function of shallow intertidal habitats. The reason for this lack of zonation might be multiple and related in the first place to the specific nature of the study area. First, fishes could have been easily transported by the tidal currents to the upper parts of the mudflat, by which mobility probably was less limiting. Secondly, any positive effect of increased intertidal water temperatures on fish growth is possibly negligible because of the thermal homogenous conditions on the mudflat. Furthermore, the high turbidity in the mesohaline zone of the estuary makes predation unlikely as a strong driving force for intertidal migration. Probably the most important (and perhaps only?) trigger for intertidal migration in this area is the high intertidal food availability. The lack of zonation in the macrobenthic prey community on the mudflat may have contributed to the homogenous distribution of the fishes (Chapter 4). However, in years with high fish recruitment to the estuary and hence increased pressure on the prey population, competition may cause a zonation of the fish community by forcing the more mobile species to exploit benthic prey on the upper mudflat. The evidence presented is however insubstantial and more research is needed.

Acknowledgements

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CHAPTER 3

Taking potluck: trophic guild structure and feeding strategy of an intertidal fish assemblage

3

Maarten Stevens, Joachim Maes and Frans Ollevier

Abstract

Many estuarine fishes migrate with the tides onto the intertidal mudflats, which provide them with abundant food resources. The concentration of large numbers of juvenile fishes in these intertidal areas may give rise to competitive interactions. In this study, the seasonal change of the trophic guild on an estuarine mudflat in the Scheldt estuary was described and prey selection was compared with prey availability. Diet analysis showed that all fish species on the mudflat, without exception, fed to a more or lesser extent on *Corophium volutator*. The importance of prey species in the diet of fishes corresponded with the seasonal availability of prey in the field, confirming the generalist and opportunistic feeding nature of estuarine fishes. For all species, the niche width was larger when resources were less abundant or the population density was highest. A generalist and opportunistic feeding mode reduces the potential for competition resulting in an increasing population diet breadth with increasing population density. On the contrary, when fish abundance was lower, fish species were specializing on *C. volutator*, resulting in a narrowed niche. As most fishes were feeding on few prey species, niche overlap was generally high between species. A significant niche overlap was found between flounder and sole in summer. Direct competition for food was probably avoided by spatial niche separation, as flounder migrated relatively higher onto the mudflat when fish abundance increased. Compared to sole, flounder selected for smaller prey size classes, which suggests resource partitioning at the level of prey size. It is not clear whether this size selective strategy is adopted to reduce interspecific competition or is the result of foraging behaviour.

Keywords: *Niche overlap; Prey selection; Resource partitioning; Stomach analysis; Scheldt estuary*

Unpublished manuscript

1. Introduction

Estuaries, in particular intertidal estuarine water, are generally considered as nursery areas because they provide food and refuge from predation for juvenile marine fishes (Blaber and Blaber, 1980; Elliott and Hemingway, 2002; Baker and Sheaves, 2005). The high productivity of estuaries, compared to coastal zones and the open ocean, supports high densities of fish and macrocrustaceans (Day *et al.,* 1989). Generally, two food webs can be distinguished: a bed-dominated detrital food chain, fueled by the input of allochthonous material and a phytoplankton-based food chain, supported by autochthonous production of suspended benthic diatoms (microphytobenthos) (Heip *et al.,* 1995; Elliott *et al.,* 2002; Dauvin and Desroy, 2005). In turbid, temperate estuaries, the higher trophic levels seem to be primarily dependent on the detrital food web (Day *et al.,* 1989). Estuarine fish production is usually supported by small epi- or hyperbenthic crustaceans (amphipods and shrimps) and infaunal molluscs and annelids, which form the main link between detritus and fish (Day *et al.,* 1989; Elliott and Hemingway, 2002).

On their turn, macrobenthic and epibenthic prey are sustained by the primary productivity of the microphytobenthos on the intertidal flats (Heip *et al.,* 1995; Herman *et al.,* 1999). Intertidal areas in estuaries have a much higher productivity per unit area compared to subtidal areas. In order to exploit these resources, fish are committed to intertidal migrations at high tide (Gibson, 1994). The most important fish predators on mudflats are flatfishes. In the mesohaline zone of the Scheldt estuary, characterized by the presence of extensive mud and sand flats, flatfish species like *Pleuronectes platessa*, *Platichthys flesus*, *Solea solea* and *Limanda limanda* feed on endobenthic prey. Most species had more full stomachs in the intertidal compared to the subtidal zone. In the mesohaline zone, the hyperbenthos (mysids) reaches its maximal density and biomass (Mees *et al.,* 1993) and are also a major food source for many juvenile fish and shrimps (Hostens and Mees, 1999).

Many estuarine fishes adopt a generalist or opportunistic foraging strategy and feed on a wide range of available prey items (Moore and Moore, 1976; Elliott *et al.,* 2002). When studying the trophic interactions within an estuarine fish community and their implications on the structure of this community, prey availability in the environment plays a crucial role. As predicted by the optimal foraging theory, fish should select those prey items that maximize their net rate of energy gain (Wootton, 1992; Gill, 2004). As a result, prey species that are readily available should be preferred to prey species that have lower energy content and/or are more difficult to find, encounter and capture.

The availability of prey items is determined by both environmental and biological factors. Environmental factors that determine feeding in estuaries include turbidity, salinity and temperature (Elliott *et al.,* 2002; De Robertis *et al.,* 2003). Among the biological factors, ontogenetic niche shifts (trophic as well as spatial) and the effect of predator morphology (gape size and dentition) on prey selection are well documented (Moore and Moore, 1976; Piet *et al.,* 1998; Elliott *et al.,* 2002;

Gibson *et al.,* 2002). Although the importance of these factors is obvious, the present study focuses more on the intra- and interspecific regulators of food web structure like prey density and competition.

Competition for food may be an important factor in structuring fish communities and is usually demonstrated by measuring the dietary overlap within and between species (Connell, 1983; Munday *et al.,* 2001; Cabral *et al.,* 2002; Elliott *et al.,* 2002; Vinagre *et al.,* 2005). The greater the degree of overlap, the greater the chance for competition. However, overlap doesn't indicate competition unless it can be shown that the amount of prey available was limited to one or both species. The evidence for the structuring effect of competition in estuaries is rather conflicting, but the general consensus seems to be that direct competition is rare and avoided by resource partitioning on a trophic, temporal and spatial scale (Gibson, 1994; Munday *et al.,* 2001; Cabral *et al.,* 2002; Vinagre *et al.,* 2005; but see Thorman, 1982; Le Mao, 1986; Hostens and Mees, 1999). Furthermore, these studies argue that resources are not limiting due to the superabundance of available prey items. As a result, the fish populations remain below the carrying capacity of the system.

Although an extensive literature exists on feeding relations between estuarine fishes (see references above), most studies did not take into account the availability of prey species. As such, they lack the power to interpret the functioning of the estuarine habitat and translating their findings to competitive interactions. Food is one of the most important factors to determine habitat quality and as such has an effect on fish recruitment and survival (Gibson, 1994). Studies on trophic partitioning in food-dense habitats may help to understand the functioning of estuaries and intertidal areas, in particular.

This study combines both the availability of macrobenthic prey and the trophic relations within the fish community on an intertidal mudflat (Figure 3.1). The mesohaline part of the Scheldt estuary is characterized by extensive intertidal sand and mudflats, and is recognized as an important nursery area for fish and crustaceans (Hostens, 2000; Maes *et al.,* 1998a). The high abundance of macrocrustaceans, fishes and birds suggests that competition between predators is likely to be a community-structuring factor. We focused on the importance of the intertidal zone as a feeding ground for the estuarine brackish water fish community. Two main questions were postulated. First, which species exploit the mudflat as a feeding ground and to what extent are they dependent on this zone for their food supply? Secondly, how does trophic niche sharing affect the composition and functioning of the feeding guilds on the mudflat?

2. Materials and methods

2.1. Study site and sampling

The Scheldt estuary is a heavily urbanized and industrialized river draining in the Southern Bight of the North Sea (Figure 3.1). Samples were taken on an intertidal mudflat in the mesohaline zone of the Scheldt estuary near the Dutch-Belgian border. The mudflat is about 450 m wide, has a gentle slope and a muddy substrate with a mean grain size of 50 µm. The tidal range near the Dutch-Belgian border is up to 6 m during spring tide and 4 m during neap tide. Turbidity in this part of the estuary is generally high and is mainly dependent on the freshwater discharge (Fettweis *et al.,* l998). The water temperature ranges from 22 °C in August 2002 to 5.5 °C in January 2004; salinity fluctuates between 10 and 2 in respectively August 2002 and January 2003.

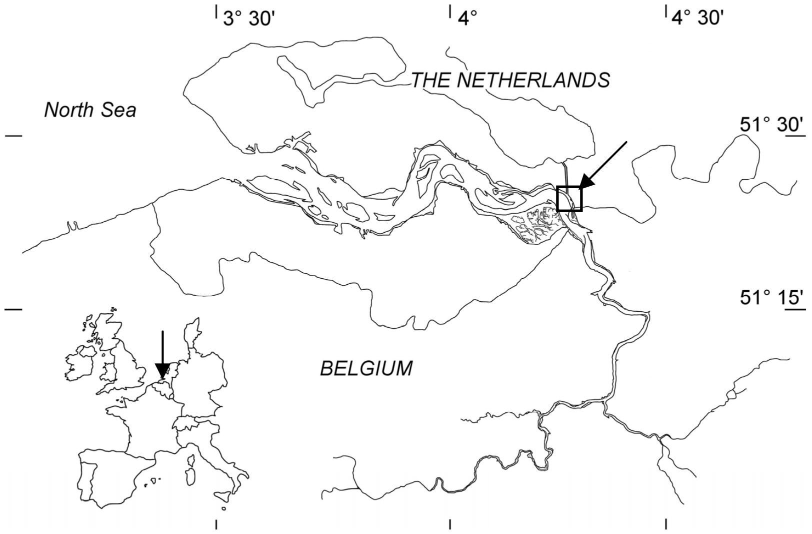

Figure 3.1. *Map of the Scheldt estuary. The study area (box) is situated on a mudflat on the right bank of the river near the Dutch-Belgian border.*

The fish community was sampled on a seasonal basis in August and October 2001 and 2002, in January 2003 and 2004 and in April 2003. Fishes were captured with directional fyke nets at three different heights on the shore. The stretched mesh size of the last hoop of the fyke, where the fish was collected, measured 1.5 cm. A detailed description of the method is given in chapter 2.

Each month the fykes were left in place for four consecutive tidal cycles. In October 2001 however only three tides were sampled. The fishes were collected immediately after the water receded, anaesthetized with benzocaine to prevent regurgitation of food and fixed in 7% formaline. In the laboratory, all fishes were measured (total length, ± 1 mm) and weighted (± 0.1 g) and their stomach content was removed for further analysis. For fish species that had no clearly defined stomach (e.g. cyprinids) the foremost first third part of the intestine was analysed. Subsamples were taken if the number of fish per fyke exceeded 10. Prey items were identified to the lowest taxonomic level possible and counted. The total dry weight (70°C; ± 0.1 mg) of each prey species was determined for up to 20 individuals per species per month. No gravimetric data are available for August 2001.

Prey size selectivity of flounder and sole was estimated by measuring the most important prey species *Corophium volutator* and *Nereis diversicolor* in the stomachs of the fishes in August and October 2002, April 2003 and January 2004. *N. diversicolor* was measured to the nearest millimeter by stretching it along a ruler. If the animal was incomplete, the width of the 10^{th} segment was measured with the ruler of a stereomicroscope and converted to the total length using the regression listed in Esselink and Zwarts (1989). Shrinking of worms through conservation in formaldehyde was corrected by multiplying by 1.47 (Esselink and Zwarts, 1989). Almost all *C. volutator* in the stomachs were intact and individuals were divided into five size classes: 0-2 mm, 2-4 mm, 4-6 mm, 6-8 mm and > 8mm.

2.2. Stomach analysis

2.2.1. Descriptive statistics

A total of 2564 stomachs of 22 species were analysed, but only the stomachs of the most abundant species were retained for further analysis. The relative importance of prey items in the diet of these species was evaluated by three indices: percentage occurrence (O%), percentage weight (W%) and percentage abundance (N%). The percentage occurrence refers to the occurrence of the specific prey item in the non-empty stomachs. Percentage weight and abundance were calculated as the contribution of a prey species to respectively the total weight or number of all prey species in the stomachs.

Inter- and intraspecific dietary interactions on the mudflat were investigated using Two Way Indicator Species Analysis (TWINSPAN) on the presence/absence data of the eight most common fish species in the fyke nets. TWINSPAN was performed on a matrix with 21 species samples and nine prey items. A species sample refers to the diet of a species for one month. For the analysis, the stomachs of fishes from the same month were combined over different years. The analysis produced a two-way array of both samples and attributes, grouping together the samples with greatest affinity (Marshall and Elliott, 1997). As the TWINSPAN groups are based on the presence or absence of a particular prey, the results are not biased by the weight of dominant species.

2.2.2. Prey selectivity and feeding strategy

Prey importance and feeding strategy were visually explored using Amundsen‘s (1996) modification of the Costello method in which the prey-specific abundance ($\%P_i$) is plotted against the percentage occurrence ($\%F_i$) in a two-dimensional graph (Figure 3.2). Prey-specific abundance is defined as the percentage a prey item contributes to the total amount of all prey items in only those stomachs in which the actual prey occurs:

$P_i = 100 (\Sigma S_{i)} (\Sigma S_{ti)})^{-1}$

where S_i = the stomach content comprised of prey i and S_{ti} = the total stomach content in only those individuals with prey i in their stomach. Prey importance (dominance) increases along the diagonal from the lower left to the upper right. The feeding strategy of a predator species (specialist vs. generalist) is described along the central vertical axis. The contribution of individual prey types to the overall population niche width is displayed along the diagonal from the upper left to the lower right corner. Prey items located in the lower right corner will have been eaten by most individuals

(High Within-Phenotypic Component), whereas prey in the upper left corner will have been consumed by a few specializing individuals (High Between-Phenotypic Component) (Amundsen *et al.,* 1996). For the analysis, prey-specific abundance and frequency of occurrence were calculated as average values per species per sampling month.

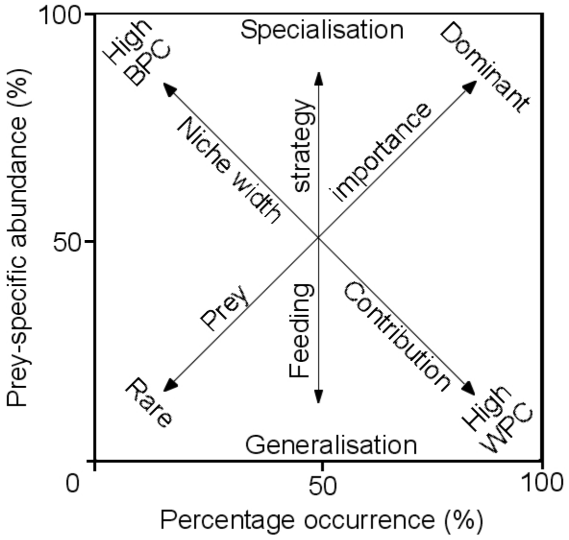

***Figure 3.2**. Descriptive diagram for the interpretation of the modified Costello graph. After Amundsen et al. (1996). BPC = Between Phenotypic Component; WPC = Within Phenotypic Component.*

To assess the relationship between diet composition of the predator species and the availability of macrobenthic prey items on the mudflat, Chesson's α value was calculated for prey species over the different sampling months (Chesson, 1983). The selectivity index was calculated for *Platichthys flesus*, *Solea solea*, *Clupea harengus* and *Dicentrarchus labrax*. Chesson's α was calculated as:

$$\alpha = \frac{r_i / p_i}{\sum_j^m r_j / p_j}, \qquad i = 1, \ldots, \mathrm{m}$$

where r_i = the relative abundance of prey species i in the stomachs, p_i equals the relative abundance of prey species *i* on the mudflat and m is the number of prey species included in the analysis. The index returns a proportion coefficient ranging from 0 to 1, with values exceeding 1/m indicating 'preference' for that prey item and values less than 1/m indicating 'avoidance' of that prey item. Chesson's α values for each fish-prey combination were analysed to be different from zero using one-sample T-tests after (α-1/m) transformation.

The size selectivity for *C. volutator* was determined in a similar way. Chesson's alpha values were calculated for the different size classes of *C. volutator* in the stomachs of *S. solea* and *P. flesus* in August and October 2002, April 2003 and January 2004. The α-1/m values were tested to be different from zero using a one-sample T-test. The difference in mean length of *C. volutator* consumed by *S. solea* and *P. flesus* in August and October 2002 was evaluated with a two-way ANOVA (factors are month and species). For *N. diversicolor* the analysis was limited to August because there were not enough specimen of this prey in the stomachs of sole in October. We corrected for the length of the fish by dividing the prey length by the length of the predator. Since the variances of the prey length distributions were not identical, the nonparametric Mann-Whitney U test was used to compare the corrected median length of *N. diversicolor* in flounder and sole.

2.2.3. Niche breadth and overlap

The niche breadth (*B*; Levins, 1968) and niche overlap (*NO*; Schoener, 1968) were calculated as:

$$B = 1 / \sum_{i=1}^{N} p_i^{\,2} \qquad NO = 1 - 0.5 \cdot \sum_{i,j}^{N} \left| p_i - q_j \right|$$

Where p_i and q_i are the proportions of the diet of respectively individual p and q that are represented by diet category i. N is the total number of prey items. A bootstrap procedure was used to calculate the mean niche breadth and associated 95% confidence intervals (Manly, 1986). Confidence intervals were based on 1000 bootstrap samples.

In order to test for significance of the niche overlap we used the Mantel test (Manly, 1986). Hereto, two symmetric $n \times n$ matrices were constructed. The first contained the overlap indices for each pair of individuals and the second, a binary matrix with a 1 for pairs of individuals from the same group and a 0 for individuals from different groups. A significant positive correlation between the two matrices indicates that two individuals from the same group tend to have a higher niche overlap than two individuals from different groups.

2.3. Macrobenthic sampling

The availability of macrobenthic prey species on the mudflat was verified by taking sediment samples on the mudflat at the same time as the fish samples. Each month 12 sediment cores (22.4 cm^2) were taken to a depth of 10 cm. The samples were sieved over a 500 µm mesh and fixed in 7% formaline with rose Bengal to color the benthic species. Species were identified and counted under a stereomicroscope. Only Oligochaeta were not identified to species level. The lengths of *N. diversicolor* and *C. volutator* were determined similarly as in the stomachs. Differences in benthic prey abundance between sampling months and years was investigated by a one-way ANOVA after $\log_{10}(x + 1)$ transformation of the data.

3. Results

3.1. Fish abundance

A total of 25 fish species was caught over the whole sampling campaign, of which seven species accounted for 95% of the total catch (all listed in figure 3.3). *Platichthys flesus*, *Solea solea*, *Clupea harengus* and *Dicentrarchus labrax* were by far the most abundant and all but *S. solea* occurred in reasonable numbers throughout the year (Figure 3.3). *Osmerus eperlanus*, *Gymnocephalus cernuus* and *P. minutus* showed a strong seasonal trend, with *P. minutus* only being caught in October 2001. In 2001, the density of most species, except for seabass, was up to fourfold the fish density in 2002.

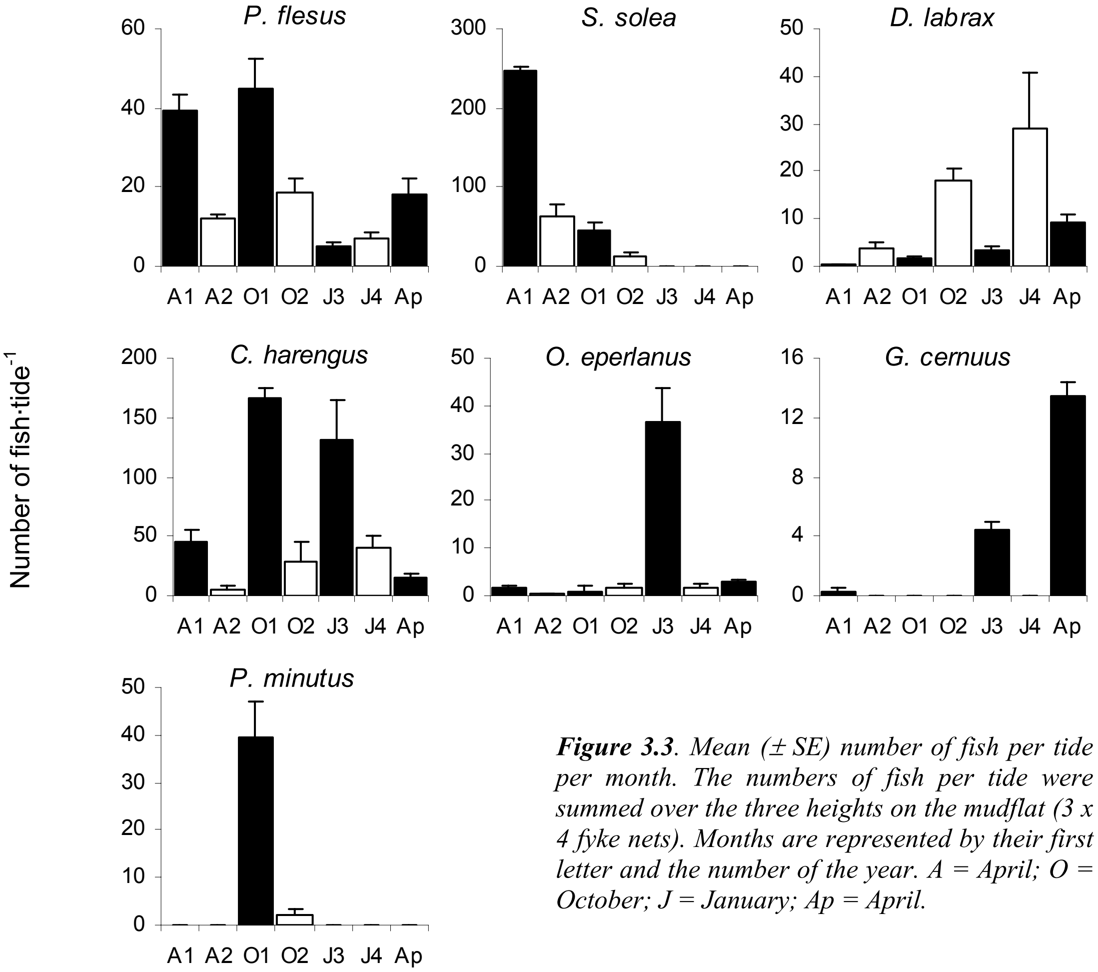

Figure 3.3. *Mean (± SE) number of fish per tide per month. The numbers of fish per tide were summed over the three heights on the mudflat (3 x 4 fyke nets). Months are represented by their first letter and the number of the year. A = April; O = October; J = January; Ap = April.*

The vast majority of species populations consisted of juveniles (mean lengths between 5 and 15 cm). Only the populations of flounder and pikeperch (*Sander lucioperca*) consisted of a significant fraction of adult specimens. A more detailed description of the abundance patterns of the intertidal fish community on the mudflat is given in chapter 2.

3.2. Macrobenthos

A complete description of the macrobenthic community is given in chapter 4; here we restrict our description to the three most dominant taxa. *Corophium volutator*, *Nereis diversicolor*, and oligochaetes made up 95% of the total number of organisms in the sediment. These taxa reach their maximal abundance in August and lowest abundance in April. In August, no difference was found between the years for *C. volutator* and *N. diversicolor*. However, in October 2002, more *C. volutator* ($F = 10.4$; $P < 0.01$) but less *N. diversicolor* ($F = 10.4$; $P < 0.01$) were found compared to October 2001. Also in January 2003 and 2004, the densities of *C. volutator* were significantly different ($F = 10.5$; $P < 0.01$; Figure 3.4).

For the oligochaetes, there were significant differences in density between years for August (F = 5.2; $P = 0.035$) and October (F = 22.7; $P < 0.01$).

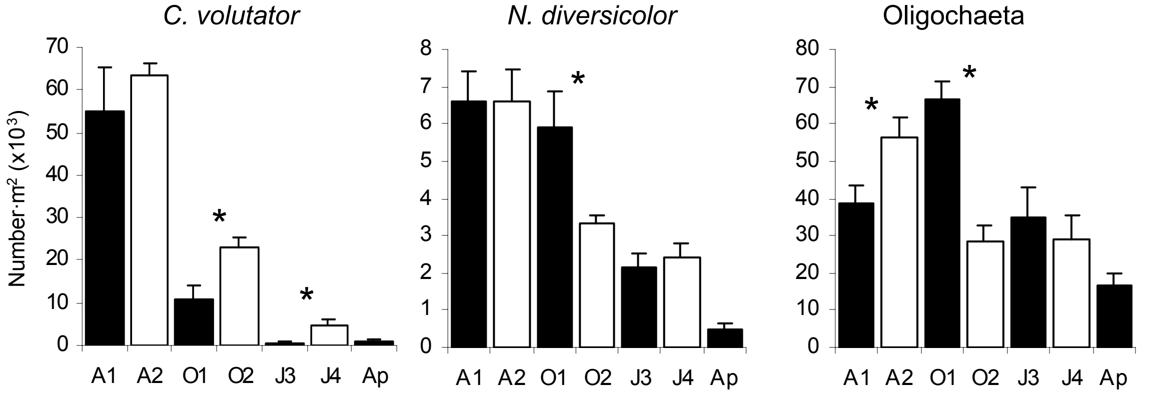

***Figure 3.4**. Mean (± SE) density of the three most abundant macrobenthic taxa on the mudflat. Months are represented by their first letter and the number of the year. A = April; O = October; J = January; Ap = April. Significant differences (one-way ANOVA: $P < 0.05$) between years are indicated with an asterisk.*

3.3. Seasonal diet composition

A total of 2562 stomachs of 22 species were analyzed of which 1718 (67%) were filled. The diets of the four dominant species on the mudflat, *P. flesus*, *C. harengus*, *S. solea* and *D. labrax*, were always included in the analysis. Some other species were seasonally abundant; they were included in the analysis for these months that they were common. Most of the stomachs and intestines of the freshwater species (*Abramis brama*, *Rutilus rutilus* and *Blicca bjoerkna*) were empty. Only in January, we caught some *A. brama* that had small amounts of *Corophium volutator*, *Nereis diversicolor* and zooplankton in their stomachs (17% full stomachs).

In total 24 different prey items were found. The amphipod *Corophium volutator* was the main prey item for most species and was found in 72% of all full stomachs, followed by *Nereis diversicolor* (23%), mysids (18%), copepods (14%) and *Crangon crangon* (12%) (Table 3.1).

In August of both years, *C. volutator* numerically and gravimetrically dominated the diet of *P. flesus* and *S. solea* (Appendix 3.1). It also accounted for 42% of the total number of prey items in *C. harengus*. The annelid *N. diversicolor* was only of significance in the diet of *S. solea* (16 W%). Mysids were important in the diets of *D. labrax*, *C. harengus* and *S. lucioperca*. The former almost exclusively fed on this species. *S. lucioperca* was the main piscivore: 78% of them had fish remains in their stomachs. Most of the prey fish that could be identified were gobies, but the digestion was already far advanced, suggesting that they had been eaten during the previous tide or during low water. No zooplankton could be found in the stomachs in August.

C. volutator remained important in the diet of most species in October. Mysids on the other hand were replaced by zooplankton (cladocerans and copepods) in the diet of *C. harengus* and by *C. crangon* in the diets of *D. labrax* and *S. lucioperca*. *P. minutus* was only caught in October and was feeding mainly on small *C. crangon* and *C. volutator*.

***Table 3.1**. Summary of all prey species identified in the stomach analysis, including their overall percentage occurrence in the non-empty stomachs (O%). The most important prey species are indicated in bold.*

Prey group	Prey species	**O%**
Annelida	***Nereis diversicolor***	23
	Heteromastus filiformis	2
	Oligochaeta	10
	Pygospio elegans	< 1%
Amphipoda	***Corophium volutator***	72
	Gammarus sp.	1
	Bathyporeia pilosa	4
Mysidacea		18
Cladocera		8
Copepoda		14
Decapoda	***Crangon crangon***	12
	Carcinus maenas	< 1%
Isopoda	*Cyathura carinata*	1
	Sphaeroma rugicauda	1
	Idotea sp.	< 1%
Mollusca	*Macoma baltica*	1
	Hydrobia ulvae	1
	Cerastoderma edule	< 1%
Ostracoda		< 1%
Teleostei (3%)	*Pomatoschistus sp.*	< 1%
	Clupeidae	< 1%
	Pleuronectidae	< 1%
	Unidentified teleosts	< 1%
Others	Terrest. Invert.	< 1%
	Plant material	< 1%

In January, *Osmerus eperlanus* and *Gymnocephalus cernuus* were found in reasonable numbers, but few had food in their stomachs (Appendix 3.1). *O. eperlanus* was feeding predominantly on zooplankton and mysids, whereas *C. volutator* was the dominant prey in the stomachs of *G. cernuus*. The highest densities of *C. harengus* were found in January, but only a minority of them had been eaten. Zooplankton was numerically the most important prey in their stomachs, but just as in October, *C. volutator* contributed significantly to the weight of the stomach content. *C. volutator* was also important in the diet of *P. flesus* and *D. labrax*. Gravimetrically however, *N. diversicolor* and Oligochaeta in particular, were more important for *P. flesus*. The relatively high contribution of teleost prey to the total weight of the stomach content of flounder was due to the presence of a single large flounder (32 cm) that had been feeding exclusively on fish. Again, the stomach content was too much digested to identify the fish species, suggesting that it was taken during low tide.

The fish density was the lowest in April, with *P. flesus*, *D. labrax*, *C. harengus* and *G. cernuus* the main predators on the mudflat. *D. labrax* fed on hyperbenthic mysids and *C. crangon*. *C. harengus* consumed mainly zooplankton, while *N. diversicolor* was an important supplementary food item. *N. diversicolor* was gravimetrically the most important prey species in *P. flesus*. The stomach contents of *G. cernuus* were dominated by *C. volutator*. Teleost fish were found in the stomachs of all fishes. However, while seabass mainly fed on gobies and herring, ruffe and flounder fed exclusively on newly settled flatfish.

From TWINSPAN, there appeared to be eight groups of species based on diet similarities (Figure 3.5). The first division separated pikeperch, which fed on teleosts, from the rest of the species. The second division can be considered as the most important one, splitting the species feeding on amphipods from the species with zooplankton and mysids in their stomachs. This group contained herring and smelt, while flatfishes and ruffe were assigned to the amphipod feeding group. Seabass was found in both clusters. Mysids were mainly responsible for the separation of the August samples, whereas annelids were indicator species for the stomach contents in January.

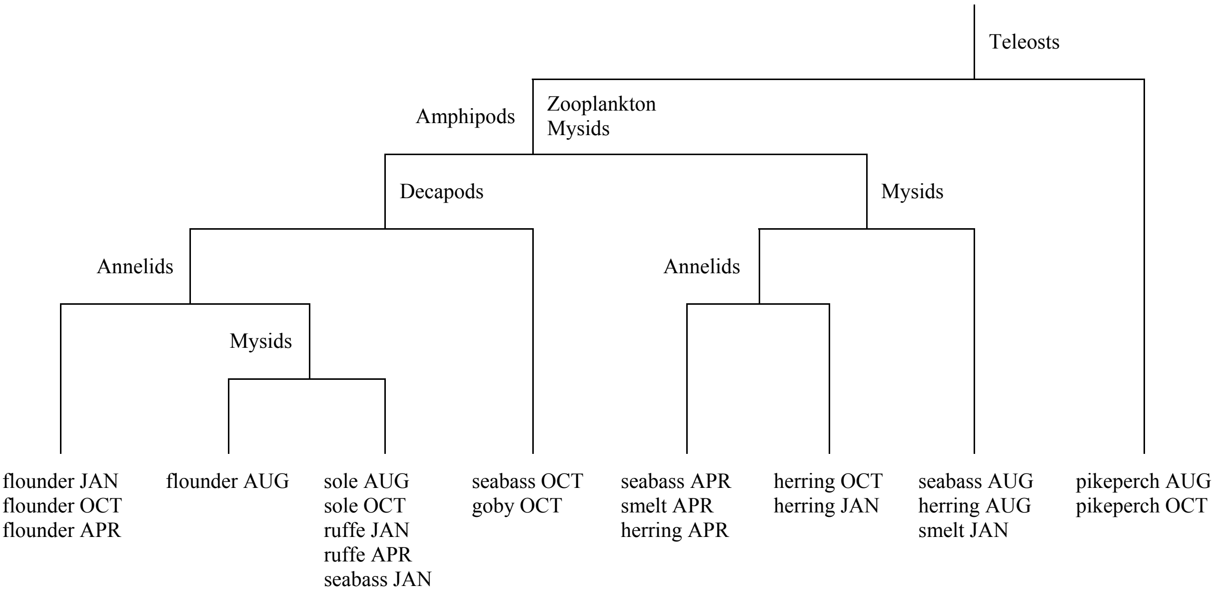

***Figure 3.5**. A dendrogram illustrating the diet similarities within the fish community on the mudflat as revealed by TWINSPAN. Indicator prey species are given for each group. Species are indicated by their common name and the first three letters of the month in which they were caught.*

3.4. Prey selectivity

The Chesson's alpha value was calculated for all macrobenthic species that were present in the sediment samples. For the sake of comparison, only the results of the four most important prey and predator species will be discussed.

On the mudflat, the densities of *C. volutator* and Oligochaeta were about equal in August and October, whereas in January and April oligochaetes were by far the most dominant group (Figure 3.4). In the stomachs on the other hand, *C. volutator* was always the main benthic prey item and Oligochaeta constituted only a small fraction of the diet. This selective feeding on *C. volutator* was also demonstrated by the significant positive selection for this prey (Table 3.2). The Chesson's alpha values for oligochaetes were always near zero, suggesting that the predator species avoided or could not utilize this prey species. The small fractions of oligochaetes in the stomachs in August and October might also suggest that in these months, they were taken as a kind of by-catch when feeding on *C. volutator*. Another important benthic prey species was *N. diversicolor*, which was recorded in relatively small amounts in the diets of sole and flounder throughout the study period.

Only in April 2003, the fish species seemed to have actively selected for *N. diversicolor* (Table 3.2). The occurrence of this species in the stomachs of herring indicates that *N. diversicolor* should have been present in the water column, where it is vulnerable to predation by more pelagic species. *Heteromastus filiformis* was found at low abundance in the environment and was virtually absent in the diet of the fish species.

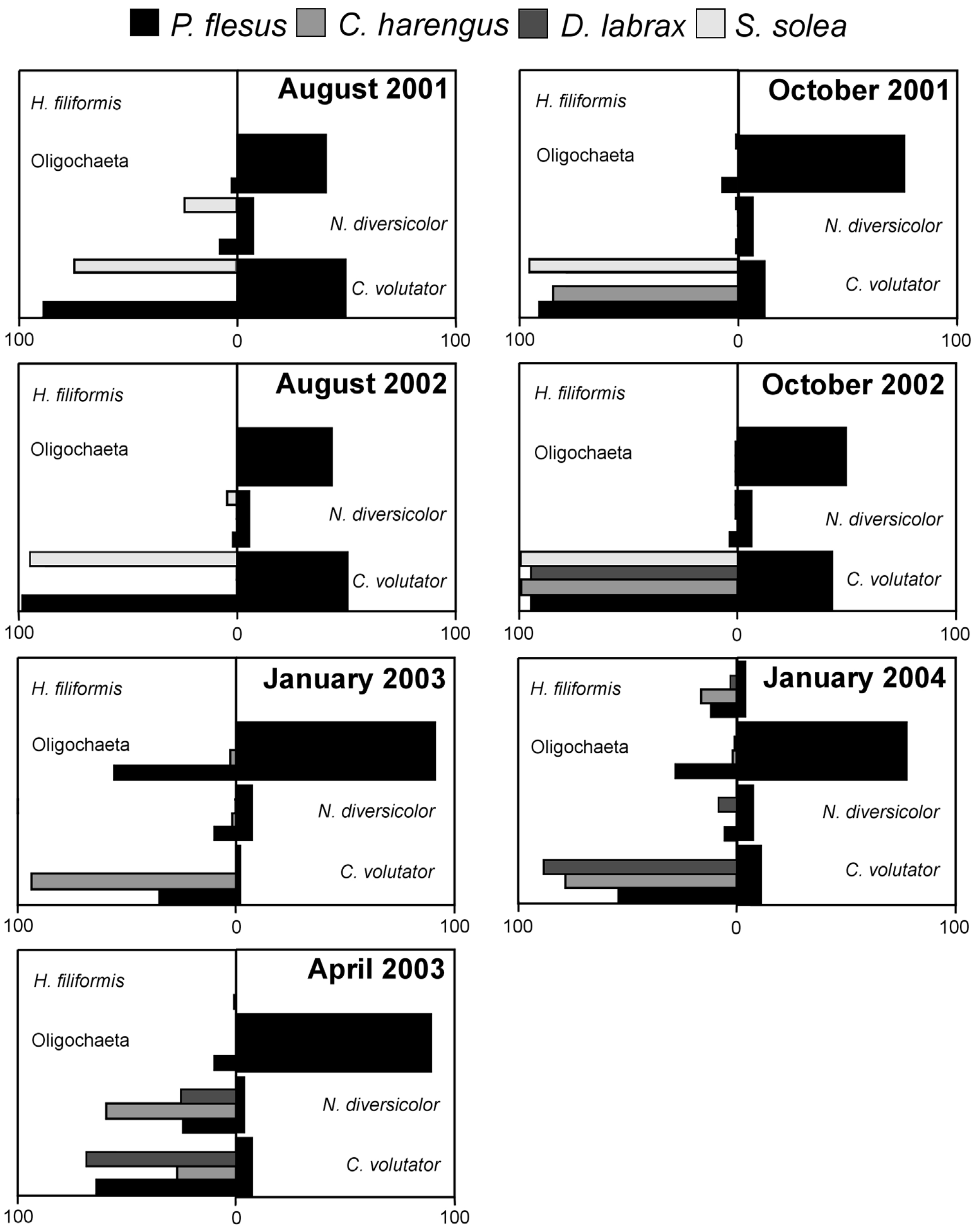

Figure 3.6. *Relative abundance (%) of the main prey items in the field (right side) and in the stomachs (left side) of the most common fish species in the different sampling months.*

Table 3.2. *Chesson's alpha value (α) for the data of figure 3.6. Negative values indicate a negative selection for that prey species. (°) Indicates that α - 1/m was not significantly different from 0 (no selection). (†) Indicates that no p-value could be calculated because none of the individuals was feeding on that prey (no variance).*

			C. volutator	***N. diversicolor***	**Oligochaeta**	***H. filiformis***
P. flesus	August	2001	0.67	0.04 °	-0.09	†
		2002	0.70	-0.02 °	-0.12	†
	October	2001	0.82	-0.12	-0.09	†
		2002	0.73	0.00 °	-0.12	†
	January	2003	0.74	-0.05	-0.06	†
		2004	0.37	-0.04 °	-0.07	0.15
	April	2003	0.47	0.25	-0.10	†
S. solea	August	2001	0.52	0.22	-0.12	†
		2002	0.71	0.02 °	†	†
	October	2001	0.85	-0.11	-0.12	†
		2002	0.85	-0.10	†	†
C. harengus	October	2001	0.87	†	-0.12	†
		2002	0.87	†	-0.12	†
	January	2003	0.84	-0.11	-0.11	†
		2004	0.66	†	-0.12	0.08 °
	April	2003	0.18 °	0.57	†	†
D. labrax	October	2002	0.84	-0.10	-0.12	†
	January	2004	0.72	-0.01 °	-0.12	-0.08 °
	April	2003	0.56	0.19 °	†	†

Prey size selection

The previous data suggests a preferential selection for *Corophium volutator* by most predator species. In order to examine whether this selection is also size specific, Chesson's alpha values were calculated for the different size classes of *C. volutator*. The selectivity indices were only calculated for the main benthivorous species flounder and sole.

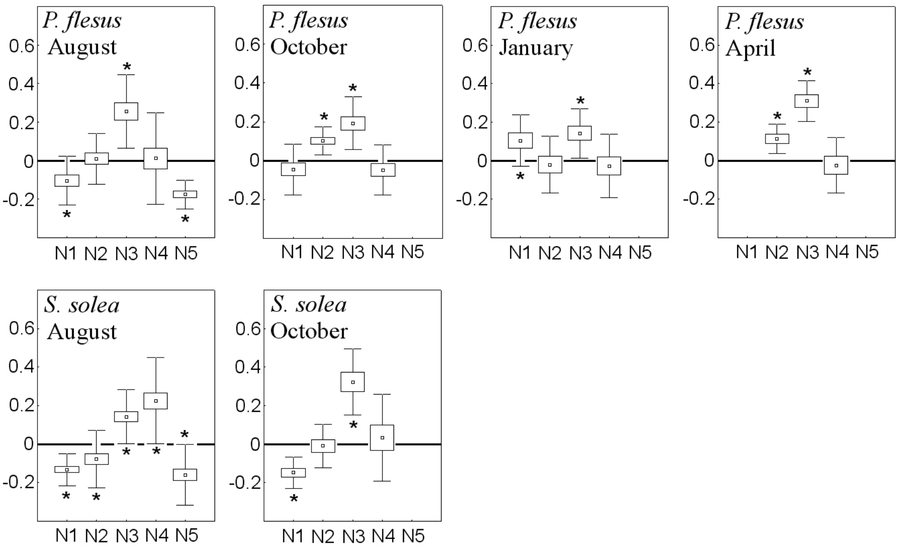

Figure 3.7. *Chesson's alpha values (α – 1/m) by length class of* C. volutator *(N1-N5) for August and October 2002, April 2003 and January 2004. Mean value, standard error (box) and standard deviation (whiskers) are given. The black line indicates the level of no selective feeding (α – 1/m = 0). Significant results of the one-sample T-test are marked with an asterisk (*P < 0.05*).*

Generally, sole and flounder selected the mid range size classes of *C. volutator* (N2-N4) by avoiding the smallest and largest ones (N1 and N5). In August, the selectivity index for size class four (6 –8 mm) was significantly different from zero for sole, but not for flounder. Furthermore, sole clearly avoided the two smallest length classes, although this was only the case for N1 in the diet of flounder in August. Hence, sole selected larger *C. volutator* compared to flounder. This was also confirmed by the results of the ANOVA, which showed that the mean length of *C. volutator* was larger in the stomachs of sole than in flounder (df = 1; F = 42.3; $P < 0.01$). The effect of the month and the interaction term (month*species) were not significant. The same trend was found for the median length of *N. diversicolor* in the stomachs, with larger worms eaten by sole compared to flounder (M-W U test; $P < 0.01$).

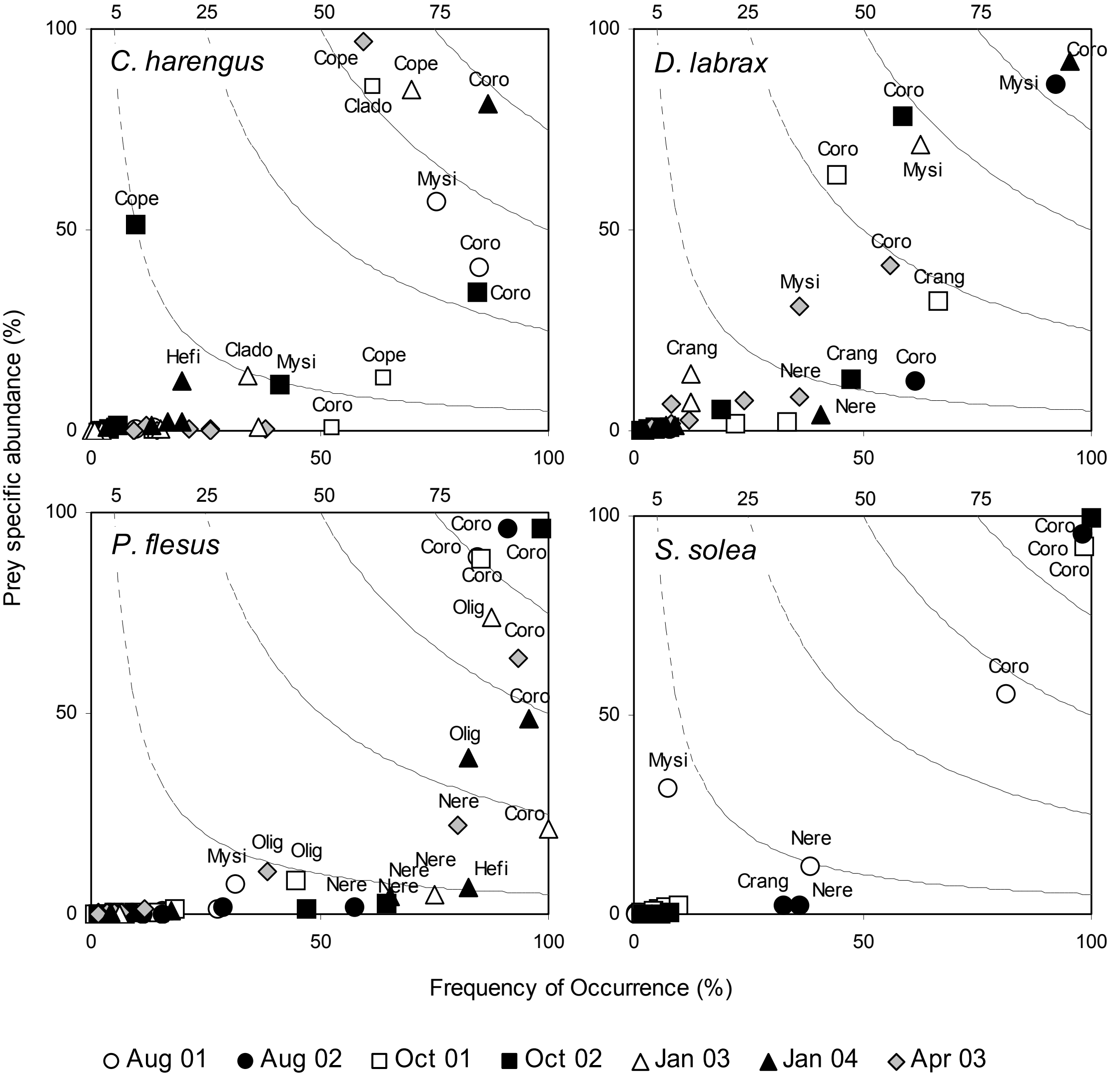

Figure 3.8. *Modified Costello graphs showing the feeding patterns of four species over several months. Isopleths (dashed lines) represent the overall abundance of prey species in the stomachs. The most important prey species are coded: Clado = cladocerans; Cope = copepods; Coro =* Corophium volutator*; Crang =* Crangon crangon*; Hefi =* Heteromastus filiformis*; Mysi = mysids; Nere =* Nereis diversicolor*; Olig = oligochaetes*

3.5. Feeding strategy and trophic interactions

The modified Costello graph (Amundsen *et al.,* 1996) allows to visually interpret the feeding strategies of the fish species. Again, only the four most important fishes were included in the analysis. The diets of all species were more or less characterized by a specialized feeding strategy as indicated by the occurrence of prey species in the upper right corner (Figure 3.8). However, for herring and seabass the dominance of a prey item in the diet differs drastically between the years and months. While *C. harengus* specializes on copepods in January 2003, it switches to *C. volutator* in 2004. *D. labrax* on its turn specializes alternately on mysids and *C. volutator*. Compared to the other species, the bulk of the dominant prey species of seabass lie more the centrally in the graph. This suggests a higher individual specialization and a more generalist feeding strategy compared to the other species.

Figure 3.8 shows a *Corophium*-specific diet for *P. flesus* and *S. solea*. Only in January and April, flounder adopts a more generalist feeding strategy. The presence of prey species in the lower right corner of the graph (high O% and moderate to low P%) indicates a high within phenotypic contribution to the niche width. This means that most individuals have a relatively large niche width and similar diets. In August 2001, when fish density was at its highest, sole seemed to adopt a more generalist feeding strategy as *C. volutator* became numerically less important in favor of mysids and *N. diversicolor*.

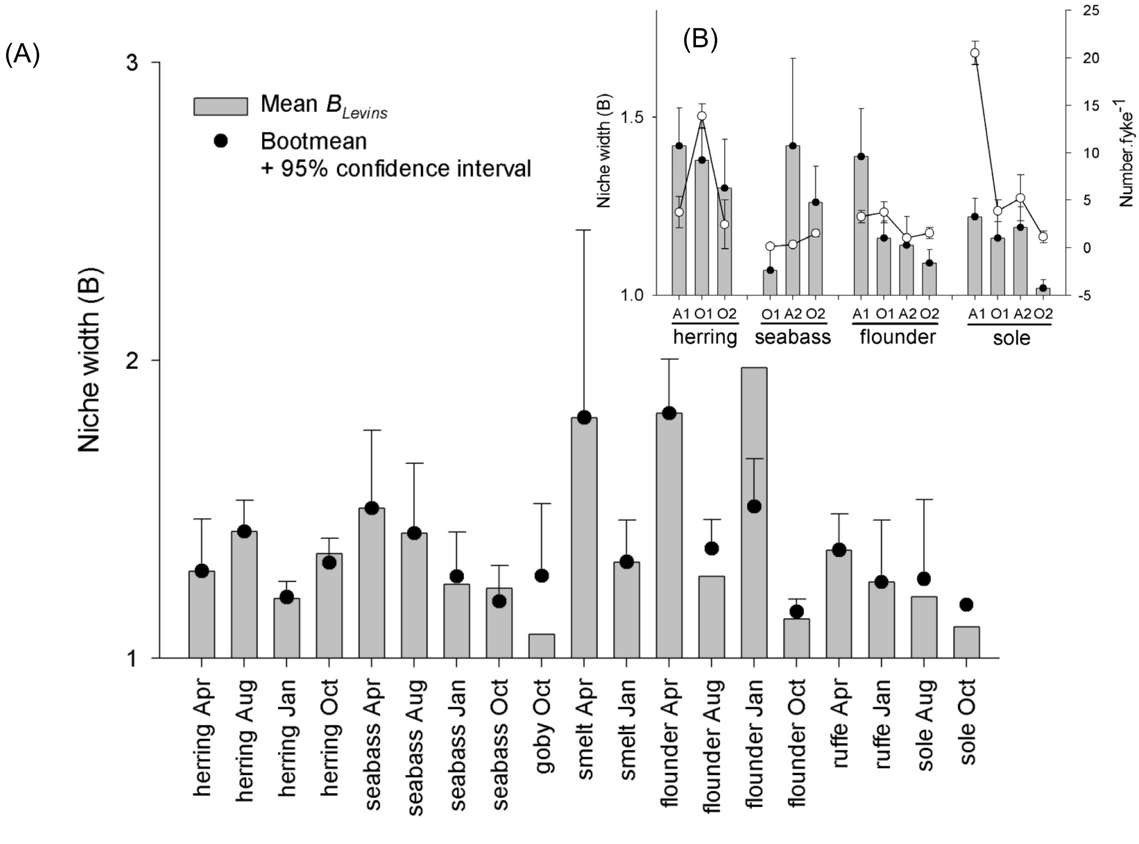

Figure 3.9. *(A) Niche width of fishes calculated by the Levins measure. Black dots and error bars are respectively the bootstrap estimator and the 95% confidence intervals. (B) Niche width for 2001 and 2002 separated. No data were available for herring in August 2002 and seabass in August 2001. The mean number of fish per fyke net (± SE) is indicated on the right Y-axis. Months are represented by their first letter and the number of the year.*

Differences in niche width were situated between periods (months and years) rather than between species. The absolute niches were quite narrow as was already suggested by the specialized feeding strategy (Figure 3.8). For most species, the niche width was largest in April and smallest in October (Figure 3.9 A). In addition, prey diversity in the stomachs seemed to be negatively correlated with population density, as the niche width was higher in years with higher fish density (Figure 3.9 B).

High values of dietary overlap were obtained among flounder, sole, herring and gobies in October and August. In August 2001, a biologically significant diet niche overlap (> 60% overlap) was found for the most abundant species, while this was not the case in 2002. A similar pattern was found for October, but here also *P. minutus* had a biologically significant overlap with seabass in 2002. However, the calculation of this overlap was based on the stomach content of only six gobies.

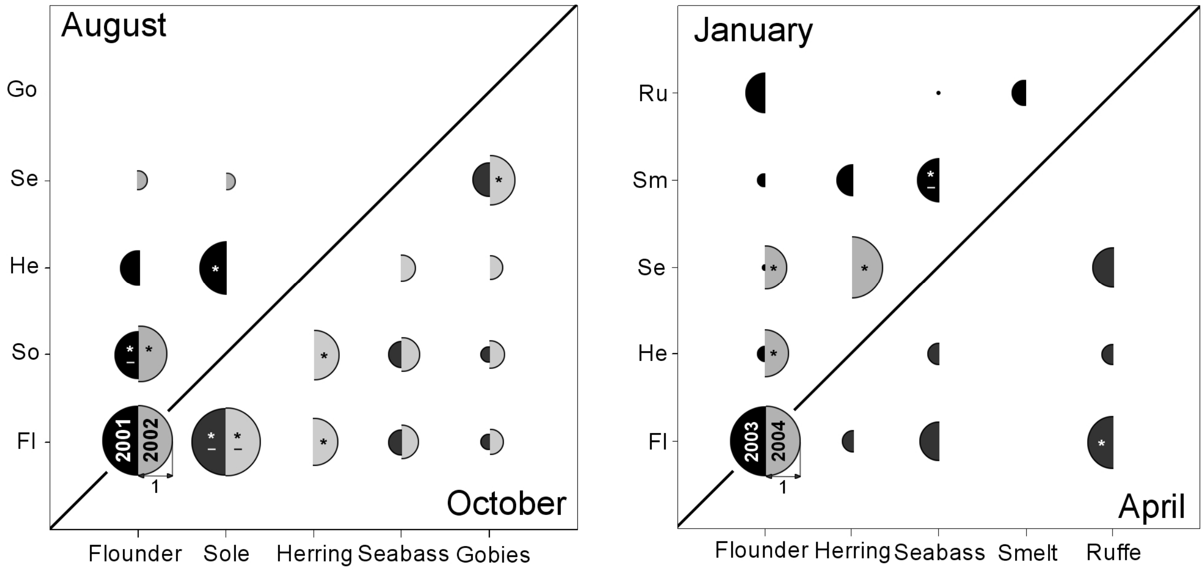

***Figure 3.10**. Niche overlap between the numerically most important species for the different sampling months. The bottom left scale indicates the maximum overlap. Niche overlaps > 60% are indicated with an asterisk. If the overlap is significant according to the Mantel test, the asterisk is underlined.*

While the niches of flounder, sole and seabass were clearly separated in January 2003, they had almost identical niches in 2004 when fish abundance was lower. In April 2003 the dietary overlap was low for most species except for flounder and ruffe, which were both feeding on *C. volutator*. The Mantel test statistics suggested that the within-species overlap was generally higher than the between-species overlap. Only the diets of flounder and sole in August and October and of smelt and seabass in January 2003 were not significantly different.

4. Discussion

Most studies on intertidal foraging focus on the foraging activities of flatfish as the main intertidal predators (Beyst *et al.,* 1999a; Cabral *et al.,* 2002; Summers, 1980; Andersen *et al.,* 2005a), few however consider the role of the other (demersal and pelagic) intertidal migrants. Here we describe the diet and feeding relations of the most abundant fish species on the mudflat. We show that prey selection is determined by prey availability, confirming the generalist and opportunistic feeding strategy of estuarine fishes. An opportunistic feeding strategy results in an increasing population diet breadth with increasing population density, which reduces the potential for competition. Niche overlap was generally high between species and in particular between flatfishes in summer. However, direct competition for food was probably avoided by spatial niche separation and resource partitioning at the level of prey size.

Over the whole sampling period, we found a relatively low diet diversity, with *C. volutator* as an important food source for most species. All fish species were feeding to a more or lesser extent on this prey. *C. volutator* is a main food source for wading birds, crustaceans and fishes on mudflats of the northern Atlantic Ocean (Pihl, 1985; Wilson and Parker, 1996; Hilton *et al.,* 2002; McCurdy *et al.,* 2005). In the Scheldt estuary the density of *C. volutator* is low in winter and spring, increases from June onwards and reached the highest values in summer. Numbers start to drop again in September (Chapter 4; Ysebaert and Herman, 2002). The importance of *C. volutator* in the diet of the fishes matched its density pattern on the mudflat. Similar observations were made elsewhere (Summers, 1980; Vinagre *et al.,* 2005; Hampel *et al.,* 2004; Andersen *et al.,* 2005a). The fact that *C. volutator* was also found in reasonable numbers in the stomachs of a pelagic species like herring, further illustrates its role as a key energy source for the higher trophic levels on the mudflat.

When the densities of benthic prey items in the field were compared with their occurrence in the fish stomachs, it was obvious that *C. volutator* was overrepresented in the stomachs of all predators. This may indicate that either fish actively select for *C. volutator* or *C. volutator* is more vulnerable to predation. While other benthic prey like *N. diversicolor*, *H. filiformis* and oligochaetes rarely emerge from the sediment, adult *C. volutator* regularly venture into the water column, where they are more susceptible to demersal feeding fish (Essink *et al.,* 1989; McCurdy *et al.,* 2005). Cattrijsse *et al.* (1993) also reported *C. volutator* as an important member of the hyperbenthic community in the Westerscheldt. These tidal excursions are sex-biased as males disperse much more often in the water column, resulting in a higher predation risk (Lawrie and Raffaelli, 1998; McCurdy *et al.,* 2005). In our study, males (> 5 mm) were present in the field in very low numbers in August and couldn't be detected in the other months. This could be partially related to the difficulty of identifying males smaller than 5 mm. However, in April males were found in the stomachs of flounder and ruffe. This indeed might be related to an increased reproductive activity of males searching for mates, given the fact that the reproductive season may start as early as the beginning of May (Wilson and Parker, 1996).

Besides benthic prey, also zooplankton, mysids and *C. crangon* were seasonally important in the diet of visitors on the mudflat. *C. harengus* is a zooplanktivore whose main prey are copepods in this part of the estuary (Hostens and Mees, 1999; Maes *et al.,* 2003). The copepod community in the Scheldt estuary is dominated by *Eurytemora affinis* in spring and by *Acartia tonsa* in late summer (Soetaert and Van Rijswijk, 1993; Tackx *et al.,* 2004). In our study, copepods were numerically the dominant prey in the diet of herring, but gravimetrically, *C. volutator* and *N. diversicolor* were almost equally important. Surprisingly, zooplankton was virtually absent in the diet of herring in August, when it was feeding on *C. volutator* and mysids. Although we have no data on the intertidal availability of zooplankton, it seems likely that its density in the area was very low.

Mesopodopsis slabberi and *Neomysis integer*, the two main mysid species in the brackish zone of the Scheldt estuary, reach their maximal abundance in summer (Mees *et al.*, 1993). In the present study, mysids were mainly eaten by seabass and herring in August. Hostens and Mees (1999) reported the importance of mysids (mainly *N. integer*) in the diet of the majority of fishes caught in the subtidal of the estuary. The former suggests that mysids are particularly important for fast moving epibenthic predators in the subtidal and represent a supplementary food item in the intertidal. *Crangon crangon* reaches its highest densities in the brackish zone of the Scheldt estuary in summer and autumn (Hostens, 2000). This coincides with the higher abundance of shrimps in the diet of seabass, gobies and pikeperch. Most of the *C. crangon* in the stomachs of species other than seabass were juvenile (< 1 cm). In the study of Maes *et al.* (2003), shrimps were only important in the diet of seabass. The broader importance of this species as prey in the intertidal guild may simply reflect its availability.

The present study clearly demonstrates the opportunistic feeding of the fishes visiting the mudflat. The diet of most species largely reflects the seasonal composition of the prey community in the brackish part of the estuary. Fish may eat the most suitable prey available within the size range they can ingest (Moore and Moore, 1976; Elliott *et al.,* 2002; Link *et al.,* 2005). The high abundance and availability of prey such as *C. volutator* stimulate a specialized feeding behaviour on these specific prey species.

Previous studies on the trophic structure of the fish community of the Scheldt estuary concluded that the feeding guild mainly forages on the hyperbenthos and zooplankton (Hostens and Mees, 1999; Maes *et al.,* 2003). On the other hand, from our perspective, the fish community mainly depends on benthic prey organisms. Although the pelagic component was probably underestimated by the choice of our sampling gear (fyke nets), we believe our results give a reliable estimate of the importance of the benthic food chain for the estuarine fish community. Intertidal mudflats are crucial feeding grounds for juvenile fishes and flatfishes in particular. By their sheltered nature, they support a high benthic productivity of readily available prey. It might however be clear that the above mentioned studies are biased to some extent by the choice of locality and sampling strategy. Consequently they suffer from a one-sided perspective on the feeding guild of the estuary. As such, there is a need to quantify the importance of the various food webs in the estuary and the energy exchange between them.

One of the central aspects of estuarine nursery areas is the low abundance of piscivorous predation, which is explained by the high turbidity and shallow character of the habitat (Blaber and Blaber, 1980; Baker and Sheaves, 2005). For the majority of the fish species we almost exclusively caught juvenile fishes (Chapter 2), which is in agreement with the hypothesis that the brackish part of the estuary functions as a nursery for young marine fishes (Maes *et al.,* 1998a; Hostens, 2000; Beck *et al.,* 2001; Able *et al.,* 2005). The main piscivore in our study was *S. lucioperca*, mainly feeding on small gobies and herring. Flatfishes occurred in the diet of *G. cernuus* in April. Most of the prey fishes, except for the flatfishes, were already digested, suggesting that they were consumed in the subtidal zone during low water. The fishes that migrated onto the mudflat were probably too big to be consumed by the piscivores present and as such were not dependent on intertidal migration to escape from predation. Gobies could have been an exception considering their size, but they were only present in October on the mudflat. They stayed in the lower intertidal zone where they still would have been vulnerable to predation. The former suggests that piscivorous predation on the mudflat is of minor importance. However, the present study doesn't give a decisive answer about the refuge hypothesis of intertidal migration (Baker and Sheaves, 2005). Therefore, the intertidal and subtidal area should be simultaneously sampled for the presence of piscivores.

To return to the first question of our objectives, it was shown that, according to their feeding dependence on the intertidal zone, the intertidal fishes could be divided into two groups. A first group includes the flatfishes that are obviously dependent on the benthic prey available on the mudflat. They are bound to intertidal migration and these areas are crucial for their growth and survival. The other group consists of facultative visitors of the mudflat, such as herring and seabass. These species take advantage of the intertidal feeding opportunities as some benthic organisms emerge from the sediment and disperse in the water column.

The fish community was sampled over two years, with the density of most fish species being considerably higher in 2001 compared to 2002. This allowed us to examine the effect of predator density on inter- and intraspecific feeding interactions (second question). The higher fish densities in 2001 were not correlated with higher benthic prey density. If prey availability wasn't higher, interference between species with similar diets must have been increased. If resources are limited, competition may act as a strong structuring factor in the fish community (Pianka, 1981; Le Mao, 1986; Piet *et al.,* 1998; Munday *et al.,* 2001). Considering the seasonal availability of prey species and the trophic characteristics of the fish species, trophic interference between the fishes of the intertidal community may be an issue in the second part of the year. The low density of benthivorous predators and the reduced metabolic activity associated with the colder water temperature may suggest that competition is less crucial in January and April. Although the benthic biomass was much lower in winter and early spring, it is not clear whether the decreased foraging activity was caused by limited prey availability or by temperature-dependent metabolic constraints (Fonds *et al.,* 1992).

For all species, the niche width was larger when resources were less available or the population density was highest. A generalist and opportunistic way of feeding reduces the potential for competition resulting in an increasing population diet breadth with increasing population density (Svanbäck and Persson, 2004). Lower values of niche width were observed when fish abundance was lower and fish species were specializing on *C. volutator*. This is in agreement with the hypothesis of MacArthur and Pianka, (1966) that species adopt a specialized feeding strategy when food is abundant, resulting in a reduced niche width (Andersen *et al.,* 2005a).

The niche overlap was strongest between flounder and sole in August and October, when they were both heavily preying on *C. volutator*. Reports of high dietary overlaps between flatfish species are common (Piet *et al.,* 1998; Beyst *et al.,* 1999a; Cabral *et al.,* 2002; Vinagre *et al.,* 2005). Hereby the absence of competition is usually explained in terms of spatial and/or temporal segregation. In chapter 2, it was demonstrated that flounder migrated relatively higher on shore in 2001 when the abundance of most species was significantly higher than in 2002. Competition with *S. solea* may force flounder to move up further on the shore to exploit the available food sources. Further evidence for enhanced competition on the lower and middle shore can be obtained from the density pattern of *C. volutator* in 2001 and 2002 (Chapter 4). In August 2001 and 2002, the density of *C. volutator* on the upper and middle shore was about equal. In October 2001 however, numbers dropped dramatically on the middle shore and stayed high on the upper shore, while they remained high at both heights in 2002. This might suggest that food limitation may occur in years with a high fish density, resulting in spatial segregation of fishes on the mudflat. A similar observation was made for plaice (*Pleuronectes platessa*) and dab (*Limanda limanda*). For these species, the utilization of shallow water by juvenile plaice is explained in function of the avoidance of competition with dab which stays in deeper water (Harlay *et al.,* 2001; Gibson *et al.,* 2002). Resource partitioning may also occur at the level of prey size, but this has rarely been quantified among coexisting fish species (Elliott *et al.,* 2002). Our study demonstrated the existence of differential prey size selection, as flounder fed on smaller *C. volutator* and *N. diversicolor* than sole. Apparently sole selected larger prey than flounder, irrespectively of the size of the individual. This can be due to differences in gape morphology or hunting strategies (Moore and Moore, 1976; Piet *et al.,* 1998). It is not clear whether this size selective strategy is adopted to reduce interspecific competition or is the result of foraging behaviour. In addition, high dietary overlap was also found between flatfishes and other fishes. However, it seems less likely that these relatively high overlap values resulted in active competitive interactions. The availability of alternative pelagic and hyperbenthic prey items should have allowed species as herring, seabass and gobies to switch easily to those prey types. Furthermore, the densities of seabass correlated negatively with other species on the mudflat. Overall species abundance peaked in 2001 and 2003, whereas seabass reached it highest densities in October 2002 and January 2004, hence avoiding competition.

Central in the discussion on competition and resource partitioning on the mudflat remains the question whether resources are limiting. The period of maximum diet similarity in our study coincided with peak abundances of wading birds on the brackish water mudflats in the estuary (Ysebaert *et al.,* 2000). Waders feed on the same benthic prey (e.g. *C. volutator* and *N. diversicolor*) and may have further increased pressure on the macrobenthic prey population. Most studies on this topic state that estuarine fish populations remain below the carrying capacity of the system, thereby avoiding strong negative competitive interactions (Thiel *et al.,* 1996; Elliott *et al.,* 2002). Although suspected, as indicated by high dietary overlap, competition in our study will probably have been minimized by the superabundance of benthic and hyperbenthic prey on the mudflat. However, it remains unclear how the predator and prey populations in the estuary are mutually affected and to what extent the higher trophic levels are dependent of the intertidal benthic production.

As pressure on the estuary will probably increase because of dyke building and dredging, a further decrease of the low dynamic area (mudflats and shallow water) is expected (Meire *et al.,* 2005). A reduction of the intertidal area may reduce the carrying capacity of the estuary, as they provide important feeding grounds for fishes and wading birds (McLusky *et al.,* 1992). Therefore, the combined effects of fish, crustacean and bird predation on the benthic community require special attention.

Acknowledgements

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Appendix 3.1

Diet composition of the most important fish species, given as percentage occurrence (FO%), numerical percentage (N%) and weight percentage (W%). The number of stomachs analyzed and the percentage non-empty stomachs are indicated between brackets under each species name (N / %). Horizontal dashes indicate that the corresponding index was not determined.

***Table A3.1**. August 2001 and 2002.*

	P. flesus			*S. solea*			*D. labrax*			*C. harengus*			*S. lucioperca*		
	(100/96)			(382/91)			(13/100)			(81/94)			(13/96)		
	FO%	N%	W%	FO%	N%	W%	FO%	N%	W%	FO%	N%	W%	FO%	N%	W%
C. volutator	88	88	83	88	94	83	62	4	3	86	42	-			
N. diversicolor	42	2	2	38	4	16	8	<1%	<1%						
Oligochaeta	14	1	<1%	1	<1%	<1%									
Mysidacea	30	<1%	1	7	<1%	<1%	92	96	97	74	48	-	33	41	-
Zooplankton										3	<1%	-			
M. baltica	5	<1%	1	1	<1%	<1%									
C. crangon	16	9	13	14	1	1	8	<1%	<1%	4	<1%	-	11	6	-
Teleosts	5	<1%	<1%							1	<1%	-	78	53	-
C. carinata							8	<1%	<1%						
B. pilosa				1	<1%	<1%				13	1	-			
Others	1	-	<1%							4	9	-	8	-	-

***Table A3.2**. October 2001 and 2002.*

	P. flesus			*S. solea*			*D. labrax*			*C. harengus*			*P. minutus*			*S. lucioperca*		
	(193/95)			(136/91)			(82/88)			(229/66)			(48/67)			(10/90)		
	FO%	N%	W%	FO%	N%	W%	FO%	N%	W%	FO%	N%	W%	FO%	N%	W%	FO%	N%	W%
C. volutator	90	76	75	99	93	89	57	14	7	63	1	36	22	35	3			
N. diversicolor	33	2	1	6	1	3	7	<1%	<1%	0								
Oligochaeta	46	14	12	3	1	1	1	<1%	<1%	1	<1%	<1%						
Mysidacea	3	1	2	4	2	<1%	17	<1%	<1%	23	<1%	5	16	15	<1%	11	<1%	<1%
Zooplankton				1	<1%	6				51	99	56						
M. baltica	2	<1%	1															
C. crangon	14	5	6	3	<1%	<1%	50	82	87	1	<1%	2	56	41	63	22	33	25
Teleosts				1	<1%	<1%	4	<1%	<1%				3	2	25	78	67	75
C. carinata	1	<1%	<1%				1	<1%	<1%									
Sphaeroma sp.	1	<1%	2				7	<1%	5				3	2	<1%			
B. pilosa	6	1	<1%	6	1	1				9	<1%	1						
Others	2	-	1	6	-	<1%	1	-	<1%	1	-	<1%	6	-	6			

Table A3.3. *January 2003 and 2004.*

	P. flesus (48/85)			*D. labrax* (77/77)			*C. harengus* (593/35)			*O. eperlanus* (153/33)			*G. cernuus* (18/50)		
	FO%	N%	W%	FO%	N%	W%	FO%	N%	W%	FO%	N%	W%	FO%	N%	W%
C. volutator	98	40	16	81	68	55	46	4	30	16	3	4	89	68	-
N. diversicolor	68	7	19	36	7	19	0	<1%	4	4	1	13	44	7	-
Oligochaeta	83	46	32	7	2	4	3	<1%	2	6	2	7			
Mysidacea	2	<1%	5	19	17	7	14	<1%	9	68	18	50			
H. filiformis	49	4	7	8	1	3	6	<1%	3						
Zooplankton		<1%	4		<1%	<1%	58	95	48	30	74	17	33	25	-
M. baltica	10	1	<1%												
C. crangon	2	2	4	5	<1%	<1%				2	<1%	<1%			
Teleosts	2	<1%	12	2	5	12				10	1	8			
C. carinata	2	<1%	<1%												
B. pilosa				2	<1%	<1%	2	<1%	<1%	2	<1%	<1%			
Others							3	-	4						

Table A3.4. *April 2003.*

	P. flesus (67/90)			*D. labrax* (33/76)			*C. harengus* (55/76)			*G. cernuus* (49/82)		
	FO%	N%	W%	FO%	N%	W%	FO%	N%	W%	FO%	N%	W%
C. volutator	93	60	35	56	18	14	21	<1%	5	85	68	59
N. diversicolor	80	25	49	36	8	17	38	1	30	23	2	2
Oligochaeta	38	9	<1%									
Mysidacea	2	<1%	<1%	36	37	21	26	<1%	5	10	<1%	<1%
H. filiformis	3	<1%	<1%									
Zooplankton							60	98	49	8	<1%	<1%
M. baltica	2	<1%	<1%									
C. crangon	12	1	<1%	24	28	36	26	<1%	2	28	17	18
Teleosts	2	1	2	12	2	5	14	<1%	9	10	13	21
C. carinata	7	4	2	8	4	4						
Sphaeroma sp.	2	<1%	<1%	4	1	2						
B. pilosa				8	1	1	10	1	<1%	5	<1%	<1%
Others		-	12				2	-	<1%			

CHAPTER 4

Direct and indirect effects of predation on an intertidal benthic community

4

Maarten Stevens, Els Cuveliers, Joachim Maes and Frans Ollevier

Abstract

Intertidal estuarine habitats are extensively used as feeding grounds by birds, fishes and crustaceans. The impact of predation on the intertidal macrobenthic community has been measurable, although not consistently. A macrobenthos monitoring study at various tidal heights on a mudflat in the Scheldt estuary revealed that when fish abundance was high, the density of the main prey species (*Corophium volutator*) was significantly reduced on the lower and middle shore, but not on the higher shore. On the lower parts of the shore, where the inundation time is longer, fish predation may reduce the density of macrobenthic prey. The effect of predation on the intertidal macrobenthic community was examined by excluding both birds and fishes from the mudflat. A first caging experiment lasted for two months and excluded both birds and fishes, while a second experiment ran for a whole year and excluded only birds. Fish and bird predation did not have a significant direct effect on the abundance of macrobenthic species. Diet analysis showed that both predators select the larger size classes of the macrobenthic species, but only birds were shown to influence the size distribution of their prey. From the exclosure experiments, fish predation turned out to be relatively unimportant as a structuring factor for the macrobenthic prey community. However, fish abundance during the caging experiments was much lower than during the monitoring study. The effects of short-term experiments may only be noticeable at higher predator densities. In the long-term experiment, the density of *C. volutator* in the cages was significantly lower than in the uncaged plots. In the absence of predation, infaunal interactions like competition may become more important and regulate the benthic community structure.

Keywords: *Exclosure; Corophium volutator; Nereis diversicolor; Flatfish; Infaunal interactions; Shorebirds*

Unpublished manuscript

1. Introduction

Intertidal mudflats are highly productive habitats in the estuarine environment. The high secondary production provides abundant food resources for estuarine fish and macrocrustaceans at high tide. At low tide, they are important foraging grounds for waterfowl (Luo *et al.,* 2001; McLusky and Elliott, 2004). Given the intense use of the intertidal resources, the question arises whether the benthic resources are limited and whether the carrying capacity of the system for both epibenthos and birds is reached (Yates *et al.,* 1996). Consequently, the effect of predators on the benthic community has received a lot of attention over the past decades. The outcome of the different studies on the structuring effect of predation in soft-bottom communities seems to vary from study to study, probably reflecting temporal and spatial differences in predator feeding pressure (Raffaelli and Hawkins, 1996). Shorebirds may deplete the densities of certain benthic prey (Boates and Smith, 1979; Baird and Milne, 1981; Azovsky *et al.,* 1999). Other studies however, suggest only a marginal effect of bird predation on the infaunal abundance (Raffaelli and Milne, 1987; van der Meer, 2001; Hiddink *et al.,* 2002). Also for epibenthic predators the results are somewhat different, with some studies indicating significant effects of fish and crustacean predation (Virnstein, 1977; Gee *et al.,* 1985; Azovsky *et al.,* 1999; Hiddink *et al.,* 2002) and others finding little or no effects (Gee *et al.,* 1985; Raffaellli and Milne, 1987; Raffaelli *et al.,* 1989; Hall *et al.,* 1990b). Furthermore, bird and epibenthic predators may influence the zonation (Hiddink *et al.,* 2002), depth distribution (Esselink and Zwarts, 1989; Quijón and Jaramillo, 1996), life history (Hilton *et al.,* 2002) and size structure (Raffaelli and Milne, 1987; Wilson, 1989) of the prey population.

To identify the role of predators in structuring soft-bottom communities, different techniques have been applied, ranging from long-term observations (van der Meer *et al.,* 2001), over modelling prey production and energetic demands of the predators (Hall *et al.,* 1990a), stomach content analysis (McCurdy *et al.,* 2005), meta-analysis (Raffaelli and Möller, 2000) and field experiments (Thrush, 1999; Raffaelli and Möller, 2000). Among those methods, the exclusion or enclosure of suspected predators is generally the most often applied. Although this technique has its limitations, care should be taken in the design and interpretation of the results (Virnstein, 1978; Hall *et al.,* 1990a; Fernandes *et al.,* 1999; Raffaelli and Möller, 2000). It remains a powerful method to examine the importance of predation as a structuring factor of benthic communities.

The aim of this study was to estimate the impact of predation on the intertidal macrobenthic community and to differentiate herein between the effects by birds and fishes. A former study on the diet of the fish community on a mudflat in the mesohaline zone of the Scheldt estuary (Figure 4.1) indicated that *Corophium volutator* and *Nereis diversicolor* were the main prey items (Chapter 3). During summer, large numbers of flounder (*Platichthys flesus*) and common sole (*Solea solea*) migrate onto the mudflat and feed almost exclusively on *C. volutator*. The mudflats in the mesohaline zone of the estuary are also important feeding areas for large numbers of waders and shelducks (*Tadorna tadorna*) (Van den Bergh *et al.,* 2005). This might suggest that predation acts as a structuring factor for the macrobenthic community. This was tested with both field surveys and exclosure experiments. In the first part we describe the seasonal and spatial (zonation and depth distribution) abundance patterns of the infaunal community. We hypothesized that:

(a) The effects of bird predation on the macrobenthos should be more pronounced on the upper mudflat (shorter inundation time), whereas the longer inundation time on the lower parts should favor fish predation.

(b) The depth distribution of the infaunal species should be related to predation pressure, as animals in the top few centimeters of the mud are more vulnerable to predation.

In the second part, the predation hypothesis is investigated using two exclosure experiments. The results were compared with the diet of the common fish (Chapter 3) and bird predators on the mudflat.

2. Materials and methods

2.1. Study site

Samples were taken on an intertidal mudflat in the mesohaline zone of the Scheldt estuary, near the Dutch-Belgian border (Figure 4.1). The mudflat is about 450 m wide, has a gentle slope and consists of mud (< 63 µm) and muddy sand (63-125 µm). Near the low water level, there is a fringe of sandy sediment (mean grain size: 175 µm). The tidal range near the Dutch-Belgian border varies from 6 m (spring tide) to 4 m (neap tide). The water temperature near the Dutch-Belgian border during the study period (2001-2004) was lowest in January 2002 (4.5 °C) and highest in August 2003 (25 °C) (http://www.waterbase.nl).

2.2. Seasonal composition of the macrobenthic community

The macrobenthic community was sampled monthly from March until November 2001 at three different heights on the mudflat (Figure 4.1C). No samples could be taken in September. The samples on the lower shore were taken in the sandy zone. At each location, four replicates were taken with a corer (Ø 5.3 cm) to a depth of 10 cm. Each sample was subdivided in two parts: the top layer (0-2 cm) and the remaining section (2-10 cm). All samples were preserved in buffered formalin (7 %) and stained with Rose Bengal for sorting.

2.3. Exclosure experiments

Two experiments were conducted in a homogenous area at mid-tidal level (Figure 4.1C). The first experiment tested for the effects of both bird and fish predation on the macrobenthic community. It started on 3 July 2003 and ran until 3 September 2003, when a storm, which is unusual at that time of the year, ended the experiment. Four different treatments were established and each treatment was replicated three times: an exclosure which excluded both fish and birds (EBF), an exclosure which excluded only birds (EB), a control area (C) and a cage control to test for cage effects (CC) (Figure 4.2). The cages enclosed an area of 1 m^2 and were 30 cm high. The fish and bird exclosures

(EBF) consisted of four wooden corner posts with a wooden frame on top and covered with a 1 cm square mesh. For the bird exclosures (EB) we used the same wooden frame, but covered it with horizontal strings spared at 10 cm interval. This allowed fish to move in and out the exclosure. The cage control (CC) was used to test for any cage effect on sediment characteristics in the treatment (EBF) that excluded both fish and birds. They were identical to the fish and bird exclosures, but two sides were removed. This allowed free access of epibenthic predators, while mimicking the effects of the cages on the environment (Wilson, 1991). The control cages were positioned with their fenced sides facing the direction of the ebb and flood currents in order to maximize sedimentation effects. The control area consisted of four stakes only. The cages were arranged in a completely randomized design within a grid of 100 m by 50 m.

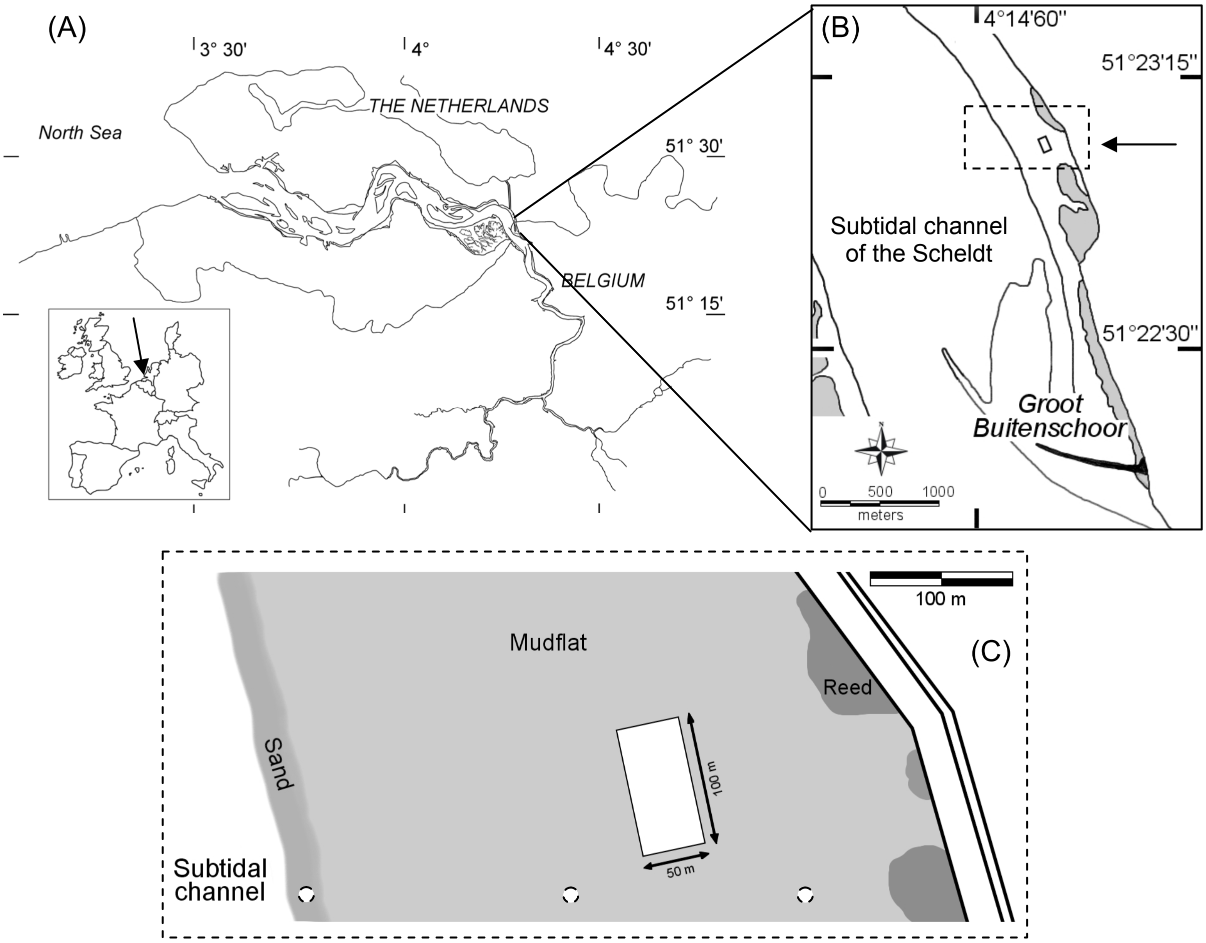

Figure 4.1. *(A-B-C) Maps of the study area on the right bank of the Scheldt estuary. (C) The white rectangle in the middle of the mudflat (50m x 100m) represents the experimental area in which the 12 cages were placed. The white dots represent the monthly sampling locations for the temporal analysis.*

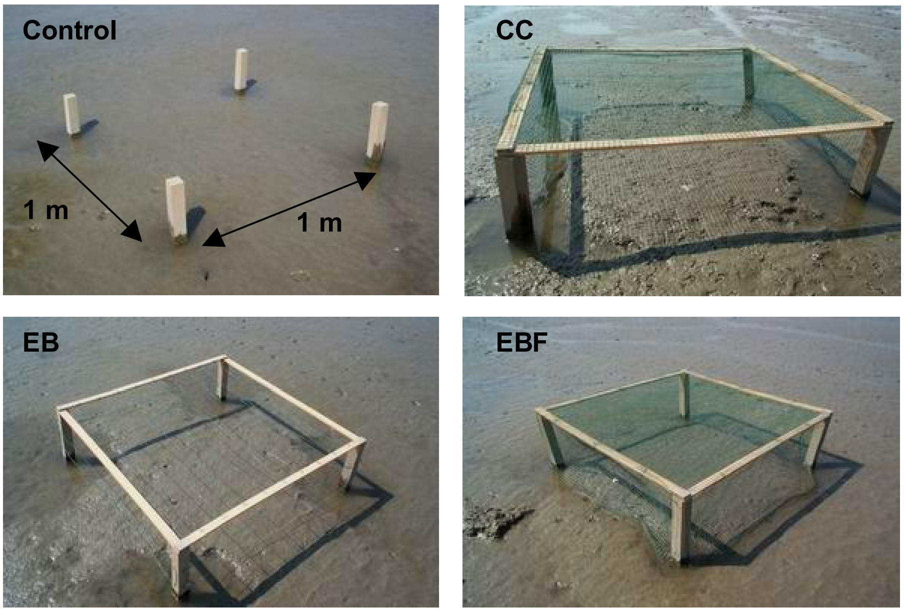

***Figure 4.2**. Design of the exclosures for the first exclosure experiment. Codes are as defined in the text under 2.3.*

The second experiment tested only for the effect of bird predation and ran from 9 October 2003 until 13 October 2004. The treatments (control and bird exclosure) were arranged in a randomized block design and replicated four times. A block consisted of a cage (EB) and a control (C). The construction of the exclosure cage was identical to the EB treatment in the first experiment. At the beginning of both experiments, the macrobenthos was sampled outside the plots to determine the initial densities on the mudflat. Treatments were only sampled at the end of the experiment to avoid sampling effects. In each cage 10 samples were taken randomly with a corer (Ø 5.3 cm) to a depth of 10 cm, avoiding the area within 20 cm of the cage perimeter. All samples were fixed in buffered formalin and coloured with Rose Bengal dye. In the first experiment, an additional core sample was taken from a randomly chosen position in each cage for sediment analysis.

2.3. Laboratory analysis

Faunal cores were sieved over a 500 μm mesh and stored in 70% alcohol. In the first experiment, the 10 cores were pooled per cage, whereas in the second experiment, they were kept separately to increase the power of the analysis. All macrobenthic organisms were identified to species level, except for nematodes and oligochaetes, and counted.

The lengths of *N. diversicolor* and *C. volutator* were only determined in the experiments. *N. diversicolor* was measured to the nearest mm by stretching it along a ruler. If the animal was incomplete, the width of the 10th segment was measured using the ruler of a stereomicroscope and converted to total length using the regression given in Esselink and Zwarts (1989):

$$\text{Total length (cm)} = 1.67 \times \text{width (mm)}^{1.48}$$

Shrinking of worms through preservation in formalin was corrected by multiplying the length by 1.47 (Esselink and Zwarts, 1989). Individual *C. volutator* were measured and assigned to one of five size classes: 0-2 mm, 2-4 mm, 4-6 mm, 6-8 mm and > 8 mm. Sediment samples were wet sieved to determine mud content.

2.4. Predator abundance and diet

During the experiment, the abundance and diet of the fishes and birds on the mudflat were monitored in order to obtain an idea of the predator selection of macrobenthic species. Fishes were sampled in August 2003 during two consecutive tides using two fyke nets. The fykes were set up below the experimental area, their mouth facing the riverbank and emptied at each low tide. The fish were anaesthetized and preserved in 7% formalin. In the laboratory, each fish was identified, weighted and the stomach removed for further analysis. Prey items were identified and counted. The lengths of *N. diversicolor* and *C. volutator* were determined the same way as they were in the sediment samples. A complete description of the method of stomach analysis is given in chapter 3. A proxy for seasonal trends in the abundance of fishes on the mudflat for the period 2002-2004 was obtained using a permanent sampling station. A fyke net was mounted near the low water level and emptied twice a week. All fish were identified, counted and the catch converted to numbers per fyke per day (Figure 4.7).

Birds were counted in July and August at low water, when foraging on the mudflat. Long-term data (1997-2003) of the monthly mean numbers of shorebirds on a nearby mudflat (Groot Buitenschoor) were provided by the NGO Natuurpunt (http://www.schorrenwerkgroep.be). In August, droppings of shelduck (*Tadorna tadorna*) were collected for faecal analysis. Shelduck was selected because it was by far the most dominant shorebird on the mudflat (Figure 4.7). The pellets were sieved over a 50 µm mesh, prey remains were identified as much as possible and counted. Digested *C. volutator* could still be recognized and were counted by their telsons. The number of *N. diversicolor* in the pellets was calculated from the number of pharyngeal jaws divided by two. To determine the total length of fragmented *C. volutator* in the faeces, a correlation was established between the width of the second antenna (near the process of article 4) and total body length (tip rostrum to end of telson). For the calculation of the regression, *C. volutator* were collected from the mud and sexed, based on sexually dimorphic characters (Appendix 4.1; Hayward and Ryland, 1990). As only animals > 4 mm could be sexed, smaller individuals were classified as juveniles. The largest individuals in the field tend to be female. Very large males are rarely observed, possibly because of their crawling behaviour and consequent higher predation risk (Fish and Mills, 1979). However, the second antenna of males is significantly larger than that of females of the same body size (Barbeau and Grecian, 2003; figure 4.3). Because of this sexual dimorphism, separate regressions were made for males and females. Juveniles were included in the calculation of both regression equations. Small sized *C. volutator* (< 2 mm) in the shelduck pellets were intact and could be easily measured with a ruler. For the larger *C. volutator*, fragmented second antennas of the same size were paired and the width of the antenna near the process (peduncle article 4) was measured (± 1 µm; Figure

4.3). The width of distinct male antennas was converted to total body length (mm) using equation 2. The length of sexually indistinct and juvenile (width < 0.4 mm) individuals was determined using equation 1.

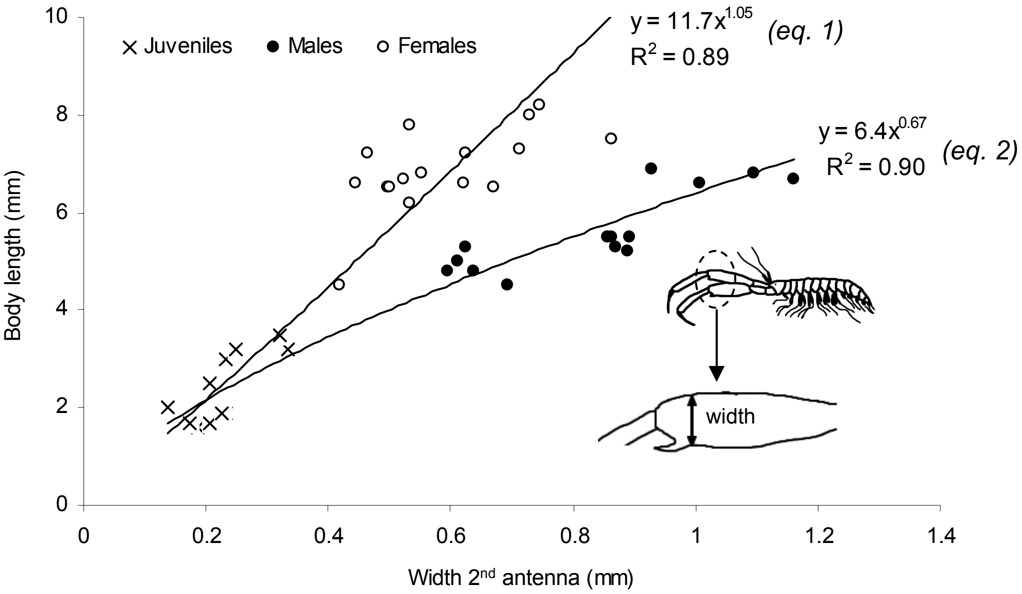

Figure 4.3. *Correlation between the width of the* 2^{nd} *antenna (mm) of* C. volutator *and the total body length (mm). The regression equation was calculated for males (N = 14; equation 2) and females (N = 15; equation 1) and is presented next to the trend line. Juveniles (N = 12) were included in the calculation of both the male and female regression equation.*

The pharyngeal jaws of *N. diversicolor* in the faeces were easily identifiable. The size of each jaw was measured and converted into the total length using the regression of Zwarts and Esselink (1989):

Body length (cm) = 9.15 x jaw (mm) $^{1.54}$

2.5. Data analysis

For each sampling month and experimental treatment, the abundance, the number of species, the Shannon-Wiener diversity index (H) and the equitability (J) were calculated. Macrobenthic abundance data were $\log_e$ transformed prior to analysis.

In the first experiment, differences in abundance between treatments were analysed with a one-way ANOVA. The mud content of the treatments was compared using a Kruskal-Wallis ANOVA. In the second experiment, we tested for the effects of bird predation on the macrobenthic abundance, species richness and diversity in a mixed-model ANOVA using PROC MIXED in SAS 9.1 (Verbeke and Molenberghs, 1997; SAS Institute, 2004). In the model, the block effect and the interaction between block and treatment were included as random factors. The mixed model can be written as

$$Y_{ij} = \mu + \alpha_i + B_j + (\alpha B)_{ij} + \varepsilon_{ij}$$

where Y_{ij} is the expected response, μ the population mean, α_i is the fixed treatment effect (control/cage), B_j (block) and the interaction term $(\alpha B)_{ij}$ are the random effects, ε_{ij} the error component and i and j represent respectively the i^{th} and j^{th} subgroup of the factors α and B.

The effect of the experimental treatment on the length distribution of *C. volutator* and *N. diversicolor* was tested with a log-linear analysis of the length-frequency tables using STATISTICA 6.0 (StatSoft, Inc., 2001). A basic method for analysing data involves crosstabulation, in which the major results of interest can be summarized in a multi-way frequency table, that is, in a crosstabulation table with two or more factors (size class and treatment). Log-linear analysis allows to test the different factors that are used in the crosstabulation and their interactions for statistical significance. The term log-linear derives from the fact that one can, through logarithmic transformations, restate the problem of analysing multi-way frequency tables in terms that are very similar to ANOVA. Specifically, one may think of the multi-way frequency table to reflect various main effects and interaction effects that add together in a linear fashion to bring about the observed table of frequencies. The model for a two-way contingency table is given as

$$\ln Y_{ij} = \mu + \alpha_i + \beta_i + \alpha\beta_{ij}$$

where Y_{ij} is the expected frequency in row i and column j, μ is the mean of the logarithms of the expected frequencies, α_i and β_i are the effects of categories i and j of factors A (e.g. size class) and B (e.g. treatment: C, EBF, EB, Fish and Birds) respectively, and the $\alpha\beta_{ij}$ interaction term expresses the dependence of category i of factor A on category j of factor B and *vice versa* (Sokal and Rohlf, 1995). Deviations of the observed frequencies from the expected frequencies were evaluated via a Pearson χ^2-test. Because a total of 10 pairwise tests was done for the first experiment, we applied a sequential Bonferroni correction to reduce the probability of Type I errors (Rice, 1989). An additional Kruskal-Wallis ANOVA (first experiment) and mixed model analysis (second experiment) was applied to test for differences in the mean length of *N. diversicolor*.

3. Results

3.1. Seasonal composition and zonation of the prey populations

A total of thirteen taxa were found during the sampling campaign in 2001, of which *C. volutator, N. diversicolor* and oligochaetes were numerically the most abundant taxa. Throughout the year, they constituted together almost 95% of the total number of organisms. Oligochaetes were not identified to species level, but Seys *et al.* (1999b) found three species on the mudflat of Groot Buitenschoor. Species richness was highest in summer and autumn and decreased towards the end of the year (Figure 4.4). The species patterns on the middle and upper shore were similar, whereas the lower shore (sandy area) was clearly impoverished in species number. The increased species richness in November in this zone was due to the single occurrence of a few species and returned an increased diversity. The Shannon-Wiener diversity (H) followed the same pattern as the species richness and was highest in late summer. The increased diversity in April was caused by a reduction in the abundance of oligochaetes. In October and November, the diversity decreased on the middle shore, while it stayed high on the upper shore. Because of the lowered density of *C. volutator* on the middle shore, the dominance of oligochaetes was more pronounced here, resulting in a lower diversity (Figure 4.4).

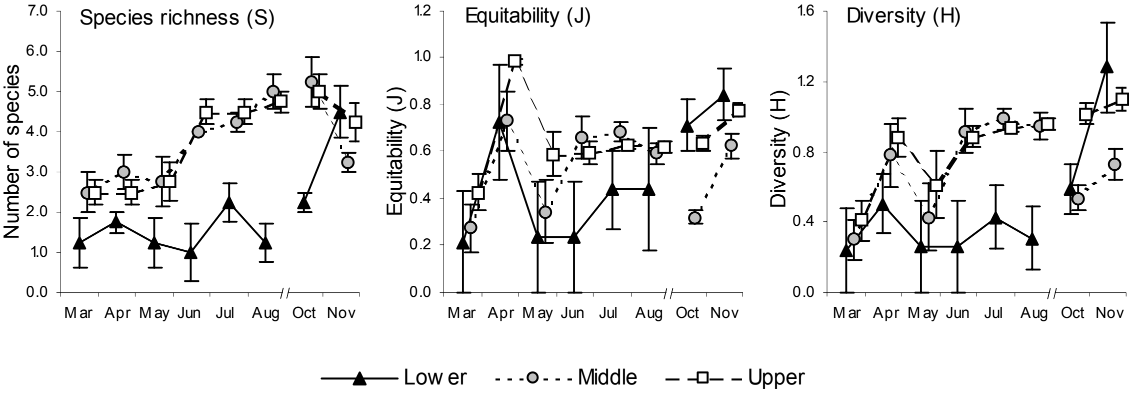

***Figure 4.4**. Seasonal and spatial variation (± S.E.) in the number of species (S), equitability (J) and the Shannon-Wiener diversity index (H) on the mudflat in 2001.*

Most species showed a clear seasonal pattern. Generally, numbers were low in winter and early spring and increased in early summer. Species reached their maximal abundance in August (*C. volutator*: 63000 m^{-2}; *N. diversicolor*: 6600 m^{-2}; Oligochaeta: 56000 m^{-2}) and densities decreased towards winter. *Manayunkia aestuarina* was only abundant in the samples of the second part of the year and reached peak abundances of 16000 individuals per square meter in October, after which numbers dropped rapidly (Figure 4.5).

For most species the abundances on the middle and the upper shore were similar. However, for *C. volutator* and to a lesser degree also for *N. diversicolor*, densities dropped in October on the middle shore whilst staying more or less the same on the upper shore (Figure 4.5). In August and October 2002 the middle and upper segment of the mudflat was also sampled, but the densities at both heights didn't differ a lot.

The density of *C. volutator* was always higher in the top 2 cm of the sediment (Figure 4.6). In October, the numbers decreased in both the top layer and the deeper layer on the middle shore. On the upper shore however, the decrease in density in the top layer was compensated by an increase in the lower layer, suggesting that *C. volutator* buried deeper in the sediment. The density of *N. diversicolor* was higher in the upper sediment layer in the summer months, but this pattern reversed in autumn, when the majority of polychaetes was found deeper in the mud (Figure 4.6).

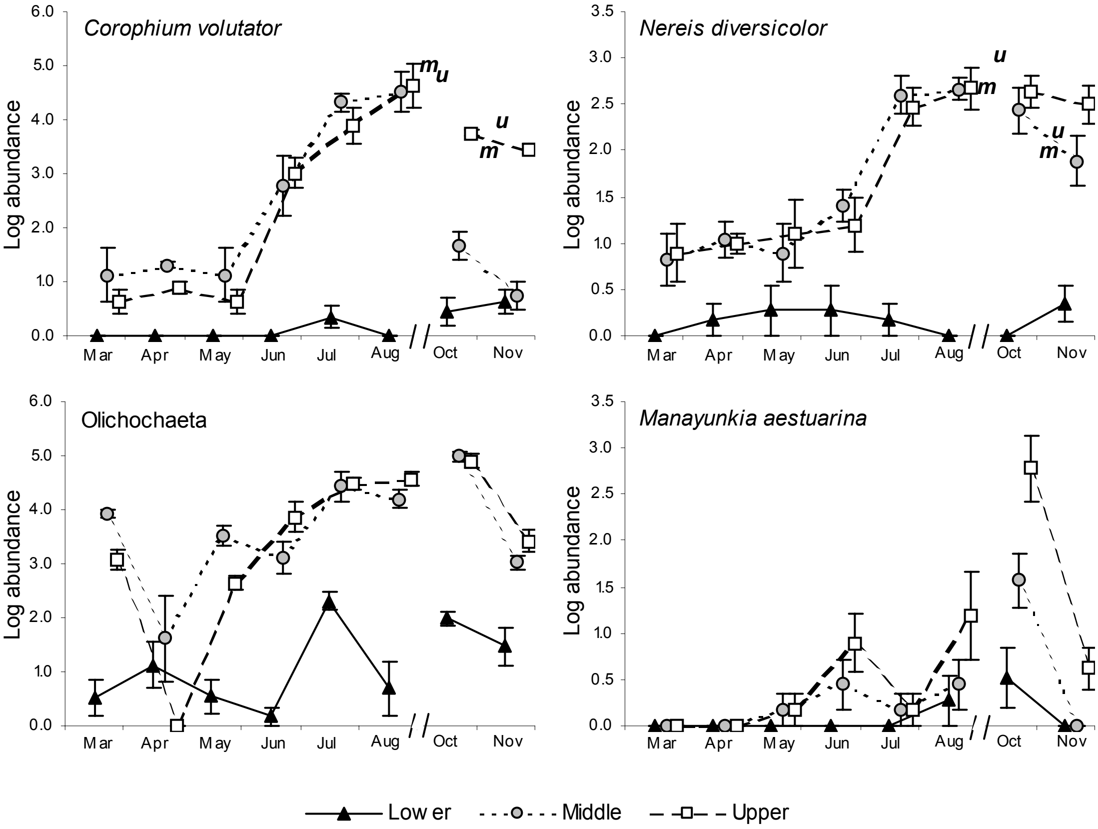

Figure 4.5. Spatio-temporal patterns in the densities (± S.E.) of the four most common macrobenthic species in 2001. No samples were taken in September. The density of C. volutator *and* N. diversicolor *in August and October 2002 is marked with the first letter of the mudflat segment (m = middle shore, u = upper shore). Abundance data (number per core) were log*$_e$ *transformed prior to analysis.*

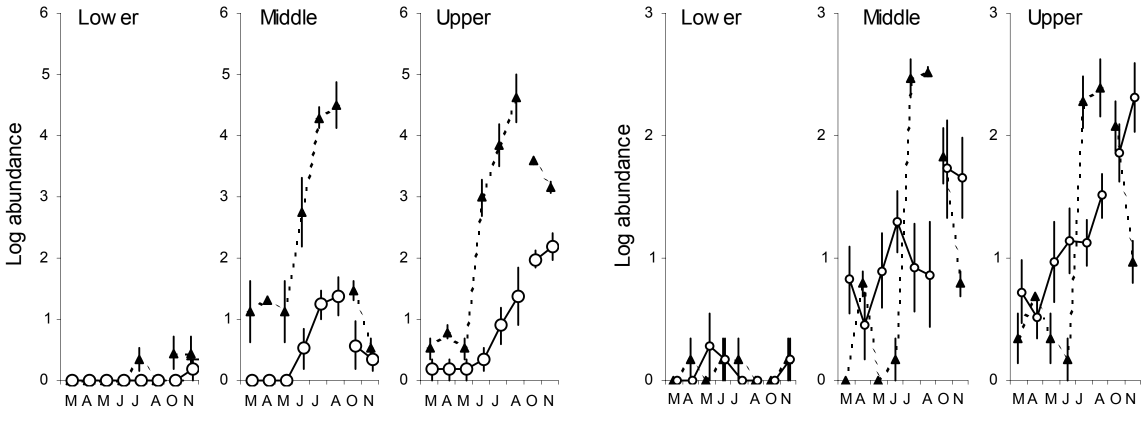

Figure 4.6. Seasonal depth distribution (log$_e$ *abundance ± S.E.) of* C. volutator *(left) and* N. diversicolor *(right) in the sediment by shore levels. Months are represented by their first letter. Black triangle = 0-2 cm layer; open circle = 2-10 cm layer. Abundance data (number per layer) were log*$_e$ *transformed prior to analysis.*

3.2. Density and stomach contents of predators

During the experiment in July and August 2003, the shorebird community on the mudflat was dominated by shelducks (*Tadorna tadorna*), which made up 60% of the total number of birds present. In summer also avocet (*Recurvirostra avosetta*), curlew (*Numenius arquata*) and grey plover (*Pluvialis squatarola*) represented a significant proportion of the avian community on the mudflat (Figure 4.7).

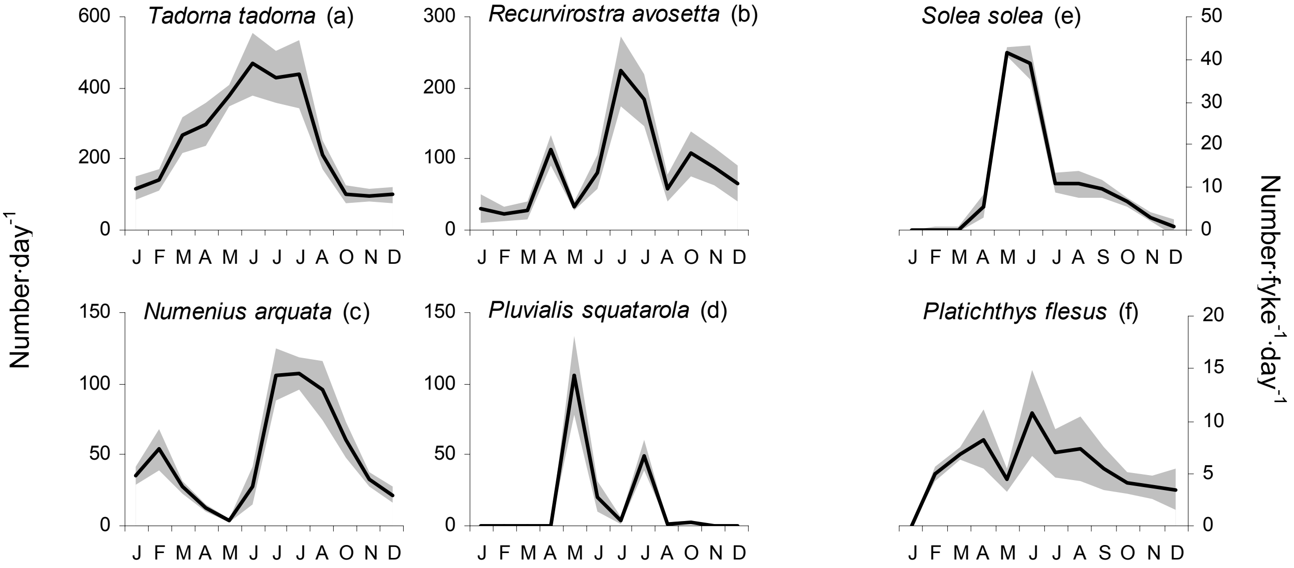

Figure 4.7. The averaged monthly abundance (birds: 1997-2003; fishes: 2002-2004) of the most common birds (a-d) and flatfishes (e-f) on the mudflat of Groot Buitenschoor in the mesohaline zone of the Scheldt estuary (Figure 4.1). The black line represents the mean abundance and the shaded zone the standard error.

Only three dietary items could be recognized in the faecal bird pellets. *C. volutator* was the most abundant prey (66%), followed by the mudsnail *Hydrobia ulvae* (29%) and *N. diversicolor* (5%). A large fraction of the smaller *C. volutator* was still intact, but the larger specimens could only be recognized by the presence of the second antenna. Of *N. diversicolor* only the jaws could be detected and these were used to estimate the total body length. Shelduck seemed to select smaller *C. volutator* and larger *N. diversicolor* (Figure 4.9; Table 4.2). It is possible however, that we underestimated the juvenile fraction of *N. diversicolor* in the diet of shelduck because their jaws were not retained on the 50 µm sieve.

Flatfishes dominated the intertidal fish community in the mesohaline zone of the estuary in summer (Figure 4.7e and 4.7f; Chapter 2). In August 2001, the fish abundance on the mudflat was up to four times higher compared to 2002 (Chapter 2). *Solea solea* (66%) and *Platichthys flesus* (18%) accounted together for 83% of the total catch in August; they fed on infaunal prey. *C. volutator* dominated the diet of both species and *N. diversicolor* represented an important supplementary food item (Chapter 3). In contrast to shelducks, the fishes selected larger *C. volutator*, whereas the length distribution of *N. diversicolor* in the stomachs wasn't significantly different from that in the field (Table 4.2; Figure 4.9).

3.3. Experiment 1

There was no evidence of silting up in the exclosures. A slight decrease in the mud percentage was observed in the cages that excluded both fishes and birds, but the differences weren't statistically significant (H = 2.79; $P = 0.42$). The mud percentage of the cage control treatment (CC) was similar to the exclosure treatments. Although bird footprints were found in one of the partial cages, the lack of any difference from the other exclosures indicates that these control cages may have functioned as complete exclosures. Given the uncertainty about the functioning of the cage controls as predator exclosures, the results of these partial cages will not be considered for further analysis.

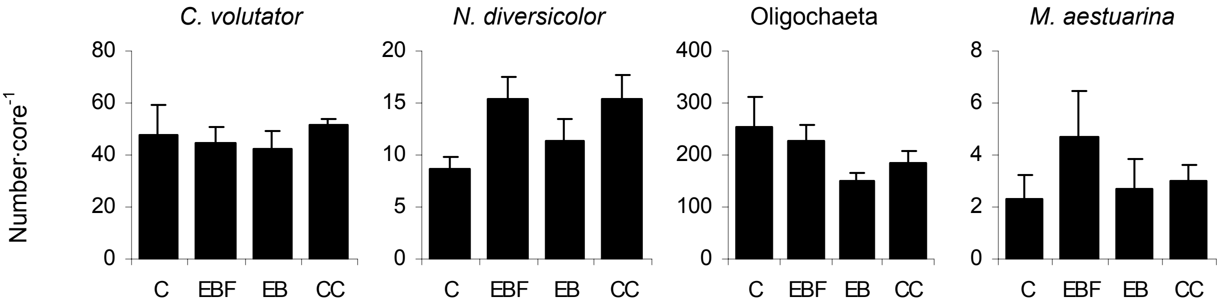

***Figure 4.8**. Mean abundance (± S.E.) of the four most common macrobenthic taxa in the different treatments of experiment 1.*

There were no statistically significant differences in the densities of macrobenthic species between all treatments (Figure 4.8; Table 4.1). Diversity was significantly lower in the control compared to the treatments.

***Table 4.1**. Experiment 1. Results of the one-way ANOVA for differences in abundance and diversity measures between treatments. Only the results for the most abundant species are given. Significant differences between treatments are listed in bold.*

	df	F-ratio	*P*
Species richness	3	1.296	0.341
Diversity (H)	3	6.933	**0.011**
Eveness (J)	3	3.263	0.075
C. volutator	3	0.250	0.859
N. diversicolor	3	2.690	0.117
Oligochaeta	3	1.810	0.223
M. aestuarina	3	0.759	0.548

The length of *C. volutator* in the treatments and in the diets of the predators was compared with a log-linear analysis of frequency tables. *C. volutator* was larger in the exclosure treatments compared to the control, but no statistically significant difference could be found among the various types of exclosures (Table 4.2). Although not confirmed by the pellet analysis, the former suggests that the combined predation on *C. volutator* by birds and fishes favoured larger individuals.

The mean length of *N. diversicolor* was slightly larger in the control treatment than in the predator-free exclosures. However, the Kruskal-Wallis ANOVA test showed no significant difference in mean length between the treatments (H = 7.6; p = 0.055). Also figure 4.9 suggests that smaller worms could be expected in the exclosures, but again, this was not supported by the results of the log-linear analysis (Table 4.2).

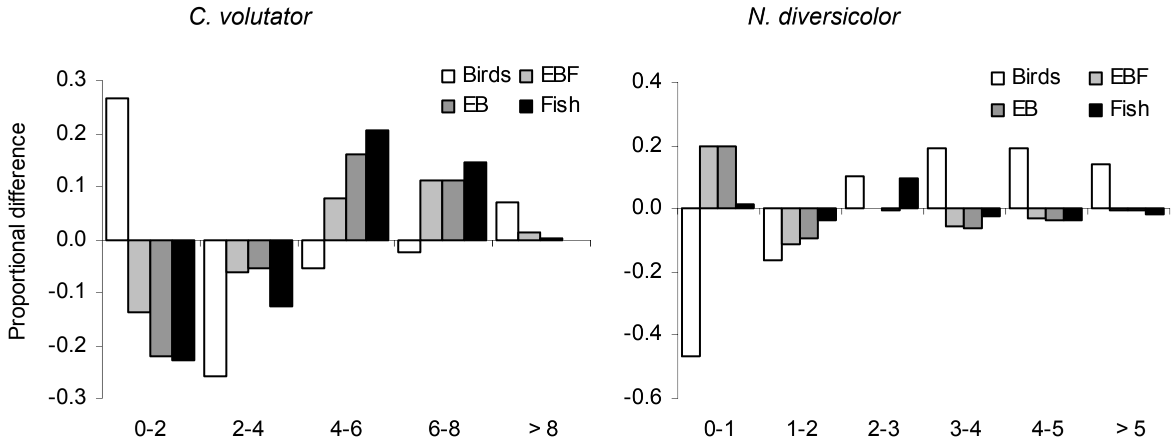

***Figure 4.9**. Experiment 1. Proportional difference in size frequency distribution between the treatments (exclusion and predator diet) and the uncaged area (control). The size classes of* C. volutator *(mm) and* N. diversicolor *(cm) are given on the x-axes. 'Birds' and 'Fish' represent respectively the proportion of a prey size class in the pellets of shelduck and in the stomachs of the fishes. For each of the four groups (EB, EBF, Birds and Fish), the proportion of a size class in the control was subtracted from the proportion of the same size class in the treatments and the predator diet. A positive value (y-axis) for a size class indicates that the proportion of that size class in the respective treatment is higher than in the control. Data are given in Appendix 4.2.*

***Table 4.2**. Experiment 1. Results of the pairwise log-linear analysis of the length-frequency tables for* C. volutator *and* N. diversicolor. *Bold* P*-values indicate significant differences between the pairs of treatments in column 1 (after sequential Bonferroni correction).*

	C. volutator		***N. diversicolor***	
	Pearson χ^2	P	**Pearson χ^2**	***P***
C-Birds	26.0	**< 0.001**	87.6	**< 0.001**
C-EB	21.7	**< 0.001**	10.4	0.064
C-EBF	12.4	0.014	9.0	0.109
C-Fish	14.5	**0.006**	8.4	0.137
EB-Birds	64.8	**< 0.001**	121.5	**< 0.001**
EBF-Birds	42.8	**< 0.001**	117.6	**< 0.001**
EBF-EB	4.6	0.331	1.0	0.962
EBF-Fish	5.2	0.272	9.3	0.097
EB-Fish	2.1	0.729	10.5	0.063
Fish-Birds	56.9	**< 0.001**	99.9	**< 0.001**

3.4. Experiment 2

Only the density of *C. volutator* differed significantly between the bird exclosures and the control treatment (Table 4.3). The abundance of *C. volutator* was noticeable lower in the plots that were protected from bird predation ($P = 0.012$; Figure 4.10).

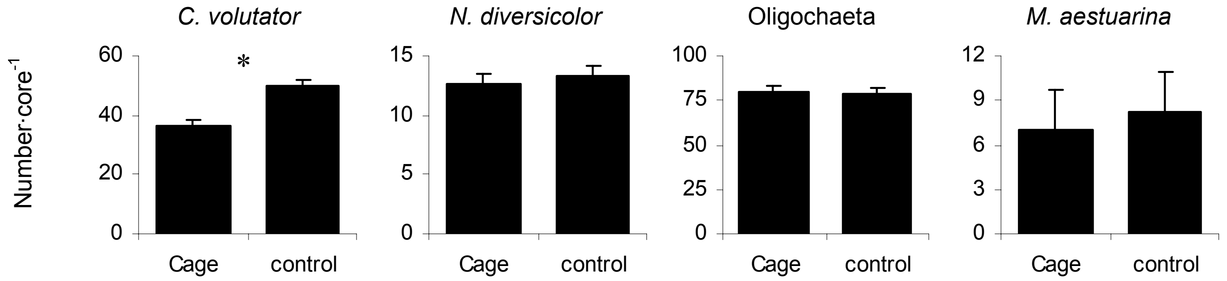

Figure 4.10*. Experiment 2. Mean abundance (± S.E.) of the four most common macrobenthic taxa in the different experimental treatments. A significant difference between the caged and uncaged treatments is indicated with an asterisk.*

Tabel 4.3*. Experiment 2. Results of the mixed model analysis for differences in diversity measures and species abundance. Only the most abundant species were included. Bold* P*-values indicate a significant difference between the exclusion and uncaged treatments.*

	df	**F-ratio**	***P***
Species richness	3	2.40	0.219
Eveness (J)	3	1.25	0.344
Diversity (H)	3	6.18	0.088
C. volutator	3	30.19	**0.012**
N. diversicolor	3	1.30	0.337
Oligochaeta	3	0.02	0.886
M. aestuarina	3	3.50	0.158

No differences were found for the size distribution of *C. volutator*. *N. diversicolor* on the other hand, was significantly larger in the exclosure plots (df = 3; F = 23.9; *P* = 0.016). Figure 4.11 shows that there were proportionally fewer small and more large worms in the cages.

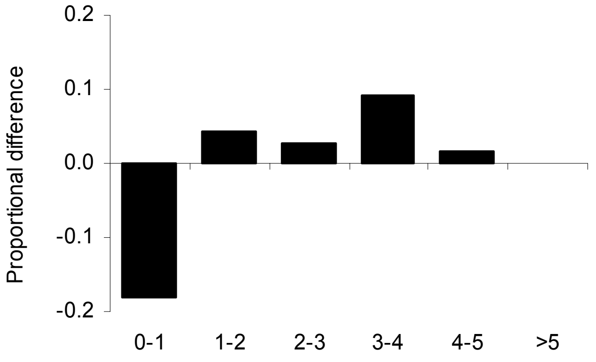

Figure 4.11*. Proportional difference in size frequency distribution between the bird exclosures and the uncaged areas (control). The size classes of* N. diversicolor *(cm) are given on the x-axis. A positive value (y-axis) indicates a higher proportion of that size class in the cages, compared to the control. Data are given in Appendix 4.2.*

4. Discussion

This study was designed to examine whether or not the macrobenthic community of an estuarine mudflat was top-down controlled by predation of fishes and/or birds. We therefore combined field observations of the zonation and the seasonal structure of the intertidal infaunal community with exclosure experiments that tested for the effect of predation on the macrobenthic assemblages. The field study indicated that the intertidal macrobenthos was dominated by three taxa, namely *C. volutator*, *N. diversicolor* and Oligochaeta. Dominance of a few euryhaline species like in our study is typical for the mesohaline zone of an estuary. Here benthic communities seldom progress beyond early benthic-community succession due to the high seasonal variation in salinity (Ysebaert *et al.,* 2003; Attrill, 2002; McLusky and Elliott, 2004).

Seasonal trend

The densities and the seasonal patterns are similar to a study on a nearby and similar mudflat (Ysebaert *et al.,* 2005). The lower abundance of most species in spring is the result of abiotic and biotic factors. Ysebaert *et al.* (2003) suggest that the decrease of the salinity during winter and spring accounts for the high mortalities in the benthic fauna (McLusky, 1967). Several surface feeding species like *C. volutator* and *N. diversicolor* feed intensively on benthic diatoms (microphytobenthos) (Gerdol and Hughes, 1994a; Smith *et al.,* 1996; Scaps, 2002). Benthic grazing is most intense during the summer and early autumn, when the density of *C. volutator* is sufficiently high to reduce the microphytobenthic populations (Gerdol and Hughes, 1994b). The reduction of the microalgae populations destabilizes the sediment and in combination with autumn storms, might stimulate the erosion of the mudflat (Daborn *et al.,* 1993; Smith *et al.,* 1996; Hughes and Gerdol, 1997). Erosion and resuspension of the sediment may destroy the shallow burrows of the amphipods and hence negatively affect the infaunal populations. Furthermore, food limitation in winter and predation may control the zoobenthic assemblage. The well-documented structuring effect of predation is discussed further on.

Depth distribution

C. volutator and *N. diversicolor* progressively inhabit deeper sediments towards winter. Probably, this reflects individual growth, as larger animals tend to bury deeper into the sediment (Meadows, 1964; Esselink and Zwarts, 1989). In addition, sediment surface temperature and predator avoidance may explain at least partly the seasonal variation in burrow depth (Esselink and Zwarts, 1989; Quijón and Jaramillo, 1996).

Zonation

The distribution of species was investigated on the lower, middle and upper intertidal zone. Species richness and abundance is expected to be highest in mid-intertidal and muddy sands (Dittmann, 2000). In our study, the lowest densities and species richness were indeed observed in the sandy zone near the low water level. Suspension feeders were only present in very low numbers and were not detected on the highest shore. The middle and higher shore were almost identical in species composition and density pattern. During autumn however, the density of *C. volutator* and to a lesser degree of *N. diversicolor*, dramatically dropped in the mid-tidal zone but stayed high on the upper shore (Figure 4.5). This pattern was not observed in 2002. Zonation of intertidal soft-bottom communities has been described by several authors and is generally attributed to the combined effect of the physical environment, species-sediment relations and species interactions (Beukema, 1976; Beukema and Flach, 1995; Raffaelli and Hawkins, 1996; Dittmann, 2000). Benthic organisms may be distributed along a depth gradient in the intertidal zone according to their tolerance for environmental conditions. Near the lower intertidal zone, the sediment becomes more sandy and unstable due to the stronger currents, which limit the distribution of tube-building species. Higher on the shore, the shorter inundation time restricts the feeding opportunities for suspension feeders and desiccation may become a limiting factor (Beukema, 1976; Raffaelli and Hawkins, 1996). On the studied mudflat, desiccation and current speed may not be limiting for organisms in the middle and upper zones. Both zones have a very high mud content and at low tide, sufficient amounts of water remain in the sediment, held by capillary forces (Raffaelli and Hawkins, 1996). The zonation of benthic animals may also be influenced by the availability of food items. Several studies reported a positive correlation between the pigment content (microalgae) and the height on the mudflat (Ysebaert *et al.,* 2005; De Jong and De Jonge, 1995). They argue that the lower reaches of the mudflat might experience stress of light limitation. The higher microphytobenthos biomass on the upper shore probably supports a higher abundance of grazing benthic invertebrates, which could, at least partially, explain the observed zonation of the zoobenthos.

In addition to physical factors and food availability, zonation may also be influenced by inter- and intraspecific interactions. Species interactions include predation, disturbance and food competition (Ölaffson and Persson, 1986; Rönn *et al.,* 1988; Wilson, 1991 and references therein; Beukema and Flach, 1995; Meziane and Retière, 2001). Among the various species interactions, predation seems the most likely candidate for the regulation of species zonation on the mudflat in our study. The abundance of most fishes was significantly higher in 2001 than in 2002 and the community was dominated by flatfishes (Figure 4.7; Chapter 2). *C. volutator* was the dominant prey item in the stomachs of sole and flounder, but also other fishes were feeding on this prey (Chapter 3). If epibenthic predation is a structuring factor for the distribution of *C. volutator*, then the effect should be stronger as the inundation time increases (i.e. on the lower parts of the mudflat). The reverse should be true if bird predation is more important (stronger effect on the upper shore). In our study, the observed density of *C. volutator* and to a lesser degree also of *N. diversicolor*, decreased in October on the middle shore while this was not the case on the upper shore. This might suggest that fish predation may be responsible for the observed decrease in abundance of these two prey species.

Exclosure experiments

To test whether predation is a structuring factor for the macrobenthic community on the mudflat, two exclosure experiments were set up. The exclosure experiments were designed to differentiate between the effects of bird and fish predation on the macrobenthic community and on the population structure of *C. volutator* and *N. diversicolor* in particular.

The first experiment, which ran for two months, could not detect any effect of predation on the density of the infauna. Different reasons can be identified for this lack of impact. First, of all, it is possible that the effect was real and that predation is not an important structuring factor on the mudflat. Fish abundance in 2003, at the time of the experiment, was much lower than during the monitoring study in 2001 and could have been too low to result in a significant effect. In this respect, it is also possible that the power of the experiment was too weak to detect any predation effect of fishes at these low densities (Hall *et al.,* 1990a). Secondly, the timing and the duration of the experiment might have influenced the outcome. The effect of predation on the infaunal density might have been masked by high benthic recruitment during the summer. The effect could have been stronger in autumn, when reproduction ceased and the populations were established. Furthermore, the experimental period might have been too short to detect any predation effects. Two months is rather short, but the duration is typical of similar experiments (see Raffaelli and Moller, 2000). Our experiment coincided with the peak abundances of both birds and fishes on the mudflat, so if predation was an issue, we should have been able to detect it. Finally, smaller shrimps (*Crangon crangon*) were able to pass through the mesh of the cages and could have undone the protecting effect of the cages. Various studies demonstrated that crustacean predation is common in intertidal areas and can significantly reduce prey densities (Virnstein, 1977; Pihl and Rosenberg, 1984; Gee *et al.,* 1985). However, the results of an exclosure experiment in the Ythan estuary (Scotland) showed that the impact of crustacean predators was not significant (Raffaelli *et al.,* 1989). The authors argued that ecological differences between study areas and the choice of (unrealistically high) predator densities in enclosure studies determine the outcome of predator-effect studies. The intertidal shrimp densities in the Scheldt estuary (4.5 ind.·m^{-2}; Hostens, 2000) are much lower than those reported in the Ythan study (140 ind.·m^{-2}), suggesting that crustacean predators in our study only had a marginal effect on the infaunal community.

We found an effect of predator exclusion on the size distribution of *C. volutator* and *N. diversicolor*. *C. volutator* was larger in both the bird exclosures and the complete exclosures, whereas *N. diversicolor* was smaller in the cages, although this difference was not significant. The lack of any difference between the birds only (EB) and both the fish and bird exclosures (EBF) suggests that birds but not fish were responsible for the observed patterns. The diet analysis on the other hand, showed that fish selected larger *C. volutator*, while shelduck consumed proportionally smaller individuals (Figure 4.9). Shelduck was targeted because it was the most abundant shorebird on the mudflat during the experiment and droppings were easy to collect. While feeding on benthic organisms, shelduck use their bill to sieve the top sediment layer (Mclusky and Elliott, 2004) and hereby might take a high number of juvenile *C. volutator* living in the top centimeter of the mudflat (Ysebaert *et al.,* 2005). Waders (curlew, grey plover and avocet) are probably more important predators of *N. diversicolor* and *C. volutator* (McLusky and Elliott, 2004). They were shown to select for the larger size classes of prey (Zwarts and Wanink, 1983; Zwarts and Esselink, 1989).

Therefore, we probably underestimated the contribution of the larger size classes in the diet of birds. Our short-term experiment couldn't detect a statistically significant effect on the length of *N. diversicolor* in the cages, although the pellet analysis clearly showed that shelduck consumed proportionally larger *N. diversicolor*. Similar results were found in several other soft-bottom exclosure experiments. These studies showed that shorebird and/or epibenthic predation had only little or no effect on invertebrate densities, but significantly affected the size structure of the infaunal species (Raffaelli and Milne, 1987; Raffaelli *et al.,* 1989; Wilson, 1989; Wilson, 1991; Sardá *et al.,* 1998). They suggested that in the absence of predation, adult-recruit competition might become more important and the adults in the cages might displace their juvenile conspecifics (Raffaelli and Milne, 1987; Wilson, 1989).

In the second experiment, which ran over a full year, only birds were excluded. In contrast to the short-term experiment, we found a significant effect of predator exclusion on the density of *C. volutator*. However, the direction of the effect was opposite to what was expected, as densities were lower in the cages than outside the cages. This time, no effect was found on the size structure of *C. volutator*. *N. diversicolor* on the other hand, was significantly larger in the cages. Since waders select the larger worms, they might have a direct effect on the size structure of *N. diversicolor* in the cages. The presence of larger worms and the reduction of the density of *C. volutator* in the cages, suggests that infaunal species interactions may become more important in the absence of bird predation. The relationship between *C. volutator* and *N. diversicolor* has been studied extensively with contrasting results. *N. diversicolor* may reduce the densities of *C. volutator* either by competition, disturbance or predation (Ölaffson and Persson, 1986; Rön *et al.,* 1988; Hughes and Gerdol, 1997). Other studies indicate that they may co-exist (Jensen and Andre, 1993). Size-selective predation by *N. diversicolor* should affect the size structure of the *C. volutator* population in the cages, but this was not observed in our study. Furthermore, food competition doesn't seem to be a probable regulator of the densities in the exclosures. If competition would be significant, it should be more likely at higher macrobenthic densities, but these were observed outside the cages. Therefore it is suggested that in the present study, disturbance by larger *N. diversicolor* is the most likely cause for the reduced abundance of *C. volutator* in the cages. *N. diversicolor* probably destroyed the burrows of the amphipods, causing their emigration (Ölaffson and Persson, 1986; Jensen and Andre, 1993)

The results of this study suggest that in high productive intertidal systems, fish and bird predation is less important as a regulating factor of the infaunal density. However, when fish densities are very high, intense episodic predation can locally reduce the zoobenthic populations. Consequently, the effects of short-term experiments in these areas may only be noticeable at much higher predator densities than in our case. Long-term exclosure experiments on the other hand, seem to reflect the indirect effects of predation, as in the absence of predation, infaunal regulation (competition and predation) becomes more important (Figure 4.12). The effect of predator exclusion was not examined at the level of primary production (microphytobenthos). Given the importance of benthic algae as both an important food item for the higher trophic levels and as a sediment-stabilizing factor, it would be worth exploring if and how cascading effects by fish and bird predation can affect habitat stability (Daborn *et al.,* 1993).

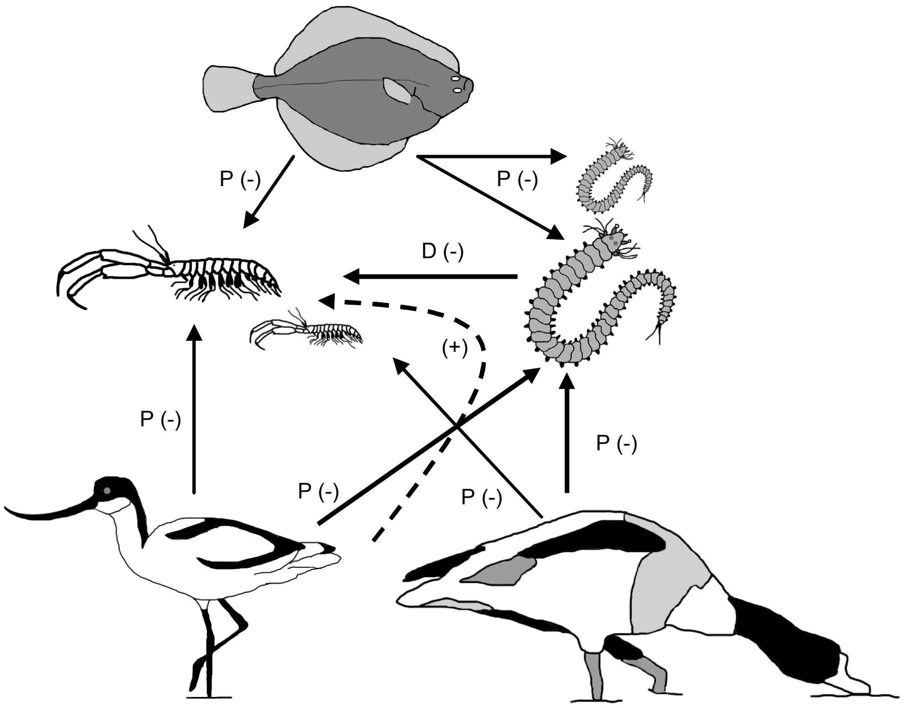

Figure 4.12. *Schematic summary of the major species interactions (- negative or + positive) on the mudflat.* P *stands for predation,* D *stands for disturbance. Depicted species (clockwise from top):* Platichthys flesus *(flounder),* Nereis diversicolor*,* Tadorna tadorna *(shelduck),* Recurvirostra avosetta *(avocet) and* Corophium volutator.

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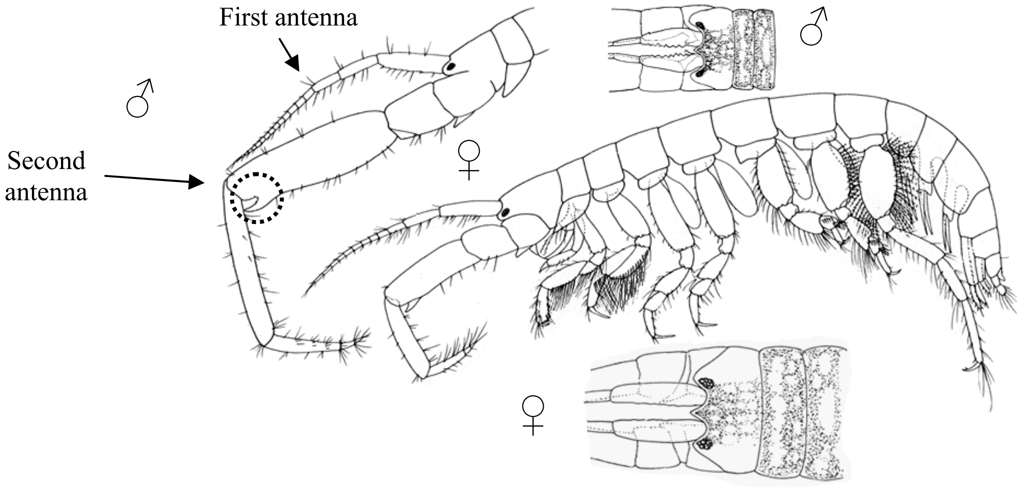
Appendix 4.1 - Sexually dimorphic characters of *Corophium volutator* (Pallas, 1766) (Hayward and Ryland, 1990). Pictures were taken from http://www.amphipoda.com/.

Description male. *Rostrum* rostrum vestigial (not reaching past lateral lobes of head), acute or subacute (less than 90 degrees). *Antenna 1* peduncular article 1 inner margins crenulate. *Antenna 2* robust (compared to antenna 1), sexually subsimilar; peduncular article 4 with posterodistal spine or spines, posterodistal spine(s) extending beyond end of segment 4, without robust setae on ventral margin.

Description female. *Rostrum* rostrum present, short (less than 1/3 length of A1 peduncular article 2). *Antenna 1* peduncular article 1 inner margins not crenulate. *Antenna 2* peduncular article 4 without posterodistal spine or spines, with robust setae on ventral margin, ventral margin with one row of robust setae.



Appendix 4.2 - Size frequencies of *Corophium volutator* and *Nereis diversicolor* in the experimental treatments and in the diet of fishes and birds on the mudflat. These data were used to calculate the proportional differences in figures 4.9 and 4.11. C = control, EBF = birds and fish exclosure, EB = bird exclosure, Birds = faecal pellets of shelduck and Fish = stomach of fishes.

Table A4.1. *Size frequencies of* Corophium volutator *(lengths in mm). The data were used in figure 4.9.*

Size class	C	EBF	EB	Birds	Fish
0-2	0.32	0.18	0.10	0.58	0.09
2-4	0.42	0.36	0.37	0.17	0.30
4-6	0.18	0.26	0.34	0.13	0.39
6-8	0.07	0.19	0.19	0.05	0.22
> 8	0.00	0.02	0.00	0.08	0.00

Table A4.2. *Size frequencies of* Nereis diversicolor *(lengths in cm). The data in the left column were used for figure 4.9, those in the right column for figure 4.11.*

Size class	C	EBF	EB	Birds	Fish	C	EB
0-1	0.47	0.66	0.66	0.00	0.48	0.78	0.60
1-2	0.29	0.18	0.20	0.13	0.26	0.08	0.13
2-3	0.09	0.09	0.08	0.19	0.19	0.06	0.08
3-4	0.10	0.05	0.04	0.29	0.08	0.07	0.17
4-5	0.04	0.01	0.00	0.23	0.00	0.01	0.03
> 5	0.02	0.01	0.02	0.16	0.00	0.00	0.00

CHAPTER 5

5

A bioenergetics model for juvenile flounder Platichthys flesus

Maarten Stevens, Joachim Maes and Frans Ollevier

Abstract

Despite the numerous physiological studies on flatfish and their economic and ecologic importance, only few attempts have been made to construct a bioenergetics model for these species. Here, we present the first bioenergetics model for European flounder (*Platichthys flesus*), using experimentally derived parameter values. We tested model performance using literature derived field-based estimates of food consumption and growth rates of an estuarine flounder population, in the Ythan estuary, Scotland. The model was applied to four age classes of flounder (age 0-3). Sensitivity of model predictions to parameter perturbation was estimated using error analysis. The fit between observed and predicted series was evaluated using three statistical methods: partitioning mean squared error, a reliability index (RI) and an index of modelling efficiency (MEF). Overall, model predictions closely tracked the observed changes of consumption and growth. The results of the different validation techniques show a high goodness-of-fit between observed and simulated values. The model clearly demonstrates the importance of temperature in determining growth of flounder in the estuary. A sex-specific estimation of the energetic costs of spawning in adult flounder and a more accurate description of the thermal history of the fish may further reduce the error in the model predictions.

Keywords: *Feeding rate; Growth; Model evaluation; Platichthys flesus; Ythan estuary*

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1. Introduction

Quantitative estimates of energy transfer in ecosystems are essential to many aspects of ecological research and different approaches are available to estimate feeding rates in animals (Rice and Cochran, 1984; Ney, 1993). Bioenergetics models quantify the allocation of consumed energy over respiratory metabolism, waste products and growth (Kitchell *et al.,* 1977). Their relatively simple structure and the fact that model input data such as water temperature and fish weights are obtained with moderate effort, make these models widespread and increasingly popular in fisheries research. Consequently, fish bioenergetics models have been applied to a wide variety of ecological disciplines and are now available for over 25 fishes, in particular pelagic species (Hanson *et al.,* 1997).

Despite their economic importance and wide geographic distribution, pleuronectid flatfishes are a taxonomic group that is underrepresented in bioenergetics literature. Worldwide, flatfish inhabit continental shelves but they are particularly abundant in estuaries and shallow coastal zones where they find plenty food and shelter against predators (Miller *et al.,* 1985; Ruiz *et al.,* 1993; Pihl *et al.,* 2002). Higher temperature and increased prey density in shallow waters promote growth and survival, especially of the juvenile life history stages. In this respect bioenergetics modelling can be used as a tool to quantify the habitat quality of nursery areas for these species. As far as we know, only two studies report on the bioenergetics of flatfish species, both occurring in the NW-Atlantic (Winter flounder *Pleuronectes americanus*: Rose *et al.,* 1996; Southern flounder *Paralichthys lethostigma*: Burke and Rice, 2002). Several authors already emphasized the need for flatfish bioenergetics models to describe the quality and quantity of habitats in space and time (van der Veer and Nash, 2001; Nash and Geffen, 2003).

This paper reports on the application of a bioenergetics model to simulate seasonal growth and consumption in flounder, *Platichthys flesus*, a European flatfish species. *P. flesus* occurs from the Baltic to the Black Sea. North Sea flounder spawn in winter followed by upstream migration to estuarine brackish and freshwater habitats (Wheeler, 1969; van der Veer *et al.,* 1991). This study is the first to estimate the energetic demands of juvenile *P. flesus*. In particular, we addressed the question whether the growth of flounder in the field can be described as a function of the ambient water temperature and body weight.

2. Model description

We applied the Wisconsin bioenergetics framework (Kitchell *et al.,* 1977; Stewart *et al.,* 1983; Hanson *et al.,* 1997) to simulate growth and consumption of flounder and used the study of Fonds *et al.* (1992) for parameter estimates in the bioenergetic equations. Bioenergetics models allocate daily consumed energy C over metabolic processes such as standard respiration R_S, active respiration R_A and specific dynamic action SDA, waste losses due to egestion F and excretion U and growth G such that

$$G = C - (R_S + R_A) - SDA - F - U$$

In this paper, we considered growth of immature fish aging less than three years (0 - 3), and thus ignoring gamete production in growth. Respiration rate equals the sum of standard (R_S) and active metabolism (R_A) and the costs of digestion (specific dynamic action, SDA). Waste products are the total of non-assimilated or egested energy (F) and assimilated energy that is excreted (U).

Individual components of the bioenergetics equation were calculated in units of energy (Joules per day) and converted to biomass (g). Modelled values of specific food consumption were expressed in g g^{-1} day^{-1} biomass (ash free dry weight, AFDW) in order to compare with observed measurements from the laboratory experiment (Fonds *et al.,* 1992).

2.1. Consumption

The specific food consumption (C, g g^{-1} day^{-1}) is modelled as a proportion of maximum daily ration $C_{\max}$. $C_{\max}$ corresponds to the consumption of a fish at weight W (g) at optimal temperature T (°C) when feeding *ad libitum*:

$$C_{\max} = a_1 \cdot W^{b_1} \cdot f(T)$$

where a_1 is the intercept of consumption (g g^{-1} d^{-1}), W denotes the wet mass of the fish (g) and b_1 is the weight-dependent exponent of consumption. $f(T)$ is a dome-shaped function of water temperature for cool- and cold-water species (Thornton and Lessem, 1978), which modifies the maximum ration. The daily consumption C necessary to account for observed growth is obtained by adjusting the realized consumption to a constant proportion P of maximum consumption rates within each simulation period.

We used Fonds *et al.* (1992) to derive parameters to assess maximum daily ration. In their laboratory experiments, Fonds *et al.* (1992) fed flounder in excess. Therefore, we assume that the daily food consumption of the laboratory fish equals $C_{\max}$ and P was set at one. This procedure yielded a value of 0.798 (-0.202 for daily specific rate) for the weight exponent b_1 and 0.186 (g g^{-1} day^{-1}) for the intercept a_1 of the allometric mass function (Table 5.1).

Maximum daily ration increases with increasing temperature to an optimal temperature and subsequently declines to zero at a temperature just below the maximum lethal temperature. We modelled the temperature dependence of maximum daily ration using the Thornton and Lessem algorithm (1978), assuming 20 °C as optimal temperature and 27 °C as lethal temperature (Waede, 1954; Fonds *et al.,* 1992).

2.2. Metabolism

We modelled metabolism (R, g O_2 g^{-1} day^{-1}) as a function of fish weight W, temperature T, fish activity ACT and specific dynamic action SDA:

$$R = a_2 \cdot W^{b_2} \cdot f(T) \cdot ACT + SDA$$

We used values reported in Fonds *et al.* (1992) for the weight dependent coefficient b_2 and the intercept (Table 5.1). The metabolic weight exponent for standard respiration of flounder estimated by Duthie (1982) was similar to the value that is used in our model. The influence of temperature on respiration was simulated by a non-linear function with a slope Q (Table 5.1). The upper lethal temperature (27 °C) was used as maximum temperature for respiration and sets the upper bounds on the system (Waede, 1954).

To account for metabolism due to swimming activity, the function for metabolism is multiplied by an activity multiplier (ACT). We estimated the cost of activity for flounder in the field to be 10 % higher than the routine metabolism as measured in the laboratory (ACT = 1.1). This assumption was based on recorded swimming speeds of flounder migrating between the river channel and intertidal mudflats (Wirjoatmodjo and Pitcher, 1984), a laboratory derived relationship between oxygen consumption and swimming speed (Duthie, 1982) and reported field observations of flounder behaviour (Raffaelli *et al.,* 1990).

Specific dynamic action (SDA) was calculated as a constant proportion of assimilated energy (consumption minus egestion) (Table 5.1). The SDA coefficient was set at 0.19 after Jobling and Davies (1980) who described the SDA coefficient in plaice *Pleuronectes platessa* as a proportion of the assimilate. It follows that SDA equals 15.7 % of the ingested energy, a value which comes close to 15.2 % found by Fonds *et al.* (1992) for flounder and 16 % found by Jobling and Davies (1980) for plaice.

2.3. Egestion and excretion

We modelled egestion as a constant proportion F_A of the ingested consumption and excretion as a constant proportion U_A of assimilated consumption (Table 5.1). The proportion of ingested energy lost through egestion and excretion is relatively constant, has low sensitivity in bioenergetics models and is regarded as a diet-specific parameter. Kelso (1972) found that walleye (*Stizostedion vitreum*) absorbed 83.5 % of a crayfish diet and 82.1 % of an amphipod diet. Crustaceans were also the most important prey in the diet of flounder (De Groot, 1971; Summers, 1974). Therefore, we assumed a value of 17 % for F_A. We used observations on nitrogen excretion in flounder by Carter *et al.* (1998) to set U_A at 10 % (Table 5.1).

Table 5.1. *Parameter values for the different equations of the bioenergetics model.*

Symbol	Parameter description	Values
Consumption		
a_1	Intercept for C_{max} ($g \cdot g^{-1} \cdot d^{-1}$)	0.186
b_1	Slope for C_{max}	- 0.202
T_1	Temperature for K_1 (°C)	2
T_2	Temperature for K_2 (°C)	20
T_3	Temperature for K_3 (°C)	21
T_4	Temperature for K_4 (°C)	27
K_1	Proportion of C_{max} at T_1	0.05
K_2, K_3	Proportion of C_{max} at T_2 and T_3	0.98
K_4	Proportion of C_{max} at T_4	0.01
Fact	Conversion factor W_{wet} to W_{AFD}	5.6
Respiration		
a_2	Intercept for maximum standard respiration ($g \cdot g^{-1} \cdot d^{-1}$)	0.0178
b_2	Slope for maximum standard respiration	- 0.218
T_m	Maximum temperature for standard respiration (°C)	27
T_o	Optimal temperature for standard respiration (°C)	21
Q	Slope for temperature dependence of std. Respiration ($\cong Q_{10}$)	2.5
SDA	Specific dynamic action coefficient	0.19
ACT	Activity multiplier	1.1
Waste losses		
F_A	Proportion of food egested	0.17
U_A	Proportion of food excreted	0.1

2.4. Predator and prey energy contents

Conversions between energy and weight in flounder were made using an energy density of 4406 J g^{-1} wet weight (Fonds *et al.,* 1992). Where appropriate, wet weight was recalculated to biomass (ash-free dry weight) using a conversion factor of 5.6 (Fonds *et al.,* 1992). Estimates of the daily energy content of flounder in the field were determined by linearly interpolating between the monthly estimates provided by Summers (1974) for the different age classes of flounder in the Ythan estuary. Age dependent seasonal changes in the diet composition as well as calorific values of different prey species were obtained using Summers (1974), Chambers and Milne (1979) and Dobrzycka and Szaniawska (1995).

3. Model calibration and comparison with field data

We estimated the proportion of maximum consumption *P* by forcing the model through weight-at-age derived from field data presented by Summers (1974). For each simulation the mean weights of flounder on January the 15th (day 0) and December the 15th (day 335) were taken as the start and end weight of a cohort (Table 5.3). For age 0 flounder the simulation started on July the 15th at a weight of 0.48 g.

Data of water temperature, diet composition and predator energy content of the field study were used as input variables to model growth and respiration of four age classes of flounder (0 - 3 group). Summers measured mean body mass, energy density, daily ration and growth of flounder on a mudflat in the Ythan estuary (Scotland). The study was carried out on the Sleek of Tarty and Forvie Bank, in the upper part of the estuary. Flounder (>0 group) were captured with a V-shaped wire mesh trap as they left the mudflat during the ebb tide. Age-0 flounder were sampled using a beach seine-net and a hand net. Growth was measured using weight frequency data of different age classes through the year. Daily ration estimates were based on mean stomach contents. Stomach analysis indicated that the amphipod *Corophium volutator* was the main prey item of flounder in the Ythan estuary. In terms of calorific equivalents, *Nereis diversicolor* was important in autumn and winter (Summers, 1980). The total prey energy content varied seasonally (Summers, 1974; Chambers and Milne, 1979; Dobrzycka and Szaniawska, 1995) between 16.2 KJ g^{-1} AFDW and 20.5 KJ g^{-1} AFDW. Monthly water temperatures of the study area were provided by the Scottish Environment Protection Agency (Figure 5.1a).

3.1. Model validation

The evaluation of model performance with independent data is a mandatory step in simulation exercises. All too often, only a visual comparison of simulated and actual data is used as a cursory technique for model validation. On the other hand, statistical techniques may give an unambiguous judgment of model performance. The validity of bioenergetics models has previously been evaluated using field (Rice and Cochran, 1984) and laboratory (Bajer *et al.,* 2003) estimates of daily food consumption, growth, predator and prey energy densities and fish thermal experience. Ideally, bioenergetics models should first be evaluated in the laboratory under standardized conditions and then in the field to ensure that model assumptions and parameters are applicable to non-captive fishes. We tested the validity of the model by comparing the model predictions field observations of the Ythan population, using three different test statistics: Partitioning mean squared error (MSE), a reliability index (RI) and an index of modelling efficiency (MEF) (Table 5.2).

Table 5.2. *Different test statistics used in the model evaluation.* $\overline{P}$*,* $\overline{O}$*,* S_P *and* S_O *are the means and the standard deviations of the predicted (P) and observed (O) series and r is their correlation coefficient. MC is the mean component, or the bias due to differences in the means of the predicted and observed values. SC is the slope component, or the error resulting from the slope deviating from unity. RC is the residual component, or the proportion of MSE due to random error.*

Test	Description	Best fit
1. Partitioning mean squared error (MSE) $1=\frac{(\overline{P}-\overline{O})^2}{MSE}+\frac{(S_P-rS_O)^2}{MSE}+\frac{(1-r^2)S_O{}^2}{MSE}$ $=\quad MC \quad + \quad SC \quad + \quad RC$	A measure of the variance of the regression points (P_i, O_i) around a perfect regression line (slope = one and intercept = zero). Dividing the decomposition by MSE yields the proportions of MSE attributed to three different sources of error (MC, SC and RC).	MSE = 0 OR MC = 0 SC = 0 RC = 1
2. The reliability index (RI) $RI=\exp\sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(\log\frac{O_i}{P_i}\right)^2}$	Quantifies the average factor by which model predictions differ from observations (Leggett and Williams, 1981)	RI = 1
3. The modelling efficiency (MEF) $MEF=\frac{\left(\sum_{i=1}^{n}(O_i-\overline{O})^2-\sum_{i=1}^{n}(P_i-O_i)^2\right)}{\sum_{i=1}^{n}(O_i-\overline{O})^2}$	Measures how well a model predicts relative to the average of the observations (Mayer and Butler, 1993)	MEF = 1

3.2. Parameter uncertainty analysis

In addition to evaluations of model performance, a sensitivity analysis evaluated the effect of a parameter perturbation on the model results by performing 250 simulations using parameters that were randomly chosen from normal distributions, with nominal parameter values as means and a coefficient of variation (CV) of 10 % as standard deviations. The effect of each parameter on model performance was determined by assessing its contribution to the explanation of the variation in simulated *P*-values. The importance of each parameter was ranked using the relative partial sum of squares (RPSS) resulting from a multiple linear regression where the proportion of maximum consumption (*P*) was related to parameter values. RPSS quantifies the independent contribution of each individual parameter to the total explained variance (Bartell *et al.,* 1986).

Table 5.3. *Start and end weights of the different age classes used in the simulations. The proportion of maximum daily ration (P-value) is given for each simulation. Growth of age I flounder was simulated using both one (a) and two (b) P-values.*

Age group	W_{start} (g)	W_{end} (g)	*P*-value
Age 0	0.48	4.5	0.54
Age 1 (a)	4.5	34	0.45
Age 1 (b_1)	4.5	38	0.52
Age 1 (b_2)	38	34	0.32
Age 2	34	141	0.42
Age 3	141	246	0.37

4. Simulation results

For each cohort, the model was forced through a final weight-at-age by adjusting the proportion *P* of maximum daily consumption. Except for age 1 flounder, growth of all cohorts was simulated using a single *P*-value (Table 5.3). The simulated weight increment of age 0 to age 3 flounder in the Ythan estuary is shown in figure 5.1b. Growth was limited to spring and early summer and ceased during fall and winter. The seasonal growth pattern of age 1 flounder (4.5 g - 34 g) is presented in more detail in figure 5.2. The model simulation using one *P*-value underestimated growth as derived from field data (dashed line). Goodness-of-fit increased if the simulation period was separated into two intervals (complete line; January-August and September-December). For all age classes, the model accurately predicted the growth of flounder in the field as shown by the Reliability Index and Modelling Efficiency which are both close to one (Table 5.4). Partitioning the mean squared error showed that most of the error was the result of random error. However, for 0 and 3 group flounder the relative high values for the mean component (0.4084 and 0.3912) indicated that a large part of the total error could still be attributed to a lack of fit between the means of observed and predicted values.

Table 5.4. *Results of the model evaluation for the field study (Summers 1974). Growth of age I flounder in the Ythan estuary was modelled using both a single P-value (a) and two P-values (b). See text for abbreviations.*

Age group	Partitioning MSE				RI	MEF	r^2
	MSE	MC	SC	RC			
Age 0	0.53	0.4084	0.0064	0.5852	1.1304	0.8285	0.9072
Age 1 (a)	47.42	0.5151	0.0776	0.4073	1.1408	0.7626	0.9076
Age 1 (b)	1.34	0.0626	0.0006	0.9368	1.0486	0.9935	0.9939
Age 2	38.50	0.0194	0.3179	0.6627	1.0430	0.9760	0.9925
Age 3	735.08	0.3912	0.0413	0.5676	1.0833	0.7021	0.8367

P-values were used to assess the daily food consumption of flounder in the field. *P*-values ranged from 0.54 for age 0 to 0.37 for Age 3 flounder and decreased with increasing fish size, indicating that younger fish fed at a higher proportion of their maximum consumption rate (Table 5.3). Figure 5.2 shows the specific consumption (J g^{-1} day^{-1}) of age 1 flounder in the Ythan. For all age classes the daily ration followed a bell-shaped curve with a maximum in May and minima in the winter months. In general, the model closely fitted the field based daily rations, but simulations slightly underestimated consumption in April and May when the water temperature rapidly increased (Figure 5.1a).

In table 5.5, we ranked the parameters included in the error analysis according to their relative contribution to model output variability. Since the modelled weight is fitted to the observed weight by adjusting the *P*-value, *P* can be regarded as a single measure of model performance. Most of the variance in *P* could be attributed to the uncertainty of the optimal temperature of standard respiration (T_o) and the intercept of maximum consumption (a_1).

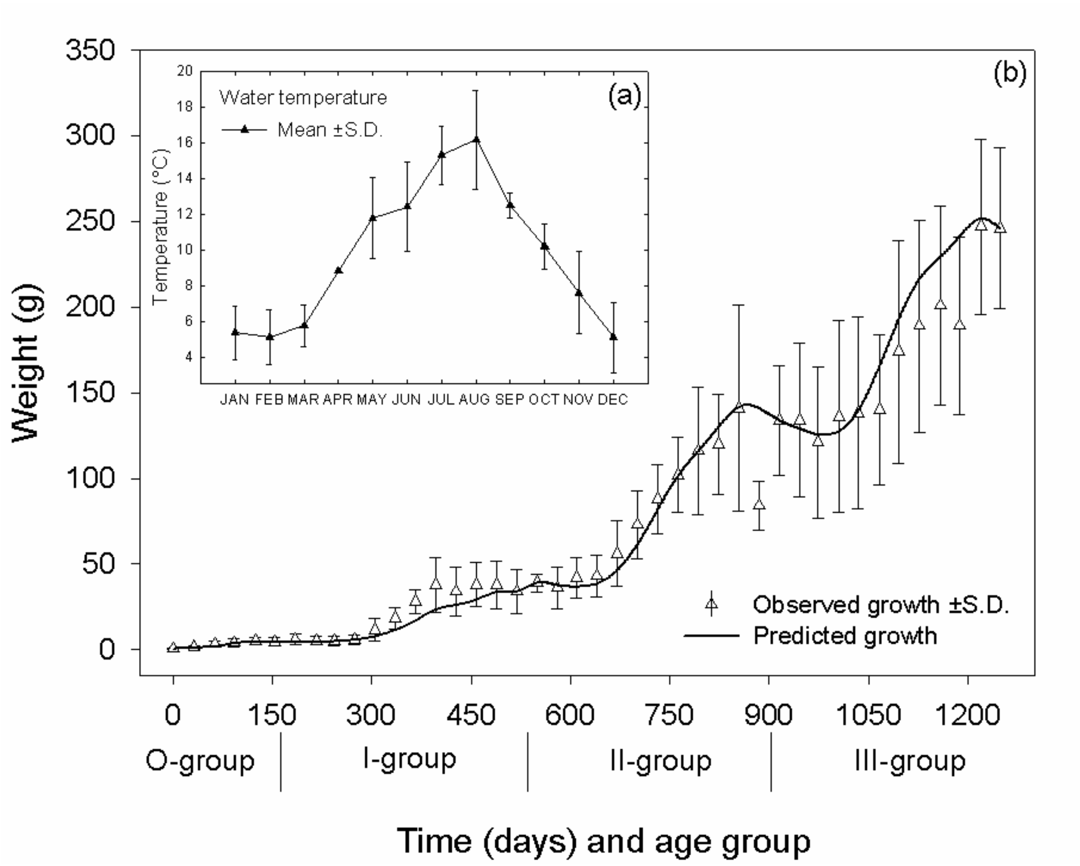

Figure 5.1. *(a) Average monthly water temperatures ± SD of the Ythan estuary near Newburgh Bridge. (b) Observed mean body mass ± SD (n = 1456) and masses predicted by the model for each year class, plotted against the cumulated days of the four simulation (day one = July 1st of age 0 simulation). For each year class, the model was fitted to a final weight at the end of each year.*

Table 5.5. *Rank order of top 10 parameters resulting from the error analysis using. Relative partial sums of squares (RPSS) were used to rank parameters. Overall performance of the multiple regression model is given by the r-square value.*

Parameter	RPSS	Rank order	Parameter	RPSS	Rank order
Consumption			*Respiration*		
a_1	0.175	2	a_2	0.079	5
b_1	0.008		b_2	0.041	8
T_1	0.004		T_m	0.001	
T_2	0.043	7	T_o	0.218	1
T_3	$< 1 \cdot 10^{-3}$		Q	0.080	4
T_4	$< 1 \cdot 10^{-3}$		SDA	0.011	9
K_1	0.005		ACT	0.078	6
K_4	$< 1 \cdot 10^{-3}$				
			Waste losses		
			F_A	0.010	10
Fact	0.105	3	U_A	0.002	

$r^2 = 0.977$

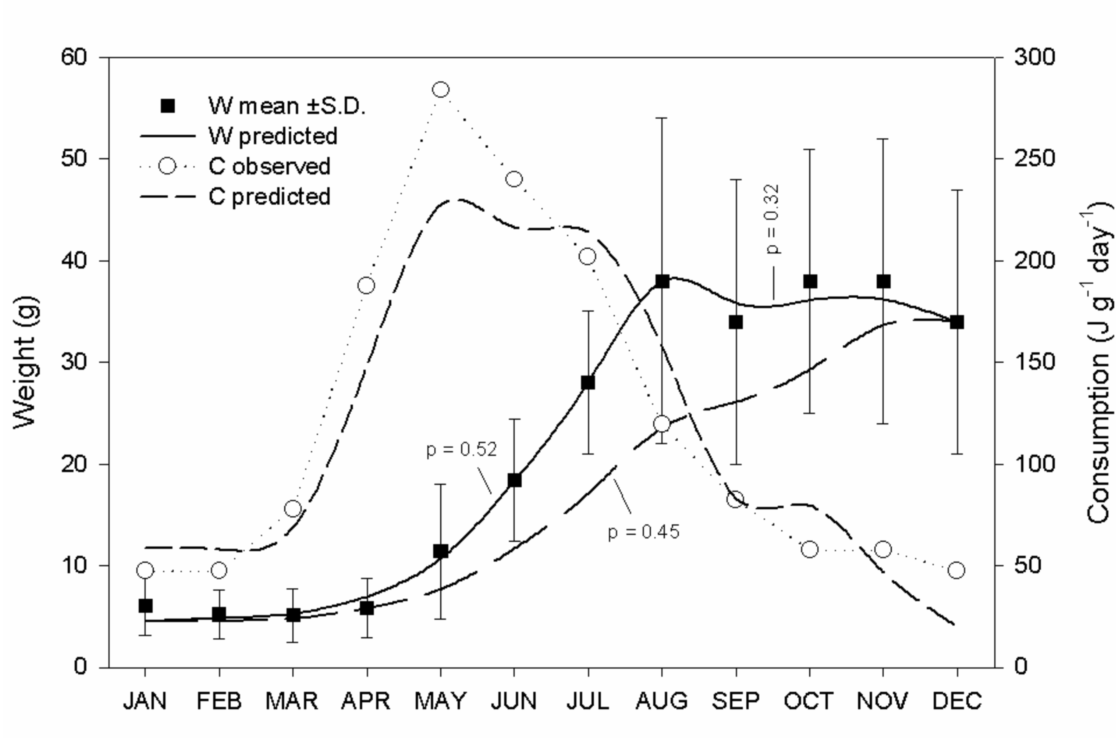

Figure 5.2. *Observed mean body mass ± SD (W) and daily food consumption (C) of age 1 flounder in the Ythan estuary (n = 555). Predicted masses estimated from as single* P*-value (0.45) or from two* P*-values (0.52 and 0.32). Predicted daily food consumption was estimated from two* P*-values, all plotted against time.*

5. Discussion

We were able to successfully describe growth and consumption of flounder in the field, based on site-specific data of diet composition and water temperatures. The different model diagnostics demonstrated the high predictive capability of the model to identify seasonal changes in the field data.

Growth was greatly influenced by seasonal changes in the rate of consumption, reflecting in the first place the seasonal fluctuation in water temperature, but also the changes in quality and quantity of the prey population. The drop in daily ration (J g^{-1} day^{-1}) from June onwards, may be explained by changes in the population structure and energy quality of the prey. Between January and August, *C. volutator* was the most abundant resource and dominated the diet of flounder (Summers, 1980). Seasonal changes in the calorific value of *C. volutator*, related to spawning activity near the end of July, accounted for the largest part of the variance in prey energy and might have caused the drop in energy intake in the period following July. From September onwards, *C. volutator* densities decreased, affecting particularly the 1 group flounder, since this cohort is largely dependent on this species.

As *P*-values indicated, young-of-the-year flounder were feeding at 54 % of maximum consumption, while the consumption in age 3 fish was reduced to only 37 % of maximum ration. The *P*-value is often interpreted as an indicator of feeding efficiency or prey availability for populations in the field, which suggests that the larger fish in our study were more food-limited.

Water temperature and prey energy sufficiently explained growth and consumption of flounder in the Ythan, confirming the importance of these factors as regulating operators in flounder energetics. Given the key role of temperature, an accurate thermal input is essential for model performance. Here, we assumed homothermous conditions in the study area. However, the temperature pattern may differ along a cross section in the river as the shallow water body on intertidal flats warms up faster than water in the main channel. This may locally affect the distribution patterns of flounder, and hence, growth and consumption rates. The underestimation of consumption in April and May may be partly attributed to faster heating of shallow mudflat water.

The model described fairly well the field data of weight and consumption of the different age classes of flounder. Moreover, dividing the simulation in two or more intervals with different *P*-values would probably increase the modelling efficiency, as demonstrated for the one-year flounder. It remains, however, questionable from a physiological point of view, whether the presented model is applicable to 0 group and >2 group flounder. Fonds *et al.* (1992) showed for plaice that there is probably a decrease of the optimum temperature for feeding and growth with increasing size of the fish. This suggests that in contrast to juveniles, larger fish avoid the higher temperatures on the mudflats. Although such relation was not found for flounder (Fonds *et al.,* 1992), it seems likely that juveniles have a higher optimum temperature for growth than adults.

In addition, flounder is known to be a catadromous species. It feeds and grows in estuaries during summer and in winter it returns to the sea where mature age groups (>2 group) spawn in early spring (Summers, 1979; Kerstan, 1991). In the Ythan, males increased gonad weight towards the end of their third year (as 2 group fish) and females towards the end of their fourth year (as 3 group fish), indicating that they would spawn for the first time as 3 and 4 group fish respectively (Summers, 1979). The weight of the gametes approximately accounts for 1 % - 3 % of the fish wet weight in males and 8 % - 16 % in females. For our simulation we assumed a resident non-spawning population, staying in the same area during the whole growing season and thus liable to the biotic and abiotic conditions of that area. Probably this only partially holds true for the population under study. However, since growth data of both sexes were combined in the field study, we were unable to allow for the cost of ovary and testis production. Spawning and the attendant migration towards the spawning grounds are an actual energetic cost for the mature age classes, through which the model probably overestimates growth and consumption in mature flounders.

The parameter uncertainty analysis demonstrated the sensitivity of model performance to parameters of consumption and respiration, showing the importance of accurate estimates of the coefficients and exponents of the allometric functions for maximum consumption and standard metabolism. In general, consumption-dependent prediction errors may be common in bioenergetics models and are probably the result of deficiencies in parameter values or assumptions within the parts of the models related to consumption (Bajer *et al.* 2003). In our study, the most critical parameters were taken from the same often-cited article (Fonds *et al.* 1992) and only in a few cases we resorted to 'species borrowing' from closely related (flatfish) species, which strengthens our believe in the robustness of our parameter choice. On the other hand, the Ythan study was not designed to check the validity of a bioenergetics model, which makes the data subject to some uncertainty (e.g. growth estimates based on length-frequency analysis). Therefore a field study in which the growth rate is based on otolith measurements may give a more reliable estimate of the field growth.

The presented model can be used as a framework for further studies on the ecology and bioenergetics of flounder and other flatfish species. A more accurate description of the thermal history of flounder in the field may further reduce the error in predicting the physiological behaviour of the fish. If other habitat characteristics such as dissolved oxygen, salinity and food availability can be incorporated, the model will be useful in spatially explicit measures of the environmental quality of nursery areas for these species (Brandt *et al.,* 1992).

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CHAPTER 6

A spatially-explicit bioenergetics model of habitat quality for flounder Platichthys flesus in the Scheldt estuary

6

Maarten Stevens, Joachim Maes and Frans Ollevier

Abstract

Habitat quality for a given fish species is determined by factors that enhance survival, growth and reproductive success of its individuals. As such fish growth rate can be used to estimate the quality of essential fish habitats like estuaries. If food is not limiting, then temperature is likely to be the main controlling factor for growth. A multivariable bioenergetics model was constructed for the European flounder to generate spatially-explicit estimates of growth in the Scheldt estuary. Digital maps of abiotic variables (temperature, oxygen concentration and salinity) for March, July and October were used as model inputs to evaluate spatial and temporal effects of water quality on growth rate potential. The model was run for two years (1998 and 2003) in order to describe any effect of improvement of the water quality on the estuarine-wide distribution of flounder. The model predicted that in March, when 0-group flounder is known to migrate upstream, growth is highest in the upper freshwater reaches of the estuary. This suggests that freshwater migration of flounder may be, at least partly, temperature driven. In July and October, low oxygen concentrations near the mouth of the main tributary probably prevent upstream migration. In summer and autumn, growth rate in the brackish part of the estuary was higher compared to the marine part. Field data on the distribution of flounder in the estuary showed that flounder abundance throughout the year was highest in the brackish part, where suitable prey items are readily available. Our results indicate that abiotic variables may be useful to predict the habitat use of diadromous species in estuaries. Given the importance of food in habitat selection, the model should be expanded with a foraging compartment to account for prey availability.

Keywords: *Bioenergetics model; Hypoxia; Migration; Platichthys flesus; Scheldt estuary*

Unpublished manuscript

1. Introduction

Estuaries are recognized as important nurseries for a wide variety of fishes, including both commercially and ecologically important species (Blaber and Blaber, 1980; Ruiz *et al.,* 1993; Paterson and Whitfield, 2000; Elliott and Hemingway, 2002). Within estuaries, a number of habitats have been identified as important fish habitats (Pihl *et al.,* 2002). High quality habitats are those where growth, survival and future reproductive potential are optimized for the species involved (Gibson, 1994; Le Pape *et al.,* 2003). Both biotic and environmental factors contribute to habitat quality. Amongst others, food availability, predators and water temperature have been identified as the most important ones. These factors influence each other and operate together or separately to affect growth and survival of a given species (Gibson, 1994; Yamashita *et al.,* 2001). The importance of environmental abiotic factors in regulating the growth of ectothermic animals is widely recognized and well documented (Fry, 1971; Yamashita *et al.,* 2001). Temperature is considered a controlling factor, setting the pace of physiological processes (Fonds *et al.,* 1992; Gibson, 1994; Yamashita *et al.,* 2001) and is an important determinant of estuarine fish abundance (Marshall and Elliott, 1998; Attrill and Power, 2004). Low oxygen concentration acts as a limiting factor by constraining food intake, conversion efficiency and metabolic rate (Bejda *et al.,* 1992; Tallqvist *et al.,* 1999). Most fishes also show avoidance behaviour when the oxygen concentration falls below a critical level (Jobling, 1994; Phelan *et al.,* 2000). Salinities that strongly deviate from plasma iso-osmotic concentration may increase the metabolic losses and hence reduce growth (Gutt, 1985; Yamashita *et al.,* 2001). In practice however, salinity seems to have only limited effects on growth and mainly controls distribution and movement of fishes (Malloy and Target, 1991; Gibson, 1994; Marshall and Elliott, 1998; Bœuf and Payan, 2001).

Under the assumption that optimal habitats are characterized by maximum growth, several authors have used growth rate of juvenile fish to evaluate habitat suitability. Some of the more common methods employed in these studies to investigate the effect of environmental factors on fish growth are enclosure studies (Phelan *et al.,* 2000; Meng *et al.,* 2001; Andersen *et al.,* 2005, Tarpgaard *et al.,* 2005), statistical modeling (Norcross *et al.,* 1997; Stoner *et al.,* 2001) and spatially-explicit bioenergetics models (Nislow *et al.,* 2000; Luo *et al.,* 2001; Tylor and Brandt, 2001; Höök *et al.,* 2003; Niklitschek and Secor, 2005). Caging studies are demanding time and effort and are usually site specific. Empirical models on their turn, often fail to explain the underlying ecological principles for habitat selection. In contrast, spatially explicit bioenergetics models incorporate the spatial distribution of fish and the physical conditions that affect foraging and growth and allow predictions over longer time.

The purpose of the present study was to develop a spatially explicit growth model to describe habitat suitability for flounder in the Scheldt estuary (Figure 6.1). The presented model only incorporates the effects of water quality parameters (temperature, dissolved oxygen and salinity) on the growth of flounder. It does not account for biotic regulators of habitat quality such as predation, prey availability, competition or foraging strategy (Nislow *et al.,* 2000; Tyler and Brandt, 2001) and should therefore be considered a baseline for further studies. Furthermore, the model describes the spatial patterns of growth along the longitudinal axis of the estuary; abiotic changes on a lateral scale (e.g. higher intertidal temperatures) are not included.

A first bioenergetics model for European flounder *Platichthys flesus* was implemented by Stevens *et al.* (2006; Chapter 5) and describes growth as a function of water temperature. In the present study, the model is extended with salinity and oxygen related parameters to describe growth changes along the environmental gradients in the Scheldt estuary. *P. flesus* is the only European flatfish that enters the freshwater reaches of estuaries (Beaumont and Mann, 1984; Weatherley, 1989; Kerstan, 1991). It has a broad osmoregulatory capacity and survives in a wide range of salinity conditions. 0-group flounder seem to prefer freshwater conditions in the field (Kerstan, 1991) as well as in the laboratory (Bos and Thiel, 2006). However, only a part of the population displays this freshwater migration and as such, this species should be classified as facultative catadromous (Bos, 2000). Several studies report that flounders in oligohaline and freshwater habitats grow faster than in marine areas (Beaumont and Mann, 1984; van Leeuwen and Vethaak, 1988). It is however difficult to separate the effects of temperature and salinity on growth in field studies, as summer water temperatures are generally highest in the upstream reaches of the estuary. In this respect, our modeling approach may help to disentangle the differential effect of both factors. *P. flesus* is one of the most common species in the mesohaline zone of the Scheldt estuary (Maes *et al.,* 2005b) and predominantly feeds on the rich benthic infauna of intertidal mudflats (Chapter 3). The freshwater distribution of flounder in the Scheldt estuary was demonstrated by catches near Gent and in the upstream reaches of tributaries (Buysse, 2003; C. Geeraerts, pers. comm.; Figure 6.1). This basin-wide distribution of flounder allows us to study the effects of abiotic environmental factors on habitat suitability in the entire Scheldt estuary.

To validate the model, we compare predicted growth with the distribution of flounder in the estuary. In this respect, we assume that flounder density will be greatest in areas with the highest suitability (growth). This concept is based in the Ideal Free Distribution (IFD) model of Fretwell and Lucas (1970). In this theoretical model, all animals are equal and free to move between habitats. The highest quality habitats are occupied first and as they fill and diminish in quality because of competition, lower quality habitats are occupied in sequence. As a result of this free movement, animals will distribute themselves over the available habitats until they all experience the same realized fitness (Gibson, 1994; Morris, 2003). It thus follows that population density should be greatest in habitats with the highest suitability. The distribution of animals may deviate from an IFD if dominant individuals that occupy the best habitats regulate subordinates into lesser habitats (Ideal Despotic Distribution; Fretwell and Lucas, 1970). As in our model the selection of suitable habitats is only determined by abiotic factors, we implicitly assume that competition for food is not important in the estuary. To test whether food availability could explain the distribution of flounder, we also compared the prey density in the estuary with the observed flounder abundance.

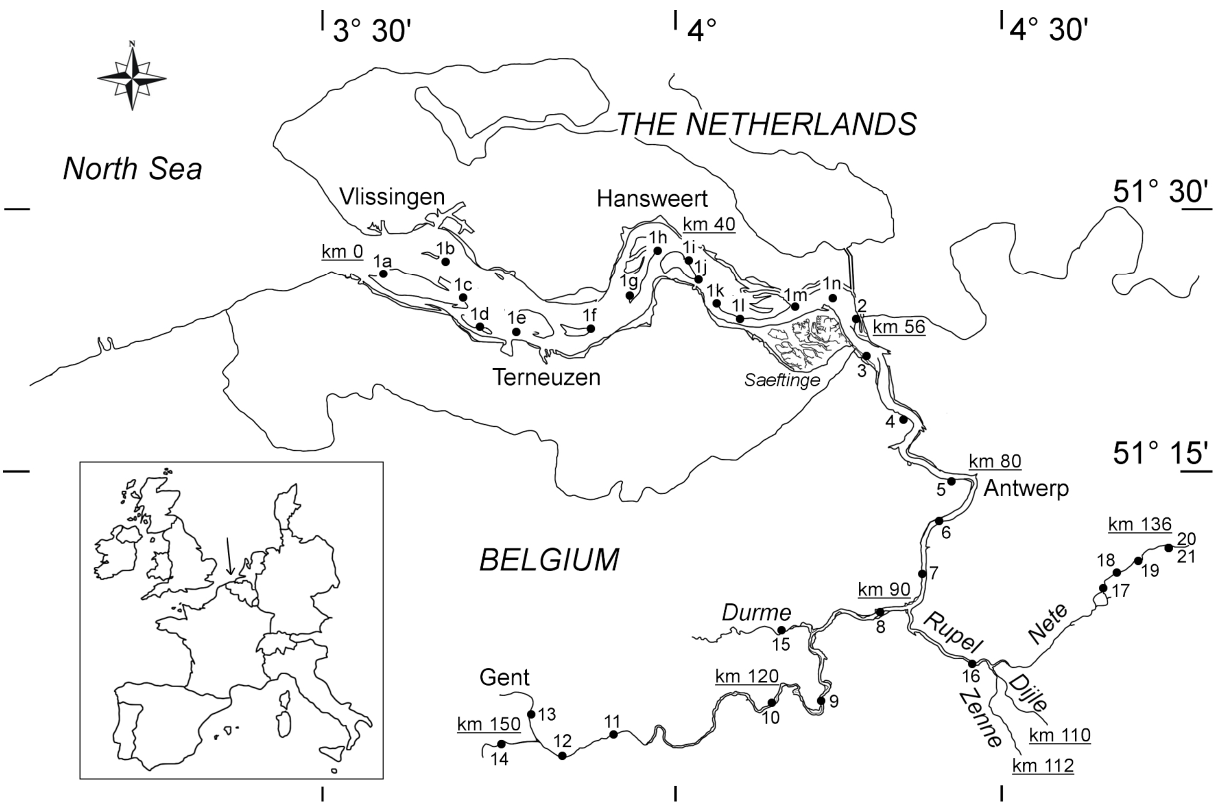

Figure 6.1. *Map of the Scheldt estuary with indication of the distance in kilometers, starting at the mouth of the estuary (Vlissingen). The major tributaries that have an open connection with the Scheldt are given in italics. Points (1-21) refer to the fish sampling locations (See also table 6.1).*

2. Materials and methods

2.1. Study area

The Scheldt is a highly urbanized river, which rises in the north of France and flows after 355 km into the North Sea near Vlissingen (The Netherlands). The river can be divided into the nontidal Upper Scheldt (from source to Gent) and the tidal part, extending from Vlissingen to Gent (150 km; Figure 6.1). The tidal part can be further divided into the Westerschelde (from Vlissingen to the Dutch-Belgian border) and the Zeeschelde (from the border to Gent). In Gent, the tidal influence is stopped by sluices. The tidal wave also enters the major tributaries Durme and Rupel. The latter receives largely untreated sewage water from the Brussels region via the River Zenne. The history of the Scheldt is characterized by pollution, eutrophication, loss of intertidal area, canalisation and habitat degradation (Meire *et al.,* 2005; Van Damme *et al.,* 2005). Between 1970 and 1980, anoxic conditions were regularly observed in the Zeeschelde. Due to wastewater treatment, oxygen concentrations increased during the eighties and the improvement continued in the nineties (Soetaert *et al.,* 2006). However, low oxygen concentrations still persist around the mouth of the Rupel (Van Damme *et al.,* 2005). Oxygen depletions in the freshwater part force fishes towards the oligohaline zone and probably prevent the building up of an established fish community in the limnetic zone (Maes *et al.,* 1998b).

2.2. Model description

The energy budget of juvenile (non-reproductive) fish is given as

$$(1)\qquad G = C - R - SDA - F - U$$

where G is the growth, C is the consumed energy, R the metabolic losses (standard + active metabolism), SDA the energy lost in metabolism for specific dynamic action and F and U the energetic losses in respectively faeces and excretion. This energy budget is affected by environmental factors such as temperature, dissolved oxygen and salinity and their interactions (Yamashita *et al.,* 2001). The growth of individual flounder in our habitat model is described as a function of fish weight (W; g), water temperature (T; °C), salinity (Sal) and oxygen concentration (DO; mg·L^{-1}).

$$(2)\qquad G = f(Sal)\cdot\left[f(DO)\cdot C - f(DO)\cdot R - f(DO)\cdot\{SDA + F + U\}\right]$$

The individual components of the energy budget equation were calculated in units of energy (Joules·day^{-1}) and converted to daily percentage weight increase (% of total fish weight). The relationship between length (cm) and weight (g) of flounder in the Scheldt estuary is given as $W = 0.013\cdot L^{2.94}$ (N = 555; R^2 = 0.95).

2.2.1. Temperature

When food is not limiting, temperature is a main factor controlling ingestion and metabolism and hence growth of fishes (Jobling, 1994). A temperature and weight-dependent bioenergetics model for growth of juvenile flounder in the Ythan estuary was developed by Stevens *et al.,* 2006 (Chapter 5). Here, the same model is applied to a flounder population in the brackish part of the Scheldt estuary. Each component of the energy budget equation is described as a function of water temperature and fish weight. A complete description of the model structure and parameters is given in chapter 5 (Stevens *et al.,* 2006). Here, we only mention that the daily consumption, necessary to account for observed growth, is obtained by adjusting the realized consumption to a constant proportion P of maximum consumption rates ($C = P\cdot C_{max}$). This estimated P-value will then be used as input in the spatially explicit habitat model.

2.2.2. Dissolved oxygen

Modeled consumption (C) and respiration (R) are modified by a proportional oxygen-dependence factor, which varies between 0 and 1. The other metabolic losses (SDA, F and U) are modeled as a proportion of the consumed energy (Chapter 5) and are modified by the same oxygen-dependence function as consumption. Decreases in DO reduce food intake and metabolism, leading to reduced growth (Jobling, 1994; Yamashita *et al.,* 2001). Generally, growth is unaffected above a critical oxygen concentration (DO_{crit}). Below this level, consumption and respiration are suppressed proportionally to the oxygen concentration. Below a minimum oxygen concentration (DO_{min}), consumption and respiration cease and fish die.

$$(3)\qquad f(DO)=\left|\begin{array}{ll} 0 & for\ DO < DO_{\min} \\ \dfrac{DO-DO_{\min}}{DO_{\min}-DO_{crit}} & for\ DO_{\min} \le DO \le DO_{crit} \\ 1 & for\ DO > DO_{crit} \end{array}\right.$$

Fish of several species are known to show avoidance reactions to waters deficient in oxygen. The avoidance response often appears to occur before the onset of respiratory distress (Jobling, 1994). Unless fish are locked under hypoxic conditions, they probably do not experience reduced growth rates due to low oxygen concentrations. The effect of oxygen on habitat quality should therefore be understood as the probability that flounder enters river sections with a given oxygen concentration. Compared to other flatfishes, flounder are quite tolerant to oxygen-poor conditions (Jørgensen and Mustafa, 1980; Tallqvist *et al.,* 1999). The critical and minimum DO levels for flounder were estimated from an experiment of Tallqvist *et al.* (1999). Both feeding activity and ventilation rate showed no effect of oxygen concentration above 4.3 $mg \cdot L^{-1}$. Below this threshold (DO_{crit}), predation and respiration decreased rapidly. We assumed that flounder stops feeding at 2 $mg \cdot L^{-1}$ (= DO_{min} for consumption) and dies at 1 $mg \cdot L^{-1}$ (= DO_{min} for respiration). Jobling (1994) suggested that it could be more appropriate to use oxygen saturation (%) rather than concentration ($mg \cdot L^{-1}$) as a criterion when examining critical oxygen requirements for growth. However, oxygen saturation data were not always available for the modeled period in the Scheldt estuary and we were confined to concentration measurements.

2.2.3. Salinity

The energetic cost of osmoregulation in euryhaline species is rather limited and is roughly estimated as 10 % of the total fish energy budget (Bœuf and Payan, 2001). In a growth experiment with juvenile flounder at a range of salinities, Gutt (1985) found that *P. flesus* grew faster at intermediary salinities (5 and 15) compared with extreme values (0 and 35). Furthermore, Bos and Thiel (2006) showed that 0-group flounder selected the lower salinity conditions in a laboratory migration experiment. In our model, we accounted for salinity by multiplying calculated growth by a salinity dependent factor. We assumed that growth is optimal at salinity 5 and decreases linearly with increasing or decreasing salinity:

$$(4)\qquad f(Sal)=\left|\begin{array}{ll} 0.033\cdot Sal+0.833 & for\ Sal \le 5 \\ -0.004\cdot Sal+1.021 & for\ Sal > 5 \end{array}\right.$$

The data of Gutt (1985) were used to estimate the effect of salinity on the proportion of maximum growth. Equation 4 predicts that growth in freshwater (< 1) and marine (35) conditions is still respectively 83 % and 87 % of the maximum rate.

2.3. Model calibration

We estimated the proportion of maximum consumption *P* by forcing the model through weight-at-age derived from field data of flounder in the brackish part of the Scheldt estuary. Data on the length (total length; mm) of flounder in 1994 and 1995 were obtained from the cooling water intake catches at the power plant of Doel (Maes *et al.,* 1998a) and fyke catches on a nearby mudflat (point 2 in figure 6.1). Size-at-age was estimated through modal progression analysis of length distributions, based on the Bhattacharya method (Bhattacharya, 1967). The software used in this analysis was FISAT II, (FAO, 2002). Because there were no data available from the fyke catches for the first three months of the year and because the larger size classes of flounder were underrepresented in the cooling water catches, we pooled the lengths of flounder of both sampling techniques. Modal progression analysis showed that there were no marked differences in the growth of different age-classes of flounder from the two samples (Figure 6.3b), which supports the merging of both data sets.

The energy content of flounder and prey were held constant throughout the year. The predator energy content was set at 4500 J·g^{-1} (Summers, 1974; Stevens *et al.,* 2006). Flounder in this part of the Scheldt estuary predominantly feed on *Corophium volutator* (Chapter 3), for which the average energy density was estimated at 18000 J·g^{-1} AFDW (Chambers and Milne, 1979; Dobrzycka and Szaniawska, 1995). Water quality parameters (temperature, salinity and oxygen concentration) were obtained from the Ministry of Transport, Public Works and Water Management of The Netherlands (http://www.waterbase.nl). Daily temperatures (365 days) used in the model simulation were determined by fitting a trigonometric function to the monthly temperatures (°C) of 1994 recorded near the Dutch Belgian border:

$$(5) \qquad T = 13.9 - 6.6 \cdot \cos(0.0175 \cdot day) - 4.2 \cdot \sin(0.0175 \cdot day)$$

where 0.0175 converts degrees to radians. As the oxygen concentration (*DO*; mg·L^{-1}) near the border was always above the critical level (4.3 mg·L^{-1}), the oxygen-dependence function was always equal to one. The model was run for juvenile (probably I-group) flounder. The mean length of flounder in January (95 mm) was taken as the start length of the cohort growth simulation. For August, November and December, too little data were available to determine a reliable estimate of the flounder length (Figure 6.2). Analysis of the length-frequency data of 0-group flounder from the same years, showed only a minor length increase in the two last months of the year (Figure 6.3b). Therefore, the length of flounder at the end of the model simulation (day 365) was set at 20 cm.

We tested the validity of the model by comparing the model predictions with field observations of the Scheldt population, using two test statistics: a reliability index (RI) and an index of modelling efficiency (MEF) (see chapter 5 for a complete description of these methods).

2.4. Spatial analysis

The growth model described above was used to develop a spatially explicit model of flounder growth in the Scheldt estuary. The model was run for flounder of 9.5 cm and the calculated *P*-value from the growth study (0.64) was used as an estimate of the proportion of maximum consumption. To be able to describe the seasonal distribution of flounder in the estuary, growth was calculated in March, July and October. This exercise was done for 1998 and 2003 in an attempt to describe any effect of improvement of the water quality of the Scheldt estuary on flounder distribution. Digital maps were constructed of water temperature, oxygen concentration and salinity in the Scheldt estuary. The Netherlands Institute of Ecology (CEMO) provided measurements of water quality of the Westerschelde. Data of the Zeeschelde and tributaries were obtained from the Flemish Environment Agency (http://www.vmm.be). Combining both datasets yielded 48 data points in 1998 and 56 in 2003 for the whole estuary. These georeferenced point values were interpolated using an Inverse Distance Weighting (IDW) method. Through this approach, we created for each month a continuous grid covering the study area. The resulting maps of the environmental variables were used as input in the growth model. All spatial analyses were done in GEOMEDIA GRID 5.2 (Intergraph Co.). The digital map of the Scheldt estuary was provided by the Research Institute for Nature and Forest (INBO).

The results of the spatially explicit growth model were compared with the field data on the distribution of *P. flesus* in the Scheldt estuary. However, these data are scattered in time and space and a combination of fishing techniques were used (Table 6.1), which makes it virtually impossible to compare the catches with each other. None of the studies describes the distribution of fishes over the complete length gradient of the estuary (Vlissingen to Gent). We therefore decided to combine all the available data and use presence/absence of flounder in the samples as an estimate of its distribution. Data on the distribution of flounder in the Westerschelde (Vlissingen to D-B border) were obtained from Hostens (2003). Fishes in the Zeeschelde (D-B border to Gent) were sampled with fyke nets (own data and Buysse, 2003) and stow nets (Maes *et al.,* 2001). The Research Institute for Nature and Forest (INBO) provided data of flounder in the Rivers Nete, Rupel and Durme (C. Geeraerts, pers. communication).

Prey availability was not incorporated in the model, but may be an important factor affecting habitat quality (Gibson, 1994). As already mentioned, *C. volutator* is the main prey item of flounder in the brackish part of the estuary (Chapter 3). In the freshwater regions however, the zoobenthic diversity is very low and the macrobenthic community is dominated by oligochaetes (Seys *et al.,* 1999a). There are no data available on the diet of flounder in the freshwater part, but it seems likely that oligochaetes make up the largest part of the flounder diet. If we assume (1) that habitat quality is determined by prey availability, (2) that prey density correlates with prey availability and (3) that population density increases with increasing habitat quality (Fretwell and Lucas, 1970), we can use prey density to test whether the distribution of flounder is affected by food availability. We therefore used data on the seasonal distribution of *C. volutator* and oligochaetes in the estuary as a substitute for prey availability. Macrobenthic biomass data (g AFDW·m^{-2}) were obtained from Sistermans *et al.* (2003) for the Westerschelde and from the Research Institute for Nature and Forest (INBO) for the Zeeschelde and tributaries. Prey biomass was converted to energy density (Joule m^{-2}) using energy contents of 18000 J·g^{-1} AFDW for *C. volutator* and 20000 J·g^{-1} AFDW for oligochaetes. Point measurements were interpolated linearly (IDW) to create a continuous grid of the study area. These prey energy maps were compared with the distribution of flounder in the estuary.

***Table 6.1**. Summary of the fishing locations in the Scheldt estuary. The points are indicated on figure 6.1. Years refer to the years in which the location was sampled.*

Location	Point	Method	1995	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
Westerschelde[4]	1a-1o	beam trawl	1989, 1990, 1991, 1992										
Bath	2	fyke net	x	x	x	x		x	x	x	x		
Schaar Ouden Doel	3	fyke net					x	x	x				
Liefkenshoek	4	fyke net					x	x	x		x	x	x
St.-Anna	5	fyke net								x	x	x	
Antwerp	6	fyke net		x	x			x	x	x	x	x	x
Antwerp[3]	6	stow net			x								
Kruibeke	7	fyke net		x									
Steendorp	8	fyke net		x	x			x	x	x	x	x	x
Kastel	9	fyke net		x	x			x	x	x	x	x	x
Dendermonde	10	fyke net		x									
Wetteren	11	fyke net		x									
Melle	12	fyke net		x									
Gentbrugge	13	fyke net		x									
Sluices Gent[2]	14	fyke net							x				
Durme	15	fyke net									x		
Rupel	16	fyke net									x		
Nete[1]	17-21	electricity								x	x		

[1] C. Geeraerts, Research Institute for Nature and Forest (INBO) (pers. communication)
[2] Buysse, 2003
[3] Maes *et al.,* 2001
[4] Hostens, 2003

3. Results

3.1. Growth of flounder in the brackish zone of the estuary

The length-frequency distribution of flounder in the brackish zone of the Scheldt estuary is given in figure 6.2. In the first part of the year, the population consists of juvenile fishes. They are probably the recruits from the previous year that stayed in the estuary during winter. From May on, the new 0-group flounder enter the estuary. In August, almost no larger flounder (> 15 cm) were caught, but they returned towards the autumn. The last part of the year is characterised by a low abundance of both juveniles and adults.

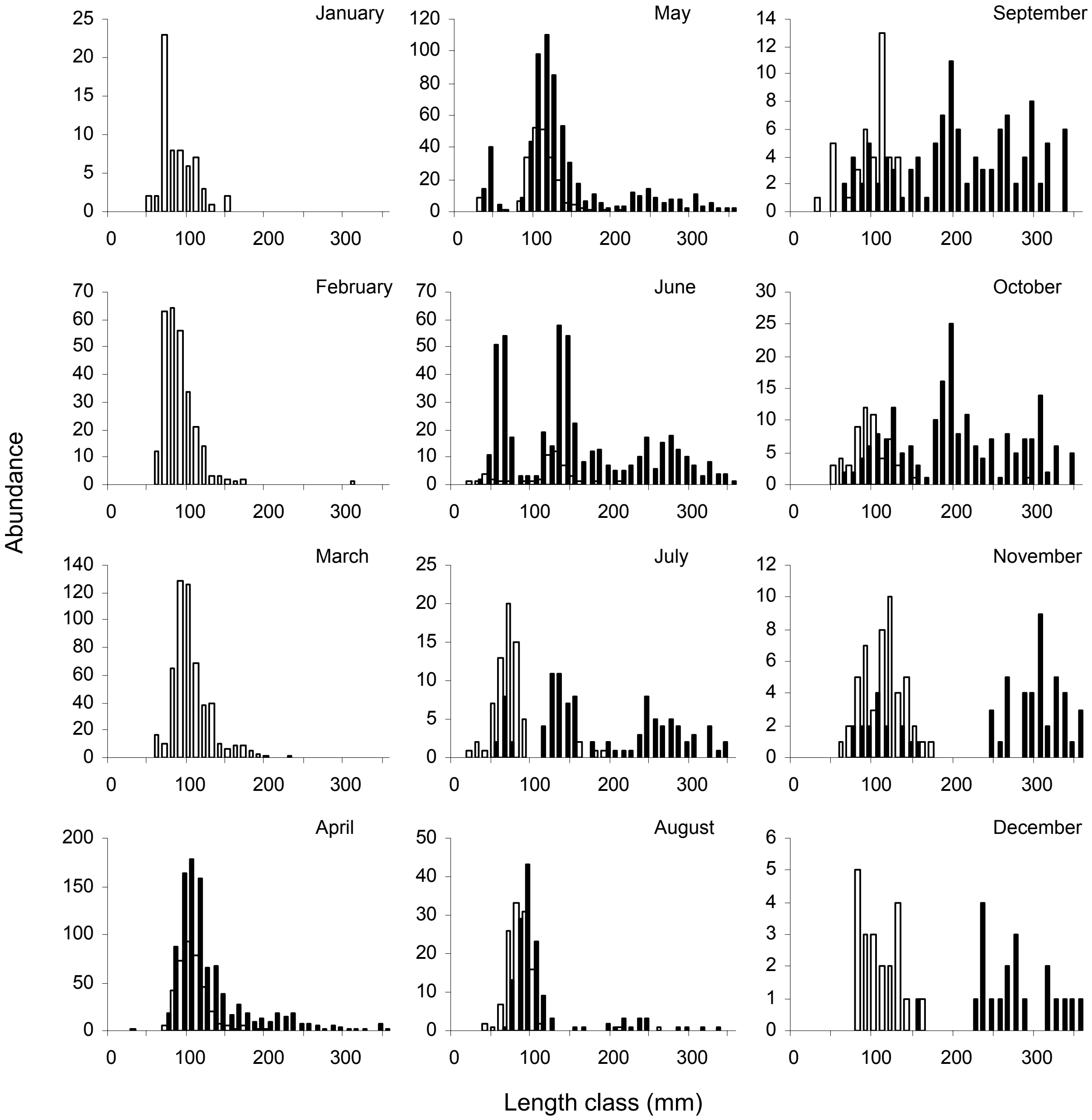

__Figure 6.2__. Monthly length - frequency distributions of flounder in the brackish part of the Scheldt estuary (1994-1995). The open bars represent the data from the catches of the power plant of Doel and the solid bars represent the fyke net catches.

The growth of *P. flesus* in the brackish zone was modelled as a function of water quality (temperature, salinity and DO) and fish weight (Figure 6.3b). The model closely fits the observed length of flounder in the field as indicated by the results of the model validation (RI = 1.03 and MEF = 0.92). The proportion *P* of maximum daily consumption was estimated at 0.64, meaning that the realised consumption was 64 % of the maximal possible consumption at the given water temperature. This proportion was further used in the spatially explicit growth model.

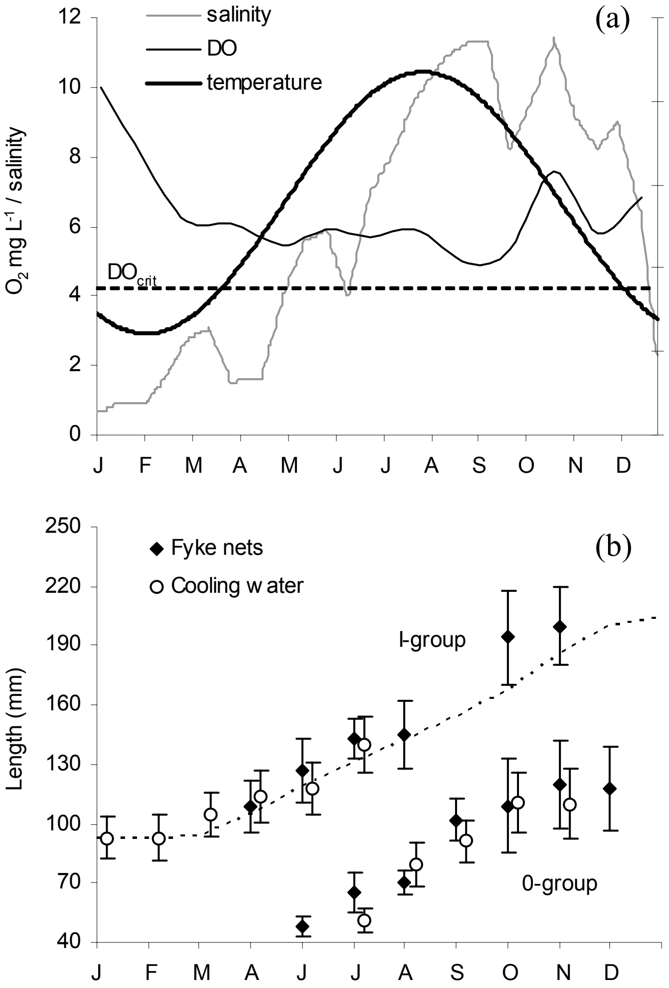

Figure 6.3.
Upper panel *(a) – Interannual variation in water quality of the Scheldt estuary near the Dutch-Belgian border in 1994. The oxygen concentration was always higher than DO_{crit} (4.3 mg·L^{-1}).*
Lower panel *(b) - Growth of flounder in the brackish part of the Scheldt estuary. Monthly mean lengths and standard errors were calculated with the Bhattacharya method from length-frequency data (figure 6.2). Data from the power plant at Doel and from the fyke net catches were lumped together for the model simulation. The dotted line represents the modelled length for I-group individuals. The coefficient of determination (r^2) for the observed and predicted values is 0.95.*

3.2. Growth of flounder in the Scheldt estuary

In the spatially explicit model, growth is affected by temperature, dissolved oxygen and salinity. As shown in figure 6.4, there were clear differences in water quality between the different zones of the estuary. In March and July, the water temperature was clearly higher in the upstream reaches of the Zeeschelde. However, the Nete, one of the larger tributaries, was generally colder than the rest of the estuary. In October, the warmest zones in the estuary were situated near Gent and, surprisingly, in the brackish section. Possibly, this could be related to the discharge of warmer (fresh)water from the harbour docks and a drainage canal near the Dutch side of the border. The temperature profiles in the estuary were similar in 1998 and 2003. In July 2003 however, the water was 2-3 °C warmer compared to 1998. The oxygen concentration was always higher in the Westerschelde and the Nete (Figure 6.4). Upstream of the D-B border, the oxygen concentration rapidly decreased towards the mouth of the Rupel tributary, which carries untreated domestic wastewater from the Brussels region (River Zenne). In 2003, the summer O_2 conditions improved towards Gent. The salinity is dependent on the freshwater discharge and shows a clear seasonal trend. The polyhaline zone (18-30 PSU) ranges from the mouth of the estuary to approximately 40 km upstream. The mesohaline (brackish; 5-18 PSU) zone is highly variable in space and time, stretching from km 37 to km 55 in

March 1998, and from km 48 to km 73 in October 2003. The freshwater zone refers to the oligohaline (0.5-5 PSU) and limnetic zone and is situated roughly upstream Antwerp.

In March of both years, the growth model of flounder in the estuary predicted weight loss for flounder near the mouth of the estuary and highest growth in the upper reaches near Gent (Figure 6.5). Apparently, the water temperature in the polyhaline zone (< 6 °C) was too low to allow growth. It should be mentioned however, that we assumed a constant *P*-value of 0.64 for the entire modelling environment. This value could be locally and temporally higher, still allowing growth where the present model predicted weight loss. Predicted growth in July and October was inhibited in a large part of the freshwater region. This was mostly due to the low oxygen concentration (< 4 $mg \cdot L^{-1}$). In July 2003, however, the negative predictions of growth in the upper reaches of the estuary were caused by the high water temperatures there. The model predicted favourable growth conditions in the River Nete throughout the year. However, access to this section of the estuary was probably prevented by the hypoxic conditions in the Rupel and the lower Zeeschelde in July and October. The same holds true for the upper Zeeschelde (between km 120 and Gent) in October 1998, when low O_2 concentration in the downstream section of the Zeeschelde hampered upstream migration to the head of the estuary. During summer, growth conditions were most favourable in the polyhaline and mesohaline zone. The higher temperatures in the brackish zone in October (Figure 6.4) resulted in the higher predicted growth rates between Saeftinge and Antwerp. The available area, where growth is optimal, was larger in 2003 because of improved oxygen conditions near Antwerp.

Biomass data of *C. volutator* and oligochaetes were transformed to the total amount of energy present per square meter (hereafter referred to as energy density). In March, energy density of the prey was much lower than in July and October. Oligochaetes were important in the first part of the year and reached their highest energy density in the lower Zeeschelde (Figure 6.6). In July, and to a lesser extent also in October, the amount of available energy was highest in the brackish zone. This was almost exclusively due to the high density of *C. volutator,* whereas oligochaetes accounted for the increased energy density in the Rupel and tributaries. The biomass of oligochaetes was determined indirectly by length conversion, which could have underestimated the actual energy density in the freshwater zone (I. Verbessem, pers. comm.).

The distribution of flounder in the catches in the estuary is represented in figure 6.6a. *P. flesus* occurs all over the estuary up to Gent and the upper reaches of the Nete, where upstream migration is prevented by barriers and sluices. Flounder is observed most in the brackish part of the estuary, where it is found in almost 100 % of the samples. The sampling effort concentrated in the brackish zone and the freshwater zone was only sampled sporadically. Because of this, we may have underestimated the distribution of flounder in the freshwater reaches. In March, flounder is sporadically caught in the freshwater part of the Zeeschelde and tributaries. In summer, flounder was never caught in the middle estuary. The flounder sample near Gent in July 2002, consisted of a large number of juveniles (Figure 6.7; Buysse, 2003). This location was monitored monthly in 2002, which showed that flounder occurred from June till September.

4. Discussion

The objective of this study was to estimate growth of flounder in the Scheldt estuary as a function of environmental abiotic parameters and to use growth to index habitat suitability. The model was not designed to predict actual values of growth rate, but to highlight the seasonal and spatial patterns in the estuary. The accurateness of the model predictions depends to a large extent on our understanding of how the different environmental variables influence habitat quality. We assumed that growth is enhanced in high quality habitats and that growth is affected amongst others by temperature, salinity and oxygen concentration. Although biotic factors such as competition, predation and prey availability are also important aspects of habitat quality, we focused on the abiotic factors as a first step. The effect of temperature on growth of flounder has been quite well studied (Fonds *et al.,* 1992; Yamashita *et al.,* 2001; Chapter 5). However, how salinity and oxygen affect growth is less well understood. The few studies that have examined the effect of oxygen concentration on growth of estuarine fishes demonstrated that reduced oxygen can significantly reduce food intake and hence growth rate (Bejda *et al.,* 1992; Jobling, 1994; Tallqvist *et al.,* 1999). As already mentioned before, fish show avoidance responses to hypoxic conditions and probably do not experience direct reduced growth rates due to low oxygen concentrations in open systems like the estuary. It is however possible that this avoidance response forces the fish to temporally retreat in lower quality habitats, where growth is lower. Salinity seems to have only limited effect on growth and is generally considered to be a controlling factor for distribution and movement (Gibson, 1994). In the model, salinity affects the habitat quality by directly regulating growth. A possible physiological explanation for this might be that the cost of osmoregulation is lower at salinities close to the fish plasma concentration (Yamashita *et al.,* 2001; Andersen *et al.,* 2005). This could explain the distribution of flounder in the mesohaline zone (18-5 PSU), but not the migration of juveniles into the (hypo-osmotic) freshwater zone.

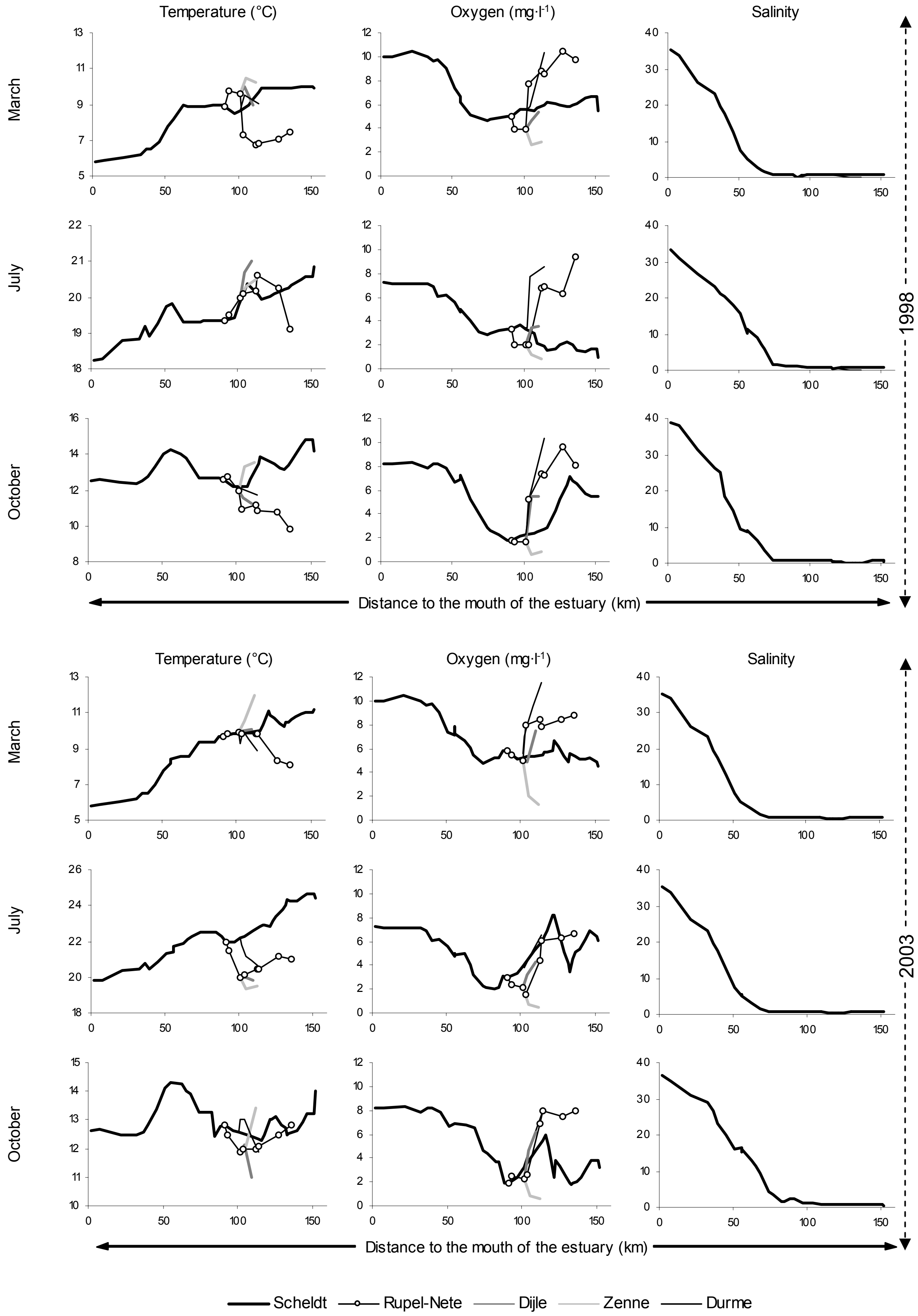

Figure 6.4. Monthly water quality parameters in the Scheldt estuary for 1998 (upper panels) and 2003 (lower panels). Tributaries enter the Scheldt after 91 km (Rupel) and 101 km (Durme) and are indicated separately.

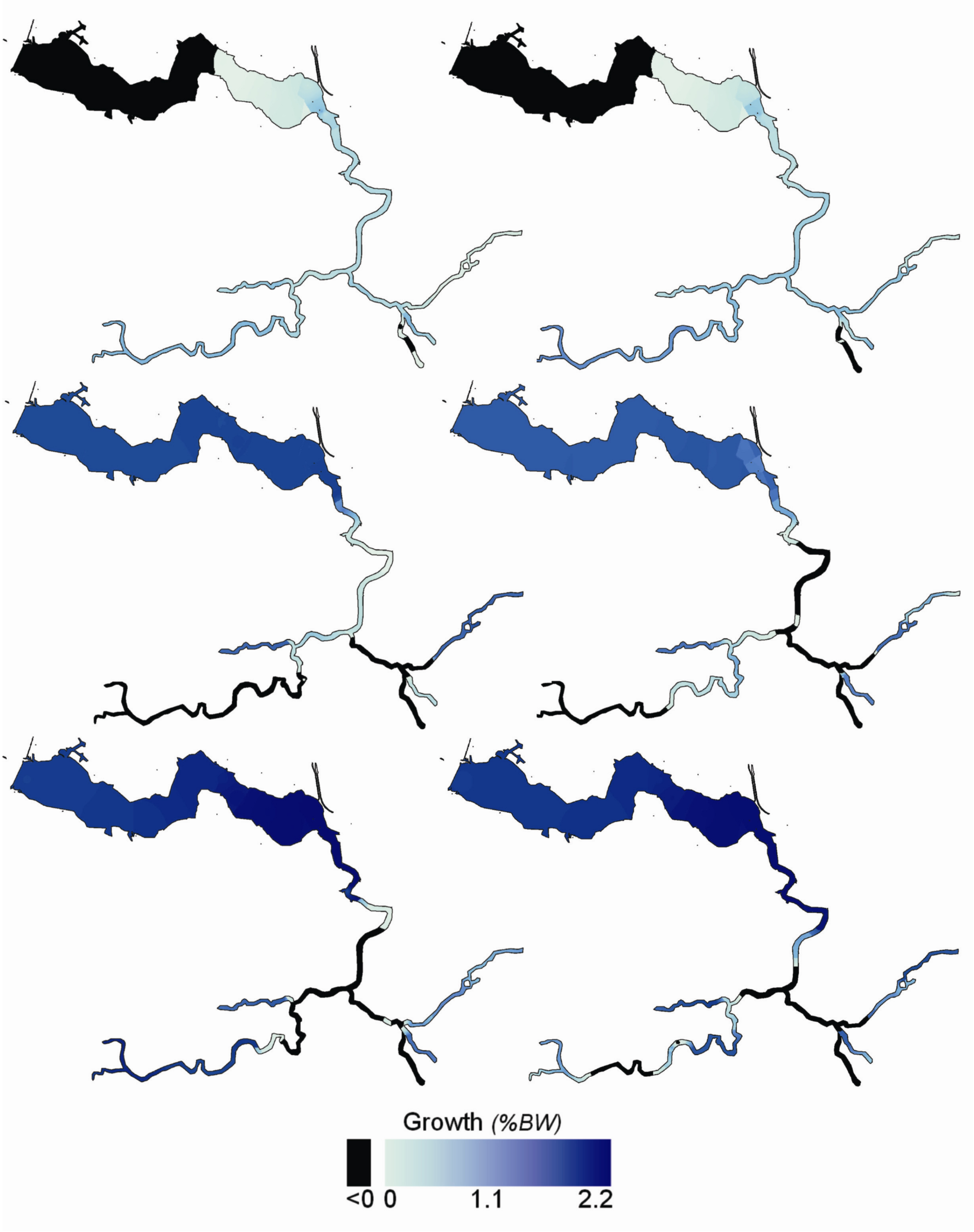

Figure 6.5. *Growth (% BW) of flounder in the Scheldt estuary, as predicted by the spatially-explicit growth model. Zones where growth was negative (weight loss) are indicated in black. Areas where growth is maximal are circled with a dotted line.*

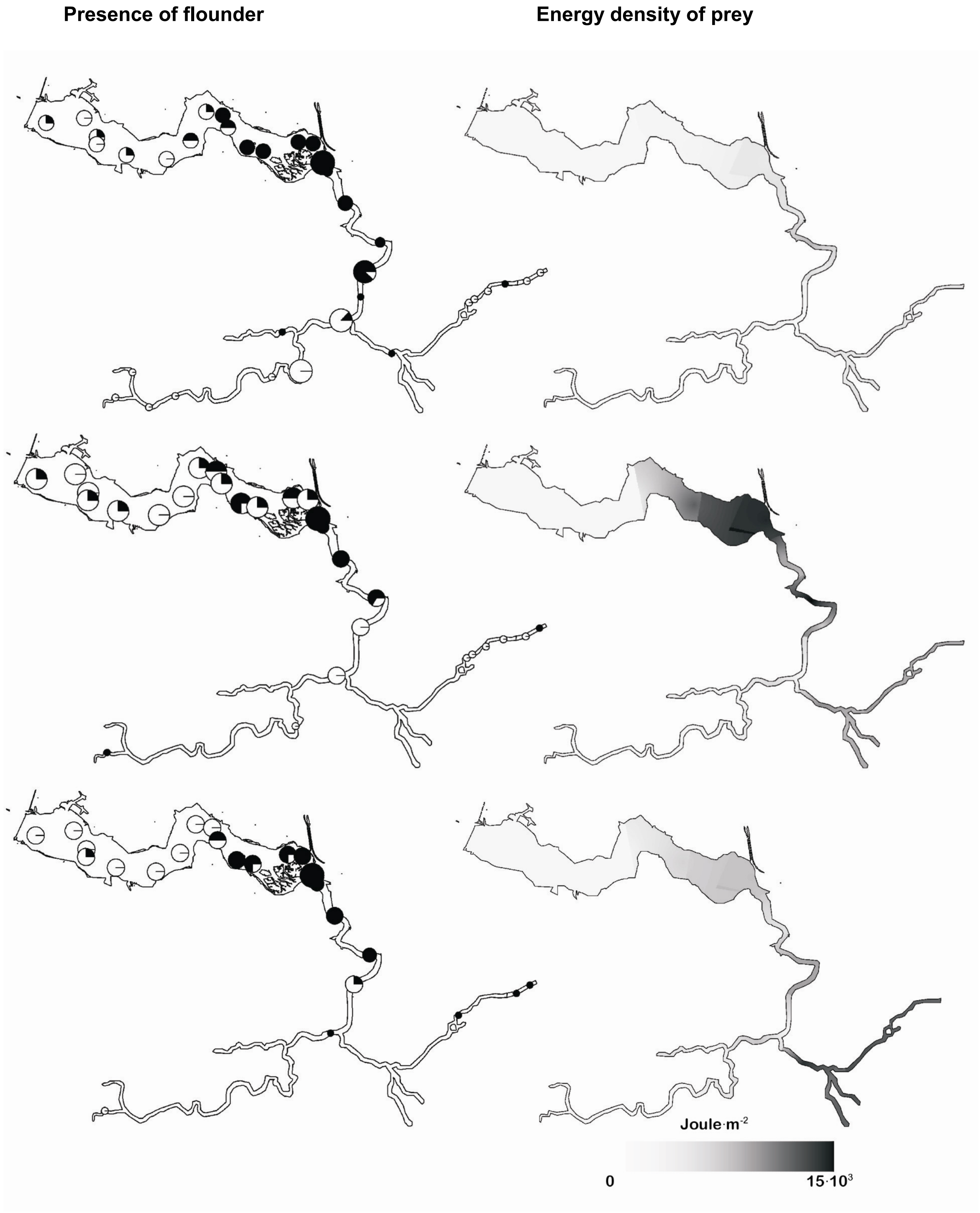

Figure 6.6. *Left panels: monthly distribution of flounder in the Scheldt estuary. Data were lumped over all years (Table 6.1). The size of the circle is proportional to the number of samples at that location. Black represents the proportion of samples in which flounder was present, white the proportion without flounder. Right panels: monthly combined energy density (J·m^{-2}) of* C. volutator *and oligochaetes.*

The results of our model demonstrate that spatial differences in water quality may determine the distribution of flounder in the Scheldt estuary. Unfavourable temperature conditions in the lower estuary and higher temperatures in the head of the estuary may trigger upstream migration of juvenile flounder during the first part of the year. Flounder are known to spawn from mid January until April in the southern North Sea (Russel, 1976; Van der Land, 1991), after which the larvae are transported to coastal nursery areas using selective tidal stream transport (STST, Jager *et al.,* 1999). The (post-)larval stages (± 10 mm) were observed in the Westerschelde in April (Beyst *et al.,* 1999b). In the estuary the metamorphosing juveniles migrate further into the brackish and freshwater reaches. Post-settlement stages of flounder (± 30 mm) are recorded in the cooling water catches of the power plant at Doel as early as May (Maes *et al.,* 1998a). During a monitoring study near the sluices in Gent in 2002, 0-group flounder were observed from July (mean length ± 45 mm) until September (mean length ± 90 mm) (Figure 6.7; Buysse, 2003). This indicates that mainly 0-group flounder migrate into the freshwater reaches and that, if temperature and DO allow, they stay there to feed and grow during summer.

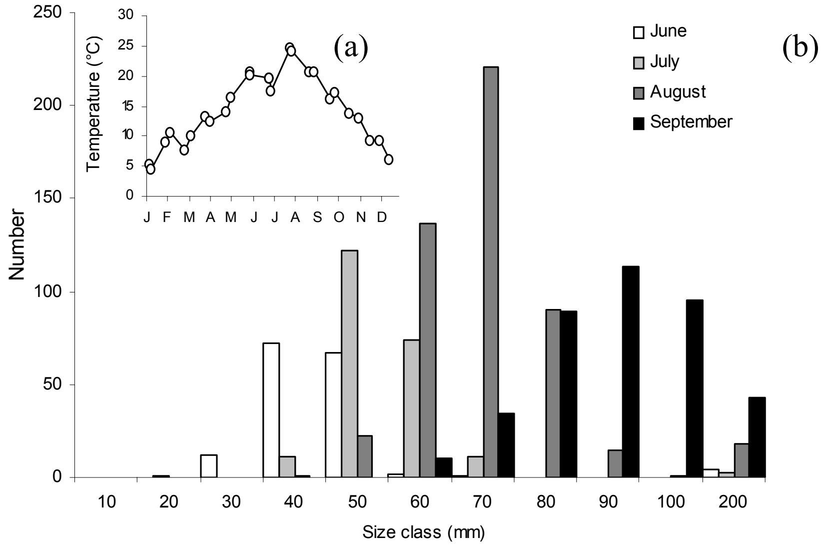

***Figure 6.7**. (a) Monthly water temperature of the upper Zeeschelde near Gent in 2002 (http://www.vmm.be). (b) Length distribution of* P. flesus *in 2002, near the sluices in Gent. Flounder were sampled monthly with fyke nets. Data courtesy of the Research Institute for Nature and Forest (Buysse, 2003).*

In summer, growth is potentially optimal in the entire estuary, if locally temperatures are not too high. However, fish are shut off from the upper estuarine reaches due to hypoxic conditions in the River Rupel and in the Zeeschelde between Antwerp and the River Durme. In this respect, it is also possible that fish, which migrated to the head of the estuary during spring and early summer, are locked in the freshwater zone by anoxic conditions in the River Rupel and the downstream parts of the Zeeschelde. In October, growth is highest in the mesohaline zone, where flounder is recorded most frequently throughout the year. The data of Buysse (2003; Figure 6.7) suggest that flounder leave the freshwater reaches in autumn. Possibly, a fast decrease of the water temperature by the end of September may trigger downstream migration to the more favourable (warmer) brackish zone. Although the overall trends in water quality were quite consistent between years, it should be stressed that high inter-annual variation in suitable habitat may occur. In warm and dry summers with low discharge, oxygen concentration and temperature may be limiting in large parts of the limnetic and oligohaline zones of the estuary (Van Damme *et al.,* 2005). As mainly 0-group flounder is observed to migrate into freshwater, warm years will probably only affect this year class.

Although it is unmistakable that abiotic factors are important in controlling growth and hence habitat quality for fish, other factors such as food, predation, water depth and habitat structure may be at least equal important (Gibson, 1994). Of the biotic factors, the availability of suitable prey items is probably the most trivial one. Food was not incorporated in the model, but was only used to interpret flounder distribution. Most habitat models that incorporate a foraging submodel were constructed for pelagic (filter) feeding fish (Brandt and Kirsch, 1993; Tyler and Brandt, 2001; Maes *et al.,* 2005a). The few foraging models available for demersal and benthic feeding fish are data hungry and require considerable information about feeding strategy and prey availability (Rose and Cowan, 1993; Rose *et al.,* 1996). Realistic results from a similar model for flounder can only be obtained if more information is available on the foraging strategy of this species. For now, prey density may be regarded as a substitute for prey availability and is considered to be positively related to habitat quality (Fretwell and Lucas, 1970). The prey densities in the Scheldt estuary were much higher in the mesohaline zone than in the freshwater zone. This is mainly due to the high productivity of *C. volutator* in the brackish zone, where it is the preferred prey item for most intertidally feeding fish (Chapter 3). Benthic biomass in the Scheldt estuary shows a clear gradient, from high biomass in the marine and brackish zone, to low biomass in the freshwater zone. Molluscs (*Cerastoderma edule*) and polychaetes (*Heteromastus filiformis*) dominate in the marine zone, *Nereis diversicolor* and *C. volutator* in the brackish zone and Oligochaetes in the freshwater zone (Ysebaert *et al.,* 1993). A high prey biomass doesn’t necessarily imply high prey availability. Prey availability is in part determined by the maximum prey size the predator can ingest and by prey detectability (Moore and Moore, 1976; Mattila and Bonsdorff, 1998). Most molluscs are probably too large for juvenile flounder to consume in total, although Mattila and Bonsdorff (1998) showed that small *Macoma balthica* (2 mm) were preferentially taken if offered together with a mobile amphipod. However, some (larger) prey can still be important for smaller fish as they are only consumed partly. Several studies indicate the importance of siphon cropping of bivalves and tail tipping of annelids in the diet of mainly larger flatfish (De Vlas, 1979; Summers, 1980; Hostens and Mees, 1999; Seys *et al.,* 1999b; Link *et al.,* 2005). Annelids are hidden in the sediment and are only available to flounder when they are present in the top centimetre of the sediment (Summers, 1980; Ysebaert *et al.,* 2005). *C. volutator* on the other hand, is probably much more available to flounder. It is present in high densities in the top centimeter of the sediment and is known to regularly disperse into the water column, where they are more susceptible to demersal feeding fish (McCurdy *et al.,* 2005). The high availability of *C. volutator* in the mesohaline zone may partly explain the distribution of *P. flesus* in summer, when the model predicts high growth rates in the entire Westerschelde, while flounder is mainly caught in the mesohaline zone.

Other biotic factors that may influence habitat suitability include competition and predation. Competition as a driving factor for migration of flounder into freshwater was suggested by Beaumont and Mann (1984). This implies that food is limiting in the other parts of the estuary. However, the general consensus seems to be that resources are not limiting in estuaries due to the superabundance of available prey and hence, competition is avoided (Chapter 3 and 4). Since this study only discusses habitat quality for flounder along the longitudinal axis of the estuary, the predator-refuge function of intertidal migration will not be further discussed here. However, predation might also influence habitat suitability along the longitudinal gradient of the estuary. By migration into freshwater, juvenile flounder may avoid their predators, which are less tolerant to lower salinities. Shrimps (Crangonidae) are considered to be one of the most important predators of small 0-group flatfish in shallow coastal waters (Taylor and Collie, 2003; van der Veer *et al.,* 1991; van der Veer, 2000b). It is suggested that flatfishes reach a size refuge for crustacean predation at a length of 25 mm (Taylor and Collie, 2003). *Crangon crangon* become abundant in the Westerschelde from June onwards (Hostens, 2003), which coincides with the occurrence of 0-group flounder in Doel and in the upper reaches (Maes *et al.,* 1998a; Buysse, 2003). However, the flounder observed in the oligohaline and freshwater zone are probably already too big (> 30 mm) to be preyed upon by shrimps. In addition, we have no indication that mortality of 0-group flounder in the estuary is significantly affected by (marine) fish predation.

Conclusion

The model presented estimates the habitat quality for juvenile flounder along the longitudinal axis of the Scheldt estuary. We assumed that high quality habitats are those in which growth of juvenile flounder is enhanced and that growth is primarily affected by abiotic factors. Our results suggest that if food is not limiting, temperature sets the pace of growth and relative prey availability may determine the distribution within the suitable habitat. Freshwater migration seems to be mainly important for 0-group flounder, which are attracted by the higher temperatures in spring, resulting in higher growth rates. Juvenile (≥ I-group) and adult flounder avoid the lower unfavourable oxygen conditions and stay in the mesohaline and upper oligohaline zone. Here, suitable prey items like *C. volutator* are superabundant and readily available. Although they were not incorporated in the model and as such we can only speculate about their role, competition and predation seem to be less important in regulating the distribution of juvenile flounder in the estuary. The model does not make quantitative predictions about the estuarine habitat use of flounder. This necessarily implies that food availability is incorporated into the model. If the model is extended with a foraging submodel, then habitat quantity for benthivorous flounder can be estimated from the available intertidal surface area. When using growth rate potential to predict the distribution of fishes in the field, one should take care about the habitat choice behavior of the studied species. Tyler and Brandt (2001) showed that bioenergetics models not always effectively predict fish growth and distribution. Individual-based models do not include the effects of habitat selection or competition on fish population predictions. Generally, the selection of a habitat by a given species is influenced by a trade-off between multiple environmental factors (e.g. food, temperature, refuge, etc.). To account for the hierarchy of these factors in habitat selection, the design of individual-based models should therefore include submodels of habitat choice (Tyler and Brandt, 2001; Wildhaber and Lamberson, 2004).

A demerit of the present study is the lack of reliable field data for the evaluation of model performance. There is an urgent need for a consistent monitoring programme in the entire estuary to evaluate habitat use by migratory fishes. Furthermore, the planned sewage treatment of the Brussels region offers a unique opportunity to investigate how the estuarine fish community, and the diadromous species in particular, respond to water quality restoration. In this respect, the presented model can be used as a tool to predict how the fish communities will respond to the improvement of the water quality. Although our model is certainly an oversimplification of the mechanisms involved in the regulation of habitat suitability and ultimately reproductive potential, it may be considered as a baseline for further definition of essential fish habitats.

Acknowledgements

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CHAPTER 7

General discussion

7

In this final chapter we summarize the major findings of the previous chapters and formulate our final analysis and conclusions. The aim of this thesis was to describe the habitat use of fishes in the Scheldt estuary on both a local (intertidal mudflat) and regional (estuary) scale. We examined the importance of intertidal migration for fishes and discussed the different factors that affect the intertidal habitat quality (**first part**). Chapter 2 described the migration and zonation patterns of the fish community on a brackish water mudflat. As foraging is one of the main triggers for intertidal migration, the diet and feeding strategy of the intertidal fishes was studied in chapter 3. The high niche overlap among flatfishes observed, suggested that competition might be a structuring force for the intertidal fish community. Competition can only occur when resources are limited, i.e. when the competing predators deplete their prey populations. The effects of epibenthic predators on the macrobenthic community were examined in two exclosure experiments (Chapter 4). In the **second part**, we described the effect of abiotic factors on the use of the Scheldt estuary by European flounder *Platichthys flesus*. In chapter 5 we constructed a bioenergetics model which describes growth of flounder as a function of temperature. This model was further extended with oxygen and salinity dependent functions to estimate the habitat quality for flounder in the Scheldt estuary (Chapter 6). The main results of these chapters can be summarized in six theses:

T. 1 Most estuarine fishes utilize the intertidal area either in an opportunistic (aided by the tidal currents) or compulsory way. Only flatfishes seem to be bounded to intertidal migration to exploit the abundant benthic food resources, whereas piscivorous predation seems not important as a trigger for intertidal migration (Chapter 2 & 3).

T. 2 Zonation of fishes on the studied mudflat, when observed, is mainly the result of species-specific differences in mobility. When fish densities are high, spatial segregation on the mudflat may arise to avoid competition for food (Chapter 2 & 3).

T. 3 The intertidal fish community is characterized by generalist feeders and their diet largely reflects the relative availability of prey species (e.g. *C. volutator*) (Chapter 3).

T. 4 Fishes and birds have a negligible effect on the abundance of their infaunal prey species, but they may affect the prey size spectrum. In the absence of predation, infaunal interactions become more important and may regulate the macrobenthic community structure (Chapter 4).

T. 5 The carrying capacity of the estuary for fishes is probably not reached, but it may happen in years with high fish recruitment (Chapter 3 & 4, this discussion).

T. 6 When food is not limiting, temperature is an important factor determining habitat selection in the estuary. Oxygen depletion limits the use of the available habitats in the freshwater reaches of the Scheldt estuary (Chapter 5 & 6).

These theses will be further developed in the following paragraphs. Given the importance of food availability as a steering factor for intertidal habitat use, we will elaborate on the trophic interactions on the mudflat and their effects on the estuarine fish community. In this respect, we discuss the possibility for the presence of a carrying capacity for fishes in the Scheldt estuary. We also present a generalized food web for the intertidal estuarine zone and quantify the major energy fluxes and compartments of this web.

1. The importance of intertidal migration for fishes

The migration of fishes in and out the intertidal zone has been studied extensively (Gibson, 1969; Gibson, 1993; Horn and Martin, 1999). The vast majority of these studies focused on marine habitats and are limited to rocky (Horn and Martin, 1999) and sandy (Gibson, 1973) shores. In contrast, intertidal migration on estuarine soft sediment habitats is less studied. Although the cues for intertidal migration on estuarine and marine shores are likely to be the same, their relative importance may be somewhat different because of the specific nature of the estuarine system. The increased turbidity and lower abundance of large piscivores in estuaries may reduce the importance of predation as a cue for intertidal migration. Other important differences between marine and estuarine shores that may cause differences in the use of the intertidal zone are the high variability of the abiotic estuarine environment and the structure of the substratum, which generally consists of muddier sediments in estuaries. In addition, the concentration of juvenile fishes in estuarine nurseries may intensify competitive interactions and hence influence the distribution of species. Intertidal fishes on rocky shores generally display a strict zonation, whereas on sandy shores, the whole intertidal is much more uniform and zonation patterns are less clear.

As our samples were representative of the fish community of the Beneden Zeeschelde, our study showed that most estuarine fishes enter the intertidal zone (Chapter 2; Maes *et al.,* 2005b). The fishes migrated onto the mudflat either actively looking for food (flatfishes) or passively transported by the tidal currents (pelagic species). Catches were dominated by flatfishes, which preyed on the dense macrobenthic species. The intertidal area seems vital for food supply (Chapter 3). The (semi-)pelagic species can be considered vagrants on the mudflat. They possibly follow their migrating epibenthic prey (e.g. mysids and shrimps) and may find a valuable supplement to their diet in the infauna that disperses into the water column (e.g. *Corophium volutator*). Feeding seems to be the most likely trigger for intertidal migration. Almost all flatfishes had macrobenthic prey in their stomachs, which are abundant and readily available in the intertidal zone. Two other factors that are known to influence intertidal migration are temperature (growth) and predation (survival). As a result of the well-mixed nature of estuaries, the vertical temperature gradients are either small or negligible. A significant temperature differential between the shallow intertidal and deeper subtidal may only be observed during windless conditions and when the air temperature is clearly different from the water temperature (warm summer or cold winter days). Furthermore, estuarine fishes are generally eurytopic species and adapted to the highly variable environment, so it seems less likely that they gain a substantial (growth) benefit from relatively small temperature increases in the intertidal zone. However, temperature differences may be important for growth along the longitudinal axis of the estuary as was shown in chapter 6.

The overwhelming presence of juveniles confirms the nursery status of this part of the estuary. Beck *et al*. (2001) defined a nursery as a habitat for juveniles of a particular species if its contribution per unit area to the production of individuals that recruit to adult populations is greater, on average, than production from other habitats in which juveniles occur. Several studies showed that survival is enhanced by migration into estuaries (Blaber and Blaber, 1980; Maes *et al.,* 2005a) and into the intertidal zone of marine sandy beaches (Burrows, 1994; Gibson *et al.,* 2002). It is however unclear whether intertidal migration in estuaries directly influences the survival of juvenile fishes by reducing the predation risk. The fishes in our study that actively migrated onto the mudflat were probably too large to be consumed by piscivorous fishes (Chapter 2). Furthermore, it was suggested that the turbidity makes predation unlikely as a strong driving force for intertidal migration in estuaries.

We had expected to find a zonation of fishes on the mudflat, according to their size or to species-specific habitat use. However, clear patterns were absent, probably because of the homogenous nature of the intertidal zone (prey distribution, abiotic conditions, absence of predation). Mobility may limit the intertidal distribution of some species, as was shown for the less mobile gobies, which were restricted to the lower and middle shore. We did find (weak) evidence for density-dependent zonation in flatfishes. In the year with high fish density, *Platichthys flesus* moved up higher on the mudflat. This might suggest that interspecific competition (in this case with *Solea solea*) can regulate the vertical distribution of fishes, especially when food is limiting. This kind of spatial resource separation was also suggested for *Pleuronectes platessa* in the Wadden Sea, which move onshore when there is competition for food (Berghahn, 1987).

2. Muddy trophic interactions

The possible structuring effect of trophic competition was further examined in a diet study of the intertidal fish community, in which we combined prey availability with prey consumption (Chapter 3). This study showed that all fish species on the mudflat, without exception, fed to a more or lesser extent on *Corophium volutator*. The importance of prey species in the diet of fishes reflected the prey availability in the field, confirming the generalist and opportunistic feeding nature of estuarine fishes. The analysis of niche overlap indicated that there was a significant dietary overlap between the two flatfish species flounder and sole. This suggests possible competition between these two species. Competition is only likely if food resources are limiting, which implies that fish predators can deplete their prey populations. In our study, the macrobenthic prey community was sampled simultaneously with the intertidal fish community (Chapter 3 and 4). The analysis of the macrobenthic samples showed that in a year with a high fish abundance (2001), the density of *C. volutator* had dropped significantly from August to October on the lower and middle shore, but not on the higher shore. This was not the case in the following year (2002), when fish abundance on the mudflat was four times lower. As the lower parts on the shore are inundated for longer periods, the macrobenthos will be more exposed to fish predation, which could explain the observed decrease in that part of the intertidal zone in the high fish density year.

We examined the effect of epibenthic predation on the infaunal community by exclosure experiments in which both fish and birds were excluded. Birds were also taken into account because they can have significant effects on the macrobenthic community (Daborn *et al.,* 1993; Goss-Custard *et al.,* 2001). Fish and bird predation did not have a significant direct effect on the abundance of macrobenthic species. Both predators select the larger size classes of the macrobenthic species, but only birds influence the size distribution of their prey. Our results also suggest that in the absence of predation, infaunal interactions like competition could become more important and can regulate the benthic community structure. From the exclosure experiments, it was found that fish predation was relatively unimportant as a structuring factor for the macrobenthic prey community. However, the fish density during those experiments was lower than in 2001 (see previous paragraph). The high interannual variation in fish recruitment makes it difficult to predict the outcome of fish exclosure studies. Probably, fishes significantly affect the prey abundance only when their population is close to the carrying capacity of the system.

The lack of clear direct effects of predation on the abundance of organisms at the lower trophic levels, suggests that the interaction strength in the benthic food web is rather weak. The strength of the interaction between two consecutive trophic levels determines the food web stability, with weak links generally supporting food web stability (Woodward *et al.,* 2005). Most well studied food webs show only a few strong interactions in a matrix of weak interactions, making the effect of trophic cascades unlikely (Raffaelli and Hall, 1992; Neutel *et al.,* 2002; Bascompte *et al.,* 2005). What determines the strength of these interactions? Emmerson and Raffaelli (2004) showed that the *per capita* effect of predators on their prey scales with **predator–prey body size ratio**, with an exponent of around 0.6. This indicates that the size of predators relative to their prey determines the strength of trophic interactions (Shurin and Seabloom, 2005). This might partly explain why the effect of shorebird predation on benthic food webs is generally more pronounced than the effect of fish predation (Quammen, 1984; Daborn *et al.,* 1993; Wootton, 1997; Hamilton, 2000; Goss-Custard *et al.,* 2001). The effect of predation is affected by the **availability of the prey** organisms. The vulnerability of prey depends on the possibility to hide in the sediment or between the vegetation (Barnes and Hughes, 1999). Organisms that burry in the sediment are often consumed only partly (e.g. tail tips of polychaetes and siphons of bivalves) by the predators that move over the surface of the sediment. The three-dimensional structure of soft sediments may also reduce the intensity of infaunal competition.

It is believed that soft sediment habitats generally **lack keystone predators** (Raffaelli and Hawkins, 1996). The attribute 'keystone' refers to the effect the predator has on a competitive superior prey species, which in the absence of predation may become dominant. As a result of the lack of strong competitive interactions, direct effects of keystone predators may be less obvious in soft sediments. Effects of predation in soft sediments may be further dampened by the complexity of the benthic food web. This complexity may, at least partly, be the result of the generalist and omnivorous diet of many of the species. It is suggested that **omnivory** dampens top-down control by predators and that greater omnivory leads to weaker trophic cascades (Shurin *et al.,* 2006; Vandermeer, 2006). Omnivory is widespread in the intertidal food web of our study (Figure 7.1). Most fishes feed on primary consumers (*C. volutator*) as well as on secondary consumers (*C. crangon*). Omnivory was also observed for fish feeding on gobies and for *N. diversicolor* feeding on *C. volutator*. The latter link was not examined in our study, but *N. diversicolor* is known to be omnivorous and feeding on *C. volutator* (Commito and Ambrose, 1985; Ölaffson and Persson, 1986).

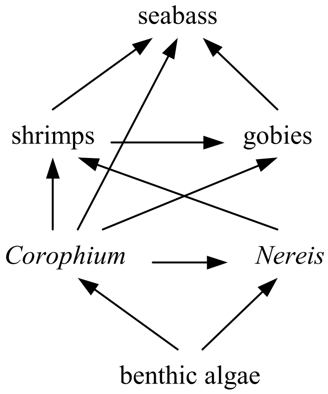


Figure 7.1. *Example of omnivory in the benthic food web of the Scheldt mudflats. Four omnivorous loops can be observed:*

1 – Benthic algae → Corophium → Nereis
2 – Corophium → Nereis → *shrimps*
3 – Corophium → *shrimps* → *gobies/seabass*
4 – Corophium → *gobies* → *seabass*

In order to estimate the efficiency of the energy transfer through the intertidal food web, we quantified the energy fluxes on the mudflat. The aims of this exercise were to obtain a first idea of the importance of the different components in the benthic food web and to estimate the amount of energy available to the higher trophic levels. Because of the illustrative nature of this exercise, the calculations were confined to only six compartments: organic matter, benthic algae, macrobenthos, shrimps, fishes and shorebirds. Where appropriate, we calculated for each component the biomass (standing stock), production (growth), consumption, respiration and metabolic losses (faeces and excretes). All the calculations, except for the fish compartment, are taken from Wilson and Parkes (1998) and adapted to the situation of the mudflat we studied, in the brackish zone of the Scheldt estuary. Details of the calculations are available in Appendix 1-3. Input data for the equations were obtained from our own study or from published studies on mudflats in the mesohaline zone of the Scheldt estuary. For the fish compartment, we applied the Wisconsin bioenergetics model (Hanson *et al.,* 1997). For *P. flesus*, this model was parameterised in chapter 4. The same model was applied to common sole (*Solea solea*), but the temperature-dependence functions of consumption and respiration were adapted to the specific nature of sole. For herring (*Clupea harengus*) we used the model of Rudstam (1988).

The parameters for the allometric functions for the seabass (*Dicentrarchus labrax*) model were taken from the striped bass model (*Morone saxatilis*; Hartman and Brandt, 1995) and the temperature-dependence functions were parameterised with data from literature (Appendix 7.3). The relative importance of the different prey categories in the diet of these four species was estimated from appendix 3.1 in chapter 3. The three prey categories included were macrobenthos, shrimps and other prey (zooplankton, mysids). All the estimates of fluxes and stocks were converted to kJ m^{-2}.

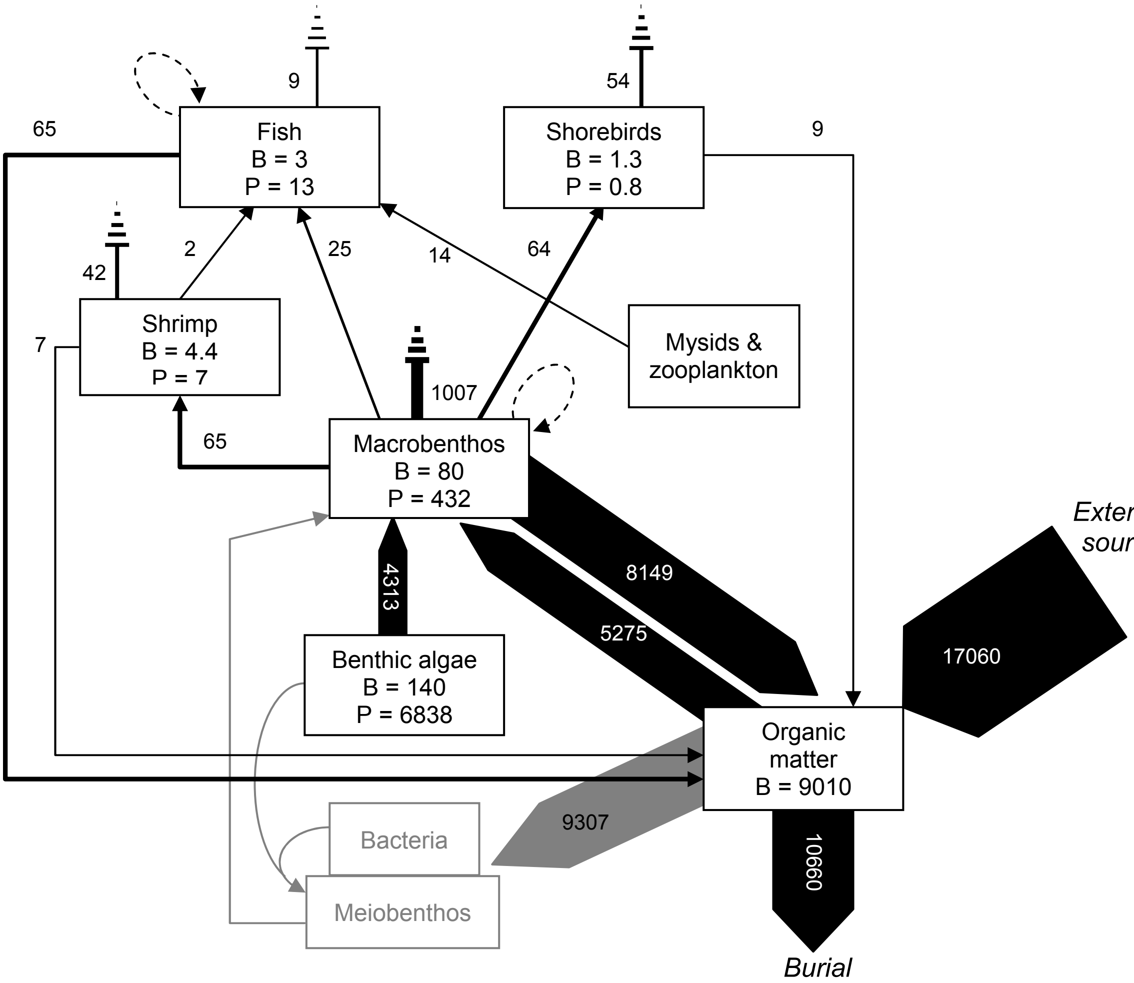


***Figure 7.2**. Food web of an intertidal mudflat in the mesohaline zone of the Scheldt estuary. The values represent the energy in biomass (*B*) (kJ m^{-2}), production (*P*) (kJ m^{-2} year^{-1}) and major fluxes in the web. Respiration is symbolized by an upward broken arrow. Loops (dashed line) represent intra-compartment predation (e.g. piscivorous fish). The compartments and fluxes that were not calculated are in grey. Arrows are proportional to the energy flux they represent. See appendix 7.1-7.3 for details about the calculation of the different components of the food web.*

The food web presented in figure 7.2 only deals with the organisms that contribute directly to the higher trophic levels. Bacteria and meiobenthos are important components of the benthic food web and process a large amount of energy, either between them or transferred to the macrobenthos (Heip *et al.,* 1995). In our food web, the energy consumed by macrobenthic deposit feeders (5275 kJ m^{-2} year^{-1}) is directed immediately from the organic matter pool. However, it should be clear that a substantial part of this energy channels through the meiobenthos and bacteria compartments. We did not quantify this loop in order to limit the complexity of the web. Figure 7.2 shows that a large part of the total production of benthic algae (microphytobenthos; MFB) is consumed by macrobenthic grazers. Although there is substantial evidence that benthic diatoms are the primary food resource of *C. volutator*, this crustacean also feeds on other microbenthic items and detritus (Gerdol and Hughes, 1994a). Consequently, the contribution of MFB to the food of macrobenthos in our web may be somewhat overestimated. However, as also meiobenthos grazers consume a significant proportion of the algae (Heip *et al.,* 1995), microphytobenthos might be top-down controlled. The reduction of the microalgae populations destabilizes the sediment and might stimulate the erosion of the mudflat (Daborn *et al.,* 1993; Hughes and Gerdol, 1997). Roughly one third of the annual macrobenthic production is consumed by the higher trophic levels. *C. volutator* accounts for about 30 % of the macrobenthic production on our mudflat, which corresponds to 130 kJ m^{-2} $year^{-1}$. If the predators (shrimps, fishes and birds) would obtain only half of their energy from *C. volutator*, they would consume about 60 % of the annual *C. volutator* production. Pihl (1985) showed that up to 98 % of the annual production of *C. volutator* in a Swedish estuary is consumed by shrimps, crabs and fish. Although the values we calculate are first approximations and the uncertainty in the calculation of the predator density is substantial, the order of magnitude of the presented fluxes is probably correct. Our calculations suggest that epibenthic predation by birds, fishes and shrimps can have significant effects on the abundance of the most available macrobenthic species on the mudflat.

3. Is prey limiting? The carrying capacity of the intertidal zone

The idea that predators can deplete their prey is closely linked to the existence of a carrying capacity for the system. The carrying capacity is usually defined as the population density of a habitat at which the *per capita* population growth rate is zero (van der Veer *et al.,* 2000a). When the carrying capacity is reached, competition becomes more intense and may result in density-dependent growth and population regulation. Density-dependent processes in the fish nurseries are thought to dampen the recruitment variability of marine fish populations (Beverton, 1995; van der Veer *et al.,* 2000b). Rijnsdorp *et al.* (1992) found a positive relationship between relative recruitment and nursery size, which raises the question whether nursery areas ever become saturated with settling larvae and reach their carrying capacity. At least for zooplanktivorous fishes in estuaries there is some evidence that their consumption may exceed prey production (Mehner and Thiel, 1999; Luo *et al.,* 2001; Maes *et al.,* 2005c). It was also shown for striped bass (*Morone saxatilis*) that its population decline in San Francisco Estuary was partly caused by a decline of the carrying capacity, following a decrease in the abundance of hyperbenthic mysids, its main prey (Kimmerer *et al.,* 2000). With regard to flatfishes, the general idea seems to be that food in nurseries is seldom limiting and that saturation of nursery grounds is rare or non-existent (Gibson, 1994; van der Veer, 2000b). Our data suggest that in years with high fish recruitment (e.g. 2001), the macrobenthic prey populations can be depleted by predation and that under those conditions, the benthic system is close to its carrying capacity. However, this conclusion is based on the assumption that the decrease of the *C. volutator* abundance can be largely attributed to (fish) predation. Furthermore, our exclosure experiments didn't show any effect of predation on the abundance of the macrobenthos. It seems therefore delicate to jump to conclusions about the carrying capacity of the estuary for benthic fish, based on our preliminary results. There are reasons to accept that intertidally foraging fishes can't fully exploit the available prey populations and hence are more likely to be food limited. At least three reasons can be given why only a fraction of the intertidal prey energy is available to benthivorous fishes:

1. The tide constraints the feeding possibilities of intertidal foraging fishes. The macrobenthic prey species are only accessible when the flats are covered by water. Pelagic feeding fishes, on the other hand, do not have this limitation and can feed continuously on zooplankton in the water column (till satiation).

2. Most of the macrobenthic species are buried in the sediment and as such reduce their vulnerability for epibenthic predators. Only a fraction of the prey population is available to the fishes, when they disperse into the water column or when they occupy in the top layer of the sediment.

3. Fishes that feed on the intertidal infauna have to share their prey with shorebirds, shrimps and, in the case of commercially harvested shellfish, also with men. This means that a substantial fraction of the total amount of energy in the intertidal food web is directed to birds and crustaceans and as such is not available for fish.

The fact that benthic prey could be limiting does not mean that a carrying capacity exists for demersal fishes in the estuary. It was shown from the diet analysis in our study (Chapter 3) that omnivory is prevalent in the estuarine food web. Consequently, fishes are flexible to switch between prey species (infaunal, epibenthic, hyperbenthic or pelagic), according to the relative availability of prey in the field.

If intertidal benthic prey is limiting for fishes, it should also be so for shorebirds and crustaceans. While food limitation for crustaceans in estuaries is less well documented, there is a body of literature available on the carrying capacity of benthic systems for shorebirds. Given the amount of published studies on this topic, one could conclude that the carrying capacity of a system is reached more frequently for birds than for fish. The research effort on this topic may be somewhat biased towards birds because of their high visibility, charismatic status and the competition of some birds (e.g. eiders, *Somateria mollissima* and oystercatchers, *Haematopus ostralegus*) with the shellfish industry (Goss-Custard *et al.,* 2004). Several studies indicate that shorebirds are able to deplete their prey to the extent that the remaining prey may become insufficient to support the population, resulting in the emigration or death of at least part of the population (Camphuysen *et al.,* 2002; Atkinson *et al.,* 2003). The effect of shorebirds on the macrobenthos may be temperature-driven. Most shorebirds arrive in the estuarine feeding areas in late autumn, when the environmental temperature is low and benthic production is dropping. The birds maintain a higher body temperature than the environment and have to build up a reserve for winter. As a result, these birds have a higher mass-specific energy demand than poikilothermic fishes. Furthermore, most of the studies that report on prey limitation for birds deal with large bivalves (mussels and cockles) as prey. These bivalves are also harvested by man and commercial shellfishery is shown to decrease the carrying capacity of the system for wintering oystercatchers (Goss-Custard *et al.,* 2004).

It may be clear from the shorebirds, that temperature and body weight are important determinants of the carrying capacity of an ecosystem. The idea that the carrying capacity of nursery areas is never reached for 0-group flatfishes (van der Veer *et al.,* 2000a), stems from the lack of evidence for density-dependent growth. This lack of evidence may be due to the inability to incorporate the effect of ambient temperatures on field estimates of growth rate (van der Veer *et al.,* 2000a). Bioenergetics modelling (Chapter 5) could offer a solution, but these models are time-consuming to construct and highly species-specific. Recently, Brown and co-workers (West *et al.,* 1997; Gillooly *et al.,* 2001; Brown *et al.,* 2004; Savage *et al.,* 2004) presented a metabolic theory of ecology (MTE) in which they describe how the metabolic rate (I) of an organism scales with body size and temperature:

$$I = i_0 M^{3/4} e^{-E/kT}$$

where i_0 is a normalization constant (varies with the organism, biological traits and environment), E is the activation energy (estimated as ≈ 0.63 eV; Gillooly *et al.,* 2001), k is Boltzmann's constant, M is the body mass and T is the absolute temperature in K.

This macro-ecological theory and derived models offer a framework to explore food web stability, patterning of energy fluxes and responses to perturbation. The model predicts that metabolic rate constrains biological processes at all levels of organization like population and community dynamics and ecosystem processes (Brown *et al.,* 2004). The carrying capacity (K; expressed as number of individuals) of a system is predicted to vary as

$$K \propto [R] M^{-3/4} e^{E/kT}$$

linearly with the supply rate or concentration of the limiting resource (R), as a power function of body mass and exponential with temperature (Savage *et al.,* 2004). This means that the carrying capacity of a system decreases with increasing temperature (body temperature in homoiotherms or environmental temperature in poikilotherms) and body size. This may be the reason why the carrying capacity for warm-blooded birds in winter is smaller than for cold-blooded fishes. The theory is still at its initial stage and the fit contains several sources of variance, which make its predictions for the present unreliable for individual-based modelling. However, it offers a consistent framework for ecosystem wide predictions and provides insight into the regulation of food web processes.

From the previous paragraphs, it is clear that a fixed estimate for a species-based carrying capacity in a highly variable environment like an estuary is unrealistic. Further steps taken to determine the carrying capacity for demersal fishes in estuaries should account for body size, ambient temperature, possible competitors (e.g. shorebirds and crustaceans) and feeding strategy of the target species. In particular, the relation between benthic prey density and prey availability needs more attention in order to construct a realistic foraging model as available for pelagic zooplanktivorous species (e.g. Maes *et al.,* 2005a).

4. How important are abiotic factors as estimators of the habitat quality of an estuary?

The availability of suitable prey items is probably one of the two most important factors determining habitat quality for juvenile fishes, the other factor being predation risk (Gibson, 1994). The size classes of flounder we modelled probably reached a size refuge for predation and consequently, habitat selection should be mainly determined by food availability and abiotic factors. If food is not limiting, which is thought to be the rule in estuarine nurseries, then abiotic factors determine the habitat selection of fishes. The predominance of temperature as a regulator of growth and hence habitat quality was demonstrated in chapter 5, where we were able to describe the growth of flounder in an estuarine environment, solely based on temperature. The growth of flounder in the Ythan estuary (Scotland) was modelled using temperature measurements of a single location in the estuary. We assumed that the population was resident and didn't migrate between the sea and the estuary or between different habitats in the estuary. One may question whether this is realistic for a facultative catadromous species like flounder. A large part of the flounder population moves into deeper coastal waters during winter and 0-group flounder are known to migrate into the freshwater reaches of estuaries (Summers, 1979; Kerstan, 1991). In both situations, they experience different temperature regimes, which probably influence their growth rate. It would therefore be more appropriate to use a dynamic state-variable model (Clark and Mangel, 2000). In dynamic modelling, the fish are allowed to respond to changes in their environment in order to maximize their fitness. In the majority of current applications of bioenergetics models it is assumed that fish choose habitats based on maximization of energy gain. However, the growth rate potential of an environment based on bioenergetics estimates does not always effectively predict fish growth and distribution (Tyler and Brandt, 2001). The authors attribute this discrepancy between predicted and observed patterns to the lack of appropriate habitat selection submodels in individual-based spatially-explicit models. If fish choose habitats based on a hierarchy of variables (e.g. temperature over food) then the application of bioenergetics models without allowing for such a hierarchy of choice can lead to erroneous conclusions (Wildhaber and lamberson, 2004).

Because of the specific nature of estuaries, other abiotic factors like oxygen concentration and salinity also contribute significantly to the quality of fish habitats. The salinity gradient and the fluctuating oxygen levels constitute a major challenge for the species that have to cope with these conditions. While salinity variation mainly determines the distribution of stenohaline species, euryhaline species like flounder are probably less affected. Furthermore, habitats that are characterized by high fluctuating salinities may even yield some advantage to tolerant species by excluding competitive interactions with less tolerant species. A factor that is usually not taken into account when evaluating the habitat quality for fishes is the distribution of parasites. The parasite community of flounder is known to change along a salinity gradient (Schmidt *et al.,* 2003). Möller

(1978) considered stenohalinity of parasites and their hosts as the main reason for a natural reduction in the parasitic fauna in brackish water. Ectoparasites are directly affected by low salinities, whereas in digeneans it is the lack of molluscs that serve as intermediate hosts. Although further information is lacking, migration into freshwater may be an adaptation to reduce parasite load and optimize associated fitness traits.

In highly urbanised estuaries, the combination of high nutrient loads from untreated sewage effluents and low river runoff in summer may seasonally cause hypoxic or even anoxic conditions. The recent history of the Scheldt estuary is characterized by pollution and eutrophication. Particularly in the Zeeschelde anoxic conditions were regularly observed in the seventies. The situation improved noticeably due to wastewater treatment, but low oxygen concentrations still persist around the mouth of the Rupel. The results of our spatially-explicit habitat model (Chapter 6) show that the low oxygen concentrations limit the migration opportunities of flounder in the estuary. In summer, hypoxic conditions prevent the upstream migration to the freshwater reaches, where the model predicts optimal growth conditions. The model further suggests that flounder may use the freshwater zone to optimise their growth rate, as a result of the higher ambient temperatures. However, if temperature would be the dominant trigger for upstream migration, it remains unclear why flounder is the only flatfish adapted to use the freshwater reaches. Beaumont and Mann (1984) suggested that competition for food and space might be a possible stimulus for flounder to move upstream. Before we answer this question, we should extend the model with a foraging compartment to account for food availability and do the same exercise for possible competing species.

The model makes predictions about the habitat selection of flounder in the entire estuary. However, we were not able to reliably falsify our results with field data on the distribution of flounder in the estuary. The lack of a consistent monitoring programme for the entire estuary makes it very difficult to give a well-founded management advice concerning conservation programmes for migrating species. Scientifically, the fish compartment of the Scheldt estuary is running (far) behind the microbial, planktonic, macrobenthic and bird compartments of the estuarine food web. To catch up, cross-border cooperation is needed and sampling programmes have to be coordinated. In addition, the sampling effort in the freshwater zone should be extended spatially as well as temporally if we want to scientifically guide the restoration of the fish community in the Zeeschelde now that the increased capacity for sewage treatment is available in the Brussels region.

Appendix 7.1 - Details of the calculation of the fluxes and compartments in the intertidal food web. For conversion from carbon to energy, the values 1 g C = 12 kcal = 50.28 kJ were used. Unless mentioned differently, all formulas are taken from Wilson and Parkes (1998).

Compartment	Biomass	Consumption	Respiration	Production/input	Metabolic wastes
Organic carbon (OC)	Herman *et al.* (2000): Weight % OC in top 1 cm = 0.64 % sediment gravity = 2.8 g cm^{-3}	/	Heip *et al.* (1995): Mineralization = 290 gCm^{-2} $year^{-1}$	Heip *et al.* (1995): Influx = 503 gCm^{-2} $year^{-1}$	Heip *et al.* (1995): Burial = 212 gCm^{-2} $year^{-1}$
Benthic algae (MFB)	Biomass (mg C) = 24.3 * Chl a (mg) + 29.3 Heip *et al.* (1995): 113 mg chlorophyll a m^{-2}	/	/	Heip *et al.* (1995): Production = 136 gC m^{-2} $year^{-1}$	/
Macrobenthos	Biomass of *N. diversicolor* (Nd) and *C. volutator* (Cv) calculated from Length-weight relationships (Zwarts and Wanink, 1993) Length data from chapter 4 Biomass (gAFDW m^{-2}) for Oligochaetes (Oli), *H. filiformis* (Hf) and *M. baltica* (Mb) from Seys *et al.* (1999) Energy values (cal $gAFDW^{-1}$) from Chambers and Milne (1979)	Respired energy (*R*) = 0.7 * assimilated energy (*A*) *A* = 0.15 * consumed energy (*C*) % dependence of MFB as food (rest = POC) Nd: 50 % Cv: 100 % Mb: 50 % Oli and Hf: 0 %	Respired energy (*R*) $\text{Log}_{10} R$ $= 0.367 + \log_{10} P$	Production (*P*) = mean energy content (kJ m^{-2}) of each species multiplied by *P:B* ratio $P{:}B = 0.525 * W^{-0.304}$	Faeces (*F*) $F = C - A$
Shrimps	Intertidal densities of *C. crangon* from Hostens, 2003 The components of the energy budget (C, R and F) were calculated for a shrimp of 30 mm	Consumed energy (*C*) $= R + G + U$ Assimilation efficiency = 85 %	Respiration (*R*) calculated from equation 8 in Taylor and Peck (2004)	Growth (*G*) calculated from data in appendix 1 from Taylor and Peck (2004): $0.572 * W(\text{g})^{0.56} * T\,(^{\circ}\text{C})$	Excretion (*U*) calculated from Taylor and Peck (2004) $U = 20.9 Jg^{-1} * R_{Ti} * R_{18^{\circ}}^{-1}$
Fish	Fish densities on the mudflat were calculated from fyke catches in 2002-2004 (chapter 4) (Appendix 7.2)	The components of the energy budget were calculated using a bioenergetics equation. The mean length of each species in the catches is given between brackets. *P. flesus* (12 cm): Stevens *et al.* (2006) (Chapter 5) *C. harengus* (8 cm): Rudstam (1988) *S. solea* (8.5 cm): appendix 7.3 *D. labrax* (9.5 cm): appendix 7.3			
Birds	Monthly mean numbers of shorebirds on the mudflat of Groot Buitenschoor (1997-2003) provided by Natuurpunt (http://www.schorrenwerkgroep.be) Bird weights were obtained from http://www.bto.org/ Energy density: 6.5 kJ g^{-1}	Assimilated energy (*A*) (kcal $bird^{-1}$ day^{-1}): $\text{Log}_{10} A$ $= 1.89 + 0.72 * \log_{10} W$ *W* = weight (kg) Assimilation efficiency = 85 %	Respired energy (*R*) (kcal $bird^{-1}$ day^{-1}): $R = 0.5244\ W^{0.7347}$ *W* = weight (g)	$P = 0.79 * R - 1.055$ *P* = production ($\log_{10}$cal m^{-2} $year^{-1}$) *R* = respiration ($\log_{10}$cal m^{-2} $year^{-1}$)	Faeces (*F*) $F = C - A$

Appendix 7.2 - Calculation of the fish density (# m^{-2}) from fyke catches on a mudflat.

Basic principle: We assumed that fishes are equally distributed in the water column on the mudflat and that their abundance in the fyke nets is proportional to the volume of water that flows through the fyke nets. In order to calculate the fish density on the mudflat, we have to multiply the abundance of fishes in the fyke with the volume of water passing through the net.

The total volume of water on the mudflat in front of the fyke net is divided in three compartments A, B and C (see bottom figure). When the water retreats from the mudflat during ebb, it passes through the vertical surface $Hm*Wf$. The fraction that goes through the fyke net is proportional to the height of the fyke net, relative to the height of the water column above the net, which changes during ebb. When the water level is equal to the height of the fyke net, all the remaining water (volume A) passes through the fyke net. In order to calculate the total volume of water filtered by the net, we have to add volume A and the fraction of B+C that passes through the fyke net.

The total volume of water on the mudflat in front of the fyke net decreases per second according to

$$Volume\ decrease\ (m^3\ \sec^{-1}) = \left\{ \frac{Wf \times vebv \times vebh}{2} + Wf \times vebv \times Lm \right\} - \{Wf \times vebv \times vebh\} \times T = a_1 + b_1 \times T$$

Of this volume, only a fraction passes through the fyke net. The rest passes through the surface above the fyke net ($Hv*Wf$). The fraction (of B+C) passing through the fyke changes during ebb and is calculated as

$$Fraction = \frac{Hf}{Hm - vebv \times T}$$

This fraction is used to calculate the volume that is filtered through the fyke net:

$$Filtered\ volume = \int_0^T \frac{Hf \times (a_1 + b_1 \times T)}{Hm - vebv \times T}\, dT$$

which is integrated over the time T (sec) it takes for the water level to drop from high water (Hm) to the height of the fyke (Hf).

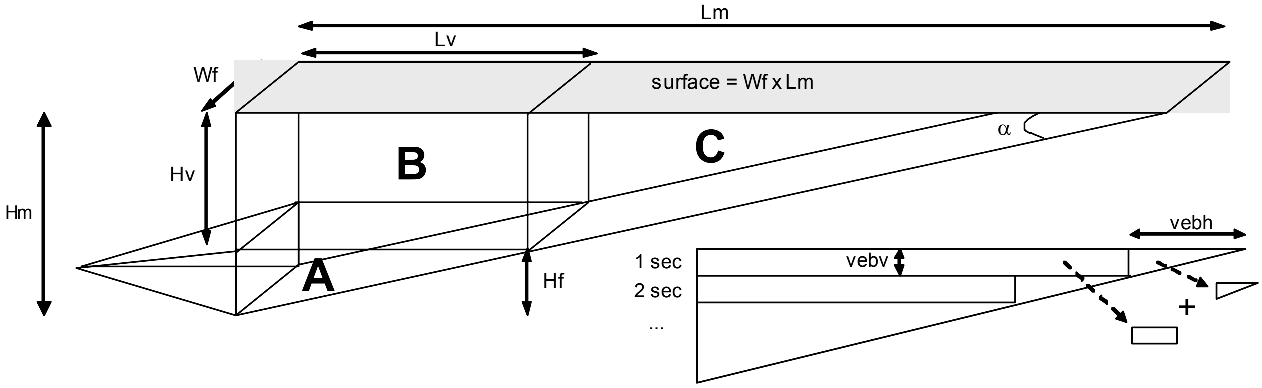
$$Filtered\ volume = \left| (b_2 \times T \times vebv - \log(Hm - vebv \times T)) \times a_2 \times vebv + \frac{\log(Hm - vebv \times T) \times Hm \times b_2}{vebv^2} \right|_0^T$$

with $a_2 = Hf*a_1$ and $b_2 = Hf*b_1$

The sum of this filtered volume and A, gives the total amount of water (m^3) passing through the fyke net.

Table A7.1. *Measurements and parameters used to calculate the volume passing through the fyke net (m^3 day^{-1}). The measurements are represented on the figure below.*

Width mudflat (*Lm*)	400 m	Volume B (*Lv***Hv***Wf*)	1171 m^3
Tidal height at high water (*Hm*)	5.4 m	Volume C (*Hm***Lm***Wf*/2-A-B)	1391 m^3
Height fyke net (*Hf*)	1.6 m	Time from high water (*Hm*) to low water in seconds (*T*)	6h 30min = 23400sec
Width fyke net (*Wf*)	2.6 m	Horizontal displacement of the tide on the mudflat in 1 sec (*vebh*)	$vebh = Lm\ T^{-1} = 0.017$ m
Height of the water column above the fyke net at high water (*Hv*)	3.8 m	Vertical displacement of the tide on the mudflat in 1 sec (*vebv*)	$vebv = Hm\ T^{-1} = 23 \cdot 10^{-5}$ m
Volume A (*Wf***Hf***Lv*/2)	247 m^3	Total volume through fyke net (day^{-1})	2835 m^3



Appendix 7.3a - Parameters from the bioenergetics equations used to calculate the different components of the energy budget of fishes in the mudflat food web (for equation see appendix 7.3b). The activity multiplier (ACT) for seabass was calculated from the swimming speed function (equation 2) for striped bass (*Morone saxatilis*; Hartman and Brandt, 1995).

	flounder[1]	**herring**[2]	**sole**[1,3,4]	**Seabass**[5-9]
Consumption	*Eq.1*	*Eq. 1*	*Eq. 1*	*Eq. 1*
CA	0.186	0.642	0.186	0.302
CB	-0.202	-0.256	-0.202	-0.252
CQ	2	1	5	6
CTO	20	15	23	24
CTM	21	17	24	27
CTL	27	25	27.2	31
CK1	0.05	0.1	0.01	0.01
CK4	0.01	0.01	0.01	0.01
Respiration	*Eq. 3*	*Eq. 2*	*Eq. 3*	*Eq. 3*
RA	0.0178	0.0033	0.0178	0.0028
RB	-0.218	-0.227	-0.218	-0.218
RQ	2.5	0.0548	3	2
RTO	21	0.03	19.7	27
RTM	27	0	27.2	32
RK1		15		
RK4		0.13		
SDA	0.19	0.175	0.19	0.175
ACT	1.1	3.9	1.1	1.6
FA	0.17	0.16	0.17	0.15
UA	0.1	0.1	0.1	0.1
F+U+SDA (C)	0.443	0.419	0.443	0.41

[1] Stevens *et al.,* 2006
[2] Rudstam, 1988
[3] Lefrançois and Claireaux, 2003
[4] Sims *et al.,* 2005
[5] Hartman and Brandt, 1995
[6] Claireaux and Lagardère, 1999
[7] Jobling, 1994
[8] Person-Le Ruyet, 2004
[9] Pickett and Pawson, 1994

Appendix 7.3b – Abbreviations and equations used in the bioenergetics models for the fish compartment of the mudflat food web. The equations for the temperature dependence functions (1-3) were taken from the manual of *Fish bioenergetics 3.0* (Hanson *et al.,* 1997). Growth was calculated as: $G = C - (R + S + F + U)$. Further information about the different components of a bioenergetics model is given in chapter 5.

- **Consumption**

$$C = C_{\max} \cdot p \cdot f(T) \qquad\qquad C_{\max} = CA \cdot W^{CB}$$

where

C	specific consumption rate ($g \cdot g^{-1} \cdot d^{-1}$)
C_{max}	maximum specific feeding rate ($g \cdot g^{-1} \cdot d^{-1}$)
p	proportion of maximum consumption
f(T)	temperature dependence function
T	water temperature (°C)
W	fish mass (g)
CA	intercept of the allometric mass function ($g \cdot g^{-1} \cdot d^{-1}$)
CB	slope of the allometric mass function (dimensionless)

Equation 1

$$f(T) = K_A \cdot K_B$$

$$K_A = (CK1 \cdot L1)/(1 + CK1 \cdot (L1 - 1)) \qquad\qquad K_B = (CK4 \cdot L2)/(1 + CK4 \cdot (L2 - 1))$$

$$L1 = e^{(G1 \cdot (T - CQ))} \qquad\qquad L2 = e^{(G2 \cdot (CTL - T))}$$

$$G1 = (1/(CTO - CQ)) \cdot \ln((0.98 \cdot (1 - CK1))/CK1 \cdot 0.02))$$

$$G2 = (1/(CTL - CTM)) \cdot \ln((0.98 \cdot (1 - CK4))/CK4 \cdot 0.02))$$

- **Respiration**

$$R = RA \cdot W^{RB} \cdot f(T) \cdot ACT \qquad\qquad S = SDA \cdot (C - F)$$

where

R	specific rate of respiration ($g \cdot g^{-1} \cdot d^{-1}$)
W	fish mass (g)
RA	intercept of the allometric mass function ($g \cdot g^{-1} \cdot d^{-1}$)
RB	slope of the allometric mass function (dimensionless)
f(T)	temperature dependence function
T	water temperature (°C)
ACT	activity multiplier (dimensionless)
S	proportion of assimilated energy lost to SDA
SDA	Specific Dynamic Action
C	specific consumption rate ($g \cdot g^{-1} \cdot d^{-1}$)
F	specific egestion rate ($g \cdot g^{-1} \cdot d^{-1}$)

Equation 2

$$f(T) = e^{(RQ \cdot T)}$$

$$ACT = e^{(RTO \cdot VEL)}$$

$$VEL = RK1 \cdot W^{RK4}$$

Equation 3

$$f(T) = V^{X} \cdot e^{(X \cdot (1 - V))}$$

$ACT =$ multiplier

$$V = (RTM - T)/(RTM - RTO)$$

$$X = (Z^2 \cdot (1 + (1 + 40/Y)^{0.5})^2)/400$$

$$Z = LN(RQ) \cdot (RTM - RTO)$$

$$Y = LN(RQ) \cdot (RTM - RTO + 2)$$

- **Egestion (F) and excretion (U)**

$$F = FA \cdot C$$

$$UA = UA \cdot (C - F)$$

Summary

Animals are influenced by both biotic and abiotic factors when selecting a habitat. The final decision of an organism to select a habitat generally depends on the fitness increase that can be expected. Within a selected habitat, interactions with conspecifics and other species also affect its quality. This thesis describes the habitat use of estuarine fishes in the Scheldt estuary. In the first part (Chapter 2 to Chapter 4) we studied the importance of intertidal migration for fishes and discussed the various factors that affect intertidal habitat quality. We also examined to what extent fishes affect their environment by predation on the intertidal infauna. The second part (Chapter 5 and 6) describes the effect of abiotic factors on the use of the Scheldt estuary by European flounder *Platichthys flesus* (L.).

The fish assemblage on a mudflat in the brackish part of the Scheldt estuary is dominated by juveniles, confirming the nursery status of this part of the estuary. The composition of the intertidal fish community reflects well the cyclicity of seasonal recruitment of species into the estuary. Flatfishes migrate actively onto the mudflat whereas the distribution of (semi-) pelagic species in the fyke nets suggests that they are transported passively by the tidal currents. Most fish species do not show a clear zonation on the mudflat and, if observed, this is mainly the result of species-specific differences in mobility. Zonation of flatfishes tends to be affected by density-dependent processes. When fish abundance in the nets is higher, relatively more flounder are caught on the upper shore. By doing so, they probably avoid competition for food or space with sole, which stays on the middle and lower zones of the mudflat. Because of the low predator abundance and high turbidity in the brackish part of the estuary, predation is probably not an important trigger for intertidal migration.

The most important (and perhaps only?) trigger for intertidal migration in turbid estuaries is the high intertidal food availability. The concentration of large numbers of juvenile fishes in these intertidal areas may give rise to competitive interactions. Diet analysis shows that all fish species on the mudflat, without exception, target the same prey species (*Corophium volutator*). The relative importance of prey species in the diet of fishes reflects the seasonal prey availability in the field, confirming the generalist and opportunistic feeding nature of estuarine fishes. For all species, the niche width is larger when resources are less available or when the population density is higher. It is suggested that a generalist and opportunistic feeding strategy reduces competition and results in a broader population diet with increasing population density. As most fishes are feeding on a limited number of prey species, niche overlap is generally high between species. A significant niche overlap was found in summer between flounder and sole. Direct competition for food, however, is probably avoided by spatial niche separation and by resource partitioning at the level of prey size.

The intensive use of the intertidal benthic resources raises the question whether or not predators deplete the macrobenthic prey populations. The results of two exclosure experiments on an estuarine mudflat indicate that fishes and birds have only a negligible effect on the abundance of their infaunal prey species. The effects of short-term experiments in these highly productive areas may only be noticeable at high predator densities. Long-term exclosure experiments on the other hand, seem to reflect the indirect effects of predation, as in the absence of predation, infaunal regulation (competition and predation) becomes more important. The lack of clear direct effects of predation on the abundance of organisms on the lower trophic levels, suggests that the interaction strength in the benthic food web is rather weak. Weak links generally support food web stability and decrease the probability of tropic cascades. These weak interactions may be the result of (1) the three-dimensional structure of soft sediments, which reduces the risk of strong competitive and predatory interactions and (2) the prevalence of omnivory in the benthic food web. The fact that benthic prey is superabundant and that fishes are flexible to switch between different prey species (infaunal, epibenthic, hyperbenthic, pelagic), suggests that the carrying capacity of the estuary for benthic fishes is only rarely reached.

If food and predation are not limiting, which is thought to be the rule in estuarine nurseries, then abiotic factors determine the habitat selection of fishes. We were able to accurately describe the growth of flounder in an estuarine environment, solely based on temperature, which demonstrates the predominance of temperature as a regulator of growth and hence habitat quality. A multivariable bioenergetics model, based on temperature, oxygen concentration and salinity, was constructed for European flounder (*Platichthys flesus*) to generate spatially-explicit estimates of growth in the Scheldt estuary. The model was run for two years (1998 and 2003) and predicts that in March, when 0-group flounder is known to migrate upstream, growth is highest in the upper freshwater reaches of the estuary. This suggests that freshwater migration of flounder may be, at least partly, temperature driven. In July and October, the unfavourable oxygen concentration in the central part of the Zeeschelde reduces growth and probably prevents upstream migration. In summer and autumn, the growth rate in the brackish part of the estuary is higher compared to the marine part. Field data on the distribution of flounder in the estuary shows that the abundance of this species is highest in the brackish part, where it also finds plenty of suitable prey items. Our results indicate that abiotic variables may be useful to predict the habitat use of diadromous species in estuaries. Given the importance of food in determining habitat quality, the model should be further extended with a foraging compartment to account for spatial differences in prey availability.

Samenvatting

Bij de selectie van een habitat worden dieren zowel door biotische als abiotische factoren beïnvloed. De uiteindelijke beslissing van een organisme om een habitat te selecteren, is meestal afhankelijk van het verwachte fitness voordeel. In het geselecteerde habitat bepalen interacties met individuen van de eigen soort en/of andere soorten de uiteindelijke fitness. Deze organismen kunnen fungeren als prooien, predatoren of concurrenten.

Voorliggende verhandeling beschrijft het habitatgebruik door vissen in het Schelde-estuarium. In deel één (Hoofdstukken 2 tot en met 4) wordt het belang bestudeerd van intergetijdenmigratie voor vissen en worden de factoren besproken die voor hen de kwaliteit van intergetijdengebieden bepalen. Daarnaast wordt onderzocht in welke mate predatie door vissen de bentische prooigemeenschap beïnvloedt. Deel twee (Hoofdstukken 5 en 6) beschrijft het effect van abiotische factoren op het gebruik van het Schelde-estuarium door de bot *Platichthys flesus* (L.).

De visgemeenschap op een slik in de brakwaterzone van het Schelde-estuarium wordt gedomineerd door juvenielen, wat de kinderkamerfunctie bevestigt van het oostelijk deel van de Westerschelde. De seizoenale samenstelling van de visgemeenschap op het slik weerspiegelt grotendeels de cycliciteit van de recrutering van mariene soorten in het estuarium. De migratiepatronen van vissen op het slik werden bestudeerd met directionele fuiken. Deze fuiken worden zodanig opgesteld dat ze elk de migrerende visgemeenschap vanuit een andere richting bemonsteren. De verdeling van soorten in de directionele fuiken suggereert dat voornamelijk platvissen actief op het slik migreren. Voor soorten als bot en tong is intergetijdenmigratie dan ook cruciaal voor hun voedselvoorziening. (Semi-) pelagische soorten zoals haring en zeebaars worden daarentegen passief door de getijdenstroom op het slik gebracht. Deze soorten kunnen beschouwd worden als opportunistische passanten voor wie intergetijdenmigratie belangrijk is maar niet cruciaal. Over het algemeen vertonen de soorten op het slik geen duidelijke zonatie. Wanneer dit toch het geval is, is dit meestal het gevolg van soort-specifieke verschillen in mobiliteit. De zonatie van platvissen lijkt beïnvloed te worden door densiteitsafhankelijke processen: bij hogere visdensiteiten worden relatief meer botten gevangen op de hoger gelegen delen van het slik. Op deze manier vermijdt bot competitie voor voedsel en plaats met tong, aangezien tong minder hoog op het slik voorkomt. Predatie is daarentegen hoogstwaarschijnlijk geen belangrijke stimulans voor intergetijdenmigratie. Eerder onderzoek toonde immers aan dat in dit deel van het estuarium de densiteit van predatoren laag is en dat hoge turbiditeit er de kansen voor visuele predatie gevoelig vermindert.

De meest waarschijnlijke (en misschien enige) stimulans voor intergetijdenmigratie in turbiede estuaria is de beschikbaarheid van voedsel. De concentratie van grote aantallen juveniele vissen in deze gebieden kan dan ook aanleiding geven tot competitie. Dieetanalyse van de belangrijkste vissoorten toonde aan dat alle vissen op het slik zich in meer of mindere mate voeden met één enkele prooisoort (*Corophium volutator*). Het belang van prooien in het dieet van deze vissen weerspiegelt dan ook grotendeels hun beschikbaarheid op het slik. Dit bevestigt de opportunistische voedingsstrategie van de meeste estuariene vissoorten. De nichebreedte is voor alle soorten groter bij lagere prooidensiteit of grotere populatiedensiteit. Het voorgaande suggereert dat door een opportunistische voedingsstrategie de nichebreedte van de populatie vergroot bij een groter wordende populatiedensiteit, waardoor de kans op competitie verkleint. Aangezien het aantal prooisoorten per vissoort beperkt is, is de nicheoverlap vrij hoog. Er werd een significante nicheoverlap gevonden voor bot en tong in de zomer. Directe competitie voor voedsel wordt waarschijnlijk echter vermeden door ruimtelijke nichesegregatie en door differentiële selectie van prooigroottes door de verschillende vissoorten.

De intense predatiedruk van vissen, vogels en crustaceeën op het macrobentos doet de vraag rijzen of deze predatoren de macrobentische prooigemeenschap uitdunnen. De resultaten van twee exclosure experimenten suggereren dat vissen en vogels slechts een verwaarloosbaar effect hebben op de densiteit van het macrobentos op het slik. Effecten van kortetermijnexperimenten in dergelijke hoogproductieve systemen zijn mogelijk alleen observeerbaar bij zeer hoge predatordensiteiten. Langetermijnexperimenten daarentegen lijken vooral de indirecte effecten van predatie op het macrobentos te weerspiegelen: in afwezigheid van predatie worden competitie en predatie binnen de macrobentische gemeenschap belangrijker als regulerende factoren. Het ontbreken van duidelijke effecten van predatie op de densiteit van organismen op de lagere trofische niveaus wijst erop dat het merendeel van de interacties in het bentisch voedselweb eerder zwak zijn. Over het algemeen bevorderen zwakke interacties de stabiliteit van een voedselweb en verminderen ze de waarschijnlijkheid van trofische cascades. Deze zwakke interacties kunnen het gevolg zijn van (1) de driedimensionale structuur van zandige substraten, waardoor het risico op competitie en predatie vermindert en (2) de hoge frequentie van omnivorie in bentische voedselwebben. De zeer hoge densiteit van bentische prooien en het relatief brede dieetspectrum van vissen, doet vermoeden dat de draagkracht van het estuarium voor bentische vissen zelden of nooit bereikt wordt.

Wanneer, zoals we vermoeden, voedsel en predatie niet limiterend zijn in estuariene kinderkamers, dan bepalen abiotische factoren de habitatselectie door vissen. In onze studie zijn we erin geslaagd om de groei van bot in een estuarium te beschrijven enkel op basis van watertemperatuur. Dit toont het belang aan van deze variabele als regulerende factor voor groei en dus ook voor habitatkwaliteit. Er werd een bio-energetisch model voor bot opgesteld, dat gebaseerd is op temperatuur, zuurstofconcentratie en saliniteit. Dit model werd geïntegreerd in een ruimtelijke dimensie om de groei van bot in het Schelde-estuarium te voorspellen. Het model voorspelde dat de groei van bot in maart het hoogst was in de zoetwaterzone. In deze periode migreren de 0-groep individuen van deze soort ook effectief stroomopwaarts. Dit alles suggereert dat de zoetwatermigratie van bot tenminste gedeeltelijk beïnvloed wordt door de watertemperatuur. Voor juli en oktober voorspelt het model dat door de lage zuurstofconcentratie in het centrale deel van de Zeeschelde de groei van bot in deze zone vertraagt. Bovendien verhindert de slechte zuurstofhuishouding in de zoetwaterzone waarschijnlijk de stroomopwaartse migratie van diadrome soorten. In de zomer en de herfst is de groei in de brakwaterzone hoger dan in de mariene zone. Veldgegevens over de verspreiding van bot in het estuarium tonen aan dat bot voornamelijk in het brakke deel voorkomt, waar tevens de prooibeschikbaarheid het hoogst is. De resultaten van ons model tonen aan dat abiotische variabelen gebruikt kunnen worden om het habitatgebruik van diadrome soorten in estuaria te voorspellen. Gezien echter het belang van voedsel voor habitatkwaliteit, zou het model verder uitgebreid moeten worden met een foerageer-submodel, waarbij de prooibeschikbaarheid mee in rekening wordt gebracht.

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