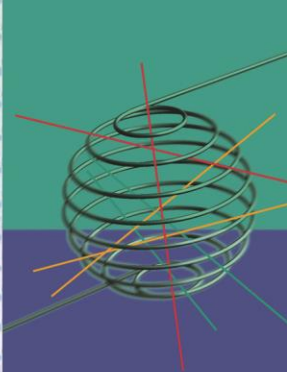




**Structural and functional  
biodiversity of North Sea  
ecosystems: species and  
their habitats as indicators  
for a sustainable  
development of the  
Belgian continental shelf**

**SUSTAINABLE  
MANAGEMENT OF  
THE NORTH SEA**





# BELGIAN SCIENCE POLICY

## SCIENTIFIC SUPPORT PLAN FOR A SUSTAINABLE DEVELOPMENT POLICY (SPSD-I)

### Programme: “Sustainable management of the North Sea”

#### **Structural and functional biodiversity of North Sea ecosystems: species and their habitats as indicators for a sustainable development of the Belgian continental shelf**

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**D/2004/1191/43**

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## **Acknowledgements**

We thank the master and crew of the RV Belgica for their skilful and patience during sampling. The same holds for the master and crew of the Oostende XI and Zeehond for their efforts during the benthic-pelagic coupling sampling campaign.

This research has been funded by the Belgian government (Belgian Science Policy). The KUL team thanks L. Bolle, E. Hunter, D. Miossec, P. Bossier, F. Argenton, A. Alcazar, P.S. Economidis, S. Zanuy, I. Palomera, R. Millner for providing us with samples.

## ABSTRACT

The Belgian Continental Shelf (BCS) is characterized by numerous subtidal sandbanks. Their close position to the coastline and the shallowness of the area make them unique, both from an ecological and geological point of view. It has recently been shown that the sandbank system in the Belgian coastal area acts as an internationally important resting and foraging area for various seabird species.

The project '***Structural and functional biodiversity of North Sea ecosystems: species and their habitats as indicators for a sustainable development of the Belgian continental shelf***' aims to assess the factors that determine and influence the marine biodiversity of the Belgian Continental Shelf.

Within this project, an emphasis is made to incorporate all existing and new biodiversity information (community, population and genetic level) on all benthic compartments, including demersal fish, their parasites and birds. Most emphasis is given on the description of the structural aspects of benthic biodiversity in order to find a pattern within the relationship between the biological and the geochemical characteristics of the sediment. The question whether the structuring of ecosystem and species diversity and its functioning is reflected in the genetic structuring of marine taxa on the Belgian Continental Shelf is addressed for the first time.

### **Monitoring of the Belgian Continental Shelf**

Clear patterns in average species number have been observed for the meiobenthos, the macrobenthos, and the hyperbenthos. The species number of benthic assemblages in inshore waters changes dramatically between the eastern and western end of the Belgian coast. A second gradient runs perpendicular to the coastline and involves an increasing species number and diversity offshore. A third gradient relating to depth within a zone needs yet to be confirmed. An offshore decrease in density has also been observed for macrobenthos and hyperbenthos. Avifauna data do not confirm these patterns even though seabird assemblages offshore differ from the inshore avifauna. The monitoring of the hyperbenthos shows that the estuarine outflow on the one hand and the hydrodynamic setting and protective function of the sandbanks on the other hand create a diverse environment along the Belgian coastal zone. The sandbanks system extends moreover in gently sloping sandy beaches. This makes the setting of the Belgian coastal sandbank system ecologically unique.

## Spatial patterns on sandbank ecosystems

### Benthos

The meiobenthos of the Flemish Banks, the Hinder Banks and the Zeeland Banks is divided over four different communities, which do not reflect the geographical position but are influenced by local differences in sedimentological characteristics within sandbanks. The sedimentological differences influenced the taxon diversity as well. Sediments with a median grain size below 300  $\mu\text{m}$  were poor in terms of densities and number of taxa, while coarser sediments were richer in taxon diversity. Sediments with a median grain size between 300-450  $\mu\text{m}$  were rich both in terms of diversity and density, while sediments with a median grain size of  $>450$   $\mu\text{m}$  still showed high diversity but lower densities. The information on the species diversity of the most dominant group within the meiobenthos, the free-living marine nematodes indicates that sandbanks can be seen as geographically isolated "islands" since tidal current patterns around these sandbanks prevent nematodes from being transported from one sandbank to another. A detailed study of the Kwintebank, which is heavily exploited for sand and gravel extraction, shows that a depression occurred in the middle of the sandbank due to gravel extraction (comparison between 1978 and 1997). In the centre of the sandbank, copepod diversity decreased and a shift was recorded from a species rich northern community to a less diverse southern community (less species, high dominance of *Paraleptastacus espinulatus*, *Leptastacus laticaudatus* s. str. and *Kliopsyllus constrictus* s. str.) as a result of changes in sediment characteristics. The altered sediment composition may be the result of an accretion of fine sediments, as a consequence of changed current patterns in the depression. The extension of the present depression due to sand extraction can become quite problematic if the human-induced physical disturbances may cause a continuing erosion and impoverishment. A thorough analysis of all available data of macrobenthos of the BCS (with an extensive survey on the sandbank systems) shows that five different macrobenthic communities (1. *Abra alba*-*Mysella bidentata*; 2. *Nephtys cirrosa*; 3. *Ophelia limacina*-*Glycera lapidum*; 4. *Eurydice pulchra*-*Scolelepis squamata* and 5. *Barnea candida* community) can be distinguished with five transitional groups in between them. All these groups are clearly linked to sediment characteristics and to their occurrence related to the geographical position on the sandbanks. These communities are ecologically important, because they are a representation of the different habitats that are characteristic for the BCS. Obviously, different macrobenthic communities and species associations show different temporal dynamics. A successful recruitment and survival of one species, for instance, will only take place in the community where this species belongs to and not in any other



community. Instead of putting a lot of effort, time and money in working out time-series of lots of macrobenthic samples, randomly spread over the area under consideration, monitoring of the macrobenthos should concentrate on the spatial distribution of the macrobenthic 'potentials'. This can be done by the identification of (1) the spatial distribution of the macrobenthic communities actually present, (2) the communities' environmental 'needs', and, finally, (3) the communities' 'potentials' by means of long-term monitoring of some selected stations within each community.

### Parasites

The study concentrates on ectoparasites belonging to the genus *Gyrodactylus* (Monogenea, Platyhelminthes) parasitizing the threespine stickleback (*Gasterosteus aculeatus*) and gobies from the genus *Pomatoschistus* (Gobioidea, Teleostei). Many new species have been found. Thanks to the available molecular techniques the detection of putative new species has become considerably easier. Both morphometric and genetic analyses are used in the species descriptions.

### Avifauna

Out of the 121 bird species encountered at sea during the period 1992-98, 23 were selected as being true marine species that occur in relatively high densities within the Belgian waters. Of these 23 species, six can be classified as 'focal species' (little gull, diver spp., common scoter, sandwich tern, common tern, little tern) and another five as 'locally important species'. A cumulative picture of the abundance and conservation value of six focal species indicates that the western Coastal Banks (Trapegeer, Nieuwpoort Bank, Stroombank, Balandbank, and Smal Bank), the Vlake van de Raan and most of the Flemish Banks are avian hotspots throughout the year. The eastern Coastal Banks (Wenduinebank and to a lesser extent the Paardenmarkt) are very important from spring till autumn, mainly as feeding grounds for the three tern species. In winter this area is of marginal importance. On comparison the deep-water zone, the Zeeland ridges and the offshore Hinder Banks are less important.

### **Ecological indicators**

In general, characteristics (density, biomass, biodiversity, species composition) of biological communities can be linked to habitat disturbance. However, since biological communities are quite complex to investigate, a set of ecological indicators is put forward as easy measures for community change due to general disturbances. Four 'easy measures' will be discussed: 1. Nematode Biomass

Spectra, 2. Occurrence of *Lanice conchilega*, 3. Speciation patterns in parasites and 4. Distribution patterns of seabirds.

Nematodes : the biomass distribution over size requires no taxonomical knowledge and can be used by non-specialist. Nematode biomass spectra (NBS) were constructed for undisturbed sandbanks, physically disturbed sandbanks (sand extraction on the Kwinte Bank), eutrophicated and less eutrophicated coastal stations and during a pulsed supply of fresh phytodetritus following a spring phytoplankton bloom. Using NBS allows in tracking changes in all the communities mentioned above.

*Lanice conchilega* : *Lanice conchilega* is a tube-building, suspension or deposit feeding polychaete, of which the tube has a length of up to 45 cm. *Lanice conchilega* typically occurs within the macrobenthos-rich *Abra alba* – *Mysella bidentata* community. On the western Coastal Banks the polychaete was also found in the *Magelona mirabilis* species association. Within both communities, a positive correlation between the macrobenthic density and the number of species per sample ( $N_0$ ), on the one hand, and the density of *L. conchilega* on the other hand was found. The increase in habitat complexity in dense patches of *L. conchilega* might be responsible for the increasing macrobenthic density and diversity.

Parasites: Parasite speciation is influenced by ecological and phylogenetic factors. By comparing host and parasite phylogenies a differentiation can be made between the different speciation modes. And in the case of strict co-speciation parasite phylogeny mirrors host phylogeny. This mirror association is absent in the present study, suggesting the important influence of ecological factors in this host-parasite system.

Seabirds: seabirds are often used as indicators of changes in the marine environment. Occupying high trophic levels they accumulate persistent chemicals and are extremely vulnerable to their effects, making them of value as sensitive indicators of marine pollution with chemicals. Monitoring of their numbers and distribution at the BCS can be a strong tool and an early warning system for changes within the marine ecosystem. Monitoring of contaminants in tern eggs and feathers can be used as an indicator of pollution in the Belgian waters. At present it is too early to use seabirds as indicators of changes in marine prey stock (pelagic fish, bivalves) since detailed knowledge on their feeding ecology and on food-chain interactions is still missing.

### **Ecological sensitive areas**

The Belgian sandbank system harbour important numbers of fish and macrocrustaceans such as sole (*Solea solea*) and the common shrimp (*Crangon crangon*). Young macrocrustaceans have their maximum abundance during summer.

Recruitment of fish larvae is well defined in time and happens for most species during April May. The Flemish Banks have the most abundant patterns in density for the young decapods larvae. This distribution pattern can be explained on the basis of the hydrodynamical regime in the area. Older decapod larvae reach highest densities on the Coastal Banks. Also the sheltered and food-enriched Coastal Banks provides suitable adult habitats. For example, zoea larvae of common shrimp *Crangon crangon* reach highest densities at the Flemish Banks, its postlarval stage is most abundant at the Coastal Banks, while the adults are dominant users of the adjacent beaches. It has also been shown that postlarval stages of *Crangon crangon* enter the Westerschelde estuary and use the nearby tidal marshes as a nursery.

The impact of sand and gravel extraction on benthos biodiversity has been demonstrated for the occurrence of copepods on the Kwintebank. Comparison between 1978 and 1997 was made and drastic changes have been found, both in sediment characteristics and the associated composition of the copepod communities.

Oil sensitive areas for seabirds: Winter and to a lesser extent early spring turn out to be the most delicate periods for oil-pollution. And the closer an oil-slick approaches the coastline the worse the presumed impact on seabirds will be. Within each season the western sandbank systems western Coastal Banks and Flemish Banks are highly oil-sensitive. During winter the eastern Coastal Banks (with the exception of the Vlakte van de Raan) are less vulnerable than the western part of Belgian inshore waters. In spring and summer the eastern Coastal Banks are most vulnerable.

Disturbance-sensitive seabirds such as scoters, divers, auks and grebes are most abundant in Belgian marine waters in winter and early spring. Higher disturbance-sensitivity values in autumn compared to summer are due to increasing numbers of sensitive species from October onwards. Most vulnerable sites in terms of disturbance are the western Coastal Banks and Flemish Banks, both in winter and early spring

### **Bentho-pelagic coupling**

The Belgian coastal zone is characterized by accumulation zones of deposited organic matter originating from primary production. The importance of the primary production in the water as structuring factor for benthic communities is investigated in a coastal, enriched station and an offshore station (less enriched). For the nematode community the response to the enhanced supply of organic matter was obvious in terms of abundance, community composition and feeding type distribution at both stations. At the offshore station the increase of freshly deposited organic matter is more episodic and favoured opportunistic nematode species. At the coastal station, a

rather continuous and sufficient supply of organic matter was present, which had an obvious impact on the total nematode association. An impact on the macrobenthos community was only observed at the coastal station, by increased densities of mainly young individuals in May. At the offshore station, enhanced densities, mainly mobile organisms, a rather seasonal effect in the summer months was present. It was hypothesised that dominant macrobenthic species were rather dependent on decomposition processes during summer months.

### **Genetic biodiversity of ecologically important fishes**

The physical structure of the BCS is characterized by the presence of a series of sand banks; this heterogeneity is also reflected in the ecosystem by the presence of species associations, mostly positioned on an inshore / offshore gradient. The question whether the structuring of ecosystem and species diversity and its functioning is reflected in the genetic structuring of marine taxa on the Belgian Continental Shelf was addressed for the first time. The main questions addressed in this study relate to a characterisation of genetic diversity among the various gobies species with various mitochondrial and nuclear (allozymes and DNA microsatellites) markers. From this genetic diversity an estimate is made of the genetic structure, the gene flow (or conversely of genetic isolation) and of the effective population size. The allozyme and microsatellite data on *P. minutus* point to a pattern of genetic homogeneity due to a substantial larval dispersal at least on a scale of tens of kilometres, with a possible effect of selective pressure at locus *GPI-1* in lozano's goby. Any genetic structure on the Belgian Continental Shelf is influenced by strong tidal currents and a northward bound residual current. We may assume that a strong mixing of pelagic larvae occurs in the area.

Marine organisms show a high potential for species-specific dispersal because of historical, physical and biological factors. Based on fishing tagging, adult flatfish have been reported to migrate over distances of 100 km and more. Eggs and larvae might become displaced over distances of 10 to 100 km by physical advection. In literature, a gradient in the genetic structure of *Solea* populations (also called genetic isolation by distance) was demonstrated. This means that genetic exchange between populations is high, a typical feature of marine organisms. Interesting is also that the core of each spawning unit along the Atlantic shelf is separated by a distance of about 100 km, which is exactly the distance where genetic structure is changing. In an attempt to detail the genetic diversity and structure of the Dover sole (*Solea solea*) on the Atlantic and Mediterranean continental shelf with markers complementary to the allozyme markers reported so far, we screened a limited number of populations across the full natural range. Only the mitochondrial marker cytochrome b (Bossier, pers. comm.), the nuclear intron (EPIC) marker aldolase and several nuclear

microsatellite markers seem to provide useful information of high quality. The mitochondrial control region (Bossier, pers. comm.), and the nuclear intron markers growth hormone, ITS and calmodulin do not satisfy our requirements for various reasons. We confirmed the previously found distinction between Mediterranean, southern Atlantic and northeastern Atlantic populations. However, we expect to find a higher resolution than reported in literature to discriminate among the populations (also called stocks by fisheries managers) and we should also obtain an idea of the phylogeography (this is historic patterns of migration and isolation) of Dover sole. This work should lead to the fine-tuning of the stock management of Dover sole, a task well needed as many commercial stocks are under growing fishing pressure. It should also facilitate ongoing and planned studies on the small-scale genetic structure and gene flow of Dover sole in the North Sea, the English Channel and the Bay of Biscay.

## I. GENERAL INTRODUCTION

The Belgian Continental Shelf (BCS) is the most southwestern part of the Southern Bight of the North Sea and is bordered by the Dover Strait and the Central North Sea. The North Atlantic drift and the freshwater supply of the rivers Schelde and Yzer characterize the water mass. Strong semi-diurnal tides and a net tidal current, running northeast parallel to the coast, keep the water column well mixed. A gyre, situated in front of the eastern part of the coast creates a low energy zone. The physical, chemical and biological characteristics of the BCS display a gradient from turbid, nutrient rich and well-mixed inshore waters towards more oceanic transparent and less productive offshore waters.

The Belgian Continental Shelf is characterized by numerous subtidal sandbanks, being grouped in a parallel pattern. They are elongated sedimentary bodies, typically tens of kilometres long, a few kilometres wide and several tens of metres high (Trentesaux *et al.*, 1993). Four sets of sandbanks cover a large part of the Belgian continental shelf: the Coastal Banks, the Flemish Banks, the Zeeland Banks and the Hinder Banks. Their close position to the coastline and the shallowness of the area make them unique, both from an ecological and geological point of view. Near-shore coastal sandbanks dissipate energy from incoming waves and are very effective in protecting the coast (Carter & Balsillie, 1983). The continuous interaction between morpho- and hydrodynamics results in a complex heterogeneous structure of the coastal area. This generates a variety of habitats for marine sea life showing patches of varying organic entrapment and shelter. In addition, it has often been hypothesized that the protective functioning of these sandbank systems might sustain nursery areas for several fish and crustacean species (Dyer & Huntley 1999).

It has recently been shown that the sandbank system in the Belgian coastal area acts as an internationally important resting and foraging area for various seabird species (Maes *et al.* 2000; Cattrijsse & Vincx 2001). Part of the area has therefore been designated as a Belgian Marine Wetland of International Importance under the Ramsar Convention (Maes *et al.* 2000).

The Belgian Continental Shelf experiences heavy human mediation such as nutrient enrichment, dredging activities and dumping of dredged material, sand and gravel extraction, ship traffic and oil pollution (Maes *et al.* 2000; Volckaert *et al.* 2000). In all, fisheries have been categorized as exerting the strongest pressure on the North Sea ecosystem (OSPAR Commission 2000).

The project ***'Structural and functional biodiversity of North Sea ecosystems: species and their habitats as indicators for a sustainable***



**development of the Belgian continental shelf'** aims to assess the factors that determine and influence the marine biodiversity of the Belgian Continental Shelf.

The extensive sandbank complex of the BCS is merging into gently sloping sandy beaches, and extends eastwards into the mouth of the Westerschelde estuary. The coincidence of these features is rare along the NW European coast, providing high habitat heterogeneity and hence shelter and feeding opportunities for various species.

Within this project, an emphasis is made to incorporate all existing and new biodiversity information (community, population and genetic level) on all benthic compartments, including demersal fish, their parasites and birds. Most emphasis is given on the description of the structural aspects of benthic biodiversity in order to find a pattern within the relationship between the biological and the geochemical characteristics of the sediment. The question whether the structuring of ecosystem and species diversity and its functioning is reflected in the genetic structuring of marine taxa on the Belgian Continental Shelf is addressed for the first time.

Benthos is here defined as the fauna that is associated with the seabed. The organisms living in the sediments (infauna) are classified as microbenthos, meiobenthos and macrobenthos on a size criterion. **Meiobenthos** are organisms living in the sediment passing a 1 mm sieve and being retained on a 38- $\mu$ m sieve. Dominant taxa are nematodes and harpacticoid copepods, which reach very high densities in soft sediments. Other taxa included kinorhynchs, turbellarians, interstitial polychaetes and many others. Meiobenthos samples are usually collected using boxcorers. These are subsequently subsampled using smaller cores (surface 10 cm<sup>2</sup>) from which the top 10-20 cm are studied. **Macrobenthos** is the infauna retained on a 1-mm sieve. The most important organisms are bivalves, polychaete worms and amphipods; next to infaunal echinoderms, decapods, gastropods and oligochaetes are less representative organisms. Burying larger crustaceans like *Callinassa* and some bivalve species like *Ensis* are common in coastal waters but often these animals bury to deep into the sediments for efficient sampling. Macrofauna in subtidal areas is mainly sampled with a Van Veen grab (sampling surface 0.1 m<sup>2</sup>) which samples the top 10 cm of the sediment.

The microbenthos (bacteria and protozoans) is not considered in this work.

The organisms living on or nearly on the seabed are called **epibenthos**. Data collected here include mobile epifauna (decapods, fish and echinoderms) while data on the sessile fauna were not available. The working definition for this benthic compartment actually defines epifauna as all organisms caught with a beam trawl, an otter trawl or an epibenthic sledge: starfish, sea urchins, squid, shrimp, crabs, benthic

and demersal fish. Only relative larger organisms are efficiently sampled since the mesh size of these gears is usually above 5 mm. The exclusion of sessile epibenthic organisms like hydroids and bryozoans that cannot be assessed quantitatively further narrows down the term epibenthos for our purpose.

The **hyperbenthos** comprises all organisms that occur in the lower water column and that live in close association with the sediment (mysids, isopods, amphipods, larval stages of infauna and epibenthos). The hyperbenthos is generally being sampled with a sledge. The definition is a working definition, which actually does not distinguish the epibenthos strictly from the hyperbenthos.

Furness' (1993) definition was applied to identify the **marine avifauna**. This includes both the seabirds sensu stricto and the high number of migrants occasionally found within the study area and not directly dependent on marine ecosystems. Divers, grebes, petrels, cormorants, skuas, gulls, terns, auks, seaducks, eider and long-tailed duck are considered as the true seabirds.

For the study of the **genetic structuring of marine taxa** the choice of model species was critical in several regards. Key species of the region represent a first choice, as they are important in differentiating the communities. However they should occur commonly and be widespread with easy sampling access. Hence macrobenthos and fishes should offer the best chances. Ultimately one fish group was chosen as the main target: the *Pomatoschistus* gobies, which are ecologically very significant due to their numerical dominance, as predator of meio- and macrobenthos and as food for the large commercial fish species. Although considerable information was available on their population dynamics, ecology and taxonomy, few studies had attempted to address the genetic structuring of *Pomatoschistus minutus*, *P. lozanoi* and *P. microps* on the Atlantic shelf (Wallis & Beardmore, 1984; Al-Hassan *et al.*, 1987) and none was made on the Belgian Continental Shelf. Three additional representative species were included as indicators of the fish fauna of the Belgian continental shelf. Each occupies a different ecological niche from the gobies and has traits not found in the other: Dover sole (*Solea solea*), three-spined stickleback (*Gasterosteus aculeatus*) and European eel (*Anguilla anguilla*).

The **ectoparasites** belonging to the genus *Gyrodactylus* (Monogenea, Platyhelminthes) parasitising the three-spined stickleback (*Gasterosteus aculeatus*) and gobies from the genus *Pomatoschistus* (Gobioidea, Teleostei) were studied as well. To uncover and discriminate among the phylogenetic and ecological speciation patterns that contributed to the enormous diversity (20.000 species), detailed studies on *Gyrodactylus* in its natural habitat are needed.

This report is divided into several chapters: all data on the monitoring of the Belgian Continental Shelf have been summarized already in Cattrijsse & Vincx (2001) but will be illustrated in this report more in detail for the hyperbenthic

component as a case study. Several aspects of the sandbank ecosystems are considered: (1) spatial and temporal distribution of the above mentioned components together with emphasis on habitat characterization within these sandbanks; (2) the ecological indicators are discussed together with the delineation of vulnerable areas. The interactions in the higher trophic levels are only briefly discussed while the benthopelagic coupling is studied in detail for the link between plankton and meio- and macrobenthos. The genetic biodiversity of ecological important fish species is explained. Finally some conclusions and recommendations will be made for biodiversity conservation and sustainable development of North Sea Ecosystems with emphasis on the Belgian coastal area and the subtidal sandbank systems.

## II. MONITORING OF THE BELGIAN CONTINENTAL SHELF

### II.a. General biodiversity patterns

The benthos of the Belgian Continental Shelf (BCS) has been monitored since 1970 and since 1986 for avifauna and sea mammals. To detect changes in biodiversity patterns, literature data were collected on diversity measures and published in a review paper 'Biodiversity of the benthos and the avifauna of the Belgian Coastal Waters. Summary of data collected between 1970-1998 (Cattrijsse & Vincx, 2001).

For this analysis, the BCS was divided into nine zones (Fig.1). The demarcation of these zones relies partly on geographical and partly on biological information.

Zone 1, the east coast, encloses the shallow inshore waters between the Belgian-Dutch border and the city of Oostende. Zone 2, the west coast, stretches between Oostende and the Belgian-French border. The northern limit of these two zones was arbitrary chosen as the southern tips of the 'Flemish Banks'. The 'Wenduine bank' is the only sandbank in zone 1. The so-called coastal banks the 'Stroombank', the 'Nieuwpoort Bank', the 'Trapegeer', and the 'Smalbank' form Zone 2. The 'Oostdyck', the 'Buiten Ratel', the 'Kwinte Bank', the 'Middelkerke Bank' and the 'Oostende Bank' constitute the Flemish Banks and form zones 3 and 4. Zone 3 represents the slopes and the tops of these subtidal sandbanks while zone 4 delineates the gullies between them. The Zeeland Banks form zone 5: the 'Vlakte van de Raan', the 'Akkaert Bank', the 'Goote Bank' and the 'Thornton Bank'. The gullies between these banks are zone 6. Still further offshore lies the Hinder Bank area (the 'Bligh Bank', the 'Oosthinder', the 'Westhinder', the 'Fairy Bank' and the 'Noordhinder').

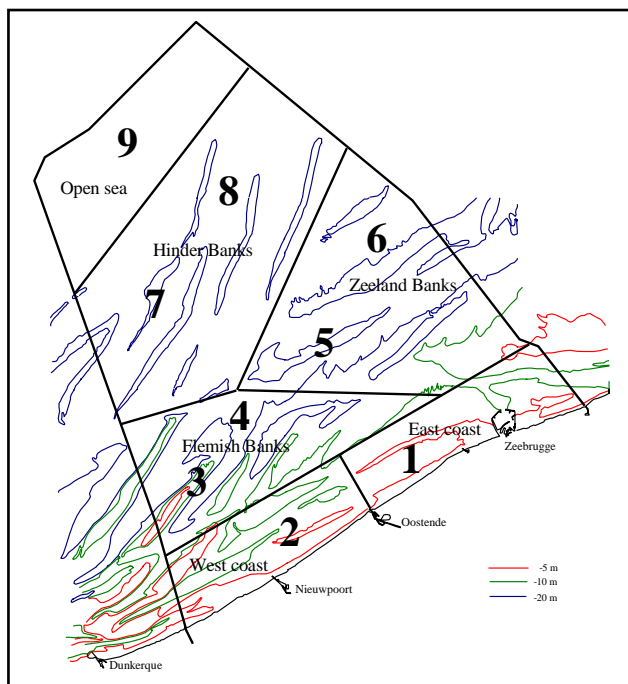


Figure 1: Belgian Continental Shelf indicating the 9 bio-geographical zones

In analogy with the Flemish and the Zeeland Banks the gullies and the banks form respectively zone 7 and 8. North of the Hinder Banks, the deepest area of the BCS is designated as zone 9 (open sea zone).

For each of these zones, data on species number and species richness were collected. Results from this analysis were summarized as follows: clear patterns in average species number have been observed for the meiobenthos, the macrobenthos, and the hyperbenthos. The species number of benthic assemblages in inshore waters changes dramatically between the eastern and western end of the Belgian coast. A second gradient runs perpendicular to the coastline and involves an increasing species number and diversity offshore. A third gradient relating to depth within a zone needs yet to be confirmed. An offshore decrease in density has also been observed for macrobenthos and hyperbenthos. Avifauna data do not confirm these patterns even though seabird assemblages offshore differ from the inshore avifauna. All observed patterns need yet to be confirmed by standardised research and monitoring, being the aim of the ongoing program.

Besides the analysis of structural and functional biodiversity data on benthos and avifauna, this synthesis also examined the knowledge on the genetic structure and the parasite fauna of fish occurring on the BCS. The limited information available on the Belgian coastal waters points at continuity between fish populations of the English Channel, the Southern Bight and the Central North Sea. Genetic differences of fish parasites have been observed in the area and may aid in addressing issues like genetic selection of the heavily fished populations.

### **II.b. Detailed monitoring survey**

As an example, recent results of the hyperbenthos monitoring will be presented (Dewicke, 2001). Information of the other benthic compartments, the avifauna and the parasites are presented in chapter III because they were also mainly related to the sandbanks.

Research on the hyperbenthos of the Belgian coastal area has started in 1993 by the Marine Biology Section of the Ghent University. An extensive spatial study, covering all sandbanks sets of the Belgian continental shelf and off Zeeland was carried out in September 1993 in order to provide good baseline data for continuous monitoring. Temporal data were achieved by monthly sampling at the Coastal, Flemish and Zeeland Banks during 1994 and 1995. The hyperbenthos has also been monitored during winter and late summer of the following years. Following text provides a summary of the major findings concerning the hyperbenthos community structure and the structuring environmental factors; for details of these studies is referred to other chapters of this report.

### II.b.1. Principal gradients in community structure

The hyperbenthos shows two gradients in community structure at the Belgian and Zeeland continental shelf: a principal onshore-offshore gradient perpendicular to the coastline and a less pronounced east-west gradient parallel to the coastline. The first gradient mainly indicates differences in density and biomass of the hyperbenthic taxa, while the second reflects species richness and diversity.

The community structure of the holohyperbenthos shows a strong change between the Coastal Banks on the one hand and further offshore waters on the other hand (Flemish Banks, Zeeland Banks, Hinder Banks).

The Coastal Banks are year-round dominated by mysids (mainly *Schistomysis kervillei*), except during the spring months, which are characterised by high densities of amphipods (*Atylus swammerdami*, *Gammarus crinicornis*, epifaunal amphipods like *Microprotopus maculatus*, *Pariambus typicus* and *Phtisica marina*) and ctenophores (*Pleurobrachia pileus*). Yearly average biomass value in this area is for more than 50 % accounted for by mysids. Species diversity is significantly lower at the E-Coast while total density and biomass are higher than at the W Coast.

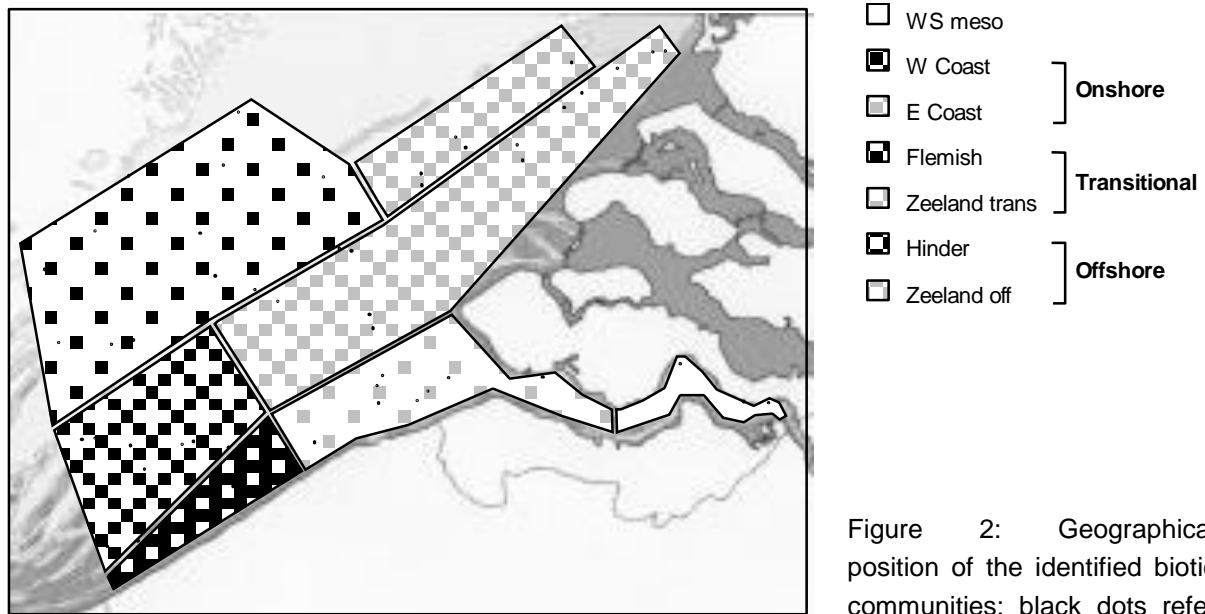
The Flemish Banks are characterised by the occurrence of planktonic species (chaetognaths, copepods, hydromedusae and ctenophores) accounting for half of the total density in this area. Bottom-dependent taxa like mysids, amphipods and cumaceans generally reach low densities in this area and preferentially occur in the swales. Yet, they might be more abundant during certain conditions, as observed in May during a *Phaeocystis* bloom and supposedly also during harsh weather conditions. The Flemish Banks harbour a more diverse community than the Coastal Banks. Yearly average densities might be comparable, though total biomass is lower in offshore waters.

The merohyperbenthos community is strongly temporally structured, due to different recruitment peaks of various postlarval fish and decapod larvae. Both species richness and total abundance of fish larvae are highest during April - May. Most abundant species are sole *Solea solea*, *Pomatoschistus* spp., flounder *Pleuronectes flesus*, clupeids and dab *Limanda limanda*. Recruitment for decapod larvae is most pronounced from late spring to late summer, with respect to both species richness and densities. Common shrimp *Crangon crangon*, swimming crab *Liocarcinus holsatus*, shore crab *Carcinus maenas*, and porcelain crab *Pisidia longicornis* are most common.

Despite the strong temporal variability in the merohyperbenthos community structure, a clear onshore-offshore gradient exists as well. Species diversity is higher at the Flemish Banks compared to the Coastal Banks. This gradient coincides with a change in ontogenetic structure of the larval decapod community. Zoea stages of nearly all species are typically found at the Flemish Banks, whereas postlarval



carideans are more abundant at the Coastal Banks. Similar as for the holohyperbenthos, diversity is clearly lower at the E Coast compared to the W Coast. The further offshore Hinder Banks were only assessed during summer 1993. Both density and diversity were significantly lower compared to the Flemish and Zeeland Banks, while the major taxonomic composition was rather similar. Also, a strong diversity gradient was found with a richer fauna off the Belgian coast as compared to the Zeeland offshore waters (Figure 2).



to sampling sites

Figure 2: Geographical position of the identified biotic communities; black dots refer

### II.b.2. Important factors influencing community structure

*Spatially:* Habitat heterogeneity and water mass flow characteristics are believed to be of major importance in regulating the hyperbenthos in the Belgian and Zeeland coastal area.

The cross-shore gradient in community structure is related to sedimentological and physico-chemical variables, with finer sediments and the influence of coastal run-off (e.g. high nutrient load, strong variability in temperature and salinity) onshore. High levels of suspended matter and the presence of a mud field at the Belgian Coastal Banks favour bottom-dependent animals in terms of food. Outflow from the Westerschelde estuary, nutrient-enriched and less saline water, most probably explains the diversity decline at the E Coast. Resistant species are however thriving in high numbers at the mouth of the estuary, despite the unpredictable fluctuations in its environment. The influence of Channel water at the offshore Flemish and Hinder Banks on the one hand and the heterogeneity in seabed structure on the other hand,

might play a role in sustaining more diverse communities in these areas as compared to the area off Zeeland.

In addition, tidal flow is hypothesized to play an important role in the spatial structuring of the hyperbenthic communities in the Belgian coastal zone. Onshore, tides are asymmetric and flood-dominated (i.e. towards the north). The relative strength of flood versus ebb current gradually decreases with offshore distance, and the ebb current (i.e. towards the south) gets dominant off the Flemish Banks. Differential swimming abilities and potential of maintaining position in flow are believed to lead to an onshore - offshore segregation among taxa.

Low current velocities at the Coastal Banks may permit settlement of the suspended matter load, enabling growth of a variety of organisms that live or feed in the near-bottom water layer. Mysids, amphipods and postlarval shrimp favour this area and have the ability to maintain their position and avoiding offshore transport by the flood-dominated flow. Suspended matter concentration is *in se* lower at the Flemish Banks, and deposition on its crests is moreover prevented by a persisting strong flow. Although of minor importance, organic matter sedimentation at the Flemish Banks may occur in the swales, which are characterised by slower currents at the turning of the tides, and thus permitting development of populations of bottom-dependent animals. High densities of planktonic organisms in this area, like cnidarians, hydromedusae and decapod zoeae, can be due to accumulation processes, induced by the particular current regime. Flood-dominance on the shore might lead to dispersal in a seawards direction, while ebb-dominated flow north of the Flemish Banks could deliver an additional supply of organisms from farther offshore waters.

*Temporally:* It is well known that recruitment of merohyperbenthic species (like larval fish and decapod larvae) is often related to temperature and probably also to phytoplankton blooms. This is confirmed for the Belgian coastal area. But, temporal variability in holohyperbenthos community structure is not clearly correlated with physico-chemical factors. Seasonality might be masked by migration behaviour performed by many species (mysids in particular) and by differential reproduction periods of the holohyperbenthic species. Some biological phenomena, like the blooming of *Phaeocystis* during spring and the growth of hydrozoan colonies during summer are believed to exert a structural influence. Nevertheless, temporal variability has been shown to be to a certain extent related to fluctuations in the residual flow. A conspicuous correlation between monthly numbers of mysid species and this residual flow could be indicated. Interannual variability, observed between the autumn months of two succeeding years could be linked to differential directions in residual flow, which is probably driven by variations in wind conditions. It is not unlikely that hydrodynamic conditions *per se* play a major role in structuring coastal hyperbenthic communities, especially with respect to larval recruitment events.

Thus, the estuarine outflow on the one hand and the hydrodynamic setting and protective function of the sandbanks on the other hand create diverse environments along the Belgian coastal zone. The sandbanks system extends moreover in gently sloping sandy beaches. This makes the setting of the Belgian coastal sandbank system ecologically unique. The area sustains rich communities of holohyperbenthic species and early life history stages when compared to the adjacent areas. Its beaches and the Westerschelde estuary have both been shown to act as nursery areas for fish and macrocrustaceans, pointing to the importance of the area.

### III. SANDBANK ECOSYSTEMS OF THE BELGIAN CONTINENTAL SHELF

#### III.a. Spatial and Temporal distribution

##### III.a.1. Meiobenthos

##### III.a.1.1. Meiobenthos community at higher taxon level

*Spatial pattern:* The meiobenthos of the sandbanks was investigated during 1997 and 1998 (Vanaverbeke *et al.* 2000). In total, 10 sandbanks have been sampled, belonging to three different geographically isolated systems: the Flemish Banks, the Hinder Banks and the Zeeland Banks (see also Figure 1). No obvious differences in sedimentological characteristics between the sandbanks were found, but biologically some differences could be detected. The more offshore Hinder Banks had the most diverse meiobenthos, while the Flemish Banks harboured the lowest number of meiobenthic taxa (Fig. 3).

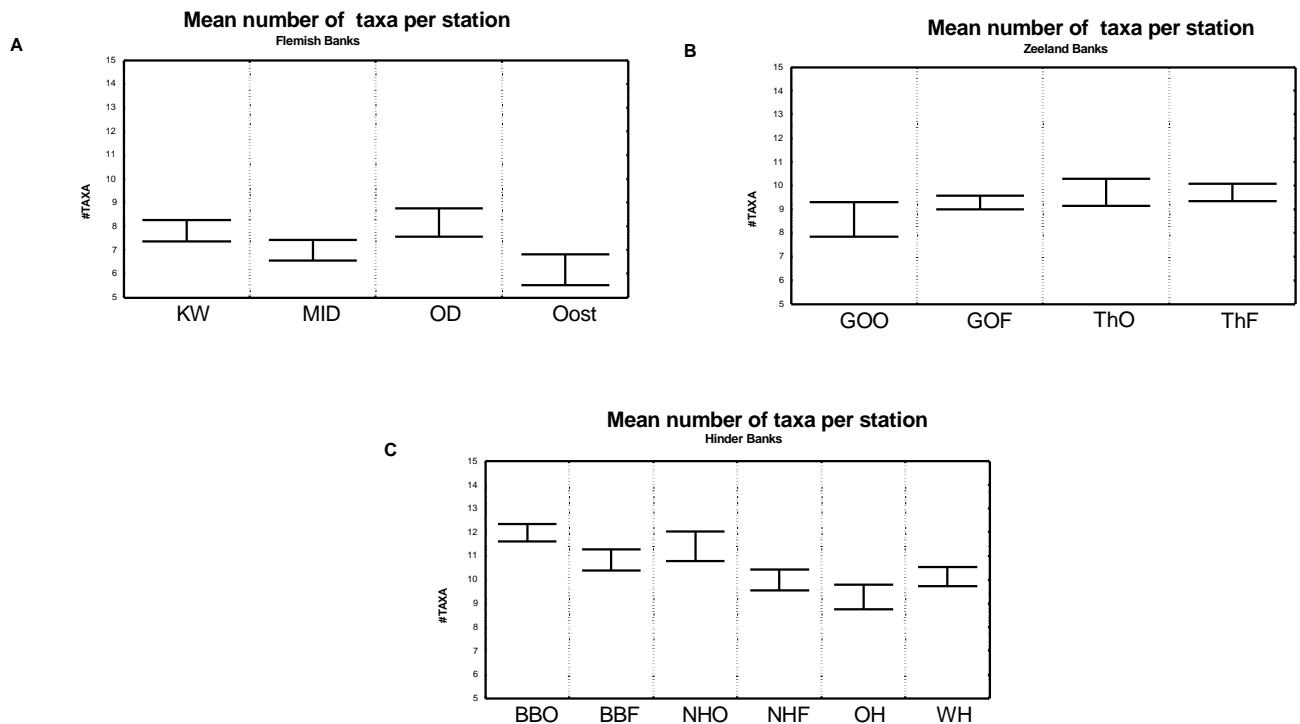


Figure 3: Meiobenthic taxon diversity per sandbank system (A: Flemish Banks; B: Zeeland Banks; C: Hinderbanks (NH: Noordhinder; BB: Bligh Bank; OH: Oosthinder; WH: Westhinder; Th: Thornton Bank; GO: Goote Bank; Kw: Kwinte Bank; OD: Oostdijck; Oost: Oostende Bank; MID: Middelkerke Bank; O: October; F: February)

*Temporal pattern* : Seasonal and regional differences in terms of densities are a result of a coupling with the primary production in the water column, October showing highest densities and the offshore Hinder Banks harbouring lowest densities (Fig. 4). The meiobenthos on the sandbanks was less dense (450-500 ind./10 cm<sup>2</sup>) than in the deeper channels between the sandbanks (1250-1600 ind./10 cm<sup>2</sup>). These differences were attributed to high hydrodynamic stress around the sandbanks, preventing phytoplankton to reach the sandbanks. Moreover, higher current speed above the sediment increases the risk for the meiobenthos of being eroded or suspended during storms.

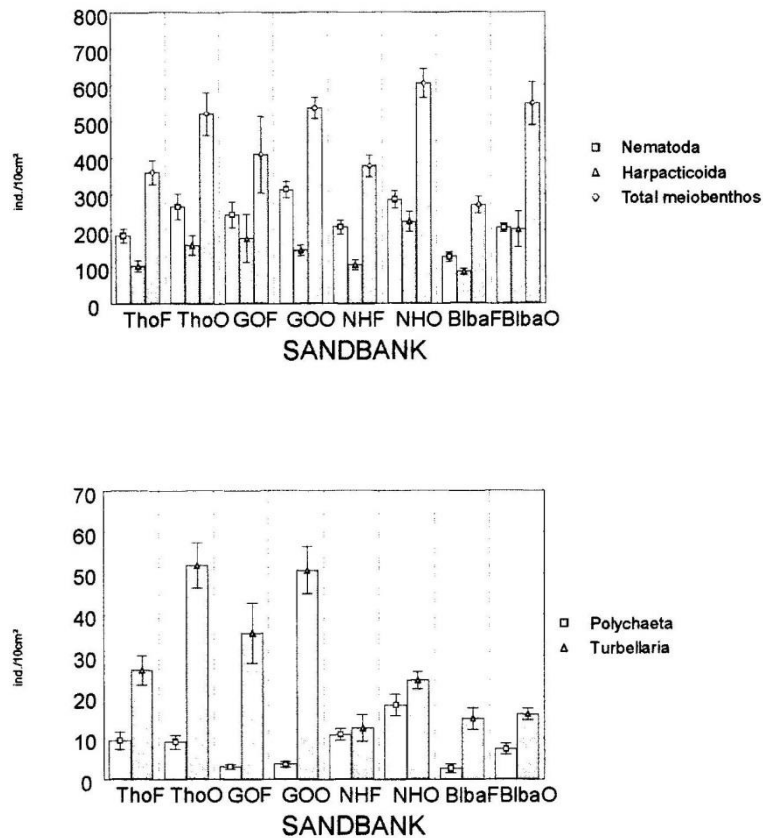


Figure 4: Mean Densities per sandbank and per sampling period ThoF: Thornton Bank February; ThoO: Thornton Bank October; GOF: Gootebank February; GOO: Gootebank October; NHF: Noordhinder February; NHO: Noordhinder October; BlbaF: Bligh Bank February; BlbaO: Bligh Bank October)

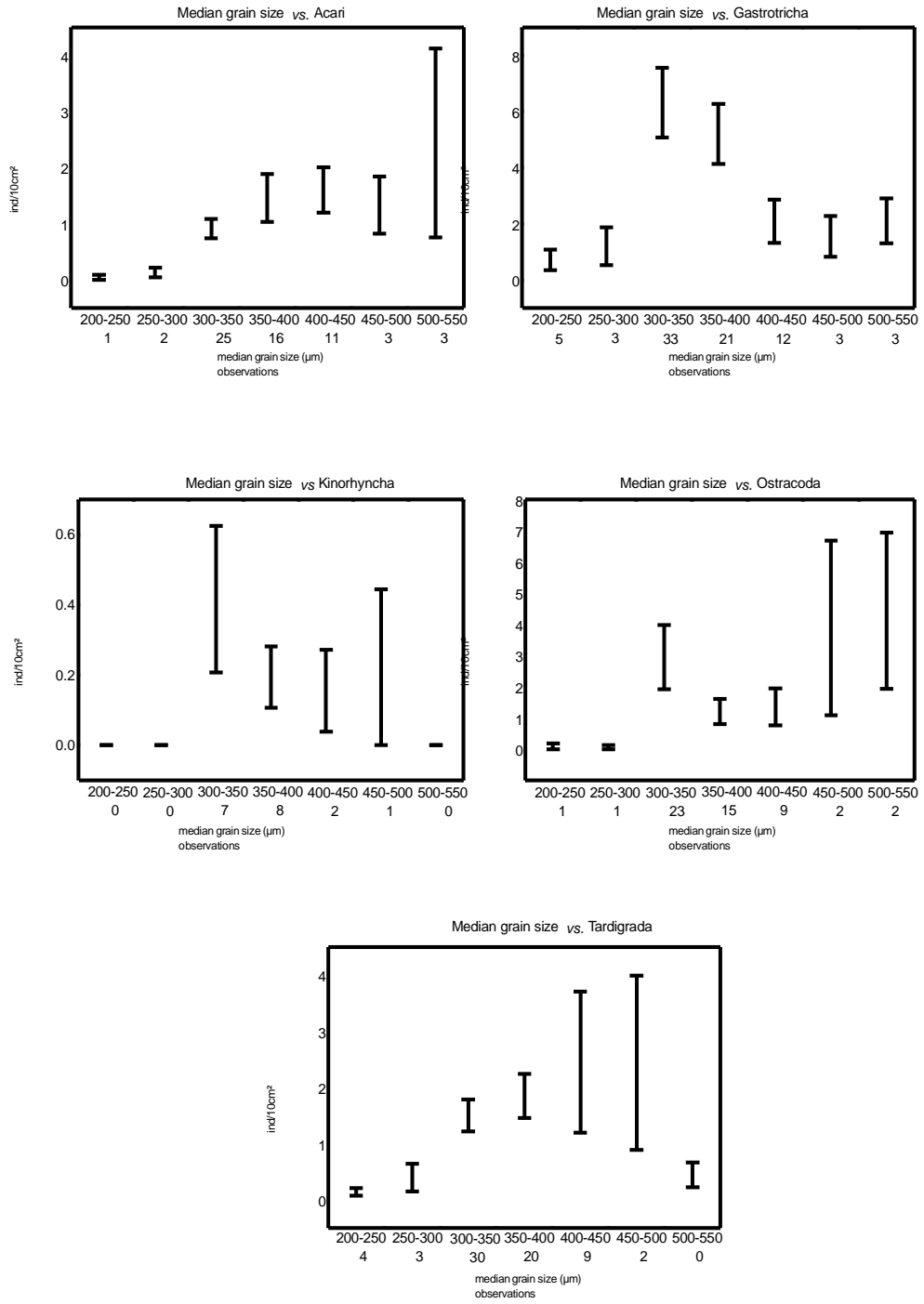


Figure 5: Densities of rare taxa vs. median grain size and number of observations per size class. Note different scaling of y-axis



Four different communities are identified which do not reflect the geographical position on the BCS but are influenced by local differences in sedimentological characteristics within sandbanks and sandbank systems.

The sedimentological differences influenced the taxon diversity as well. Sediment preferences for less abundant taxa were investigated in several ways, and results indicated that sediments with a median grain size below 300  $\mu\text{m}$  were poor in terms of densities and number of taxa, while coarser sediments were richer in taxon diversity (Fig. 5, 6 and Table I). Sediments with a median grain size between 300-450  $\mu\text{m}$  were rich both in terms of diversity and density, while sediments with a median grain size of >450  $\mu\text{m}$  still showed high diversity but lower densities.

Table I: Xi per taxon. Chance of finding a taxon in a certain size class assuming that all stations (also without considered taxon) are equally distributed among size classes.

	Gastro- tricha	Acari	Ostra- coda	Turbel- laria	Kino- rhynch a	Tardi- grada	Nema- toda	Poly- chaeta	Harpac- ticoida
200-250 $\mu\text{m}$	10.00	3.35	4.07	14.59	0.00	9.35	14.29	12.88	14.29
250-300 $\mu\text{m}$	10.50	9.37	5.70	14.59	0.00	13.09	14.29	12.02	14.29
300-350 $\mu\text{m}$	15.20	15.42	17.26	13.06	18.72	17.22	14.29	15.02	14.29
350-400 $\mu\text{m}$	15.31	15.62	17.82	13.98	33.87	18.18	14.29	15.02	14.29
400-450 $\mu\text{m}$	14.00	17.18	17.11	14.59	13.55	13.09	14.29	15.02	14.29
450-500 $\mu\text{m}$	17.50	23.43	19.01	14.59	33.87	14.54	14.29	15.02	14.29
500-550 $\mu\text{m}$	17.50	15.62	19.01	14.59	0.00	14.54	14.29	15.02	14.29

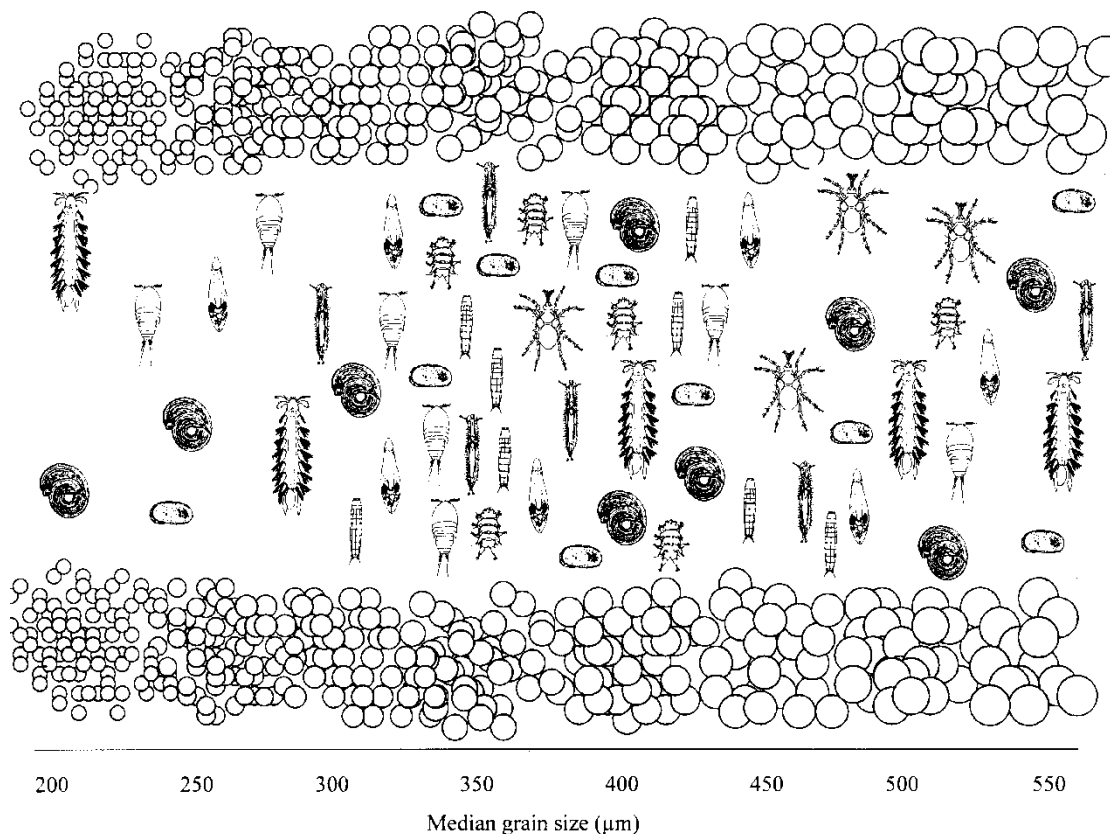


Figure 6: Biodiversity of meiobenthos in relation to grain size of the sediment

### III.a. 1.2. Nematoda

The nematode communities from four sandbanks belonging to different sandbank systems were investigated in order to describe the spatial patterns over the sandbanks.

Multivariate analysis (both Twinspan and DCA) revealed the existence of six nematode communities. The Noordhinder (Twin 1) and Bligh Bank (Twin 2) nematode communities were clearly separated from the Goote (Twin 3) and the Kwinte Bank (Twin 4, 5, 6). The latter sandbank harboured three nematode communities: Twin 4 comprised the stations located at the northern part of the sandbank together with the channel station 12, while the southern stations were grouped in Twin 5. As a final twin group (Twin 6), another channel station was identified. No seasonal trend could be detected.

Diversity values (expressed as Hill numbers) were clearly different between the Twin groups (Fig. 7).

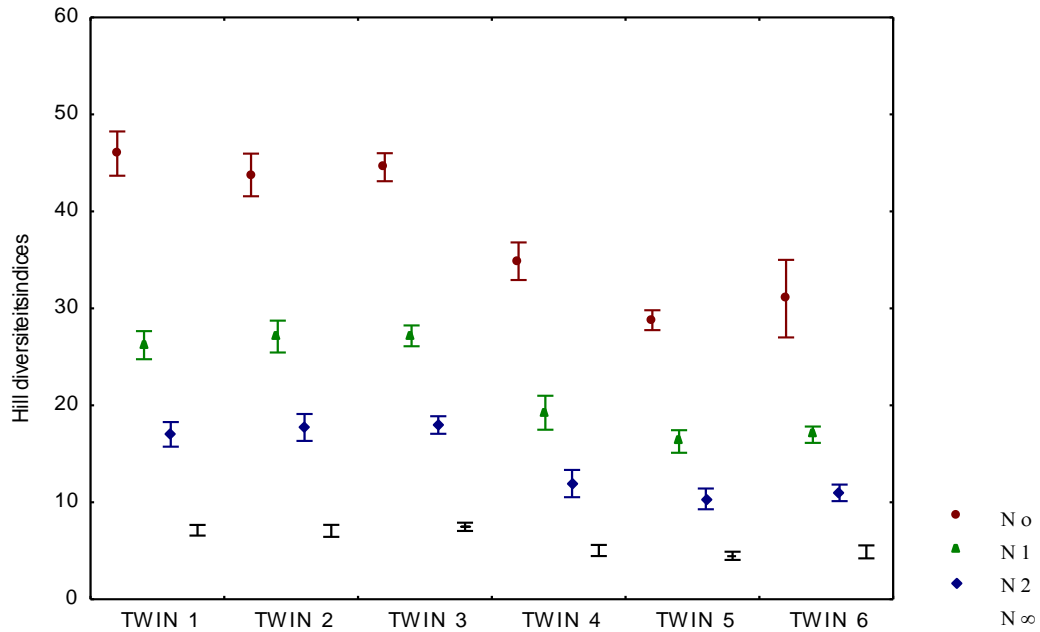


Figure 7: Mean Hill numbers per Twin group (error bars represent standard errors)

The Kwinte Bank (Twin 4, 5 and 6) had significantly lower diversity values compared to the other sandbanks. The increase of diversity with distance to the coast seems to be a general feature for nematode communities at the BCS (see Vanaverbeke *et al.* 2001 for an overview).

Figure 8 clearly shows that sediments in the channel station of Twin 6 were clearly finer when compared to sandbank sediments.

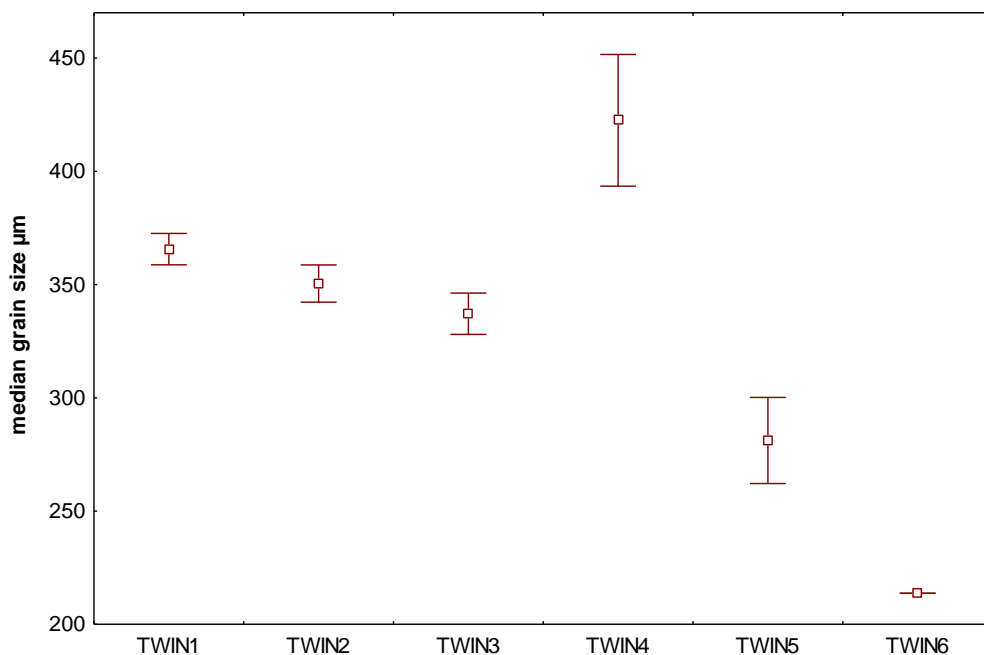


Figure 8: Mean median grain size per Twin group (error bars indicate standard errors)

Median grain size was coarser at Twin 4 compared to Twin groups 2 and 3, while finer sediments were found in Twin 5 stations.

Within the Kwinte Bank, the northern stations (Twin 4) were significantly coarser than those from Twin 5, revealing the coarsening effect of sand extraction. The differences in sediment composition from the Kwinte Bank stations are responsible for the existence of three nematode communities. Three completely different nematode communities exist on the other sandbanks, while sediment characteristics are quite similar.

In conclusion, sandbanks can be seen as geographically isolated “islands” since tidal current patterns around these sandbanks prevent nematodes from being transported from one sandbank to another (Gheskiere 2000).

### **III.a.1.3. Copepoda**

Density, diversity and community structure of harpacticoid copepods were analysed on the Kwinte Bank and related to sediment characteristics and sand extraction intensity. Because of its location near the coast, the appropriate grain size of the sand and the low lime content, more than 95% of the sand extraction on the Belgian Continental Shelf occurs on the Kwinte Bank. To relate the structure of different harpacticoid communities to environmental factors, it's important to take the intensity of dredging into account, as this can be an important structuring factor. The communities of sandbanks and sand waves in areas with a high amount of exposure, as is the case on the Kwinte Bank, are adapted to continuous changing conditions. Since 1978 the Kwinte Bank has been the subject of several benthic research projects, making it possible to compare the present day community analyses with the situation in the seventies (Claeys, 1979), before the start of intensive sand extraction.

In the northern part of the Kwinte Bank (station 1 to 4) mean densities were comparable and counted 63 to 65 ind./10 cm<sup>2</sup> (Fig. 9). Mean density was highest at station 7 (105 ind./10 cm<sup>2</sup>) and decreased to the south of the bank. A very low mean density was recorded in the centre for station 6 (10 ind./10 cm<sup>2</sup>) and in the gully stations, significantly differing from all the other sandbank stations.

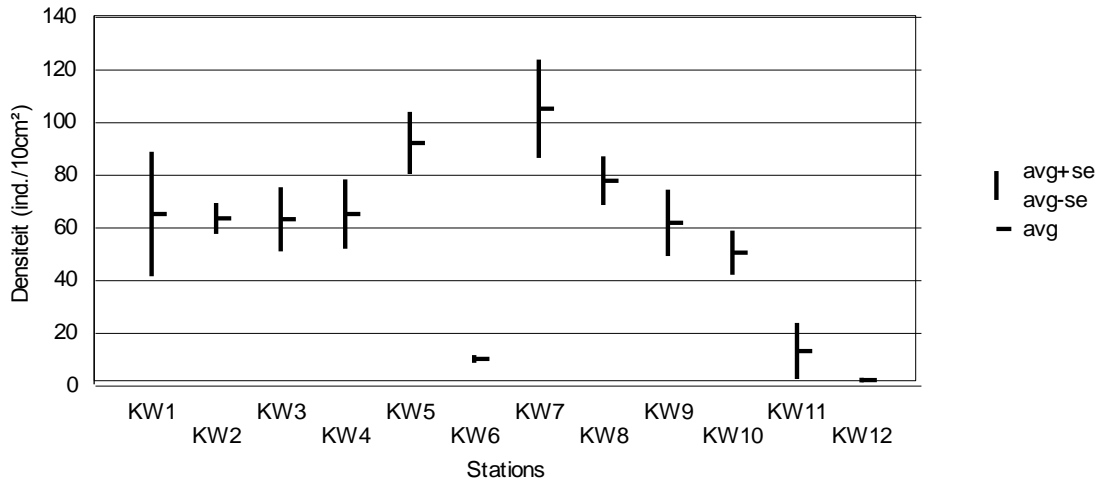


Figure 9: Mean total densities per station

Diversity indices of Hill (Fig. 10) are significantly higher in the northern part of the sandbank (stations 1,2 and 3) and highest at the northern tip (station 1).  $N_1$  and  $N_2$  are negatively correlated with fine sand ( $p < 0.05$ ) and  $N_2$  and  $N_\infty$  positively with the median grain size ( $p < 0.05$ ).

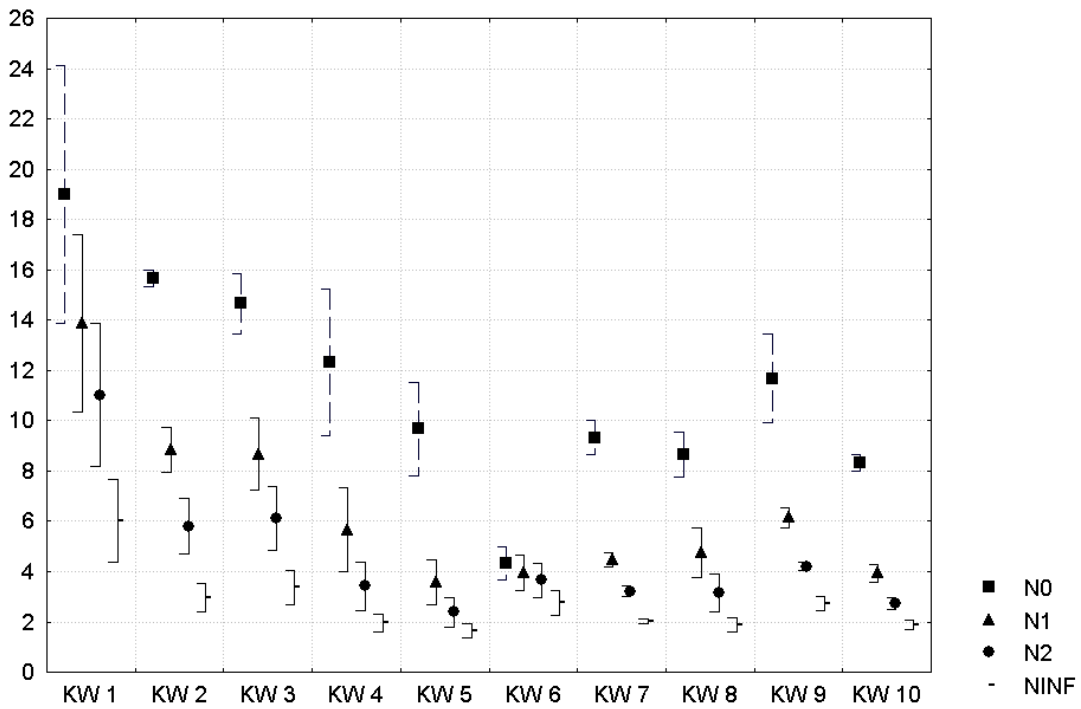


Figure 10: Diversity indices of Hill per station

The high diversity of microhabitats of coarse sediments and the dynamic conditions due to the impact of the strong currents, create a very diverse and dynamic biotope for meiofaunal organisms in the northern part of the sandbank. Diversity decreased linearly to the south of the sandbank. Surprisingly the lowest species richness ( $N_0$ ) was recorded at station 6, although a high species richness was expected due to the high percentage (15 %) of coarse sands in this station.

#### *Long-term trend in sedimentology*

In 1978 the median grain size showed a linear trend and decreased from north to south (Willems *et al.*, 1982). The gradient still existed in 1997 but the difference in grain size between north and south in 1997 was not as pronounced as in 1978, mainly because of dramatic changes at station 5 and because the percentage of medium sand increased to a great extent in the south of the sandbank (from 18 % to 45 %). The domination of this area by fine sands was reduced from 71 % in 1978 to 47 % in 1997. At deeper stations in the north, fine sands were more abundant in 1978 but also gravel and very coarse material was present, creating a very diverse biotope for meiofaunal organisms. However, the heterogeneous sediment of station 1 is much more homogenised in 1997. The gravel, very coarse and fine sands, were replaced by medium sands. The former sediment composition of sand waves still applied to station 3 and 4 in 1997 but the abundance of fine sands declined and gravel and very coarse sands increased remarkably. The biggest changes were recorded for station 5. In 1978 it was a coarse station with a dominance of coarse sand of 46 %, it changed into a fine sand station with a dominance of fine sand of 73 %. In station 6 the sediment coarsened from 4 % to 14 % of coarse sand (Table II).

#### *Changes in the copepod community*

Densities were significantly higher ( $p < 0.05$ ) in 1978 than in 1997. In 1978 the lowest densities were recorded at the only two stations with a very high percentage of fine sands (92 %); density was highest in station 5, characterised by the highest percentage of coarse sand (46 %). Because of a different taxonomic resolution at species level between 1978 and 1997, diversity indices of Hill of both years were compared at genus and family level, although this resulted in an underestimation of genus diversity in station 3 in 1978. In 1978 genus and family richness were higher in the centre of the sandbank ( $p < 0.05$  for station 4 and  $p < 0.01$  for station 5) compared to 1997. Family richness in 1978 was also significantly higher at station 1. A remarkable but not significant trend at genus level is shown by the higher values for  $N_1$ ,  $N_2$  and  $N_\infty$  in 1997 in the northern part (stations 1 to 3) and at stations 7 and 8. At family level this trend is also obvious for station 6, but not for station 3.

During the year 1997, 95 % of all harpacticoids on the Kwinte Bank were interstitial copepods, 3 % were epibenthic and 2 % endobenthic ones. At the northern



tip (station 1) density and species richness of epibenthic copepods were significantly lower in 1997 than in 1978. In station 3, density of epibenthic species was significantly higher ( $p < 0.05$ ) in 1997 because of the high abundance of a *Metacyclops* species, a small paramesochrid-like cyclopoid. Densities, species richness and  $N_2$  of endobenthic species were significantly lower ( $p < 0.05$  and  $p < 0.01$ ) in 1997 compared to 1978, while the densities of interstitial species were significantly higher ( $p < 0.05$ ) in 1997. Within this interstitial group the diversity indices of Hill increased significantly for the entire sandbank except for the centre (stations 5 and 6), where a decrease of species diversity ( $N_0$ ) is found.  $N_1$  and  $N_2$  decreased only in station 5.

When the data of benthic copepods of 1997 and 1978 are compiled in a DCA, the identified copepod communities of 1978 and 1997 are clearly separated along the first axis, of length 4.08, but no environmental gradient is significantly correlated with this pattern. The absence of *Robertgurneya ilievecensis* in 1997 was selected by TWINSPLAN as the most important characteristic to distinguish both years. The southern communities are separated from the northern communities for both years along the second axis, of length 3.0. Differences in fine and coarse sand content were important along the second axis. In the northern community a higher variability was observed in 1978 in comparison with 1997. The similarity, indicated by cluster analysis, within the northern part increased with 30 % from 1978 to 1997, while the southern community of 1978 (stations 6, 7, 8, 9 and 10) and of 1997 (stations 5, 7, 8, 9 and 10) still shows a high similarity after 20 years.

Table II: Differences in granulometric and biological characteristics between 1978, 1997 and 2000 at stations 5 and 6, situated in the depression in the centre of the Kwinte Bank

	Depression in the centre			
	Station 5		Station 6	
	1978	1997	1978	1997
Very coarse sand (%)	6	0.3	3	4
Coarse sand (%)	46	1	4	14
Medium sand (%)	43	25	57	54
Fine sand (%)	5	73	35	27
Very fine sand (%)	0.1	0.5	0.5	0.2
Sediment change	refining (by accretion of fine sediments in the recently formed depression ?) ↓		coarsening (by erosion ?) ↓	

	1978	1997	2000	1978	1997
Density (ind./10cm <sup>2</sup> )	463	100	39	101	10
Number of species	20 <sup>(1)</sup>	10	10	8 <sup>(2)</sup>	4
Epi- and endobenthic species (%)	21	1	0	21	5
Interstitial species (%)	79	99	100	77	95
<i>Paraleptastacus espinulatus</i> (%)	0	64	33	27	25
	decreasing density and diversity community shift impoverishment			decreasing density and diversity impoverishment	

<sup>(1)</sup> 13 % of individuals were not identified

<sup>(2)</sup> 21 % of individuals were not identified

On the multibeam-image of the Kwinte Bank, performed by the Geological Survey of Belgium in 2000, the sand waves in the north of the sandbank, the kink in the centre and the flat elevated plateau of the south are easily recognisable (Figure 26, chapter IIIc). But the centre of the sandbank shows a disturbed topography. A depression is formed in the central part beneath the kink in the longitudinal profile of the sand bank, in which stations 5 and 6 are situated and which was not present in the seventies. In this area, the centre of the bank, diversity decreased: a shift was recorded from a species rich northern community to a less diverse southern community (less species, high dominance of *Paraleptastacus espinulatus*, *Leptastacus laticaudatus* s. str. and *Kliopsyllus constrictus* s. str.) as a result of changes in sediment characteristics. The altered sediment composition may be the result of an accretion of fine sediments, as a consequence of changed current patterns in the depression. The extension of the present depression due to sand extraction can become quite problematic if these human-induced physical disturbances may cause a continuing erosion and impoverishment.

### III.a.2. Macrobenthos

Within the frame of different research projects, a large number of stations on the Belgian Continental Shelf were sampled for the macrobenthos between 1994 and 2000. These samples cover a diverse range of habitats: from the sandy beaches to the open sea, from the gullies in between sandbanks to the top of the sandbanks, and from coarse sandy to clay sediments. To investigate the large-scale spatial

distribution of the macrobenthos of the BCS, the data of all these research projects - 740 sample places in total - were combined and analysed. By means of several multivariate techniques (Two Way Indicator Species Analysis, Detrended Correspondence Analysis and Cluster analysis), 10 sample groups were distinguished. Each sample group is found in a particular physico-chemical environment and contains a specific set of species (Figure 11). Four sample groups are differing drastically, both in habitat and species composition, and are considered to represent four macrobenthic communities.

A first community, the *Abra alba* – *Mysella bidentata* community (group 1 and 2), is found at sites, situated mainly in the gullies of the western Coastal Banks and the Southern Flemish banks (Kwinte and Middelkerke bank), with fine sandy sediments and relatively high mud contents. The high macrobenthic diversity ( $N_0$ : 25 species/sample on average) and densities (5356 ind/m<sup>2</sup> on average) are characteristic for this group. The dominant species are the bivalves *Abra alba*, *Spisula subtruncata* and *Mysella bidentata*, the polychaetes *Lanice conchilega* and *Sthenelais boa*, and the crustacean *Pariambus typicus*.

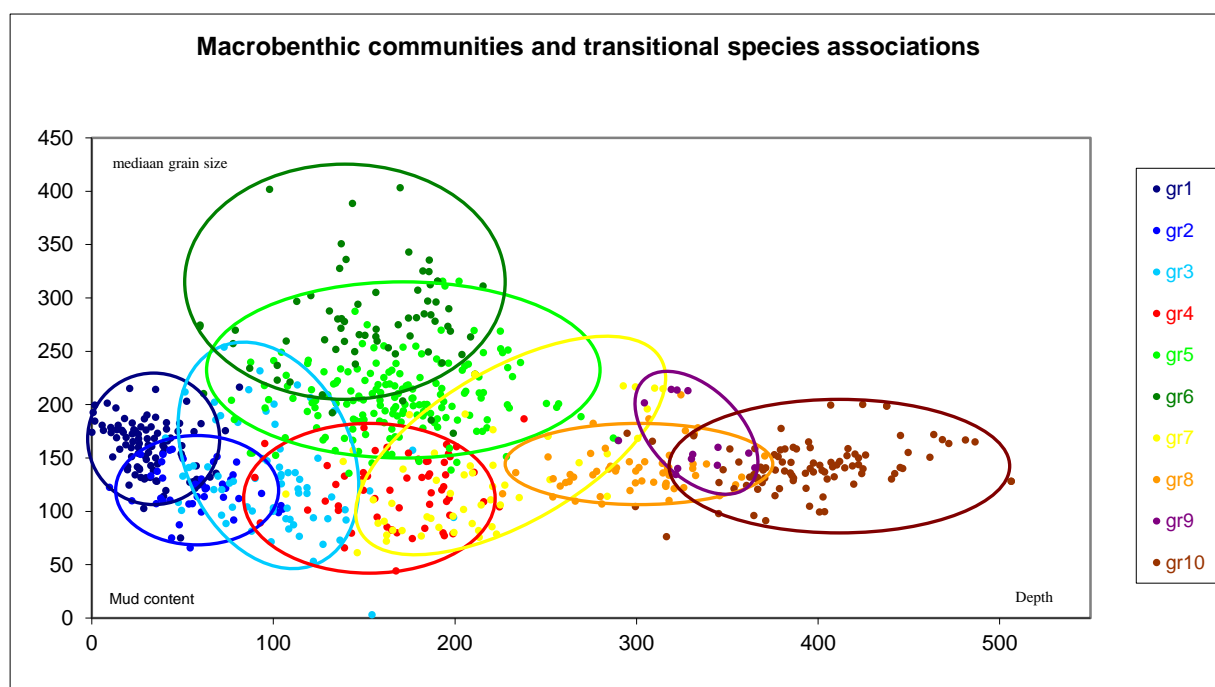


Figure 11: The macrobenthic communities and the transitional species associations of the Belgian Continental Shelf. The *Barnea candida* community isn't shown.

The second community, *Nephtys cirrosa* community (group 4), is found in stations with medium sandy sediments and a very low mud content. This group is less diverse ( $N_0$ : 7 species/sample on average) and the densities are low (402 ind/m<sup>2</sup> on average).

The macrobenthic diversity and densities of the third community, the *Ophelia limacina* – *Glycera lapidum* community (group 6), are very low, respectively 5 species/sample and 180 ind/m<sup>2</sup> on average. This community is found at stations with coarse sandy sediments and is typically situated on the sandbanks further offshore (Flemish banks, Hinder banks and Zeeland banks).

The fourth community, the *Eurydice pulchra* – *Scolelepis squamata* community (group 10), is exclusively found at the high intertidal sandy beaches and is characterized by a low diversity ( $N_0$ : 5 species/sample on average) and high densities (305 ind/m<sup>2</sup> on average). Typical species are the crustaceans *Eurydice pulchra* and *Bathyporeia spp* and the polychaete *Scolelepis squamata*.

The fifth macrobenthic community is typical for compact mud bottoms (median grain size: 14  $\mu$ m) and containing high numbers of the bivalve *Barnea candida*: the *Barnea candida* community. There are a few reports of this community.

Of course these macrobenthic communities are not isolated from each other and five specific transitional species associations were found. The transition group (group 3) between the *Abra alba* – *Mysella bidentata* community and the central community, the *Nephtys cirrosa* community, is characterized by a reduction in the mud content and is dominated by *Magelona mirabilis*. The transition (group 5) between the *Nephtys cirrosa* and the *Ophelia limacina* – *Glycera lapidum* community is characterized by decreasing densities and coincides with a gradual transition between medium and coarse sandy sediments. From the *Nephtys cirrosa* to the *Eurydice pulchra*–*Scolelepis squamata* community, transitional species associations (group 7,8 and 9), related to the transition from the subtidal (group 7) to the intertidal (group 8 and 9) environment, were found.

Four highly different communities, ‘connected’ with each other by means of gradual transitional species associations, characterize the spatial distribution of the macrobenthos of the BCS. These communities are ecologically important, because they are a representation of the different habitats that are characteristic for the BCS.

Obviously, different macrobenthic communities and species associations show different temporal dynamics. Because each community is composed of a very specific combination of species, the temporal variation of the complex biological interactions, due to the coexistence of these species, will differ from one community to another. A successful recruitment and survival of one species, for instance, will only take place in the community where this species belongs to and not in any other community. This recruitment may trigger a chain reaction of changing biological interactions (competition, predation, etc.), causing major shifts in the species composition and density of the community: formerly abundant species may become rare or even disappear and less abundant or even new species may become dominant.

### *Implications for management*

Designating an ecological value to a macrobenthic community, based on the results out of a single sampling campaign, can lead to misinterpretation of the community's importance. Two sampling campaigns already give some more information on the 'potentials' of the community, but in order to know all possible dynamics of a community long-term studies are necessary. Knowledge of the dynamics and the environmental 'needs' of all communities of an area under consideration may provide information on the ecological 'potentials' of a particular place with known physical environment. The possibility to predict the existence of a macrobenthic community only knowing the physical characteristics of a certain environment, would be an extremely powerful tool in coastal zone management. Furthermore, instead of putting a lot of effort, time and money in working out time-series of lots of macrobenthic samples, randomly spread over the area under consideration, monitoring of the macrobenthos should concentrate on the spatial distribution of the macrobenthic 'potentials'. This can be done by the identification of (1) the spatial distribution of the macrobenthic communities actually present, (2) the communities' environmental 'needs', and, finally, (3) the communities' 'potentials' by means of long-term monitoring of some selected stations within each community.

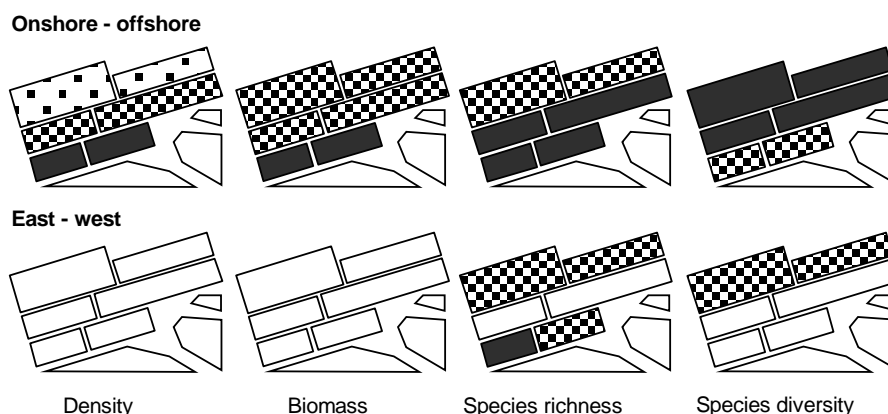
## **III.a.3 Hyperbenthos**

### **III.a.3.1. Spatial pattern**

The hyperbenthos of the Belgian continental shelf, the Dutch continental shelf off Zeeland and the Westerschelde estuary was sampled within a two-week period at 62 locations in the summer of 1993. A total of 109 taxa were recorded, half of which were amphipods and decapods. Mysids were the most important taxon in terms of density and biomass. In all, seven hyperbenthic communities were identified (Fig. 2).

Their geographical occurrence was correlated with the presence of isolated sandbank systems in the area. The community in the mesohaline reaches of the Westerschelde estuary was different from the shelf hyperbenthos and was characterized by the highest density and biomass and lowest diversity. Two gradients in community structure were detected on the continental shelf: the principal onshore-offshore gradient perpendicular to the coastline and a less pronounced east-west gradient parallel to the coastline (Fig. 12). The first gradient mainly indicated differences in density and biomass, while the second reflected species richness and diversity.

### Holohyperbenthos



### Merohyperbenthos

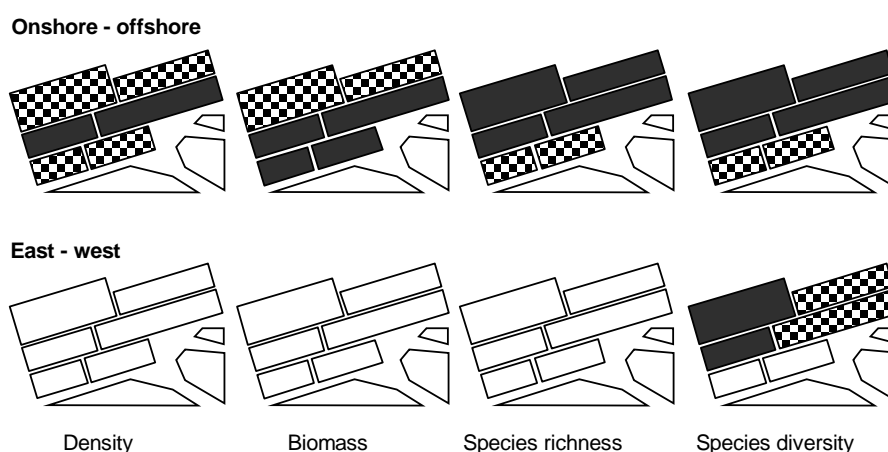


Fig. 12: Summarising scheme of onshore-offshore and east-west gradients for the holo- and merohyperbenthos in the study area. The boxes represent the 6 communities on the continental shelf; highest grey-shade corresponds with highest values.

The most diverse communities were found at the Flemish and Zeeland Banks. The holohyperbenthos (i.e. animals that spend variable periods of their adult life in the hyperbenthal zone) reached high densities at the Coastal Banks and consisted nearly exclusively of mysids (Fig. 13A); *Schistomysis spiritus* and *S. kervillei* were the most common species. The high levels of suspended matter and mud in this onshore area probably advantage this motile fauna in terms of food. Community composition altered with distance from the shore and planktonic species such as hydromedusae, ctenophores and chaetognaths gained in abundance. Outflow from the Westerschelde estuary may cause a decline of species richness at the East Coast, but favours high densities of resistant species.

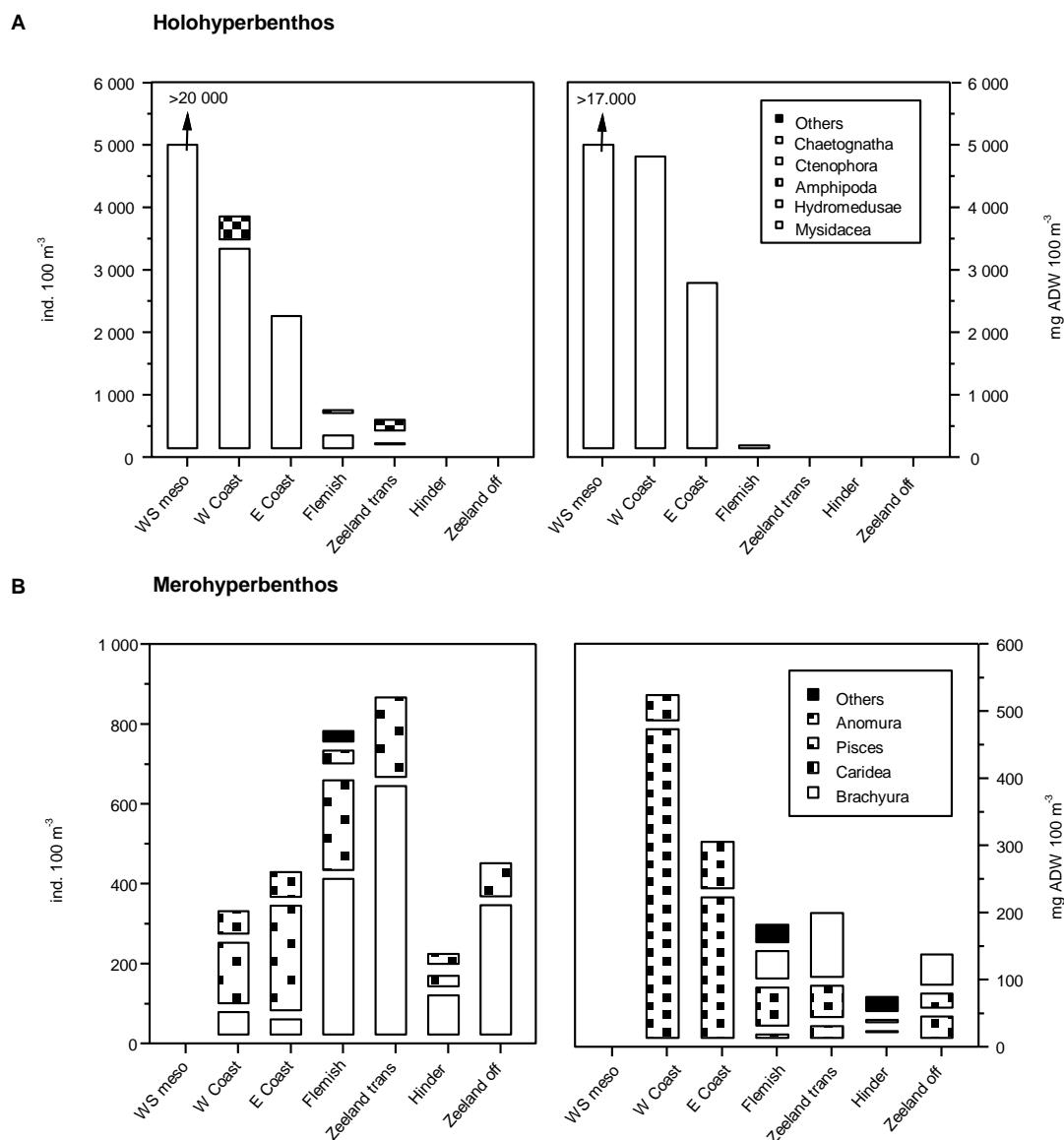


Fig. 13: Mean total density (left) and biomass (right) per community and per major taxonomic group for (A) holohyperbenthos and (B) merohyperbenthos.

The merohyperbenthos (early life history stages) was most abundant at the Flemish and Zeeland Banks and was mainly composed of a variety of larval decapods (e.g. *Liocarcinus holsatus*, *Crangon crangon*) (Fig. 13B). Biomass peaked at the Coastal Banks with a dominance of postlarval gobies. A strong diversity gradient was found for the merohyperbenthos with a richer fauna off the Belgian coast as compared to the Zeeland offshore waters. This was mainly due to a variety of larval decapods occurring in considerable numbers (e.g. *Pilumnus hirtellus*, *Pisidia longicornis*, *Galathea intermedia*) in addition to a number of species that were rather sporadically encountered (e.g. *Alpheus glaber*, *Pagurus prideauxi*). Habitat heterogeneity and water mass flow characteristics are believed to be the most important structuring environmental factors.

### III.a.3.2. Temporal pattern

In order to evaluate the importance of the sandbank system for the hyperbenthic fauna, monthly surveys (from September 1994 until December 1995) were carried out at the Coastal and Flemish Banks (13 sites in total).

#### Holohyperbenthos

A total of 93 holohyperbenthic species were recorded. Spatial structure, corresponding to a major cross-shore and secondary alongshore gradient, persisted throughout the year. The Flemish Banks harboured the most diverse community, characterised by planktonic species (chaetognaths, copepods, hydromedusae and ctenophores) accountable for half of the total density in this area (Fig. 14). Scarce food supply and strong flow in this offshore area are less favourable for bottom-dependent animals compared to onshore waters. Mysid dominance typified the Coastal Banks, and community structure changed along the east-west direction. Densities were highest for the East Coast but diversity was low. This is believed to be due to the nearby mouth of the Westerschelde estuary leading to variable fluctuations in the environment.

Temporal variability in community structure was most pronounced during spring, with increased amphipod densities and a conspicuous ctenophore bloom. The amphipod *Atylus swammerdami* showed a distinct density increase at the Flemish Banks in May, coinciding with the decaying phase of a *Phaeocystis* bloom. In addition, various epifaunal amphipods (e.g. *Microprotopus maculatus*, *Pariambus typicus*, *Phtisica marina*) were encountered in association with hydrozoan colonies at the Coastal Banks. *Gammarus crinicornis*, being confined to and strongly dominant on the E Coast, similarly co-occurred with hydrozoans. Its persistence during subsequent months probably indicates detritivory after collapse of the hydrozoan populations.



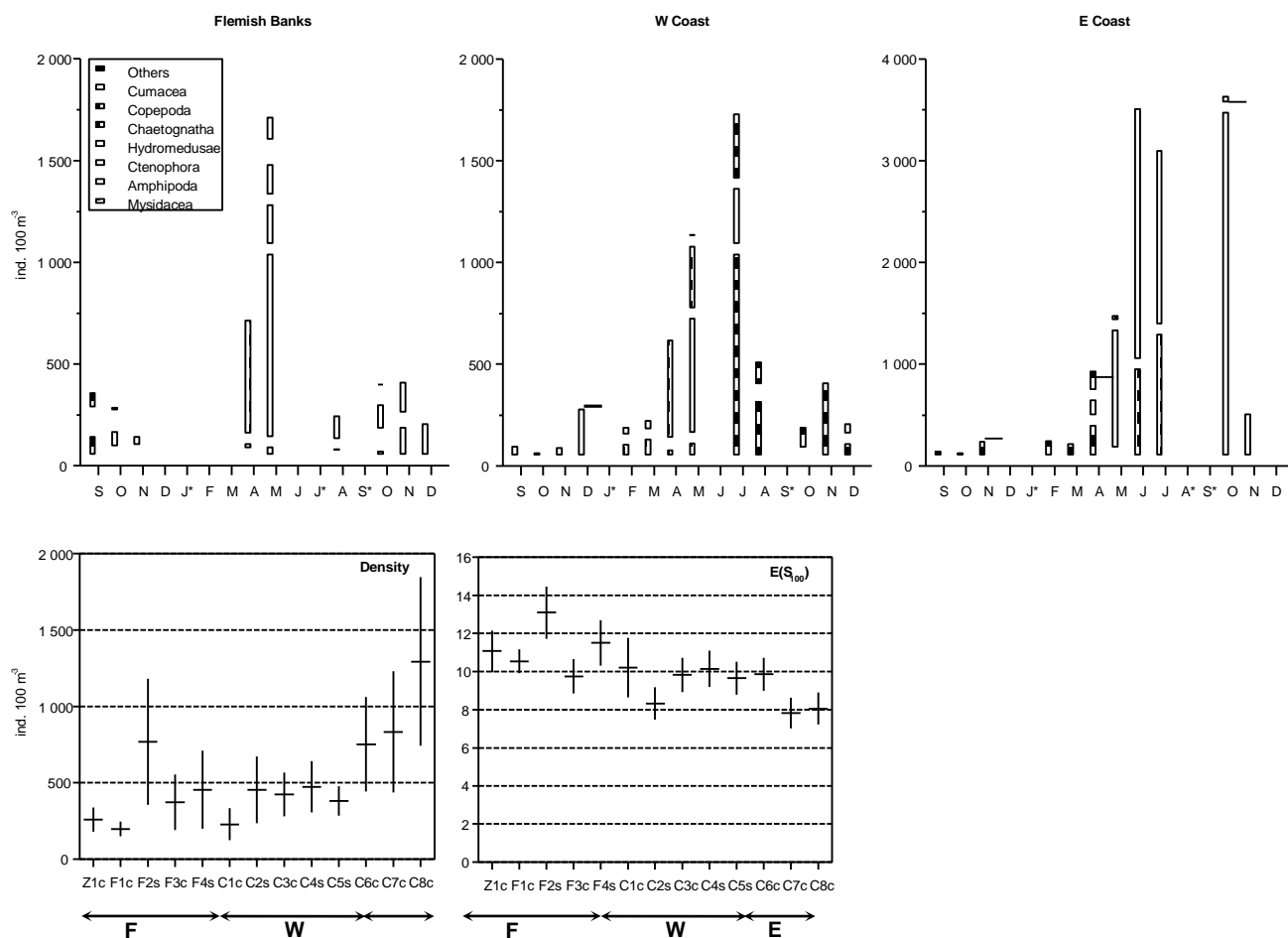


Figure 14: (upper) Taxonomic composition and monthly density fluctuation of the holohyperbenthos; (lower) Annual mean density and diversity (Hurlbert's  $ES_{(100)}$ ) per site. Data are separately presented for the Flemish Banks (F), W Coast (W) and E Coast (E), (\*) no sampling was carried out at these months.

Mysids were most abundant during summer. However, density fluctuations were species-specific and fairly irregular. The dominant mysid *Schistomysis kervillei* (annual mean density of 293 ind.  $m^{-3}$  at the E Coast) occurred year-round at the Coastal Banks and appears to seek out turbid environments, which are favourable for feeding. *Schistomysis spiritus* is believed to be more sensitive to extremes conditions, like strong wave action, and probably migrates to more sheltered areas. The most extreme temporal fluctuation in density was found in *Mesopodopsis slabberi*, possibly caused by migrations between the adjacent biotopes of the coastal area. Several mysid species only occurred during a limited period; total species numbers tended to be maximal during winter.

## Merohyperbenthos

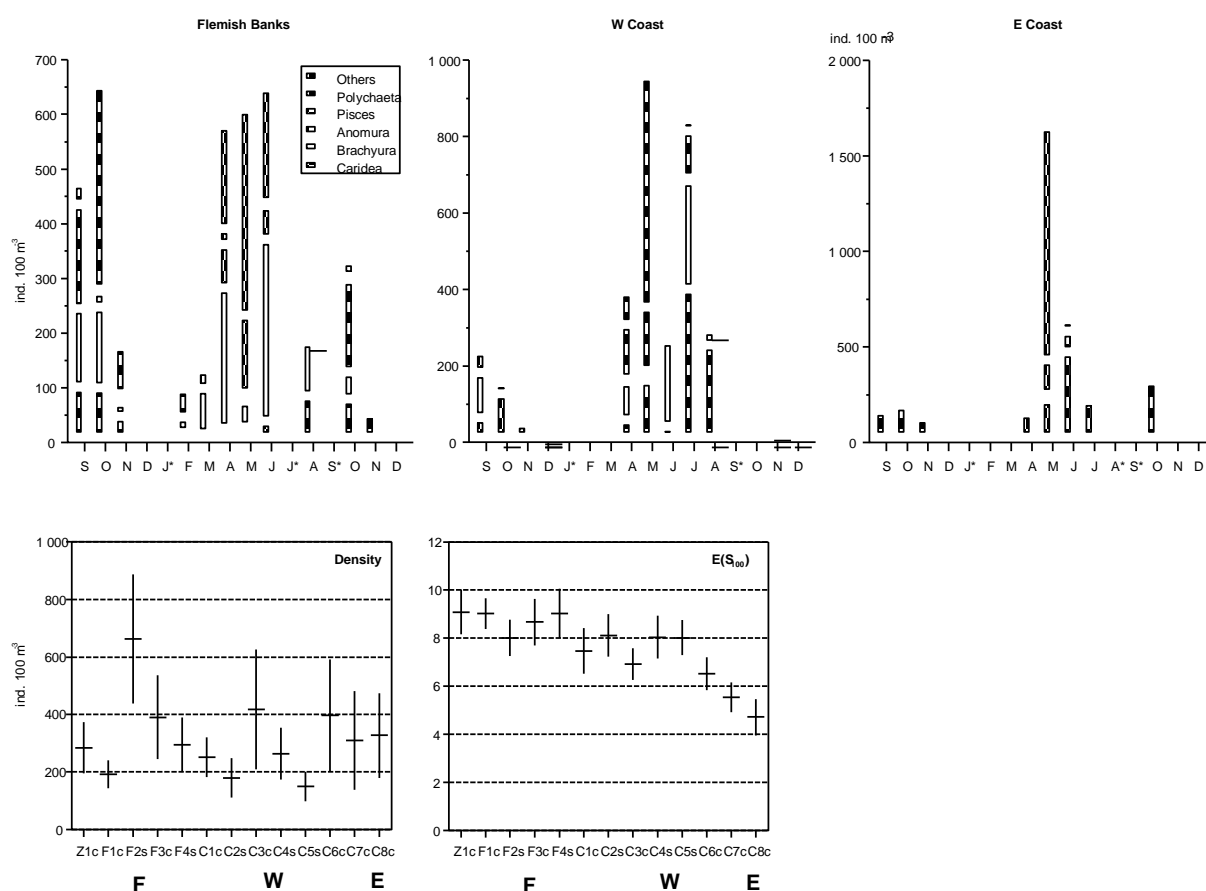


Figure 15: (upper) Taxonomic composition and monthly density fluctuation of the merohyperbenthos; (lower) Annual mean density and diversity (Hurlbert's  $ES_{(100)}$ ) per site. Data are separately presented for the Flemish Banks (F), W Coast (W) and E Coast (E), (\*) no sampling was carried out at these months.

The merohyperbenthos community was predominantly temporally structured, due to differential recruitment peaks of various postlarval fish (at least 21 species) and decapod larvae (at least 29 species). Both species richness and total abundance of fish larvae were highest during April - May (Fig. 15). Species successions were conspicuous, recruitment often being restricted to a single month. Sole *Solea solea* (confined to May) was the most abundant fish species throughout the study area with densities at times exceeding 100 ind.  $100\text{ m}^{-3}$ . Other common species were *Pomatoschistus* spp. (all year with peaks in May and July), flounder *Pleuronectes flesus* (confined to April), clupeids (peak in February and May) and dab *Limanda limanda* (confined to May). Recruitment for decapod larvae was most pronounced from late spring to late summer, with respect to both species richness and densities. Common shrimp *Crangon crangon* (annual mean density of 137 ind.  $\text{m}^{-3}$  at the E

Coast), swimming crab *Liocarcinus holsatus*, shore crab *Carcinus maenas*, and porcelain crab *Pisidia longicornis* were most abundant.

Masked by this strong temporal pattern, an underlying onshore-offshore gradient for the merohyperbenthos community existed as well. The Flemish Banks were most diverse; several species, like most anomurans and certain fish species, preferentially occurred in this area. Moreover, decapods showed ontogenetic differences in spatial distribution. Zoea stages of nearly all species were typically found at the Flemish Banks, whereas postlarval carideans were more abundant at the Coastal Banks. Diversity was distinctly lower at the East Coast. *Crangon crangon* and *Lanice conchilega* larvae strongly dominated in the latter area (together more than 80 % of the total density), whereas Brachyura were poorly represented. Merohyperbenthos community structure appears to be largely regulated by the hydrodynamical setting and morphodynamical characteristics of the area.

In conclusion, the estuarine outflow on the one hand and the hydrodynamic setting and protective function of the sandbanks on the other hand create diverse environs along the Belgian coastal zone, sustaining rich communities of holohyperbenthic species and early life history stages when compared to the adjacent areas.

#### **III.a.4. Parasites**

The study concentrates on ectoparasites belonging to the genus *Gyrodactylus* (Monogenea, Platyhelminthes) parasitising the threespine stickleback (*Gasterosteus aculeatus*) and gobies from the genus *Pomatoschistus* (Gobioidea, Teleostei). The Monogenea is one of the largest groups of Platyhelminthes, containing some 20,000 species (Rohde, 1996), among which the genus *Gyrodactylus* is one of the most species rich groups (Brooks and McLennan, 1993). To uncover and discriminate among the phylogenetic and ecological speciation patterns that contributed to this enormous diversity, detailed studies on *Gyrodactylus* in its natural habitat are needed. However, many host species have hardly been sampled (Harris, 1993). Moreover, these small sized organisms (200 - 1200  $\mu\text{m}$ ) are easily overlooked. An example of such an “unexplored” host group is the genus *Pomatoschistus*. The only descriptions in the literature are made by Gläser (1974) and Geets *et al.* (1998).

Samples were collected throughout the natural geographical distribution of the fish hosts ranging from the Mediterranean Sea along the North Eastern European coasts up to the Norwegian Sea, depending on the *Pomatoschistus* species. The two-spotted goby *Gobiusculus flavescens* and the three-spine stickleback *Gasterosteus aculeatus* were collected as an outgroup. Fish were brought alive to the laboratory and directly examined for *Gyrodactylus*-infection using a stereomicroscope. Some gyrodactylid specimens were fixed in ammonium picrate

glycerin as described by Malmberg (1970), to examine the haptoral sclerites by phase contrast microscopy or in glutaraldehyde for scanning electron microscopy. Drawings were made with a drawing tube on an Olympus microscope (100x oil immersion objective). All parasites were identified morphologically to species level prior to DNA analysis. Each specimen was individually placed in 5  $\mu$ l of milli-Q water and stored at -20°C until required. DNA extractions, amplification and sequencing were performed as described by Zietara *et al.* (in press). For data analysis see chapter VI (genetic biodiversity of ecologically important fish).

Geets (1998) described *G. longidactylus* on the gills from *P. lozanoi*. During our parasitological survey a very similar *G. cf. longidactylus* species is found on the gills and fins from *P. norvegicus*. Morphometric and genetic analyses are currently in progress. Gläser (1974) described *G. rugienis* and *G. micropsi* on both *P. minutus* and *P. microps*. Our study added *P. pictus* Malm, 1865 and *P. lozanoi* de Buen, 1923 as new host species. Subsequent molecular analysis of the ITS rDNA region revealed that the parasite species represent a complex of two cryptic species, one restricted to *P. microps* and the other shared by *P. minutus*, *P. lozanoi* and *P. pictus*. Morphometric analyses were conducted on seventeen features of the opisthaptoral hard parts of specimens collected from all host species. Standard discriminant analysis showed a clear separation of both genotypes by significant differences in marginal hook and ventral bar features. Based on molecular, morphological and statistical analyses a new species, *Gyrodactylus pseudorugiensis* is described. All parasite species found in this study are listed in Table III.

In conclusion: many new species have been found during the study. Thanks to the available molecular techniques the detection of putative new species has become considerably easier. Both morphometric and genetic analyses will be used in the species descriptions.

Table III: *Gyrodactylus* fauna of gobies from the genus *Pomatoschistus* found to date.

<i>Gyrodactylus</i> species	Host species	Location on host
<i>G. rugiensis</i>	<i>P. microps</i>	Fin
<i>G. cf. harengi</i> 1*	“	Fin
<i>G. sp</i> 1a*	“	Gill
<i>G. micropsi</i>	“	Fin
<i>G. sp.</i> 1a*	<i>P. marmoratus</i>	Gill
<i>G. cf. harengi</i> 2*	<i>P. marmoratus</i>	Fin
<i>G. cf. micropsi</i> 1*	<i>P. minutus/P. lozanoi</i> /	Fin
<i>G. cf. micropsi</i> 2*	“	Fin
<i>G. sp.</i> 4*	“	Gill.fin

<i>G. pseudorugiensis n. sp.</i>	<i>P. minutus/P. lozanoi/ P. pictus</i>	P. Fin
<i>G. sp. 2*</i>	<i>P. pictus</i>	Gill
<i>G. longidactylus</i>	<i>P. lozanoi</i>	Gill
<i>G. cf. longidactylus*</i>	<i>P. norvegicus</i>	Gill/fin
<i>G. sp. 3*</i>	"	Gill/fin
<i>G. sp. 5*</i>	<i>G. flavescens</i>	Gill/fin
<i>G. arcuatus</i>	<i>G. aculeatus</i>	Gill/fin
<i>G. branchicus</i>	"	Gill

\* non-described species

### III.a.5. Avifauna

#### III.a.5.1. Selection of species

Out of the 121 bird species encountered at sea during the period 1992-98, 23 were selected as being true marine species that occur in relatively high densities within the Belgian waters. Of these 23 species six can be classified as 'focal species' and another five as 'locally important species' (Table IV). Focal species are defined here as seabirds that are included in the highest priority lists of international conservation instruments (EC-Birds Directive, Bern Convention or Bonn Convention) and attain at least 1% of the flyway population in Belgian marine waters in a particular season. Locally important seabird species also reach the 1%-limit, but are not included in the highest priority lists of the legislative instruments mentioned above. The remaining 12 species listed in table I are common birds at the BCP, but do not reach the 1%-limit.

Table IV: Selection of focal seabird species in Belgian marine waters based on 1) maximal numbers observed during ship-based or aerial surveys, land-based counts or counts in the breeding colony, 2) the size of the flyway population and 3) their global status as indicated by international conservation instruments/agreements (BD = Birds Directive; Bern = Bern Convention; Bonn = Bonn Convention). Focal and locally important species outnumber the 1%-norm, outlined by Rose and Scott (1997) or 1% of the estimated population as published by Lloyd *et al.* (1991), Hildén and Tasker (1997) and Harris (1997).

Species	Maximal numbers	1% of flyway population	BD	Bern	Bonn
<i>FOCAL SPECIES</i>					
<i>Red-throated Diver Gavia stellata</i>	1453	750	I	II	II

Common Scoter <i>Melanitta nigra</i>	15528	16000		III	II
Little Gull <i>Larus minutus</i>	3670	750		II	
Sandwich Tern <i>Sterna sandvicensis</i>	3300	1500	I	II	II
Common Tern <i>Sterna hirundo</i>	3900	1800	I	II	
Little Tern <i>Sterna albifrons</i>	860	340	I	II	II
<i>LOCALLY IMPORTANT SPECIES</i>					
Great-crested Grebe <i>Podiceps cristatus</i>	3736	1500		III	
Great Skua <i>Stercorarius skua</i>	439	272		III	
Lesser Black-backed Gull <i>Larus fuscus</i>	15608	4500	II	X	
Herring Gull <i>Larus argentatus</i>	19272	14000		III	
Great Black-backed Gull <i>Larus marinus</i>	5727	4800	II	X	
<i>OTHER SEA- AND COASTAL BIRDS</i>					
Razorbill <i>Alca torda</i>	3791	4820		III	
Fulmar <i>Fulmarus glacialis</i>	1312	100000		III	
Northern Gannet <i>Morus bassanus</i>	3714	8920		III	
Cormorant <i>Phalacrocorax carbo</i>	381	3200		III	
Eurasian Wigeon <i>Anas penelope</i>	7956	12500		III	II
Greater Scaup <i>Aythya marila</i>	1940	3100	II/III	III	II
Common Eider <i>Somateria mollissima</i>	4953	15000		III	II
Velvet Scoter <i>Melanitta fusca</i>	4220	10000	II	III	II
Black-headed Gull <i>Larus ridibundus</i>	4220	50000		III	
Common Gull <i>Larus canus</i>	6927	16000	II	III	
Kittiwake <i>Rissa tridactyla</i>	5648	84000		III	
Common Guillemot <i>Uria aalge</i>	13101	19900		III	

### III.a.5.2. Spatial distribution

In the next section the spatial distribution of the six focal seabirds (Table I) is discussed. For each species hotspots are given in terms of sandbanks.

*Little Gull* At sea Little Gulls are usually seen in small flocks (< 10 birds), although sometimes larger groups may form (Camphuysen and Leopold 1994, Keijl and Leopold 1997). The southernmost part of the North Sea appears particularly attractive for the formation of larger flocks (see also Van Impe 1966, Bulteel and van der Vloet 1969). At several occasions flocks of 200-500 birds were encountered on the French side of the Flemish Banks. In autumn groups of 50-250 individuals are not rare in the vicinity of the harbours of Oostende and Zeebrugge. Further hotspots for Little Gull in

Belgian marine waters are situated on the Flemish Banks (autumn, winter and spring), in the Stroombank-area (autumn), at the Vlakte van de Raan (autumn) and at the Goote Bank (autumn and winter).

*Diver spp.* Divers occur solitary or in small flocks in the study-area. Major hotspots for divers are the western Coastal Banks (in particular the Trapegeer and Smal Bank), the Vlakte van de Raan and the Flemish Banks area. The Middelkerke Bank and Smal Bank show the highest densities.

*Common Scoter* Common Scoters have a strictly coastal distribution and traditional wintering grounds are found in the western Coastal Banks area. Most important hotspots are the Trapegeer-area, Stroombank-Balandbank-Grote Rede area and the Nieuwpoortbank area.

*Sandwich Tern* Sandwich Terns breeding at Zeebrugge fly as far as 45 km to the feeding grounds. The main foraging areas are situated around the harbour of Zeebrugge, on the Flemish Banks, the western Coastal Banks and at the Goote Bank. During migration most Sandwich Terns are encountered at the western and eastern Coastal Banks, the Flemish Banks and the Vlakte van de Raan, although some individuals appear to migrate much further from the coast.

*Common Tern* Regular boat-trips in the proximity of Zeebrugge indicate that local Common Terns do not feed beyond 5 km away from the nesting site. Within the harbour itself and at the dock entrance large feeding flocks (500-1000 birds) are often observed in the wake of in- and outgoing ships (mainly ferries) (Van Waeyenberge *et al.* 2000). During migration the harbour-areas of Oostende and Zeebrugge, the entire coastal zone (western and eastern Coastal Banks) and the Flemish Banks are important.

*Little Tern* The great majority of the Little Terns nesting in the harbour of Zeebrugge remains within the harbour itself. Very few Little Terns are seen at sea. The species migrates very close to the shoreline and is subsequently missed at sea surveys.

### **III.a.5.3. Hotspots for seabirds**

A cumulative picture of the abundance and conservation value of six focal seabird species in Belgian marine waters (Fig. 16) indicates that the western Coastal banks (Trapegeer, Nieuwpoort Bank, Stroombank, Balandbank, and Smal Bank), the Vlakte van de Raan and most of the Flemish Banks are avian hotspots throughout the year.

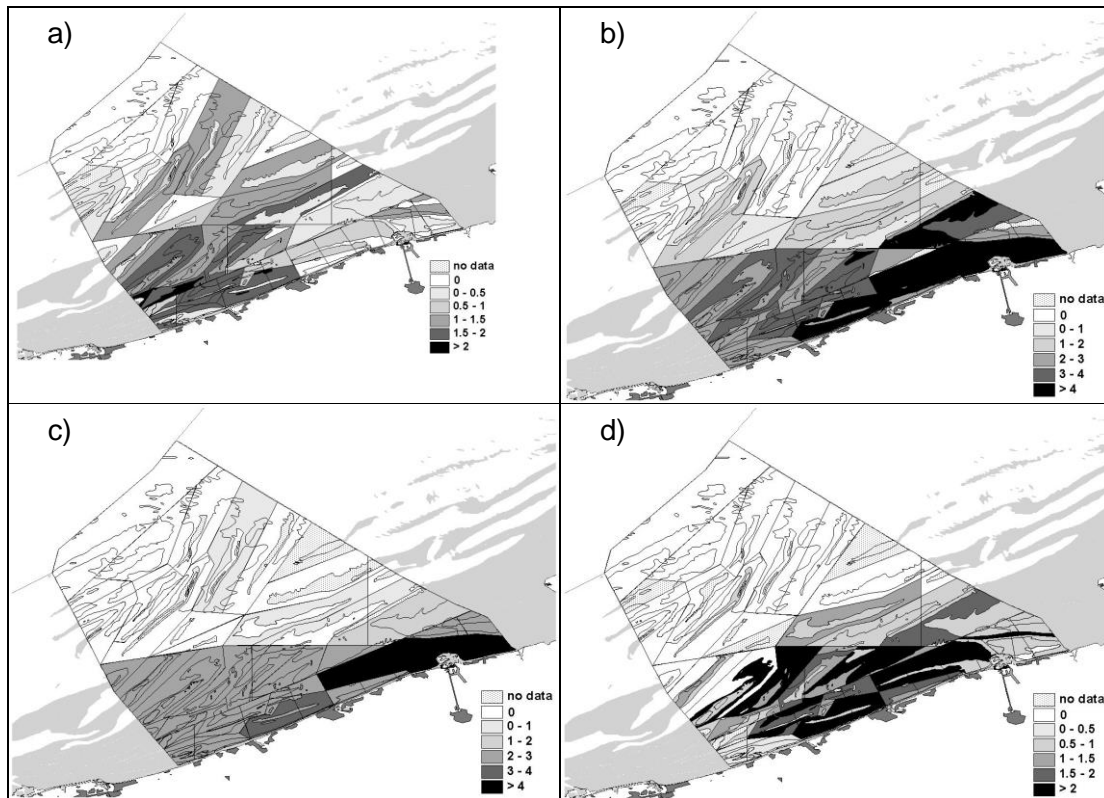


Figure 16: Hotspots for focal seabird species in Belgian marine waters in a) winter, b) spring, c) summer and d) autumn. Shading is based on the relative abundance of six focal species times a weight factor (see Seys *et al.* 2001a for more details).

The eastern Coastal Banks (Wenduinebank and to a lesser extent the Paardenmarkt) are very important from spring till autumn, mainly as feeding grounds for the three focal tern species. In winter this area is of marginal importance. On comparison the deep-water zone, the Zeeland ridges and the offshore Hinder Banks area are less important for the six species.

#### III.a.5.4. Seasonal pattern

Figure 17 shows the temporal distribution of five focal seabirds at the BCS (the temporal pattern of Little Tern is not shown as this species is rarely encountered at sea). Red-throated Diver and Common Scoter are basically wintering species. Red-throated Diver occurs in high densities at the BCP from December to February, while Common Scoter normally peaks in April-March. Little Gull shows important numbers in all seasons except summer and peaks during March-April and September-November. Three tern species breed in the outer harbour of Zeebrugge since the late 1980s and use the Belgian marine waters as feeding grounds. Their maximum breeding numbers amount to more than 2 % of the flyway population. The first Common and Sandwich Terns arrive in the colony at the end of March – early April.



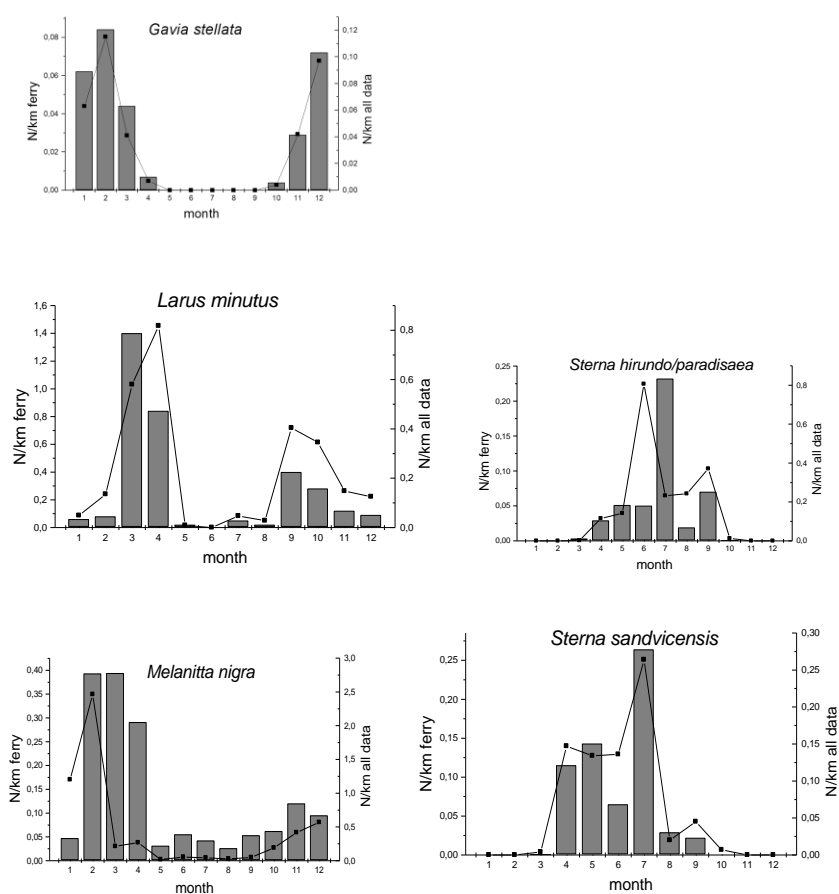


Figure 17: Seasonal pattern of occurrence ( $N.km^{-1}$ ) of Red-throated Diver *Gavia stellata*, Common Scoter *Melanitta nigra*, Little Gull *Larus minutus*, Sandwich Tern *Sterna sandvicensis* and 'Commic Terns' (undifferentiated Common/Arctic Terns) *Sterna hirundo/paradisaea* in the southernmost part of the North Sea during 1992-98. The pattern based on ferry data - collected on fixed routes - is indicated separately (grey bars) from 'all data' (line).

Resident Common Terns from the Zeebrugge colony cause a peak in the numbers observed per km during ship-based surveys in June. A peak in July during surveys on board ferries to the UK results from migratory movements. Migration of Sandwich Tern peaks in April-May and again in July. The Little Tern is usually observed at the Zeebrugge colonies from the end of April - early May onwards and leaves the area in July (De Putter & Orbie 1990). The latter species mainly feeds within the harbour of Zeebrugge and is rarely seen at sea. It probably migrates very close to the shoreline.

### III.b. ECOLOGICAL INDICATORS

In general, characteristics (density, biomass, biodiversity, species composition) of benthic communities can be clearly linked to habitat disturbance (Huston, 1994). However, since benthic communities are quite complex to investigate, a set of ecological indicators are put forward as an easy measure for community change due to general disturbances (including anthropogenic disturbance as well).

#### III.b.1. Meiobenthos : Nematoda biomass spectra

Nematodes are widely accepted as suitable organisms for monitoring changes in sediments due to their high abundances (only a small sample is needed), their high diversity (species ranging from very sensitive to highly tolerant to disturbances), continuous reproduction and the absence of pelagic life stages. However, the species identification of nematodes requires taxonomically trained scientists and is rather time-consuming. This is the major reason that nematode community studies have not made their way in routine monitoring programmes. An alternative method involves the study of a functional descriptor of communities: the biomass distribution over size. This offers the most extensive and powerful generalisation that can be used in ecological studies (Peters 1983). Moreover, this method requires no taxonomical knowledge and therefore it offers an alternative and sensible technique for describing and comparing benthic communities. Recent evidence suggests a change in macrobenthic biomass spectra in stressed ecosystems (González-Oreja & Saiz-Salinas 1999), while in undisturbed sediments, complete benthic size spectra seems to be conservative: no changes in the spectra were observed when the biomass spectra of different geographical areas or sediments were examined (Drgas *et al.*, 1994, Duplisea & Drgas 1999, Duplisea 2000). Moreover, experimental approaches (Leaper *et al.* 2001) failed to prove differences in body-size distributions when clearly different artificial substrates were used.

Nematode biomass spectra (NBS) were constructed for undisturbed sandbanks, physically disturbed sandbanks (sand extraction on the Kwinte Bank), eutrophicated and less eutrophicated coastal stations (Station 702 vs. station 790) based on Steyaert *et al.* 1999) and during a pulsed supply of fresh phytodetritus following a spring phytoplankton bloom (Station 330)(Vanaverbeke *et al.* subm).

Three different methods for constructing NBS non-normalised NBS, normalised NBS, cumulative NBS and a Pareto-type approach were examined. Non-normalised NBS proved to yield better results than normalised NBS since the ecological relevant information was retained. Normalising the spectra caused elevated biomass values and peaks to disappear, introducing bias in interpreting the distribution of biomass over spectra. Cumulative nematode biomass spectra proved

to be useful to evaluate differences in a statistical way, using the slope of the regression line of the cumulative biomass to the nominal value of a  $\log_2$  based size class. Constructing Pareto-type graphs and regressions produced results that were not straightforward in interpretation.

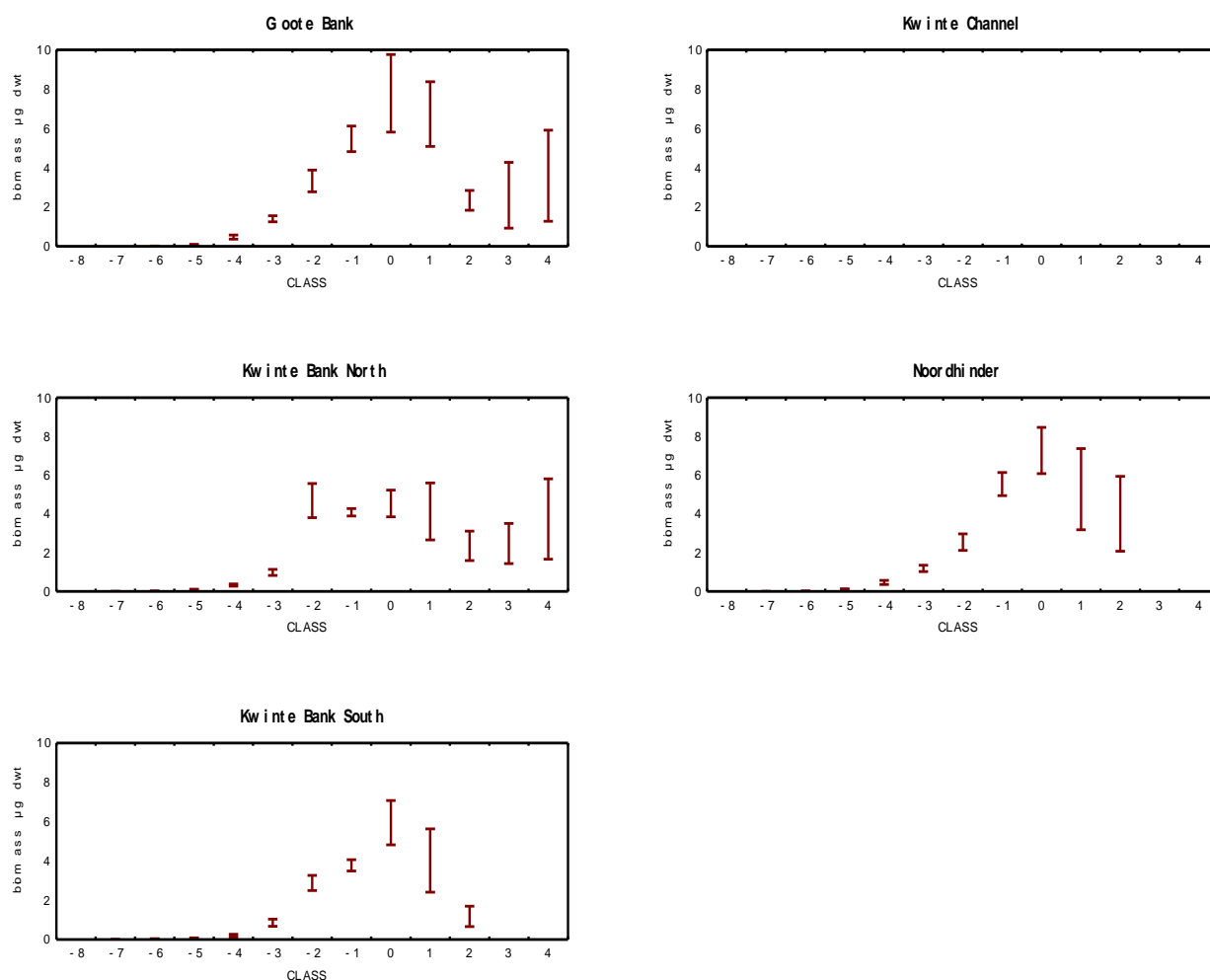


Figure 18: Mean Nematode Biomass Spectra for different nematode communities discerned by TWINSPLAN and DCA

NBS (Fig. 18) and cumulative NBS (Fig. 19) constructed for nematode communities from undisturbed sediments proved to be conservative: no differences in size distribution were found (Fig. 18). Moreover, physical disturbance, introduced by sand extraction did not affect NBS and regression slopes of cumulative NBS.

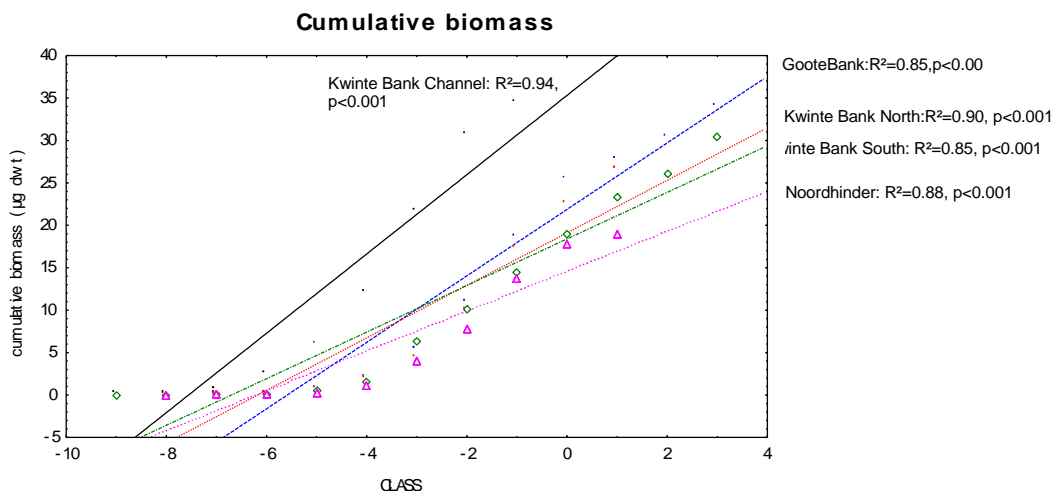


Figure 19: Cumulative Nematode Biomass Spectra per TWIN group

Eutrophication clearly had an impact on NBS, resulting in single class biomass peaks due to the strong dominance of a single nematode species, well adapted to the impoverished sediment quality. This was reflected in significant differences in the regression slopes of the cumulative NBS (Fig. 20).

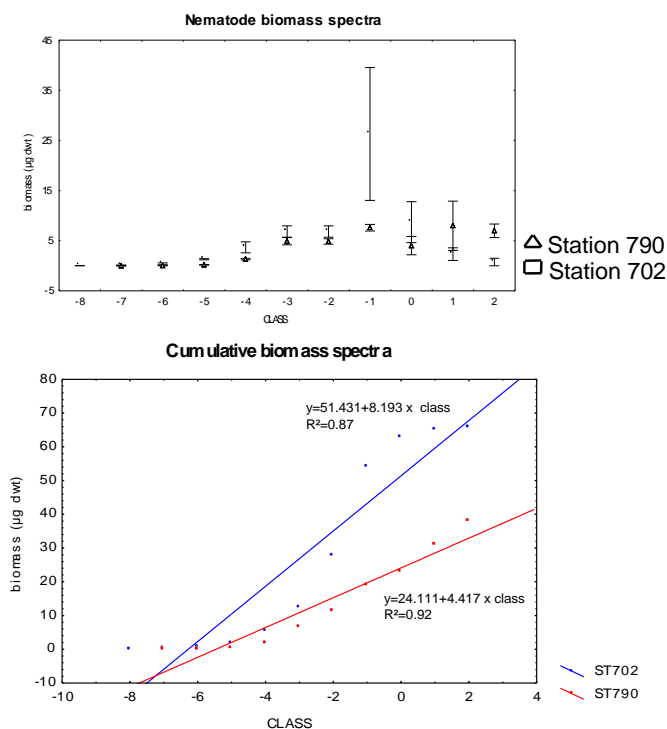


Figure 20: Nematode Biomass Spectra and cumulative Nematode Biomass Spectra for Station 702 (eutrophicated) and Station 790 (not eutrophicated)

Phytoplankton sedimentation during a spring bloom resulted in shifts in peaks in NBS due to a change in age structure of the nematode communities; and biomass values increased significantly as a result of a higher food supply to the benthos (Fig. 21).

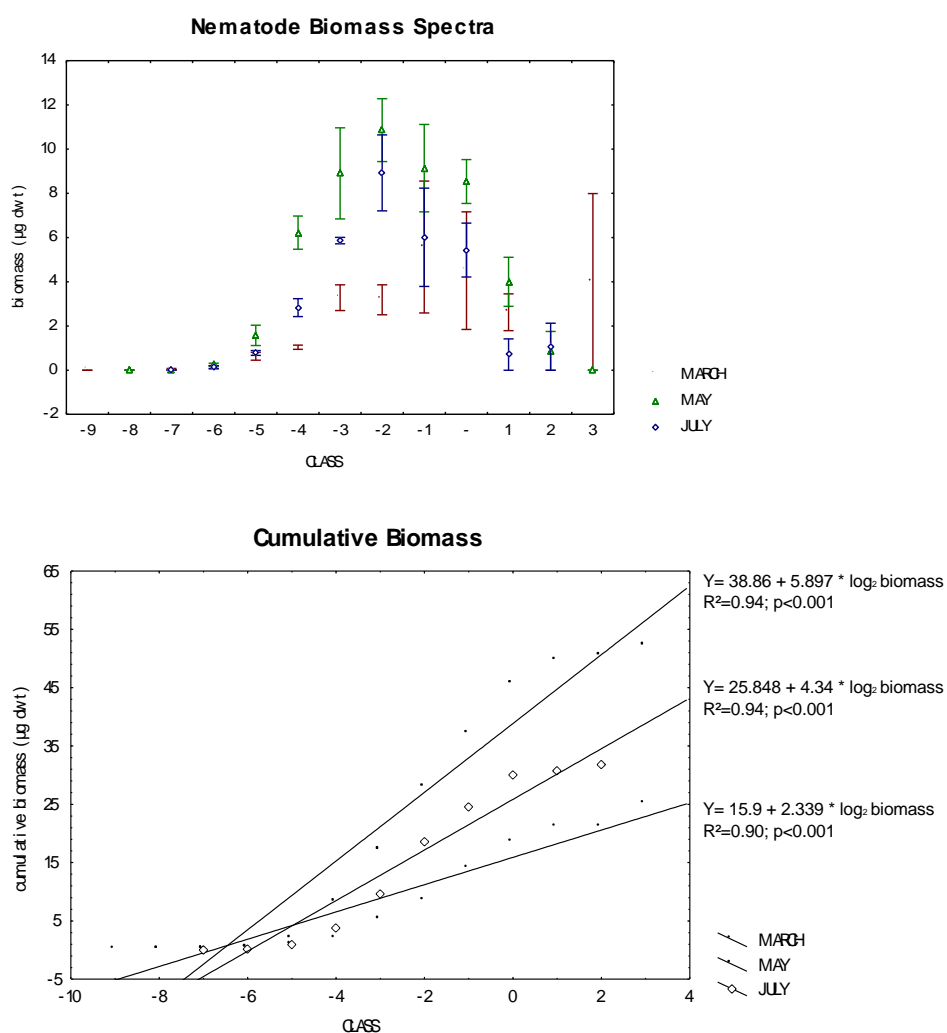


Figure 21: Changes in Nematode Biomass Spectra and cumulative Nematode Biomass Spectra during a spring phytoplankton bloom at Station 330

This study clearly demonstrated the use of biomass size spectra in tracking changes in nematode communities due to natural or human induced eutrophication events.

### III.b.2. Macrobenthos: *Lanice conchilega*

*Lanice conchilega* is a tube-building, suspension or deposit feeding polychaete, of which the tube has a length of up to 45 cm. The lower part of the tube (35 to 40 cm) is dug within the sediment, while 1 to 5 cm is extending into the water column and often ends in a fringe (Buhr and Winter, 1976). The tube is constructed of fine to coarse grained sediment and fragmented shell material (Hartmann-Schröder, 1996). The fringes of the tubes, together with the palps of the animal, have a high sediment

catching capacity. If present in high density patches, these patches of tubes thus create a hydrodynamically benign micro-climate in which suspended material is trapped. Consequently, a local heightening of the sediment surface might be observed (Holtmann *et al.*, 1996). This sediment-catching capacity of *L. conchilega* allows the polychaete to increase the amount of deposited organic matter and, thus, its food resources. Because of this heightening, the high density patches are detectable with several acoustic techniques (e.g. side-scan sonar), allowing a time- and cost-efficient, full-coverage study of the distribution of these patches (for more information see: Degraer *et al.*, 2001). Furthermore, the presence of the extending tubes, including their fringe, are responsible for a local increase in habitat complexity, compared to the fringing flat bottom soft substrates. Because of the direct relationship between habitat complexity and biodiversity, a detailed study of *L. conchilega* on the BCS was performed. The study aimed at the description of the spatial distribution, the habitat preferences, and ecological importance of *L. conchilega*.

#### **III.b.2.1. Spatial distribution**

Based on data collected from 1994 till 2001, the distribution of *L. conchilega* is restricted to the gullies nearby the Flemish Banks, Middelkerke and Kwintebank, and the gullies and flanks of the Coastal Banks (e.g. Westdiep) (Figure 22.). No individuals were found within the areas of the Hinder Banks nor Zeeland Banks. Furthermore, some observations on the presence of *L. conchilega* within the gullies of sandy beaches (e.g. Zeebrugge bad: up to highest elevated gully) were made. Taking into account observation from the period 1975-1985, it seems that the spatial distribution of *L. conchilega* is restricted to the western part of the BCS. The absence of the polychaete along the eastern part of the Belgian coast, might be explained by the high mud concentration in the area, which is due to the Westerschelde. North of the mouth of the Westerschelde, *L. conchilega* is found in front of the Dutch Delta area. During both periods, very high densities of the polychaetes were detected at the western Coastal Banks (Trapegeer – Broersbank – Potje – Westdiep complex).

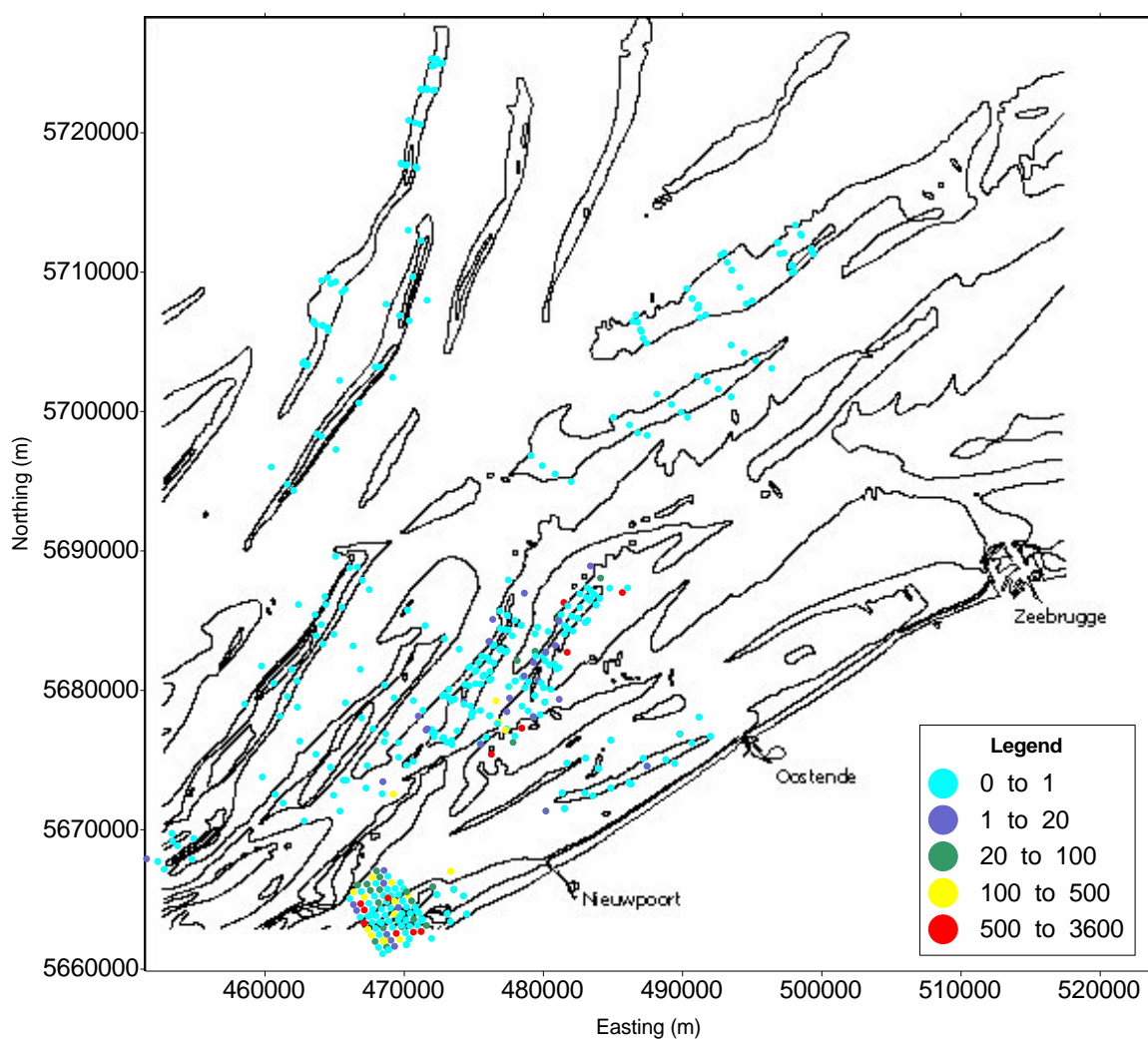


Figure 22: Map showing the spatial distribution of the *Lanice conchilega* on the BCS during the period 1994 – 2000

### III.b.2.2. Habitat preferences

Analysis of the MS-Access *MacroDat* database revealed the presence of *L. conchilega* at a large range of depth (0 – 30 m below MLWS). Obviously, depth is not an important factor to explain the spatial distribution of *L. conchilega* on the BCS. Yet, *L. conchilega* showed clear sediment grain size preferences. Almost all samples containing *L. conchilega* had a median grain size of 180 – 225  $\mu\text{m}$ . The highest densities were found in sediments with a median grain size of about 200  $\mu\text{m}$ , containing 5 – 20 % of mud. The fact that (1) very coarse sand and shell fragments are only present in low percentages and (2) the tubes are containing an important fraction of very coarse sand and shell fragments, demonstrates the capability of *L. conchilega* to select the grains to build its tube.

### III.b.2.3. Ecological importance

*Lanice conchilega* typically occurs within the macrobenthos-rich *Abra alba* – *Mysella bidentata* community. On the western Coastal Banks the polychaete was also found in the *Magelona mirabilis* species association. Within both communities, a positive correlation between the macrobenthic density and the number of species per sample ( $N_0$ ), on the one hand, and the density of *L. conchilega* on the other hand was found: Spearman rank correlation:  $R = 0.70$  &  $0.75$ , respectively;  $p < 0.0001$  (Degraer *et al.*, 2001). The increase in habitat complexity in dense patches of *L. conchilega* might be responsible for the increasing macrobenthic density and diversity. Although patches of *L. conchilega* may be short-lived, their habitat-structuring and -stabilising capacity has similar ecological consequences as e.g. *Sabellaria* reefs (e.g. increasing macrobenthic density and diversity). Although juvenile *Sabellaria* species were found on the Western Coastal Banks, *Sabellaria* reefs were never found on the BCS in recent years (since 1994).

### III.b.2.4. Implication for management

The importance of *L. conchilega* as an ecological indicator is twofold. At first, because of the local heightening of the sediment surface at dense patches of *L. conchilega*, its presence allows a time- and cost-efficient, full-coverage study of the spatial distribution of the macrobenthos-rich *A. alba* – *M. bidentata* community, using acoustic techniques (e.g. side-scan sonar). Secondly, because of its habitat structuring capability, *L. conchilega* is responsible for local, but drastic, increases in biodiversity. Using acoustic techniques to map the distribution of *L. conchilega* – and consequently the *A. alba* – *M. bidentata* community – will further provide important information on biodiversity hotspots on the BCS.

### III.b.3. Hyperbenthos : species composition

The hyperbenthos comprises a group of highly motile species, which are rather sensitive to characteristics of the water column and to short-term hydrodynamic changes. Mysids, the most abundant hyperbenthic taxon, perform species-specific migrations that are presumably a response to various or even a combination of environmental stimuli. Therefore, it remains a difficult task to define small ranges of species' ecological preferences, as such that they can be used as indicators species.

Notwithstanding their motile behaviour, well-defined hyperbenthic communities are identified on the Belgian coastal area. They are structured along a principal onshore-offshore-gradient and a secondary east-west gradient (see Chapter III.a.3).



Community structure of the hyperbenthos shows a strong temporal pattern for most species.

### III.b.4. Parasites : speciation patterns

Parasite speciation is influenced by ecological and phylogenetic factors. By comparing host and parasite phylogenies a differentiation can be made between the different speciation modes (Brooks and McLennan, 1993). On the one hand, the direct life-cycle and the high host-specificity enforce a tight relationship of *Gyrodactylus* with its host, promoting co-evolution. On the other hand, their ability for auto-infection increases the chance for sympatric speciation and speciation by host switching.

The reconstruction of the parasite phylogeny was performed as described under Chapter III.a (Spatial and temporal distribution). The reconstruction of the host phylogeny was performed as described in chapter VI genetic biodiversity of ecologically important fish.

The comparison of host and parasite phylogeny is shown in Figure 23. Except in the case of the closely related *P. minutus* and *P. lozanoi* each host species harboured a specific gyrodactylid species. The crossing lines point to historical host switching events. In the case of *P. microps* and *P. marmoratus* co-speciation is possible since each host species appeared infected with one species of two closely related species pairs.

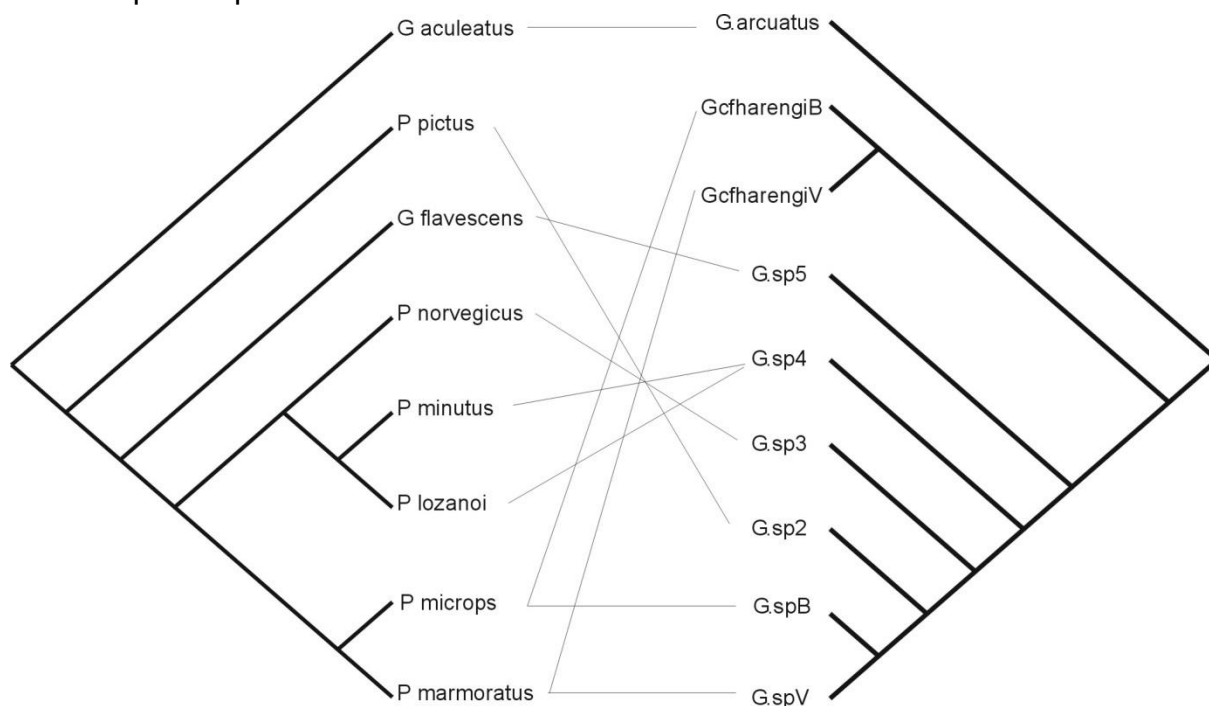


Figure 23: Comparison of host and parasite phylogenies (constructed with PAUP\*, Swofford *et al.*, 1999). Lines connect hosts and their parasites.

However, *G. sp. B* and *G. sp. V* are more closely related to the parasites of the other fish species than they are to *G. cf harengiB* and *G. cf harengiV*. This indicates that both species pairs are not the result of sympatric speciation.

In conclusion : in the case of strict co-speciation parasite phylogeny mirrors host phylogeny (known as Fahrenholz' rule, see Ridley, 1996). This mirror association is absent in the present study, suggesting the important influence of ecological factors in this host-parasite system. However, the parasite fauna of the *P. microps* complex could be the result of co-speciation. To address this question a molecular clock for both parasite and host phylogeny is used (in progress).

### III.b.5. Seabirds

Seabirds are often used as indicators of changes in the marine environment (Furness and Greenwood 1993). Occupying high trophic levels they accumulate persistent chemicals and are extremely vulnerable to their effects (Furness 1993, Debacker *et al.*, 1997, Becker *et al.*, 2001), making them of value as sensitive indicators of marine pollution with chemicals. Patterns in oil-rates of beached seabirds can be used to detect changes in oil pollution at sea (Camphuysen and van Franeker 1992, Seys *et al.*, 2001c, Seys *et al.*, 2001d). Many seabirds are extremely vulnerable to fluctuations in the availability of their food. Shifts in the horizontal distribution of their food, the composition of the food stock or altered food availability (e.g. through changes in the abundance of the food or changes in water clarity) can have strong effects on the distribution, numbers and behavioural ecology of seabirds. Hence indices of seabirds can be useful and early indicators of changes in stocks of pelagic fish or shellfish (Montevecchi, 1993). Finally, seabirds can be used as indicators of climatic changes (Jarvis, 1993, Barrett, 2001).

The indicator value of a seabird not only depends on its international importance and local densities (Table IV), but also on its feeding strategy and behavioural ecology. All six species that are selected as focal species for Belgian marine waters (Chapter III.a.5., Table IV) are extremely suitable ecological indicators. Except for Common Scoter, all focal species mainly depend on marine fish. Little Gull, Little Tern, Common Tern and Sandwich Tern capture fish in the upper few meters of the water layer by dipping to the surface or by plunge diving, while Red-throated Diver can reach depths up to 25 m depth by pursuing the fish under water. Indices based on the distribution and densities of these focal species might be particularly useful as indicators of fluctuations in pelagic fish. In particular terns are suitable biomonitors for the marine environment because there is a good knowledge of their ecology, they breed in high numbers at the Belgian coast, they occupy high positions within the marine food-chains and they are used as such in other European countries. In

contrast, Common Scoters depend on small molluscs (preferably *Spisula*) present in shallow waters. Outside the breeding season Common Scoters are highly gregarious and stay in shallow coastal areas where they actively trace sites with high abundance of edible clams, cockles or mussels. Strong shifts in the distribution of Common Scoters are often the result of prey depletion, either caused by intensive predation by the birds themselves or by fishery (Leopold *et al.* 1995). Hence, the abundance and distribution patterns of Common Scoter are valuable indicators for the presence of certain types of macrozoobenthos (Degraer 1999). All locally important seabirds (Table IV) largely depend on marine fish, but their scavenging (gulls) or kleptoparasitic (Great Skua) behaviour make them poor indicators of the availability of their prey. The distribution of Lesser Black-backed, Herring and Great Black-backed Gull very much depends on the distribution of commercial fishing vessels (Offringa *et al.* 1996, Seys *et al.* 2001e). Among the locally important seabirds only Great-crested Grebe does not feed behind ships. As this species occupies a specific niche among the fish-eating seabirds (it feeds by pursuing fish under water and its distribution is restricted to the coastal area) it certainly has an added value as bio-indicator.

Concluding, terns, Little Gull, Red-throated Diver, Great-crested Grebe and Common Scoter can be selected as valuable ecological indicators of changes in the marine environment. Monitoring of their numbers and distribution at the BCS can be a strong tool and an early warning system for changes within the marine ecosystem. Monitoring of contaminants in tern eggs and feathers can be used as an indicator of pollution in the Belgian waters. At present it is too early to use seabirds as indicators of changes in marine prey stock since detailed knowledge on their feeding ecology and on food-chain interactions is missing.

### III.c. ECOLOGICAL IMPORTANT AND SENSITIVE AREAS

The Belgian continental shelf is a highly exploited system. During the project, discussion started about the installation of Marine Protected Areas (MPA's) along the Belgian West Coast. Although the awareness in the need of marine protected areas is well accepted, the definition of criteria to which such areas should fulfill is still in progress in scientific research. The goal of the establishment of marine protected areas is to provide long-term sustainability. Its choice needs to be underpinned by scientific research and asks insight into the biotic components of the considered ecosystem and their functional interactions. Moreover, the degree of anthropogenic pressure should be balanced. Priority should be given to natural systems with high landscape diversity, rather than to artificial systems or to rare species. Interactions with adjacent areas should be considered as such that selected area is large enough in order to minimise effects of negative events in adjacent waters. Moreover, processes such as the supply and dispersal of larvae, both spatially and temporally (recruitment periods) should be considered.

During the network program, we were able to identify some vulnerable areas on the Belgian continental shelf based on biological and ecological indicators.

For macrobenthos data related to the topic of the Belgian west coast, we refer to the report of Degraer *et al.*, 2001 (Intensive evaluation of the evolution of a protected benthic habitat: HABITAT).

#### III.c.1. Importance of the Belgian sandbank system for fish and macrocrustaceans

The Belgian subtidal waters harbour a great variety of early life history stages of fish and macrocrustaceans. Larvae of at least 21 fish species (Fig. 24) and 29 decapod species (Fig. 25) occur in the area, which was revealed by monthly sampling surveys of the hyperbenthic fauna (see Chapter III.a.3). Recruitment of fish larvae is generally well defined in time, and happens for most species during April-May (Fig. 24). The most abundant species in the study area (even on an annual basis) is sole *Solea solea*; its density often exceeds 100 ind. 100 m<sup>-3</sup>. Decapod larvae show in general a more extended recruitment period. They are present in the Belgian coastal area from March till November with a maximum abundance during summer (Fig. 25). Common shrimp *Crangon crangon* is the most abundant species with an annual mean density of 137 ind. 100 m<sup>-3</sup>.

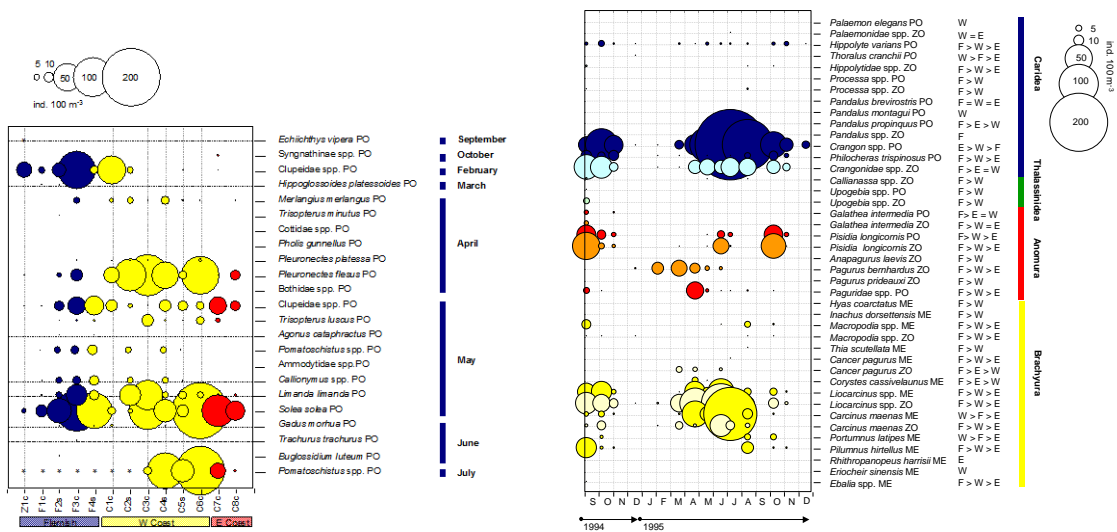


Fig. 24: Spatio-temporal variability for fish larvae. The bubble chart represents densities per site for the month in which the species attained a maximum average abundance. Postlarva of clupeids and *Pomatoschistus* spp. are represented twice since they showed a bimodal annual fluctuation. Sites are shown along the x-axis and the species, grouped per month, on the y-axis.

Fig. 25: Spatio-temporal variability for all decapod larvae caught. The bubble charts represent mean densities per month over all sites. Zoea (ZO) and postlarva (PO) or megalopa (ME) are separately presented. Months are shown along the x-axis, scaled in Julian days. Note that no sampling was carried out in January and September 1995. Taxa and life stages are grouped per infra-order and presented along the y-axis. Spatial occurrence is ranked for the three areas according to highest mean abundance; Flemish (F), W Coast (W), E Coast (E).

### III.c.2. Key role of the Flemish Banks in dispersal of macrocrustaceans

The earliest life stages of decapods, i.e. zoea larvae, are most abundant at the Flemish Banks (Fig. 25). This might be explained by the particular hydrodynamic characteristics of the sandbank system of the Belgian continental shelf. Tidal flow is strongly flood-dominated (higher current velocities towards the N than to the S) at the Coastal Banks. Early larval stages hatched in this area are thus most probably dispersed in the offshore direction. Flood-dominance decreases with distance off the shore and tidal flow becomes ebb-dominated off the Flemish Banks (higher current velocities towards the S than to the N). The ebb-dominated tidal flow off the Flemish Banks might prevent further offshore dispersal, therefore leading to an accumulation of early larval stages. Larval supply from farther offshore waters (e.g. from the Hinder Banks) might moreover be supported by the ebb-dominated current.

Later larval stages (megalopae or postlarvae) of certain species reach highest densities on the Coastal Banks. These stages generally have a better retention behaviour and may gradually reach their juvenile / adult habitat by selective tidal stream transport (Shanks 1995). It seems that the sheltered and food-enriched area

of the Coastal Banks provides suitable adult habitats for many species. For example, zoea larvae of common shrimp *Crangon crangon* reach highest densities at the Flemish Banks, its postlarval stage is most abundant at the Coastal Banks, while the adults are dominant users of the adjacent beaches (Beyst *et al.* 2001). It has also been shown that postlarval stages of *Crangon crangon* enter the Westerschelde estuary and use the nearby tidal marshes as a nursery (Cattrijsse *et al.* 1997).

### **III.c.3. Transit area (Flemish Banks) and nursery potential (Coastal Banks) for fish larvae**

Horizontal transport of fish larvae from spawning ground towards nursery area is largely determined by the hydrodynamics of the area. Spawning sites for the fish larvae encountered at the Belgian coastal zone are most probably located in the English Channel, judging from the residual circulation (Daan *et al.*, 1990; Knijn *et al.*, 1993; Woehrling & Le Fèvre-Lehoërf 1998; Grioche *et al.*, 1999). Nevertheless, the Belgian coast is known as a major spawning site for sole and probably also for flounder (Knijn *et al.*, 1993; Woehrling 1985). As fish larvae grow, they gain competency to regulate their dispersal by selective tidal stream transport. This ability of performing tide-dependent vertical migrations leads to an efficient and fast transport towards coastal nursery grounds.

The tidal current system of the Flemish Banks is hypothesised to favour fish larvae in their transport from offshore waters towards suitable nursery grounds in the Belgian coastal area. Most species encountered in the sandbank system do occur as juveniles in the nearby nurseries, such as the surf zone (Beyst *et al.*, 2001) or the Westerschelde estuary (Hostens 2000). Furthermore, the Coastal Banks harbour high numbers of fish larvae. Densities for several species exceed the ones of adjacent biotopes and certain species do preferentially occur at the Coastal Banks during their larval stage. This automatically reveals the issue of the potential of the Coastal Banks as a nursery area. A nursery should meet three conditions, i.e. provide larval supply and transport, food, and refuge (Boesch & Turner 1984). As illustrated by the high numbers and variety of fish larvae and the hydrodynamic characteristics discussed above, it is concluded that the first condition is met. The other conditions still have to be assessed. We strongly presume that the area has a great nursery value for sole. Densities of larval sole are far exceeding the values reported for adjacent shallow areas as the Dutch Voordelta, the surf zone and the Westerschelde estuary. Caught sole larvae have been observed to consume large numbers of copepods (A. Dewicke pers. observ.), pointing to the value of the area as feeding ground. During their growth, sole larvae may still remain in the area. Quantitative data of the occurrence of juvenile sole is unfortunately lacking for

subtidal waters, it has however been shown that they are abundant in the adjacent surf zone (Beyst *et al.* 2001)

**Conclusions :** The Flemish Banks are regarded as playing a key role in the supply of larvae from offshore waters towards more sheltered onshore areas suitable for settlement. The Coastal Banks at the W Coast harbour high densities of larval fish and macrocrustaceans, potentially being of nursery value for several species. Moreover, the proximity of the Westerschelde estuary and the extensive sandy beaches – known nurseries for fish and macrocrustaceans – makes the setting of the sandbanks unique. Several organisms perform ontogenetic migrations between these biotopes and might fulfil their entire lifecycle in this shallow coastal ecosystem. The Flemish Banks and the Coastal Banks of the W Coast, together with its adjacent gently sloping beaches are therefore considered as most vulnerable areas, in particular during the peak period of larval recruitment (April-May).

### III.c.4. Impact of sand and gravel extraction on benthos biodiversity

The biological data of the Kwinte Bank were compared with information of sand extraction intensity and erosion.

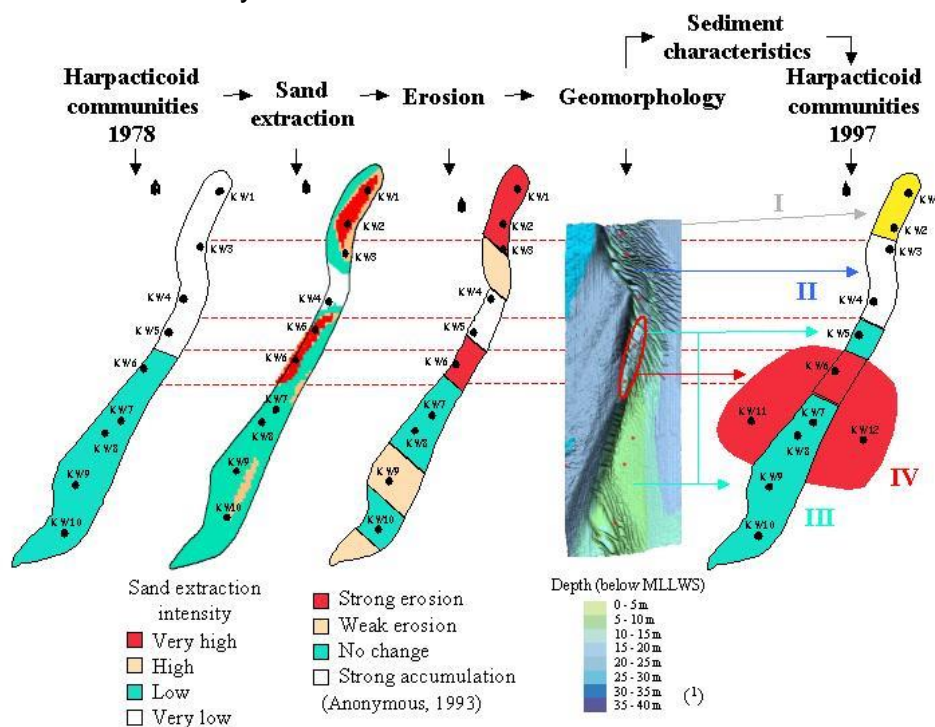


Figure 26: (1) Multibeam recording: Geological Survey of Belgium - Continental Shelf Team + Research Unit for Marine and Coastal Geomorphology, University of Gent.

On the Kwinte Bank, three levels of sand extraction intensity can be distinguished: 1) the centre (stations 5 and 6) and the northern tip (stations 1 and 2) of the sandbank are very intensively exploited areas, 2) station 3 and the southern part of the sandbank are exploited much less frequently and 3) at station 4 and the gully stations (almost) no extraction occurs.

Analogies could be found in the occurrence of erosion and extraction areas and the occurrence of harpacticoid communities on the sandbank (Figure 26). In the nineties the Kwinte Bank was split up in 4 communities while only two parts could be distinguished in the seventies: a high variable northern part and a southern part with a high similarity and stable in time. In the northern part diversity remained high in the nineties but the species composition altered in favour of interstitial species. In the centre of the bank, the most intensive exploited area, copepod density and diversity decreased dramatically as explained before. In Figure 27 conclusions are summarised in a scheme with hypothetical harpacticoid-environment interactions. Natural conditions define which harpacticoid species and communities occur but sand extraction can influence species composition both on a direct and an indirect way. The northern tip and the centre of the Kwinte Bank are strongly impacted areas. The sand exploitation on the Kwinte Bank is very patchy and much too intensive in the centre.

Spreading the extraction activities over the different sandbanks in the concession zone will help decreasing disturbance frequency and intensity.

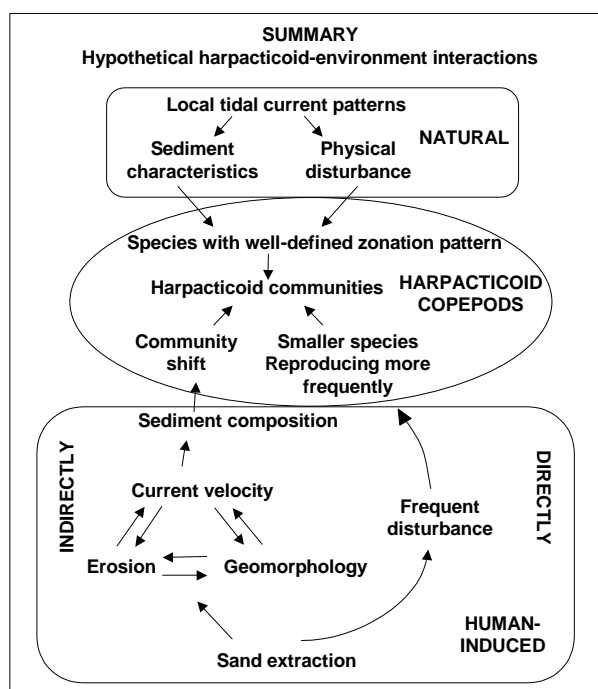


Figure 27: Hypothetical harpacticoid-environment interactions.



### III.C.5. Oil sensitive areas for seabirds

By focussing on six focal seabirds and by using species-specific vulnerability indices for oil pollution and traffic disturbance (see Seys *et al.* 2001a for more details) we have strong tools at our disposal to designate vulnerable areas within the Belgian marine waters.

By incorporating a measure for oil-vulnerability, seabird hotspots can be compared in terms of sensitivity for oil-pollution. Winter and to a lesser extent early spring turn out to be the most delicate periods for oil-pollution (Fig. 28).

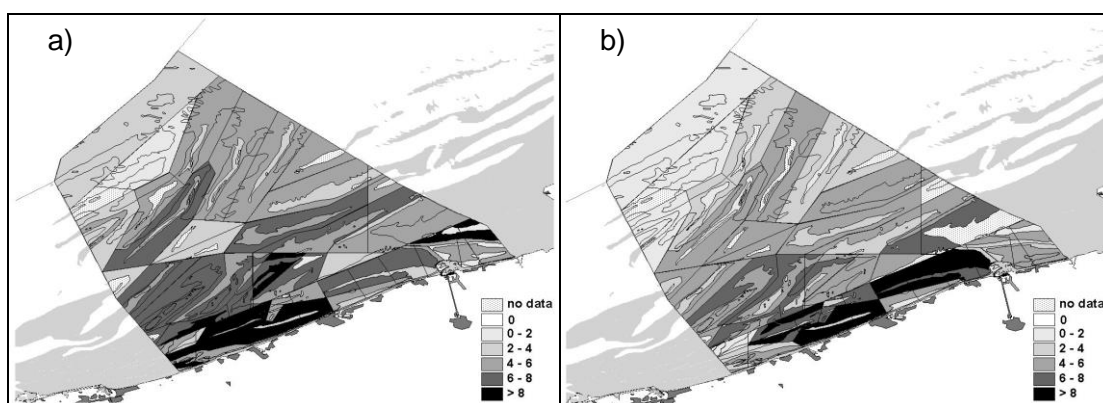


Figure 28: The oil-vulnerability for sea- and coastal birds in Belgian marine waters in a) winter and b) spring. Shading is based on an oil vulnerability value, calculated from the relative abundance and the oil vulnerability index of the 23 most abundant species (see Seys *et al.* 2001a for more details).

The high oil-vulnerability indices for some wintering seabirds (e.g. auks, scoters and divers) in comparison with those of terns are the main reason for the bias towards winter. Generally spoken, the closer an oil-slick approaches the coastline the worse the presumed impact on seabirds will be (Fig. 28). Within each season the western sandbank systems western Coastal Banks and Flemish Banks are highly oil-sensitive. During winter (Fig. 28a) the eastern coastal sandbanks (with the exception of the Vlakte van de Raan) are less vulnerable than the western part of Belgian inshore waters. In spring (Fig. 28b) and summer the eastern Coastal Banks are most vulnerable.

### III.c.6. Disturbance-sensitive areas for seabirds

Disturbance-sensitive seabirds such as scoters, divers and (to a lesser extent) auks and grebes are most abundant in Belgian marine waters in winter and early spring. Higher disturbance-sensitivity values in autumn compared to summer are due to increasing numbers of sensitive species from October onwards. Most vulnerable sites in terms of disturbance are the western Coastal Banks and the Flemish Banks, both in winter and early spring (Fig. 29).

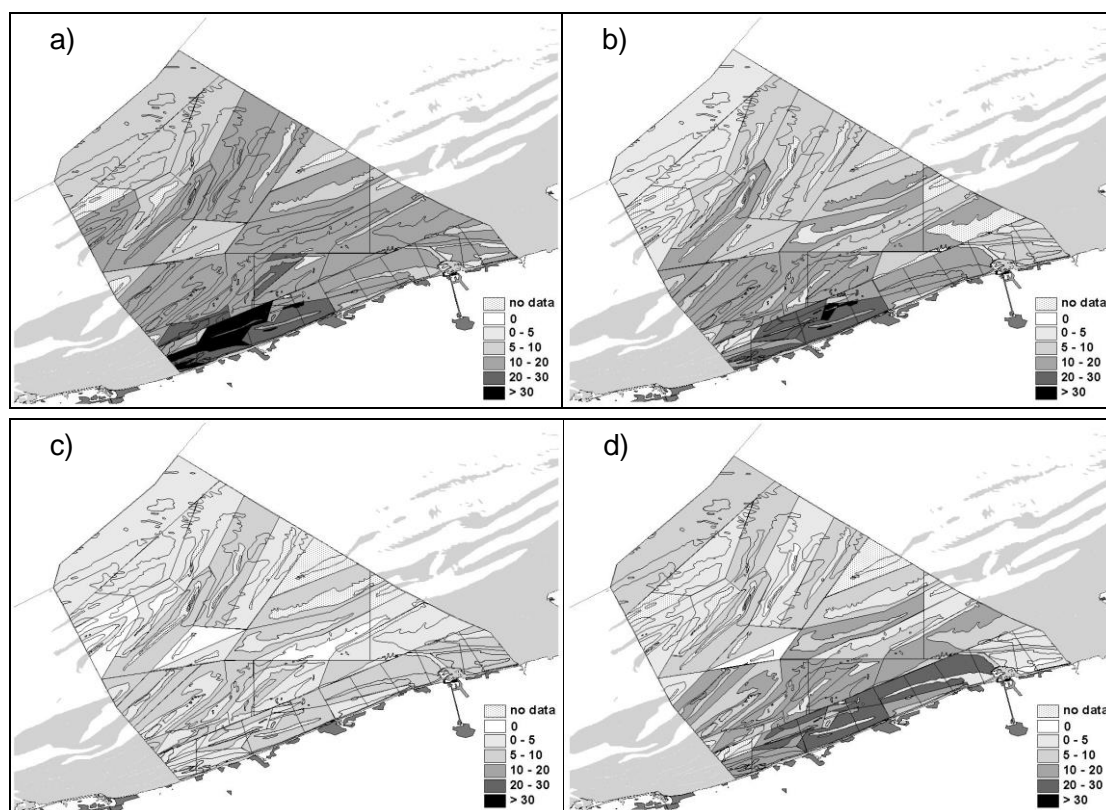


Figure 29: The disturbance-sensitivity for seabirds in Belgian marine waters in a) winter, b) spring, c) summer and d) autumn. Shading is based on a disturbance sensitivity value, calculated from the relative abundance and the disturbance sensitivity of the 23 most abundant species (see Seys *et al.* 2001a for more details).

Disturbance sensitivity scores are low from April till October. Very sensitive spots (disturbance sensitivity value >30) in winter and/or spring are Nieuwpoort Bank, Trapegeer, Stroombank and Balandbank, closely followed (value 20-30) by Ravelingen, Smal Bank-north and Middelkerke Bank (Fig. 29).

#### IV. BENTHO-PELAGIC COUPLING

The Belgian coastal zone is characterized by accumulation zones of deposited organic matter originating from primary production during several days (weeks) of the year. The importance of the primary production in the water as structuring factor for benthic communities is investigated. The structural and functional response of the meio- and macrobenthos to the input of natural organic enrichment is examined.

Meio- and macrobenthos, together with abiotic and biotic environmental factors, were weekly sampled from February 1999 till July 1999 in two stations (station 330 and 115) on the Belgian Continental Shelf. The macrobenthic samples were taken with a Van Veen grab on board of the RV *Belgica*. Meiobenthos and samples for environmental analysis (chlorophyll-, fucoxantin, nutrient, grain size) were taken with a Reineck boxcorer. Surface water for pigment and nutrient analysis was sampled by means of a Niskin bottle.

Station 115bis is characterised by an enhanced accumulation of organic matter (Fig. 30a and 30b) resulting from the prevailing hydrodynamic conditions, as it is located in between the Coastal and Flemish banks. The sediment consists of fine sand with an average median grain size of 185  $\mu\text{m}$ . Station 330 is an offshore station, characterised by medium sand with an average median grain size of 344  $\mu\text{m}$ .

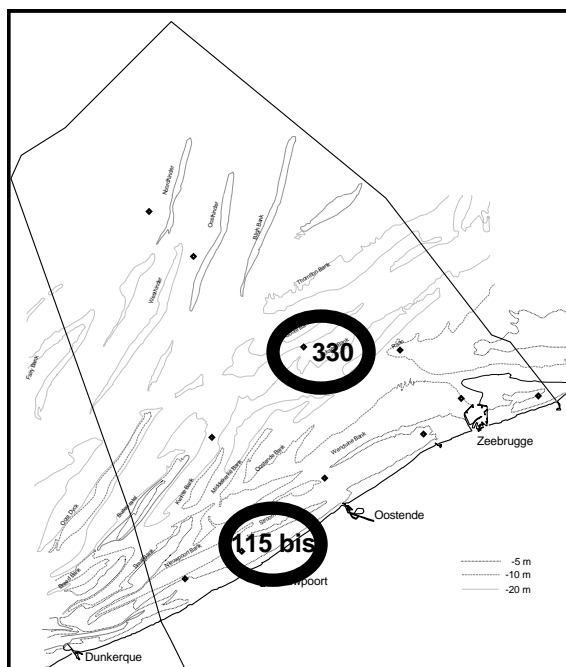


Figure 30 a : position of the two sampling stations

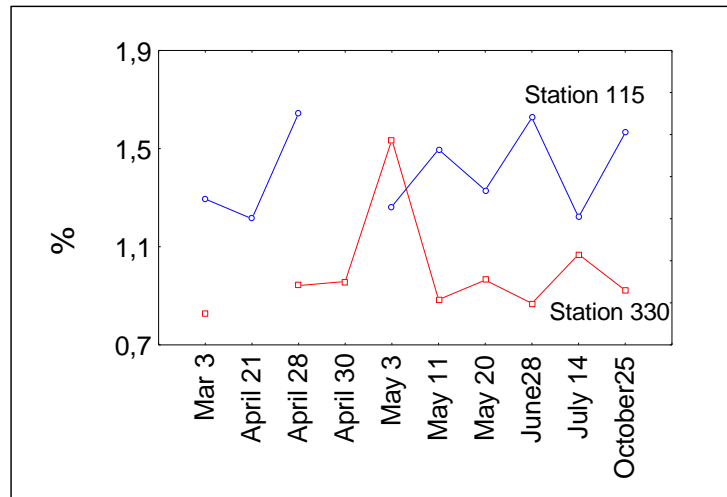


Figure 30b: Total organic matter at station 115 and 330

In response to the N and P anthropogenically-enriched nutrient delivery, the coastal waters of the Southern Bight of the North Sea are highly eutrophicated (Rousseau 1999). This eutrophication is characterised by the occurrence of recurrent short-living spring blooms of the non-siliceous colony-forming haptophyte *Phaeocystis pouchetti* succeeding to a moderate early spring diatom growth.

The *Phaeocystis pouchetti* bloom, succeeding the diatom bloom, started in April and continued in May 1999 at station 330, was indicated by enhanced chlorophyll *a* concentrations and diatom biomass in the water column (data of VUB, Fig. 31; Rousseau, 1999).

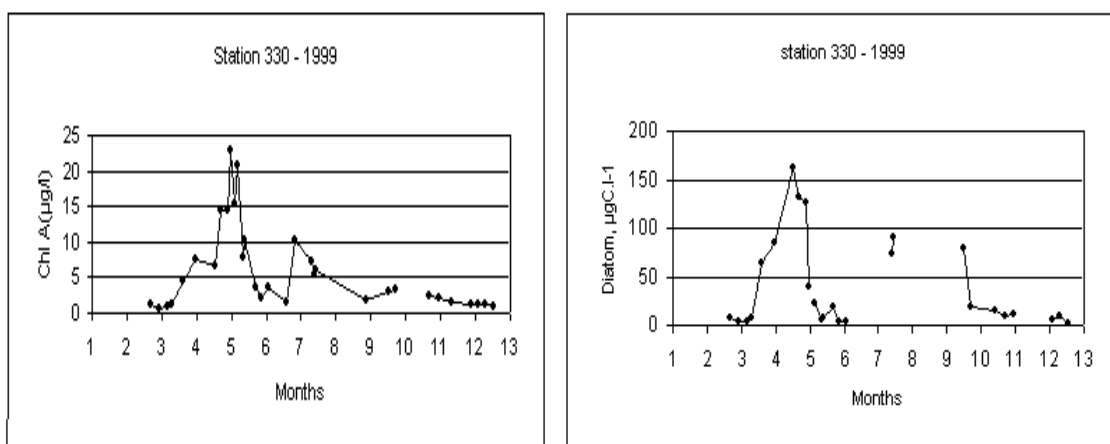


Figure 31: Chlorophyll *a* concentrations and biomass of diatoms in the water column at station 330

Chlorophyll *a* measurements for station 115 were much scantier; diatom biomass measurements were not available (data from VUB, Fig. 32). The analysis indicated a slightly earlier *Phaeocystis* bloom, as chlorophyll *a* concentrations started to increase in March and reached maximum values at the end of March.

The phytoplankton produced in the water accumulated and subsequently reached the sea floor before remineralisation in both stations. This was obvious in station 115 (Fig. 32) by the high chlorophyll *a* concentrations (2000 ng/g) in the sediment and to a lesser extent at station 330 (90 ng/g at the beginning of May).

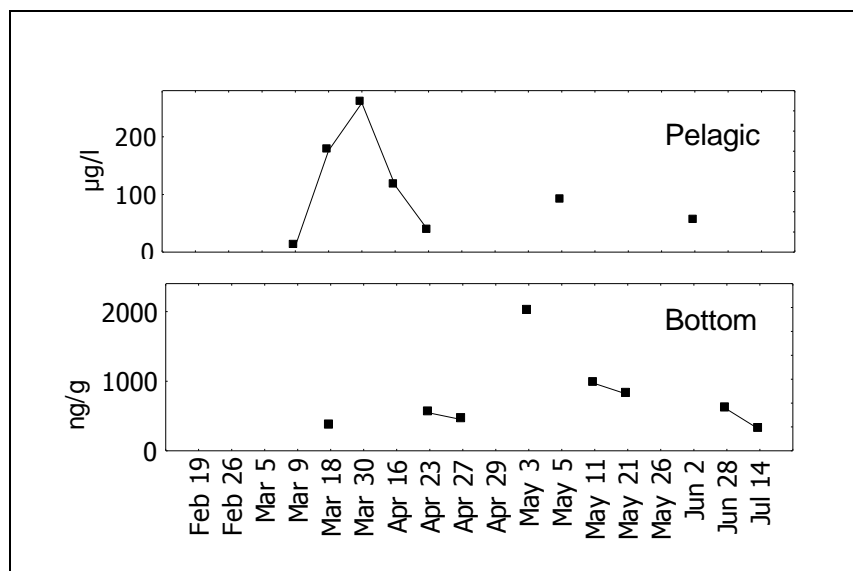


Figure 32: chlorophyll *a* concentrations at station 115

#### IV.a. Meiobenthos

Meiobenthic communities of both stations were analysed at following dates:

##### A. Station Station 330

9/3/1999	9/3/1999
24/4/1999	24/4/1999
12/5/1999	12/5/1999
2/6/1999	28/6/1999
14/7/1999	12/7/1999

The different characteristics of both stations were described by their environmental parameters; nematode communities, the dominant taxon, were also different. The coastal station showed high densities, low species diversity (92 nematode species, mainly Comesomatidae and Xyalidae) with mainly of non-selective deposit feeders. At the offshore station 330 a lower density and a higher species diversity (166 species, mainly Chromadoridae, Xyalidae and Microlaimidae) was recorded. Here, all feeding guilds were well represented.

At both stations, a clear but different response of the nematode community to an enhanced supply of organic matter was observed. At station 115 nematode densities

were low before the onset of spring bloom and increased towards summer months (Fig. 33). At the offshore station 330, an obvious increase was noticed immediately after spring bloom, however one month later densities returned to initial values (Fig. 33). However, this increase in nematode density is situated in deeper sediment layers in station 115 and in surface layers in station 330 (not illustrated in this chapter).

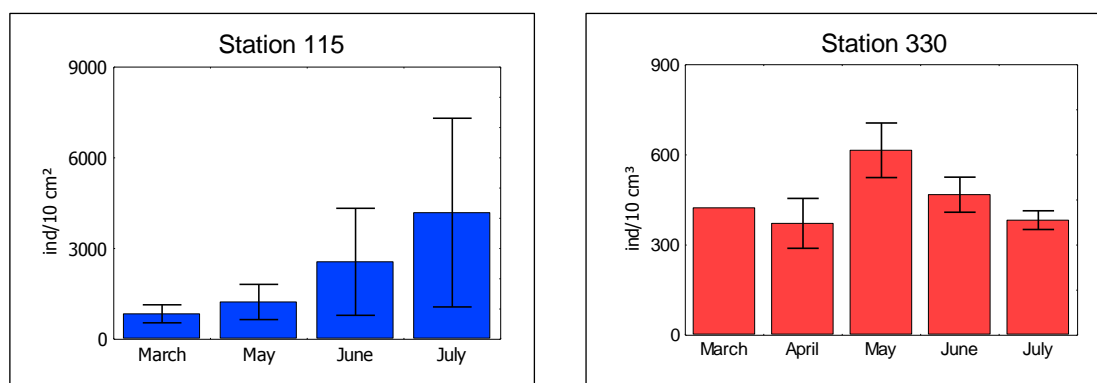


Figure 33: total nematode abundance at station 115 and 330

At station 330, dominant nematode species could be classified into 3 groups, according to their maximum abundance over time (Fig. 34). Eight species, *Actinonema celtica*, *Chromadorita* n.sp.2, *Epsilonema* sp.2, *Epsilonema pustulatum*, *Microaimus marinus*, *Onyx perfectus*, *Richtersia inaequalis* and *Tricoma* sp.1 reached maximum values before the onset of spring bloom. Four species, *Desmodora schulzi*, *Ixonema sordidum*, *Richtersia quemer* and *Sabatieria celtica* were maximum abundant during and 5 species, *Dichromadora cucullata*, *Neochromadora angelica*, *N. munita*, *Prochromadorella ditlevseni* and *Theristus denticulatus* were maximum abundant after the spring bloom.

At the coastal station 115, no grouping of nematode species according to their species abundance patterns over time could be recognised.

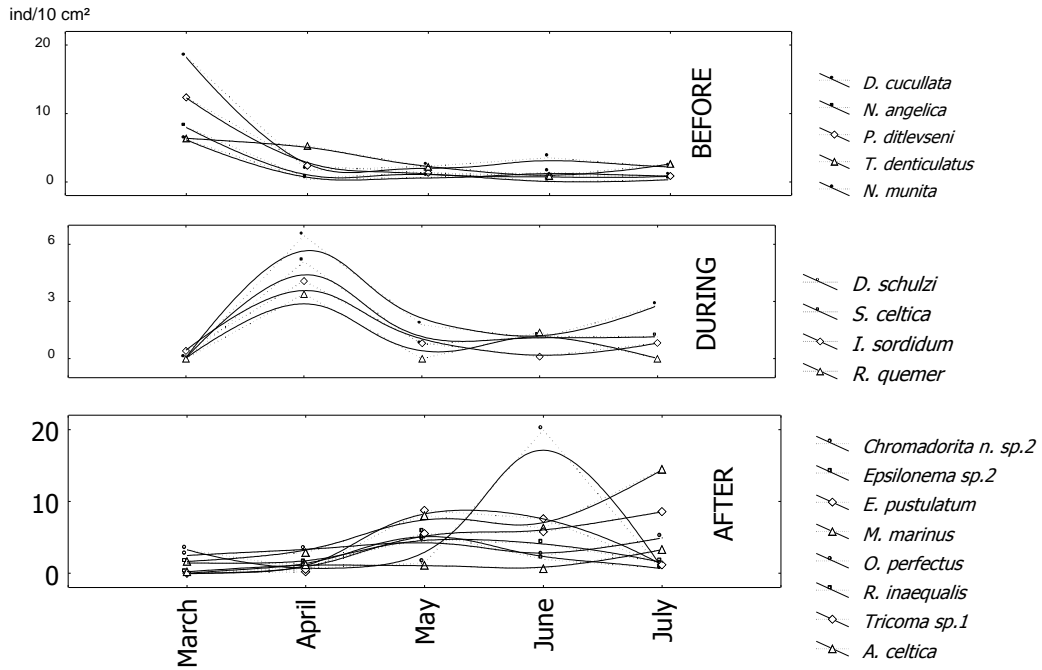


Figure 34: abundance patterns of dominant species at station 330

An impact on species diversity ( $N_0$ ,  $N_1$ ,  $N_2$  and  $N_{inf}$  Hill indices) was only observed at station 330, where all indices were obviously higher during and after the spring bloom (Fig. 35).

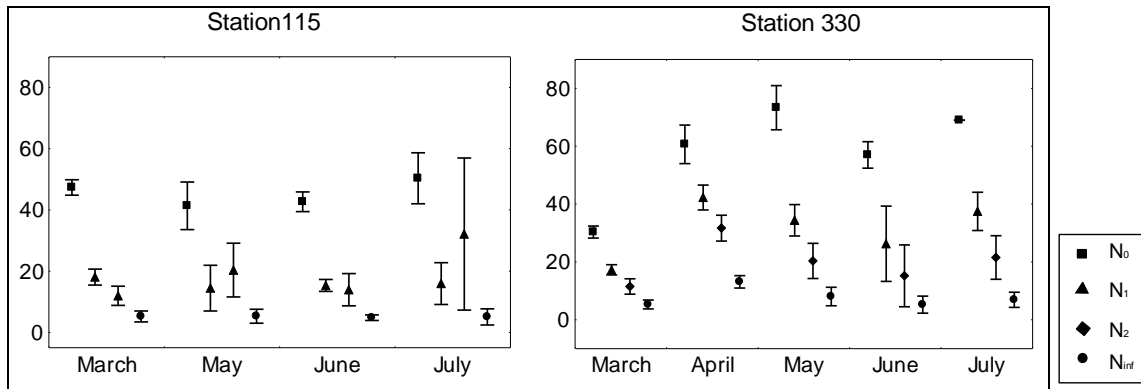


Figure 35: nematode species diversity at station 115 and 330

#### IV.b. Macrobenthos

At station 115 an *Abra alba* – *Mysella bidentata* community (Van Hoey *et al.* in prep.) was observed, characterised by a high density (80000 ind/m<sup>2</sup>) and diversity (28 species). The dominant species at this station were polychaete species (*Nephtys hombergii*, *Magelona mirabilis*, *Lanice conchilega*, *Spio filicornis*) and bivalve species (*Abra alba*, *Spisula subtruncata*, *Fabulina fabula*). The dominant feeding-types in this community were deposit feeders and filter feeders (possibly combined). At station 330 an *Ophelia limacina* – *Glycera lapidum* community (Van Hoey *et al.* in prep.) was observed, characterised by a lower density (1000 ind/m<sup>2</sup>) and diversity (max No: 15 species). This station was dominated by polychaetes (*Nephtys cirrosa*, *Ophelia limacina*, *Spiophanes bombyx*) and crustacean (*Urothoe brevicornis*), with deposit feeders-grazers and omnivores as dominant feeding types.

At station 115 densities increased significantly in time towards the end of May (Fig. 36). Total densities were correlated with chlorophyll *a* concentrations in the bottom. At station 330 a seasonal effect was observed, with maximum abundances in June-July. The food supply after a phytoplankton bloom seems to be important for the growth and reproduction for a lot of invertebrates (Christensen and Kannevorff, 1985). Biomass increased at both stations towards the summer. No correlation was found with chlorophyll *a* concentrations.

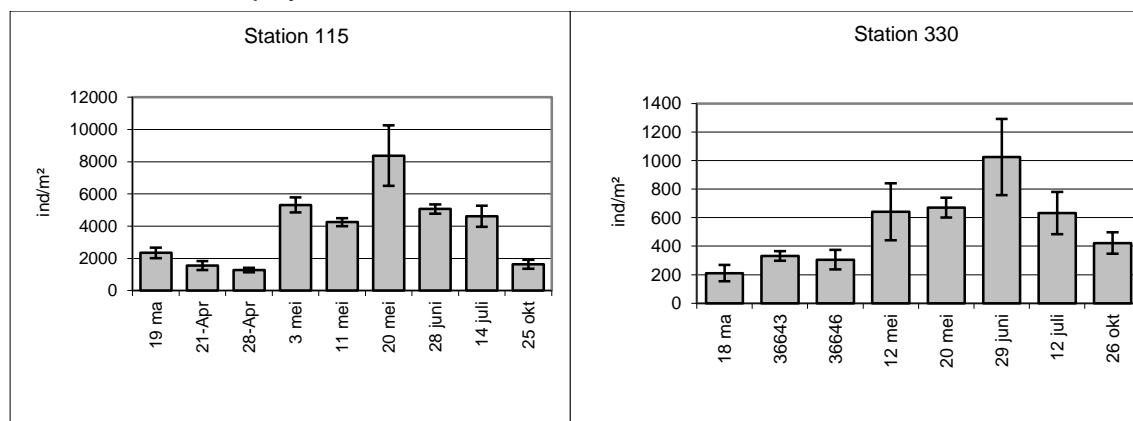


Figure 36: total macrobenthos abundance at station 115 and 330

Besides an increase of the species diversity, the settling of young individuals caused increased densities at station 115 after the phytoplankton bloom. The highest densities of juveniles (*Lanice conchilega*, *Anaitides maculata-mucosa*, *Nephtys* spp.) were observed in May (Fig. 37). There was also a correlation between juvenile densities and chlorophyll *a* concentrations in the bottom. The highest density of juvenile *Nephtys cirrosa* at station 330 was reached at June-July (Fig. 38). Overall, the settling of young individuals of species can be directly linked to an increased



influx of organic matter (station 115), however this could not be demonstrated at station 330.

The length-frequency distribution of *Abra alba*, *Spisula subtruncata* and *Fabulina fabula* at station 115 showed the settling of young individuals in May, with a strong growth towards the summer.

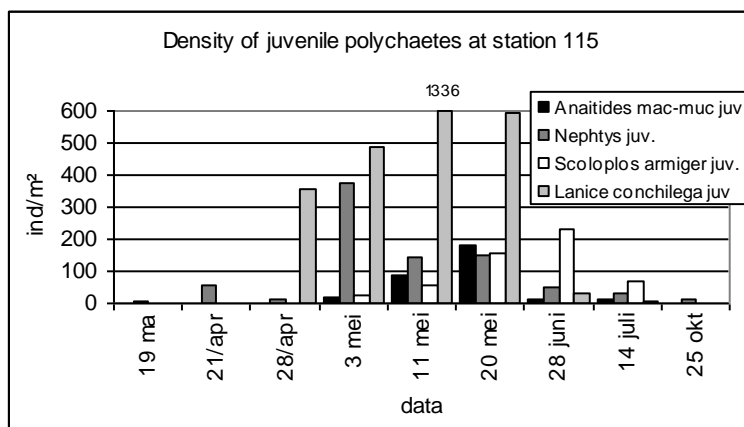


Figure 37: total abundance of juvenile polychaetes at station 115

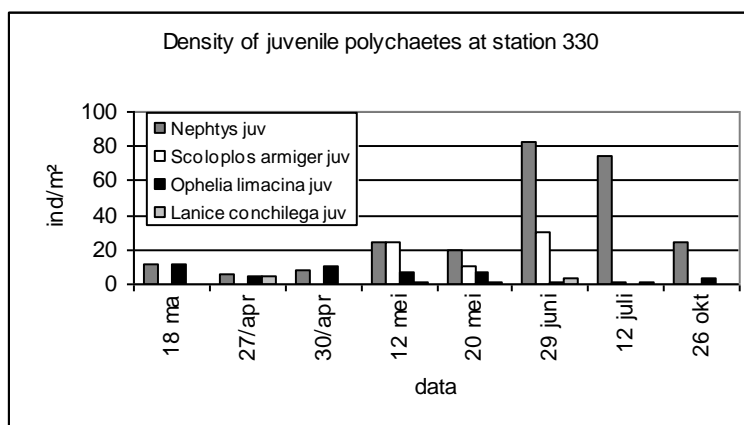


Figure 38: total abundance of juvenile polychaetes at station 330

#### IV.c. Conclusion

A differential response of nematode- and macrobenthos communities to an enhanced natural supply of organic matter originating from the primary production was observed.

The coastal station 115 harboured a species poor, but abundant nematode community and a species rich macrobenthos community compared to the offshore station 330, which was characterised by a highly diverse nematode community and relatively poor macrobenthos community.

For the nematode community the response to the enhanced supply of organic matter was obvious in terms of abundance, community composition and feeding type

distribution at both stations. At the offshore station the increase of freshly deposited organic matter is more episodic and favoured opportunistic nematode species. At the coastal station, there existed a rather continuous and sufficient supply of organic matter, which had nevertheless an obvious impact on the total nematode association. An impact on the macrobenthos community was only observed at the coastal station, by increased densities of mainly young individuals in May. At the offshore station 330, enhanced densities, mainly mobile organisms, a rather seasonal effect in summer months was present. It was hypothesised that dominant species were rather dependent on decomposition processes during summer months (Van Hoey, 2000).

## V. GENETIC BIODIVERSITY OF ECOLOGICALLY IMPORTANT FISHES

### V.a Genetic biodiversity of ecologically important fishes.

#### V.a.1. Introduction

The North Sea ecosystem sustains a high productivity due to its shallowness, strong tidal mixing and high terrestrial nutrient input. Its species diversity is characteristic of temperate continental shelf systems with moderate species diversity and the dominance of few taxa. The Belgian Continental Shelf differentiates from the North Sea by the presence of a series of Pleistocene sand banks, and the influence of the Schelde estuary in its northeastern section. Hence the physical structure is reflected in the ecosystem by the presence of seven clearly identifiable groups, mostly positioned on an inshore / offshore gradient (Cattrijsse & Vincx, 2001). The question whether the structuring of ecosystem and species diversity and its functioning is reflected in the genetic structuring of marine taxa on the Belgian Continental Shelf was addressed for the first time.

The choice of model species to address local genetic diversity and structure was critical in several regards. Key species of the region represent a first choice, as they are important in differentiating the communities. However they should occur commonly and be widespread with easy sampling access. Hence macrobenthos and fishes should offer the best chances. Ultimately one fish group was chosen as the main target: the *Pomatoschistus* gobies, which are ecologically very significant due to their numerical dominance, as predator of meio- and macrobenthos and as food for the large commercial fish species. Although considerable information was available on their population dynamics, ecology and taxonomy, few studies had attempted to address the genetic structuring of *Pomatoschistus minutus*, *P. lozanoi* and *P. microps* on the Atlantic shelf (Wallis & Beardmore, 1984; Al-Hassan *et al.*, 1987) and none was made on the Belgian Continental Shelf. Three additional representative species were included as indicators of the fish fauna of the Belgian continental shelf. Each occupies a different ecological niche from the gobies and has traits not found in the other: Dover sole (*Solea solea*), three-spined stickleback (*Gasterosteus aculeatus*) and European eel (*Anguilla anguilla*). The flatfish sole represents a major component of the large demersal fish fauna and is the economically most valuable species for the Belgian fishery. The anadromous three-spined stickleback shows a complicated relationship between fresh and salt water and is a superior model species due to its detailed biological knowledge. It has been the focus of an associated project funded by the Ministry of the Flemish

Government (AMINAL – VLINA/96), is still the focus of evolutionary research in the region and hasn't been further reported in this document. The European eel uses the continental shelf in transit as glass eel between the spawning ground and the continental waters, and as silver eel in the reverse sense, although evidence is growing that a large number of yellow eel mature in coastal waters. This project has been funded by various sources, most recently by the European Union (Q5RS-2001-01806-EELREP), and hasn't been further reported in this document.

The main questions addressed in this study relate to a characterisation of genetic diversity among the various gobies species with various mitochondrial and nuclear (allozymes and DNA microsatellites) markers. From this genetic diversity an estimate is made of the genetic structure, the gene flow (or conversely of genetic isolation) and of the effective population size. The results point to highly diverse populations of gobies and sole on the Belgian Continental Shelf with a high amount of gene flow, suggesting in some cases reproductive isolation.

## **V.a.2. Material and methods**

### *Sampling*

Sampling on the Belgian Continental Shelf (BCS) was carried out with R/V 'Belgica'. Fish were caught with a 3-m beam trawl (outer net mesh size of 20 mm stretched and a mesh size of 12 mm for the cod end). The samples from the Schelde estuary were taken in Doel at an industrial cooling water intake. Mesh size of the nets used was 4 mm. The samples were frozen in dry ice or liquid nitrogen immediately after capture and kept in a  $-80^{\circ}\text{C}$  freezer until analysis. Gobies were identified morphologically on the basis of the dermal papillae of the head according to Miller (1986), and biochemically according to Wallis and Beardmore (1984a, b).

### *Allozyme electrophoresis*

Liver, eye and muscle tissues were dissected and ground in distilled water. The samples were subjected to cellulose acetate allozyme electrophoresis (Richardson *et al.*, 1986) using two buffer systems: Tris-maleate (pH 7.8) and Tris-glycine (pH 8.8) as described by Hebert & Beaton (1989). Loci were stained according to recipes described by Hebert & Beaton (1989) and Richardson *et al.* (1986). The slowest migrating locus was designated 1 or A according to the nomenclature of Shaklee *et al.* (1990).

### *Microsatellite analysis*

DNA samples were extracted from pectoral fins using a Chelex extraction protocol (Walsh *et al.* 1991). Reactions were run in a Biometra III DNA thermal cycler. PCRs were carried out in 10 $\mu$ l containing 1 $\mu$ l of tris-HCL buffer, 1 $\mu$ l of DNTPs (2mM), 0.2-0.4 $\mu$ l of Mg (50mM), 0.25-1 $\mu$ l of primers (1-2.5 $\mu$ M) and 0.05 $\mu$ l of Taq DNA polymerase (5U/ $\mu$ l). Cycling conditions included an initial denaturation step of 2 minutes at 95°C, followed by 25 cycles of 1 minute at 95°C, 1 minute at annealing temperature (62°C for *Pmin-05*, 57°C for *Pmin-07*, *Pmin-08*, *Pmin-09* and 60°C for *Pmin-11*) and 1 minute at 72°C. Finally, the PCR reaction stopped after 3 minutes at 72°C. PCR products were diluted with 5 $\mu$ l a 99% formamide solution and were electrophoresed on 6% polyacrylamide gels on an automated sequencer (LICOR) using the software E-seq (Westburg, version 2.00). Alleles were scored using the software Gene ImagIR ver. 4.3 (Westburg). Four primer sets were designed and tested for PCR reaction in the sand goby. They were applied on 10 samples of sand goby consisting of 516 individuals from Belgian and Netherlands coasts.

### *Data analysis*

Allele frequencies and the observed and unbiased expected heterozygosity were calculated in GENETIX version 4.0 (Belkhir, 1999). Hardy-Weinberg equilibrium (HWE) was calculated and tested for significance in GENEPOP (Raymond & Rousset, 1995). Significance levels were adjusted with a sequential Bonferroni test (Rice, 1989). Wright's single-locus F-statistics (Wright, 1951) were calculated from allele frequencies of all loci examined for each population according to Weir and Cockerham (1984) in GENETIX. Standard deviations of single-locus  $F_{ST}$  values were obtained by jackknifing over all populations according to Weir (1990). Significance of multilocus  $F_{ST}$  was assessed with permutation tests (1000 replicates). Pairwise genetic distance (Nei, 1978) was calculated in GENETIX and significance was assessed with permutation tests (1000 replicates). A Mantel test (Mantel, 1967) was performed in GENETIX for testing whether any isolation-by-distance could be observed. An Analysis of Molecular Variance (AMOVA) (Schneider *et al.*, 2000) was carried out in Arlequin version 2.0 for assessing how much of the observed variation was due to within versus between population effects.

### Sequencing

DNA extraction was performed following the protocol for microsatellites. A total of 356bp of the 12S mtDNA region was amplified for six *Pomatoschistus* species, *Gobiusculus flavescens* and *Gasterosteus aculeatus* using the primers 12Sa and 12Sb (Kocher *et al.*, 1989). The total PCR reaction volume (25 µl) consisted of: 1x PCR buffer (Eurogentec), 0.5mM MgCl<sub>2</sub> (Eurogentec), 200 µM of each dNTP (Amersham Pharmacia Biotech, Sweden), 1 µM of each primer (Eurogentec), 1 µl template, 1 unit *Taq* polymerase (Eurogentec) and mQ-H<sub>2</sub>O. The mixtures were layered with mineral oil, heated for 4 min at 97°C and subjected to 35 cycles as follows: 95°C for 30s, 55°C for 30s and 72°C for 45s and then cooled at 4°C. The PCR products were purified by means of GFX columns according to the manufacturer's instructions (Amersham Pharmacia). Sequencing was done following the protocol of SequiTherm EXCEL II (Epicentre Technologies). The reaction products were separated on a LICOR 4200 system and visualised on a 6% Long Ranger gel (FMI BioProducts).

### Data analysis - sequencing

The 12S sequences were aligned using Clustal W (version 1.7, Thompson *et al.*, 1994). Modeltest 1.05 was used to select the model of DNA evolution that best fits the data based on log likelihood scores (Posada and Crandall, 1998). Maximum parsimony (MP), maximum likelihood and distance-based methods (PAUP\* v. 4.01b, Swofford *et al.*, 1999) were used to infer a phylogeny. With maximum parsimony we conducted the exhaustive search method and bootstrapped (n=1000) with the branch and bound algorithm. In these analyses gaps were treated as missing data and all sites were equally weighted. The maximum likelihood analysis was performed using the parameters estimated under the best-fit model. For the minimum-evolution distance method, the distance matrix was calculated using the maximum likelihood distances.

## V.a.3. Results

### Allozymes and microsatellites

The number of *P. minutus* and *P. lozanoi* analysed amounts to a total of 875 specimens for allozyme analysis. Five hundred sixteen *P. minutus* were subjected to microsatellite analysis.

An overview of the sampling sites at the Belgian Continental Shelf with the number of fish analysed is presented in Table V.

Table V: Sampling sites on the sand banks of the Belgian Continental Shelf with coordinates and numbers of fish examined.

Habitat	Sampling site	Code	Period	Numbers of <i>P. minutus</i>	Numbers of <i>P. lozanoi</i>	Numbers of <i>P. minutus</i>
				•	<i>Allozymes</i>	<i>Micro-satellites</i>
<b>Estuary</b>	Doel 3	D3	March 1998	-	34	-
	Doel 10	D10	October 1998	71	-	53
<b>Coast</b>	Westerschelde	Ws	August 2001	-	-	52
	Kwintebank 2	Ki2	February 1997	68	-	-
	Stroombank	Sb	February 1997	83	-	-
	Kwintebank 10	Ki10	October 1997	161	42	58
	Raan 3 (780)	Ra3	March 1997	50	-	-
	Zeebrugge (702)	Ze	October 1997	-	78	-
	Ostend (790)	Os	October 1997	52	50	-
	Weststroom-bank (120)	Wsb	October 1998	35	-	-
	Kaloo (GC1)	Gc-1	February 1999	-	23	-
	Botkil (250)	250	February 1999	-	13	-
Kwintebank	Ki8	August 2000	-	-	53	
Oostduinkerke	Od1	August 2000	-	-	54	
Zuid-Steenbank (350)	Zst	August 2000	-	-	53	

<b>Off-shore</b>	Raan 8 (BO3)	Ra8	August 2000	-	-	53
	Oostduinkerke	Od2	August 2001	-	-	52
	Oosthinder	Oh	October 1997	51	31	36
	Bligh Bank	Bli	February 1998	28	61	-
	Noordhinder	Nh	October 1998	-	46	-

### *P. minutus*

*P. minutus* showed no interpopulation differences in mean heterozygosity, number of alleles per locus or levels of polymorphism with allozyme electrophoresis. Observed and expected levels of heterozygosity were about twice as high in *P. minutus* as in *P. lozanoi*, with the latter species also showing a lower percentage of polymorphic loci and fewer alleles per locus.

Microsatellite analysis yielded a comparable level of heterozygosity across all populations. Variability was high with mean number of alleles ranging from 12 to 24 but no geographical trend was observed.

Results of allozymes showed that all polymorphic loci were in HWE with the exception of *LDH-C* showing a strong heterozygote deficit across all populations examined. Exact tests of pairwise population differentiation indicated significant differences ( $P < 0.05$ ) between most populations, but when locus *LDH-C* was excluded no significant differentiation was observed anymore ( $P > 0.05$ ). Hierarchical analysis of molecular variance (AMOVA) revealed that within-population effects explained all of the observed variation.

Table VI: Number of alleles per population for each microsatellite locus

	Doel- 97	Ki-97	Od1	Zst	Raan 8	Oh	Ki10	Od2	Ws	Total num- ber
SG5	45	56	44	33	39	33	37	33	38	85
SG7	7	6	5	6	6	3	6	5	5	9
SG10	27	28	24	14	26	19	32	26	28	41
SG11	6	7	6	6	7	5	5	7	5	8



Comparing allele frequencies of *P. minutus* between samples taken at approximately the same site but in different seasons showed no differentiation either. Microsatellite analysis revealed a very strong heterozygote deficit in all populations, depending on the locus under study. Nei's (1978) and Cavalli-Sforza's (1967) genetic distances revealed significant pairwise population differentiation. However, AMOVA results showed that virtually all of the variation present was due to within-population effects.

### *P. lozanoi*

No departures from Hardy-Weinberg equilibrium or linkage disequilibrium were observed. Both summer (Oosthinder, Ostend, Noordhinder and Zeebrugge) and winter (Bligh Bank, Doel and Kaloo) samples showed significant interpopulation differentiation ( $F_{ST} = 0.017$  and  $F_{ST} = 0.061$  respectively), which could be allocated entirely to differences in the allele frequencies at locus *GPI-1*. Locus *GPI-1* has two alleles in both species,  $^{100}GPI-1$  and  $^{113}GPI-1$ . Whereas it is almost monomorphic in *P. minutus*, it is the most polymorphic locus in *P. lozanoi*.

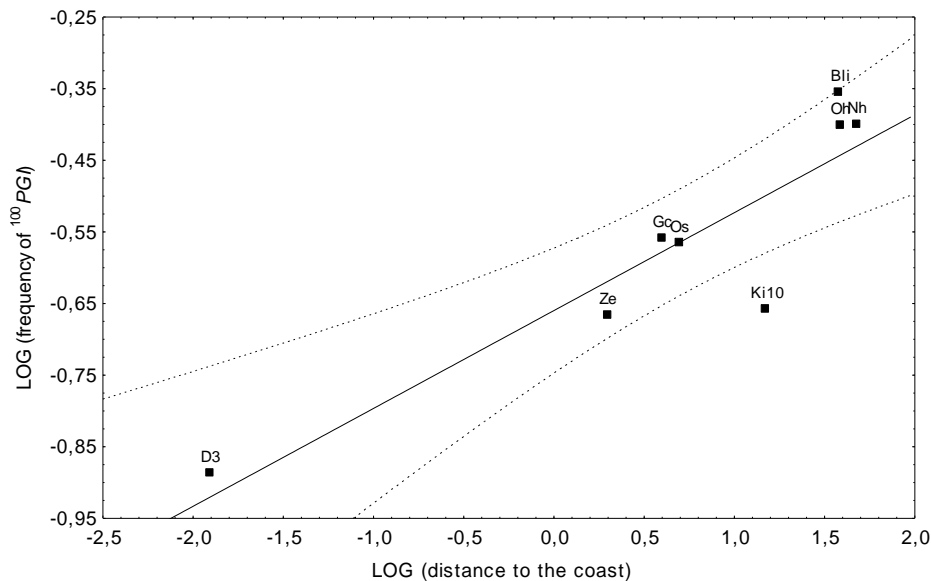


Figure 39: Linear regression with 95% confidence interval of the log-transformed distance to the coast and the frequency of  $^{100}GPI-1$  for *P. lozanoi* for the respective sampling stations.  $r^2_{adj.} = 0.79$ ,  $P < 0.00$ . As Doel is situated east of the coastline, its distance to the coast was given a negative sign relative to the reference point at Vlissingen. For abbreviations see Table I.

A linear regression (Fig. 39) of the frequency of  $^{100}GPI-1$  against the distance from the coast was highly significant ( $r^2_{adj.} = 0.79$ ,  $P < 0.01$ ).

A Mantel test showed a significant correlation between geographic and genetic distance ( $r = 0.501$ ,  $p = 0.019$ ). Populations pooled showed a clear differentiation between estuarine, coastal and offshore populations with a multilocus  $F_{ST}$  value of 0.027. Pairwise genetic distances (Nei, 1978) also revealed a similar differentiation. AMOVA results also showed that a small (5 %) but significant ( $P = 0.005$ ) percentage of the observed variation was due to the difference between the estuarine (Doel), coastal (Kwintebank, Zeebrugge, Ostend and Kaloo) and offshore group (Bligh Bank, Noordhinder and Oosthinder).

The differences at the *GPI-1* locus for *P. lozanoi* were stable over the period during which the sampling has been carried out; although several stations were sampled in consecutive years and different seasons, allele frequencies at *GPI-1* in the coastal and off-shore groups of samples remained similar.

### Sequencing

The maximum parsimony tree is represented in Fig. 40. All different treebuilding methods resulted in the same topology with resembling bootstrap values. Although belonging to a different genus, *Gobiusculus flavescens* clusters clearly within the *Pomatoschistus* group. Its position however, remains unresolved. Based on the genetic distances *G. flavescens* appeared most closely related to *P. microps* and *P. marmoratus*. The distances between the members of the *P. minutus* complex (*P. minutus*, *P. lozanoi* and *P. norvegicus* ranged from) amounted to 1.5%, the distances between *P. microps* and *P. marmoratus* amounted to 3%. The differentiation between two complexes ranged from 5-6%. Both *P. pictus* and *G. aculeatus* differed from the other species in 12-13%.

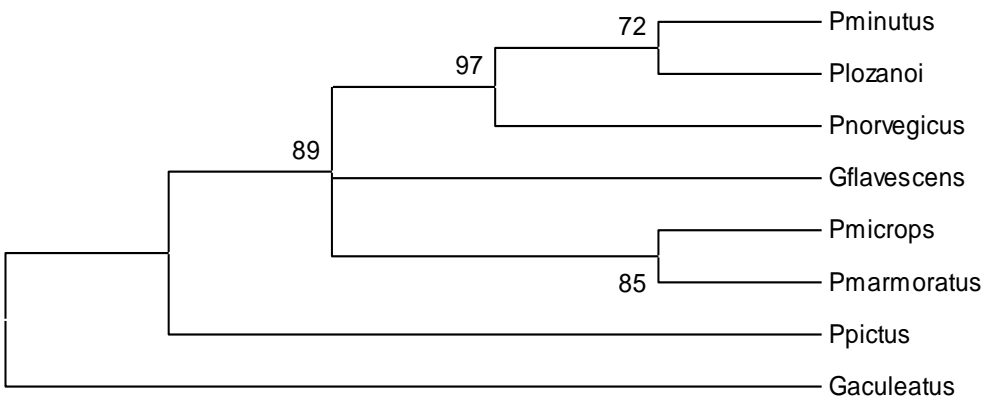


Figure 40: Maximum parsimony tree based on the 12S sequences of six *Pomatoschistus* species and *Gobiusculus flavescens*. The threespined stickleback *G. aculeatus* is used as outgroup.

#### V.a.4. Discussion

##### *P. minutus*

At first view results from allozyme electrophoresis and microsatellite analysis seem conflicting as no genetic structure is observed with the former, while microsatellites do point to a significant degree of populations. However, a closer look to the microsatellite data reveals that almost all of the observed variation is due to within-population effects as the number of alleles found is very high and only few alleles are shared between populations. Hence, based on the available information from these two kinds of genetic markers, we cannot draw any conclusions regarding genetic structure of the sand goby on the BCS. This lack of structure might be due to either the high variability of the microsatellite markers, the lower resolution of the allozymes, or the high potential for dispersal of the sand goby.

Moreover, other factors such as mating strategy of the fish and the sampling strategy might further complicate the study of the sand goby 's population structure.

##### *P. lozanoi*

A significant difference exists between the marine, inshore and offshore samples of *P. lozanoi*. As the only differentiation was found at locus *GPI-1*, this was probably not due to random genetic drift. A possible explanation might be the effect of differential selection pressure exerted on *P. lozanoi* populations living in the inshore and offshore habitats. This might be due to differences in ecosystem dynamics or in environmental variables (Mork & Sundnes, 1985; Turan *et al.*, 1998; Lemaire *et al.*, 2000). The *GPI* locus is known to be under selective pressure (Watt, 1983; Al-Hassan *et al.*, 1987; Katz & Harrison, 1997). There might be a clinal change in allele frequencies with increasing distance from the Scheldt estuary due to selective pressure of any environmental factor changing gradually with distance from the estuary (such as salinity, turbidity, and nutrient concentrations). However, a few well-known exceptions notwithstanding (e.g. Watt, 1983; Powers *et al.*, 1991), it remains very difficult to prove any clear-cut association between allele frequencies and environmental variables.

### *Molecular phylogeny based on 12S mtDNA*

The only known studies about the phylogeny of *Pomatoschistus* gobies are based on morphology and allozymes (Wallis and Beardmore, 1984b; McKay and Miller, 1997). However, both topologies are conflicting with each other. Penzo *et al.* (1998) state that the morphological characters used in systematic analyses of Gobiidae are likely to be insufficient to correctly infer phylogenetic relationships. Direct sequencing remains the most reliable method. Our phylogeny generated from 12S sequencing from 6 *Pomatoschistus* species is more or less in agreement with the phylogeny from Wallis and Beardmore (1984b). An important difference however, is the position of *P. pictus* which is highly differentiated from the other goby species. Another apparent result is the clustering of *G. flavescens* within the *Pomatoschistus* complex. In the study by McKay and Miller (1997) this species adopts an uncertain position. According to the treebuilding method this species clusters outside the *Pomatoschistus* group or together with *P. microps* and *P. canestrinii*.

#### **V.a.5. Conclusions**

Any genetic structure on the Belgian Continental Shelf is influenced by strong tidal currents and a northward bound residual current. We may assume that a strong mixing of pelagic larvae occurs in the area. Our allozyme and microsatellite data on *P. minutus* point to a pattern of genetic homogeneity due to a substantial larval dispersal at least on a scale of tens of kilometres, with a possible effect of selective pressure at locus *GPI-1* in lozano's goby.

We are currently sequencing a 600bp region of the 16S mtDNA and more goby species will be included aiming to resolve the present uncertainties in the gobiid phylogeny.

## V.b. Genetic structure and gene flow of the Dover Sole (*Solea solea*) on the Belgian and European Continental Shelf

### V.b.1. Introduction

Marine organisms show a high potential for species-specific dispersal because of historical, physical and biological factors. Based on fishing tagging, adult flatfish have been reported to migrate over distances of 100 km and more. Eggs and larvae might become displaced over distances of 10 to 100 km by physical advection.

Kotoulas *et al.* (1995) and Exadactylos *et al.* (1998) have shown that there is a gradient in the genetic structure of *Solea* populations (also called genetic isolation by distance). This means that genetic exchange between populations is high, a typical feature of marine organisms. Interesting is also that the core of each spawning unit along the Atlantic shelf is separated by a distance of about 100 km, which is exactly the distance where genetic structure is changing.

### V.b.2. Material and Methods

#### 2.1 Sampling

The aim was to collect samples that were equally distributed along the whole geographic area. For the moment there are 6 samples from the Mediterranean and 14 samples from the Atlantic Ocean. On each sampling site we collected at least 20 adult animals usually during the spawning season. There are still three additional samples expected: Cadiz, Vigo and Stavanger.

Table VII: Sampling sites from *Solea solea* (date and collector included).

No	Origin	Basin	Date	Collector
1.	Palavas (F)	Gulf of Lions	03.02.99	F.Volckaert
2.	Brown Ridges	North Sea	03.03.99	L. Bolle (RIVO) & Ewan Hunter (CEFAS)
3.	Oostelijk Kanaal	English Channel	03.99	D. Miossec
4.	Venetië (I)	Adriatic Sea	23.03.99	F. Argenton
5.	Murcia (ES)	Mediterranean	31.03.99	A. Alcazar

6.	North Sea	North Sea	02.04.99	L. Bolle
7	Grote Rede	North Sea	23.03.99	P. Bossier – D. Delbarre
8	Wenduine B	North Sea	30.03.99	P. Bossier – D. Delbarre
9	Grote Rede	North Sea	06.04.99	P. Bossier – D. Delbarre
10	Thessaloniki	Egeic Sea ??	04.99	P.S. Economidis
11	Oostelijk Kanaal (Vlild)	English Channel	11.04.99	R. Millner – J. Dann
12	Kenitra (MO)	Atlantic Ocean	02.05.99	F. Volckaert
13	Noirmoutiers	Gulf of Biskaje	20.05.99	Léauté
14	Baie de Gironde	Gulf of Biskaje	26.05.99	Léauté
15	Sète	Gulf of Lions	16.06.99	F. Volckaert
16	Baie de Seine (F)	English Channel	2-5.07.99	D. Miossec
17	Barcelona	Mediterranean	18.02.99	I. Palomera
18	Castellon	Mediterranean	18.01.00	S. Zanuy
19	Oostduinkerke	North Sea	04.00	F. Volckaert
20	Lissabon	Atlantic Ocean	07.00	P. Bossier
21	Cadiz	Atlantic Ocean	Expected	G. Martinez
22	Vigo	Atlantic Ocean	Expected	
23	Stavanger	North Sea	Expected	

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## 2.2 DNA analysis

*DNA extraction:* DNA is prepared with the 'Dneasy Tissue Kit' (Qiagen, Westburg). The final elution volume is 200 µl AE buffer of which 1 µl is used for the PCR reaction. Six different sampling sites were extracted. These sites were distributed along the whole sampling area: Venice, Murcia, Kenitra, Baie de Gironde, Noirmoutiers and Baie de Seine. From each sample 10 individuals were purified except Kenitra (only two available).

*EPIC-PCR reaction:* 4 different nuclear primer combinations were tested: Calmodulin (Chow, 1998), ITS (Sajdak and Philips, 1997), Growth Hormone (Venkatesh *et al.*, 1999) and Aldolase (Lemaire, pers. comm.). The reaction volume is 25 µl: 1 µl of the DNA solution, 2.5 µl of 10XPCR buffer, 2.5 µl of 2mM dNTP's, 1 µl of each primer (20 µM), 1 µl of 50 mM MgCl<sub>2</sub>, 0.1 µl of the Taq polymerase (5 U/µl) (Silverstar, Eurogentec) and 16

µl of double distilled water. The conditions of the 4 reactions are almost the same: 3 min denaturation at 97°C, followed by 35 cycles of 30 s denaturation at 95°C, 30 s at annealing temperature (55°C for Calmodulin , ITS and for Growth Hormone and 54°C for Aldolase) and 45 s at 72°C. The reaction is finished with an extra elongation at 72°C for 7 min. The reaction products are separated on a 1.5% agarose gel in 0.5xTBE buffer. The length of the different fragments: 426 bp for Calmodulin, 900 bp for ITS and 370 bp for aldolase. There was no amplification of the GH locus. Finally the fragment was purified with the 'GFX PCR DNA and Gel Band Purification' kit from Amersham Pharmacia Biotech.

*DNA microsatellites:* Beside these intron markers we also tested 3 microsatellites (Iyengar *et al.*, 1999). The reaction volume is 10 µl: 1 µl of the DNA solution, 1 µl of 10XPCR buffer, 1 µl of 2mM dNTP's, 1 µl of each primer (2 µM), 0.4 µl (for F8-I) or 0.3 µl (for F13 and F8-II) of 50mM MgCl<sub>2</sub>, 0.1 µl of the Silverstar Taq polymerase (5 U/µl) (Eurogentec) and 5.6 µl or 5.7 µl of double distilled water. The annealing temperatures are 61°C for F13 and 56°C for F8-I and F8-II. The reaction products were separated on an automatic sequencer (LICOR) and analysed with the Gene ImagIR (Scanalytics).

*Sequencing:* Sequencing reactions were carried out in 6 µl reactions following the supplier's instructions (Sequi Therm Excell II kit, Epicentres Technologies). The reaction products were separated on an automatic sequencer (LICOR).

*SSCP (Single Strand Conformational Polymorphism):* This technique represents a reliable and fast way to analyze large numbers of samples. The purpose is to sequence only those fragments that give a different pattern based on SSCP. Three and a half µl of the PCR reaction is added to 3.5 µl of formamide stop solution, followed by a 5 min denaturation at 95°C. These samples were loaded on a ready to use gel ('Excel gel DNA analysis' kit, Amersham Pharmacia Biotech) and run on a horizontal electrophoresis system at 7°C (Multiphor II system, Amersham Pharmacia Biotech). The fragments are visualized by silver staining (DNA silver staining kit, Amersham Pharmacia Biotech).

### **V.b.3. Results**

#### *V.b.3.1 Sequencing*

The calmodulin fragment has been sequenced completely (426 bp) and contains 6 polymorphic sites (1.4%). The ITS fragment has been sequenced from the MD1 primer site and has a length of 445 bp. There are 2 polymorphic sites (0.7% nucleotide diversity). In both cases the variability is really low; as a consequence these intron markers cannot be used as a nuclear marker for population genetic analysis. A third

genetic marker, growth hormone (GH) didn't amplify in *Solea solea*. The aldolase primers gave a fragment of 270 bp. We developed a new primer to amplify a short fragment of 170 bp, which is suitable for analysis by SSCP. This fragment has 13 polymorphic sites (7.6%). Because of the diploid status, it is necessary to clone the fragments before sequencing. In some cases there are more than two alleles per individual, which might be attributed to mismatching during polymerization.

### V.b.3.2 Genotyping

Three primers were used for microsatellite amplification in the Dover sole.

Table VIII. Characteristics of 3 microsatellites in 6 populations of the Dover sole. NA= number of alleles, H<sub>E</sub>= expected heterozygosity, H<sub>O</sub>= observed heterozygosity, size relates to the size rang of the alleles.

LOCUS	F13				F8-I				F8-II			
	N	H <sub>E</sub>	H <sub>O</sub>	Size	N	H <sub>E</sub>	H <sub>O</sub>	Size	N	H <sub>E</sub>	H <sub>O</sub>	Size
Sampling sites	A				A				A			
Venice (Italy)	3	0.3	0.4	169- 64	3	0.4	0.6	92- 60	4	0.5	0.5	138- 90
Murcia (Spain)	3	0.5	0.6	165- 00	5	0.7	0.4	94- 45	6	0.6	0.5	138- 94
Noirmoutiers (France)	5	0.5	0.5	173- 85	2	0.3	0.2	100- 75	6	0.7	0.9	132- 50
Baie de Gironde (France)	5	0.6	0.3	169- 00	5	0.4	0.4	90- 80	5	0.6	0.9	138- 20
Baie de Seine (France)	6	0.6	0.5	169- 00	3	0.2	0.3	92- 65	6	0.7	0.7	138- 25
Kenitra (Morocco)	2	0.3	0.5	175- 75	2	0.3	0.5	92- 75	2	0.7	0.5	132- 50
Total	9			165- 205	8			90- 110	9			132- 158

They were applied on 6 samples of 52 individuals from the Mediterranean, the Atlantic shelf and the North Sea.

We constructed a neighbour joining tree using the Genetix software (Belkhir *et al.*, 1999) to calculate the Nei genetic distances between the populations (figure 1). At one side the French Atlantic samples and at the other side the Mediterranean samples group



together, although the exact relationship between the samples remains to be confirmed due to the very small sample sizes.

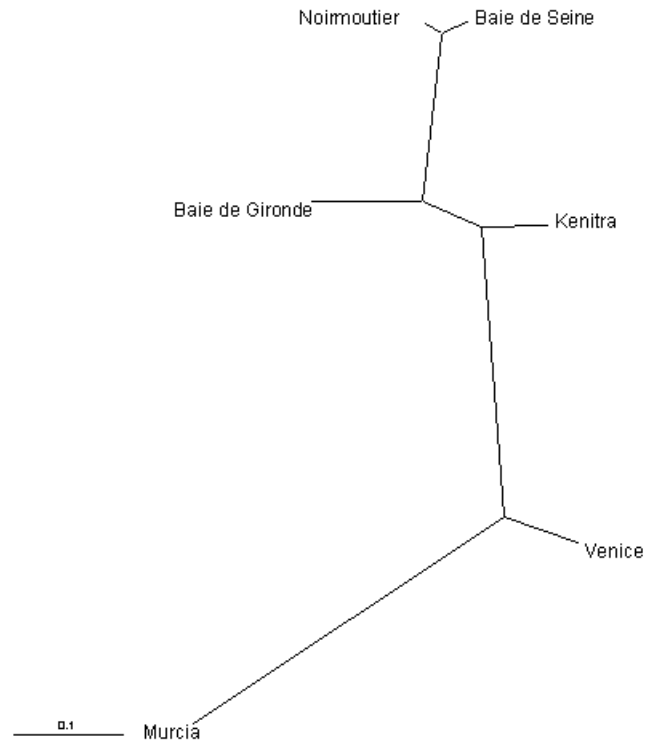


Figure 41. Neighbour joining tree based on Nei genetic distances calculated from the genotypes at 3 microsatellite loci from six populations of the Dover Sole.

### V.b.5 Discussion

In an attempt to detail the genetic diversity and structure of the Dover sole (*Solea solea*) on the Atlantic and Mediterranean continental shelf with markers complementary to the allozyme markers reported so far (Kotoulas *et al.*, 1995; Exadactylos *et al.*, 1998), we screened a limited number of populations across the full natural range. Only the mitochondrial marker cytochrome b (Bossier, pers. comm.), the nuclear intron (EPIC) marker aldolase and several nuclear microsatellite markers seem to provide useful information of high quality. The mitochondrial control region (Bossier, pers. comm.), and the nuclear intron markers growth hormone, ITS and calmodulin do not satisfy our requirements for various reasons.

At the moment screening of a large number of populations is in progress with microsatellite markers (this study) and the *cyt b* marker (Bossier, pers. comm.). The first results point to a contamination of two samples with *Solea senegalensis* (a related but morphologically distinct species sharing the southern range of Dover sole but living inshore) (see also Sotelo *et al.*, 2001). We also confirmed the previously found distinction between Mediterranean, southern Atlantic and northeastern Atlantic populations. These results are not unexpected in view of the isolation by distance pattern detected by Kotoulas *et al.* (1995), Exadactylos *et al.* (1998) and Exadactylos & Thorpe (2001). However, we expect to find a higher resolution than reported in these papers to discriminate among the populations (also called stocks by fisheries managers) and we should also obtain an idea of the phylogeography (this is historic patterns of migration and isolation) of Dover sole.

Our work should lead to the fine-tuning of the stock management of Dover sole, a task well needed as many commercial stocks are under growing fishing pressure. It should also facilitate ongoing and planned studies on the small-scale genetic structure and gene flow of Dover sole in the North Sea, the English Channel and the Bay of Biscay (EU proposal SOLid and the ongoing IFREMER programme DéfiGasco).

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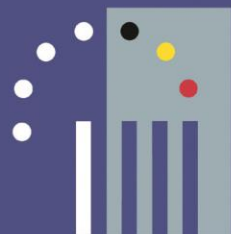
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