SCIENTIFIC SUPPORT PLAN FOR A SUSTAINABLE DEVELOPMENT POLICY

SPSD II

HIGHER TROPHIC LEVELS IN THE SOUTHERN NORTH SEA (TROPHOS)

M.VINCX, E. KUIJKEN, F. VOLCKAERT

PART 2

GLOBAL CHANGE, ECOSYSTEMS AND BIODIVERSITY

ATMOSPHERE AND CLIMATE

MARINE ECOSYSTEMS AND BIODIVERSITY

TERRESTRIAL ECOSYSTEMS AND BIODIVERSITY

NORTH SEA

ANTARCTICA

BIODIVERSITY

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(SPSD II)

Part 2:
Global change, Ecosystems and Biodiversity

FINAL REPORT

HIGHER TROPHIC LEVELS IN THE SOUTHERN NORTH SEA
"TROPHOS"

EV/25

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ABSTRACT

This study investigated the marine ecosystem on the Belgian Continental Shelf (BCS) at three levels. At a first level, processes occurring at the sediment-water interface where investigated with respect to the remineralisation of pelagically produced organic matter and its structuring role for bacterial and benthic communities. In addition, food-web studies using stable isotope techniques revealed information on the use of various food sources for the benthos. A second part concentrated on the dispersal of selected invertebrates, fish species and their parasites using molecular approaches. The third part concentrates on the link between breeding success of seabirds and the availability of their prey items (pelagic fishes) in the water column.

Benthic pelagic coupling

Processes related to benthic-pelagic coupling were investigated at two contrasting sites which are representative of the majority of sediments encountered at the BCS. Station 115bis is located close to the coast in the Westdiep area and has fine sanded sediments. Sedimentation of phytodetritus here results in oxygen stress at deeper sediment layers, and the establishment of strong vertical gradients in pigment, oxygen and nutrient concentrations (used here as a proxy for labile organic matter) and can be considered a "depositional" station. Station 330 is located more offshore in the vicinity of the Gootebank and has coarser, permeable sands. In these sediments, pore water is flushed each incoming and outgoing tide by advective currents. This results in year-round well oxygenated sediments without the strong profiles occurring in finer sediments.

We show that both sediments have a different ecology. In the fine sanded sediments, mineralization processes are delayed with respect to the peak sedimentation event following the spring phytoplankton bloom. In these sediments, water temperature affects the magnitude of the processes. On the contrary, a fast response to phytoplankton sedimentation was observed in the permeable sediments, as a consequence of the prevailing lateral advective currents, providing the sediment column with oxygen and removing toxic byproducts of the mineralization processes.

Mineralisation rates were largest in the finer sediment. However, as a consequence of methodological constrains, oxygen consumption rates measured at Station 330 should be considered absolute minimum estimates, *in situ* fluxes probably being higher.

Experimental work showed that short-term mineralization rates in contrasting sediments were comparable although densities and diversity of meio- and macrobenthos at both stations were very different. This suggests that densities and diversity should not be used as the sole means to judge the ecological value of

selected areas on the BCS. In the coastal zone, the macrobenthos is an important structuring component since the remineralisation of the phytodetritus is linked to bioturbation and bio-irrigation by the macrobenthos. However, we have no idea yet on the relative importance of macrobenthic species, functional groups and their interactions for the magnitude and intensity of the processes.

Dispersal of marine organisms

Genetic research revealed that the North Sea, despite the strong hydrodynamics and the seemingly continuity of the water masses represents a discontinuous habitat for pelagic organisms and their populations. Organisms with an inshore life style (eg. *Pomatoschistus* gobies) show fragmented populations while more offshore life styles seem to promote connectivity between populations. Population genetic diversity of all species investigated is high. As the North Sea is among the most productive shelves on the planet, our findings have important consequences for fisheries management, habitat management and implementations for Marine Protected Areas. The BCS has to be managed as a landscape in order to maintain its populations sustainability.

The differential survival of cohorts not only affects cohort strength, but also the genetic make-up. Since adult older individuals of commercial fish species have become scarce or absent, genetic diversity of these populations is eroding. This has negative effects on the adaptive potential and sustainability of these species and their populations. Sustainable fisheries management therefore should aim at maintaining a well-balanced population structure of young and old fishes of both gender.

Our first attempts to model the dispersal of living particles (e.g. fish larvae) on the BCS stimulated a more comprehensive approach to connectivity in the oceans. It should allow for the realistic quantification of dispersal in time and space. It is a promising tool towards the sustainable management of our fish populations.

Seabirds as top predators

We focussed research on the Common and Sandwich Tern, two species breeding in Zeebrugge harbour. The breeding colony is of high international importance and has been designated a Special Protected Area under the Bird Directive. Protecting both tern species will require local management (Common Tern) and concerted international efforts (Sandwich Tern). The highly productive Common Tern colony is largely self supporting but breeding output will probably decrease in the future as the carrying capacity of the area has been reached. Sandwich Terns recently experienced several years of poor breeding success in combination with exchange of individuals over a much larger distance than Common Tern.

The fledging success and relative population growth of Common Tern seems to be a good monitoring tool for the condition of small pelagic fish in the vicinity of

Zeebrugge. Detailed investigations on the reproductive success of Sandwich Tern showed that the survival of chicks depends on the availability of suitable prey. When a specific length-class in their prey (young clupeids) is missing, continuous chick growth is disturbed. Parent Sandwich Terns cannot correct for this by increasing their foraging effort towards smaller prey items. In such years, chicks grow initially at normal years but starve to death at a certain age when the required food items are not available.

Overall, the significance of all these findings for the Belgian Continental Shelf relate to a better understanding of its functioning and its significance for the North Sea ecosystem.

1. INTRODUCTION

TROPHOS builds on the results obtained within the SPSD I project "Structural and functional biodiversity and North Sea ecosystems: species and their habitats as indicators for a sustainable development of the Belgian Continental Shelf". Within this project, the spatial and temporal patterns of the benthos (with emphasis on meiobenthos and macrobenthos) and seabirds were investigated in detail, in combination with the genetic characteristics of selected invertebrates, fishes and their parasites.

The benthic studies revealed that different sediments on the Belgian Continental Shelf (BCS) showed strongly differential responses to the sedimentation of the spring phytoplankton bloom. In fine-grained sediments, this event triggered oxygen stress in the sediment coinciding with the build-up of ammonium in the deeper sediment layers. Nematode response in terms of densities was delayed and species-specific with respect to timing and sediment horizon. No changes in diversity were observed at this station (Steyaert et al. subm.) In coarser grained sediments in the vicinity of the Gootebank, nematode response was fast both in terms of diversity and density (Vanaverbeke et al 2004b) and was most noticeable in short (<700 µm) and stout (Length/Width ratio <15) nematodes (Vanaverbeke et al. 2004a). In these sediments, oxygen was present and there was no build-up of reduced substances in the sediment. These results led us to the hypothesis of strongly differing benthic ecosystems within a limited geographical distance. We considered fine-sanded sediments as depositional areas, where sedimentation of phytodetritus would lead to steep gradients in labile organic matter in the sediment and where diffusion processes could be responsible for the distribution of this organic matter in the sediment. On the other hand, the coarser sediments were considered as permeable sediments. Here, pore water would be flushed during every ebb and flood tide as a consequence of lateral advective currents through the sediment, resulting in the removal of toxic substances and the continuous presence of oxygen even at deeper sediment horizons. In order to test these hypotheses and quantify these processes, a monthly sampling was set up, during which Sediment Oxygen Consumption, macrobenthic- and meiobenthic densities, changes in bacterial communities and stable isotope signals in both pelagic and benthic organic matter and metazoan benthic fauna was assessed. This allowed explaining the patterns observed by Steyaert et al (subm.) and Vanaverbeke et al (2004 a,b) and elucidating if the structure of the benthic food web is indeed different in both sediment types.

In addition, lab incubations where both diatoms and *Phaeocystis* were labelled with the stable isotope ^{13}C revealed that both items were incorporated in the benthic food web..

Molecular work within SPSD I revealed evidence for the presence of genetically structured populations of marine fishes (e.g. *Pomatoschistus* gobies and sole) on the BCS. On a short time scale, this genetic structure of marine organisms is determined by gene flow and selection. Gene flow is the process where genetic information is exchanged between populations by various processes while selection is the process where individuals in a population adapt and survive on the basis of their fitness. As marine populations often have huge sizes, genetic drift should be of minor importance. Most populations of marine organisms often lack a clear delimitation and oceans show a high degree of connectivity. Therefore, the genetic diversity is generally higher while their genetic structure is weaker than their terrestrial conspecifics. Nevertheless, marine organisms do show genetic structure, sometimes more then expected. Within TROPHOS, we focussed on the genetic diversity, structure, gene flow and life history of selected key species (the invertebrates *Mesopodopsis slabberi* and *Gyrodactylus*) and the vertebrates (*Pomatoschistus* gobies and common sole) of the BCS. There was evidence for local genetic structure among all these taxa, which provides insight into marine comparative metapopulation dynamics and the responses of species to the environment on medium scales. We developed a particle tracking model for the BCS in order to model passive drift of planktonic stages (eggs and larvae) in the water column and their dispersal. Hydrodynamical and particle dispersal models provide unique opportunities to gain insight in the transport mechanism of these pelagic life stages from the spawning to the nursery grounds and increase our knowledge concerning connectivity on the BCS.

The establishment of a highly important breeding colony of terns in the outer harbour of Zeebrugge at the end of the 20st century, created an opportunity to fill in a gap in the ecological knowledge on the functioning of the pelagic ecosystem in Belgian coastal waters. At the start of TROPHOS, the pelagic ecosystem was poorly studied and hence our knowledge on that ecological niche was comparably poor. The reproductive performance of the terns could possibly act as a mirror reflecting changes in the pelagic ecosystem. Terns are small seabirds with short foraging ranges who feed predominantly on small fish species. Their expensive feeding methods make them highly sensitive species that react instantaneously to changes in feeding conditions and feeding resources (Stienen 2006). The feeding habits of terns are relatively easy to study because terns breed together in dense colonies and parents carry the food crosswise in their bills to feed the chicks in the colony, clearly visible for researchers. All this makes them very good and useful monitoring species for the health of the upper part of the coastal ecosystem (Stienen 2006). During TROPHOS, we tried to acquire the necessary knowledge to use and measure the indicative value of terns as ecosystem indicators. A first step was to gain more insight in the diet preferences of the birds trough a detailed study on their feeding biology. A second step was then to measure the range of breeding parameters that had a high potential to act as monitoring tool for the health of the local pelagic ecosystem.

Since many of the research questions investigated during TROPHOS were generated as a result of an SPSD I project, we depended greatly on the availability of the SPSD-I data. However, the majority of the scientific personnel involved in SPSD I was not employed on a permanent basis and moved towards other positions in Belgium or Europe. Therefore, the scientific teams within the TROPHOS network were supported by the data management team of the Flemish Marine Data Centre (VMDC) of VLIZ. In a first step, all data collected during SPSD I were collected and integrated in single project database. During the course of TROPHOS, all new data was added to the project database, resulting in complete and consistent database that guaranteed the use of quality checked data by all TROPHOS researchers. Moreover, this database was used as a basis for a smooth transfer of all collected data to the IDOD database of the Belgian Marine Data Centre.

2. BENTHIC PROCESSES AT IN CONTRASTING SEDIMENTS ON BELGIAN CONTINENTAL SHELF

INTRODUCTION

Results obtained during the first SPSD programme "Structural and Functional Biodiversity of North Sea Ecosystems: species and their habitats as indicators for a sustainable development of the Belgian Continental Shelf" suggested strongly differing benthic ecosystems on the Belgian Continental Shelf (BCS). The response of benthic organisms to phytoplankton sedimentation was investigated at two stations (Fig. 1), representative for the majority of sediment types prevailing at the BCS.

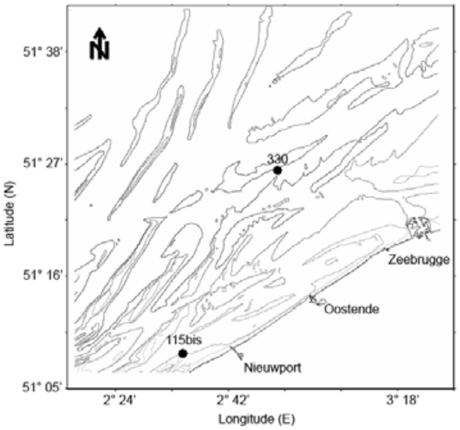

Figure 1 Location of sampling stations on the Belgian Continental Shelf

Sediments at Station 115bis (51°09.2N; 02°37.2 E, 13 m depth) consist of fine sandy sediments (according to the classification scale of Buchanan (1984) with a median grain size of 185 µm) containing a small fraction of mud (4% < 63µm) (Steyaert et al. subm.). At Station 330, sediments are classified as medium sands (median grain size between 321 and 361 µm in spring 1999) (Vanaverbeke et al. 2004a) devoid of mud. This difference in sedimentological characteristics has important consequences for the biogeochemistry and hence for the organisms living in close contact with their environment, e.g. the free-living nematodes. The differences in both biogeochemical profiles and nematode response to phytodetritus sedimentation let us to assume that at the BCS very different ways of ecosystem functioning prevailed at only a relatively small geographical distance. Quantifying and modeling these ecosystem processes (including food-web reconstruction) was therefore the next step to increase the understanding of the structuring factors for benthic biodiversity. In addition, information about the benthic fate of the harmful algae *Phaeocystis* after sedimentation to the sea floor is needed to support a sustainable management of the marine ecosystem at the Belgian Continental Shelf.

MATERIAL AND METHODS

Sampling and measurements

On a monthly basis, sediment samples were taken with a Reineck box corer for measurement of sediment community oxygen consumption (SOC) rates, for chlorophyll and degradation products, organic carbon and nitrogen, carbon and nitrogen isotope concentrations of particulate organic matter (sediment and water) and the main benthic fauna, macro- and meiofauna density and bacterial biomass, community composition and diversity. Sampling started in October 2002 and lasted until October 2003. During three intensive campaigns (February, April, October), additional samples were collected for nutrient fluxes and vertical profiles.

Cores, used for flux measurements were immediately put in a temperature-controlled water bath. Bottom (1 m above seafloor) and surface water (3 m depth) was sampled with a Niskin bottle mounted on the CTD wire approximately 1 m above bottom. SOC measurements were performed in two 70.88cm^2 cylindrical cores with a magnetic type of horizontal stirring to constantly homogenise the water in the cores. The decline in oxygen concentration was monitored with electrodes, measuring at minute intervals and oxygen consumption rates calculated from the regression of oxygen concentration versus time.

Cores for meiobenthic and pigment analysis (10 cm^2) were sliced in 1 cm slices dow to a depth of 10 cm. Sediment slices for pigment analysis were preserved at -20°C on board and stored at -80°C at the laboratory. Meiofauna was extracted from the sediment using the Ludox centrifugation method (Heip et al. 1985). 120 nematodes were picked randomly from every sample and mounted on slides. Nematode length and maximum width were measured using a Quantimet 500+ image analyser. Biomass was calculated according to Andrassy (1956) and converted to µg Carbon.

Chlorophyll *a* (Chl *a*) and its degradation products Phaeophytin and Phaeophorbide from the water column and the sediment were analysed by HPLC (Gilson) following Wright and Jeffrey (1997). The ratio phaeopigments to the sum of phaeopigments and Chl *a* (PAP ratios) was calculated as an indication of the freshness of the organic material (Boon et al. 1998).

Samples for the analysis of bacterial diversity were obtained by subsampling 1ml of sediment from the 0-1 and 4-5 sediment layers. DNA was extracted following Demba Diallo et al. (subm.) and amplified (see Franco et al. subm. for details). Denaturating Gradient Gel Electrophoresis was applied to reveal bacterial community structure and diversity (Muyzer et al. 1993).

Bacterial counts were obtained following Starink et al. (1994). All particles >0.2 µm on a slide were counted and allocated to size class using a Leica confocal microscope connected to QWIN software. Bacterial biomass (gC m^{-2}) was estimated by calculating bacterial volume per size class and using a conversion factor of 310 fg

During the intensive campaigns, benthic solute fluxes of ammonium and nitrate, and corresponding concentration-depth profiles were measured in the lab. Sediment cores of 78.54cm^2 inner diameter were incubated at *in situ* temperature in the dark and under constant aeration. Incubations lasted between 22-24 hours, during which 5 to 6 samples were drawn at regular intervals during the day, but the cores were left unsampled at night. The benthic flux was calculated from the linear regression of concentration change over time. Oxygen profiles were established by a microelectrode, measuring at 0.5 mm depth intervals. For station 115bis, the oxygen electrode was inserted till the depth at which O_2 was exhausted. This was not always possible for station 330, due to deeper O_2 penetration here and the danger of micro-electrode breakage caused by the presence of coarse fragments in the sediment.

At the start and end of the incubations, 2 or 3 sediment cores (78.54cm^2) were sliced for measurement of nutrient concentration profiles. Slicing and squeezing of the pore water out of the sediment occurred in a glove box under constant flushing with N_2, to avoid oxidation of ammonium to nitrate. The pore water was filtered through cellulose-acetate filters (0.4 µm) and collected in acid-rinsed polyethylene vials. Cores were sectioned in 1 cm slices down to 4 cm depth, followed by 2 cm slices down to 10 cm depth. Samples for nitrogen and carbon content in the solid phase were taken from separate cores and at 0-1 and 4-5 cm depth only. Determination of nutrients (ammonium, nitrate+nitrite) in bottom water, flux- and pore water samples was performed by using an auto-analyzer. NO_3 was determined after reduction to nitrite (NO_2).

Samples for pore water content were taken in February; they were sectioned in 1 cm slices till 4 cm, and 2 cm slices till 10 cm depth, and water content estimated from weight loss induced by oven drying.

Meiofauna and macrofauna were hand-picked and used for measurement of organic C, N, ^{13}C and ^{15}N. In most cases, several individuals were combined into one sample for stable-isotopic determinations. Freeze-dried sediment, animal or filter samples were analysed for organic carbon and nitrogen using a Carlo Erba elemental analyzer (Nieuwenhuize et al. 1994); the carbon and nitrogen isotopic composition of the samples was determined using a CN analyser coupled on line to a mass spectrometer. The carbon and nitrogen isotope ratios are expressed in the delta notation $\delta^{13}C$ and $\delta^{15}N$.

All multivariate analysis en calculation of diversity indices were done in PRIMER 5, the STATISTICA software package was used for parametric or non-parametric testing for significant differences between stations or sampling dates.

RESULTS

Pigment dynamics in the water column en sediment

Chlorophyll *a* concentrations in the water column at both stations started rising in February, peaked in April (48 µg.l^{-1} at station 115bis and 32µg.l^{-1} at station 330) and decreased afterwards (Fig. 2). Smaller peaks were also observed in July for both stations and in September only at station 330,

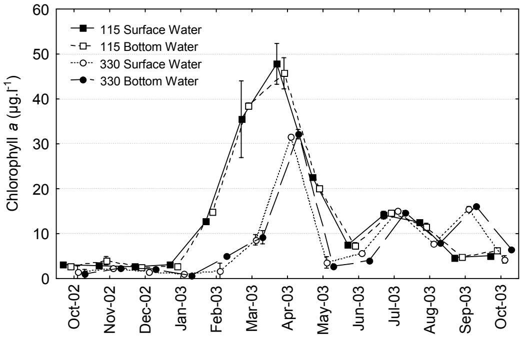

Figure 2: Chlorophyll a concentration in the surface and bottom water (µg.l-1) at stations 115bis and 330 on the period from October 2002 to October 2003. The vertical bars represent the standard error.

At the sediment surface Chl *a* concentrations were about ten times higher at station 115bis than at station 330 throughout the sampling period and followed the patterns observed in the water column (Fig. 3).

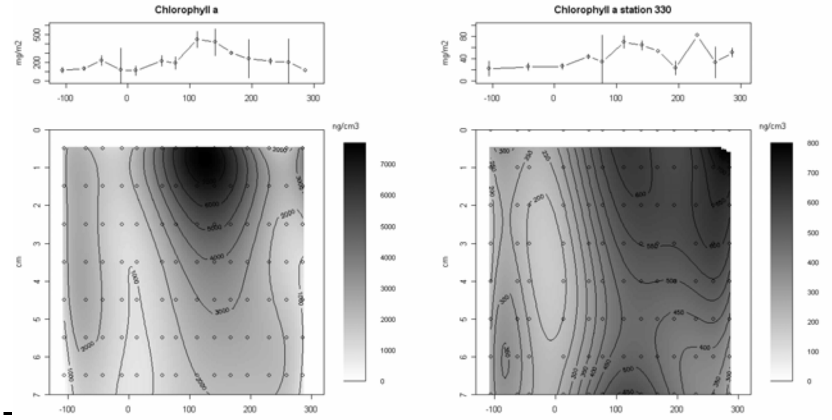

Figure 3: Vertical profiles of Chl *a* concentration in the sediments of Station 115bis and Station 330 (Lower Panels) and total Chl *a* concentrations in the sediment (Upper Panels) during the study period.

At both stations peak spring values were observed in April. At station 330 another peak was observed in August 2003 and at the sediment surface in October 2003 corresponding to the deposition of the late summer and autumn blooms (Fig. 3).

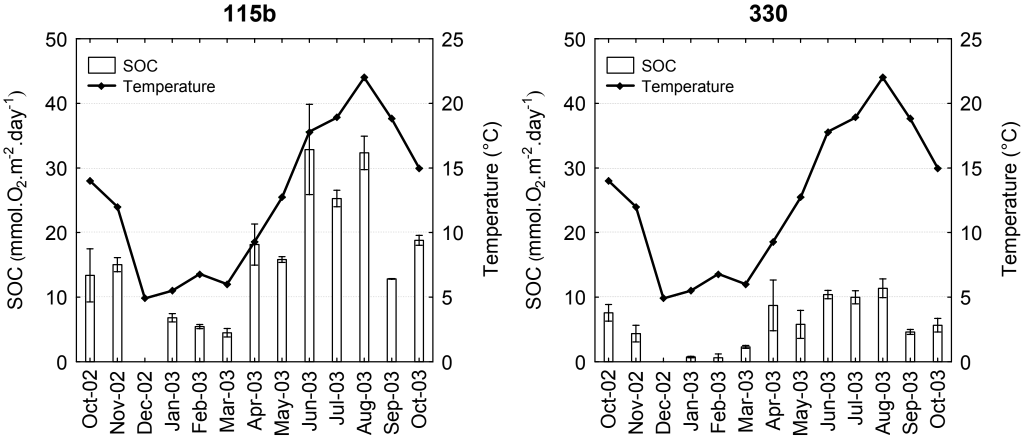

Figure 4. Sediment Oxygen Consumption and water temperature (°C) at stations 115bis and 330 on the period from October 2002 to October 2003. The vertical bars represent the standard error.

In general, SOC was higher at Station 115bis compared to Station 330. SOC increased after the arrival of fresh phytodetritus at the seafloor, but peak oxygen consumption values at Station 115bis are observed only in June (Fig. 4). The response in SOC to the spring sedimentation at Station 330 was immediate and values remained relatively constant until August. In both stations, SOC valued decreased after August.

Biogeochemical fluxes

At station 115bis, there was a significant in influx of nitrate in February (0.97 ± 0.09 mmol m^{-2} d^{-1}), and of ammonium in October (0.715 ± 0.14 mmol m^{-2} d^{-1}), whilst immediately after the bloom deposition in April, an efflux of ammonium (-1.14 ± 0.23 mmol m-2 d-1) was observed. For Station 330, we measured influxes of nitrate in February (0.34 ± 0.15 mmom m^{-2} d^{-1}) and October (0.38 ± 0.15 mmol m^{-2} d^{-1}) and influx of ammonium in October (0.26 ± 0.07 mmol m^{-2} d^{-1}). No significant sediment-water exchange was measured in April.

Biogeochemical modeling

As the algal material is deposited on the sediment, it triggers a response of the benthic organisms that feed on this source of organic matter. Through their activity, the organic matter is mixed in the sediment where it is decomposed (mineralized) and this consumes oxygen and releases part of the nutrients. We investigate three types of responses (1) benthic uptake and respiration of organic matter, (2) burrowing and movement activity of larger benthic animals (bioturbation) and (3) the ventilation of animal burrows, such as to ensure sufficient oxygen supply (bio-irrigation) for the benthic animals.

Three models were implemented that allow inferring each of these activities. Each consecutive model builds upon the results from the previous model. Explaining the models in detail would take too far, thus we will mention only the rationale of each model and its main results.

Model 1.

The first, and simplest mathematical description of the system is a dynamic 0-dimensional model that describes how algal carbon and chlorophyll in the water column (as estimated from monthly samples) sinks to the bottom where it becomes incorporated in the sediment. In the sediment, both are decaying with a rate that depends on temperature. This model uses as input (forcing function) the chlorophyll concentration in the water column and the water column temperature. A simplified representation of the model is:

$$\frac{dC}{dt} = Flux - k \cdot f(T) \cdot C$$

Where t is time, T is temperature, C is the (carbon or chlorophyll) concentration, dC/dt is the rate of change, Flux is the deposition (calculated from the chlorophyll concentration in the water column), k is the decay rate and f(T) is the temperature function.

The model output was fitted to the monthly total chlorophyll concentrations in the sediment, and the SOC measurements. It allowed inferring (1) the decay rates (k) of organic matter and of chlorophyll, (2) the total amount of organic matter and chlorophyll deposited and mineralized on the sediment and (3) the temperature dependence of the decay rate. As the reactivity of the organic matter is relatively low (average 0.0045 d^{-1}), the uptake and respiration of the organic matter significantly lags behind its deposition (Fig. 5). Note the prominent effect of temperature on the sediment metabolism.

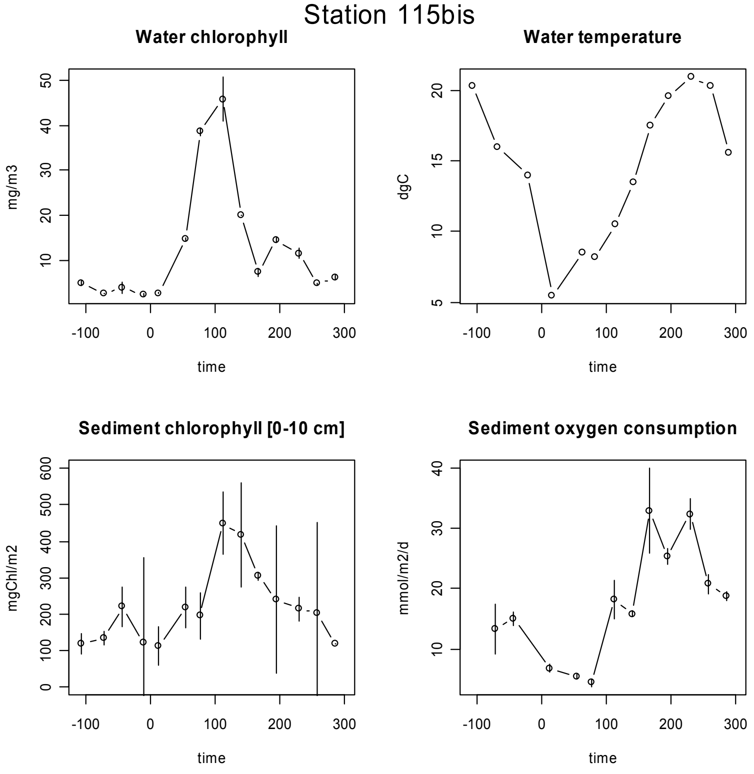

Figure 5. Concentrations of chl a in the water column and sediment at Station 115bis (Left Panels), Water temperature and SOC (right panels)

Model 2.

The aim of the second model is to infer the rate at which benthic animals mix the sediment, i.e. to quantify the bioturbation coefficient. For this purpose, we implemented a dynamic 1-dimensional model that describes the chlorophyll concentration in 100 thin (1 mm) vertical layers into the sediment. The model takes as input the chlorophyll deposition (as derived from the first model). Its output is fitted against the chlorophyll-depth profiles that were measured each month.

To understand the principle of this model, consider a simplified version of the model, where we assumed steady-state.

$$Chl_z = Chl_0 \cdot e^{-\frac{k \cdot f(T)}{Db} \cdot z}$$

The formula shows that the concentration of chlorophyll at depth z in the sediment is a function of the surface concentration (Chl_0) and exponentially decays with increasing sediment depth (z). The exponent equals the ratio of the temperature dependent decay rate (k.f(T)) and the mixing coefficient (Db). Thus in this simple model the fitting of the chlorophyll profile allows to estimate the ratio of the decay rate versus the mixing coefficient. As the decay rate of chlorophyll was derived by the first model, we can then calculate the bioturbation rates. Note that the model we used was significantly more complex.

We found that bioturbation is generally high and significantly increases after the phytoplankton bloom.

Model 3.

Due to the high bioturbation rates, the organic matter penetrates deep into the sediment at station 115. In general, the increase of ammonium at large depth into the sediment depends on the amount and the depth of the organic matter mineralization. With increasing mixing rates, the organic matter is decomposed deeper into the sediment, and the pore waters tend to become more enriched in ammonium at these depths. For station 115bis, we do not observe high concentrations of ammonium, despite the high mixing rates. They average about 100-150 mmol m^{-3}, whereas, concentrations up to one order of magnitude higher would be expected. This indicates that another process, the bio-irrigation, is counteracting the buildup of ammonium. Large animals need oxygen to respire, and when living deep into the sediment, they ensure a supply of oxygen by ventilating their burrows, i.e by pumping water in and out of the sediment. This not only brings oxygen into the sediment, but also removes reduced substances (such as ammonium). The relatively low concentrations of ammonium in the sediment thus indicate that bio-irrigation is important in this station. Bio-irrigation affects the profiles of all dissolved substances (including oxygen and ammonium) in the sediment. The third model is devised to estimate the extent of this bio-irrigation. It is a one-dimensional, so-called 'diagenetic' model that describes the penetration of organic matter, oxygen and ammonium vertically into the sediment. It uses as input the bioturbation rates and organic matter deposition and decay rates as estimated from the two previous models. Its sole unknown parameters are related to the bio-irrigation. The output of the model will be fitted against the fine-scaled vertical oxygen profiles and the ammonium concentration at depth. Results obtained with this model are still premature at the time of writing this report.

The response of bacterial communities to phytoplankton deposition

Bacterial biomass showed different seasonal patterns when comparing both stations (Fig. 6). Data from Station 115bis were only available from January 2003 onwards. Bacterial biomass values at Station 115bis were much more variable than at Station 330. Peak values were reached in March 2003, intermediate biomass values were observed in April and May and values decreased towards October.

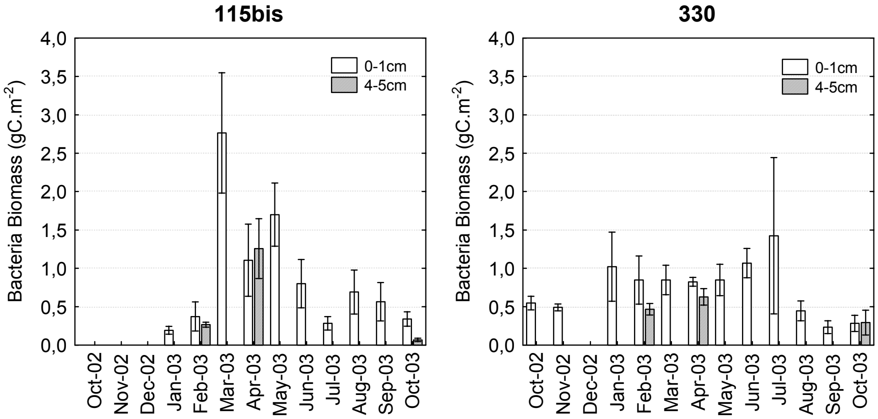

Figure 6. Bacterial biomass at the first cm layer from Station 115bis and Station 330.On Values for 4-5 cm for February, April and October 2003 are also indicated.

Biomass values at Station 330 were lower than at Station 115bis and fluctuated around 1g C m-2 from January until July 2003. Afterwards, values decreased. Bacterial biomass at the 4-5 cm layers showed similar trends in both stations.

For both stations, bacterial biomass showed no correlation with either chl *a* concentrations or temperature ($p>0.05$).

Bacterial community composition was analysed using DGGE. Each band on a DGGE gel (Fig. 7) represents one "Operational Taxonomical Unit" (OTU). The relative band intensity per OTU is used as input variable for multivariate analysis (MDS) and calculation of the Shannon-Wiener diversity index using the PRIMER 5 software package. A one-way ANOSIM considering all the samples, showed that there were highly significant differences between the two sampling stations (Global R = 0.721; $p < 0.05$). MDS carried out for both stations separately revealed seasonal and vertical differences in bacterial community composition (Fig. 7). When considering station 115bis a two-way crossed ANOSIM showed that there were significant differences between the sampling months (Global R = 0.991; $p < 0.05$) and sediment depths (Global R = 0.788; $p < 0.05$). All sampling months were significantly different from each other ($p < 0.05$) in pairwise tests. At Station 330 two-way crossed ANOSIM showed again significant differences between sampling months (Global R = 0.884; $p < 0.05$) and between the depth layers (Global R = 0.726; $p < 0.05$). Pairwise tests revealed that bacterial communities in October were significantly different ($p < 0.05$) from those in February and April, while no differences were found when comparing the communities from the latter months.

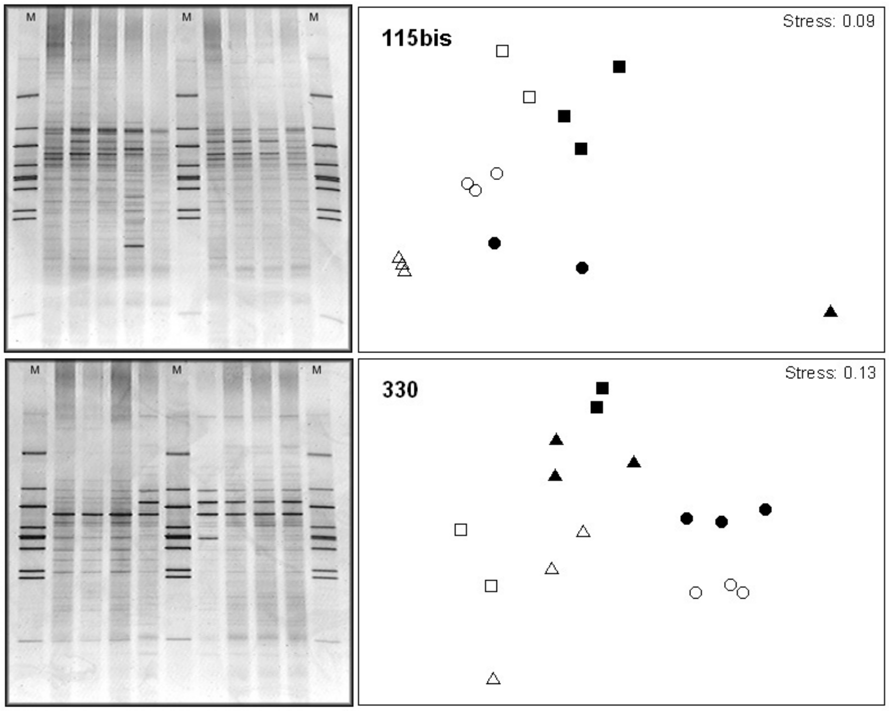

Figure 7 Example of DDGE gels and corresponding MDS analysis of bacterial community composition at Station 115bis and Station 330. Squares: February; triangles: April; circles: October. Open symbols: 0-1 com; solid symbols: 4-5 cm.

The bacterial community composition was significantly influenced by the Chl. *a* concentration in the sediment as shown by the RELATE test ($\rho = 0.26$; $p < 0.05$).

Bacterial diversity (not depicted) was significantly higher at Station 115bis (1-way ANOVA: F = 6.789, $p<0.05$). A 2-way ANOVA per station was then performed to test for the effects of month (February, April and October), sediment layer and the interaction term. Significant differences were observed between months (F =7.945, $p<0.05$), sediment depth (F = 12.926, $p<0.05$) and the interaction term (F = 7.872, $p<0.05$) for bacterial diversity at Station 115bis. Post-hoc comparison for the interaction term (Tukey HSD test) showed that only the 4-5 cm layer from April was different from the others. At Station 330, only significant differences between depth layers (F = 14.31, $p<0.05$) were observed. Similar analyses on OTU richness confirmed these results for both stations.

Meiobenthic response to phytoplankton sedimentation

Although the response of the meiobenthic communities was taxon-specific, we will concentrate on the dynamics within the nematode communities of Station 115bis and Station 330, since this taxon is the dominant taxon at both stations. Nematode densities at Station 115bis were always 4-5 times higher than at Station 330 (Fig. 8).

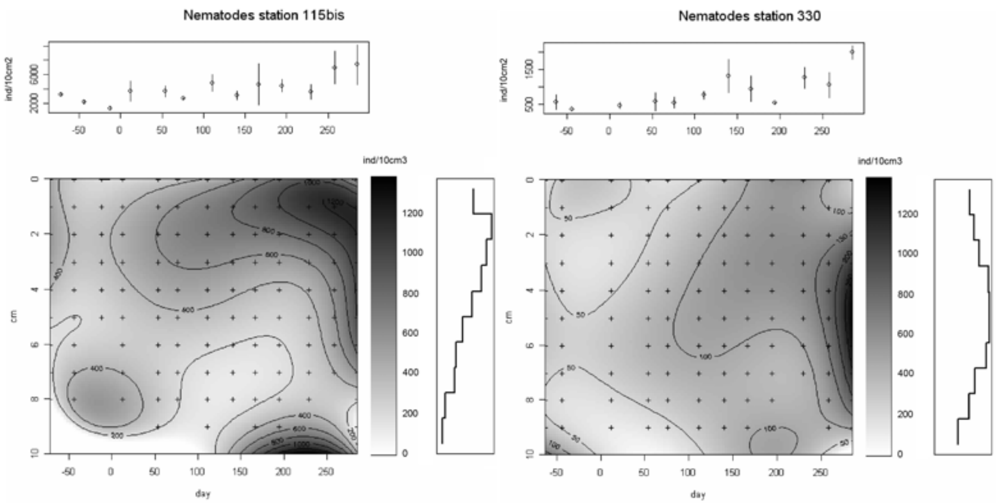

Figure 8. Nematode total densities, vertical distribution and average vertical distribution from Station 115 bis (Left) and Station 330 (Right)

Nematode densities were lowest during winter at both stations and increased in April, after the sedimentation of the phytoplankton bloom. At Station 330, nematode densities increased further and reached peak values in May. Afterwards densities decreased in June – July and increased again during late summer. In October, a new peak value was observed. A different pattern was observed in Station 115bis: densities remained stable from April to August and increased drastically towards September and October.

Vertical distribution patterns at both stations were different as well (Fig. 8). The bulk of the nematode communities were found at the upper 4cm during the year. Only in October, relatively high densities were observed at 6-7 cm depth. Vertical distribution patterns were different at Station 330, where subsurface maxima were encountered.

A "split-plot" ANOVA, analyzing temporal differences in vertical distribution patterns with a station, revealed no significant differences for the effect of time ($F_{2,4}$= 0.59; p>0.05), sediment depth ($F_{4,8}$=1.56; p>0.05) nor for the interaction term ($F_{8,16}$= 0.97; p> 0.05). At station 330, there were significant differences for the effect of time ($F_{2,4}$= 12.78; p<0.05), sediment depth ($F_{4,8}$= 4.14; p<0.05) and the interaction term depth x depth ($F_{8,16}$= 2.64; p<0.05).

Food sources of benthic invertebrates.

The possible food sources and relative position in the benthic food web were investigated using the natural occurring stable isotopes 13C and 15N in both sediment and pelagic OM and in the benthos (meio- and macrobenthos). Meiobenthic organisms were picked from 2 depth layers (0-1 cm and 4-5 cm) with the highest possible detail, while the macrobenthos was analysed at the genus level.

At station 115bis, sediment OM δ13 values showed significant differences between sediment depth (F=5.34, df=1;p<0.05) and sampling events (F= 15.53, df=2; p<0.001) (Fig. 9) whereas only time-related differences were observed for Station 330 (F=32.15; df=2; p<0.001). In February δ^{13}C values for *Richtersia* and *Sabatieria* from both sediment layers and "other nematodes" from the upper cm were about 3 units higher than the corresponding OM signatures, while the deeper dwelling "other nematodes" showed intermediate values. In April, deep-dwelling "other nematodes" were more depleted than both OM values, while *Richtersia, Sabatieria* and surface-living "other nematodes" showed signatures slightly above or resembling the OM readings. A similar pattern was observed in October for *Sabatieria* while "other nematodes" showed lower values compared with the OM signal at both sediment layers. A very depleted value for both ^{13}C and ^{15}N was observed for surface-living harpacticoid copepods in October.

Sediment OM δ^{13}C values at Station 330 (Fig. 9) from both sediment layers in October where higher when compared to the February and April. During the latter months, all benthic groups from both sediment layers showed δ^{13}C values between 2 and 4‰ higher than the OM values in the sediment while in October both OM and faunal values ranged between -19‰ and -17‰.

Kruskal-Wallis analysis by ranks revealed that significant differences in δ^{13}C values existed between the meiobenthic taxa. When the extreme values of the copepods were omitted, these differences disappeared. Isotopic signals were not different between sediment depths, however a time effect was observed. At station 330, significant differences in isotopic signals were observed between the taxa and with time. δ^{13}C values were not significantly different between the sediment layers.

δ^{15}N values for the meiobenthos were similar for both stations. At Station 115bis, only copepods showed aberrant values from the majority of the taxa. No significant differences were detected by the Kruskal-Wallis analysis by ranks between sediment layers and sampling occasions. At Station 330, significant differences in δ^{15}N values were detected between sampling occasions, however not between sediment layers.

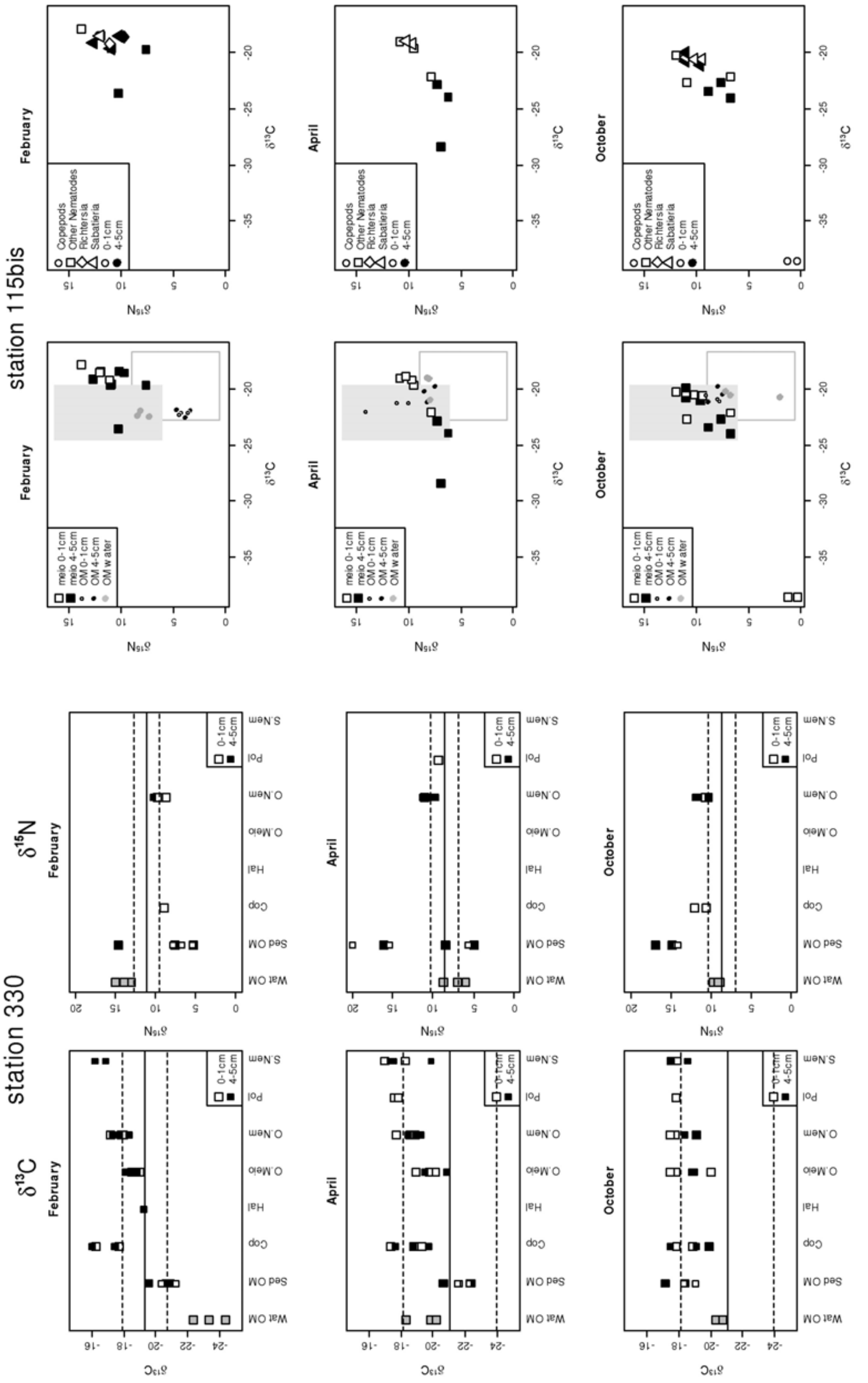

Figure 9. $\delta^{13}C$ and $\delta^{15}N$ values of organic matter (OM) in the water column, sediment and meiobenthic taxa

Integrated carbon isotopic signals of the macrobenthos from Station 115bis were always less depleted than the meiobenthic values in the corresponding months (Fig. 10). However, when compared to the surface-living meiobenthic taxa, this difference becomes less obvious. The high δ^{13}C values in October are due to omnivorous feeding macrobenthic organisms. Although many feeding types are present within the analysed macrobenthic organisms, no clear separation of food sources can be inferred from the δ^{13}C signal. The δ^{15}N values within the majority of the macrobenthos are generally at the same level as the meiobenthic organisms. In April and October, clearly higher δ^{15}N values were observed in the macrobenthos.

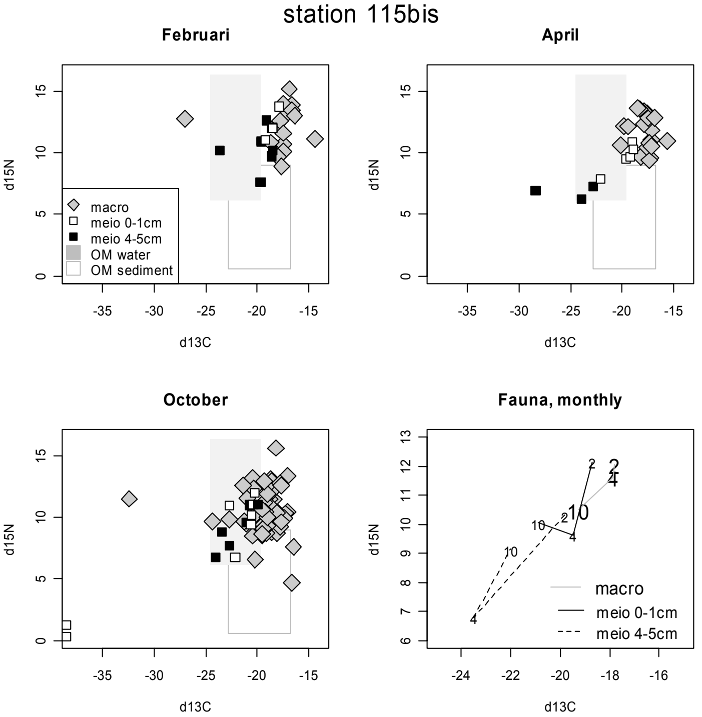

Figure 10. Natural occurring stable isotope values in the meiobenthos and macrobenthos of Station115bis.

These elevated values were drawn from predators and suspension feeding organisms (not depicted) while surface-deposit feeding and deposit feeding macrobenthos showed δ^{15}N values similar to the meiobenthos.

At Station 330 (not depicted), patterns were less clear due to the low densities of macrobenthos at this station. Here, clearly higher trophic levels were present in April and October while slightly higher δ^{13}C signals were observed in April and October. In February, δ^{13}C signals were 7 to 20 ‰ higher.

The importance of pelagic diatoms versus *Phaeocystis* as benthic food sources

We tested the importance of the harmful algae *Phaeocystis* and the natural occurring diatom *Skeletonema* as a possible food source for benthic organisms by incubating undisturbed sediment from both stations in temperature controlled climate room in dark conditions with pre-labeled food sources. It was not possible to culture axenic *Phaeocystis* colonies. Incubations with pre-labeled *Phaeocystis* were only done for Station115bis. Experimental units were maintained for 2 weeks. Results are still preliminary but are mentioned here as indication of the importance of decaying *Phaeocystis* colonies for the benthic environment.

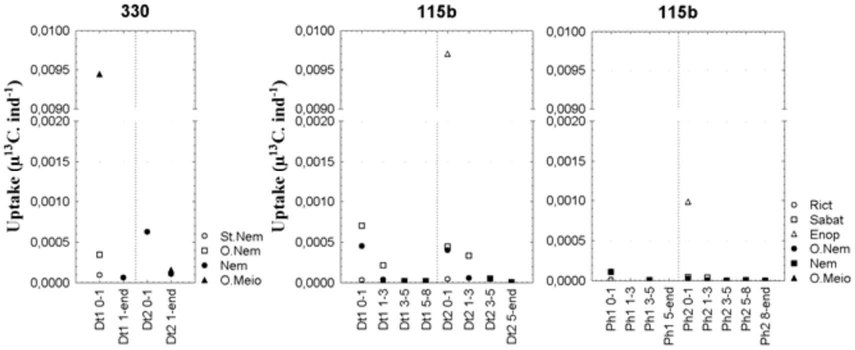

Figure 11. Uptake (µg ^{13}C per ind.) of pre-labeled food source (Dt= diatom, Ph= *Phaeocystis*) in different depth horizons of both sediments.

Uptake of labeled algal material was highest in the upper cm-layer at both stations (Fig. 11). At station 330, especially organisms not belonging to the nematodes were responsible for the uptake, when present. At Station 115bis, *Sabatieria* and *Enoploides* (when present) used the labeled diatoms as food source, while the other nematodes were labeled to a lesser extent. *Phaeocystis* uptake by nematodes (as a group) was observed in low quantities in 1 replicate from Station 115bis, whereas *Enoploides* was labeled considerably in the other experimental unit.

The general response of C-respiration to the addition of diatom carbon is comparable at both stations, after 1 day and 1 week (Fig. 12): at both station, 20 to 25 % of the added algal carbon was respired.

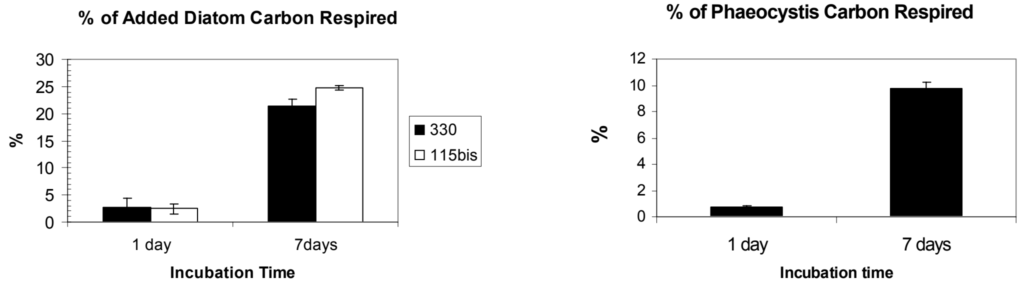

Figure 12. Respiration of added diatom C in Station 115bis and Station 33 (Left panel) and *Phaeocystis* at Station 115bis in temperature controlled dark incubations.

Respiration of *Phaeocystis* derived labeled carbon was relatively lower, however 10% of the added *Phaeocystis* label was respired by the benthic communities of Station 115bis.

Food web models

The biogeochemical models discussed above focus on the cycles of carbon, oxygen and nutrients and the activity of benthic animals is captured in a few parameters: their respiration and excretion is represented by the decay rate of organic matter; one bioturbation coefficient approximates their crawling and feeding activity, whilst the burrow ventilation is captured in the bio-irrigation coefficient. Thus, the biogeochemical approach completely disregards the biological complexity.

Via inverse food web modeling, the transfer of organic matter through the main biotic compartments will be assessed. The basic information that enters the food web model is (1) the biomasses of the main components and (2) the sediment oxygen consumption rates. The former put bounds on the magnitude of the many food web flows by physiological constraints, whilst the latter is an effective measure of the respiration of all organisms summed.

Note that this type of modeling can only be initiated as all data become available, and as some quantities have been estimated by biogeochemical model. Thus, the results from this model are preliminary at the time of writing. In Table 1 are the biomass input data that have been collected for station 115bis. Note that the bacterial biomass was only measured in two slices of one cm, and then extrapolated to 10 cm. Two measures of organic carbon are present; edible carbon was estimated based on the results of the biogeochemical model nr 1. The other constitutes all organic carbon, including the fraction that is not eaten by the organism.

group	FEBRUARI	APRIL	OCTOBER
Depositfeeder	0.0	1.2	6.8
Omnivore	1.2	0.3	5.9
Predator	3.2	0.1	5.4
Surface deposit feeder	2.6	5.8	9.0
Suspension feeder	0.01	70.5	68.3
Unknown	0.5	0.4	1.9
nema 10cm	0.5	0.8	0.7
Bact 10cm	3.0	12	3.0
TotalC[0-10cm] %Cbased	228.4	2202.9	281.1
ReactiveC SCOC-based	26.8	71.1	42.2

Table 1. Biomass values (gC m-2) used as input values for inverse food web modeling at Station 115bis

DISCUSSION

Response to phytoplankton deposition

The response of both stations to phytoplankton sedimentation was very different and resembled the patterns described in Steyaert et al. (subm.) and Vanaverbeke et al 2004a, b. Concentrations of Chl *a* in the sediment were much higher at Station 115bis when compared to Station 330. After sedimentation of the spring bloom, a strong vertical profile was established at Station 115bis with high concentrations in the upper sediment layers and lower concentrations deeper down the sediment. Sedimentation of labile organic matter from the water column also resulted in oxygen stress in most of the sediment column.

At Station 330, sediment Chl *a* concentrations were much lower although water column concentrations were similar at both stations. Clear vertical profiles, as present at Station 115bis were never observed at Station 330 and the sediment column remained completely oxic during our sampling period. The lower concentrations of organic matter at Station 330 are probably caused by (1) a lower sedimentation rate of phytodetritus as a consequence of higher bottom water currents in combination with (2) the permeability of the sediment. Indeed, recent work clearly showed that lateral advective currents *through* the sediment induce Chl *a* profiles as observed at Station 330 (Huettel & Rush 2000, Ehrenhauss & Huettel 2004, Ehrenhauss et al. 2004). Moreover, these lateral advective currents accelerate the aerobic degradation of organic matter and the recycling of nutrients (Huettel &

Rush 2000, Janssen et al 2005, Bühring et al. 2006) preventing the establishment of vertical gradients in organic matter as observed at Station 115bis.

These differences were clearly reflected in the response to phytoplankton sedimentation in both benthic ecosystems. Degradation of organic matter, as measured by sediment oxygen consumption, at station 115bis was delayed with about one month compared to the organic matter content (as measured by chlorophyll concentrations). As SOC peaked about one month earlier than the water-column temperature, it is likely that benthic activity is affected both by temperature and food concentration. In contrast, SOC values at station 330 reach higher values from April onwards and do not increase towards summer, showing a fast response to the arrival of fresh organic matter at the seafloor compared to station 115bis. Moreover, SOC values at Station 330 are remarkably lower. This could be due to the lower sedimentation rates at these stations, caused by a reduced primary production at this site. However, observed values at Station 330 are probably underestimates of the natural situation. The advective currents through the sediment were not mimicked during the lab measurements and this has recently been shown to provoke an underestimation by a factor of 1.4 compared to *in situ* situations (Janssen et al. 2005), or even a factor 2 to 3 when diatoms were added to experimental sediments (Ehrenhauss & Huettel 2004). Multiplying the observed values at Station 330 with the factors calculated in the mentioned papers still would result in values lower than those observed at Station 115bis. However, given the fact that most of the sediments at the BCS are comparable with those at Station 330, the overall importance of these sediments for the functioning of the marine ecosystem should not be neglected.

Differences between the stations were not only reflected in timing and magnitude of organic matter degradation, bacterial and meiobenthic patterns were different as well. Both bacterial communities (this study) and nematode communities show differences between stations in terms of standing stock and community composition (Steyaert et al. subm, Vanaverbeke 2004b). Seasonal differences in bacterial community composition were stronger at Station 115bis, probably as a result in the larger variation in organic matter availability in this station compared to Station 330. However, in both stations, bacterial community composition is related to Chl *a* concentrations in the sediment.

Nematode communities at Station 330 show lower densities when compared to values observed at Station 115bis, but diversity in Station 330 is much higher (compare Steyaert 2003 and Vanaverbeke et al. 2004b). This is explained by the biogeochemical response to phytoplankton sedimentation. This event triggers oxygen stress in the sediments of Station 115bis, creating an environment in which only a limited number of well-adapted species survive. The high concentration of labile food provides an opportunity for these opportunistic species to reach the high densities observed in this study and Steyaert et al. (subm.). Timing of nematode peak densities coincides with the timing of the degradation of Chl *a* at Station 115bis as described by Provoost et al. (in prep.). In addition, the burial of degradation products, as a result of diffusion, bio-irrigation and bioturbation triggers a depth-specific and species specific response of the nematode communities (Steyaert et al. subm.). At Station 330, the timing of the nematode response is closely related to the sedimentation event, rather than time-lagged. Here both densities and diversity increase, as a result of the fast remineralisation processes in these permeable sediments (Vanaverbeke et al. 2004b) triggering the fast emergence of short and stout nematodes (Vanaverbeke et al. 2004a).

Field studies using the natural abundance of stable isotope ^{13}C and ^{15}N in both organic matter in the water column, sediment and meio- and macrobenthos further confirmed the difference in functioning of the benthic ecosystem at both stations. At both stations, $\delta^{13}C$ values observed in the meiobenthic taxa were different from the signal obtained in the sediment particulate organic matter (POM) indicating that bulk organic matter cannot be considered an appropriate food source. Moreover, there is no obvious fluctuation in the isotopic signal of the organisms in the upper cm. This reflects a constant food source throughout the year. We hypothesise that the taxa living on the upper sediment horizons depend on a constant but limited supply of fresh algal material from the water column. Indeed, diatoms are present in the water column throughout the year (Rousseau et al. 2002). Since the BCS is a well-mixed part of the North Sea (Brussaard et al. 1995) there is always a certain amount of fresh material that can reach the sediment which sustains the meiobenthic communities. An increase in available phytodetritus after the spring bloom sedimentation is then triggering the increase in densities observed at both stations. The absence of vertical differences in the $\delta^{13}C$ values of both sediment POM and organisms reflects the permeability of the sediments at station 330 and suggest that the benthic food web solely depends on fresh phytoplankton. At station 115bis, vertical differences in the "other nematodes" group reveal the use of different food sources, with the deeper-dwelling nematodes being part of a food web based on older , more fractionated and decomposed organic material. The extremely low value observed in the copepods in October suggests the existence of a chemoautotrophic food source (Felbeck & Distel 1999) based on sulfur-oxidizing bacteria.

Macrobenthic signatures at both stations (Van Oevelen et al. in prep) showed selective feeding on organic matter as well. In contrast with the meiobenthos, a larger variation in ^{15}N values was observed, reflecting the presence of multiple trophic levels within the size group. On the other hand, the presence of vertically segregated food sources revealed by the meiobenthic organisms was not observed within the macrobenthos, suggesting that analysis of isotopic signals of meiobenthic organisms is more suitable for studying small scale patterns in fine sandy sediments.

Experimental work on the relative importance of pelagic diatoms and *Phaeocystis* confirmed the general accepted idea that benthic ecosystems are largely driven by sedimentation of pelagic diatoms from the water column. However, we showed for the first time ever that *Phaeocystis* derived carbon can be used as a food source for nematodes. Since the analysis of the stable isotope values of the macrobenthos and bacteria is ongoing, the full ecological importance of *Phaeocystis* sedimentation for the benthic food web is still unclear. However, since 10% of the added *Phaeocystis* carbon was respired after 1 week, we suggest that sedimentation of decaying *Phaeocystis* colonies indeed affects the functioning of the benthic ecosystem.

Estimations of mineralization rates of added diatom carbon at both sites showed little differences between both stations. This is surprising, since both meiobenthic and macrobenthic biomass and diversity is very different. This indicates that mineralization processes are largely driven by bacterial communities, whereas larger metazoan organisms facilitate these processes by bio-irrigation and bioturbation activities which provide the sediment with oxygen and remove toxic byproducts.

CONCLUSIONS

Our research confirmed our initial idea that strongly different benthic ecosystems were present at the Belgian Continental Shelf. Both processes and diversity and densities of bacterial, meiobenthic and macrobenthic communities were strongly related to the sedimentation and hence availability of labile organic matter from the water column. In the fine sanded sediments, which are mainly present in the coastal zone, sediment remineralisation of organic matter is rather intense, but delayed with respect to the major sedimentation event in spring. Here, oxygen consumption (as a proxy for remineralisation processes) is a function of temperature and macrobenthic activities. These processes require oxygen, resulting in oxygen stressed deeper sediment layers. As a consequence, different food sources for the meiobenthos were identified at different sediment layers, explaining the observed nematode responses by Steyaert et al (subm.). Surface living organisms depend year-round on fresh algal material derived from the water column whereas deeper-dwelling organism feed on more degraded material. The presence of chemo-autotrophic food sources was shown for the fine-sanded sediment as well. The more off-shore permeable sediments show a fast response both in terms of densities and diversity of benthic organisms and mineralization processes. This is due to the prevailing lateral advective currents through the sediment, providing the deeper sediment layers with oxygen and removing toxic byproducts of the mineralization processes.

Mineralization rates were largest in the fine-sanded sediments and peaked during summer. Permeable sediments show an immediate response to phytoplankton sedimentation, although the recorded values were lower. It should be noted that the mineralization rates in the permeable sediments recorded during TROPHOS should be considered as absolute minimum estimates, these values are probably higher in field situations. Given the fact that the majority of the sediments at the Belgian Continental Shelf consists of these coarser sediments, the ecological value of these sediments for the functioning of the marine ecosystem should not be underestimated. Experimental work with pelagic diatoms and *Phaeocystis* as possible food sources for sediment inhabiting organisms clearly showed the importance of pelagically produced diatoms for the functioning of the benthic ecosystem. However, we showed for the first time that *Phaeocystis* can be remineralised in the sediment and cascades in the benthic foodweb. These experiments further showed that short-time mineralization rates in both sediment types are comparable, despite the strong differences in density, diversity and biomass of bacteria, meio- and macrofauna.

3. LIFE HISTORY AND DISPERSAL OF MARINE FISHES AND INVERTEBRATES, A MOLECULAR APPROACH

INTRODUCTION

The spatial characteristics of the habitat of marine organisms and the connectivity between their habitats are of fundamental importance to understand their population dynamics. These patterns have been shaped throughout the Pleistocene, where climate cycling and sea level stands have strongly affected shallow seas such as the North Sea. The long glacial periods during the Middle to Late Pleistocene coincided with low sea water levels and a retreat of the ocean up to the shelf edge. Interglacial periods were warmer and saw a reflooding of the shelves. Almost all marine organisms have expanded or stabilized their populations in response to these processes. The current populations of marine organisms integrate this history together with the ongoing postglacial equilibrium between hydrodynamics, habitat availability, ecological niche and anthropogenic factors such as fisheries, pollution and climate change.

Here we report on the historical and current patterns of two invertebrate taxa (mysid shrimps and *Gyrodactylus* flukes) and two vertebrate taxa (*Pomatoschistus* gobies and common sole). We also initiated a modeling analysis of their patterns in the North Sea and English Channel through particle tracking.

Our genetic diversity and phylogeography study of planktonic invertebrates focuses on a widespread mysid species (*Mesopodopsis slabberi*) along the European coasts. From an evolutionary point of view mysids are interesting model organisms as their dispersal capacities are rather restricted (brood protection and absence of pelagic larvae), and hence they often show a significant population genetic structuring. As an omnivore, which mainly utilizes mesozooplankton and detritus carbon pools (Fockedey & Mees 1999), and as an important prey for demersal and pelagic fish and larger epibenthic crustaceans (Hostens & Mees 1999), they are believed to be key species in the ecosystems of estuaries and coastal regions.

One of the most significant radiations of plathyhelminth parasites is documented in the monogenean "supergenus" *Gyrodactylus*. The high diversity on a wide host range can be linked with their remarkable reproduction mode accelerating speciation. The tight host-parasite relationship promotes coevolution, while the ability to reproduce clonally enchances sympatric speciation and speciation through host-switching. We test whether this is not only the case at the species level (Huyse & Volckaert 2005) but also at the population level.

Sand gobies prefer coastal habitats, have small sizes and are very abundant, they play a key role in the food web of the coastal zones of temperate seas. The combination of brood care, a short pelagic larval stage and a demersal life style during their short life makes them suitable for an in depth genetic study. Gysels et al. (2004a, b) observed historical patterns in the North Sea. A small scale study of Pampoulie et al. (2004) revealed a moderate differentiation between estuarine, coastal and marine samples, suggesting that historical events (eg the Holocene sea level rise) and contemporary habitat fragmentation contributed to the pattern. Moreover, samples were assigned to two rather complex breeding units (Westcoast and Easternscheldt).

Adult sole 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. Homing behaviour and the presence of discrete spawning areas have been observed (Symonds & Rogers 1995). Kotoulas et al. (1995) have shown that there is a gradient in the genetic structure of *Solea solea* populations, which means that genetic exchange between populations is high but limited. The core of each spawning unit along the Atlantic shelf is separated by a distance of about 100 km. The question rises to what the nature is of the gene flow between the spawning populations, historically and contemporaneously. Since there are many biological data available, our own analysis can be put in a broad context.

During the planktonic stage, eggs and larvae drift in the water column. Hydrodynamics, ecosystem dynamics, life-history characteristics, interannual variability and chance affect survival and dispersal. High-quality hydrodynamical and particle dispersal models provide a unique opportunity to gain insight in the transport mechanisms of the larvae from the spawning to the nursery grounds. We have started to adapt and validate such model to simulate the transport of larvae on the Belgian Continental Shelf (BCS).

Our results point to major communalities between taxa, where dispersal is usually less than if only passive dispersal would be invoked, that population dynamics strongly influence genomic variation, and that metapopulations represent a fair concept to describe their patterns.

MATERIALS AND METHODS

Mesopodopsis slabberi

Specimens of the mysid *Mesopodopsis slabberi* were collected from 15 Northeastern Atlantic and 9 Western Mediterranean populations (Fig. 13). In addition, a detailed spatial and temporal sampling was conducted on the scale of the BCS, where mysids were collected at 3 stations (115, 701 and 790) in 2001, 2003, 2004 & 2005. Specimens of the congeneric species *M. wooldridgei*, *M. aegyptia*, *M. orientalis* and *M. africana* were used as close outgroups in the phylogenetic analyses. Samples from each location were collected with a hand net or a hyperbenthic sledge (mesh size 1 mm).

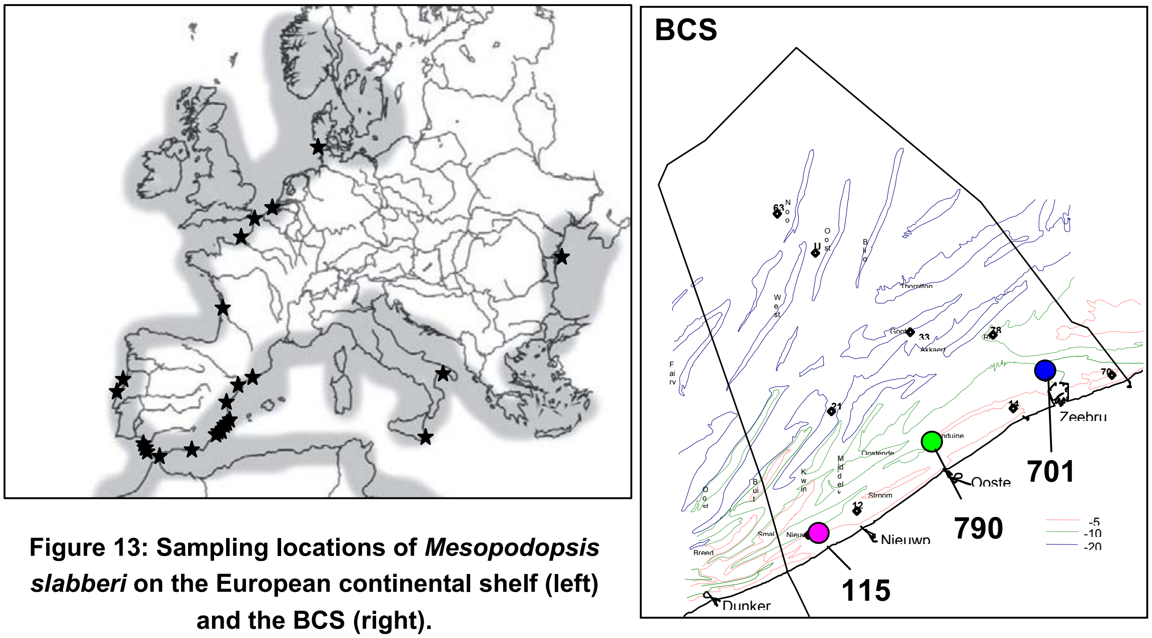

Figure 13: Sampling locations of *Mesopodopsis slabberi* on the European continental shelf (left) and the BCS (right).

After collection, the samples were stored in ethanol (70 – 95%) or acetone at 4°C. DNA was extracted from mysid tissue using a modified CTAB protocol (see Remerie et al. 2006a). Small aliquots of extracted nucleic acids (1 µl) were used as template for polymerase chain reaction amplification (PCR) of the mitochondrial cytochrome oxidase I (COI) and the 16S rRNA genes. The PCR conditions and thermocycle profile are described by Remerie et al. (2006a). PCR products were purified with exonuclease I (10 U μl^{-1}; Amersham) and shrimp alkaline phosphatase (1 U μl^{-1}; Amersham). Purified products were cycle sequenced using BigDye Terminator Mix (PE Applied Biosystems) and electrophoresed on a Perkin-Almer ABI Prism 377 DNA sequencer and an ABI 3130 capillary sequencer.

Temporal and spatial *M. slabberi* samples of the BCS were analysed with SSCP (Single Strand Conformation Polymorphism). Mutations in a smaller fragment of the

COI gene (252 bp) were screened with this technique. SSCP analysis were performed using 0.5 mm thick nondenaturing polyacrylamide gels (250 x 110 mm) (T = 12.5%, C = 2%) and an electrophoresis at a constant power of 8 W at 20°C for for 3.5 h. Bands were visualized with a DNA silver staining kit (Amersham Biosciences) and scored by their relative mobility.

Gyrodactylus

We quantified differentiation between populations of three *Gyrodactylus* species on a European scale. DNA extraction and PCR optimization of the COI mitochondrial DNA locus were done as specified in Huyse et al. (in prep.). New primer sequences were developed to amplify a smaller COI fragment (330 bp). These fragments were sequenced according to standard protocols.

Pomatoschistus gobies

An extensive sampling campaign on gobies was initiated along the Southern Bight of the North Sea and the Western Scheldt (Fig. 14). In total 35 sampling sites were sampled in 2003 and 2004 with a hyperbenthic sledge, beam trawl and the water intake of a power plant, and this as well at sea (fishing and research vessels) as on land (beach and water intake). For the moment 1030 (year 2003) and 545 (year 2004) gobies have been identified to estimate the density over the whole region and to collect tissue samples for genotyping, isotope and otolith analysis (the latter are not included in this project). The aim to sample extensively during one year has been reached. The second year was used to obtain data of an even higher quality.

In the coastal zone sand gobies were sampled over a track of 5 km offshore, especially in the vicinity of the sand banks. Estuarine migrations were tracked with various techniques. Gobies were frozen on site for shipping to the laboratory. In the lab fish were identified to the species level on biological characteristics and if necessary based on mitochondrial DNA genotyping. Standard length (SL), total length, weight and sex were determined. Fin clips of the tail fin were collected for molecular identification. Full details of the samples are available on request.

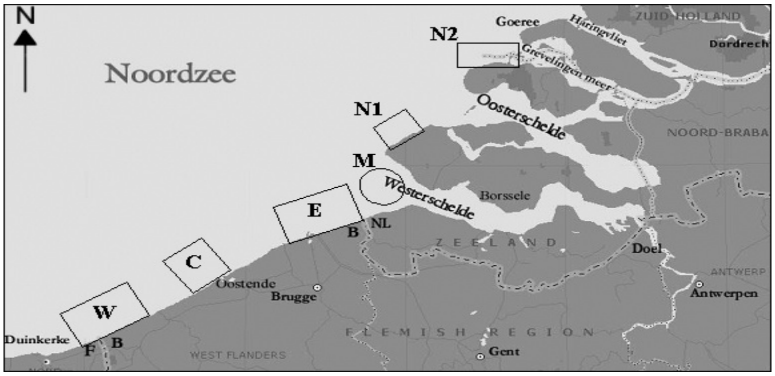

Figure 14. Study range Six coastal zones have been targeted between Dunkirk and Goeree: W, C, E, M, N1 and N2. In the Westernscheldt estuary are Borssele (polyhaline zone) and Doel (oligohaline zone) listed.

DNA was extracted with Proteinase K. Primer loci used for *Pomatoschistus minutus* included the published Pmin-10 (Jones et al. 2001) and Pmin-06, Pmin-07, Pmin-08 and Pmin-11 (Pampoulie et al. 2004). Especially for this project loci Pmin-09, Pmin-16-2, Pmin-13 and Pmin-20 were developed (Larmuseau et al. 2006; Table 2).

Locus	Primer sequence (5' to 3')	# of alleles	Range	Repeat
Pmin-06	R: CGCATTAGAATTATTAGGCC F: TCANTNCTACTCACTAACCT	47	91-199	(CA)(AA)(CA)
Pmin-08	R: GTTCGCCACCATGCACC F: AGTCTTCCACCGCTCACG	56	152-286	(CA)(CG)(CA)(CG)(CA)
Pmin-09	R: GGGATGTGTGAGTGTGCAAG F:GCCGTGGATGCATTATCAGT	37	196-310	$(TG)_2CG(TG)_{20}$
Pmin-11	R: CCGACCCAGAAATGGACAA F: GATTCGCCAACACAGATTCAA	14	100-120	$(TGGA)_6$
Pmin-13	R: CGCTACCGGATTCTAAAGTG F: CGCAGATTCTACTACTCCCCTCTT	70	88-284	$(CTACT)_7(CTGCT)_6(CTACT)_{10}$-$(CTGCT)(CTACT)_7(TTACT)_2CTTTT(CTACT)_3$
Pmin-16-2	R: TGAGACATGAGAGGGGGAAG F: CTTTTAGAGTGAGCAGAAAAGAGTG	24	144-188	$(AT)_3GTAT(GT)_4GC(GT)_8$
Pmin-20	R: GGCCACAGATACGACCTAGC F: CAGATCTGTGGAAATCCAACC	55	216-340	$(GT)_3GC(GT)_4GC(GT)_8$
Pmin-10	R: AACCGCCCAATCCACAAC F: GAATGTCCCGAGAAACTGGAG	9	142-202	(GT)
Pmin-07	R: TTTCAGCTGTATAGTCGCTGC F: TCGACAAACTCAAACTCACC	45	162-178	(GA)

Table 2: Overview of the microsatellite loci of *Pomatoschistus minutus*

Loci were amplified in a standard PCR-mix including the following ingredients: milliQ-water, PCR-buffer (10x), dNTPs, primer, *Taq*-polymerase and $MgCl_2$ (see Table 3).

	Reaction cocktail A	Reaction cocktail B	Reaction cocktail C
Reaction buffer (10 x)	1 µl	1 µl	1 µl
dNTPs (2 mM)	1 µl	1 µl	1 µl
mQ water	5.8 µl	5.7 µl	5.6 µl
$MgCl_2$ (50 mM)	0.2 µl	0.3 µl	0.4 µl
Taq-polymerase (5U/µl)	0.08 µl	0.08 µl	0.08 µl
Primer solution (2 µM)	1 µl	1 µl	1 µl

Table 3: Concentrations and volumes of the components of a standard PRC-cocktail of one sample.

Further details on the PCR reaction protocol are available on request.

DNA Fragments were separated on polyacrylamide gel electrophoresis on a double laser Li-COR system and automatically analysed on the GeneImagIR 4.03 software (Scanalytics, 2001).

Length and weight data were analysed with STATISTICA version 6.0 (Statsoft, 2001) and MS EXCEL 2000 (Microsoft, 1999). Condition was calculated with $RCF=W'/(a^*L^b)$. Growth parameters *a* and *b* represent the intercept and direction coefficient, respectively, of the log L-log W regression of all samples.

The quality of the genotypes was assessed for null alleles, stuttering and large allele dropout with the software package Micro-Checker version 2.2.0. (Van Oosterhout et al. 2004). Reading errors could be picked up through factorial correspondence analysis implemented in the software package GENETIX version 4.04 (Belkir et al. 2002). Genetic variability was calculated from the genotype and allele frequencies and included heterozygosity (observed and expected), number of alleles, inbreeding coefficient and mean number of alleles (implemented in GENETIX). Genetic differentiation was calculated with 3-dimensional factor analysis, pair-wise F_{ST}-waarden, estimated as θ (theta), G_{ST}-values (implemented in GENETIX) and R_{ST}-values, estimated as ρ (rho) (implemented in SPAGEDI version 1.1 - Hardy & Vekemans 2002). Also principal component analysis was used as implemented in PCA-GEN (Goudet 1999) based on the F_{ST}-values. The number of migrants between all populations was calculated as N_em under GENETIX. Pair-wise genetic distance (D_{CE}) was calculated according to Cavalli-Sforza & Edwards (1967) in GENETIX and visualised in a phylogenetic tree with the with the "Neighbour joining" algorithm. Isolation-by-distance was tested with the non-parametric Mantel test (as implemented in GENETIX). To test for the hierarchical structure of the samples we used AMOVA-tests (analysis of molecular variance - as implemented in ARLEQUIN version 2.000 (Schneider et al. 2000). We analysed the grouped spring and fall populations, as well as Scheldt and North Sea populations.

Solea solea

Samples were collected along the whole distributional range in the Mediterranean Sea (n=8) and the Atlantic Ocean (n=28) and at the mitochondrial cytochrome *b* locus and six microsatellite loci. Atlantic samples consist of eight samples from the Gulf of Biscay and 16 from the North Sea. Twenty additional samples are available from the Southern Bight of the North Sea (not listed).

DNA was purified using either the 'Dneasy Tissue Kit' (Qiagen, Westburg) or the 'NucleoSpin Kit' (Machery-Nagel, Düren, Germany). Final elution is done in 200 µl milliQ water of which 1 µl is used for the PCR reaction. Six DNA microsatellite primer pairs were tested, i.e., F8-I, F8-II, F8-III, F8-IV, F13 and F-14 (Iyengar et al. 2000). The reaction volume is 10 µl: 1 µl of the DNA solution, 1 µl of 10× PCR buffer, 1 µl of 2mM dNTP's, 1 µl of each primer (2 µM), 0.4 µl (for F8-I), 0.3 µl (for F13 and F8-II) or

0.2 µl (for the other three) of 50 mM $MgCl_2$, 0.1 µl of the Silverstar *Taq* polymerase (5 u/µl) (Eurogentec) and milliQ water is added until the total volume is 10 µl. The annealing temperatures are 61°C (F13), 60 °C (F8-III and -IV), 58 °C (F14), and 56°C (F8-I and -II) respectively. The reaction products were separated on an automatic sequencer (LICOR) and analysed with the Gene ImagIR (Scanalytics) software.
Data are analysed similarly as the *Pomatoschistus* gobies.

Modelling

The transport model uses the three-dimensional (3-D) hydrodynamic model OPTOS-BCZ, that covers the BCS. It is based on the COHERENS model (Luyten *et al.* 1999). The COHERENS model is a three-dimensional hydrodynamic multi-purpose model for coastal and shelf seas, which resolves mesoscale to seasonal scale processes. The program was developed between 1990 and 1998 by a multinational European group (EU-MAST projects PROFILE, NOMADS and COHERENS).

The 3-D model solves the continuity and momentum equations on a staggered Arakawa-C grid, with sigma coordinates over the vertical. The model uses an explicit mode-splitting treatment of the barotropic and baroclinic modes. The OPTOS-BCZ model covers an area between 51°N and 51.92°N in latitude and between 2.08°E and 4.2°E in longitude. The horizontal resolution is about 800 m and the vertical has 20 sigma levels. Bathymetry is presented in Figure 15. Boundary conditions are water elevation and depth-averaged currents provided by the OPTOS-NOS model, which is also based on the COHERENS code, but covering the whole North Sea and part of the English Channel. The boundary conditions of the OPTOS-NOS model itself are provided by the OPTOS-CSM model, a two-dimensional hydrodynamic model of the entire Northwest European Continental Shelf. Meteorological surface forcing is from the forecasts of the UK Meteorological Office at Bracknell and available every 6 h. Current velocities of the OPTOS-BCS model have been validated using about 400 h of ADCP current profiles collected during 12 R/V *Belgica* campaigns from September 2002 onwards (Van Lancker *et al*., 2004). The validation leads to the conclusion that the norm and the direction of the current profiles are satisfactory represented by the hydrodynamic model. The Root-Mean-Square-Error of the norm of the currents is usually less than 15 $cm.s^{-1}$ and the relative error of the direction less than 15 % (Fig. 16).

The transport model MU-TRANS is based on a particle tracking method. This has the advantage that subgrid scale information can be obtained and that a great flexibility in adding different processes and properties for the particles is offered. The MU-TRANS model has been used independently from the hydrodynamic model. By doing so, different simulations can be carried out for specific hydrodynamic conditions. A typical run uses 100 to 1000 particles to represent a population.

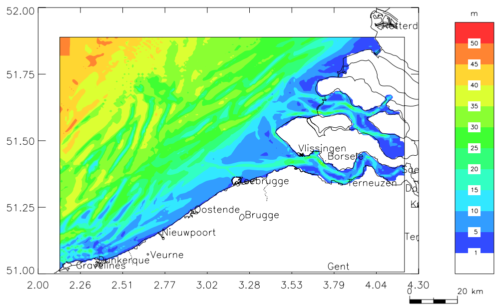

Figure 15: Bathymetry of the OPTOS-BCZ model.

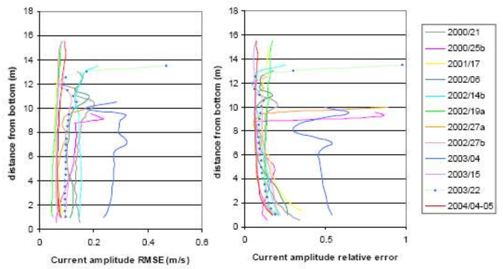

Figure 16: Validation results of the hydrodynamic model OPTOS-BCZ using ADCP current profiles.

Particles can be advected by the depth-averaged currents or by full 3-D currents. The horizontal diffusion of the particles is represented by a random Gaussian distributed displacement. For the vertical migration, different possibilities have been implemented in the model. For diurnal migration the particles will move to the surface at night and to the bottom in daylight, or inversely. Directional vertical migration can also be applied. In this case the particles will move to the surface when the currents are in a certain direction and will move to the bottom, when the currents are in an opposite direction. In this way, it is possible for the particles to move in a preferred direction.

RESULTS

Mesopodopsis slabberi

- Phylogeographic patterns

A total of 195 specimens of *M. slabberi* were analysed for mtDNA COI variation, and an additional 41 mtDNA 16S rRNA sequences were obtained. Genetic diversity was overall very high, with a total of 156 COI and 36 16S haplotypes. The phylogeny of the 16S haplotypes clearly shows the presence of 6 diverged monophyletic clades (Fig. 17). Remarkably, the presence of only five of these clades was corroborated by the COI phylogeny (tree not shown). The nucleotide divergence between those clades ranged from 1.1% – 19.6% (16S) and 5.7 – 34.9% (COI), the intra-clade divergence was significantly lower (16S: 0.4% - 1.5%; COI: 1% - 2.2%). The congeneric species *M. aegyptia* (Mediterranean Sea) and *M. wooldridgei* (South-Africa) appeared to be sister species to the *M. slabberi* clades. Interestingly, the divergence of the *M. slabberi* clades with these sister species ranged up to similar levels as the inter-clade divergence.

The geographic distribution of the *M. slabberi* clades is depicted in Fig. 5. Two Atlantic clades are observed, with one ('Atlantic') having a wide distribution and the other ('MO B') restricted to Southern European brackish water habitats. Within the Western Mediterranean, three clades are observed: one restricted to the Alboran Sea ('Medit 2b'), one restricted to a seagrass population near Alicante ('Medit 2a') and one more widely observed in the Balearic, Ionian and Southern Adriatic Sea ('Medit 1'). Finally, a distinct clade was observed in the Black Sea.

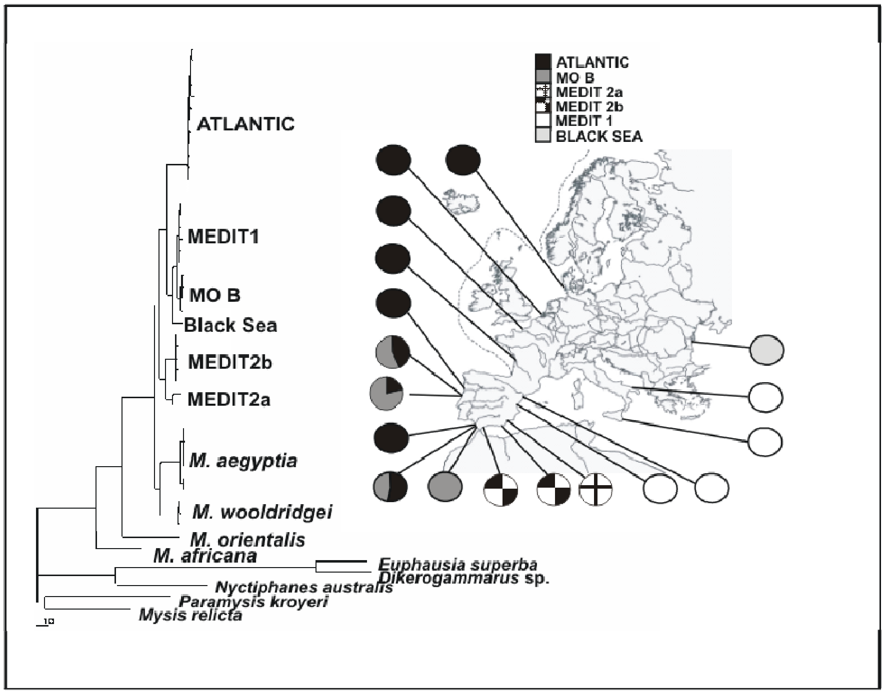

**Figure 17: Left: Maximum Parsimony tree of the 16S haplotypes.
Right: geographic distribution of the *M. slabberi* clades.**

- Spatial and temporal patterns on the BCS

Small-scale spatial and temporal samples from *M. slabberi* were analysed from the BCS. A total of 207 mysids were analysed with SSCP from three sampling stations (115, 701 and 790) in four different years (2002, 2003, 2004 and 2005). The haplotype diversity within each sampling station was very high (h = 0.67 – 0.9), except in the 2001 sample of station 790 (h = 0.5) and the 2003 sample of station 115 (h = 0.29), probably caused by artifacts in the SSCP analysis. Hence, these samples were excluded from further analyses. There were no significant differences in genetic diversity between the different spatial and temporal samples. A 2-level AMOVA was performed in order to detect spatial and temporal patterns of genetic variation in our dataset. The spatial variation seemed to be non-significant (F_{CT} = 0.004; $P = 0.33$), while the (limited) temporal variation was highly significant (F_{SC} = 0.078; $P < 0.001$). However, still most variation (91.76%) was observed within each sample. The nMDS plot corroborates these results; with exception of the 2001 samples all samples of the same year are grouped together and interconnected in the MST (see Fig. 18).

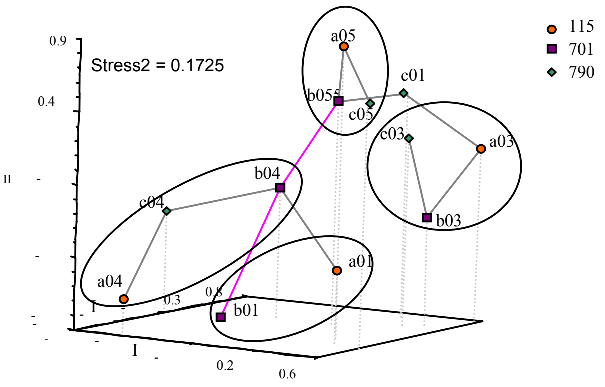

Figure 18: Minimum spanning tree (MST) superimposed on a nMDS plot of the DCE distances between all temporal and spatial samples. Codes: circle = st. 115 (a), square = st. 701 (b), triangle = st. 790 (c).

Gyrodactylus

The gill and fin parasites *Gyrodactylus branchialis* and *G. ostendicus* collected on *Pomatoschistus microps* and *P. marmoratus* cluster in two homogenous groups: Mediterranean and Atlantic (Fig. 19). The Atlantic group includes samples from Texel and Yerseke (NL), Ambleteuse (F), Ostend and Scheldt (B). This contrasts with *G. gondae* (collected on *P. lozanoi* and *P. minutus*), who show different haplotypes, independent of the sampling site (NL, B or N) and the host on which they reside. The group is represented in a network (Fig. 20). The species seem to evolve very fast (genetic differentiation on a small scale) in contrast to other species. A similar pattern was found on *G. arcuatus*, which parasitizes the threespined stickleback. The species is already differentiated within the Flemish river basins. It has also been found in Stockholm (Sw) on *P. microps* (vector host); divergence between these specimens and those from Belgium amounted to 2 % of the COI mtDNA fragment.

The third species, *G. rugiensoides*, has only been partially analysed on samples from Norway (*P. minutus* and *P. lozanoi*). Samples from Texel (NL), the North Sea and Ostend (B) are being processed. Preliminary results show that the parasites infecting *P. minutus* are identical in their COI mtDNA fragment to those infecting *P. lozanoi*. Previous studies showed that *G. rugiensoides* parasites of *P. pictus*, despite an identical ITS fragment, were different in certain characteristics of the haptorial organ (a trait used for identification). We conclude that both morphotypes represent one single species, which infects all three hosts.

When comparing these results with the phylogeographical pattern of the host (allozymes and cyt *b* mtDNA; Gysels et al. 2004b), *P. microps* turns out to be differentiated in an Atlantic and Mediterranean group, similar to its parasites *G. ostendicus* and *G. branchialis*. In the Atlantic Ocean the host is more structured than its parasites (eg Texel). This means that the factors structuring the host populations have no isolating effect on their parasites. *G. gondae* seems to be evolving faster than its host because it is differentiated on a smaller scale (within the BCS). This is expected because the parasites have a shorter generation time (24 h) in comparison with the host (1 to 2 y).

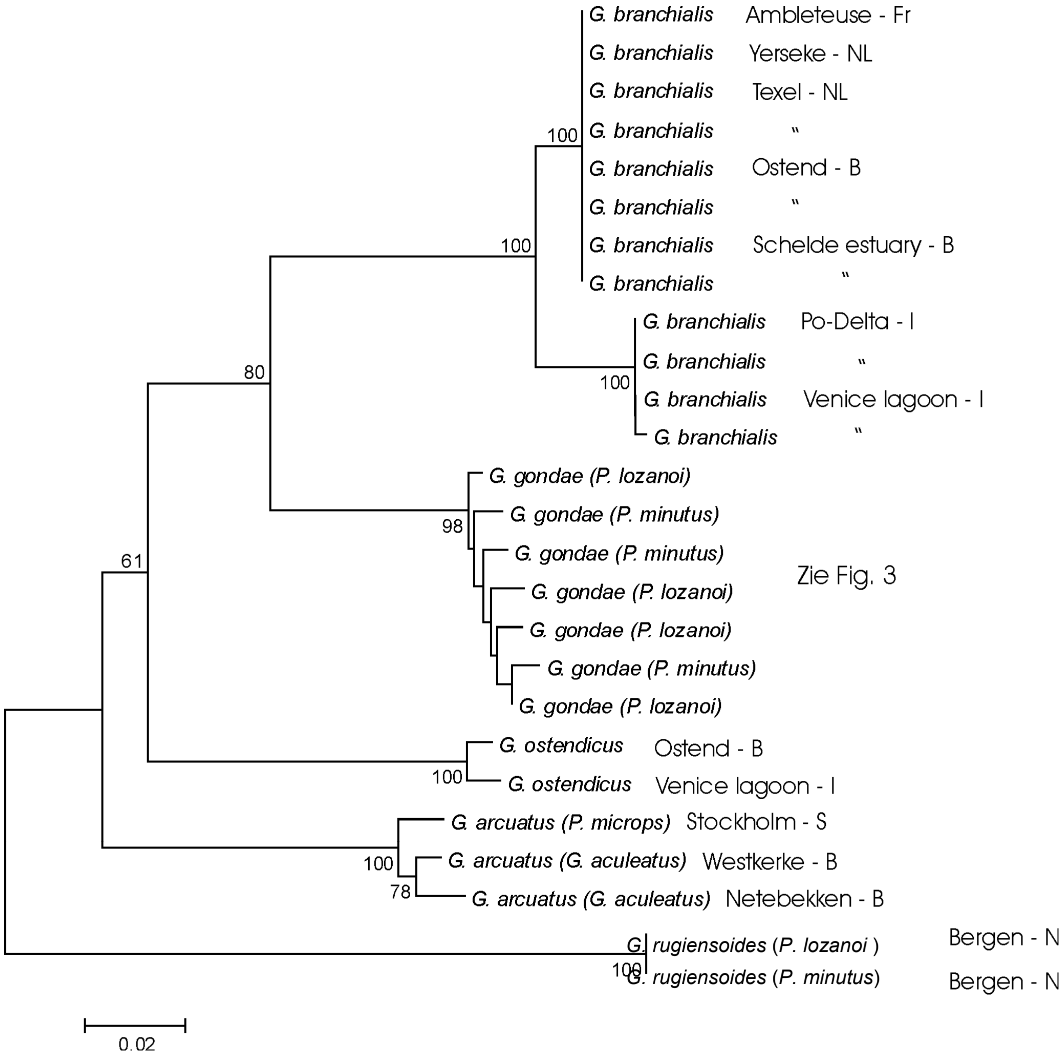

Figure 19. Neighbour joining tree based on COI sequences of three *Gyrodactylus* species collected on marine gobies of the genus *Pomatoschistus*.

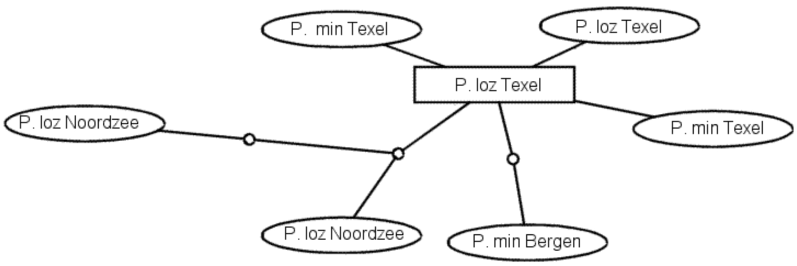

Figure 20. Network based on COI haplotypes of *Gyrodactylus gondae* colled on the BCS, Texel (NL) and Bergen (N). Parasites are named by their host: *Pomatoschistus lozanoi* (P. loz) or *P. minutus* (P. min).

Pomatoschistus gobies

A comparable but more evolved small-scale field study between De Panne and Renesse (The Netherlands) based on seven DNA microsatellites complements the research of Pampoulie et al. (2004) by controlling for site (five coastal stations and two estuarine stations were sampled) and season (samples were collected during the spring spawning season and fall) (Larmuseau 2005). The spatio-temporal distribution of the densities shows low densities at the start of the sampling campaign (April - May 2003), at the time the sand gobies are spawning As soon as the new generation appears in May (less in W so than in C), density increases, especially along the Westcoast. Many postlarval and juvenile gobies were observed along the Eastcoast, possible as a consequence of transport by the dominant NE current. Small gobies of the first density maximum are observed in brackish water in June for the first time. Average length in the estuary increases monthly up to October, in parallel with growth along the coast; it varies among sites and season (Fig. 21).

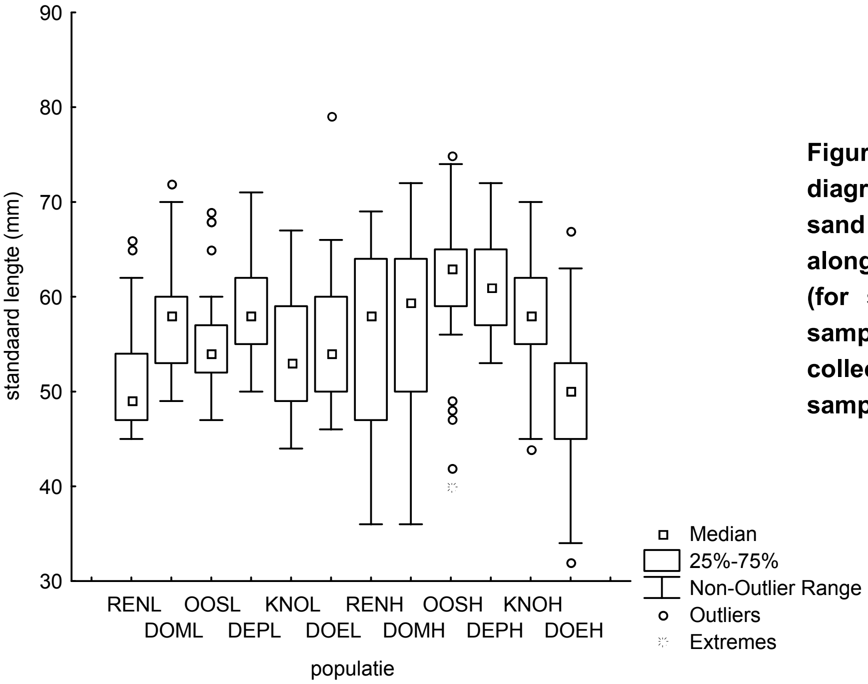

Figure 21: Site - length diagrams (SL in mm) of sand gobies collected along the Southern Bight (for sites see Fig. 1). L samples have been collected in spring, H samples in fall.

Genotypic variation at all seven microsatellite loci was high as judged from various parameters (Table 4).

	RENL	DOML	OOSL	DEPL	KNOL	DOEL	RENH	DOMH	OOSH	DEPH	KNOH	DOEH	TOTAL
pmin-06													
n	44	47	50	48	36	40	44	46	44	46	48	46	-
A	19	19	24	26	21	19	21	19	24	25	24	24	-
H_e	0.878	0.886	0.919	0.922	0.906	0.912	0.890	0.907	0.927	0.913	0.898	0.885	0.904
h_o	0.750	0.617	0.720	0.729	0.667	0.625	0.636	0.739	0.796	0.870	0.833	0.870	0.738
F_{IS}	0.147	0.306	0.218	0.210	0.267	0.317	0.288	0.186	0.143	0.048	0.073	0.017	0.182

Table 4: Genetic variation at one of the seven microsatellite loci scored on 12 populations of *Pomatoschistus minutus*; number of individuals analysed (n), number of alleles (A), average number of alleles (MNA), expected heterozygosity (H_e), observed heterozygosity (H_o) and F_{IS}.

Genetic differentiation between all populations was weak (F_{ST} = 0.009) but statistically highly significant (Fig. 22). Significant temporal differentiation was observed within the populations; spring populations turned out to be more distinct than fall populations, suggesting that spawners belong to distinct groups while progeny tend to mix on the nursery grounds in fall. This study proposes that the "adopted-migrant" hypothesis with a high degree of exchange between neighbouring populations provides a realistic hypothesis to explain the present population genetic structure of sand gobies. As expected, there is evidence that the Scheldt estuary is a nursery area for juvenile sand gobies (Larmuseau 2005).

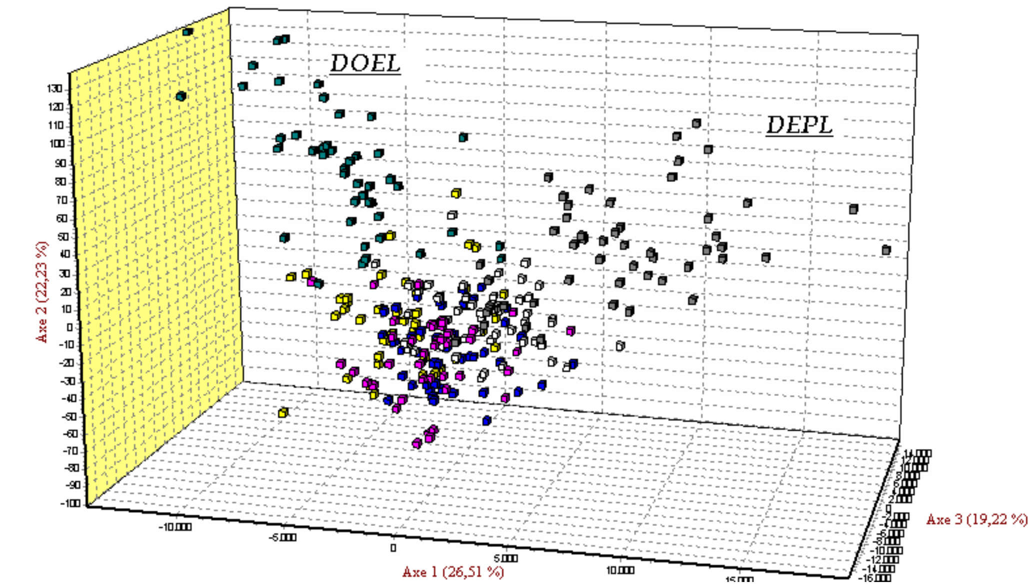

Figure 22: Factorial correspondence analysis based on six spring populations of *P. minutus*.

These results point indeed to the presence of small-scale patterns (named patchy metapopulation), despite the strong hydrodynamics and seemingly continuity of the water masses. It also establishes the scale at which gobies establish their habitat (10 – 100 km) for the first time. But we still don't understand very well (1) the link between spawning ground, nursery and feeding ground, (2) the variation in population structure and functioning between years and (3) the adaptive potential (or the capacity to adjust to environmental and biological variation) of an organism and even a population, be it a sand goby, or any organism on the BCS.

Solea solea

Six primer sets were used for microsatellite amplification in Common sole. For the loci F8-I, -II, -III, -IV, F13, and F14, respectively, 11, 14, 8, 17, 20, and 19 alleles were detected. The observed number of alleles per population increased with sampling size, except that for the Venice and Thessaloniki populations the number of detected alleles is low compared to populations of similar sampling size. This is also reflected in the level of heterozygosity of these two populations, which is lower than in the other populations. Fig. 23 points to clear evidence for isolation-by-distance.

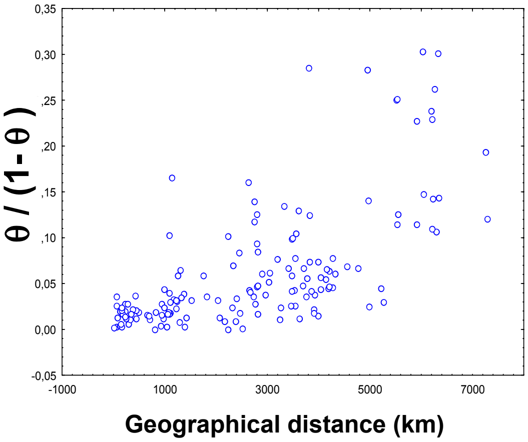

Figure 23: *Solea solea*: Genetic distance (y-axis) against geographical distance (x-axis) showing isolation-by-distance (Note: populations 4-7, 11-17, 20-22, 24, 26 were not included). Mantel test: r = 0.67; p = 0.001.

3-D factor analysis of all 36 populations clearly separates the Atlantic from the Mediterranean samples (Fig. 24). Separation within the Atlantic is less obvious. The four groups (Aegean, Adriatic, West Mediterranean, and Atlantic) are confirmed when θ values of populations are compared pair-wise (not listed). Within the Atlantic not much differentiation is detected, except that the Westdiep population seems to be quite distinct from most Atlantic populations.

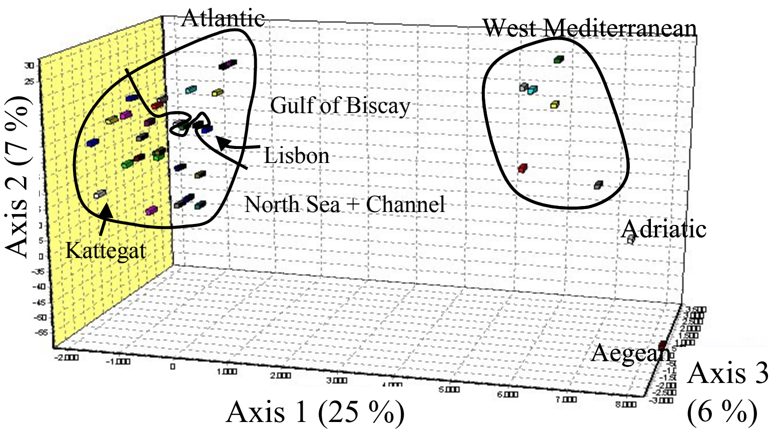

Figure 24:*Solea* solea: Factor analysis of Mediterranean and Atlantic populations. Each dot represents one population.

Modelling

In Figure 25 the residual water transport in the southern North Sea is presented as calculated by the OPTOS-BCZ model. Water movements were calculated over a full spring-neap tidal cycle, *i.e.* from March 2nd 2004, 6h30 till March 17th 2004, 0h00. Water transport generally directed towards the northeast. Depending on the vertical migration regime, larvae are transported differentially. Figure 26 and 28 list the particle tracks of larvae from two different starting points.

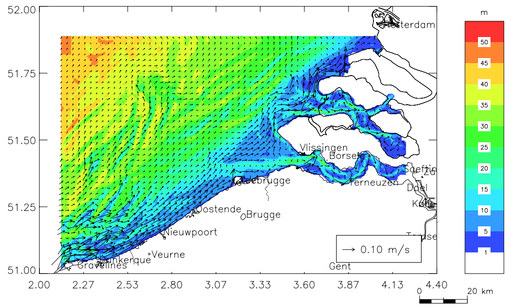

Figure 25: Residual transport vectors in the southern North Sea. A single vector is shown for each nine vectors in the OPTOS-BCZ model.

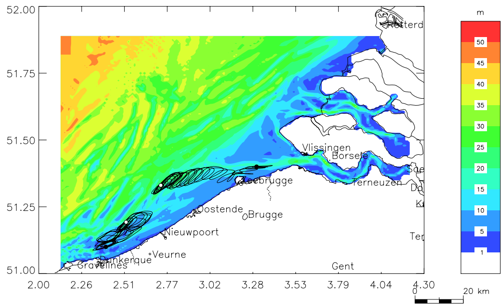

Figure 26: Particle tracks of larvae released at two different starting points. The black dot is the starting point, the white point the ending point after one spring-neap cycle. No vertical migration is implemented.

In Figure 26 no vertical migration is applied; the particle released in the western starting point on the BCS is transported to the east, while the particle released in the point east of the Zeebrugge harbour is moving westwards. In Figure 27 release of the particles is at the same starting points, but with directional vertical migration.

Particles move to the surface, when the direction of the currents is to the southeast and to the bottom during other directions. The influence on the particle tracks is clear. In Figure 28 the release of 200 particles east of Zeebrugge is simulated. A horizontal diffusion of 100 m².s^{-1} without vertical migration is implemented. Particles generally move to the west again, to an area in the centre of the BCS.

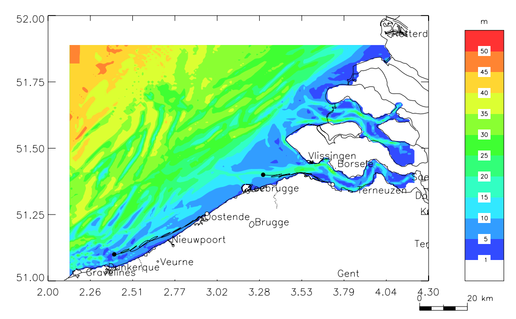

Figure 27: Particle tracks of larvae released at two starting points (off Zeebrugge and Dunkerque). The black dot is the starting point, the white point the end point after one spring-neap cycle. Directional vertical migration is applied.

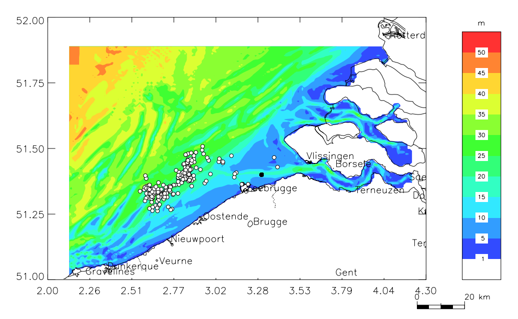

Figure 28: Particle tracks for 200 larvae, released at the black dot. The white points are the ending point after one spring-neap cycle. No vertical migration is applied.

DISCUSSION

Until recently, the physical continuity of the Southern Bight of the North Sea and the English Channel made scientists assume that populations of marine organisms had continuous distributions. However, there is sufficient evidence that, in addition to a high genetic diversity, so typical of marine organisms, populations are structured in space and time.

The mysid *Mesopodopsis slabberi*, common sole, *Pomatoschistus* gobies (Gysels et al. 2004a, b) and their coevolving Gyrodactylus parasites show evidence for historical phylogeographic patterns with a high level of diversity and in some cases (mysid) cryptic speciation (Remerie et al. 2006a). Offshore species (eg *M. slabberi, P. minutus*) tend to be less differentiated than inshore species (eg *Neomysis integer*, *P. microps*) Remerie et al. 2006b). Current North Sea populations reflect postglacial recolonisation in the late Pleistocene from the Gulf of Biscay along an eastern (English Channel) and western (Atlantic) route. In some cases the suture zone between both recolonisations (evidenced by the cooccurrence of divergent haplotypes) is located in the Southern Bight of the North Sea.

Spatio-temporal patterns on the BCS are more subtle and if they exist can only be resolved with high-resolution genetic markers. No significant spatial differentiation was observed yet for *M. slabberi* and common sole, but we managed to differentiate for the sand goby between a Westcoast and Grevelingen/Easternscheldt population. Temporal differentiation has been observed among cohorts of common sole (although in the Gulf of Biscay), sand goby and European eel (Maes et al. 2006). Temporal population fluctuations may influence the effective population size (*Ne*) and hence influence genetic drift. Fishing pressure on commercial species such as common sole negatively influences *Ne* and adaptive variation (Rijnsdorp, pers. comm.).

The modelling exercise provided a first local attempt for calculating the transport and the fate of larvae on the BCS. Preferential areas where the larvae will be transported on the BCS can be calculated. The model will be expanded in the future, to include the full life cycle of the fish larvae, from passive pelagic transport as eggs to selective tidal stream transport as larvae. The water temperature could be the driving force for the transition from one stage to the next.

In conclusion, the sand banks on the BCS and the estuaries along the coast seem to function as landscape elements promoting isolation, while the strong tidal currents so typical of the southern North Sea influence but do not fully mix the populations. Those are rather novel conclusions for organisms that have a pelagic larval stage lasting weeks to months. The future will bring us further details on the ecological meaning of small scale genetic patterns, and the inclusion of adaptive variation thanks to progress in genomics.

4. POPULATION DYNAMICS OF GULLS AND TERNS

METHODS

Population size, reproductive parameters and diet composition of Common Tern *Sterna hirundo* and Sandwich Tern *Sterna sandvicensis* were studied in the Zeebrugge colony (51°21'N, 3°12'E) during four successive breeding seasons in the period 2002-2005. Reproductive performance was measured in at least 25 nests that were enclosed by 50 cm high wire netting to prevent the chicks from leaving the colony site (more details in Stienen 2006). For comparison and for a broader understanding of spatio-temporal patterns, additional data were used that were derived in earlier years in Zeebrugge as well as data obtained in the colonies on Griend (53°15'N, 5°15'E), Dutch Wadden Sea, in 1992-2000 and Hirsholm (57°29N, 10°38'E) in the Danish Kattegat in 1997.

Within the enclosures all chicks were ringed with a unique metal ring and about 20 chicks were colour dyed to further enhance individual recognition. Observations on food transport to the colour dyed chicks were performed from an elevated hide placed at a few meters from the enclosure. We noted prey species (distinguishing clupeids *Clupeidae*, sandeel *Ammodytidae*, and other prey species), prey size (relative to the bill length of the parent carrying the prey) and fate of the prey (eaten by the chick, robbed by Black-headed Gull *Larus ridibundus*, prey lost when out the observer's view, eaten by parent, robbed by species other than Black-headed Gull and other losses).

Observations on courtship behaviour were conducted between 5 and 21 May on Hirsholm, between 16 April and 6 July on Griend and between 2 and 9 May in Zeebrugge. Most observations concerned ground displaying males that were observed from a hide placed at a few metres from a roost or a settling group. In Zeebrugge and in a minority of the sightings at Griend and Hirsholm, the food of aerial displaying males was identified using binoculars. Because of the very low sample size of fish used in courtship displays in Zeebrugge (8 and 4 fishes, respectively in 2003 and 2004), the two years were treated as one sample.

Diet of incubating adult Sandwich Terns was studied by food remains present in the faeces. On Hirsholm and in Zeebrugge, faeces samples were collected from the central part of the colonies and on Griend from the centre of main subcolony. Sampling took place 1-2 days before hatching of the first egg in the involved (sub)colony to ensure that the faeces originated from adults only. In each colony, 10-20 nests that were not neighboured by species other than Sandwich Tern were selected at random. The guano layer surrounding the nest was removed and stored dry for later analysis. Given the high nest density of the terns in the colony centre, a sample contained excrements from parents of the sampled nest as well as from neighbouring couples. Therefore, the samples collected in a specific (sub)colony were pooled together. In the laboratory, the pooled material was carefully washed with water, parts of vegetation were removed, and remains of animal material were retained and stored dry. The fish otoliths were later identified using Härkönen (1986) and Leopold et al. (2001). Most otoliths showed very little sign of wear due to erosion by gastric acids and 97.8% of a sub sample of 2741 otoliths found on Griend and Hirsholm were classified as 'apparently complete with irregular perimeters and clearly visible sulcus' (wear class 1 after Leopold et al. 1998).

Prey energy content was computed by applying the allometric relationships between length of *Ammodytidae* or *Clupeidae* and prey energy content given by Stienen & Brenninkmeijer 2002a. For prey species other than *Ammodytidae* and *Clupeidae* we used the relationship given for *Ammodytidae*. This seems justified since 'other prey' always concerned prey of lower energy density than *Clupeidae*, and were thus more comparable to sandeel. Also the category 'other prey' accounted for only a small proportion of chicks' diet and often concerned small prey (mainly Gobidae), so that this will have only a minor influence on the final results.

To calculate daily delivery rates of prey and energy only data of first hatchlings were used since second hatchlings received almost no food and normally starved to death soon after hatching. Daily observation bouts of individual chicks lasted between 0.3 and 10.4 h (average 4.3 h, SD = 1.7 h). Prey and energy delivery rates were converted into daily values by assuming a daylight period of 18 h.

For readability of the INBO-results, we chose to discuss the most important and most coherent results in this final report. More details on diet composition of beach-washed seabirds, foraging distribution of Sandwich and Common Terns and breeding biology and population dynamics of terns and gulls can be found in (Stienen et al. 2002, Van Wayenberge et al. 2002, Braarup Cuykens 2003, Brabant 2004, Courtens & Stienen 2004, Del Villar D'Onofrio 2005, Stienen et al. 2005, Verstraete 2006).

RESULTS

Population dynamics of terns

Since the first settlement of Common Tern in the outer port of Zeebrugge in 1987, breeding numbers show a steady increase (Fig. 29). Peak numbers of 3052 pairs were reached during the study period in 2004, corresponding to 4.8% of the total biogeographical population. Only in 2005 numbers decreased, probably due to food shortage hampering settlement and chick growth of Common Terns in large parts of the southern North Sea (this study; also Meininger et al. 2006). The Sandwich Tern shows a much more erratic occurrence in Zeebrugge, with peak numbers of > 1500 pairs in 1993, 2000, 2004 and 2005. Maximum numbers of Sandwich Tern (4067 pairs in 2004) correspond with 7.2% of the biogeographical population. Since

Zeebrugge regularly supports more than 1% of the biogeographical population of these tern species, the area is of high international importance. For that reason, parts of the outer port of Zeebrugge as well as the main feeding areas at sea were recently assigned as Special Protected Area under the Bird Directive.

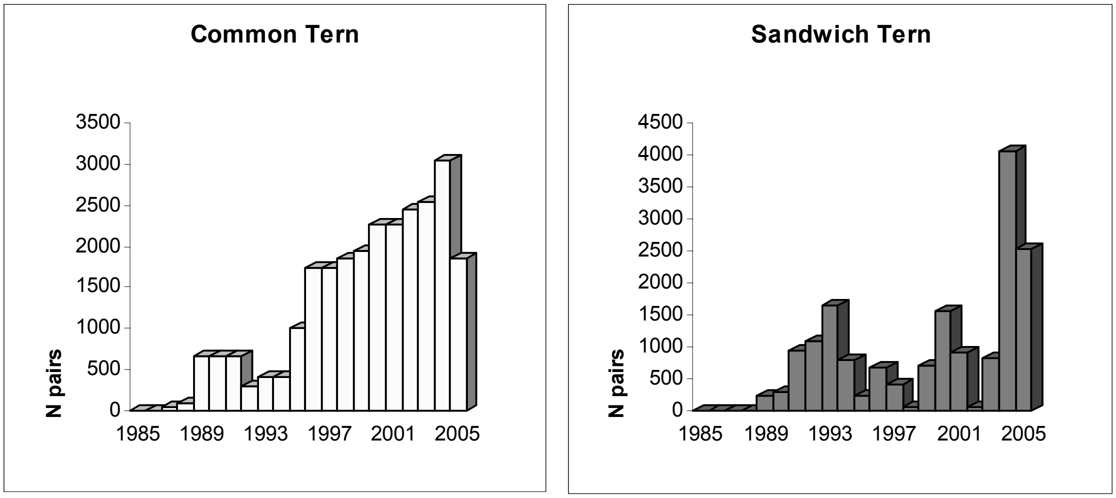

Figure 29. Fluctuations in the breeding numbers of Common and Sandwich Tern in the outer port of Zeebrugge since 1985.

Year	Clutch size	Hatching success	Fledging success	Breeding success
1997	2.4	78	50	1.2
1998	2.5	77	61	1.2
1999	2.5	78	67	1.3
2000	2.3	91	37	0.8
2001	2.3	80	74	1.4
2002	2.2	79	8	0.1
2003	2.6	87	74	1.7
2004	2.1	81	38	0.7
2005	2.0	80	36	0.6

Table 5. Reproductive parameters of Common Tern in Zeebrugge in 1997-2005. Listed parameters are: clutch size (i.e. N eggs per nest), hatching success (proportion of eggs that successfully hatched chicks), fledging success (i.e. proportion of chicks that

Common Tern

Long term data on reproductive performance of Common Terns breeding in Zeebrugge show that reproductive output greatly varied between 0.1 and 1.7 fledged chicks per pair, respectively in 2002 and 2003 (Table 5). Reaching an average breeding success of 1.0 fledglings per pair during the past 9 years, Zeebrugge ranks among the most productive breeding sites in Europe (compare Becker & Ludwigs 2004), although at most sites data is not gathered in a similar standardised way and it is not easy to compare with our results.

Historical data collected in the colony on Griend suggest rather low productive performance of the population during the 1980s (Stienen & Brenninkmeijer 1992). Standardised measurements in the 1990s (similar to the method as used in Zeebrugge) suggest persistently poor success rates and present declines of this population are in line with this (Becker et al. 1997, Lutterop & Kasemir 2006). Also compared to standardised measurements of breeding success in various German Wadden Sea colonies (Becker 1998), parents in Zeebrugge reach high reproductive outputs.

Why then are Common Terns so successful in Zeebrugge? In the first place feeding conditions in and around the Zeebrugge port seem to be very good (although no sampling data on pelagic fish is available that can support this) and secondly, due to less competition for food and space, newly settled, growing colonies may reach higher outputs than established colonies. A strong relationship between the size of the Zeebrugge population and the body conditions of the chicks (Fig. 30) certainly suggests that competition may play an important role. Only data gathered in 2002 do not fit the relationship (Fig.30). In that year adverse feeding condition set in at the end of the chick-rearing period.

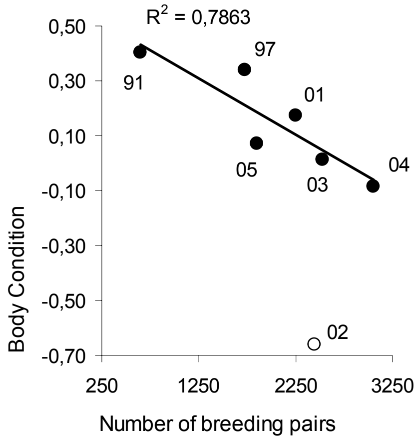

Figure 30. Relationship between the population size and the body condition index of chicks in Zeebrugge in 1991-2005. The body condition index is a measurement of body mass relative to the size of chick and was adapted from Stienen & Brenninkmeijer 2002b. Note that the year 2002 was omitted from the regression analysis.

Further investigation of the data on reproductive performance of Common Terns in Zeebrugge suggests that the chicks' probability to survive until fledging, but not the hatching success of the eggs, determines the final breeding success of the terns (Fig. 31). In other words: it seems that environmental factors acting during the chick-rearing period (and not so much factors acting during incubation) influence the reproductive performance of the parents. Hatching success of the eggs did not vary much and was always higher than 75%. Remarkably, clutch size also correlates (although less strongly) with breeding success (Fig. 31). These findings suggest that the terns' reproductive performance is already influenced at the onset of breeding season and that the influence lasts throughout the chick-rearing period. The most likely factor influencing the terns' reproductive abilities in Zeebrugge is the availability of their food, since this directly influences the energy acquisition by the adults. In poor years, parents may not find enough food to invest sufficient amounts of energy in egg production as well as in the rearing of their offspring. Apparently, in those poor years parents experienced no difficulties in incubating and defending their eggs, although these costs can be high in birds and may incur high fitness costs, but these might be manifested only at the chick-rearing phase (Cichoń 2000).

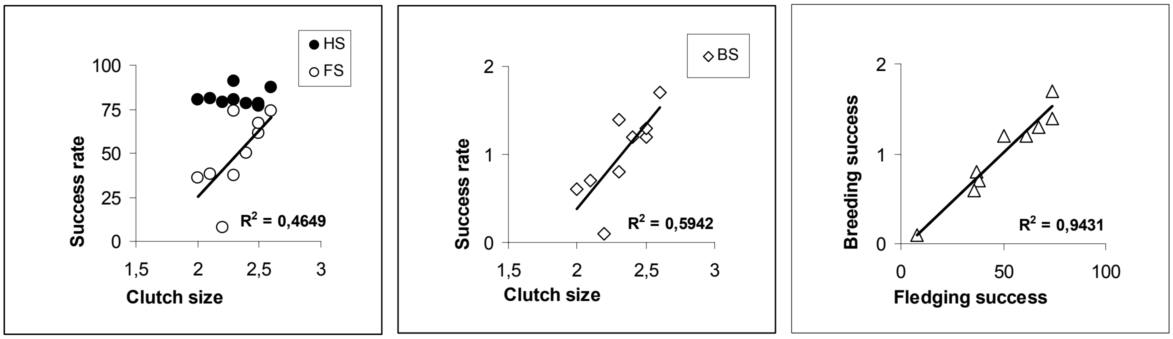

Figure 31. Correlations between various reproductive parameters of Common Terns in Zeebrugge in 1997-2005 using Pearson regression on data from Table 1. HS = hatching success, FS = fledging success and BS = breeding success.

Data on reproductive performance as those gathered in this study make it possible to predict the growth of the population 3 years later when the birds recruit to the colony. Although such predictions may not be very accurate since crude assumptions have to be made on survival probabilities, it may help to pinpoint particular bottlenecks hampering the actual population development. Here data on breeding success (Table 5) was fed into a very simple population model that uses fixed survival rates of adults and juveniles (adult survival rate of 0.90, a survival to recruitment of 0.27 and assumed first breeding when 3 years old; extracted from Becker & Ludwigs 2004) to predict the population size. This exercise shows that, except for 2005, observed population size closely follows the expected size of the Zeebrugge population (Fig. 32). In other words: the study data suggest that Zeebrugge's Common Tern population is largely self-supporting and that population growth primarily results from recruitment of own birds that were born in the Zeebrugge colony, rather than from immigration of foreign birds to the Zeebrugge colony.

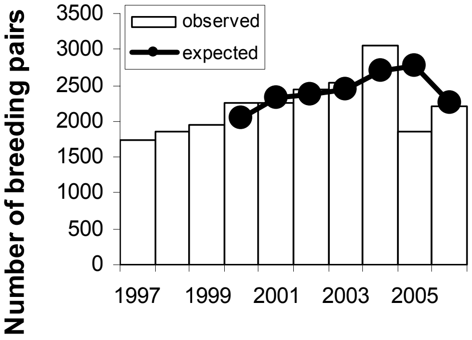

Figure 32. Observed and predicted fluctuations in the number of Common Terns breeding in Zeebrugge from 1997-2006.

This view is further supported by data on ring recoveries. Of 167 Common Terns ringed as pullus and recovered by INBO during a subsequent breeding season (period May - July), 86.8% were ringed as a chick in the Zeebrugge colony itself. In total 40 recoveries concerned birds ringed as adults of which 95.0% was ringed in Zeebrugge. Non-Zeebrugge birds all originated from nearby colonies in the Dutch Delta area and predominantly from colonies in the southern part of the Westerscheld (Fig. 33). With a high proportion of young birds (50% of the population is less than 5 years old; Fig. 6) the age composition of the Zeebrugge colony is rather typical for a self-sustaining seabird colony (compare Stienen & Brenninkmeijer 1992).

Figure 33. Ringing locations of Common Terns (left panel) and Sandwich Terns (right panel) recovered in Zeebrugge during the breeding season (May-July) and ringed as pullus (dots) or adult (stars) elsewhere. The size of dots gives an indication of the number of birds originating from that specific site (1-5 individuals in left panel and 1-14 in right panel).

Sandwich Tern

Of 202 Sandwich Terns ringed as pullus and recovered by INBO during a subsequent breeding season (period May - July), 83.7% originated from the Zeebrugge colony itself. In total 17 recoveries concerned birds that were ringed as adults and of these 82.4% was ringed in Zeebrugge, whereas the remaining 3 individuals were ringed during migration or wintering. Ringed immigrants predominately originated from Dutch colonies (Hooge Platen and Hompelvoet in the Delta area and Griend in the Dutch Wadden Sea) and to a lesser extent from colonies in the UK and Ireland (Shoeburness, Farne Islands and Lady's Island Lake; Fig. 33).

At present, the Zeebrugge colony seems not to be self-sustaining, but probably is part of a larger metapopulation that might be self-sustaining in itself. Breeding success of Sandwich Terns was rather poor in some years (Table 6) and the average breeding success in Zeebrugge (0.4 fledglings per pair) clearly is below self-sustaining levels. The breeding numbers fluctuate heavily (Fig. 29) and unlike in other colonies (compare for example Brenninkmeijer & Stienen 1992), the age composition of the Zeebrugge population is very much skewed towards older individuals (Fig. 34). In contrast to Common Tern, in this species clutch size was not indicative for the survival of eggs or chicks (Pearson regression, not significant in all cases).

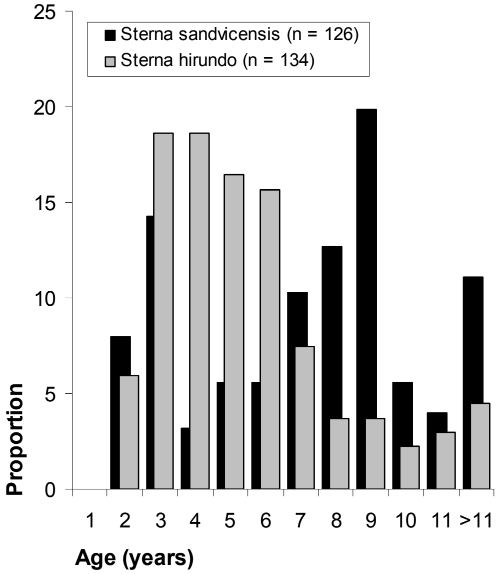

Figure 34. Age composition of 126 Sandwich and 134 Common Terns ringed as pullus (either in Zeebrugge or elsewhere) and recovered in Zeebrugge during a subsequent breeding season (May - July).

Year	Clutch size	Hatching success	Fledging success	Breeding success
1997	1.5	58	13	0.1
1998				
1999				
2000	1.7	80		
2001	1.1	74	70	0.6
2002	1.1	nihil	43	nihil
2003	1.3	90	66	0.8
2004	1.5	90	52	0.7
2005	1.2	57	28	0.2

Table 6. Reproductive parameters of Sandwich Terns in Zeebrugge during the period 1997-2005. Listed parameters are: clutch size (i.e. N eggs per nest), hatching success (proportion of eggs that successfully hatched chicks), fledging success (i.e. proport

Distribution of gulls

Recovery data on 481 Herring Gulls *Larus argentatus* and 409 Lesser Black-backed Gulls *Larus fuscus* that were colour-ringed as a chick in the Zeebrugge colony in 1999 and in 2006 were used to compare spatio-temporal distribution patterns outside the breeding area. Both species were present in or near the breeding grounds during the period April-July, but started migration from august onwards. Typically, Lesser Black-backed Gulls moved much further away from the colony than Herring Gulls. In January Lesser Black-backed Gulls were on average 1765.2 km (N = 23, SD = 1125.2) away from the colony, whereas Herring Gulls reached an average maximum distance of 75.3 km (N = 395, SD = 61.5) in October (Fig. 35).

Dispersion was in all directions, although in both species there was a marked tendency for southwestward movements. Herring Gulls were recovered as far north as Schleswich-Holstein in Germany and the most northern Lesser Black-backed Gulls were seen in the Dutch province of Drenthe (Fig. 36). Most recoveries to the north of the Zeebrugge colony are from the Dutch Delta area and the southeast of the UK. This phenomenon of overshooting the colony might be more common in juveniles and subadults (Campbell & Lack 1985, Vercruijsse 1999, Van Wayenberge et al. 2002), but adults of the Zeebrugge colony were also found to the north of their colony of birth. In the literature, there is some discussion on whether or not adults and sub-adults exhibit different migration patterns. Some studies suggest that non-adults winter farther south than adults (a.o. Parsons & Duncan 1978, Vauk & Pruter 1987), whereas other studies suggest hat there is no difference in wintering areas between young and adult birds but only a difference in the duration that birds stay at the wintering grounds (Coulson & Butterfield 1985, Vercruijsse 1999, Calladine 2002, Van Wayenberge et al. 2002). A graph showing the average distance from the colony in relation to the age of gulls from Zeebrugge (Fig. 37) rather suggest the opposite, namely that immature gulls (and particularly young Herring Gulls) on average stay closer to the colony than adults. In their first year, Herring Gulls from Zeebrugge stayed at more than 50 km from the colony, but progressively moved closer to the colony. In accordance with earlier findings of Coulson & Butterfield (1985), but in contrast to Kilpi & Saurola (1983, 1984), immature Herring Gulls of the Zeebrugge colony on average do not show the typical circular movement pattern away from the colony after the breeding season and back in winter. Only when they reached an age of 3 years a circular pattern evolves and at an age of 4 years more pronounced movements were recorded (Fig 37). Our data suggest that Lesser Black-backed Gulls already exhibit circular movements during their first years (although less pronounced than in adults), but the data are not sufficient to analyse further migration patterns of sub-adults in this species. Similar to Herring Gulls, adult Lesser Black-backed Gulls from Zeebrugge seem to wander further south than immatures, which is in contrast to birds from Britain and Ireland (Rock 2002 and Baker 1980 therein). Further investigations of newly ringed cohorts are necessary to confirm these regional differences.

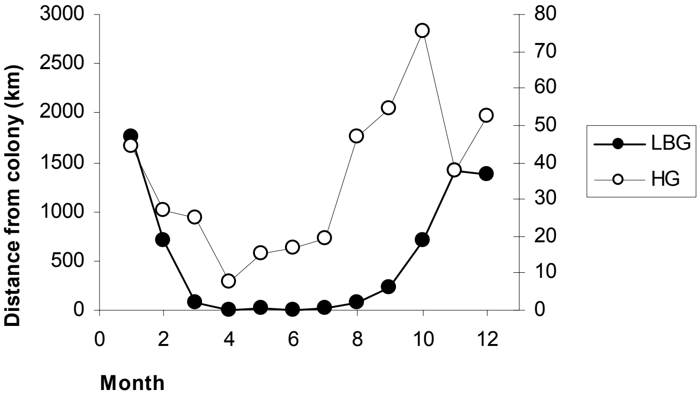

Figure 35. Seasonal variation in the average distance away from the colony of Herring (HG projected on the right axis) and Lesser Black-backed Gulls (LBG on left axis) from Zeebrugge.

The most southern Herring Gulls were reported from the Seine estuary, but the majority stayed along the coastlines of Northern France, Belgium and the Dutch Delta area during winter. Lesser Black-backed Gulls were reported wintering as far south as Mauritania, but the majority was found wintering at the Vendée (France) and in Iberia. Both species were predominantly reported from coastal areas; reports outside the coastline nearly all concern rubbish dumps. More details on the distribution and breeding biology of Belgian gulls are presented in Stienen et al. 2002 and Van Wayenberge et al. 2002).

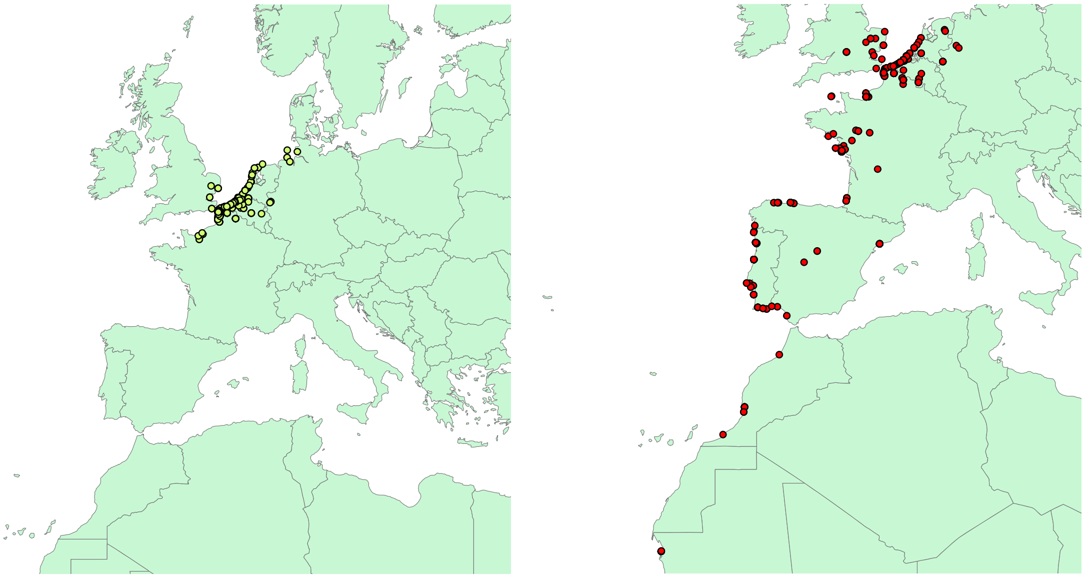

Figure 36. Recovery locations of Herring (left graph) and Lesser Blacked Backed Gulls (right graph) that were ringed as chicks in Zeebrugge.

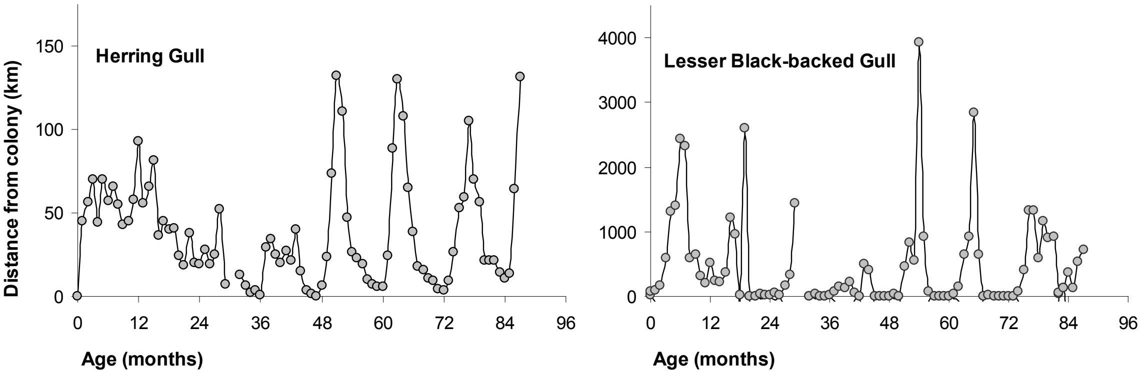

Figure 37. Average distance away from the Zeebrugge colony in relation to the age of Herring and Lesser Blacked Backed Gulls.

Food availability of terns

Sandwich Terns are known as highly specialised seabirds that predominantly feed on an exquisite diet dominated by a few marine fish species of high nutritive value. Very occasionally other prey items are reported such as squids, crustaceans, insects and worms (Cramp 1985, Glutz von Blotzheim & Bauer 1982, Hallet 1984, Del Hoyo et al. 1996, Leopold et al. 1996, Stienen et al. 2000). In the North Sea and the North Atlantic, *Ammodytidae* and *Clupeidae* are the predominant prey fish of Sandwich Terns. Normally these prey constitute more than 95% of the chicks' diet (Langham 1968, Pearson 1968, Veen 1977, Garthe & Kubetzki 1998, Stienen et al. 2000, Stienen 2006). In line with these findings, also Zeebrugge chicks were fed high proportions of *Ammodytidae* and *Clupeidae*, with clupeids being the most dominant prey group in all study years (Fig. 38). Relatively to other colonies Zeebrugge chicks were fed higher proportions of other fish species. These species consisted of Gobidae, Atlantic Cod *Gadus* morhua, Whiting *Merlangius merlangus, Trisopterus* spp., Pipefish *Sygnathus spp.* and Sea Lamprey *Petromyzon marinus*. In Zeebrugge, the proportional importance of the category 'other species' in the chicks' diet fluctuated between 5.8 and 20.7%, and was strongly, negatively correlated with the proportion of clupeids in the chicks' diet (Pearson regression, $r^2 = 0.90$, $p < 0.001$). By contrast, the proportion of Ammodytidae was rather constant and fluctuated between 21.3 and 23.7%. Only in 2001, chicks were fed a lower proportion (13.8%) of sandeel. It thus seems that the availability of Clupeidae in the terns' feeding areas strongly varied between years and that in poor clupeid years less nutritive prey were included in the chicks' diet. In particular in 2002 the proportion of clupeids in the chicks diet was low and this coincided with very low numbers of breeding Sandwich Terns (Fig.29) and poor survival of the chicks (Table 6).

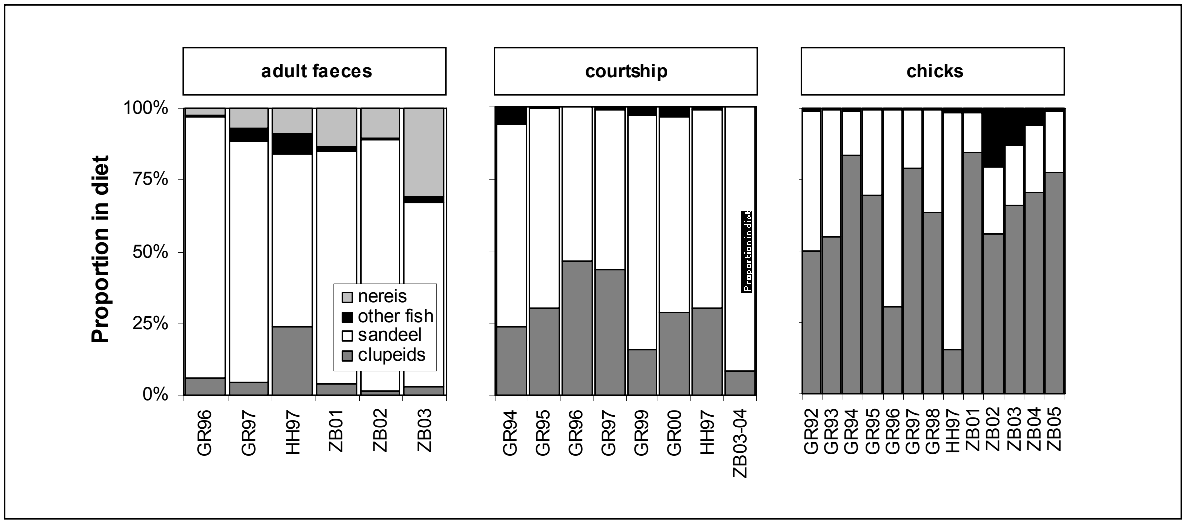

Figure 38. Diet composition of self-feeding adult Sandwich Terns as reflected by prey remains found in the faeces (adult faeces) compared with observations on adults courtship feeding their mate (courtship) and parents feeding their chicks (chicks). GR96 = Griend 1996, HH97 = Hirsholm 1997, ZB01 = Zeebrugge 2001, etc.

In the winter quarters, European Sandwich Terns switch to other species, but small marine fish remain to predominate the diet (Dunn 1972, Grimes 1977, Brenninkmeijer et al. 2002). In the breeding areas the diet of adults remains poorly studied. During the pre-breeding period most information on the food of adults relates to observations of males feeding their mates during courtship display. In the North Sea and North Atlantic, *Ammodytidae* and *Clupeidae* are commonly used by adults in courtship display and are supplied to incubating mates (Taylor 1975, Fuchs 1977, Veen 1977).

There are only a few quantitative studies that describe the diet of adult Sandwich Terns (Blus et al. 1979, Shealer 1998, Favero et al. 2000, McGinnis & Emslie 2001) in the East Atlantic and there is only one dated European study (Collinge 1925). Not surprisingly most of these studies found that adult Sandwich Terns feed on a diet dominated by energy-rich fish, but some found remarkably high proportions of non-fish items. Here we present data on 3 colonies dispersed over Northwest Europe (Fig. 35).

It shows that in all three colonies and all study-years self-feeding adults feed on an entirely different diet than when provisioning chicks or mates. Faeces samples of self-feeding adults contained very high proportions of sandeel. Other fish prey mainly consisted of Sprat *Sprattus sprattus* and Herring *Clupea harengus*, and few otoliths of Cod, Whiting, Bib *Trisopterus luscus* and Poor-cod *Trisopterus minutes* were also found. Remarkably, in all colonies and all years, faecal samples contained jaws of ragworms (*Nereidae*). In Zeebrugge 2003, nereid jaws even made up 30.8% of all prey remains found in the faeces samples. Also the distribution of prey length differed between the various stages of the breeding cycle. Self-feeding adults exhibited a broader choice of prey lengths, but on average took larger prey than when feeding partners or chicks (Fig. 39). Transporting energy rich fish prey (Clupeidae have higher energy densities than Ammodytidae; Stienen & Brenninkmeijer 2002a) to the colony to feed chicks and females, whereas Ammodytidae and nereid worms of lower energy values were included in the diet of self-feeding adults is consistent with an optimal foraging strategy of birds that are restricted to return to a central place to feed their young or mates (Lessel & Stephens 1983). Also broadening of the diet when self-feeding fits the suppositions of the optimal foraging theory. Similar results have been found in a wide variety of seabirds. Several alcid species take smaller or lower-quality prey when self-feeding than when provisioning their chicks (Bradstreet & Brown 1985, Baird 1991, Davoren & Burger 1999, Mehlum 2001) and Great Skuas *Chataracta skua* preferentially feed their young more energy rich fish (Furness & Hislop 1981, Votier et al. 2003). Similarly, Fulmars *Fulmarus glacialis* and many Laridae seem to selectively feed their chicks on an energy rich diet (Spaans 1971, Pedrocchi et al. 1996, Shealer 1998, Ojowski et al. 2001). However, such strong contrast in diet between adults and chicks seems unique for Sandwich Terns. We hypothesise that the dietary difference between adults and chicks is larger in single prey loaders that must return to the colony to feed their chicks or mates each time they catch a fish and particularly large in species that experience high flight costs due to long travelling time.

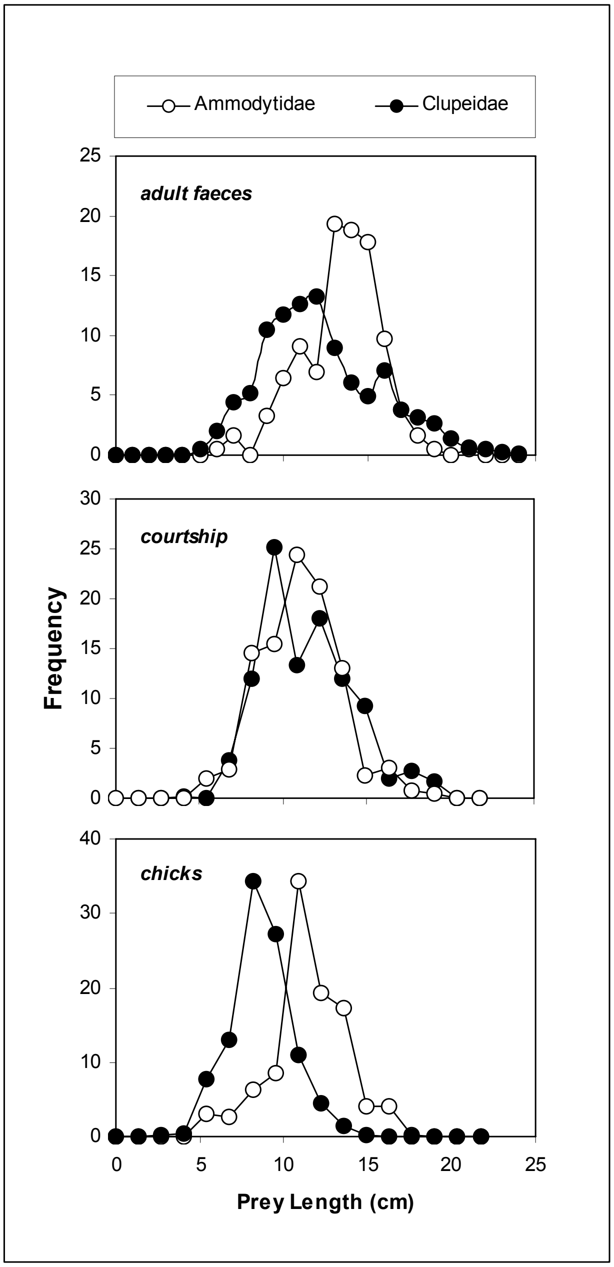

Fig. 39. Length distribution (in cm) of Ammodytidae and Clupeidae found in adult faeces (upper graph), observed in courtship behaviour and during chick feeding of Sandwich Terns (pooled data of Griend, Hirsholm and Zeebrugge).

Comparable to earlier studies in The Netherlands (Griend), also in Zeebrugge Sandwich Tern parents fed their offspring with increasingly large prey items to meet their increasing energy demands of their growing chicks (Fig. 40). Both in Zeebrugge and on Griend, and throughout the chick-rearing period, sandeel were larger than clupeids. However, in Zeebrugge parents fed larger sandeel and smaller clupeids to the chicks than on Griend. The smaller clupeid size was noticeable during the first two weeks after hatching. Given that during this period clupeids dominated the chicks' diet, parents in Zeebrugge compensated for their small size by an increased prey transport (Fig. 12). Prey delivery rate strongly decreased from 14.8 prey/chick/day at hatching to an average of 8.4 fishes/chick/day after day 10. In contrast, on Griend prey delivery rate was constant throughout the chick-rearing period (about 9-10 fish/chick/day according to Stienen et al. 2000).

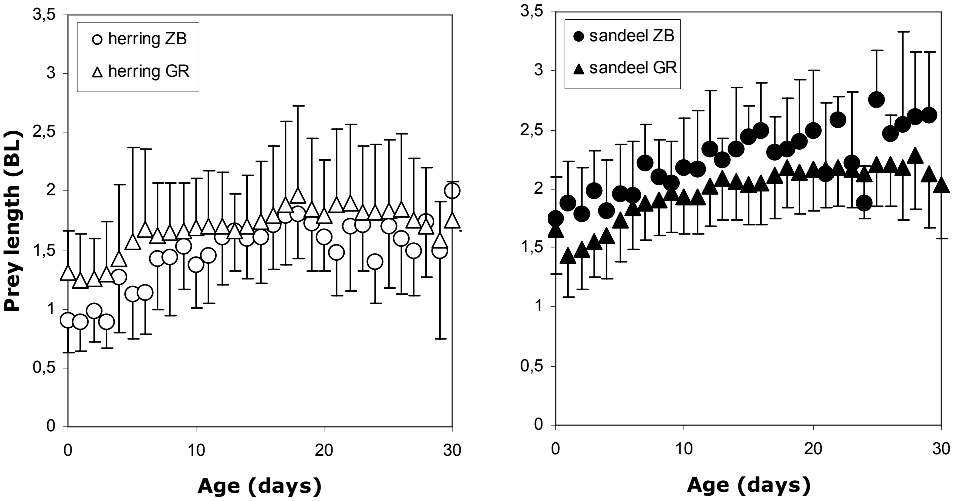

Figure 40. Effect of the age of the tern chicks and the length of Clupeidae (mean ± SE, left graph) and Ammodytidae (mean ± SE, right graph) brought to the colonies in Zeebrugge (dots) and Griend (triangles). Prey length is expressed in bill length of the parent measuring on average 5,43 cm.

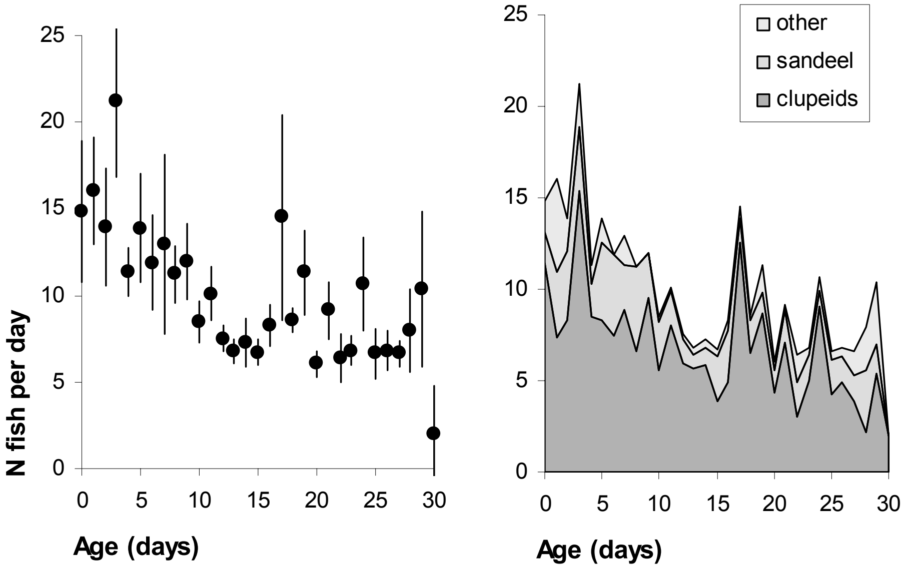

Figure 41. Relationship between the age of the chicks and prey delivery rate (mean ± SE) in Zeebrugge (left graph). The right graph shows changes in the diet composition throughout the chick-rearing period.

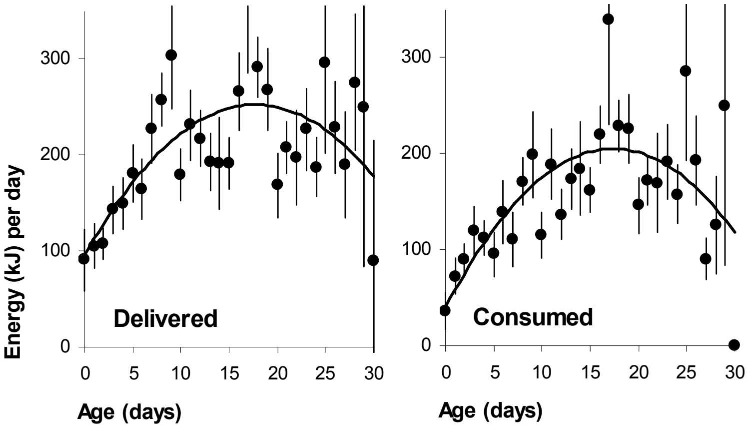

Figure 42. Effects of the age of the chicks on energy delivery rate (mean ± SE) and energy consumption rate (mean ± SE) in Zeebrugge.

The combined increase in prey length and decrease in prey numbers with the age of the chicks translates into a parabolic relationship between the age of the tern chicks and the amount of energy transported to the Zeebrugge colony (Fig. 42). On Griend, the constant prey delivery rate and an increased prey length resulted in a steady increase of energy transport to the colony with the age of the chicks (Stienen et al. 2001). A parabolic curve of energy transport is more in line with the development of the energy demands of chicks reaching a maximum when about two weeks old (Klaassen et al 1992). Why than do we find different curves of energy transport for Griend and Zeebrugge? The reason is that not all energy that is brought to the tern colony is actually consumed by the chicks. The ratio between energy supply and energy consumption is much more favourable in Zeebrugge than on Griend. On Griend, a few days after hatching more than 25% of the supplied energy is actually not consumed by the chicks and this proportion even increases to more than 30% after day 13. In Zeebrugge, the proportion of energy loss is generally lower than 20%. The reasons for not consuming food that parents deliver to the colony are very different in Zeebrugge and on Griend (Fig. 43). On Griend, a very high proportion of the food is lost to kleptoparasitising Black-headed Gulls. With the aging of the chicks and the increasing length of prey, the gulls steal an increasing proportion of the food that is actually meant for the tern chicks. Since the gulls prefer to rob the larger prey items, this has an even higher impact on the proportion of the energy that is actually consumed by chicks. The present food situation around Griend in combination with the high rates of kleptoparasitism causes a lot of food stress for Sandwich Tern parents (Stienen & Brenninkmeijer 2002) undernourishment is consequently an important cause of death for Sandwich Tern chicks (Stienen & Brenninkmeijer in press). The numbers of Black-headed Gulls breeding in Zeebrugge are much smaller than on Griend (maximum size during the study period in Zeebrugge was 2390 pairs, whereas Griend harbours > 20.000 pairs). More important, in Zeebrugge egg-laying of the gulls on average starts about 2 weeks before the first tern eggs are laid, while at Griend Sandwich Terns breed much more synchronised with Black-headed Gulls. As a result, kleptoparasitism is not very pronounced in Zeebrugge, whereas the loss of food for other reasons is comparable to the Griend situation (Fig. 43).

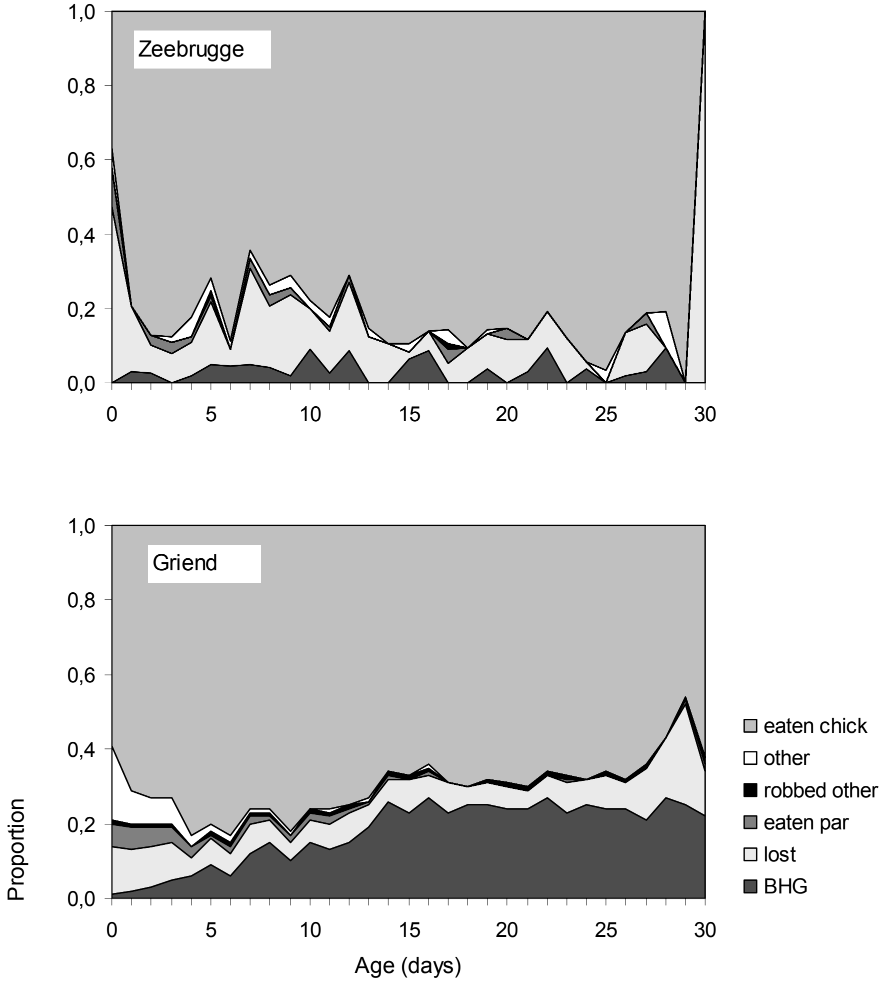

Figure 43. Relationship between the age of Sandwich Tern chicks and the fate of the prey delivered to the colonies in Zeebrugge and Griend. BHG = prey robbed by Black-headed Gull, lost = lost when out of the observer's view, eaten par = consumed by the parent, robbed other = robbed by a species other than Black-headed Gull, eaten chick = consumed by the chick and other = other reason.

Concluding, in Zeebrugge only a marginal portion of the food is lost to prey robbing gulls. Therefore parents can afford to bring less food and smaller prey to the colony than is the case on Griend, while the chicks still reach comparable high energy intake rates. The most pronounced effect of the absence of high rates of kleptoparasitism can be found in the amount of time that parents allocate to foraging. In both colonies, small chicks were always attended by at least one parent while the partner went foraging (Fig. 44). Small tern chicks still need brooding (Klaassen et al. 1994), but older chicks can be left alone without much energetic consequences and are furthermore less prone to be caught by avian predators (Veen 1977). On Griend, older chicks were often left unattended at the nest. Parents left the chicks unattended at the nest more often in years when food losses to kleptoparasitising gulls were high (Stienen & Brennikmeijer 2002a), and by doing so they compensated for the food loss by increasing their foraging efforts. Instead in Zeebrugge, chicks were almost never left alone at the colony (Fig. 44).

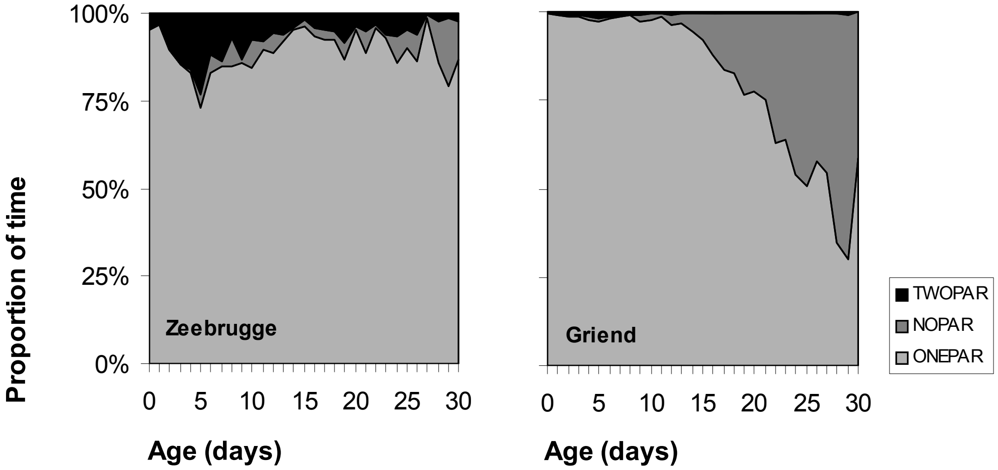

Figure 44. Development of parental nest attendance with the age of Sandwich Tern chicks in Zeebrugge (averaged values from the period 2001-2005) and on Griend (1992-1998). Onepar = one parent, nopar = no parent and twopar = two parents present with the chick.

Apparently, in Zeebrugge Sandwich Terns enjoy a more leisurely living compared to terns breeding on Griend. Zeebrugge terns do not have to cope with a high incidence of food robbery and do not have to put too much effort into foraging. Therefore, one might expect chick growth and survival to be much higher in Zeebrugge, but the opposite turns out to be true. As shown in Table 2, fledging success can be very poor in Zeebrugge and often much lower than the success rates measured on Griend in the 1990s (Stienen & Brenninkmeijer 2002). Also, the averaged yearly body condition index of the chicks in Zeebrugge can be directly linked to their survival probabilities (Fig. 45), which suggests that chicks starved to death because a lack of food.

This contradicting result can be aptly put as "*the riddle of Zeebrugge*". Why do parent terns on Griend very efficiently compensate for an imminent food shortage by increasing their foraging effort so that no effects on growth and survival of the chicks are present, whereas in Zeebrugge where parents have much wider margins to adjust their foraging behaviour, parents do not compensate for an imminent food shortage. Even in years with poor chick survival rates (2002 and 2005) the chicks were almost never left unattended at the colony (Fig. 46). The riddle can not yet be solved, but the answer can probably be found by an in-depth study of the spatio-temporal patterns in food availability and in particular by studying changes in the length distribution of the available prey stock in front of our coast. The results of this TROPHOS-study indicate that small anomalies in the length distribution of clupeids may be responsible for the poor fledging success recorded in 2002 and 2005. In all other years the length distribution curves are smooth indicating that a range of prey length was equally available for the growing chicks (Fig. 47). Both in 2002 and 2005 the curves show anomalies, meaning that a certain prey length was lacking from the chicks' diet. We therefore hypothesise that growing Sandwich Tern chicks need a complete spectrum of prey lengths, ranging from very small prey (5-6 cm long clupeids) for freshly hatched chicks to fish of more than 10 cm for nearly fledged chicks.

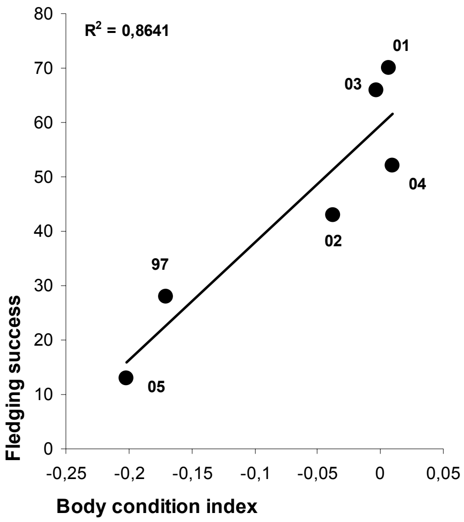

Figure 45. relationship between the chicks' body condition and fledging success of sandwich Tern chicks in Zeebrugge during the period 1997-2005. Numbers near dots show the various years.

If, however, for one reason or another a certain length-class is (temporarily) missing from the spectrum, parents can not correct this effectively by increasing their foraging effort. In such years, chicks may initially grow at normal rates, but suddenly get into trouble and starve to death.

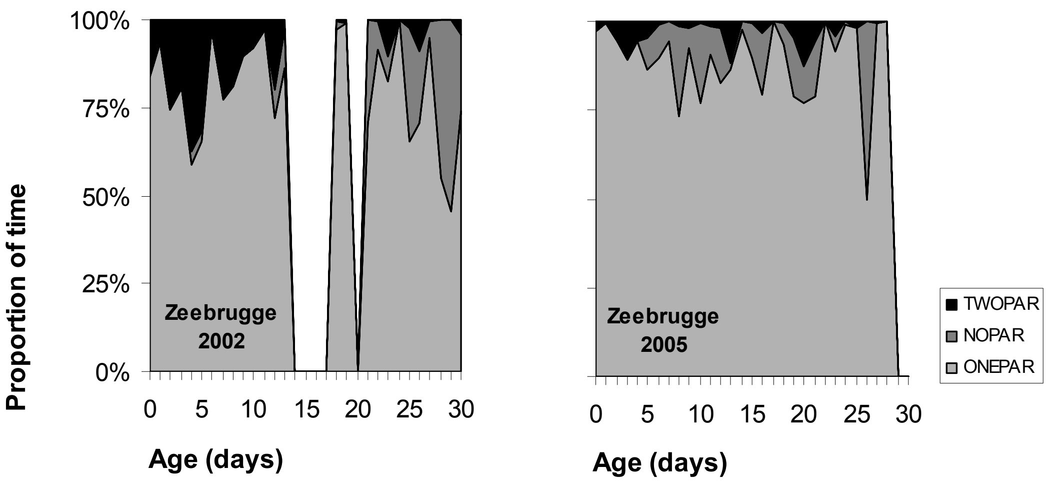

Figure 46. Development of parental nest attendance with the age of Sandwich Tern chicks in Zeebrugge in 2002 and 2005. Onepar = one parent, nopar = no parent and twopar = two parents present with the chick.

This seemed to be the case in 2002, when older chicks and also young Common Tern chicks (that start breeding later in the season) suddenly got into trouble. In 2005, smaller prey were lacking so that young chicks experienced difficulties, but when chicks survived the first days post-hatching body condition normalised.

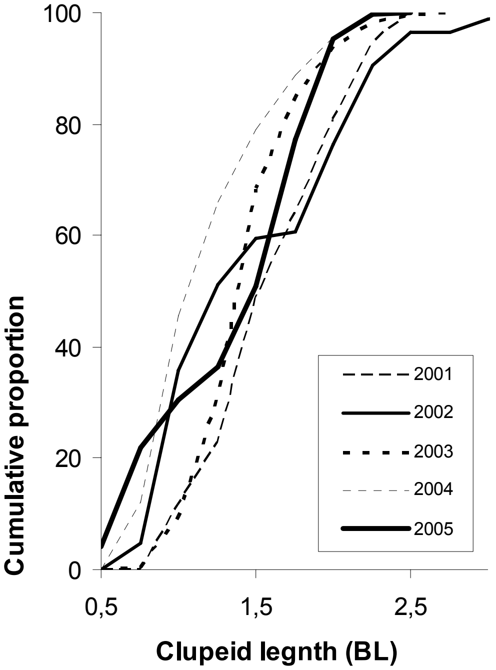

Figure 47. Length distribution of herring fed to Sandwich Tern chicks in Zeebrugge in 2001 - 2005.

5. GENERAL CONCLUSIONS AND RECOMMENDATIONS

Research within TROPHOS was essentially fundamental, and therefore our primary output is directed towards high level scientific journals. However, as outlined below we are convinced that our results can be used directly and indirectly in decision building processes. We formulate suggestions for further research in order to improve our knowledge on the functioning of the marine ecosystem off of our coast. Only a sound scientific understanding of the processes occurring at the Belgian Continental Shelf can and will support a further sustainable management.

Benthic-pelagic coupling

The benthic-pelagic coupling can be considered as one of the most important processes occurring in a marine ecosystem: after a phytoplankton bloom, dead cells sink to the seafloor where they are remineralised. As a result of this process, nutrients are released again to the pelagic phase, where they fuel the next phytoplankton bloom. As this phytoplankton bloom is at the basis of the pelagic food web, a sound understanding of the timing and magnitude of these processes is very important for a sustainable management of the marine ecosystems.

Our results clearly showed that at the BCS strongly different benthic ecosystems were present. In the fine sanded sediments, mineralisation processes are delayed with respect to the mass sedimentation of phytodetritus after the phytoplankton bloom. The more offshore permeable sediments show a fast response both in terms of densities and diversity of benthic organisms and mineralization processes. This is due to the prevailing lateral advective currents through the sediment, providing the deeper sediment layers with oxygen and removing toxic byproducts of the mineralization processes.

Therefore it is very important not to carry out activities which disturb the benthic processes when these are acting at their maximum rates. In the coastal zone, sediments are most vulnerable during summer, while the more offshore coarser sediments show the largest activity in April-May.

Mineralization rates were largest in the fine-sanded sediments while permeable sediments show lower values. However, we stress that mineralization rates in the permeable sediments recorded during TROPHOS should be considered as absolute minimum estimates; these values are probably higher in field situations. **Given the fact that the majority of the sediments at the Belgian Continental Shelf consists of these coarser sediments, the ecological value of these sediments for the functioning of the marine ecosystem should not be underestimated.**

In the coastal zone, the remineralisation of phytodetritus is linked to bioturbation and bio-irrigation of macrobenthic fauna. However, we have no idea as yet about the relative importance of macrobenthic species, functional groups and their interactions for the magnitude and intensity of the processes. **Further research should focus on the biodiversity-ecoystem functioning link in order to provide the managers and stakeholders of the Belgian Continental Shelf with scientific information about the role and importance of these organisms in the intensity of the benthic processes. This information is extremely useful in order to predict changes in the magnitude of the processes as a consequence of eg. decreasing species densities as a consequence of human disturbances. In addition such information is invaluable for assessing the impact of invasive species in marine benthic environments.**

Short-term mineralisation in contrasting sediments was comparable; however densities and diversity of meiobenthos and macrobenthos were very different between these sediments. **Therefore we stress that diversity and density <u>alone</u> are not at all good proxies for assessing the value of marine ecosystems. We therefore suggest stimulating the incorporation of process studies when developing tools supporting decision processes.**

For the first time ever, we showed the incorporation of *Phaeocystis* derived organic matter in benthic biomass and the capacity of the bentic ecosystem to remineralise degraded *Phaeocystis* colonies. As these findings are the result of a single time-consuming experiment, **cooperation between research groups investigating the marine primary producers in general and *Phaeocystis* in particular should be promoted.**

Dispersal of marine organisms

Against the general perception, the North Sea represents a discontinuous habitat for living organisms and their populations. More inshore life styles seem to induce the fragmentation of populations, while more offshore life styles tend to promote connectivity between populations. The consequences for fisheries management (in the case of commercial species), habitat management and the implementation of Marine Protected Areas are major. **The BCS has to be managed as a landscape**, with sufficient attention to details and full awareness of its unique properties. The North Sea is among the most productive shelves across the blue planet.

The differential survival of cohorts does not only affect cohort strength (a well known feature of fisheries), but also the genetic make-up (a novel discovery). As the population truncation of commercially exploited stocks has become a sad reality (older fish have become almost absent), there are clear indications that genetic diversity is eroding and is compromising the adaptive potential and indeed sustainability. This trend can only be reversed by **maintaining a well balanced population structure** of young and old fishers, of males and females.

Particle tracking represents an effective tool to model the dispersal of living particles (micro-algae, zooplankton and fish larvae) in the ocean. It quantifies the dispersal processes at any given time and place for the highly dynamic BCS. Our preliminary results have stimulated a more **comprehensive approach to connectivity** in the ocean. It is expected that the model will provide a real-time management tool in the near future.

Finally, the concept and effectivity of **Marine Protected Areas** (MPAs) is well understood in the scientific community. Time has come to implement it in the management plans of the BCS on a large scale. MPAs are only effective if they cover a third of the target area, which indeed represents a large surface. The biological richness of the BCS strongly supports the implementation of clear management decisions, including the delineation of MPAs.

Seabirds as top predators

The tern colony in Zeebrugge is of high international importance and has recently been designated as a Special Protected Area under the Bird Directive. Scientific knowledge as has been gathered during the THROPHOS project is crucial for the preservation of these species and to develop a proper management plan for the breeding site. The study reveals that protecting the two tern species requires both local management (Common Tern) as well as concerted international and cross-North Sea management (Sandwich Tern). The two species are closely related and breed in close proximity to each other, but exhibit very different life-history traits. In Zeebrugge, Common Terns are largely self-supporting although there is some exchange of individuals with nearby colonies in the Dutch Delta-area. At present, the young Common Tern colony (first settlement in 1987) is highly productive. However, breeding output will probably decrease in future as carrying capacity of the area will be reached. In contrast, Sandwich Terns already experienced several years of poor breeding success in combination with strong fluctuations in breeding numbers and exchange of individual with colonies over a much larger distance than Common Terns.

Both species may serve as indicators for the state of the marine environment. The fledging success and relative population growth of Common Terns prove to be good candidates to serve as a proxy for the condition of small pelagic fish in the surroundings of Zeebrugge. For Sandwich Terns various potential indicators have been worked out in detail and their value is compared with earlier studies on Griend in the Dutch Wadden Sea. Unlike in the Wadden Sea, parental effort put into foraging was of poor indicative value in Zeebrugge. Here only a marginal portion of the food is lost to prey robbing gulls. Therefore parents can afford to bring less food and smaller prey to the colony than is the case on Griend, and still chicks reach comparable high energy intake rates. This is translated into a very low foraging effort of parents in Zeebrugge. As Zeebrugge birds live more leisurely one might expect chick growth and survival to be much higher in Zeebrugge than on Griend, but the opposite turns out to be true. This contradicting result can be aptly put as "*the riddle of Zeebrugge*". Why do parent terns on Griend very efficiently compensate for an imminent food shortage by increasing their foraging effort so that no effects on growth and survival of the chicks are present, whereas in Zeebrugge where parents have much wider margins to adjust their foraging behaviour, parents do not compensate for an imminent food shortage. We found evidence that in some years a certain length-class is (temporarily) missing from the prey spectrum that is needed for continuous chick growth. Apparently parents can not correct this effectively by increasing their foraging effort by shifting towards smaller prey. In such years, chicks may initially grow at normal rates, but suddenly get into trouble and starve to death.

Where the TROPHOS study showed marked reflections of seabirds that were presumably caused by changes in the underlying marine food web, further research should focus more on the direct links with the underlying pelagic ecosystem. When developing predictors to indicate changes in the marine environment it is extremely important that one understands the driving forces that cause the changes and one must validate the magnitude of the underlying process.

The incorporation of a data management team/outreach team within the network had several advantages. First of all, data gathered during the project are compiled in a single well-managed system, ensuring long-term data management. Integrating different datasets in a single database ensures consistency of the data across datasets, and facilitates multi-disciplinary analysis. Increasing the data availability and providing more elaborate possibilities for exchange of data towards other projects (and towards the scientific community in general) was one of the main objectives within the TROPHOS data management. This was realized through the development of two major databases: the metadata database and the integrated project database. Moreover, during TROPHOS, all data collected during a previous SPSD project "Structural and functional biodiversity of North Sea ecosystems

(1997-2001)" were integrated in the TROPHOS database, which now contains a total of 70 000 measured values. It became very clear during the course of the project that successful data management can only be achieved trough a very intense cooperation between data managers and data providers. This cooperation has to be based on a mutual understanding and willingness to learn the specific language, needs and timing of both the data managers and the scientists. One of the most important lessons learned concerns the timing of the transfer of data from scientists to the data management team. As long as each partner works with his own data system, it proves to be very difficult to put a deadline on the delivery of data to the centralised data system. Datasets evolve constantly: every scientist does a lot of quality control on his/her data before and during analysis of the dataset. Transferring intermediate datasets is often for both the scientist as the data manager a pointless and frustrating activity; final submission to the archive can better be postponed till the end of the project. Of course, it remains important to have close collaboration between scientists and data managers throughout the duration of the project.

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