Diensten van de Eerste Minister

Federale diensten voor wetenschappelijke, technische en culturele aangelegenheden

Tweede plan voor wetenschappelijke ondersteuning van een beleid gericht op duurzame ontwikkeling

Deel II: *"Global change, Ecosystemen en Biodiversiteit"*

Netwerkovereenkomst EV/02/25A

HOGERE TROFISCHE NIVEAUS IN DE ZUIDELIJKE NOORDZEE "TROPHOS"

Universiteit Gent
Sektie Mariene Biologie

NIOO-CEME

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Instituut voor Natuurbehoud

-

Katholieke Universiteit Leuven
Laboratorium voor Aquatische Ecologie

WETENSCHAPPELIJK VERSLAG
voor de periode van 01/02/2003-31/01/2004

Dynamics of meiobenthos and bacteria at two contrasting sites at the Belgian Continental Shelf

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Material and Methods

Study area

The sampling stations, 155bis (51°09.1 N; 2°37.1 E) and 330 (51°26.0 N; 2°48.5 E), are located in the Belgian Continental Shelf, the first one in the coastal area (Westdiep) and the second in the open sea near the Gootebank respectively.

Sampling campaigns

The two sampling stations were sampled monthly from October 2002 to October 2003 from the research vessels Zeeleeuw or Belgica. Detailed campaigns were conducted in February, April and October 2003

Water samples at the sea surface (3 m depth) and 1 m above the bottom were collected using 10 l niskin botlles, which were subsampled for the analysis of Dissolved Organic Carbon (DOC), nutrients,pigments and phytoplankton composition. The water samples for DOC and nutrient analysis were kept at -20°C on board an in the laboratory. 0.5 l of water was filtered on Whatman GF/f filters (i.d 4.7 cm) for pigment analysis. Three replicate filters per sampling occasion and water depth were obtained at both stations. The filters were stored at -20°C on board and transferred to -80°C in the laboratory.

Macroafauna was sampled by means of 5 replicate Van Veen grab deployments. A Reineck boxcorer (surface area 180 cm²) was used to collect undisturbed sediment for meiobenthic sampling. Ar each occasion, the boxcorer was deployed three times. After each deployment, the Reineck boxcorere was subsampled using Perspex cores (i.d 3.6 cm) for meiofauna, C/N and pigment analysis. All sediment cores were sliced per cm until a depth of 10cm. Meiobenthic samples were preserved in a hot (70°C) neutral 4% formaldehyde tap-water solution. C/N and pigment samples were stored at -20°C on board, at the laboratory they were kept at -20°C and -80°C respectively. From each boxcorer, an additional core (i.d. 6 cm) was retained for the analysis of bacterial diversity.

In the detailed campaigns (Feb., Apr. and Oct. 2003) a box corer was used. The same sampling scheme was followed, but a forth core (i.d 3.6 cm) was collected to analyse sediment porosity. These cores were equally sliced in 1cm slices until a maximum of 10cm, preserved in a –20°C freezer and stored in a –20°C freezer at the laboratory. Extra sediment samples were also taken for the study of the natural abundance of stable isotopes in the benthos. These samples were stored at –20°C at the laboratory.

In December 2002 it was not possible to sample station 330.

Meiofauna analysis

Meiobenthic organisms were extracted from the sediment by centrifugation with Ludox (Heip et al. 1985). Macrofauna was excluded using a 1 mm sieve. All animals retained on the 38 μm sieve were stained with Rose Bengal. The samples were sorted into higher taxa and counted under a binocular microscope.

120 nematodes were picked randomly and transferred to glycerol, following De Grisse (1965, 1969), and mounted on Cobb slides for identification to species level. When less then 120 nematodes were present in a sample, all nematodes were removed.

Diversity of the bacterial community

Samples

The cores for the study of the temporal variation in the biodiversity of the bacterial community were taken to the laboratory, retaining the overlaying water by carefully closing the cores. In a bench flow the upper centimetre of sediment was sub-sampled using a syringe from which the tip was removed. A 1ml sub-sample was equally divided into two 2ml ependorfs for further DNA extraction. This was replicated twice per core in order to obtain 4 sub-samples from each of the three cores taken at each sampling site. From the more detailed campaigns (Feb., Apr. and Oct. 2003) the 4-5cm layer was sampled as well. All samples were frozen at −80°C until DNA extraction.

DNA extraction

Total DNA was extracted from about 3 g of sediment following the procedure of Demba Diallo *et al.* (2003).

PCR amplification of 16S rDNA

6μl (ca 100 ng) of the purified DNA was amplified in a Genius temperature cycler. The PCR mixture contained: 6μl of template DNA, 0.5 μM of each of the appropriate primers, 200 μM of each deoxynucleoside triphosphate, 5 μl of 10 x PCR buffer (100 mM Tris-HCl (pH 9); 500 mM KCl; 15 mM MgCl2, 20 ng of bovine serum albumine and 2.5 U of Taq DNA polymerase (Ampli-Taq Perkin Elmer). Each mixture was adjusted to a final volume of 50 μl with sterile water (Sigma). Two "touchdown" PCR (Muyzer *et al.* 1993; Don *et al.* 1991) were performed starting with 5 minutes at 94 °C, followed by 20 cycles of denaturation at 94°C for 1 min, annealing at 65°C (the temperature was decreased by 0.5°C every cycle until the touchdown temperature of 56°C was reached) for 1 min, and primer extension at 72°C for 1 min. Ten additional cycles were carried out at an annealing temperature of 55°C. The tubes were then incubated for 10 min at 72°C. The products of these two PCR's were pooled together and the DNA reconcentrated.

DGGE (Denaturing Gradient Gel Electrophoresis) analysis

The DGGE technique was carried out using the D-Code System from Bio-Rad Laboratories. The PCR products were loaded onto 8% (w/v) polyacrylamide gels of 1 mm thickness, in 1 x TAE buffer [20

mM Tris-acetate with pH 7,4; 10mM acetate; 0.5mM disodium EDTA]. The denaturing gradient contained 35% to 70% denaturants (100% denaturant corresponded to 7 M urea and 40% (vol/vol) deionized formamide). The total lane intensity was normalised between the samples on 400 ng of DNA. Electrophoresis was performed at a constant voltage of 75 V for 16 h and at a constant temperature of 60°C. After electrophoresis, the gels were stained for 1 h in 1X TAE containing ethidium bromide (0.5 mg.l-1). The bands were visualized on a UV transillumination table equipped with a digital CCD camera.

As standards, we used a mixture of DNA from 9 clones (Van Der Gucht *et al.* 2001).

Results

Meiofauna

The meiofauna samples of station 115bis have been sorted until the first samples of July 2003 while samples from station 330 are elaborated until April 2003. The average abundance of the different meiobenthic taxa are shown in Table 1 and 2 for station 115bis and 330 respectively.

Table 1: Average abundance of the meiobenthic taxa (ind. / $10cm^2$) in station 115bis.

115bis	Oct 02	Nov 02	Dec 02	Jan 03	Feb 03	Mar 03	Apr 03	May 03	Jun 03	Jul 03
Copepoda	10.0	1.0	1.3	2.0	1.7	2.7	9.3	9.7	4.3	4.0
Cumacea	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Gastrotricha	103.3	84.0	30.0	197.7	102.0	94.7	65.3	130.3	100.3	116.0
Halacaroidea	0.3	0.0	0.0	0.0	0.3	0.7	0.3	0.7	0.7	0.0
Kinorhyncha	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Loricifera	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.0	0.0	0.0
nauplii	1.0	0.0	0.7	0.3	6.7	5.0	1.0	26.0	12.7	3.0
Nematoda	3212.3	2133.7	1251.3	3705.3	3641.7	2649.0	4841.3	3087.7	4645.3	5753.0
Oligochaeta	0.3	1.0	0.0	2.3	1.7	0.3	7.3	0.3	1.7	3.0
Ostracoda	0.3	0.0	0.0	0.0	0.7	0.0	0.3	0.0	0.3	1.0
Polychaeta	3.7	2.0	0.3	0.0	1.0	1.7	1.0	6.0	7.0	8.0
Tardigrada	0.3	0.0	0.0	0.0	0.3	0.0	3.0	0.0	0.3	0.0
Turbellaria	54.00	87.67	19.67	87.00	68.67	49.00	20.00	20.00	29.33	15.00

Table 2: Average abundance of the meiobenthic taxa (ind. / $10cm^2$) in station 330.

330	Oct 02	Nov 02	Dec 02	Jan 03	Feb 03	Mar 03	Apr 03	May 03	Jun 03	Jul 03
Copepoda	63.33	188.33		176.33	111.33	140.67	249.00			
Cumacea	0.00	0.00		0.00	0.00	0.00	0.00			
Gastrotricha	7.00	15.33		12.33	31.33	16.00	32.67			
Halacaroidea	4.67	4.67		5.00	7.00	5.67	11.33			
Kinorhyncha	0.33	0.33		0.33	0.33	0.00	0.00			
Loricifera	0.00	0.00		0.00	0.00	0.00	0.00			

nauplii	14.33	90.00	73.33	77.33	127.00	116.67
Nematoda	561.67	349.33	468.33	578.00	553.33	766.33
Oligochaeta	1.00	5.00	2.00	8.67	7.33	0.67
Ostracoda	1.33	2.00	1.33	2.33	2.00	10.00
Polychaeta	10.67	11.67	2.33	1.67	3.67	9.00
Tardigrada	4.33	4.67	22.33	106.67	190.67	55.00
Turbellaria	11.33	22.67	16.67	22.00	26.67	18.00

Until now, considering both stations the following meiobenthic taxa were found: Harpacticoid copepods, Cumacea, Gastrotricha, Halacaroidea, Kinorhyncha, Loricifera, nauplii, Nematoda, Oligochaeta, Ostracoda, Polychaeta, Tardigrada, and Turbellaria

The dominant groups in both stations are reported in Table 3. In station 115bis Nematoda were very dominant with an average abundance of 95% (±1.86). The only other groups reaching relative abundances higher then 1% were Gastrotricha and Turbellaria, (3% (±1) and 1% (±1) respectively). In station 330, nematodes were again dominant, but with considerably lower values (60% (±11)). The following taxa were present for more then 1% as well: Harpacticoida, Turbellaria, Gastrotricha, Polychaeta and nauplii.

Table 3: Average dominance of the meiofaunal groups (%) in both stations.

	115bis			330	
	%	± Stdev		%	± Stdev
Nematoda	95.20	± 1.86	Nematoda	60.10	± 10.56
Gastrotricha	2.79	± 1.00	Copepoda	16.73	± 5.84
Turbellaria	1.48	± 1.14	Turbellaria	9.34	± 3.61
nauplii	0.20	± 0.29	Gastrotricha	6.76	± 6.74
Copepoda	0.12	± 0.08	Polychaeta	2.21	± 0.61
Polychaeta	0.07	± 0.06	nauplii	2.12	± 0.92
Oligochaeta	0.04	± 0.03	Tardigrada	0.77	± 0.56
Loricifera	0.02	± 0.05	Halacaroidea	0.40	± 0.29
Halacaroidea	0.01	± 0.01	Ostracoda	0.33	± 0.30
Ostracoda	0.01	± 0.01	Oligochaeta	0.30	± 0.21
Tardigrada	0.01	± 0.01	Kinorhyncha	0.02	± 0.02
Cumacea	0.00	± 0.01	Cumacea	0.01	± 0.03
Kinorhyncha	0.00	± 0.00	Loricifera	0.01	± 0.01

Comparing both sampling sites, station 115bis presents fine sediments (medium grain size: 47.2 – 143.8µm) with a high percentage of mud (36.1–83.6%) (Steyaert *et al*, subm.), while station 330 consists in medium sand (medium grain size: 329.3–360.7µm) with no mud content (Vanaverbeke *et al.*, 2003.). As a consequence anoxic sediments can be found very close to the surface at station 115bis (Steyaert *et al.*, subm.) while at station 330 redox values remain positive throughout the sediment

column (Vanaverbeke *et al.*, subm .). Due to the anoxic conditions at station 115bis, a clear dominance of the nematodes is expected, since nematodes present higher tolerance to low oxygen conditions (Moodley *et al.*, 2000b). In station 330, other meiobenthic groups more sensitive to anoxic conditions could also co-exist and consequently nematodes dominance was lower.
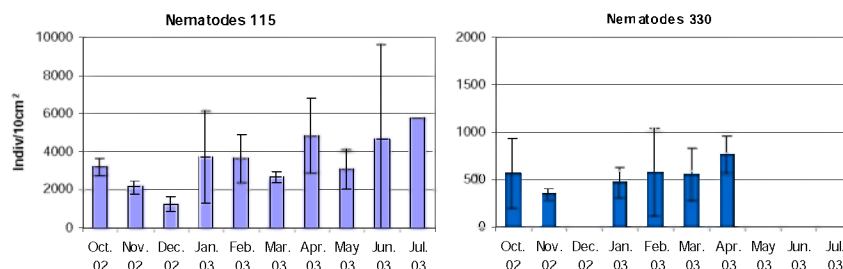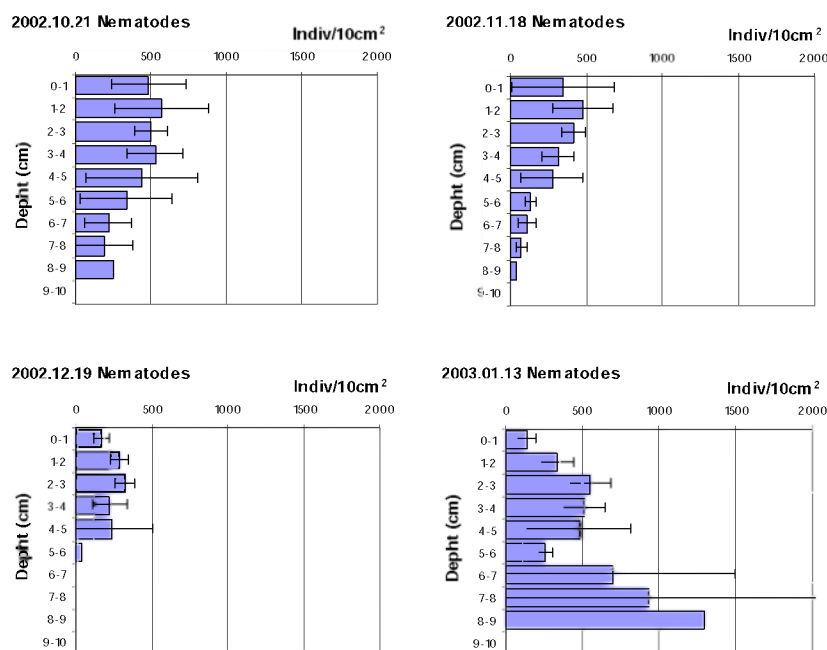

Figure **1**. Nematoda mean abundance in stations 115bis and 330.

Nematode abundance varied in the same way in both stations althou**gh with different values (fig. 1**). Densities decreased from October to December, increasing afterwards with an abundance peak in April and a second peak (shown only in station 115bis) in July.

This difference in nematodes density might reflect differences in food availability at both stations, since values of Chlorophyll *a* in station 115bis (Steyaert *et al.*, subm.) are much higher then in station 330 (Vanaverbeke *et al.*, subm.).

The vertical distribution of the nematodes was different when comparing both stations: nematodes were generally concentrated in the upper centimetres of station 115bis (fig. **2**), while no trend was visible in station 330 (fig. **3**).
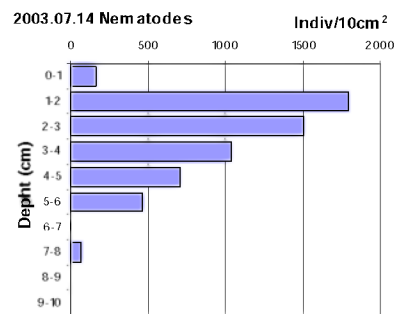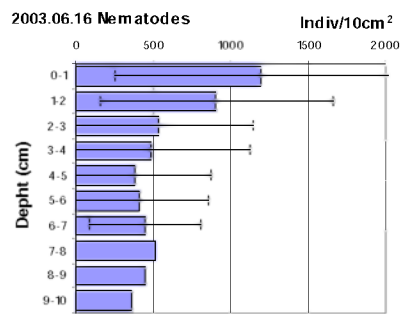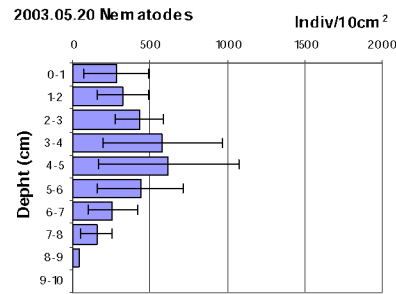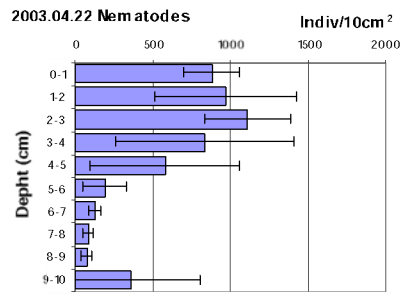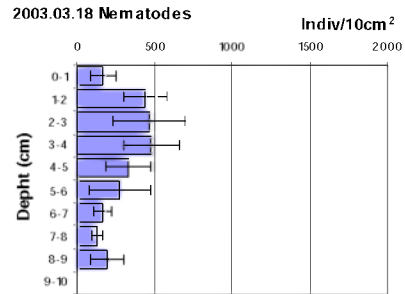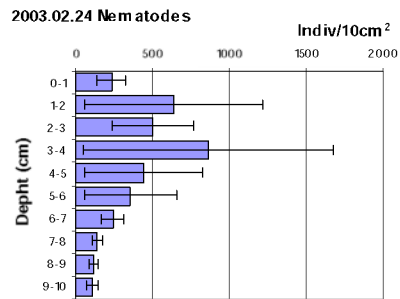
Figure 2 Nematode vertical profiles at Station 115bis: October – July

2003.01.13 Nematodes   Indiv/10cm²

2002.02.24 Nematodes   Indiv/10cm²

2002.03.18 Nematodes   Indiv/10cm²

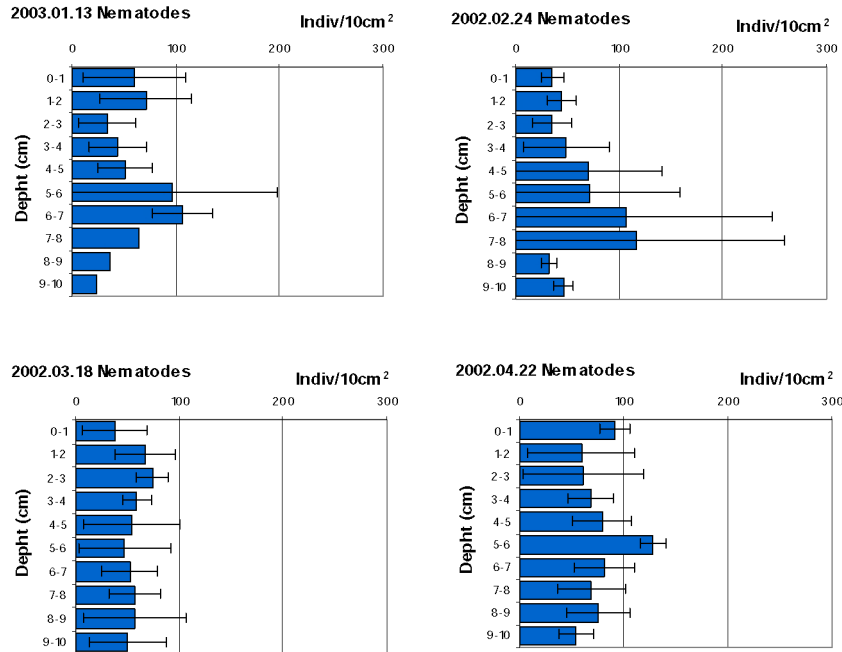**2002.04.22 Nematodes**   Indiv/10cm²

Figure 3. Nematodes vertical profiles on station 330 in the sampling months from October to April.

This again reflects differences between oxygen conditions and/or food availability. In station 115bis the oxygenated sediment layers or layers with a higher Chlorophyll *a* concentration can be found at the surface layers (Steyaert *et al.*, subm.). In station 330 Vanaverbeke *et al.* (subm.) found no clear vertical profile for redox potential, and Chlorophyll *a* vertical profile showed clear subsurface peaks around 3-5cm deep, probably due to the high permeability of the sediment (Vanaverbeke *et al.*, subm.). Therefore the nematodes should respond to such oxygen and food availability gradients that are present at station 115bis but absent at station 330.

Bacteria

Preliminary results indicate that bacterial diversity is higher at station 115bis than at station 330. This may be related to higher food availability in station 115bis. No clear changes in the number of bands were observed in station 115bis with time while in station 330 there is an increase in the number of bands (species) around spring. This suggests a response of the bacterial community to the spring bloom triggering an input of organic matter in this oligotrophic station.

At station 115bis, vertical differences between the bacterial communities of the 0-1 cm and the 4-5 cm layers were observed in samples from the detailed campaigns; however no such clear differences could be seen in station 330. This is probably due to the absence of clear vertical gradients in oxygen and food in the latter station, which are very obvious at station 115bis.

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Benthic-pelagic coupling and sediment metabolism at the Belgian Continental Shelf.

Karline Soetaert, Dick van Oevelen & Leon Moodley (NIOO-CEME)

Introduction

It is generally believed that benthic regeneration of nutrients is an important factor in maintaining the high rates of primary production in coastal areas (Nixon et al., 1980). Due to the shallowness of the Belgian Continental Shelf (BCS), organic matter remineralization in the sediment is also assumed to play a significant role here, but as yet its magnitude remains to be assessed. In addition, there is controversy about the fate of the pelagic primary production in the area: is it mainly remineralised in the water column or do significant amounts fuel the sediment metabolism.

Our view of sediment functioning has changed drastically in the last decades. Traditionally biogeochemical and biological research focussed on sediments with high organic loading (biogeochemists) and high benthic biomasses (biologists). The more barren sandy sediments were considered to be less active. We now know that physically-forced sediments are not only widespread but they may interact with the overlying water column at much higher rates than can be deduced from their organic matter content.

The aim of the first year of investigation by NIOO, in cooperation with Ghent University was to:
Quantify the benthic-pelagic exchange rates in two sites of the BCS
Track the fate of pelagic production organic matter into the sediment
Establish the impact of physical forcing on sediment functioning.

The investigations were performed in two stations in the Southern Bight of the North Sea: station 115bis and station 330. Both stations share the sandy nature of the sediments, but differ significantly in how they interact with the water column. Station 330, is located near the Goote Bank, in a high-energy environment. The sediment is predominantly physically-forced, and pore-water transport is driven by strong water currents. In contrast, at station 115bis, located near the Belgian Coast, sediment-water exchanges are driven by molecular diffusion and biotic irrigation.

Assessing the benthic metabolism.

The benthic metabolism was assessed by measuring oxygen and nutrients (Nitrate, ammonium, silice and phosphate exchange rates in laboratory dark benthic flux chamber incubations.
Sediment community oxygen consumption rates (SCOC) are good measures for the total mineralisation rates in sediments. This is because oxygen is consumed either directly by oxic mineralisation, or indirectly to re-oxidise reduced substances formed upon anoxic mineralisation. Moreover, a time-series
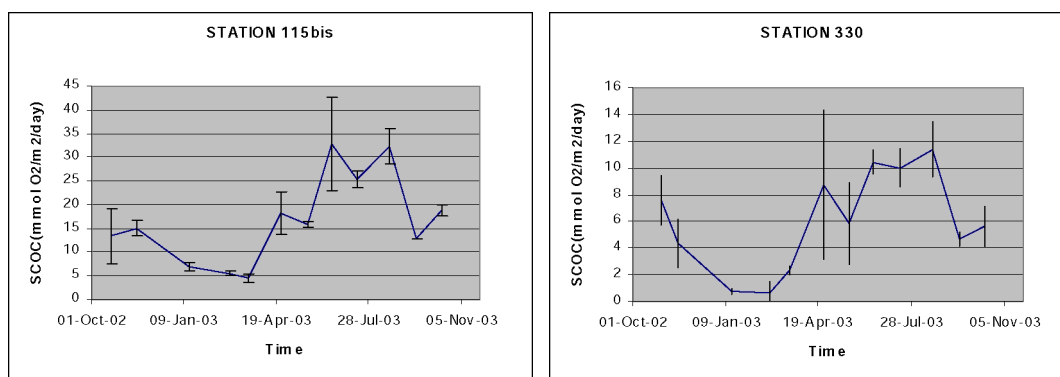
may give information about the reactivity (freshness) of organic matter consumed within the sediment: the higher the amplitude the more reactive. Nutrient fluxes and sediment-depth profiles may give indication upon and allow quantifying the metabolic pathways (oxic, suboxic, anoxic) that prevail in the sediments.

Replicate SCOC measurements were performed monthly in the lab (October 2002 – October 2003) by NIOO. Nutrient exchange rates were estimated jointly by NIOO and UG on three occasions (February, April and October 2003), and by fitting a 24 hour time-series of the nutrient concentration in the overlying water. After sampling and till the end of the incubation the oxygen concentration in the overlying water was kept saturated by air bubbling. Nutrient and oxygen profiles were measured, at the start of the incubations, and after one day of incubation (UG).

SCOC and nutrient flux measurements generally started about one day after sampling. All data are processed.

SCOC in the two stations demonstrated a clear and consistent and similar seasonal signal (Figure): oxygen consumption rates start increasing in April and reach maximum values in June-August, after which they drop again; minimum values are recorded in January till March. The amplitude (Maximal / minimal SCOC value) is 7 and 19 for stations 115bis and 330 respectively which points to the highly reactive nature of the organic matter in the sediments.

Oxygen consumption rates can be converted to carbon mineralisation, assuming a O:C conversion ratio of 1. We calculate a yearly-averaged carbon mineralisation of 16.8 mmolC m^{-2} d^{-1} for station 115bis, 6.0 mmolC m^{-2} d^{-1} for station 330. This is equivalent to 73.7 and 26.1 gC m^{-2} yr^{-1} mineralised in the sediments of station 115bis and 330 respectively. (Compare that to an estimated pelagic primary production rate of about 200 gC m^{-2} yr^{-1} in the area).

The nutrient fluxes are summarised in the tables below:

Station 115

mmol/m2/d	Februari average	stdev	April average	stdev	Oktober average	stdev
NO3+NO2	-0.25	0.03	-0.10	0.21	0.16	0.10
NO2	-0.01	0.01	-0.01	0.07	0.00	0.01
NH4	-0.03	0.02	1.23	0.42	-0.08	0.23
PO4	0.16	0.15	-0.02	0.11	-0.05	0.00
Si	-0.29	0.20	4.92	5.07	0.31	0.14
O2	2.89	4.09	18.14	4.49	18.80	1.10

Station 330

mmol/m2/d	Februari average	stdev	April average	stdev	Oktober average	stdev
NO3+NO2	-0.03	0.08	0.07	0.13	0.19	0.01
NO2	-0.02	0.01	-0.05	0.05	-0.02	0.00
NH4	-0.12	0.06	0.39	0.54	0.02	0.14
PO4	-0.01	0.01	-0.19	0.12	-0.06	0.02
Si	-0.11	0.04	0.33	0.11	0.09	0.00
O2	0.61	0.86	8.73	5.56	5.65	1.48

CO2 fluxes are derived from the oxygen fluxes and were described above. We note small fluxes of phosphate, overall larger fluxes at station 115 compared to 330, consistent with the oxygen consumption rates. Biogeochemical budgets will be drawn from these data. Tentative budgets demonstrate the larger importance of denitrification (consumption of nitrate) at station 330.

Tracking the fate of pelagic production into the sediment

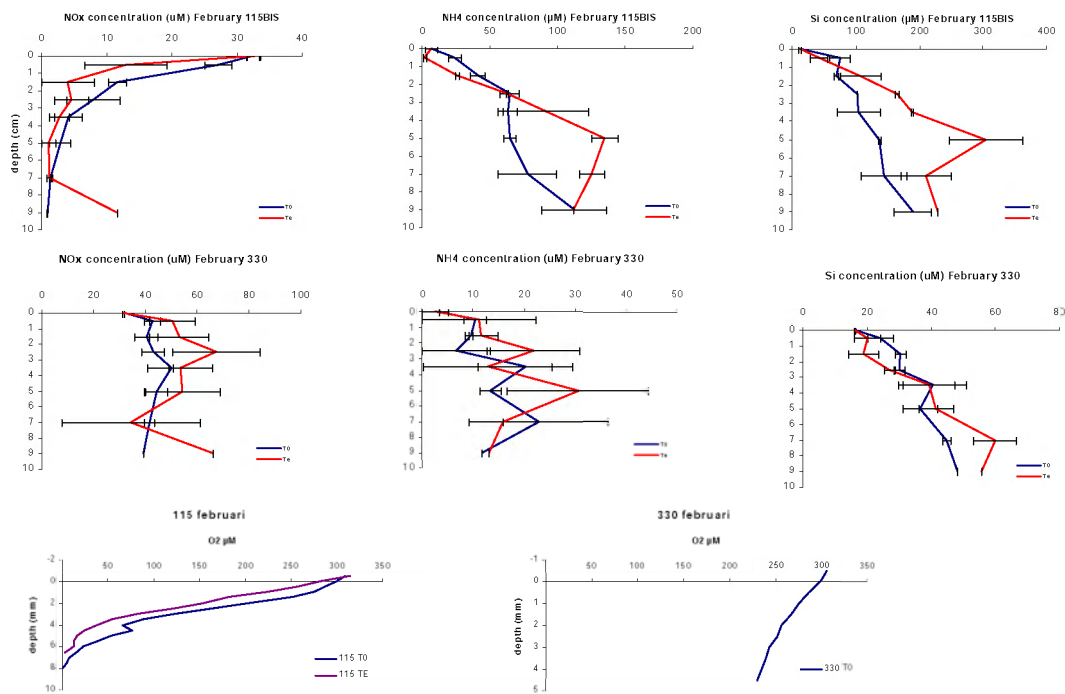Each month water samples were procured 1m.a.b. and near the water surface and analysed for total suspended and organic matter content and the isotopic signature. Similar measurements were performed in the upper 1 cm of the sediment beneath (monthly) and the 4-5 cm layer (three intensive campaigns). Results are available till June 2003. Samples from July-October 2003 are still awaiting laboratory analysis.

Results for station 115bis show clearly a change in the ^{13}C content of the water samples near the surface and 1 metre above the bottom. The increase in $\delta^{13}C$ is first apparent in March (due to appearance of phytoplankton) and is followed about 1 month later with similar changes in the signature in the upper cm of the sediment. This clearly demonstrates the pelagic origin of the organic matter at that period. The signature of the organic matter at 4-5 cm depth is not affected as much. These trends are not as clear in station 330.
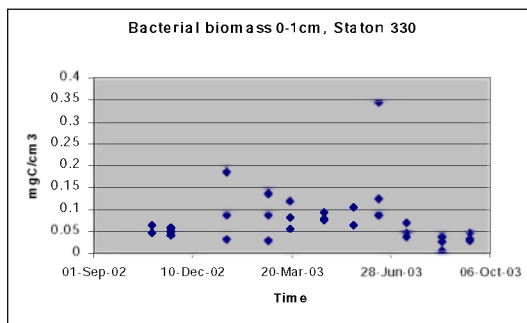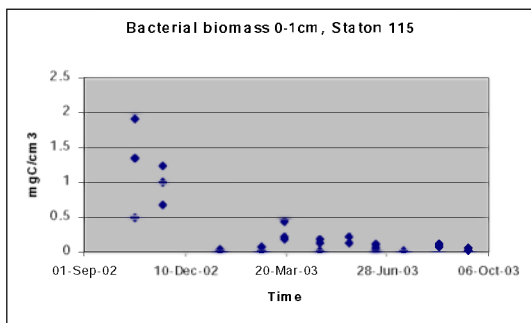
Impact of physical forcing on sediment functioning.

Representative nutrient profiles and oxygen microprofiles, obtained in the Februar**y cruise are in the figure below**– note the differences in scale.

The higher concentration of ammonium at depth in the sediment, the rapid consumption of nitrate within the upper part of the sediment and the exhaustion of oxygen at short distances below the sediment-water interface (not shown) point to the importance of anoxic pathways in station 115bis. On the other hand, the constancy of the nitrate vertical profile, the high concentration of oxygen and the lower concentration of ammonium demonstrate the relative unimportance of anoxic pathways and the predominance of physical mixing at station 330. Higher build-up of Si at station 115bis demonstrates that this station is also more active in the dissolution of detrital Si than station 330.

Bacterial cell densities in different size-classes were estimated and used to calculate bacterial biomass. Results (awaiting quality-control) are in the figure below:

Bacterial biomass 0-1cm, Staton 115

Bacterial biomass 0-1cm, Staton 330

Genetic structure and gene flow of the Common sole (Solea solea L.) on the Belgian and European Continental Shelf

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1. Introduction

The spatial and temporal scale over which populations can be identified and the strength of gene flow among them, are of fundamental importance to the conservation and management of organisms. Such knowledge is poorly developed in marine fishes because of the connectivity and vastness of their habitat.

Based on tagging experiments, adult flatfish have been reported to migrate over distances of 100 km and more. Eggs and larvae might become displaced over distances of 10 to 100 km by physical advection. Although marine organisms have a high potential for dispersal, physical and behavioural factors often limit dispersal and facilitate the maintenance of population structure.

Kotoulas et al. (1995) and Exadactylos et al. (1998) have shown that there is a gradient in the genetic structure of *Solea solea* populations (also called genetic isolation by distance). This means that genetic exchange between populations is high, a typical feature of marine organisms. Interesting is also that the core of each spawning unit along the Atlantic shelf is separated by a distance of about 100 km, which is exactly the distance where genetic structure is changing. Homing behaviour and the existence of discrete spawning areas have been observed in *S. solea* (Rijnsdorp et al. 1992, Symonds & Rogers 1995). The question rises to what degree gene flow occurs between different spawning populations of Common sole. This study aims to determine the scale of population structure in *Solea solea* (Teleostei; Soleidae) in the Atlantic using high-resolution genetic markers, i.e., microsatellites. It is hypothesized that in spring when adults are at the spawning grounds more structure is detectable than outside the spawning season.

2. Materials and Methods

Sampling

Samples were collected along the whole distributional range. For the moment 8 samples from the Mediterranean and 28 samples from the Atlantic Ocean have been genotyped for six microsatellite loci (Table 1). The Atlantic samples consist of eight samples from the Gulf of Biscay and 16 from the North Sea. Twenty more samples are available from the Southern Bight of the North Sea (not listed).

Tabel 1: List of genotyped *Solea solea* samples (01-28; Atlantic from North to South and 29-36; Mediterranean from West to East). ??: Data still to be received or confirmed from collectors. Avg # alleles / loc. = Average number of alleles per locus. H_e = expected heterozygosity. H_o = observed heterozygosity.

Nr.	Sample name	Basin	n	latitude	longitude	Collection date (dd-mm-yy)	Avg # alleles / loc.	H_e	H_o
01	Kattegat	Kattegat	50	57.07	11.15	??	9.0	0.69	0.72
02	North Sea	North Sea	30	??.??	??.??	02-04-99	6.5	0.64	0.65
03	Brown Ridges	North Sea	27	52.25??	03.45??	03-03-99	7.0	0.64	0.65
04	Steendiep	North Sea	60	51.35	03.15	13-03-03	8.2	0.66	0.61
05	Sierra Ventana	North Sea	52	51.28	03.03	17-09-02	8.3	0.62	0.58
06	Bol van Knokke 1	North Sea	58	51.25	03.19	17-04-02	9.5	0.66	0.62
07	Bol van Knokke 2	North Sea	51	51.25	03.22	16-09-02	7.8	0.62	0.61
08	Wenduine	North Sea	16	51.19	03.03	30-03-99	6.0	0.67	0.72
09	Grote Rede 1	North Sea	28	51.14	02.52	23-03-99	7.8	0.66	0.63
10	Grote Rede 2	North Sea	17	51.14??	02.52??	06-04-99	6.8	0.67	0.66
11	Kleine Rede	North Sea	52	51.13	02.50	18-09-02	8.0	0.65	0.62
12	Stroombank	North Sea	60	??	??	19-06-03	9.0	0.66	0.61
13	Oostduinkerke 1	North Sea	60	51.08	02.40	06-05-03	9.7	0.66	0.62
14	Oostduinkerke 2	North Sea	60	51.09	02.39	15-04-02	7.8	0.65	0.65
15	Westdiep	North Sea	48	51.09	02.36	18-09-02	8.3	0.58	0.53
16	Potje	North Sea	60	51.06	02.32	14-06-02	8.8	0.68	0.59
17	Thames	North Sea	52	51.30	01.02	20-09-02	8.3	0.65	0.65
18	Eastern Channel	Channel	30	50.47	00.35	11-04-99	8.2	0.68	0.71
19	Seine	Channel	30	49.27	-00.58	02-06-99	7.7	0.63	0.62
20	Vilaine 1	Gulf of Biscay	30	47.28	-02.35	23-09-00	7.7	0.66	0.69
21	Vilaine 2	Gulf of Biscay	30	47.28??	-02.35??	07-09-02	7.7	0.66	0.69
22	Loire	Gulf of Biscay	30	47.12	-02.18	22-09-00	7.2	0.62	0.58
23	Noirmoutiers	Gulf of Biscay	28	47.11	-03.46	20-05-99	7.5	0.65	0.63
24	Pertuis de Breton	Gulf of Biscay	30	45.15	-01.15	15-09-00	7.3	0.66	0.68
25	Gironde 1	Gulf of Biscay	30	45.36	-01.33	26-05-99	8.2	0.65	0.63
26	Gironde 2	Gulf of Biscay	30	45.50	-01.20	10-09-00	8.2	0.63	0.58
27	St. Jean de Luz	Gulf of Biscay	39	43.25	-01.41	??	8.2	0.61	0.65
28	Lisbon	Atlantic Ocean	23	38.35	-09.35	??	6.8	0.63	0.65

29	Murcia	West Mediterranean	10	37.54	-00.40	31-03-99	4.7	0.57	0.62
30	Castellon	West Mediterranean	27	40.00	00.12	18-01-00	7.3	0.63	0.63
31	Barcelona	West Mediterranean	16	41.23	02.33	18-02-99	5.7	0.66	0.73
32	Palavas	Gulf of Lion	27	43.31	3.56	03-02-99	7.0	0.66	0.69
33	Sète 1	Gulf of Lion	28	43.05	04.10	16-06-99	6.8	0.64	0.62
34	Sète 2	Gulf of Lion	50	43.05??	04.10??	??	8.5	0.64	0.64
35	Venice	Adriatic Sea	29	45.22	12.29	23-03-99	5.3	0.55	0.57
36	Thessaloniki	Aegean Sea	24	40.37	22.55	??-04-99	4.8	0.43	0.44

DNA analysis

DNA extraction: DNA is purified using either the 'Dneasy Tissue Kit' (Qiagen, Westburg) or the 'NucleoSpin Kit' (Machery-Nagel, Düren, Germany). The final elution is done in 200 µL milliQ waterr of which 1 µL is used for the PCR reaction.

DNA microsatellites: Six microsatellite primer pairs were tested, i.e., F8-I, F8-II, F8-III, F8-IV, F13 and F-14 (Iyengar et al., 1999). 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 50mM $MgCl_2$, 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 analysis: Data are analysed using the software package GENETIX 4.02 (Belkhir et al. 2001) Population differentiation is calculated according to Weir and Cockerham's (1984) estimator of F_{ST}; θ. Isolation-by-distance is examined by plotting pair-wise θ/(1-θ) values against geographical distances and tested using the Mantel test (as implemented in GENETIX).

3. Results

Six primer sets were used for microsatellite amplification in Common sole. They were applied on all samples listed in Table 1.1. 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 (Table 1). Figure 1 demonstrates the occurrence of isolation-by-distance.
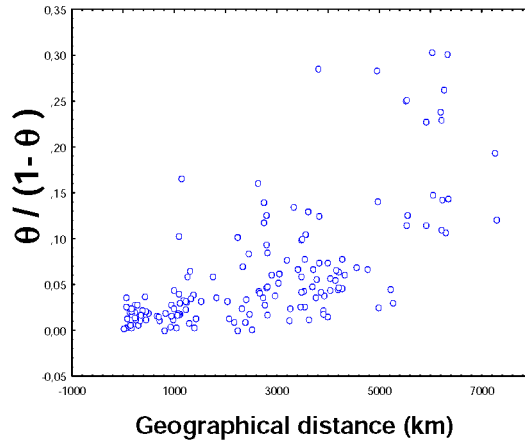
Fig. 1: Genetic distance (y-axis) against geographical distance (x-axis) showing isolation-by-distance (Note: populations 4-7, 11-17, 20-22, 24, 26 were not included). Mantel test: r = 0.67; p = 0.001.

Figure 2 shows the result of a 3-dimensional factor analysis of all 36 populations, clearly separating the Atlantic from the Mediterranean samples. Separation within the Atlantic is less obvious. The four groups in Fig. 2 (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. After sequential Bonferroni correction this is not the case anymore. However, sequential Bonferroni becomes highly conservative when more than five comparisons are made.
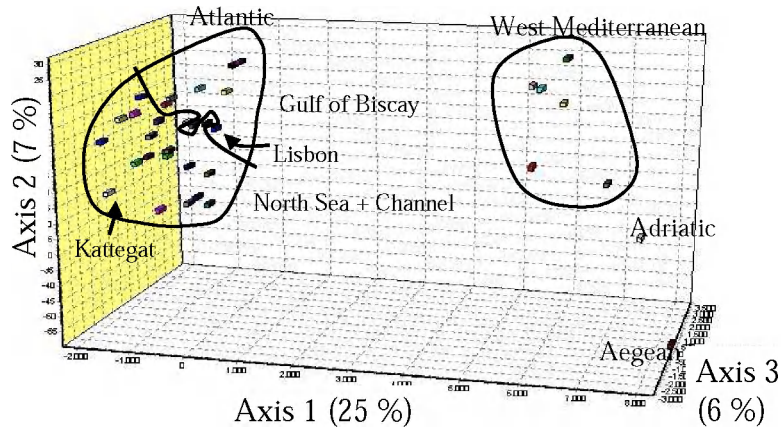

Fig. 2: Factor analysis of all populations listed in Table 1. Each dot represents one population.

Discussion

The six microsatellite loci used in the present study confirmed the previously found distinction between Mediterranean, Biscay and North Sea populations. These results are not unexpected in view of the isolation by distance pattern detected by Kotoulas et al. (1995), Exadactylos et al. (1998) and

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Exadactylos & Thorpe (2001). However, we expected to find a higher resolution than reported in these papers to discriminate among the populations (also called stocks by fisheries managers). The distinction between the Atlantic populations is not very clear; more powerful statistical approaches should be included. Adding extra microsatellite loci, rather than larger sampling sizes, may also add to a better resolution.

Acknowledgements

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Evolution, foraging behaviour and reproductive output of coastal breeders at Zeebrugge

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Seabird populations in Zeebrugge

The creation of new land in the outer harbour of Zeebrugge in 1985 attracted large number of coastal breeding birds. Each species shows its own specific evolution in breeding numbers since the creation of new suitable nesting habitat. As a pioneer species the population of Little Tern initially increased up to a maximum of 425 pairs in 1997. Because of habitat loss and succession of the vegetation numbers decreased afterwards. The Common Tern population shows a gradual increase up to 2510 pairs in 2003, while the number of Sandwich Terns strongly fluctuated. The latter species shows peaks in occurrence in 1993 and 2000. The reasons for these strong fluctuations are not clear. All three species of gulls (Black-headed Gull, Herring Gull and Lesser Black-backed Gull) show a strong increase up to 2001. The Lesser Black-backed Gull and Herring Gull further increased in 2003. For all species the outer harbour of Zeebrugge is by far the most important site within Flanders. Also at the international level, the numbers of Little Tern, Common Tern, Sandwich Tern and Lesser Black-backed Gull are extremely important (2.4% - 4.0% of the total geographical population!). European law protects all the three tern species because their populations are vulnerable for extinction. In 2004 measures will be taken to further protect the unique and internationally important ornithological value of the outer harbour of Zeebrugge.

Reproductive parameters

Being highly specialised piscivorous birds, terns are often used as bio-indicators for example to predict the abundance of specific species of fish or the presence of toxicants in the coastal marine environment. To serve as bio-indicator one must, however, know precisely how fluctuations in the marine environment translate into changes in the biology of the investigated species. This requires the monitoring of a range of parameters for several years. For this reason, the reproductive output of Common Terns at Zeebrugge is monitored since 1997 by enclosing a part of the colony with chicken wire to prevent the chicks to walk away from the study site (Table 1). Up to and including 2001 clutch size (i.e. the average number of eggs per nest), hatching success (i.e. the proportion of eggs that actually hatched) and fledging success (i.e. the proportion of hatched eggs that actually fledge) of the Zeebrugge Common Terns was high. This resulted in a high reproductive output averaging 1.2 fledglings/pair during the period 1997-2001. Such output is by far sufficient to maintain a stable population and is high when compared to foreign colonies. The high reproductive success suggests a high availability of food at Zeebrugge. Surprisingly in 2002 none of the parents were able to fledge a chick. Clutch size and hatching success in 2002 were comparable to those in earlier years, but chick

mortality was exceptional high. Data on food composition, food intake rate and growth of the chicks were gathered, but were not yet analysed. We got the impression that a combination of food shortage during the chick rearing period and high predation rates by Herring and Lesser Black-backed Gull caused the failure of the 2002-breeding season. Poor breeding success was also recorded in nearby colonies in the Dutch Delta area, suggesting a lack of clupeids in the entire southern North Sea. After all, clupeids are the major food source for Common Tern chicks. Apparently the food situation greatly improved in 2003 when fledging success and breeding success was amongst the highest measured since the onset of the monitoring programme.

Table 1 Reproductive parameters of Common Terns in Zeebrugge compared to colonies in The Netherlands (Griend) and Germany (Baltrum, Minsener Oldeoog and Trischen). Source: Becker *et al.* 1997.

Colony	Year	N pairs	Clutch size (N eggs)	Hatching success (%)	Fledging success (%)	Breeding success (N fledged young/pair)
Griend	1993	25	2.5 (62)	87	24	0.5
	1994	21	2.6 (55)	80	39	0.8
	1995	23	2.6 (60)	82	31	0.7
Baltrum	1993	18	2.9 (53)	87	37	0.9
	1994	22	2.9 (64)	92	68	1.8
	1995	16	2.9 (47)	83	9	0.3
Minsener Oldeoog	1993	21	2.7 (56)	88	0	0.0
	1994	25	2.9 (73)	96	47	1.3
	1995	18	2.9 (52)	96	48	1.3
Trischen	1993	35	1.5 (50)	0	0	0.0
	1994	33	2.4 (79)	0	0	0.0
	1995	35	1.1 (39)	0	0	0.0
Zeebrugge	1997	78	2.4 (185)	78	50	1.2
	1998	185	2.5 (466)	77	61	1.2
	1999	90	2.5 (230)	78	67	1.3
	2000	52	2.3 (121)	91	37	0.8
	2001	35	2.3 (82)	80	74	1.4
	2002	34	2.2 (75)	81	0	0.0
	2003	36	2.6 (94)	87	74	1.7

Food availability

Earlier research on terns in The Netherlands suggests that foraging trip duration (i.e. the time it takes a parent from leaving the colony until it returns with a fish) is a good indicator for the amount food available to the terns. In 2001 and 2002 (and also in 2003 but these results were not yet analysed) foraging trip duration was measured at the Zeebrugge Sandwich Tern colony. The time a parent is

absent from the nest is positively related to the size of the prey it brings back to colony (Fig. 1). Compared to Europe's largest colony, which is established at the isle of Griend in The Netherlands, it took parents at Zeebrugge less time to return with a fish. At Zeebrugge in particular small clupeids were brought to nest in a relatively short time. Even compared to a colony at Hirsholm (Denmark) where the availability of clupeids appeared to be very high, foraging trip duration of small clupeids was even shorter at Zeebrugge. In other words: at Zeebrugge there seems to be a superabundance of small clupeids. There were no large differences in foraging trip duration between 2001 and 2002, suggesting that the availability of clupeids did not decline in 2002. This seems to be in contradiction with our above conclusions that for Common Terns the availability of clupeids has drastically decreased in 2002. There is, however, a major difference in the timing of breeding season between Common and Sandwich Terns, which can explain this seemingly contrast. The latter species hatches most chicks during the last week of May, while most Common Tern chicks hatch around the second half of June.
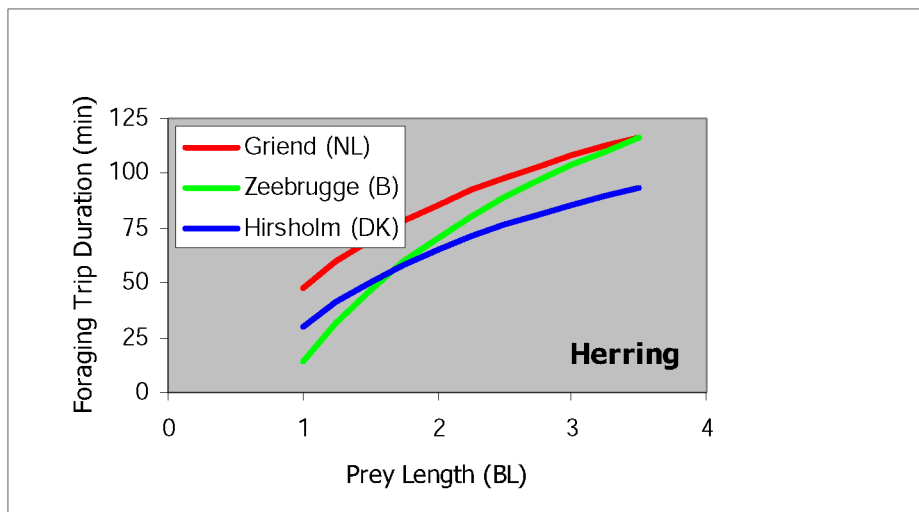

Figure 1 Foraging trip duration in relation to the length of the clupeids brought to Sandwich Tern chicks at Griend, Hirsholm and Zeebruge

Diet composition

Food requirements of seabirds are mainly known from observations at the breeding site. In many cases it is very difficult to obtain knowledge on the food preferences of adult seabirds. Therefore, it is often assumed that the composition of food supplied to the chicks also reflects the diet composition of adults. For this reason Sandwich are Terns are characterised as being highly specialised fish-eaters that almost exclusively feed on Clupeidae and Ammodytidae. In this study samples of faeces were taken around the nests of Sandwich Terns. The faeces were sampled just before hatching of the eggs to be sure that they only contained adult food remains. The samples were carefully washed and bony structures, otoliths and remains of invertebrates were sorted out under a microscope. Otoliths were specified and the length of the prey fish was back-calculated using allometric relationships described in literature. It

appears that there are major difference in species composition between adult Sandwich Terns and their offspring. Observations from a hide placed at a few meters from a colony in 2001 and 2002, showed that chicks at Zeebrugge were mainly fed with Clupeidae. Ammodytidae and other species (mainly Gonidae) were less common prey items. In contrast, the diet of adult Sandwich Terns predominantly consisted of Ammodytidae, whereas Clupeids (Sprattus sprattus) were rarely found in the faeces. Surprisingly the faeces of the adults contained relatively high proportions of Nereis jaws. Judged from the size of these jaws it can be excluded that they stem from the stomach of the fish that was eaten by the birds. In other words the polychaete might be an important food item for adult for Sandwich Terns during the incubation period, which certainly is unexpected for a strictly piscivorous seabird.
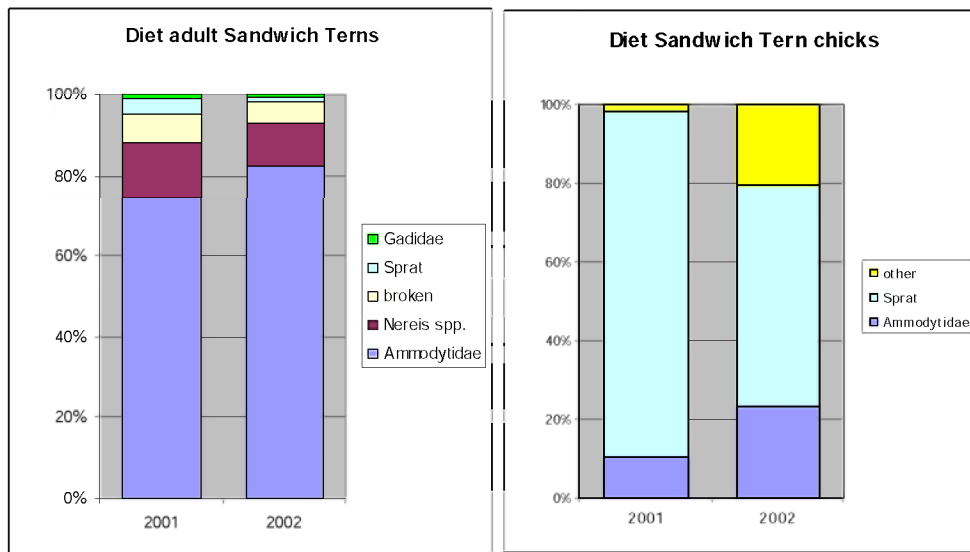

Figure 2 Diet composition of adult Sandwich Terns (based on numbers of otoliths and Nereis jaws found in faeces) compared to that of chicks in the outer harbour of Zeebrugge in 2001 and 2002.