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 cm^2) 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 $MgCl_2$, 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.

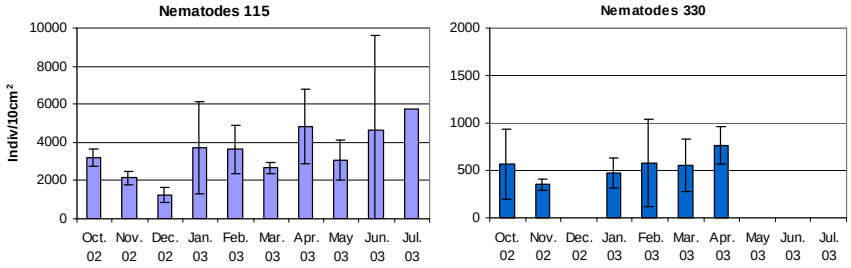
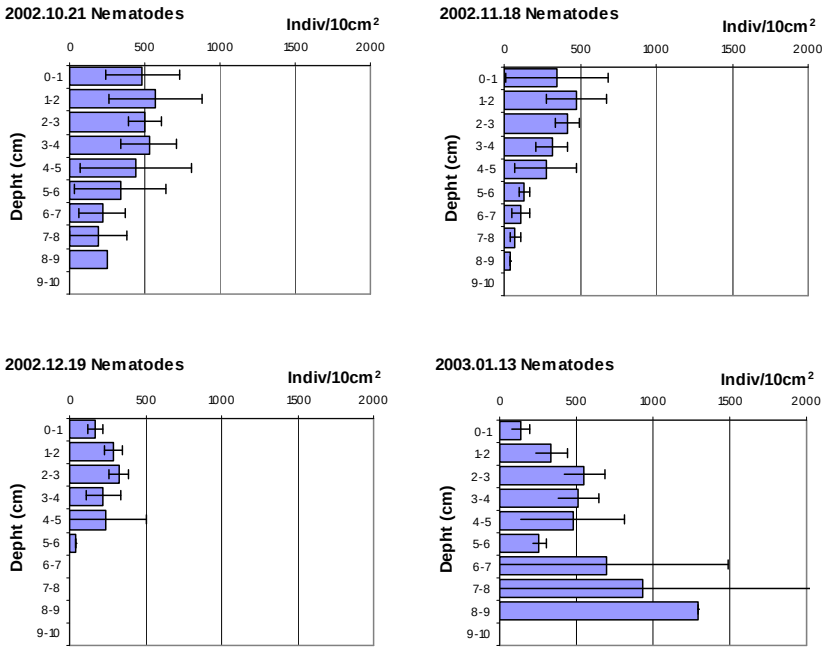


Figure **1**. Nematoda mean abundance in stations 115bis and 330.

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**).



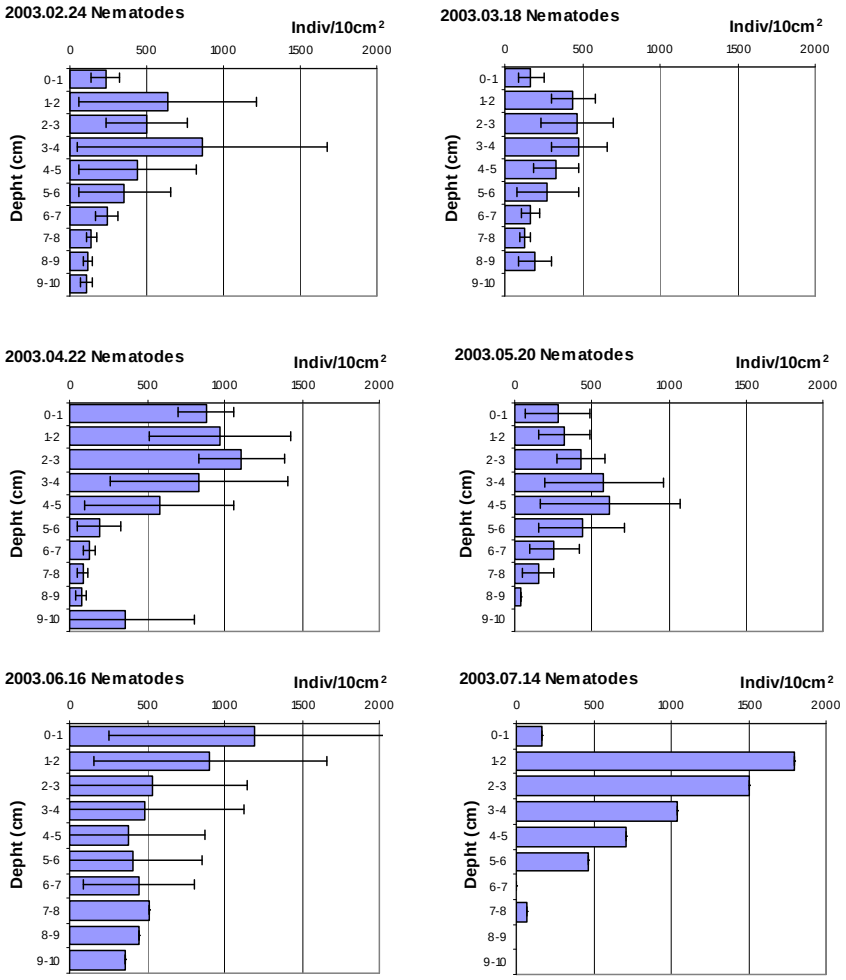
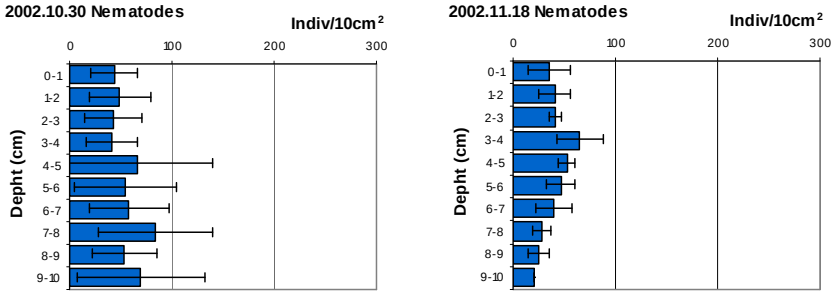


Figure 2 Nematode vertical profiles at Station 115bis: October – July



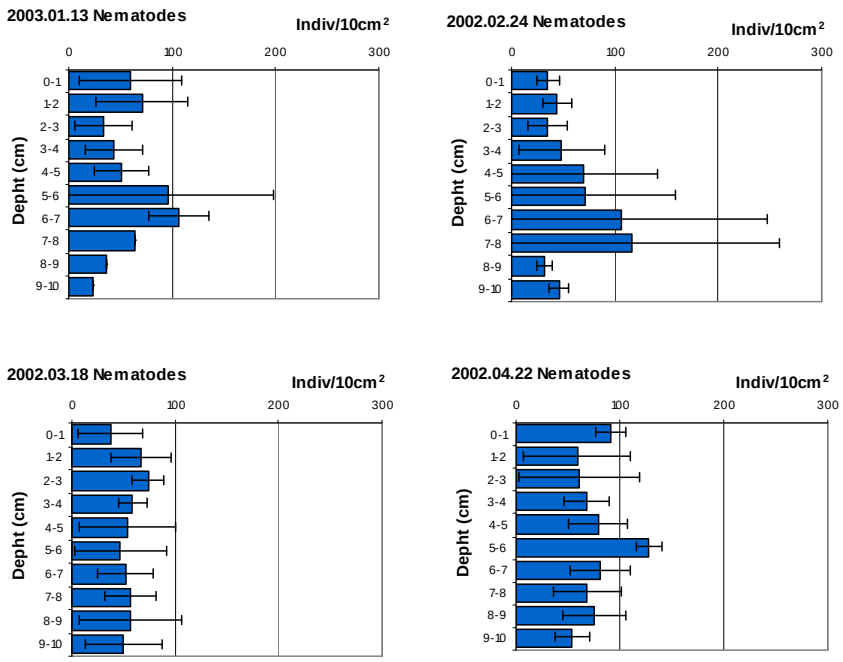


Figure 3. Nematodes vertical profiles on station 330 in the sampling months from October to April.

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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