

Nematode biomass spectra as descriptors of functional changes due to human and natural impact

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ABSTRACT: Nematode biomass spectra (NBS) for different nematode communities—subject to different forms of stress and enrichment—from the Belgian continental shelf have been constructed and analysed. These analyses showed that non-normalised NBS yield better results for comparisons of nematode assemblages than normalised NBS (in which the biomass in a weight class is divided by its corresponding weight interval) since the ecologically relevant information is retained. Normalising the spectra caused elevated biomass values and peaks to disappear, introducing bias when interpreting the distribution of biomass over spectra. Cumulative nematode biomass spectra proved to be useful in evaluating statistical differences, using the slope of the regression line of the cumulative biomass to the nominal value of a \log_2 -based size class. Interpreting Pareto-type graphs and regressions was not straightforward. We suggest a combined use of both NBS and the regression approach for the analysis of NBS. NBS and cumulative NBS constructed for nematode communities from undisturbed sediments proved to be conservative: no differences in size distribution were found for communities from different locations. Physical disturbance, introduced by sand extraction, did not affect the regression slopes of cumulative NBS. However, a shift in peak biomass values towards lower size classes was observed in the regular NBS. This was attributed to an alteration of the nematode communities due to the frequent physical disturbance of the sediments. At an oxygen-stressed site, we observed a single class biomass peak, due to the presence of a single nematode species well adapted to the impoverished sediment quality. Phytoplankton sedimentation during a spring bloom corresponded to shifts in peaks in NBS due to a change in age structure of the nematode communities. Biomass values probably increased as a result of a higher food supply to the benthos.

KEY WORDS: Nematodes · Biomass · Size spectra · Physical disturbance · Oxygen stress · Phytoplankton sedimentation

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INTRODUCTION

In traditional benthic ecological research, communities are described by structural variables such as density, species composition and diversity indices. This approach is generally time-consuming and requires taxonomically trained researchers. An alternative method involves the study of a functional descriptor of communities, the biomass distribution over size. This offers the most extensive and powerful generalisation that can be used in ecological studies (Peters 1983). In addition, this method requires no taxonomical knowl-

edge and therefore it offers an alternative and sensible technique for describing and comparing benthic communities (Sprules & Manuwar 1986, Drgas et al. 1998, Duplisea & Drgas 1999, González-Oreja & Saiz-Salinas 1999, Duplisea 2000). Recent evidence suggests a change in macrobenthic biomass spectra in stressed ecosystems (González-Oreja & Saiz-Salinas 1999), while in undisturbed sediments, complete benthic size-spectra seems to be conservative; no changes in the spectra were observed when the biomass spectra of different geographical areas or sediments were examined (Drgas et al. 1998, Duplisea & Drgas 1999,

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Duplisea 2000). Moreover, experimental approaches (Leaper et al. 2001) failed to document differences in body-size distributions when clearly different artificial substrates (glass beads of respectively 1.5 to 2 and 0.055 to 0.1 mm median particle diameter) were used.

Schwinghamer (1981, 1983, 1985), in a series of papers, was the first to describe the benthic size spectra as the distribution of equivalent spherical diameters (ESD) over a \log_2 scale. He found 3 distinct heterotrophic size groups, independent of the location of the sampling sites, corresponding with bacteria, meiobenthos and macrobenthos. Differences in size were related to the way organisms perceive the sedimentological environment: bacteria live on the sand grains (grain surface dwellers), meiobenthos live in between the sand grains (interstitially) while the macrobenthos experience the sediment at the macroscopic scale (the sediment-water interface). Warwick (1984) described a species size spectrum and found 2 distinct peaks when studying the complete metazoan spectrum. Peaks corresponded with meio- and macrobenthos and were explained as the optimal size for optimising life-history and feeding traits within both macro- and meiobenthos. Neither finding contradicts the other, which indicates that species and biomass distributions might be controlled by different external factors.

Since the pioneering work of Schwinghamer and Warwick, most of the recent benthic studies reporting biomass spectra included the complete benthic community (Drgas et al. 1998, Duplisea & Drgas 1999, Duplisea 2000). This study reports on the biomass spectra of a single taxon within the meiobenthos—the nematodes. Nematodes are generally the dominant taxon within the meiobenthos, and it can be assumed that the general shape of the meiobenthic part of the spectrum is determined by nematode biomass (e.g. Fig. 1 in Duplisea & Hargrave 1996). Drgas et al. (1998) also identified nematodes as the main contributors to the biomass in the lower weight classes of benthic size spectra constructed for Baltic Sea sediments.

Nematodes are generally considered to be a good tool for sediment monitoring due to their high diversity, short generation times, ubiquitous distribution and direct benthic development (Heip et al. 1985). However, nematode studies are not yet regularly implemented in monitoring studies, due to (1) the relatively large amount of work (and therefore money) that is required in comparison to macrobenthic studies and (2) the rather high taxonomic skills needed for nematode species identification. Therefore, constructing nematode biomass spectra (NBS) can provide an easy way for monitoring changes in the sediments due to anthropogenic or natural stress, since this is less time-consuming and can be performed by non-specialists.

This paper has 2 major aims: (1) to analyse the response of NBS to 3 distinct stressors upon the sediment habitat. Firstly, spectra from undisturbed communities were compared to spectra constructed for physically disturbed communities (due to sand extraction). Secondly, coastal NBS from reduced sediments were compared to spectra from an oxidised coastal station; and thirdly, the changes in the spectra during deposition of the spring phytoplankton bloom were followed. The null hypothesis tested is that the spectra will not show differences due to disturbance or food enrichment. (2) Since some debate exists about the appropriate statistical method for comparing size spectra (see e.g. Vidondo et al. 1997, Baca & Threlkeld 2000), routine comparisons of size spectra have not found their way into benthic ecology. Therefore, nematode size spectra were analysed in different ways and the advantages and disadvantages of the various methods are discussed. The advantage of using NBS instead of complete benthic spectra will also be discussed.

MATERIALS AND METHODS

Sampling sites. Sandbanks: Three sandbanks on the Belgian continental shelf belonging to 3 different sandbank systems were sampled (Fig. 1). The Kwintebank (Flemish Banks) was sampled in February 1997. Both the Gootebank (Zeeland Banks) and the Noordhinder (Hinder Banks) were visited in February 1998. The crests of the Flemish Banks are situated some 4 m below MLLWS, while the crests of the other sandbanks are deeper (Zeeland Banks: below the 10 m depth line; Hinder Banks: well below the 10 m depth line) (Maes et al. 2000). A more detailed description of the sandbank systems on the Belgian continental shelf is given in Vanaverbeke et al. (2000).

On each sandbank, 7 stations were sampled: 5 of them were located at regular distances on the crest of the sandbank, while 2 stations were situated at the 10 m depth line on the flanks of the sandbanks. On the Kwintebank, 12 stations were sampled: 2 stations were located in the gullies next to the sandbanks, while 10 stations at the crest of the sandbank correspond with the stations listed in Willems et al. (1982). In the northern part of the Kwintebank, regular sand extraction occurs in the area comprising Stns Kw1 to Kw6, while in the remaining area (south, Stns Kw7 to Kw10) sand extraction activities are significantly lower (Bonne & Vincx unpubl.).

Samples for meiobenthos and sedimentological analysis (10 cm²) were obtained by subsampling the same Reineck boxcorer at all stations. Sediments were analysed using a Coulter LS100 particle size analyser. Sediment fractions <1000 µm are expressed as volume

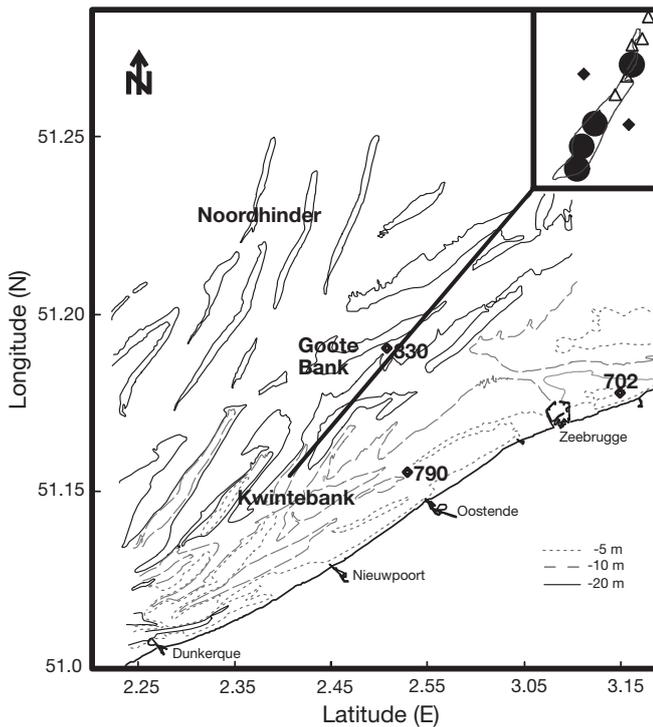


Fig. 1. Belgian continental shelf with indication of the sampling stations. Kwintebank stations (triangles) are high sand extraction activities; also shown are low sand extraction activities (dots); and gully stations (diamonds)

percentages, while the fractions between 1000 and 2000 μm and $>2000 \mu\text{m}$ are mass percentages.

Coastal area: Data for this part of the study were collected from 2 stations in the coastal area of the Belgian continental shelf. Stn 702 ($51^{\circ}09.1' \text{N}$, $02^{\circ}36.1' \text{E}$; water depth: 10 m) is located near the mouth of the Westerschelde area and is influenced by organically polluted water coming from the estuary. Stn 790 ($51^{\circ}16.0' \text{N}$, $02^{\circ}65.0' \text{E}$; water depth: 8 m) is situated in the central part of the Belgian coastal area. Stn 702 was sampled in December 1994, while samples from Stn 790 were collected in March 1994. Sediments were clearly different; Stn 702 had a fine sand sediment with a low mud content (clay-silt fraction: 11.4%, recalculated from Steyaert et al. 1999), whereas at Stn 790, sediments consisted of medium sand almost devoid of mud (clay-silt fraction $<0.1\%$, recalculated from Steyaert et al. 1999). Redox potential values at both stations were recorded with a mV-meter. Concentrations of nitrate plus nitrite and ammonia in the interstitial water were measured through an automatic chain (SAN^{plus} segmented flow analyser, SKALAR). The concentration of nitrate, nitrite and ammonia, together with redox potential measurements, were used to evaluate the oxidation status of the sediment. Redox potential values are reported in 4 classes representing

strongly oxidised ($>100 \text{ mV}$), oxidised ($0 \text{ mV} < x < 100 \text{ mV}$), reduced ($0 \text{ mV} < x < -100 \text{ mV}$) and strongly reduced ($<-100 \text{ mV}$) sediment after Steyaert et al. (1999).

Open sea: The functional response of nematode communities to natural phytoplankton sedimentation following a spring bloom was studied at the open sea site Stn 330 ($51^{\circ}26.0' \text{N}$, $02^{\circ}48.5' \text{E}$; water depth: 20 m) at the Belgian continental shelf. The sediment consisted of medium sand. Chl *a* values at the sea surface were obtained from Rousseau (2000). The supernatant water in the Reineck boxcorer was carefully siphoned off, and 0.5 l was filtered on Whatman GF/C filters. Filters were stored in the freezer until processing. Chl *a* values were obtained by HPLC (Gilson) using a slightly modified method of Mantoura & Llewellyn (1983). Samples for meiobenthos were obtained on March 9, May 12 and July 12, 1999.

Sampling and treatment of samples. Sampling at all sites took place aboard RV 'Belgica'. Sediment was collected at the sandbank stations and at Stn 330 using a modified Reineck boxcorer. The boxcorer was deployed 3 times per station. From each boxcorer, one perspex core (10 cm^2) was used for faunal analysis up to 10 cm sediment depth. The coastal stations were sampled using a boxcorer (surface area 804 cm^2), which was subsequently subsampled with similar perspex cores (see Steyaert et al. 1999 for more details). Additional cores were used at all sites for the determination of environmental variables, which are reported in Vanaverbeke et al. (2000) for the sandbanks and Steyaert et al. (1999) for the coastal stations. At the sandbank stations, the complete sediment column was fixed using a hot (70°C), neutral formaldehyde tap-water solution (4%), whereas at Stns 330, 790 and 702, sediments were sliced vertically at 1 cm intervals to 10 cm depth before fixation, in order to study the vertical distribution of the meiobenthos (Steyaert et al. 1999, J. Vanaverbeke & M. Steyaert unpubl.). The upper 2 cm of Stn 330 were sliced every 0.5 cm. In the laboratory, all animals passing a 1 mm sieve and retained by a $38 \mu\text{m}$ sieve were extracted from the sediment by centrifugation with Ludox (Heip et al. 1985). After staining with Rose Bengal, nematodes were removed randomly and mounted on Cobb slides for identification and measurements. From the sandbank samples and sliced sediments, 200 and 120 nematodes per slice were used, respectively.

Nematode community analysis and diversity. All nematodes were identified to species level. The nematode communities from the sandbanks were grouped according to their geographical position and sand extraction history. Vanaverbeke et al. (2002) showed clearly that nematode communities from the different sandbanks were different from each other. On the

Kwintebank, a further subdivision was made based on the sand extraction history at each station (Bonne & Vincx unpubl.). Stns 1 to 3 and 5 to 7 were grouped together (Kw H) since high sand extraction activities were recorded at these stations (1000 to 8000 m³ yr⁻¹ are removed). A low extraction occurred at Stns 4 and 8 to 10 (Kw L); here 1200 to 1400 m³ yr⁻¹ of sand is removed. Both gully stations (Kw 12 and Kw 13) were put together in a final group (Kw Gul).

Nematode community structure for the temporal study at the open sea site, Stn 330, was evaluated using TWINSPAN (Hill 1979a) and canonical analysis (CA) (Hill 1979b). All analyses were performed on mean nematode densities per sediment slice for each sampling date. Data were reduced by eliminating all species with a maximum abundance $\leq 1\%$ in all stations, resulting in a data matrix of 34 stations by 138 species. Cut levels applied in TWINSPAN were 0, 0.35, 0.5, 1 and 3. Groups discerned by TWINSPAN and CA were subjected to multi-response permutation procedures (MRPP) in order to test their significance (Mielke et al. 1976). All analyses were performed using the PC-Ord package. These analyses were not performed for the coastal stations since Steyaert et al. (1999) accurately documented community differences.

Construction and analysis of nematode biomass spectra. Nematode length (excluding filiform tails, if present) and maximal width were measured using an image analyser (Quantimet 500+). Nematode biomass was calculated from Andrassy's formula (Andrassy 1956) and a dry-to-wet-weight ratio of 0.25 was assumed. For the sandbank study, all nematodes from 1 replicate were measured, while at the coastal stations, all nematodes from all sediment slices were measured from 3 replicates.

NBS were constructed using log₂ groupings of nematode dry weight (μg) on the *x*-axis and total biomass per size class (dry weight, μg) on the *y*-axis. The log₂ weight-class represents the organism weight within the class; e.g. the biomass in Size Class 0 represents the sum of the biomass of all organisms in the dry weight range $\geq 2^0$ to $< 2^1$ (i.e. ≥ 1 to < 2 μg). For each nematode assemblage, NBS were constructed considering all stations within a group as replicates. NBS for the sliced sediments were constructed after pooling the measurements of 1 sediment column into 1 replicate, thereby providing a spectrum for the nematodes living in the complete sediment column.

When possible, a univariate 'split-plot' ANOVA design was constructed in order to test for differences of biomass per size class with location (or time) \times class. Replicates were nested within 'location (time)'; however, not within 'class'.

Studies on biomass spectra in the pelagic field are often normalised (Ahrens & Peters 1991, Rojo &

Rodriguez 1994) in order to find suitable regressions between biomass and size (Platt & Denman 1978, Sprules & Munawar 1986). Normalising is performed by dividing the biomass in a size class by the size class width. Normalised NBS have been constructed here in order to discuss their relevance in benthic ecology.

As a second way of interpreting biomass spectra, mean cumulative NBS for each group were constructed here. The mean cumulative NBS were described by regression of the cumulative biomass in each size class against the log₂ of the upper limit of the size class. Slopes of these regression lines were used to characterise the NBS. The slopes of these regressions were compared according to Zar (1984). If differences were significant ($p \leq 0.05$), a multiple comparison approach was applied following Zar (1984).

Vidondo et al. (1997) offered a third way of analysing size spectra. In order not to lose information by pooling a large number of observations in discrete classes, it was assumed that size distribution follows an underlying Pareto distribution. The characteristics of this underlying distribution can be obtained by plotting the probability that size *s* of a particle at random will be greater than size *S* ($\text{prob}[s>S]$) as a function of *S* on a double-logarithmic scale. In practice, the term $\text{prob}(s>S)$ is calculated for each nematode as the fraction of all nematodes larger than or equal to itself. If the nematode biomass is distributed according to a Pareto model, this graph will display a straight line. Fitting a least-squares regression line through these points will produce the necessary statistics to evaluate the parameters of the underlying Pareto distribution. By doing this, each individual nematode-biomass value contributes 1 point in the plot and all the information in the observations is used. For a theoretical explanation, the reader is referred to Vidondo et al. (1997). Pareto-type distributions were only plotted for the coastal study.

RESULTS

Physical disturbance study (sandbanks)

Study area and community analysis

Mean granulometric variables are listed in Table 1. All sediments were classified as medium sand (median grain size between 250 and 500 μm). Finest sediments were found at the gully stations in the vicinity of the Kwintebank. No obvious differences in the median grain size between the different sand extraction areas at the Kwintebank were noted. A detailed description of the nematode communities is beyond the scope of this paper and is discussed elsewhere (Vanaverbeke et al. in press). However, it should be noted that the

Table 1. Disturbance study. Mean sedimentological variables of the different groups

	Median grain size (μm)	% clay	% silt	% very fine sand	% fine sand	% medium sand	% coarse sand	>1000 μm	>2000 μm
Noordhinder	375.23	0	0	0	8.28	74.985	16.77	0	0
Gootebank	341.96	0.02	0.16	0.40	18.26	67.22	13.94	0	0
Kw H	361.05	0.03	0.09	0.29	23.55	54.98	21.06	5.25	2.38
Kw L	355.75	0	0	0.12	23.66	59.18	17.04	8.22	2.03
Kw G	266.2	1.06	4.08	1.68	42.45	43.01	7.74	5.13	3.02

nematode assemblages were clearly different. Only *Neochromadora munita* was listed among the 10 dominant species in more than 2 Twin groups.

Nematode biomass spectra

Mean NBS (not normalised) per group are shown in Fig. 2. The general trend was similar in all groups: biomass increased with body size up to Size Class -1 or 0, and decreased again at higher size classes. At the gully stations, biomass peaked at Size Class 1, and higher biomass values were recorded here in comparison with the other groups. Normalised NBS (not depicted) were very similar, but normalised biomass peaked at Size Class -3 for most of the spectra. A split-plot ANOVA could not be performed since the number of replicates differed between groups.

Regressions of cumulative biomass per size class against \log_2 of the upper limit of the corresponding size classes (Fig. 3) were all highly significant ($p < 0.001$ for all ANOVAs) with high r^2 values (between 0.90 and 0.94). However, slopes of the regressions were not significantly different.

Oxygen stress (coastal stations)

Study area and community analysis

Redox-potential values and vertical profiles of the nitrogen compounds from the 2 coastal stations were clearly different (Fig. 4). Sediments at Stn 790 were completely oxidised, with high redox potential values (>100 mV throughout the sediment column) and a stable nitrogen compound depth pattern. At Stn 702, sediments were only oxidised in the upper cm, and strongly negative redox potential values were recorded deeper than 2 cm, associated with a buildup of ammonia. Nitrate/nitrite concentrations dropped severely at this depth. Multivariate analysis of the nematode community composition of the 2 coastal stations was not performed since differences in communities have been adequately described in Steyaert et al. (1999). In short,

the nematode communities in the reduced sediments of Stn 702 harboured only 45 species, and the communities were dominated by *Sabatieria punctata*. In contrast, 98 species were found at Stn 790 with no strong dominance of a single genus or species.

Nematode biomass spectra

Both regular NBS and normalised NBS for the 2 stations are depicted in Fig. 5. Visual inspection of the graph shows that biomass values from Stn 702 are higher. The regular NBS shows a single great peak in Size Class -1. This peak disappears when spectra are normalised, but clearly higher values were obtained for all size classes. In the right hand part of the spectrum, differences between size classes are less obvious. ANOVA results confirmed the difference between the regular NBS ($F_{10,5} = 2.11$; $df = 10$; $p \leq 0.05$).

Regressing cumulative biomass per size class to the \log_2 value of the upper size limit yielded significant regressions ($p < 0.001$) with high r^2 values (Fig. 6). The slope from the regression for Stn 702 was steeper compared to the slope for Stn 790. Statistical comparison of the slopes following Zar (1984) revealed significant differences ($p < 0.001$).

Pareto-type graphs are displayed in Fig. 7. In both cases, a significant regression was fitted with relatively high r^2 values. The higher biomass in Stn 702 was reflected in a gentler slope when compared to the slope of Stn 790. Again, regression slopes were significantly different ($p < 0.001$).

Food pulse study (open sea)

Study area and community analysis

Chl *a* values in both the surface and near bottom waters are depicted in Fig. 8. Surface chl *a* concentrations revealed a peak in the phytoplankton bloom at the end of April/beginning of May. The pattern at the surface was closely followed by the pigment concentrations in the bottom water, indicating sedimentation

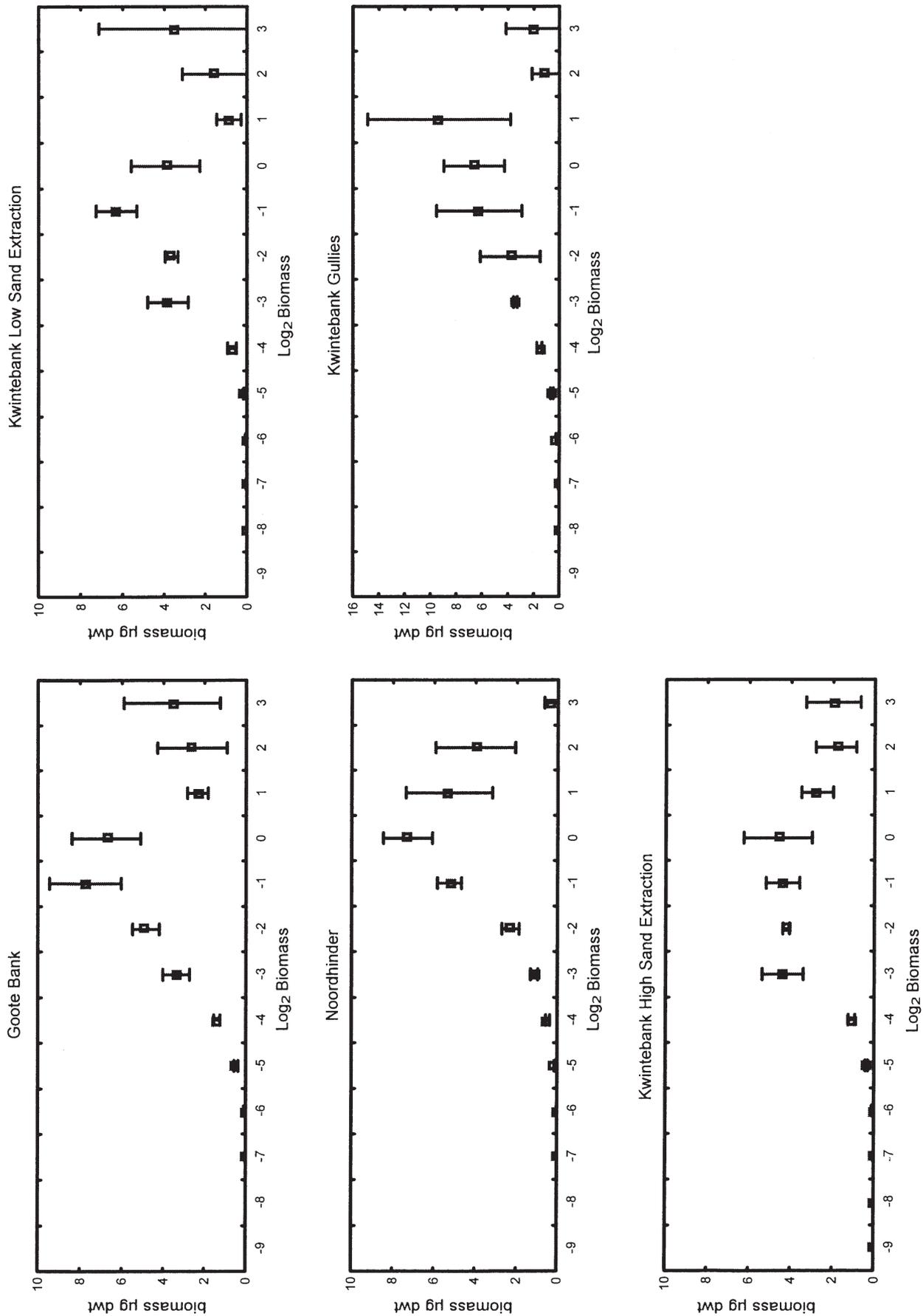


Fig. 2. Disturbance study. Mean nematode biomass spectra (NBS) (error bars: SE of mean biomass values per size class) of different groups on the Belgian continental shelf

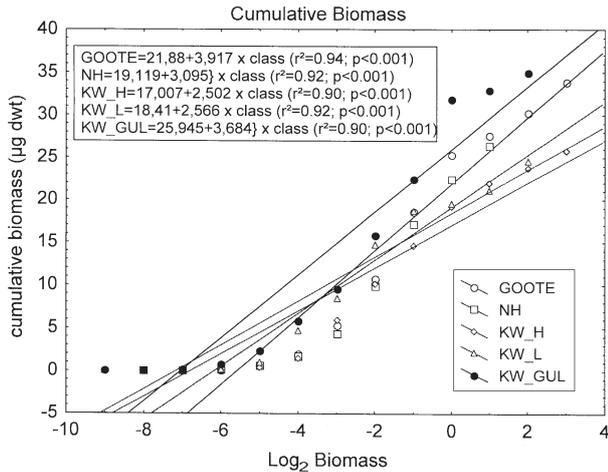


Fig. 3. Disturbance study. Cumulative nematode biomass spectra (NBS) of different groups on the Belgian continental shelf. Goote: Gootebank, NH: Noordhinder, KW_H: Kwintebank High Sand Extraction, KW_L: Kwintebank Low Sand Extraction, KW_Gul: Kwintebank Gullies

of phytoplankton to the seafloor. At the date of the first sampling for the meiobenthos (March 9), rather high chl *a* values in the bottom water were observed as well.

Nematode communities clearly showed a change in community composition from March till July (Fig. 9). Communities in March were separated from May and July (although 1 sediment slice from July was classified in this group as well). May and July were subsequently separated as well, resulting in 3 groups, each representing 1 sampling date. A Canonical Analysis (not depicted) confirmed these patterns. Although a detailed description of the nematode communities is not the aim of this paper, it should be pointed out that changes in the number of species did occur (ANOVA: $F_{2,5} = 64.14$; $p < 0.001$).

Nematode biomass spectra

NBS were similar in May and July, with maximum values found for Size Class -2. NBS peaked in the -1 size class during March (Fig. 10). Biomass values were much higher in May than in March, with intermediate biomass values in July. Differences are most obvious in the middle size classes. The general pattern remained the same in the normalised NBS. Here again, the peak size class shifted towards lower size classes compared to simple NBS, and differences were more pronounced in the lower biomass values of the spectrum. Spectra did not show single class peaks as in the reduced Stn 702, but elevated biomass values are spread over several size classes. The 'time × class' interaction term was significant ($F_{24,72} = 2.16$; $df = 24$; $p < 0.01$) in the ANOVA split-plot analysis of the regular NBS.

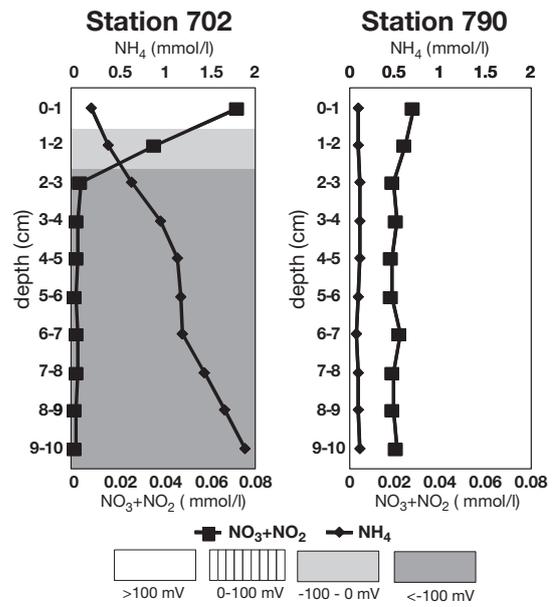


Fig. 4. Redox potentials and nitrogen compounds at Stns 702 and 790 on the Belgian coast

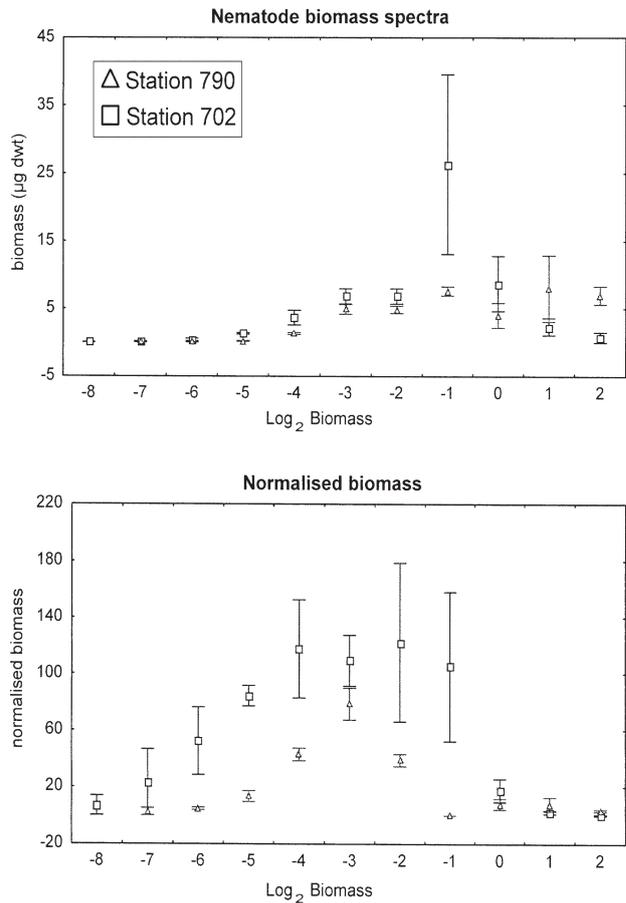


Fig. 5. Nematode biomass spectra (NBS) (upper panel) and normalised NBS (lower panel) for Stns 702 and 790 on the Belgian coast

Cumulative biomass regression slopes (Fig. 11) were significantly different ($p < 0.001$). Multiple comparisons revealed significant differences between the slopes for March and May ($p < 0.001$) and March and

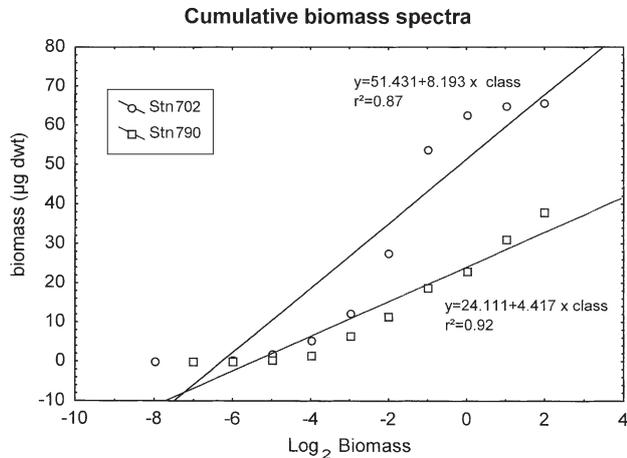


Fig. 6. Cumulative biomass spectra for Stns 702 and 790 on the Belgian coast

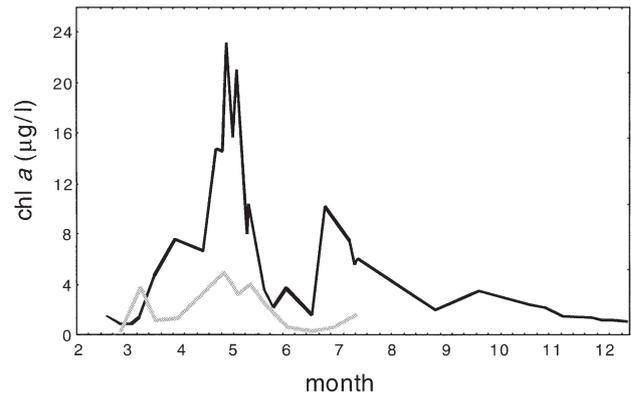


Fig. 8. Chl a concentrations in the water column at Stn 330 on the Belgian continental shelf. Black line: surface water; grey line: bottom water

July ($p < 0.001$). No significant differences were detected between the slopes of the regressions for May and July.

Pareto-distributions (not depicted) gave similar pictures as for the coastal stations. Characteristics of the regressions are listed in Table 2. All regressions were highly significant ($p < 0.001$) with high corresponding r^2 values (>0.83). Here, the accumulation of biomass in higher size classes in May and July again resulted in more negative slopes compared to March, but differences between the slopes of May and July were not obvious. The slope of the July spectrum was even slightly steeper than for the spectrum constructed for May.

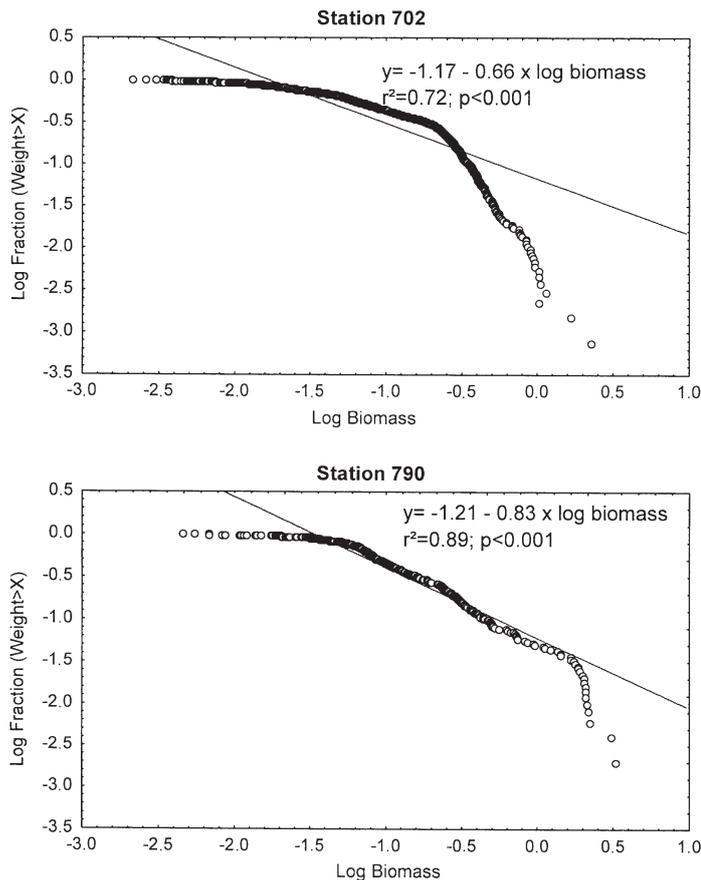


Fig. 7. Pareto-type biomass distributions for Stns 702 (upper panel) and 790 (lower panel) on the Belgian coast

DISCUSSION

Functional response of nematodes to environmental changes

Sandbank study

Slopes of the cumulative spectra were not significantly different, indicating that no differences exist among biomass spectra from different localities. However, careful examination of the nematode biomass

Table 2. Regression slope parameters with indication of the significance level (F -test) for the regression. Pareto-distributions for the phytoplankton study, Belgian continental shelf

	a	b	R ²	p
March	-1.218	-0.653	0.83	<0.001
May	-1.497	-0.919	0.88	<0.001
July	-1.462	-0.923	0.85	<0.001

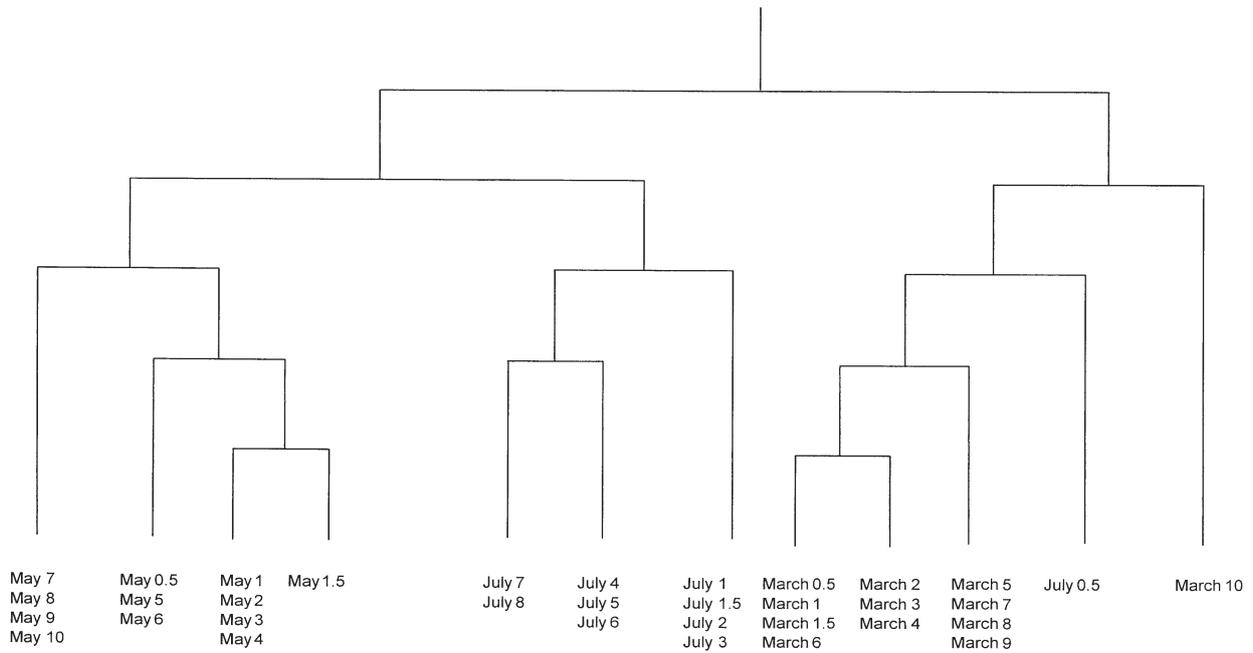


Fig. 9. Dendrogram with TWINSpan results: phytoplankton sedimentation study (numbers indicate sediment layers) on the Belgian continental shelf

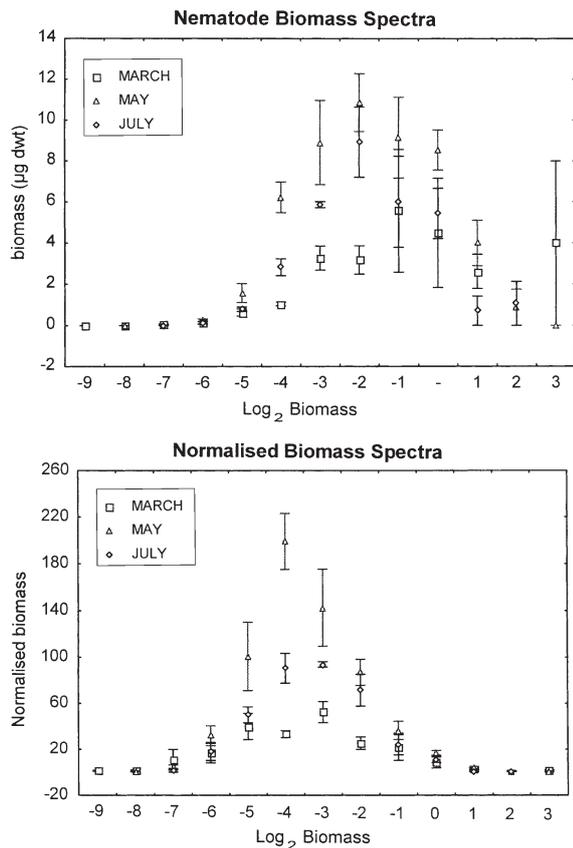


Fig. 10. Phytoplankton sedimentation study. Nematode biomass spectra (NBS) (upper panel) and normalised NBS (lower panel) on the Belgian continental shelf (error bars: SE of mean biomass values per size class)

spectra in Fig. 2 reveals that spectra from the high sand extraction area on the Kwintebank and the gully stations exhibit some differences when compared to the other spectra. Biomass at the Kwintebank high sand extraction sites peaked earlier in the spectrum. This might be an alteration of the nematode communities due to the frequent physical disturbance of the sediment in this area; smaller species seem to be more resilient to sediment removal, resuspension and changes in overlying water currents. Moreover, smaller organisms can often be designated as 'colonisers' since they show rapid growth and early reproduction, which are often associated with frequently disturbed habitats (Newell et al. 1998)

In contrast, biomass at the gully stations peaked at a higher size class. This is due to the fact that one of the gully stations had a rather fine sediment (median grain size: 171 µm). Field observations at this station revealed black sediments at a few cm depth, indicating reduced and oxygen-depleted sediments. This sediment was dominated by 2 *Sabatieria* species, rather large nematodes that can withstand poor oxygen conditions in the sediment (see below). This dominance caused the biomass peak at Size Class 1. This points to the differences in the ability of the 2 methods to compare NBS. The regression of the cumulative biomass values is influenced by the total biomass in the system and integrates all data points (e.g. each biomass value and its corresponding x-axis value) in a single regression equation. Visual inspection of the NBS explores the total biomass values in each class separately. The number of indi-

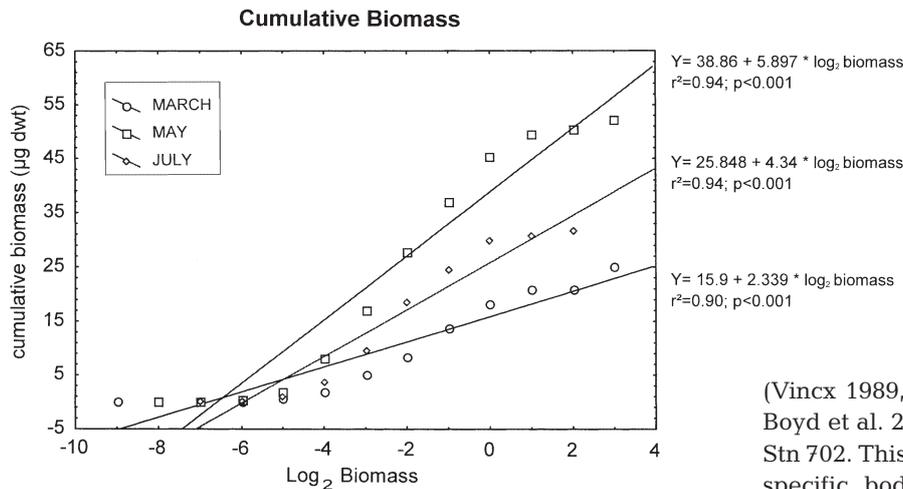


Fig. 11. Phytoplankton study. Cumulative biomass spectra on the Belgian continental shelf

viduals falling within the size-class range determines these values. Therefore, responses on the species level (e.g. smaller species more resilient to physical disturbance; dominance of *Sabatieria* in reduced sediments) are more likely to be expressed in different NBS. Whether these results confirm the earlier findings that no differences exist among biomass spectra from different localities (Drgas et al. 1998, Duplisea & Drgas 1999, Duplisea 2000) remains unclear. NBS from the undisturbed sandbank locations (Goote Bank, Noordhinder) or low impacted location (Kwintebank low sand extraction area) point in this direction, but since no split-plot ANOVA could be performed, this result should be confirmed by a more balanced sampling scheme. Disturbance, both physical or related to anoxia, seems to alter the NBS, as a result of responses on the species level in the nematode communities.

Oxygen stress

Nematode community composition at the 2 coastal stations reflected differences in sediment composition, but the high dominance of the single species *Sabatieria punctata* at Stn 702 is attributed to the highly reduced conditions at that site (Vincx 1990, Steyaert et al. 1999). This is reflected in the NBS, which shows a very large biomass peak at a single size class (Fig. 5). ANOVA confirmed the difference in the shape of the NBS. Tita et al. (1999) explored the relationship between nematode size and nematode metabolism. Since the reduced sedimentological environment at Stn 702 causes difficult situations for metazoans to survive, only organisms that are specifically adapted to these conditions will remain. One of these adaptations can be a specific body length:width ratio (Tita et al. 1999). Since *S. punctata* can tolerate unstable, highly polluted environments

(Vincx 1989, Heip et al. 1990, Vanreusel 1991, Boyd et al. 2000), it shows a high dominance at Stn 702. This high abundance, together with the specific body length:width ratio (parameters needed to calculate individual biomass) resulted in a single size class biomass peak (Fig. 5).

Diversity at Stn 790 was higher and no clear dominance of single species or genera was observed. These findings can be explained by the coarser sediment together with the more oxic conditions compared to Stn 702 (M. Steyaert et al. 1999). The different nematode feeding types were more evenly distributed over the communities (Steyaert unpubl.). This resulted in the absence of a single size-class peak: relatively high biomass values were encountered in Size Class -3 to Size Class -1. The significant differences in the slope of the cumulative NBS confirmed the existence of higher biomass in the reduced sediments; therefore the hypothesis, that no differences in biomass distribution over size occur when sediments become reduced, can be rejected. The overall higher biomass in Stn 702 can possibly be explained by higher food abundance in that station compared to Stn 790 (Steyaert et al. 1999).

Although the differences in NBS and the regression slopes were obvious, it should be pointed out that these results are based on only 2 sampling stations. Spatial and temporal replication of both situations (oxic vs reduced sediments) is needed to avoid pseudoreplication (see Hurlbert 1984). The composition of the nematode communities at both stations are known from time series since 1976 (Vincx 1986, Steyaert et al. 1999). The nematode communities always reflected the difference in sediment characteristics, and *Sabatieria* species dominated the nematode community at Stn 702. However, an experimental approach, in which comparable sediments are subjected to different oxygen regimes, would enhance our understanding of the variables responsible for the observed shift. This would confirm the oxygen stress hypothesis, since other related variables (e.g. organic loading, historical pollution, sulphide production) might play an important role in shaping NBS.

Food pulse study

The deposition of fresh organic material from the water column clearly influenced structural aspects of the nematode communities, since both community composition and diversity showed drastic changes. The rather high chl *a* values in the bottom water at the beginning of March might point to the sedimentation of an earlier phytoplankton bloom. However, we have no surface chl *a* values to support this finding. Since the response time of the benthic community to food supply is on the order of 2 wk to 2 mo (Graf 1992), it was assumed that NBS from March reflect the pre-bloom situation. Indeed, chl *a* concentrations in the nearbottom water seemed to be minimal in the period preceding our sampling ($0.19 \mu\text{g l}^{-1}$ on February 26).

A more detailed description of these changes has been studied by Vanaverbeke et al. (unpubl.). In addition, an increase of nematode biomass suggested a functional response to organic input. Nematode biomass increased drastically when fresh remineralisable organic matter reached the sediment and gradually decreased after a period of remineralisation. Data on biomass responses to changes in food availability in shelf seas are lacking, but several deep-sea studies have shown lower nematode biomass values in areas with a lower food supply (Vanreusel et al. 1995, Vanaverbeke et al. 1997). Sommer & Pfannkuche (2000) reported significant correlations between mean individual nematode biomass with chl *a* concentration in the sediment, when comparing different sites in the deep Arabian Sea. Soltwedel et al. (1996) reported similar evidence and found a relationship between the size structure of deep-sea nematode communities and seasonal supply of organic matter to the seafloor.

A higher biomass during summer was associated with enhanced deposition of particulate organic carbon during this period. This was explained by growth and reproduction during periods of enhanced deposition. It was followed by deaths of adults and the emergence of a next generation, explaining a decrease in biomass when many juveniles were present in September. The age structure of the nematode communities at Stn 330 also showed shifts. The relative contribution of juveniles and females with eggs varied over time; the proportion of juveniles increased significantly with time, while the opposite was the case when gravid females were considered. This shift might be responsible for the shift in peak biomass in the NBS; in March this peak was situated in Size Class -1, while the higher amount of juveniles resulted in a peak biomass situated in Size Class -2 in May and July. The absence of a large peak in a single size class is again related to the high diversity in the nematode communities in association with the even distribution of the

feeding types present (J. Vanaverbeke unpubl.). Higher biomass values and steeper slopes in the cumulative NBS can then be attributed to higher food availability (directly or indirectly via the microbial loop) as reported in deep-sea studies (Vanreusel 1995, Soltwedel et al. 1996, Vanaverbeke et al. 1997).

Comparison of techniques

Biomass spectra have been reported in the past in many different ways: as regular biomass spectra (Duplisea & Drgas 1999, Duplisea 2000), as normalised biomass spectra (Drgas et al. 1998, Saiz-Salinas & Ramos 1999), relative biomass contribution (González-Oreja & Saiz-Salinas 1999), cumulative proportion of biomass (Baca & Threlkeld 2000), cumulative frequency distributions (Vanreusel et al. 1995), equivalent spherical diameters (Schwinghamer 1981, 1983, 1985) and Pareto-models (Vidondo et al. 1997).

Attempts were made to relate biomass spectra to \log_2 size classes using regression analysis of the form $B = a(S)^b$, where B = biomass, S = size and a and b are constants. However, this model was flawed by the differential width imposed by the logarithmic nature of the size classes, with small size classes containing organisms of similar sizes (e.g. on a μm scale) and larger size classes comprising organisms ranging meters in size (Blanco et al. 1994). The normalised spectrum, where the biomass in different size classes is scaled to the width of the size class, was proposed to overcome this problem (Platt & Denman 1978, Sprules & Manuwar 1986). The slope of the regression line of normalised biomass to the \log_2 scale has since been used to assess ecosystem health in many lake studies (Sprules & Manuwar 1986, 1991, Rojo & Rodriguez 1994). However, when only a limited part of the spectrum of benthic animals is considered (e.g. NBS), a different picture emerges. First of all, both regular NBS and normalised NBS were not linearly related to the \log_2 scale of the size classes, making a regression of the form $B = a(S)^b$ useless. In addition, these comparisons reveal that normalisation can significantly alter the interpretation of NBS. When biomass values are higher in reduced situations or when more food is available, this clearly results in an increase in biomass values at a single size class (Fig. 5) or in a number of size classes at the right part of the spectrum (Fig. 10). In the case of the reduced sediments (Fig. 5), the single size class peak disappeared, and higher values in the left part of the spectrum showed up; this was also the case in the phytoplankton sedimentation study (Fig. 10). This is a result of dividing the biomass values by increasingly smaller values when shifting to the left part of the spectrum. This clearly results in a loss of

ecologically relevant information, since the real total biomass values in an ecosystem are of key importance in assessing productivity. Spectrum normalisation was introduced in order to overcome the problem of differences in size of large magnitudes (from μm to m). This problem does not arise when limiting the study to a single taxon. Therefore, we suggest using the non-normalised NBS for studying various sources of impact to the sediments. Possible changes in NBS can be tested using an ANOVA 'split-plot' design. This approach was introduced by Steyaert et al. (2001) for testing differences in vertical profiles of nematodes over time. Here, biomass values were compared, using 'time' (food pulse study) or 'location' (coastal station) and 'class' as dependent variables. However, a balanced design is needed, and planned comparisons cannot be made.

Although NBS show an obvious response to changes in the sediment (oxygen stress and food pulse study), a mathematical expression of the spectra is not straightforward. In order to overcome this problem, we constructed cumulative biomass spectra, and linear regressions to the \log_2 scale were applied, resulting in highly significant regressions corresponding with high r^2 values. Baca & Threlkeld (2000) proposed a similar approach but they used the cumulative proportion of biomass distributions in order to standardise the y-axis. This again resulted in a loss of information on the total biomass in the distributions. Since we considered this as a disadvantage, the true cumulative biomass values were used in the regression. Accumulation of biomass in higher size classes always resulted in steeper slopes. This method allows for statistical comparison of slopes and the possibility of relatively easy multiple comparisons among slopes when following Zar (1984).

Vidondo et al. (1997) proposed the use of Pareto-type distributions to describe size spectra. This approach has the advantage of using all individual biomass measurements, thereby increasing the power of possible regressions. Pareto-type distributions have been made both for the redox state study (Fig. 7) and the food pulse study (Table 1). Although all regressions were highly significant, it can be seen from the graphs that a linear regression is not the best way to describe the patterns. A better model would be obtained by fitting a polynomial regression, but Vidondo et al. (1997) propose to fit these kind of data by a Pareto distribution of the second order: $\log(\text{prob}[s>S]) = c \log(K+D) - c \log(S+D)$. Estimators for the parameters K , c and D can be obtained with an iterative non-linear regression algorithm. However, this calculation is not straightforward, and the extra parameters make a simple comparison between distributions difficult. Moreover, Vidondo et al. (1997) clearly state that there will

always be data sets for which both Type I and Type II distributions will be inappropriate. Hence, forcing data to these specific distributions is ill-founded and can be highly misleading.

We therefore suggest that biomass spectra of nematode communities should be examined using a combination of simple NBS (reflecting possible single size class peaks and/or shifts in the position of the peak) and cumulative biomass spectra, making it possible to statistically test differences in size distributions. NBS from undisturbed sediments do indeed seem to be conservative (e.g. no differences in NBS or cumulative NBS were observed for the non-impacted sites in the sandbank study); hence deviations from this distribution can be a result of a changed environment as shown in both the oxygen and the food pulse study. This suggests that NBS could be used as an indicator of ecosystem disturbance. In many studies in freshwater lakes (Sprules & Manuwar 1986, 1991, Rojo & Rodriguez 1994), studies on normalised size spectra are used to establish ecosystem health. A departure from a regression slope of -1 is used as an indication of ecosystem disturbance. This is not possible when using regression slopes of cumulative NBS, since a reference of how the spectrum looks when no disturbance has occurred is needed. Therefore, NBS can be of great use in impact studies when an *a priori* spectrum is known, or well-documented control sites are available. NBS and cumulative NBS can be an easy and fast way to detect functional changes in a highly sensitive group such as the nematodes. However, the diagnostic accuracy and probability of Type I and II errors in the method should be evaluated before using it as a risk management tool.

Advantages of NBS in ecological studies

Most studies on benthic size spectra have focussed on the entire size spectrum, from small meiobenthos to large meiobenthos and sometimes including epibenthic fish. In order to cover this relatively broad range, various sampling methods have been used within one study (Gerlach et al. 1985, Drgas et al. 1998), sieves with different mesh sizes have been applied (Ramsay et al. 1997), or a combination of different gear and different mesh size were used (Duplisea & Drgas 1999, Duplisea 2000). Using different sampling gear might introduce bias in a spectrum since a single type of gear is designed to effectively sample organisms within a certain size range. Problems might arise in deciding which animal groups are sampled correctly by which gear. Edgar (1990) pointed to the fact that the use of sieves with a different mesh size can lead to the overestimation of biomass in lower size classes. The con-

struction of NBS addresses some of these problems. First of all, biomass within the meiobenthic part of the benthic size spectrum is often dominated by nematodes. The study by Drgas et al. (1998) shows that nematodes have a minimum contribution of 46.2% to the total biomass in weight class 501 ng C to 1 µg C, but in all other weight classes (to 500 ng C), almost 100% of the biomass is attributed to nematodes. A close inspection of the figures in Duplisea & Hargrave (1996) reveals the same pattern. Therefore, constructing NBS would reveal the same picture as if all animals in the meiobenthic sample had been measured, especially since nematodes cover the complete size range in which other meiobenthic taxa are found (see Drgas et al. 1998). Measuring all meiobenthic animals requires more time, energy and also money than constructing NBS, since all animals must be removed from the sample and measured.

Moreover, in order to construct reliable NBS, the use of 1 type of accurate sampling equipment is sufficient, as is the use of only 1 mesh size (the lower limit for meiobenthic samples). This reduces not only bias in the spectra introduced by the use of different sampling equipment and various sieves, but also the energy needed to process a rather large amount of samples and sieve contents.

CONCLUSIONS

(1) This study demonstrates the value of NBS in assessing functional changes in nematode communities as a result of a changing environment. Non-normalised NBS, in combination with regressions of cumulative biomass to the nominal value of the size classes, were able to detect these changes in a straightforward way. Moreover, changes in the biomass distribution could be tested statistically. Therefore, these methods are preferred above normalising spectra and Pareto-type approaches.

(2) Nematode communities exhibited functional responses to oxygen stress and phytoplankton sedimentation events. In both cases, cumulative biomass regressions showed a steeper slope, but the underlying mechanisms causing these changes were probably different. Reduced sediments triggered a situation with low diversity accompanied by a higher biomass, while in the food pulse case, higher diversity was associated with higher biomass. The difference in the oxygenation of the sediments (reduced circumstances in Stn 702 vs oxygen in the coarser sediments of Stn 330) resulted in different responses of the nematode communities. Sand extraction resulted in a peak in smaller size classes, possibly an adaptation of the nematode communities to frequent physical disturbance.

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