UNIVERSITEIT
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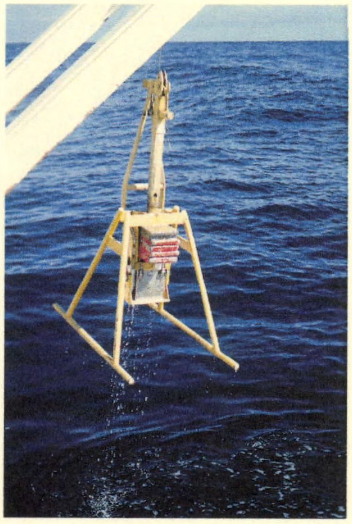
Faculteit Wetenschappen

MARINE BIOLOGY SECTION
GHENT UNIVERSITY. BIOLOGY DEPARTMENT

Structural and functional diversity of the meiobenthos on the Belgian Continental Shelf, with emphasis on nematode communities

Structurele en functionele diversiteit van het meiobenthos op het Belgisch Continentaal Plat, met nadruk op de nematodengemeenschappen



Jan Vanaverbeke

Promotor: Prof. Dr. Magda Vincx

Proefschrift voorgelegd tot het behalen van
de graad van Doctor in de Wetenschappen:
Biologie
Academiejaar 2002-2003

Ghent University. Biology Department. Marine Biology Section
Krijgslaan 281 / S8. B-9000 Gent Belgium

Mariene Biologie
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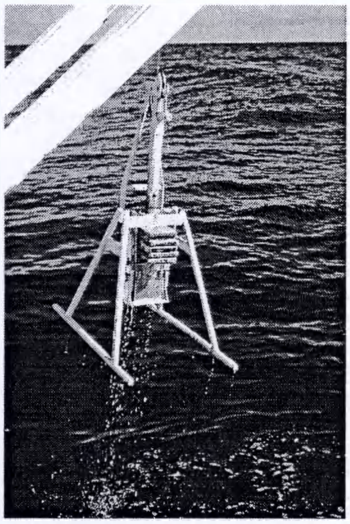

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Mariene Biologie
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Promotor: Prof. Dr. Magda Vincx

Other members reading committee:

Prof. Dr. Ann Vanreusel
Prof. Dr. Carlo Heip
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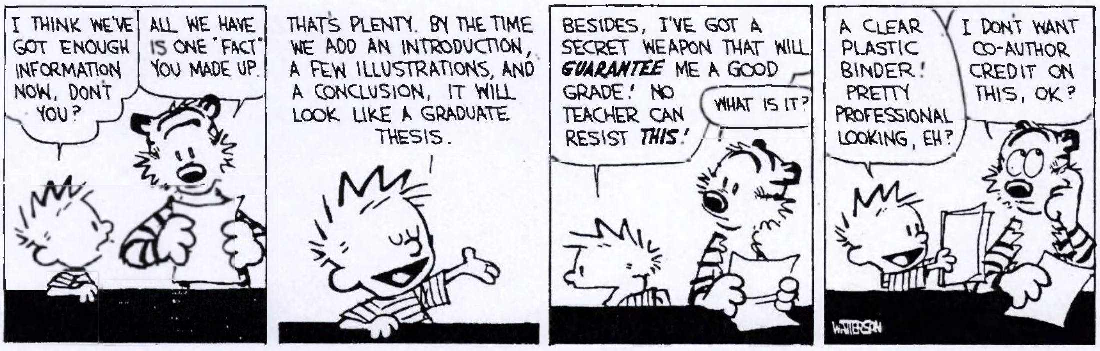
I THINK WE'VE GOT ENOUGH INFORMATION NOW, DON'T YOU?
ALL WE HAVE IS ONE "FACT" YOU MADE UP.
THAT'S PLENTY. BY THE TIME WE ADD AN INTRODUCTION, A FEW ILLUSTRATIONS, AND A CONCLUSION, IT WILL LOOK LIKE A GRADUATE THESIS.
BESIDES, I'VE GOT A SECRET WEAPON THAT WILL GUARANTEE ME A GOOD GRADE! NO TEACHER CAN RESIST THIS!
WHAT IS IT?
A CLEAR PLASTIC BINDER! PRETTY PROFESSIONAL LOOKING, EH?
I DON'T WANT CO-AUTHOR CREDIT ON THIS, OK?
WATTERSON

TABLE OF CONTENTS

Dankwoord

Voila, het zit er op! Het resultaat van zes jaar "zwoegen" staat samengevat op amper 125 bladzijden...Nu rest me alleen nog dat stukje te schrijven dat met argusogen zal bekeken worden.
Laten we maar beginnen bij het begin: ik wil mijn promotor, Prof Dr. *Magda Vincx* bedanken voor alle steun en vertrouwen die ik hier op de Mariene Biologie heb genoten. Ik geloof niet dat ze ooit gedacht had dat ik een doctoraat zou schrijven, waarin twee hoofdstukken handelen over biomassa en vorm van nematoden. De wetenschappelijke vrijheid die ik echter altijd heb gekregen, heeft er voor gezorgd dat ik steeds enthousiast naar mijn "beesten" bleef kijken.
Een doctoraatstudent moet ook eten, en om aan die primaire behoefte te voldoen heb ik uiteindelijk drie doctoraatsonderwerpen aangesneden. Ik hou er dan ook aan om Prof. Dr. *Carlo Heip* en Prof. Dr. *Schminke* te bedanken om mij gedurende de beginperiode van mijn "carriere" doctoraatsonderwerpen aan de hand te doen, en ook om mij daarvoor financiële ondersteuning te geven.
Ann Vanreusel, ondertussen ook al Prof. Dr. heeft me bijgestaan bij mijn eerste stappen in de wereld van de nematoden, eerst als begeleidster van mijn licentiaatsverhandeling, daarna tijdens mijn werk als "diepzeemeiobentholoog". Ook tijdens het schrijven van dit verhaal kon ik steeds op haar rekenen om eens deftig te discussiëren over het hoe en waarom van het nematodenwereldje.
Karline Soetaert heeft mij ook van in het prille begin - ten tijde van OMEX I - tot nu helpende handen toegestoken waar en wanneer nodig. Ik moet zeggen dat het soms wel schrikken was om manuscripten herleid te zien tot minder dan de helft van de oorspronkelijke tekst, maar uiteindelijk bleek alles dan toch wel in orde te komen. Ook nog eens bedankt om juist mijn data te gebruiken om databaseworkshops te houden, waardoor ik in een klap kon beschikken over een pico bello database...
Een doctoraat maak je nooit alleen, dat is me ondertussen al wel ongelooflijk duidelijk geworden. Ik vrees dat ik een halve hectare bos zou nodig hebben om genoeg papier te produceren om iedereen te bedanken die zijn/haar steentje heeft bijgedragen tot het welslagen van dit proefschrift. Ik ga dus mensen vergeten, maar bij deze zijn jullie allemaal van harte bedankt voor de hulp en inspanningen. Zo is het onbegonnen werk om iedereen te bedanken die tot een gat in de nacht op het dek van de Belgica heeft gestaan, soms nog met 7-8 beaufort en een fris hagelbuitje om de vermoeide en koude ledematen nog wat extra te doen kraken. Niet alleen mensen van de Mariene hebben zich op de Belgica vuil gemaakt, ook toevallige passanten van de KUL (*Els Gysels*, *Tine Huyse*) en het IN (onder wie *Henk Offringa*, *Jan Seys*, *Jeroen Van Waeyenberghe*) hebben menige core in een Reineck geduwd. De vaste "kliek" van de Mariene Biologie wil ik hier zeker niet onvernoemd

laten: *Dr. Dré, Dirk Van Gansbeke, Guy De Smet, Wendy Bonne, Johan van de Velde, Danielle Schram, Annick Van Kenhove*,...en in latere tijden *Tom Gheskiere, Thomas Remerie, Gert Van Hoey, Bernard Timmerman*,...(straks heb ik hier toch een half bos nodig).

André Cattrijsse verdient een extra woord van dank, om altijd de campagnes deftig voor te bereiden (denk maar aan de wekelijkse campagnes van 1999), op mijn wensen in te gaan, en steeds rustig en kalm te blijven (o zoete ironie) als het opnieuw eens niet van een leien dakje liep.

Stalen nemen is één ding, ze uitwerken een ander...De mensen van het "tweede" op de Ledeganck hebben me enorm geholpen met opspoelen, preparaten maken, sedimentanalyses, scheikundige analyses,...enorm bedankt. Ook *Wies Ghyselinck* verdient een woordje, want zij was voor een groot deel verantwoordelijk voor het in preparaat brengen van de nematoden.

Bepaalde tradities sterven stilletjes uit, maar Dr. (wie had dat ooit gedacht) *Kris Hostens* verdient hier ook nog een plaatsje, om in lang vervlogen tijden mijn computer draaiende te houden en commentaar op de kleuren van mijn presentaties te geven. Huidige probleempjes worden keurig opgelost door het onvolprezen duo *Guy De Smet* en *Tim Deprez*, ook bedankt.

Als je wint, heb je vrienden...maar als je aan je doctoraat schrijft ben je een slechte vriend. Dus geachte leden mijner vriendenkring, binnenkort sta ik er weer, en dan gaan we mijn periode van isolement eens goed doorspoelen...Ik hou er wel aan om speciaal *An* en *WD* te vermelden, die altijd klaar stonden om grote en/of kleine ongemakken te verhelpen. Ook alle mensen die ons op een of andere manier gesteund hebben, op Lukja gepast hebben,...verdienen een dikke mersie.

Ilse De Mesel en *Maaike Steyaert* verdienen een huizenhoog standbeeld voor wat ze de laatste jaren gepresteerd hebben, niet alleen als collega's maar eerst en vooral als fantastische mensen!

Lukja zal ook wel heel tevreden zijn dat ik niet meer ganse dagen moet vertrekken naar het "gebouw" met "maai - ke" en "iese"...als dit alles echt achter de rug is, krijg je opnieuw de papa die je verdient!

De ereplaats van dit dankwoord is voorbehouden aan *Bregje*. Het valt met geen woorden te vatten wat in welke mate Bregje heeft bijgedragen aan dit verhaal...en ik zal het dan ook niet proberen. Maar recht uit mijn hart: dank u wel voor alles!! Ik denk dat het nu een beetje mijn beurt is...

Woensdag 7 mei 2003

SUMMARY - SAMENVATTING

Summary

In the first chapter, the aims of the thesis are presented and put in their global perspective. For each chapter, the hypotheses that were tested are presented, and a short introduction to the research strategy is provided.

Chapter 2 describes structural aspects of the meiobenthos on the subtidal sandbanks of the Belgian Continental Shelf (BCS). The BCS is characterised by a set of isolated sandbanks, which greatly increased the habitat heterogeneity of the area. Ten sandbanks belonging to three geographically isolated sandbank systems (Flemish Banks, Hinder Banks and Zeeland Banks) were sampled during 1997 and 1998. Most of the sandbanks were visited only once, whereas the Bligh Bank, Noordhinder, Thornton Bank and Gootebank were visited both in spring and autumn in the same year.

Sedimentological differences between sandbanks were not observed, but biological differences did occur. Seasonal and regional differences in terms of meiobenthic densities were related to primary production in the water column. The lowest densities were recorded on the most offshore Hinder Banks, coinciding with the lower primary production in the offshore areas of the BCS. Densities were higher in October in comparison with the values in February, which was attributed to the sedimentation of phytoplankton after termination of the spring and summer bloom. Meiobenthic densities on the sandbanks (450-500 ind. 10 cm^{-2}) were much lower than in the deeper channels surrounding the sandbanks (1250-1600 ind.10 cm^{-2}). Higher hydrodynamic stress around the sandbanks prevents phytodetritus from reaching the sandbanks, rendering sandbank sediments poorer in food content compared to the gullies. Moreover the high current speed above the sandbank sediments increases the risk for the meiobenthos being eroded or suspended.

Concerning the meiobenthic community composition on the taxon level, four different communities were identified using multivariate statistics. These communities did not reflect the geographical position on the BCS, but seemed to be influenced by local differences in sedimentological characteristics within sandbanks and sandbank systems. Meiobenthic taxon diversity was influenced by the absence/presence of less abundant taxa. Sediment preferences for these taxa were investigated in several ways, and results indicated that sediments with a median grain size below 300 µm were poor in densities and number of taxa. Sediments with a median grain size between 300-450 µm were rich both in terms of diversity and density, while sediments with a median grain size of >450 µm still showed a high diversity, but lower total meiobenthic densities.

Chapter 3 deals with the structural diversity of the nematode communities from the sandbanks. The nematodes from four sandbanks (Bligh Bank, Noordhinder, Gootebank and Kwintebank) were identified to species level. Multivariate analysis revealed that the assemblages from the various sandbanks were characterised by different species composition patterns, in contrast with studies on macrobenthos or meiobenthic taxa. Although the sediments of all the stations were classified as medium sand, a detailed analysis of all granulometric variables using Multiple Discriminant Analysis suggested that median grain size and the proportions of median sand and very fine sand were most successful in explaining the difference in nematode community composition. These results emphasise the strong relationship between the relative abundance of nematode species and sediment composition. Sand extraction on the Kwintebank seemed to result in a coarsening of the sediments, which had a direct effect on the nematode community structure. Diversity was not affected in the sand extraction area, indicating that nematodes inhabiting highly dynamic environments are well adapted to physical disturbance. In general, nematode diversity in sandbank sediments is not necessarily different from the surrounding areas, since in the more offshore parts of the BCS, clean and rather coarse sands prevail, and the differences in sediment composition are not sufficient to induce large differences in diversity.

A functional aspect of nematode communities, the distribution of nematode biomass over size is explored in **Chapter 4**. Since this distribution is one of the most powerful generalisations that can be used in ecological studies (Peters 1983), it was used to develop a method for using nematodes as a tool for assessing disturbances to sediments. Several characteristics of nematode life in marine sediments make this taxon one of the most suitable animal groups for environmental monitoring. However, identification of these animals is not easy, which hampers the regular use of nematode studies in monitoring programmes. The use of nematode biomass spectra (NBS) to detect changes in the communities as a consequence of various sources of stress was explored in this chapter. NBS were analysed in several ways in order to propose a straightforward way of analysing and interpreting such data. Non-normalised NBS yielded 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). Normalising the spectra made elevated biomass values and peaks disappear, causing bias when interpreting the distribution of biomass over spectra. Cumulative NBS 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. Pareto-type graphs, in which the information of each single measurement is retained, and associated regressions were not straightforward in analysing NBS. The combined use of both NBS and the regression approach for the cumulative NBS for the analysis of biomass distributions is suggested. NBS and cumulative NBS constructed for nematode communities from undisturbed sediments proved to be conservative: no differences in size distributions could be found among locations. Physical disturbance, introduced by sand extraction on the Kwintebank did not affect the regression slopes of the cumulative NBS, but a shift in peak biomass toward 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, a single size class biomass peak was observed due to the presence of a single nematode species well adapted to the impoverished sediment quality. The sedimentation of a phytoplankton bloom corresponded to shifts in peaks in NBS due to a change in age structure of nematode communities. Biomass values probably increased as a result of a higher food supply to the benthos.

In order to improve the understanding of the responses of nematode communities to the sedimentation of phytoplankton, size related aspects were studied in **Chapter 5**. Nematode length, width and Length/Width ratios (L/W ratios) were assumed to reflect functional characteristics of species, since these features reflect life history, physiology, energy requirements and biotic and abiotic interactions (Calder 1984, Peters 1983, Schwinghamer 1983). As the optimal size of organisms is linked with the food input

(Sebens 1987, Rex & Etter 1998), changes in the morphometric characteristics were expected during a pulsed food supply. A rather unique dataset was constructed, since nematode identifications were coupled with the length and width measurements per individual.

During the peak of the spring phytoplankton bloom of 1999 at Station 330, several small species (adult length <700 µm) emerged. Most prominent was the appearance of a "stout" nematode assemblage characterised by low L/W ratios. Most of these small nematode species were virtually absent before the peak blooming and they decreased in abundance shortly after deposition of phytoplankton to the sea floor. This indicates the opportunistic behaviour of these nematodes, which is consistent with their small length, enabling them to rapidly reach adulthood. The net increase of the stout nematodes during the bloom was estimated at 6.4 % day^{-1}, which is much larger that the estimated net rate of 1.5% day^{-1} for the total nematode community. The species composition of the stout nematode assemblage was different from similar stout assemblages described for continental slope and deep-sea areas. At Station 330, Epsilonematidae were dominant while in deeper areas, Desmoscolecidae were prominent. These differences are probably related to the relatively strong hydrodynamic forces prevailing at the North Sea site.

The small species in the North Sea were only present in reasonable densities shortly after the spring bloom sedimentation, while they seem to represent a consistent member of deep-sea nematode communities. It is hypothesised that this is caused by the quality of organic matter reaching the sea floor, coupled with differences in sedimentology and temperature, influencing the duration of the presence of suitable food items for these nematodes.

The structural and functional response of the nematode communities to this pulsed food supply was investigated in **Chapter 6**. Nematode densities, diversity, vertical distribution and community composition were considered as structural aspects, while functional characteristics included feeding type distributions and the number of species within the feeding groups. Both structural and functional characteristics showed considerable changes shortly after the arrival and subsequent remineralisation of fresh organic matter at the sediments of Station 330. A general increase in densities and diversity was related to changes within the selective deposit feeding and epistrate feeding nematodes (*sensu* Wieser 1953). Although temporal variability was significant for total nematode densities and deposit feeding nematodes, spatial variability (in the order of 100 of meters between replicate Reineck boxcorer deployments) was high when single species were concerned. It is hypothesised that the sedimentation and remineralisation of fresh organic matter during and after the phytoplankton bloom results in an increase of suitable food items (both living and dead). This, combined with the permanent availability of oxygen and the high habitat heterogeneity at the sampling location (both at a scale of meters and over the sediment depth profile) create circumstances in which many nematode species can co-exist.

General conclusions

Based on the results presented in this thesis, some general conclusions on the functional and structural diversity of the meiobenthos from the Belgian Continental Shelf (BCS) can be put forward:

1. The distribution of meiobenthos on the taxon level does not reflect the geographical isolation of sandbanks/sandbank systems. Dominant meiobenthic taxa (nematodes and harpacticoid copepods) can be found in all types of sediments within the range of 200-550 µm. Most other taxa (except turbellarians and small polychaetes) prefer sediments with a median grain size >300 µm. When median grain size exceeded 450 µm, diversity was still high but total densities declined. These findings indicate that sedimentological features should be considered when planning human activities at sea, since these features govern the diversity of the meiobenthos.
2. Although nematodes as a taxon are rather indifferent toward sediment granulometry, nematode species are certainly not. This is not a new finding, but statements on the relationship between nematode community composition and sediment granulometry were mainly based on comparisons of very different sediments. Results in this thesis proved that even within very comparable

sediments, nematode community composition is related to small sedimentological differences. This implies (1) that even minor changes in the sediment can have an impact on the composition (and therefore most probably on the functioning) of nematode communities, and (2) that research on nematode communities should include as much sedimentological variables as possible.

3. Functional aspects of nematode communities can be used in assessing changes in the environment by using the biomass distribution over size. However, when anomalies in size spectra are detected, information on the species composition is still required to relate these changes to human/natural impact.
4. The response of nematode communities to phytoplankton sedimentation in oxygenated sediments can be related to morphological adaptations. Being short or plump enables nematode species an opportunistic response to a pulsed food supply. This seems to be a consistent feature in marine sediments, but the composition of the stout community and the timing of its presence are governed by the local conditions.
5. The study of size/biomass related aspects of nematode communities clearly provides new insights in meiobenthic ecology, and should be more regularly included in research programmes.
6. The sedimentation of organic material from the water column after a phytoplankton bloom triggers structural and functional changes in nematode communities from oxygenated sediments. These changes are related to the increase in quality and amount of possible food resources, both living and dead. The high nematode diversity in this kind of sediments is regulated by food availability, habitat heterogeneity and the presence of oxygen.

Samenvatting

Deze studie behandelt de functoniële en structurele diversiteit van het meiobenthos op het Belgisch Continentaal Plat (BCP). Het meiobenthos wordt hier gedefinieerd als alle meercellige organismen die doorheen een 1 mm zeef gaan en die worden weerhouden op een zeef van 38 µm. De nadruk van deze studie ligt op de nematodengemeenschappen, omdat ze het dominante taxon zijn binnen het meiobenthos.

In het **eerste hoofdstuk** worden de doelstellingen van dit werk naar voor gebracht en wordt het onderzoek gesitueerd in zijn globale context. De hypotheses die voor elk hoofdstuk werden vooropgesteld, worden kort weergegeven. Tevens wordt de onderzoeksstrategie bondig toegelicht.

Het **tweede hoofdstuk** beschrijft structurele aspecten van de meiobenthische gemeenschappen op de subtidale zandbanken van het BCP. Het BCP wordt namelijk gekenmerkt door de aanwezigheid van een aantal geïsoleerde zandbanken die de habitatheterogeniteit van het gebied sterk vergroten. Er werden stalen genomen op 10 zandbanken die behoren tot drie geografisch geïsoleerde zandbankgebieden (de Vlaamse Banken, de Hinderbanken en de Zeelandbanken). Deze staalnames vonden plaats in 1997 en 1998. De meeste zandbanken werden slechts eenmalig bemonsterd, maar van de Bligh Bank, de Noordhinder, de Thornton Bank en de Gootebank werden zowel in de lente als in de herfst van hetzelfde jaar stalen verzameld.

Alhoewel er geen duidelijk onderscheid was tussen de sedimentologische karakteristieken van de zandbanken, bleken er toch biologische verschillen te bestaan. Seizoenale en ruimtelijke veranderingen in de dichtheden van het meiobenthos waren gerelateerd aan de primaire productie in de waterkolom. De laagste densiteiten werden opgetekend op de verst afgelegen Hinderbanken, wat samenvalt met de lagere primaire productie in die gebieden van het BCP. De sedimentatie van fytoplankton na de lente- en zomerbloei resulteerde in hogere dichtheden in oktober in vergelijking met februari. Op de zandbanken zelf werden veel lagere dichtheden (450-500 ind.10 cm^{-2}) genoteerd in vergelijking in de diepere geulen rond de zandbanken (1250-1600 ind.10 cm^{-2}). Dit is te wijten aan het feit dat de stromingen rond de zandbankflanken sterker zijn waardoor de

aanvoer van voedsel vanuit de waterkolom verhinderd wordt. Zandbanksedimenten zijn daardoor armer aan voedsel dan de sedimenten in de geulen. Daarenboven is er op de zandbanken een verhoogd risico voor het meiobenthos om gesuspendeerd of geërodeerd te worden.

Na de analyse van de samenstelling van het meiobenthos met behulp van multivariate statistiek, werden vier gemeenschappen onderscheiden. Lokale variatie in sedimentologische kenmerken (binnen dezelfde zandbank of binnen de zandbankgebieden) bleken belangrijker dan de geografische positie op het BCP om deze verschillen in gemeenschapsstructuur te verklaren.

Het aan- of afwezig zijn van minder abundante taxa beïnvloedde sterk de diversiteit van het meiobenthos. Daarom werden de sedimentpreferenties voor deze taxa op verschillende manieren geanalyseerd. Sedimenten met een mediane korrelgrootte <300 µm bleken slechts kleine aantallen aan meiobenthos te herbergen, zowel voor wat betreft de dichtheden als het aantal taxa. Sedimenten met een mediane korrelgrootte begrepen tussen 300 en 450 µm waren rijk in termen van diversiteit en densiteit, maar in grovere sedimenten werden opnieuw lagere dichtheden gevonden terwijl de diversiteit op hetzelfde peil bleef.

De structurele diversiteit van de nematodengemeenschappen van de zandbanken wordt behandeld in **hoofdstuk 3**. De nematoden van vier zandbanken (de Bligh Bank, de Noordhinder, de Gootebank en de Kwintebank) werden tot op soort gedetermineerd. Door middel van multivariate analyses werd aangetoond dat de gemeenschappen van de verschillende zandbanken van elkaar verschilden in hun soortensamenstelling, wat niet het geval is voor het macrobenthos of het meiobenthos op taxonniveau. Alhoewel de sedimenten van alle stations behoorden tot hetzelfde tye ("medium zand"), bleek na een Multiple Discriminant Analyse dat de relatieve hoeveelheid aan medium zand en zeer fijn zand de belangrijkste variabelen waren om de verschillen in de nematodengemeenschap te verklaren. Deze resultaten benadrukken de sterke samenhang tussen de relatieve abundantie van nematodensoorten en de sedimentsamenstelling. De zandextractie op Kwintebank zou een vergroving van het sediment kunnen veroorzaken, wat een effect had op de samenstelling van de nematodengemeenschap. De diversiteit in dit gebied werd niet beïnvloed, wat er op wijst dat de nematoden die in dergelijke sterk dynamische gebieden voorkomen sterk aangepast zijn aan fysische verstoring. De nematodendiversiteit van zandbanksedimenten in het algemeen blijkt niet sterk af te wijken van de diversiteit die wordt aangetroffen in de aangrenzende gebieden. In de ver afgelegen gebieden van het BCP bestaat het sediment ook uit niet vervuilde, relatief grove sedimenten, zodat de verschillen in sedimentsamenstelling tussen de zandbanken en de tussenliggende gebieden niet van die orde zijn om grote veranderingen in diversiteit te induceren.

Hoofdstuk 4 behandelt een functioneel aspect van nematodengemeenschappen: de verdeling van nematodenbiomassa over nematodengewicht. Omdat deze verdeling een van de krachtigste veralgemeningen omvat die in ecologische studies kan worden gebruikt (Peters 1983), werd deze hier aangewend om een methode te ontwikkelen waarbij nematoden kunnen worden ingezet als instrument om verstoringen op sedimenten in te schatten. Vele kenmerken van het nematodenleven in mariene sedimenten zorgen er voor dat dit taxon een van de meest geschikte diergroepen is om aan monitoring te doen. Het op soort brengen van nematoden is echter niet gemakkelijk, wat verhindert dat ze op regelmatige basis worden gebruikt bij monitoringsprogramma's. In dit hoofdstuk werd nagegaan of veranderingen in gemeenschappen als gevolg van verschillende vormen van stress, kunnen worden teruggevonden in de biomassaspectra van nematoden (NBS). NBS werden op verschillende wijze geanalyseerd ten einde een relatief gemakkelijke manier voor te stellen, waarop dergelijke spectra kunnen worden onderzocht en geïnterpreteerd. De niet-genormaliseerde NBS bleken beter geschikt voor de vergelijking van de nematodengemeenschappen dan de genormaliseerde NBS (waarbij de biomassa in een grootteklasse wordt gedeeld door het overeenkomende gewichtsinterval). Door de spectra te normaliseren verdwenen eventuele pieken en hoge waardes, waardoor de interpretatie bemoeilijkt wordt. Statistische verschillen werden best geanalyseerd met cumulatieve NBS, door de helling te berekenen van de regressielijn van de cumulatieve biomassawaarde met de nominale waarde van de gewichtsklasse op een $\log_2$ basis. Het gebruik van grafieken van het Pareto-type (waarbij de informatie van elke meting wordt behouden) en de geassocieerde regressies bleek niet te voldoen. Er werd voorgesteld om een combinatie te gebruiken van de gewone NBS en de regressies van de cumulatieve NBS om biomassaspectra te analyeren. De NBS en cumulatieve NBS die werden opgesteld voor nematodengemeenschappen afkomstig uit onverstoorde sedimenten bleken conservatief te zijn: er werden geen verschillen gevonden in de spectra van de verschillende locaties. Fysische verstoring, in dit geval als gevolg van zandontginning, had geen effect op de helling van de regressielijn van de cumulatieve NBS. Er werd wel een verschuiving opgemerkt van de hoogste biomassawaardes naar lagere gewichtsklasses in de gewone NBS. Dit is een gevolg van een veranderde nematodengemeenschap als gevolg van de frequente fysische verstoring van de zeebodem. In een station dat wordt gekenmerkt door zuurstofstress in het sediment, werd een biomassapiek waargenomen die beperkt bleef tot slechts 1 gewichtsklasse. Deze piek werd veroorzaakt door het dominante voorkomen van slechts 1 soort die goed aangepast is aan het leven in deze ongunstige omstandigheden. De sedimentatie van een fytoplanktonbloei viel samen met verschuivingen van de NBS als gevolg van veranderingen in de leeftijdsstructuur van de nematodengemeenschappen. De biomassa steeg hoogstwaarschijnlijk als een gevolg van de hogere voedselbeschikbaarheid voor het benthos na de sedimentatie van organisch materiaal uit de waterkolom.

Om de respons van nematodengemeenschappen op de sedimentatie van een fytoplanktonbloei beter te begrijpen, werd in **hoofdstuk 5** dieper ingegaan op aspecten die te maken hebben met de morfometrie van de nematodengemeenschappen. De lengte, breedte en Lengte/Breedte verhoudingen (L/B ratio's) van de nematoden werden verondersteld om functionele aspecten van nematodensoorten te reflecteren, omdat deze maten en verhoudingen een weerspiegeling zijn van de levensgeschiedenis, fysiologie, energiebehoeftes en zowel biotische als abiotische interacties van soorten (Calder 1984, Peters 1983, Schwinghamer 1983). Omdat die ideale lichaamsbouw van organismen gerelateerd is aan de toevoer van hun voedsel (Sebens 1987, Rex & Etter 1998) werden veranderingen in de morfometrische karakterisatie van de gemeenschappen verwacht tijdens een periode van geconcentreerde voedseltoevoer. Om dit na te gaan, werd een vrij unieke dataset opgebouwd, waarbij de identificaties van de nematoden werd gekoppeld aan de lengte- en breedtegegevens per individu.

Tijdens het hoogtepunt van de voorjaarsbloei van 1999 op Station 330 verschenen een aantal kleine nematodensoorten (adulte lengte <700µm) in de gemeenschappen. Het opduiken van een "dik" nematodentype, gekenmerkt door lage L/B ratio's, was hierbij opvallend. Deze kleine soorten waren virtueel afwezig voor de fytoplanktonbloei en verdwenen heel snel uit de gemeenschappen na de sedimentatie van het fytoplankton op de zeebodem. Dit wijst op sterk opportunistisch gedrag van deze soorten, wat in overeenstemming is met hun korte lichaamslengte die hen in staat stelt om snel volwassen te worden. De netto toename van de "dikke" nematoden tijdens de voorjaarsbloei werd geschat op 6.4% dag^{-1}, wat heel wat meer is dan de geschatte netto toename van 1.5% dag^{-1} voor de totale gemeenschap. De soortensamenstelling van de "dikke" gemeenschap in de Noordzee verschilde van de samenstelling van gelijkaardige gemeenschappen uit de diepzee en langsheen continentale hellingen. In Station 330 werd deze gemeenschap gedomineerd door Espilonematidae, terwijl in de diepzee Desmoscolecidae dominant zijn. Dit verschil is waarschijnlijk te wijten aan de sterkere hydrodynamiek boven het sediment van ons station.

De kleine sooorten waren in de Noordzee slechts in belangrijke mate aanwezig gedurende de korte tijdsspanne die volgt op de sedimentatie van de fytoplanktonbloei, terwijl ze in de diepzee permanent vertegenwoordigd zijn in de nematodengemeenschap. Dit wordt verklaard door verschillen in de kwaliteit van het organisch materiaal dat de zeebodem bereikt, gekoppeld aan het onderscheid in sedimentologie en temperatuur. Deze factoren samen beïnvloeden de tijdsduur waarin geschikte voedselbronnen voor deze kleine nematoden aanwezig zijn.

De functionele en structurele respons van de nematodengemeenschap op deze geconcentreerde toevoer van voedsel werd onderzocht in **hoofdstuk 6**. Densiteiten, diversiteit, verticale distributie en gemeenschapssamenstelling werden beschouwd als structurele aspecten, terwijl de functionele karakteristieken werden gedefinieerd als de relatieve verdeling van de voedingstypes en het aantal soorten per voedingstype. Zowel de functionele als de structurele kenmerken vertoonden belangrijke veranderingen kort na de aankomst en de daaropvolgende mineralisatie van vers organisch materiaal op het sediment van Station 330. De algemene stijging in densiteit en diversiteit was gekoppeld aan veranderingen binnen de selectieve detritivoren en epistratumeters (*sensu* Wieser 1953). Alhoewel de temporele variabiliteit significant was voor de totale nematodendichtheden, moet vermeld worden dat de ruimtelijke variatie (in de orde van honderden meters tussen het neerlaten van gerepliceerde boxcorers) hoog was indien afzonderlijke soorten onder de loep genomen werden. Er werd besloten dat de sedimentatie en mineralisatie van vers organisch materiaal gedurende en na de fytoplanktonbloei een stijging van het aantal geschikte voedselpartikels (zowel levend als dood) tot gevolg heeft. Dit, gecombineerd met de permanente aanwezigheid van zuurstof in het sediment en de hoge habitatheterogeniteit (zowel horizontaal en in de diepte in het sediment) zorgt voor omstandigheden waarin een groot aantal nematodensoorten kan samenleven.

Algemene conclusies

1. De geografische spreiding van de verschillende zandbanken/zandbankgebieden op het BCP wordt niet weerspiegeld in de samenstelling van het meiobenthos op taxonniveau. Dominante taxa (nematoden en harpacticoide copepoden) kunnen gevonden worden in alle sedimenten met een mediane korrelgrootte tussen 200 en 550 µm. De meeste andere taxa (met uitzondering van Turbellaria en kleine Polychaeta) verkiezen sedimenten met een mediane korrelgrootte >300 µm. Als de mediane korrelgrootte groter wordt dan 450 µm blijft de diversiteit hoog, maar totale dichtheden dalen. Deze resultaten geven aan dat sedimentologische kenmerken zouden moeten worden onderzocht wanneer menselijke activiteiten op zee worden gepland, omdat deze de diversiteit van het meiobenthos als groep bepalen.
2. Alhoewel nematoden als taxon geen voorkeur vertonen voor bepaalde sedimenten, kan dat voor de afzonderlijke soorten niet gezegd worden. Dit gegeven is niet nieuw, maar de meeste bevindingen betreffende de relatie tussen de samenstelling van de nematodengemeenschappen en de granulometrie van het sediment zijn veelal gebaseerd op vergelijkingen van sterk verschillende

sedimenten. De resultaten van het huidige onderzoek hebben aangetoond dat de samenstelling van de gemeenschappen gekoppeld is aan kleine sedimentologische verschillen, zelfs binnen sterk vergelijkbare sedimenten. Dit heeft als gevolg dat (1) zelfs minieme verschuivingen in de samenstelling van het sediment een impact kan hebben op de samenstelling (en hoogstwaarschijnlijk ook op het functioneren) van de gemeenschappen, en (2) onderzoek naar nematodengemeenschappen daarom zoveel mogelijk sedimentologische variabelen in rekening zou moeten brengen

3. Functionele aspecten van nematodengemeenschappen kunnen worden gebruikt om veranderingen in hun omgeving vast te stellen. Hiervoor kan de verdeling van de biomassa van de gemeenschap over gewichtsklassen worden gebruikt. Als afwijkingen in het verloop van de spectra worden aangetroffen, blijft informatie over de samenstelling van de gemeenschappen (soortsniveau) een vereiste om deze verandering te koppelen aan een mogelijke menselijke of natuurlijke impact.
4. De respons van nematodengemeenschappen op de sedimentatie van fytoplankton in zuurstofrijke sedimenten kan worden gekoppeld aan morfologische adaptaties van enkele soorten. Korte en dikke soorten hebben het voordeel dat ze als opportunist kunnen reageren op een geconcentreerde voedseltoevoer. Dit blijkt een constant gegeven te zijn in mariene sedimenten, maar de samenstelling van de "dikke" gemeenschap, en de tijdsspanne waarin ze aanwezig is, wordt bepaald door lokale omstandigheden.
5. Onderzoek naar de grootte/biomassa aspecten van nematodengemeenschappen biedt duidelijk nieuwe inzichten in de ecologie van het meiobenthos, en zou daarom meer moeten worden geïmplementeerd in onderzoeksprogramma's.
6. De sedimentatie van organisch materiaal uit de waterkolom na een fytoplanktonbloei veroorzaakt structurele en functionele veranderingen in de nematodengemeenschappen van zuurtofrijke sedimenten. Deze veranderingen zijn te wijten aan een toename van de kwaliteit en de hoeveelheid geschikte voedselpartikels, zowel levend als dood. De hoge diversiteit aan nematoden in dit type sediment wordt gereguleerd door de voedselbeschikbaarheid, de habitatheterogeniteit en de aanwezigheid van zuurstof.

CHAPTER I

GENERAL INTRODUCTION AND OUTLINE

Abstract

This thesis presents results on the functional and structural diversity patterns of the meiobenthos on the Belgian Continental Shelf. Nematode communities represent the focus, since they are the dominant metazoans within this animal group. In this chapter, the research is situated in its global perspective. The general aims are put forward and the research strategy is briefly explained.

Introduction and background

The results presented in this thesis have been collected in the framework of an OSTC-sponsored programme “Functional and structural biodiversity of North Sea ecosystems: Species and their Habitats as Indicators for a sustainable Development of the Belgian Continental Shelf”. This project was implemented in the larger “Scientific Support Plan for a Sustainable Development Policy” and was conducted between 1996 and 2001. The main goal of the project was to describe the functional and structural diversity of the benthos (all animals living in or in association with the sediments), and to use this knowledge to develop ecological indicators for a sustainable management of the Belgian part of the North Sea.

This thesis focuses on the meiobenthos (*all metazoans living in the sediment, passing a 1 mm sieve and being retained on a 38µm sieve*), and more specifically on the nematodes. Nematodes tend to be the dominant taxon within the meiobenthos and this is certainly the case on the Belgian Continental Shelf (Vanaverbeke et al. 2001). Other frequently found members of the meiobenthos include harpacticoid copepods (Harpacticoida), Ostracoda, Turbellaria and Acari; the remaining meiobenthic taxa are found occasionally (eg. Gastrotricha, Kinorhyncha, Tardigrada, Tanaidacea,…) (Fig. 1).

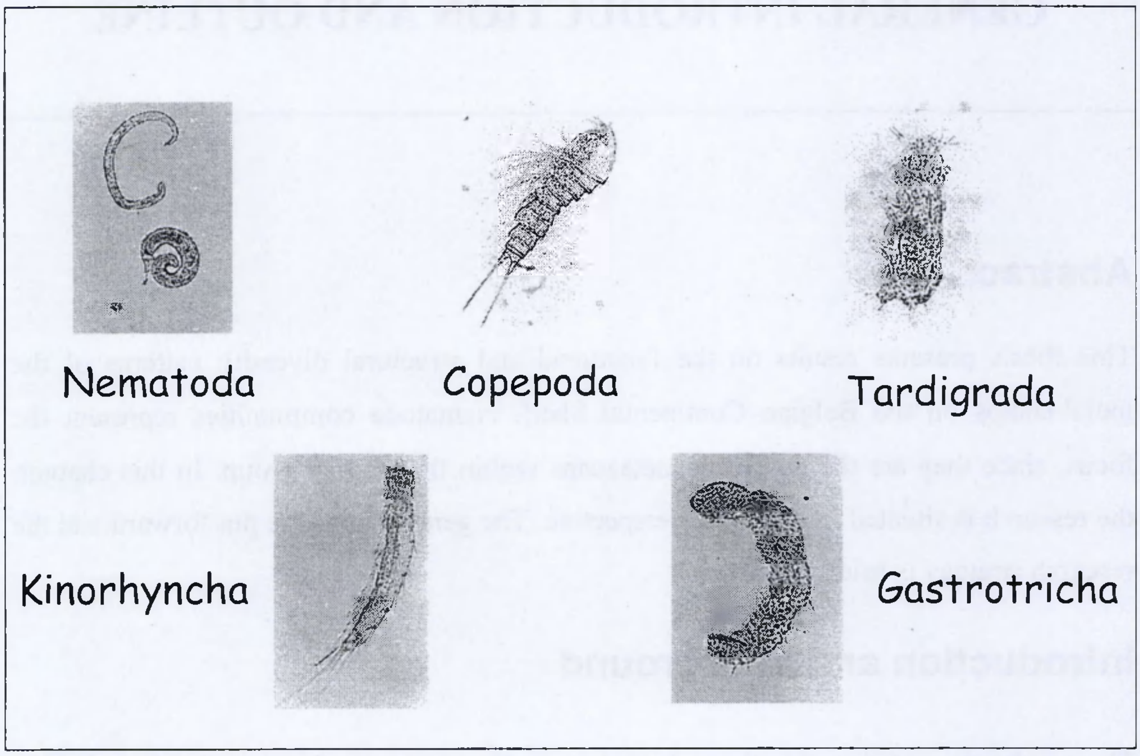

Figure 1. Examples of meiobenthic taxa

Nematodes are theoretically an excellent taxon to use as ecological indicators for benthic environments (Heip et al. 1985, Kennedy & Jacoby 1999, Schratzberger et al. 2000). They reach very high abundances, hence a small sediment sample yields enough animals to make scientific sound statements. They have a ubiquitous distribution, a very high diversity (with a wide range from very sensitive to very tolerant species), short generation time and a continuous reproduction. Moreover, they are restricted to the sediment throughout their life. Therefore, the composition of the nematode communities will reflect the recent history of the benthic environment, including possible human induced and/or naturally occurring disturbances. Understanding the environmental factors structuring these communities will therefore contribute to a sustainable use of the world's oceans in general and the Belgian Continental Shelf (BCS) in particular.

Study site

The Belgian Continental Shelf (BCS) is located in the most south-western part of the Southern Bight of the North Sea and is bordered by the Dover Strait and the Central North Sea (Fig. 2). The North Atlantic Drift and the fresh water supply of the rivers Schelde and Yzer characterise the water masses. Strong semi-diurnal tides and a net tidal current, running north-east parallel to the coast keep the water column well mixed. A gyre, situated in front of the western part of the coast creates a low energy zone. The physical, chemical and biological characteristics of the BCS display a gradient from turbid, nutrient rich and well-mixed inshore waters towards the more oceanic transparent and less productive offshore waters (Cattrijsse & Vincx 2001).

Figure 2. Map of the North Sea with indication of the Belgian Continental Shelf (after Maes et al. 2000)

Geologically, the BCS is characterised by the presence of four sandbank systems (Fig. 3): the Coastal Banks, the Flemish Banks, the Zeeland Banks and the Hinder Banks. The shallow Coastal Banks were not considered during this study, since logistic reasons (research vessel depth) make it rather difficult to sample the sandbanks from this area. The Flemish Banks are situated on the eastern part of the BCS, some 10-30 km offshore. Sandbank length varies between 15 and 25 km. The crests of these sandbanks are situated about 4 m below MLLWS (Maes et al. 2000). Within this thesis, four sandbanks (Kwintebank, Middelkerkebank, Oostendebank and Oostdijck) were sampled.

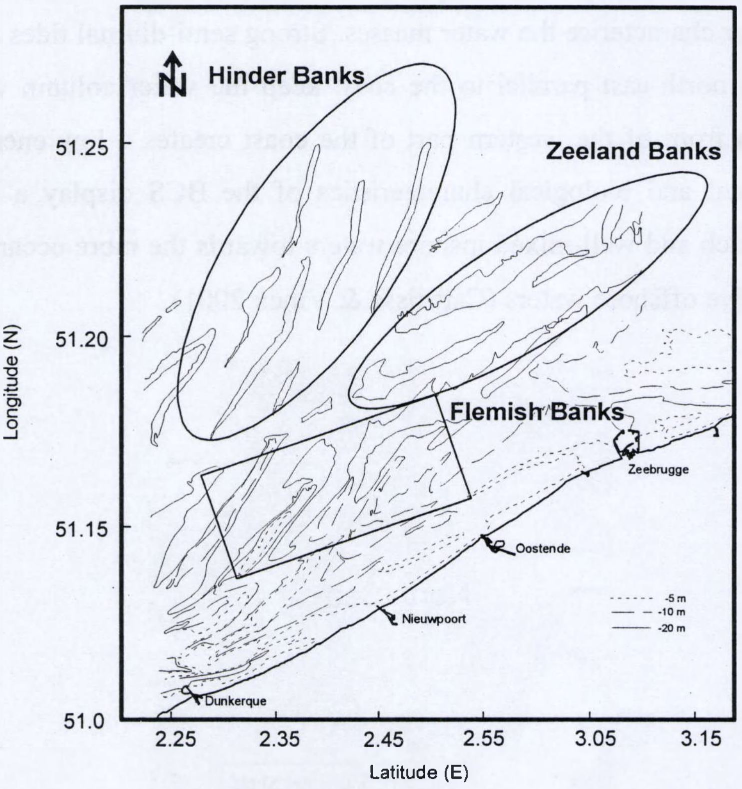

Figure 3. Map of the Belgian Continental Shelf with indication of the sandbank systems

The Zeeland Banks are situated further offshore (15-30 km). Their orientation is parallel to the coastline and their crests are below the 10-m depth line (Maes et al. 2000). Both Thornton Bank and Gootebank were sampled in the framework of this PhD.

The most offshore sandbanks, the Hinder Banks, are situated in the deepest part of the BCS. The crests of the Hinder Banks are situated well below the 10-m depth line. Samples were collected on the Noordhinder, Oosthinder, Westhinder and Bligh Bank.

The sandbanks are built up recently and therefore a continuous interaction between water movement, sediment transport and sediment morphology is required to keep the sandbanks in position (Van de Meene & Stolk 1993). The general position of a sandbank does not change on a human time scale (Van Cauwenberghe 1971, Vannieuwenborgh 1982), but the topography of the crest of the sandbanks changes regularly (Seys et al. 1993). Superimposed bed forms, ranging from small to large dunes, migrate over the sandbanks, and the direction of the movements changes over relatively short time intervals (Trenteseaux 1993, Houthuys et al. 1994, Lanckneus 1994). Storm events and high wind speeds can affect the presence, size and shape of the dunes, even if the tops of those dunes are situated deeper than 15 m water depth (Houthuys et al. 1994). Moreover, stormy weather causes an irregular distribution of sand grains along the sandbanks (Houthuys et al. 1994). All these circumstances make the sandbanks a special environment for the benthos to inhabit, since such a highly dynamic environment is rather unique in coastal areas.

Almost all sandbank systems represent internationally important bird areas (Maes et al. 2000). The Flemish Banks are a key site for wintering Little Gull, Red-throated Diver, Razorbill and Guillemot and the same species are known to concentrate on the Hinder Banks and Zeeland Banks as well. On the other hand, the BCS is an area with a high level of human activity: fishing, shipping, military exercise zones (!), dredging and dumping,…The most influential activity, from the point of view from the benthos, however is the sand and gravel extraction. On the BCS, sand and gravel extraction is done mainly on the sandbanks in front of the coast: the Thorntonbank and the Gootebank are part of an extraction zone which is reserved for the Ministry of Public Works (Maes et al. 2000). The second extraction zone, situated on the Kwintebank, Buiten Ratel and Oostdijck is exploited by private companies. About 1.5 million m^3 per year is extracted, mainly from the Kwintebank.

Many data were collected at Station 330 (51° 26.0 N, 02° 48.5E), located in the vicinity of the Gootebank. This station was visited weekly from March 1999 to July 1999 to study to what extent phytoplankton sedimentation influenced the benthic nematode communities. This Station was chosen as a reference, since the same exercise was performed at the enriched coastal zone Station 115bis (Steyaert 2003) and at a

Kwintebank station (Bonne 2003). In addition, long term data on the phytoplankton dynamics at Station 330 were available from the ULB (Rousseau 2000) and we aim at setting up a complementary long term database concerning the benthos at Station 330.

Aims and outline

One of the first goals of the above mentioned project was to summarise all available data on the benthos of the BCS up to 1998. Data on the meiobenthos were described by Vanaverbeke et al. (2001). Many authors studied the meiobenthos on the BCS, but most of the information was buried in grey literature. The bulk of the information on nematodes was gained in the framework of the PhD of Vincx (1986), published in Vincx (1990) and Vincx et al. (1990). Vanaverbeke et al. (2001) showed that most of the meiobenthic research up to 1998 was restricted to the coastal zone (about 70 stations on the east coast, almost 60 stations at the western part of the coastal zone). Very few information was available from the offshore areas. Information on sandbank nematodes was limited to the single study of Willems et al. (1982) on the Kwintebank.

In general, no clear inshore-offshore or east-west gradients in meiobenthic densities on the BCS were observed, but on the Kwintebank, much lower densities were found compared to the deeper gullies surrounding the Flemish Banks. On the contrary, nematode species richness showed a drastic increase from the coastal zone to the deeper waters. Species richness on the Kwintebank was much higher compared to the surrounding gullies. Vincx et al. (1990) and Vincx (1990) described the nematode community composition of 102 stations in the Southern Bight of the North Sea, mainly situated on the BCS and the Dutch Continental Shelf. According to these results, the BCS can be divided in 5 areas: a coastal area is separated from deeper water by a transition zone (gullies around Flemish Banks and Zeeland Banks). Within the transition zone, the Kwintebank was recognised as a separate unit. Further offshore, 2 areas were distinguished from east to west, the latter one continuing into the deeper coastal waters in front of the Dutch coast. Differences in nematode community composition were related mainly to sedimentological features; however other environmental variables (e.g. temperature, salinity, chl *a* content, NO^{3-} and NH^{4+}) influenced the spatial distribution of nematodes as well.

Summarising the above mentioned data, it became clear that there was an enormous lack of data on the meiobenthos of sandbank ecosystems. Extracting patterns proved to be rather difficult since different sampling strategies, sampling gear, subsampling procedures… have been used, indicating the need for standardised protocols, both at sea and in the lab. And last but not least, many data were purely descriptive, since they were analysed before powerful computers enabled scientists to use apply sophisticated number crunching statistical packages.

Therefore, the first goal of this thesis was to sample all sandbanks on the BCS in a standardised way, if possible in spring and autumn of the same year to account for temporal variability.

In Chapter 2 entitled **The meiobenthos of subtidal sandbanks on the Belgian Continental Shelf (Southern Bight of the North Sea)** the results gathered from these sampling campaigns were analysed. (*Hypotheses: (1) No spatial and temporal patterns exist within the meiobenthos from the different sandbanks/sandbank systems on the BCS; (2) Meiobenthic taxa do not show preferences for certain granulometric characteristics of the sediment*). This chapter was published as *Vanaverbeke J, Gheskiere T, Vincx M (2000) The meiobenthos of subtidal sandbanks on the Belgian Continental Shelf (Southern Bight of the North Sea) Est Coast Shelf Sci 51: 637-649*. The spatial and temporal patterns of the meiobenthos were described on the taxon level. In order to obtain an idea about the most valuable areas within the sandbank systems (here defined as the areas supporting the highest taxon diversity), the sediment preferences of all taxa belonging to the meiobenthos were determined. By compiling both sediment preferences and densities of the various taxa, a schematic model is proposed which illustrates the relationship between sediment granulometry and the meiobenthic taxon composition.

From a limited number of sandbanks, all nematodes were identified up to the species level. Sandbanks were selected according to their geographical position (one Flemish Bank, one Zeeland Bank and two Hinder Banks) and sampling frequency (two campaigns within the same year). Results are presented in Chapter 3 (**Nematode assemblages from subtidal sandbanks in the Southern Bight of the North Sea: effect of small sedimentological differences**), which was published as *Vanaverbeke J, Gheskiere T, Steyaert M, Vincx M (2002) Nematode assemblages from subtidal sandbanks in the*

Southern Bight of the North Sea: effect of small sedimentological differences. J Sea Res 48: 197-207. Three major questions were tackled in this chapter.

The first hypothesis that was investigated was that *nematode communities from isolated sandbanks/sandbank systems would show no differences in terms of diversity and assemblage structure.* Secondly, the relation between sediment granulometry and nematode communities (H_0: *differences in nematode communities are not related to differences in sediment composition*) was assessed using Multiple Discriminant Analysis, a statistical technique rarely used in marine ecology. It was widely accepted that sediment granulometry affects nematode diversity and community structure, but these statements were mainly made based on results from very contrasting sediments (e.g. comparisons of fine sand with a lot of clay/silt with medium or coarse sands devoid of mud). However, our approach aimed at identifying very specifically those granulometric variables that were responsible for structuring nematode communities in very comparable sediments. As a third item in this chapter, we filled the gap in knowledge considering nematode species richness already mentioned before (H_0: *Nematode communities from the sandbanks and the surrounding seabed do not differ in terms of diversity and assemblage structure*).

It was already mentioned that the deliverables of the project "Structural Functional and structural biodiversity of North Sea ecosystems" included the proposition of ecological indicators for a sustainable management of the North Sea. As pointed out above, nematodes as a taxon fulfil all criteria to be used as such. 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 we decided not to look for nematode indicator species within the framework of this PhD, but to develop an alternative and more cost-effective way to use nematodes as a taxon in routine environmental assessment programmes.

This alternative was found by studying one of the most powerful and extensive generalisations that can be used in ecological studies: the biomass distribution over size (Peters 1983). Indeed, body size reflects many aspects of animal life such as life history, physiology, energy requirements, biotic and abiotic interactions (Calder 1984, Peters

1983, Schwinghamer1983). Nematode body size can be assessed by measuring the nematodes length and maximal width (Andrassy 1956) and can be performed by non-specialists. The use of size spectra to assess ecosystem health, overall productivity and fisheries yield is widely used in pelagic environments (see Silver & Platt 1978, Sprules & Munawar 1986), where deviations from the slope and intercept of a normalised biomass spectrum point to an impacted ecosystem. However, this cannot be simply transferred to the benthic environment, since the sediment itself constrains benthic body size distributions (Schwinghamer 1981, 1983, 1985). As a second disadvantage, sampling the wide range of benthic animal life (from microbiota to large macrobenthos and epibenthic fish), requires a variety of sampling methods. The use of different 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 can arise in deciding which animal group is sampled correctly by which gear. As a third difficulty, there was considerable debate on an appropriate statistical method for comparing size spectra (see eg Vidondo et al. 1997, Baca & Threlkeld 2000).

In Chapter 4 "**Nematode biomass spectra as descriptors of functional changes due to human and natural impact**" we limited the analysed part of the complete benthic biomass spectrum to that part occupied by the meiobenthic nematodes. Nematode biomass spectra (NBS) from different areas on the BCS were constructed and compared in order to investigate the effect of disturbance on the spectra (H_0: *Nematode biomass spectra do not change as a result of human or natural disturbances*). The Kwintebank nematode communities were analysed in order to study the effect of sand extraction (physical disturbance) on the NBS. The effect of oxygen stress (anoxia probably as a consequence of human induced eutrophication) and a natural, seasonal occurring enrichment of sediments during the sedimentation of a spring phytoplankton bloom on the NBS were investigated as well. The analyses of the biomass distribution over size were done in several ways, in order to propose an appropriate and straightforward method to analyse these functional descriptors of nematode communities. These results were published as *Vanaverbeke J, Steyaert M, Vanreusel A, Vincx M (2003) Nematode biomass spectra as descriptors of functional changes due to human and natural impact. Mar Ecol Prog Ser 249: 157-170.*

The remaining two chapters deal with the response of nematode communities to a pulsed food supply during the sedimentation of spring phytoplankton bloom. Phytoplankton blooms in the North Sea are initiated early March and generally peak at the end of April (Boon et al. 1998, Rousseau 2000). The organic material produced in the water column will be deposited on the sediment where it fuels benthic life (Graf 1992). The remineralisation of this organic material can cause fast and drastic changes in the sediments, such as oxygen depletion accompanied by the production of toxic substances for animals (see Graf 1992 for a review). On the other hand, the products of the remineralisation processes are partly released into the water column where they provide nutrients for the next phytoplankton bloom. Therefore it is of major importance to understand the processes in the sediments and the responses of benthic animals to this natural enrichment of their environment.

Soetaert et al. (2003) showed that within nematode communities along continental slopes and deep-sea areas, different morphological adaptations to specific life styles existed, independently of phylogenetic relationships between those species. Very short and thick species (“plump” nematodes) all seemed to have developed cuticular ornamentation (spines, ridges…). Being plump enabled these nematodes to behave in an opportunistic way; the ornamentation prevented them from being an easy prey. However, these species suffer from a reduced mobility and can’t withstand anoxic conditions. Therefore they are always encountered in the upper cm of the sediment, where oxygen is available. Within the regular nematode body type (the “slender” nematodes), morphological adaptations in length or maximal width were explained as modifications enabling these species to survive in oxygen stressed deeper sediment layers. This all resulted in vertical gradients in nematode length, width or Length/Width ratio coinciding with the oxygen profiles in the sediment. We adopted the same approach at sampling Station 330 (51° 26.0 N, 02° 48.5E). This station was sampled weekly from early March to July 1999 to follow the effect of sedimentation of the spring phytoplankton bloom on the nematode communities. Since body size reflects many functional aspects of animal life, it was hypothesised that the increase of organic matter in the sediments would influence the morphometric characteristics of the nematode assemblages (H_0: *the morphometric characteristics of nematode communities are independent of food supply in sandy, oxygenated sediments*).

Nematodes from this sampling campaign were not only identified up to species level, but all of them were measured as well. The combination of these datasets is rather unique, and it was assumed that careful analysis would provide insight in the functional dynamics of nematode responses to a pulsed food supply. It also allowed for testing the hypothesis of Soetaert et al. (2003) in fully oxygenated sediments. These results are presented in Chapter 5 "**Changes in morphometric characteristics of nematode communities during a spring phytoplankton bloom deposition**". This manuscript (authors: Vanaverbeke J, Soetaert K, Vincx M) has been submitted to Marine Ecology Progress Series.

In the last chapter entitled "**Changes in structural and functional diversity of nematode communities during a spring phytoplankton bloom in the Southern North Sea**" a more traditional approach was followed. Both structural (density, diversity and community composition) and functional (feeding type distributions and diversity within feeding groups) characteristics were investigated to complete our picture of the benthic response to natural enrichment in a fully oxygenated environment. (H_0: S*tructural and functional aspects of nematode communities inhabiting oxygenated sandy sediments are not influenced by the sedimentation and remineralisation of a spring phytoplankton bloom*). It can readily be assumed that different nematode feeding types react differently to the deposition of fresh organic material, both in densities as in species composition, since resource partitioning can enable different specialised species to co-exist in a limited volume of sediment. This manuscript (*Vanaverbeke J, Steyaert M, Soetaert K, Rousseau V, Van Gansbeke D, Parent J-Y, Vincx M. Changes in structural and functional diversity of nematode communities during a spring phytoplankton bloom in the Southern North Sea*) has been submitted to Marine Ecology Progress Series.

Remark

Each chapter of this work is intended to be an autonomous part of work, which is published or submitted to scientific journals. Therefore each chapter can be read separately from the rest. On the other hand, considerable overlap will exist in the sections describing the study sites and material and methods. In order to save some trees, only one general reference list is included at the end of this thesis. All data are available upon request and will be deposited to Flanders Marine Data Centre (VMDC) at Flanders Marine Institute (VLIZ).

5343

CHAPTER II

The meiobenthos of subtidal sandbanks on the Belgian Continental Shelf (Southern Bight of the North Sea)

Results presented in:

Vanaverbeke J, Gheskiere T, Vincx M (2000) The meiobenthos of subtidal sandbanks on the Belgian Continental Shelf (Southern Bight of the North Sea). Est Coast Shelf Sci 51: 637-649

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Abstract

The Belgian Continental Shelf (BCS) is characterised by a set of isolated subtidal sandbank systems, which greatly increase the habitat heterogeneity of the area.

The meiobenthos of these sandbanks was investigated during 1997 and 1998. In total, 10 sandbanks have been sampled, belonging to three different geographically isolated systems: the Flemish Banks, the Hinder Banks and the Zeeland Banks. No obvious differences in sedimentological characteristics between the sandbanks were found, but biologically some differences could be detected. The more offshore Hinder Banks had the most diverse meiobenthos, while the Flemish Banks harboured the lowest number of meiobenthic taxa. Seasonal and regional differences in terms of densities are a result of a coupling with the primary production in the water column, with October showing highest densities and the offshore Hinder Banks harbouring lowest densities. The meiobenthos on the sandbanks was less dense (450-500 ind.10 cm^{-2}) than that in the deeper channels between the sandbanks (1250-1600 ind.10 cm^{-2}). These differences were attributed to high hydrodynamic stress around the sandbanks, preventing phytoplankton to reach the sandbanks. Moreover, higher current speed above the sediment increases the risk for the meiobenthos of being eroded or suspended during storms.

Four different communities could be identified. These did not reflect the geographical position on the BCS but seem to be influenced by local differences in sedimentological characteristics within sandbanks and sandbank systems. These sedimentological differences influenced the taxon diversity as well. Sediment preferences for less abundant taxa were investigated in several ways, and results indicated that sediments with a median grain size below 300 µm were poor in terms of densities and number of taxa, while coarser sediments were richer in taxon diversity. Sediments with a median grain size between 300-450 µm were rich both in terms of diversity and density, while sediments with a median grain size of >450 µm still showed high diversity but lower densities.

Introduction

The Belgian Continental Shelf (BCS), situated in the Southern Bight of the North Sea is characterised by the presence of four sandbank systems: the Coastal Banks, the Flemish Banks, the Zeeland Banks and the Hinder Banks. These sandbanks form a geologically unique area and they are important areas for both marine and seabird life since they act as internationally important resting and foraging areas for various seabird species (Skov et al. 1995), feeding on small pelagic fish and macrobenthos (Degraer et al. 1999, Skov et al. 1995). The rich marine and birdlife makes them suitable area for the designation as marine protected areas, as already recognised in other areas (*e.g.* the Baltic Sea (Andrulewicz and Wielgat 1999).

The sandbanks on the BCS are built up recently and therefore a continuous interaction between water movement, sediment transport and sediment morphology is required to keep the sandbanks in position (Van de Meene & Stolk 1993). Indeed, the position of the sandbanks does not change on a human time scale (Van Cauwenberghe 1971, Vannieuwenborgh 1982) but regular changes in the topography of the crest of the sandbanks do occur. Superimposed bedforms, ranging from small to large dunes, migrate over the sandbank and the direction of migration changes over relatively short time intervals (Trenteseaux 1993, Houthuys et al. 1994, Lanckneus et al. 1994). From a study on the Middelkerkebank (Houthuys et al. 1994), it became clear that even storm events and high wind speeds affect the presence, size and shape of the dunes, even if the depth of the tops of the large dunes exceeded 15m. The same study indicated a much less regular distribution of sand grains along the sandbank after a storm passed by.

These geological settings indicate that life on a sandbank is different from life at "regular" seabeds, especially for organisms living in the sediment. Surprisingly, the benthos of the sandbanks is poorly investigated, and for the meiobenthos only three studies are available (Willems et al. 1982, Vincx 1990, Vincx et al. 1990) and all of them addressed the same sandbank (Kwintebank). Therefore this study was designed in order to get information about the meiobenthos inhabiting these highly dynamic sandbank sediments. It aims at 1) presenting the first data on densities and composition of meiobenthic communities on subtidal sandbanks; 2) explaining differences in densities of the meiobenthos and dominant taxa and diversity patterns between different sandbank systems and 3) comparing meiobenthic densities with surrounding sediments in deeper channels.

Material and methods

Study area and treatment of meiobenthic samples

The Flemish Banks system (Fig. 1) is situated on the eastern part of the BCS, 10 – 30 km offshore. Sandbank length varies between 15 to 25 km. The crests of the sandbanks are situated some 4 m below MLLWS (Maes et al. 2000). Four sandbanks were sampled: The Kwintebank (February 1997), The Middelkerkebank, the Oostendebank (October 1997) and the Oostdijck (February 1998).

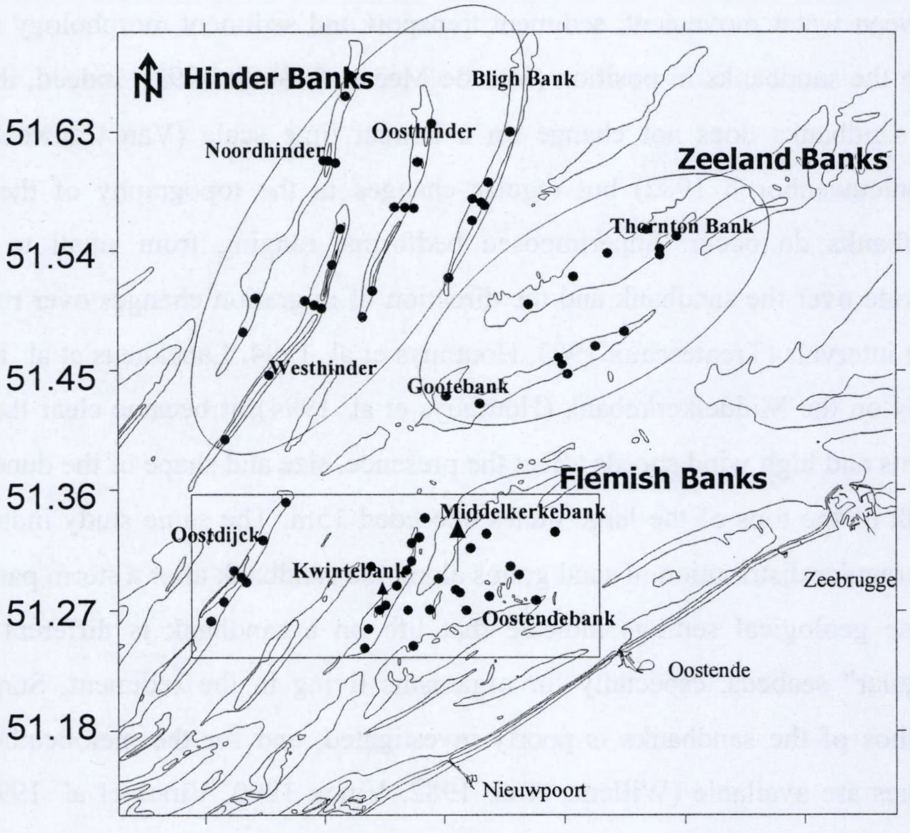

Figure 1. Map of the Belgian Continental Shelf showing the sampling stations

The Zeeland Banks are situated further offshore (15-30 km) and are orientated parallel to the coastline. Almost all of the sandbanks have crests below the 10-m depth line (Maes et al. 2000). Here, the Thornton Bank and the Gootebank were sampled both in February and October 1998.

The Hinder Banks are situated in the deepest part of the BCS. Distance to the coastline is 35-60 km. Again, the crests of the sandbanks are situated well below the 10-m depth line (Maes et al. 2000). The Westhinder and Oosthinder were visited in October 1997, while samples from the Noordhinder and Bligh Bank were obtained both in February and October 1998.

On most of the sandbanks, seven stations were sampled, using a modified Reineck boxcorer. Five stations were situated on regular distances along the crest of the sandbank, while two additional stations were situated on the sides of the bank. On the Kwintebank, ten stations along the top of the sandbank were sampled, corresponding with the stations listed in Willems et al. (1982). Since the Oostendebank is a rather small sandbank, only six stations were sampled (four along the crest and two on the flanks of the bank). The Reineck boxcorer was deployed three times per station. From each boxcorer, one subsample for the meiobenthos was taken with a plastic core (10 cm²)*, and fixed with a hot (70°C), neutral formaldehyde tap-water solution (4%). Another core was used to collect sediment for sediment analysis. Metazoan meiobenthic organisms were extracted from the sediment by centrifugation with Ludox (Heip et al. 1985). Macrofauna was excluded by means of a 1-mm sieve. All animals retained on a 38-µm sieve were stained with Rose Bengal, counted and identified to the taxon level (Phylum, Classis, Subclassis or Ordo).

Sediment analysis was performed using a Coulter LS100 Particle Size Analyser. Sediment fractions up to 1000 µm are expressed as volume percentages, while the fractions between 1000-2000 µm and >2000 µm are mass percentages. Sediment fractions are defined according to the Wentworth scale (Buchanan 1984).

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* about 10 cm of sediment was collected in all cores

Multivariate analysis

In order to compare the densities of all meiobenthos and the dominant taxa (Nematoda, Harpacticoida, Polychaeta and Turbellaria) between sandbanks, ANOVA's were performed on log (x+1) transformed data in order to meet the assumptions for ANOVA. Since not all sandbanks were sampled twice in the same year or in the same season, different datasets were subjected to the analyses. The sandbanks, which have been visited twice in one year (Bligh Bank, Noordhinder, Thornton Bank and Gootebank), were included in a first analysis to test the effect of both time and geographical position. Differences between the other sandbanks were tested within one sampling period. When overall significant differences were detected, sandbanks were compared two by two using the planned comparison option in STATISTICA.

The meiobenthic community composition was analysed by means of TWINSPAN (Hill 1979a). Only meiobenthic groups occurring in all stations (Nematoda, Harpacticoida, Polychaeta, Turbellaria, Ostracoda, Tardigrada, Acari, Gastrotricha and nauplii) were included in the dataset (cutlevels: 0, 1, 3, 10, 35, 200). A Detrended Correspondance Analysis (DCA, Hill 1979b) on fourth root transformed data was applied in order to confirm the patterns detected by the TWINSPAN analysis.

Sediment preferences

For all organism groups, occurence within a possibly limited sediment grain size range was investigated by plotting the abundance data originating from all sandbanks *vs.* median grain size. Densities and number of observations within a certain median grain size range then give a first indication of possible sediment prefences. Differences in densities were tested using ANOVA on log (x+1) transformed data. When transformation was not sufficient to meet the assumptions for ANOVA, the non-parametric Kruskal-Wallis test was applied. When densities were significantly different, pairwise comparisons between sediment classes were made using the planned comparison option in ANOVA (parametric data) or multiple comparisons (non-parametric data) (Conover 1971). However, since most of the stations had a median grain size range of about 350 µm, the highest number of observations for all taxa falls close to this value. Therefore, the sediment fraction between 200 and 550 µm was divided in 7 classes with a width of 50 µm (e.g. class 1: 200-250 µm, class 2: 250-300 µm,...; class 7:500-550 µm). In order to exclude the fact that most stations have sediments with a median grain size around 350 µm, the data were transformed according to Vanreusel (1991):

$$Xi=\frac{Nxi}{Nti}\left(\sum_{i=1}^{k}\frac{Nxi}{Nti}\right)^{-1} * 100$$

where Xi: the number of stations with taxon K; N_{xi} the number of subsamples in class i with taxon K; N_{ti} the total number of subsamples in class i and k=the number of classes. X_i is then expressed as a percentage of the total number of stations with taxon K, assumed that all stations (also those without taxon K) are equally spread over the different classes.

Results

The sediments from all sandbanks can be described as medium sand (median grain size between 250 and 500 µm) (Table 1).

Sandbank	sampling month	median grain size (µm)	Meiobenthos (ind/10cm²)	Nematoda (ind/10cm²)	Harpacticoida (ind/10cm²)	Polychaeta (ind/10cm²)	Turbellaria (ind/10cm²)
Kwintebank	February	214-527	427 (106-2244)	357 (84-2232)	42 (0-457)	3 (0-10)	16 (3-60)
Middelkerkebank	October	204-425	856 (161-2707)	785 (121-2663)	41 (13-88)	1 (0-3)	23 (9-55)
Oostendebank	October	215-340	215 (97-442)	165 (79-386)	10 (1-15)	1 (0-2)	37 (15-62)
Oostdijck	October	323-486	333 (151-467)	138 (55-311)	139 (44-245)	4 (2-13)	15 (0-26)
Bligh Bank	February	127 (318-407	271 (148-434)	127 (72-246)	87 (45-101)	3 (0-10)	15 (7-31)
Bligh Bank	October	330-430	549 (401-968)	209 (154-509)	205 (119-562)	7 (1-6)	16 (9-19)
Noordhinder	February	320-412	379 (246-471)	210 (92-290)	106 (68-177)	11 (8-16)	12 (4-28)
Noordhinder	October	338-381	606 (446-729)	285 (198-436)	224 (125-340)	18 (6-30)	24 (15-28)
Oosthinder	October	316-531	629 (453-787)	396 (233-666)	150 (78-253)	12 (7-17)	43 (20-89)
Westhinder	October	305-545	508 (266-727)	227 (87-348)	187 (109-356)	11 (5-24)	32 (20-49)
Thornton Bank	February	313-478	361 (279-586)	187 (107-354)	104 (63-146)	9 (3-18)	27 (15-38)
Thornton Bank	October	291-400	523 (332-932)	266 (152-492)	160 (72-356)	9 (3-21)	52 (28-89)
Gootebank	February	301-400	401 (153-1192)	243 (90-472)	178 (24-602)	3 (0-5)	36 (9-98)
Gootebank	October	298-362.	537 (410-686)	315 (211-389)	147 (80-236)	4 (0-5)	51 (34-70)

Table 1. Summary of main variables per sandbank, range between brackets

However, some exceptions are present: on the Kwintebank, two stations had fine sand (median grain size: 125-250 µm) and the sediment in one station consisted of coarse sand (median grain size: 500-1000 µm). On the Middelkerkebank and the Oostendebank, two fine sandy stations were present, while coarse sand was found in one station on both the Oosthinder and the Westhinder.

Within the meiobenthos, 18 taxa were recognised. Nematodes were the dominant taxon but in some stations they were replaced by harpacticoid copepods as the dominant group. Polychaeta and Turbellaria were subdominant (Table 1), while Ostracoda, Gastrotricha, Acari, Kinorhyncha, Tardigrada and nauplii were found less frequently. Other taxa (Oligochaeta, Tanaidacea, Asteroidea, Cnidaria, Amphipoda, Isopoda, Echinoidea and Cumacea) were sometimes noted.

Meiobenthic taxon diversity is shown in Fig. 2. Diversity was highest on the Hinder Banks, followed by the Zeeland Banks, while the lowest values originated from the Flemish Banks.

On the Hinder Banks, taxon diversity was higher in October compared to February, while this was not the case at the Zeeland Banks. ANOVA results, from the comparisons between the sandbanks for densities of the total meiobenthos and the most important taxa (Nematoda, Harpacticoida, Turbellaria and Polychaeta), are listed in Tables 2 and 3.

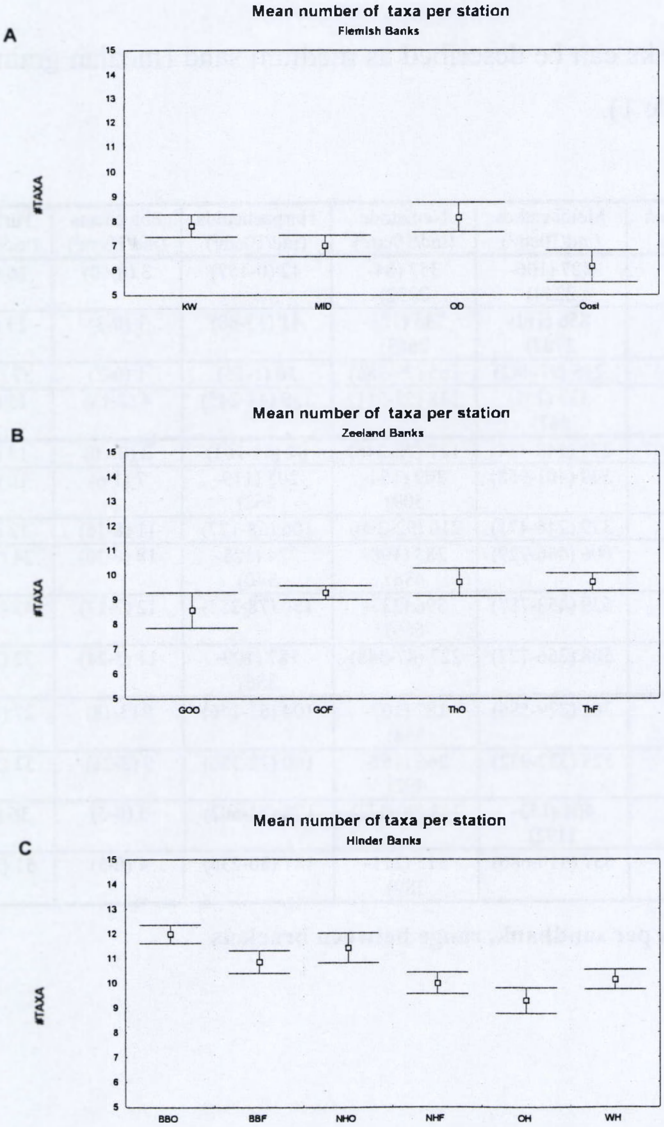

Figure 2. Meiobenthic taxon diversity per sandbank system (A: Flemish Banks; B: Zeeland Banks; C: Hinder Banks. (NH: Noordhinder;BB: Bligh Bank; OH: Oosthinder; WH: Westhinder; Tho: Thornton Bank; Kw: Kwintebank; OD: Oostdijck; OB: Oostendebank; MB: Middelkerkebank; O: October; F: February)

When testing the sandbanks that were sampled twice, significant differences were found between the sampling dates and between the sandbanks, but no significant differences were obtained for the combined effect. The sandbanks were significantly different when considering nematode, polychaete and turbellarian densities ($p<0.001$) and significant differences for all he taxa and the total meiobenthic densities were found between the sampling dates ($p<0.001$) (Fig. 3).

Table 2 shows that not all the taxa differed in the same way on all the sandbanks, but generally total meiobenthic densities were higher in October. When spatial patterns are considered, it seems that nematodes, turbellarians and polychaetes where significantly more abundant on the Zeeland Banks in both seasons. An exception occurred at the Noordhinder, which had a significantly higher polychaete density.

	Month (Sandbank)	Sandbank comparison
Total meiobenthic densities	October>February (NH, BB, THO, GO)	ns
Nematoda	October>February (BB, NH, GO)	THO, GO>BB
Harpacticoida	October>February (BB, NH)	ns
Polychaeta	October>February (BB)	NH>BB, GO
Turbellaria	October>February (NH, THO, GO)	THO, GO>BB, NH

Table 2. Two-Way ANOVA: Planned comparisons. Summary of significant differences between months and sandbanks.

A second analysis compared the sandbanks sampled only in February. Significant differences were found for the densities of nematodes ($p<0.001$), harpacticoid copepods ($p<0.001$), polychaetes ($p<0.001$) and turbellarians ($p<.01$). Again, densities of nematodes, polychaetes and turbellarians were higher on the Zeeland Banks in comparison with the Hinder Banks (Table 3). When the Zeeland Banks are compared to the Flemish Banks, higher densities are found at the Zeeland Banks, while no clear differences can be detected when comparing the Hinder Banks with the Flemish Banks. The Kwintebank showed a higher number of nematodes, while harpacticoid copepods appeared in higher densities on the Bligh Bank.

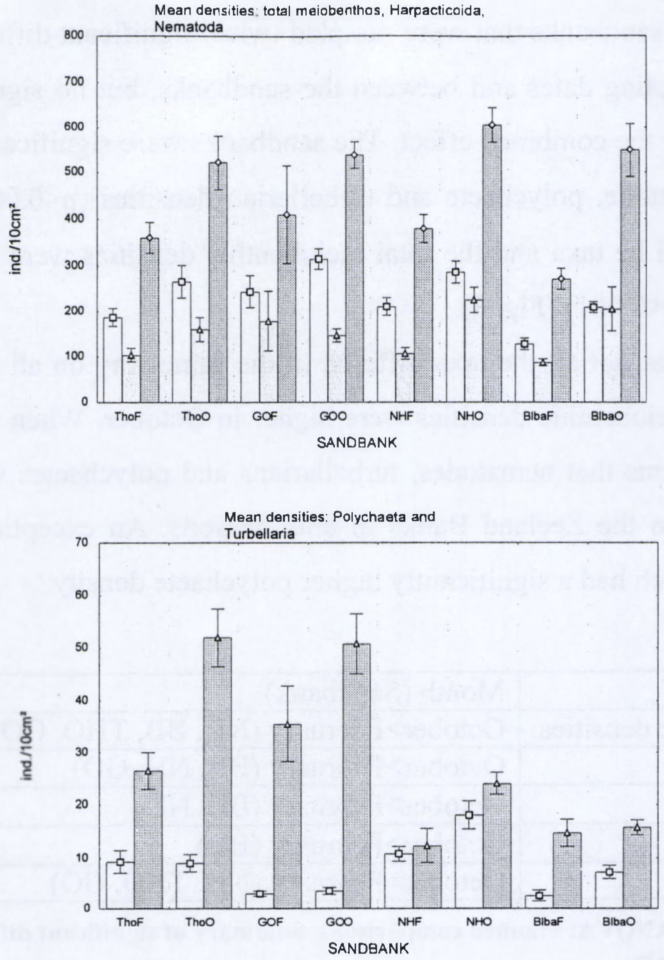

Figure 3. Mean densities per sandbank and per sandbank period. ThoF, Thornton Bank February; ThoO, Thornton Bank October; GOF: Gootebank February; GOO, Gootebank October; NHF: Noordhinder February; NHO: Noordhinder October; BlbaF: Bligh Bank February, BlbaO: Bligh Bank October. (ao Total meiobenthos (diamonds); Nematodea (squares); and Harpacticoida (triangles). (b) Polychaeta (squares); and Turbellaria (triangles)

Similar results were obtained when the sandbanks sampled in October were compared (Table 3). Significant differences were found for nematodes ($p<0.01$), turbellarians ($p<0.01$) and total meiobenthic densities ($p<0.05$). The Zeeland Banks showed higher densities than the Hinder Banks, while no clear differences with the Flemish banks could be detected. The latter banks differed significantly from the Hinder Banks; a higher amount of nematodes and total meiobenthos was present on the Flemish Banks.

	February	October
Total meiobenthic densities	ns	THO, GO>OH OB, MB>WH, OH
Nematoda	GO>BB, OD Kw>BB	THO, GO>NH THO>WH OB>GO, WH, NH, OH MB>OH
Harpacticoida	THO,GO>Kw BB>Kw	ns
Polychaeta	THO>BB, OD, Kw GO>NH	ns
Turbellaria	THO, GO>NH, OD, Kw	THO, GO>OH OB, MB>WH, OH

Table 3. One-way ANOVA: Planned comparisons for stations sampled during February or October: summary of significant differences

Summarising, it can be stated that the Zeeland Banks showed the highest densities, both in February and October. In February, no clear differences between the Flemish Banks and the Hinder Banks were found, while in October higher densities were present at the Flemish Banks. Here, the differences between the Flemish Banks and the Zeeland Banks disappeared.

The meiobenthic community composition was analysed using both TWINSPAN and DCA. TWINSPAN discerned four groups (Fig. 4) and did not reflect geographically isolated sandbank systems or sandbanks. TWIN 1 included mainly Hinder Bank stations, while both TWIN 2 and TWIN 3 were a mixture of Hinder Bank and Zeeland Bank stations. TWIN 3 included four stations from the Flemish Banks as well. Most Flemish Bank stations were listed in TWIN 4, together with a single Hinder Bank station and the rest of the Zeeland Bank stations. The picture revealed by DCA (not depicted) confirmed this pattern, although less clearly.

The division between TWIN 1 and TWIN 2 on the one hand, and TWIN 3 and TWIN 4 on the other hand was based on the occurrence of taxa appearing usually in low abundances within meiobenthic communities (Acari, Ostracoda and Tardigrada) together with the relatively high abundances of nauplii in TWIN 1 and TWIN 2, suggesting a higher taxon diversity within these stations compared to the TWIN 3 and TWIN 4 stations.

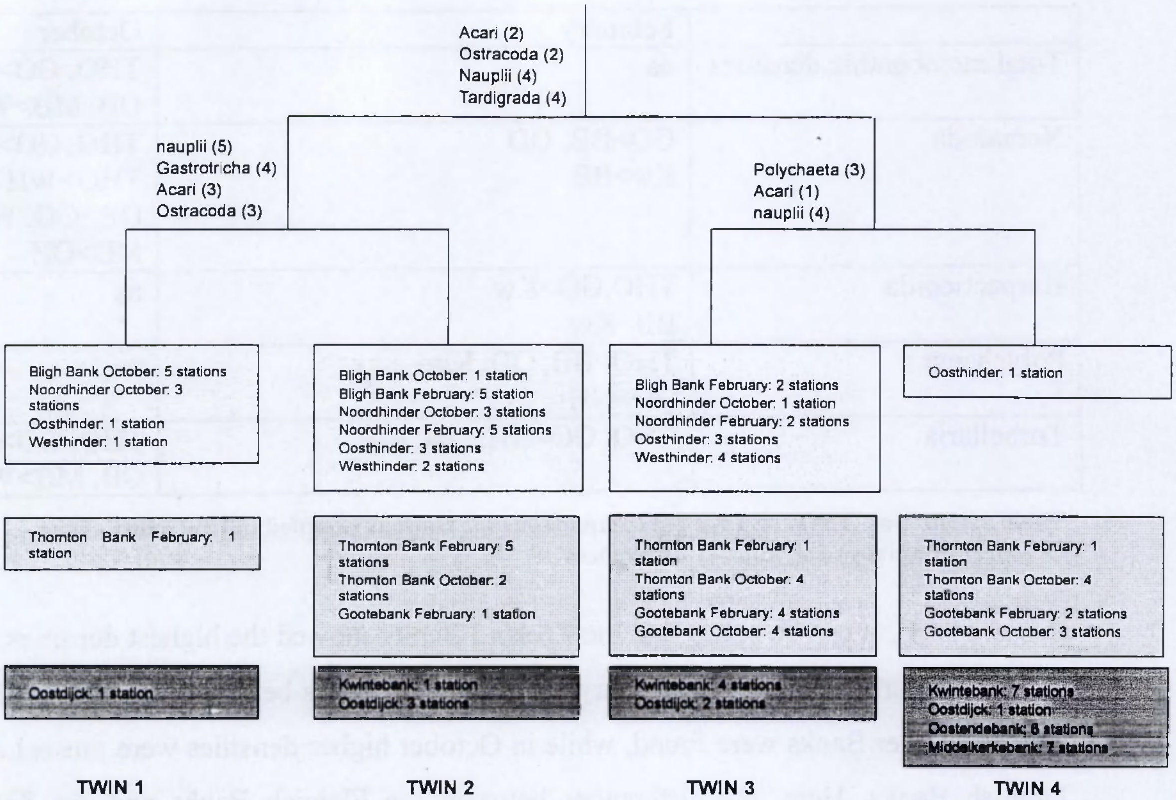

Figure 4. Dendrogram with TWINSPAN results

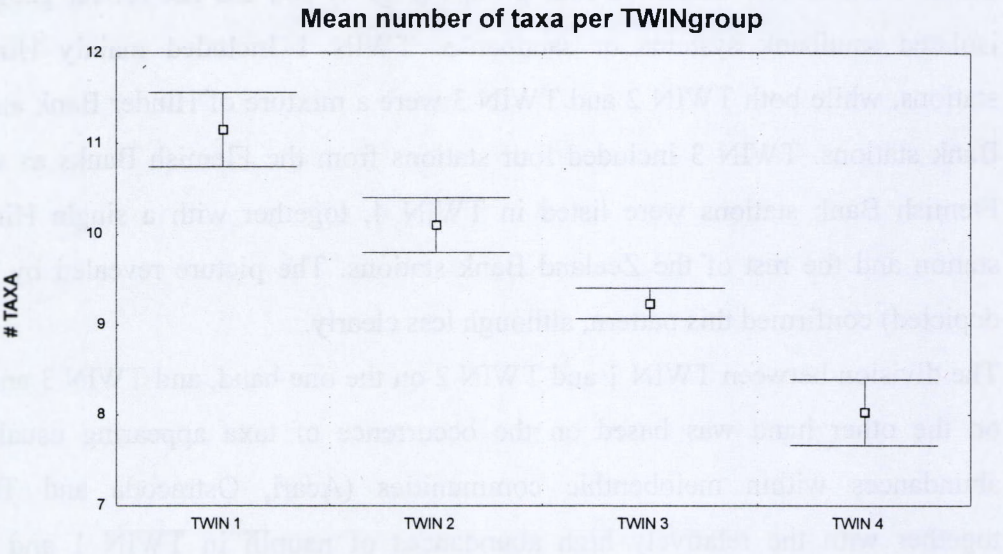

Figure 5. Meiobenthic taxon diversity per TWINgroup (defined in Figure 4)

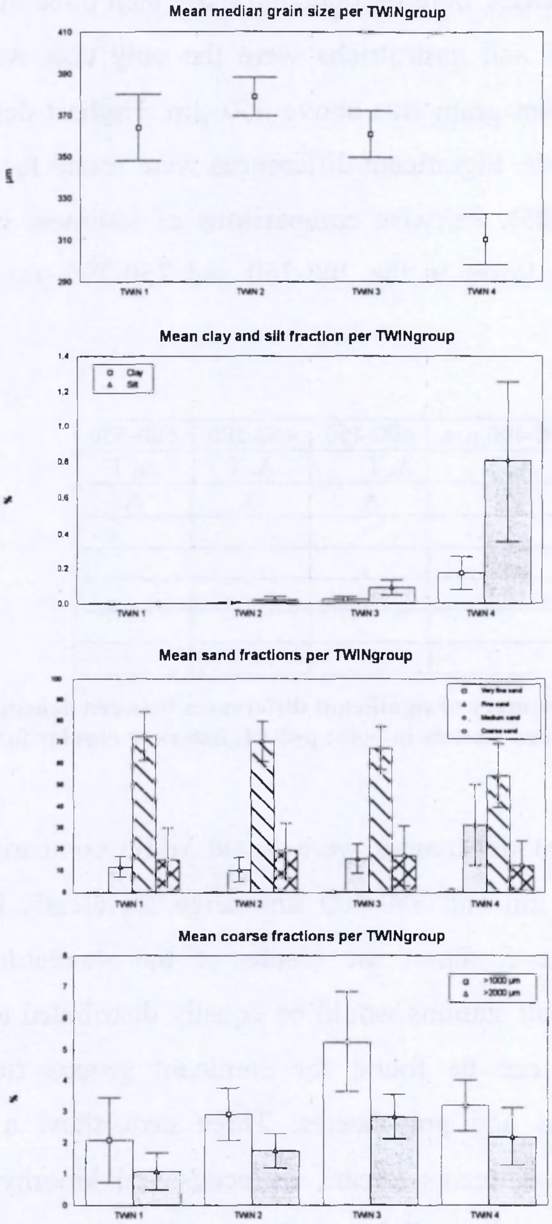

Figure 6. Mean sediment characteristics per TWINgroup (defined in Figure 4)

A Kruskal-Wallis test showed the taxon diversity to be significantly different between the TWIN groups (p<0.001). Meiobenthic taxon diversity was highest in TWIN 1 (Fig. 5). TWIN 4 showed the lowest values, TWIN 2 and TWIN 3 showed intermediate values. Mean median grain size per TWIN group was lowest in TWIN 4 (Fig. 6) and highest in TWIN 2, while TWIN 1 and 3 had comparable intermediate values. Although the amount of clay and silt was always lower than 1%, it was clear that the amount of fine particles was higher in the stations belonging to TWIN 4. Differences in sand fractions between the TWIN groups are less obvious. Medium sand dominated the sand fraction in all the TWIN groups, with comparable values in TWIN 1 to 3, and lower percentages at TWIN 4. Here, the amount of fine sand was higher compared to the other groups. The relative amount of coarse sand fractions increased from TWIN 1 to TWIN 3, while in TWIN 4 stations, these sediment fractions appear in comparable amounts as in TWIN 2.

Since the division of the TWIN groups was merely based on the occurrence of less abundant taxa, possible sediment preferences were investigated in different ways. First of all, the numbers of observations within certain intervals was studied, together with the mean densities in these intervals (Fig. 7). Most observations were made in sediments with a median grain size between 300 and 400 µm and to a lesser extinct in the 400-450 µm range. Only gastrotrichs and tardigrades were encountered more then three times in the 200-300 µm interval, while Acari and gastrotrichs were the only taxa with >5 observations in sediments with a median grain size above 450 µm. Highest densities were encountered from 300 µm onwards. Significant differences were found for Acari and tardigrades (Kruskal-Wallis, $p<0.05$). Pairwise comparisons of sediment classes show that densities were significantly lower in the 200-250 and 250-300 µm range (Table 4).

	250-300 µm	300-350 µm	350-400 µm	400-450	450-500	500-550
200-250 µm		A, **T**	**A, T**	**A**, T	A, T	A, T
250-300 µm			A	A	A	A
300-350 µm						
350-400 µm						
400-450 µm						
450-500 µm						
500-550 µm						

Table 4. Results of multiple comparisons. Summary of significant differences between densities in different size classes. A= Acari, T= Tardigrades. Letters in bold: $p<0.01$, letters in regular font: $p<0.05$. Blank indicates no difference

Considering gastrotrichs, no significant differences were found while comparing all classes, but densities in the 300-350 µm and 350-400 µm range are clearly higher compared to the other classes. Table 5 shows the results of the standardisation according to Vanreusel (1991). When all stations would be equally distributed among the sediment classes, no preference can be found for dominant groups such as nematodes, harpacticoids, turbellarians and polychaetes. Three taxa show a clear negative preference for relative fine sediments (Acari, ostracods and kinorhynchs). Gastrotrichs on the other hand showed a slight preference for coarser-grained sediments.

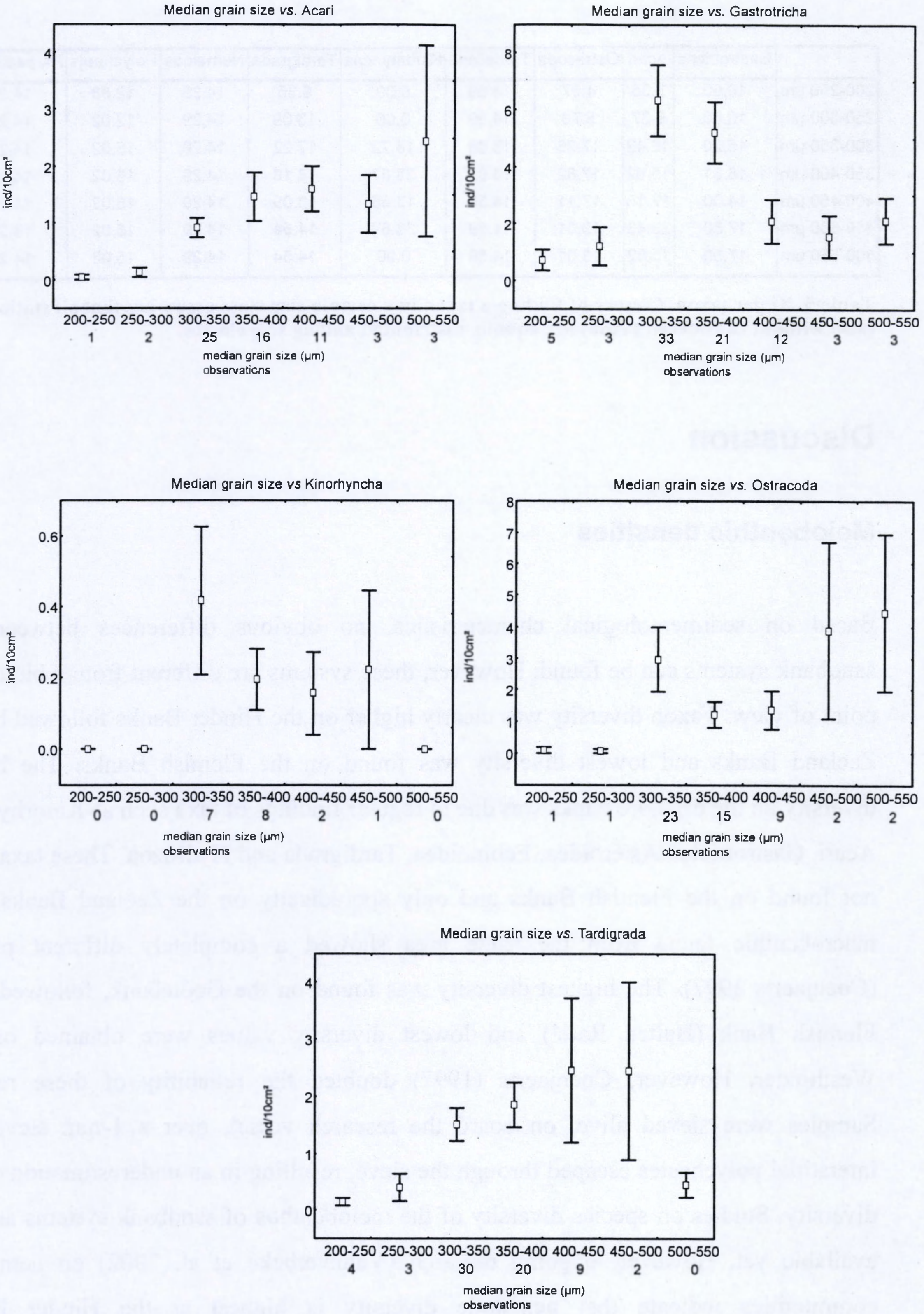

Figure 7. Densities of rare taxa vs. median grain size and number of observations per size class. Note different scaling of y-axis.

	Gastrotricha	Acari	Ostracoda	Turbellaria	Kinorhyncha	Tardigrada	Nematoda	Polychaeta	Harpacticoida
200-250 µm	10.00	3.35	4.07	14.59	0.00	9.35	14.29	12.88	14.29
250-300 µm	10.50	9.37	5.70	14.59	0.00	13.09	14.29	12.02	14.29
300-350 µm	15.20	15.42	17.26	13.06	18.72	17.22	14.29	15.02	14.29
350-400 µm	15.31	15.62	17.82	13.98	33.87	18.18	14.29	15.02	14.29
400-450 µm	14.00	17.18	17.11	14.59	13.55	13.09	14.29	15.02	14.29
450-500 µm	17.50	23.43	19.01	14.59	33.87	14.54	14.29	15.02	14.29
500-550 µm	17.50	15.62	19.01	14.59	0.00	14.54	14.29	15.02	14.29

Table 5. Xi per taxon. Chance of finding a taxon in a certain size class assuming that all stations (also without considered taxon) are equally distributed among size classes.

Discussion

Meiobenthic densities

Based on sedimentological characteristics, no obvious differences between the sandbank systems can be found. However, these systems are different from a biological point of view. Taxon diversity was clearly higher on the Hinder Banks followed by the Zeeland Banks and lowest diversity was found on the Flemish Banks. The higher diversity on the offshore banks was due to regular findings of taxa such as Kinorhyncha, Acari, Gastrotricha, Asteroidea, Echinoidea, Tardigrada and Hydrozoa. These taxa were not found on the Flemish Banks and only sporadically on the Zeeland Banks. The macrobenthic fauna from the same area showed a completely different picture (Coenjaerts 1997). The highest diversity was found on the Gootebank, followed by a Flemish Bank (Buiten Ratel) and lowest diversity values were obtained on the Westhinder. However, Coenjaerts (1997) doubted the reliability of these results. Samples were sieved alive, on board the research vessel, over a 1-mm sieve and interstitial polychaetes escaped through the sieve, resulting in an underestimation of the diversity. Studies on species diversity of the meiobenthos of sandbank systems are not available yet. However, ongoing research (Vanaverbeke et al. 2002) on nematode communities indicate that nematode diversity is highest at the Hinder Banks (Noordhinder and Bligh Bank), intermediate at the Zeeland Banks (Gootebank) and lowest on the Flemish Banks (Kwintebank). This is in agreement with the general picture that both nematode and copepod species diversity increases when distance to the coast increases (Vanaverbeke et al. 2001).

Comparing the meiobenthic densities and community composition with other studies on sandbanks or surrounding areas is quite difficult, since most of the information is buried in grey literature. Vanaverbeke et al. (2001) summarised the available literature for the BCS. A mean meiobenthic density of ± 500 ind.10 cm^{-2} is given for the Flemish Bank area, based on two studies (Chen 1987, Willems et al. 1982). Values found in the present study were comparable. The extremely high values of 2244 ind.10 cm^{-2}on the Kwintebank and 2707 ind.10 cm^{-2} on the Middelkerkebank come from stations in deeper channels between the sandbanks (Kwintebank) or from the side of the bank (Middelkerkebank). When these values were excluded, a mean meiobenthic density of 262 ind.10 cm^{-2}on the Kwintebank and 547 ind.10 cm^{-2} on the Middelkerkebank can be recognised. Lower mean densities were also found on the Oostendebank and the Oostdijck. No data were available from the Hinder Banks and the Zeeland Banks so far. Mean meiobenthic densities (calculated over all stations and both sampling periods) for the Zeeland Bank are 457 ind.10 cm^{-2}, while for the Hinder Banks this amounts 490 ind.10 cm^{-2}. Similar values (200-495 ind.10 cm^{-2}) in coarse sands have been found by Coull (1970), Gerlach (1971) and Soyer (1971). The sandbank systems do not show much difference, but the difference from the deeper channels between the sandbanks is striking. Mean meiobenthic densities in the channels at the Flemish Bank area are about 1250 ind.10 cm^{-2}, at the Zeeland Bank area this is about 1600 ind.10 $cm^{-2}$and around the Hinder Banks some 1420 ind.10 cm^{-2} are reported (Vanaverbeke et al. 2001). Although these figures are based on a limited number of stations, it is clear that meiobenthic densities at the sandbanks are much lower. This could be explained by sandbanks forming a highly dynamic environment, with relatively high hydrodynamic stress and subsequently less settlement of food particles for the benthos. Moreover, it has repeatedly been shown that enhanced hydrodynamic stress reduces meiobenthic densities and influences their vertical distribution. Palmer & Brandt (1981) and Palmer & Gust (1985) demonstrated a higher risk for the meiobenthos to be eroded when friction velocity increases as a result of tidal currents. Thistle et al. (1995) showed that harpacticoid copepods were suspended during winter storms at a 18 m deep site, and even in the deep sea strong currents reduced the abundance of meiobenthos (Thistle & Levin 1998) and influenced the vertical distribution of several taxa (Thistle et al. 1999). The seasonal pattern resulting in higher densities in October is probably a result of a higher primary production during spring and summer in the water column (Joiris et al. 1982), which has a pronounced effect of the inventory of chlorophyll *a* in the sediments (Cramer 1990, Boon et al. 1998). Indeed, nutrient measurements in the water column during the sampling campaign revealed a significantly lower amount of nutrient concentrations in October compared to February (Verween 1999) and this was attributed to nutrient use by phytoplankton during a bloom preceding the sampling. Sediment phytoplankton acts as fresh food for the benthos and the higher densities in October could be a result of the coupling between meiobenthic densities and food input in earlier months. By February, this extra food input can be depleted completely, resulting in lower numbers in the benthos. Measurements of benthic metabolism in the North Sea before, during and after the spring bloom confirm this patterns, with lowest metabolisms measured in February and highest values in May decreasing towards August (Boon et al. 1999)

ANOVA results showed that the Hinder Banks harboured lower densities than both the Zeeland Banks and the Flemish Banks. Again, this can be attributed to differences in primary production in the water column, since Joiris et al. (1982) found an inshore-offshore gradient in chl *a* concentrations in the BCS, with the highest values recorded in the coastal zone. This gradient was particularly sharp during the phytoplankton bloom. A similar patterns was described by Verween (1999) with highest chlorophyll *a*/*c* and fucoxanthine concentrations on the Flemish Banks, follewed by the Hinder Banks and the Zeeland Banks.

This pattern disappears during winter, explaining the absence of differences in meiobenthic densities between the Flemish Banks and the Hinder Banks in February. It is more difficult to find an explanation for the differences found between the Zeeland Banks and the Flemish Banks in February, since these differences disappear in October. According to Joiris et al. (1982), there is an east-west gradient in chl *a* concentrations, but this gradient should be more expressed in the October situation. The most reasonable explanation would be annual variation. Most of the samples from the Flemish Banks originated from October 1997, while sampling on the Zeeland Banks took place in October 1998. Two explanations can be put forward. Firstly, the differences could be due to interannual variation. The Zeeland Banks were sampled in 1998, while most of the Flemish Banks were visited in 1997. Interannual variability in sandbank benthos has been described by Philips (1998) for macrobenthos on the Flemish Banks. A clear decrease in macrobenthic densities between similar periods in 1995 and 1997 was recorded. As a second reason, climatological circumstances might have impacted the sandbank benthos. Storms do have an impact on the shape of superimposed bedforms on sandbanks (Houthuys et al. 1994). Large dunes and sand ripples can be moved, lowered or even swept out during heavy weather. It is clear that such events have a drastic impact on the meiobenthos since they live in close relation with the sediment

Community composition and diversity

Using TWINSPAN, four different station groups could be discerned, which did not coincide with the geographical position of the sandbank systems. This picture was confirmed by the DCA, but less clear, indicating considerable overlap between groups. The division in four different groups is nevertheless meaningful, since it reflects differences in sediment structure (Fig. 6) even within sandbanks. The macrobenthic studies of both Coenjaerts (1997) and Philips (1998) revealed the same pattern. No clear distinction between sandbanks or sandbank systems could be made when analysing the macrobenthic communities. Degraer et al. (1999) sampled two separated areas within the Coastal Banks on the BCS and again multivariate analysis reflected sedimentological differences where species replace each other gradually along a gradient in sediment characteristics.

The differences in sediment characteristics seem to influence the composition and the diversity of the meiobenthic community at the higher taxon level. Higher taxon diversity in coarser sediments has been shown a few times in North Sea studies (Chen 1997, Huys et al. 1992, Steyaert et al. 1994). These sediments harbour more microhabitats and sediment particles >300 µm show more flat surfaces compared to smaller particles, hence a wider variety of bacterial colonies can colonise these areas (Giere 1993). This larger variation attracts more meiobenthic species (Marcotte 1986).

According to Giere (1993), some meiobenthic taxa (e.g. Gastrotricha, Oligochaeta, Ostracoda and Kinorhyncha) prefer coarser sediments. This was confirmed by the present study, since densities, number of observations and standardised occurence was low in sediments with a median grain size below 300 µm. The relationship between meiobenthic diversity and density on sandbanks can be summarised as follows: sediment with a median grain size below 300 µm seemed to be low in taxon diversity, sediments comprised between 300 and 400 µm showed both high densities and diversity, while coarser sediments contained a similar amount of taxa but in lower densities.

This explains the low taxon diversity in TWIN 4, since median grain size here was about 310 µm. However, not only the median grain size affects the meiobenthic diversity, since highest taxon diversity was found within TWIN 1, while highest median grain size was found in TWIN 2. Moreover, TWIN 1 and TWIN 3 have comparable median grain size values, but quite different taxon diversity. The main factor explaining the taxon diversity on the sandbanks is the sorting* of the sediment: although minor differences between the distribution of the various sediment fractions exist, clear differences in taxon diversity do occur. The main difference in sediment characteristics between TWIN 1 and TWIN 3 is the relative amount of particles larger then 1 mm. In TWIN 1-stations, this is about 3%, while in TWIN 3, some 8 % of the particles are within this size range. This indicates that the general rule predicting higher taxon diversity in coarser sediments should be treated with caution. This also explains why the four TWIN groups fail to reflect the geographical position of the sandbank systems: within a sandbank, minor differences between the stations are very likely to exist, resulting in a classification within another TWIN group. The distribution of the stations originating from one sandbank or sandbank system over the different TWIN groups therefore reflects the sedimentological diversity of the sandbanks.

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* "Sorting is not well chosen here: it would be better to change "sorting" into "the proportions of the various granulometric characteristics"

29173

CHAPTER III

Nematode assemblages from subtidal sandbanks in the Southern Bight of the North Sea: effect of small sedimentological differences

Results presented in:

Vanaverbeke J, Gheskiere T, Steyaert M, Vincx M (2002) Nematode assemblages from subtidal sandbanks in the Southern Bight of the North Sea: effect of small sedimentological differences. J Sea Res 48: 197-207

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Abstract

Nematode assemblages from four subtidal sandbanks belonging to different sandbank systems on the Belgian Continental Shelf (BCS) were investigated both in spring and fall. The assemblages were characterised by different species composition patterns on the different sandbanks. This is in contrast to earlier studies which showed that neither meiobenthic taxa not macrobenthos differed between sandbanks. Although the sediments on these sandbanks could be classified as medium sand, the use of Multiple Discriminant Analysis (MDA) suggested that median grain size and the proportions of medium sand and very fine sand were the variables explaining the difference in nematode community composition. These findings emphasise the strong relationship between the relative abundance of nematode species and sediment granulometry. The influence of sand extraction on these sandbanks resulted in coarsening of the sediment, which had a direct effect on the nematode species composition. Diversity was not affected, indicating that nematodes inhabiting highly dynamic environments are well adapted to physical disturbance.

The diversity at sandbanks is not necessarily very different from the surrounding areas, since in more offshore parts of the Belgian Continental Shelf clean and rather coarse sand prevail and the difference in sediment composition is not sufficient to induce large differences in diversity.

Introduction

The Belgian Continental Shelf (BCS), located in the Southern Bight of the North Sea, is characterised by the presence of extensive sandbank systems. These sandbanks differ considerably from the regular seabed, since strong hydrodynamic currents induce changes in the topography of these sandbanks. These changes are irregular (e.g. migration direction of superimposed bed forms changes over relatively short time intervals) (Trenteseaux 1993; Houthuys et al. 1994; Lanckneus et al. 1994) and can be influenced by high wind speeds (Houthuys et al. 1994). These unique geological circumstances create an environment that is unique, especially for sediment-inhabiting animals. Moreover, these sandbanks act as internationally important areas for seabirds (Maes et al. 2000), indicating them as valuable potential marine protected areas. Alternatively, some of the sandbanks are concession areas for sand extraction (Maes et al. 2000), and the gullies between the sandbanks are important for fisheries, creating a conflict situation between human and natural interests.

Given the importance of the sandbanks for the functioning of the North Sea ecosystem on the Belgian Continental Shelf (e.g. nursery area for fish (Dewicke 2001, feeding and wintering area for seabirds (Maes et al. 2000), baseline data describing benthic life and explaining distribution patterns are needed in order to propose a sustainable management policy for these areas. Meiobenthic communities, and especially nematodes, are accepted to be very suitable for providing this kind of data. Nematodes show high abundances, a ubiquitous distribution, rapid generation times and are restricted to the sediment throughout their lives. (Heip et al. 1985, Kennedy & Jacoby 1997, Schratzberger et al. 2000).

Vanaverbeke et al. (2000) published data concerning the composition of meiobenthos on taxon level of all sandbank systems (Flemish Banks, Hinder Banks and Zeeland Banks). However, on nematode species level, only three studies have been published (Willems et al. 1982, Vincx 1990, Vincx et al. 1990). All of these focused on the same sandbank, Kwintebank, located in the Flemish Banks area. The nematode communities from this sandbank were clearly different from the communities sampled in the regular seabed, and diversity at the sandbank was significantly higher than at the adjacent area, but similar to the more offshore part of the BCS (Vincx 1990).

This paper aims to fill the gap in knowledge of benthic life in very dynamic environments in shallow coastal waters by 1) describing nematode communities from isolated sandbanks/sandbank systems (H_0: Nematode communities originating from different sandbanks are not different in terms of diversity and assemblage structure) 2) relating nematode communities to sediment granulometry (H_0: Differences in nematode communities are not related to differences in sediment composition) and 3) describing differences between nematode communities from the individual sandbanks and surrounding flat sea beds (H_0: nematode communities from the sandbanks and the surrounding seabed do not differ in terms of diversity and assemblage structure).

Material and methods

Study area

Sandbanks on the BCS are grouped into three major sandbank systems: the Flemish Banks, the Zeeland Banks and the Hinder Banks (Fig.1). Detailed information about depth and orientation of these sandbanks can be found in Vanaverbeke et al. (2000). 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 10m depth line; Hinder Banks: well below 10 m depth line) (Maes et al. 2000). Of each sandbank system, at least one sandbank was sampled for meiobenthos using a modified Reineck boxcorer. The Kwintebank, located within the Flemish Banks, was visited in February 1997, while the Noordhinder and Bligh Bank (both Hinder Banks) and the Gootebank (a Zeeland Bank) were sampled in February and October 1998. Stormy weather prevented an autumn sampling on the Kwintebank in 1997. At all sandbanks, seven stations were sampled: five of them were located at regular distances along the crest of the sandbanks; two additional stations were situated on the sides of the banks, thereby avoiding spatial pseudoreplication.

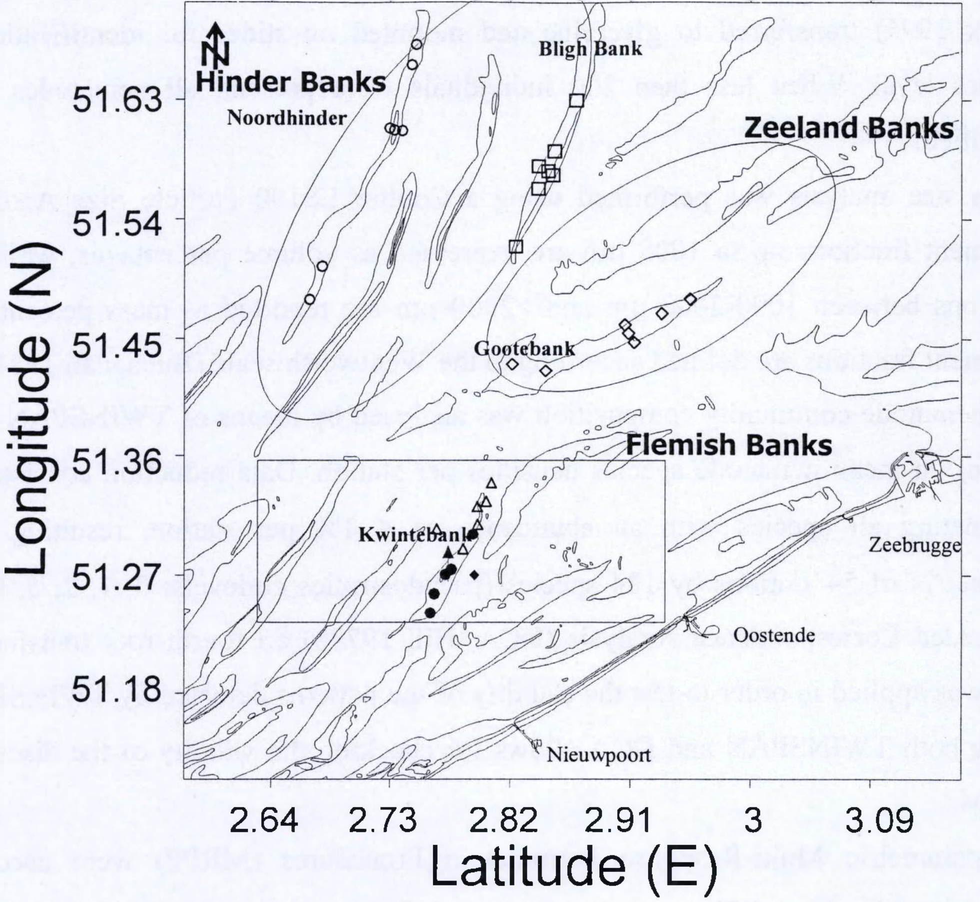

Figure 1. Map of the Belgian Continental Shelf with indication of the sampling stations. Symbols refer to nematode communities as deduced from multivariate analysis (Fig. 2)

On the Kwintebank, 10 stations were located on the crest of the sandbank, and two additional stations were sampled in the deeper channels neighbouring the sandbank. Station locations corresponded to those mentioned in Willems et al. (1982).

Sample collection and processing

The Reineck boxcorer was deployed three times per station and from each boxcorer, one subsample for meiobenthos and another one for sediment analysis were obtained using a perspex core (10 cm²). Meiobenthos was fixed with a hot (70°C) neutral formaldehyde tap-water solution (final concentration: 4%). Metazoan 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 a 38 µm-sieve were stained with Rose Bengal counted and classified to the taxon level. Results on meiobenthic taxon distribution patterns were published in Vanaverbeke et al. (2000). From two replicates, 200 nematodes were picked at random, (following Vincx 1996) transferred to glycerine and mounted on slides for identification to species level. When less then 200 individuals were present, all nematodes were identified.

Grain size analysis was performed using a Coulter LS100 Particle Size Analyser. Sediment fractions up to 1000 µm are expressed as volume percentages, while the fractions between 1000-2000 µm and >2000 µm are reported as mass percentages. Sediment fractions are defined according to the Wentworth scale (Buchanan 1984).

The nematode community composition was analysed by means of TWINSPAN (Hill 1979a) on mean nematode species densities per station. Data reduction consisted of eliminating all species with an abundance of ≤ 1% per station, resulting in a datamatrix of 54 stations by 184 species (pseudospecies cutlevels: 0, 1, 2, 5, 9). A Detrended Correspondence Analysis (DCA, Hill 1979b) on fourth root transformed data was applied in order to test the stability of the patterns detected by TWINSPAN. Using both TWINSPAN and DCA allows for checking the validity of the discerned groups.

Non-parametric Multi-Response Permutation Procedures (MRPP) were used for testing multivariate differences among pre-defined groups (Mielke et al. 1976, Whaley 1983, Zimmerman et al. 1995).

Diversity per TWIN group was calculated and expressed as Hill numbers of the order of 0, 1, 2 and $+\infty$ (Hill 1973), as recommended by Heip et al (1988). The indices differ in their tendency to include or ignore the relatively rare species: the impact of dominance increases and the influence of species richness decreases with an increasing order of the diversity number. Differences in diversity between TWIN groups were tested using the non-parametric Kruskal-Wallis test since transformation of the data was not sufficient to meet the assumptions for ANOVA. When significant differences were observed, multiple comparisons between the TWIN groups were made, following Conover (1971). Differences in diversity between seasons per sandbank for each TWIN group were tested by a Mann-Whitney U test.

To assess the role of different sediment components in structuring the nematode communities, a Multiple Discriminant Anaysis (MDA) was performed. This approach has rarely been used in marine data but has been proven to be powerful in unravelling patterns that are difficult to analyse using ANOVA or non-parametric alternatives

(Shin & Fong 1999). MDA allows determining which variables discriminate between two or more naturally occurring groups. These groups were the station assemblages based on the multivariate analysis of the nematode communities. Before entering the abiotic variables in the data matrix, correlated variables were removed in order to prevent ill conditioning of the matrix. Finally, median grain size and proportions of clay, very fine sand, medium sand, >1000 µm and >2000 µm were retained. All variables were tested for ANOVA assumptions prior to the analysis. A forward selection procedure was performed in order to identify the most important variables. Only statistics for variables significantly incorporated in the model are reported. Squared Mahalanobis Distances were calculated to detect significant differences between groups (StatSoft 1995), while factor structure coefficients identify those variables that are most associated with the discriminant functions. Analyses were performed using the Statistica software package (StatSoft 1995).

Results

Nematode community composition and diversity

Both TWINSPAN and DCA indicated the existence of 6 different nematode communities (Fig. 2).[*] TWIN 1 comprised all stations from the Noordhinder, while the Bligh Bank stations were grouped in TWIN 2. The Gootebank stations were put together in a third group (TWIN 3). Kwintebank stations were divided in three groups: TWIN 4 comprised stations located at the northern part of the sandbank (plus channel station Kw12), while the southern stations were grouped in TWIN 5. TWIN 6 was a single station group, containing the other channel station Kw13. MRPP showed these groups to be significantly different at $p<0.001$. Table 1 lists the 10 dominant species per TWIN group and their relative abundance. This table clearly shows the difference in the nematode communities: *Leptonemella aphanothecae* is dominant in

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[*] A preliminary analysis, using all replicates as stations, indicated that replicates were plotted closely together in a DCA analysis (see Gheskiere 2000 Structurele diversiteit van nematodengemeenschappen van de Bligh Bank (Zuidelijke bocht van de Noordzee). Universiteit Gent, unpublished licentiates thesis, 92 pp.). The TWINSPAN analysis separated Twin 1 and 2 from the remaining groups at the first division. The second division discerned Twin 1 from Twin 2 and Twin 3 from the Twin 4-6. At the third level, the distinction between the various Kwintebank groups became clear (Gheskiere 2000)

both the Hinder Bank TWIN groups (TWIN 1 and 2) and *Neochromadora munita* is dominant in TWIN 4 and TWIN 5 (both Kwintebank), but differences in the species lists among all the groups are obvious.

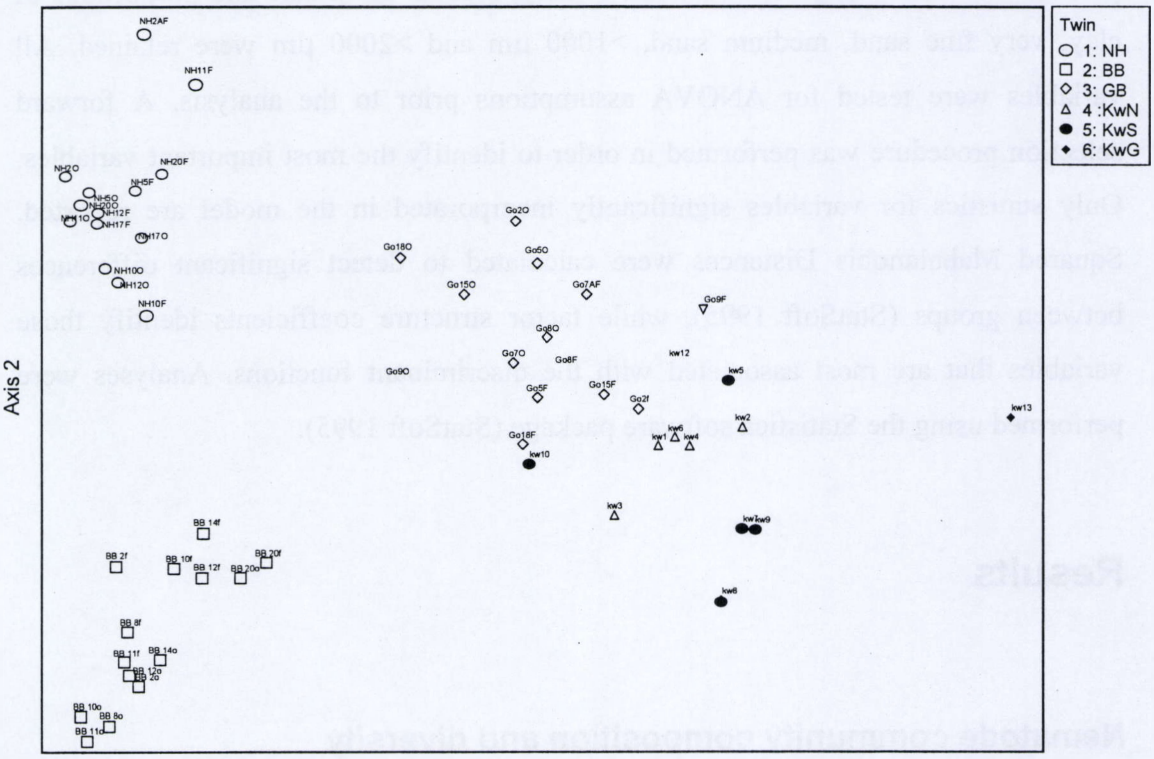

Figure 2. Detrended Correspondence Analysis (DCA) output with indication of the Twin groups (NH: Noordhinder, BB: Bligh Bank; GB: Gootebank; KnN: Kwintebank Northern area; KwS: Kwintebank Southern area; KwG: Kwintebank gully). Eigenvalue Axis1: 0.64; Eigenvalue Axis 2: 0.40

Hills' diversity indices (with the exception of N∞) were significantly different between the 6 groups (Kruskal-Wallis test: N_0: $p<0.01$; N_1: $p<0.001$; N_2: $p<0.01$). Multiple comparisons revealed that these differences were mainly due to a significantly lower diversity in the nematode communities of the Kwintebank groups compared to communities inhabiting the other sandbank sediments. No significant differences (at $p<0.05$) were recorded when diversity indices from February and October per sandbank were compared.

Twin1		Twin2		Twin3	
Leptonemella aphanothecae	12.93	*Leptonemella aphanothecae*	13.70	*Onyx perfectus*	7.10
Microlaimus marinus	8.72	*Xyala striata*	4.81	*Chromaspirina pellita*	5.52
Sigmophoranema rufum	4.71	*Onyx perfectus*	3.54	*Xyala striata*	5.50
Desmodora schulzi	3.71	*Tubolaimoides* aff. *tenuicaudatus*	3.36	*Neochromodora munita*	3.86
Chromaspirina parapontica	3.34	*Calomicrolaimus parahonestus*	3.21	*Microlaimus marinus*	3.50
Chromaspirina sp. 2	2.88	*Stephanolaimus elegans*	2.93	*Prochromadorella septempapillata*	3.34
Neochromadora minuta	2.77	*Microlaimus marinus*	2.85	*Odontophora rectangula*	2.47
Rhynchonema quemer	2.65	*Chromaspirina pellita*	2.80	*Theristus denticulatus*	2.35
Ptycholaimellus vincxae	2.63	*Desmodora schulzi*	2.60	*Rynchonema moorea*	2.21
Stephanolaimus elegans	2.61	*Theristus denticulatus*	2.60	*Dichromodora cuculata*	1.94

50 <50

Twin4		Twin5		Twin6	
Neochromodora munita	13.82	*Neochromodora munita*	16.52	*Sabatieria celtica*	16.56
Theristus bastiani	12.53	*Metadesmolaimus pandus*	12.81	*Sabatieria punctata*	13.38
Theristus maior	4.33	*Bathylaimus capacosus*	8.81	*Synonchiella riemanni*	12.74
Enoploides spiculohamatus	3.54	*Viscosia franzii*	8.81	*Microlaimus acinaces*	4.78
Onyx perfectus	3.15	*Theristus maior*	3.60	*Microlaimus marinus*	4.78
Chromadorita sp. 2	2.92	*Cyatholaimide* sp.	3.30	*Daptonema* sp.	4.14
Cyatholaimide sp.	2.92	*Bolbolaimus teutonicus*	2.80	*Metalinhomoeus* sp.2	3.50
Odontophora exharena	2.64	*Pomponema loticum*	2.70	*Metalinhomoeus* sp.1	2.87
Calomicrolaimus parahonestus	2.47	*Enoploides spiculohamatus*	2.60	*Odontophora exharena*	2.87
Metadesmolaimus pandus	2.25	*Microlaimus ostracion*	1.80	*Terschellingia longicaudata*	2.87

Table 1. Dominant nematode species per Twin group and their relative abundance (in %)

Relation with sediment granulometry

Mean sediment characteristics per station group are shown in Fig. 3. Although significant differences between all the variables (except % silt) were found (using ANOVA or Kruskal-Wallis), an explanation of the differences in community composition between the sandbanks/sandbank systems is not straightforward.

Therefore, forward stepwise MDA was applied, elucidating the relative importance of the different sedimentological variables in discriminating between the nematode communities. TWIN 6 (Kwintebank station 13) was excluded from the analysis since this was a single station group, and caused ill conditioning of the data matrix. However, Fig. 3 clearly shows that sediments at this channel station are much finer compared to other sediments at the sandbank proper.

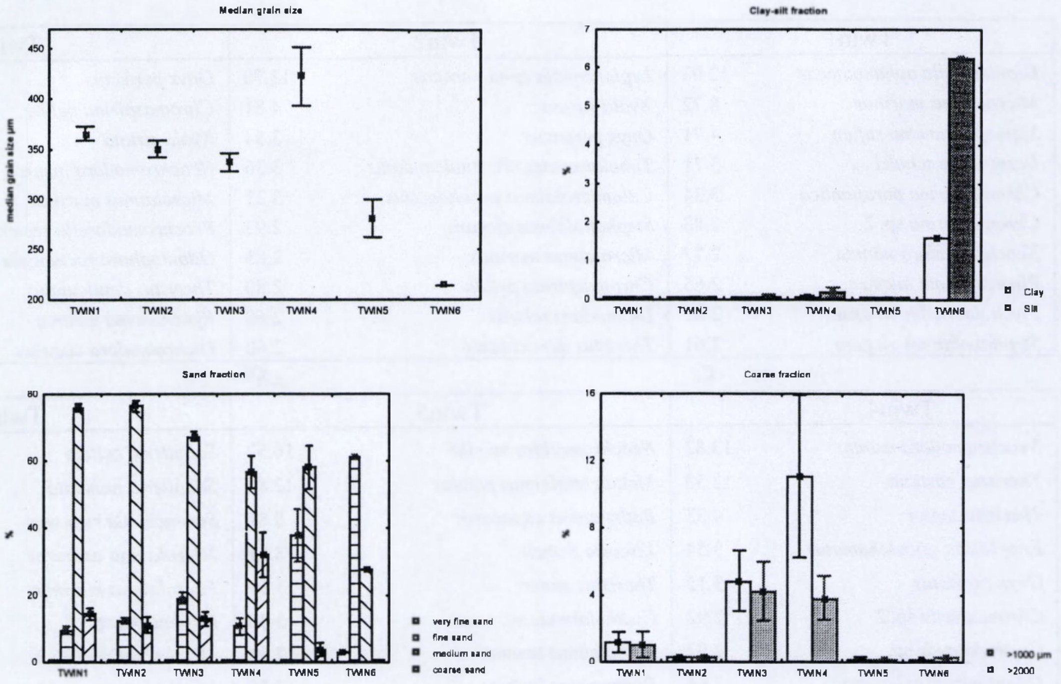

Figure 3. Mean sediment characteristics (following Buchanan (1984)) per Twin group

This coincides with the presence of a relative large amount of clay-silt in the sediment, a feature almost absent at the other sediments. The first two discriminant functions explained 87% of the variation (Table 2), and since the eigenvalues of discriminant function 3 and 4 are low, only the first two roots will be reported upon.

Discriminant function	**1**	**2**	**3**	**4**
eigenvalue	1.81	0.92	0.38	0.0012
cumulative separation	0.58	0.87	0.99	100

variable	**Factor structure coefficients (correlations between variables in the model and the discriminant functions)**			
% medium sand	-0.68	-0.48	0.41	-0.39
median grain size	-0.34	0.85	-0.18	0.36
% very fine sand	0.42	0.31	0.29	-0.8
%>2000 µm	0.16	0.32	0.49	0.8

Table 2. Summary of the stepwise multiple discriminant analysis of the sedimentological variables of the Twin groups

Four variables were retained in the model (Wilks' Lambda: 0.13, p<0.001) and % medium sand and % very fine sand seemed to be the most important variables in discriminant function 1, while the second root is influenced by median grain size and % medium sand (Table 2, Fig.4).

Squared Mahalanobis distances and their significance level are listed in Table 3. Both Fig. 4 and Table 3 show that Kwintebank sediments are quite different from sediments found at the other sandbanks (Kwintebank southern stations along root 1; Kwintebank northern stations along root 2). Differences between sediments from the Gootebank and the Hinder Banks (Bligh Bank and Noordhinder) were significant as well, whereas no significant differences were found between the Hinder Bank sediments (Noordhinder and Bligh Bank).

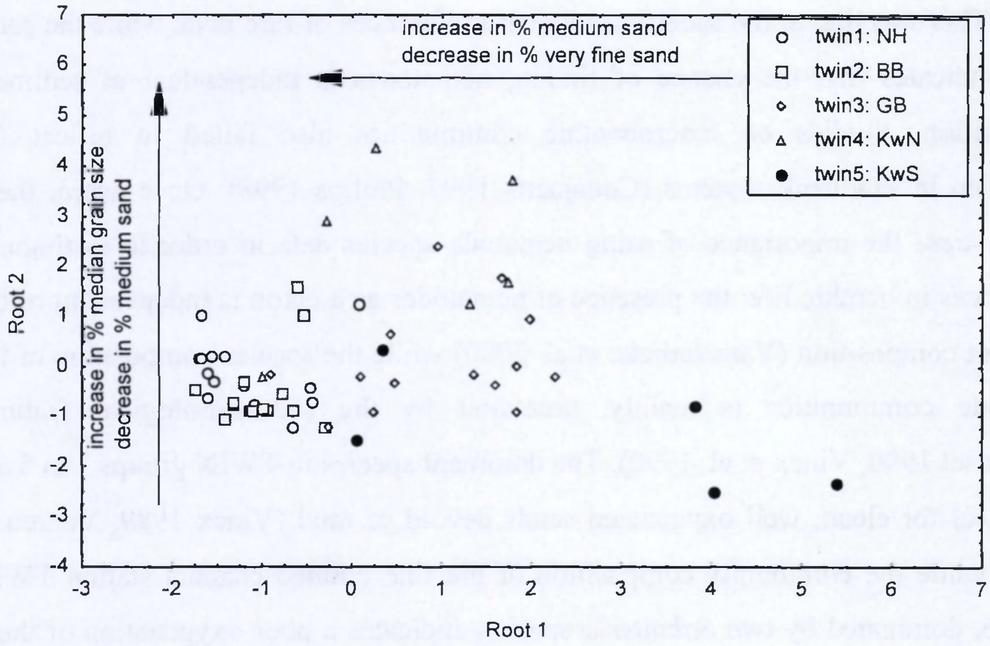

Figure 4. Plot of location groups from stepwise MDA based on sediment granulometry

	Twin 1	Twin 2	Twin 3	Twin 4
Twin 1				
Twin 2	0.21 ns			
Twin 3	6.21 ***	5.92 ***		
Twin 4	10.34 ***	11.71 ***	8.01 ***	
Twin 5	19.53 ***	16.89 ***	9.42 ***	19.59 ***

Table 3. Squared Mahalanobis distances between the Twin groups with indication of the significance (*p<0.05; **: p<0.01; ***: p<0.001).

Discussion

Nematode community structure in relation to sediment granulometry

Both multivariate methods (TWINSPAN and DCA), discerned six different nematode assemblages on the sandbanks of the BCS. These groups were significant as revealed by MRPP, resulting in the rejection of the first null hypothesis (no difference in nematode communities from different sandbanks/sandbank systems).

However, a study of all meiobenthic communities from the sandbanks on the BCS, indicated that geographical position (e.g. sandbank/sandbank system) was not important in structuring the communities: stations originating from the same sandbank were distributed over different groups in multivariate analyses (Vanaverbeke et al. 2000). This was due to the specific sediment preferences of rare taxa, while the same study indicates that the chance of finding nematodes is independent of sediment composition. Studies on macrobenthic communities also failed to reflect the difference in sandbank systems (Coenjaerts 1997, Philips 1998). Once again, these results stress the importance of using nematode species data in order to distinguish differences in benthic life: the presence of nematodes as a taxon is independent of the sediment composition (Vanaverbeke et al. 2000) while the species composition of the nematode communities is mainly structured by the sedimentological features (Vanreusel 1990, Vincx et al. 1990). The dominant species in TWIN groups 1 to 5 are all typical for clean, well oxygenated sands devoid of mud (Vincx 1989, Vanreusel 1991), while the community composition of the fine grained channel station TWIN group 6, dominated by two *Sabatieria* species, indicates a poor oxygenation of these sediments (Steyaert et al. 1999, Boyd et al. 2000). This points to a main difference in the ecology of sandbank sediments compared to the regular seabed in certain areas.

Personal observations confirmed these results: sediment cores obtained from the channel station showed black sediments at a few cm depth, indicating reduced and oxygen depleted sediments, while this was never the case on the sandbank crest.

Although generally, the different communities reflect the geographical position of the sandbanks, this position alone offers a poor explanation for the existence of well-delineated groups. The existence of different nematode communities in the Southern Bight of the North Sea is often linked to differences in sediments (eg. Vincx 1990, Vincx et al. 1990 and references therein), but in their studies a wide variety of sediments, from clay-silt to coarse sand, were compared. The present study investigated nematode communities from sediments belonging to a single sediment class: medium sand (median grain size: 250-500 µm (Buchanan 1984)).

Therefore, MDA was applied to elucidate the relative importance of the different sedimentological features. A graphical presentation of root 1 *vs.* root 2, explaining 87% of the variation in the sedimentological data (Fig. 4) reveals that groupings based on sedimentological data match the biological groupings based on nematode species abundance data. Squared Mahalanobis distances confirm these findings, emphasising the great differences between the Kwintebank groups and the other sandbanks, the close resemblance between the Hinder Bank groups and the intermediate position of the Gootebank. The identical groupings of both the biological and sedimentological data are due to the structuring effect of the sediment granulometry on the nematode community composition.

The separation of the Kwintebank stations in a northern and a southern group based on the nematode assemblages is confirmed by granulometric differences associated with the second discriminant function (median grain size and % medium sand). Indeed, median grain size reached highest values in this TWINgroup (Fig. 3). These coarser sediments are probably a result of intensive sand extraction activities, occurring in this area, where up to 20 cm of sediment is removed each year (Bonne pers. comm.).

Sand extraction is known to induce coarsening of the sediment (Anonymous 1993). Although these activities have a severe impact on macrobenthic communities (Kenny & Rees 1996, Desprez 2000), the influence on nematode diversity seems to be less drastic. Diversity in this area is not significantly different from the southern part of the sandbank, indicating that these nematode species surviving in this area are well adapted to a frequent physical disturbance of their environment. Their continuous reproduction strategy enables the community to be less vulnerable than for instance. the macrobenthos, where a disturbance event during the recruitment period can destroy the population until the next recruitment. Another advantage of the continuous reproduction strategy is the time-round presence of individuals, facilitating recolonisation of disturbed areas, while recolonisation of a sediment extraction area by macrobenthos can take more than 2 years (Kenny & Rees 1996). Moreover, Schratzberger & Warwick (1999) demonstrated that nematodes living in a coarse sandy environment are less influenced by physical disturbance in comparison with nematode species inhabiting muddy sediments. Since sandbank sediments can be considered a dynamic environment, nematode communities surviving here must be adapted to a regularly changing environment.

Differences between the other sandbanks are mainly associated with the first root, showing that sediments at the Gootebank have intermediate lower medium sand content, compared to the Kwintebank and both Hinder Banks. Differences between these Hinder Banks are not significant. The nematode communities from the latter sandbanks also show the largest level of resemblance (Table 1): among the 10 dominant species, 4 are common (*Leptonemella aphanothecae, Microlaimus marinus, Desmodora schulzi* and *Stephanolaimus elegans*) or closely related (species within *Chromaspirina*). Although all these communities are living within the same sediment category (medium sand) the second null hypothesis (differences in nematode community composition is not related to sediment granulometry) should be rejected, again emphasising the extreme way in which the presence/absence or relative abundance of nematode species is influenced by minor differences in sediment composition.

Nematode diversity and differences with surrounding channels

Nematode diversity followed the general pattern already described in Vanaverbeke et al. (2001): diversity of nematode communities at the BCS increases with distance to the coast. This was attributed both to sedimentological differences, higher offshore diversity being associated with clean, coarser sand in comparison with the finer grained coastal sediments where anoxia as a result of eutrophication can have a drastic impact on the nematode communities, especially at the east coast (Steyaert et al. 1999). Lowest diversity in this study was indeed associated with the finest sediments (Twin groups 5 and 6) at the Kwintebank. Comparing nematode species numbers from sandbanks and the surrounding area was done using the new data presented in this study and the compilation of data on the BCS listed in Vanaverbeke et al. (2001) (Fig. 5). Statistical comparison of these data is not recommended, since differences in data collection might influence the reliability of the results of such testing, but Fig. 5 clearly demonstrates that differences between the sandbanks and the surrounding areas are very obvious in the Flemish Bank area. This is probably because differences in sediment composition between the sandbanks and gullies are largest in that area. Since a strong hydrodynamic regime occurs around sandbanks, sediments are coarser and saturated with oxygen, while in the channels, fine sediment particles can settle to the seabed, and oxygen is not refreshed as often as in the more dynamic sandbank sediments

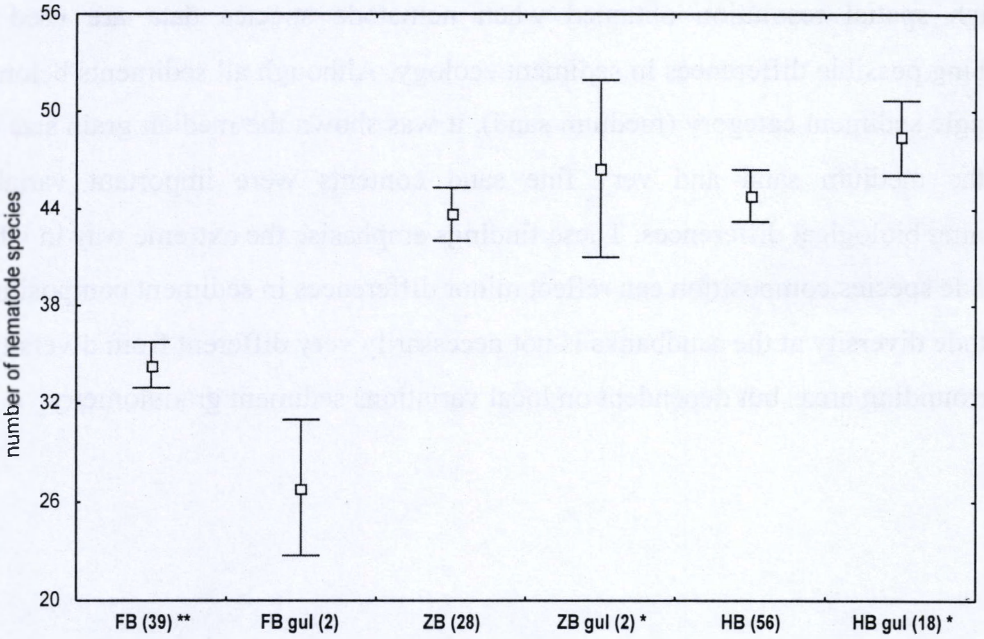

Figure 5. Mean nematode species number (□ SE) for different areas on the BCS (FB: Flemish Banks; FB gul: Flemish Banks gullies; ZB: Zeeland Banks; ZB gul: Zeeland Bank gullies; HB: Hinder Banks; HB gul:Hinder Bank gullies).*: Data from Vanaverbeke et al. (2001).

The lower diversity in fine-grained sediments, associated with low oxygen content or oxygen depletion has been documented already (Steyaert et al. 1999). When sediments at the channels consist of clean sands (e.g. those sediments in the Hinder Bank area and the Zeeland Bank area), nematode diversity increases and can be even slightly higher in comparison to the nematode diversity at the sandbanks, possibly a result of the stronger hydrodynamic circumstances prevailing at the sandbanks. In these areas, the third hypothesis (no difference in nematode diversity between sandbanks and surrounding seabed) should not be rejected, while at the Flemish Bank area the compiled data indicate the existence of marked differences.

Conclusions

This study shows that nematode assemblages from isolated sandbanks differ. Such differences were not found when investigating spatial patterns on the same sandbanks using data on the meiobenthic and macrobenthic taxon distribution. This emphasises the high spatial resolution obtained when nematode species data are used for describing possible differences in sediment ecology. Although all sediments belonged to a single sediment category (medium sand), it was shown the median grain size and both the medium sand and very fine sand contents were important variables explaining biological differences. These findings emphasise the extreme way in which nematode species composition can reflect minor differences in sediment composition. Nematode diversity at the sandbanks is not necessarily very different from diversity in the surrounding areas but dependent on local variations sediment granulometry.

CHAPTER IV

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

Results presented in:

Vanaverbeke J, Steyaert M, Vanreusel A, Vincx M (2003) Nematode biomass spectra as descriptors of functional changes due to human and natural impact. Mar Ecol Prog Ser. 249:157-170

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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 comparing 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 was retained. Normalising the spectra caused elevated biomass values and peaks to disappear, introducing bias in interpreting the distribution of biomass over spectra. Cumulative nematode biomass spectra proved to be useful 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 increased probably as a result of a higher food supply to the benthos.

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 knowledge and therefore it offers an alternative and sensible technique for describing and comparing benthic communities (Sprules & Manuwar 1986, González-Oreja & Saiz-Salinas 1999, Drgas et al. 1998, Duplisea & Drgas 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, 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 1.5-2 and 0.055-0.1 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 three 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 lives in between the sand grains (interstitially) while the macrobenthos experiences the sediment at the macroscopic scale (the sediment–water interface). Warwick (1984) described a species size spectrum and found two 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 two major aims: (1) to analyse the response of NBS to three 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, nematodes 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.

Material 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 10 m depth line; Hinder Banks: well below 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 two stations were situated at the 10 m depth line on the flanks of the sandbanks. On the Kwintebank, 12 stations were sampled. Two 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 the stations Kw1 to Kw 6 while in the remaining area (South stations Kw7 to Kw10), sand extraction activities are significantly lower (Bonne & Vincx unpublished). 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 percentages, while the fractions between 1000 and 2000 µm and >2000 µm are mass percentages.

Coastal area. Data for this part of the study were collected from two stations in the coastal area of the Belgian continental shelf. Station 702 (51°09.1'N, 02°36.1'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. Station 790 (51°16.0'N, 02°65.0'E; water depth: 8 m) is situated in the central part of the Belgian coastal area.

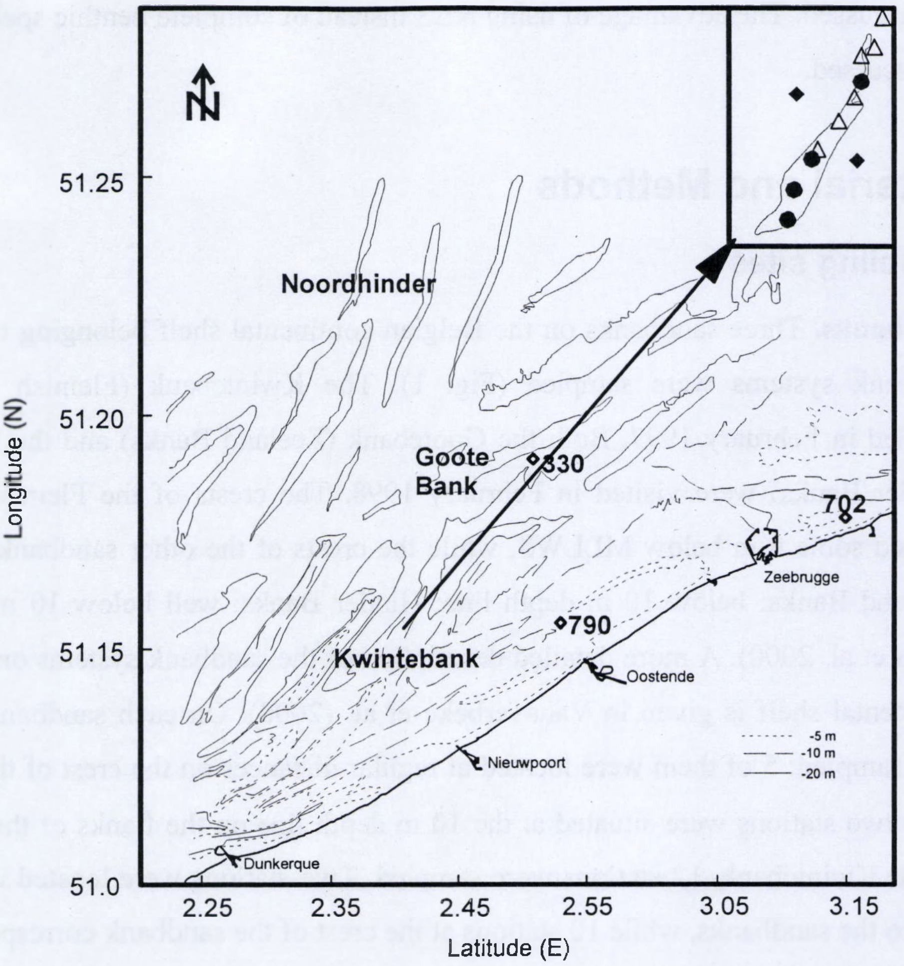

Figure 1. Map of the Belgian continental shelf with indication of the sampling stations. Kwintebank stations indicated as triangles: high sand extraction activities; as dots: low sand extraction activities; as diamonds: gully stations

Station 702 was sampled in December 1994, while samples from Station 790 were collected in March 1994. Sediments were clearly different; Stations 702 had a fine sand sediment with a low mud content (clay-silt fraction: 11.4 %, recalculated from Steyaert et al. 1999), whereas at Station 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 four classes representing strongly oxidised (>100 mV), oxidised (0mV<x<100mV), reduced (0mV<x<-100 mV) and strongly reduced (<-100 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 Station 330 (51°26.0'N, 02°48.5'E; water depth: 20 m) at the Belgian continental shelf. The sediment consisted of medium sand. Chlorophyll *a* (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 at March 9th, May 12th and July 12th 1999.

Sampling and treatment of samples

Sampling at all sites took place aboard RV Belgica. Sediment was collected at the sandbank stations and at Station 330 using a modified Reineck boxcorer. The boxcorer was deployed three times per station. From each boxcorer, one perspex core (10 cm²) was used for faunal analysis up to 10 cm sediment depth. The coastal stations were sampled using a boxcorer (surface area 804 cm²), 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 Stations 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 Station 330 were sliced every 0.5 cm. In the laboratory, all animals passing a 1-mm sieve and retained by a 38 µm-sieve were extracted from the sediment by centrifugation with Ludox (Heip et al. 1985). After staining with Rose Bengal, nematodes were picked out randomly and mounted on Cobb slides for identification and measurements. From the sandbank samples and sliced sediment, 200 and 120 nematodes 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 unpublished). Stations 1 to 3 and 5 to 7 were grouped together (Kw H) since high sand extraction activities were recorded here (1000-8000 m^3 yr^{-1} are removed). A low extraction occurred at stations 4 and 8 to 10 (Kw L); here 1200 to 1400 m^3 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, Station 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. Cutlevels 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 $\log_2$ groupings of nematode dry weight (µg) on the *x*-axis and total biomass per size class (dry weight, µg) on the *y*-axis. The $\log_2$ 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 one sediment column into one 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) x 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 $\log_2$ of the upper limit of the size class. The 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 loose 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 the size (s) of a particle at random will be greater than size S [prob($s \geq S$)] as a function of S on a double-logarithmic scale. In practice, the term prob($s \geq S$) 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 could be 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. 2002). However, it should be noted that the nematode assemblages were clearly different. Only *Neochromadora munita* was listed among the 10 dominant species in more then 2 Twin 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	21.04	8.22	2.03
Kw_G	266.2	1.06	4.08	1.68	42.45	43.01	7.74	5.13	3.02

Table 1. Mean sedimentological variables of the different groups: disturbance study

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.

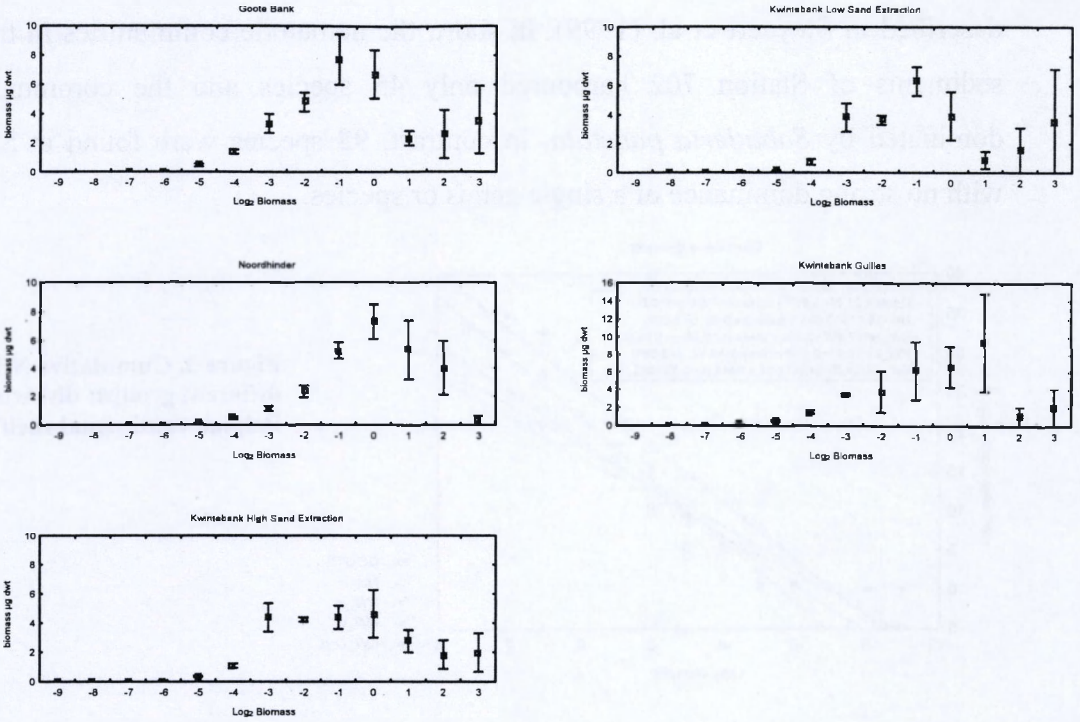

Figure 2. Mean NBS of different groups: disturbance study, Belgian continental shelf

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 two coastal stations were clearly different (Fig. 4). Sediments at Station 790 were completely oxidised, with high redox potential values (>100 mV throughout the sediment column) and a stable nitrogen compound depth pattern. At Station 702, sediments were only oxidised in the upper cm, and strongly negative redox potential values were recorded deeper than 2 cm, associated with a build up of ammonia. Nitrate/nitrite concentrations dropped severely at this depth. Multivariate analysis of the nematode community composition of the two 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 Station 702 harboured only 45 species and the communities were dominated by *Sabatieria punctata*. In contrast, 98 species were found at Station 790 with no strong dominance of a single genus or species.

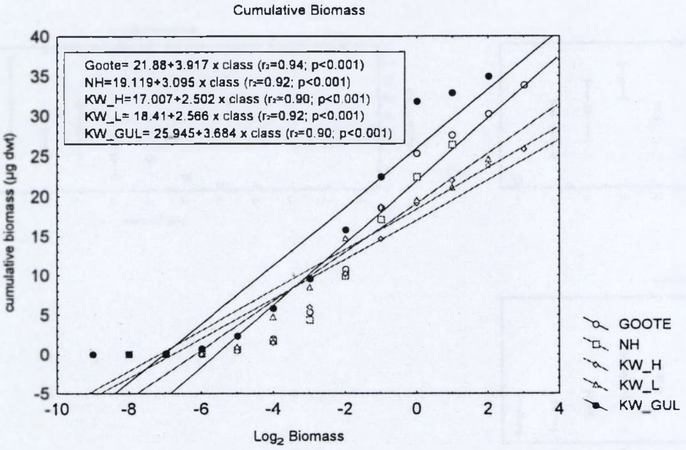

Figure 3. Cumulative NBS of different groups: disturbance study, Belgian continental shelf

Nematode biomass spectra. Both regular NBS and normalised NBS for the two stations are depicted in Fig. 5. Visual inspection of the graph shows that biomass values from Station 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≤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 Station 702 was steeper compared to the slope for Station 709. 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 Station 702 was reflected in a gentler slope when compared to the slope of Station 790. Again, regression slopes were significantly different (p<0.001).

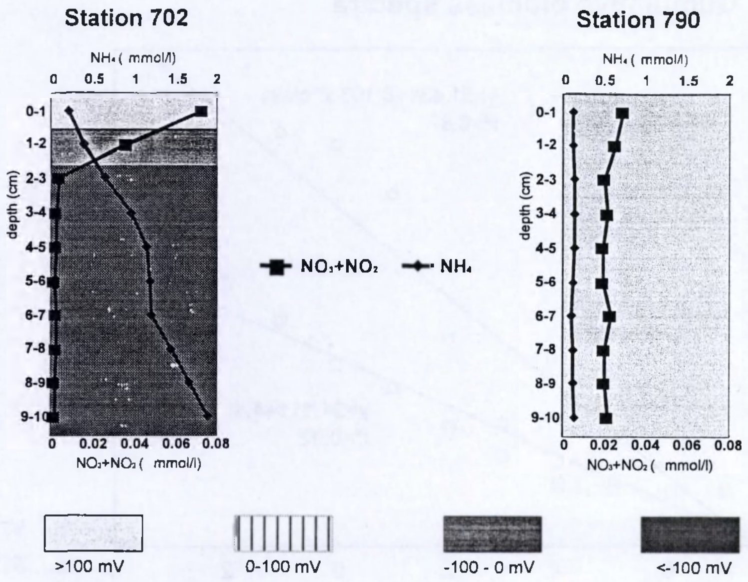

Figure 4. Redox potentials and nitrogen compounds at Station 702 and Station 709, Belgian coast

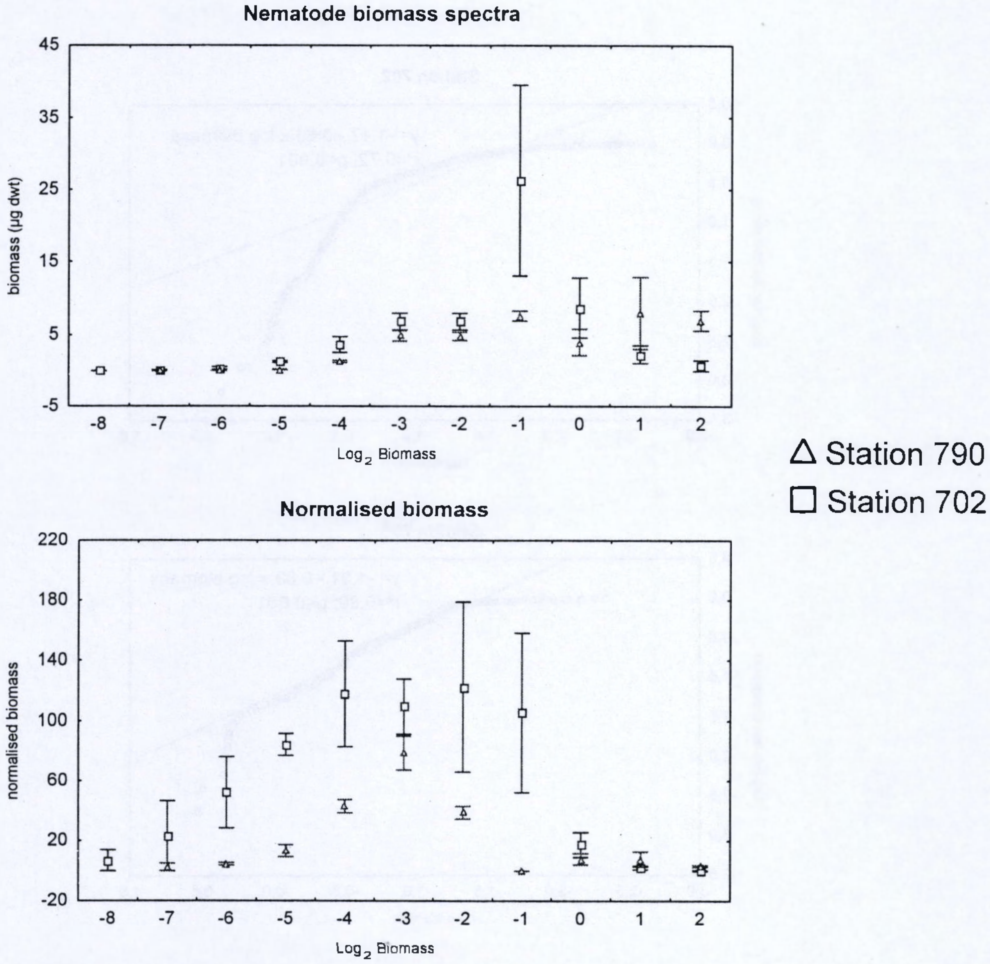

Figure 5. NBS (upper panel) and normalised NBS (lower panel) for Station 702 and Station 790, Belgian Coast

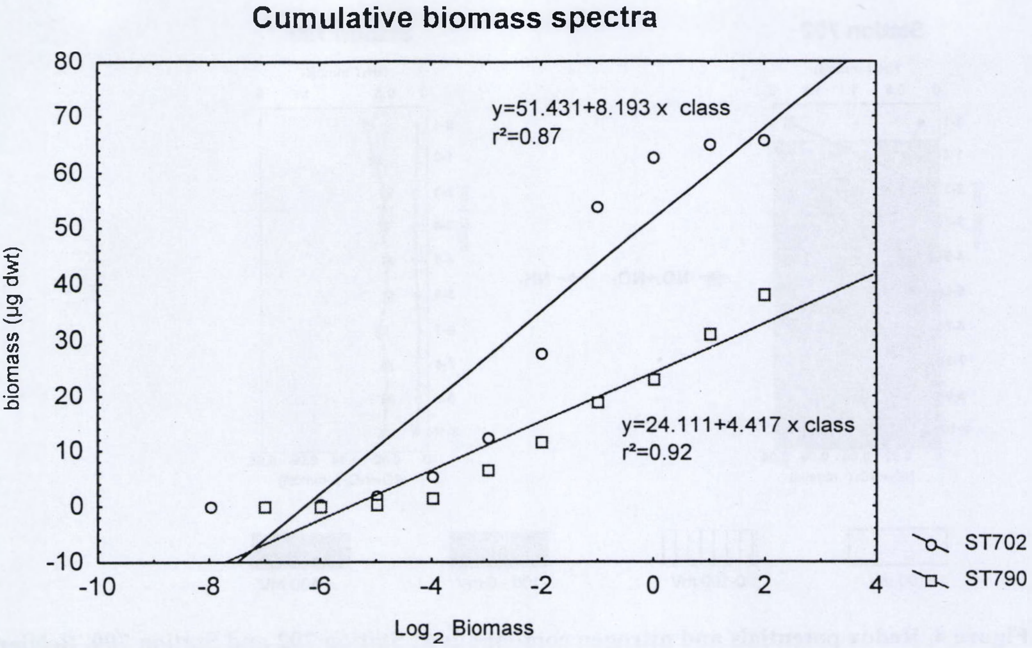

Figure 6. Cumulative biomass spectra for Station 702 and Station 790, Belgian Coast

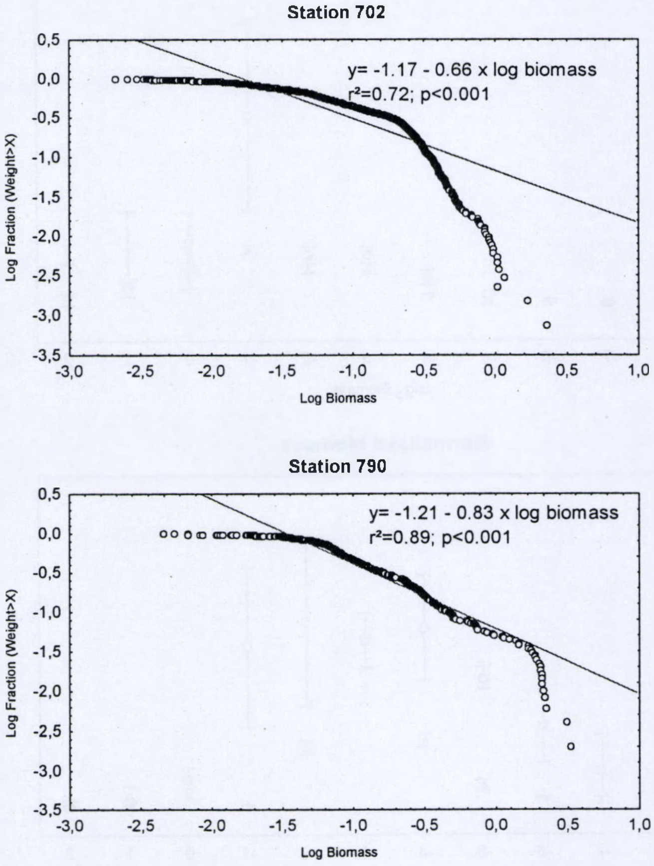

Figure 7. Pareto-type biomass distributions for Station 702 (upper panel) and Station 790 (lower panel), Belgian Coast

Food pulse study (Open Sea)

Study area and community analysis. Chlorophyll *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.

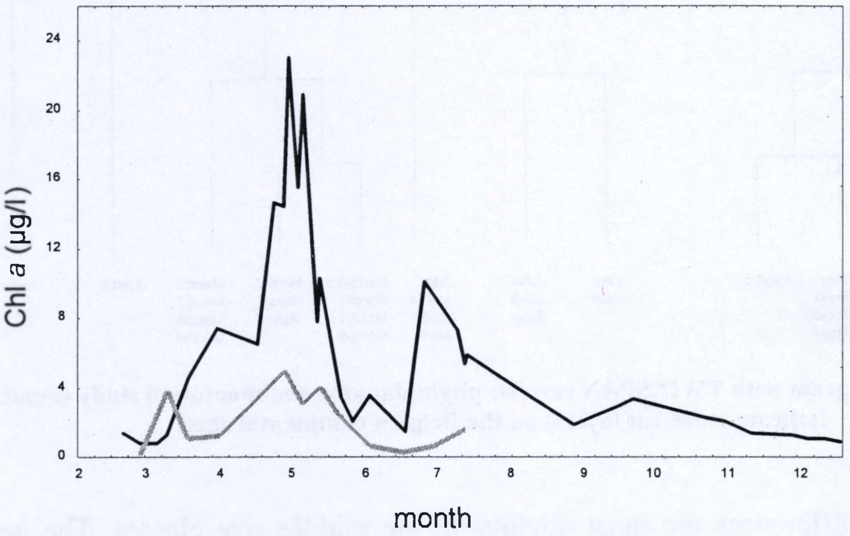

Figure 8. Chl *a* concentrations in the water column at Station 330 on the Belgian Continental Shelf. Black line: surface water; grey line: bottom water

The pattern at the surface was closely followed by the pigment concentrations in the bottom water, indicating sedimentation 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 one sediment slice from July was classified in this group as well). May and July were subsequently separated as well, resulting in three groups, each representing one 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

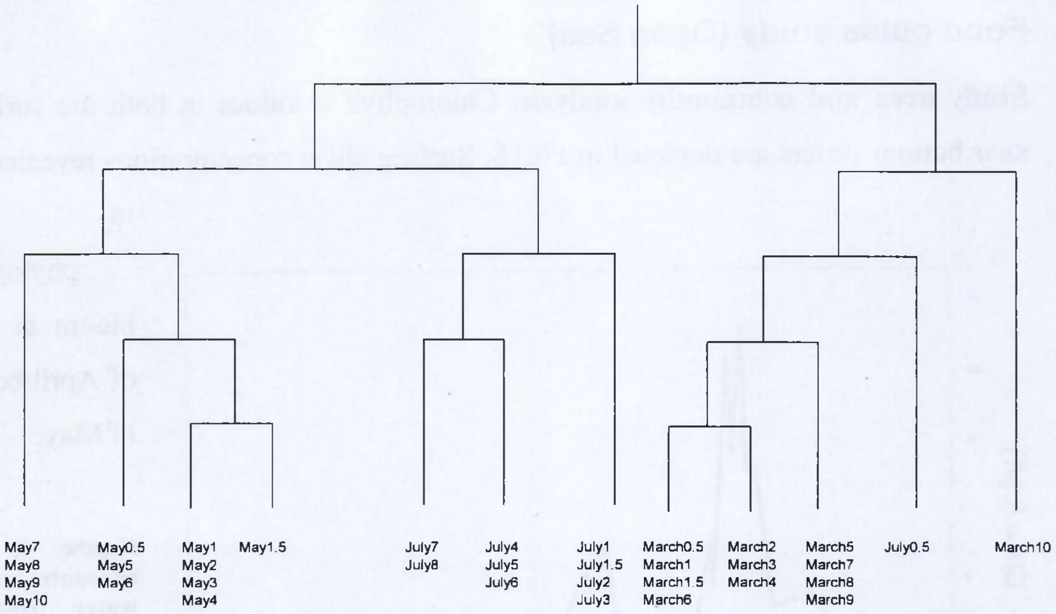

Figure 9. Dendrogram with TWINSPAN results: phytoplankton sedimentation study (numbers indicate sediment layers) on the Belgian continental shelf

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 Station 702, but elevated biomass values are spread over several size classes. The "time x 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.

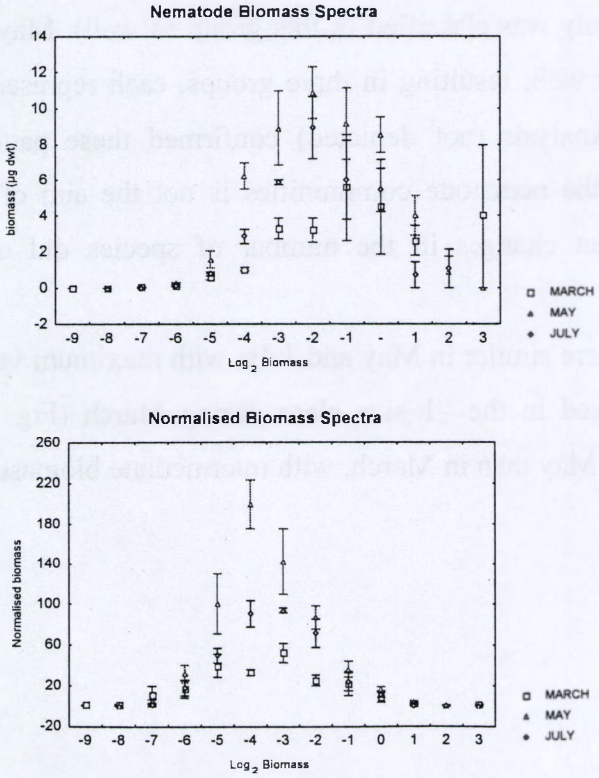

Figure 10. Phytoplankton sedimentation study. Nematode biomass spectra (NBS (upper panel) and normalised NBS (lower panel) on the Belgian continental shelf (error bars: SE of mean biomass values per size class)

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

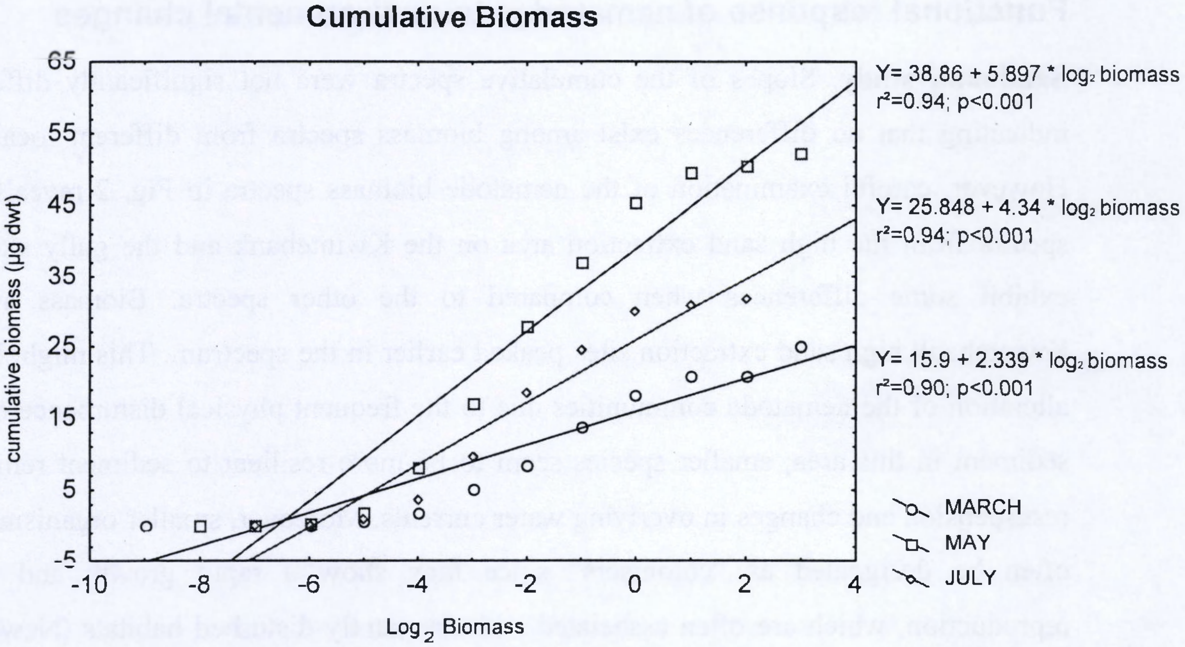

Figure 11. Phytoplankton study. Cumulative biomass spectra on the Belgian continental shelf

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).

	a	b	R²	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

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

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.

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 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 a 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 (eg. 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 individuals falling within the size-class range determines these values. Therefore, responses on the species level (eg. 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 physically 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 *S. punctata* at Station 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 Station 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 Station 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 Station 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 Station 702 (Steyaert et al. 1999). The different nematode feeding types were more evenly distributed over the communities (M. Steyaert unpublished). 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 Station 702 can possibly be explained by higher food abundance in that station compared to Station 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 Station 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 (eg. 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 in the order of 2 weeks to 2 months (Graf 1992), it was assumed that NBS from March reflect the pre-bloom situation. Indeed, chl *a* concentrations in the near-bottom water seemed to be minimal in the period preceding our sampling (0.19 µg/l on February 26th).

A more detailed description of these changes will be published elsewhere (Vanaverbeke & Steyaert in prep). 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 Station 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 (Vanaverbeke unpublished).

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 and 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 $B=a(S)^b$ were B = biomass and S = size. 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 (eg. 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. 9). 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 in a system. Spectrum normalisation was introduced in order to overcome the problem of differences in size of large magnitudes (from µm to meters). 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 standardize the y-axis. Here, again, this 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: $\log[\text{prob}(s \geq S)] = c \log(K+D) - c \log(S+D)$*. 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 test statistically differences in size distributions. NBS from undisturbed sediments 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 (Rojo & Rodriguez,

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* This formula is a transformation of $B=a(S)^b$ (Herman PMJ, pers. comm)

1994, Sprules & Manuwar 1986, 1991), 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 type 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 sample effectively organism 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 construction 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 to 1µg 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 picked out 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 Station 702 *vs.* oxygen in the coarser sediments of station 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.

ADDENDUM

Biomass spectra are often log-normally distributed (Warwick (1984)). The cumulative biomass spectra indeed point in that direction. When a log normal distribution is log-transformed, values correspond to the normal distribution, and the spectra can be described by the mean and variance of this distribution, and the total biomass as a third parameter. Differences between the fitted means can be compared with ANOVA if the variances are homogeneous among the different samples. However, this should be carefully tested as differences in variance could also be important indications of biological effects.

This approach may result in a description of the spectra by a simpler model, in comparison to e.g. the split-plot ANOVA. Moreover, this approach enables the use of all data entries.

The datasets used to describe the NBS for the oxygen-stress study and the food pulse study were both re-analysed to test the usefulness of a simple ANOVA to describe the NBS and to detect possible differences between the spectra. Both datasets indeed matched the assumptions for ANOVA after a log transformation.

ANOVA indicated significant differences between the means of the biomass spectra from the food pulse study (F= 3.3, $p<0.05$) but no significant differences (F= 1.40 p= 013) were detected when analysing the biomass spectra from the coastal stations (oxygen stress). Pairwise comparisons (Tukeys HSD for unequal N) failed to detect significant differences between the monthly biomass spectra of the food pulse study.

Although a split-plot design is more complicated, it seems to be more powerful when analysing NBS. In short (and simplified); the split plot analysis treats the different size classes as treatments, and checks if the biomass within those "treatments" is significantly different. This has the advantage that the "height" of the spectrum is included directly in the analysis, while a regular ANOVA tests differences between the means The "height" of the spectrum is not incorporated in a simple ANOVA comparison of the means. The theoretical disadvantage of the split-plot ANOVA is that size class cannot be considered a model I treatment, as it is in no way fixed by the experimenter. In fact, the individual weight of the nematode determines in which class it will fall, so the result variable is confused with a planned treatment (P.M.J. Herman, pers. comm.)

Comparing the NBS from the oxygen stressed site (Station 702) with the more oxygenated site (Station 790) in Figure 5 of Chapter IV shows that the general "shape" of both spectra are very similar: both are peaking in size class -1, and show elevated values from size class-3 onwards. The main visual difference between the NBS is the huge peak in size class -1 for Station 702, corresponding to the *Sabatieria* dominance in that area. This difference in "height" between the spectra is not reflected in the regular ANOVA (which only compares means, assuming variances are homogeneous). Only when higher values are reached in more size classes (see Figure 10 in Chapter IV), the differences are reflected using ANOVA, however not in the planned comparisons.

Considering the elegance of fitting log-normal distributions to the NBS, and the theoretical problems associated with the split-plot ANOVA design, it seems advisable to develop a new way of analysing differences in NBS. A possible approach is outlined by Clarke & Warwick (1984), who designed a maximum-likelihood based test for bimodality of benthic size spectra. Along similar lines, and given two or more samples, one could compare the goodness-of-fit of a single log-normal distribution fitted to all data together, with a set of sample-specific log-normal distributions. The latter will inevitably fit the data better, but the improvement of the fit can be tested for significance, taking into account the increase of the number of parameters in the second fit.

CHAPTER V

Changes in morphometric characteristics of nematode communities during a spring phytoplankton bloom deposition.

Results presented in:

Vanaverbeke J, Soetaert K, Vincx M (submitted) Changes in morphometric characteristics of nematode communities during a spring bloom deposition

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Abstract

Nematode body size was investigated in terms of body length, width and length/width (L/W) ratios, before, during and shortly after a spring phytoplankton bloom deposition in the Southern North Sea. Sediments consisted of medium sand (median grain size: 333 μm), were devoid of mud and always fully oxygenated. During the peak of the spring phytoplankton bloom in May 1999, several small-sized species (adult length < 700 μm) emerged. Most prominent was the appearance of a "stout" nematode assemblage characterised by low L/W ratios. Most of these small nematode species were virtually absent before the peak blooming and they decreased in abundance shortly after deposition of phytoplankton to the seafloor. This indicates the opportunistic behaviour of these nematodes, which is consistent with their small length, enabling them to rapidly reach adulthood. The net rate of increase of the stout nematodes during the bloom was estimated at 6.4% day^{-1}. This is much larger than the estimated net rate of 1.5% day^{-1} for the total nematode community.

The species composition of the stout nematode assemblage differed from similar stout assemblages described before for continental slope and deep-sea areas. In the Southern North Sea, Epsilonematidae were dominant while members of the Desmoscolecidae were prominent in offshore deeper areas. Possibly these differences relate to the relatively strong hydrodynamic forces prevailing at the North Sea site.

The small species in the North Sea were only present in reasonable densities shortly after the spring bloom, while they seem to be a consistent member of deep-sea nematode communities. We hypothesise that this is caused by the quality of organic matter reaching the sea floor, together with differences in sedimentology and temperature, influencing the duration of the presence of suitable food items for these nematodes.

Introduction

Research on the response of benthic communities to food enrichment mostly focuses on changes in species composition and/or biomass patterns. A combined approach, where species data are linked with the corresponding body size parameters has rarely been published. This is surprising, since body size influences many aspects of animal life such as: life history, physiology, energy requirements, biotic and abiotic interactions (Calder 1984, Peters 1983, Schwinghamer 1983). As the optimal size of an organism is linked to food input (Sebens 1987, Rex & Etter 1998), changes in the size structure of a community are expected during a food deposition event.

Nematodes are amongst the most abundant metazoan organisms in marine sediments and, as their length and width are easily measured using non-destructive methods (Soetaert et al. 2002) they are especially suited for analysing body size distributions. Moreover, the ratio of the nematodes' length to the maximal width (L/W) offers a quantitative measure of their shape. Whereas most nematodes have a typical snake-like body, some species are conspicuously plumper. Ratsimbazafy et al. (1994) were the first to report on the existence of these two different nematode morphotypes in fine sandy sediments (median grain size: 130-160 µm) of the North Sea. Soetaert et al. (2002) analysed nematode morphology from various continental slope areas around the world and confirmed the existence of both morphotypes. As this group included members of distantly related taxa, they concluded that these groups arose as an adaptation to evolutionary conflicting constraints. Based on indirect evidence, they hypothesised that the short, plump type could persist due to its more rapid development compared to longer nematodes, whereas the presence of 'armor' and its thickness were effective against predation pressure. The concomitant lowered mobility and a reduced capacity to withstand anoxic conditions however precluded these plump organisms to live in the well-oxygenated surficial layers of the sediment (Soetaert et al. 2002).

Our study investigates the morphometry of nematode communities as triggered by a spring bloom phytoplankton deposition in a well-oxygenised North-Sea sampling station. We describe the changes in length, width and L/W ratios as a result of the changing food availability in the sediment. We examine whether the two morphotypes can be discerned and how both respond to food pulse and in the absence of oxygen gradients.

Material and methods

Study site, sampling and treatment of samples

Samples were obtained from the open sea site Station 330 (51°26.0'N; 02°48.5'E) (Fig 1) at the Belgian Continental Shelf (Southern Bight of the North Sea). Sampling was performed weekly from March 1999 until July 1999 (Vanaverbeke et al. in prep). Nematode species were identified on a monthly interval (March 9th, April 27th, May 12th , June 28th and July 12th 1999) (Vanaverbeke et al. in prep.). Morphometric analysis of the communities was performed on the samples from March, May and July, i.e. before, during and after the spring phytoplankton bloom.

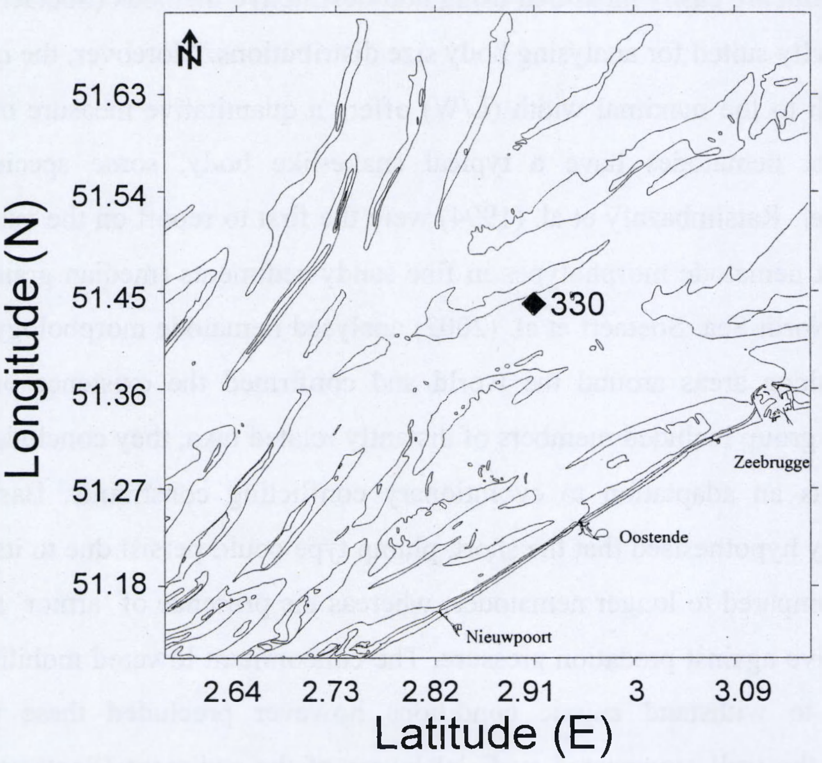

Figure 1. Map of the Belgian continental shelf with indication of the sampling station

Sampling took place aboard of the RV Belgica using a modified Reineck boxcorer. The boxcorer was deployed three times at each sampling occasion. From each boxcorer, one perspex core (10 cm²) was used for faunal analysis up to 10 cm sediment depth. Additional cores were used for the analysis of sediment characteristics, pigment concentrations and nutrient profiles (Vanaverbeke & Steyaert, unpublished). In July, only two cores for faunal analysis were available. Sediments were sliced vertically: the upper 2 cm in 5mm intervals, the deeper layers per cm. A hot (70°C), neutral formaldehyde tap-water solution was used for fixation. At the laboratory, all metazoans passing a 1-mm sieve and retained by a 38 µm sieve were extracted from the sediment by centrifugation with Ludox (Heip et al. 1985). Per slice, 120 nematodes were picked out randomly and mounted on Cobb slides for identification and measurements of length and maximal width. When less than 120 individuals were present, all nematodes were picked out. Measurements were performed using an image analyser (Quantimet 500+). From each individual, age, gender and presence of eggs in adult females was recorded. Only nematodes from the March, May and July samples were measured.

Sediments were analysed using a Coulter LS100 Particle Size Analyser. The redox potential of the sediment column was recorded with a mV meter.

Chlorophyll *a* (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).

Results

Study site

Sediments at the sampling station could be classified as medium sand (median grain size ranging from 329.3 µm in May to 360.7 µm in June) (Buchanan 1984), devoid of mud. Chl *a* values in the water column reached their highest values on April 29th and May 5th, reflecting the peak phytoplankton bloom (Rousseau 2000). The pattern at the surface was closely followed by the pigment concentrations in the bottom water (Fig. 2), indicating sedimentation of phytoplankton from the end of April. At the date of the first sampling, rather high chl *a* values in the bottom water were observed as well. Redox values remained positive (>100 mV) during the complete sampling period and at all sediment depths.

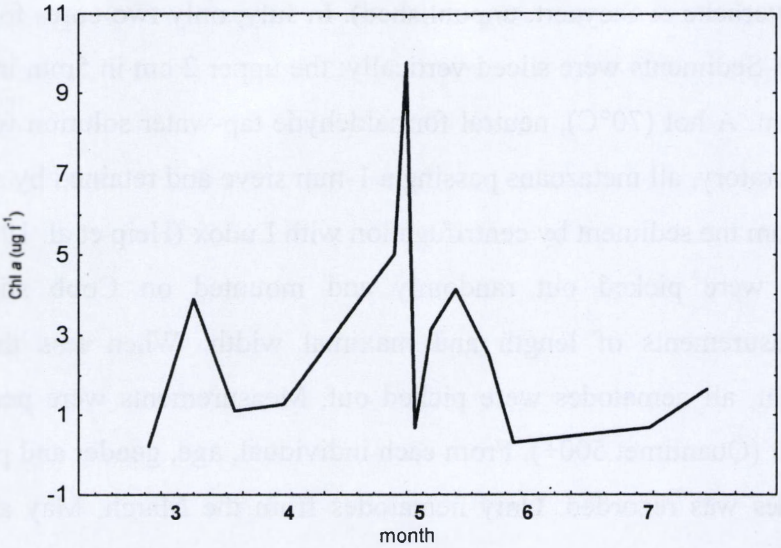

Figure 2. Chlorophyll a concentrations in the bottom water at Station 330 (Belgian Continental Shelf – spring and summer 1999)

Morphological diversity of the nematode communities

All measurements of all replicates and sediment slices per month were pooled before analysing the morphometry of the nematode communities. In March, May and July, respectively 491, 1441 and 528 nematodes were measured.

Figure 3 shows the length-width plots on a log scale per month, and the corresponding length/width frequency distribution. Fig. 4 gives the length distribution for the adult nematode community before, during and after the bloom deposition. At the onset of the bloom in March, nearly all nematodes (>98%) are of the slender type (Fig. 3), with L/W ratios well over 15 and peaking at L/W of 32. One species, *Dichromadora cucullata* dominated the community (18%) and is responsible for the adult peak size at 800-900 µm (Fig. 4). Four other, larger, nematode species (*Neochromadora angelica, N. munita, Prochromadorella ditlevseni* and *Pomponema multipapillatum*) represent another 12% of the total community.

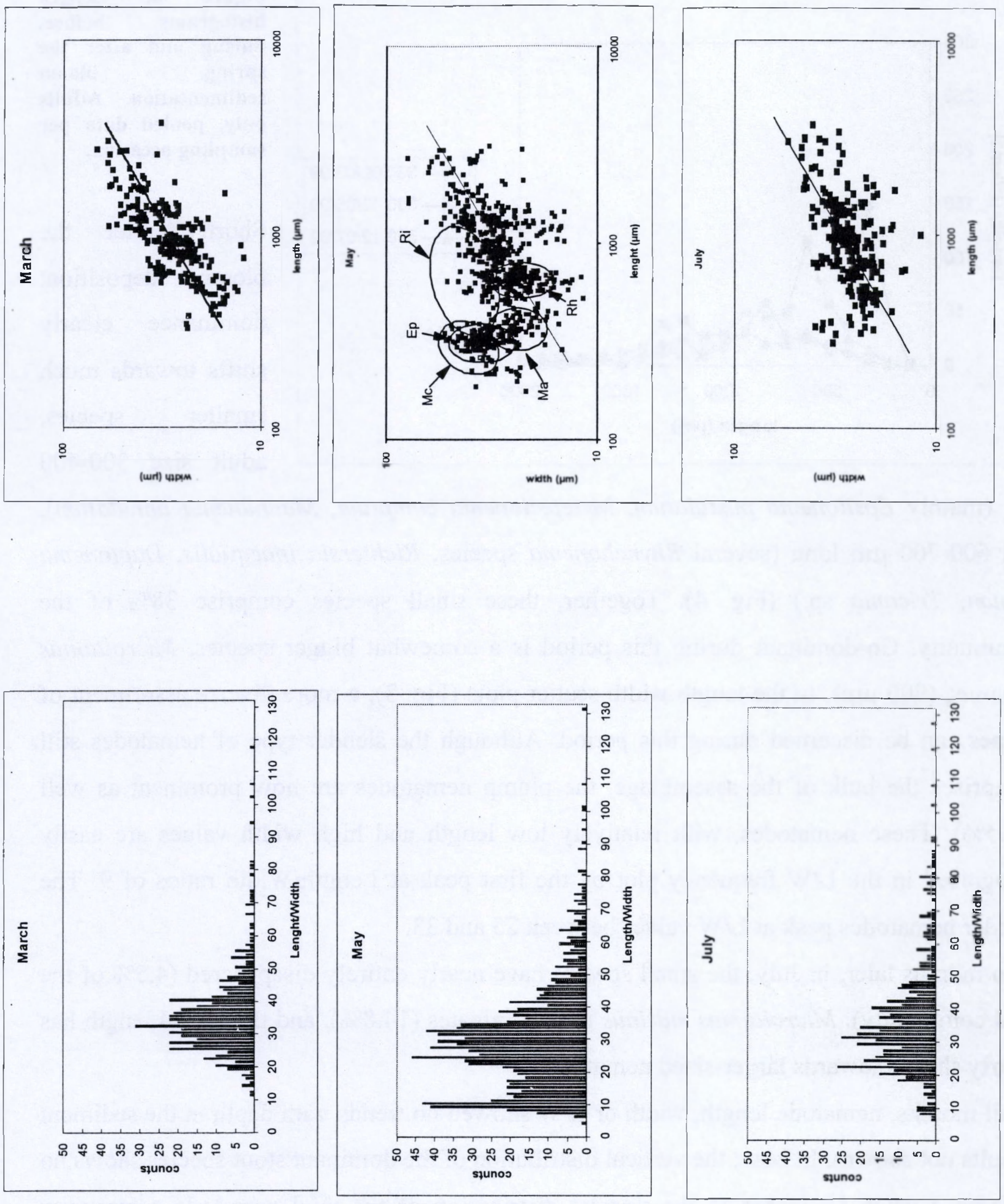

Figure 3. Morphological landscape of North Sea nematodes before, during and after spring bloom deposition. Right panels: Length/Width histograms (Ri: *Richtersia inaequalis*; Ep: *Epsilonema pustulatum*; Mc: *Metepsilonema comptum*; Ma: *Manunema annulatum*; Rh: *Rhynchonema* species). Left panels: body width vs body length on a log scale. All data per month combined. The regression line in the right panels corresponds to the line fitted to the March data in order to facilitate comparisons.

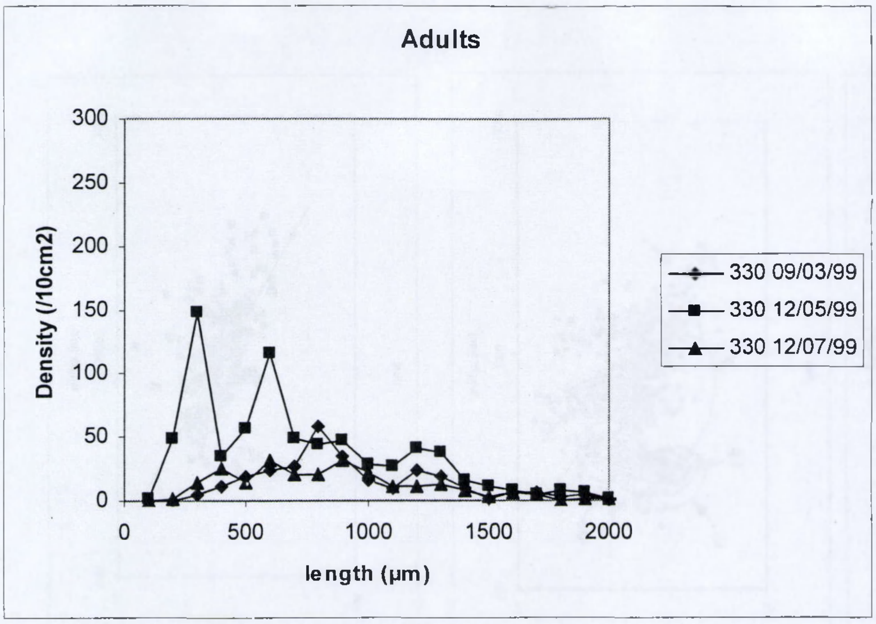

Figure 4. Length histograms before, during and after the spring bloom sedimentation. Adults only, pooled data per sampling occasion.

Shortly after the bloom deposition, dominance clearly shifts towards much smaller species, adult size 300-400 µm (mainly *Epsilonema pustulatum*, *Metepsilonema comptum*, *Mannunema annulatum*), and 600-700 µm long (several *Rhynchonema* species, *Richtersia inaequalis*, *Daptonema nanum*, *Tricoma* sp.) (Fig. 4). Together, these small species comprise 38% of the community. Co-dominant during this period is a somewhat bigger species, *Microlaimus marinus*, (900 µm). In the length width scatter plots (Fig. 3), a more diverse assortment of shapes can be discerned during this period. Although the slender type of nematodes still comprises the bulk of the assemblage, the plump nematodes are now prominent as well (21.5%). These nematodes, with relatively low length and high width values are easily recognised in the L/W frequency plot by the first peak at Length/Width ratios of 9. The slender nematodes peak at L/W values between 25 and 33.

Two months later, in July, the small species have nearly entirely disappeared (4.5% of the total community). *Microlaimus marinus* now dominates (14.8%), and the modal length has clearly shifted towards larger-sized nematodes.

In all months, nematode length, width or L/W showed no trends with depth in the sediment (results not shown). In May, the vertical distribution of the dominant stout species shows no difference when compared to the vertical distribution of the total nematode community (Fig. 5). Except for *M. comptum*, about 40% of the stout species is found in the upper 2 cm of the sediment.

In order to understand the appearance of the stout and small species in May, the distribution of age/sex characteristics of the assemblages (sediment slices and replicates pooled) is depicted in Fig. 6. Since both stout (L/W <15) and small (Length<700 µm) nematodes were virtually absent in March, the distribution for the entire community is shown.

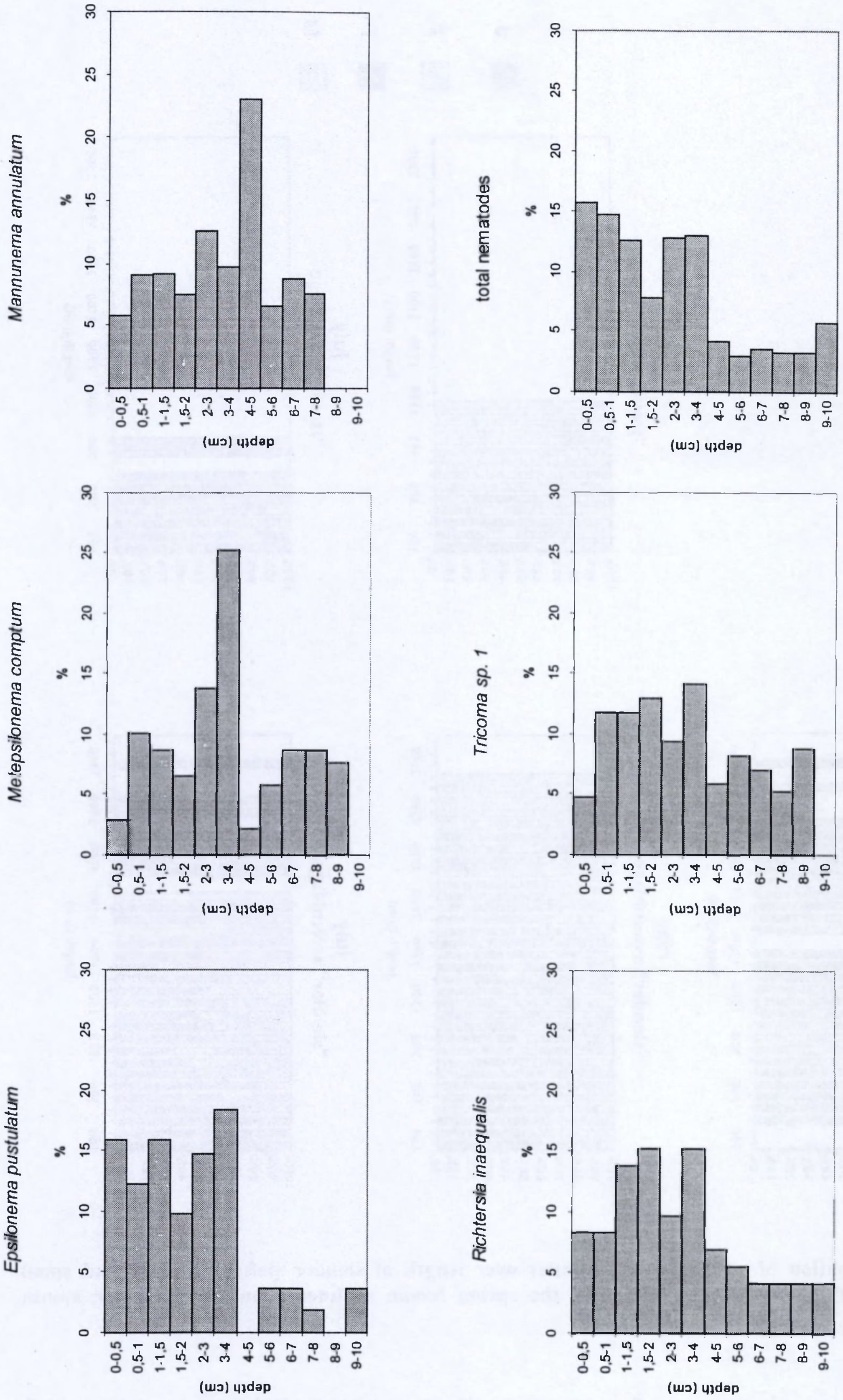

Figure 5. Vertical distribution of dominant stout nematode species and the total nematode community (stout and short nematodes excluded) in May at Station 330. Relative abundances per sediment layer, mean of three replicates.

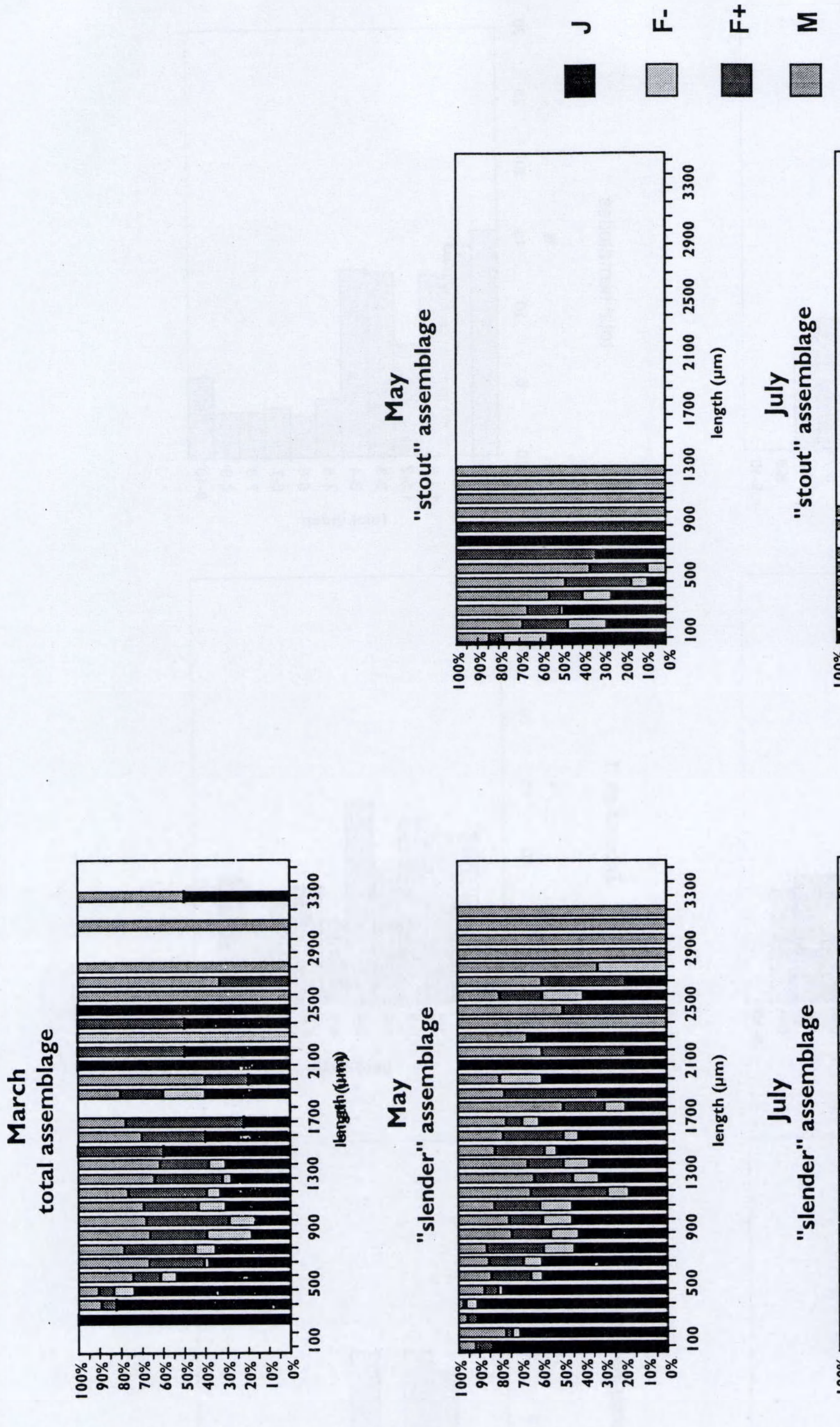

Figure 6. Distribution of age/sex characteristics over length of slender (left) and stout and small nematodes (right) before, during and after the spring bloom sedimentation. All data per month combined.

Comparing the age distribution of stout and small nematodes (individuals identified as *E. pustulatum*, *M. comptum*, *M. annulatum*, *Tricoma* sp., *R. inaequalis* and all *Rhynchonema* sp.) with the slender nematodes in May and July reveals the lower proportion of juveniles present in the lower size classes of the former assemblage. In May, at least 50% of all stout and short species is adult for lengths >200 µm (except one length class). In July, adults constitute >60% of the stout and short nematodes when length exceeds 300 µm. No obvious trends were observed when comparing the slender assemblage throughout the sampling period.

Discussion

Temporal patterns

In coastal areas, a significant fraction of primary production may end up in the sediments, where it is food for the benthic animals (Heip et al. 1995). At our study site, phytoplankton starts to grow in early March; algal densities peak at the end of April after which the algal biomass rapidly declines (Rousseau 2000). As similar temporal patterns are observed for the chlorophyll of the overlying bottom water, at least part of the algal bloom settles on the sediment (Vanaverbeke et al. 2003.

Nematode densities, although generally low, varied almost 4-fold over the 5 month sampling period (March-July). Both small-scale spatial (order of 100 metres) and temporal effects (order of months) contributed to this variability, but the temporal signal explained most of the variation (87%, F=15.1, p=0.0005). Moreover, the low variability in sediment composition between dates (median grain 330–360 µm) and the consistently positive redox over the entire sediment depth (<10 cm) indicate that the same station was always sampled. Mean nematode densities increased steadily from about 200 individuals 10 cm^{-2} in March to more than 600 individuals 10 cm^{-2} in May after which they decreased sharply again to about 380 individuals 10 cm^{-2} in July (Fig. 7) (Vanaverbeke , this thesis: Chapter VI).

The rapid increase was largely attributed to the emergence of small species, where adult lengths peaked at 300-400 µm and at 600-700 µm. In May more than 50% of the adult nematodes were smaller than 700 µm, whereas this was only 20% in March, 30% in July. One group of thick nematodes (defined with Length /Width ratios < 15) was almost totally absent in March but reached quite high densities (>130 ind 10cm^{-2}) in May. In June, few members of this morphotype were present (17 ind 10cm^{-2}).

Although our data do not allow estimates of nematode growth rates in the field, we can roughly assess the net rate of increase, from March to May and the net rate of decrease, from May to June, by fitting an exponential growth model:

$$\frac{dN}{dt} = aN$$

which can be solved as:

$$N_t = N_0\, e^{at}$$

with N_t: nematode density at time t; t: time in days and a: the net increase day^{-1}.

Results indicate that stout nematodes (L/W < 15) increased their densities at a rate of 6.5% d^{-1}, which is four times faster, compared to the total community. Short species (length<700µm) increased densities with a double speed, 3% d^{-1} (Table 1).

Rate of net increase	March to May	May to July
Total community	0.015 d^{-1}	-0.007 d^{-1}
Short adults (L<700µm)	0.03 d^{-1}	-0.017 d^{-1}
Stout community (L/W<15)	0.065^{-1}	*-0.03d^{-1}*

Table 1. Results of exponential growth model dN/dt = aN. All p<0.001; *p=0.013*

The same differences were observed when densities decreased from May to July. All this indicates that stout and short species are at an advantage when the bloom settles on the sediment surface. Smaller species not only have larger growth rates (Peters 1983), and therefore reach adulthood faster, but they also have higher reproduction rates (Kooijman 1986). This is consistent with the larger rates of increase (Table 1) and the higher dominance of adults in the stout or short species (compare contribution of adults to total assemblages in left and right panels of Fig. 6). The small and stout species therefore have the ability to react in an opportunistic way to a pulsed food supply. On the other hand, they also disappear at a rate much higher than that of the average nematode. Chl *a* values in the sediment decrease strongly after the peak bloom (Vanaverbeke , this thesis, Chapter VI), indicating that the organic material is remineralised rather fast, and it is probable that the decrease of both stout and short nematodes can partly be attributed to food shortage. Being plump or small *in se* infers that the life span would be rather short. Moreover, small individuals have relatively larger maintenance costs and less storage material, which may make them more vulnerable to food shortages. During summer or late summer, a second diatom bloom occured in Belgian coastal waters (Rousseau 2000), providing the sediments again with fresh organic matter when the bloom was terminated. This second input of food sources probably enabled the stout communities to maintain their populations year round.

The stout and small nematode assemblages

Our results confirm the findings of Ratzimbazay et al. (1994) and Soetaert et al. (2002), on the existence of different nematode morphotypes. Soetaert et al. (2002) explained the existence of the stout morphology as the result of evolutionary selection amongst distantly-related taxa rather than resulting from phylogenetic lineage. Our results confirm this. Whereas on the Ligurian shelf and slope (Mediterranean Sea), the stout nematode assemblage consisted of desmoscolecids (*Tricoma*: 26% and *Desmoscolex*: 24%) and *Richtersia* (30%) (Soetaert et al. 2002), at our study site, epsilonematids dominated the stout assemblage, followed by desmoscolids and *Mannunema annulatum*. These belong to three different suborders within the Chromadorida. The predominance of the epsilonematids at our study site is probably due to the rather coarse sediments, in contrast to the finer grained deep-sea sediments of the stations used in the analysis of Soetaert et al. (2002). Members of the epsilonematids are usually found in sandy sediments, on sandbanks (Willems et al. 1982) and in open-sea sediments (Vincx 1986) where hydrodynamic forces can be substantial.

Soetaert et al. (2002) further hypothesized that the stout nematode communities evolved as an adaptation towards reducing predation pressure, as they combined large body width with armored cuticulas. Similarly, the representatives of the stout nematodes at our site combine the plump shape with armor. The ε-like body shape of epsilonematids, together with the presence of long setae will make it difficult for a predator to swallow the nematode, while *Manunema annulatum* has stout somatic setae on peduncles all over the body. The somewhat longer *Rhynchonema* species, which also show an opportunistic response to phytoplankton sedimentation, similarly have cuticular ornamentation.

In the deep-sea or continental slope stations, the Length/Width ratio of the nematodes showed consistent patterns with depth in the sediment, with stout nematodes always living in the upper layers of the sediment. Soetaert et al. (2002) argued that their large width precluded them from inhabiting the suboxic and anoxic layers deeper into the sediment. Our results also corroborate this: in the absence of oxygen related gradients no patterns in length, width or Length/Width ratio were observed.

Our findings however contrast with the results of Soetaert et al. (2002) in one important manner. Whereas the existence of the plump nematodes in the continental slope and deep-sea areas appears to be a persistent feature (they were found at all margin sites examined), their presence is clearly a transient phenomenon in our sampling site. Boon & Duineveld (1998) and Boon et al. (1998) showed that, in North Sea sediments comparable with our sampling station, bacterial activity is very much related to the input of organic matter from the water column: it increases drastically after a food pulse and decreases again when the easily degradable fraction of the organic matter is broken down. Such pulses in bacterial activity are probably not as pronounced along continental slopes and at deep-sea sites, where the quality of organic material reaching the sea floor decreases with increasing water depth (Heip et al. 2001) and where the reduced quality and availability of the organic matter, together with the lower temperatures causes a prolonged remineralisation process (Soetaert et al. 1996). Except for the *Richtersia* species, the stout and short nematode species have very small buccal cavities, forcing them to feed selectively on small food items, such as bacteria (Wieser 1953). Moens et al. (1999) showed that even closely related nematode species show clear species-specific preferences for bacterial strains, bacterial densities and bacterial age. This might indicate that the preferential food source for this type of nematode at deep-sea sites is present over a longer time span than in coastal areas.

Conclusions

- In a sandy site in the North Sea, the spring bloom opens a window of opportunity for small nematode species whose densities rise vigorously immediately after the deposition of fresh organic matter. A couple of months later, their densities decline almost as quickly as they rose. This quick response can be explained by the life-history characteristics typical of these species.
- Many of these small nematodes are similar in shape to the ‘stout nematode assemblage’ as described previously for ocean margin sites. In addition, they share morphological similarities by the presence of armour. However, being dominated by Epsilonematidae, the composition of the stout assemblage in the southern North Sea sediment differs from margin sites where Desmoscolecidae were more prominent.

- Whereas in the southern North Sea sediment, the presence of plump species is a transient feature, in continental slope areas they seem to persist, possibly relating to the continuous presence of suitable food items in these areas.

CHAPTER VI

Changes in structural and functional diversity of nematode communities during a spring phytoplankton bloom in the Southern North Sea

Results presented in:

Vanaverbeke J, Steyaert M, Soetaert K, Rousseau V, Van Gansbeke D, Parent J-Y, Vincx M (submitted) Changes in structural and functional diversity of nematode communities during a spring phytoplankton bloom in the Southern North Sea

Acknowledgements

We would like to thank the master and crew from the RV *Belgica* , *Zeehond* en *Oostende XI* for their skilful help during the sampling. Annick Van Kenhove and Wies Ghijselinck are acknowledged for making of all the slides. Many people from the Marine Biology Section gave a a helping hand during the sampling period. This research was funded by Ghent University (BOF, 1998-2003) and OSTC (Belgium Sustainable Development-North Sea Project MN/DD[40-41-42] and TROPHOS Contract nr EV/02/25A. This is publication nr xxxx from NIOO-CEME.

Abstract

The response of nematode communities to the sedimentation of a spring phytoplankton bloom in a sandy, well-oxygenated sediment in the Southern North Sea is investigated from early March to July 1999 with monthly intervals. Both structural (nematode density, diversity, vertical distribution and community composition) and functional (feeding type distributions, number of species within feeding groups) characteristics showed considerable changes shortly after the arrival of fresh organic material at the sediment surface. The general increase in density and diversity was related to changes within selective deposit feeding and epistrate feeding nematodes. Although temporal variability was significant for total nematode densities and deposit feeding nematodes, spatial variability (in the orders of 100 of meters) was high when single species were concerned. It is hypothesised that sedimentation and subsequent remineralisation of fresh organic matter during the spring phytoplankton bloom results in an increase of suitable food items (both living and dead). This, combined with the availability of oxygen and the high habitat heterogeneity at the sampling location (both in the order of meters and over the sediment depth profile), create conditions in which many nematode species can co-exist.

Introduction

Shelf seas are areas with a high primary production in the euphotic zone and about 20-50% of this net phytoplankton production is deposited on the sediment (Jørgensen 1983) where it fuels benthic life (Graf 1992). The meiobenthos represents the smaller-sized (< 1mm) animals in the sediment and their response to the sedimentation of phytodetritus has been the subject of many studies. Results were equivocal: in certain cases, a clear response was lacking (*e.g.* Warwick & Buchanan 1971, Boucher 1980, Fleeger et al. 1989), while other studies showed prominent responses to phytoplankton deposition (*e.g.* Bovée & Soyer 1974, Rudnick et al. 1985, Ólafsson & Elmgren 1997, Ólafsson et al. 1999, Steyaert et al. subm). These differences seem to indicate that site-specific processes control the signal of the response to this pulsed food supply from the water column.

Few studies have examined the meiobenthic communities on the species level, although data on the major taxon level may conceal the more subtle changes occurring at the species level (Gooday et al., 1996). For instance, in a study of the nematodes, the dominant taxon within the meiobenthos, Steyaert et al. (subm) showed that closely related species inhabiting a fine-grained, anoxic North-Sea sediment, respond differently to the changes in the sediment caused by sedimentation of a spring phytoplankton bloom.

As the trophic composition of nematode assemblages reflects the quality and quantity of their food sources (Moens & Vincx 1997, Danovaro & Gambi 2002), changes in the distribution of feeding types are also expected.

Another factor that may change in response to pulsed food deposition is the way in which benthic animals are distributed vertically in the sediment. The remineralisation of fresh, high quality organic material results in changes in the biogeochemical environment of the sediment, at short time scales. Nematodes are particularly sensitive to the sediment biogeochemical conditions (Boyd et al. 2000, Hendelberg & Jensen

1993, Steyaert et al. 1999), and vertical profiles can change rapidly in time, while density and diversity are not yet affected (Steyaert et al. in press).

In previous papers we have documented the changes in biomass spectra and nematode shape, during and after sedimentation of phytodetritus and subsequent remineralisation in our sampling station (Vanaverbeke et al. 2003, Vanaverbeke et al. subm). Here we take a closer look at structural (densities, diversity and vertical profiles) and functional (feeding habit, number of species per feeding type) changes. We chose nematodes as a study object because they are the most dominant metazoan animals and because of the ease at which the morphology (Soetaert et al. 2002) and trophic position (Wieser, 1953) can be determined. Moreover, because of their high turnover rates and continuous reproduction (Heip et al. 1985), a clear signal is expected.

As the ability to track temporal changes not only depends on the time scale at which sampling was performed, but also on the degree of spatial variability, both temporal and spatial variability are compared.

Material and methods

Study site and sampling

Samples were obtained from the open sea site Station 330 (51°26.0'N; 02°48.5'E) (Fig. 1) on the Belgian Continental Shelf (Southern Bight of the North Sea). Sampling was performed weekly from March 1999 until July 1999.

Sampling took place from the *RV* Belgica, Zeehond or Oostende XI, using a modified Reineck boxcorer. At each sampling occasion, the boxcorer was deployed 3 times. Each boxcorer was sampled by means of 2 identical perspex cores (i.d. 3.6 cm) and one larger core (i.d. 6 cm). All cores were sliced vertically: the upper 2 cm in 5 mm intervals, the deeper layers per cm. In July, only two good boxcores were retrieved.

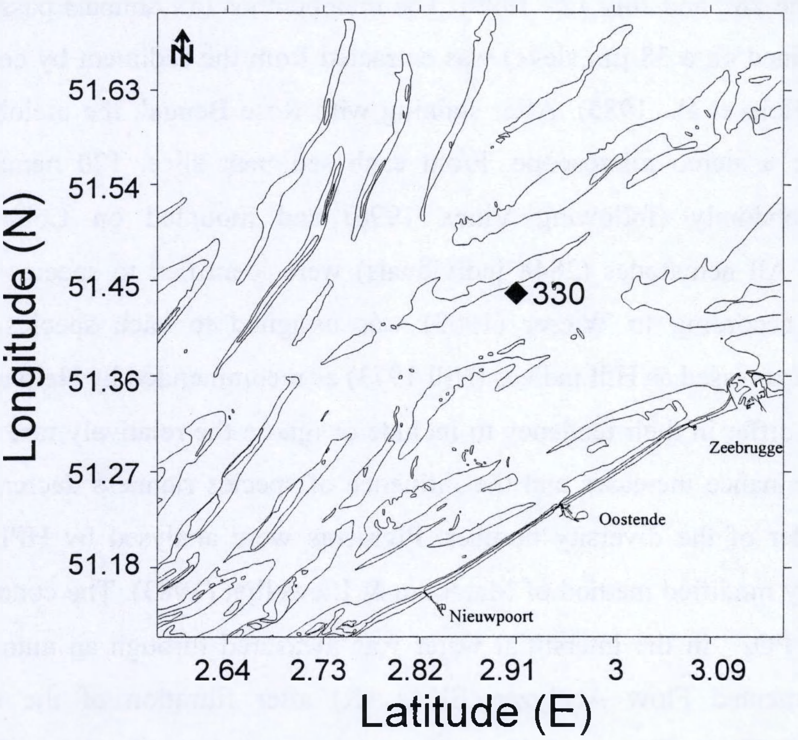

Figure 1. Map of the Belgian continental shelf with indication of the sampling station

Sediments from one core were stored in a hot (70°C), neutral formaldehyde tap-water solution for later faunal analysis.

From the remaining 10 cm² core, 1 ml of sediment was subsampled using a syringe from which the tip was removed. These samples were frozen for pigment analysis. Sediment slices for nutrient analysis were obtained from the larger cores and frozen until analysis. On board, the redox potential of the sediment was measured using a mV meter.

Chlorophyll *a* (Chl *a*) values at the sea surface were obtained from Rousseau (2000). Bottom water Chl *a* values were obtained from the supernatant water of the Reineck boxcorer, which was carefully siphoned off, and 0.5 l was filtered on Whatman GF/C filters. Filters were stored in the freezer until processing.

Laboratory treatment of samples

Samples for faunal analysis were processed on a monthly interval (March 9th, April 27th, May 12th , June 28th and July 12th 1999). The meiobenthos (all animals passing a 1 mm sieve and retained on a 38 µm sieve) was extracted from the sediment by centrifugation with Ludox (Heip et al.. 1985). After staining with Rose Bengal, the meiobenthos was counted under a stereo microscope. From each sediment slice, 120 nematodes were picked out randomly (following Vincx 1996) and mounted on Cobb slides for identification. All nematodes (2848 individuals) were identified to species level and a feeding type according to Wieser (1953) was assigned to each species. Nematode diversity was expressed as Hill indices (Hill 1973) as recommended by Heip et al. (1988). These indices differ in their tendency to include or ignore the relatively rare species: the impact of dominance increases and the influence of species richness decreases with an increasing order of the diversity number. Pigments were analysed by HPLC (Gilson) using a slightly modified method of Mantoura & Llewellyn (1983). The concentration of NH_4^+, Si and PO_4^{---} in the interstitial water was measured through an automatic chain (SAN^{plus} Segmented Flow Analyzer, SKALAR) after filtration of the samples on Whatman GF/F filters. The remaining sediment was used for grain size analysis with a Coulter Counter LS Particle Size Analyser. Sediments were defined according to the Wentworth scale (Buchanan 1984).

Statistical Analysis

Variation in total nematode densities, feeding type distribution and feeding type densities, number of species per feeding type and diversity indices per month were analysed using Analysis of Variance (ANOVA). Nematode densities were root-root transformed in order to meet the assumptions for ANOVA. Densities per feeding type required a log (x+1) transformation. When overall significant differences were detected, Tukeys Honest Significance Test for unequal N was used for pairwise comparisons. When the assumptions for ANOVA were not met, even not after transformation (Feeding Type distribution), the non-parametric Kruskal-Wallis test was used. Overall significant differences were compared pairwise following Conover (1971).

In order to elucidate to what degree total nematode densities, feeding type densities and species densities are impacted by small-scale spatial heterogeneity (i.e. between replicate deployments of the Reineck boxcorer) or by temporal effects, we calculated the % variation among dates (Sokal & Rohlf 1997) in ANOVA on root-root (total and species densities) or log (x+1) (densities per feeding type) transformed densities.

Changes in nematode densities with time, sediment depth and time x depth were tested by constructing a univariate 'split-plot' ANOVA design on root-root transformed densities, following Steyaert et al. (2001). Replicates were nested within 'time'; however, not within depth.

Nematode community structure was analysed by means of a Detrended Correspondence Analysis (DCA) (Hill 1979). Species occurring less than 3 times in all sediment slices from all dates were eliminated from the dataset. Analyses were performed on mean abundances per sediment slice per date.

Results

Study site and environmental variables

Sediments at Station 330 were classified as medium sand (median grain size ranging from 329.3 µm in May to 360.7 µm in June) (Buchanan 1984), devoid of mud. Chl *a* values in the water column were maximal at April 29th and May 5th, reflecting the peak phytoplankton bloom (Rousseau 2000). Pigment concentrations in the water overlying the sediment closely followed the pattern at the surface (Fig. 2), indicating sedimentation of phytoplankton from the end of April.

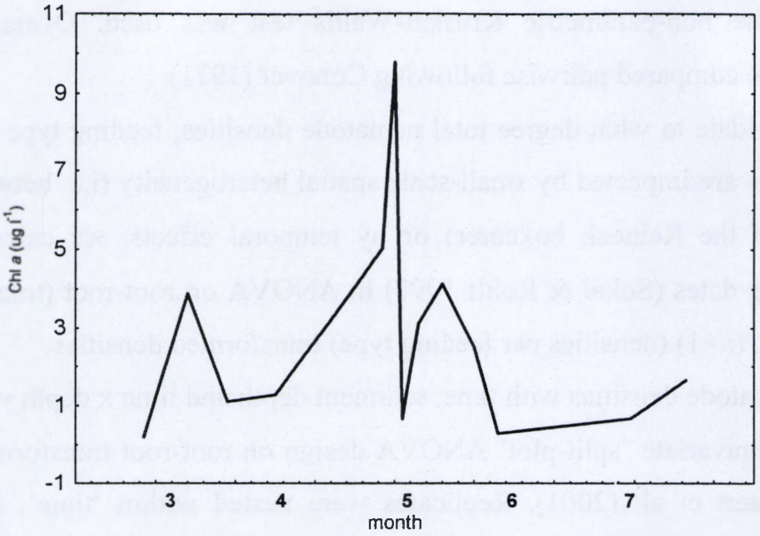

Figure 2. Chlorophyll a concentrations in the bottom water at Station 330 (Belgian Continental Shelf –spring and summer 1999)

Chl *a* values in the sediment (Fig. 3) were highest at the beginning of March (Chl *a* concentrations in all sediment layers > 100 ng/g on March 9th), and decreased toward the end of March. From April until the end of the sampling period, mean concentrations per sediment layer ranged between 50 and 150 ng/g. Only on May 26th were higher concentrations recorded. Many chlorophyll versus depth profiles demonstrated clear subsurface peaks, around 3-5 cm deep.

Ammonium concentrations, averaged over the first 4 cm increased from March till May and then decreased (Fig. 4). Redox values remained positive (>100 mV) during the complete sampling periods and at all sediment depths (Fig. 5).

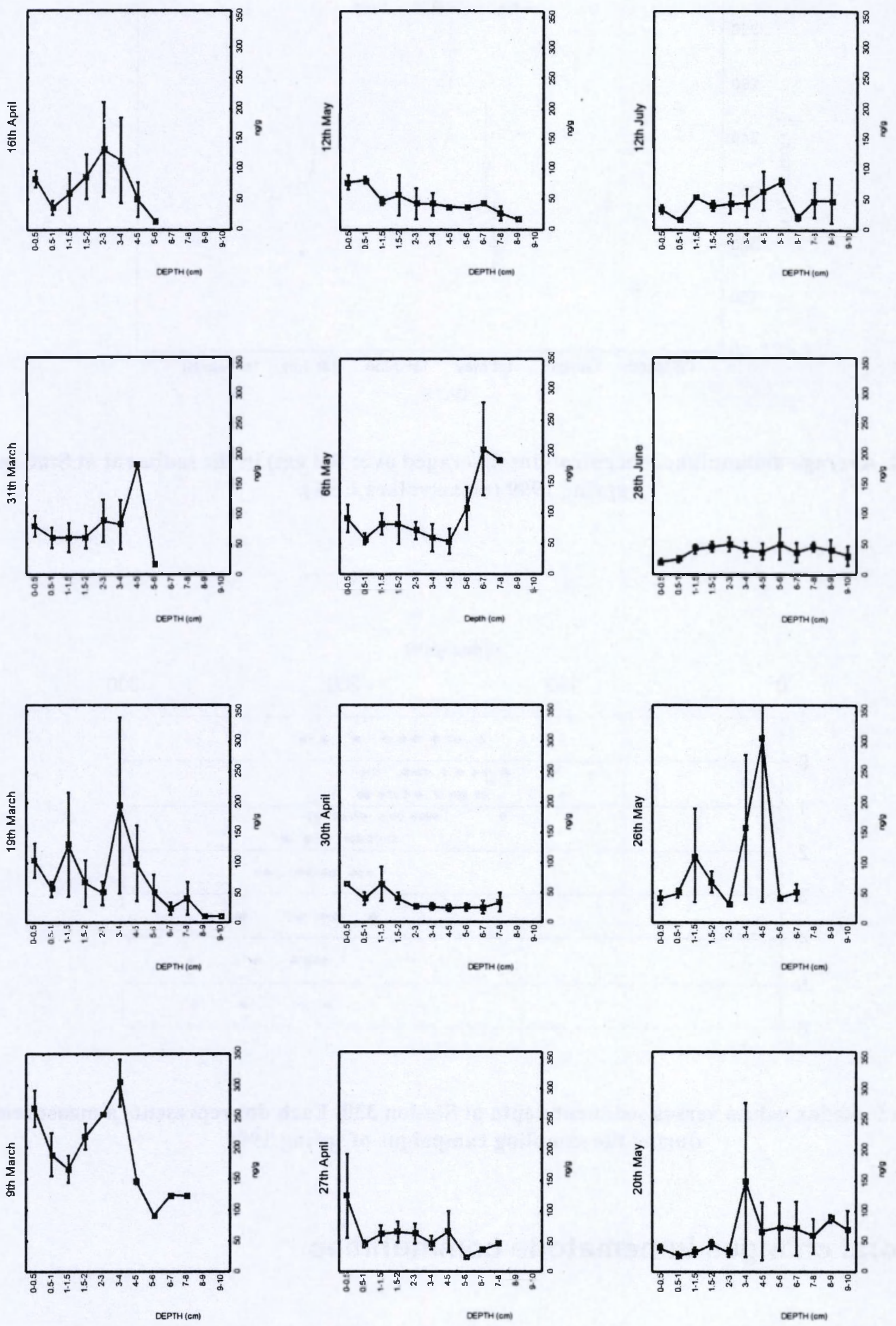

Figure 3. Chlorophyll a depth profiles in the sediment at Station 330: spring and summer 1999 (mean values ± SE).

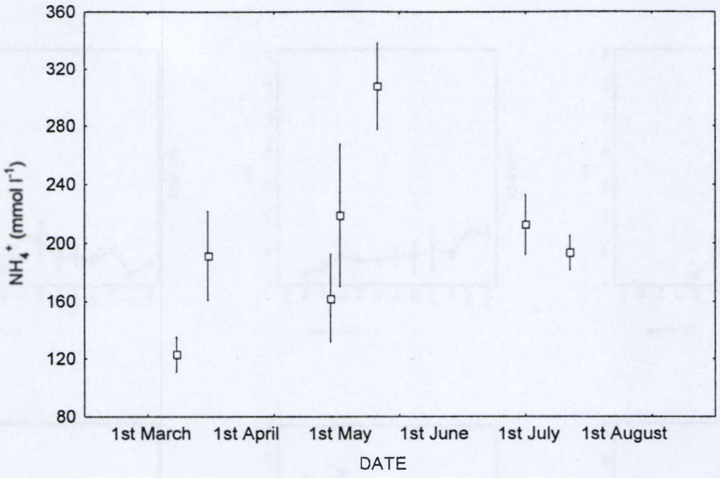

Figure 4. Average ammonium concentrations (averaged over 0-4 cm) in the sediment at Station 330: spring 1999 (mean values ± SE).

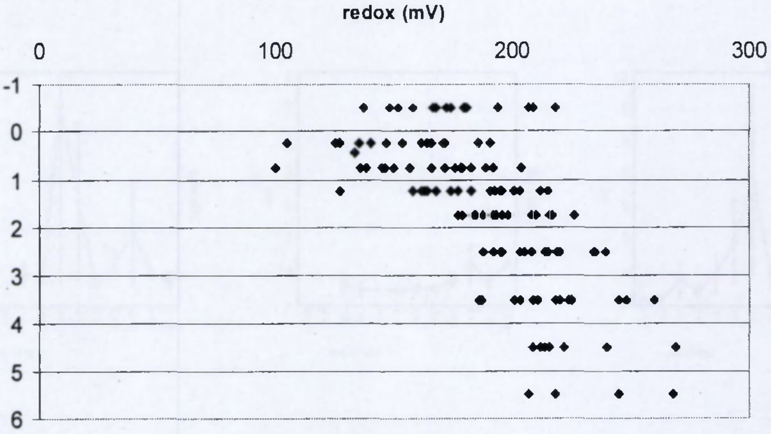

Figure 5. Redox values versus sediment depth at Station 330. Each dot represents a measurement during the sampling campaigns of spring 1999.

Temporal changes in nematode communities

Nematode densities (Fig. 6) increase steadily from March till May, afterwards values decreased towards July. An ANOVA on root-root transformed values resulted in significant differences ($F_{4,9}$=16.43; p<0.001).

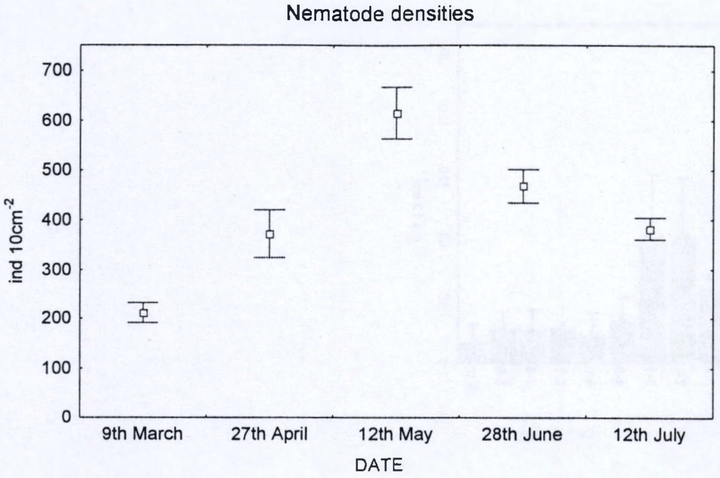

Figure 6. Total nematode densities at Station 330: spring and summer 1999 (mean values ± SE)

Pairwise comparisons revealed significantly lower densities in March compared to all other months, while the densities in April were significantly lower than in May (Table 1).

	April	May	June	July
March	*	***	**	*
April		*	NS	NS
May			NS	NS
June				NS
July				

Table 1. Significant differences between monthly nematode densities at Station 330 (spring 1999). Results of Tukeys HSD for unequal N (*:p<0.05; **p<0.01; ***p<0.001; NS: not significant).

Nematodes tended to be concentrated in the upper 4 cm of the sediment in May (Fig. 7). In June, highest values were recorded at the upper 0.5 cm slice. The ANOVA 'split-plot' analysis demonstrated a significant effect for sediment depth ($F_{11,22}$= 9.49, df= 11, p<0.01) and the interaction term time x depth ($F_{44,110}$= 2.51,df= 44, p<0.001). Densities per sediment layer were not significantly affected by time ($F_{4,8}$= 1.25, df= 4, p=0.36).

Hill's diversity numbers (Fig. 8) of all orders were lowest in March. ANOVA's only reflected significant differences for N_0 ($F_{4,9}$=15.35; p<0.001) and N_1 ($F_{4,9}$=3.90; p<0.05). Pairwise comparisons (Tukeys HSD for unequal N) showed that the number of species (N_0) in March was significantly lower compared to all other months. Concerning N_1, values in March differed only significantly from April.

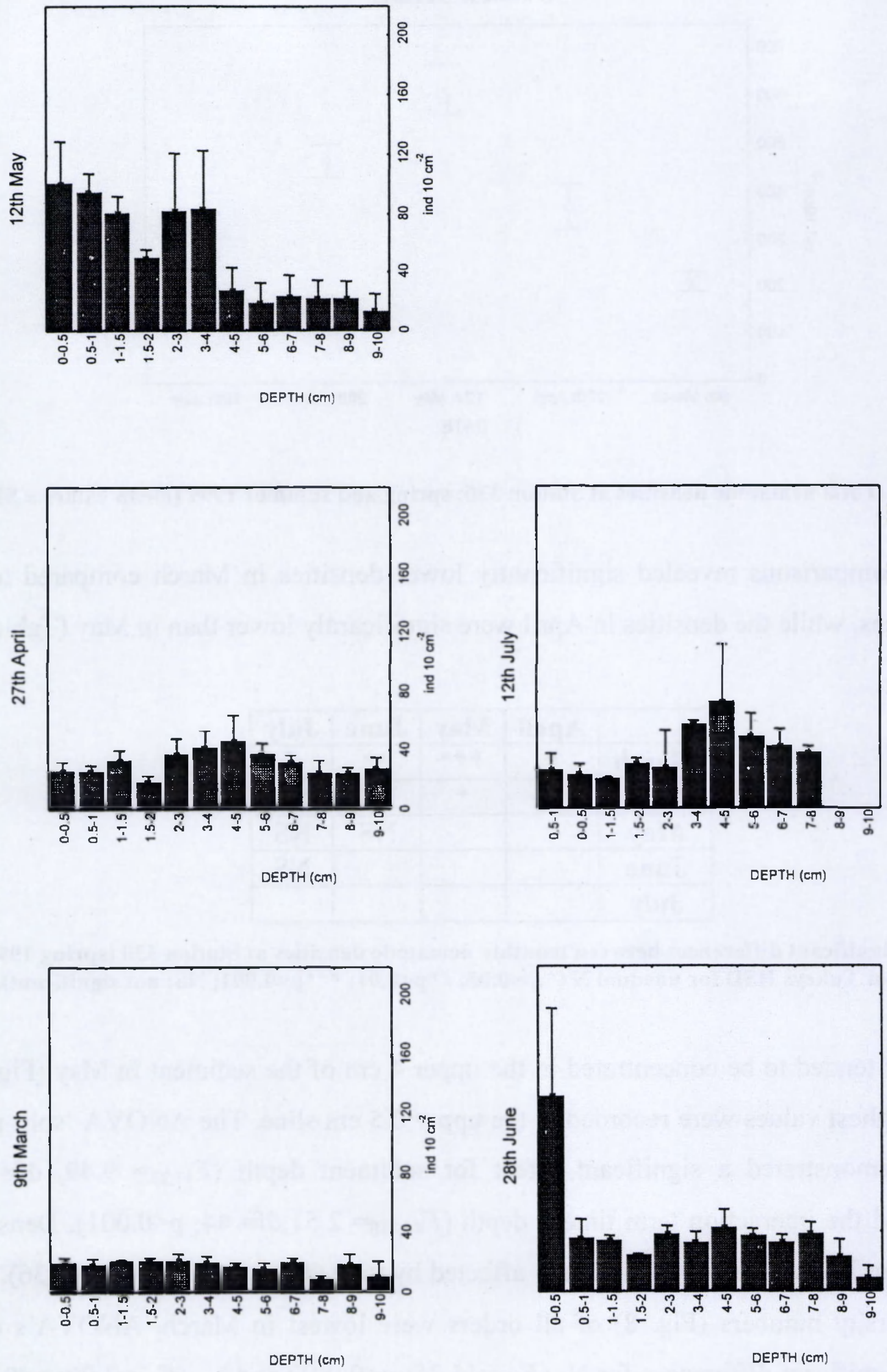

Figure 7. Vertical distribution of nematodes at Station 330: spring and summer 1999 (mean values ± SE).

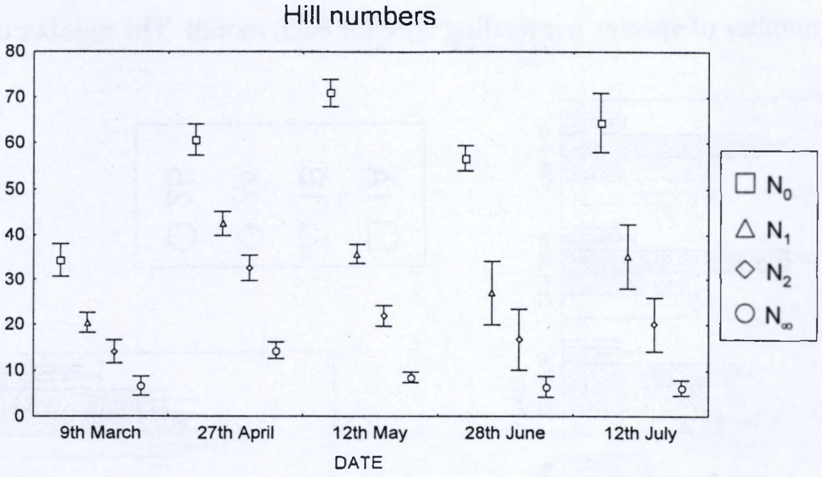

Figure 8. Diversity indices (Hill numbers) of the nematode communities at Station 330: spring and summer 1999 (mean values ± SE)

The densities from all feeding types changed during the sampling period (Fig. 9A). This was significant for 1A ($F_{4,9}$= 21.97, p<0.001) and 1B ($F_{4,9}$= 12.16, p<0.01) nematodes. Densities generally increased from March till May (Fig 9A, Table 2) but selective deposit feeders increased at a higher rate.

	April	May	June	July
March	**1A, 1B**	*1A,1B*	*1A*,1B	**1A**, 1B
April		1A	NS	NS
May			NS	NS
June				NS
July				

Table 2. Significant differences between monthly densities per feeding type at Station 330 (spring 1999). Results of Tukeys HSD for unequal N. (no formatting: p<0.05, bold:p<0.01; italic:p<0.001; NS: not significant).

Thus, the relative contribution of feeding types (Fig. 9B) showed a significant increase of the proportion of 1A nematodes (selective deposit feeders) during the sampling period (Kruskal-Wallis analysis by rank, p<0.01). Pairwise comparisons revealed that the proportion of the selective deposit feeders in March was significantly lower than in May, June and August (p<0.05), and April values were significantly lower than in May (p<0.05) No significant differences were found for the other feeding types. Fig. 9C depicts the number of species per feeding type for each month. The number of species

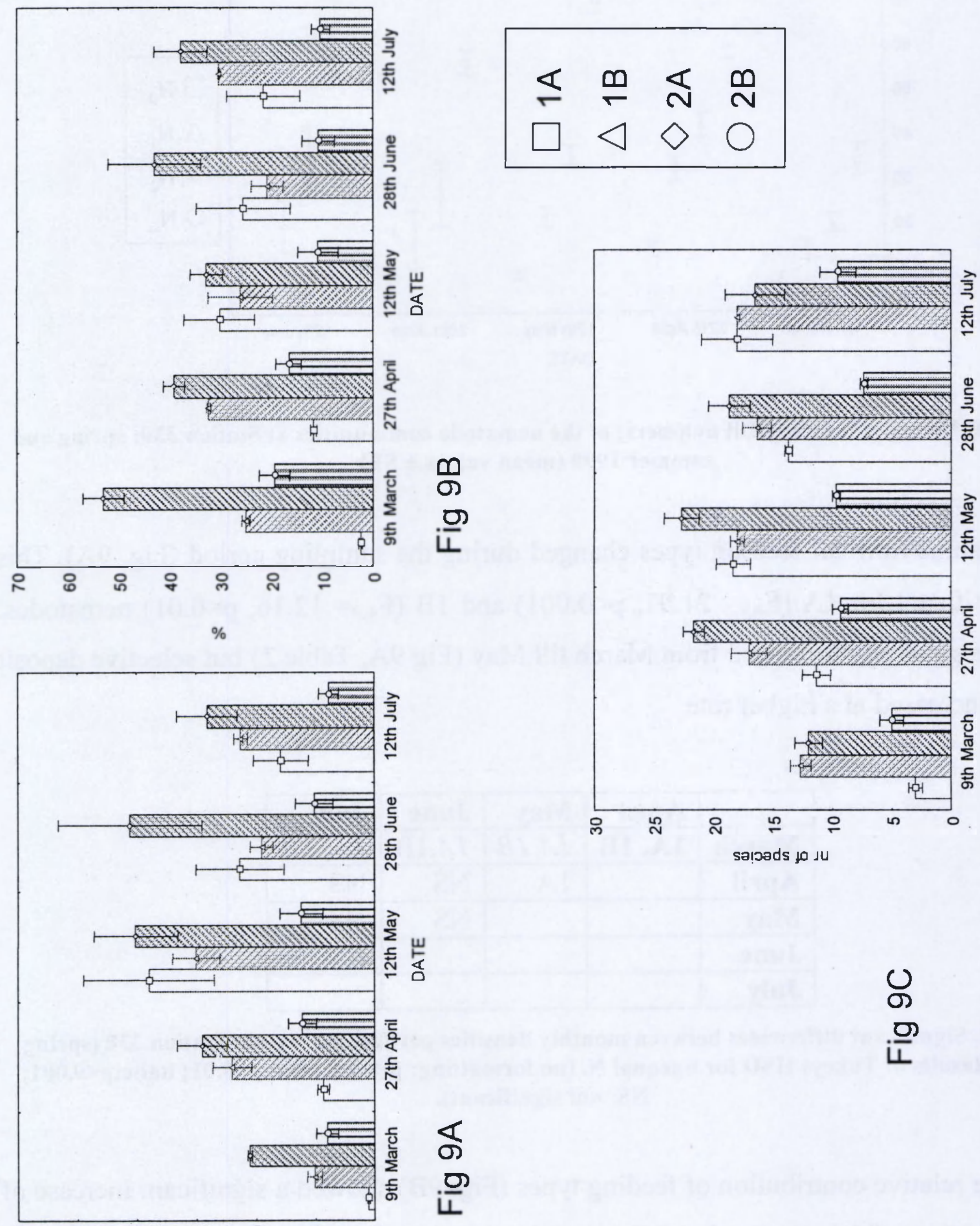

Figure 9. A: Densities per feeding type at Station 330: spring and summer 1999. B: Relative contribution of feeding types per sampling date. C: Number of species per feeding type per sampling date. All values: mean values ± SE

were significantly different for each feeding type between months (ANOVA, Table 3).

Feeding Type	**$F_{4,9}$**	**p**
1A	24	***
1B	4.3	*
2A	8.6	**
2B	7.2	**

Table 3. Significant differences between the number of species per feeding type per month at Station 330 (spring 330). Summary of ANOVA results (*:$p<0.05$; **$p<0.01$; ***$p<0.001$).

Especially in March, the number of species per feeding type was significantly lower compared to the other months (Tukeys HSD for unequal N, Table 4). Strongest differences were observed for the 1A and 2A feeding types.

	April	**May**	**June**	**July**
March	**1A; 2A**; 2B	*1A*, 1B, **2A**, 2B	**1A**	*1A*, 2B
April		1A		
May				
June				
July				

Table 4. Significant differences between the number of species per feeding type per month at Station 330 (spring 1999). Results of Tukeys HSD for unequal N. (no formatting: $p<0.05$, bold:$p<0.01$; italic:$p<0.001$)

The variance structure of the 25 most abundant species was compared to the variance of the total nematode densities and the total densities of the feeding types (Table 5). The variation between sampling dates was significantly larger than the spatial variation for the total nematode densities and those nematodes feeding on bacteria and detritus (1A and 1B nematodes). Such large temporal versus spatial variation was not the case for all dominant species, since significant results were only obtained for 14 of the 25 dominant species.

Species (n=14, a=5)	**Mean**	**Standard Deviation**	**% among dates**	**p**
Microlaimus marinus	28.0	27.0	76	*
Chromadorita n. sp. 2	20.8	58.7	33	
Tricoma sp.1	20.0	21.7	84	**
Metepsilonema comptum	19.2	32.1	73	*
Dichromadora cucullata	17.4	15.1	65	*
Onyx perfectus	14.1	15.1	21	
Richtersia inaequalis	13.1	15.2	60	
Enoploides spiculohamatus	11.8	10.3	36	
Theristus denticulatus	11.4	6.5	63	*
Camacolaimus longicauda	11.4	6.3	78	**
Manunema annulatum	9.6	9.9	50	
Epsilonema pustulatum	9.3	15.1	67	*
Desmodora schulzi	9.3	9.2	94	***
Prochromadorella ditlevseni	8.5	9.9	25	
Daptonema nanum	8.4	6.2	86	***
Pomponema multipapillatum	7.6	8.3	79	**
Rhynchonema sp1	7.4	5.2	57	
Sabatieria celtica	6.7	7.5	75	**
Desmoscolex sp.1	6.7	5.4	84	***
Trichotheristus mirabilis	6.5	6.8	45	
Paracyatholaimoides multispiralis	5.5	5.3	90	***
Rhynchonema moorea	5.5	8.5	76	**
Neochromadora munita	5.5	5.6	42	
Calomicrolaimus parahonestus	5.4	4.8	43	
Theristus heterospiculoides	5.3	3.9	53	
1A Feeding Type	86.6	81.9	92	***
1B Feeding Type	105.1	40.1	86	**
2A Feeding Type	160.8	65.1	50	
2B Feeding Type	51.3	21.8	15	
TOTAL density (n=14, a=5)	413.6	152.6	87	***

Table 5. Mean densities, standard deviation and percentage of variation due to temporal effects of the 25 most abundant species, feeding types and total nematode community at Station 330 (spring 1999). Significant ANOVA results: *:p<0.05; **p<0.01; ***p<0.001

A Detrended Correspondance Analysis (Fig. 10) separated all sediment slices from March from the other months along the first axis (eigenvalue: 0.23). Along the second axis (eigenvalue: 0.13) a distinction between April samples and all other months can be noted.

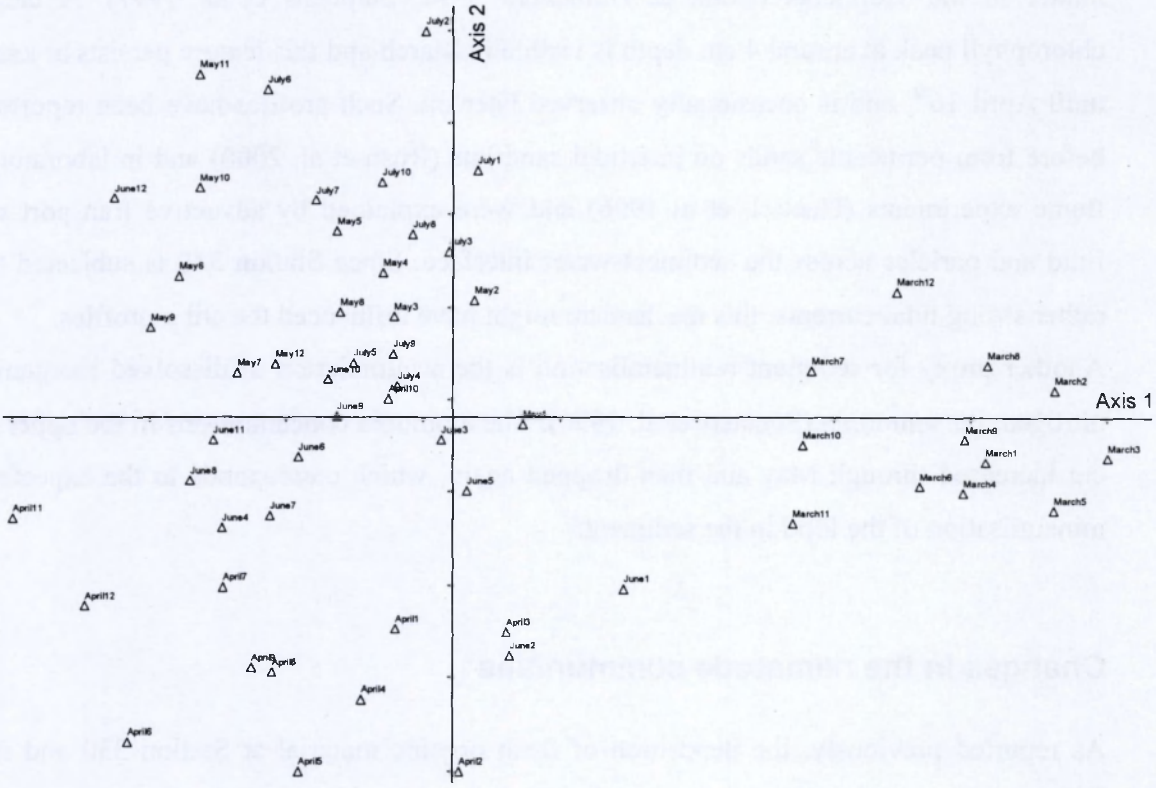

Figure 10. Results of Detrended Correspondance Analysis (DCA): axis 1 vs axis 2. Numbers indicate sediment layers (March 1: March 0-0.5 cm,…March5:March 2-3cm,…,March 11: March 8-9 cm).

Discussion

Environmental changes

The Chl *a* concentrations at the sea surface reflect the phytoplankton bloom as described previously for Station 330 (Rousseau 2000) and the North Sea in general (Boon et al. 1998). The bloom is initiated in early March and peaks at the end of April; later on, algal biomass declines rapidly. Since a similar pattern can be observed in the chlorophyll concentrations of the overlying bottom water, the bloom settles at least partially on the sediment (Vanaverbeke et al. 2003). In certain studies, the Chl *a* inventories in the sediment have been used successfully as a proxy for the amount of labile fresh organic matter in the sediments (Boon & Duineveld 1998, Stephens et al. 1997). A clear chlorophyll peak at around 4 cm depth is visible in March and this feature persists at least until April 16th, and is occasionally observed later on. Such profiles have been reported before from permeable sands on intertidal sandflats (Rush et al. 2000) and in laboratory flume experiments (Huettel. et al 1996) and were explained by advective transport of fluid and paricles across the sediment-water interface. Since Station 330 is subjected to rather strong tidal currents, this mechanism might have influenced the chl *a* profiles.

Another proxy for sediment remineralisation is the accumulation of dissolved inorganic nitrogen, i.e. ammonia (Soetaert et al. 1996). The ammonia concentrations in the upper 4 cm increased through May and then dropped again, which corresponds to the expected mineralisation of the food in the sediment.

Changes in the nematode communities

As reported previously, the deposition of fresh organic material at Station 330 and its subsequent mineralisation had a considerable effect on the nematode biomass spectra (Vanaverbeke et al. 2003) and morphometric characteristics (Vanaverbeke et al. subm). Temporal changes in densities and vertical distribution were clearly present as well (this study). Total densities showed significant differences between the sampling dates, and relatively low small-scale variation (i.e 100 of meters between replicate drops of the Reineck boxcorer). This low degree of spatial variability was not the case for several dominant nematode species and for those nematodes feeding on algae and other animals (the epistratum and predatory feeders sensu Wieser, 1953). Here the temporal features, if present, were masked or overwhelmed by small-scale variation. The reason for this is unclear although it may relate to the patchiness of the deposition and subsequent burial of organic matter in sediments as already reported (Boon et al. 1998 and references therein). Tidal currents, which can be rather strong at our sampling site, can cause high variability in chl *a* content of the sediment (Jennes & Duineveld 1985). The variation in chl *a* (Fig.

3) and ammonia (Fig. 4) concentrations indeed reflect substantial variation of sources of organic matter and remineralisation activities between replicates.

Both structural (total densities, vertical profiles, diversity indices N_0 and N_1) and functional (feeding type distribution, species richness per feeding type) aspects of the nematode communities changed most drastically from March to April (ANOVA). Sedimentation of organic material from the water column in this period could be deduced from the chlorophyll concentrations of the bottom water and the increased ammonia loading of the sediments. Significant differences between April and May were also observed, but these were less prominent. These trends were corroborated by the multivariate analysis, which clearly showed similar changes in nematode community composition.

The increase in densities from March onwards is mainly due to the opportunistic behaviour of a limited number of species (mainly *Metepsilonema comptum*, *Epsilonema pustulatum*, *Manunema annulatum*, *Richtersia inaequalis* and several *Rhynchonema* species) with aberrant morphometric characteristics (Vanaverbeke et al. subm). Almost all of these species belong to Wiesers' selective deposit feeding (1A) nematodes, animals with small buccal cavities that feed on bacterial-sized organisms (Moens & Vincx 1997). The rise of these small, thick species partly explains the increasing importance of this feeding group within the total nematode community. However, later on, larger members of selective deposit feeders become more abundant: in July only 4.8 % of the total nematode community is comprised by the thick species (Vanaverbeke et al. subm), whereas 20% of all nematodes still belong to the 1A group. This indicates that selective deposit feeders benefit most from increased amounts of fresh organic material in the sediment by feeding on the remineralising bacteria (Graf 1992) that respond to this inputted material. Similar findings were reported from Baltic Sea sediments (Ólafsson & Elmgren 1997).

Diversity (indicated by Hill numbers N_0 and N_1) increased after sedimentation of the spring phytoplankton bloom. Since significant differences were restricted to these indices, diversity increased as a result of the appearance of "rare" species in the nematode communities. The species numbers increased in all feeding types, possibly in response to the "diversity" of organic particles in sediments (e.g. Whitlatch 1981, Danovaro &

Gambi 2002). At Station 330, the arrival of fresh organic material activates remineralising bacteria (Graf 1992), which are a food source for deposit feeding nematodes (Moens & Vincx 1997). Both increases in the amount and quality of the living (bacterial) and non-living (detrital) food sources of nematodes, and the "diversity" of the deposited and decomposing organic particles in the sediments probably affect the number of species that can co-exist within the deposit feeding nematode guilds (1A+1B nematodes). Experimental evidence has demonstrated specific responses of closely related nematode species to the quantity and quality of bacterial food items (Moens et al. 1999). Therefore, we hypothesise that the observed increase in species richness, especially within the deposit-feeding nematodes is a result of an increase of the quantity and diversity of its food resources following sedimentation of the spring phytoplankton bloom.

Diversity within the epistrate feeding nematodes (2A group) also almost doubled after the sedimentation of phytoplankton. These nematodes have buccal armature that is used to either scrape off particles from a substrate, or to damage and open food items before emptying them (Moens & Vincx 1997). They often feed on diatoms and other microalgae that are cracked or pierced using the large dorsal tooth, before the contents is sucked and digested (Nehring 1992, Moens & Vincx 1997). Recently, it has been shown that a considerable amount of chl *a* present in sediments after phytoplankton bloom sedimentation can be attributed to a large quantity of living pelagic diatoms (Hansen & Josefson 2001, 2003). The spring bloom at Station 330 is dominated by the same diatom genera (*Chaetoceros*, *Thalassiosira* and *Skeletonema*) as in the study of Hansen & Josefson (2003) (Rousseau 2003) and pelagic diatoms were observed in the samples (JV, pers obs). Hence living diatoms possibly constitute an addition to the diet of these epistrate feeders and this could be a factor explaining the increase of species diversity within this feeding guild.

The increase of diversity after increasing the organic loading of the sediment is not a consistent feature: in an organic-poor estuarine sediment, Schratzberger & Warwick (1998) observed a decrease in species richness possibly due to anoxia and the release of toxic products under high doses of organic enrichment. Similarly, Steyaert et al. (subm) could not find an effect of bloom deposition on nematode species diversity in an oxygen stressed station at the Belgian continental shelf during the same period as this study. However, increasing diversity under mild food input and in oxic conditions is consistent with the general scheme presented by Levin et al. (2001), who put forward food supply, the availability of oxygen and sediment heterogeneity, disturbance and bottom current flow as key factors regulating species richness of local communities in the deep sea.

The vertical distribution of the nematode communities also changed during the sampling period, as indicated by the significant “time x depth” interaction term in the split-plot ANOVA. Nematodes tended to be concentrated in the upper sediment layers in May and in June, and this could be due to active migration (Graf 1992; Schulz, 1983) or net increase of the nematode communities within these layers. In Station 330, the concentration of nematodes in the upper sediment layers is mainly caused by the epistrate feeders (47.5% of all nematodes in the upper 0.5 cm in May, 55.6% in June), probably in response to the deposition of pelagic diatoms at the sediment-water interface. The vertical distribution of other feeding types did not show specific trends (not depicted) indicating that no vertical segregation of their preferred food items existed.

In conclusion:

Both small-scale spatial and temporal variability affect the nematode communities in a sandy station in the Southern Bight of the North Sea. For many nematode species, the small-scale spatial variability masked any temporal trend. However, the nematodes as a group and those feeding on detritus or bacteria responded significantly to the deposition of organic matter to the sediment. Nematodes that feed on fresh algal material did not respond significantly in terms of density, but were clearly seen to migrate upward when bloom deposition occurred. The sedimentation and subsequent mineralisation of the phytoplankton bloom caused an increase in suitable food items (living and dead), enabling many species to co-exist. The larger diversity was most prominent within the selective deposit feeders and the epistratum feeders.

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