UNIVERSITEIT GENT

Faculteit Wetenschappen

MARINE BIOLOGY SECTION

GHENT UNIVERSITY, BIOLOGY DEPARTMENT

# Spatial and temporal scales of nematode communities in the North Sea and Westerschelde

## Ruimtelijke en temporele patronen van nematodengemeenschappen in de Noordzee en Westerschelde

**Maaike Steyaert**

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

**Promotor: Prof. Dr. Magda Vincx**

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Mariene Biologie

Vakgroep Biologie

Biodiv.

⊛ 5000 soorten beschreven – 10000 nog in beschrijven.

waarvan 800 Noordzee.
hoeveel in deze studie? hoeveel BKP-WS

⊛ jul–okt: dens hoger in diepere lagen = 1.5–2 cm!
onmiddellijk hersteld?
in deze lagen in die dynamische systemen niet verstoord ??
door stromingen, visserij, etc...
overzwemmende, rustende?
overvarende vissersboten
catastrofale gebeurtenissen
visserij, baggeren...
hoe snel herstelt dit na verstoring ??

vert. profiel analyse

①

⊛ migratie v. soorten op welke plaats in celt aantonend!

②

⊛ eerste 2cm in 0.5cm schijven
indien 0.5cm schijven in in rekening → geen patronen te zien.
misschien is dit nog te groot?
gaat er nog info verloren door te dikke schijven?
en patronen in diepere lagen?

① ⊛ fluff-laag
→ zitten daar nema's in ?

nooit onder binoculair bekijken?

of. wanneer aanwezig alles in top 0.5cm.

(Aims)

Begrijpen & behoud

④ • summary p V. Understanding & conservation / management of the bentho-pelagic environment. waar behoud en beheer?

ook p 10: management.

thesis is contr. tot begrijpen relatie tss benthos en pelagos,

• p40: Mat & Met: CA following DCA was applied. species rarer than Fmax/5 downweighted.

• p82: waarom inversie van de sedimentatie? extra food added: 2mg diatom C ↦ waarom dit?

③ • p102. use of nematodes as ecological indicators for anthr. impacts remains highly problematic.
- why is this?
- what is your contribution?
- what is the way forward? wat zou je de meeste om dit te betrachten?

interesting = trends over

• p108: shift over 10 yrs: ‖ diversity ↗↗ near Westerschelde.
‖ diversity ↘↘ in western part BCP.

↳ = result of fining of sed.

hoe betrouwbaar is dit gezien grote patchiness gezien ook 115 → 115b.

= result of ‖ pollution effects
dredging activities
sediment transport.

② • General. wat is uw bijdrage tot de wetenschap? wat weten we nu meer over nematoden na het beëindigen v. uw werk.

Ook is van deze gel. willen eens grijpen om u voorleest…

~ Felicitaties: met gepresenteerde wet. werk, met de kwaliteit prod. & met presentatie van vandaag. | vertaler v. dit onderz. vr. een groot publiek toe.

Een publiek van leken – en ik kan mijzelf daar ook toe rekenen =

~ Dit gezegd zijnde:

[illegible]

① bekentenis: eerste meio/nema thesis die ik lees. Daarom ook volgende vraag, en begrip voor ~~[illegible]~~ mijn onwetendheid

| aangenaam verrast.

the. met duidelijke focus, goed gedocumenteerd

compact, in te veel detail, waardoor ontoegankelijk, voor n-specialisten.

misschien elke goede thesis nu meer vragen gegenereerd dan ze beantw. heeft.

aanleiding! achteraf.

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36174

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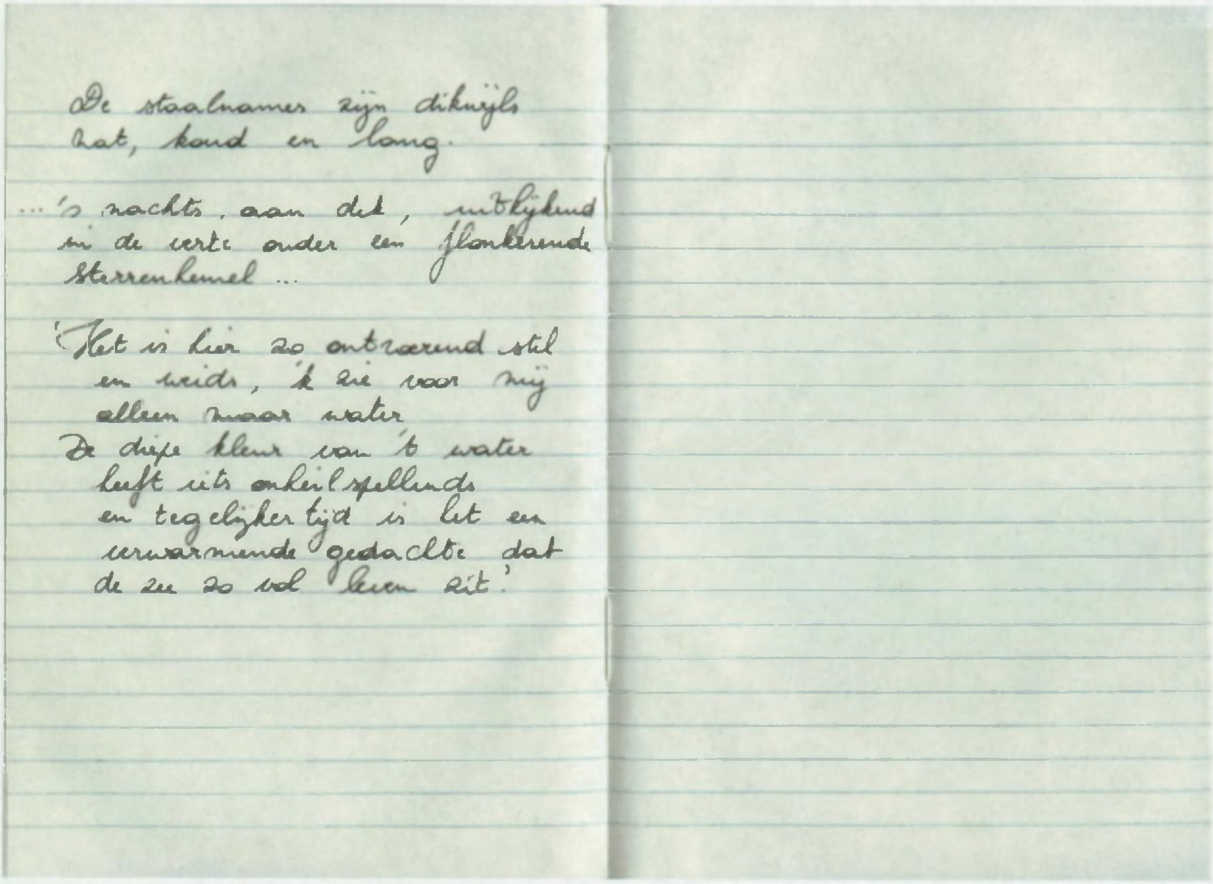

De staalnames zijn dikwijls
nat, koud en lang.

…'s nachts, aan dek, uitkijkend
in de verte onder een flonkerende
sterrenhemel …

'Het is hier zo ontroerend stil
en weids, 'k zie voor mij
alleen maar water
De diepe kleur van 't water
heeft iets onheilspellends
en tegelijker tijd is het een
verwarmende gedachte dat
de zee zo vol leven zit.'

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# Acknowledgements – Dankwoord

Ik begin heel graag mijn dankwoord met mijn promotor, Prof. Dr. Magda Vincx, en niet in het minst omdat zij steeds onvoorwaardelijk achter mij (en al de mensen van 'de mariene') stond. Magda, jij hebt mij ook - negen jaar geleden - geduldig geleerd hoe je al die nematoden op soort moest determineren. 't Is er uiteindelijk de aanzet van geweest dat ik zelf 59.040 nematoden gedetermineerd heb voor deze thesis.

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Andy, we shared a nice period on the Discovery. Although this sampling campaign was not in the framework of this thesis, during this campaign you guided me through the labyrinth build by the North Sea story.

Ilse, je vrolijke babbels hebben mij dikwijls opgemonterd. En Bregje, bedankt voor de uitrustende momenten in vermoeide tijden... Jan, de spanning was soms tastbaar de laatste weken. Maar 't zal helemaal niet dat zijn wat ik me vooral herinner van de laatste 9 jaar. In tegendeel, we hebben veel geploeterd in de 'morre', ook in data sets en geschreven teksten en we hebben 'netjes' congressen bijgewoond en practicum gegeven. 't Waren hele leuke momenten; je hebt er mij dikwijls bovenop gebracht.

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# Summary

Investigation of the meiobenthos (metazoans passing a 1 mm sieve and retained on a 38 μm sieve) communities on the Belgian Continental Shelf (BCS) and the Westerschelde started in the early seventies and mainly focussed on the identification of large horizontal distribution patterns within the area (*e.g.* Heip & Decraemer 1974, Heip *et al.* 1983, Van Damme *et al.* 1984, Heip *et al.* 1985, Vincx 1986, Herman 1989, Vincx & Herman 1989, Heip *et al.* 1990, Vincx *et al.* 1990, Vincx 1990). Nematodes were the dominant meiobenthic taxon, especially in coastal regions of the BCS and Westerschelde sediments. Vincx (1986) carried out the first extensive species-level survey of nematodes in the Southern Bight of the North Sea. She reported a decreasing nematode diversity towards the coastal zone and related this trend to a combination of factors, including reduced habitat heterogeneity and organic mater enrichment along the Belgian coast. Within the coastal zone, there was a tendency for nematode diversity to decrease towards the eastern part, probably related to the difference in sediment composition and sediment quality, originating from Westerschelde effluents (Vincx & Herman 1989, Heip *et al.* 1995). Later, Li (1993) and Soetaert *et al.* (1994, 1995) described the nematode communities at the species level in the Westerschelde estuary. Both studies investigated temporal and spatial aspects, with an emphasis on horizontal gradients and depth profiles in the sediment. The stability and standing stock of nematode communities increased from the upstream area to the estuarine mouth (Li *et al.* 1996). Intertidal communities showed a well-developed depth gradient in the sediment, whereas subtidal and channel communities sometimes exhibited a distorted pattern. This was probably related to sediment disturbance caused by higher current velocities and dredging activities in subtidal and channel regions (Soetaert *et al.* 1994).

The overall aim of the present thesis is to explain temporal (seasonal and tidal) and spatial (horizontal and vertical in the sediment) patterns of nematode communities in the coastal zone of the North Sea and on a tidal flat in the Westerschelde estuary, based on both field observations and experimental data. The significance of high-resolution vertical profile analysis in detecting environmental alterations is evaluated and compared to the horizontal (*i.e.* regional) analysis of faunal characteristics based on the comparison of bulk characteristics. An important objective is to test the extent to which interpretation of the temporally changing vertical distributions of nematodes can provide insights into the nature of the benthic-pelagic environment and its conservation.

Based on field observations, a description of the temporal (seasonal, tidal) and spatial (on different horizontal and vertical scales) patterns of nematode communities is presented in Chapter 2 and 3 (North Sea) and Chapter 4 and 5 (Westerschelde). Chapter 6 provides information on the direct structuring role of oxygen in the distribution of nematodes, based on experimental data.

Environmental controls on species diversity and vertical distribution within North Sea sediments were investigated by evaluating the abundance, species composition, diversity and trophic composition, and relating these community parameters to sediment composition, redox state and food sources (Chapter 2). The nematode assemblages at fine-grained subtidal sites (Station 115 and 702) were dominated by *Daptonema tenuispiculum* and *Sabatieria punctata.* For both species, the vertical distribution in the sediment seemed to be independent of the redoxchemistry (formerly believed to be important in the case of *Sabatieria punctata*) and primarily influenced by food availability. This also applied to *Ixonema sordidum* and *Viscosia langrunensis,* the most abundant nematodes at a coarse sandy site (Station 790).
Comparison of horizontal differences between the North Sea sites suggested that nematode diversity was regulated primarily by sediment granulometry. Coarser sediments yielded more diverse communities than fine sediments, although variations in sediment penetration were not obvious. Furthermore, the higher abundance, the lower diversity and the higher dominance found at the two silty sites of the eastern and the western part of the Belgian coast, indicated a stressed, organically enriched environment.

The importance of vertical distribution profiles in the sediment in explaining the horizontal macroscale variability was demonstrated for tidal flat communities of the Westerschelde (Chapter 4). This chapter addressed the question of whether regional (km's) variability was more important than local (m's) variability. Differences in community structure were analysed at a kilometre scale at three sites that differed in their chemico-physical characteristics. The differences in geochemical and physical conditions at larger (regional) spatial scales were reflected in the species composition and trophic structure of the nematode communities, and to a much lesser extent in their total abundance and species diversity.

Detailed investigation of vertical depth profiles showed more pronounced differences between environmentally divergent sites. Sediment granulometry appears to be important in controlling the fauna in the upper sediment layers. In deeper layers, similar faunal assemblages were found irrespective of sediment granulometry, suggesting that other environmental parameters were more important.

Vertically, nematode species showed depth distribution patterns that were linked to sediment characteristics related to the site-specific hydrodynamic regime. Pronounced vertical segregation of nematode species was observed within sandy sediment under strong hydrodynamic and food-stressed conditions. A surface-dwelling nematode community of large predatory enoplids was separated from a deposit feeding xyalid-microlaimid community in deeper sediment layers (beneath 2 cm). This segregation is believed to be due to species interactions, feeding strategies and/or physical disturbance. In the finest sediments with a high silt content, almost all nematode species were confined to the upper sediment layers (1.5 cm) and a sharp decline occurred in density and diversity with depth. This distribution pattern may be related to the limited oxygen penetration in surface layers and the occurrence of sulphide in deeper sediment layers. Between these two end-points of the hydrodynamic and granulometric spectrum, a gradual change in the vertical structure of the nematode community was observed, with dominant nematode species reaching maximum densities at specific depths.

Chapter 2 and 3 consider seasonal fluctuations in North Sea communities at silty coastal sites. Low-resolution sampling (Chapter 2: March – June – August) indicated that the overall diversity was higher in March, when the sediment column was more oxidised, and showed a positive relationship to the mud content, which varied with depth into the sediment. This positive relationship is explained by an enhanced deposition of organic matter associated with the accumulation of fine particles near the river mouths of the Westerschelde and Ijzer rivers. When seasonal variation was analysed on a monthly basis (Chapter 3) in relation to pelagic primary production, no effect on general species diversity was observed. Sedimentation of the spring bloom against a background of high levels of organic matter led to a switch of dominant species over time without affecting nematode species diversity. Thus the organically enriched coastal areas of the North Sea appear to harbour a nematode community, where niches are successively occupied by dominant species. The nematode community response to phytoplankton sedimentation (measured as chlorophyll *a* inventories), was also evident in the distribution of faunal abundances and feeding types which were related to changes in food availability. When fresh food was available on the seafloor, nematodes were concentrated in the top two centimetres of the sediment. The subsequent burial and remineralisation of the algal material generated highest nematode densities deeper in the sediment. The dynamics of the total nematode community was reflected in temporal changes in the abundance of epistrate feeders, and to a lesser degree of the non-selective deposit feeders.

A differential response was observed for the species belonging to the two dominant genera, *Sabatieria* and *Daptonema*. In April, when fresh food was supplied to the benthic environment, interspecific competition between *Sabatieria* and *Daptonema* species resulted in vertical depth segregation. In summer and late summer, decomposed algal material was available in excess at depths, which enabled species to co-exist.

The tidal dynamics of nematode communities from an estuarine tidal flat area (Westerschelde, SW Netherlands) were described in Chapter 5. The observed distribution profiles indicated that vertical migrations occurred and were species-specific. The predatory species *Enoploides longispiculosus* and the deposit-feeding species *Daptonema normandicum* migrated upwards at incoming tide and downwards when the flat became exposed, while another deposit feeder, *Daptonema setosum*, exhibited the opposite behaviour. Several abiotic and biotic factors may contribute to these observed patterns. Hydrodynamics, pore water drainage and episodic steep increases in temperature during low tide exposure, as well as vertical movements of prey organisms may have been important parameters at the time and site of sampling. However, the impact of each of these factors needs further investigation.

Chapter 6 describes microcosm experiments to assess the direct structuring role of oxygen in the vertical distribution of nematodes. In these manipulation experiments, the vertical distribution of the nematode species was examined following sediment inversion, with or without the presence of oxygen in the bottom layers.

The introduction of oxygen to deep layers significantly altered the vertical distribution of total nematode densities; highest numbers were recorded in the artificially aerated bottom layer. However, the distribution of the majority (~ 79 %) of the species in this case study was not directly linked to oxygenation. Dominant species were *Terschellingia communis, Microlaimus tenuispiculum* and *Sabatieria pulchra*. Only a small fraction (~ 2 %) of the nematode community was restricted to oxygenated sediment layers, irrespective of sediment depth. Six species belonged to this group (*Aegialoalaimus elegans, Axonolaimus helgolandicus, Bathyeurystomina sp., Desmoscolex sp., Dorylaimopsis sp.* and *Monoposthia mirabilis*) and their distribution evidently was directly governed by oxygenation. The majority of the species from the examined nematode community are highly tolerant of short-term anoxic conditions.

# Samenvatting

De studie van de meiobenthos (metazoa die door een 1 mm zeef gaan en achterblijven op een 38 µm zeef) gemeenschap van het Belgisch Continentaal Plat en van het Westerschelde estuarium startte in de jaren zeventig en concentreerde zich op de identificatie van horizontale, grootschalige verspreidingspatronen (*e.g.* Heip & Decraemer 1974, Heip *et al.* 1983, Van Damme *et al.* 1984, Heip *et al.* 1985, Vincx 1986, Herman 1989, Vincx & Herman 1989, Heip *et al.* 1990, Vincx *et al.* 1990, Vincx 1990). Nematoden vormden steeds de grootste fractie binnen het meiobenthos. In vergelijking met het gehele BCP was deze dominantie heel groot in de Belgische kustzone en de Westerschelde, beiden studiegebied van dit proefschrift. Vincx (1986) voerde een eerste intensieve studie op nematode soortniveau uit in de Zuidelijke bocht van de Noordzee. De dalende diversiteit van open zeegebied naar de kustzone werd gekoppeld aan een combinatie van factoren waarvan de belangrijkste een gereduceerde habitatheterogeniteit en een organisch aangerijkte bodem in de Belgische kustzone zijn. Binnen de kustzone nam de diversiteit af van de west- naar oostkust. Een oorzakelijk verband hiervoor was de aanwezigheid aan de oostkust van fijner, organisch belast sediment dat aangevoerd werd door de Westerschelde (Vincx & Herman 1989, Heip *et al.* 1995). In een latere fase werden de nematodengemeenschappen van het Westerschelde estuarium beschreven op soortniveau door Li (1993) en Soetaert *et al.* (1994, 1995). Beide studies omvatten zowel temporele als ruimtelijke (horizontale gradiënten in het estuarium en vertikale profielen in het sediment) verspreidingspatronen. Li *et al.* (1996) beschreef een grotere stabiliteit en een verlaagde standing stock van de nematodengemeenschappen volgens een stroomafwaarts gericht gradiënt. Intertidale gebieden werden gekenmerkt door gradueel wijzigende nematodengemeenschappen, in tegenstelling tot subtidale gebieden en geulen, waar verstoorde diepteprofielen aangetroffen werden. Sedimentverstoring, veroorzaakt door hogere stroomsnelheden en baggeraktiviteiten in de subtidale gebieden en de geulen werden aangehaald als belangrijke causale factoren (Soetaert *et al.* 1994).

In dit proefschrift wordt getracht de temporele (seizoensgebonden en tidale) en ruimtelijke (horizontaal en vertikaal in het sediment) verspreidingspatronen van nematodengemeenschappen in de kustzone van de Noordzee en op een getijdenplaat in de Westerschelde te beschrijven. Veldstudies en experimentele data moeten leiden tot het inschatten van de meerwaarde die de analyse van gedetailleerde verticale profielen kunnen leveren op gemeenschapsanalyse die de horizontale variabiliteit vergelijkt, vertrekkende vanuit bulkkarakteristieken. Verder wordt nagegaan in welke mate de interpretatie van temporeel variërende verticale profielen van nematoden, in relatie met de structurerende factoren, bijdraagt tot het begrijpen en het behoud van de bentho-pelagische omgeving.

In Hoofdstuk 2 en 3 (Noordzee) en Hoofdstuk 4 en 5 (Westerschelde) worden de temporele (seisoensgebonden, tidaal) en ruimtelijke (horizontaal en vertikaal in het sediment) verspreidingspatronen van de nematodengemeenschap beschreven op basis van veldstudies. In Hoofdstuk 6 wordt de rechtstreeks strukturerende rol van zuurstof in de verspreiding van nematoden toegelicht, op basis van proefondervindelijke gegevens.

In het Noordzee onderzoek werd de structurerende rol van omgevingvariabelen op soortendiversiteit en vertikale verspreiding in het sediment bepaald door de densiteit, de soortensamenstelling van de gemeenschap, de diversiteit en de trofische samenstelling te analyseren in relatie tot de sedimentsamenstelling, de redoxtoestand en de voedselbronnen (Hoofdstuk 2). De nematodengemeenschappen in de fijnkorrelige, subtidale stations (Station 115 en 702) werden gedomineerd door *Daptonema tenuispiculum* en *Sabatieria punctata*. De vertikale verspreiding van beide soorten in het sediment werd niet bepaald door de redoxchemie, zoals eerder vooropgesteld voor *Sabatieria punctata*, maar voornamelijk door voedselbeschikbaarheid. Dit werd ook bevestigd voor de twee dominante soorten uit grofzandig sediment (Station 790), *Ixonema sordidum* en *Viscosia langrunensis*.

De relatie soortendiversiteit – sedimentgranulometrie volgde uit een vergelijking van de bulkkarakteristieken van de Noordzee stations (horizontale variatie): gemeenschappen in grovere sedimenten waren diverser in vergelijking met fijnere sedimenten. Deze relatie werd echter niet bevestigd bij een vertikaal profielinventarisatie. De hogere densiteit, de lagere diversiteit en de hogere dominantie in de gemeenschappen van de fijnkorrelige sedimenten aan de Belgische oost- en westkust (Station 115 en 702) wijzen op een gestresseerde, organisch belaste omgeving.

In Hoofdstuk 4 werd onderzocht aan de hand van nematodengemeenschappen op een getijdenplaat (Westerschelde) in welke mate de horizontale variatie werd vertaald in verticale distributiepatronen in het sediment. In dit hoofdstuk werd het belang van de macroschaal (km's) variatie boven microschaal (m's) variatie geprevaleerd. De verschillen in gemeenschapsstructuur op kilometerschaal werden geanalyseerd in drie chemisch-fysisch verschillende stations. De horizontale verschillen in geochemische en fysische condities

werden weerspiegeld in soortensamenstelling en in trofische structuur van de nematodengemeenschappen en in mindere mate in de totale densiteit en soortendiversiteit. De grote verscheidenheid tussen de drie onderzochte stations was meer uitgesproken in de verticale diepteprofielen. De sedimentsamenstelling was de structurerende factor in de bovenste sedimentlagen. In diepere lagen werden vergelijkbare gemeenschappen aangetoond, wat een indicatie vormt voor andere structurerende omgevingsvariabelen.
De distributie van nematodensoorten in het sediment werd beïnvloed door sedimentkarakteristieken die indirect bepaald werden door het hydrodynamische regime. Een uitgesproken verticale segregatie van soorten werd waargenomen in zandige sedimenten die gedomineerd worden door een sterke dynamiek en voedselarme condities. Een nematodengemeenschap van grote, predatorische enopliden in de bovenste sedimentlagen werd gescheiden van een dieper (onder 2 cm) voorkomende gemeenschap, bestaande uit xyalide-microlaimide 'depositeters'. Onderliggende factoren voor deze verticale segregatie zijn de soorteninteracties, voedingsstrategieën en/of fysische verstoring van het milieu. In de fijnste sedimenten met een belangrijke slib fractie werd het grootste aantal soorten teruggevonden in de bovenste sedimentlagen (1.5 cm); een scherpe afname van densiteit en diversiteit in de diepte werd waargenomen. De belangrijkste structurerende factoren werden gerelateerd aan de beperkte zuurstof penetratie in het sedimentoppervlak en het voorkomen van sulfide in diepere lagen. Bij intermediaire hydrodynamische en granulometrische condities werd een gradueel wijzigende nematodengemeenschap geobserveerd volgens een diepteprofiel. Daar waren de dominante soorten maximaal aanwezig op verschillende dieptes.

Seizoensgebonden variaties van de gemeenschappen uit kuststations van de Noordzee werden beschreven in Hoofdstuk 2 en 3. In Hoofdstuk 2 werd een seizoenaliteit (maart – juni – augustus) gevonden in slibbige stations. Een hogere diversiteit werd gemeten in het geoxideerde sediment (10 cm) in maart. De variatie in het diepteprofiel werd positief gecorreleerd met de slib fractie (< 63 μm) en wordt verklaard door een verhoogde accumulatie van fijn, organisch belast materiaal nabij de monding van de Westerschelde en de Ijzer. In het onderzoek naar het effect van sedimentatie van een algenbloei in een organisch aangerijkt station aan de westkust (Hoofdstuk 3), werd geen seizoensgebonden variatie van de soortendiversiteit waargenomen. Maandelijkse observaties toonden een opeenvolgende dominantie aan van nematodensoorten, naarmate organisch materiaal, afkomstig van de pelagische primaire produktie, geïncorporeerd werd in het sediment. Deze studie suggereert een stabiele nematodengemeenschap in een organisch aangerijkt station aan de Belgische westkust, waarbij de ecologische niches successief ingenomen worden door dominante soorten tijdens sedimentatie van een algenbloei. De waargenomen verschillen in densiteit en in verspreiding van de voedingstypes werden voornamelijk toegeschreven aan de gewijzigde voedselkwaliteit. Nematoden concentreerden zich in de bovenste sedimentlagen wanneer vers voedsel bezonken was op de zeebodem. Naarmate dit materiaal geïncorporeerd en gemineraliseerd werd in het sediment, werden hogere densiteiten gevonden dieper in het sediment. De dynamiek van de totale nematodengemeenschap werd weerspiegeld in de dynamiek van de dominant vertegenwoordigde voedingstypes, nl. de 'epistratum feeders', en in mindere mate, de 'niet-selectieve deposit feeders'.

Een differentiële respons werd waargenomen voor de soorten van de twee dominante genera, nl. *Sabatieria* en *Daptonema*. In april, wanneer de benthische omgeving werd bevoorraad van levend materiaal, kwamen *Sabatieria* en *Daptonema* soorten verticaal gescheiden voor in het sediment, om zo interspecifieke competitie te minimaliseren. In de zomer en late zomer was er een overvloed aan gemineraliseerd phytoplankton materiaal, waardoor dezelfde dieptehorizonten konden bezet worden, in afwezigheid van competitie voor voedsel.

De tidale dynamiek van een nematodengemeenschap, afkomstig van een estuariene getijdenplaat (Westerschelde, ZW Nederland), werd besproken in Hoofdstuk 5. De waargenomen distributie profielen toonden aan dat nematoden verticaal migreren in functie van de getijden en dat deze dynamiek soort-specifiek is. *Enoploides longispiculosus*, een predator, en *Daptonema normandicum*, een 'deposit-feeder', migreerden naar boven bij inkomend getij, en naar beneden wanneer de plaat droog kwam te liggen. Een omgekeerde trend werd waargenomen voor *Daptonema setosum*, eveneens een 'deposit-feeder'. Verscheidene causale abiotische en biotische factoren kunnen bovenvermelde patronen verklaren; de hydrodynamiek, het uitwateren van de interstitiën, de episodisch sterke temperatuursstijging bij droogtij, en op- en neerwaartse migraties van prooiorganismen. Doch de individuele impact van deze factoren vereist verder onderzoek.

In Hoofdstuk 6 werd de direct structurerende rol van zuurstof in de verticale distributie van nematoden nader bepaald aan de hand van microcosmos experimenten. In deze manipulatie experimenten werd het verspreidingspatroon van nematodensoorten onderzocht na inversie van het sediment, waarbij al dan niet zuurstof toegevoegd werd aan de bodemlaag. Concluderend kan worden gesteld dat de introductie van zuurstof in diepere sedimentlagen een wijzigend verticaal nematoden profiel in de hand werkt. Hogere densiteiten werden geobserveerd in de artificieel beluchtte bodemlaag. Niettegenstaande werd voor het overgrote deel van de soorten uit deze case studie (~ 79 %) geen direct verband gevonden tussen hun verspreiding en oxygenatie van het sediment. Dominante soorten waren *Terschellingia communis, Microlaimus tenuispiculum* en *Sabatieria pulchra*. Slechts een minieme fractie van de soorten (~ 2 %) uit de testgemeenschap werden gelimiteerd in hun verspreiding tot geoxygeneerde sedimentlagen, onafhankelijk van de diepte van het sediment. Zes soorten behoorden tot deze groep (*Aegialoalaimus elegans, Axonolaimus helgolandicus, Bathyeurystomina sp., Desmoscolex sp., Dorylaimopsis sp.* en *Monoposthia mirabilis*). Dit experiment illustreert dat het overgrote deel van de soorten uit de bestudeerde nematodengemeenschap tolerant is voor kortstondige anoxische situaties.

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# Chapter I

## General introduction and aims

## Introduction

Ecological research on the meiobenthos (metazoans passing through a 1 mm sieve and retained on a 38 µm sieve) of the Belgian Continental Shelf and the Westerschelde started in the early seventies and adressed the relationship of biodiversity and various types of sediment (*e.g.* Heip & Decraemer 1974, Heip *et al.* 1983, Heip *et al.* 1985, Vincx 1986, Herman 1989, Vincx & Herman 1989, Heip *et al.* 1990, Vincx 1990, Vincx *et al.* 1990). These studies revealed that nematodes were the dominant meiobenthic taxon within the area. Based on horizontal distribution patterns of nematodes, the Southern Bight of the North Sea was divided into 6 sub areas with decreasing diversity towards the coastal zone (Vincx 1986b, 1990, Vincx *et al.* 1990). The decreased coastal diversity is probably caused by a combination of factors such as a reduced habitat heterogeneity due to the more highly sorted sand fraction and the increased amount of fine particles, and organic matter enrichment close to the Belgian coast (Vincx 1990). The Belgian coastal zone was subdivided in a species rich western part and an impoverished eastern part (Heip *et al.* 1984, Herman *et al.* 1985, Herman 1989, Vincx & Herman 1989), reflecting differences in sediment composition and sediment quality. Effluents from the Westerschelde estuary probably lead to concentrations of mud and a high amount of organic and inorganic pollution at the Westerschelde mouth (Vincx & Herman 1989, Heip *et al.* 1995).

Interest in high-resolution vertical patterns started with the study of Platt (1977). Important studies of the distribution of nematode species within the sediment were those of Jensen (1983), Warwick & Gee (1984), Ólafsson & Elmgren (1991) and Soetaert *et al.* (1994). These were all based on the idea that the vertically changing geochemical gradients within the sediment may cause more substantial faunal changes than those associated with regional-scale horizontal gradients. For example, Vanaverbeke *et al.* (1997) observed that changes in nematode assemblages with depth in the sediment were larger than those occurring along a depth transect of more than 2000 meter of the Goban Spur area in the NE Atlantic.

Nematode communities have often been considered to be particularly well suited for the impact assessment of disturbance. This is due to their high abundances, their ubiquitous distribution, their high and continuous reproduction, their short generation time and their complete benthic life style (Vincx & Heip 1991). Recently, autoecological and life cycle information have revealed that nematodes are not only highly diverse taxonomically, but also occupy a wide range of ecological niches, as exemplified by the different feeding habits observed by Moens & Vincx (1997). Moreover, generation times and reproductive capacity (Heip *et al.* 1985, Vranken *et al.* 1986), the ability to migrate (Soetaert *et al.* 2002) and tolerance to stressed environments (*e.g.* Boyd *et al.* 2000) seem to be species-specific characteristics in nematodes. Natural occurring or anthropogenically-induced alterations in the geochemical environment will inevitable cause a differential response in nematode species distribution. The interpretation of the temporally changing vertical distribution patterns of nematodes in relation to environmental forcing factors will yield information of value for the understanding and conservation of the benthic-pelagic environment.

## Study area

The North Sea study sites (Station 702, Station 790, Station 115, Station 115b) are located off the Belgian coast in the Southern Bight of the North Sea (Fig. 1). The Belgian coast, 65 km long, is remarkably free of muddy sediments, except at river mouths. Earlier studies have revealed an extensive development of muddy sedimentation off the eastern Belgian coast, mostly caused by suspended material discharged from the Westerschelde estuary. The nematode communities of these silty sediments are much less diverse than those from other areas with the same sediment type in the North Sea and in the rest of Europe, possibly because of higher levels of pollution (Vincx *et al.* 1989). Earlier research indicated that the presence of the Westerschelde estuary seems to induce a gyre off the eastern part of the Belgian coast, where the freshwater from the Westerschelde persists for some time (Nihoul & Ronday 1975). As a consequence of this hydrographic regime, fine polluted material is deposited near the mouth of the Westerschelde (Govaere *et al.* 1980). During the 1970s and 1980s, nematode communities along the Belgian coast showed a decline in species diversity towards the Westerschelde mouth (Vincx *et al.* 1990). Recently it has been shown that the influence of the Westerschelde reached much further than was formerly described by Nihoul & Ronday (1975). The Westerschelde plume is variable in shape and can even extent from the open sea area on the Belgian Continental Shelf (Station 330) as far as the Dutch coast, depending on the prevailing current regime (Yiang 1998).

**Figure 1. Map of the Belgian Continental shelf with location of the sampling stations**

Based on current knowledge of benthic faunal patterns and bottom topography, the Belgian Continental Shelf can be divided into nine zones (Fig. 2, Cattrijsse & Vincx 2001). The research presented in this thesis was concentrated in two of these nine zones, namely the eastern coastal zone and the western coastal zone. The eastern coastal zone comprises the shallow inshore waters between the Belgian-Dutch border and the city of Oostende. The western coastal zone stretches between Oostende and the Belgian-French border. The northern limit of these two zones was arbitrary chosen as the southern tips of the 'Flemish Banks'. Although one sandbank (the 'Wenduine Bank') is situated in the eastern and three sandbanks (the 'Stroombank', the Nieuwpoort Bank', the 'Trapegeer') in the western coastal zone, these areas fall beyond the present study area.

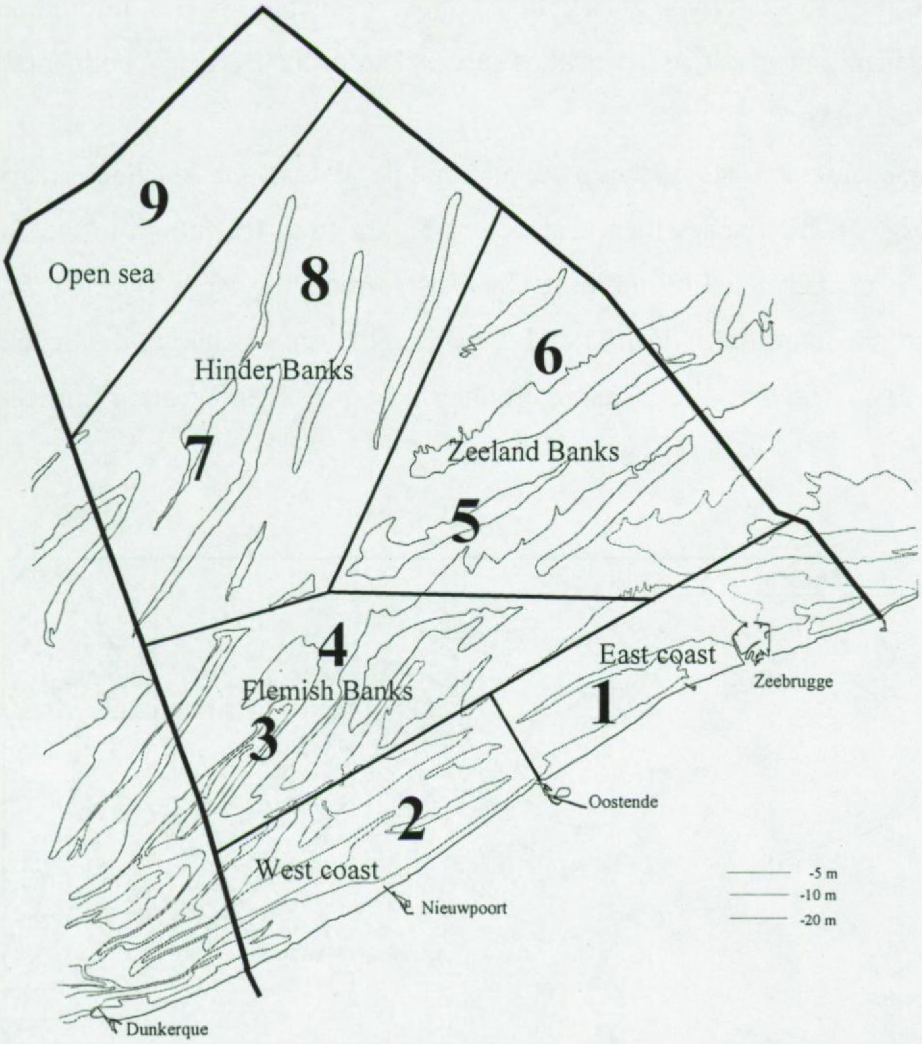

**Figure 2. Designation of the nine zones on the Belgian Continental Shelf.**

Station 115 (Chapter 1) and Station 115b (Chapter 2) are both situated within a part of the western coastal zone designated as a Belgian Marine Wetland of International Importance under the Ramsar Convention in 1984 (the 'Vlaamse Banken', 1900 ha). The 'Vlaamse Banken' site itself lies within an area of 17000 ha, which has been proposed as a Special Conservation Area under the EC Habitats Directive (Maes *et al.* 2000).

The marine biota of the Belgian coastal area, and of the Belgian Continental Shelf in general, has been intensively studied. Previous investigations addressing nematode diversity and distribution patterns along the Belgian coast include those of Heip & Decraemer (1974) and Heip *et al.* (1983, 1984), Willems *et al.* (1982), Vincx (1990) and Vincx *et al.* (1990). Within the framework of the OSTC project 'Sustainable Management of the North Sea' (MN/DD/[40-41-42]) all scientific information on the benthos and the avifauna since 1970 has been synthesised in order to identify patterns of structural and functional biodiversity in the area (Cattrijsse & Vincx 2001). In addition, available species information has been compiled, generating an extensive benthos species list for the Belgian Continental Shelf.

The Westerschelde consists of the lower and middle parts of the Schelde estuary and covers 310 km² (Fig. 3). It stretches for a distance of 55 km from the mouth to the Dutch-Belgian border and is characterised by multiple channels surrounding large intertidal flats. Due to a large amount of domestic, industrial and agricultural waste discharge, the Schelde estuary is among the most heavily polluted and eutrophicated estuaries in Western Europe (Abril *et al.* 2002).

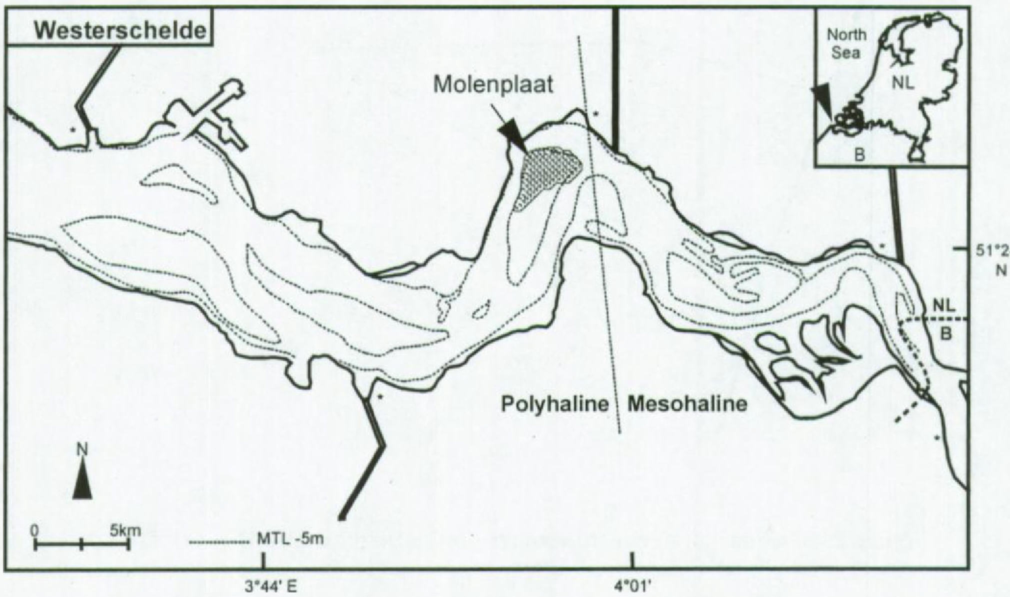

**Figure 3. Map of the Westerschelde with location of the Molenplaat**

The macrotidal character of the Westerschelde results in large intertidal areas which are important for the accumulation and mineralisation of organic matter (Heip *et al.* 1995). In the Westerschelde estuary, the intertidal sediments are estimated to account for about 25 % of total carbon retention (Middelburg *et al.* 1996). The Molenplaat, (Chapter 3 and 4) is an intertidal sandflat, situated in the western, marine part of the Westerschelde (Fig. 3). Over its entire extent, the Westerschelde is a highly productive ecosystem, characterised by high heterotrophic bacterial production (Goosen *et al.* 1997). The structural and temporal aspects of the nematode (Soetaert *et al.* 1994, 1995, Li *et al.* 1993, 1996, 1997), and the meiobenthos communities in general (*e.g.* Soetaert *et al.* 1994, 1995), in the Westerschelde estuary are well described. Based on a time dynamic model in the brackish zone of the Westerschelde, Li *et al.*

(1996) found that the stability and standing stock of nematode populations increased from the upstream area to the estuarine mouth. The intertidal communities exhibited clear and consistent changes with depth into the sediment. The vertical structure of subtidal and channel stations was different from that of the intertidal stations and in some cases showed a distorted pattern, probably caused by sediment disturbance due to higher current velocities and dredging activities in these regions (Soetaert *et al.* 1994). All benthic compartments are well documented and several recent PhD investigations devoted to the Westerschelde ecosystem illustrate its ecological importance. These studies address a variety of faunal groups including the protozoa (Hamels 2003), meiobenthos (Li 1993), macrobenthos (Ysebaert 2000), epibenthos (Hostens 2003) and hyperbenthos (Mees 1994).

## Objectives and outline of the thesis

The present thesis presents information on temporal (seasonal, tidal) and spatial (on different horizontal and vertical scales) patterns of nematode communities in the North Sea (Chapter 2 and 3) and the Westerschelde (Chapter 4 and 5) based on field observations. Chapter 6 tackles the question of the direct structuring role of oxygen in the distribution of nematodes, based on experimental data.

Chapter 2 ('**Nematode communities from the North Sea: environmental controls on species diversity and vertical distribution within the sediment**') is concerned with the seasonal variation of nematode communities at three localities on the Belgian coast in the southern part of the North Sea. The main aims are to describe patterns of vertical distribution and species diversity and to relate these patterns to: (1) particular natural environmental parameters including redox chemistry, sediment granulometry, bacterial densities, chlorophyll *a* concentrations; and (2) the broader question of anthropogenic influences. The selection of three localities (Station 702, Station 790, Station 115) effluents from ongoing long-term monitoring research in representative sites for the western and eastern part of the Belgian coast. The analysis of data from 1972 to 1984 was subject of the PhD of Vincx (1986) and is published in Vincx (1990) and Vincx *et al.* (1990). Present research was carried out within the framework of the Federal Impuls Programme Sea (MS/02/080; 1992-1996) of the Belgian Ministry of Science, which is an interdisciplinary project aiming at the conservation and the sustainable management of the North Sea and the Schelde estuary and to the scientific support of the national policy. This chapter was published as *Steyaert M, Garner N, Van Gansbeke D,* *Vincx M (1999) Nematode communities from the North Sea: environmental controls on species diversity and vertical distribution within the sediment. J Mar Biol Ass UK 79:253-264.*

The ecological implications of the phytoplankton bloom sedimentation for a nematode community in an organically enriched area were studied in Chapter 3 ('**Bentho-pelagic coupling: changes in nematode community structure during sedimentation of pelagic primary production in an organically enriched coastal environment (North Sea)**'). It was expected that deposition of phytoplankton bloom impacts the present nematode communities directly by changes in food supply as well as indirectly as reflected in vertical distribution patterns of nematode species in relation to the redox status of the sediment. The results presented in this chapter are collected within the OSTC-sponsored project 'Functional and structural biodiversity of North Sea ecosystems: Species and their habitats as indicators for a sustainable development of the Belgian Continental Shelf' (Belgium Sustainable Development-North Sea project MN/DD/[40-41-42]; 1999-2001). The main goal of this project was to describe the functional and structural diversity of the benthos and to use this knowledge to develop ecological indicators for a sustainable management of the Belgian part of the North Sea. The sampling site of this chapter (Station 115b: 51° 09.1'N, 2° 37.1'E) deviated slightly from Station 115 (51° 09.1'N, 2° 36.1'E), which was extensively studied in Chapter 2, in order to minimise variety in grain size (pers. comm. Van Lancker). This manuscript is submitted as *Steyaert M, Vanaverbeke J, Van Gansbeke D, Rousseau V, Parent J-Y, Vanreusel A, Vincx M. Bentho-pelagic coupling: changes in nematode community structure during sedimentation of pelagic primary production in an organically enriched environment (North Sea)* to *Marine Biology*.

Chapter 4 ('**The importance of fine-scale, vertical profiles in characterising nematode community structure**') deals with the spatial heterogeneity of nematode associations on the Molenplaat, which is a small intertidal flat in the Westerschelde estuary. Differences in structure of the communities were initially analysed in terms of depth-integrated characteristics for a high number (5) of replicate samples (collected at a m scale) at three geographically separated (at km scale) and chemico-physically diverging sites. It is examined whether changing environmental conditions over a small system like the Molenplaat are reflected in the vertical distribution pattern of nematodes species and result in shifting community characteristics with depth in the sediment. Investigations of both Chapter 4 and 5 are carried out within the EU-funded multidisciplinary project ECOFLAT (The eco-metabolism of an intertidal flat) (ENV 4-CT96-0216; 1996-1999), which aims at bridging the gap between the small scales at which biological and biogeochemical processes in tidal flats operate and are measured, and the large scales (estuarine scale, regional scale) at which the environmental problems are posed. This chapter is published as *Steyaert M, Vanaverbeke J, Vanreusel A, Barranguet C, Lucas C, Vincx M (2003) The importance of fine-scale, vertical profiles in characterising nematode community structure. Estuar Coast Estuar Coast Sh Sci 56:1-14.*

In Chapter 5 ('**Tidal migration of nematodes on an estuarine tidal flat (the Molenplaat, Schelde Estuary, SW Netherlands)**') the tidal migration of nematodes on the species as well as on the community level was investigated. It was tested to which extent the community level approach masks species-specific patterns. The choice of the study site (Site 3 in Chapter 4 or MP 4 in Herman *et al.* 2000) related to the assumption that nematode communities will be more pronounced impacted by tidal periodicity, compared to the other investigated sites at the Molenplaat (Site 1 and 2 in Chapter 4). Exposure time (about 7 hours during each tidal cycle) was longest, sediments (median grain size 172 µm) were coarsest and bottom shear stress (1.15 Pa) was highest compared to Site 1 and 2. This chapter is published as *Steyaert M, Herman PMJ, Moens T, Widdows J, Vincx M (2001) Tidal migration of nematodes on an estuarine tidal flat (the Molenplaat, Schelde Estuary, SW Netherlands). Mar Ecol Prog Ser 244:299-304.*

Although the importance of oxygen in nematode distribution has often been mentioned in above chapters, direct evidence of the structuring role is not provided. In Chapter 6 ('**The direct role of oxygen in the vertical distribution of nematodes: microcosm experiments**') the direct role of oxygen on the vertical distribution of nematodes was investigated by means of experiments in which different oxygen conditions were imposed on sediments. The migratory activity of the nematode community and of nematode species was evaluated as function of oxygen availability. It was tested if changing oxygen conditions influence the vertical distribution of the nematode assemblages and nematode species (*e.g.* nematodes do not migrate to favourable oxygen conditions). This research was supported by the FWO project 'Food, oxygen and bioturbation: an experimental study of meiofauna community structure' (2002-2004), carried out within a Flemish-Dutch co-operation. The main objective of this project is to elucidate the role of the food, oxygen conditions and macrofauna as structuring agents for meiofauna biodiversity patterns and hereby increasing the value of meiofauna as potential indicators of particular environmental conditions. This manuscript is submitted as *Steyaert M, Moodley L, Vanaverbeke J, Vandewiele S, Vincx M. The direct role of oxygen in the vertical distribution of nematodes: microcosm experiments* to *Hydrobiologia.*

In the general conclusions, key issues are derived from the main results of the different chapters in order to elucidate on the significance of temporal and spatial high-resolution research of nematodes for the understanding and the management of the bentho-pelagic environment.

Each chapter of this thesis is intended to be an autonomous part, which can be read separately from other chapters. As a result there may be some overlap in the sections on study site, and materials and methods. The summary/samenvatting summarises the major results and conclusions, which are drawn from this thesis. References are generalised and listed at the end of the work. Raw data (species lists, abundances, environmental data) are available on request on cd-rom. It is intended to deposit the data for archival and make the data available through re-distribution in the Flanders Marine Data and Information Centre of the Flanders Marine Institute (VLIZ, Vlaams Instituut voor de Zee).

## General remarks

Data for this dissertation were gathered over a period of nine years (1993 - 2002), within the framework of four different projects. During this relatively long period, sampling and analytical methods improved considerably. However, this meant that comparison and integration of the results of this study needed to be done with caution. As an example, one of the main objectives of the 'Impuls project Sea' (MS/02/080) was to continue long-term monitoring which started in the seventies. In order to make results comparable, the samples collected in 1993 had to be taken in a similar way to earlier samples. Pseudoreplication was an important and inevitable consequence of this requirement. This highlights once more the need for standardised sampling and analytical methods.

Both Chapter II and III concerned the nematode community at the western Belgian coast, however sampling sites deviated a few hundred meters from each other (Station 115 and Station 115b). The shift of the sampling site was performed in order to improve the chance of sampling homogeneous sediment during the effect study of the sedimentation of the algae bloom.

Extrapolation of experimental results to field situations remains problematic. The complete simulation of field conditions in the laboratory is a complex task that is logistically often difficult to achieve. Nevertheless, laboratory experiments, in addition to field observations, are necessary in order to understand how environmental factors influence the benthic ecosystem. Micro- and mesocosm-scale experiments provide the possibility to simulate field situation in a controlled way, for example, to isolate the role of single environment factors. The analysis of the extensive dataset summarised in Chapters 2 to 5 made it possible to address some important questions, in particular regarding the direct role of oxygen in structuring benthic ecosystems. The experiment described in Chapter 6 is one of the first in a series dealing with the role of the food, oxygen concentrations and macrofauna as structuring agents for meiofaunal biodiversity patterns. These experiments will be conducted as part of the FWO project 'Food, oxygen and bioturbation: an experimental study of meiofauna community structure' (Flemish-Dutch co-operation).

The field studies, both at sublittoral sites of the North Sea and intertidal sites of the Westerschelde led to the conclusion that intertidal environments provide more satisfactory study areas in the sense that one can locate the sampling site much more accurately than is possible in the case of sampling using deployments of box- or Reineckcorers. In addition, direct visual observations of the habitat are possible during sampling of exposured intertidal areas.

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# Chapter II

# Nematode communities from the North Sea: environmental controls on species diversity and vertical distribution within the sediment

Results presented as

Steyaert M, Garner N, Van Gansbeke D, Vincx M (1999) Nematode communities from the North Sea: environmental controls on species diversity and vertical distribution within the sediment. J Mar Biol Ass UK 79: 253-264

## Abstract

Nematode assemblages were sampled seasonally at three subtidal stations along the Belgian coast. The stations were characterized by muddy sediments (Station 115), fine sand (Station 702) and fine to coarse sand (Station 790). The forces structuring vertical distribution were investigated by evaluating abundance, species composition, diversity and trophic composition, and relating these to sediment composition, redox state and food sources.

The nematode assemblages at the two finer grained stations (115, 702) were dominated by *Daptonema tenuispiculum* and *Sabatieria punctata.* For both species, the vertical distribution in the sediment seemed not dependent on the redoxchemistry, as former believed for *Sabatieria punctata*, but primarily influenced by food availability. This feature could also be recognized for *Ixonema sordidum* and *Viscosia langrunensis,* the most abundant nematodes at the coarse sandy station (790).

In general, nematode diversity was regulated primarily by sediment granulometry. Coarser sediments (Station 790) yielded more diverse communities compared to the fine sediments (Station 115, 702), however seasonal fluctuations and variations with depth into the sediment were not obvious. At the silty stations, when the sediment column was more oxidised in March, overall diversity was higher and showed a positive relationship to the mud content which varied with depth into the sediment. This positive relationship is probably explained by an enhanced deposition of organic matter associated with the accumulation of fine particles near the river mouths. Furthermore, the higher abundance, the lower diversity and the higher dominance found at the two silty stations of the eastern and the western part of the Belgian coast, pointed to a stressed, organically enriched environment.

The results demonstrate that controls on nematode community structure are complex and that information at both species and community level are required to properly evaluate the effects of natural and anthropogenic impacts.

# Introduction

Meiobenthos often shows an aggregated spatial distribution within the sediment, both horizontally and vertically. The causes of this patchiness are often complex and involve a variety of biological, physical and chemical variables, including granulometry, salinity, oxygen tension, food availability and chemical compounds in the pore water (*e.g.* Giere 1993, Li & Vincx 1993, Ndaro *et al.* 1995). The processes that generate and maintain the vertical distribution patterns in different localities are particularly poorly understood and form an important challenge for contemporary ecological research[1]. For nematodes, oxygen and hydrogen sulphide are thought to be of prime importance (Platt 1977, Giere 1993, Hendelberg & Jensen 1993, Wetzel *et al.* 1995) at least in shallow waters. Moreover, both of these ecofactors indirectly or directly determine all other biogeochemical characteristics of the sediment such as nutrient concentrations. In addition to these natural variables, anthropogenic impacts, including eutrophication, pollution and physical disturbance, may have a substantial influence on meiofauna, particularly in coastal areas. As the meiobenthos lives in the interstitial spaces of the sediment, and thus in direct contact with the pore water and its pollutants, the impact of pollution also needs attention when considering the structural forces on the vertical distribution of the meiobenthos.

The present investigation is concerned with nematode communities at three localities on the Belgian coast in the southern part of the North Sea. The main aims are to describe patterns of vertical distribution and species diversity and to relate these patterns to: (1) particular natural environmental parameters including redox chemistry, sediment granulometry, bacterial densities, chlorophyll *a* concentrations; and (2) the broader question of anthropogenic influences. The results demonstrate that controls on nematode community structure are complex and that information at both species and community level are required to properly evaluate the effects of natural and anthropogenic impacts. Previous studies, addressing nematode diversity and distribution patterns along the Belgian coast include those of Heip *et al.* (1974, 1983, 1984), Willems *et al.* (1982), Vincx (1990) and Vincx *et al.* (1990).

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[1] Within the framework of this research a first attempt was made to unravel the role of nematodes in the sediment, which was of prime importance to contemporary meiobenthic ecological research. The exploration of the vertical distribution of the nematodes in their biogeochemical environment enabled to formulate hypotheses about the generality of the profiles and possible interactions between the organisms and their biogeochemical environment.

# Material and methods

## The study area

The study sites are located on the Belgian coast in the Southern Bight of the North Sea (Fig. 1). This long straight coast, 65 km long, is remarkably free of muddy sediments, except at river mouths. Earlier studies have revealed an extensive development of muddy sedimentation off the eastern Belgian coast, mostly caused by suspended material discharged from the Westerschelde estuary, as well as by local effluents from the River Ijzer. The nematode communities of these silty sediments are much less diverse than those from other areas of the same sediment type in the North Sea and in the rest of Europe, possibly because of higher levels of pollution (Vincx 1990).

Figure 1. Location of the sampling stations

The presence of the Westerschelde estuary seems to induce a gyre off the eastern part of the Belgian coast, where the freshwater from the Westerschelde resides for some time (Nihoul & Ronday 1975). As a consequence of this current regime, fine polluted material precipitates near the mouth of the Westerschelde (Govaere *et al.* 1980). During the 1970s and 1980s, nematode communities along the Belgian coast showed a decreasing species diversity towards the Westerschelde mouth (Vincx 1990). The present study describes the nematode community of a station within this impoverished area (Station 702), which has been monitored for almost 20 y (Fig. 1; Table 1). At the western end of the Belgian coast, off the Flemish sandbanks, the sediments tended to be more sandy, compared to the eastern zone, during the seventies and eighties.

| Station | Coordinates | Depth (m) |
|---|---|---|
| 115 | 51°09.1' N 2°36.1' E | 15 |
| 702 | 51°23.2' N 3°19.4' E | 10 |
| 790 | 51°16.0' N 2°65.0' E | 8 |

**Table 1. Sampling sites with the coordinates and the water depth**

This was reflected in a more diverse nematode association (Vincx 1990). The second station (115) was selected in this western zone, around Nieuwpoort (Fig. 1; Table 1). To compare extreme sediment types, a third station (790), which was situated in the central coarse sandy area of the coast, was sampled (Fig. 1; Table 1). The present study indicates a major change in nematode diversity trends since the work of Vincx (1990).

## Sample collecting

The three subtidal stations were sampled seasonally - in March, June and August 1993 - to cover the periods of high and low organic input into the sediment. Samples were taken with a boxcorer (surface area 804 cm²) which was subsequently subsampled with Perspex cores (diameter 3.6 cm) on board of the RV 'Belgica'. For all abiotic and biotic determinations, the sediment was divided into ten slices, each one centimetre thick.

## Chemical analysis

The redox potential of the first 10 cm was recorded simultaneously[2] with a mV-meter.

For the determination of bacterial abundances two subsamples of 1 ml each were removed from one core and fixed in a 2% formaldehyde solution. To release bacteria from the sediment particles, the samples were sonicated ten times for one second. Subsequently, the bacteria were stained with acridine orange and counted by epifluorescence microscopy, according to the method of Hobbie *et al.* (1977). Three subsamples were removed from one core and frozen immediately for pigment analysis. In the laboratory, the concentration of chlorophyll *a* was determined by chromatography, with a Gilson high-performance liquid chromatography-chain, using a slightly modified method of Mantoura & Llewellyn (1983). For the analysis of pore water nutrients, three cores of 6 cm diameter were processed and frozen immediately after

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[2] Ten redox measurements were carried out at the same time on the first 10 cms, with an interval of 1 cm.

slicing. In the laboratory, the samples were passed through Whatman GF/C filters. The 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 important oxidised (nitrate and nitrite) and reduced (ammonia) nitrogen compounds, 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 (0 mV< x <100 mV), reduced (0 mV< x <-100 mV) and strongly reduced sediment (<-100 mV). Triplicate samples were processed with a Coulter Counter LS particle size analyser to measure the median grain size and the fraction of mud (<63 µm).

### Faunal analysis

Triplicate cores were processed for metazoan meiofauna. The sediment slices were fixed in hot (70°C) 4% formaldehyde solution. Meiofaunal organisms 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). All meiofaunal taxa were counted after staining with Rose Bengal. For each sample, 120 nematodes were picked randomly and mounted on Cobb slides for identification to species level. According to the feeding type classification of Wieser (1953), a distinction is made between selective deposit feeders (1A), non-selective deposit feeders (1B), epistratum feeders (2A) and omnivores (2B). The influence of biogeochemistry on the vertical distribution of the nematodes was analysed seasonally in terms of abundance patterns, dominance and species diversity. Dominance is expressed as the relative abundance of the most abundant species (R1D), *i.e.* the first point on a k-dominance curve (Lambshead *et al.* 1983). The diversity is expressed as the $N_1$ diversity index (Hill 1973), which is a normalisation of the Shannon Wiener index H and recommended for meiofaunal assemblages by Heip *et al.* (1988). It summarizes the structure of the community, taking into account the most common as well as the most rare species. Because diversity indices are dependent on the sample size as well as on the number of analysed species, $N_1$ values are given as the means of three replicates, rather than being calculated from the sum of three replicates. This allowed comparison with earlier research on the Belgian coastal zone. The distribution patterns of the most abundant species were followed during the field study. Non-parametric Spearman rank correlation coefficients were calculated ($p<0.05$) to determine a relationship between diversity and environmental variables along a depth gradient.

## Results

The main characteristics of the nematode assemblages and the abiotic environments are summarized in Table 2.

| Station | Abundance (ind. 10 cm$^{-2}$) | No. of species | R1D (%) | Mud (% <63 μm) | Median (μm) |
|---|---|---|---|---|---|
| 115 | 1847, 2805, 1156 | 56.0 | 73.3 | 36.1 - 83.6 | 47.2 - 143.8 |
| 702 | 270, 786, 2326 | 45.0 | 51.4 | 5.9 - 53.3 | 76.5 - 198.1 |
| 790 | 141, 247, 622 | 98.0 | 24.7 | 0.0 - 0.3 | 402.5 - 487.1 |

**Table 2. Main characteristics for the three stations (mean values over depth): abundance for March, June and August respectively, number of species and Rank 1 Dominance (R1D) (mean values), mud fraction and median grain size (lowest highest value)**

### The abiotic environment

Station 115 had fine sediments with a higher percentage of mud compared to other stations. Redox potential profiles and nitrogen compounds indicate that the top 5 cm of the sediment in winter or early spring was oxidised (Fig. 2). The sediment was nearly anoxic from the top downwards in June and returned back to a somewhat less reduced situation in August. Fig. 3 shows the vertical distribution of bacterial densities and chlorophyll *a* content. The chlorophyll *a* concentration remained very low during March compared to June and August, when a gradual decrease within depth is recorded. The bacterial densities were greatest in deeper sediment layers in March, while in June and August, the densities were highest in the surface layer and decreased gradually within depth.

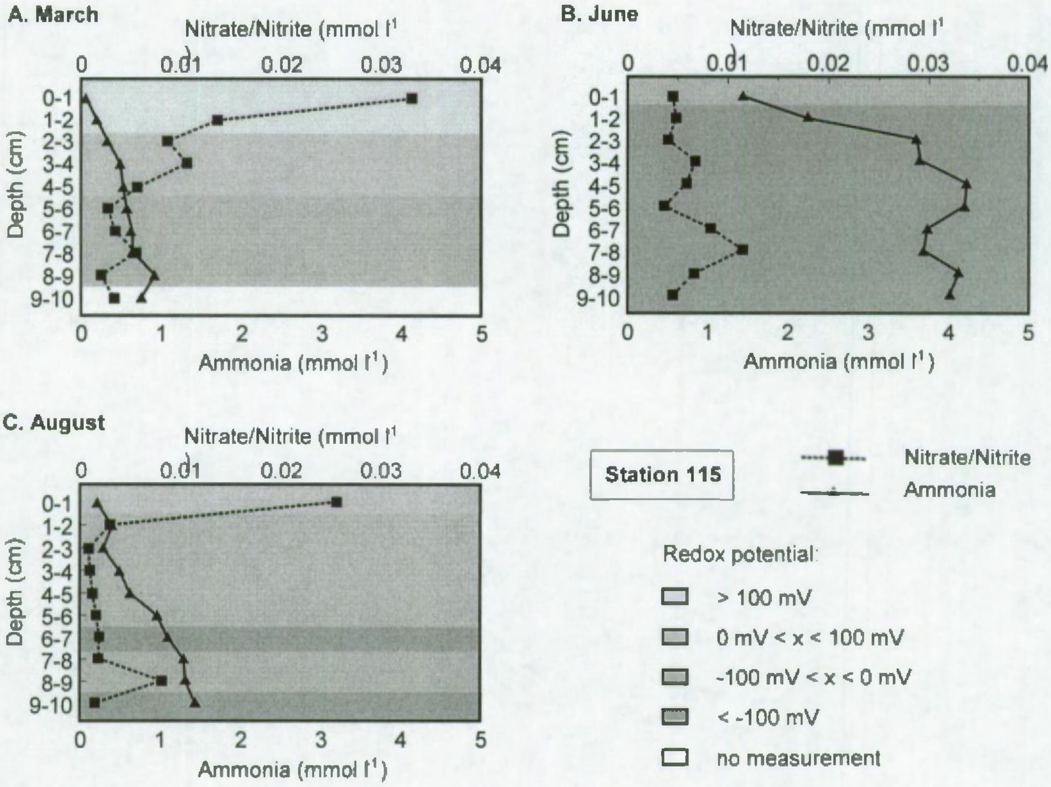

**Figure 2. Redox potentials and nitrogen compounds at Station 115.**

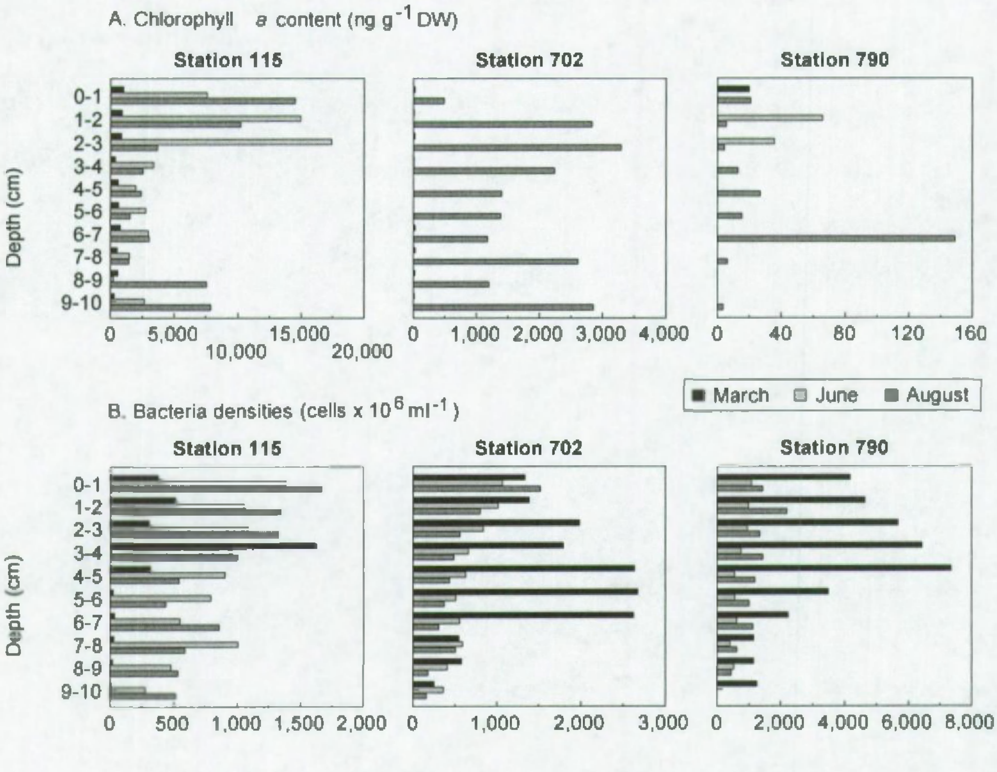

**Figure 3. Chlorophyll *a* concentrations and bacterial density**

Sediments at Station 702 consisted mainly of fine sand with a low mud content. The sediments were fully oxidised in March with nitrate concentrations not lower than 0.035 mmol $l^{-1}$ and very low ammonia concentrations (<0.078 mmol $l^{-1}$) (Fig. 4). An intermediate situation existed in June with only the upper 3 cm oxidised and lower layers strongly reduced. In August, however, the sediment was completely reduced. Zones of higher redox potential shown in Fig. 4 may be related to burrows. No positive redox potential values were measured and the ammonia concentrations were extremely high (up to 4.09 mmol $l^{-1}$). The chlorophyll *a* concentrations were rather low in March and June (Fig. 3). Concentrations were higher in August, and fluctuated considerably with depth. The bacterial profiles were similar to those of Station 115, apart from the higher densities in March compared to those in June and August.

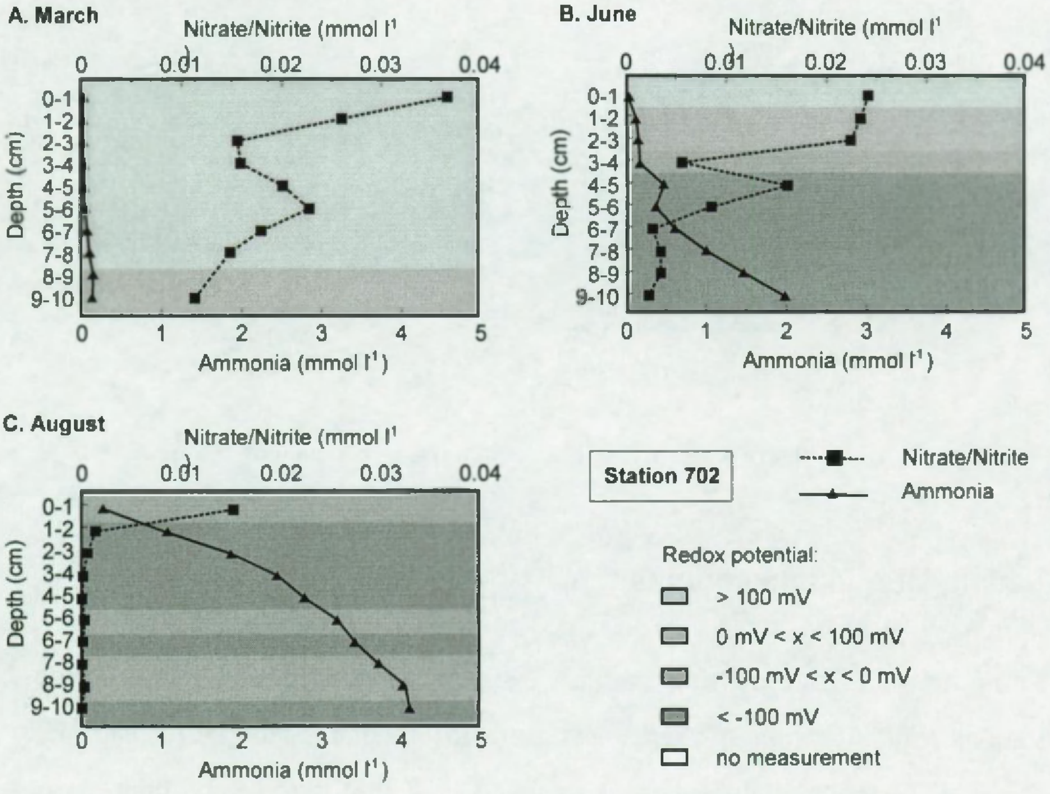

**Figure 4. Redox potentials and nitrogen compounds at Station 702**

At Station 790 the sediment consisted of fine to coarse sand, almost devoid of mud and characterized by large interstitial spaces. Redox potential values were mostly positive (Fig. 5) and nitrogen compounds exhibited a fairly stable seasonal depth pattern. Chlorophyll *a* concentrations were rather low and constant with depth in March. In June, the pigment concentration started to increase within the upper sediment layers, and reached the highest values in August. However, in August, pigment maxima shifted towards deeper sediment layers. Bacterial densities showed the same pattern as at the other two stations. However, the difference in overall density between March on one hand, and June and August on the other hand, were more obvious.

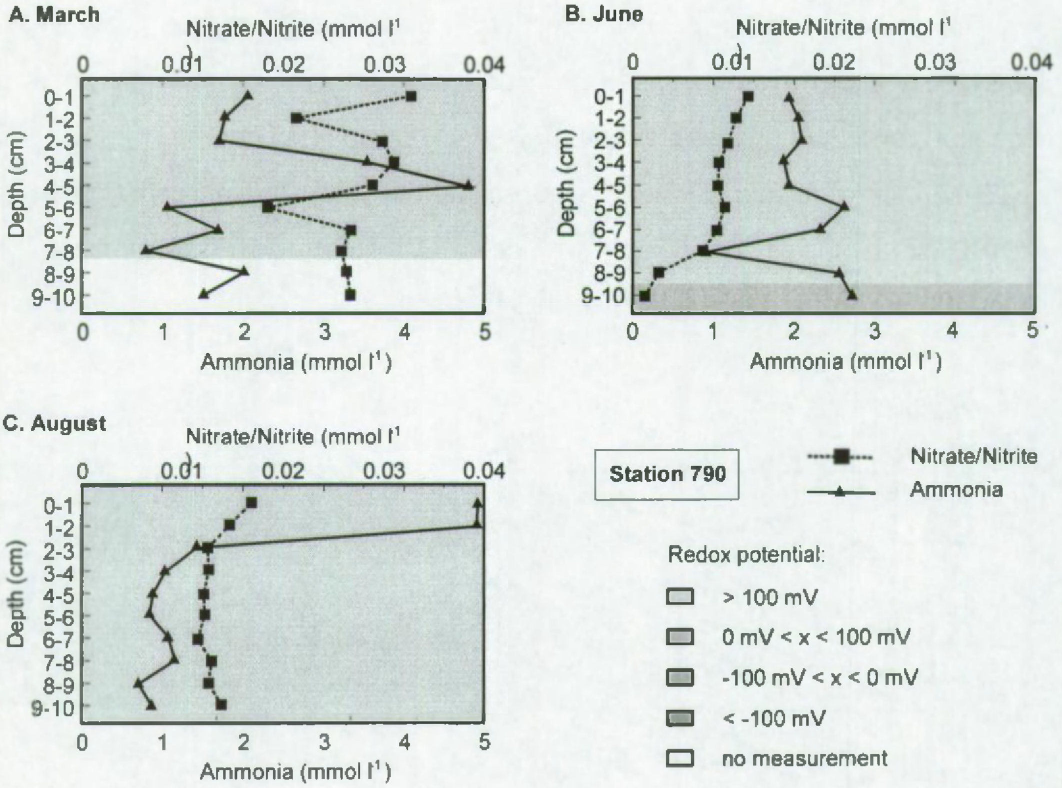

**Figure 5. Redox potentials and nitrogen compounds at Station 790**

## Density and faunal composition

Based on mean total densities, a clear distinction could be made between the three subtidal stations (Fig. 6). Highest abundances were recorded in Station 115, especially in June. At Station 702 lower abundances were found, except for the very high August value. The meiofauna exhibited lowest values in Station 790. Here, as well as at Station 702, densities increased from March to August. Average relative abundances of species constituting >1 % of the fauna at the three stations are shown in Table 3.

*Sabatieria punctata* was most abundant at the fine sandy, subtidal stations (115 and 702), followed by *Daptonema tenuispiculum*. In the coarse sandy station (790), *Ixonema sordidum* was the most important species, followed by *Rhynchonema quemer* (Boucher, 1974), *Viscosia*

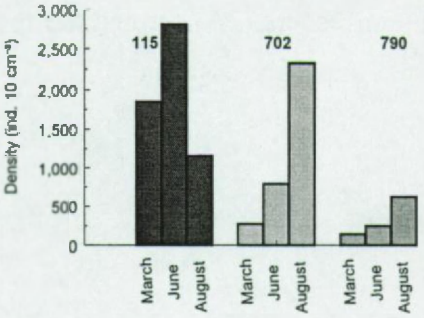

**Figure 6. Mean total densities**

| | Trophic group | Relative abundance of most important species (>1%) | | |
|---|---|---|---|---|
| Species | (Wieser 1953) | Station 115 | Station 702 | Station 790 |
| *Ascolaimus elongatus* | 1B | 2.1 | 7.7 | |
| *Chromadorina demani* | 2A | | | 2.4 |
| *Cyartonema elegans* | 1A | | | 3.0 |
| *Daptonema sp.* | 1B | 5.3 | | 1.1 |
| *Daptonema normandicus* | 1B | 2.3 | | |
| *Daptonema tenuispiculum* | 1B | 6.5 | 11.2 | |
| *Daptonema trichinius* | 1B | | 1.6 | |
| *Desmodora sp.* | 2A | | | 1.3 |
| *Desmolaimus zeelandicus* | 1B | | 1.1 | |
| *Enoploides sp.* | 2B | | | 2.9 |
| *Ixonema sordidum* | 2A | | | 24.7 |
| *Metalinhomoeus sp.* | 1B | 2.3 | | |
| *Microlaimus sp.* | 2A | | | 1.5 |
| *Microlaimus conothelis* | 2A | | | 2.2 |
| *Neochromadora hyalocheile* | 2A | | | 7.1 |
| *Odontophora phalarata* | 1B | | 2.2 | |
| *Onyx perfectus* | 2B | | | 1.6 |
| *Paracanthonchus sp.* | 2A | | 1.0 | 1.8 |
| *Rhynchonema quemer* | 1B | | | 8.1 |
| *Richtersia sp.* | 1B | | 1.5 | |
| *Richtersia inaequalis* | 1B | | 7.2 | |
| *Sabatieria punctata* | 1B | 73.3 | 51.4 | 2.4 |
| *Sigmophoranema rufum* | 2B | | 1.6 | 1.7 |
| *Spirinia parasitifera* | 2A | 1.3 | | |
| *Syringolaimus sp.* | 2B | | 1.8 | 1.5 |
| *Syringolaimus caspedum* | 2B | | | 2.8 |
| *Tubolaimoides sp.* | 1A | | | 4.1 |
| *Viscosia langrunensis* | 2B | | 4.4 | 7.9 |
| *Xyala imparis* | 1B | | | 1.9 |
| Total | | 93.1 | 92.6 | 80.1 |

**Table 3. Composition of nematode assemblages at subtidal stations (115, 702 and 790)**

*langrunensis* and *Neochromadora hyalocheile*. Except for *Viscosia langrunensis*, which has also been recorded in Station 702, these species were found only at Station 790.

Stations 115 and 702 were dominated (>80 %) by non-selective deposit feeders (1B), whereas at Station 790 the epistratum feeders (2A) formed the most important trophic group (>40 %; Table 3).

## Diversity and dominance

In total, 56 species were found at Station 115, 45 species at Station 702 and 98 species at Station 790. Rank 1 dominance (R1D) values were higher at Stations 115 (73.3%) and 702 (51.4%) than at Station 790 (24.7%).

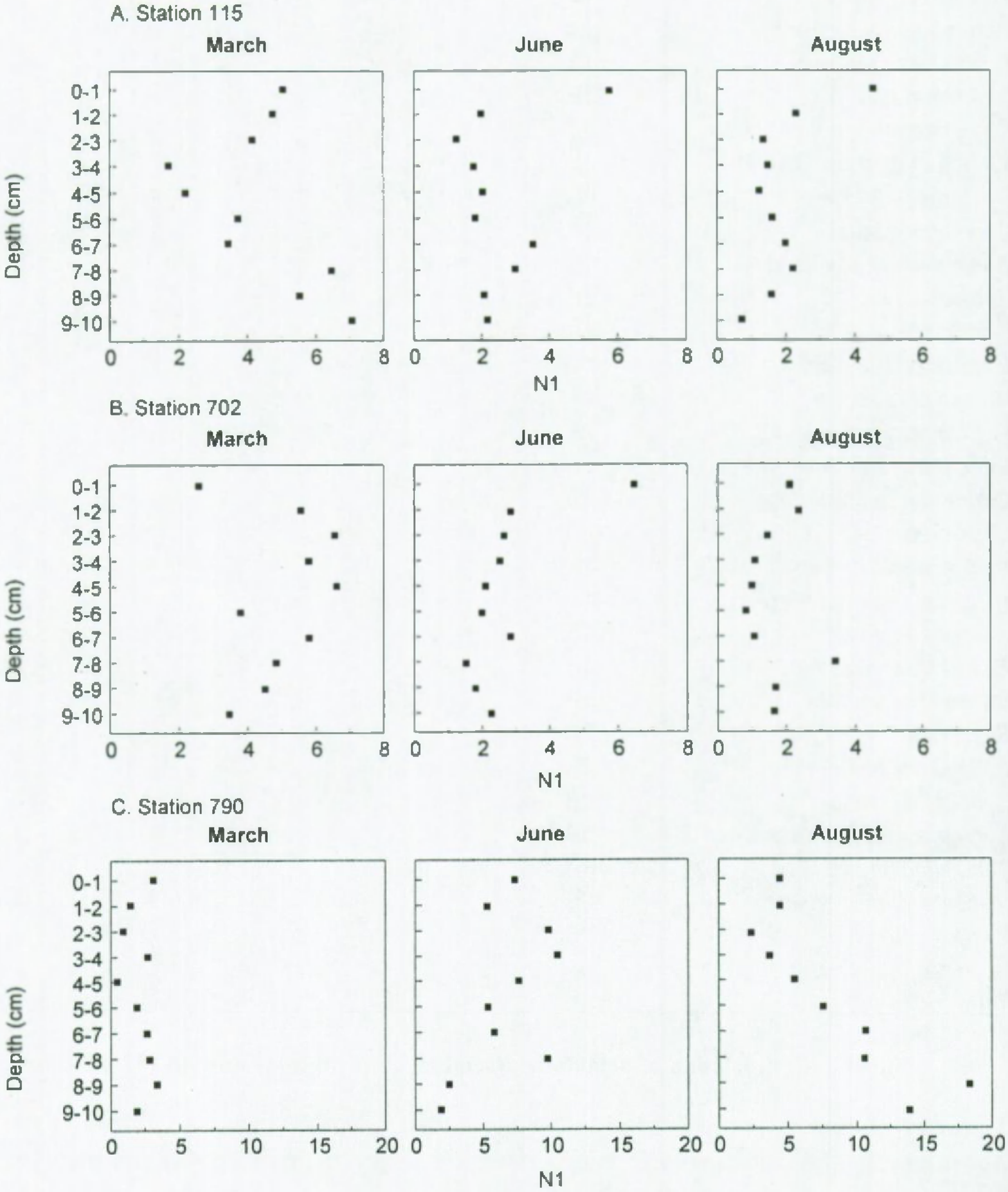

**Figure 7. Variability of $N_1$ species diversity in each station (average of three replicates)**

The overall species diversity ($N_1$) was similar at both muddy to fine sandy Stations (115 and 702), but lower than June and August values at Station 790, where the sediment consisted of coarse sand (Fig. 7). At the former two stations, the highest species diversity was recorded in March and the lowest in August. This contrasts with Station 790, where diversity levels increased from March to August.

## Vertical trends within the sediment

*Abundance trends.* The vertical distribution of nematodes within the sediment varies between stations and seasons. At Station 115, nematodes were most abundant at 3 to 4 cm depth during the three sampling periods. Except in June, when the sediment column was most strongly reduced, a second peak was developed at the surface. At Station 702, densities were rather low and nearly constant with depth in March, but higher during June and August, reaching maximum values in subsurface sediment layers (1 to 2 cm) and thereafter decreasing gradually with depth. At Station 790, nematode abundance was low and constant with depth in March whereas in June, there was a clear decrease with depth. In August, there was no distinct trend.

*Diversity trends*[3]. At Station 115 in March, $N_1$ diversity decreased down to 8 cm depth below which an abrupt increase occurred to a level even higher than in the top centimetre (Fig. 7). These higher $N_1$ values were attributed to five species, *Daptonema normandicum* (de Man 1890), *Leptolaimus* sp. (de Man 1876), *Metalinhomoeus* sp. (de Man 1907), *Microlaimus conothelis* (Lorenzen 1973) and *Molgolaimus cuanensis* (Platt 1973), which became more important in these deep sediment layers.

| Station | Month | | Spearman (*r*) | p level |
|---|---|---|---|---|
| 702 | March | % mud | 0.661 | 0.04 |
| | June | % mud | 0.744 | 0.01 |
| | | Ammonia | -0.709 | 0.02 |
| 115 | March | % mud | 0.915 | 0.0002 |
| 790 | August | Bacteria | -0.855 | 0.002 |

**Table 4. Spearman rank order correlations. Only significant correlations of the different sediment layers between species diversity ($N_1$) of total nematode community and particular environmental factors are shown**

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[3] Since diversity indices are dependent on sample size, and abundances are often low (< 120 ind. 10 $cm^{-2}$), especially in deeper sediment layers, the interpretation of depth profiles of diversity indices need to be done with care.

A positive correlation between $N_1$ species diversity and the mud content (<62 µm fraction) of different layers could be demonstrated using a Spearman rank test (Fig. 7; Table 4). There was a similar trend between the diversity of non-selective deposit feeders and the amount of mud in the sediment (Table 5). In June and August, diversity decreased with depth at this station, showing a sharp contrast between the first centimetre and the remainder of the sediment column, where the diversity was much lower.

| Station | Month | Feeding type | | Spearman (*r*) | *p* level |
|---|---|---|---|---|---|
| 702 | June | 1B | Redox potential | 0.786 | 0.04 |
| | | | Nitrate/nitrite | 0.644 | 0.04 |
| | | | Ammonia | -0.673 | 0.03 |
| 115 | March | 1A | % mud | 0.720 | 0.03 |
| 790 | March | 2A | Chlorophyll *a* | 0.937 | 0.00006 |
| | August | 1B | Bacteria | -0.879 | 0.0008 |
| | | 2A | Bacteria | -0.891 | 0.0005 |

**Table 5. Spearman rank order correlations. Only significant correlations of the different sediment layers between species diversity ($N_1$) within trophic groups and particular environmental factors are shown**

Sediment samples obtained from Station 702 in March exhibited a high degree of oxidation and a very gradual decrease in $N_1$ species diversity with depth (Fig. 7). Here, too, a positive correlation could be found between $N_1$ values and mud fraction (Table 4). In June, the overall diversity pattern with depth at this station resembled that of Station 115 in June and August. Only species diversity of non-selective deposit feeders, the dominant trophic group of Station 702, was positively correlated with the redox potential (Table 5). In August, when the sediments at Station 702 were most strongly reduced, a nearly constant, low species diversity prevailed throughout the sediment, except at 7 to 8 cm depth, where a peak of higher diversity could be noticed. Although this higher diversity coincided with a higher redox potential, no correlation with any measured abiotic factor could be established.

At Station 790, species diversity levels remained relatively low and nearly constant with depth in March and were not correlated with any abiotic factor. However, diversity of epistratum feeders was positively correlated with chlorophyll *a*, suggesting a relation with food resources. The fluctuating pattern in species diversity, which was found in June, could not be linked to any measured abiotic factor. In August, an extraordinary increase in species diversity with depth occurred. This trend was also reflected in the species diversity of different trophic groups. Thus, negative correlations with bacteria densities were found between $N_1$ species diversity of the total community, $N_1$ diversity of epistratum feeders and, somewhat surprisingly, $N_1$ diversity of non-selective deposit feeders (Tables 4 and 5).

*Species distribution.* The depth distribution of the most important species, *Sabatieria punctata, Daptonema tenuispiculum, Ixonema sordidum* and *Viscosia langrunensis* are summarised in Fig. 8. *V. langrunensis* is included in this list of species because it is particularly abundant in one sampling period.

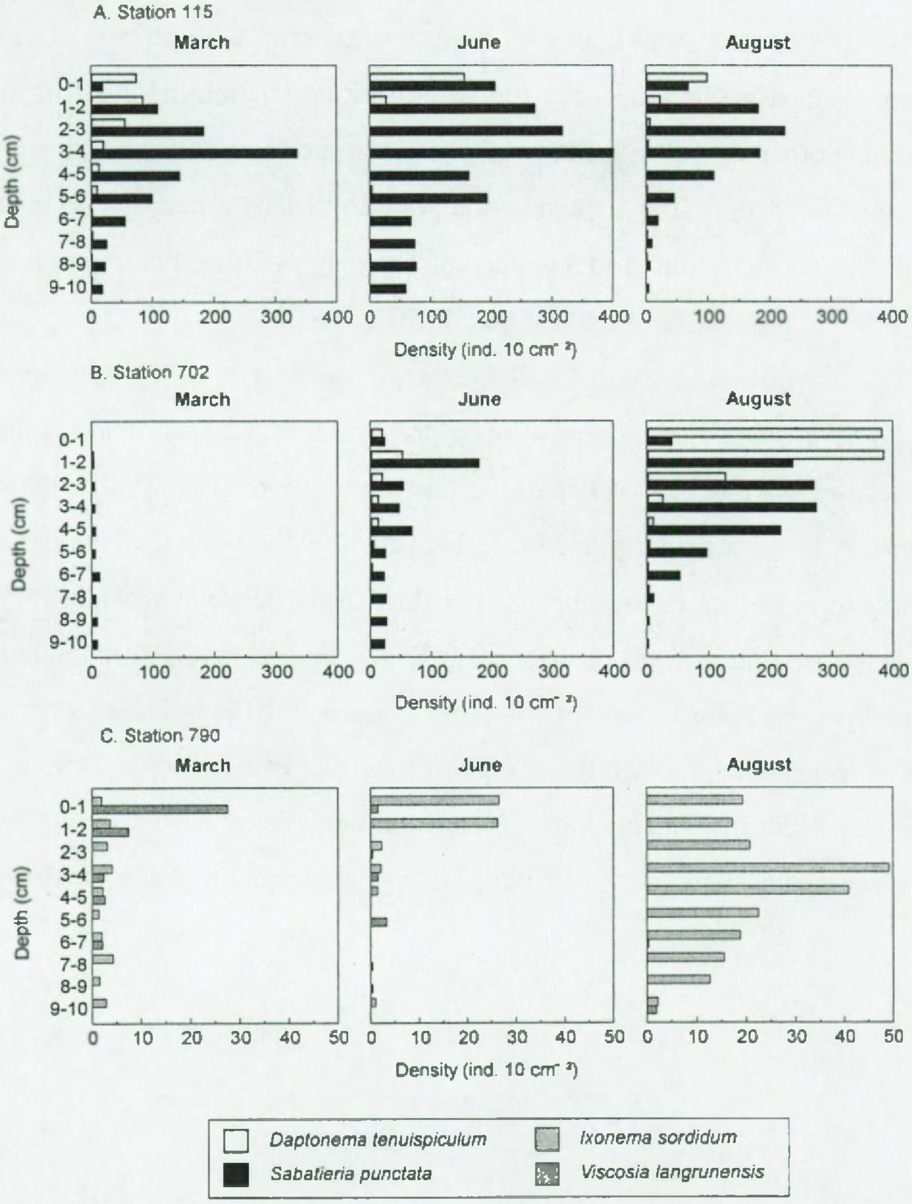

**Figure 8. Depth distribution of *Daptonema tenuispiculum, Ixonema sordidum, Sabatiera punctata* and *Viscosia langrunensis***

The highest overall abundances (total sediment column) of *Daptonema tenuispiculum* and *Sabatieria punctata* were recorded in June at Station 115 and August in Station 702, when sediments at these stations were most reduced (Figs. 2, 4, 8). Extremely low abundances were recorded for both species at Station 702 in March, when sediments were highly oxidised. Despite their fluctuating abundance, the two species exhibited similar distribution patterns with depth at both stations and during different seasons, apart from the aberrant March sample from Station 702 when abundance were too low to determine patterns reliably. The maximum densities for *D. tenuispiculum* always occurred in the surface layers. From 3 to 4 cm downwards, densities were halved or even reached zero. Only in two cases, March at Station 115 and August at Station 702, were significant correlations found with redox compounds. Concerning other variables, the only other correlation was a positive one with bacteria densities for August at Station 702. *S. punctata* appears to penetrate deeper into the sediment; highest densities were found from 3 to 4 cm depth. The only significant correlation was found with the sediment mud content for August at Station 702.

The two most abundant species at the coarse sand station (790), *Ixonema sordidum* and *Viscosia langrunensis,* constitute a lower proportion of the total fauna at that station, than do *D. tenuispiculum* and *S. punctata* at the fine sand stations (Fig. 8). The density of *I. sordidum* was mostly low, particularly in March. However, in June samples, the density of this species was six times higher in the upper 2 cm than in deeper layers, while in August samples it exhibited a peak at a depth of 4 to 5 cm. For *V. langrunensis,* on the other hand, abundance was high in the upper layers of samples obtained in March, but a constant low abundance prevailed otherwise. Though the oxidation state of the sediment at Station 790 did not show any obvious seasonal fluctuations, an apparent periodicity existed in the depth distribution of both species. This could not be attributed to any single abiotic factor measured in this study.

# Discussion

## Limitation of the data set

At a particular station, characterization of the nematode fauna during each month (May, June, August) was made on three subcores. However, the sets of subcores were not strictly replicates since they were each taken from a simple boxcore. Hurlbert (1984), discussing pseudoreplication *vs* the design of ecological field experiments, stated that replication reduces the effects of 'noise' or random variation or error, thereby increasing the precision of an estimate, *e.g.* the mean of a treatment or the difference between two treatments. On the other hand pseudoreplication was defined as the use of inferential statistics to test for treatment effects with data from experiments where either treatments are not replicated (though samples may be) or replicates are not statistically independent. In the case of this study, pseudoreplicates are justified because the mathematical analysis adopted did not demand statistical independent samples.

The aim of this study was to establish micro scale vertical distribution patterns in relation to different biogeochemical variables. To do this, replicates of the same sediment type, from the same microenvironment were needed. Although the Belgian coastal zone is, in general, rather fine grained, compared to the offshore North Sea, the sediment granulometry within this narrow zone is heterogeneous and variable, especially in highly dynamic regions such as the Westerschelde mouth. Only sampling on a detailed horizontal scale, *i.e.* within one boxcore, ensured uniformity of sediment type at each station. This is the most important reason why the boxcore, as a treatment, could not be replicated. It should also be noted that a total of 10,800 nematodes (3 stations × 3 subsamples × 10 slices × 120 nematodes) were separated and identified, which obviously indicates the enormous cost of analysing vertical distribution patterns of nematodes at species level.

## Controls on species vertical distribution profiles

Literature data on the autoecology of *Daptonema tenuispiculum*, *Ixonema sordidum, Sabatieria punctata, Viscosia langrunensis,* and indeed of most free-living nematode species, are very scarce. However, *D. tenuispiculum* and *S. punctata,* both non-selective deposit feeders, are known to be typical of silty sediments. They are eurytopic species, which can tolerate unstable, highly polluted environments (Vincx 1989a, Heip *et al.* 1990, Vanreusel 1991). This study suggests *D. tenuispiculum* to be a typical surface dweller, which is not affected by the redox state. *Sabatieria pulchra* (Schneider 1906), a brackish water species related to *S. punctata* (Vincx 1986a, Jensen 1981), is also very abundant in disturbed environments, independent of the sediment composition. Several authors have suggested that *S. pulchra* is physiologically well adapted to stressed conditions (Heip & Decraemer 1974, Tietjen 1980, Heip *et al.* 1984, Jensen 1984). It can even survive as a facultative anaerobic species in deoxygenated sediments (Jensen 1984). However, it is incorrect to state that *Sabatieria* species in general exhibit tolerance to long periods of anoxia (Hendelberg & Jensen 1993). Based on morphological characteristics and microhabitat preferences, a distinction can be made between a *S. pulchra* group and a *S. ornata* (Ditlevsen 1918) group. The *S. pulchra* group consists of *S. pulchra, S. punctata, S. granulosa* (Vitiello & Boucher 1971) and *S. claviculata* and lives in reduced sediments, while the *S. ornata* group consists of *S. ornata, S. proabyssalis* (Vitiello & Boucher 1977) and *S. abyssalis* (Filipjev 1918) and is limited to oxidised sediments. Furthermore, some authors found that *S. pulchra* and closely related *Sabatieria* species living in muddy sediments had their population maximum at the RPD-layer (Jensen 1981, Bouwman *et al.* 1984, Platt & Lambshead 1985, Jensen *et al.* 1992). The present study shows that *S. punctata* is able to penetrate deep into the sediment, having its highest density at a depth of 3 – 4 cm in both reduced and oxidised sediments. The vertical distribution of this species into the sediment seems to be dependent on food availability, rather than on oxygen concentrations. Clearly, *Sabatieria* species display a range of ecological preferences, making identification at the species level essential.

The distribution profiles of *Ixonema sordidum* and *Viscosia langrunensis* could not be linked to any measured geochemical characteristic. On the other hand, similar seasonal fluctuations in *I. sordidum* densities and the chlorophyll *a* content of the sediment suggest a close relationship between the species distribution and the availability of food sources. Before the spring bloom, both were rather uncommon. In June, as algal concentrations in the sediment started to increase, *I. sordidum* became more abundant and reached highest densities in late summer (August), when algae were most abundant. However, it is interesting to note that the maximum abundancies of chlorophyll *a* and *I. sordidum* within the sediment profile do not coincide in August. This may be because these nematodes avoid competition for food and space in densely populated sediment layers rich in algae. *Ixonema sordidum* is a rather small nematode, which can move easily within the interstitial spaces. Furthermore, its cuticle is always covered with agglutinated grains and presumable also food particles such as algae. This suggests that it is able to 'carry' its own food reserve to sediment layers where competition from other meiofaunal organisms is less intense.

## Controls on diversity

Stations 115 and 702 had similar sedimentological characteristics. As eutrophication will be indirectly reflected through the redoxstate of the sediment, the seasonal samples collected at these stations can be arranged in the following order ranging from low to high eutrophication impact: March, August, June (Station 115), March, June, August (Station 702). In general, Station 115 was less reduced than Station 702 and the situation in June at Station 115 was comparable to the situation in August at Station 702.

With these characteristics in mind, three models relating diversity to the redox state of silty sediments can be put forward.

(1) In strongly oxidised sediments (March at Stations 115 and 702), an obvious positive correlation existed between mud content of the sediment and $N_1$ diversity of nematodes. This positive correlation could also be recognized for $N_1$ species diversity within non-selective deposit feeders (Station 115)

(2) In oxidised sediments, with the redox potential discontinuity layer (RPD layer) situated somewhat higher in the sediment column (June at Station 702), $N_1$ diversity was coupled both onto mud content and ammonia concentrations. Furthermore, there is a relation between $N_1$ values of non-selective deposit feeders and the redox state of the sediment

(3) In reduced sediments with an oxidised layer of only about 1 cm (June and August at Station 115 and August at Station 702), $N_1$ values were always low and were not influenced by sediment grain size or by redox values.

Although a relationship between species diversity and sediment grain size has been suggested many times in the literature, some controversy about this issue still exists. Heip & Decraemer (1974) found that nematode species diversity was positively correlated with median grain size and negatively correlated with the silt-clay content of the sediment. They ascribed this to the wider range of microhabitats available for meiofauna in sandy sediments compared to muddy sediments. These findings were confirmed by several other authors (see Giere (1993) for an overview), and are consistent with the observation that overall species diversity is higher at the coarse sand station (790) than at the finer grain stations (115, 702). However, the present study suggests that the opposite trend between species diversity and mud content of the sediment may occur within individual cores, especially in oxidised sediments, since $N_1$ values were positively correlated with the mud content at both silty stations in March and at Station 702 in June. The contrast between this result and the conclusions of the previous authors may arise from differences of scale. Earlier studies considered diversity differences between localities, while this study addresses variations within the sediment[4].

When interpreting a correlation between two variables, for example, the diversity of non-selective deposit feeders and the mud content of the sediment, one has to be aware that a significant mathematical relation between both does exist. However, in themselves, correlations do not prove the existence of any causal connection between two variables. The accumulation of mud could be associated with a variety of unknown environmental factors, which create a highly favourable habitat for the non-selective deposit feeders. The sediment may have become finer grained because of the enhanced deposition of organic matter, which in turn, could explain the diversification of non-selective deposit feeders. In this sense mud would have an indirect effect on the non-selective deposit feeders.

Diversity in the strongly reduced silty sediments was low and not related to any of the redox components of the pore water. A plausible explanation is that only a few species can tolerate these conditions. Alternatively, the biochemical composition of the pore water may change over relatively short periods leaving the meiofauna out of phase with the changes.

The coarse sandy sediment of Station 790 had a minute fraction of mud which implied the existence of large interstitial spaces, deep oxygen penetration, and a relative stable redox chemistry spatially (with depth) as well as seasonally. Consequently, the nematode fauna at this site was highly diverse and the vertical distribution of species was not affected by redox processes.

## Anthropogenic effects and spring bloom food inputs

Hitherto, the nematode fauna of the eastern Belgian coast (Westerschelde estuary) has been considered poorer (less diverse) than the fauna of the western part of the Belgian coast Vincx

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[4] The comparison of diversity indices based on 'within core' variability (this thesis) with diversity indices based on 'between stations' variability (Heip & Decraemer 1974) is not recommended considered the difference in sample size.

(1990). The present study, however, suggests that a shift to an enriched nematode assemblage near the Westerschelde estuary has occurred over a period of almost 20 y, as indicated by enhanced species diversity values (Table 6).

| | Eastern | | Western | |
|---|---|---|---|---|
| | June 1977 | June 1993<br>This study | June 1977 | June 1993<br>This study |
| $N_1$ diversity | 1.79 (0.7) | 3.89 | 8.61 ( - ) | 3.44 |
| Number of species (per 10 $cm^{-2}$) | 4 (5) | 40 | 19 ( - ) | 39 |
| Number of individuals (per 10 $cm^{-2}$) | 45 (177) | 745 | 98 ( - ) | 2573 |

**Table 6. Diversity of the eastern and the western Belgian coast. Values between brackets are of June 1978[5].**

On the other hand, the nematode fauna of the western part of the Belgian coast (Station 115) clearly shows a decreasing trend in diversity, presumably related to the presence of finer sediments. The changes in sediment types along the Belgian coastal zone is probably the result of the transport of sediments by current activity (Anon., study IHE-BMM 1993). In addition, the sediment in this region became finer-grained during a period of 1979 – 1993, probably as a result of dredging activities (Vyncke *et al.*1994).

The nematode association is more diverse at Station 790 than at Stations 115 and 702. This is certainly the result of differences in sediment granulometry (see above). However, features such as higher abundance, fewer species, higher dominance (R1D) and lower $N_1$ diversity at Stations 115 and 702 (Table 2) suggest a close association with stressed, organically enriched environments. As both silty stations are located close to the mouths of highly polluted rivers (Westerschelde, Ijzer), the influence of eutrophication on the vertical structure of nematode communities is important at both the eastern and western ends of the Belgian coast. Assuming a seasonally constant input of anthropogenic organic pollutants, the nematode fauna will be most affected in early spring, before the onset of the spring bloom. In this strongly oxidised situation, diversity of the nematode community seems not to be dependent on the redox chemistry of the sediment. However in early summer, after an increased sedimentation of organic material, originating both from anthropogenic and natural sources, the reduced sediment layer slightly shifts towards the surface, influencing the distribution of nematodes and causing a decrease in species diversity. A tight coupling of the vertical distribution of a

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[5] Values of June 1978 (only available for Station 702) were added after publication of the manuscript to allow comparison of indices, obtained with similar methodology.

nematode assemblage to seasonal hypoxic bottom water was also observed by Hendelberg & Jensen (1993) in sediments from a muddy, sheltered bay at the Swedish west coast. As a consequence of oxygen deficiency and sulphide poisoning induced by hypoxic bottom waters during late summer, a mortality of the surface fauna and an upward migration by the subsurface fauna were observed.

## Conclusions

This study of nematode communities at three different sites on the Belgium coast leads to the following conclusions.

(1) Caution must be exercised when lumping *Sabatieria* species into groups according to their morphological characteristics and their affinity for a certain redox state. *Sabatieria punctata*, a species classified previously within the *S. pulchra* group, which is considered to be associated with reduced sediment layers, was abundant in oxidised as well as in reduced sediment layers. The vertical distribution patterns of *Daptonema tenuispiculum, Ixonema sordidum* and *Viscosia langrunensis* also show no relation to redox chemistry. It is suggested that food sources have a more important impact on the vertical distribution of *S. punctata* and *I. sordidum.*

(2) A comparison of the three stations confirms the important influence of sediment granulometry on the diversity of nematode communities. Coarser sediments give rise to a more diverse nematode community, while finer grained sediments are associated with reduced diversity. However, diversity profiles within individual cores indicate that this relationship is not necessary valid at small spatial scales. These profiles showed the inverse relationship, *i.e.* the finer the sediment becomes, the more diverse the nematode community, especially the non-selective deposit feeders. This may be explained by an enhanced deposition of organic matter associated with fine-grained sediments. In the case of coarse sediments with large, well-aerated interstitial spaces, the diversity is high and rather constant with depth and not affected by the redox state of the sediment.

(3) There have clearly been major shifts in the diversity of nematode communities since the work of Vincx (1990). These changes are attributed to a fining of the sediment due to sediment transport, pollution effects and dredging activities.

(4) Anthropogenic impact on the whole community along the Belgium coast is reflected in the

species diversity of nematode communities. In early spring, before the algal bloom, the sediment pore waters were strongly oxidised, allowing the development of high species diversity deep into the sediment. In these situations, diversity was higher in deeper, muddy sediment layers because of the diversification of non-selective deposit feeders. In early summer, as the sediment became more reduced, diversity values decreased with depth. This could be attributed to more favourable redox conditions in the superficial layers, enabling more species to coexist. For the purpose of assessing anthropogenic impacts, the distribution profiles of single nematode species were much less informative than information on diversity profiles of the whole nematode community. The use of nematodes as ecological indicator still remains highly problematic.

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# Chapter III

36176

# Bentho-pelagic coupling: changes in nematode community structure during sedimentation of pelagic primary production in an organically enriched coastal environment (North Sea)

Results presented as

Steyaert M, Vanaverbeke J, Van Gansbeke D, Rousseau V, Parent J-Y, Vanreusel A, Vincx M (submitted) Bentho-pelagic coupling: changes in nematode community structure during sedimentation of pelagic primary production in an organically enriched coastal environment (North Sea)

# Abstract

The response of a nematode community to phytoplankton sedimentation was investigated at an organically enriched station on the Belgian coast. Monthly observations reveal a structural and functional response, mainly related to changes in food availability. During periods of enhanced fresh food supply to the bottom (measured as chlorophyll *a*), nematodes were concentrated in the top two centimetres of the sediment. The subsequent burial of the algal material and mineralisation processes generated highest nematode densities deeper down into the sediment. The dynamics of the total nematode community was reflected in temporal changes in the abundance of epistrate feeders and, to a lesser degree, of the non-selective deposit feeders.

A differential species response was observed within the two dominant genera, *Sabatieria* and *Daptonema*. In April, when living material was supplied to the benthic environment, interspecific competition between *Sabatieria* and *Daptonema* species resulted in vertical depth segregation. In summer and late summer, decomposed algal material was available in excess at depths, enabling species to co-exist.

Sedimentation of a spring bloom event against a background of high levels of organic matter led to a succession of dominant species over time, but did not affect the general species diversity.

## Introduction

Sedimentation of an algal bloom results in a substantial export of particulate organic matter from the pelagic to the benthic ecosystem (Bianchi *et al.* 2002, Hansen & Josefson 2003). This organic fraction of particle input is a potential energy source for benthic organisms. In general, nematodes are known to respond to organic enrichment (Moore & Bett 1989, Soetaert *et al.* 1997, Vanaverbeke *et al.* 1997, Schratzberger & Warwick 1998) and should be able to benefit from phytodetrital pulses (Graf 1992, Middelburg *et al.* 2000), either directly or indirectly. Nematodes feed largely on bacteria, algae and, particulate and dissolved organic matter (Heip *et al.* 1985, Jensen 1987, Giere 1993, Moens & Vincx 1997, Moens *et al.* 1999a, b). Thus, even if they do not exploit phytodetritus directly, they may consume bacteria in superficial sediments, which show often an immediate biomass response to phytoplankton sedimentation (Meyer-Reil 1983, Graf 1992).

Some controversy exists concerning metazoan meiobenthic community responses to a pulsed organic matter supply in sublittoral settings. At shallow depths within the euphotic zone, benthic primary production may be an important factor in regulating community structure (Rudnick *et al.* 1985, Graf 1992, Ólafsson & Elmgren 1997, Vanaverbeke *et al.* subm.). Schulz (1983 in Graf, 1992) found evidence for the upward migration of infaunal meiofauna following organic matter inputs in some sublittoral habitats. Ólafsson & Elmgren (1997) studied nematode species responses to phytoplankton sedimentation and reported a significant increase in subsurface, selective deposit feeders and surface, epistrate feeders after the spring bloom. In contrast, Fleeger *et al.* (1989) found no increase in meiofaunal biomass and abundance following sedimentation events. Data at the major taxon level or community level, however, may conceal more subtle changes at the species level (Fleeger & Shirley 1990, Gooday *et al.* 1996). Species data in relation with phytoplankton sedimentation remain rare but are required to obtain this information to fully understand how benthic communities respond to pulsed organic matter inputs (Shimanaga *et al.* 2000).

In this paper we explore the ecological implications of the phytoplankton bloom sedimentation for a nematode community in an organically enriched area. It is expected that deposition events impact nematode community parameters directly through changes in food availability as well as indirectly, as reflected in vertical distribution patterns of nematode species in relation to redox boundaries. We evaluate both structural (abundance, community composition and species diversity) and functional (trophic composition) aspects of the nematode community on a monthly basis.

## Study site

The sampling locality (Station 115b, 51° 09.1'N, 2° 37.1'E; water depth 10 m) is located in the western zone of the Belgian coast, off the city of Nieuwpoort (Fig. 1). Water temperature varied from 8.0 ° C to 19.8° C and salinity from 32.8 psu to 34.5 psu. This station is located within a half-open gully bounded by sandbanks (Coastal and Flemish banks).

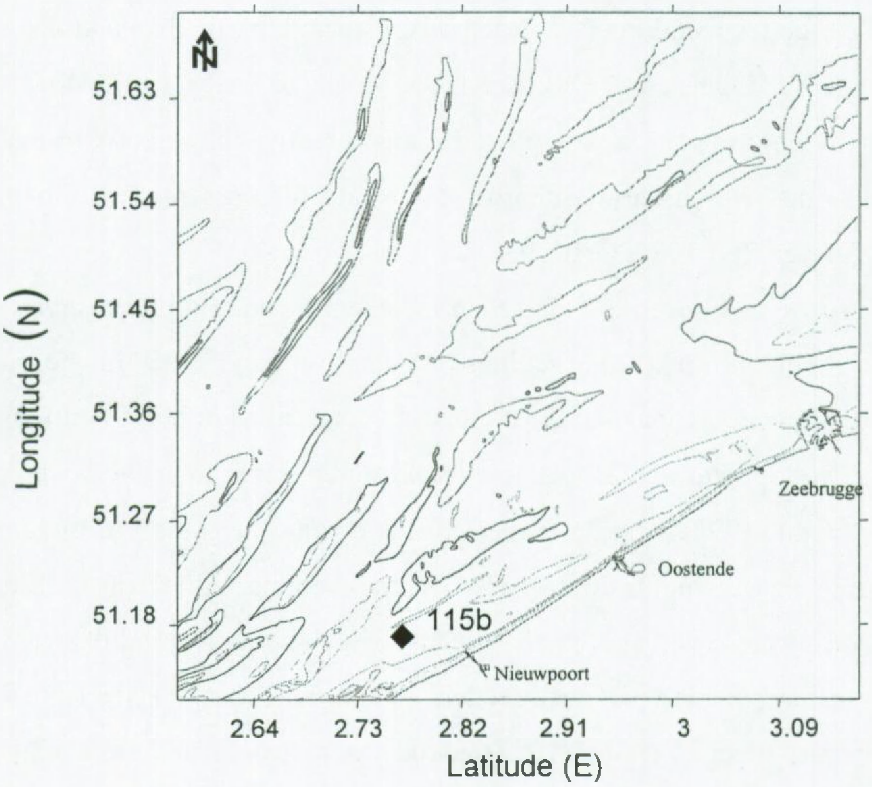


**Figure 1. Map of the Belgian Continental Shelf with indication of the sampling station**

The prevailing hydrodynamic conditions have an important impact on the input and output of material from the surrounding area and regulate concentrations of inorganic and organic matter. As a result, this coastal station is characterised by an enhanced accumulation of organic matter, originating from residual and tidal currents and from riverine sources (Schelde and Ijzer) (Boon *et al.* 1998, VMM 2000).

## Material and methods

### Sample collection

Sampling was conducted monthly from February 1999 to July 1999 from the research vessel *RV* Belgica, Zeehond or Oostende XI. Additional samples were taken in October 1999. A modified Reineck boxcorer (surface area 180 $cm^2$) was deployed 3 times on each sampling occasion. The boxcorer was subsampled using Perspex cores (i.d. 3.6 cm; 6 cm for grain size and nutrients). These subcores were divided into seven slices: 0-0.5 cm, 0.5-1 cm, 1-1.5 cm, 1.5-2 cm, 2-3 cm, 3-4 cm, 4-5 cm. The sediment slices were fixed in a hot (70°C) 4% formaldehyde solution for analysis of the nematode communities. For pigment analysis, one ml of sediment was subsampled using a cut-off syringe. These subsamples were frozen immediately. From April to May, samples were taken approximately weekly in order to assess accurately the timing of the sedimentation and the burial events. Before and after this period samples were analysed at monthly intervals. For the analysis of pore water nutrients, large cores (i.d. 6 cm) were processed and frozen immediately after slicing. The redox potential was recorded simultaneously with a mV-meter.

The phytoplankton community was analysed by examining diatoms and *Phaeocystis* colonies in surface waters. Water samples were obtained with a bucket in order to avoid the disruption of *Phaeocystis* colonies. Samples were preserved with 1% (final concentration) lugol-glutaraldehyde solution and stored at 4°C in the dark until analysis.

### Laboratory analysis

Faunal analysis was carried out at monthly intervals (March 9th, April 21st, May 12th, June 2nd, July 14th, October 25th). Meiofauna (all metazoans passing 1 mm sieve and retained on 38 µm sieve) were extracted from the sediment by centrifugation with Ludox (Heip *et al.* 1985). Nematodes were enumerated after staining with Rose Bengal. Identification to species level was done on the first 120 nematodes (Vincx 1996), mounted on Cobb slides. According to the feeding type classification of Wieser (1953), a distinction is made between selective deposit feeders (1A), non-selective deposit feeders (1B), epistratum feeders (2A) and omnivores (2B). Nematode diversity was expressed as Hill indices $N_0$, $N_1$, $N_2$ and $N_\infty$, (Hill 1973), as recommended by Heip *et al.* (1988).

The concentration of pore water chlorophyll *a* was determined by HPLC (Gilson) using a method slightly modified from that of Mantoura & Llewellyn (1983). The concentrations of nitrate plus nitrite and ammonia in the interstitial water were measured using 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 and processed with a Coulter Counter LS Particle Size Analyser. The median grain size and the fraction of mud (<63 µm) were defined according to the Wentworth scale (Buchanan, 1984).

The C-biomass of diatoms in the water column was determined according to the procedure described in Rousseau *et al.* (2002). The C-biomass of *Phaeocystis* colonies in the water column was calculated according to Rousseau *et al.* (1990).

### Statistical analysis

Univariate two-way Analysis of Variance (ANOVA) was used to test for differences, in relation to time, depth and time *vs* depth, of total nematode density and diversity, the densities of 5 nematode species and feeding type distribution. A 'split-plot' design was constructed following Steyaert *et al.* (2001) with replicates nested within 'time' but not within 'depth'. Densities were square root transformed prior to analysis.

In order to determine the degree to which total nematode density distribution is impacted by small-scale spatial heterogeneity and by temporal effects, the % variation among dates was calculated in ANOVA, following Vanaverbeke *et al.* (subm.).

Correspondence Analysis (CA) following Detrended Correspondence Analysis (DCA) was applied to assess total community variability based on mean values of non-transformed relative abundances. Species rarer than $F_{max}/5$ (with $F_{max}$ the frequency of the commonest species) were downweighted in proportion to their frequency. Species commoner than $F_{max}/5$ were not downweighted.

## Results

### Study site and environmental data

Phytoplankton carbon biomass (diatoms and *Phaeocystis*) in the surface waters, showed that a substantial spring bloom occurs from mid-March to early May (Fig. 2). The spring succession was initiated in March by diatoms that were present at low levels of abundance (max. 0.26

mgC $l^{-1}$) throughout the entire spring period. In April, *Phaeocystis* colonies developed and reached a maximal biomass of 1.27 mgC $l^{-1}$ at the beginning of May before declining rapidly to low levels by mid-May (Fig. 2).

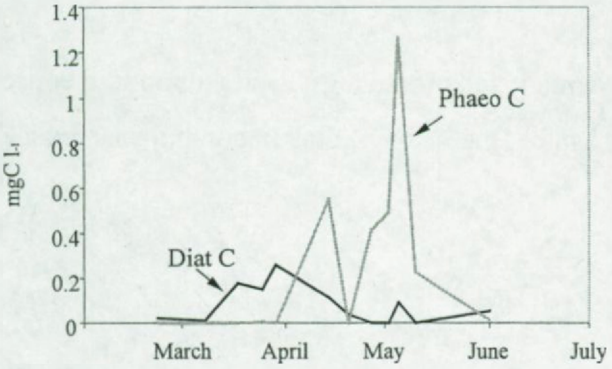
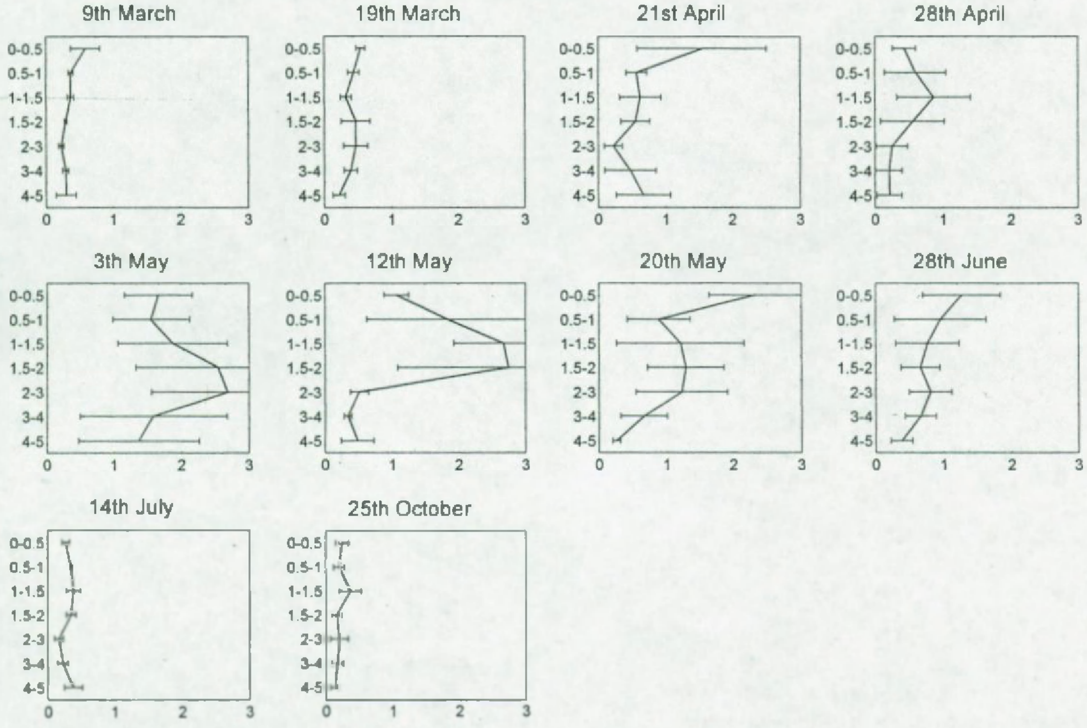


**Figure 2. *Phaeocystis sp.* carbon biomass and diatom carbon biomass in the water column**

Sediments consisted of fine sand (median grain size 185 ± 1.5 μm) with a small fraction of mud (4% < 63μm). Chlorophyll *a* concentrations in the sediment were low in March (< 588 ng $g^{-1}$ in all sediment layers) (Fig. 3).


9th March

19th March

21st April

28th April

3th May

12th May

20th May

28th June

14th July

25th October

**Figure 3. Chlorophyll *a* depth profiles in the sediment at Station 115b (mean values SE) (μg $g^{-1}$)**

From 21st April, there was a general increase, initially observed in the surface layer (21st April). A subsurface peak at 1-3 cm formed progressively, starting at the end of April and reaching maximum values in mid May, one week before the second surface input. From June onwards, chlorophyll *a* concentrations declined to their original values. Lowest concentrations were recorded in October.

Depth profiles of redox potential, and nitrate/nitrite and ammonium concentrations indicate that, except for the 0-0.5 cm in March, the whole sediment column was reduced at all sampling dates (Fig. 4).

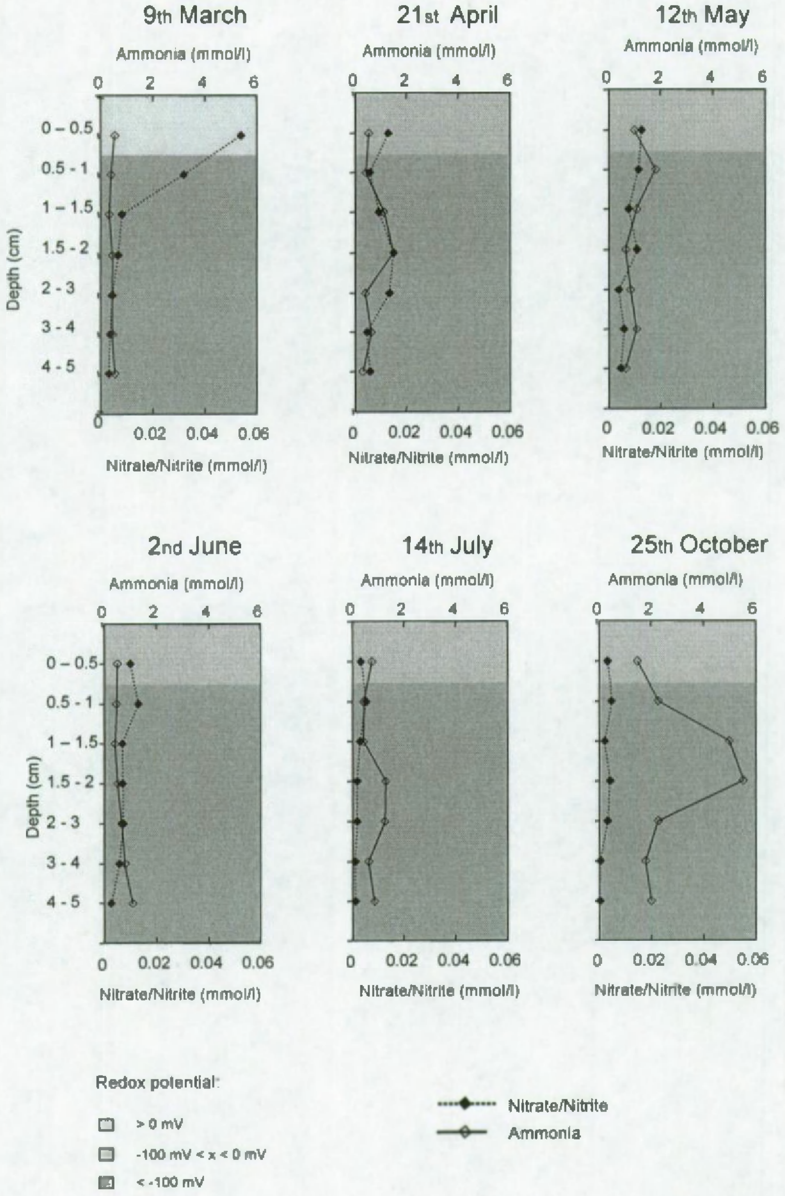


**Figure 4. Redox potentials and nitrogen compounds for the 6 sampling events**

## Nematode community

Mean total nematode densities varied from 840 ± 172 ind. 10 $cm^{-2}$ (March) to 3970 ± 1884 ind. 10 $cm^{-2}$ (July), although no statistical differences were found between monthly densities (Fig. 5).

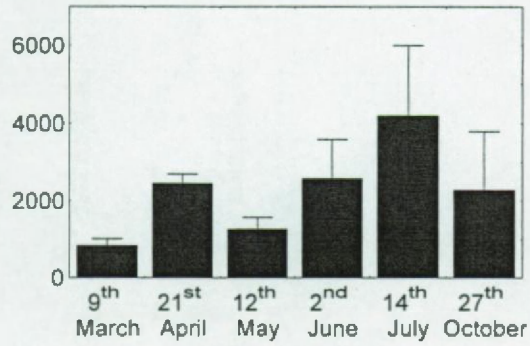


**Figure 5. Nematode abundance on the 6 sampling events (ind. 10 $cm^{-2}$)**

The proportion of variation among dates is 52 % (Table 1). In total, 115 nematode species were identified.

| | Mean | Standard Deviation | % among months | p |
|---|---|---|---|---|
| Total density | 2033.2 | 1106.7 | 52 | 0.136 |
| 1A | 153.6 | 119.2 | 75 | 0.008 |
| 1B | 1213.6 | 734.8 | 53 | 0.129 |
| 2A | 664.4 | 536.2 | 72 | 0.013 |
| 2B | 144.7 | 78.8 | 50 | 0.170 |

**Table 1. Mean densities, standard deviation and percentage of variation due to temporal effects of the feeding types and total nematode community**

Further analysis is restricted to the total nematode community and to 5 dominant species (all non-selective deposit feeders) belonging to the two most important genera: *Daptonema fistulatum* (8 %), *D. proprium* (3 %), *D. riemanni* (5 %), *Sabatieria celtica* (19 %) and *S. punctata* (8 %). Except in the case of *S. punctata,* a significant difference was found between sampling events (Table 2).

| | Month (df=5,10) | | Depth (df=6,12) | | Month x Depth (df=30,72) | |
|---|---|---|---|---|---|---|
| | *F* | p | *F* | p | *F* | p |
| Total nematode community | 3.008 | 0.065 | 13.677 | < 0.001 | 1.691 | 0.036 |
| *Daptonema fistulatum* | 3.993 | 0.030 | 3.137 | 0.044 | 1.528 | 0.074 |
| *D. proprium* | 13.982 | < 0.001 | 7.197 | 0.002 | 0.752 | 0.805 |
| *D. riemanni* | 9.263 | 0.002 | 6.415 | 0.003 | 1.722 | 0.032 |
| *Sabatieria celtica* | 10.621 | < 0.001 | 0.816 | 0.578 | 2.549 | < 0.001 |
| *S. punctata* | 2.184 | 0.137 | 2.828 | 0.059 | 1.299 | 0.183 |

**Table 2. Univariate ANOVA tests**

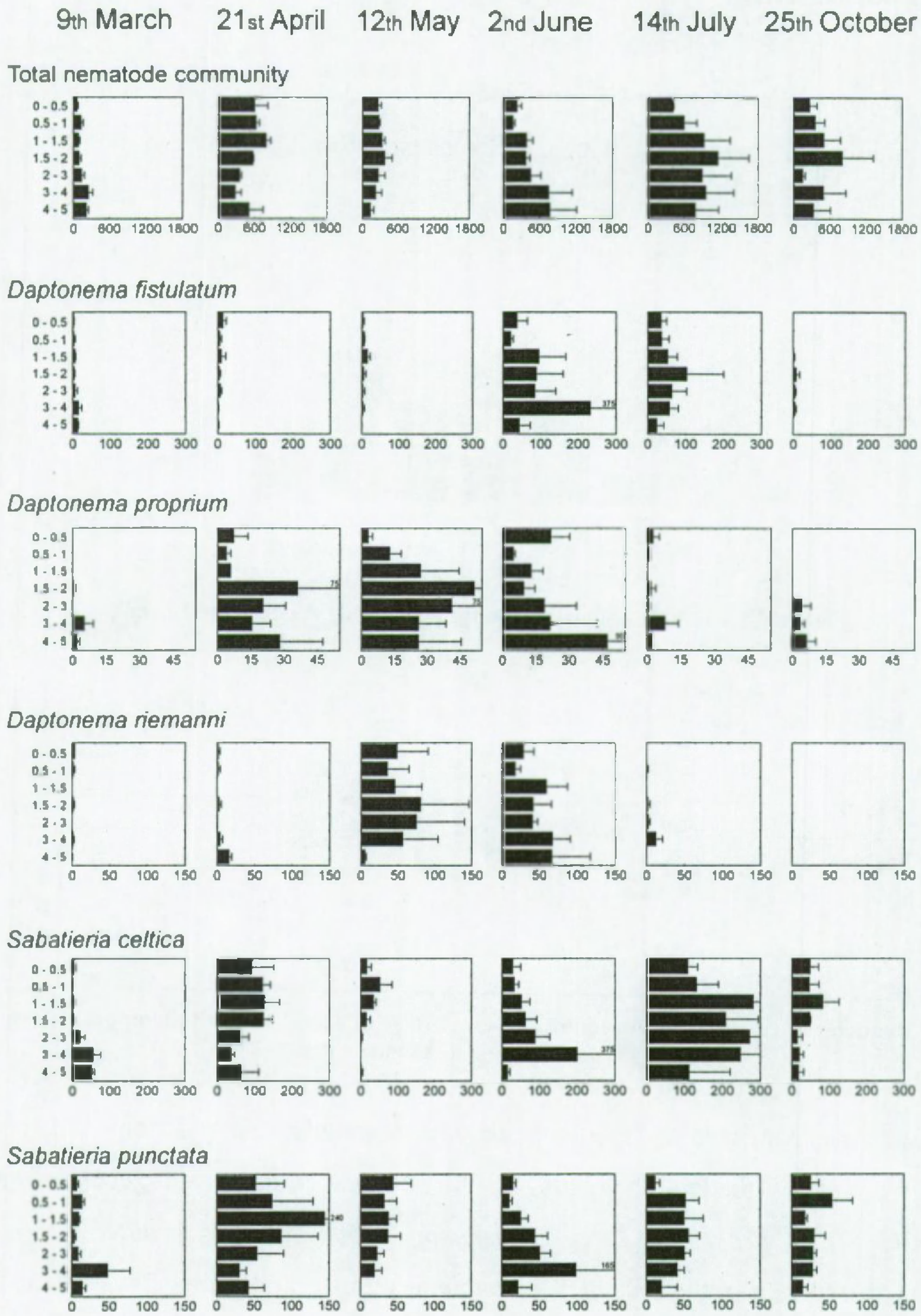


**Figure 6. Depth distribution of the nematode species and the total nematode community for the 6 sampling events (horizontal axes: ind. 10 cm$^{-3}$, vertical axes: depth in cm)**

Nematode vertical profiles changed significantly during the sampling period (Fig. 6, Table 2). In March, no obvious pattern was observed, while in April and May, nematodes tended to be concentrated in the surface layers (68 % between 0-2 cm). In June, highest density was recorded in the 4-5 cm layer, while in July and October, highest densities were found at 1.5-2 cm depth.

Split plot ANOVA for the time × depth interaction was only significant for two of the five species (Table 2) proving that the depth distribution of these species was changing significantly with time. The vertical distribution trends for *S. celtica* and *S. punctata* were similar to those observed for total nematodes. The *Daptonema* species were present in considerable densities during a limited part of the sampling period, and preferred the deeper sediment layers.

Correspondence Analysis (Fig. 7) demonstrates that temporal changes in nematode composition occurred in all depth layers (from 0-5 cm). The different vertical layers were grouped according to the months (eigenvalue of the first and second axes: 0.313 and 0.267). In general, March samples are plotted separately from all other samples.

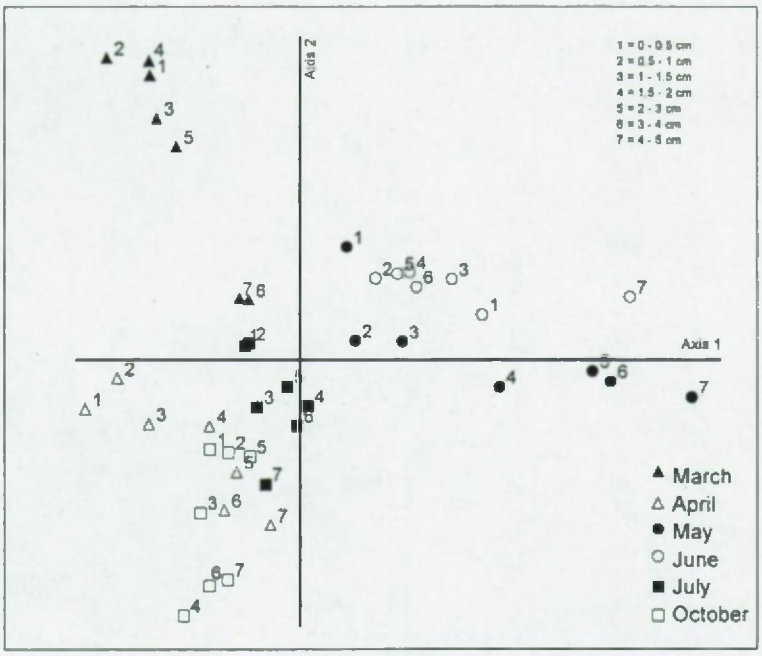


**Figure 7. Results of Correspondence Analysis**

Total nematode diversity was constant over time, as reflected by the Hill indices which showed no significant differences between months, depths or months × depths.

No significant differences were found for the relative contribution of feeding types. In average, non-selective deposit feeders (58 %) represented the dominant feeding group and epistrate feeders (28 %) were the second most important feeding group. Significant differences between months ($F_{5,10} = 5.20$; $p \leq 0.01$) and month × depth ($F_{30,72} = 1.87$; $p \leq$

0.02) were found only for epistrate feeders. Total densities were obviously higher April, July and October (Fig. 8).

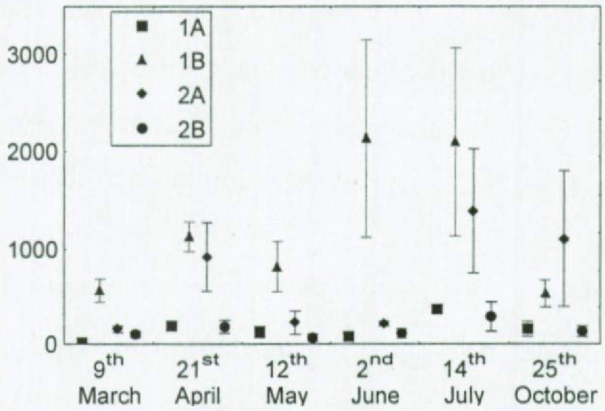


**Figure 8. Mean densities per feeding group for the 6 sampling events (ind. 10 cm$^{-2}$)**

In April, numbers of 2A-nematodes were clearly higher in the top sediment layers, relative to those present in July and October (Fig. 9).

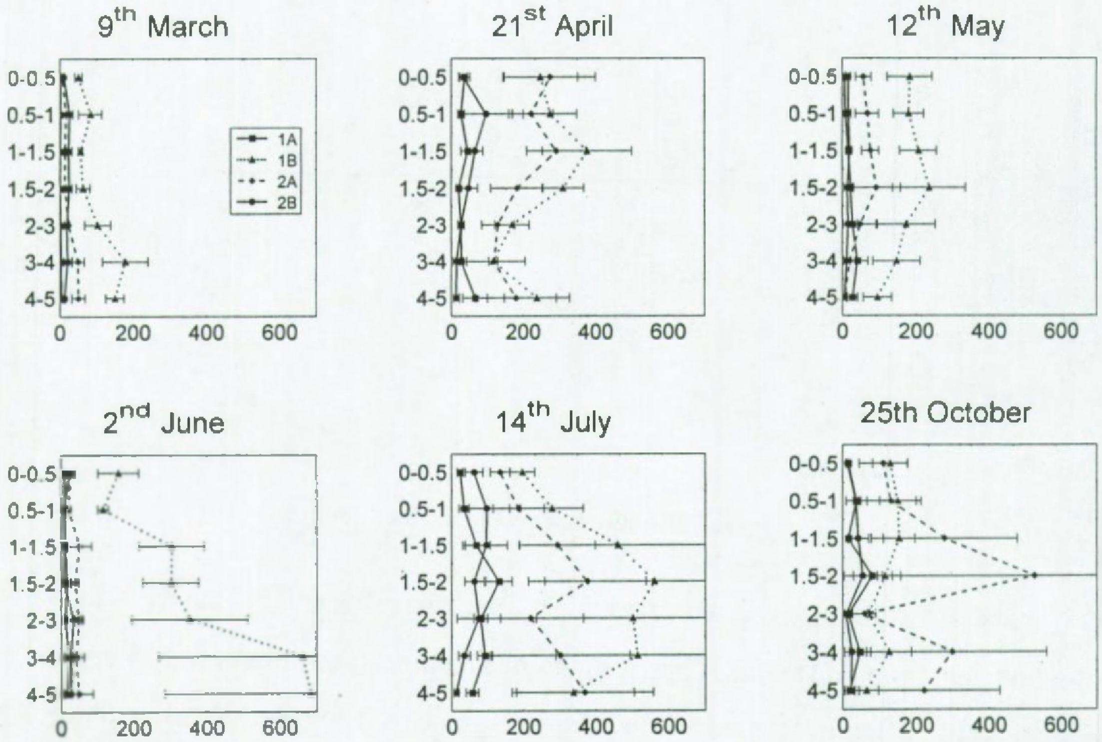


**Figure 9. Feeding type distribution in depth (horizontal axes: ind. 10 cm$^{-3}$; vertical axes: depth in cm)**

# Discussion

## Environmental changes

The observed phytoplankton patterns at Station 115b follows the general pattern described for the Belgian Continental Shelf (Rousseau *et al.* 2002) and the North Sea in general (Boon *et al.* 1998). Pelagic diatoms are important contributors to the algal C-content at the sea surface in March and until *Phaeocystis* becomes dominant at the beginning of May. These changes in the pelagic phytoplankton communities corresponded well benthic trends. The sediment surface chlorophyll *a* peak at 21st of April coincided with a crash in algal C in the water column, suggesting the settling of labile organic matter on the seafloor (Boon *et al.* 1998). Burial of this organic matter was observed in subsequent weeks. A second sedimentation peak was then observed on the 20th of May, preceding a crash of *Phaeocystis* in the water column. As indicated by the chlorophyll *a* inventory, our chlorophyll *a* data show an accumulation of chlorophyll *a* in spring. This spring input may fuel a late summer benthic mineralisation, demonstrated by an accumulation of ammonium (Soetaert *et al.* 1996). A similar pattern of carbon accumulation in spring and mineralisation in late summer is described by Boon & Duineveld (1998).

## Response of the nematode community

Despite the fourfold higher mean values in July, no significant differences between months in nematode abundances was found. This may have resulted either from a gradual change of densities over months or from the large small-scale spatial variation of nematode densities (cfr. large standard errors) which masked possibly temporal effects. Spatial variation is especially pronounced in June, July and October, when all organic matter is incorporated and mineralised in the sediment, and is most probably related to a patchy distribution of food material (Moens *et al.* 1999a). A patchy distribution and incorporation of algal material into the sediment has also been invoked to explain the small-scale variation of nematode communities in sandy sediments on the Belgian Continental Shelf (Vanaverbeke *et al.* subm.). The dynamics of the total nematode community at Station 115b is clearly reflected in the temporal variation of the epistrate and the non-selective deposit feeders. Both of these feeding types are likely to be strongly affected by the sedimentation of the phytoplankton bloom (Ólafsson & Elmgren 1997, Danovaro & Gambi 2002). Evidence comes from their food sources; non-selective deposit feeders ingest food particles such as larger bacteria, detritus and diatoms (Wieser 1953, Nehring 1992, Moens & Vincx 1997), whereas epistrate feeders feed primarily on diatoms and other algae, which are scraped off from sand grains or which are pierced with one or more teeth (Wieser 1953, Moens & Vincx 1997). The occurrence of large quantities of pelagic diatoms in our samples confirms the deposition of living diatoms on the sediment surface. This would favour epistrate feeders (mainly *Microlaimus conothelis*). A significant increase of the number of viable planktonic diatoms in sediment samples is also reported by Hansen & Josefson (2003). A fast colonisation of the deposited algal material by mineralising bacteria (Meyer-Reil 1983) may consequently favour non-selective deposit feeders.

The vertical distribution profile of the total nematode community was significantly different between months. The dominance of individuals in the surface sediments in April is mirrored in the distribution of epistrate feeders and can be explained by enhanced temperature dependant reproduction in the upper sediment layers or by migration from deeper to upper sediment layers. An upward migration is often found in sublittoral environments and is probably related to the supply of fresh food (Schulz 1983 in Graf 1992) or a shallowing of the chemocline (Graf 1992). The latter explanation is unlikely at Station 115b where the sediment was almost continuously reduced over the entire sampling period. The coincidence of high surface densities of 2A-nematodes with the chlorophyll *a* surface peak in April, support the idea that they are closely related to the arrival of fresh diatoms. Interestingly, in July and October, when all labile organic matter was mineralised, 2A-nematodes (mainly *M. conothelis*) were more abundant in deeper sediment layers. This suggests the existence of trophic plasticity among 2A-nematodes. Such species feed preferentially on diatoms. However, when diatoms are not available, they can take advantage of the large reservoir of mineralised material associated with bacteria, as described by Moens & Vincx (1997) and Danovaro & Gambi (2002). Changes in the vertical distribution of 1B-nematodes is reflected in the total nematode distributions and is most likely related to food source distribution, *i.e.* the burial and decomposition of algal C results in a highly heterogeneous mass of detrital particles and associating mineralising bacteria at depth within the sediment.

A differential response to phytoplankton sedimentation was observed in the case of 3 *Daptonema* species and 2 congeneric *Sabatieria* species, all of which belong all to the 1B-feeding guild (Wieser 1953). The vertical distribution of two morphologically similar *Sabatieria* species, namely *S. celtica* and *S. punctata,* was very similar, although significant differences with month and month × depth were only observed for *S. celtica*. Both species were dominant at the study site, and hence their vertical distribution patterns were similar to those of the total nematode community and 1B-nematodes and may be generated by similar causal factors. This is consistent with observations made at a nearby site (Steyaert *et al.* 1999) which indicated that the vertical distribution of *S. punctata* is not primarily related to the redox status of the sediment, but is controlled by food availability. This is the opposite conclusion to that reached by some other authors (Jensen 1981, Bouwman *et al.* 1984, Platt & Lambshead 1985, Jensen *et al.* 1992). *S. punctata* is well adapted to stressed environments (Vincx 1989b, Hendelberg & Jensen 1993) and is able to penetrate to a depth of 4 cm in both reduced and oxidised sediments (Steyaert *et al.* 1999). Moreover, the observations of Vincx (1989b) demonstrate that *S. punctata* feeds on bacteria. The 3 *Daptonema* species (*D. fistulatum*, *D. propium* and *D. riemanni*) were present in considerable densities during certain months but consistently preferred deeper sediment layers. Since both *Sabatieria* species and *Daptonema* species are 1B-nematodes, their different distribution may be a response to interspecific competition for food. This vertical segregation is only necessary when fresh food is delivered to the upper sediment layers in April. From May onwards, the freshly arrived organic matter is buried and progressively mineralised deeper in the sediment. The striking similar distribution profiles of *Sabatieria* and *Daptonema* species in summer and late summer suggest that excess food is available, making competition for this resource unnecessary.

### Productivity versus diversity

Our study area is anthropogenically enriched with relatively high levels of organic material (Steyaert *et al.* 1999). The community might be expected to respond to the high levels of food supply after bloom sedimentation with a decrease in diversity. Moreover, the decrease in habitat heterogeneity associated with these high levels of organic enrichment could lead to an increased dominance by a few species (Steyaert *et al.* 1999, Boyd *et al.* 2000, Levin *et al.* 2001). Our study clearly demonstrates that Station 115b harbours a nematode community, where niches are successively occupied by dominant species. However, the number of species and their relative abundance did not change over time.

## Conclusions

This study of the response of a nematode community at a subtidal station (Station 115b) on the Belgian coast to phytoplankton sedimentation leads to the following conclusions:

(1) In a general sense, the nematode community was food limited in this organically enriched site. Phytoplankton sedimentation generated changes in abundance, community composition and feeding type distribution, largely driven by changes in food availability. The response was mainly expressed as a shift in vertical distribution within the sediment. At periods of fresh food supply to the bottom, animals were more concentrated in the top two centimetres of the sediment. Later, as the algal material was buried and progressively mineralised, highest densities were recorded in deeper sediment layers. These vertical shifts reflected the temporal changes among epistrate feeders and, to a lesser degree, the non-selective deposit feeders.

(2) Species of the 2 dominant genera, *Sabatieria* and *Daptonema* exhibited a differential response to inputs of labile food. In April, when fresh food was supplied to the sediment surface, *Sabatieria* and *Daptonema* species were vertically segregated in the sediment. In summer and late summer, buried and progressively mineralised algal-C became available in excess, enabling species to co-exist in the same depth horizons.

(3) At Station 115b niches are successively occupied by dominant species, although neither the number of species nor their relative abundance differed over time.

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# Chapter IV

36177

# The importance of fine-scale, vertical profiles in characterising nematode community structure

Results presented as

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## Abstract

In this study the spatial heterogeneity of the nematode community on an intertidal flat (the Molenplaat) in the Westerschelde estuary (SW Netherlands) was investigated. It was tested to what extent macroscale (km's) variability was more important than microscale (m's) variability. In addition the importance of vertical distribution profile in the sediment in explaining the horizontal macroscale variability was evaluated. Differences in structure of the community were analysed at a kilometre scale at three sites that differed in chemico-physical features. The differences in geochemical and physical conditions on a horizontal scale were reflected in species composition and trophic structure of the nematode communities, and to a much lesser extent in their total abundance and species diversity.

Detailed investigation of vertical depth profiles showed more pronounced differences between environmentally divergent sites. Sediment granulometry appears to be important in controlling the fauna in the upper sediment layers. At depths similar faunal assemblages were found irrespective of sediment granulometry, suggesting that other environmental features are more dominant.

Vertically, nematode species showed depth distributions that were indicative of sediment characteristics related to the site-specific hydrodynamic regime. Pronounced vertical segregation of nematode species was observed within sandy sediment under strong hydrodynamic and food-stressed conditions. A surface-dwelling nematode community of large predatory enoplids was separated from a deposit feeding xyalid-microlaimid community in deeper sediment layers (beneath 2 cm). Causal factors for this segregation are thought to be species interactions, feeding strategies and/or physical disturbance. In the finest sediments, with high silt content, almost all nematode species were confined to the upper sediment layers (1.5 cm). A sharp decline in density and diversity with depth was observed. Key factors for this distribution pattern are possibly related with the limited oxygen penetration in surface layers and the occurrence of sulphide in deeper sediment layers. At intermediate hydrodynamic and granulometric conditions, a gradual shifting nematode community was observed with depth, with dominant nematode species maxima present at specific depth layers.

## Introduction

Knowledge of spatial patterns of benthic organisms and the scale of these patterns contribute to a better understanding of benthic community structure and functioning. Such information is often the best, if not the only way for assessing interspecific interactions, which to a considerable extent determine community structure. Comparisons between the spatial patterns of consumers and resources provide information on trophic interactions and the spatial scales at which these interactions occur (Pinckney & Sandulli 1990, Sandulli & Pinckney 1999).

On a vertical scale of centimetres, the effect of abiotic characteristics of sediments (*e.g.* oxygen, water content, proximity to surface) on community structure is as important as the other abiotic variables (such as salinity, sedimentological and geomorphological variables), which act on a horizontal scale of hundreds of metres. Moreover, the ecological understanding of the functioning of meiobenthic communities is enhanced by knowledge of animal vertical distribution (Soetaert *et al.* 1994). It has been shown that many nematode species exhibit a typical vertical distribution which often relates to a variety of biological, physical and chemical variables (Warwick & Gee 1984, Giere 1993, Hendelberg & Jensen 1993, Wetzel *et al.* 1995, Soetaert *et al.* 1994, Steyaert *et al.* 1999). It has been argued that the vertical segregation of species will reduce the number of (competitive or predative) interactions, and this could explain the very high number of species that coexist in a certain small patch (Joint *et al.* 1982).

The spatial patterns of temperate nematode communities on different horizontal scales have already been investigated extensively in different estuaries. Most of these studies related structural patterns of the nematode assemblages to environmental variables as sedimentary and latitudinal gradients, food resources, salinity, disturbances of different nature (*e.g.* Warwick & Gee 1984, Soetaert *et al.* 1995, Li *et al.* 1997, Guo *et al.* 2001, Neilson & Boag 2002, Tita *et al.* 2002). The spatial patterns of nematode communities are well documented in intertidal and subtidal zones of the Westerschelde. Soetaert *et al.* (1994) found maximum abundance of the majority of the species in the intertidal zone. Intertidal communities exhibited a well-developed community gradient with sediment depth, whereas the subtidal and channel communities showed distinct and in some cases distorted community patterns associated with large socio-economic pressure by dredging, pollution and consequently oxygen depletion.

This study deals with the spatial heterogeneity of nematode associations on a small intertidal, estuarine flat. Differences in structure of the communities were initially analysed in terms of depth-integrated characteristics for a high number (5) of replicate samples (collected at a m scale) at three geographically separated (at km scale) and chemico-physically diverging sites. In addition to the comparison of bulk characteristics, spatial differences in community structure are established by micro-scaled vertical profile analysis of the same community parameters. We examined whether changing environmental conditions over a small system like the Molenplaat are reflected in the vertical distribution pattern of nematodes species and result in shifting community characteristics with depth in the sediment.

## Study site

The Molenplaat (51°26 N, 3°57 E) is a small intertidal flat (2 to 3 km²), located in the turbid, nutrient-rich and heterotrophic Westerschelde estuary (Fig. 1). Salinity in this region of the estuary varies between 20 and 25 psu (Herman *et al.* 2000). The flat is characterised by a high diversity of sediment types over a small distance. The ecology of the tidal flat has been studied during the project 'Eco-metabolism of an estuarine tidal flat' (ECOFLAT), and detailed background information on pigment distributions (Barranguet *et al.* 1997, Lucas & Holligan 1999), microphytobenthos production (Barranguet *et al.* 1998, Barranguet *et al.* 2000), photosynthetic activity (Kromkamp *et al.* 1998), microphytobenthos resuspension (Lucas *et al.* 2000), nematode feeding behaviour (Moens *et al.* 1999a, 2000, Hamels *et al.* 2001), nematode tidal migration (Steyaert *et al.* 2001) and microbenthic (Hamels *et al.* 1998) and macrofaunal (Herman *et al.* 2000) communities is available.

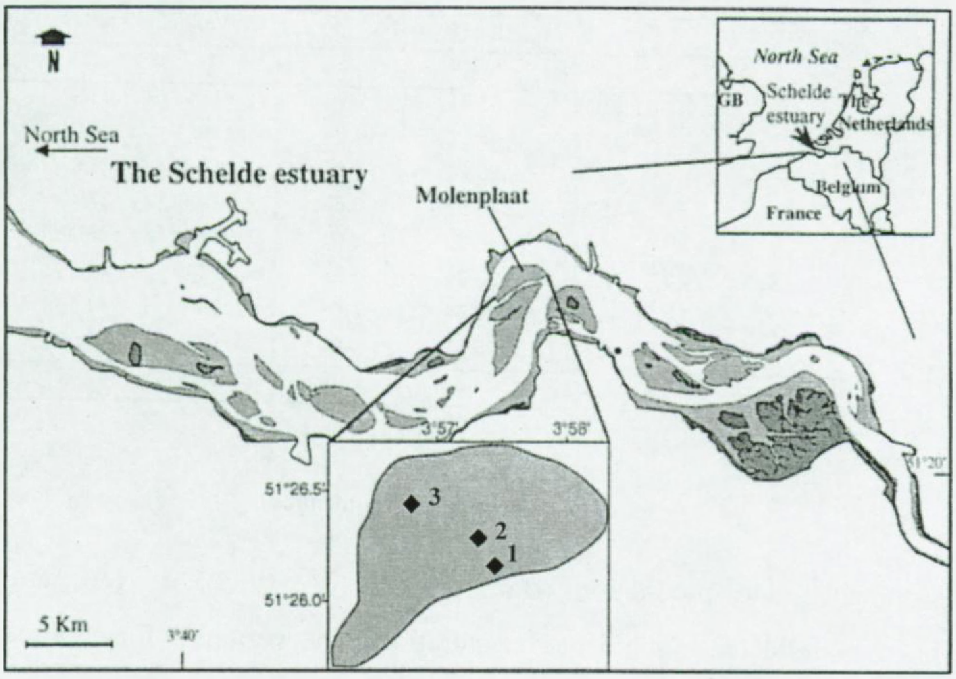

**Figure 1. Location of the sampling sites on the Molenplaat**

The three sites were selected on the basis of their sediment characteristics (Table 1). Site 2 (57°2 N, 26°3 E) has the finest sediment, Site 3 (56°8 N, 26°4 E) is more dynamic and sandier and Site 1 (57°3 N, 26°15 E) has intermediate characteristics (Herman *et al.* 2000, Widdows *et al.* 2000). Estimated of bottom shear stress (maximal value during a tidal cycle) were produced by a hydrodynamic model. Values are 0.36 Pa for Site 2, 0.43 Pa for Site 1 and 1.15 Pa for Site 3 (Van de Koppel *et al.* 2001). The average period of emersion varies between 4.5 h (Site 1) and 7 h (Site 2 and 3) per tidal cycle.

| Depth (cm) | Median grain size (µm) | | | Fine sand fraction (%) | | | Medium sand fraction (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| Site | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| 0-1 | 136.79 | 76.95 | 169.58 | 49.31 | 24.46 | 80.71 | 6.13 | 0.55 | 6.97 |
| 1-2 | 147.62 | 65.61 | 173.14 | 57.31 | 22.78 | 81.12 | 7.26 | 0.53 | 7.47 |
| 2-3 | 127.63 | 44.81 | 173.14 | 43.77 | 12.37 | 79.18 | 6.87 | 0.40 | 8.46 |
| 3-4 | 132.13 | 68.87 | 167.24 | 45.98 | 15.85 | 78.98 | 7.06 | 1.14 | 7.39 |
| 4-5 | 132.13 | 0.01 | 173.14 | 46.61 | 1.57 | 77.87 | 6.56 | 0.04 | 9.33 |
| 5-6 | 161.54 | 87.78 | 176.78 | 45.83 | 22.20 | 78.52 | 18.23 | 1.70 | 10.18 |
| 6-7 | 192.11 | 95.39 | 171.94 | 48.11 | 27.71 | 77.18 | 24.94 | 0.75 | 9.17 |
| 7-8 | 196.15 | 96.72 | 174.34 | 49.72 | 27.80 | 73.61 | 25.74 | 0.88 | 11.95 |
| Mean | 153.26 | 67.02 | 172.41 | 48.33 | 19.34 | 78.40 | 12.85 | 0.75 | 8.87 |

**Table 1. Sediment characteristics for the three sites (data out of the ECOFLAT database, Herman *et al.* 2001)**

| Depth (cm) | Silt (%) | | | Very Fine sand fraction (%) | | |
|---|---|---|---|---|---|---|
| Site | 1 | 2 | 3 | 1 | 2 | 3 |
| 0-1 | 24.13 | 43.32 | 3.96 | 19.74 | 31.67 | 8.14 |
| 1-2 | 11.73 | 48.67 | 4.04 | no data | 28.01 | 7.20 |
| 2-3 | 17.05 | 59.26 | 3.82 | 31.44 | 27.97 | 8.10 |
| 3-4 | 15.35 | 45.51 | 3.88 | 30.74 | 37.49 | 9.42 |
| 4-5 | 14.25 | 0.42 | 3.68 | 31.81 | no data | 8.47 |
| 5-6 | 9.59 | 28.34 | 3.22 | 26.29 | 47.58 | no data |
| 6-7 | 7.24 | 26.94 | 3.67 | 19.71 | 44.56 | 9.26 |
| 7-8 | 6.71 | 26.61 | 4.03 | 17.82 | 44.66 | 9.15 |
| Mean | 13.26 | 34.88 | 3.79 | 25.36 | 37.42 | 8.53 |

**Table 1. (continued)**

Microphytobenthic production (Barranguet *et al.* 1998), as well as microphytobenthic biomass, as reflected in pigment concentrations, was very high for all three sites in June 1996 (Table 2).

| Depth (cm) | Chlorophyll *a* | | | Chlorophyll *c* | | | Fucoxanthin | | |
|---|---|---|---|---|---|---|---|---|---|
| Site | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| 0-1 | 939.39 | 175.20 | 16.48 | 123.77 | 22.95 | 2.96 | 465.62 | 140.34 | 8.55 |
| 1-2 | 79.14 | 94.88 | 22.42 | 15.10 | 16.33 | 4.89 | 48.53 | 64.05 | 12.63 |
| 2-3 | 130.06 | 50.14 | 10.03 | 23.53 | 7.18 | 2.77 | 68.46 | 30.92 | 7.90 |
| 3-4 | 195.93 | 25.96 | 6.29 | 34.79 | 2.48 | 1.12 | 93.23 | 14.84 | 5.61 |
| 4-5 | 51.03 | 11.42 | 2.59 | 8.30 | 0.98 | 0.31 | 23.60 | 7.16 | 2.61 |
| 5-6 | 103.12 | 7.13 | 1.35 | 16.61 | 0.62 | no data | 44.03 | 4.30 | 1.20 |
| 6-7 | 12.89 | 1.15 | 0.72 | 2.23 | 0.06 | no data | 9.38 | 2.37 | 0.78 |
| 7-8 | 12.36 | 5.50 | 1.48 | 1.66 | 0.35 | no data | 10.04 | 4.66 | 1.31 |
| mean | 190.49 | 46.42 | 7.67 | 28.25 | 6.37 | 2.41 | 95.36 | 33.58 | 5.07 |

**Table 2. Photosynthetic pigments for the three sites (in mg $m^{-2}$); chlorophyll *a* data from Hamels *et al.* (1998)**

The distribution of chlorophyll *a* in the sediment was reported by Hamels *et al.* (1998). All pigments (chlorophyll *a*, chlorophyll *c*, fucoxanthin) were negatively correlated ($p < 0.05$) with depth in the sediment. Maximum chlorophyll *a* concentrations were recorded at Site 1; Site 2 had intermediate values and Site 3 had the lowest values. For the three sites, the bulk of the algal pigments was present in the top 2 cm of the sediment. For Site 2 and 3 pigment concentrations decreased gradually with depth whereas for Site 1 a more distorted depth pattern was recorded (Hamels *et al.* 1998).

## Materials and methods

In June 1996, the three sites (at km scale) were sampled during low water at daytime (sediments were exposed to air). At each of the three sites, 5 cores (3.6 cm diameter) were taken at 10 m intervals. The samples were divided into 12 horizontal slices (0-0.5 cm, 0.5-1 cm, 1-1.5 cm, 1.5-2 cm, 2-3 cm, 3-4 cm, 4-5 cm, 5-6 cm, 6-8 cm, 8-10 cm, 10-15 cm, 15-20 cm) immediately after sampling and fixed in a hot, neutral (70° C) 4 % formaldehyde solution. Meiofaunal organisms retained on a 38 μm sieve were extracted from the sediment by centrifugation with Ludox (density 1.18) (Heip *et al.* 1985). All macrobenthos were excluded by a 1 mm sieve. For each slice, all nematodes were counted after staining with Rose Bengal and 120 nematodes were picked out randomly and mounted on Cobb slides for identification to species level. The nematodes were grouped into 4 feeding guilds, according to the feeding type classification of Wieser (1953).

Samples for the pigment analyses were taken from contiguous cores for meiofauna samples. Particle size distribution was determined by laser diffraction using a Malvern particle sizer. Analytical techniques for determination of the pigment content were described in Hamels *et al.* (1998), for the organic carbon content in Herman *et al.* (2000).

Horizontal and vertical patterns in nematode abundance and community composition were analysed using ordination techniques from the PC-ORD for Windows package (version 4.20, McCune & Mefford 1999). Through ordination samples are ordered along axes according to their resemblances. A detrended correspondence analysis (DCA) was applied on vertically integrated densities (summation of all depth layers) to test the variability between replicates. Subsequently, another DCA was used to assess total community variability based on non-transformed relative abundances. Species rarer than $F_{max}/5$ ($F_{max}$ is the frequency of the commonest species) down-weighted in proportion to their frequency. Nematode diversity was expressed as Hill indices $N_0$ and $N_1$ (Hill 1973). In order to test for significant differences in depth integrated (total sediment column) density and diversity between the three sites, the non-parametric Kruskal-Wallis analysis by rank and pairwise multiple comparison tests were used (Conover 1971). If assumptions were met, a univariate two-way analysis of variance (ANOVA) was used to test for significant differences in depth distribution between the three sites. A 'split-plot' design was constructed with replicates nested within 'site', however, not within 'depth', following Steyaert *et al.* (2001). All data were log (x+1) transformed prior to analysis. Non-parametric Spearman rank correlation coefficients were calculated to determine relationships between diversity and environmental variables along a depth gradient.

## Results

### Density and species composition

*Horizontal.* A significant difference in total (depth-integrated) nematode densities ($p \leq 0.05$) was found when comparing all three sites (Table 3). A post hoc multiple comparison revealed only significant differences between Site 1 and 2.

| | Site 1 | Site 2 | Site 3 |
|---|---|---|---|
| Total number of species | 52 | 41 | 54 |
| Nematodes density | 2990 ± 818 | 1560 ± 699 | 2090 ± 666 |
| $N_0$ diversity | 28.4 ± 3.58 | 25.2 ± 1.92 | 32.0 ± 2.90 |
| $N_1$ diversity | 9.1 ± 0.94 | 8.0 ± 2.15 | 8.6 ± 0.77 |

**Table 3. Total number of species, vertically integrated nematode density (ind. 10 $cm^{-2}$) and vertically integrated nematode diversity for the three sites (mean values from 5 samples ± standard deviation)**

Highest abundances were recorded at Site 1, lowest abundances at Site 2 and intermediate abundances at Site 3. In addition, detrended correspondance analysis, based on species densities of bulk samples, separated all three sites and illustrated the high similarity between the five replicates of each site (Fig. 2). Eigenvalues were 0.71 and 0.05 for respectively the first and second axes.

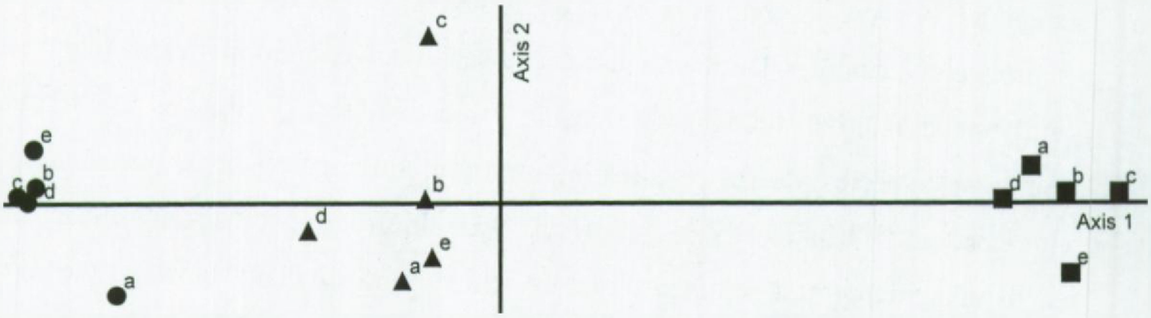

**Figure 2. Results of Detrended Correspondence Analysis axes 1 and 2, based on species abundances (Site 1 triangles, Site 2 squares, Site 3 circles, letters represent replicates)**

Total species number was in the same range at all three sites. In total, 76 nematode species were identified: 52 at Site 1, 41 at Site 2 and 54 at Site 3. Site 1 had 11 species exclusive to that site while Site 2 and 3 had respectively 9 and 17 unique species. Average relative abundances for the dominant species are shown in Table 4.

| | Feeding type | Site 1 | Site 2 | Site 3 |
|---|---|---|---|---|
| *Theristus blandicor* | 1B | **20.1** | **0.6** | **30.5** |
| *Viscosia viscosa* | 2B | **9.5** | **15.3** | **4.0** |
| *Ascolaimus elongatus* | 1B | **26.0** | 2.6 | **3.1** |
| *Eleutherolaimus amasi* | 1B | **14.4** | 0.2 | **3.9** |
| *Theristus pertenuis* | 1B | **5.2** | 0.1 | **5.6** |
| *Microlaimus marinus* | 2A | **2.7** | | **2.7** |
| *Trefusia helgolandica* | 1A | **1.0** | | **2.3** |
| *Enoploides longispiculosus* | 2B | 0.5 | 0.1 | **30.1** |
| *Daptonema riemanni* | 1B | 0.1 | 0.2 | **3.9** |
| *Microlaimus acinaces* | 2A | 0.3 | | **2.2** |
| *Tripyloides gracilis* | 1B | 2.9 | **40.1** | 0.2 |
| *Ptycholaimellus ponticus* | 2B | 0.3 | **12.4** | 0.1 |
| *Daptonema tenuispiculum* | 1B | 0.8 | **9.7** | |
| *Calyptronema maxweberi* | 2B | 1.6 | **2.8** | |
| *Theristus acer* | 1B | 0.1 | **3.1** | 0.1 |
| *Sabatieria pulchra* | 1B | 1.4 | **0.9** | 0.1 |

**Table 4. Relative abundances and feeding type (Wieser 1953) of the dominant species (values in bold are the dominant species per site)**

Nematode species were termed dominant when present in at least 25 of the 180 slices or in at least 7 slices with a minimum relative abundance of 40 % (restrictions based on practical considerations). Based on the dominant species, three different species assemblages could be recognised at the three sites: *Theristus blandicor*, *Ascolaimus elongatus* and *Eleutherolaimus amasi* were most dominant in Site 1 (60.5% of total community); *Tripyloides gracilis*, *Viscosia viscosa* and *Ptycholaimellus ponticus* in Site 2 (67.8%); *T. blandicor* and *Enoploides longispiculosus* in Site 3 (60.6%).

*Vertical profiles.* ANOVA 'split-plot' analysis (Table 5) showed significant differences in depth distribution of total nematode densities (Fig. 3) and of densities for each dominant species (Fig. 4, 5, 6) among the three sites. Nematode density at Site 1 (Fig. 3) was highest at the sediment surface and decreased gradually with depth. The maximum density of each of the dominant species at Site 1 occurs at different depth layers (Fig. 4), which indicates a gradual shifting of the nematode community with depth in the sediment at Site 1. Maximum abundance of *V. viscosa* was found in the upper sediment layer (0-0.5 cm). *Ascolaimus elongates*, *E. amasi* and *Theristus pertenuis* showed peak abundance at 0.5 to 1 cm depth; *Microlaimus marinus* and *T. helgolandica* at intermediate sediment depths (respectively 2-3; 6-8 cm), and finally *T. blandicor* at 5 to 10 cm depth in the sediment (Fig. 4).

| | Site | | Depth | | Site × Depth | |
|---|---|---|---|---|---|---|
| | *F* | p | *F* | p | *F* | p |
| Nematode community | 8.982 | 0.009 | 32.725 | 0.000 | 11.301 | 0.000 |
| *Ascolaimus elongatus* | 58.182 | 0.000 | 47.698 | 0.000 | 26.079 | 0.000 |
| *Calyptronema maxweberi* | 16.247 | 0.002 | 12.628 | 0.000 | 3.637 | 0.000 |
| *Daptonema tenuispiculum* | 107.517 | 0.000 | 23.619 | 0.000 | 13.099 | 0.000 |
| *Eleutherolaimus amasi* | 30.397 | 0.000 | 5.511 | 0.000 | 7.896 | 0.000 |
| *Enoploides longispiculosus* | 147.509 | 0.000 | 34.552 | 0.000 | 18.075 | 0.000 |
| *Microlaimus acinaces* | 20.651 | 0.001 | 3.320 | 0.005 | 2.763 | 0.001 |
| *Microlaimus marinus* | 57.632 | 0.000 | 8.647 | 0.000 | 8.104 | 0.000 |
| *Ptycholaimellus ponticus* | 205.658 | 0.000 | 49.261 | 0.000 | 28.031 | 0.000 |
| *Sabatieria pulchra* | 10.489 | 0.006 | 5.446 | 0.000 | 3.431 | 0.000 |
| *Theristus acer* | 40.125 | 0.000 | 14.292 | 0.000 | 16.890 | 0.000 |
| *Theristus blandicor* | 12.632 | 0.003 | 22.656 | 0.000 | 7.444 | 0.000 |
| *Theristus pertenuis* | 41.311 | 0.000 | 6.514 | 0.000 | 5.858 | 0.000 |
| *Theristus riemanni* | 9.746 | 0.007 | 5.386 | 0.000 | 4.449 | 0.000 |
| *Trefusia helgolandica* | 9.802 | 0.007 | 8.781 | 0.000 | 2.823 | 0.000 |
| *Tripyloides gracilis* | 23.913 | 0.000 | 49.971 | 0.000 | 15.236 | 0.000 |
| *Viscosia viscosa* | 17.188 | 0.001 | 40.367 | 0.000 | 7.210 | 0.000 |
| | | | | | | |
| $N_0$ diversity | 3.929 | 0.065 | 8.664 | 0.000 | 6.227 | 0.000 |
| $N_1$ diversity | 5.806 | 0.028 | 10.686 | 0.000 | 5.605 | 0.000 |

**Table 5. Univariate ANOVA tests (df = 2, 11, 18 for respectively Site, Depth, Site × Depth)**

At Site 2, the total nematode density was very high at the sediment surface and decreased gradually with depth (Fig. 3). Here 95 % of the nematode community was confined to the upper 2 cm of the sediment, compared to 73 % in Site 1. This steep gradient in depth is also reflected in the individual species distributions (Fig. 5). All dominant species, *Calyptronema maxweberi, Daptonema tenuispiculum, P. ponticus, Theristus acer, T. gracilis* and *V. viscosa*, showed maximum abundances in the top 1 cm of the sediment, except for *Sabatieria pulchra* and *T. blandicor*, which were more uniformly distributed downcore. For Site 3, total nematode density remained low and relatively constant until 10 cm depth (Fig. 3).

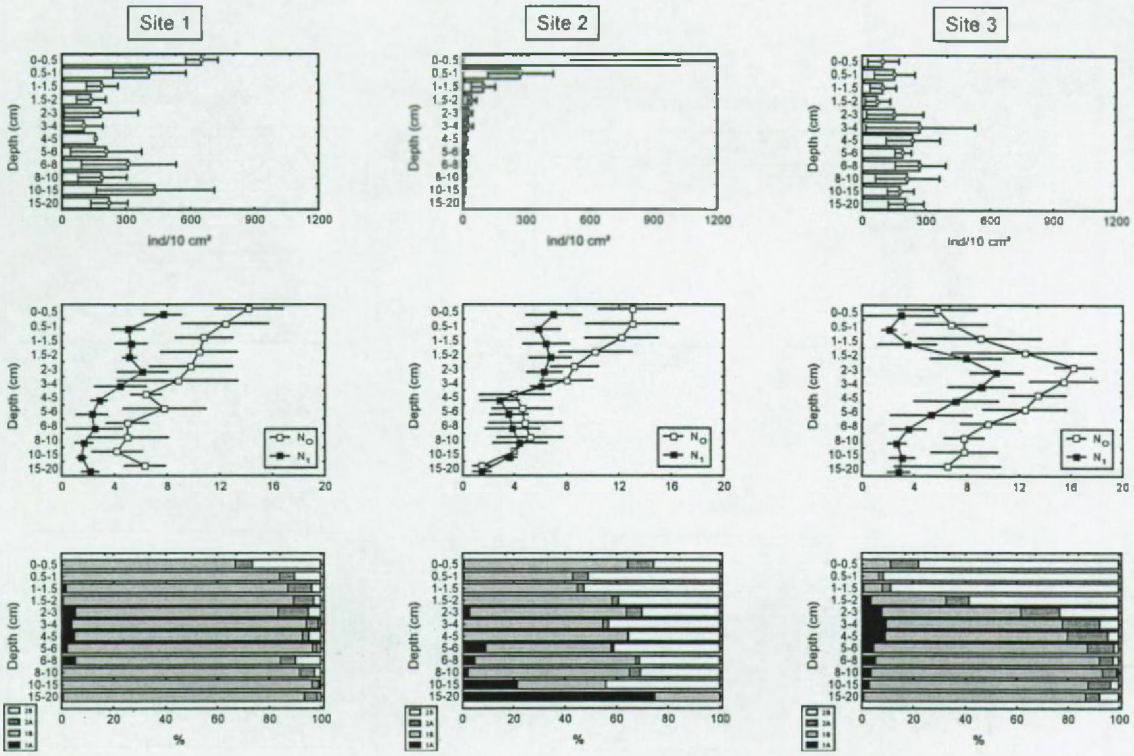

**Figure 3. Nematode abundance, nematode diversity and feeding type distribution with depth**

Deeper down, the abundance tended to decrease very slowly. The individual species (Fig. 6) belong to two vertically segregated species assemblages. The '*Enoploides longispiculosus*-assemblage' situated at the top 1.5 to 2 cm of Site 3, is characterised by high abundances of *E. longispiculosus*, with a maximum abundance recorded at 0.5 to 1 cm depth and other species that also conform to this zonation. The '*Theristus blandicor*-assemblage' is located in deeper sediment layers (from 3 cm onwards). The most important species here is *T. blandicor*, which has its maximum abundance at 5 to 6 cm depth. A similar feature is found for the less numerous species *A. elongatus, E. amasi, M. marinus, M. acinaces, T. helgolandica, T. pertenuis* and *Theristus riemanni* however species' preferences are not monotonic in this zone. Their maximum abundance is between 8 to 10 cm. All species, except *V. viscosa*, can be attributed to one of both subcommunities. *V. viscosa* has highest abundance between 1.5 and 2 cm depth.

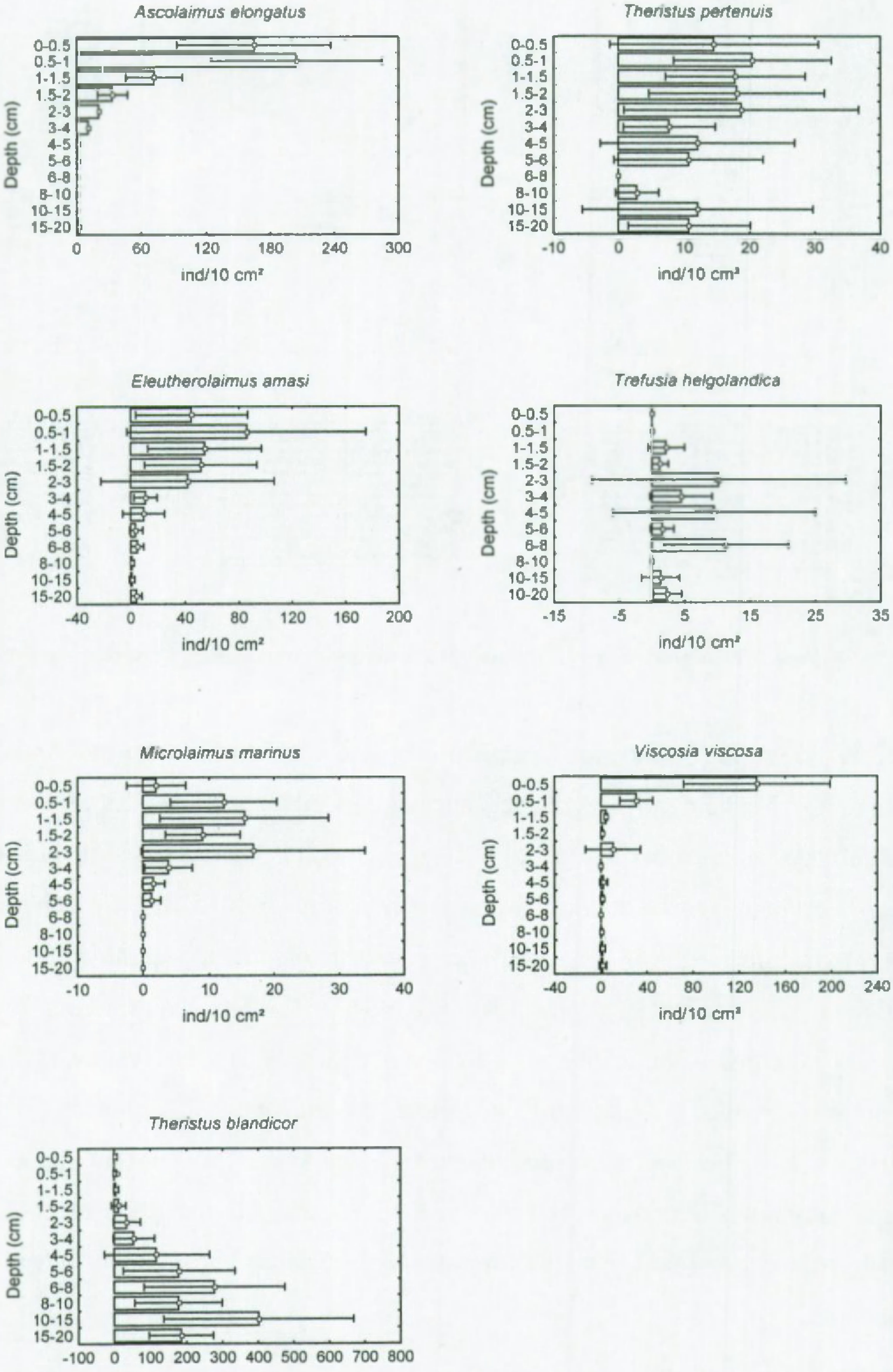

Figure 4. Depth distribution of the dominant species in Site 1 (note different width in sediment slices on Y axes)

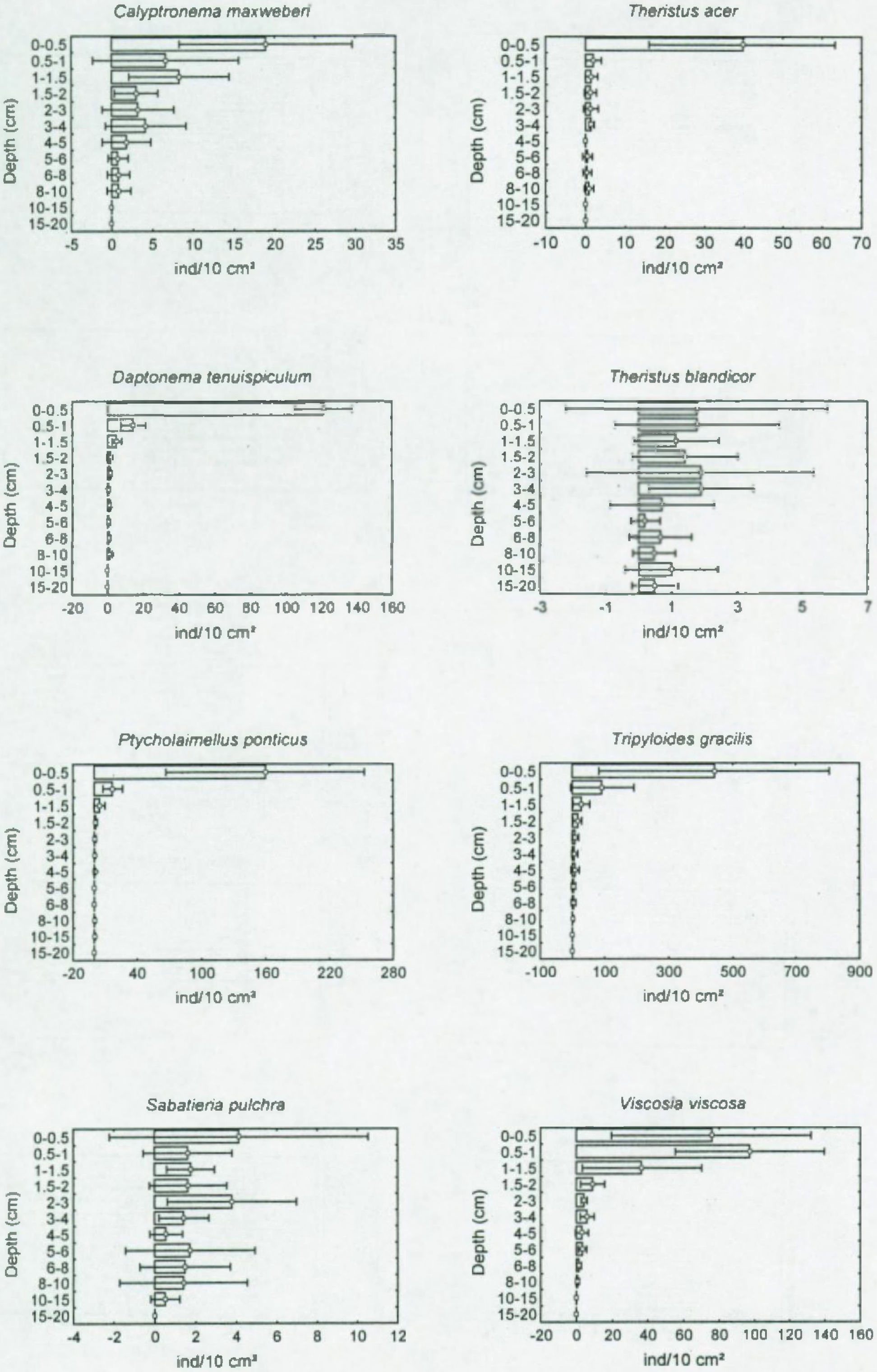

**Figure 5. Depth distribution of the dominant species in Site 2 (note different width in sediment slices on Y axes)**

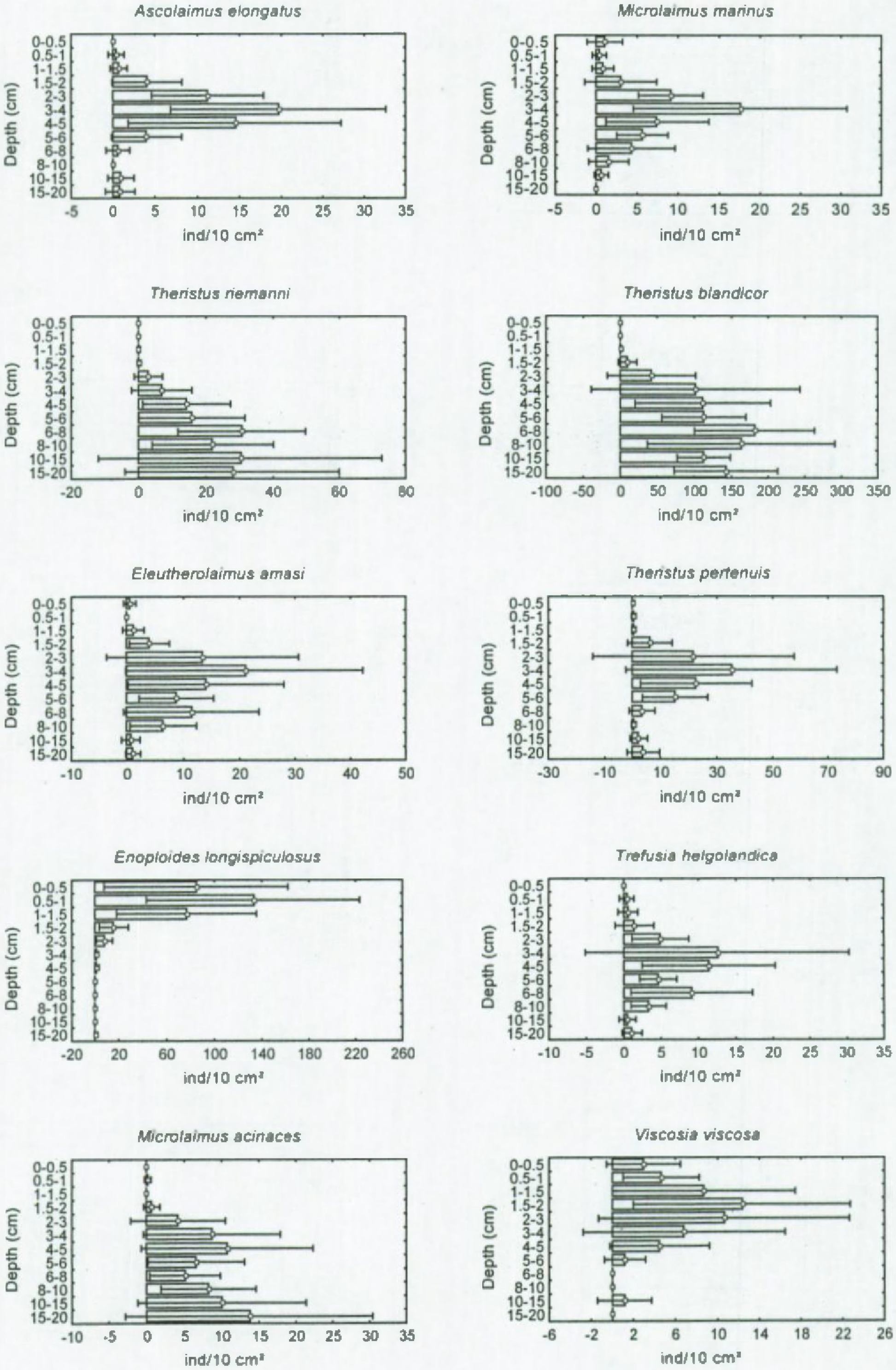

**Figure 6. Depth distribution of the dominant species in Site 3 (Note different width in sediment slices on Y axes)**

The existence of three different nematode assemblages at the three sites (based on dominant species and depth profiles) is confirmed by the DCA (Fig. 7). The first ordination axis has an eigenvalue of 0.787; the second axis has an eigenvalue of 0.42.

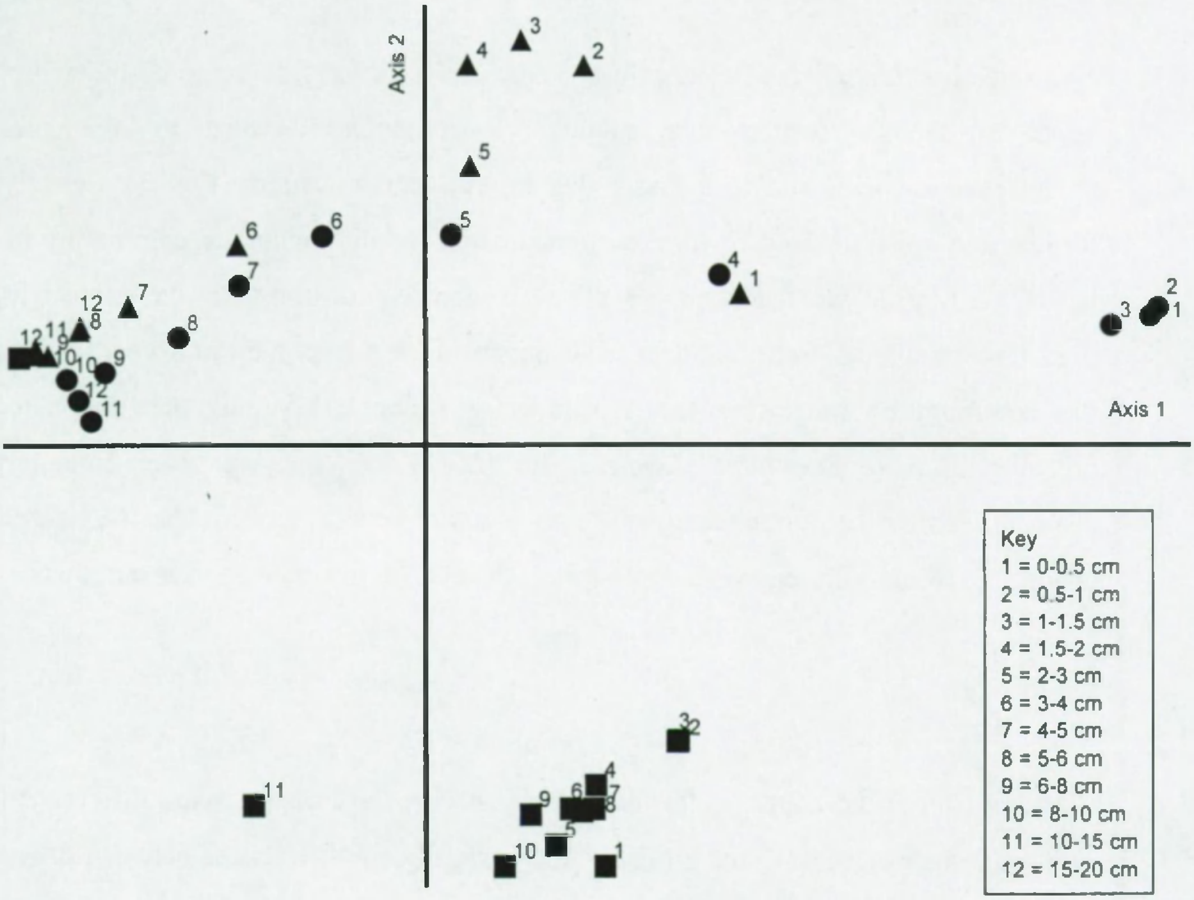

**Figure 7. Results of Detrended Correspondence Analysis axes 1 and 2, based on relative abundances (Site 1 triangles, Site 2 squares, Site 3 circles)**

The third axis (not depicted) has a low eigenvalue (0.098) and yields no additional information. Site 1 consists of a gradually shifting nematode community with depth. This is illustrated by the samples, which are placed along a depth gradient parallel to the first axis. It should be noticed that depth layers are plotted relatively far from each other (especially in upper depth layers). The samples of upper sediment layers of Site 3 (0-0.5; 0.5-1; 1-1.5 cm) appear separate from those of deeper sediment layers (Fig. 7), pointing again to the existence of two vertically separated subcommunities at Site 3. The analysis further indicates that there is a similarity between deeper layers of Site 1 and 3 (beneath 2 cm) and the deepest layer of Site 2 (15-20 cm), all dominated by *T. blandicor*. Site 2 is characterised by a surface nematode association that declines with depth. Vertically sectioned samples, exclusive the deepest sediment layer (15-20 cm), of Site 2 are clustered closely together and species associations seem to be similar at all depth layers.

### Feeding type distribution

Non-selective deposit feeders dominated Site 1. In the surface layer of the sediment, non-selective deposit feeders are slightly less important, due to the presence of predators/omnivores, and to a lesser degree, epistratum feeders (Fig. 3). Selective deposit feeders and epistrate feeders form a small fraction of the nematode community in all depth layers. At Site 2, two feeding types are well represented: non-selective deposit feeders and predators/omnivores. Selective deposit feeders were only important in deeper layers. At Site 3, the communities inhabiting the top layers (to about 1.5 cm) are dominated by the predator/omnivore *Enoploides longispiculosus*. The deeper layers, from 2 cm onwards are characterised by the dominance of the non-selective deposit feeder, *Theristus blandicor*. The changes in trophic strategy with depth reflect the change in single species dominance at Site 3.

### Diversity

For total (integrated depth layers) number of species ($N_0$), a significant difference ($p \leq 0.05$) was found between Site 2 and 3 (Table 3). No significant differences between sites existed for $N_1$ diversity. The ANOVA 'split-plot' analysis demonstrated significant differences between the three sites with respect to the variation in diversity with depth (Fig. 3, Table 5). Site 3 is clearly different from the other sites. At Site 3, $N_0$ and $N_1$ are low at the surface of the sediment and a maximum diversity exists at around 3 to 4 cm depth precisely where there is a change from a surface community to a deeper community. At Sites 1 and 2, species diversity within the sediment exhibited a different profile. At both sites, diversity was highest at the sediment surface and decreased with depth. At Site 2, there was a sharper decrease of diversity below 8 cm compared to Site 1; this can be linked to a decrease in density.

At Site 1 there was positive correlation between diversity and chlorophyll *a*, silt and a negative correlation between medium sand content (Table 6). At Site 2, diversity was positively correlated with chlorophyll *a* and silt content. At Site 3, no significant correlations were found between diversity and any of the environmental factors.

| | | Site 1 | | Site 2 | | Site 3 | |
|---|---|---|---|---|---|---|---|
| | | Spearman R | p | Spearman R | p | Spearman R | p |
| Chlorophyll *a* | $N_0$ diversity | **0.865** | **0.003** | **0.728** | **0.026** | 0.134 | 0.731 |
| Chlorophyll *a* | $N_1$ diversity | **0.762** | **0.017** | **0.711** | **0.032** | 0.251 | 0.515 |
| Organic C | $N_0$ diversity | 0.470 | 0.202 | 0.117 | 0.764 | -0.287 | 0.454 |
| Organic C | $N_1$ diversity | 0.371 | 0.325 | 0.134 | 0.731 | -0.226 | 0.558 |
| Silt | $N_0$ diversity | **0.848** | **0.004** | **0.828** | **0.006** | -0.444 | 0.232 |
| Silt | $N_1$ diversity | **0.895** | **0.001** | **0.862** | **0.003** | -0.326 | 0.391 |
| Fine sand | $N_0$ diversity | -0.136 | 0.728 | 0.142 | 0.715 | 0.251 | 0.515 |
| Fine sand | $N_1$ diversity | -0.134 | 0.731 | 0.126 | 0.748 | 0.318 | 0.404 |
| Medium sand | $N_0$ diversity | **-0.763** | **0.017** | -0.176 | 0.651 | -0.159 | 0.683 |
| Medium sand | $N_1$ diversity | **-0.828** | **0.006** | -0.192 | 0.620 | -0.226 | 0.559 |

**Table 6. Spearman Rank Order Correlations for the three sites**

# Discussion

## Nematode densities

Previous studies addressing nematode community structure on intertidal flats, deal with diversity, distribution on different scales and production (Ott 1972, Warwick & Price 1979, Van Es *et al.* 1980, Hogue & Miller 1981, Joint *et al.* 1982, Pickney & Sandulli 1990). In general, extremely high abundances of meiofauna, with nematodes always the dominant taxon, are characteristic of sheltered muddy regions of estuaries (Heip *et al.* 1985). This study shows a significant difference in total nematode abundance between the muddy site (Site 2, lowest densities) and Site 1, which has fine sandy sediment with high silt content and the highest nematode densities. The higher densities in Site 1, together with the higher benthic primary production (Herman *et al.* 2001), the higher autotrophic biomass (Hamels *et al.* 1998) and the larger macrobenthos stock (Herman *et al.* 2000), all point to a higher productivity in Site 1. This site is located at the border of the tidal flat and the open water and it is therefore exposed to stronger hydrodynamic forces compared to Site 2 (Widdows *et al.* 2000). As such it is likely that the input and output of fresh organic material may be larger and is the basis of the highly productive system.

Also the presence of macrofauna may affect the nematode densities although in different ways. Besides alterations of the chemical and physical properties of the sediment by macrobenthos, their effect be also linked to feeding activities (*e.g.* Ólafsson *et al.* 1993, Austen *et al.* 1998). Site 3, and in minor degree, Site 1, with higher current velocities and bottom shear stress (Van De Koppel *et al.* 2001) are characterised by higher densities of surface deposit feeders, while Site 2, where sedimentation rates were high (Schmidt *et al.* 1999 in Herman *et al.* 2001), is characterised by suspension feeders (Herman *et al.* 2001). Deposit feeders may have a predatory effect due to coincidental consumption of nematodes whilst feeding. Alternatively, the disturbance activity may stimulate microbial growth and increased sediment oxygenation, providing an increase in food and spatial resources, which in turn stimulated the nematodes (Reise 1983). Suspension feeders may stimulate nematode abundance through biodeposition of organic carbon. However it remains unclear what the macrofauna-meiofauna interaction is at the Molenplaat.

The differences in sediment characteristics, hydrodynamical conditions (reflected by current velocity and bottom shear stress) and productivity did not affect diversity of the three investigated sites and only partially total nematode abundance (only two of the three sites differed in nematode abundance). Detailed investigation of vertical depth profiles however, revealed differences that may relate to environmental factors.

Generally, the vertical distribution pattern of nematodes in silty sediments is well established: abundances are extremely high at the sediment surface or subsurface and subsequently decrease steeply with depth (for an overview, see Heip *et al.* 1985). This trend was evident in Site 1 and was even more pronounced in Site 2 of the Molenplaat. In the more sandy conditions of Site 3, nematode abundance remains generally lower and fluctuates greatly with depth. Oxygen penetration and the occurrence of sulphide have been coupled many times to the depth distribution of nematodes (Platt 1977, Heip *et al.* 1985, Giere 1993, Hendelberg & Jensen 1993, Wetzel *et al.* 1995). In the sediment of Site 3, which is highly bioturbated by macrobenthos, oxygen penetration might be several centimetres in the proximity of burrows, whereas Site 2 is the least bioturbated (pers. comm. C. Barranguet). Besides a number of biotic (*e.g.* resource availability and distribution) and abiotic (*e.g.* compaction of sediment) interactions, oxygen distribution is thought to be one of the important regulating factors in explaining the obvious discrepancy in vertical distribution patterns on the Molenplaat.

## Community composition

Some studies have dealt with the vertical distribution of free-living nematodes at the species level (*e.g.* Joint *et al.* 1982, Blome 1983, Warwick & Gee 1984, Jensen 1987, Hendelberg &

Jensen 1993, Soetaert *et al.* 1994, Steyaert *et al.* 1999). From these studies it is clear that some nematode species show a consistent depth distribution in different areas, which suggests species-specific depth preferences. As the auto-ecological information on free-living nematodes is still very scarce, the causal factors for this depth preference are not yet completely clear. It has been suggested that the biogeochemical properties of the sediment might control the depth distribution of some species (Jensen 1981, 1987, Bouwman *et al.* 1984, Platt & Lambshead 1985, Jensen & Aagaard 1992, Steyaert *et al.* 1999). This might explain the surface dominated community of the silty sediment (Site 2), where oxygen penetrated only into the upper millimetres of the sediment (pers.comm. C. Barranguet).

The factors determining the vertical distribution in the fine sandy sediments may act in combination with biotic interactions. Joint *et al.* (1982) argued that interspecific competition gives rise to vertical niche segregation. As such, fine scale vertical stratification may play a role in allowing species with similar food requirements and feeding behaviour to co-exist in the same locality. This study supports this finding for the highly productive system of Site 1. Here, a gradual shifting of the nematode community was recorded, as a result of the succession of maximum density peaks of dominant species with depth. This sediment was characterised by a high percentage of non-selective deposit feeders (*e.g. Theristus blandicor, Ascolaimus elongatus, Eleutherolaimus amasi, Theristus pertenuis*).

In the sediment of Site 3, two vertically segregated species assemblages were observed. The upper assemblage (to about 1.5 cm depth) was dominated by *Enoploides longispiculosus*. The lower assemblage (below 2 cm) was dominated by *T. blandicor* and a number of less common species (Fig. 6). In earlier studies, these consistent depth profiles were also reported for some species: *E. longispiculosus* being a true surface-dweller (Soetaert *et al.* 1994) while *T. blandicor, M. marinus, T. pertenuis* and *T. riemanni* were considered as 'deep-dwelling' species (Blome 1983, Soetaert *et al.* 1994). The existence of these two vertically segregated assemblages on the Molenplaat is probably due to a combination of factors, of which the most important might be related to food preferences and the strong hydrodynamic regime at the site. As sediment granulometry appears to be of more importance in controlling the fauna in the upper sediment layers, the similarity of the deeper nematode communities - caused by the dominance of *T. blandicor* - at Sites 1 and 3 is particularly interesting. The environmental regime at depth seems to result in similar faunal assemblages irrespective of sediment granulometry. From its dominance in deeper sediment layers at Site 1 and 3 and the apparent lack of depth preference in Site 2 it can be concluded that *T. blandicor* persists in sediments with restrictive conditions for other nematode species. It appears that this species is capable of surviving anoxic conditions and is able to exploit the available food resources at depth. Such life conditions have often been described for *Sabatieria* species, which are typical inhabitants for deeper sediment layers of muddy intertidal and subtidal sediments (Hendelberg & Jensen 1993, Soetaert & Heip 1995, Steyaert *et al.* 1999). One *Sabatieria* species, *S. pulchra*, is also observed in this study.

## Diversity

Nematode community diversity has been associated with sediment composition, oxygen, salinity, stress and organic enrichment (Giere 1993, Warwick & Clarke 1993, Soetaert *et al.* 1994, Warwick *et al.* 1997, Austen *et al.* 1998, Essink & Keidel 1998, Steyaert *et al.* 1999). This study documented a high nematode species richness on a small area (the Molenplaat) of approximately 2-3 km². This high diversity may be largely the result of the heterogeneous geochemical and physical characteristics on the tidal flat. Moreover, the twofold difference in total (over the whole sediment column) number of species and averaged (over five replicates) total number of species per site, illustrates also the importance of small-scale effects. Thus, besides the mean differences, found on a large scale (between the three sites), local effects (on 10-m scale) of sedimentological characteristics are evident.

The difference in vertical profile of species richness found between Site 3 and both Site 1 and 2, is expected as a result of the wider range of microhabitats available for meiofauna in sandy sediments (Site 3) compared to muddy sediments (Site 1 and 2) (Heip & Decraemer 1974). Within the sediment column diversity in Site 3 is strongly variable and is only high at a depth of 2 to 5 cm. These higher values can be explained by the occurrence of both species from the upper '*Enoploides longispiculosus*-community' and the lower '*Theristus blandicor*-community'. The lower diversity values in the uppermost layers of the sediment are related to low densities and probably result from the strong hydrodynamic regime and the tidal disturbance that prevail at the upper 2 cm of the sediment at Site 3 (Herman *et al.* 2000, Widdows *et al.* 2000). Therefore it is suggested that the hydrodynamic regime, which influences sediment granulometry, will predominantly affect the number of nematode species at the Molenplaat.

In many studies, correlation tests have been used to illustrate possible relationships between meiobenthos, in particular nematodes and copepods, and possible food sources (*e.g.* Findlay 1981, Blanchard 1990, Pickney & Sandulli 1990, Danovaro *et al.* 1995, 1996 Santos *et al.*

1996, Moens *et al.* 1999a, Steyaert *et al.* 1999). These studies considered, in most cases, horizontal variation. When visualising vertical distribution patterns in the sediment, correlations between individual nematode species or feeding types and biotic variables are in many cases misleading. Moreover, correlation does not imply causation. The vertical distributions of factors such as pigments, bacteria and nutrients often change with depth in the sediment. Therefore caution needs to be used when correlating depth profiles of different variables. In this study the diversity indices of Site 1 and 2, both sediments characterised by a high silt content, were positively correlated with this silt content. Such a strong positive correlation of diversity and silt content with depth has also been found in muddy sediments of the Belgian coastal zone (Steyaert *et al.* 1999). In general, sediment granulometry exerts an important influence on the diversity of nematode communities. On a broad, horizontal scale, coarser sediments will enhance nematode diversity by creating a broad range of microhabitats (Heip & Decraemer 1974). Diversity on a small spatial scale, within the sediment, is inversely related to the sediment granulometry of muddy sediments. The finer the sediment becomes, the more diverse the nematode community.

## Conclusions

Differences in sediment composition in association with different hydrodynamic conditions at separate sites of an estuarine intertidal flat are reflected in total nematode abundances and in species composition. The heterogeneity was much higher at km than at m scales, at which level environmental conditions seemed more consistent. Species richness in combination with equitability did not differ among the three sites, when integrated over 20 cm depth in the sediment. In contrast, microscale vertical profile analysis illustrated the presence of three distinctly different distribution patterns of species associations on the tidal flat: (1) in sandy sediment under strong hydrodynamic and food-stressed conditions a surface-dwelling nematode community of mainly a large predatory enoplid was observed living above a deposit feeding xyalid - microlaimid community in deeper sediment layers. These extreme environmental conditions resulted in low density and diversity. Diversity was highest at the interface between the two vertically separated communities as a result of co-existence of species from the upper and lower community. (2) In the finest sediment most nematode species were confined to the surface layers. Only a few could occasionally penetrate into deeper layers, resulting in a sharp decline in diversity and density with depth in the sediment. (3) At intermediate hydrodynamic and granulometric conditions there is a gradual shift from a diverse and abundant nematode community at the surface to a less diverse and less abundant one in the deeper sediment layers. The vertical changes in nematode composition resulted in a community similar to that found in the deeper sediment layers of the sandy site in terms of abundances, diversity and composition. Vertical profile analysis provides additional information over bulk sampling that is key to understanding horizontal patterns and their relation with environmental characteristics in nematode communities.

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# Chapter V

# Tidal migration of nematodes on an estuarine tidal flat (the Molenplaat, Schelde Estuary, SW Netherlands)

Results presented as

Steyaert M, Herman PMJ, Moens T, Widdows J, Vincx M (2001) Tidal migration of nematodes on an estuarine tidal flat (the Molenplaat, Schelde Estuary, SW Netherlands). Mar Ecol Prog Ser 224:299-304

# Abstract

The vertical distribution patterns of the nematode community and of the ten most dominant nematode species on an intertidal flat in the Schelde estuary (Molenplaat, The Netherlands) are described at specific time intervals over a tidal cycle. The observed distribution profiles indicate that vertical migrations occur and are species-specific. The predatory *Enoploides longispiculosus* and the deposit-feeding *Daptonema normandicum* migrated upwards at incoming tide and downwards when the flat became exposed, while another deposit feeder, *Daptonema setosum*, did the opposite. Several abiotic and biotic factors may contribute to the observed patterns. Hydrodynamics, pore water drainage and episodic steep increases in temperature upon low tide exposure, as well as vertical movements of prey organisms may have been of particular relevance at the time and site of sampling. However, the impact of each of these factors needs further investigation. The present study corroborates the dynamic nature of vertical distribution profiles of nematodes in intertidal sediments, highlighting the importance of sampling time with respect to the tide, as well as a species approach.

## Introduction

Meiobenthos often shows an aggregated vertical distribution within the sediment. The causes of this patchiness are often complex. In intertidal habitats, the tides can be expected to have an important dynamic impact on the chemical and physical environment of organisms living in the sediment. Factors such as temperature, wave action, currents, organic input, percolation of interstitial water, light intensity, compaction of the sediment and others will change periodically with the tidal cycle. This fluctuating environment provides a challenge to benthic organisms and forces them to adapt to an unstable environment or to migrate to deeper sediment layers.

Earlier research has demonstrated tidal migrations for nematodes and linked these to a variety of both biotic and abiotic environmental factors (Renaud-Debyser 1963, Boaden & Platt 1971, Palmer & Gust 1985, Palmer 1986, 1988, Fegley 1987). These studies mainly concentrated on the total nematode community, however. There are few studies at the species level (Rieger & Ott 1971).

Here we investigate tidal migration of nematodes on the species as well as on the community level, and test whether the community level approach masks species-specific patterns. This study was carried out within the frame of a multidisciplinary research project, ECOFLAT, which aims to study the ecometabolism of the Molenplaat, SW Netherlands. On this tidal flat, the spatial structure of nematode communities has been studied intensively in a previous phase of the project[1].

## Study site

The Molenplaat is a tidal flat in the polyhaline reach of the Schelde estuary, with a surface area of approximately 1.5 km$^2$. Considerable heterogeneity in sediment characteristics is found on this tidal flat, with sediments ranging from fine sand with a high silt fraction to medium sand without silt. Most of the tidal flat is located between –1 and +1 m relative to mean tidal level (approximately 2.75 m to mean low low water) and is subjected to a tidal amplitude of about 5 m (Middelburg *et al.* 2000). Exposure time is about 7 hours each tidal cycle (Herman *et al.* 2000). Sediments collected for the present study mainly consist of fine

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[1] Steyaert *et al.* (2003)

sand (median grain size 172 µm - Malvern particle analyser), with only a minor silt fraction (<5 %) (J.J. Middelburg pers. comm.). The site is characterised by a relatively high bottom shear stress (1.15 Pa) (MP 4 in Herman *et al.* 2000), and was selected because tidal migration of nematodes could be expected to be more pronounced in hydrodynamically impacted sediments.

## Materials and methods

### Sampling strategy

On the 11th of June 1997, triplicate 3.6 cm i.d. perspex cores were taken at five different sampling events (Fig. 1) during daytime. The first sampling (event S1) was performed at the end of the ebbing tide (remaining water cover of about 20 cm). Sampling events 2, 3 and 4 (respectively events E2, E3, E4) took place at different moments during low-tide exposure.

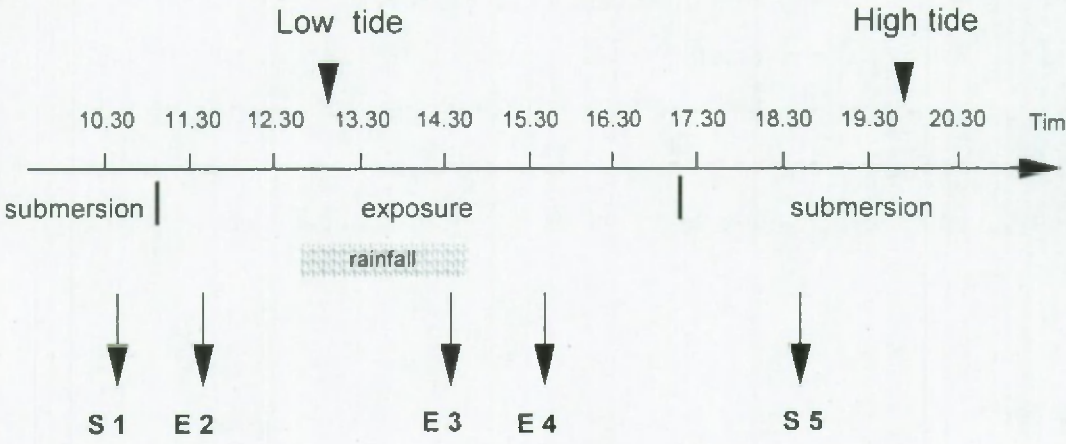

**Figure 1. Sampling time of the five events (S1, E2, E3, E4 and S5)**

The last series of samples (event S5) was collected 70 min. before high tide (1.5 m water cover). Measurements on the 7th of June 1997 at the same site indicated current velocities were highest 70 min before and after high tide (respectively 33 and 40 cm/s), and lowest during high tide (Fig. 2). Heavy rainfall occurred before E3 between 12:40 and 13:10 h.

All cores were carefully subdivided into depth slices (0-0.5 cm, 0.5-1 cm, 1-1.5 cm, 1.5-2 cm, 2-3 cm, 3-4 cm) in order to minimise disturbance. Samples were further treated as described in Steyaert *et al.* (1999).

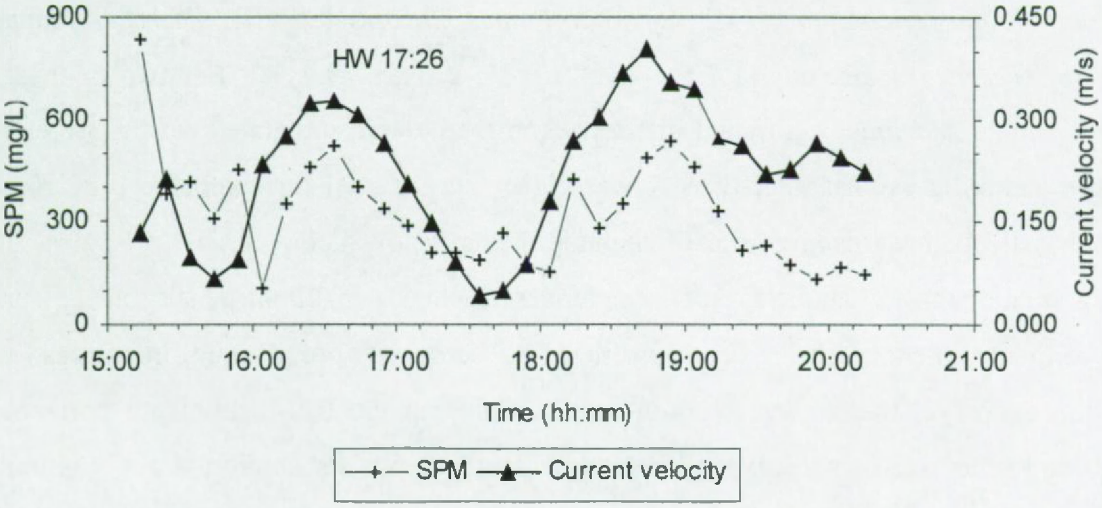

**Figure 2. Suspended particular matter (SPM) and current velocity during submersion (7 June 1997)**

The temperature at the sediment surface measured 25.8 °C and 30.7 °C, respectively 1 and 4 h after exposure. At a depth of 2 cm, the corresponding values were 22.9 and 28.9 °C, and at a depth of 3.5 cm temperature never exceeded 26.9 °C. Water temperature on the day of sampling averaged 17.3 °C (0.5 m depth).

### Statistical analysis

Univariate two-way Analysis of Variance (ANOVA) was used to test for differences of total nematode densities, as well as of the densities of the ten most dominant nematode species, with time, depth and time × depth. A 'split-plot' design was constructed with replicates nested within 'time', however, not within 'depth'. All data were log(x+1) transformed prior to analysis. Subsequently, a multivariate two-way ANOVA was performed in order to test if the relative species composition of the nematode community changed as a function of time, depth and time × depth. This MANOVA test, using Wilkinson's lambda, was based on the same design as for the univariate ANOVA.

## Results

The analysis is restricted to the total nematode community and to the following ten most abundant species: *Ascolaimus elongatus* (1.3 %), *Cyatholaimus gracilis* (1.9 %), *Daptonema* *normandicum* (15.3 %), *Daptonema setosum* (3.6 %), *Enoploides longispiculosus* (40.2 %), *Hypodontolaimus trichophora* (4.2 %), *Microlaimus marinus* (2.2 %), *Theristus blandicor* (10.2 %), *Theristus pertenuis* (1.4 %) and *Viscosia viscosa* (9.4 %). For two of these, *C. gracilis* and *M. marinus*, significant differences in total, depth-integrated density were found between sampling events; their density was higher during tidal flat exposure (Fig. 3). This probably reflects upwards migration of nematodes from below 4 cm.

During submersion (S1 and S5), total nematodes reached a maximal density in the surface layer of the sediment, with the depth profile of S5 being less pronounced. In contrast, upon low tide exposure, the highest fraction was recorded in the 0.5-1 cm depth horizon. An exception to this rule was E3, when the total nematode depth distribution strongly resembled that during submersion. The abundance of total nematode community (Table 1) changed significantly in depth, while no significant variation in time was found. Neither was the interaction between time and depth significant. Hence, the depth profile of total nematode density did not fluctuate significantly with time.

| | | Time | | | Depth | | | Time × Depth | |
|---|---|---|---|---|---|---|---|---|---|
| Univariate tests | df | *F* | p | df | *F* | p | df | *F* | p |
| Nematode community | 4 | 0.998 | 0.452 | 5 | 15.019 | < 0.001 | 20 | 0.875 | 0.616 |
| | | | | | | | | | |
| *Ascolaimus elongatus* | 4 | 1.342 | 0.320 | 5 | 8.471 | < 0.001 | 20 | 2.093 | 0.018 |
| *Cyatholaimus gracilis* | 4 | 0.960 | 0.470 | 5 | 8.068 | < 0.001 | 20 | 0.999 | 0.480 |
| *Daptonema normandicum* | 4 | 2.063 | 0.161 | 5 | 46.475 | < 0.001 | 20 | 1.232 | 0.269 |
| *D. setosum* | 4 | 0.755 | 0.577 | 5 | 47.695 | < 0.001 | 20 | 3.234 | 0.000 |
| *Enoploides longispiculosus* | 4 | 1.628 | 0.242 | 5 | 79.455 | < 0.001 | 20 | 1.971 | 0.027 |
| *Hypodontolaimus trichophora* | 4 | 3.222 | 0.061 | 5 | 15.211 | < 0.001 | 20 | 2.490 | 0.005 |
| *Marinus marinus* | 4 | 3.802 | 0.039 | 5 | 25.520 | < 0.001 | 20 | 1.588 | 0.094 |
| *Theristus blandicor* | 4 | 2.024 | 0.167 | 5 | 33.721 | < 0.001 | 20 | 2.443 | 0.005 |
| *T. pertenuis* | 4 | 6.025 | 0.010 | 5 | 20.974 | < 0.001 | 20 | 3.943 | 0.000 |
| *Viscosa viscosa* | 4 | 0.372 | 0.824 | 5 | 16.942 | < 0.001 | 20 | 2.324 | 0.008 |
| | | | | | | | | | |
| Multivariate test | 40.5 | 3.339 | 0.074 | 50.19 | 10.574 | < 0.001 | 200.4 | 2.163 | 0.001 |

**Table 1. Univariate and multivariate ANOVA tests**

Similar vertical distribution trends as for total nematodes were observed for the predacious *Enoploides longispiculosus.* This nematode showed a preference for the upper sediment layers during submersion, while during exposure, it was most abundant in the subsurface layers (Fig. 3). Note that there are minor differences between the two submerged sampling events. During S5 this species showed a slight subsurface (0.5-1 cm) peak. However, it was far less pronounced than in the foregoing exposed situation, E4. *Daptonema normandicum*, a deposit feeder, also reached a maximal abundance in the surface layer during submersion, while

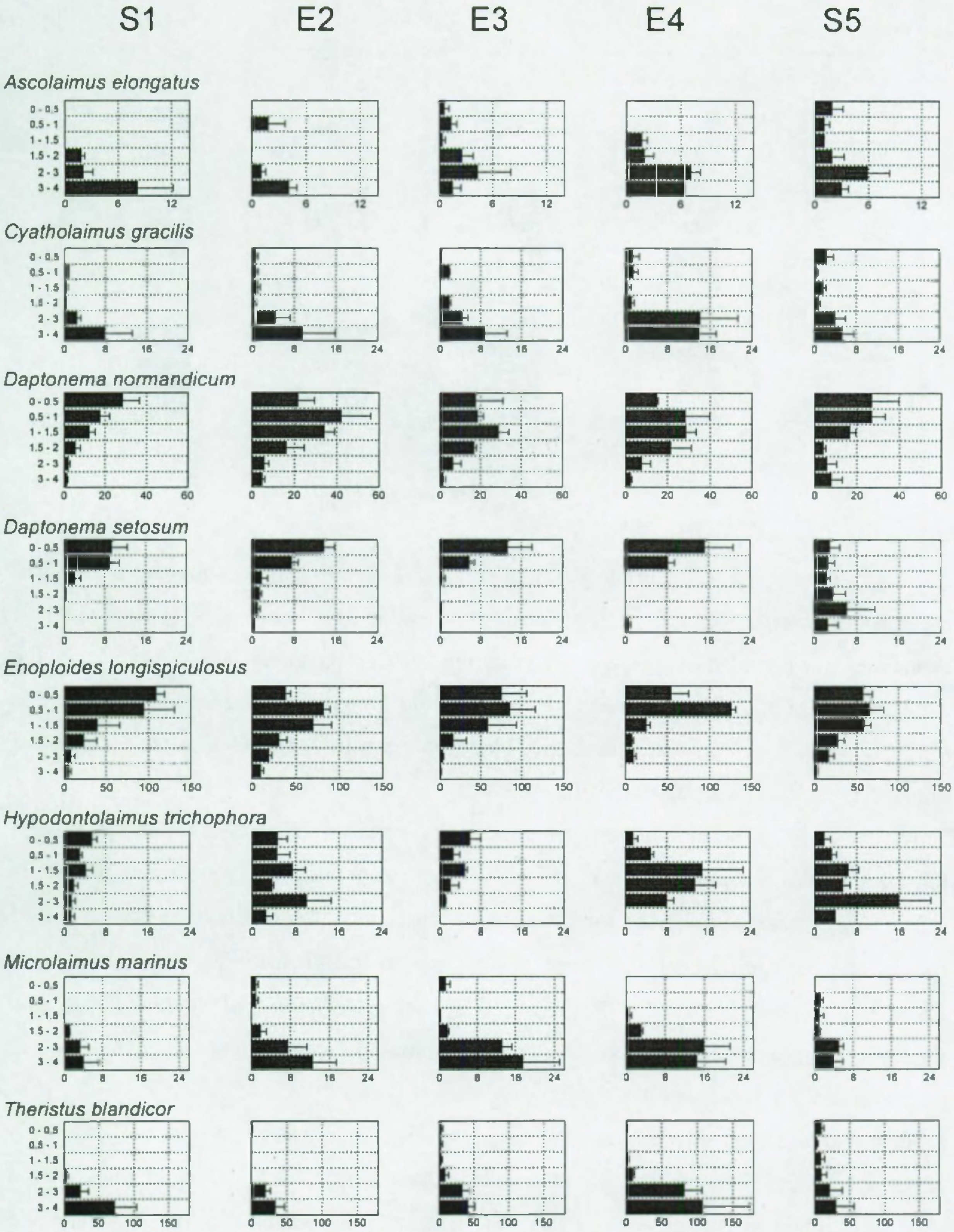

**Figure 3. (Above and next page) Depth distribution of the nematode species and the total nematode community for the five sampling events (horizontal axes: ind. 10 $cm^{-2}$; vertical axes: depth in cm)**

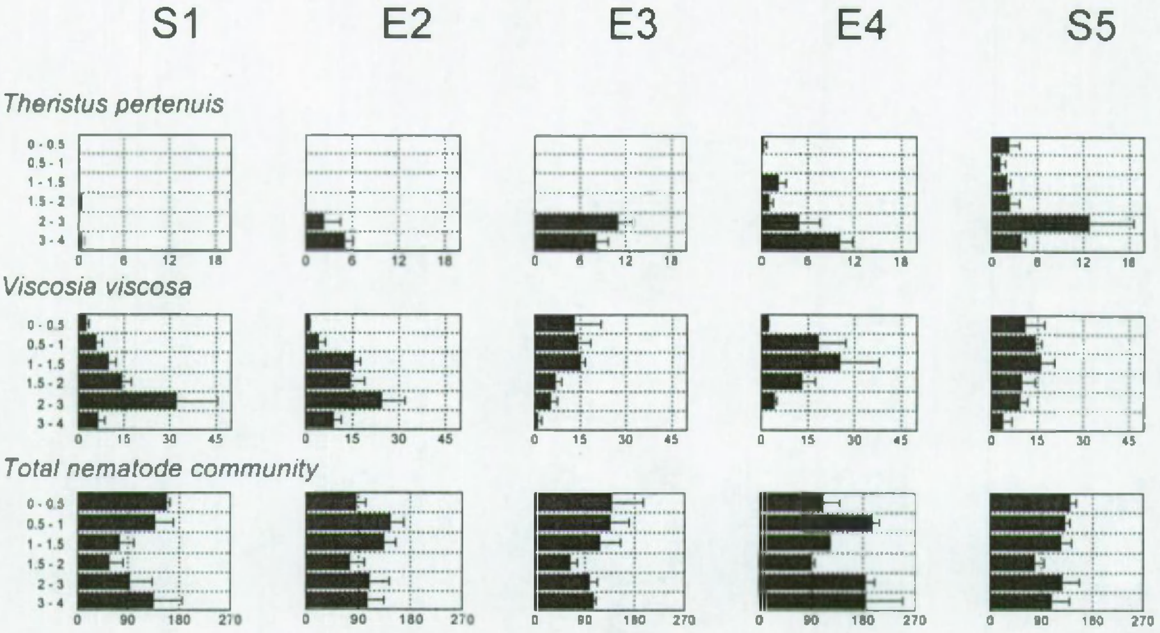

**Figure 3. (continued)**

showing a downward shift during exposure (Fig. 3). Interestingly, its congener *D. setosum* presented a similar vertical profile during submersion. However, this species' highest abundance was recorded in the upper layers during low tide exposure (Fig. 3). Here again, the depth profiles of both *D. normandicum* and *D. setosum* were less pronounced during high tide (S5) compared to ebbing situation (S1). None of the other species showed a consistent migration trend as a function of the tide.

Univariate tests on the distribution of the individual nematode species revealed that, except for *Microlaimus marinus* and *Theristus pertenuis*, the abundances were not significantly influenced by time. As for the total nematode community, the distribution profiles of all species altered significantly with depth. In contrast to total nematodes, the time × depth interaction was significant for 7 out of 10 species: *Ascolaimus elongatus, Daptonema setosum, Enoploides longispiculosus, Hypodontolaimus trichophora, T. blandicor, T. pertenuis* and *Viscosia viscosa.*

Multivariate analysis showed that nematode species composition was not significantly affected by time. Composition, however, did exhibit a significant effect of depth and a significant effect of the interaction term time × depth. The combination of univariate and multivariate analyses demonstrate that, while total nematode density does not reveal a tidal signal, tidal migrations of nematodes exist and are significant at the community level, *i.e.* the structure of the nematode community varies with depth over a tidal cycle.

# Discussion

The results of this study do not corroborate the general hypothesis that nematodes migrate deeper into the sediment as the tide comes in: submersion of the sampling site provoked upward movement of *Daptonema normandicum, Enoploides longispiculosus* and 'total nematodes' in general. In contrast, the behaviour of *D. setosum* resembles that of *Microlaimus marinus* as noted by Rieger & Ott (1971): a downward migration during submersion.

Several abiotic and biotic factors may explain the observed vertical displacements. Increased current velocities are thought to be of prime importance in inducing downward movement as an avoidance strategy against being eroded (Palmer & Gust 1985, Palmer 1986, Fegley 1987). In this respect, Boaden & Platt (1971) found that nematodes generally occurred deeper in the sediments during both flooding and ebbing when current velocities were highest. At the Molenplaat, current velocity peaks and extremely high loads of suspended particulate matter (500 mg $l^{-1}$) occur *ca* 1 h just before and after high tide, thus indicating strong hydrodynamics during these periods (Fig. 2). Hence, it is expected that the strongest downward migrations would occur on either side of high tide. This study partly confirms this hypothesis; *Daptonema normandicum, D. setosum, Enoploides longispiculosus, Hypodontolaimus trichophora,* and total nematodes were obviously less concentrated in top sediment layers just before high tide (S5), compared to the former measured submersion event (S1). Nevertheless, avoidance of erosion and resuspension cannot serve to explain the downward migration during exposure and subsequent re-entering of the more superficial layers during submersion, as observed for *E. longispiculosus, D. normandicum* (this study) and *D. leviculum* (Rieger & Ott 1971). The upper submerged sediment layers may be more favourable (S1) only when current velocities are below the critical threshold of erosion (0.23 m/s at this site; J.W. pers. comm.).

Intertidal areas are subject to cyclic changes of the groundwater level, which is not more than 2 cm below the surface for intertidal flats of the Westerschelde (Oenema *et al.* 1988). This will have important implications for highly mobile nematodes such as *Enoploides longispiculosus*, which need to actively catch their prey. It may in part explain the migration of *E. longispiculosus* to deeper sediment layers during exposure. *E. longispiculosus* is a voracious predator of nematodes and oligochaetes and is probably prey-limited in the upper stratum of the sediment of this study site (Moens *et al.* 2000). Its presence near the sediment surface during submersion may also enable it to exploit additional sources of prey, e.g.

temporary and permanent meiofauna transported through the water column. A recent study indicates that *E. longispiculosus* also forages on protozoan prey (I. Hamels *et al.* pers. comm.)[1], which are abundant at the studied site (I. Hamels pers. comm.) and may migrate upward during submersion (Fegley 1987). Additionally, the downward migration during submersion and upward movement during exposure of *Daptonema setosum* may be associated with tidal migrations of its food source, epipelic diatoms (Hopkins 1963, Joint *et al.* 1982, Pinckney *et al.* 1994).

Forster (1998) demonstrated that estuarine nematodes can tolerate significant changes in salinity, an ability which is highly species-specific and related to the nematodes' distribution in the littoral. Compared to the rainfall-induced salinity changes in this study, the salinity increases observed on the Molenplaat during exposure (and in bright summer weather) were minor (from 24 PSU one hour after emersion to a maximum of 28.5 PSU just prior to inundation) and unlikely to have provoked the vertical migration of nematodes at this site.

Temperature, however, increased significantly during low tide exposure, reaching maximal values at the sediment surface. The temperature values which exceeded optima and perhaps even upper tolerance limits of many free-living aquatic nematode species (Heip *et al.* 1985, Moens & Vincx 2000a, and references therein). In between optimal and upper lethal temperatures, even subtle temperature changes can impact reproductive and/or metabolic activity (Heip *et al.* 1985, Moens & Vincx 2000a,b). Hence, the recorded temperature differences between sediment surface and subsurface layers may provide a significant stimulus for vertical migration, although this stimulus is probably only episodically important during sunny spring and summer weather.

The design of the present study does not allow us to identify the causes of the observed migrations, nor to assess their generality. It does, however, suggest hypotheses about flow, groundwater and temperature to be tested in the future. It also clearly demonstrates the necessity of a species-level approach for accurate assessment of tidal migrations of meiofauna.

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[1] Hamels *et al.* (2001)

postdoctoral fellow with the Fund for Scientific Research – Flanders (FWO). Johan Van de Velde, Annick Van Kenhove and Steven Degraer are acknowledged for their assistance during sampling and sample processing, and the crew of the research vessel 'Luctor' for transport to and from the Molenplaat. Ms B.J. Schimmelpennink (Rijkswaterstaat Direktie Zeeland, Meetnet ZEGE) is acknowledged for the water temperature data. We thank Jan Vanaverbeke, Ann Vanreusel and 2 anonymous referees for critically reading and commenting on an earlier version of this manuscript.

# Chapter VI

36178

# The direct role of oxygen in the vertical distribution of nematodes: microcosm experiments

Results presented as

Steyaert M, Moodley L, Vanaverbeke J, Vandewiele S, Vincx M (submitted) The direct role of oxygen in the vertical distribution of nematodes: microcosm experiments

# Abstract

The direct role of oxygen in structuring the vertical distribution of an intertidal nematode community was investigated in manipulation experiments with sediments collected from the Oosterschelde (The Netherlands). In these experiments, the vertical distribution of the nematode species was examined in response to sediment inversion with or without oxygen.

The introduction of oxygen to deep layers significantly altered the vertical distribution of the nematode densities; highest numbers were recorded in the artificially aerated bottom layer. However, the distribution of the majority of the species (~ 79 %) was not directly linked to oxygenation. Dominant species were *Terschellingia communis, Microlaimus tenuispiculum* and *Sabatieria pulchra*. Only a small fraction (~ 2 %) of the nematode community was restricted to oxygenated sediment layers, irrespective of sediment depth. Six species belonged to this group (*Aegialoalaimus elegans, Axonolaimus helgolandicus, Bathyeurystomina sp., Desmoscolex sp., Dorylaimopsis sp.* and *Monoposthia mirabilis,*) and their distribution was evidently directly governed by oxygenation. The majority of the species examined are highly tolerant to short-term anoxic conditions.

# Introduction

Vertical distribution patterns of nematodes are often species-specific and established as a response to biogeochemical properties of the sediment (Jensen 1981, 1987, Bouwman *et al.* 1984, Platt & Lambshead 1985, Jensen & Aagaard 1992, Hendelberg & Jensen 1993, Steyaert *et al.* 1999). The oxygen supply of the pore water is many times suggested as the predominant structuring factor, coupled to a complex system of interacting environmental factors. However, the mechanistic understanding of the relationship between oxygen with nematode distribution is limited.

Surface dwellers as well as deep infaunal occurring species prevail in all sediment types, ranging from coarse sands to fine mud. Fine sediments harbour in general more surface dominating species (*e.g. Daptonema tenuispiculum, Ptycholaimellus ponticus*), which are primarily directed by steep oxic-sulphidic gradients. Subsurface distribution may be coupled to an indirect positive effect of low oxygen (Neira *et al.* 2001) through decreasing predatory and competitive activity, and preservation of organic matter leading to high food availability and quality. Bioturbating processes have a massive influence on population structure as oxygen and particulate organic matter are episodically inserted deeper down in the sediment. Given their aerobic respiration, the effect of bioturbation may especially be of relevance for deep infaunal nematode species of fine-grained sediments. In coarse, well-aerated sediments, biotic interactions may be rather important in structuring nematode communities *e.g.* species are vertically segregated in order to minimise competitive or predative interactions (Joint *et al.* 1982, Steyaert *et al.* 2003).

The oxygen availability of sediments influences structural as well as functional aspects of the nematode communities (Vanaverbeke *et al.* 2003). Due to their small size and short generation times, their benthic life cycle and their ubiquitous distribution, nematodes are potential biological monitors to evaluate oxygen depletion in intertidal areas.

The direct role of oxygen on the vertical distribution of nematodes was investigated by means of experiments in which different oxygen conditions were imposed on sediments from the Oosterschelde (The Netherlands). The migratory activity of the nematode community and of nematode species was evaluated as function of oxygen availability. The null-hypothesis to be tested is that the vertical distribution of the nematode assemblages and nematode species is not influenced by changing oxygen conditions (*e.g.* nematodes do not migrate to favourable oxygen conditions).

# Material and methods

Sediments (mean median grain size 92 µm) were collected on 8th March 2002 from an intertidal area (51°32.0'N; 03°52.5'E) of the Oosterschelde (The Netherlands) at low tide. Perspex cores (i.d. 3.6 cm) were used to sample the sediment up to a depth of 5 cm. Cores were transported to the lab and kept in the dark at 12°C. The cores were filled with fresh Oosterschelde water and continuously aerated. This was the control treatment (two C-cores). In two cores (An-cores), the sediment was turned upside down, imposing anoxic conditions on the original 0-1 cm depth layers. Similarly, in two other cores (Ox-cores), the sediment was inverted but with the presence of an air flushed silicone tubing running through the bottom of the core. Inversion of the sediment enabled to distinguish between oxygen-mediated and food-mediated interactions. Oxygen diffuses through silicone, thereby introducing oxygen to this otherwise anoxic sediment (Moodley *et al.* 1998). In all manipulated cores (Ox-cores and An-cores), the original 4-5 cm depth layer became the surface layer, oxygenated by the aerated overlying water (Fig. 1). Extra food was added (approximately 2 mg diatom C) on top of the C-cores and at the bottom of each manipulated core. All cores were incubated in the dark for one week. Separate cores were used to measure sediment oxygen concentration and redox potential after one week incubation, using micro-electrodes (UNISENSE). At the end of the experiment, cores were sliced in 1 cm slices and stored in a neutral 4% hot formaldehyde solution. The top layer of 2 mm was sampled with a pipette. The presence of living nematodes was verified prior to preservation.

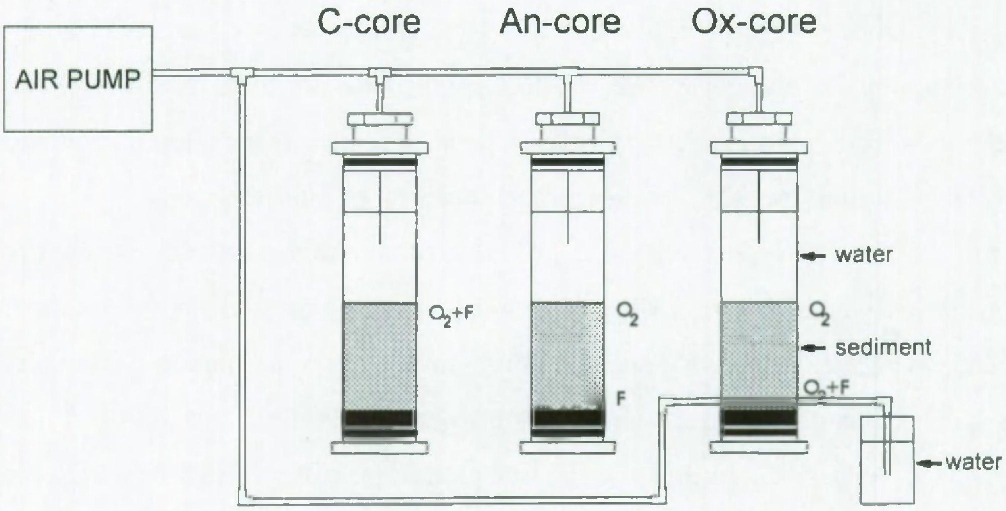

**Figure 1. Schematic of the experimental set-up. The water in the cores was continuously bubbled with air and circulated. $O_2$ and F indicate were oxygen and food is added. One replicate of each treatment is shown.**

Nematodes were extracted from the sediment by centrifugation with Ludox (Heip *et al.* 1985) and stained with Rose Bengal. Nematodes were enumerated from all slices. Identification to species level was done for the upper (0-1 cm) and the deepest (4-5 cm) sediment layer on the first 120 nematodes, mounted into Cobb-slides. For comparison with field nematode density profiles, two cores were sliced and processed, as described, directly upon sampling.

To test for variation in total nematode densities with treatment, with depth and with treatment × depth, a univariate 2-way analysis of variance (ANOVA) was used. A 'split-plot' design was constructed with replicates nested within treatments, following Steyaert *et al.* (2001). All data were log (x+1) transformed prior to analysis in order to met ANOVA assumptions.

Ordination techniques from the PC-ORD for Windows package (version 4.20, McCune & Mefford 1999) were used to examine similarities between 0-1 and 4-5 cm layers of the different treatments, based on the species composition of the communities. A Detrended Correspondence Analysis (DCA) was applied for the determination of the length of gradient, followed by a Correspondence Analysis (CA) using non-transformed relative abundances. Species rarer than $F_{max}/5$ ($F_{max}$ is the frequency of the commonest species) were down-weighted in proportion to their frequency. For all analyses, nematode values of the 0-1 cm surface layer included the upper 2 mm that was sampled separately.

## Results

The oxygen profiles of the un-manipulated and the inverted sediment were similar; the oxygen content dropped from 305 µmol $l^{-1}$ at the surface to 19.4 µmol $l^{-1}$ at 250 µm sediment depth (Fig. 2).

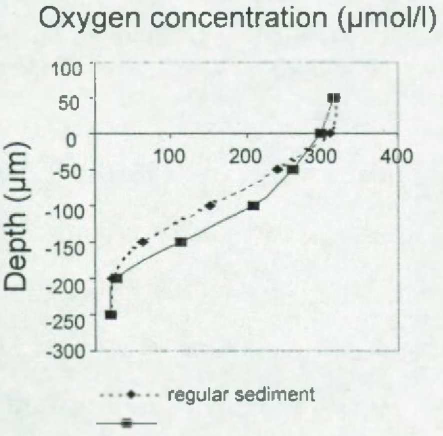

**Figure 2. Oxygen profiles in un-manipulated and inverted sediment.**

Sediment redox values of both the un-manipulated and the inverted sediment indicated that only the uppermost 2 mm was oxidised, evidenced as a light coloured top layer (Fig. 3). The surface layers of An-cores and Ox-cores (the original 4-5 cm layer) were initially dark brown and smelled strongly of sulphide. Oxygen profiles were not made around the silicone tube (Ox-core), but a clear oxidised layer of approximately 2 mm was clearly visible around the tubing during slicing.

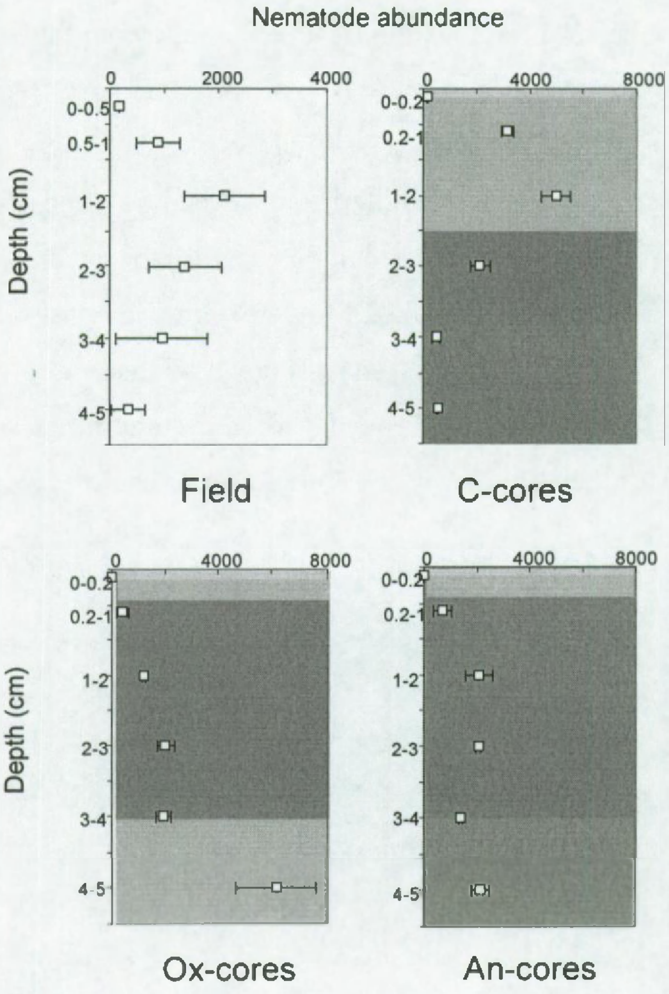

**Figure 3. Depth distribution of the total nematode community for the field situation and the different treatments (shading represents visual observation of sediment colour).**

Total nematode abundance of the field situation was 5850 ± 971 ind. 10 $cm^{-2}$. After one week, total nematode abundance of C-cores, Ox-cores and An-cores were respectively 11550 ± 305, 11820 ± 906 and 8390 ± 440 ind. 10 $cm^{-2}$. ANOVA 'split-plot' analysis demonstrated that nematode densities numbers between treatments ($F_{2,2} = 4.68$; $p > 0.05$) and between treatments and field situation ($F_{3,3} = 1.69$; $p > 0.05$) were not significantly different.

Investigation of the different life stages (juveniles/ females/ males) revealed no changes of juveniles with time.

Similar to field situation, in the C-cores, a subsurface density peak was recorded at a depth of 1-2 cm (Fig. 3). Densities decreased gradually deeper down the sediment. ANOVA 'split-plot' analysis demonstrated no significant differences between nematode depth distribution of the C-cores and the field situation ($F_{4,12} = 0.449$; $p > 0.05$). The distributional pattern in the An-cores showed no obvious peak. In the Ox-cores, peak values were recorded in the 4-5 cm sediment layer (the original surface layer). Total nematode distribution changed significantly in depth ($F_{5,5} = 73.89$; $p < 0.001$) for all cases. The interaction between treatment and depth was significant ($F_{10,15} = 18.56$; $p < 0.001$). The distributional patterns of total nematode community were significantly different between the three sets of cores.

In total 44 species were distinguished: 31, 33 and 32 in respectively the C-cores, Ox-cores and An-cores (Table 1). The species from the different cores were categorized according to their presence/absence in the upper (0-1 cm) and/or deeper (4-5 cm) sediment layer.

| | | C-cores | | | Ox-cores | | | An-cores | |
|---|---|---|---|---|---|---|---|---|---|
| **Number of species** | 0-1 | | 4-5 | 0-1 | | 4-5 | 0-1 | | 4-5 |
| Total | 27 | (31) | 16 | 29 | (33) | 20 | 26 | (30) | 23 |
| Common in both layers | | 12 | | | 13 | | | 19 | |
| Only present in 0-1 | | 15* | | | 16* | | | 9* | |
| Only present in 4-5 | | 4 | | | 4* | | | 5 | |
| **Relative abundance** | | | | | | | | | |
| Total | | 100.0 | | | 100.0 | | | 100.0 | |
| Common in both layers | | 79.3 | | | 94.7 | | | 95.0 | |
| Only present in 0-1 | | 19.3* | | | 0.7* | | | 1.6* | |
| Only present in 4-5 | | 1.4 | | | 4.6* | | | 3.4 | |

**Table 1. Occurrence of species in top and/or bottom layers of the different treatments (*: species present in oxygenated layers).**

Irrespective of the treatments in the cores, two striking features were evident (Table 1): (1) only a minor fraction of the nematode communities (4 – 5 species or 1.4 - 4.6 %), were confined to the 4-5 cm layer, irrespective of oxygenation; (2) the largest fraction, 79 - 95 % of the nematode community occurred both in the upper and deeper layers, irrespective of oxygenation. In order to identify species that were rigorously restricted to oxygenated areas, the species confined to oxygenated layers within a treatment (4 species in the 4-5 cm layer of the Ox-cores and 15 species in the 0-1 cm layer of the C-cores, Table 2), were examined for their occurrence in anoxic layers. In total, 6 species, *Aegialoalaimus elegans* (De Man 1907), *Axonolaimus helgolandicus* (Lorenzen 1971), *Bathyeurystomina sp.*, *Desmoscolex sp.*, *Dorylaimopsis sp.* and *Monoposthia mirabilis* (Schulz 1932), were restricted to these oxic horizons.

| | C-core | | Ox-core | | An-core | |
|---|---|---|---|---|---|---|
| | 0-1 | 4-5 | 0-1 | 4-5 | 0-1 | 4-5 |
| **Ox-core: 4-5 cm** | | | | | | |
| *Anoplostoma viviparum* | * | | | * | * | * |
| *Axonolaimus helgolandicus* | | | | * | | |
| *Dorylaimopsis sp.* | | | | * | | |
| *Monoposthia mirabilis* | * | | | * | | |
| **C-core: 0-1 cm** | | | | | | |
| *Aegialoalaimus elegans* | * | | | | | |
| *Anoplostoma viviparum* | * | | | * | * | * |
| *Bathyeurystomina sp.* | * | | | | | |
| *Chromadora macrolaima* | * | | * | * | * | * |
| *Chromadorella circumflexa* | * | | | | * | * |
| *Chromadorella sp.* | * | | * | | | * |
| *Chromadorita sp.* | * | | * | * | * | * |
| *Cyartonema sp.* | * | | | | | * |
| *Desmoscolex sp.* | * | | | | | |
| *Eleurolaimus sp.* | * | | * | | * | * |
| *Halalaimus sp.* | * | | * | | * | |
| *Hypodontophora sp.* | * | | | | * | * |
| *Monoposthia mirabilis* | * | | | * | | |
| *Sphaerolaimus sp.* | * | | * | | | |
| *Viscosia franzii* | * | | * | * | * | * |

**Table 2. Presence of species from (a) the 4-5 cm layer of the Ox-cores and (b) the 0-1 cm layer of the C-cores, in other treatments.**

| | C-core | | Ox-core | | An-core | |
|---|---|---|---|---|---|---|
| | 0-1 | 4-5 | 0-1 | 4-5 | 0-1 | 4-5 |
| **C-core: 4-5 cm** | | | | | | |
| *Daptonema sp.* | | * | * | | | |
| *Metalinhomoeus sp.* | | * | | | * | |
| *Siphonolaimus sp.* | | * | | | | |
| *Spirinia parasitifera* | | * | * | * | * | |
| **An-core: 4-5 cm** | | | | | | |
| *Calyptronema sp.* | | | | | | * |
| *Chromadorella sp.* | * | | * | | | * |
| *Chromadorella circumflexa* | * | | | | * | * |
| *Cyartonema sp.* | * | | | | | * |
| *Theristus sp.* | | | | | | * |

**Table 3. Presence of species from (a) the 4-5 cm layer of the C-cores and (b) the 4-5 cm layer of the An-cores, in other treatments.**

A similar analysis for the possible preference for anoxic layers revealed that only 3 species, namely *Calyptronema sp., Siphonolaimus sp.* and *Theristus sp.*, are strictly confounded to anoxic horizons (Table 3). The remaining 35 species are recorded in both oxic and anoxic horizons of the different treatments. Most abundant species were *Terschellingia communis* (De Man 1888), *Microlaimus tenuispiculum* (De Man 1922) and *Sabatieria pulchra* (Schneider 1906) totally accounting for about 45 % of the nematode assemblage.

Similarities between of 0-1 and 4-5 cm sediment layers from different treatments are shown in the CA plot (Fig. 4). The first ordination axis had an eigenvalue of 0.309; the second and the third (not depicted) axes had very low eigenvalues of respectively 0.141 and 0.126.

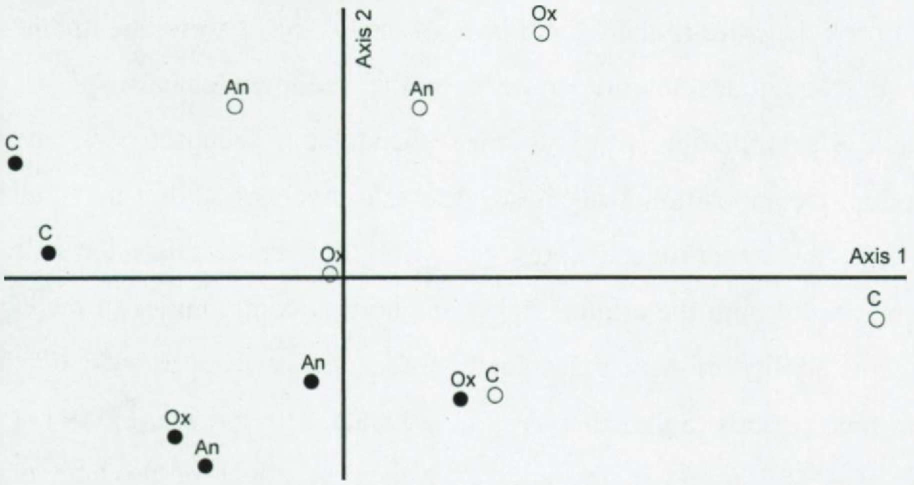

**Figure 4. Results of Correspondence Analysis. C: C-cores, Ox: Ox-cores, An: An-cores. Full circles: 0-1 cm layers, open circles: 4-5 cm layers.**

Along the first axis, samples of the 0-1 cm layer of the C-cores are clearly separated from the 4-5 cm layer of the C-cores (only one replicate). All samples of manipulated cores and one replicate sample of the 4-5 layer of the C-cores are plotted in between. The analysis further indicated the similarity between all original upper layers and all original deeper layers, since 0-1 layers of the C-cores and 4-5 layers of the An-cores and Ox-cores are plotted on one side of axis 2; 4-5 layers of the C-cores and 0-1 layers of the An-cores and Ox-cores on the other side of axis 2. Characteristic species for the upper sediment layer of the C-cores were *Bathyeurystomina* sp., *A. elegans, Desmoscolex* sp. and *Sphaerolaimus* sp.; for the 4-5 cm layer of the C-cores *Siphonolaimus* sp., *Daptonema* sp., *Metalinhomoeus* sp..

## Discussion

Oxygen profiles were not measured around the silicone tube but the presence of the oxidised layer around it confirmed the introduction of oxygen into this layer. Sediments in the experiment were not sieved or homogenised so that an unequal number of species may be attributed to patchiness. Additionally, macrofauna possibly present may have influenced the distribution patterns by introducing oxygen to deeper sediment layers (*e.g.* Wetzel *et al.* 1995, Fenchel 1996). Irrespective, it was evident that oxygenated sediments represent favourable conditions for nematodes; nematodes were concentrated in the oxygenated bottom layer. Both in the field situation and in the control cores, there was a subsurface peak in densities suggesting that peak densities in the 4-5 cm layer of the Ox-cores were due to migration from the layer above. Nematodes migrate upward in the sediment as a response to oxygen deficiency, often in combination with $H_2S$ stress (Hendelberg & Jensen 1993 and references therein). Community composition analysis suggests that inversion and/or manipulation of the oxygen regime in the experimental cores gave rise to vertical migration of nematodes, resulting in a mixing of both the original upper and bottom communities of the experimental cores. Despite the ability of nematodes to migrate actively over a wide depth range in relatively short time periods (Schratzberger *et al.* 2000a,b, Steyaert *et al.* 2001), a fraction of the nematodes was still found in the anoxic layers (but effect of possible macrofauna). Evidence for a slow recovery (years) of nematode assemblages after a hypoxic period was given by Austen *et al.* (1991).

In the experiment most nematode species (79 %) had a ubiquitous distribution relative to oxygen availability (group 2) and are evidently tolerant to short-term anoxic conditions and simultaneously also burial. This partly explains the minor changes in nematode community composition when sediment cores were inverted. The dominance of this group of species can be linked to the nature of the sediment. In general, fine sediments with steep gradients of oxygen and toxic sulphide harbour less sensitive species compared to well-aerated coarse sandy sediments (Steyaert *et al.* 1999, Vanaverbeke *et al.* 2003). The dominant species prevailing at our sampling station (*Terschellingia communis*, *Microlaimus tenuispiculum* and *Sabatieria pulchra)* are characteristic species for muddy suboxic or anoxic environments (Warwick & Gee 1984, Ólafsson 1992, Hendelberg & Jensen 1993, Modig & Ólafsson 1998, Boyd *et al.* 2000, Schratzberger *et al.* 2000a, Wetzel *et al.* 2002). In contrast, *Monoposthia* and *Desmoscolex*, which were only recorded in oxygenated conditions in the experiment, are common in well-aerated sandy sediments (*e.g.* Kim & Shirayama 2001, Gheskiere *et al.* 2002). In this experiment, the occurrence of *Monoposthia mirabilis, Desmoscolex sp.* and 4 other species, *Aegialoalaimus elegans*, *Axonolaimus helgolandicus, Bathyeurystomina sp.* and *Dorylaimopsis sp.,* seemed to be directly determined by the oxygen supply and may be potential bio-indicators of oxygenated intertidal sediments. The third group of species, defined as species which were not recorded in oxic environments, included *Calyptronema sp.*, *Siphonolaimus sp.* and *Theristus sp.*. There are however, no reports of these species being strict anaerobes and we have no explanation for their confinement to anoxic sediment layers.

Oxygenated sediment layers are clearly favourable habitats for nematodes and therefore, the subsurface peaks of nematodes generally found in intertidal sediments (Soetaert *et al.* 1994) reflect other structuring factors. This may include inter-intra specific competition, resource partitioning, reducing risks of predation *etc.*

Similar experiments with coarse sandy, well-aerated sediments and associated nematode communities may provide more insight into the direct role of oxygen. It is expected that species like, *Richtersia* sp. or *Desmoscolex* sp., typical of sandy sediments may be more sensitive to anoxic conditions. Experiments incubating known densities of potential oxyphilic species in a set of different oxygen regimes could elaborate on the controlling role of oxygen.

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# General conclusions

The investigations on the spatial and temporal scales of nematode communities in the North Sea and Westerschelde lead to the following conclusions:

1. The recognition of horizontal trends across North Sea sites confirms the important influence of sediment granulometry on the diversity of nematode communities. Coarser sediments yielded more diverse nematode communities, finer grained sediments were associated with reduced diversity. However, this relationship did not necessary apply at small spatial scales, as indicated by diversity profiles within individual cores. These profiles showed the inverse relationship, *i.e.* the finer the sediment becomes, the more diverse the nematode community. This may be explained by the higher organic matter content of fine-grained sediments. In the case of coarse sediments with large, well-aerated interstitial spaces, the diversity is high, rather constant with depth, and not affected by the redox profiles within the sediment. In contrast to the North Sea sites, a horizontal gradient in diversity was not obvious at the Molenplaat sites. Detailed investigation of vertical depth profiles showed more pronounced differences between environmentally divergent sites. Vertical profile analysis yields additional information that cannot be obtained by bulk sampling and provides a key to understand regional patterns and their relation with environmental characteristics in nematode communities.

2. The seasonal (March – June – August) inventory of the nematode communities at three coastal North Sea stations demonstrates that controls on nematode community structure are complex and that information at both species and community level is required to properly evaluate the effects of natural and anthropogenic impacts. Anthropogenic impacts on the whole community along the Belgian coast are reflected in the species diversity of nematode communities. In early spring, when the sediment pore waters were strongly oxidised, species diversity was high deep into the sediment. Under these conditions, diversity was higher in deeper, muddy sediment layers because of the diversification of non-selective deposit feeders. In early summer, as the sediments became more reduced, diversity values decreased with depth. This could be attributed to more favourable redox conditions in the superficial layers, enabling more species to co-exist. For the purpose of assessing anthropogenic impacts, the distribution profiles of single nematode species were much less

informative than information on diversity profiles of the whole nematode community. The use of nematodes as ecological indicators still remains highly problematic.

3. In general, the lower diversity at the coastal sites (this study) compared to the diversity at offshore sites (Vincx 1990, Cattrijsse & Vincx 2001, Vanaverbeke *et al.* 2002) may be largely the result of the polluting impact of coastal waste and river discharges. Nevertheless, a shift to an enriched nematode assemblage near the Westerschelde estuary was demonstrated over a period of almost 20 y (1970 - 1993). In addition to the relatively rich nematode assemblages at the Molenplaat (Westerschelde estuary) sediments, this may relativize the polluting impact of the Westerschelde river on the North Sea sediments. On the other hand, the nematode fauna of the western part of the Belgian coast clearly shows a decreasing trend in diversity, which is attributed to a fining of the sediment due to sediment transport, pollution effects and dredging activities.

4. Phytoplankton sedimentation in the organically enriched Station 115b generated changes in abundance, community composition and feeding type distribution. The response was expressed mainly as a shift in vertical distributions in the sediment. At periods of fresh food supply to the bottom, animals were more concentrated in the top two centimetres of the sediment, but later on, as the algal material was buried and progressively mineralised, highest densities were recorded deeper in the sediment column. These shifts in the total nematode community reflected the temporal changes of the epistrate feeders, and to a lesser degree, the non-selective deposit feeders. At Station 115b niches are successively occupied by dominant species, although neither the number of species nor their relative abundance differed over time.

5. A tidal survey of nematode communities on the Molenplaat demonstrated the dynamic nature of vertical distribution profiles in intertidal sediments. The fact that tidal migration patterns among nematodes are species-specific highlights both the importance of sampling in relation to the tidal cycle and investigating communities at the species level. Though tidal induced sediment transport has been demonstrated in subtidal areas along the Belgian coast (Van Lancker 1999), the effect of tides at subtidal sites has not been considered in this thesis. As such it remains an open question whether the effect of tides is as drastic in subtidal areas compared to intertidal areas.

6. Experimental evidence demonstrated that oxygenated sediments are favourable for nematodes. The manipulative introduction of oxygen to deep layers significantly altered the vertical distribution of overall nematode densities. However, the distribution of the majority of the species present in intertidal fine-grained sediment was not directly linked to oxygenation. It is hypothesised that nematode species living in coarse sandy sediment are potentially more sensitive to anoxic conditions. Experiments incubating known densities of potential oxyphilic species in a set of different oxygen regimes could clarify the role of oxygen in controlling the vertical distribution patterns of nematodes.

7. A comparison of the nematode assemblages at Station 115 and 115b, both located within the gully but separated by a few hundreds of metres, demonstrated differences in community composition as well as in diversity. Though yearly variation has not been established within the area, this suggests a substantial sediment heterogeneity of the gully between the Coastal and Flemish Banks which is reflected in the nematode communities.

8. The analysis of vertical distribution patterns, and efforts to explain their causality, yielded important autoecological information about different nematode species.
    - *Sabatieria punctata, S. celtica* and *S. pulchra* are well adapted to stressed environments. Both *S. punctata* and *S. celtica* were characteristic species in the fine-grained subtidal stations off the Belgian coast; *S. pulchra* was dominant in the fine-grained intertidal stations of the Molenplaat. Caution must be exercised when lumping *Sabatieria* species into groups according to their morphological characteristics and their affinity for a certain redox state. *Sabtieria punctata*, a species classified previously within the *S. pulchra* group, was abundant in both oxidised and reduced sediments, despite the fact that members of this group are generally considered to be associated with reduced sediments. Investigation of the spring-bloom-related seasonal dynamics of both *S. punctata* and *S. celtica* demonstrated the role of food availability in structuring their vertical distribution in the sediment. The presence of *S. pulchra* in the intertidal area has not been linked to the redox status of the sediment, though experimental evidence suggested that the distribution of this species was not primarily related to oxygenation of the sediment. In contrast, it is clear that *S. pulchra* is resistant to burial and highly tolerant of anoxic conditions. These characteristics are consistent with its evenly vertical distribution in the Molenplaat sediment.

- The importance of oxygen in structuring the vertical distribution of nematodes is less evident in fine-grained sediments. Experimental data indicated a high tolerance to short-term anoxic conditions for the majority of intertidal nematode species from fine sediments. Key species that illustrate this strategy were *Terschellingia communis, Microlaimus tenuispiculum* and *Sabatieria pulchra*. Oxygen appeared to be a controlling factor for only a limited fraction of the nematode community (*e.g. Aegialoalaimus elegans, Axonolaimus helgolandicus, Bathyeurystomina sp., Desmoscolex sp., Dorylaimopsis sp.* and *Monoposthia mirabilis*). Field evidence also demonstrated an affinity for deeper sediment layers in the case of xyalid-species (*Daptonema fistulatum, D. proprium, D. riemanni, Theristus blandicor*). Although a direct link with oxygen concentrations could not be established, the sediment microhabitats in which *Daptonema* species lived were shown to be completely reduced. Their tolerance of the extreme conditions prevailing in deeper sediment layers (*e.g.* sediment compaction, micro amounts of oxygen) enabled the xyalid-community to develop high densities and to live segregated by depth from an upper *Sabatieria* community (in the case of *D. fistulatum, D. proprium, D. riemanni*) or an enoplid community (in the case of *T. blandicor*).
- Food availability is believed to be a predominant factor controlling the vertical distribution of several nematode species in the sediment. Direct evidence was provided by the study of responses to phytoplankton sedimentation. When fresh food was supplied to the sediment surface, a vertical depth segregation of *Sabatieria* (*S. punctata, S. celtica*) and *Daptonema* (*D. fistulatum, D. proprium, D. riemanni*) species minimised interspecific competition for food. Later, the excess of mineralised algal-C in deeper sediment layers enabled species to co-exist in the same depth horizon without competition. The tidal dynamics of *Enoploides longispiculosus* also suggest that vertical distribution patterns are related to food availability. This large predator migrated upwards during the incoming tide and downward when the flat became exposed, tracking the tidal-related dynamics of ciliates, which formed an important food source at the Molenplaat site.

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