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Nematode Response to Changing Food Conditions in Shallow Marine and Estuarine Sediments

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Summary

During spring Belgian coastal waters are clearly dominated by intense *Phaeocystis* blooms co-occurring with diatom blooms. The effect of the deposition of a phytoplankton bloom on the meiobenthic community, with special focus on the nematode community was investigated in two contrasting sites in the southern North Sea: one station (station 115bis) is situated close to the coastline and is characterised by fine grained sediments while a more off-shore station (station 330) had permeable sediments. These two stations were sampled from October 2002 to October 2003 for chlorophyll *a* concentration in the water and sediment, bacterial biomass and abundance, and meiobenthos. During detailed sampling campaigns in February, April and October, corresponding to a situation prior to, during and after the spring phytoplankton bloom, samples were collected to determine the bacterial community composition and diversity, the natural carbon and nitrogen stable isotopic signatures of meiobenthos, Suspended Particulate Mater (SPM) and Particulate Organic Mater (POM) and also macrobenthos samples were taken. Also for these sampling dates Sediment Community Oxygen Consumption (SCOC) was measured as well as water temperature.

The biogeochemistry of the sediments differed considerably. In fine sediments, there was an accumulation of phytodetritus at the sediment surface after deposition of the phytoplankton bloom, which remained in the sediment for a long period, getting buried with time. A steep vertical gradient of chlorophyll *a* (as an indicator of labile organic matter (OM)) was observed after sedimentation, with mineralisation of the fresh OM leading to anoxic conditions in the sediment. In permeable sediments chlorophyll *a* concentration in the sediment was about 10 times lower than in finer sediments. The strong bottom water currents and advective transport did not allow the establishment of vertical gradients, with sub-surface peaks of chlorophyll *a* being reported. The sediment remained oxic through the whole time at the studied depths.

Previous studies reported bacterial-feeding nematodes to be among the first to respond to the deposition of phytodetritus. Therefore the bacterial community composition and diversity were investigated by means of Denaturing Gradient Gel Electrophoresis (DGGE). Two sediment horizons were investigated (0-1 cm and 4-5 cm). Bacterial community composition appeared to be primarily dependent on the amount of labile OM available. Stronger shifts on food availability in fine grained sediments induced stronger seasonal differences in community composition when compared to coarser sediments. The higher food availability in fine grained sediments supported a more diverse bacterial community. Vertical differences in bacterial community composition were also observed at both stations; however the probable causes for such differences were not the same. In fine sediments, two different communities could be distinguished due to strong vertical gradients in OM and oxygen concentrations in the sediment. Both communities showed similar diversities. In coarser sediments some bacterial populations were not able to establish themselves at the sediment surface as a consequence of the higher hydrodynamic stress, leading to a less diverse community at the sediment surface. Seasonal shifts in bacterial diversity were therefore not detected for both stations. This could not be the reason behind changes in the nematode community, as previously hypothesised, but shifts in biomass and bacterial community composition could.

Nematode samples were sliced in 1 cm thick slices up to a depth of 10 cm. Nematode densities were higher in fine grained sediments where maximum abundance values were about 4 times higher than the highest nematode densities in coarser sediments. Different responses of the nematode community to the sedimentation event were observed. In coarse sediments nematodes responded fast in terms of densities to deposition of phytodetritus while in fine sediments this response was delayed until late summer. The natural signatures of the stable isotopes ^{13}C and ^{15}N in the nematode community and sediment POM were investigated in two different sediment horizons (0-1 cm and 4-5 cm). Changes in the nematode stable

isotopic signatures did not reflect changes observed in SPM or POM, indicating a certain degree of selectivity on their feeding strategy. In fine grained sediments *Sabatieria* and *Richtersia* showed an opportunistic behaviour, migrating to the sediment surface to feed, while other deep dwelling nematodes feed on OM available in deeper sediment layers. The so called "stout nematodes", which have been previously reported to opportunistically react first after the arrival of phytodetritus to the coarser sediments, did not revealed any different feeding strategies than the other nematodes. In fine sediments copepods revealed stable isotopic signatures clearly indicating the exploitation of a chemosynthetic derived food source, which may also be exploited by deep-dwelling nematodes.

In a following experiment, ^{13}C labelled *Phaeocystis* and *Skeletonema costatum* diatoms were supplied to both sediment types. In coarser sediments, even though nematode biomass was lower, a higher uptake of algal material per nematode carbon unit was observed, indicating that this nematode community is better adapted to react fast to an input of OM. In both sediments the measured carbon uptake was not nearly enough to sustain the nematode community carbon demands. Similar results have been reported previously, and it might be related to methodological limitations and/or exploitation of different food sources by the nematode community. *Phaeocystis* was incorporated into nematode tissues at low but similar rates as the diatoms, indicating that *Phaeocystis* is a potential food source for nematodes that might had been overlooked in the past, since it was believed *Phaeocystis* would complete its cycle on the water column.

In an attempt to determine the relative importance of the nematode community in the benthic system, respiration was estimated for bacteria, nematodes and macrobenthos at both sediment types, prior to, during and after the phytoplankton bloom sedimentation event. Bacterial respiration was estimated using a fixed bacterial growth efficiency of 20 %. Nematode and macrobenthic respiration were estimated by means of allometric relations previously

described in literature. Comparable bacterial biomass was observed at both stations, while a higher nematode and macrobenthic biomass was observed in fine grained sediments, supported by a higher amount of labile OM. In permeable sediments the nematode and macrobenthic response in terms of biomass to the deposition event was fast while in fine grained sediments this response was delayed. Heterotrophic respiration shares of SCOC revealed a higher importance of anaerobic pathways in fine grained sediments. In fine sediments, bacterial biomass increased fast after deposition of the phytoplankton bloom while nematodes and macrobenthos response was delayed. In coarser sediments, nematodes and macrobenthos also showed a fast response. The respiration shares of SCOC in fine sediments were initially dominated by macrobenthos. After sedimentation occurred nematodes and macrobenthos had similar shares, with macrobenthos recovering their higher relative importance later. In coarser sediments SCOC was clearly dominated by bacterial respiration, with nematodes and macrobenthic respiration representing generally small shares of SCOC. The relative importance of the nematode community in subtidal fine grained sediments might therefore become more significant whenever anoxic conditions arise.

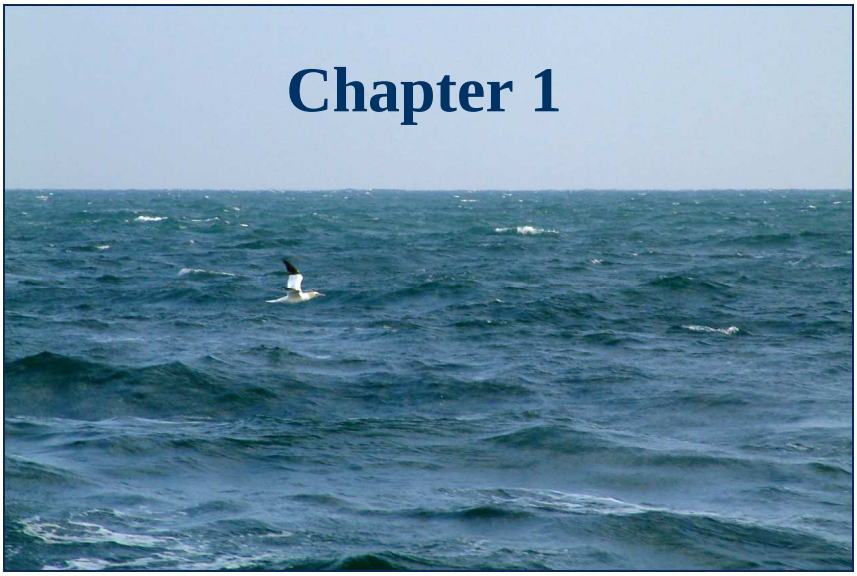
While in the BCS the nematode response to a **natural** input of OM was investigated, a short term experiment in the Tagus estuary was conducted to assess the effect of **anthropogenically** added carbon (dead discards from beam trawl fishing) on the nematode community. Comparably to the North Sea situation in fine sediments, after settling of OM at the sediment surface, anoxic conditions developed at the sediment surface due to bacterial activity. After adding shrimp carcasses to the sediment surface, simulating the settling of dead discards onto the sediment, the nematode community vertical distribution was monitored after 2, 4 and 6 h (average exposure time of an intertidal mud flat) in different sediment horizons (0-0.5; 0.5-1; 1-1.5; 1.5-2, 2-3 and 3-4 cm). Two major responses were observed: nematodes sensitive to high sulphidic condition migrated deeper in the sediment away from the anoxic

patches (*Chromadora* and *Ptycholaimellus*), while *Sabatieria*, more tolerant to such conditions, showed an opportunistic behaviour occupying the left empty niche at the sediment surface. Other nematodes remained unaffected by the experiment, which also reveals a good adaptation, in short terms, to the imposed conditions (*Daptonema*, *Metachromadora*, and *Terschellingia*).

As in the BCS, *Sabatieria* revealed an opportunistic behaviour, migrating towards better food conditions.

The response of the nematode community and other benthic organisms to the deposition event of the spring phytoplankton bloom on the BCS was, at all levels investigated, sediment specific. The benthic community inhabiting coarse sediments is therefore strongly influenced by the permeability of the sediment.

Chapter 1



General introduction and aims

Meiobenthos in shallow marine and estuarine sediments

In shallow marine and estuarine sediments the coupling between the processes in the water column and the dynamics in the sediments is well documented (see Graf, 1992; Ritzrau *et al.*, 2000 and references therein). In marine environments, the benthic communities below the euphotic zone are supported by primary production from pelagic or benthic photic areas, with a few exceptions of hot vents and cold seeps. The magnitude of this supply of organic matter (OM) is determined by temporal and spatial patterns (Ritzrau *et al.*, 2000). Sedimentation events of phytoplankton blooms represent a major source of organic matter for the benthic system (Billen *et al.*, 1990; Graf, 1992). However, the source and the nature of the organic material sedimenting on the substrate are important for the benthic dynamics. Also the fate of the organic material which arrives to the sea floor is dependent on the nature of the sediment (Graf, 1992). Bentho-pelagic coupling is the transfer of organic matter from the euphotic zone to the seafloor as well as the benthic reaction to this input (Ritzrau *et al.*, 2000).

The benthic system comprehends a highly diverse community, composed of bacteria, micro-, meio- and macrobenthos, with the classification of benthic organisms generally relying on the organisms' size. Meiobenthos is here defined as all metazoan sediment inhabiting organisms, passing a 1 mm mesh size sieve and being retained on a 38 µm mesh size sieve. Microbenthos is therefore the organisms smaller than 38 µm (e.g. flagellates and ciliates), and macrobenthos the organisms retained in a 1 mm sieve (e.g. polychaetes, bivalves, crustaceans, gastropods and echinoderms).

Meiobenthos is highly diverse and include organisms from a wide variety of taxa like Nematoda, Copepoda, Turbellaria, Gastrotricha, Rotifera, Tardigrada, Kinorhyncha (*see* Higgins & Thiel (1988) for a complete list). Frequently, abundance values of about 10^6 ind m^{-2} of sediment surface, versus 10^4 ind m^{-2} for macrobenthos are found (Coull, 1988;

Miller *et al.*, 1996). Biomass generally varies between 1-2 g m^{-2} in shallow waters, with the highest values reported for estuarine mud flats (Bouwman, 1987; Coull, 1988). Meiobenthos is particularly important within estuarine systems since it facilitates biomineralization, support various higher trophic levels and shows a high sensitivity to anthropogenic actions, making them excellent organisms for estuarine pollution bio-monitoring (Coull, 1999).

Meiobenthic organisms exploit the interstitial matrix of marine soft sediments and their small size and low mobility makes them very sensitive to the biogeochemical characteristics of their environment. Since meiobenthos shows high species diversity, short generation times, a direct benthic development and ubiquitous distribution, they are considered a very good biological tool to monitor changes in the benthic environment (Kennedy & Jacoby, 1999).

Free-living nematodes are the most abundant animals in most marine sediments (Heip *et al.*, 1985; Riemann, 1988) and therefore generally dominant within the meiobenthos. Nematodes are considered to feed mostly on bacteria, microalgae, ciliates and detritus with some nematodes being predators (Moens & Vincx, 1997). Wieser's (1953) classification of nematodes into four trophic groups according to the morphology of their buccal cavity has been traditionally used. Wieser (1953) distinguished selective deposit feeders (1A-group, nematodes without a developed buccal cavity that can only ingest small particles, mainly bacteria) non-selective deposit feeders (1B-group, nematodes with a buccal cavity but without teeth, being able to ingest bigger particles including diatoms) epigrowth feeders (2A-group, nematodes with small teeth in the buccal cavity that allow them to break cells and suck its content or graze surfaces) and the predators (Wieser, 1953) or omnivores (Wieser, 1960) (2B group, nematodes with teeth or powerful mandibles that allow them to capture preys and ingest them or their contents). Moens & Vincx (1997) proposed a new classification in six trophic groups: microvores feeding exclusively on bacteria; ciliate feeders feeding mainly on ciliates but also on bacteria; deposit feeders feeding mostly on bacteria, diatoms and other

microalgae; epigrowth feeders ingesting mainly diatoms and other microalgae; facultative predators that can feed on various items including detritus as well as other nematodes; and finally the predators feeding mainly on nematodes and oligochaetes. This classification was based on observations of the feeding behaviour of living estuarine nematodes. The abundance of each different trophic group can vary considerably according to the study site and prevailing environmental conditions.

Meiobenthic trophic interactions

Many aspects of food web trophic interactions can be investigated more directly and precisely using a variety of methods currently available to ecologists, as molecular methods, biomarkers (e.g. fatty acids) and biological tracers (e.g. stable isotopes).

A range of molecular techniques allow prey remains to be identified, often to the species and even stage level. These techniques include enzyme electrophoresis, a range of immunological approaches using polyclonal and monoclonal antibodies to detect protein epitopes, and polymerase chain reaction (PCR)-based methods for detecting prey DNA (Symondson, 2002; Sheppard & Harwood, 2005).

Biomarkers are specific biochemical compounds that may be transferred conservatively to a higher trophic position and recognized in consumers. Fatty acids have been used as qualitative markers to trace or confirm predator prey relationships, becoming an important tool for resolving trophic interactions in marine environments (Dalsgaard & St. John, 2004; Dalsgaard *et al.,* 2003). The principle behind the fatty acid biomarker approach is that consumers deposit fatty acids obtained from their food sources without modifying their structure, providing an integrated record of the major food items in their diet (Dalsgaard & St. John, 2004).

Stable isotopes techniques were used to investigate the meiobenthic trophic interaction in the North Sea. Stable isotopes of carbon and nitrogen are increasingly used in marine ecosystems for ecological and environmental studies (Lepoint *et al.*, 2004). Stable isotopes are used as tracers in food-web dynamics studies in both naturally occurring levels and experimentally enriched abundances. Isotopes are any forms of an element with a different atomic mass. The isotopes of an element have a nucleus with the same number of protons (same atomic number) but different numbers of neutrons and therefore a different atomic mass. The use of stable isotopes techniques requires a minimum of biomass, which can be a problem when working with very small animals like meiobenthos in general and nematodes in particular. Dual stable isotopic signatures of nematodes have been mostly reported on a community level (e.g. Riera *et al.*, 1996, 1999; Iken *et al.*, 2001; Riera & Hubas, 2003) and detailed studies determining the trophic role of different nematode taxa are still scarce (Carman & Fry, 2002; Moens *et al.*, 2005).

Stable isotopes can also be used as tracers in food web research. A resource's isotopic composition is deliberately changed and therefore its transfer along the food web can be monitored. The incorporation of the modified isotopic ratio from the resource is the difference between the new isotopic ratio from the sample and the background isotopic ratio.

When using labelled food sources as tracers, nematodes have been mostly handled as a community (e.g. Widboom & Frithsen, 1995; Middelburg *et al.*, 2000; Urban-Malinga & Moens, 2006; Van Oevelen *et al.*, 2006b) with only a few studies where some distinction was made within the nematode community (Ólafsson *et al.*, 1999; Carman & Fry, 2002; Moens *et al.*, 2002).

The nematode contribution to total sediment activity is still poorly quantified and limited to a few studies. To our knowledge, no information for shallow subtidal sediments is available yet. The nematode contribution to total carbon turnover was highest (13% at the most) at the

shallowest station, (206 m deep) of a depth gradient along the continental slope of the Goban Spur in the NE Atlantic Ocean (Soetaert *et al.*, 1997). In another study, on intertidal estuarine sediments, only less then 1% of total respiration was attributed to nematodes, which depended primarily on microphytobenthos as a food source (Van Oevelen *et al.*, 2006c). Hubas *et al.* (in press) estimated production for bacteria, meio- and macrobenthos for contrasting intertidal sediments. The shares on total heterotrophic production varied considerably between the different components and also when comparing the contrasting sediments.

Nematode communities from different environments can have different roles in the benthic carbon cycle. Such differences might be a reflection of differences not only in the organic matter quality and quantity but also in the biogeochemistry of the sediment (Hubas *et al.*, in press).

Investigating the trophic interactions within nematode communities as well as its role in the benthic food web can help in achieving a better understanding of the overall importance of this community on shallow subtidal benthic systems.

Study sites

Belgian Continental Shelf

The Belgian Continental Shelf (BCS), located in the south-eastern part of the Southern Bight of the North Sea has an area of 3600 km^2, with a coastline of 67 Km (Fig. 1.1). This area is characterized by the presence of extensive sandbanks and it is a relatively shallow area, with deepest waters of about 40 m in the northwest area.

The BCS is a well mixed nutrient rich part of the North Sea (Brussaard *et al.*, 1995) characterized by high primary production and algal biomass (Joint & Pomroy, 1993) with a strong seasonality. During spring Belgian coastal waters are dominated by intense blooms of *Phaeocystis* colonies co-occurring with diatom blooms (Reid *et al.*, 1990; Joint & Pomroy,

1993; Brussaard *et al.*, 1995). *Phaeocystis* blooms occur between April and May and last for 20 to 40 days, while diatoms can be present throughout the year with dense blooms occurring as early as February and smaller blooms as late as September (Reid *et al.*, 1990; Rousseau *et al.*, 2002). The sedimentation of phytoplankton in the North Sea represents the bulk of organic matter reaching sediments (see Billen *et al.*, 1990).

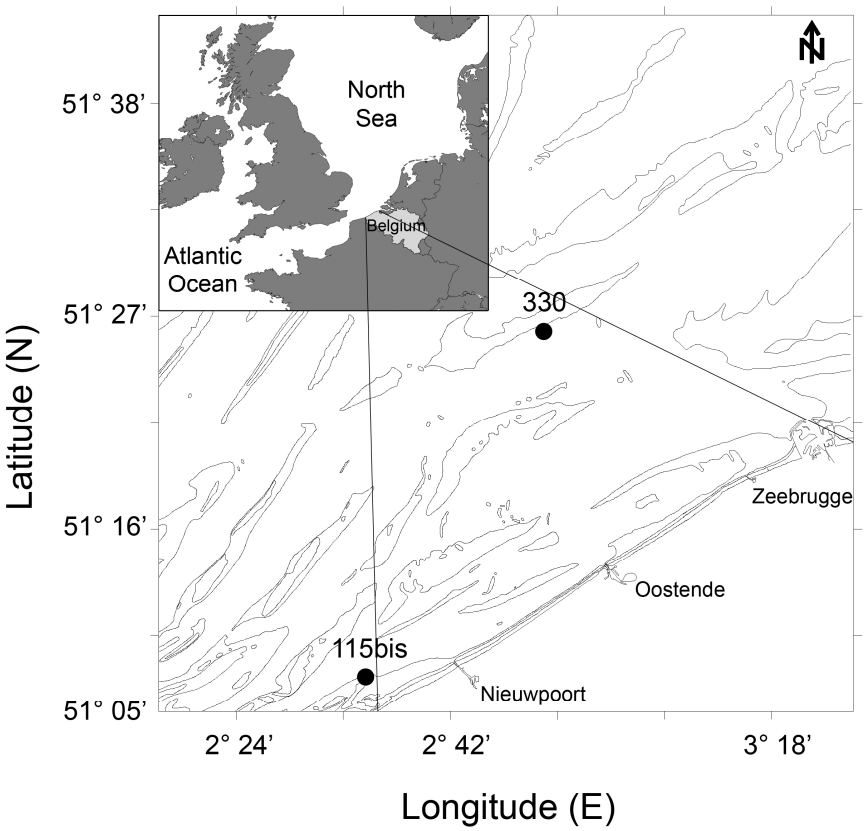


Figure 1.1: Location of the sampling stations 115bis and 330 on the Belgian Continental Shelf.

Two study sites were chosen on the BCS: station 115bis situated close to the coast line at depth of ca. 13 m, and station 330, located more offshore at depth of ca. 20 m (Fig. 1.1). Station 115bis is a deposition station, characterized by the presence of fine sediments (median grain size: 185 µm) with a small fraction of mud (4 %). After sedimentation of the spring phytoplankton bloom, chlorophyll *a* tends to accumulate at the sediment surface, getting buried with time; the whole sediment column becomes reduced, remaining that way as late as

October, which together with the low nitrite/nitrate and high ammonium concentrations indicates the importance of anoxic pathways (Steyaert *et al.,* subm.). In fine-grained depositional sediments the mineralisation of the newly arrived carbon is often associated with oxygen stress (Graf, 1992) and breakdown of this OM can be retarded until late summer (Boon & Duineveld, 1998; Provoost *et al.*, in prep.).

Station 330 consists of medium sand (median grain size: 329-361 µm) without mud. Chlorophyll *a* concentrations in the sediment are much lower then at station 115bis and subsurface peaks tend to occur frequently. The redox values of the sediment column remain positive during a phytoplankton deposition event (Vanaverbeke *et al.*, 2004a,b).

The permeability of the sediment is the capacity of the sediment to transmit fluid, controlled by size and interconnectedness of interstitial pores, and the tortuosity of fluid flow paths; such factors are in turn controlled by the grain-size distribution, grain shape, and porosity (Spinelli *et al.*, 2004).

Using the Carman-Kozeny relation (Rusch *et al.*, 2001; Forster *et al.*, 2003) it is possible to estimate the sediment permeability:

$$k_{CK} = d_{50}^2 \, \phi^3 \, / \left(180 \, (1-\phi)^2\right)$$

d_{50} is the median grain size and ϕ the sediment porosity (vol/vol). However once compared to real sediment permeability measurements, this relation clearly overestimates sediment permeability (Rusch *et al.*, 2001; Forster *et al.*, 2003) by a factor of ca. 5 (Rusch *et al.*, 2001). Applying this relation to the studied areas, corrected by a factor of 5, both sediment types are considered as highly permeable sands ($k > 10 \times 10^{-12}$ m^2, Forster *et al.*, 2003) with permeability being twice as high in station 330. However sediment at station 115bis does not behave as being permeable, since it is situated between two sand banks (Vanaverbeke pers. com.) and it behaves as deposition sediments.

In permeable sediments, advective water currents through the sediment transport oxygen into the sediment (Ziebis *et al.*, 1996; Janssen *et al.*, 2005) and induce a fast removal of decomposition products (Huettel *et al.*, 1998), factors accelerating the mineralization of organic carbon and the recycling of nutrients (Huettel & Rusch, 2000; Janssen *et al.*, 2005; Bühring *et al.*, 2006). Moreover, the advective currents are responsible for the observed subsurface peaks of chlorophyll *a* (Ehrenhauss & Huettel, 2004; Ehrenhauss *et al.*, 2004a; Vanaverbeke *et al.*, 2004b).

Recently some research has been focusing on the biogeochemistry of permeable sediments and the role of advective pore water transport in these systems, mostly through experimental studies (e.g. Huettel *et al.*, 1996; Huettel *et al.*, 1998; Ehrenhauss & Huettel, 2004; Precht & Huettel, 2003; Precht *et al.*, 2004; Meysman *et al.*, 2006), but also with some subtidal *in situ* studies (Ehrenhauss *et al.*,2004a; Precht & Huettel, 2004; Janssen *et al.*, 2005). Even though the transport processes of permeable sediments and its consequences on nutrients exchanges and mineralisation are now better understood (see references above), studies concerning functional responses of meiobenthic communities inhabiting these sediments are scarce, both in the intertidal (e.g. Urban-Malinga & Moens, 2006) and the subtidal (e.g. Vanaverbeke *et al.*, 2004a,b; Urban-Malinga *et al.* 2006).

Tagus estuary

The Tagus estuary (38°44'N, 9°08'W) is the largest Portuguese estuary and one of the largest of Western Europe (Fig. 1.2). It covers an area of about 320 Km^2, of which 40 % is intertidal (mudflats and salt marshes).

The tidal amplitude is ca. 4 m. The Tagus estuary has two morphologically distinct areas, the upper part, wide and shallow, with extensive intertidal flats and salt marshes, subjected to

intense sediment deposition and erosion; and the lower part of the channel, ca. 30 m deep (Brogueira & Cabeçadas, 2006).

Natural oxygen concentrations in muddy sediments of the Tagus estuary reach already extremely low values in the first millimetre, and become undetectable at 14 mm deep (Cartaxana & Lloyd, 1999).

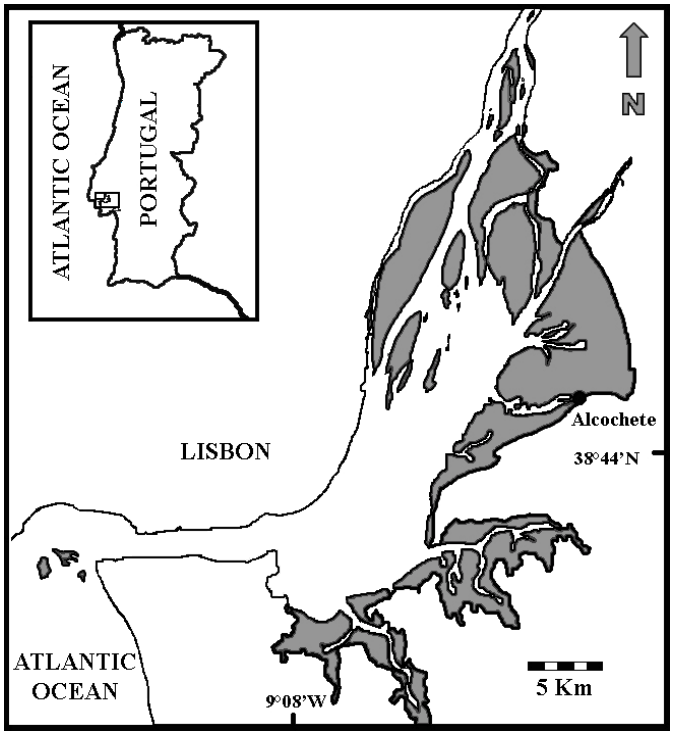


Figure 1.2: Location of the study area in the Tagus estuary, Portugal. The intertidal areas are shown in grey.

The Tagus estuary is presently the only Portuguese estuary where beam-trawl fishing is still allowed and it is common fishing practice in the area. It was traditionally directed to the shrimp *Crangon crangon*, (Linnaeus, 1758) but due to the lowering commercial value of this species, other species, like the soles *Solea solea* (Linnaeus, 1758) and *S. senegalensis* Kaup, 1858, became the real target species. *C. crangon* is mostly discarded and the dead portion of the discards represents an input of organic matter to the sediment (Cabral *et al.*, 2002).

Framework

This Ph.D. research project is part of an interdisciplinary biodiversity project within the framework of the project "TROPHOS: higher trophic levels in the Southern North Sea", funded within the Belgian research programme "Scientific Support Plan for a Sustainable Development Plan" (SPSD II, 2002-2006). TROPHOS is the follow-up of the SPSD I project: "Structural and Functional Biodiversity of North Sea Ecosystems: species and their habitats as indicators for a sustainable development of the Belgian Continental Shelf" (1997-2001). In the SPSD I project, the limited knowledge on the temporal variability of the benthos was identified as a major gap in the understanding of the ecology of the BCS. Therefore, two stations, one in coastal (Station 115bis) and the other (Station 330) in more offshore waters (Fig. 1.1), were sampled weekly from February to July 1999 for all size classes of the benthos combined with an extensive set of environmental variables. Sediments at these stations were very different: at Station 115bis sediments consist of fine sand with a mud percentage of 4 % (Steyaert *et al.*, subm). Sediments at Station 330 are classified as medium sand devoid of mud (Vanaverbeke *et al.*, 2004a,b). Both sediments are considered representative of the sediments prevailing at the BCS. Analysis of detailed temporal data of meiobenthos revealed different patterns for the two contrasting sediments. This difference in sedimentologic characteristics has important consequences for the biogeochemistry and hence for the meiobenthic organisms living in close contact with their environment. Nematode responses to phytodetritus sedimentation at both sites differed significantly leading to assume that at the BCS very different ways of ecosystem functioning prevailed at only a relatively small geographical distance. Quantifying and modelling these ecosystem processes (including food-web reconstruction) was therefore the next step to increase the understanding of the structuring

factors for benthic biodiversity and one of the major goals of both this PhD project and the TROPHOS project.

In the SPSD I project, massive sedimentation of the algae *Phaeocystis* to the seafloor was observed for the first time, which contrasted the commonly accepted idea that the *Phaeocystis* cycle was completed within the water column only (Rousseau, 2000). This indicated the need for assessing the fate of *Phaeocystis*, both in the water column and marine sediments.

PhD thesis objectives

This PhD project aimed at providing an explanation for the temporal patterns in meiobenthic communities. Research was focussed on the meiobenthic communities' responses to phytodetritus sedimentations at the contrasting stations 115bis and 330. In this sense we investigated

i) a possible change in bacterial diversity and community composition as a possible cause for the changes in nematode community composition after bloom sedimentation as described by Vanaverbeke *et al.* (2004b) and Steyaert *et al.* (subm.). Since nematodes are known to feed on bacteria (Moens & Vincx, 1997) and since there is evidence that nematode species show preferences to feed on specific bacterial strains (Moens *et al.*, 1999b; De Mesel *et al.*, 2004), it was hypothesised that changes in the characteristics of bacterial communities could be responsible for the observed shifts in nematode communities (Vanaverbeke *et al.* 2004b);

ii) the nematode response to phytodetritus sedimentations at the stations 115bis and 330, studied by means of stable isotopes techniques, using natural ^{13}C and ^{15}N signatures. By comparing ^{13}C and ^{15}N signatures in nematodes (genus or group level) and other meiobenthic taxa and in sediment particulate organic matter (POM) and water

suspended particulate matter (SPM), we aim at clarifying the different responses observed at the BCS following a spring phytoplankton bloom. We also aim to determine if there are different feeding strategies among different nematode genera or groups;

iii) the nematode response to phytodetritus sedimentations at the stations 115bis and 330, studied by means of enrichment experiments with ^{13}C labelled diatoms and *Phaeocystis* as a possible food sources for the meiobenthos. By using a labelled food source it is possible to trace its path through the food web and to determine the extent of the use of this food source by consumers. In this sense we try to determine the exploitation of a food source by the different nematode genera or groups and especially to determine the trophic fate of *Phaeocystis*. The use of *Phaeocystis* by higher trophic levels is still unknown and only recently it was observed that *Phaeocystis* reached the sediment in high quantities. By adding labelled *Phaeocystis* to the sediment it is possible to determine if meiobenthos use *Phaeocystis* as a food source and to compare its importance with another food source as diatoms;

iv) the share of nematodes in the total mineralization process, by comparing estimated respiration of bacteria, nematodes and macrobenthos with total Sediment Community Oxygen Consumption (SCOC). So far there is little information available on the role of nematodes in sediment processes in subtidal sediments. The nematode contribution in total carbon turnover has been determined along a depth gradient in ocean margin sediments (Soetaert *et al.*, 1997). For intertidal estuarine sediments the nematodes and meiobenthos contribution to total respiration has also been investigated (Van Oevelen *et al.*, 2006c). The role of nematodes in shallow subtidal sediments still needs to be investigated since there are great differences from shallow subtidal to deep sea and estuarine systems.

Moreover, in estuarine systems, nematodes can show different reactions to an increased food input. In a preliminary study in the Tagus estuary, nematodes migrated differently towards or away from an OM input (Franco, 2002). The spring phytoplankton bloom sedimentation in the North Sea represents a natural change in food supply to the benthic system which depends on it as a major source of OM (Billen *et al.*, 1990). In estuarine systems, nematodes depend mostly on microphytobenthos and bacteria as a food source (Van Oevelen *et al.*, 2006c). An anthropogenic input of OM to a system that does not entirely depend on it might trigger different responses from the nematode community then the ones investigated in the North Sea. The nematode response to the phytoplankton bloom sedimentation in the North Sea is investigated primarily in a long term situation, one year long, and also in relatively short term experiments (two weeks). In intertidal sediments the nematode community has to react fast since following sediment exposure the rising tide can displace the food source and/or make it available to other consumers. In this context a short term experiment (6 h) was conducted in the estuarine system of the Tagus to mimic the exposure time of an intertidal flat. A set of experiments were designed to investigate the impact of dead discards of beam-trawl fishing (*Crangon crangon*) on the nematode community from the Tagus estuary.

PhD thesis outline

In this thesis we focus on the nematode response to changes in the food conditions, both in contrasting subtidal coastal sediments from the North Sea (chapters 2-5) and in estuarine intertidal sediments from the Tagus estuary (chapter 6). Other meiobenthic organisms were investigated as well. We further included research on bacteria and macrobenthos in this thesis in order to maximise our understanding of the biological responses to the sedimentation of phytoplankton. The thesis comprises five major chapters (Chapter 2-6) in which different

aspects of this research are presented with the major conclusions assembled in a final conclusive chapter (chapter 7).

In chapter 2 we deal with the bacterial communities from stations 115bis and 330 on the BCS. Denaturing Gradient Gel Electrophoresis (DGGE) techniques were used to analyse the bacterial community composition of both stations in three different periods in time: prior, during and after the spring phytoplankton bloom sedimentation. Two sediment horizons were studied, the surface layer (0-1 cm) and a deeper layer (4-5 cm). Bacterial biomass was also analysed and based on the DGGE fingerprints the community diversity and composition was studied. We tested the hypotheses that there were no differences in bacterial community composition, diversity and biomass (i) between the two stations, (ii) at the surface and at a deeper sediment layer within a station and (iii) between different seasons.

In chapters 3 and 4 the trophic position of the meiobenthic communities from stations 115bis and 330 on the BCS was investigated by means of stable isotopes techniques.

In chapter 3 the natural abundance of the stable isotopes ^{13}C and ^{15}N in the meiobenthic community was measured and compared with the natural stable isotopic values from the water SPM and sediment POM. The meiobenthos was separated in major taxa and some nematode genera or functional groups were analysed separately. Three different periods in time were investigated: prior, during and after the spring phytoplankton bloom sedimentation in two sediment horizons (0-1 cm and 4-5 cm) of both stations. We aim to clarify the responses of the nematode community to the sedimentation of the spring phytoplankton bloom previously observed at these stations. At station 115bis the nematodes *Sabatieria celtica* and *S. punctata* responded fast and concentrated in the top 2 cm of the sediment shortly after the peak sedimentation event, while the deep-dwelling *Daptonema riemanni* and *D. fistulatum* showed a time-lagged response, coinciding with the burial and degradation of fresh OM (Steyaert *et al.*, subm). At station 330 nematode diversity and densities increased drastically in a short

time frame mainly due to the opportunistic response of stout and short nematodes (Vanaverbeke *et al.*, 2004a,b).

In chapter 4 two potential food sources of meiobenthos, the diatom *Skeletonema costatum* and *Phaeocystis* were enriched with the stable isotope ^{13}C. We simulated a bloom deposition event by incubating both algae in sediment cores from station 115bis while at station 330 only diatoms were incubated. Meiobenthos was separated in major groups and again some nematode genera or functional groups were picked. We aim to investigate (i) the potential use of *Phaeocystis* as a food source for meiobenthos; (ii) possible different patterns in incorporating algal carbon in the meiobenthic food web in contrasting sediments and (iii) describe the importance of different meiobenthic taxa/nematode genera or functional groups in assimilating fresh OM resulting from a phytoplankton bloom sedimentation.

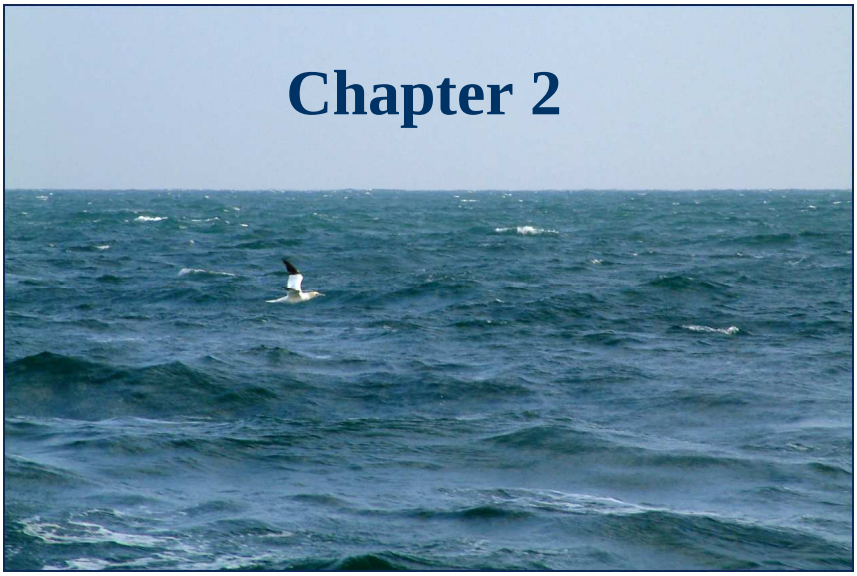
In chapter 5 the respiration of bacteria, nematodes and macrobenthos was estimated for stations 115bis and 330 on the BCS at three different periods in time: prior, during and after the spring phytoplankton bloom sedimentation and related to the actual measured SCOC. Here we aim to investigate the contribution of nematodes to the overall benthic respiration and compare its importance with other compartments of the benthic food web (bacteria and macrobenthos) in different seasons and contrasting sediments.

In chapter 6 an experiment dealing with the impact of dead discards reaching the sediment in the nematode community was set up in a mud-flat from the Tagus estuary in Portugal. The response of the nematode community to the addition of decomposing *Crangon crangon* was investigated in terms of changes of vertical distribution of the different nematode groups over time.

We believe that the combination of field observations and experimental work, both in the subtidal North Sea and the intertidal estuarine environment will lead (i) to a better understanding of the temporal patterns observed in nematode communities in shallow coastal

seas and (ii) is an important step in the understanding of biological processes in subtidal permeable sediments, further discussed in a final chapter (chapter 7).

Chapter 2



Impact of phytoplankton bloom deposition on benthic bacterial communities at two contrasting sediments in the Southern North Sea

MA Franco, I De Mesel, M Demba Diallo, K Van der Gucht, D Van Gansbeke, P Van Rijswijk, MJ Costa, M Vincx, J Vanaverbeke. *Accepted in Aquatic Microbial Ecology.*

Abstract

The response of benthic bacterial community composition, diversity and biomass to phytoplankton deposition was investigated in two contrasting sites at two different sediment horizons in the Southern North Sea. Differences in bacterial community composition between stations were observed. Seasonal differences in bacterial community composition were significant as well and were stronger in fine sediments, probably related to stronger fluctuations in food availability. Variation in community composition over the vertical sediment profile was different for both stations. In coarser sediment the difference was mostly due to the absence of certain Operational Taxonomic Units (OTUs) at the surface, while in fine sediment two distinct communities were present. A RELATE test revealed that bacterial community composition was influenced by the amount of labile organic matter (estimated through chlorophyll *a* concentration in the sediment). Diversity in terms of OTUs richness and Shannon-Weaver diversity index was higher in finer grained sediments. In coarser sediments diversity at the surface layer was lower which might be related to stronger hydrodynamic pressure at this station. These differences were not observed at the other station. Seasonal changes in diversity were not detected at both stations. Bacterial biomass was slightly higher in finer sediments. Bacterial biomass was not correlated with either chlorophyll *a* or temperature. Seasonal differences in bacterial biomass followed the ones observed for community composition while no vertical differences were detected.

Introduction

Situated in a well mixed nutrient rich part of the North Sea (Brussaard *et al.*, 1995), the Belgian Continental Shelf (BCS) supports a high primary production and algal biomass (Joint & Pomroy, 1993). Phytoplankton shows clear seasonal pattern, with Belgian coastal waters being dominated in spring by strong blooms of *Phaeocystis* simultaneously with diatom blooms (Reid *et al.*, 1990; Joint & Pomroy, 1993; Brussaard *et al.*, 1995). During April and May *Phaeocystis* blooms occur lasting for 20 to 40 days; dense blooms of diatoms can occur as early as February with smaller ones occurring as late as September (Reid *et al.*, 1990; Rousseau *et al.*, 2002).

The sedimentation of the phytoplankton bloom represents a major source of organic matter (OM) for the benthic system (*see* Billen *et al.*, 1990; Graf, 1992). In areas with a strong seasonal cycle of phytoplankton, as in coastal areas, a corresponding seasonality is to be expected in the sedimentation pattern and therefore in the input of organic matter into the benthic system.

It has previously been reported that nematodes responded to a spring bloom deposition by an increase in both density and diversity, especially among the selective deposit-feeding nematodes (1A-nematodes, Wieser, 1953) (Vanaverbeke *et al.*, 2004b) and deposit-feeding nematodes, the 1B-nematodes (Wieser, 1953) (Steyaert *et al.*, subm.). This was partially explained by changes in bacterial diversity (Vanaverbeke *et al.*, 2004b), since both 1A and 1B nematodes feed on bacteria (Wieser, 1953) and nematodes can selectively feed on particular bacterial strains (Moens *et al.*, 1999b; De Mesel *et al.*, 2004).

Bacteria have been reported to react fast on phytoplankton sedimentation in terms of biomass production (bigger cells), cell division, and activity which results in an increase in biomass, density and productivity (Graf *et al.*, 1982; Meyer-Reil, 1983; Goedkoop & Johnson, 1996;

Boon *et al.*, 1998). This response of the bacterial community to an input of OM seems to be influenced by the co-variation of both food supply and temperature (Graf *et al.*, 1982; Boon *et al.*, 1998). Van Duyl & Kop (1994) indicated temperature and substrate availability for bacteria as the most important factors influencing bacterial production.

Data concerning possible changes in benthic bacterial community composition and diversity after a mass input of OM are, however, lacking so far. Applying Denaturing Gradient Gel Electrophoresis (DGGE) in microbial ecology allows for simultaneous analyses of multiple samples, which enables monitoring the complex dynamics that microbial communities may undergo by diel and seasonal fluctuations or after environmental perturbations (Muyzer, 1999). This technique has been applied successfully to document changes in planktonic bacteria communities during phytoplankton bloom events (e.g. Van Hannen *et al.*, 1999a; Riemann *et al.*, 2000; Fandino *et al.*, 2001; Riemann & Winding, 2001; Van der Gucht *et al.*, 2001; Muylaert *et al.*, 2002; Rooney-Varga *et al.*, 2005).

The present study aims to examine the bacterial response to a pulsed food supply in terms of community composition, diversity and biomass on two well studied contrasting sites on the BCS. The two study sites, station 115bis and 330, are biogeochemically different: 115bis is a deposition station, characterized by the presence of fine sediments with 4 % of mud (Steyaert *et al.*, subm.), while station 330 has permeable sediment containing medium sand, devoid of mud (Vanaverbeke *et al.*, 2004a,b). This results in strong differences in vertical profiles of chlorophyll *a*, which is a proxy for the availability of labile OM (Boon & Duineveld, 1996). Especially in depositional stations, the differences in availability of labile OM between sediment depths during and after phytoplankton sedimentation can be striking (Graf, 1992), creating very different biogeochemical conditions. After sedimentation of phytodetritus the sediment at station 115bis can become anoxic and remain so until mid autumn (Steyaert *et al.*,

subm.). As this affects metazoan meiobenthic vertical distribution patterns (Steyaert *et al.*, 1999; Steyaert *et al.*, subm.) it probably also has important consequences for bacterial life.

In this paper we therefore aim to test whether there are significant differences in bacterial community composition and biomass (1) between the two stations, (2) at the surface and at a deeper sediment layer within a station and (3) whether there are significant seasonal shifts related to the spring bloom deposition.

Materials and Methods

Study area and sampling

Samples were taken from the BCS stations 115bis, located close to the coast (51°09.2'N; 02°37.2'E; 13 m depth) and 330, located further offshore (51°26.0'N; 02°48.5'E; 20 m depth) (Fig. 1.1). The sampling sites were never located on slopes or tops of sand banks.

Station 115bis is a deposition station, characterized by the presence of fine sediments (median grain size: 185 µm) with a small fraction of mud (4 %) (Steyaert *et al.*, subm.) while station 330 consists of medium sand (median grain size: 329-361 µm) with no mud content (Vanaverbeke *et al.*, 2004a,b).

Sampling at both stations was conducted monthly, from October 2002 until October 2003 from the research vessels Zeeleeuw or Belgica. Sampling took place on the same days at both stations. In December 2002 it was not possible to sample station 330 due to bad weather and sea conditions. In February only two box corers were obtained for station 330.

The water column was sampled at 3 m depth and 1m above the sea floor for phytoplankton analysis using 10 l Niskin bottles. To obtain pigment samples, 500 ml of water from each depth was filtered onto GF/F glass microfibre filters (i.d. 4.7 cm) using a vacuum pump. Three replicate samples were obtained. Temperature of the water was recorded

simultaneously. The samples were kept in the dark and preserved at -20°C on board and stored at -80°C in the laboratory.

Sediment was sampled using a Reineck boxcorer (surface area 180 cm^2) or another box corer with a greater surface area (campaigns of February, April and October 2003). The box corer was deployed three times at each sampling station. In February only two samples were obtained for station 330.

From each box corer 2 perspex cores (i.d. 3.6 cm) were taken: one for pigment analysis and another for bacterial counts and biomass analysis. The pigment analysis cores were sliced in 1 cm slices down to 10 cm. Pigment samples were preserved at -20°C on board and stored at -80°C in the laboratory. Bacterial counts and biomass samples were analysed monthly at the 0-1 cm layer and in the months of February, April and October 2003 also at the 4-5 cm layer. The sediment samples for bacterial counts and biomass analysis were preserved in a 4 % formaldehyde-tap water solution, thoroughly shaken and stored in the fridge until further processed.

During the February, April and October 2003 campaigns additional cores for bacterial community composition analysis (i.d. 6 cm) were taken. The bacterial cores were carefully closed in order to retain the overlaying water and preserved at 4°C until further processing in the laboratory.

Laboratory treatment of samples

The sediment samples for pigment analysis were weighted and pigment contents (chlorophyll *a*, phaeophytin and phaeophorbid) were analyzed by HPLC (Gilson) following Wright and Jeffrey (1997). The ratio of phaeopigments to the sum of chlorophyll *a* with phaeopigments (PAP ratio) was calculated as an indication of the freshness of the material deposited on the sediment (Boon *et al.*, 1998).

Samples for bacteria counting were prepared following Starink *et al.* (1994). Milli-Q water was added to the bacterial sample until a volume of 9.5 ml and 0.5 ml $Na_2P_2O_7$ (0.2 M) was added to reach a final concentration of $Na_2P_2O_7$ of 0.01 M. This mixture was sonicated five times for 30 seconds using a Soniprep 150 (10 Watt). In between sonication pulses the sample rested on ice for 30 seconds. Before staining samples were diluted 10 to 1000 times using TRIS. A subsample was filtered onto 0.2 µm polycarbonate filter and stained with Sybrgold (Molecular Probes). Filters were then mounted on a slide. Counting of bacterial cells was performed using a Leica confocal microscope connected to QWIN software. From every image 16 scans were made 0.2 µm vertically apart from each other. In such a way, a 3.2 µm thick image was analysed. From each scan, all particles >0.2 µm were counted and allocated to a size class. Per slide, 50-100 images were analysed. The volume of the bacteria was calculated as a sphere volume ($4\times\pi\times r^3/3$; r = radius), with a radius estimation of half the average of lower (L) and upper (U) diameters, and was converted to carbon content (bacterial biomass) by using a conversion factor of 310 fg of C μm^{-3} (Fry, 1990). Biomass was therefore calculated according to: $(4\times3.141593\times((L+U)/4)^3/3)\times310$ where L and U are lower and upper diameter of the bacteria. Data from station 115bis were only available from January 2003 onwards.

In a flow bench two sub-samples were taken from each of the sediment cores using a sterile syringe from which the tip had been removed. From each of the two sub-samples the layers between 0-1 and 4-5 cm (1 ml each) were preserved at -80°C until further processing.

Although on most occasions 3 replicates per station and sediment layers were obtained, methodological problems prevented a complete sampling for station 115bis in February (2 replicates at 0-1 cm layer) in April (1 replicate at 4-5 cm layer) and in October (2 replicates at 4-5 cm layer).

DNA extraction

Total DNA was extracted from about 1.5 g of sediment following the procedure of Demba Diallo (2003). Sediment (1.5 g) was mixed with 1.5 ml Na_2HPO_4 (0.1 M) and washed by shaking for 30 min at room temperature. After centrifugation at 7000 g (10 min at 4°C), the supernatant was removed. The pellet was resuspended in 500 µl of lysis buffer [2 % CTAB (Cetyltrimethylammonium bromide); 0.15 M NaCl; 0.1 M Na_2EDTA (pH8); 1 % PVPP (PolyVynil PolyPyrrolidone)], and 7.5 mg lysozyme (Sigma) was added. Samples were incubated overnight at 37°C. 25 µl of proteinase K (20 mg ml^{-1}, Boehringer, Mannheim) was added and the tubes were incubated at 50°C for 40 min. The temperature was increased to 65°C for 20 min and 300 µl extraction buffer (0.2 M NaCl; 0.1 M Tris-HCl pH 8; 2 % SDS) was added. Then, the mixture was incubated at 65°C for another 10 min. After addition of 350 µl of 5 M NaCl the samples were cooled on ice for 15 min. The supernatant was collected after centrifugation (7000 g, 10 min, 4°C) and transferred into 2 ml centrifuge tubes. To precipitate the crude DNA, 75 µl of 5 M potassium acetate and 250 µl 40 % polyethylene glycol 8000 (PEG) were added and the mixture was incubated at -80°C for 1 h. The pellet, obtained by centrifugation (13000 g for 15 min at 4°C) was resuspended in 900 µl 2 x CTAB (2 % CTAB; 1.4 M NaCl; 0.1 M Na_2EDTA) and incubated for 15 min at 68°C. After addition of 900 µl of chloroform, the solution was gently mixed and centrifuged at 13000 g for 10 min at room temperature. The DNA was precipitated by addition of 1 ml of isopropanol and incubated for at least 15 min at 20°C. The pellet (13000 g for 15 min at 4°C) was dissolved in 450 µl 2.5 M ammonium acetate (NH_4OAc) and subsequently, the DNA was precipitated by the addition of 1000 µl 95 % ethanol and then incubated for at least 15 min at -20°C. The pellet of DNA was obtained by centrifugation at 13000 g for 15 min at 4°C and resuspended in 200 µl sterile water (Sigma). For each sediment sample, we performed two independent 1.5 g sediment preparations, the purified DNA-samples of which were pooled and stored in

one single vial. A 100 µl aliquot of the crude extract was further purified using the Wizard® DNA CleanUp kit (Promega, Madison, WI USA).

PCR amplification of 16S rDNA

6 µl (ca 100 ng) of the purified DNA was amplified in a Genius temperature cycler. For each sample two PCRs were performed. The PCR mixture contained: 6 µl of template DNA, 0.5 µM of each of the appropriate primers, 200 µM of each deoxynucleoside triphosphate, 5 µl of 10 x PCR buffer (100 mM Tris-HCl (pH9); 500 mM KCl; 15 mM $MgCl_2$), 20 ng of bovine serum albumine and 2.5 U of Taq DNA polymerase (Ampli-Taq Perkin Elmer). Each mixture was adjusted to a final volume of 50 µl with sterile water (Sigma). The primers are: F357GC(5'CGCCCGCCGCGCCCCGCGCCCGGCCCGCCGCCCCCGCCCCCCTACGGGAGGCAGCAG-3') and R518 (5'-ATTACCGCGGCTGCTGG-3'). Both primers designed by Muyzer *et al.* (1993) are used to amplify the 16S rDNA region corresponding to positions 341 to 534 in *E. coli*. Primer F357GC that contains a GC-rich clamp is specific for most bacteria and R518 is specific for most *Bacteria, Archaea,* and *Eucarya* (Van Hannen *et al.* 1999b). In order to improve the specificity of the amplification and to reduce the formation of spurious by-products, a "touchdown" PCR (Muyzer *et al.* 1993; Don *et al.* 1991) was performed starting with 5 minutes at 94°C, followed by 20 cycles of denaturation at 94°C for 1 min, annealing at 65°C (the temperature was decreased by 0.5°C every cycle until the touchdown temperature of 56°C) for 1 min, and primer extension at 72°C for 1 min. Ten additional cycles were carried out at an annealing temperature of 55°C. The tubes were then incubated for 10 min at 72°C. The presence of PCR products and their concentration were determined by analysing 5 µl of PCR-product on a 2 % agarose gel. A molecular weight marker (Smartladder-Eurogentec, SA Belgium) was included.

DGGE (Denaturing Gradient Gel Electrophoresis) analysis

DGGEs were carried out using the D-Code System from Bio-Rad Laboratories. The PCR products were loaded onto 8 % (w/v) polyacrylamide gels of 1 mm thickness, in 1 x TAE buffer [20 mM Tris-acetate with pH7.4; 10 mM acetate; 0.5 mM Na_2 EDTA]. The denaturing gradient contained 35 % to 70 % denaturants (100 % denaturant corresponded to 7 M urea and 40 % (vol/vol) deionised formamide). The total lane intensity was normalised between the samples at 400 ng of DNA. Electrophoresis was performed at a constant voltage of 75 V for 16 h and at a constant temperature of 60°C. After electrophoresis, the gels were stained for 1 h in 1X TAE containing ethidium bromide (0.5 mg l^{-1}) (Muyzer *et al.*, 1993). The bands were visualized on a UV transillumination table equipped with a digital CCD camera.

As standards, we used a mixture of DNA from 9 clones (Van Der Gucht *et al.*, 2001). On each gel, three standard lanes were analyzed in parallel to the samples, to facilitate comparison between gels.

The banding patterns were then analyzed using Bionumerics 5.1 (Applied Maths BVBA, Kortrijk, Belgium). This software, through measuring of an optical density profile through each lane (corresponding to a single sample), identifies the band positions and calculates the contribution of the intensity of each band to the total intensity of the lane. This procedure yields a matrix with the relative intensity of each band in all samples.

Bacterial Biodiversity

Ecological diversity is considered a function of the number of different classes (richness) and the relative distribution of individuals among these classes (evenness) (Washington, 1984). By treating each band as an individual "Operational Taxonomic Unit" (OTU), the richness (number of OTUs present) and the Shannon-Weaver diversity index (H') (Shannon & Weaver, 1963) were calculated using PRIMER v.5 (Primer-E Ltd., Plymouth, Great Britain). As rare populations (less than 1% of the total community) might not be represented in a DGGE gel

(Muyzer *et al.*, 1993) the bacterial community richness (total number of OTUs detected) and diversity (Shannon-Weaver diversity index) calculated here only refer to the dominant bacteria populations and should be interpreted as an indicator of the minimum diversity of the bacterial community.

Statistical analysis

All correlation tests were done using Spearman rank R. The STATISTICA 6 software was used and a confidence level of 0.05 was considered in all test procedures.

The analysis of the banding profiles of the DGGE gels was done by multivariate tests using PRIMER v.5 (Primer-E Ltd., Plymouth, Great Britain). All tests were carried out on log (x+1) transformed data. The Bray-Curtis index was used as similarity coefficient. A MDS analysis places samples in a multi-dimensional space, based on the similarities between them. In the 2D ordination plots similar samples are therefore placed together (Clarke, 1993). The relative intensity of each band was taken into account, which appears to be more appropriate than using only presence/absence data (Muylaert *et al.*, 2002).

One-way ANOSIM (Analysis of Similarity) was used to test for statistical differences between the community composition of the two stations (115bis and 330). For each station a two-way crossed ANOSIM was used to test for statistical differences between the community composition at different depths (0-1 cm and 4-5 cm) and the sampling months (February, April and October 2003) (Clarke, 1993). Whenever significant differences were found, pairwise tests were done.

The relationship between chlorophyll *a* and the community structure was assessed by calculating rank correlations between similarity matrices derived from chlorophyll *a* concentration in the sediment and the banding profiles (RELATE procedure) using the software package PRIMER v.5 (Primer-E Ltd., Plymouth, Great Britain). The same RELATE

test was used between similarity matrices derived from bacterial counts per size classes and bacterial biomass per size class.

One-way and two-way Analyses of Variance (ANOVA) were performed in order to test for statistical differences in the Shannon-Weaver diversity index and OTUs richness and in the bacterial counts and biomass for the months of February, April and October 2003. Homogeneity of variances was tested using Bartlett χ^2 and data were transformed whenever necessary (log transformed on the diversity index case and inverse transformed on the bacterial counts and biomass case). Whenever significant differences were found post hoc Tukey HSD tests were performed. One-way ANOVA was used to test for significant differences between the two stations. A two-way ANOVA was used to test for the effect of depth, time and their interaction for bacterial counts and biomass at both stations and for the Shannon-Weaver diversity index and OTUs richness at station 330. At station 115bis due to the lack of replication in April at layer 4-5 cm, a two-way ANOVA could not be performed on the Shannon-Weaver diversity index and OTUs richness. One-way ANOVA was then used to test for significant differences between the sampling dates in layer 0-1 cm; a *t*-test was used to test for significant differences between the sampling dates February and October for layer 4-5 cm; and a one-way ANOVA was performed in order to test for statistical differences between the two sampling depths.

The STATISTICA 6 software was used and a significance level of 0.05 was considered in all test procedures.

Results

Environmental variables

At both stations the surface and bottom water showed comparable concentrations of chlorophyll *a*, indicating a well-mixed water column (Fig. 2.1). At station 115bis the

chlorophyll *a* concentration in the water was on most occasions higher than at station 330. Chlorophyll *a* concentrations in the water at both stations started rising in February, peaked in April (48 mg m^{-3} at station 115bis and 32 mg m^{-3} at station 330) and decreased afterwards (Fig. 2.1). Smaller peaks were observed in July for both stations and in September only at station 330, reaching values no higher than 17 mg m^{-3}.

At the sediment surface chlorophyll *a* concentrations were considerably higher at station 115bis than at station 330 (Fig. 2.2) throughout the sampling period, and followed the patterns observed in the water column, with a peak in April. At station 330 other smaller peaks were observed in August 2003 and October 2003 corresponding to the deposition of the late summer and autumn blooms. For both stations chlorophyll *a* in the bottom water was correlated with chlorophyll *a* at the sediment surface (115bis: $R = 0.73$; $p < 0.05$; 330: $R = 0.62$; $p < 0.05$).

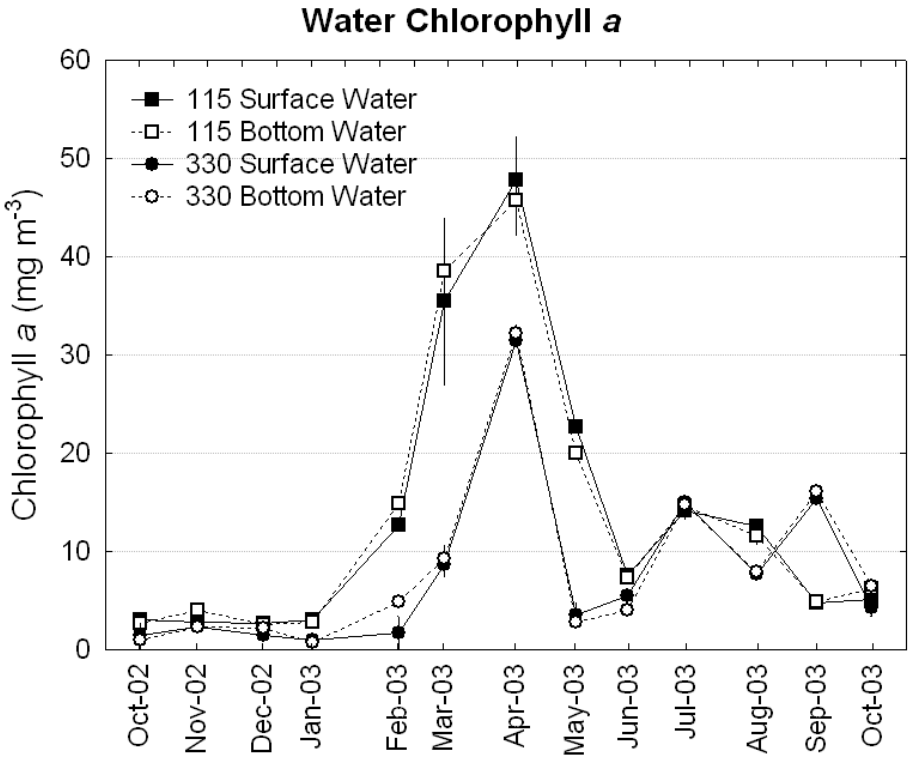


Figure 2.1: Chlorophyll *a* concentration in the surface and bottom water (mg m^{-3}) at stations 115bis and 330 for the period from October 2002 to October 2003. Vertical bars represent the standard error.

At station 115bis the PAP ratios were relatively low in winter, started increasing in April, and remained relatively stable until they reached the highest values in October 2003. At station 330 the PAP ratios varied more abruptly: they showed similar values in winter, decreased abruptly in April and peaked two months later. From June onwards PAP ratio decreased gradually (Fig. 2.2).

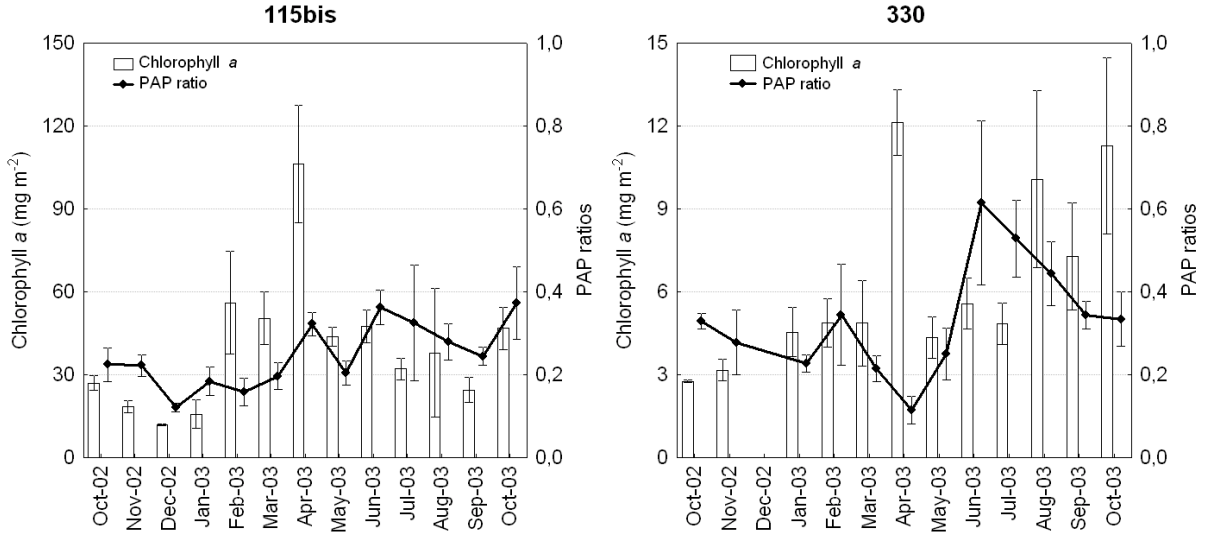


Figure 2.2: Chlorophyll *a* concentration in the sediment (mg m^{-2}) and the ratio of phaeopigments to chlorophyll *a* + phaeopigments (PAP ratio) for the 0-1 cm layer, at stations 115bis and 330 for the period from October 2002 to October 2003. Vertical bars represent the standard error. Note different scale on left *y*-axis.

At station 115bis chlorophyll *a* concentrations in the sediment were always highest at the surface (Fig. 2.3). This profile was most evident in April 2003 while deposition was occurring; PAP ratios showed the opposite trend and increased with depth. At station 330 no clear vertical gradient could be found for either chlorophyll *a* concentration or PAP ratios.

Water temperature was lowest in December (4.9°C), remained low until March and increased from April onwards. Maximum values were observed in August (22°C), after which temperature decreased gradually.

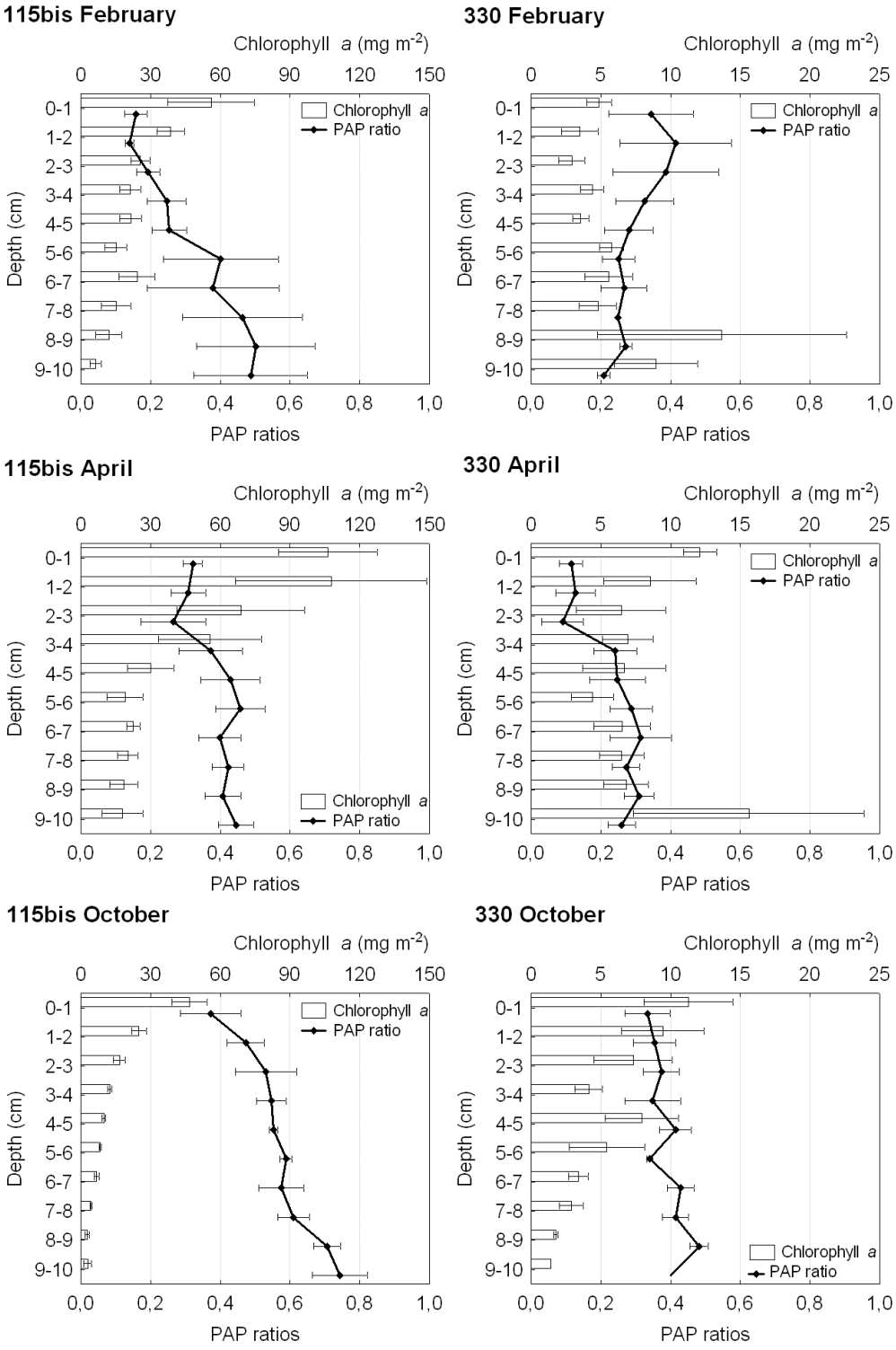


Figure 2.3: Vertical profiles of chlorophyll *a* concentration (mg m^{-2}) and ratio of phaeopigments to chlorophyll *a* + phaeopigments (PAP ratio) in the sediment at stations 115bis and 330 in February 2003, April 2003 and October 2003. Horizontal bars represent the standard error. Note different scale on top *x*-axis

Bacterial biomass

Bacterial counts varied between 1.9 (± 0.3 SE) and 14.1 (± 0.9 SE) $\times 10^{12}$ m^{-2} at station 115bis and between 2.0 (± 0.9 SE) and 10.6 (± 4.6 SE) $\times 10^{12}$ m^{-2} at station 330 (Fig. 2.4). Bacterial counts were strongly correlated with bacterial biomass ($R = 0.94$; $p < 0.001$). Moreover when taking into account the bacterial counts and biomass distributed per size class a RELATE test revealed a strong relationship between both variables ($\rho = 0.90$; $p < 0.01$). Both tests indicated that bacterial counts and bacterial biomass varied in the same way. Therefore results are focussed on bacterial biomass.

The bacterial biomass ranged from 0.2 (± 0.1 SE) to 2.8 (± 0.8 SE) gC m^{-2} at station 115bis and from 0.2 (± 0.1 SE) to 1.9 (± 0.8 SE) gC m^{-2} at station 330, showing different seasonal patterns when comparing both stations (Fig 2.4). Bacterial biomass at station 115bis was much more variable than at station 330. Bacterial biomass was low in January and February 2003. Peak values were reached in March 2003 with intermediate values in April and May 2003. A progressive decrease towards initial values was then observed. Bacterial biomass in station 330 was lower compared to station 115bis, fluctuating around 1 gC m^{-2} from January to July 2003. Prior to and after that period, bacterial biomass values were considerably lower. Bacterial biomass at 4-5 cm resembled that in the upper cm at all seasons at both stations (Fig. 2.4).

Biomass showed no correlation with either chlorophyll *a* or temperature ($p > 0.05$) in any of the stations.

Analysing bacterial biomass for the sampling dates of February, April and October 2003, no statistical differences were observed between both stations ($F = 0.243$; $df = 1$; $p > 0.05$). At station 115bis significant differences were found between sampling dates ($F = 5.24$; $df = 2$; $p < 0.05$) but not between layers ($F = 0.08$; $df = 1$; $p > 0.05$) nor for the interaction term time × depth ($F = 0.35$; $df = 2$; $p > 0.05$). Post-hoc comparisons showed that April was

significantly different from February and October. At station 330 significant differences were found between sampling dates ($F = 5.99$; $df = 2$; $p < 0.05$) but not between depth layers ($F = 0.56$; $df = 1$; $p > 0.05$) nor for the interaction term ($F = 0.77$; $df = 2$; $p > 0.05$). Post-hoc comparisons showed that April and October were significantly different.

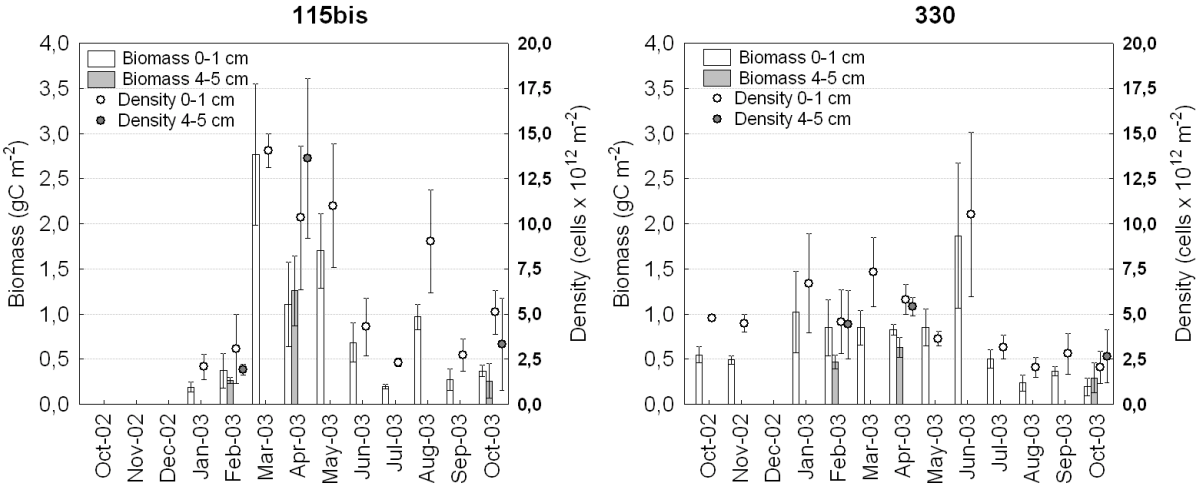


Figure 2.4: Bacterial biomass (gC m^{-2} of sediment) at the first cm layer from station 115bis and 330 on the period from October 2002 to October 2003. On the months of February 2003, April 2003 and October 2003 the values for the 4-5cm layer are also indicated. Vertical bars represent the standard error.

Bacterial community composition

Each band on the DGGE represents one "Operational Taxonomic Unit" (OTU) (Fig. 2.5). In this study 52 different OTUs were identified. Only 25 of these OTUs were detected at both stations: 21 were found only at station 115bis and 6 only at station 330. 21 of the 52 OTUs were detected on the three sampling campaigns, while 15 were restricted to 1 sampling period. 16 OTUs were detected in only one of the two depth layers. Five OTUs were only detected once.

A one-way ANOSIM considering all the samples, showed that there were significant differences between the two sampling stations (Global $R = 0.721$; $p < 0.05$).

MDS for both stations separately revealed seasonal and vertical differences in bacterial community composition (Fig. 2.5). When considering station 115bis a two-way crossed ANOSIM showed that there were significant differences between the sampling months (Global R = 0.991; $p < 0.05$) and sediment depths (Global R = 0.788; $p < 0.05$). All sampling months were significantly different from each other ($p < 0.05$) (Table 2.1). At station 330 the two-way crossed ANOSIM again showed significant differences between the sampling months (Global R = 0.884; $p < 0.05$) and depth layers (Global R = 0.726; $p < 0.05$). Pairwise tests (Table 2.1) revealed that communities in October were significantly different ($p < 0.05$) from those in February and April, while no differences were found between the latter two months ($p > 0.05$).

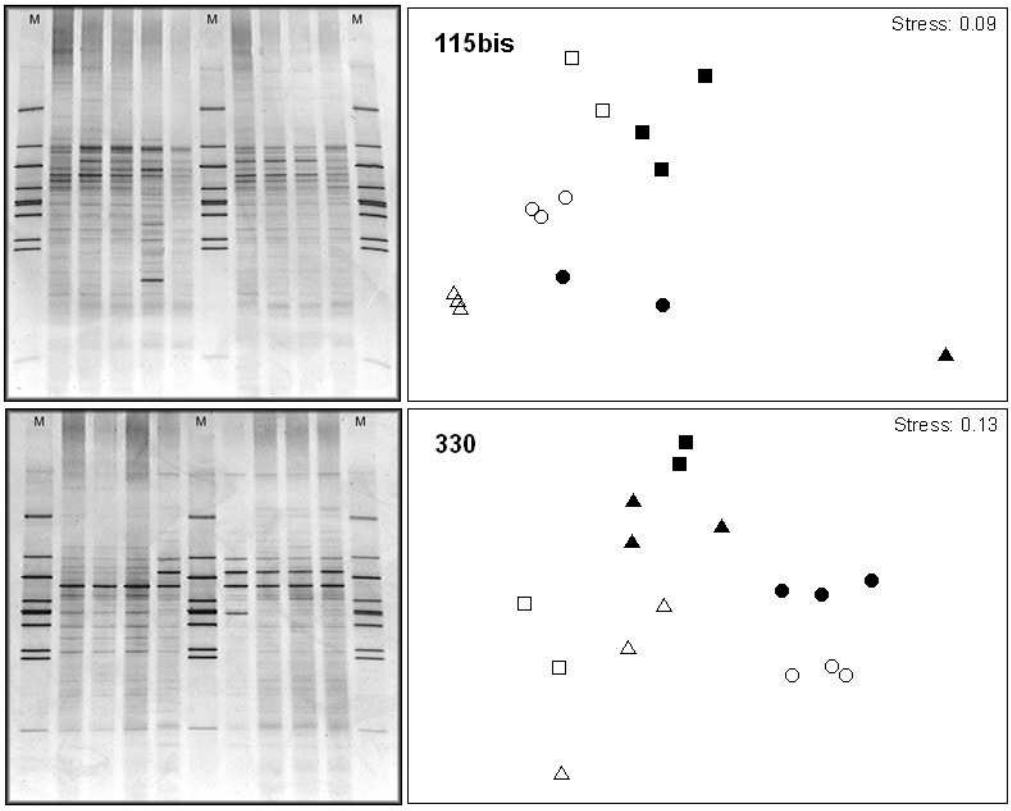


Figure 2.5: Example of DGGE gels and MDS analysis of the microbial community from station 115bis and 330 of the sampling campaigns on February 2003 (squares), April 2003 (triangles) and October 2003 (circles), at 0-1 cm (white) and 4-5 cm (black) sediment layers. (M: marker).

The bacterial community composition at both stations at all times and depths was significantly influenced by the chlorophyll *a* concentration in the sediment as (RELATE test, $\rho = 0.26$; $p < 0.01$).

Bacterial biodiversity

Both OTUs richness and Shannon-Weaver diversity index were relatively higher at station 115bis then at station 330 (Fig. 2.6). At station 115bis OTUs richness and Shannon-Weaver diversity index were similar for both sediment layers and for all the seasons, with lower values only noticed in the 4-5 cm layer in April. The OTUs richness and Shannon-Weaver diversity index of the communities at station 330 were lower at the surface layer than at the 4-5 cm layer and showed minor changes with time.

Table 2.1: Pairwise test for bacterial community composition in the different sampling months for both stations. *significantly different ($p < 0.05$).

	115bis		330	
	R statistics	Significance level	R statistics	Significance level
February *versus* April	0.958	0.010 *	0.500	0.080
February *versus* October	1.000	0.025 *	1.000	0.010 *
April *versus* October	1.000	0.033 *	0.944	0.010 *

The sample from April 4-5 cm at station 115bis was removed from the statistical analysis since it had no replication. A one-way ANOVA performed on the Shannon-Weaver diversity index showed that there were significant differences between the two stations ($F = 15.20$; $df = 1$; $p < 0.001$). At station 115bis no significant differences were found between sampling dates for layer 0-1 cm ($F = 0.035$; $df = 2$; $p > 0.05$); a t-test showed no significant differences between February and October for layer 4-5 cm (t-value = 0.071; $p > 0.05$); and a one-way ANOVA showed no significant differences between the two depth layers ($F = 0.129$; $df = 1$;

p > 0.05). At station 330 a two-way ANOVA showed that there were significant differences between the depth layers ($F = 14.310$; $df = 1$; $p < 0.05$) but not between sampling months ($F = 0.540$; $df = 2$; $p > 0.05$) nor when combining both effects ($F = 1.312$; $df = 2$; $p > 0.05$). The same analyses performed on the OTUs richness showed the same significant differences as the ones shown by the bacterial Shannon-Weaver diversity index.

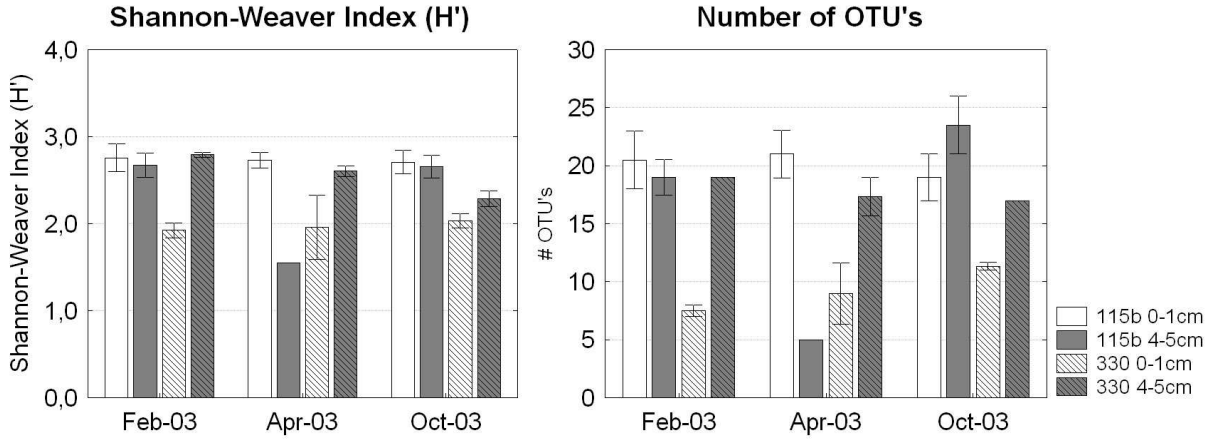


Figure 2.6: Shannon-Weaver index (H') and number of OTUs identified in the bacterial community from station 115bis and 330 on the sampling campaigns on February 2003, April 2003 and October 2003, at 0-1cm and 4-5cm sediment layers. Vertical bars represent the standard error.

Discussion

Environmental variables

As described previously (Vanaverbeke *et al.*, 2004a,b; Steyaert *et al.*, subm.) a strong phytoplankton bloom occurred in spring as indicated by the seasonal pattern of the concentration of chlorophyll *a* in the water column. The higher chlorophyll *a* concentration in the water column at station 115bis during the spring phytoplankton bloom was to be expected, since primary production is higher closer to the coast (Joint & Pomroy, 1993).

Patterns of chlorophyll *a* concentrations in the sediment also showed a strong seasonal signal (Vanaverbeke *et al.*, 2004a,b; Steyaert *et al.*, subm.), but in contrast to the water column values, differed considerably between the stations. Chlorophyll *a* concentration at the sediment surface at station 115bis was about 10 times higher than at station 330. This, together with the absence of clear vertical profiles in the sediment at station 330 corroborated the idea that this station has more permeable sediment where stronger bottom water currents prevent the deposition of sedimenting phytodetritus (Precht & Huettel, 2004) and induce sub-surface chlorophyll *a* peaks (Ehrenhauss & Huettel, 2004; Ehrenhauss *et al.*, 2004a). At station 115bis vertical gradients were very obvious, especially during the deposition of the phytoplankton bloom corroborating that 115bis is a deposition station.

The pattern of the PAP ratios showed little seasonal variation at station 115bis compared to station 330 (Fig. 2.2). The comparatively higher PAP ratios at station 330 following bloom senescence indicate a rapid degradation of OM, typical for permeable coastal sands (Ehrenhauss *et al.*, 2004b). Advective transport allows fast removal of decomposition products (Huettel *et al.*, 1998) resulting in an up and down fluctuation of the PAP ratios at station 330. At station 115bis there was a carbon build-up (phytodetritus derived) in the sediment during spring and subsequent degradation and mineralization in late summer as previously reported in the North Sea for this type of sediment (Boon & Duineveld, 1998; Provoost *et al., in prep*). Therefore PAP ratios did not show major fluctuations but slowly increased towards late summer and autumn.

Bacterial biomass

Bacterial counts make no distinction between dead, active or inactive bacteria. There is a large fraction of dead or inactive bacteria in coastal marine sediments (Luna *et al.*, 2002) and the percentage of active bacteria may change rapidly (Créach *et al.*, 2003). In deep Mediterranean sediments the number of active bacteria depends on the organic substrate in the

sediment derived from the photic layer (Luna *et al.*, 2004). However according to the same study no relationship was observed between total bacterial counts and organic content of the sediment. In our study bacterial biomass, calculated from bacterial counts, also had no correlation with either temperature or chlorophyll *a* (as indicator of the amount of labile OM) at any of the stations. Both temperature and food availability seem to be key factors influencing the response of benthic bacterial communities to an input of OM (Graf *et al.*, 1982; Boon *et al.*, 1998) and a relationship between bacterial community composition and chlorophyll *a* concentration in the sediment was detected in this study. Bacterial biomass and/or counts alone may not be reliable indicators of changes in bacterial activity; however, coupled with other data, like DGGE banding profiles, they to achieve a better understanding of changes in the bacterial community. A significantly higher bacterial biomass was detected in April at station 115bis (compared to February and October) which is probably coupled with the seasonal shifts in bacterial community composition observed at this station (still discussed further on). Also a significantly lower bacterial biomass detected in October at station 330 is probably related to the fact of this community composition being significantly different from February and April (still discussed further on).

Methodological considerations

As any PCR and DNA based techniques, DGGE of PCR amplified 16S rDNA (Muyzer *et al.*, 1993) has its own methodological limitations, like inhibition of PCR amplification by co-extracted contaminants, deferential amplification or formation of artefactual PCR products or contaminating DNA, and 16S rRNA sequence variations. Co-migration of DNA, single bands representing more then one bacterial strain or slightly different rRNA gene sequences resulting in multiple bands are problems that can occur in DGGE (Nübel *et al.*, 1996; Palys *et al.*, 1997; Vallaeys *et al.*, 1997). Nevertheless PCR-mediated analysis of 16S rRNA is a powerful tool for the determination of microbial diversity of environmental ecosystems (von

Wintzingerode *et al.*, 1997) and DGGE techniques are quite effective when characterizing a bacterial community structure, namely for monitoring changes in occurrence and/or relative frequency of the different bacterial populations (Fromin *et al.*, 2002). Since samples were treated in the same way during the whole study the method allows for a proper comparison of the results beyond a mere qualitative approach (Fromin *et al.*, 2002). Shannon-Weaver index is the most common diversity index used by ecologists (Washington, 1984) and has also been applied to DGGE fingerprints to estimate bacterial diversity (*e.g.* Nübel *et al.*, 1999; Boon *et al.*, 2002; Dilly *et al.*, 2004; Haack *et al.*, 2004; Gafan *et al.*, 2005; Xia *et al.*, 2005; Lagacé *et al.*, 2006) even though in a DGGE gel populations representing less than 1% of the total community may not be represented (Muyzer *et al.*, 1993).

Bacterial community composition and diversity

Our results showed that bacterial community composition was significantly different at both stations. Only 6 of the 52 OTUs were exclusively detected at station 330 while 21 OTUs were found exclusively at station 115bis (data not shown). OTUs richness and Shannon-Weaver diversity index were mostly higher at station 115bis than at station 330, especially at the sediment surface. This may be a reflection of higher food availability at station 115bis, since substrate availability affects bacterial community composition (Van Hannen *et al.*, 1999a; Muylaert *et al.*, 2002) and a high concentration of potentially available substrate can sustain a higher bacterial diversity (Luna *et al.*, 2004). The results on the dominant OTUs (relative density higher than 1%) indicate a relatively more diverse community at station 115bis.

In the bacterioplankton both free-living bacteria and particle-associated bacteria can occur as two distinct communities (Fandino *et al.*, 2001; Riemann & Winding, 2001; Rooney-Varga *et al.*, 2005), although this can not be generalised (Sapp *et al.*, 2007). If this would be true for the sediment as well, one would expect that the establishment of free-living bacteria populations would become more difficult at station 330 due to the high bottom water currents

and the permeability of the sediment. This would likely be more evident at the surface of the sediment, where our results show the lowest bacterial diversity for station 330. At station 115bis, with no such currents, both free-living and attached bacteria can coexist as in the plankton.

Due to water flow through the sediment bacterial cells can also be transported, in fact Rusch *et al.* (2001) observed a subsurface peak in bacterial densities (2-4 cm deep) as a consequence of advective pore water flows. The coupling of benthic bacterial communities and bacterioplankton communities should be stronger at station 330, which would not be the case at station 115bis. This would contribute to a stronger differentiation between the benthic bacterial communities from both stations.

Another factor that could be responsible for different community composition at both stations is the possible coexistence of aerobic and anaerobic bacteria at station 115bis. At this station the sediment becomes reduced after a spring bloom (Steyaert *et al.*, subm.) while at station 330 sediments at the depths studied here are well aerated (Vanaverbeke *et al.*, 2004a,b).

Significant vertical differences in bacterial community composition were observed at both stations, which may have different causes at each station. Only one OTU was found exclusively at the surface layer at station 330. This OTU was also found exclusively at the surface layer at station 115bis. All other OTUs encountered at the surface were also found at 4-5 cm, generally with relatively higher abundances. 12 OTUs were found exclusively at 4-5 cm at station 330, half of which were also observed at the sediment surface at station 115bis (data not shown). The differences in community composition between the two layers at station 330 were a consequence of the non-detection of certain bacterial populations at the surface of the sediment. Bacterial populations in the upper cm of station 330 may not be able to deal with the high hydrodynamic stress (advective currents through the sediment) prevailing there (Precht & Huettel, 2004), which results in both a lower Shannon-Weaver

diversity index, a lower number of OTUs, and a different bacterial community composition compared to deeper layers.

At station 115bis 10 OTUs were found exclusively at the surface and 8 OTUs only at the 4-5 cm layer (data not shown), indicating that at this station different communities may develop in each layer. When omitting the sample from April 4-5 cm layer at station 115bis, no significant differences were observed in Shannon-Weaver diversity and OTUs richness between layers, even though community composition was different. On this sampling occasion, it was only possible to obtain one replicate for the 4-5 cm layer. The DNA extraction and subsequent PCR was not as successful as for other samples. Since bacterial biomass was not lower this might be related to chemical inhibition. Since there was no replication, results referring to station 115bis in April 4-5 cm layer should be handled with care.

Steep vertical gradients in chlorophyll *a* concentration and PAP ratios were registered at station 115bis. Moreover, deep sediment layers at this station showed strong negative redox potential values and a build up of NH_4^+ (Steyaert *et al.*, subm.). Vertical differences in the benthic bacterial community have previously been reported (Luna *et al.*, 2004) in Mediterranean sediments with oxygen depletion problems in which the vertical differences in the bacterial community were closely related to redox potential changes (Urakawa *et al.*, 2000).

The bacterial community composition at both stations also changed with time. In the planktonic system shifts in plankton species composition are responsible for changes in the attached bacterial community composition (Rooney-Varga *et al.*, 2005). Carbon-rich mucilage sedimentation, as secreted by *Phaeocystis*, can be a post-bloom food source (Riebesell *et al.*, 1995). Experimental degradation of such compounds in agar from different sources and under different oxygen conditions presented very different microbial

communities with only a few overlapping species (Janse *et al.*, 2000). Changes in OM quality and quantity can induce shifts in bacterial community structure (Luna *et al.*, 2004). Therefore seasonal changes in food availability and oxygenation are likely to produce distinct bacterial communities. This was supported by the significant RELATE test observed between bacterial community composition and the concentration of chlorophyll *a* in the sediment.

Temporal differences in bacterial composition were more pronounced at station 115bis, where all sampling months were significantly different from each other. Also the percentage of OTUs which were present on the three sampling campaigns was lower at station 115bis than at station 330 (data not shown), indicating that there were more pronounced temporal shifts of the bacterial populations at this station. Bacterial biomass also showed shifts from February to April and again from April to October. Stronger variations in chlorophyll *a* concentration in the sediment at station 115bis would imply higher temporal differences in the bacterial community composition at station 115bis.

Besides food availability, the oxygenation of the sediment can also change more drastically at station 115bis (Steyaert *et al.*, subm.), in contrast to station 330 where the redox potential remains positive throughout the studied sediment depths and throughout the year (Vanaverbeke *et al.*, 2004a,b).

At station 330 only October's bacterial community composition was different from the other two sampling dates, and that was also the case for bacterial biomass. Temporal variability was also present at this station but not so strongly.

Even though the bacterial community composition changed with time, such differences were not detected in Shannon-Weaver diversity and OTUs richness at station 330, nor at station 115bis if the sample from April 4-5 cm layer is omitted. At each station we observed that with time OTUs were disappearing, new ones emerging and others were always present but their density changed. The regular seasonal environmental changes may be responsible for

maintenance of diversity since environmental fluctuations can provide temporal niche opportunities, allowing species coexistence (Chesson & Huntly, 1997). It does not mean that the same bacteria were not always present but changes in dominance may push certain bacteria above the detection limit (Hedrick *et al.*, 2000).

Generaly bacterial dynamics is still considered in ecosystem models as a simple consequence of abiotic factors and nutrient availability which may oversimplify the true nature of microbial ecological functions. However their distribution and abundance is regulated not only by abiotic factors and food availability (as discussed above), but also by biotic factors (e.g., competition with other species, predation by viruses) (Fuhrman *et al.*, 2006). The extent to which the interactions between abiotic and biotic processes govern bacterial community dynamics remains difficult to assess in the absence of high resolution microbial biodiversity information.

Changes in bacterial community may also be of importance when considering higher trophic levels. Changes of nematode community composition after bloom sedimentation towards feeding types that feed on bacteria have been observed in these two stations (Vanaverbeke *et al.*, 2004b; Steyaert *et al.*, subm.). Vanaverbeke *et al.* (2004b) hypothesized that an increase in nematode species richness could have been caused by an increase in bacteria diversity. An increase in bacterial diversity was not observed, however, a shift in bacterial community composition and biomass was observed and those changes can indeed be coupled with changes in the nematodes communities.

Conclusions

We have shown that bacterial community composition differed when comparing fine with coarser marine sediment and our results indicate that diversity in general tends to be higher in finer sediments. Bacterial community composition also differed vertically within the sediment but not in the same way for both sediment types. In the fine sediment two relatively distinct

communities developed at the surface and deeper in the sediment. In coarser sediments, vertical differences were related mostly to the non-detection of certain bacteria populations at the surface of the sediment where hydrodynamic stress is stronger. Seasonal patterns in food availability played a key role in the bacterial community composition, which changed more drastically within fine sediment, where the input of OM was stronger.

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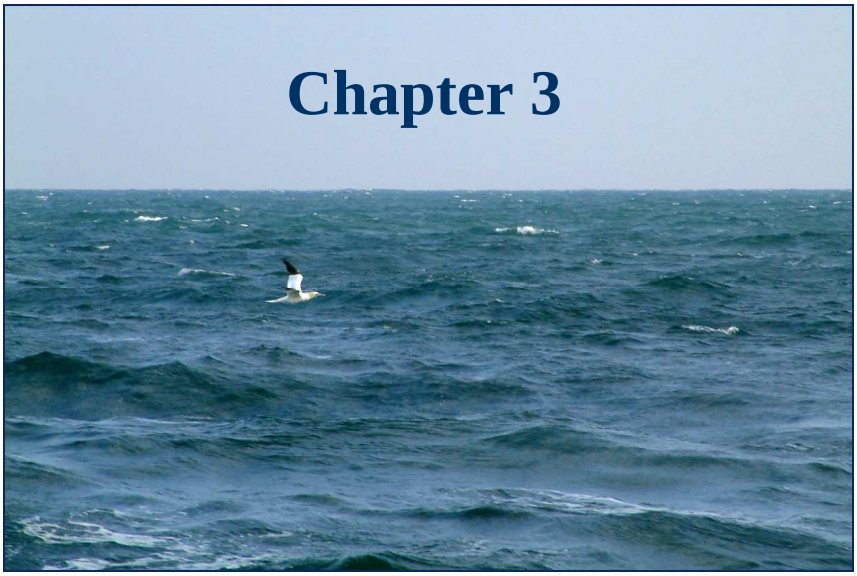
Chapter 3



Trophic resource and position of metazoan meiobenthos at contrasting subtidal sediments: carbon and nitrogen stable isotope analysis

MA Franco, K Soetaert, D Van Oevelen, D Van Gansbeke, MJ Costa, M Vincx, J Vanaverbeke. *Accepted in Marine Ecology Progress Series.*

Abstract

The response of meiobenthic communities in terms of densities and vertical distribution to the sedimentation of phytoplankton was investigated in two contrasting sites in the southern North Sea: one with fine grained sediment close to the coastline and another with highly permeable sediments. Pigments in water column and sediment and meiobenthic densities were measured monthly from October 2002 until October 2003. The stable isotopes ^{13}C and ^{15}N signatures were analysed in sediment Particulate Organic Matter (POM), water Suspended Particulate Matter (SPM) and in different meiobenthic taxa at three different sampling periods (prior, during and after the spring bloom deposition) at two sediment depths (0-1 and 4-5 cm). Differences in nematode response to the sedimentation event were obvious and related to the different biogeochemical processes at both stations. In permeable sediments the nematode response was fast after deposition occurred, while in the fine grained station, nematode response in terms of densities was delayed in time.

In general meiobenthic ^{13}C signatures remained relatively constant with time and were not coupled with changes observed on the water SPM and sediment POM. Vertical differences were observed in meiobenthic ^{13}C signatures in fine sediments. Surface-dwelling nematode species had similar signatures as the genera *Sabatieria* and *Richtersia* independent of the sediment horizon they were encountered in, indicating migration of these genera to the surface to feed. In permeable sediments such vertical differences were not present. $\delta^{13}C$ and $\delta^{15}N$ values of copepods clearly indicated a chemoautotrophic food source within the fine grained sediment, which has not been previously reported for such environments.

Introduction

The Belgian Continental Shelf (BCS), a well mixed nutrient rich area in the North Sea (Brussaard *et al.*, 1995) is characterized by a high primary production and high algal biomass (Joint & Pomroy, 1993). Phytoplankton blooms have a strong seasonal signal and during spring intense blooms of *Phaeocystis* colonies dominate Belgian coastal waters, occurring between April and May and lasting for 20 to 40 days; strong blooms of diatoms also occurring as early as February and smaller blooms as late as September (Reid *et al.*, 1990; Joint & Pomroy, 1993; Brussaard *et al.*, 1995; Rousseau *et al.*, 2002). The sedimentation of this phytoplankton bloom represents a major source of organic matter (OM) for the benthic system where it fuels benthic life (Graf, 1992).

The fate of the labile OM arriving at the sea floor is dependent on the receiving sediment type. In fine-grained depositional stations, sharp vertical profiles of labile OM (measured as chlorophyll *a* concentrations) can emerge (e.g. Steyaert *et al.*, subm.) after the sedimentation of phytodetritus in spring. Mineralisation of this newly arrived carbon often provokes oxygen stress (Graf, 1992) and breakdown of this OM can be retarded until summer or late summer (Boon & Duineveld, 1998; Provoost *et al.*, in prep.). In coarser sediments, these sharp vertical gradients can be absent and subsurface peaks of chlorophyll *a* are regularly reported (Jenness & Duineveld, 1985; Ehrenhauss & Huettel, 2004; Ehrenhauss *et al.*, 2004a; Vanaverbeke *et al.*, 2004b). Rapid degradation of OM within these sediments is often the case (Ehrenhauss *et al.*, 2004b; Vanaverbeke *et al.*, 2004b; Janssen *et al.*, 2005; Bühring *et al.*, 2006). The different biogeochemical conditions in such contrasting sediments affect the response of the residing benthic organisms. The response of the nematode communities to spring phytoplankton bloom sedimentation in 1999 was investigated at such contrasting sediments at the BCS (Vanaverbeke *et al.*, 2004a,b; Steyaert *et al.*, subm.). At a fine-grained depositional

station on the BCS (station 115bis), the expected vertical gradients of chlorophyll *a* developed in association with oxygen stress shortly after the peak sedimentation of the spring bloom in April (Steyaert *et al.*, subm.). Nematode densities increased gradually until July and a change in vertical profiles over time of the total nematode communities and the dominant species was reported. *Sabatieria celtica* and *S. punctata* responded fast and concentrated in the top 2 cm of the sediment, while deep-dwelling *Daptonema riemanni* and *D. fistulatum* showed a time-lagged response, coinciding with the burial and degradation of the fresh food. At a coarser grained station (station 330), a completely different picture emerged (Vanaverbeke *et al.*, 2004a,b). Here chlorophyll *a* was rather uniformly distributed and oxygen remained present at all sediment depths during and after sedimentation of the bloom. Moreover, mineralisation was fast and peaked in May. Nematode diversity and densities increased drastically in a short time frame, mainly due to the opportunistic response of stout (length to width ratio < 15) and short (adult length < 700 μm) nematodes (as defined in Vanaverbeke *et al.*, 2004a), including species as *Epsilonema pustulatum*, *Metepsilonema comptum*, *Mannunema annulatum*, *Richtersia inaequalis*, *Tricoma sp.*, *Daptonema nanum*, and several *Rhynchonema* species (Vanaverbeke *et al.*, 2004a). From May onwards, densities decreased again coinciding with a drastic reduction in densities of these stout and short nematodes. These results suggest that the response of nematode communities to sedimentation of phytodetritus depends both on the type of sediment and on the sediment depth.

The stable isotopes of carbon (^{13}C) and nitrogen (^{15}N) provide powerful tools to estimate carbon flows to consumers and their respective trophic positions in food webs (*see* Post, 2002). By comparing the stable isotopic signatures of carbon and nitrogen in nematodes (genus or group level) and other meiobenthic taxa and in sediment particulate organic matter (POM) and suspended particulate matter (SPM) we aim at clarifying the different responses of nematode assemblages to the deposition of the spring phytoplankton bloom observed at the

BCS. Stable isotope analysis were conducted (i) in two different sediment types, (ii) at two sediment depths, (iii) at three different moments in time, before, during and after the peak sedimentation of phytodetritus.

Material and Methods

Study site and sampling

Samples were taken from the BCS stations 115bis, located close to the coast (51°09.2'N; 02°37.2'E; 13 m depth) and 330, located further offshore (51°26.0'N; 02°48.5'E; 20 m depth) (Fig. 1.1).

Station 115bis is a deposition station, characterized by the presence of fine sediments (median grain size: 185 µm) with a small fraction of mud (4 %) (Steyaert *et al.*, subm.), while station 330 consists of medium sand (median grain size: 329-361 µm) without mud (Vanaverbeke *et al.*, 2004a,b) and considered as having highly permeable sediments.

Sampling at both stations was conducted monthly from October 2002 until October 2003 from the RV Zeeleeuw or Belgica. In December 2002 it was not possible to sample station 330 due to bad weather and rough sea.

The water column was sampled 3 m below the air-sea interface and 1 m above the sea floor using 10 l Niskin bottles. To measure pigments, 500 ml of water from each depth was filtered onto GF/F glass microfibre filters (i.d. 4.7 cm) using a vacuum pump. This procedure was repeated three times. The samples were kept in the dark, preserved at –20 °C on board and stored at –80 °C at the laboratory.

Sediment was sampled using a Reineck boxcorer (surface area 180 cm^2) or another box corer with a larger surface area (campaigns of February, April and October 2003). The box corer

was deployed three times at each sampling station. In February only two box corers were obtained for station 330.

From each box corer 2 perspex cores (i.d. 3.6 cm) were taken for meiobenthos and pigment analysis. These cores were sliced in 1 cm slices until a maximum of 10 cm. Samples for pigment analysis were preserved at -20 °C on board and stored at -80 °C at the laboratory. The meiobenthos samples were preserved with a neutral hot 4 % formaldehyde-tap water solution.

During three detailed sampling campaigns (February, April and October 2003) extra cores were taken for the study of carbon and nitrogen stable isotope signatures in the sediment and in the meiobenthos. The samples were kept frozen at -20 °C until they were processed. For the stable isotope signatures in the SPM, the water column 1 m above the sea floor was sampled and filtered as described above.

Laboratory treatment of samples

The sediment samples for pigment analysis were weighted and chlorophyll *a* concentration in the sediment was analyzed by HPLC (Gilson) following Wright & Jeffrey (1997).

Meiobenthos (the animals passing a 1 mm sieve and retained on a 38 µm sieve) was extracted from the sediment by centrifugation with a LUDOX HS-40 solution (Heip *et al.*, 1985). After staining with Rose Bengal all organisms were counted and sorted into higher taxa under a binocular microscope.

Stable isotope analysis

Each meiobenthic sample was defrosted and the specimens were hand-picked with a fine needle under a binocular microscope. The organisms were rinsed, initially in filtered seawater (0.2 µm filters), then in filtered Milli-Q water (0.2 µm filters) to remove adhering particles and transferred to tin capsules. The capsules were oven dried, pinched closed and stored

(-20 °C) until further analysis. The same procedure was repeated for 10 capsules containing no organisms for blank values (5 for carbon and 5 for nitrogen).

Different capsules were filled for carbon and nitrogen analysis respectively, with ca. 60 nematodes per capsule for ^{13}C analysis and ca. 160 nematodes per capsule for ^{15}N analysis.

The taxonomic resolution of the samples taken for stable isotope signatures depended on the amount of biomass present. When sufficient biomass was available, nematodes from the genera *Sabatieria* and *Richtersia* were picked separately from the other nematodes from the samples at station 115bis. "Stout nematodes" (as defined by Vanaverbeke *et al.*, 2004a) were picked separately from the rest of the nematodes at station 330, and other meiobenthic taxa (copepods, Halacaroidea, polychaetes) were also picked separately. When biomass was insufficient to analyse a specific group of nematodes or a specific meiobenthic taxon separately, these were included in the bulk nematode or meiobenthic sample. Replicates were not always obtained for lack of sufficient biomass. For the same reason, it was not always possible to estimate the $\delta^{15}N$ of "stout nematodes" at station 330.

Stable isotope ratios of sediment POM, SPM (filters) and meiobenthos were measured by elemental analyzer–isotope ratio mass spectrometry (EA-IRMS) (Middelburg *et al.*, 2000). Data are expressed in standard δ- unit notation, where $\delta X = [(R_{sample}/R_{reference}) - 1] \times 10^3$, where R_{sample} is either the $^{13}C:^{12}C$ ratio or the $^{15}N:^{14}N$ ratio in the sample, and $R_{reference}$ is the isotope ratio of the reference material. These ratios are reported as per mille deviations from standards: the carbon isotope ratio of Vienna Pee Dee Belemnite ($R_{VPDB} = 0.0112372$) and the nitrogen isotope ratio of air N_2 ($R_{AIR} = 0.0036765$). As trophic fractionation is low for carbon isotopes (mean 0.4 ‰, SD 1.3 ‰) and high for $\delta^{15}N$ (mean 3.4 ‰, SD = 1 ‰), a consumer will have a $\delta^{13}C$ value similar to its food source whilst the $\delta^{15}N$ value will be enriched by on average 3.4 ‰ relative to its food source (Post, 2002).

Data analysis

Spearman rank R tests were used to investigate the correlation between the nematode densities and the chlorophyll *a* concentration in the sediment. Changes in nematode densities with time, sediment depth and time × depth in the upper 5 cm were tested by constructing a univariate split-plot ANOVA design, following Steyaert *et al.* (2001). Replicates were nested within "time", however not within depth. This analysis was restricted to the upper 5 cm since changes in quantity and quality of OM were most obvious here (Provoost *et al.*, in prep.; present study). Variation in the ^{13}C signal of sediment POM with time, depth and time × depth was analyzed using a two-way ANOVA. Kruskal-Wallis (ANOVA by ranks) tests were conducted on $\delta^{13}C$ and $\delta^{15}N$ values of meiobenthos to test for differences between the meiobenthic taxa, sediment depth and sampling dates. All tests were performed using the STATISTICA 6 software package.

Results

Environmental variables

Chlorophyll *a* concentrations in the water column at both stations started rising in February (day 54), peaked in April (day 111; 48 mg m^{-3} at station 115bis and 32 mg m^{-3} at station 330) and decreased afterwards (Fig. 3.1). Smaller peaks were also observed in July (day 194) for both stations and in September (day 258) only at station 330, reaching values no higher than 17 mg m^{-3}. The sediment chlorophyll *a* concentrations were higher at station 115bis (Fig 3.2) than at station 330 (Fig. 3.3) throughout the sampling period and generally followed the patterns observed in the water column. At both stations peak values were observed in April (day 111). At station 330 other peaks were observed in August 2003 (day 229) and October 2003 (day 285) reflecting the deposition of the late summer and autumn blooms (Fig. 3.3).

At station 115bis chlorophyll *a* concentration in the sediment was highest near the sediment surface and decreased with sediment depth, especially after deposition of phytodetritus in April (day 111; Fig. 3.2). At station 330 chlorophyll *a* concentrations in the sediment were more homogeneously distributed with depth except for October 2003 (day 285) when higher values were observed near the sediment surface (Fig. 3.3).

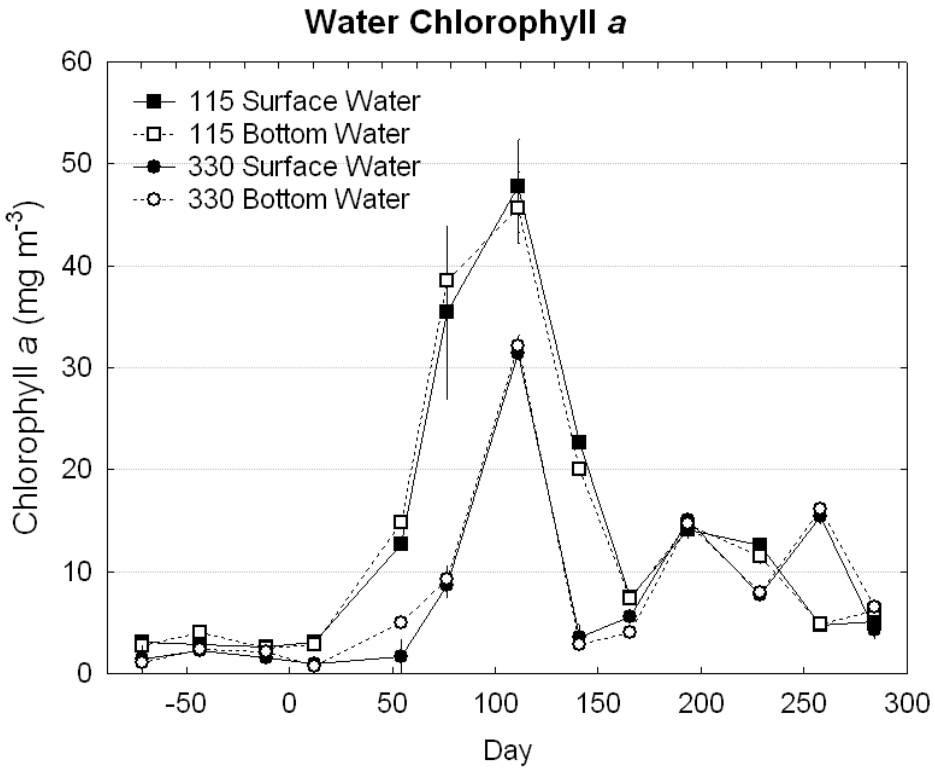


Figure 3.1: Chlorophyll *a* concentration in the surface and bottom water (mg m^{-3}) at stations 115bis and 330 for the period from October 2002 to October 2003 (day 0 = 1st January 2003). Vertical bars represent the standard error.

Temporal patterns in meiobenthic densities

In total 12 meiobenthic taxa were found (Nematoda, harpacticoid Copepoda and nauplii, Cumacea, Gastrotricha, Halacaroidea, Kinorhyncha, Oligochaeta, Ostracoda, Polychaeta, Tardigrada and Turbellaria) from which only Cumacea was never observed at station 330.

In station 115bis Nematoda were highly dominant with an average density of 96 %. Nematodes dominated in station 330 as well, but with considerably lower values (64 %). Here other taxa were present with relatively high densities, e.g. harpacticoid Copepoda and nauplii (15 % and 11 % respectively).

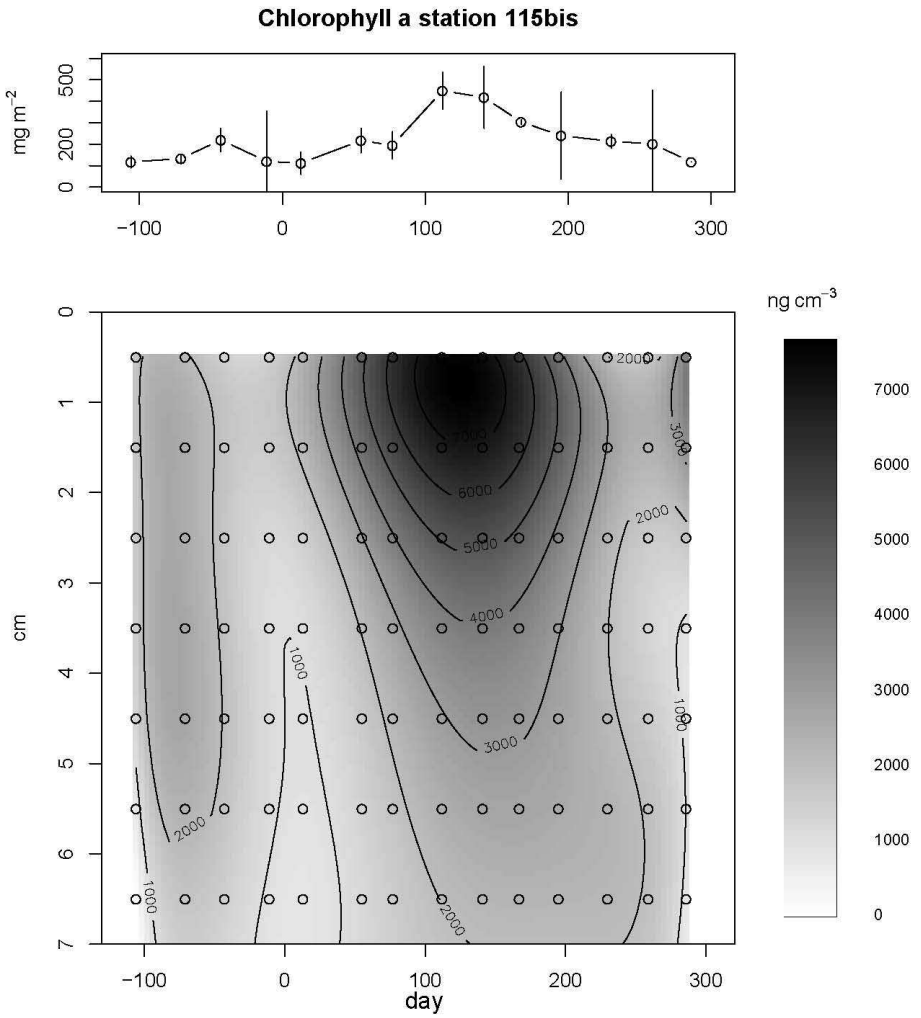


Figure 3.2: Chlorophyll *a* concentration in the sediment (ng cm^{-3}) at station 115bis for the period from September 2002 to October 2003 (day 0 = 1st January 2003) from 0 to 7cm deep. The upper graph shows the chlorophyll *a* concentration in the sediment (mg m^{-2}) in the whole vertical profile for the same dates. Vertical bars represent the standard error.

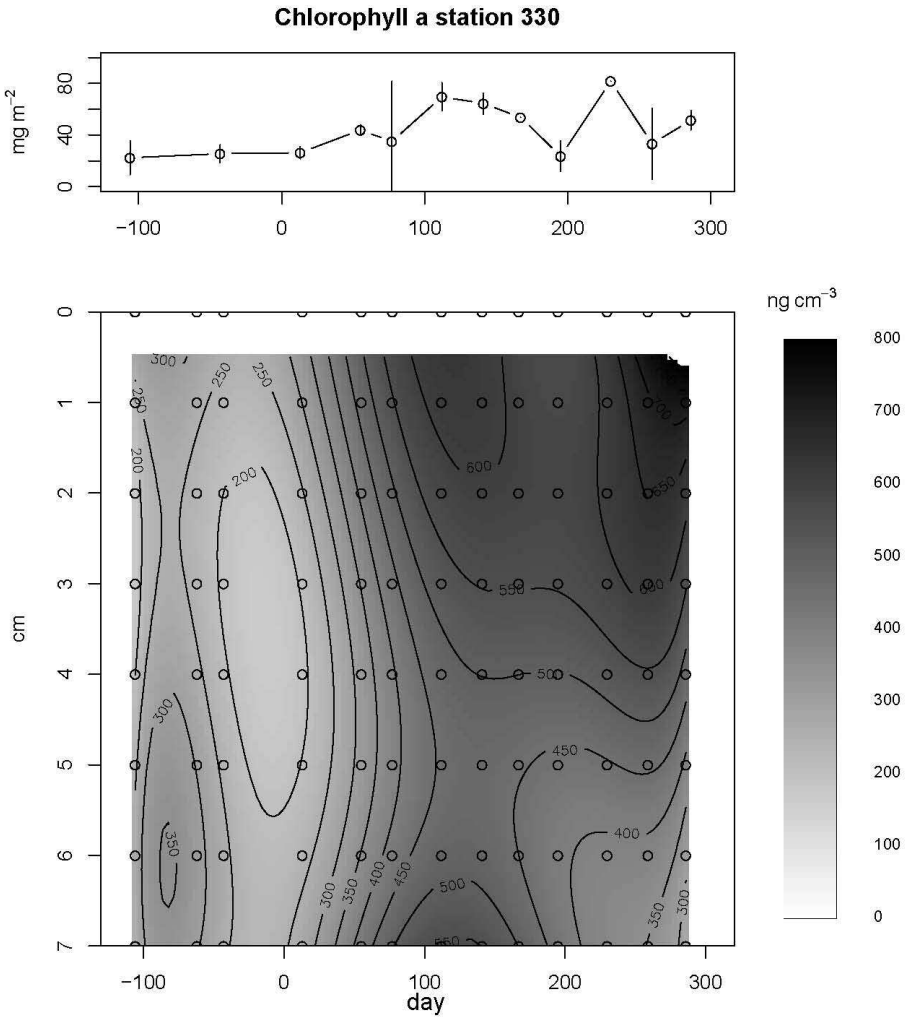


Figure 3.3: Chlorophyll *a* concentration in the sediment (ng cm^{-3}) at station 330 for the period from September 2002 to October 2003 (day 0 = 1st January 2003) from 0 to 7cm deep. The upper graph shows the chlorophyll *a* concentration in the sediment (mg m^{-2}) in the whole vertical profile for the same dates. Vertical bars represent the standard error.

At station 115bis nematode densities were lowest in winter (1251 ind $10cm^{-2}$ in December; day -12) and increased towards April (day 111; 4841 ind $10cm^{-2}$) (Fig. 3.4). No clear changes were observed in the following months until densities increased with highest values in September (day 258) and October 2003 (day 285; 7385 ind $10cm^{-2}$ in October). Nematode densities were not correlated with chlorophyll *a* in the sediment ($R = 0.30$; $p > 0.05$). Vertically, the bulk of the nematode community was found in the upper 4 cm throughout the

year, except for October 2003 (day 285) when relatively high densities (804 ind 10cm^{-2}) were still found down to 7 cm depth (Fig. 3.4). From June (day 166) until September (day 258) higher densities were also found in the 9-10 cm layer.

At station 115bis the ANOVA "split-plot" analysis performed on the nematode densities of the top five sediment layers showed no significant effect of time ($F_{2,4} = 0.59$; $p > 0.05$), sediment depth ($F_{4,8} = 1.56$; $p > 0.05$) nor of the interaction term time × depth ($F_{8,16} = 0.97$; $p > 0.05$).

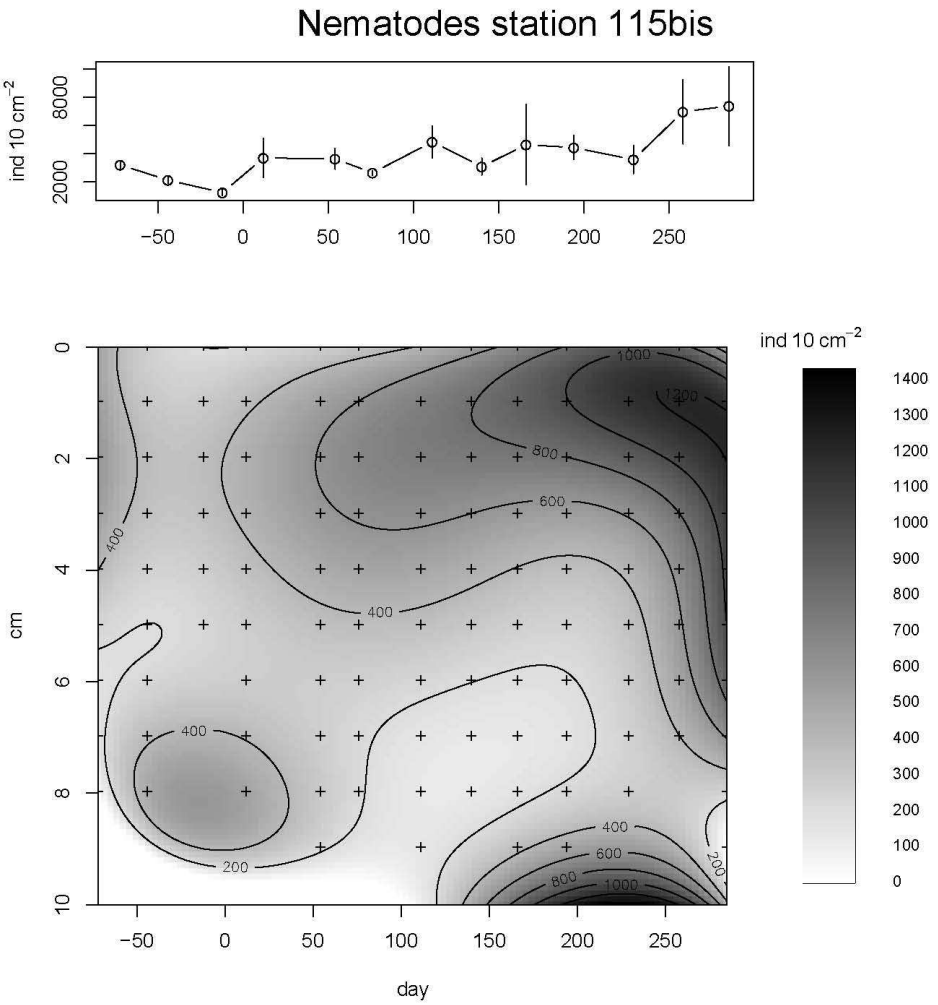


Figure 3.4: Nematode densities (ind 10cm^{-2}) at station 115bis for the period from October 2002 to October 2003 (day 0 = 1st January 2003) from 0 to 10cm deep. The upper graph shows the nematode densities (ind 10cm^{-2}) summed over the vertical profile. Vertical bars represent the standard error.

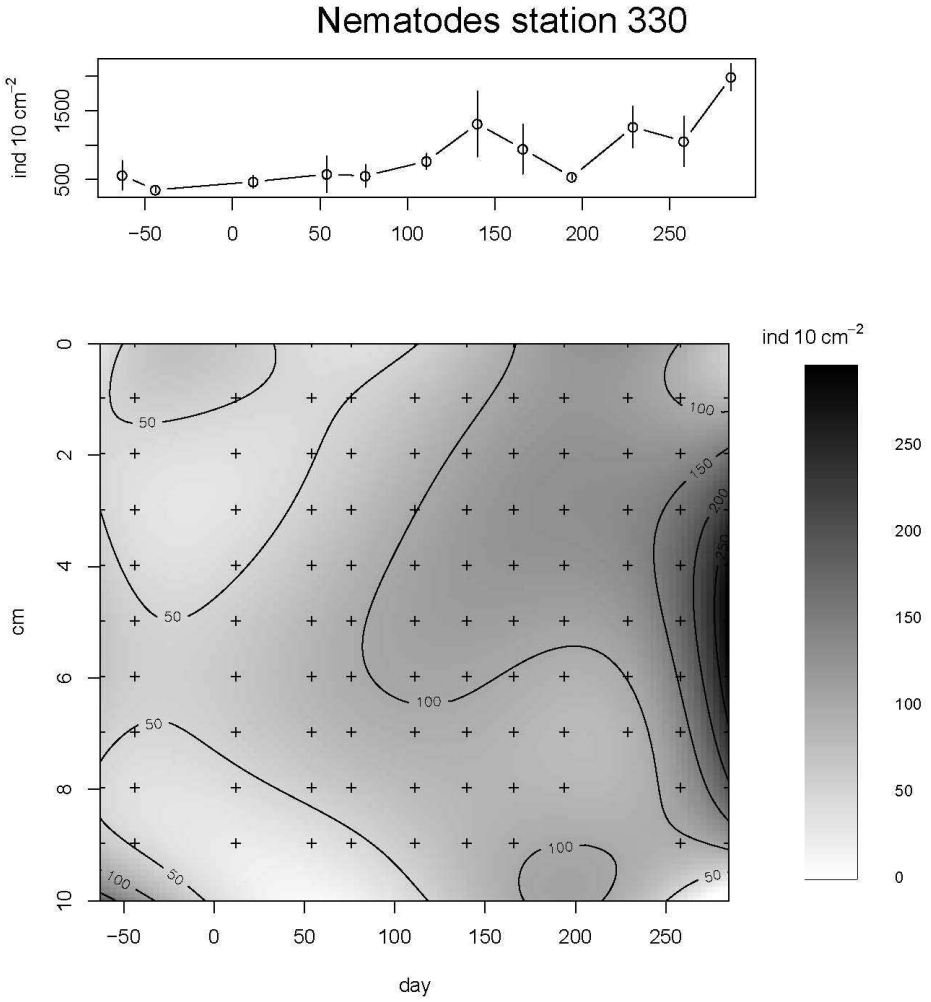


Figure 3.5: Nematode densities (ind 10cm^{-2}) at station 330 for the period from October 2002 to October 2003 (day 0 = 1st January 2003) from 0 to 10cm deep. The upper graph shows the nematode densities (ind 10cm^{-2}) summed over the vertical profile. Vertical bars represent the standard error.

Nematode densities at station 330 were much lower than at station 115bis. Lowest values were recorded in November (day -44; 349 ind 10cm^{-2}), followed by a spring peak in May (day 140; 1310 ind 10cm^{-2}) (Fig. 3.5). Densities decreased towards July (day 194) and increased again in August (day 229) and October (day 285) with the highest values observed in October 2003 (day 285; 1989 ind 10cm^{-2}). At station 330 nematode densities were correlated with chlorophyll *a* in the sediment ($R = 0.36$; $p < 0.05$). Subsurface maximum values were usually

encountered, especially in October 2003 (day 285) when densities were highest (Fig. 3.5). The ANOVA "split-plot" analysis performed on the nematode densities of the top five sediment layers showed a significant effect of time ($F_{2,4} = 23.78$; $p < 0.05$), sediment depth ($F_{4,8} = 4.14$; $p < 0.05$) and of the interaction term time × depth ($F_{8,16} = 2.64$; $p < 0.05$).

Carbon and nitrogen stable isotope signatures

At station 115bis, sediment OM $\delta^{13}C$ values were similar to the SPM values. Sediment POM $\delta^{13}C$ values showed significant differences between sediment depth and sampling events (Fig. 3.6 and 3.7 – Table 3.1). Lowest values (≈ -22 ‰) were observed in February in both sediment layers (0-1 and 4-5 cm). In April, values increased slightly and this increase was more prominent in the upper cm of sediment. Sediment OM $\delta^{13}C$ values in October were similar in both layers and resembled the values noted in April in the surface layer. In February $\delta^{13}C$ values for *Richtersia* and *Sabatieria* from both sediment layers and "other nematodes" from the upper cm were about 3 units higher than the corresponding OM signatures, while the deeper dwelling "other nematodes" showed more depleted values (Fig. 3.6 and 3.7). In April, deep-dwelling "other nematodes" were more depleted than both OM values, while *Richtersia* and *Sabatieria* (from both sediment horizons) and surface-living "other nematodes" showed signatures slightly above or resembling the OM readings. A similar pattern was observed in October for *Sabatieria* while "other nematodes" showed lower values compared to the OM signal at both sediment layers. Very depleted values for both ^{13}C (average value -38.5 ‰; n = 2; SE = 0.003) and ^{15}N (average value 0.78 ‰; n = 2; SE = 0.484) were observed for surface-living harpacticoid copepods in October.

At station 330 $\delta^{13}C$ values of sediment OM did not follow the $\delta^{13}C$ isotope values of SPM (Fig. 3.8). There was a significant difference for the $\delta^{13}C$ values of sediment OM between sampling dates (Fig. 3.8 – Table 3.1).

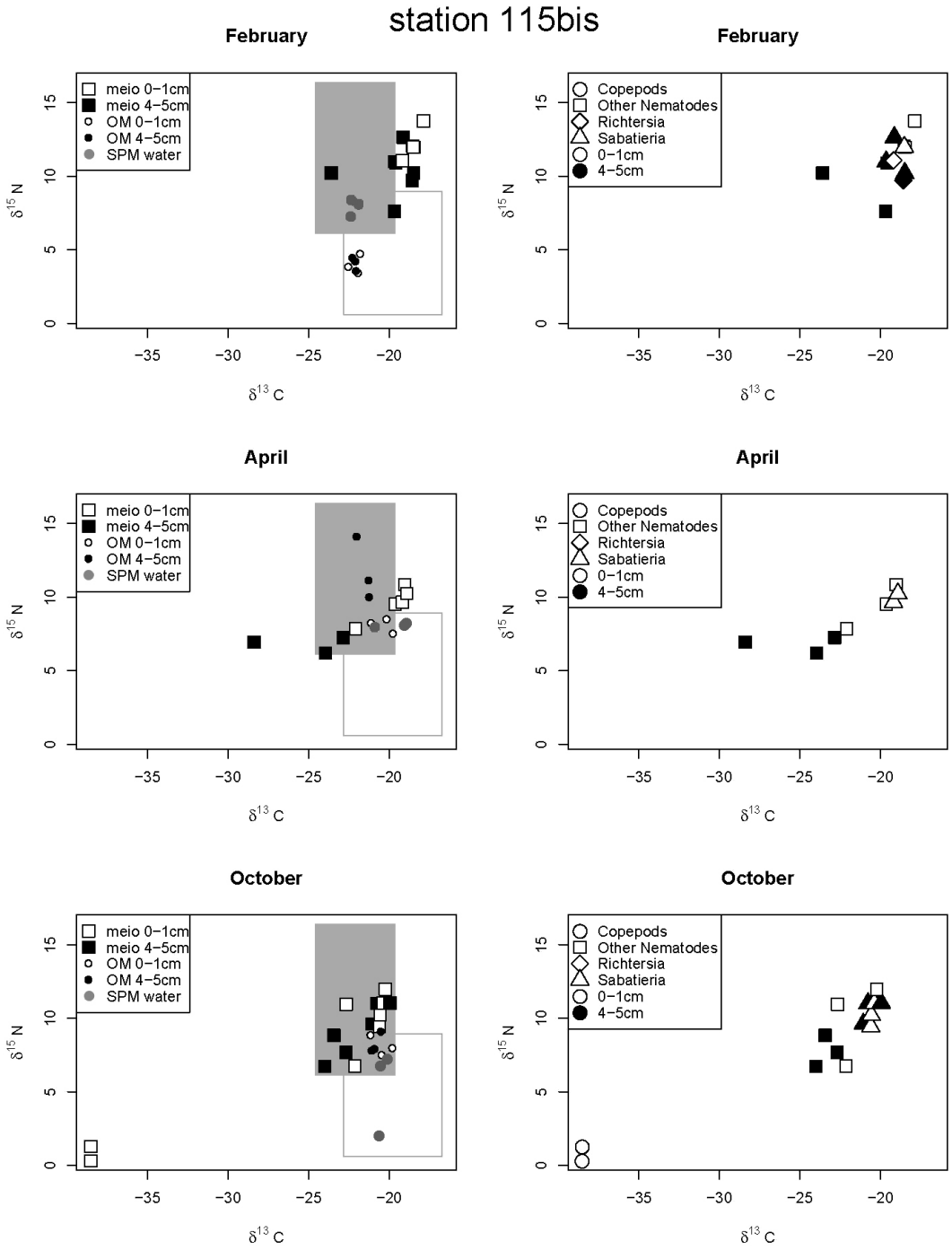


Figure 3.6: $\delta^{13}C$ vs. $\delta^{15}N$ values at station 115bis for the sampling dates of February, April and October 2003. Figures on the left show $\delta^{13}C$ vs. $\delta^{15}N$ values of SPM in the water (1m above the floor), OM in the sediment and meiobenthos at 0-1 and 4-5 cm deep. Grey-coloured box: ranges of isotopic values of SPM in the water column (1 m above the floor) based on the monthly samples. Uncoloured box: ranges of isotopic values of sediment OM (0-1 cm) based on the monthly samples. Figures on the right show $\delta^{13}C$ vs. $\delta^{15}N$ values of the different meiobenthic groups at 0-1 and 4-5 cm.

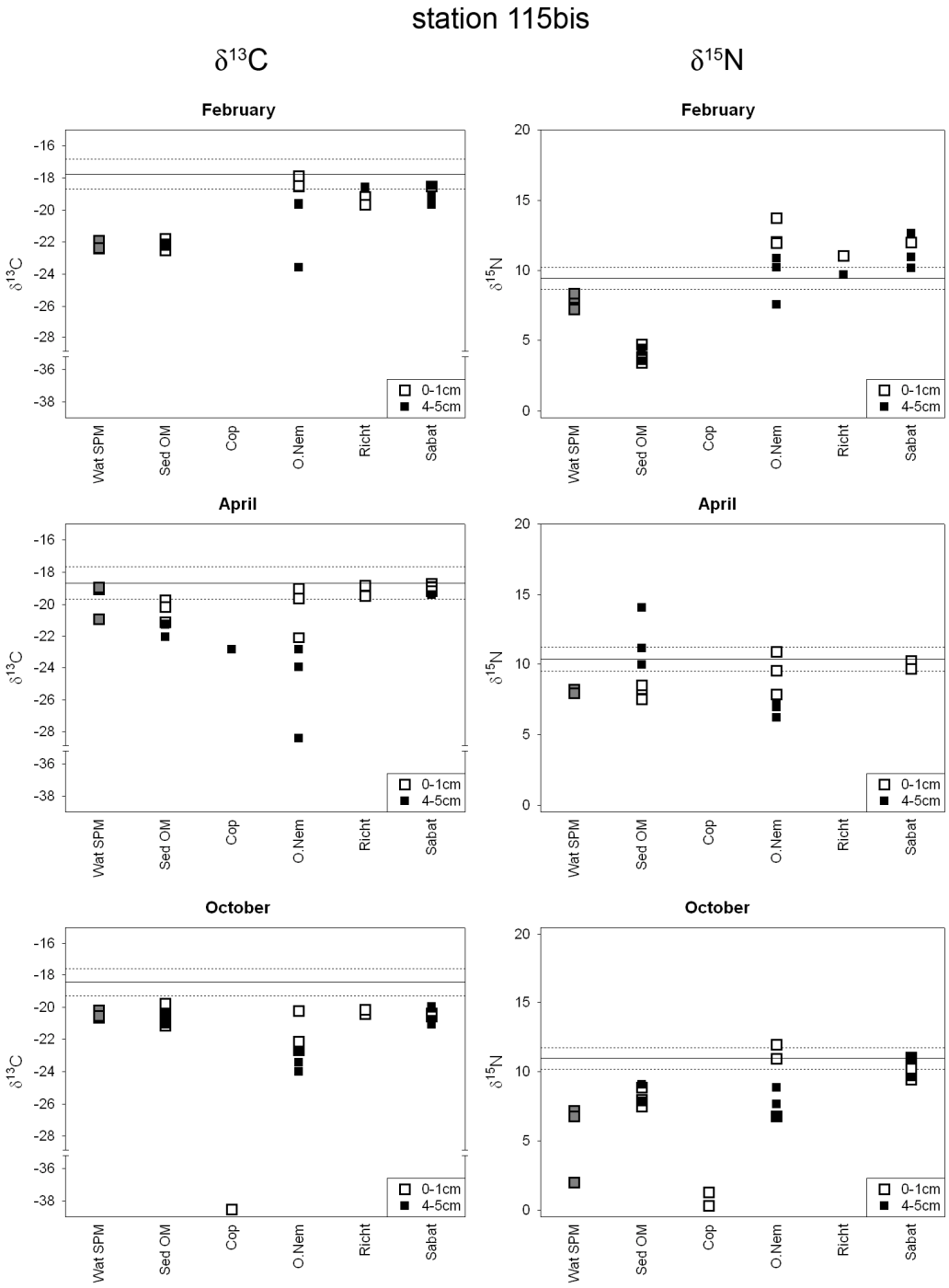


Figure 3.7: $\delta^{13}C$ (left side) and $\delta^{15}N$ (right side) signatures at station 115bis for the sampling dates of February, April and October 2003 from SPM in the water (1 m above the floor; grey squares), and OM in the sediment and different meiobenthic groups at 0-1 cm (open squares) and at 4-5 cm (closed squares) deep. Horizontal line: mean (solid) and mean ± SD (dashed) of animals in station 330. Cop: copepods; O.Nem: other nematodes; Richt: *Richtersia*; Sabat: *Sabatieria*.

Table 3.1: Results of ANOVA tests concerning the effects of Depth (0-1 cm or 4-5 cm), Time (February, April and October 2003) and the interaction between these two (Depth x Time) in the $\delta^{13}C$ values of POM from the sediment for both station (115bis and 330). The null hypothesis is that there were no significant differences. ns: not significant.

		115b			**330**		
Variable	Effect	F	df	p	F	df	p
$\delta^{13}C$ POM	Depth	5.34	1	<0.05	2.08	1	ns
$\delta^{13}C$ POM	Time	15.53	2	<0.001	32.15	2	<0.001
$\delta^{13}C$ POM	Depth × Time	2.10	2	ns	0.52	2	ns

In October, higher values were observed for both sediment layers when compared to February and April. During February and April, all benthic groups from both sediment layers showed $\delta^{13}C$ values between 2 and 4 ‰ higher than the OM values in the sediment while in October both OM and faunal values ranged between -19 ‰ and -17 ‰.

Significant differences in $\delta^{13}C$ values between sampling dates and among meiobenthic taxa were noted at both stations (Table 3.2), while no significant differences between sediment layers were observed. When the extremely low $\delta^{13}C$ values for the copepods were excluded from the analysis at station 115bis, $\delta^{13}C$ values of the meiobenthic taxa were no longer significantly different and significant differences with depth were observed.

$\delta^{15}N$ values for the meiobenthos were similar for both stations (Fig. 3.6, 3.7 and 3.8) and for the different meiobenthic taxa, except for the copepods at station 115bis in October (Fig. 3.6 and 3.7). This was confirmed by a Kruskal-Wallis test that showed no significant differences between the meiobenthic taxa at both stations, if these specific copepod samples were removed (Table 3.2). No significant differences between the two sediment layers were found for both stations (Table 3.2). $\delta^{15}N$ values showed no differences with time at station 115bis while such differences were present at station 330 (Table 3.2).

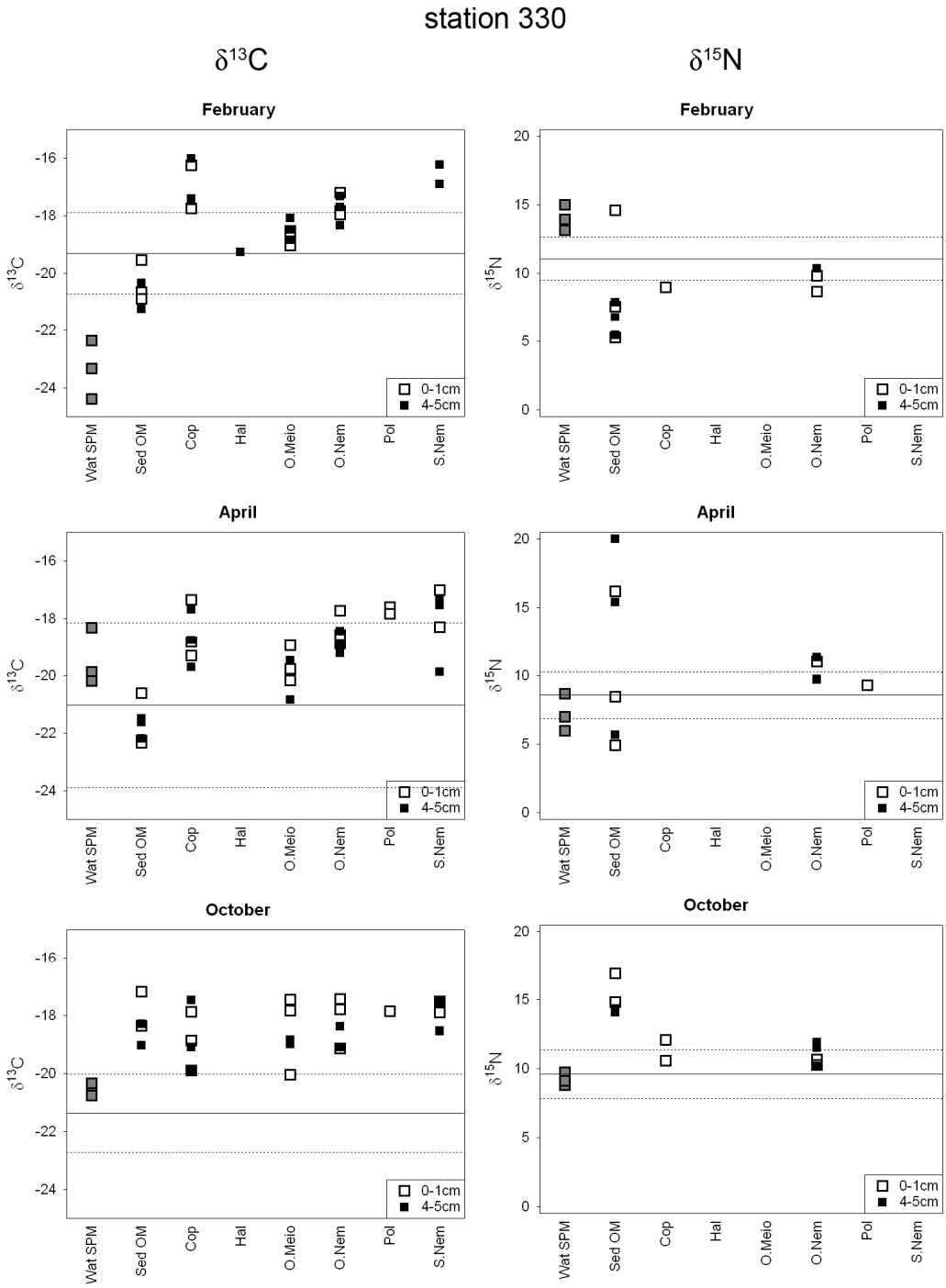


Figure 3.8: $\delta^{13}C$ (left side) and $\delta^{15}N$ (right side) signatures at station 330 for the sampling dates of February, April and October 2003 of SPM in the water (1 m above the floor; grey squares), and OM in the sediment and different meiobenthic groups at 0-1 cm (open squares) and at 4-5 cm (closed squares) deep. Horizontal line: mean (solid) and mean ± SD (dashed) of animals in station115bis (excluding copepods in October). Cop: copepods; Hal: Halacaroidea; O.Meio: other meiobenthos; O.Nem: other nematodes; Pol: polychaetes St.Nem: stout nematodes.

Table 3.2: Results of Kruskal-Wallis (ANOVA by ranks) tests concerning the effects of the Depth (0-1 cm or 4-5 cm), Time (February, April and October 2003) and the different meiobenthic groups on the $\delta^{13}C$ and $\delta^{15}N$ values of meiobenthos (Meio.) for both station (115bis and 330). The null hypothesis is that there were no significant differences. [1] with copepods. [2] without copepods. n.s.: not significant.

Station	Variable	Effect	H	p
115bis[1]	$\delta^{13}C$ Meio.	Group	11.47	<0.01
	$\delta^{13}C$ Meio.	Depth	3.26	n.s.
	$\delta^{13}C$ Meio.	Time	15.60	<0.001
115bis[2]	$\delta^{13}C$ Meio.	Group	5.03	n.s.
	$\delta^{13}C$ Meio.	Depth	4.88	<0.05
	$\delta^{13}C$ Meio.	Time	13.90	<0.001
330	$\delta^{13}C$ Meio.	Group	13.30	<0.05
	$\delta^{13}C$ Meio.	Depth	$0.16E10^{-3}$	n.s.
	$\delta^{13}C$ Meio.	Time	7.80	<0.05
115bis[1]	$\delta^{15}N$ Meio.	Group	8.31	<0.05
	$\delta^{15}N$ Meio.	Depth	1.46	n.s.
	$\delta^{15}N$ Meio.	Time	9.38	<0.01
115bis[2]	$\delta^{15}N$ Meio.	Group	3.19	n.s.
	$\delta^{15}N$ Meio.	Depth	3.69	n.s.
	$\delta^{15}N$ Meio.	Time	9.07	<0.05
330	$\delta^{15}N$ Meio.	Group	1.60	n.s.
	$\delta^{15}N$ Meio.	Depth	0.35	n.s.
	$\delta^{15}N$ Meio.	Time	5.88	n.s.

Discussion

Environmental variables

As described previously (Vanaverbeke *et al.*, 2004a,b; Steyaert *et al.*, subm.; Muylaert *et al.*, 2006) the chlorophyll *a* concentrations in the water column showed a strong phytoplankton bloom in spring at both stations. The high chlorophyll *a* concentrations at station 115bis can be explained by the position of this station closer to the coast (Joint & Pomroy, 1993).

Although sediment chlorophyll *a* concentrations indicated a seasonal signal as well, considerable differences between the stations were obvious. Pigment concentrations at station 115bis were about 10 times higher than at station 330 (Fig. 3.2).

At station 115bis phytodetritus accumulated near the sediment surface during spring, until mineralization in late summer (Provoost *et al.*, in prep) as reported before for other fine-sandy North Sea stations (Boon & Duineveld, 1998).

In contrast, no clear vertical gradients of chlorophyll *a* in the sediment were observed at station 330. Relatively strong bottom water currents can prevent the deposition of sedimenting phytodetritus at the sediment surface of permeable sediments (Huettel & Rusch, 2000; Precht & Huettel, 2004). Due to advective water flow, sedimenting phytoplankton cells can penetrate deeper in the sediment inducing subsurface peaks (Huettel & Rusch, 2000; Ehrenhauss & Huettel, 2004; Ehrenhauss *et al.*, 2004a). Such peaks could be observed in at station 330. Advective transport of oxygen into the sediment (Ziebis *et al.*, 1996; Janssen *et al.*, 2005) and fast removal of decomposition products (Huettel *et al.*, 1998) accelerate POM degradation resulting in a fast mineralization of organic carbon and recycling of nutrients (Huettel & Rusch, 2000; Janssen *et al.*, 2005; Bühring *et al.*, 2006). These processes prevent a build up of labile OM and the establishment of clear vertical gradients as observed at station 115bis.

Temporal patterns in meiobenthic densities

Both stations also demonstrated different nematode responses to phytoplankton deposition. A fast increase in terms of densities at station 330 coincided with sedimentation of labile OM from the water column. In 1999 at the same station highest densities of ca. 600 ind $10cm^{-2}$ were also reported in May (Vanaverbeke *et al.*, 2004b), however sampling only lasted until summer so further responses of nematodes to late summer blooms (if present) were not investigated. This response of the nematode community was mainly due to a fast increase in densities of short and stout nematodes (Vanaverbeke *et al.*, 2004a). These nematodes belong

to the selective deposit feeders *sensu* Wieser (1953), having bacteria as an important food source (Moens & Vincx, 1997). The rapid incorporation of algal biomass into bacteria biomass in these sediments (Bühring *et al.*, 2006) allows this opportunistic response of the nematode communities, whose densities were correlated with chlorophyll *a* concentrations in the sediment. Moreover there was a nematode increase in densities after each deposition event, indicating rapid and independent responses by the nematode community after each event.

The significant differences in vertical distribution patterns are probably a result of an upward migration of the nematodes towards their food source, already reported for station 330 (Vanaverbeke *et al.*, 2004b).

This was not the case at station 115bis: no significant differences in total densities or vertical distribution were detected here, although clear sedimentation of phytodetritus occurred. In these finer grained sediments, peak mineralisation of newly arrived OM is retarded until summer (Boon *et al.*, 1998, Provoost *et al.*, in prep) allowing for a more gradual but extended increase in nematode densities. That is why no correlation between total nematode densities and chlorophyll *a* was observed at this station. At the same station following the phytoplankton bloom of 1999 a gradual increase in nematode densities was also observed, with a first peak in April (ca. 2500 ind $10cm^{-2}$) and the highest densities in July, slightly above 4000 ind $10cm^{-2}$ (Steyaert *et al.*, subm.). At both stations nematode densities were lower in 1999 than in 2003, however seasonal changes were similar.

The absence of clear temporal trends in the vertical distribution of the nematode community as a whole was previously reported for the same station (Steyaert *et al.*, subm.). However, vertical distribution patterns of dominant species did show species-specific temporal patterns, related to food source partitioning among the dominant nematode species (Steyaert *et al.*, subm.). *Sabatieria celtica* and *S. punctata*, which were the dominant species during periods of low food availability, increased rapidly in densities shortly after the arrival of fresh food. Both

species concentrated at the sediment surface at that period. In May and June higher densities of, respectively, *Daptonema riemanni* and *D. fistulatum* were encountered at greater depths. This coincided with the decomposition and burial of the fresh OM, triggering a seasonally timed reproduction (Steyaert *et al.*, subm.).

Carbon and nitrogen stable isotope signatures

The $\delta^{13}C$ values of sediment POM were within the range reported in other studies (Herman *et al.*, 2000; Moens *et al.*, 2002; Moens *et al.*, 2005; Usui *et al.*, 2006). Marine phytoplankton $\delta^{13}C$ values are typically near -22 ‰ (Boutton, 1991). Middelburg & Nieuwenhuize (1998) reported $\delta^{13}C \approx$ -18 ‰ and $\delta^{15}N \approx 9$ ‰ for OM of marine origin in the Schelde estuary (The Nederlands) which is near our study area. Megens *et al.* (2001) observed that $\delta^{13}C$ values in POM from the North Sea were higher in spring and summer when the samples correspond almost exclusively to fresh phytoplankton; winter showed the lowest $\delta^{13}C$ values. At both stations SPM $\delta^{13}C$ values were lowest in February (winter) and the highest values were registered in April, during spring phytoplankton bloom. This pattern was reflected in sediment POM at station 115bis, while at station 330 sediment POM $\delta^{13}C$ values changed irrespectively of changes in SPM as a consequence of the high permeability of the sediment. $\delta^{13}C$ values of *Phaeocystis* during spring blooms in two locations in the North Sea showed $\delta^{13}C$ values of -17.5 ±0.3 ‰ (Van Dongen *et al.*, 2002) which were higher than the ones observed in SPM in April at both stations, probably due to the mixture of *Phaeocystis* with other OM present in the water.

At station 115bis in April, higher POM $\delta^{13}C$ values were observed at the sediment surface compared with the 4-5 cm layer, indicating sedimentation of phytodetritus. In October values at both sediment layers became more similar probably as a result of downward transport and mineralisation during summer and late summer (Provoost, *in prep*). At the coarser grained

station 330, vertical differences in $\delta^{13}C$ POM values were less obvious, since the permeability of the sediment prevents the vertical separation of fresh and older OM (Huettel *et al.*, 1998).

$\delta^{13}C$ signals observed in the meiobenthos were generally more depleted than previously reported in literature (e.g. Couch, 1989; Riera *et al.*, 1996; Carman & Fry, 2002; Moens *et al.*, 2002; Moens *et al.*, 2005). Only Riera & Hubas (2003) showed $\delta^{13}C$ values ranging from -27.0 to -14.8 ‰. All these studies have been conducted in intertidal habitats, where microphytobenthos, which has higher $\delta^{13}C$ values (≈ -15 ‰; Currin *et al.*, 1995; Moens *et al.*, 2005) compared to marine phytoplankton (see above) is also available as food source. Since meiobenthos is able to graze on microphytobenthos (e.g. Moens & Vincx, 1997), organisms feeding on this food source will have a heavier signal than organisms which are restricted to feed on pelagically produced OM or its degradation products.

Even though at both stations $\delta^{13}C$ values of meiobenthos changed significantly with time (Table 3.2), these changes did not reflect the changes observed in sediment POM or water SPM. Excluding copepods and deep-dwelling "other nematodes" from station 115bis (still discussed further on), the meiobenthic $\delta^{13}C$ signals at both stations showed little variation with time (when compared to water SPM and sediment POM) and did not differ much at both stations (-18.81 ±0.18 ‰, -19.48 ±0.34 ‰ and -20.82 ±0.26 ‰ in February, April and October respectively at station 115bis; -17.76 ±0.21 ‰, -18.67 ±0.21 ‰ and -18.45 ±0.18 ‰ in February, April and October respectively at station 330). If there were significant changes on their C-sources through out the year these were not reflected on the ^{13}C signatures.

In mesocosm experiments lasting for 5 or 6 moths, meiobenthic taxa has shown preference for fresh detritus, even if not responding quantitatively (Rudnick, 1989; Widbom & Frithsen, 1995). The feeding preferences of the meiobenthic taxa might have also remained similar during the studied period in the North Sea.

At station 115bis the copepods in October showed an average $\delta^{13}C$ value of -38.5 ‰ (n = 2; SE = 0.003) which deviated considerably from other meiobenthic signatures. Similar $\delta^{13}C$ signatures were found in macrobenthos carrying symbiotic sulphur-oxidizing chemoautotrophic bacteria or having chemosynthesis-based nutrition (Robinson & Cavanaugh, 1995; Tsutsumi *et al.*, 2001; Levin & Michener, 2002). Chemoautotrophic bacteria generate organic molecules from CO_2 and energy from the oxidation of inorganic substrates (Fenical & Jensen, 1993). Generally, it has been suggested that an isotope ratio between about -12 to -24 ‰ indicates a photoautotrophic food source, while lower values down to -45 ‰ reflect a chemoautotrophic source of carbon. Carbon isotope ratios even lower than -45 ‰ are considered to indicate methanotrophy (Felbeck & Distel, 1999). The $\delta^{13}C$ signal of these copepods indicates the exploitation of a chemosynthetic derived food resource. Similar $\delta^{13}C$ signatures have been reported for a nematode species, dominant in sulphidic microbial mat sediments in a cold methane-venting seep while even more depleted $\delta^{13}C$ values for a copepod species indicated the exploitation of a methane-derived food source (Van Gaever *et al.*, 2006). Sediments at station 115bis were generally reduced during spring 1999 (Steyaert *et al.*, subm.) and during our sampling period (unpublished data based on oxygen penetration depth data), hence may contain sulphur-oxidizing bacteria. Levin & Michener (2002) observed for some chemosynthetic bacterial mats an average $\delta^{15}N$ of -1.50 ‰. A consumer feeding on bacteria with such a $\delta^{15}N$ value would have a $\delta^{15}N$ signature 3.4 ‰ heavier (see references above) bringing it close to the values we observed for copepods from station 115bis in October, confirming their chemosynthetic food source.

The specificity of copepods from station 115bis in October for such a food source indicates its importance, at least in a specific period of time. To our knowledge, such a clear indication of an exploitation of an chemosynthetic derived food source by meiobenthic organisms, other

than in cold seeps, as not been previously reported. Even though it can be an important food source, at least in a certain period, its relevance for meiobenthic organisms remains unknown.

When copepods values were excluded at station 115bis, meiobenthic $\delta^{13}C$ signatures showed significant differences with depth. Rudnick (1989) also suggest for muddy sediments the existence of 2 distinct food webs, among meiobenthic organisms, segregated by depth. Among the meiobenthic taxa, "other nematodes" revealed the strongest vertical differences in $\delta^{13}C$ values, especially in April. Species belonging to the genus *Sabatieria* (e.g. *S. celtica* and *S. punctata*) are known to migrate toward their food sources, while other dominant species belonging to other genera (e.g. *Daptonema riemanni* and *D. fistilatum*) are restricted to deep sediment horizons (Steyaert *et al.*, subm.). The continuous lower $\delta^{13}C$ values in deep-dwelling "other nematodes" species indicate the existence of vertical segregated food webs, with deep-dwelling nematodes exploiting other C-sources. It is also possible that, as the copepods, some deep-dwelling "other nematodes" species were also exploiting a chemosynthetic derived food source, however this could be restricted only to some nematode species and their more depleted signal would be therefore diluted.

At station 330 no vertical differences within the meiobenthic $\delta^{13}C$ values or in sediment POM were detected. This results from the permeable nature of the sediment, inhibiting the build up of vertical gradients in POM quality and quantity. However the stout nematodes, which showed the strongest response to phytodetritus deposition at this station (Vanaverbeke *et al.*, 2004a) showed similar $\delta^{13}C$ values as the other nematodes, copepods and polychaetes, so our results do not indicate differences in carbon sources and/or feeding strategy.

Except for the copepods at station 115bis in October 2003, $\delta^{15}N$ values showed no significant differences between the different meiobenthic groups at both stations. Riera *et al.* (1996) reported similar $\delta^{15}N$ values for nematodes. Slightly lower values than the ones we observed have also been reported (Couch, 1989; Carman & Fry, 2002) as well as slightly higher values

(Riera & Hubas, 2003). The higher $\delta^{15}N$ values reported in Moens *et al.* (2005) coincide with the predatory ecology of the corresponding species. These species, if present in our samples, would be found within the "other nematodes". Even though the relative importance of their signal depends on the relative amount of biomass of such predators (which we have no information about), generally predators are not dominant (in terms of relative densities) within the nematodes at the studied sites (e.g. Steyaert *et al.*, subm.; Vanaverbeke *et al.*, 2004b), so probably their signature would be diluted in the "other nematodes" signature.

In conclusion the nematode community response to the deposition of the phytoplankton bloom differed according to the biogeochemistry of the sediment: in fine sediments the prolonged presence of OM in the sediment resulted in a gradual response of the nematode community with highest densities long after the deposition of phytodetritus. Depth-related differences in food-webs and feeding strategies were observed in these sediments. In permeable sediments the whole system responded fast and OM reaching the sediment was quickly mineralised. However in general the meiobenthic ^{13}C signatures remained similar with time and were not coupled with changes observed in the water and sediment OM.

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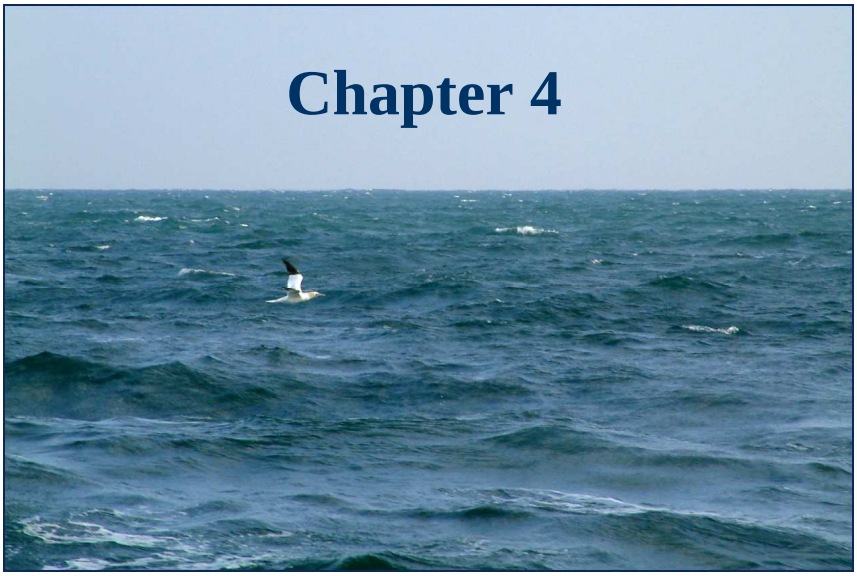
Chapter 4



Uptake of phytodetritus by meiobenthos using ^{13}C labelled diatoms and *Phaeocystis* in two contrasting sediments from the North Sea

MA Franco, K Soetaert, MJ Costa, M Vincx, J Vanaverbeke.

Abstract

Meiobenthic uptake of ^{13}C labelled diatom *Skeletonema costatum* and Prymnesiophyte *Phaeocystis* was investigated in permeable and fine grained depositional sediments from the Southern Bight of the North Sea at different sediment horizons. Both the diatom and *Phaeocystis*–derived organic matter (OM) cascaded into meiobenthic biomass in low but similar percentages and label uptake was highest at the upper cm in both stations. *Phaeocystis*–derived OM might be as important as diatoms as a food source for the meiobenthos.

Meiobenthic biomass in the coarse sediment was lower than in the fine sediment, however label uptake per unit of organismal carbon was higher. This was explained as an adaptation to an environment where only low amounts of labile OM are present. In this station, the so-called "stout nematodes" (length to width ratio < 15, Vanaverbeke *et al.*, 2004a) showed lower uptake compared to the slender nematodes. Therefore their previously reported opportunistic response to a pulsed food supply must rely then on their life-history characteristics.

In finer sediments *Sabatieria* showed the highest uptake at the 1-3cm layer, reflecting migration to the sediment-water interface to feed on freshly deposited labile OM. *Richtersia* ^{13}C uptake was fairly low, indicating the exploitation of another carbon source either than the one derived from *Skeletonema costatum* or *Phaeocystis*. *Enoploides* presented the highest uptake, indicating that these predacious nematodes have another food source besides meiobenthic preys.

Generally total uptake was low and not nearly sufficient to maintain nematodes feeding requirements.

Introduction

In spring Belgian coastal waters are dominated by intense blooms of *Phaeocystis* colonies co-occurring with diatom blooms (Reid *et al.*, 1990; Joint & Pomroy, 1993; Brussaard *et al.*, 1995; Rousseau *et al.*, 2000). *Phaeocystis* blooms last for 20 to 40 days between April and May, dominating the Belgian waters, while diatoms can be present the whole year with dense blooms occurring early in February and smaller ones can still occur in late September (Reid *et al.*, 1990; Rousseau *et al.*, 2002). Strong sedimentation events as a consequence of the settling of these phytoplankton blooms represent a major source of organic matter (OM) for the benthic system supporting benthic life (Graf, 1992).

During the spring phytoplankton bloom in the Southern North Sea, *Phaeocystis* can contribute with more than 99 % of the autotrophic biomass (Hamm & Rousseau, 2003). It has been suggested that most of the *Phaeocystis*–derived production in the Belgian coastal waters is remineralised in the pelagic realm (Rousseau *et al.*, 2000; Hamm & Rousseau, 2003). However, large amounts of *Phaeocystis* colonies have been observed at the sediment surface of undisturbed box cores in our study area (Vanaverbeke pers. com.). Recent modelling revealed that 20 % of the ungrazed primary production is deposited on the sediment, and that in spring half of the sedimentation consists in *Phaeocystis* colonies while only 12 % is of diatom origin (Lancelot *et al.*, 2005). These observations raise the question of how important *Phaeocystis*–derived OM could be for the subtidal benthic food web. So far, grazing on settled *Phaeocystis* colonies by benthic gastropods has been observed in tidal flats (Cadée, 1996) but we have no knowledge of observations on the trophic fate of *Phaeocystis* in the subtidal benthic ecosystem and its relative importance in comparison with diatom-derived OM.

The receiving sediment type is another important factor determining the fate of labile OM: in fine-grained depositional stations, accumulation and sharp vertical profiles of labile OM can emerge after the sedimentation of phytodetritus in spring (e.g. Steyaert *et al.*, subm.; Franco *et al.*, in press a) while in coarser permeable sediments rapid degradation of OM is often the case (Ehrenhauss *et al.*, 2004b; Vanaverbeke *et al.*, 2004b; Janssen *et al.*, 2005; Bühring *et al.*, 2006). The different biogeochemical processes in such contrasting sediments affect the response of the residing benthic bacteria (Franco *et al.*, in press a) and organisms (Vanaverbeke *et al.*, 2004a,b; Steyaert *et al.*, subm.; Franco *et al.*, in press b) to a pulsed sedimentation event.

Meiobenthos is known to exploit pelagical production; either directly by feeding on the sedimented microalgae or indirectly by feeding on the decaying OM and/or associated bacteria (see Heip *et al.*, 1985, 1995). Species-specific uptake of labelled organic matter has already been observed in meiobenthic feeding ecology studies (e.g. Ólafsson *et al.*, 1999; De Troch *et al.*, 2005).

Although isotopic tracing is now a widely applied technique in food web studies (Post, 2002), it is still taking its first steps within meiobenthic studies. Due to the small individual biomass of these organisms, picking sufficient amounts for isotopic analysis remains difficult and time consuming. Among marine benthic isotopic studies conducted so far using a labelled food source, only a few deal with the whole meiobenthic community (Widbom & Frithsen, 1995; Ólafsson *et al.*, 1999; Carman & Fry, 2002; Moodley *et al.*, 2002; Van Oevelen *et al.*, 2006b; Urban-Malinga & Moens, 2006). Most studies deal with a taxon in particular or with a few species within a specific group, e.g. nematodes (Middelburg *et al.*, 2000; Moens *et al.*, 2002), copepods (Pace & Carman, 1996; Buffan-Dubau & Carman, 2000; De Troch *et al.*, 2005), Foraminifera (Moodley *et al.*, 2000; Nomaki *et al.*, 2005) or ostracods (Buffan-Dubau & Carman, 2000; Modig *et al.*, 2000).

In this study we investigate the detailed response of subtidal meiobenthos to the addition of ^{13}C labelled diatoms and *Phaeocystis* in contrasting sediments. This will allow to (i) clarify the relative importance of *Phaeocystis* vs. diatoms as a food source (ii) increase the understanding of the ecology of permeable vs. depositional sediments and (iii) assess the response of meiobenthic taxa to phytoplankton sedimentation.

Material and Methods

Study site and sampling

Samples were taken from the Belgian Continental Shelf (BCS) stations 115bis, located close to the coast (51°09;2'N; 02°37;2'E; 13m depth) and 330, located further offshore (51°26;0'N; 02°48;5'E; 20m depth) (Fig. 1.1).

Station 115bis is a deposition station, characterized by the presence of fine sediments (median grain size: 185 µm) with a small fraction of mud (4 %) (Steyaert *et al.*, subm.), while station 330 consists of medium sand (median grain size: 329-361µm) with no mud (Vanaverbeke *et al.*, 2004a,b).

Sampling at both stations was conducted in April 2004 from the RV Belgica. Sediment was sampled using a box corer deployed several times at each sampling station to obtain sufficient cores to run the experiment.

At station 115bis and 330 5 and 3 intact cores (i.d. 14.5 cm) respectively with overlying water were collected. The cores were allowed to stabilize in a temperature controlled room in the dark at in situ temperature (12°C) for approximately 1 week. During storage the overlaying water was gently aerated.

Experimental design

Skeletonema costatum, a pelagic diatom naturally present in the Southern Bight of the North Sea (Rousseau *et al.*, 2002), was cultured in axenic conditions at 14°C in artificial sea water containing 30 % ^{13}C-enriched bicarbonate (Moodley *et al.*, 2002) that produced algal carbon consisting of 19.3 % ^{13}C. *Phaeocystis* labelling was accomplished by adding ^{13}C labelled $NaHCO_3$ to natural seawater, producing algal carbon consisting of 39.1 % ^{13}C. It should be noted that culturing *Phaeocystis* in axenic conditions was not possible.

Diatoms and *Phaeocystis* were concentrated by centrifugation, washed several times to remove non-incorporated ^{13}C-bicarbonate, and stored frozen until initiation of the experiment. The axenic state of the diatom culture was verified microscopically, by separate PLFA analysis of the freeze-dried diatoms and by a 0 h sediment incubation experiment (Moodley *et al.* 2002). Prior to inoculation 1 core from each station was sampled so natural values of meiobenthic stable isotopes could be determined. The remaining cores were inoculated: 2 cores from station 330 with labelled diatoms at a concentration of 1000 mg carbon of S. *costatum* m^{-2} (193 mg ^{13}C m^{-2}); 2 cores from station 115bis with labelled diatoms at a concentration of 1000 mg *S. costatum* m^{-2}; 2 cores from station 115bis with labelled *Phaeocystis* at a concentration of 128 mg C *Phaeocystis* m^{-2} (50 mg ^{13}C m^{-2}). Diatoms and *Phaeocystis* were thawed and added to cores with the help of a long pipette depositing the organic tracer gently onto the sediment surface. Cores were fitted with a lid equipped with a magnetic stirrer being continuously aerated during the 14 days incubation period.

After the 14 days, overlying water was carefully removed and sediment extracted by pushing it up with a piston and sliced into layers of 0-1 cm, 1-3 cm, 3-5 cm, 5-8 cm and 8 cm-end. Each layer separately was gently homogenized and sub-sampled for different components including meiobenthos (approx. 10 cm^3).

The meiobenthos sediment samples (10 cm^3 of sediment) were fixed in a 4 % formaldehyde solution stained with rose Bengal. Meiobenthos (animals passing a 1 mm sieve and retained on a 38 µm sieve) was extracted from the sediment by centrifugation with a LUDOX HS-40 solution (Heip *et al.*, 1985) and kept in a 4 % formaldehyde solution, stained with Bengal rose.

Meiobenthos sorting and isotopic analysis

Each meiobenthic sample was washed and the organisms were hand picked with a fine needle under binocular microscope. The organisms were then rinsed in filtered Milli-Q water (0.2 µm filters) to remove adhering particles and transferred to tin capsules. The capsules were oven dried, pinched closed and stored (-20 °C) until further analysis. The same procedure was repeated for 5 capsules containing no organisms for blank values.

Nematodes from the genera *Sabatieria, Richtersia* and *Enoploides* were picked separately from the "other nematodes" for station 115bis when present in sufficient biomass. For station 330 "stout nematodes" (length to width ratio < 15, as defined by Vanaverbeke *et al.*, 2004a) were picked separately from the "other nematodes" when sufficient biomass was available. Other meiobenthic taxa were picked separately as well when sufficient biomass was present. When there was not enough biomass to analyse a specific group of nematodes or a specific meiobenthic taxa separately, they were included in the bulk "nematodes" or "other meiobenthos" samples. Replicates were not obtained systematically due to the lack of sufficient biomass.

When there was not enough animal biomass present in the deeper layers of station 330, the organisms from the layers below 1 cm were pooled in a sample 1 cm-end layer.

Stable isotope ratios of meiobenthos were measured by elemental analyzer–isotope ratio mass spectrometry (EA IRMS) (Middelburg *et al.*, 2000). Data are expressed in standard δ- unit notation, where $\delta^{13}C = [(R_{sample}/R_{VPDB}) - 1] \times 10^3$, where R is the ^{13}C:^{12}C ratio. These values

are reported relative to the Vienna Pee Dee Belemnite standard (VPDB) with an isotopic ratio of $R_{VPDB} = 0.0112372$.

Data analysis

Incorporation of ^{13}C is reflected as excess (above background) ^{13}C and is expressed as total uptake in mg ^{13}C m^{-2}, calculated as the product of excess ^{13}C (E) and total biomass (organic carbon). E is the difference between the fraction ^{13}C of the control ($F_{reference}$) and the sample (F_{sample}), where $F = {}^{13}C/({}^{13}C + {}^{12}C) = R/(R + 1)$. The carbon isotope ratio (R) was derived from the measured $\delta^{13}C$ values as $R = (\delta^{13}C/1000 + 1) \times R_{VPDB}$. This total uptake was further standardised and expressed per individual (E × individual biomass) and per unit of carbon.

One-way ANOVA was used to test for significant differences between the two stations in total uptake and total biomass. Homogeneity of variances was tested using Bartlett χ^2. Whenever necessary uptake data were inverse-transformed and biomass was log-transformed to obtain homogeneity of variances. The STATISTICA 6 software was used and a significance level of 0.05 was considered in all test procedures.

Results

The natural $\delta^{13}C$ values of the different meiobenthic groups varied between -19.544 and -23.372 ‰ at station 115bis and between -19.623 and -25.118 ‰ at station 330 (table 4.1). After incubation for 14 days with labelled diatoms (Stations 115bis and 330) and *Phaeocystis* (station 115bis) $\delta^{13}C$ values in meiobenthos changed in all experiments, especially at the surface layer of the sediment (Fig. 4.1).

At station 330 $\delta^{13}C$ values varied between 11.87 and 1737.58 ‰ (Fig. 4.1a). At station 115bis $\delta^{13}C$ values ranged between -7.386 and 340.919 ‰ at the sediment surface of the diatom incubated cores (Fig. 4.1b). Surface values of $\delta^{13}C$ at the *Phaeocystis* cores were

between -15.243 and 164.788 ‰ (Fig. 4.1c). At the deeper sediment layers, $\delta^{13}C$ values were closer to the background values except for *Sabatieria* in both replicates for the diatom cores at 1-3 cm depth and "other nematodes" in replicate 2 from the same treatment, at the same depth.

Table 4.1: Natural $\delta^{13}C$ values (‰) in the meiobenthic taxa at both stations (330 and 115bis) at different sediment depths (cm). O.Nem.: other nematodes; O.Meio.: other meiobenthos.

115b			**330**		
Taxa	**Depth**	**$\delta^{13}C$**	**Taxa**	**Depth**	**$\delta^{13}C$**
Enoploides	0-1	-19,544	Stout Nem.	0-1	-22,910
Richtersia	0-1	-21,973	O. Nem.	0-1	-20,758
Sabatieria	0-1	-20,339	Copepoda	0-1	-21,393
O. Nem.	0-1	-21,643	Polychaeta	0-1	-19,623
Polychaeta	0-1	-23,372	O. Meio.	0-1	-20,392
O. Meio.	0-1	-20,848			
Sabatieria	1-3	-20,719	Nematodes	1-3	-20,459
O. Nem.	1-3	-23,259	O. Meio	1-3	-20,336
O. Meio.	1-3	-20,307			
Nematodes	3-5	-22,473	Nematodes	3-5	-
Nematodes	5-8	-22,884	Nematodes	5-8	-25,118
Sabatieria	8-end	-21,109			
O. Nem.	8-end	-22,732			

The individual uptake of carbon of the different meiobenthic taxa was highest at the surface layer for all experimental units (Fig. 4.2). At station 330 the highest uptake was observed within "other meiobenthos" at the 0-1 cm layer of core Dt1 with a value of 48.9×10^{-3} µgC ind^{-1} (Fig. 4.2a). The other groups showed relatively lower uptake values between 0.48×10^{-3} and 3.23×10^{-3} µgC ind^{-1} at the surface layer and between 0.34×10^{-3} and 0.81×10^{-3} µgC ind^{-1} for the deeper sediment. Stout nematodes showed lower individual uptake when compared with "other nematodes". The treatment with labelled diatoms for station 115bis sediments showed highest individual uptake from *Enoploides* at the 0-1 cm layer of core Dt2 (50.3×10^{-3} µgC ind^{-1}) (Fig. 4.2b). The other groups showed lower

individual uptake values which decreased with sediment depth. Apart from the comparatively much higher value for *Enoploides*, the highest individual uptake values were recorded for *Sabatieria*. *Richtersia*, being only present with sufficient biomass at the surface layer, showed almost no uptake. Individual uptake was lower in the incubation with labelled *Phaeocystis* (Fig. 4.2c). The highest individual uptake was from *Enoploides* at the 0-1cm layer of core Ph2 with a value of 2.51×10^{-3} µgC ind^{-1}. The other groups showed low individual uptake values between 0 and 0.30×10^{-3} µgC ind^{-1}. *Sabatieria* and "other nematodes" showed some uptake in the 0-1 and 1-3 cm layers while *Richtersia* was only present with sufficient biomass at the surface layer where it showed almost no uptake.

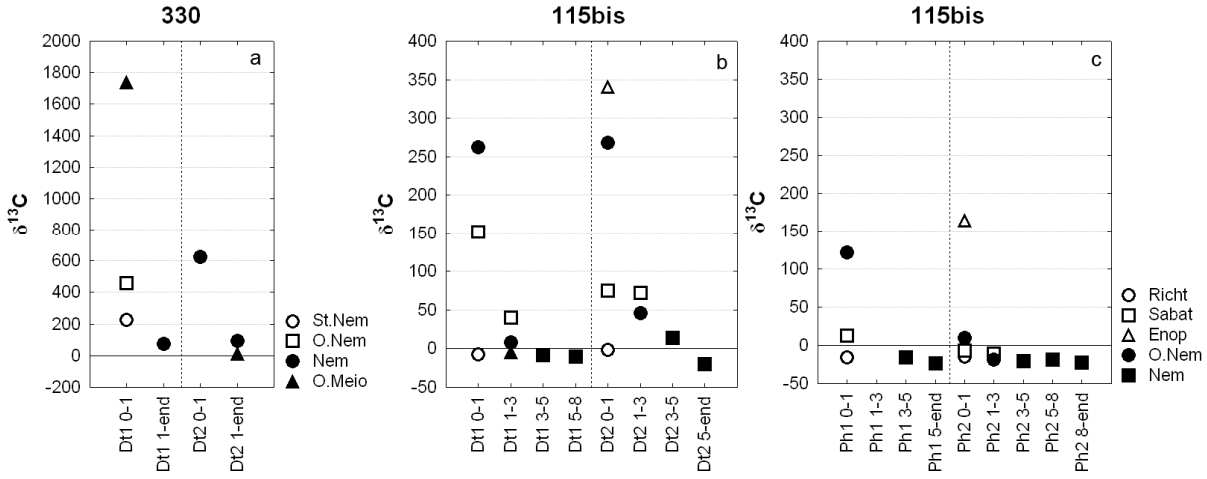


Figure 4.1: $\delta^{13}C$ values after incubation for 14 days with the labelled diatoms *S. costatum* (Dt) at stations 330 (a) and 115bis (b) and labelled *Phaeocystis* (Ph) at station 115bis (c) for the different meiobenthic taxa at different sediment depths. *S. costatum* and *Phaeocystis* treatments are not comparable due to different amount to ^{13}C added in each treatment. Note different scale on left *y*-axis. St.Nem: stout nematodes; O.Nem: other nematodes; Nem: nematodes; O.Meio: other meiobenthos; Richt: *Richtersia*; Sabat: *Sabatieria*; Enop: *Enoploides*.

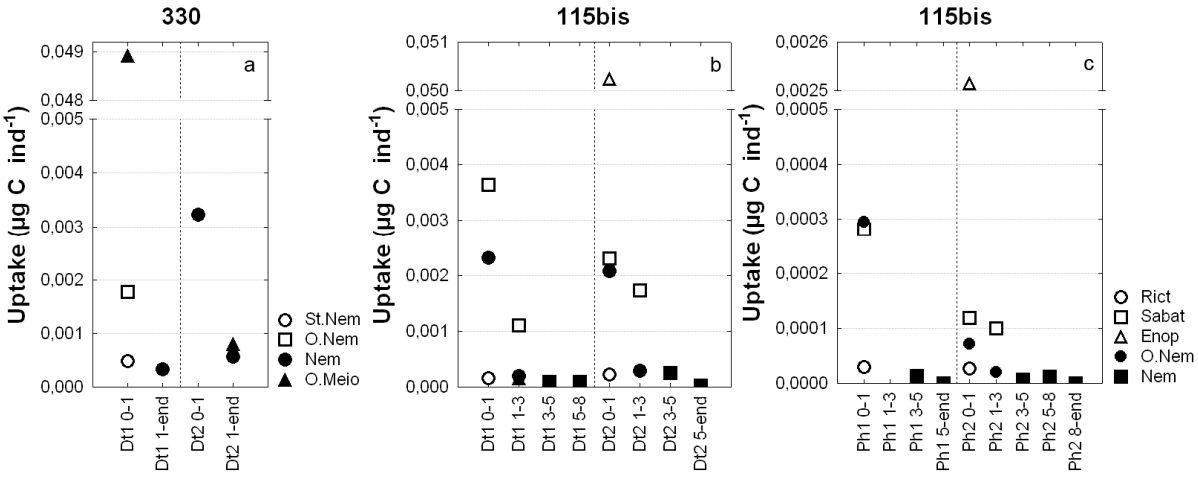


Figure 4.2: Total uptake per individual (µgC ind^{-1}) after incubation for 14 days with labelled diatoms *S. costatum* (Dt) at stations 330 (a) and 115bis (b) and labelled *Phaeocystis* (Ph) at station 115bis (c) for the different meiobenthic taxa at different sediment depths. Note different scale on left *y*-axis. St.Nem: stout nematodes; O.Nem: other nematodes; Nem: nematodes; O.Meio: other meiobenthos; Richt: *Richtersia*; Sabat: *Sabatieria*; Enop: *Enoploides.*

The uptake of carbon per unit of organismal carbon was clearly higher at the surface layer for all treatments (Fig. 4.3). Relatively higher values were observed at station 330 (Fig. 4.3a). Here "other meiobenthos" presented the highest value at the 0-1 cm layer of core Dt1 (98.2×10^{-3} µgC µgC^{-1}). The other meiobenthic groups presented lower uptake values, ranging between 14.2×10^{-3} and 36.6×10^{-3} µgC µgC^{-1} for the surface layer and between 1.83×10^{-3} and 6.60×10^{-3} µgC µgC^{-1} in the deeper sediment. "Other nematodes" presented an uptake per unit of carbon twice as high as the "stout nematodes". Uptake per unit of carbon for the incubation with labelled diatoms at station 115bis varied between 0.83×10^{-3} and 20.5×10^{-3} µgC µgC^{-1} at the surface layer, between 0.92×10^{-3} and 5.29×10^{-3} µgC µgC^{-1} for the 1-3 cm layer and between 0.13×10^{-3} and 2.07×10^{-3} µgC µgC^{-1} for deeper sediment layers (Fig. 4.3b). *Enoploides* showed slightly higher uptake values compared to "other nematodes". *Sabatieria* showed lower uptake than *Enoploides* and "other nematodes" at the

surface layer, but showed the highest uptake values at the 1-3 cm layer. *Richtersia* showed almost no uptake. When labelled *Phaeocystis* was added, uptake per unit of carbon occurred almost exclusively at the surface layer (Fig. 4.3c). Uptake values varied between 0.19×10^{-3} and 5.17×10^{-3} µgC µgC^{-1} at the 0-1 cm layer and between 0 and 0.28×10^{-3} µgC µgC^{-1} at deeper layers. *Enoploides*, and "other nematodes" presented the highest uptake per unit of carbon, followed by *Sabatieria*. *Richtersia* showed almost no uptake.

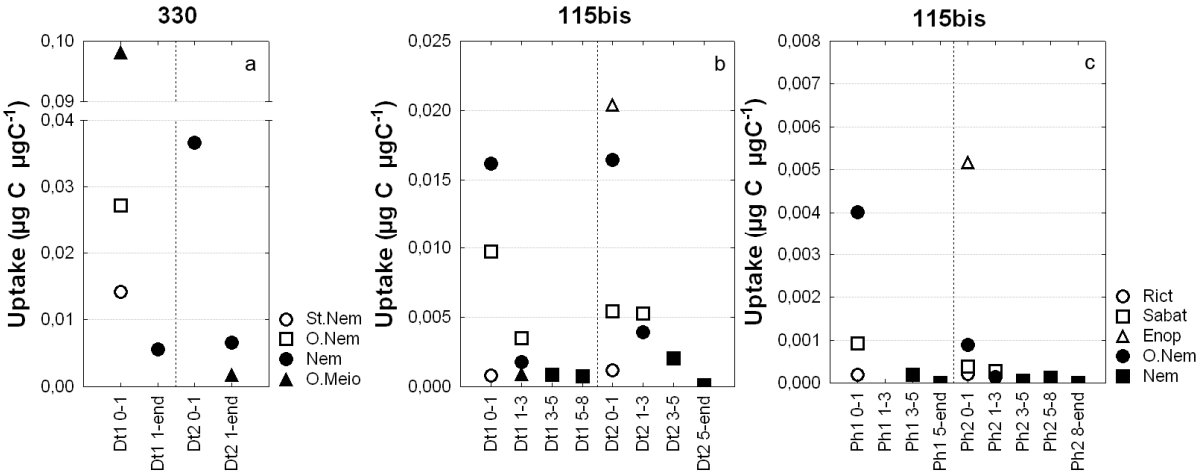


Figure 4.3: Total uptake per unit of carbon (µgC µgC^{-1}) after incubation for 14 days with labelled diatoms *S. costatum* (Dt) at stations 330 (a) and 115bis (b) and labelled *Phaeocystis* (Ph) at station 115bis (c) for the different meiobenthic taxa at different sediment depths. Note different scale on left *y*-axis. St.Nem: stout nematodes; O.Nem: other nematodes; Nem: nematodes; O.Meio: other meiobenthos; Richt: *Richtersia*; Sabat: *Sabatieria*; Enop: *Enoploides.*

The total uptake per sediment area was highest at the sediment surface for all incubations (Fig. 4.4). Nematodes were responsible for the major uptake except for station 330 at the 0-1 cm layer of core Dt1 where "other meiobenthos" was responsible for 84 % of the total uptake (Fig. 4.4a). Both stations showed comparable total uptake values when labelled diatoms were added. An ANOVA test for these two experiments on the total uptake per area, integrated over all sediment layers and taxa, revealed no significant differences between both stations

(F = 0.38; df = 1; p > 0.05). The same result was obtained when only nematode uptake was considered (F = 0.0003; df = 1; p > 0.05). The *Phaeocystis* treatment at station 115bis showed lower total uptake values compared to the *Skeletonema* treatment (Fig. 4.4b,c).

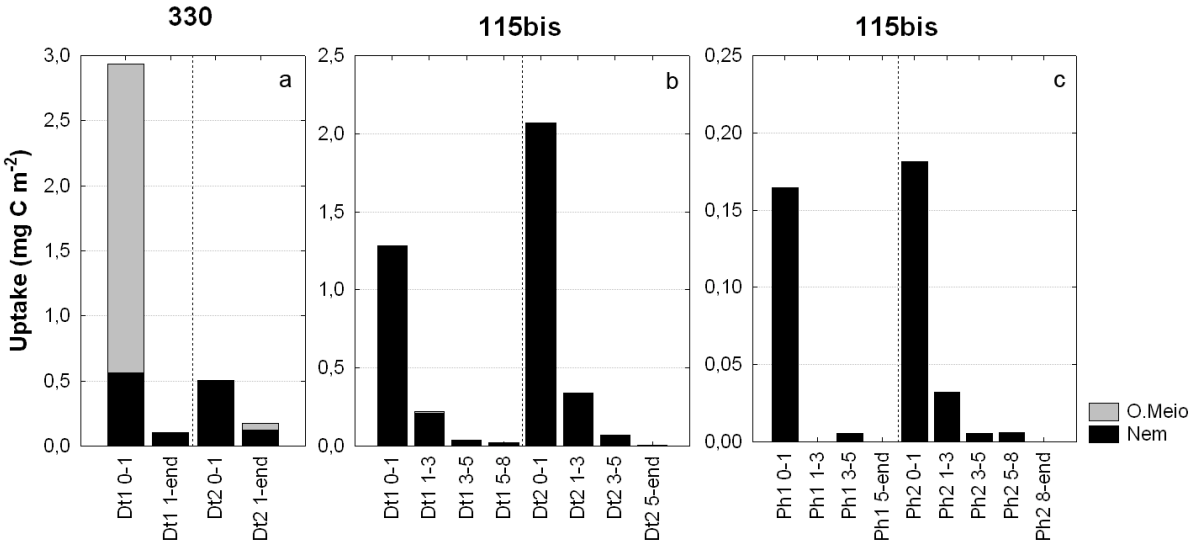


Figure 4.4: Total uptake (mg C m^{-2}) after incubation for 14 days with labelled diatoms *S. costatum* (Dt) at stations 330 (a) and 115bis (b) and labelled *Phaeocystis* (Ph) at station 115bis (c) for the nematodes (Nem.) and Other Meiobenthos (O.Meio) at different sediment depths. Note different scale on left *y*-axis.

On average, considering the whole core, 0.20 (±0.05 SE) and 0.15 % (±0.02 SE) of the carbon added was found in the nematodes in the *Skeletonema* and *Phaeocystis* treatments respectively at station 115bis, while at station 330 it was 0.06 % (±0.001 SE) for the *Skeletonema* treatment. This corresponds to a carbon uptake of 0.144 (±0.033 SE) and 0.014 mgC m^{-2} day^{-1} (±0.002 SE) for the nematodes in the *Skeletonema* and *Phaeocystis* treatments, respectively, at station 115bis, and of 0.046 mg C m^{-2} day^{-1} (±0.001 SE) for the *Skeletonema* treatment at station 330.

Total biomass was higher at station 115bis than at station 330 (Fig. 4.5). At station 115bis total biomass was higher at the surface layer and decreased with depth while at station 330 no vertical gradient was observed. An ANOVA test on the total meiobenthic biomass per area as

the sum of all layers showed that there were significant differences between both stations ($F = 15.8$; $df = 1$; $p < 0.05$) also when only nematode biomass was considered ($F = 28.08$; $df = 1$; $p < 0.01$).

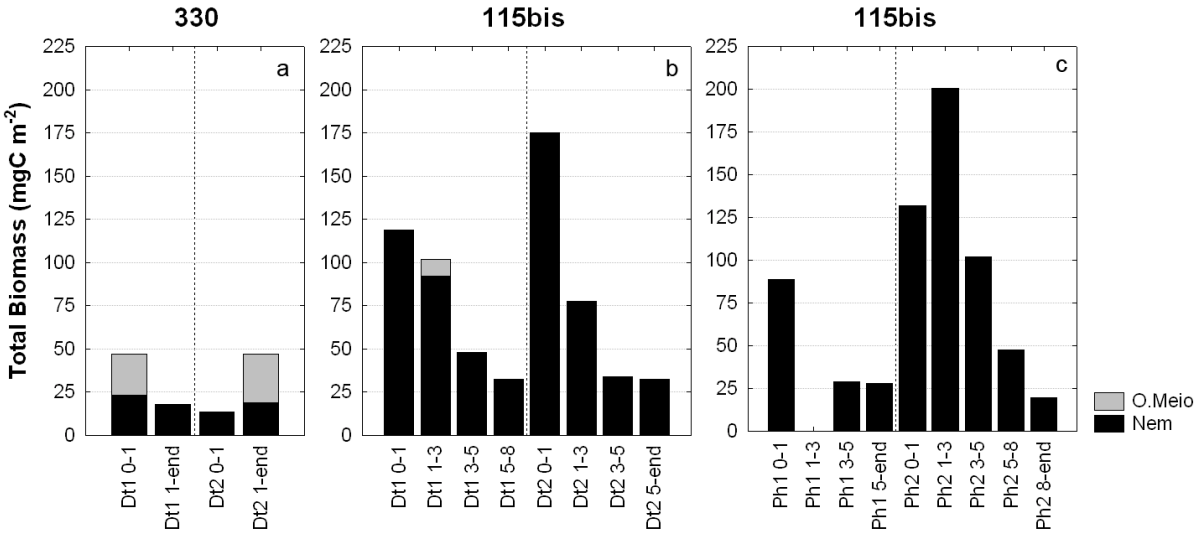


Figure 4.5: Total biomass (mgC m^{-2}) after incubation for 14 days with labelled diatoms *S. costatum* (Dt) at stations 330 (a) and 115bis (b) and labelled *Phaeocystis* (Ph) at station 115bis (c) for the nematodes (Nem.) and Other Meiobenthos (O.Meio) at different sediment depths.

Discussion

Meiobenthic natural $\delta^{13}C$ values (Table 4.1) were consistent with the range observed in the previous year for the same stations (Franco *et al.*, in press b). However these values were generally more depleted than those reported in the literature (e.g. Couch, 1989; Riera *et al.*, 1996; Carman & Fry, 2002; Moens *et al.*, 2002; Moens *et al.*, 2005). Only Riera & Hubas (2003) showed $\delta^{13}C$ values ranging from -27.0 to -14.8‰. All these studies were conducted in intertidal habitats, where microphytobenthos is also available as food source. Microphytobenthos shows higher $\delta^{13}C$ values (≈ -15 ‰) (Currin *et al.*, 1995; Moens *et al.*, 2005) compared to marine phytoplankton (typically near -22 ‰) (Boutton, 1991). Since

meiobenthos is able to graze on microphytobenthos (e.g. Moens & Vincx, 1997) it is expected that organisms partly feeding on microphytobenthos will have a heavier signal than organisms restricted to feeding on pelagical production.

After two weeks of incubation, $\delta^{13}C$ values changed considerably, and this variation was more obvious at the surface layer for both stations. Under natural conditions the vertical difference in label incorporation would be expected for station 115bis, however not for station 330. Station 115bis is a deposition station where steep vertical gradients in chlorophyll *a*, oxygen concentrations and nitrogen compounds occur (Steyaert *et al.*, subm.; Franco *et al.*, in press a). Natural abundance of stable isotopes also showed vertical differences in stable isotopic signatures at this station, revealing different C-sources for vertically segregated meiobenthic organisms (Franco *et al.*, in press b). Burial and mineralisation of fresh phytodetritus take a relatively long time at station 115bis (Provoost *et al.*, in prep.). Therefore it is expected that, in this sediment type, labelled algae are almost exclusively available for the organisms living at the sediment surface or that are able to migrate towards the surface to feed. At station 330 in natural conditions we have the opposite picture. The sediment at this station is quite permeable and the strong water currents and advective transport through the sediment result in deep penetration of phytoplankton cells (Huettel & Rusch, 2000; Ehrenhauss & Huettel, 2004; Ehrenhauss *et al.*, 2004a). In a field situation, this results in the absence of vertical gradients within the sediment at station 330, where chlorophyll *a* profiles often show subsurface peaks (Vanaverbeke *et al.*, 2004b; Franco *et al.*, in press b). Therefore the labelled diatoms should be available to deeper sediments layers fast after deposition in natural conditions. However, our laboratory conditions did not mimic such natural conditions of strong bottom water currents and the deep penetration of phytodetritus into the sediment might not occur as fast as in the natural environment.

After 2 weeks of the experiment 25 % of the diatom organic carbon and 10 % of the *Phaeocystis* carbon had been respired at both stations (L. Moodley pers. com.) indicating that food availability was not limiting during the whole experiment period. Labelled diatoms at both stations and *Phaeocystis* at station 115bis were incorporated into the meiobenthos, either directly by ingestion or indirectly through feeding on bacteria. Although total meiobenthic biomass was much lower at station 330, total uptake values were comparable at both stations. The uptake per individual was similar but the uptake per unit of carbon showed higher values at station 330. This suggests that meiobenthic organisms at station 330 are probably more effective or quicker incorporating phytodetritus-derived OM, which might reveal an adaptation to an environment with a low quantity of OM. Urban-Malinga & Moens (2006) reached a similar conclusion, with the specific uptake ^{13}C by meiobenthic organisms, and nematodes in particular, being higher in coarser sediment than in finer sediment. However the effect of dilution of the added carbon in the natural OM pool present in the sediment should also be taken into account. Even using the lowest chlorophyll *a* to carbon ratios reported in literature (e.g. Redalje, 1983; Tada *et al.*, 2000) applied to chlorophyll *a* concentration in the sediment at the same stations in April 2003 (Franco *et al.*, in press b), the natural carbon content of the sediment would exceed the carbon content of the added algae. The sediment at station 115bis would have higher carbon content than station 330 and therefore the effect of dilution could be higher.

At station 330 short (adult length <700µm) and stout (length to width ratio <15) nematode densities increase fast after the arrival of fresh material, decreasing shortly after that event, being almost absent the rest of the year (Vanaverbeke *et al.*, 2004a). Although stout nematodes at station 330 might respond faster than other nematodes in terms of densities (Vanaverbeke *et al.*, 2004a) they showed lower uptake than the other nematodes both per individual and per unit of carbon. Background stable isotopic composition of the same

organisms at the same station did not indicated differences in their isotopic signatures (Franco *et al.*, in press b). Therefore the opportunistic behaviour of these nematodes must rely then on their life-history characteristics, as suggested by Vanaverbeke *et al.* (2004a). Smaller species have higher growth rates (Peters, 1983; Soetaert *et al.*, 2002) with faster maturation and higher reproduction rates (Kooijman, 1986) which is consistent with the observations of Vanaverbeke *et al.* (2004a) of higher rates of density increase and higher contribution of adults among the "stout nematodes" community at station 330 following the deposition of a spring phytoplankton bloom. Stout nematodes have reduced mobility which limits their ability to search for food and also a lower capacity to withstand oxygen stress which confines them to the surface of the sediment (Soetaert *et al.*, 2002). Food availability might be a structuring factor for the nematode communities at station 330. Due to their low mobility, stout nematodes may not be able to compete with the more mobile slender nematodes in situations of lower food availability, but once food availability is high, short and stout nematodes could compete and their densities could rise faster due to higher growth, maturation and reproduction rates.

Both *Enoploides* and "other nematodes" showed a higher uptake per unit of carbon than *Sabatieria* at the surface layer of the diatom treatment of station 115bis. However at the 1-3 cm layer *Sabatieria* showed the highest uptake and these values were relatively closer to the ones observed for this genus at the surface layer, if compared with the difference between the "other nematodes" values for the same layers. This indicates the ability to migrate towards the surface to feed. Natural stable isotopic signatures also revealed the same migratory behaviour for *Sabatieria* (Franco *et al.*, in press b). *Sabatieria*'s ability to migrate towards recently deposited OM in combination with its tolerance of unfavourable oxygen conditions probably explains its opportunistic response to phytoplankton sedimentation as described by Steyaert *et al.* (subm.). Other deep-dwelling nematodes do not migrate to the surface layers

after the arrival of phytoplankton to the sea floor (Franco *et al.*, in press b). Their increase in densities is therefore delayed in time, as observed by Steyaert *et al.* (subm.).

In any of the experiments *Richtersia* did not show a significant uptake, therefore they feed on another food source which is not derived, directly or indirectly, from the labelled diatoms or *Phaeocystis* added to the experimental cores. Both *Sabatieria* and *Richtersia* belong to the same feeding type *sensu* Wieser (1953) (1B-group, non-selective deposit fedders) which are nematodes able to explore broad range of food sources, like bacteria, ciliates, microalgae and detritus. Previous results on dual stable isotopic values indicated that at station 115bis *Richtersia* was exploring food sources with similar $\delta^{13}C$ signals as *Sabatieria* and also displaying the same vertical migration behaviour (Franco *et al.*, in press b). However, the results presented here indicated that *Richtersia* did not explore the same food sources as the other nematodes, including *Sabatieria*, from which *Richtersia* desplayed a different feeding strategy although belonging to the same feeding type. Carman & Fry (2002) also observed that nematodes with comparable background stable isotope values showed different $\delta^{13}C$ values in experiments with ^{13}C labelled microalgae, indicating different feeding strategies which were not reflected on the natural stable isotopes signatures.

Enoploides, when present with sufficient biomass at station 115bis, showed the highest $\delta^{13}C$ values (fig. 4.1). *Enoploides* is considered as having a fairly strict predatory feeding behaviour (Moens *et al.*, 1999c) which has been confirmed by dual stable isotopic values (Moens *et al.*, 2005). Middelburg *et al.* (2000) observed for *Enoploides*, similar uptake of ^{13}C of labelled mycrophytobentos origin as other nematodes, with a stroger delay on label incorporation, attributed to their predatory behaviour. However from our results it can be inferred that, besides other meiobenthic preys, *Enoploides* fed as well on a more enriched food source, since the other meiobenthic animals analysed at station 115bis presented lower $\delta^{13}C$ values than *Enoploides*. In fact this nematode has been reported to prey on other

nematodes, ciliates and perhaps other meio- to microsized benthic organisms (Moens *et al.*, 2002; Hamels *et al.*, 2001a; Gallucci *et al.*, 2005; Moens *et al.*, 2005).

Total uptake of carbon was fairly low in all the treatments. Nematodes had carbon uptake representing ca. 0.66 % (*Skeletonema*) and 0.06 % (*Phaeocystis*) of nematode respiration rates at station 115bis and 2.96 % at station 330 (nematode respiration rates estimated in Franco *et al.*, in prep). Van Oevelen *et al*. (2006a) observed that ^{13}C excess in meiobenthos was highest after ca. 10 days, after adding labelled glucose to the sediment surface, and after 14 days ^{13}C excess levels did not differ much. Nematode's carbon uptake per day might therefore be a little underestimated; however it was clearly insufficient to maintain nematode's carbon balance.

Such low uptake of carbon by meiobenthic organisms derived from experiments where a ^{13}C labelled food source is supplied to the sediment is not unusual. Ólafsson *et al.* (1999) reported only 0.04 % of label stored in meiobenthic tissue 1 month after incubation with labelled *Skeletonema costatum*. Urban-Malinga & Moens (2006) reported meiobenthos to incorporate 0.48 (coarser sediment) and 0.81 % (finer sediment) of average daily ^{13}C-losses, with nematodes contributing only 0.5 % of the total meiobenthic uptake. Meiobenthos processed more than those percentages of labelled algae, when taking into consideration meiobenthic respiration, multiple gut passage times and mortality over the duration of the experiment (Ólafsson *et al.*, 1999). Moreover assimilation efficiencies (assimilation/consumption) of e.g. nematodes it is generally assumed to be low, ca. 20-25 % (Herman & Vranken, 1988; Urban-Malinga & Moens, 2006). Also a considerable part of the label might be lost due to fixation with formaldehyde (Moens *et al.*, 1999d). Although methodological implications might lead to an underestimation of meiobenthic carbon uptake, meiobenthos can also be exploiting other carbon sources not derived from the labelled ones, which are naturally available in the sediment cores.

The percentage of carbon uptake was similar in both *Phaeocystis* and *Skeletonema* treatments at station 115bis, indicating meiobenthos would explore both resources similarly. *Phaeocystis* culture was not axenic, due to methodological impediments which did not allow culturing enough *Phaeocystis* in the same conditions as the diatom *Skeletonema costatum*. However in both treatments some uptake was observed and since Belgian coastal waters are dominated by intense blooms of both *Phaeocystis* colonies co-occurring with diatom blooms (Reid *et al.*, 1990; Joint & Pomroy, 1993; Brussaard *et al.*, 1995) it was important to test both food sources.

The role of *Phaeocystis* on the benthic system has been previously overlooked; even though relatively high amounts of *Phaeocystis* derived OM can reach the sea floor (Lancelot *et al.*, 2005). Although label incorporation in the meiobenthos was relatively low, mineralisation rates in the *Phaeocystis*-incubated cores were about half of those observed in the diatom cores (Moodley *et al.*, in prep.), suggesting that *Phaeocystis* may contribute substantially to the benthic ecosystem.

Acknowledgements

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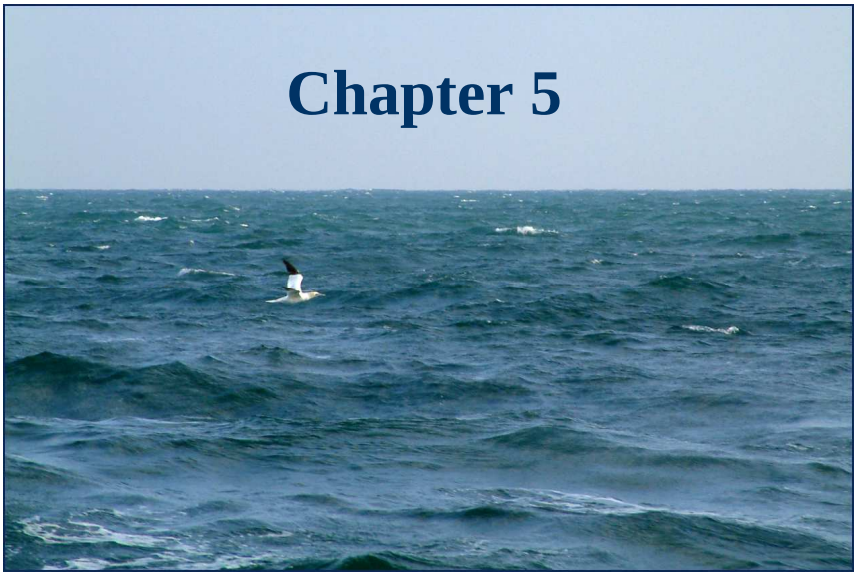
Adenddum

A carbon budget was calculated for the different meiobenthic groups at both stations (table 4.2). Based on individual biomass, individual respiration was calculated as described in chapter 5 for the nematodes. The percentage of individual uptake of the different nematode groups in relation to individual respiration was then determined. Also taken into account the relative densities of each group in each sediment layer, a percentage of each meiobenthic group uptake on total uptake was calculated.

Table 4.2. Individual respiration (µg C ind^{-1} day^{-1}), percentage of individual uptake in relation to individual respiration (%) and percentage of of total uptake of each meiobenthic group at the different sediment depths (cm) for both stations (115bis and 330). St.Nem: stout nematodes; O.Nem: other nematodes; Nem: nematodes; O.Meio: other meiobenthos; Richt: *Richtersia*; Sabat: *Sabatieria*; Enop: *Enoploides*.

Station	Depth (cm)	Meiobenthic Group	Ind. Resp. (µg C ind^{-1} day^{-1})	Ratio Ind. Uptake to Ind. Resp. (%)	Percentageof Total Uptake (%)
115bis	0-1	Rict	0.0114	0.10	2
115bis	0-1	Sabat	0.0194	1.34	26
115bis	0-1	O.Nem	0.0086	1.94	72
115bis	1-3	Sabat	0.0168	0.47	40
115bis	1-3	O.Nem	0.0070	0.21	56
115bis	1-3	O.Meio			4
115bis	3-5	Nem	0.0067	0.10	100
115bis	5-8	Nem	0.0080	0.09	100
115bis	0-1	Rict	0.0110	0.15	1
115bis	0-1	Sabat	0.0216	0.76	18
115bis	0-1	Enop	0.0968	3.71	31
115bis	0-1	O.Nem	0.0077	1.94	51
115bis	1-3	Sabat	0.0174	0.72	38
115bis	1-3	O.Nem	0.0049	0.43	62
115bis	3-5	Nem	0.0075	0.24	100
115bis	5-end	Nem	0.0134	0.02	100
115bis	0-1	Rict	0.0091	0.02	2
115bis	0-1	Sabat	0.0161	0.12	20
115bis	0-1	O.Nem	0.0048	0.44	77
115bis	1-3	Nem	-	-	-
115bis	3-5	Nem	0.0046	0.02	100
115bis	5-end	Nem	0.0066	0.00	100
115bis	0-1	Rict	0.0079	0.02	2
115bis	0-1	Sabat	0.0167	0.05	9
115bis	0-1	Enop	0.0242	0.74	65
115bis	0-1	O.Nem	0.0053	0.10	23
115bis	1-3	Sabat	0.0185	0.04	22
115bis	1-3	O.Nem	0.0087	0.02	78
115bis	3-5	Nem	0.0072	0.01	100
115bis	5-8	Nem	0.0061	0.01	100
115bis	8-end	Nem	0.0068	0.00	0
330	0-1	St.Nem	0.0025	1.38	3
330	0-1	O.Nem	0.0044	2.91	17
330	0-1	O.Meio	-	-	81
330	1-end	Nem	0.0041	0.59	100
330	0-1	Nem	0.0056	4.09	100
330	1-end	Nem	0.0055	0.73	71
330	1-end	O.Meio	-	-	29

Chapter 5



Bacterial, nematode and macrobenthic activity following a phytoplankton bloom in two contrasting sediments on the North Sea

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Abstract

Respiration rates of bacteria, nematodes and macrobenthos were estimated in relation to the deposition of the spring phytoplankton bloom in two contrasting sites in the southern North Sea: one with fine grained sediment close to the coastline and another with highly permeable sediments. Sediment Community Oxygen Consumption (SCOC) was also measured. Bacterial biomass was relatively similar at both stations, while nematodes and macrobenthic biomass were higher in fine grained sediment. In fine sediments, bacterial biomass increased fast after deposition of the phytoplankton bloom while nematodes and macrobenthos response was delayed. In coarser sediments, nematodes and macrobenthos also showed a fast response.

The respiration shares of SCOC in fine sediments were initially dominated by macrobenthos. After sedimentation occurred nematodes and macrobenthos had similar shares, with macrobenthos recovering their higher relative importance later. In coarser sediments SCOC was clearly dominated by bacterial respiration, with nematodes and macrobenthic respiration representing generally small shares of SCOC. The relative importance of the nematode community in subtidal fine grained sediments might therefore become more significant whenever anoxic conditions arise.

Introduction

The Belgian Continental Shelf (BCS) is characterised by a high primary production and algal biomass, supported by high nutrient loads (Joint & Pomroy, 1993; Brussaard *et al.*, 1995). In spring, the Belgian coastal waters become dominated by intense blooms of *Phaeocystis* colonies which co-occurring with diatom blooms (Reid *et al.*, 1990; Joint & Pomroy, 1993; Brussaard *et al.*, 1995). *Phaeocystis* blooms dominate the Belgian waters between April and May, lasting 20 to 40 days, while diatoms blooms can occur as early as February with smaller ones occurring as late as September (Reid *et al.*, 1990; Rousseau *et al.*, 2002). After these blooms a high amount of phytodetritus settles onto the sediment (e.g. Vanaverbeke *et al.*, 2004a,b; Steyaert *et al.*, subm; Franco *et al.*, in press b) where it represents a major source of organic matter (OM) for the benthic system (Graf, 1992).

Sediment type has a profound influence on the processing of sedimented labile OM. In fine-grained sediments accumulation of phytodetritus, after sedimentation in spring, causes steep vertical profiles of labile OM (e.g. Steyaert *et al.* subm.). Remineralisation of newly arrived OM can induce hypoxic/anoxic conditions (Graf 1992) and the breakdown of this OM is often delayed (Boon & Duineveld 1998, Provoost *et al.* in prep.). In coarser, permeable sediments, such vertical gradients in the sediment are often absent and subsurface peaks of chlorophyll *a* are common (Jenness & Duineveld 1985, Ehrenhauss & Huettel 2004, Ehrenhauss *et al.* 2004a, Vanaverbeke *et al.* 2004b). These sediments are typically characterised by a rapid degradation of OM (Ehrenhauss *et al.* 2004b, Vanaverbeke *et al.* 2004b, Janssen *et al.* 2005, Bühring *et al.* 2006).

The different biogeochemical processes in such contrasting sediments can affect the response of the resident benthos. Hubas *et al.* (in press), for instance, observed different relative

contributions of bacteria, meiobenthos and macrobenthos to total heterotrophic production in intertidal stations with contrasting sediment characteristics.

Nematode communities from different environments can have different roles in the benthic carbon cycle, depending on differences in OM quality and quantity as well as in the biogeochemistry of the sediment (Hubas *et al.*, in press). In general, however, the nematode contribution to benthic metabolic activity is poorly known. Only few studies have estimated the nematode contribution to benthic carbon cycles. The highest contribution of nematodes to total carbon turnover (13 %) along a depth gradient at the continental slope of the Goban Spur (NE Atlantic Ocean) was found at the shallowest station (206 m deep) (Soetaert *et al.*, 1997). In an estuarine tidal flat sediment, less than 1% of the total respiration was attributed to nematodes, which depended primarily on microphytobenthos as a food source (Van Oevelen *et al.*, 2006b).

The present study aims to partition sediment community oxygen consumption into respiration by bacteria, nematodes (the predominant meiobenthic taxon) and macrobenthos in two contrasting continental shelf sediments in the North Sea. By comparing respiration of bacteria, nematodes and macrobenthos prior to, during and after the major spring phytoplankton bloom, we aim at clarifying (i) the relative importance of the different benthic compartments (bacteria, meio- and macrobenthos) in the mineralization of the OM that reaches the sediment, (ii) the response in biomass and metabolic activity of these three major heterotrophic compartments of the benthos to the OM input from the spring phytoplankton bloom deposition, and (iii) differences in the response and relative importance of bacteria, meio- and macrobenthos between contrasting sediment types.

Material and Methods

Study sites and sampling

Samples were taken from the BCS stations 115bis, located close to the coast (51°09.2'N; 02°37.2'E; 13 m depth) and 330, located further offshore (51°26.0'N; 02°48.5'E; 20 m depth) (Fig. 1.1).

Station 115bis is a deposition station, characterized by the presence of fine sediments (median grain size: 185 µm) with a small fraction of mud (4 %) (Steyaert *et al.*, subm.), while station 330 consists of medium sand (median grain size: 329-361 µm) without mud (Vanaverbeke *et al.*, 2004a,b). Sampling at both stations was conducted in February, April and October 2003 from the RV Belgica.

The sediment was sampled for Sediment Community Oxygen Consumption (SCOC), meiobenthos and bacterial density and biomass, and bacterial production using a box corer, deployed three times at each sampling station. From each box corer 1 perspex core (i.d. 9.5 cm) was taken for SCOC measurement; 1 perspex core (i.d. 3.6 cm) for meiobenthic density and biomass analysis; 1 perspex core (i.d. 3.6 cm) for bacterial density and biomass analysis and 1 perspex core (i.d. 14 cm) for bacterial production measurements.

The SCOC and bacteria production cores were filled with water collected 1m above the sea floor using 10 l Niskin bottles, closed and transported to the laboratory. The meiobenthic cores were sliced in 1cm slices down to a maximum depth of 5 cm, and the samples preserved with a neutral hot 4 % formaldehyde-tap water solution. The bacterial counts and biomass cores were sliced in 1cm slices down to a maximum depth of 5 cm, and the samples preserved in a 4 % formaldehyde-tap water solution, thoroughly shaken and stored in the fridge.

Macrobenthos was sampled using a Van Veen grab (surface area 0.12 m^{-2}), deployed 5 times at each station. Macrobenthos samples were washed on board over a 1mm sieve and the

retained organisms were stained with Rose Bengal and preserved with a neutral 8 % formaldehyde-tap water solution.

Laboratory treatment of samples

Sediment Community Oxygen Consumption

Measurements of SCOC were performed in two replicates per station and sampling date. The SCOC cores were placed in an acclimatised room at in-situ temperature. They were carefully closed with a detachable lid, containing an YSI 5739 oxygen electrode and a Teflon-coated magnetic stirrer, which were mounted in a predetermined depth to ensure a fixed volume of water. The stirrer mixed the water without any visible disturbance of the sediment surface. The oxygen concentration in the water was continuously monitored. SCOC, expressed as mmol O_2 m^{-2} h^{-1}, was calculated by means of linear regression of the decrease in oxygen concentration over time (Moodley *et al.*, 1998). All measurements were done in the absence of light. Due to methodological problems only one replicate was available at station 330 in February.

Bacterial biomass

Samples for bacterial counts were prepared following Starink *et al.* (1994). Milli-Q water was added to the sample until a volume of 9.5 ml and 0.5 ml $Na_2P_2O_7$ (0.2 M) was added. This mixture was sonicated five times for 30 seconds using a Soniprep 150 (10 Watt). In between sonication pulses the sample rested on ice for 30 seconds. Before staining samples were diluted 10 to 1000 times using TRIS. A subsample was filtered onto a 0.2 µm polycarbonate filter and stained with Sybrgold (Molecular Probes). Filters were then mounted on slides. Counting of bacterial cells was performed using a Leica confocal microscope connected to QWIN software. From every image 16 scans were made 0.2 µm vertically apart from each other. In such a way, a 3.2 µm thick image was analysed. From each scan, all particles >0.2 µm were counted and allocated to a size class. Per slide, 50-100 images were analysed.

Bacteria were assumed to be spherical and their volume was calculated as $4\times\pi\times r^3/3$ (r = radius), with a radius estimation of half the average of the largest (L) and smallest (S) diameter. Bacterial volume was converted to biomass (carbon content) using a conversion factor of 310 fg of C μm^{-3} (Fry, 1990). Biomass was therefore calculated according to: $(4\times3.141593\times((L+S)/4)^3/3)\times310$.

Since at both stations bacterial biomass and densities did not differ between the two sediment layers, bacterial biomass and densities 0-5 cm deep were calculated as the value for the 0-1 cm layer multiplied by 5.

Bacterial production

Bacterial production was measured using the ^{3}H-leucine incorporation method as modified from Van Duyl & Kop (1994) and Hamels *et al.* (2001b). After transport to the laboratory, the sediment cores were incubated in the dark at in situ temperature and allowed to stabilize for 24h. Overlying water was then gently siphoned off; the sediment was removed from the corers by allowing it to slide down gently. The 0-1 cm and 4-5 cm horizons were sliced off and retained for bacterial production measurements. 200 µl aliquots of sediment were pipetted into 2 ml eppendorf tubes using an automatic pipette with cut-off tips, and 100 µl of a leucine solution was added. The tubes were again incubated in the dark at in situ temperature for 40-60 min, after which the incubation was stopped through the addition of 1.5 ml ice-cold 5 % trichloroacetic acid (TCA). Isotope dilution was determined using three different leucine concentrations, containing 0.2, 0.4 and 0.6 nmol leucine per 100 µl, respectively. The amount of labelled leucine (L-[4,5-3H]leucine, 50 Ci/mmol, ICN Biomedicals) was always 0.02 nmol and thus independent of the total amount of leucine added. We used three replicates and one blank for each leucine concentration. Bacteria in the blanks were killed with TCA (as above) before leucine addition.

Samples on TCA were stored overnight and then washed through four consecutive centrifugation (3 min at 8000 g) - resuspension cycles in ice-cold 5 % TCA. Macromolecules were subsequently extracted in 1.5 ml of a solution of NaOH (0.3 M), EDTA (25 mM) and SDS (0.1 %) for 24-48 h at room temperature. Sediment particles were removed by centrifugation (5 min at 1000 g), and the supernatant was transferred to glass scintillation vials where it was mixed with 10 ml of a scintillation cocktail (Lumasave Plus, Lumac). Radioactivity was measured as disintegrations per minute (dpm) on a Beckman LS-6000 liquid scintillation counter. Quenching was corrected for by automatic external standardization. After subtraction of blanks, bacterial production (in mg C m^{-2} d^{-1}) was calculated as:

$$\text{Production (mgC ml}^{-1}\text{ h}^{-1}) = dmp \times \frac{60}{t} \times \frac{1}{\left(2.2 \times 10^{12}\right)} \times M \times \frac{1}{\%Leu} \times C/Prot \times 10 \times \frac{total\ Leu\ added\ (\text{nmol}) + isotope\ dilution\ (\text{nmol})}{SA \times \left[{}^{3}H\right] Leu\ added\ (\text{nmol})}$$

where t = incubation time (min); 1 Ci = 2.2 x 10^{12} dpm; M = the molecular weight of leucine; *% Leu* = 0.073, i.e. the fraction of leucine in protein; *C/Prot* = 0.86, i.e. the carbon to protein ratio (Simon & Azam, 1989); *SA* = specific activity of the added leucine in Ci/mmol.

Meiobenthos

Meiobenthos (the animals passing a 1 mm sieve and retained on a 38 µm sieve) was extracted from the sediment by centrifugation with LUDOX HS-40 at a specific density of 1.18 (Heip *et al.*, 1985). After staining with Rose Bengal, nematodes were counted under a binocular microscope. Processing the meiobenthic samples proved to be time consuming so only two replicates per station and sampling date were processed. From each sediment slice, 120 nematodes were randomly picked (following Vincx, 1996) and mounted on Cobb slides for measurement of length and maximal width. When less than 120 individuals were present, all

nematodes were picked out. Measurements were performed using an image-analyser (Quantimet 500+).

Nematode individual biomass was calculated from Andrassy's (1956) formula:

$$WW = \frac{w^2 \times l}{1.6 \times 10^6}$$

where WW is the individual Wet Weight (µg) and *w* and *l* the nematode length (µm) and maximum width (µm). We assumed a dry-to-wet-weight ratio of 0.25 (Wieser, 1960) and a carbon content of 51 % of dry weight (de Bovée, 1987).

Macrobenthos

Macrobenthos samples were sorted, counted and the organisms picked up separately according to taxa. The organisms were washed with distilled water and paper dried and then weighed to determine wet weight (WW). They were dried at 60 °C in an oven for 4 days to determine the dry weight (DW) and dried once again at 500 °C in a muffle furnace for 2 h, to determine the ash free dry weight (AFDW).

Respiration estimates

Bacterial respiration

Bacterial respiration (BR, expressed in mg C m^{-2} d^{-1}) was estimated from bacterial production (BP) using: (i) a fixed bacterial growth efficiency (BGE) of 20 % (Osinga *et al.*, 1997) and (ii) the model I regression proposed by del Giorgio & Cole (1998) as the best predictor of BR from measured BP in aquatic microbial communities:

$$BR = 3.7 \times BP^{0.41}$$

BR was converted to oxygen units assuming a respiratory quotient (RQ) of 1 (del Giorgio & Cole, 1998). Since at station 115bis oxygen is only present in the 0-1 cm layer (Steyaert *et al.*, subm), all oxic bacterial respiration at this station was assumed to be limited to the 0-1 cm layer. At station 330, oxygen is present throughout the whole sediment depth studied (Vanaverbeke *et al.*, 2004b). Bacterial production did not differ between the 0-1 cm and

4-5 cm sediment layers (data not shown). Therefore bacterial respiration for the 0-5cm depth stratum was obtained by multiplying the respiration of the 0-1 cm layer by 5.

When comparing the different groups respiration and calculating the respective shares in SCOC, BR calculated using a fixed BGE of 20 %.

Nematode respiration

Total nematode respiration was estimated based on the summed individual DW following de Bovée & Labat (1993), assuming a Q_{10} expression for temperature dependence, and extrapolated to the total number of nematodes present in a sample:

$$R(T) = 0.0449 \times DW^{0.8544} \times \exp^{\frac{\ln(Q10)}{10}(T-20)}$$

where R is respiration (µg C day^{-1}), DW is individual dry weight (µg C) and T is temperature (°C).

Macrobenthos respiration

For macrobenthos an organic carbon content of 50 % of AFDW was assumed (Wijsman *et al.*, 1999). Respiration was estimated based on the AFDW using the Mahaut *et al.* (1995) formula for shallow water macrobenthos:

$$R = 0.0174 \times W^{0,844}$$

where R is respiration (mg C day^{-1}) and W is the mean individual AFDW (mg C), valid for the temperature range of 15–20 °C. Respiration was then corrected for temperature assuming a Q_{10} of 2.

Respiration rates of meio- and macrobenthos were converted to oxygen consumption assuming an RQ of 0.85 (Hargrave, 1973).

Statistical analysis

One-way and two-way Analyses of Variance (ANOVA) were performed on biomass, respiration and SCOC percentages for bacteria, nematodes and macrobenthos and also on the length to width ratio of nematodes in order to test for differences between sampling stations

and dates. Whenever significant differences were found, post hoc Tukey HSD tests were performed. Homogeneity of variances was tested using Bartlett χ^2 and data were fourth root, inverse or arcsine (in the case of relative date) transformed whenever necessary. Whenever homogeneity of variances was not achieved, even upon transformation, Kruskal-Wallis (one-way ANOVA by ranks) tests were conducted. Whenever significant differences were found Mann-Whitney U tests were conducted on each pair of groups and the p value adjusted with the Bonferroni method (Salkind, 2007). The STATISTICA 6 software package was used.

Results

Biomass and densities

Bacterial biomass did not differ significantly between stations (two-way ANOVA, $F = 0.29$; $df = 1$; $p > 0.05$; Fig. 2), ranging from 1.6 g C m^{-2} ±0.6 SE to 5.9 g C m^{-2} ±1.2 SE at station 115bis, and from 1.2 g C m^{-2} ±0.7 SE to 3.6 g C m^{-2} ±0.3 SE at station 330. Differences between sampling dates ($F = 11.9$; $df = 2$; $p < 0.01$) and in the interaction term station × date ($F = 4.27$; $df = 2$; $p < 0.05$), however, were significant. Differences between sampling dates were most pronounced at station 115bis, where bacterial biomass in April was significantly higher than in February and October. Temporal differences in station 330 were not statistically significant. Bacterial densities varied in the same way as biomass at both stations, ranging from 12.4 cells × 10^{12} m^{-2} (±5.5 SE) to 59.9 cells × 10^{12} m^{-2} (±13.6 SE) at station 115bis and from 11.8 cells × 10^{12} m^{-2} (±5.9 SE) to 28.0 cells × 10^{12} m^{-2} (±3.2 SE) at station 330.

Total nematode biomass was much higher at station 115bis than at station 330 (Fig. 5.1). At station 115bis biomass was lowest in February (0.42 g C m^{-2} ±0.02 SE), highest in April (1.46 g C m^{-2} ±0.99 SE) and again lower in October (0.62 g C m^{-2} ±0.38 SE).

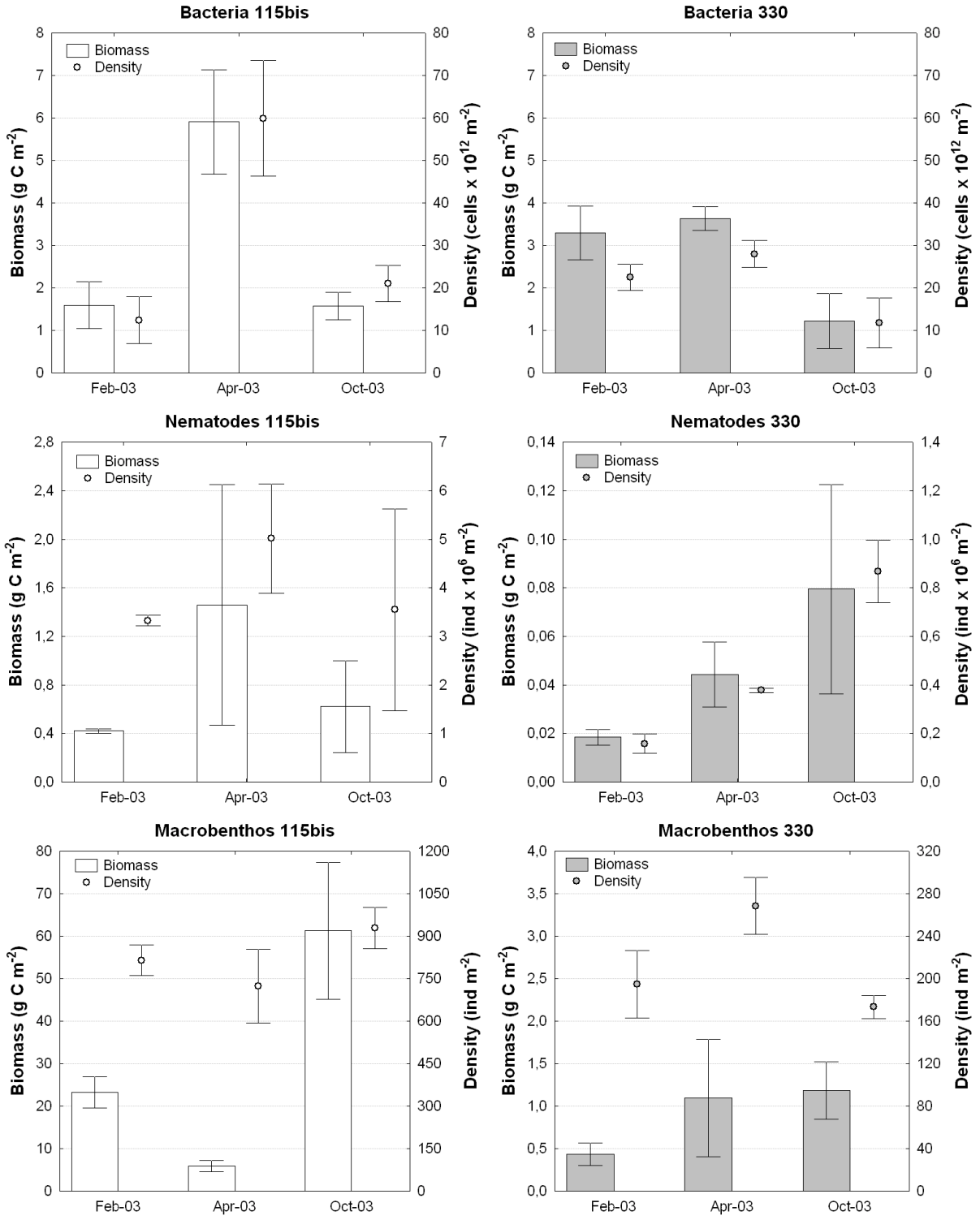


Figure 5.1: Biomass (g C m^{-2}) and densities of bacteria (cells × 10^{12} m^{-2}), nematodes (ind × 10^{6} m^{-2}) and macrobenthos (ind × 10^{3} m^{-2}) from station 115bis and 330 on the sampling campaigns of February, April and October 2003. Vertical bars represent the standard error. Note the different scales of graphs.

At station 330, nematode biomass was lowest in February (0.018 g C m^{-2} ±0.003 SE) and increased with time up to 0.079 g C m^{-2} ±0.043 SE in October. Nematode densities varied in the same way as biomass at both stations, ranging from 3.32 ind × 10^6 m^{-2} (±0.11 SE) to 5.01 ind × 10^6 m^{-2} (±1.12 SE) at station 115bis and from 0.16 ind × 10^6 m^{-2} (±0.04 SE) to 0.87 ind × 10^6 m^{-2} (±0.13 SE) at station 330. A two-way ANOVA on nematode biomass revealed significant differences between both stations ($F = 24.8$; $df = 1$; $p < 0.01$) but not between sampling dates ($F = 1.05$; $df = 2$; $p > 0.05$) or in the interaction factor station × date ($F = 0.62$; $df = 2$; $p > 0.05$).

Macrobenthos biomass (fig. 5.1) was higher at station 115bis than at station 330 (two-way ANOVA, $F = 134.9$; $df = 1$; $p < 0.001$). At station 115bis macrobenthos biomass was 23.2 g C m^{-2} (±3.70 SE) in February, reaching lowest values in April (5.83 g C m^{-2} ±1.34 SE) and increased up to a maximum of 61.3 g C m^{-2} (±16.1 SE) in October, with temporal differences being significant between April and October. At station 330 lowest values were observed in February (0.44 g C m^{-2} ±0.13 SE) and highest in October (1.18 g C m^{-2} ±0.34 SE), with intermediate values in April (1.10 g C m^{-2} ±0.69 SE); however these differences were not significant. Macrobenthos densities followed a relatively similar pattern as biomass except at station 330 in April and October, when biomasses were similar while densities were considerably higher in April (Fig. 5.1). Macrobenthic densities varied between 723 ind m^{-2} (±130 SE) and 928 ind m^{-2} (±73 SE) at station 115bis and between 173 ind m^{-2} (±11 SE) and 268 ind m^{-2} (±27 SE) at station 330.

Sediment Community Oxygen Consumption

Wtaer temperature was of 6 and 6.4°C in February at station 115bis and 330 respectively, of 9.3°C in April at both stations and of 15.1 and 15°C in October at station 115bis and 330 respectively. SCOC at station 115bis varied between 5.5 (±0.3 SE; February) and

18.8 mmol O_2 m^{-2} day^{-1} (±0.8 SE; October), with April showing SCOC values very close to the October ones (Fig. 5.2). At station 330 SCOC ranged between 1.2 (February) and 8.7 mmol O_2 m^{-2} day^{-1} (±3.9; April), with October showing intermediate values. Due to absence of replication at station 330 in February, a two-way ANOVA was not possible for the whole dataset. A one-way ANOVA on SCOC revealed no significant differences between both stations ($F = 5.09$; $df = 1$; $p > 0.05$). At station 115bis, SCOC showed significant differences between sampling dates (one-way ANOVA, $F = 17.7$; $df = 2$; $p < 0.05$). Post hoc tests revealed that values recorded in February were significantly lower than in April and October (Fig. 5.2). SCOC did not differ significantly between April and October at either of the stations (station 330, one way ANOVA, $F = 0.57$; $df = 1$; $p > 0.05$; station 115bis Tukey HSD test, $p > 0.05$).

Respiration and partitioning of SCOC among benthic size groups

Bacterial production and respiration were higher at station 115bis than at station 330 at the 0-1 cm layer (table 5.1), however when assuming a 0-5cm depth, BR was higher at station 330 (Kruskal-Wallis ANOVA: $H (1, N = 39) = 12.4$; $p < 0.001$) (Table 5.1; fig. 5.2).

Respiration values of nematodes at station 115bis were lower than those of bacteria and macrobenthos (Kruskal-Wallis ANOVA: $H (2, N = 33) = 11.98$; $p < 0.01$), while the latter two groups did not differ from each other. At station 330 bacteria showed clearly higher respiration values than nematodes and macrobenthos (one-way ANOVA, $F = 144$; $df = 2$; $p < 0.001$) (Fig. 5.2). Macrobenthos respiration was also significantly higher than nematode respiration. Bacterial respiration at station 115bis (7.7 to 10.0 mmol O_2 m^{-2} day^{-1}) was lower than at station 330 (from 8.3 to 22.9 mmol O_2 m^{-2} day^{-1}) ($H(1, N = 39) = 12.4$; $p < 0.001$) (Fig. 5.2).

A one-way ANOVA performed on each station revealed significant differences between sampling dates for both stations (station 115bis: $F = 6.41$; $df = 2$; $p < 0.01$; station 330:

F = 61.79; df = 2; p < 0.001). At station 115bis bacterial respiration in February was significantly lower than in April, while at station 330 all sampling dates were significantly different from each other.

Table 5.1: Bacterial production (mg C m^{-2} day^{-1}) and respiration (mg C m^{-2} day^{-1}) for the sediment layer 0-1 cm and bacterial respiration (mmol O_2 m^{-2} day^{-1}) for a sediment depth of 5 cm, at stations 115bis and 330 in February, April and October 2003. Bacterial respiration estimated using: [1] a fixed BGE of 20 % and [2] model I by del Giorgio & Cole (1998). (Mean ±SE).

		Production (0-1 cm)	Respiration[1] (0-1 cm)	Respiration[2] (0-1cm)
Station	Date	mg C m^{-2} day^{-1}	mg C m^{-2} day^{-1}	mg C m^{-2} day^{-1}
115bis	Feb 03	23.4 ±0.9	93.8 ±3.7	13.5 ±0.2
115bis	Apr 03	30.0 ±1.9	120.2 ±7.4	14.9 ±0.4
115bis	Oct 03	23.1 ±1.5	92.2 ±6.2	13.4 ±0.4
330	Feb 03	5.0 ±0.7	19.8 ±2.9	7.0 ±0.5
330	Apr 03	9.2 ±0.5	36.7 ±2.2	9.2 ±0.2
330	Oct 03	13.7 ±0.3	54.9 ±1.3	10.8 ±0.1
			Respiration[1] (0-5 cm)	Respiration[2] (0-5 cm)
			mmol O_2 m^{-2} d^{-1}	mmol O_2 m^{-2} d^{-1}
115bis	Feb 03		7.8 ±0.3	1.1 ±0.02
115bis	Apr 03		10.0 ±0.6	1.2 ±0.03
115bis	Oct 03		7.7 ±0.5	1.1 ±0.03
330	Feb 03		8.3 ±1.2	2.9 ±0.19
330	Apr 03		15.3 ±0.9	3.8 ±0.09
330	Oct 03		22.9 ±0.6	4.5 ±0.04

Total nematode respiration was significantly higher at station 115bis than at station 330 (two-way ANOVA: F = 40.1; df = 1; p < 0.001) (Fig. 5.2). At station 115bis nematode respiration was lowest in February (0.94 mmol O_2 m^{-2} day^{-1} ±0.03 SE), highest in April

(3.56 mmol O_2 m^{-2} day^{-1} ±2.23 SE) and lower again in October (2.50 mmol O_2 m^{-2} day^{-1} ±1.50 SE), but these differences were not statistically significant. At station 330 nematode respiration was lowest in February (0.041 mmol O_2 m^{-2} day^{-1} ±0.004 SE) and increased up to October (0.34 mmol O_2 m^{-2} day^{-1} ±0.17 SE), differences again not being statistically significant.

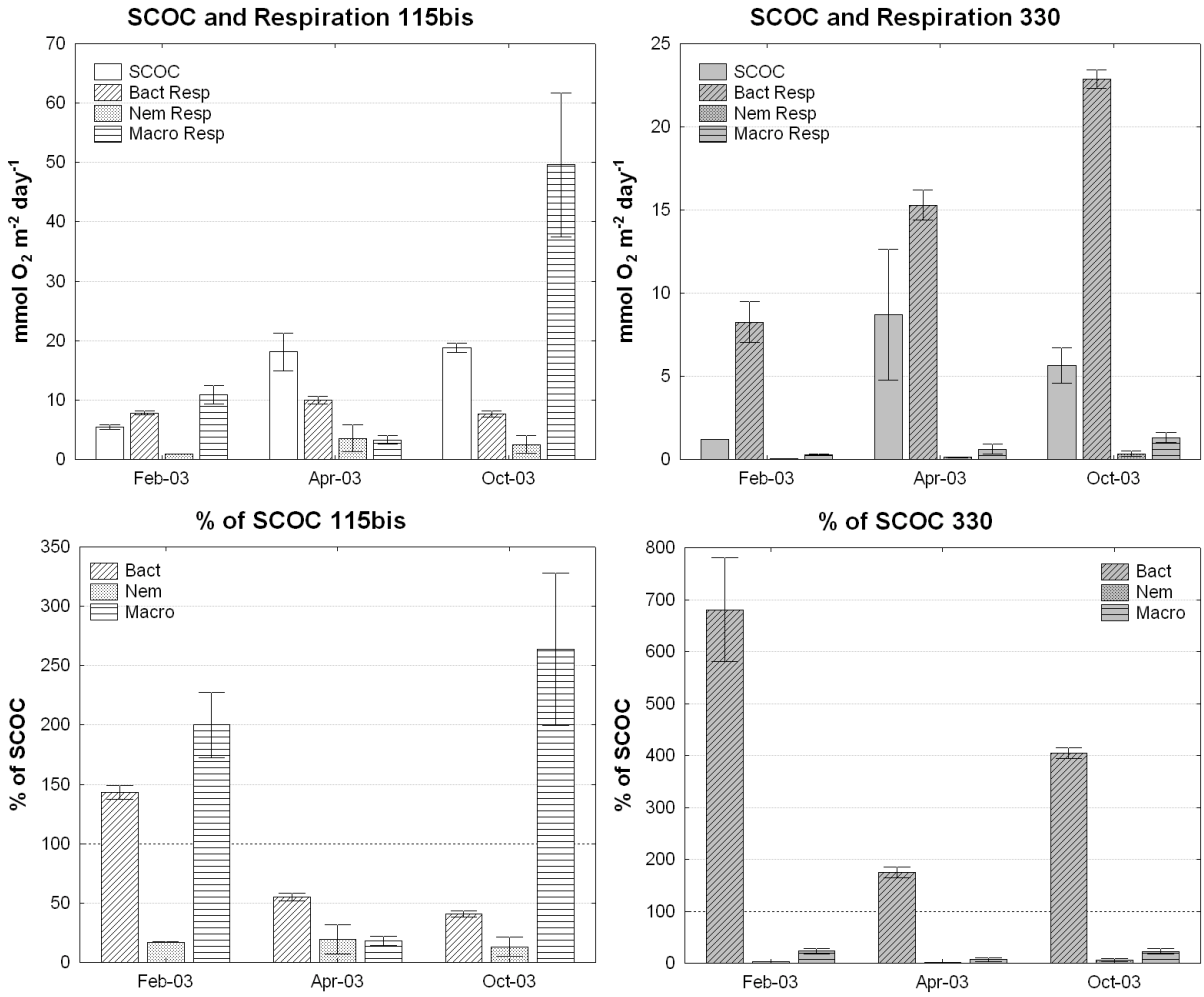


Figure 5.2: Sediment Community Oxygen Consumption (SCOC, mmol O_2 m^{-2} day^{-1}), respiration rates (mmol O_2 m^{-2} day^{-1}) and respiration shares (%) from SCOC of bacteria, nematodes and macrobenthos from station 115bis and 330 on the sampling campaigns of February, April and October 2003. Vertical bars represent the standard error.

Total macrobenthos respiration was higher at station 115bis than at station 330 (Kruskal-Wallis ANOVA: $H (1, N = 30) = 19.9$; $p < 0.001$) (Fig. 5.2). At station 115bis macrobenthic respiration decreased from February to April (when it reached a minimum of 3.57 mmol O_2 m^{-2} day^{-1} ±0.76 SE), increasing afterwards with highest values in October (53.6 mmol O_2 m^{-2} day^{-1} ±12.8 SE) ($F = 28.5$; $df = 1$; $p < 0.001$). All sampling dates were significantly different from each other. At station 330 macrobenthic respiration was lowest in February (0.08 mmol O_2 m^{-2} day^{-1} ±0.05 SE), and highest in October (0.70 mmol O_2 m^{-2} day^{-1} ±0.38 SE) ($F = 4.65$; $df = 1$; $p < 0.05$), with significant differences only between these two sampling dates.

The relative shares of each group in the SCOC differed between the two stations (Fig. 5.2). At station 115bis bacteria and macrobenthos had a very similar relative importance in February (ca. 140 % and 200 % respectively) with nematodes showing a much lower value (ca. 17 %). In April nematode and macrobenthos community respiration became very similar (ca. 20 % and 18 %, respectively) but were both largely exceeded by bacterial respiration (ca. 55 %). In October macrobenthos represented ca. 260 % of measured SCOC, with much lower contributions for bacteria and nematodes (ca. 40 % and 13 %, respectively). At station 330 SCOC was dominated by bacterial respiration throughout the year. Assuming a fixed BGE of 20 %, bacterial respiration clearly exceeded measured SCOC representing in February, April and October ca. 680 %, 175 % and 400 %, respectively, of measured SCOC values. Nematodes contributed very little to SCOC at station 330, with a maximum of about 6 % in October. Macrobenthos also showed a small contribution to measured SCOC, ranging from 7 % (April) to 23 % (February and October).

Summed contributions of bacterial, nematode and macrobenthic respiration always exceeded measured SCOC at both stations, except at station 115bis, in April, when the sum of estimated heterotrophic respiration reached 93 %.

Discussion

Methodological considerations

Summed estimates of heterotrophic respiration rates exceeding measured SCOC values have been reported before (e.g. Schwinghamer *et al.*, 1986) and are likely the result of methodological shortcomings. Bacterial production rates, for instance, may have been overestimated if leucine addition, and/or exposure of otherwise anoxic sediments to oxygen during experimental incubations, stimulated bacterial metabolism. The latter explanation can be ruled out in case of the well-oxygenated sediments of station 330, and the former is not supported by the results of the measured isotope dilution series (data not shown). Further potential methodological biases associated with BP measurements have been discussed by Kemp (1990). However, our bacterial production estimates are well within the range of values reported from 15 other North Sea stations by Hondeveld *et al.* (1995). Another difficulty is in the conversion of bacterial production to respiration. When using the model I regression proposed by del Giorgio & Cole (1998), only the bacterial respiration at sta 330 in February exceeded measured SCOC values. However, application of this regression consistently yielded bacterial growth efficiencies between 45 and 67 %, which is far above the range of values proposed for bacteria growing on detritus (del Giorgio & Cole 1998, Osinga *et al.*, 1997) Bacterial respiration rates exceeding total SCOC have repeatedly been reported (Van Duyl & Kop, 1990; Cammen, 1991; Alongi, 1995). On the other hand, in permeable sediments, like at station 330, advective pore water transport can increase sediment oxygen uptake by a factor of 1.38 to 3 (Ehrenhauss & Huettel, 2004; Janssen *et al.*, 2005). SCOC measurements were performed under hydrodynamic conditions different from those to which the sediment is normally exposed. The diffusive uptake of oxygen by the sediment depends considerably on the thickness of the diffusive boundary layer, which in turn strongly depends

on hydrodynamic conditions above the sediment surface (Polerecky *et al.*, 2005). Experimental stirring can provoke advective currents in the sediment, or not, depending on the stirrer settings. Certain stirring speeds can be enough to mix the overlaying water but not to induce advective currents (Janssen *et al.*, 2005). Although the water was stirred during our SCOC measurements, stirring may not have been sufficient to induce advective currents, potentially resulting in underestimates of SCOC at station 330 by a factor of up to 3. This alone would bring our summed estimates of bacterial, meiobenthic and macrobenthic respiration closer to or even below measured SCOC at this station.

It is obvious that a number of uncertainties are involved in the respiration estimations of both bacteria and meio- and macrobenthos. Many assumptions have been made and allometric relations between body mass and respiration for meio- and macrobenthos, even though based on a wide range of species, will mostly only give an idea of the order of magnitude of respiration rates for those communities, and should therefore only be used on the impossibility of using other methods.

In addition nematodes living deeper in the sediment at station 115bis occupy anoxic areas so either they use oxic microsites or they use oxidants other than oxygen, which would result in an overestimation of nematode respiration rates for nematodes living in such conditions.

Even though our respiration estimates are based on the use of allometric relations we are very confident that general trends can be observed when comparing both sediment types and general temporal changes.

Biomass and densities

Bacterial biomass was within the range of values reported for other subtidal North Sea stations (Van Duyl *et al.*, 1993; Van Duyl & Kop, 1994). Bacterial densities and biomass were fairly similar for stations 115bis and 330, in contrast with the general expectation that bacterial densities and biomass increase from coarser to finer sediments (Köster & Meyer-

Reil, 2001). In April, however, bacterial densities and biomass at station 115bis were substantially higher than at station 330. This is probably linked to the high chlorophyll *a* concentration (as an indicator of labile OM), which is known to stimulate bacterial growth (Goedkoop *et al.*, 1997; Ding & Sun, 2005). At the sediment surface at that time and site, chlorophyll *a* concentration was twice as high as on the other sampling dates at station 115bis and ca. 10 times higher than the corresponding values at station 330 (Franco *et al.*, in press a). The temporal fluctuations in bacterial densities and biomass at our stations, and their links to sedimentary chlorophyll *a* concentrations are mirrored by shifts in bacterial community composition, which differed for all three sampling events at station 115bis, but only between October and February + April at station 330 (Franco *et al.*, in press a).

Nematode biomass and densities differed considerably between the two stations. The higher OM availability at station 115bis supports a more abundant nematode community. At station 330, the permeability of the sediment results in low standing stocks of OM (Franco *et al.*, in press b). The nematode community at this station responds rapidly to inputs of OM (Vanaverbeke *et al.*, 2004a,b; Franco *et al.*, in press b), as illustrated by the increase in nematode biomass and densities from February to April and again in October, when a second deposition event occurred at this station (Franco *et al.*, in press b). At station 115bis phytodetritus accumulates at the sediment surface and is buried gradually (Provoost *et al.*, in prep), thus remaining in the sediment much longer than at station 330. The temporal differences in nematode density and biomass at this station have to be interpreted carefully, since ca. 80 % of the nematode community was found in the upper 5 cm of the sediment in April (the depth stratum considered in the present study), compared to only 60 % in October. Total depth-integrated nematode densities were even higher in October than in April (Franco *et al.*, in press b).

Macrobenthic biomass and densities were also much higher at station 115bis than at station 330. The higher food availability at station 115bis - not only labile OM from deposition of phytodetritus but also higher meiobenthic densities available as potential prey (Franco et al., in press b) - probably supports a more abundant macrobenthic community with a higher biomass compared to station 330. At station 115bis higher macrobenthic biomasses in October indicate that macrobenthos showed a delayed response to the deposition of the phytoplankton bloom, similar to the response of the nematode community (considering total depth-integrated nematode densities). At station 330, macrobenthos already showed an increase in biomass in April, maintaining similar values in October. This would imply a faster response to the deposition of phytodetritus, again comparable to the response of the nematode community at that station.

Even though macrobenthic densities were slightly lower in April than in February, biomass was considerably lower, indicating that smaller organisms were present. This might represent some sensibility of the macrobenthic community to anoxic conditions normally present at this station after major sedimentation events (Steyaert *et al.*, subm.). Some mortality may have occurred and younger and therefore smaller organisms are recolonising the sediment. In October the increase in biomass was much higher than the increase in densities, representing bigger organisms.

Sediment Community Oxygen Consumption

SCOC showed a similar seasonal pattern to other stations in the North Sea with comparable sediment types and water temperatures (Boon & Duineveld, 1998; Boon *et al.*, 1998). However, the SCOC values at both our study sites only resembled those previously published for coarse sediments and were much lower than values for fine grained sediments (up to 30 and 50 mmol O_2 m^{-2} day^{-1} in April and October, respectively) (Boon & Duineveld, 1998; Boon *et al.*, 1998).

In sediments with a thin oxic surface layer, a substantial part of sediment oxygen uptake is not caused by aerobic respiration, but rather by the reoxidation of reduced inorganic metabolites close to the oxic/anoxic interface (Kristensen, 2000). This is likely the case at station 115bis, particularly after the phytoplankton bloom deposition in April (Steyaert *et al.*, subm.), and may explain why at this time and site not all SCOC could be attributed to the heterotrophic compartments considered here. However, not all heterotrophic benthic compartments have been accounted for in the present study. Meiobenthic taxa other than nematodes are scarce and relatively more important at station 330 (Franco *et al.*, in press b), and hence could not have contributed significantly to SCOC at station 115bis. Densities and biomass of microbenthos (ciliates and flagellates) were not determined in the present study. Modelled respiration of microbenthos at a silty tidal flat station equalled or even exceeded that of macrobenthos and was substantially higher than that of meiobenthos, but these values were poorly constrained in the model (Van Oevelen *et al.*, 2006c).

Although oxygen penetrates deeper into the coarser and more permeable sediments, the presence of sulphate-reducing bacteria has been reported, indicating the presence of suboxic to anoxic niches in these environments (Bühring *et al.*, 2005). That may be the reason why lower percentages of aerobic respiration were observed in April at station 330.

Respiration rates

Bacterial production values were among (station 115bis) or below (station 330) low production values reported for several other subtidal stations on the North Sea (Van Duyl *et al.*, 1993; Van Duyl & Kop, 1994). Bacterial production and respiration on a volume basis were higher at station 115bis, however when considering depth-integrated values, bacterial aerobic respiration was higher at station 330 (Fig. 5.2). In coastal sediments there is a large fraction of dead or inactive bacteria (Luna *et al.*, 2002) and the percentage of active bacteria

may change rapidly (Créach *et al.*, 2003). Therefore, production is a better measure of bacterial activity than cell densities or biomass.

Most of the sediment column at station 115bis became reduced after sedimentation of the spring phytoplankton bloom and remained so until as late as October. In combination with the low nitrite/nitrate and high ammonium concentrations, this illustrates that anaerobic pathways become predominant (Steyaert *et al.*, subm.). At station 330, oxygen is available for heterotrophic respiration throughout the upper 5 cm of sediment (Vanaverbeke *et al.*, 2004b). Hubas *et al.*, (in press) estimated bacterial production for two contrasting intertidal sites. Applying a fixed BGE of 20 % to the bacterial production data from Hubas *et al.* (in press), and extrapolating in the same way to a 0-5 cm sediment depth stratum, bacterial respiration for fine intertidal sediments would range from 8 to 32 mmol O_2 m^{-2} day^{-1} and from 0 to 120 mmol O_2 m^{-2} day^{-1} in coarse sediments. Our subtidal bacterial respiration rates were slightly lower and the difference between the coarse and fine site less spectacular, but the emerging picture is that differences in bacterial respiration rates between fine and coarse sediments may be quite consistent in subtidal and intertidal systems.

Nematode respiration rates at station 115bis were high when compared to deeper subtidal stations where nematode densities were comparable to those at station 115bis (de Bovée *et al.*, 1996). Comparisons of respiration rates of nematodes from station 115bis with those of muddy estuarine sediments give variable results: Kennedy (1994) reported 3 times lower respiration rates for a nematode community with half the density found at station 115bis; Tita *et al.* (1999) estimated individual nematode respiration which, extrapolated to the densities at station 115bis, would account for values even higher than the measured SCOC for station 115bis; and Warwick & Price (1979), studying a nematode community about 3 times more abundant than at station 115bis, observed nearly 5 times higher respiration rates. The high nematode respiration in April and October at station 115bis follows nematode biomass trends,

which in turn reflect shifts in both nematode densities and average body shapes. At station 330 respiration rates of nematodes were similar to values reported at deeper subtidal stations (0.05 to 0.39 mmol O_2 m^{-2} day^{-1}, de Bovée *et al.*, 1996) and in estuarine intertidal sandy sediments (Kennedy, 1994).

We estimated nematode respiration rates assuming a Q_{10} of 2 in the whole temperature range covered by the present sampling. Q_{10} values can be highly species-specific for nematodes (e.g. 0.99 to 2.45; Kim & Shirayama, (2001)) and may substantially exceed a value of 2 in opportunistic fast-growing species (Warwick, 1981; Moens & Vincx, 2000). Moreover, Q_{10} values may vary over large temperature ranges (Wieser & Schiemer, 1977). Price & Warwick (1980) regarded Q_{10} values of 2 or higher as an adaptation to fluctuating food resources, while Q_{10} values close to 1 would be associated with a seasonally stable food supply. In view of this argument, and for reasons of comparability with data on macrobenthic respiration, we used a Q_{10} of 2 in the present study.

In our study the contribution of nematodes and macrobenthos to SCOC was much higher at station 115bis than at station 330. Similarly, Boon & Duineveld (1998) concluded that the contribution of macrobenthos to sediment respiration in the North Sea is higher in silty and fine sandy stations than at medium-coarse sandy stations. Tita *et al.* (1999) also measured higher nematode biomass and respiration rates in muddy intertidal sediment than in sandy sediment. The opposite apparently holds for bacteria. The contribution of bacterial production to total benthic community production increases with increasing grain size in intertidal (Hubas *et al.*, in press) and subtidal (this study) sediments. Since, when assuming a fixed BGE, higher bacterial production rates imply higher bacterial respiration rates, and since total nematode and macrobenthic respiration is lower in coarser sediments, the contribution of bacterial respiration to SCOC will also increase with increasing grain size. The bacterial

communities inhabiting sands are regarded as highly active (Rusch *et al.* 2003; Ehrenhauss *et al.* 2004a).

Macrobenthos is frequently assumed to have a higher share in benthic respiration than the meiobenthos (e.g. Moodley *et al.*, 1998; Heip *et al.*, 2001; Van Oevelen *et al.*, 2006c). However, Schwinghamer *et al.* (1986) estimated higher meiobenthic than macrobenthic respiration rates during most of the year in intertidal muddy sediments. Hubas *et al.* (in press) also estimated higher meiobenthic than macrobenthic production in both muddy and sandy intertidal sediments. For the BCS, Heip *et al.* (1985) estimated that total meiobenthic production equals or even greatly exceeds macrobenthic production. Our results attribute a contribution of up to 20 % of SCOC at the fine sandy station to nematodes, an estimate which exceeds maximal estimates hitherto published (13.7 %: De Bovée *et al.* (1996), 13%: Soetaert *et al.* (1997)). Even though in fine sediments macrobenthos dominated SCOC in February and October, nematodes and macrobenthos had similar contributions in April. Upon arrival of OM to the sediment, in contrast to the nematodes, macrobenthos densities lowered, probably as a consequence of the anoxic conditions, and then recovered later on. Even though the respiration rates and relative % of SCOC cannot be directly compared due to methodological limitations, the general trend pointed to a lowering of the macrobenthic contribution upon anoxic conditions while the nematode contribution rose.

Conclusions

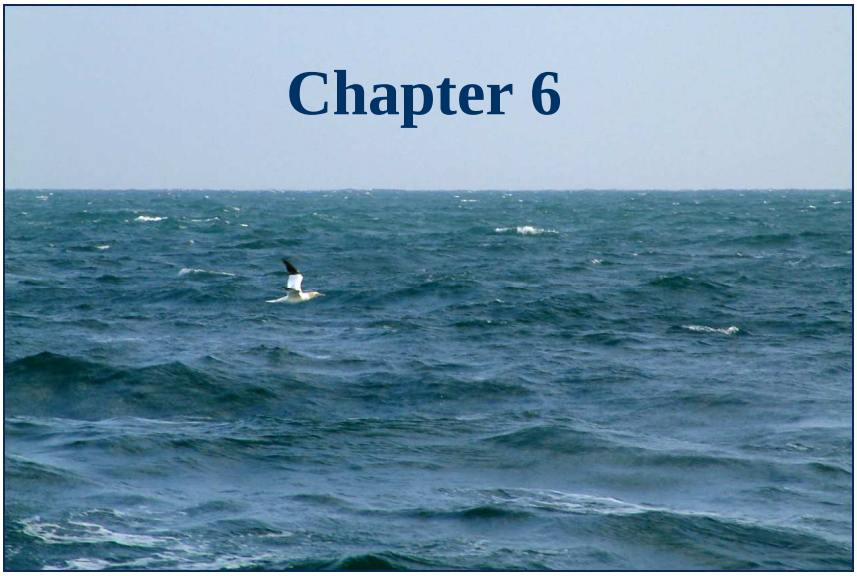
The benthic community at the BCS was highly affected by the deposition of the spring phytoplankton bloom. In fine sediments (station 115bis), deposition of the phytoplankton bloom results in a greater importance of anaerobic pathways. Following sedimentation of the phytoplankton bloom, nematode respiration may account for about 44 % of the estimated heterotrophic respiration (station 115bis in April). Later, macrobenthic respiration becomes more important again. In well aerated coarser sediments bacteria account for by far the largest

share of aerobic respiration. The importance of nematodes in carbon turnover at the BCS thus varies considerably with sediment type; in fine sediments it is more significant than in coarser sediment.

Acknowledgements

We would like to thank the master and crew of the RV Belgica for their skilful help during sampling. This research was funded by the Belgian Science Policy (TROPHOS – Contract nr. EV/02/25A). UGENT-BOF project 01GZ0705 Biodiversity and Biogeography of the Sea (BBSea) (2005-2010). M. A. Franco was financially supported by a PhD grant from the Foundation for Science and Technology (FCT - SFRH/BD/10386/2002). T. Moens is a postdoctoral fellow with the Flemish Fund for Scientific Research (FWO). The authors acknowledge the support by the MarBEF Network of Excellence 'Marine Biodiversity and Ecosystem Functioning' which is funded by the Sustainable Development, Global Change and Ecosystems Programme of the European Community's Sixth Framework Programme (contract no. GOCE-CT-2003-505446).

Chapter 6



Impact of discards of beam trawl fishing on the nematode community from the Tagus estuary (Portugal)

MA Franco, J Vanaverbeke, M Vincx, MJ Costa, M Steyaert.

Abstract

The impact of dead discards, originating from beam trawl fishing, on the nematode community from the Tagus estuary, was investigated in terms of vertical distribution of the dominant nematode groups. Sediment cores were collected from a mud-flat from the Tagus estuary. *Crangon crangon* (Linnaeus, 1758) carcasses were added to the surface of the cores, simulating the settling of dead discards on the sediment. The vertical distribution of the dominant nematode groups was determined up to 4 cm deep at four different moments in time post deposition (0, 2, 4 and 6h) and compared to control cores. The *C. crangon* addition to the sediment led to the formation of black spots and therefore oxygen depleted areas at the sediment surface. The *Chromadora / Ptycholaimellus* group, normally dominant at the surface layer, migrated downwards due to their high sensibility to toxic conditions. *Sabatieria* presented the opposite trend and became the dominant group at the surface layer. Since *Sabatieria* is tolerant to oxygen stressed conditions and high sulphide concentrations, we suggest that it migrated opportunistically towards an unoccupied niche. *Daptonema*, *Metachromadora* and *Terschellingia* did not show any vertical migration, reflecting their tolerance to anoxic and high sulphidic conditions. Our study showed that an accumulation of dead discards at the sediment surface might therefore alter the nematode community vertical distribution. This effect is apparently closely related to toxic conditions in the sediment, induced by the deposition of *C. crangon* at the sediment surface. These alterations might be temporal and reflect an adaptation of the nematode community to dynamic intertidal environments.

Introduction

Since fishing activities are an important source of food, employment and income, an increasing number of studies focus on the side effects of fishing activities on the marine ecosystem (Kaiser *et al.*, 2000; Jennings *et al.*, 2001). One of these side effects is the generation of "bycatch", which are accidentally caught species of no commercial interest (Hall, 1996). This bycatch is often returned to the sea as discards (Jennings *et al.*, 2001).

The estimated total amount of fishery discards in the upper part of the Tagus estuary is approximately 1500 tonnes per year, which represents ca. 90 % of the captures, corresponding to an input of particulate organic matter of more than 140 tonnes of carbon and 35 tonnes of nitrogen per year (Cabral *et al.*, 2002). Beam trawl fishing in the Tagus estuary is mainly targeted towards the soles *Solea solea* (Linnaeus, 1758) and *Solea senegalensis* Kaup, 1858 and discards are dominated by *Crangon crangon* (Linneaus 1758) (Cabral *et al.*, 2002). Since mortality of *C. crangon* can be high and reach up to 96 % (Gamito & Cabral, 2003), a large portion of the discard consists of carcasses which can attract different necrophageous species such as demersal fish and benthic invertebrates (Nickell & Moore, 1992; Kaiser & Spencer, 1996; Kaiser & Ramsay, 1997; Gamito & Cabral, 2003), pelagic fishes and dolphins (Hill & Wassenberg, 1990) or marine birds (Blaber *et al.*, 1995; Garthe *et al.*, 1996; Oro & Ruiz, 1997).

Decomposition of *C. crangon* carcasses deposited on the sediment also creates anoxic patches in the sediment (pers. observation), probably as a result of the oxygen consumption of the microbes responsible for the decomposition of these organisms. It has previously been reported that fine grained sediments easily become anoxic after the settling of organic matter (OM) at the sediment surface (e.g. Ólafsson, 1992; Van Duyl *et al.*, 1992; Bickford, 1996; Kristensen, 2000; Steyaert *et al.*, subm).

Since oxygen plays an important role in the vertical distribution of intertidal nematode communities (Steyaert *et al.*, 2005), we investigated the effect of the addition of *C. crangon* carcasses on the surface of Tagus sediments. Nematodes are known to migrate both horizontally and vertically. Horizontal migration was observed in recolonisation experiments (Schratzberger *et al.*, 2000a; Schratzberger *et al.*, 2004), while vertical migrations were described as a response to tides (Steyaert *et al.*, 2001) or changing oxygen concentrations (Steyaert *et al.*, 2005). Here, we test the hypothesis that discards of *C. crangon* (through the triggering of anoxic patches in the sediment during the decomposition process) will not affect the vertical distribution of intertidal nematode communities.

Material and Methods

Study site and experimental set-up

Sampling was conducted in an intertidal mudflat of the Tagus estuary (38°44'N, 9°08'W) near Alcochete (Fig. 1.2) in March 2005 during ebb tide. The sediment temperature was 16°C and the salinity of the interstitial water was 28 ‰.

From previous local beam trawl fishing campaigns sufficient amounts of the shrimp *Crangon crangon* were kept frozen (-20°C) until the experiment. 9 *Crangon crangon* units, each one comprising 3 shrimps, were thawed and weighed previous to the experiment, with an average of 2.302 g (± 0.048 SE) per shrimp unit.

In the field 3 perspex cores (i.d. 10-15 cm) were collected for shrimp incubation. The shrimp units were placed at the surface of the sediment and left there for 4h until the experiment started (time 0h) in order to initiate the decomposition of the carcasses following a preliminary experiment (Franco, 2002). The surface of the sediment was sampled for pigment analysis, grain size and Total Organic Matter (TOM) and water content. For each analysis

triplicates of 5 ml of sediment was collected. These samples were stored in the cold in the field and frozen (-80°C) in the laboratory until further processing.

In addition, 21 perspex cores (i.d. 3.6 cm) were used to collected sediment to a depth of 10 cm, closed, and transported to the laboratory together with the shrimp incubation cores. After 4h of shrimp incubation, three "Reference Cores" (Time 0 h) were sliced in following layers: 0-0.5; 0.5-1; 1-1.5; 1.5-2, 2-3 and 3-4 cm and the samples were fixed in a 4 % formaldehyde tap water solution. 9 of the remaining cores were then incubated with the decomposing shrimps (3 shrimps per core – Shrimp Cores) and 9 cores were left without shrimps (Control Cores). Since the surface of the cores was 10 cm^2, decomposing shrimp covered almost the complete surface of the shrimp cores. The experiment ran for 6h in total, which is the average exposure time of an intertidal flat. After 2, 4 and 6 hours, 3 shrimp cores and 3 control cores were treated as described above. Ambient room temperature was 23°C during the duration of the experiment and resembled the outside temperature.

Laboratory treatment of samples

Chlorophyll *a* was analyzed by HPLC (Gilson) following Wright & Jeffrey (1997). Water content was estimated comparing sediment wet weight and dry weight measured after 24h at 60 ºC. TOM content was determined by comparing dry weight and ash free dry weight after burning in a muffle furnace (2 h at 450ºC). Grain size analysis was performed using a Mastersizer 2000 Hydro G particle size analyser.

Meiobenthos (animals passing a 1 mm sieve and retained on a 38 µm sieve) was extracted from the sediment by centrifugation with a LUDOX HS-40 solution (Heip *et al.*, 1985) and kept in a 4% formaldehyde solution, stained with Bengal rose. For counting and identification the meiobenthos samples were rinsed into a 100 ml graduated measuring cylinder which was topped with water until 100 ml. The samples were stirred on a stir plate which resuspended the organisms in the water. During stirring 10 times 1 ml of the solution (a total of 10 ml

corresponding to 10 % of the sample) was removed with a pipette at different water heights into a counting dish. The nematodes were then counted and identified and sorted into groups under binocular and high power microscope. On average 71 nematodes (± 3 SE) were identified per sample. The nematodes were sorted into the following groups: (1) *Chromadora / Ptycholaimellus* group; (2) *Daptonema* group; (3) *Sabatieria* group; (4) *Metachromadora* group; (5) *Terschellingia* group and (6) an "other nematodes" group.

Data analysis

In order to investigate if mortality occurred during the experiment a one-way ANOVA was used to test for statistical differences in total densities (sum of all layers) between the reference, control and shrimp cores. The effect of treatment (control and shrimp cores), time (2h, 4h and 6h) and treatment × time on nematode densities in each layer was tested using a two-way ANOVA.

A two-way ANOVA was used to test the effect of treatment, time and treatment × time on the relative abundance of each nematode group in the whole core (integrating all layers) and per sediment layer. Homogeneity of variances was tested using Bartlett χ^2. Data was transformed when needed, with relative data being Arcsine transformed. Whenever homogeneity of variances was not achieved, Kruskal-Wallis (ANOVA by ranks) tests were conducted for the effect of treatment and time. The STATISTICA 6 software was used and a confidence level of 0.05 was considered in all test procedures.

Results

Sediment characteristics and visual observations

The sediment had a medium grain size of 4.79 µm (± 0.02 SE) with a water content of 63.4 % (± 0.09 SE) and a TOM content of 9.23 % (± 0.12 SE). The chlorophyll *a* concentration at the sediment surface was 29.2 µg g^{-1} (± 1.33 SE).

The shrimps covered almost the whole surface of the treatment cores. When the shrimps were removed from the sediment surface, black sediment spots were observed underneath the shrimp covered area. Shrimps were decomposing and slowly sinking in the sediment.

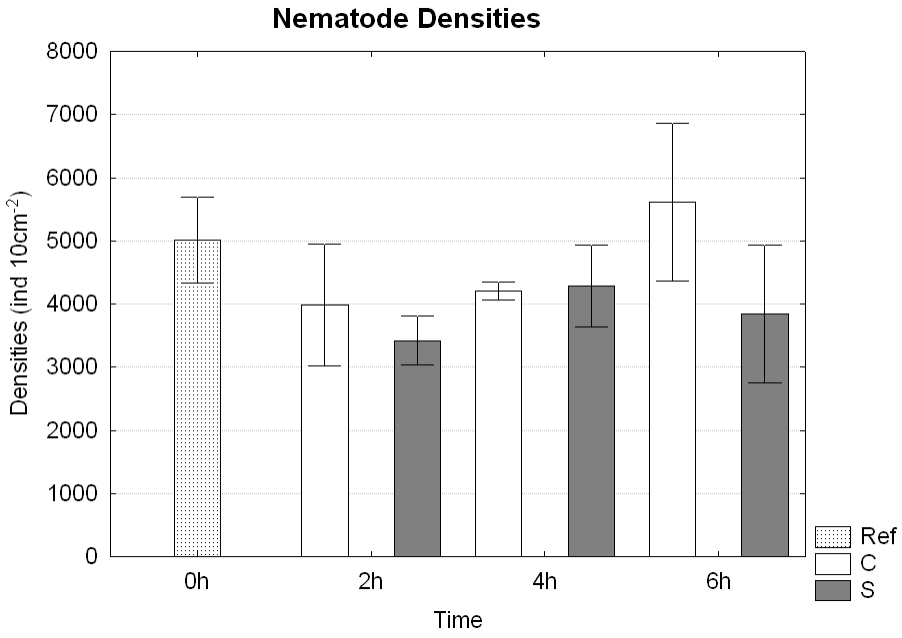


Figure 6.1: Total nematode densities at 0-4 cm (ind 10 cm^{-2}) for all treatments (Ref: reference, C: control and S: shrimp) at different times (0, 2, 4 and 6h). Vertical bars represent the standard error.

Total nematode communities

Total nematode densities in the upper 4 cm, ranged between 1774 ind $10cm^{-2}$ and 8103 ind $10cm^{-2}$, averaging 4338 ind $10cm^{-2}$ (± 302 ind $10cm^{-2}$ SE) considering all the cores (Fig 6.1). No significant differences were found between the reference, control and shrimp cores (one-way ANOVA, $F = 1.09$; $df = 2$; $p > 0.05$).

Total nematode densities were highest at the surface layer of the sediment in the control cores, while in the shrimp cores densities were highest at the 0.5-1.0 cm after 2 h, at 1.0-1.5 cm after 4 h and at 1.5-2.0 cm after 6 h (Fig. 6.2).

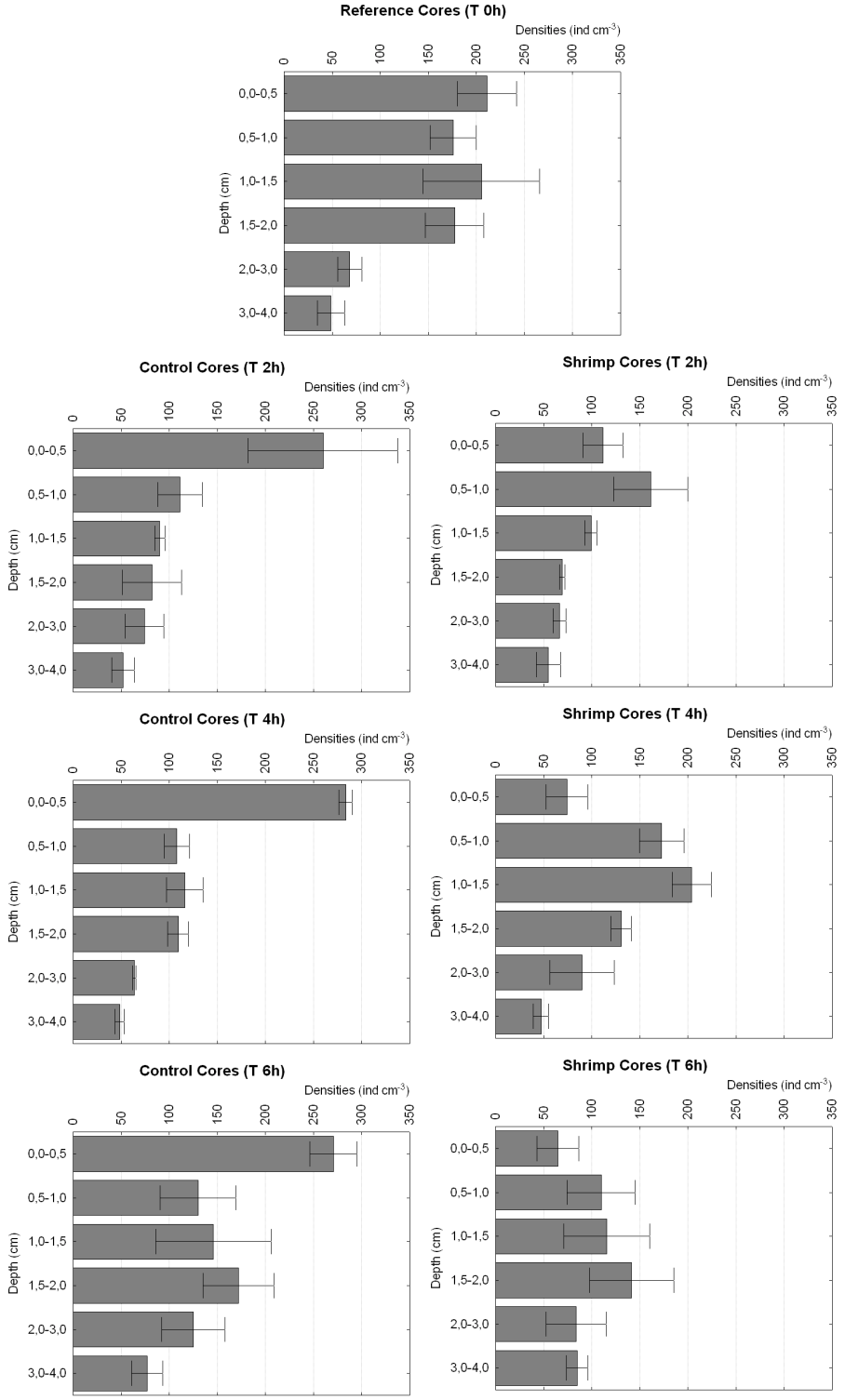


Figure 6.2: Nematode densities (ind cm^{-3}) for all treatments (Ref: reference, C: control and S: shrimp) at different times (0, 2, 4 and 6h) for all the sediment layers (0.0-0.5, 0.5-1.0, 1.0-1.5, 1.5-2.0, 2.0-3.0 and 3.0-4.0 cm). Horizontal bars represent the standard error.

Significant differences in nematode densities between treatments were only detected for the 0.0-0.5 cm layer ($F = 39.4$; $df = 1$; $p < 0.001$). The effect of time was only significant at the 1.5-2.0 cm ($F = 4.44$; $df = 2$; $p < 0.05$) and 3-4 cm ($F = 5.02$; $df = 2$; $p < 0.05$) sediment layers. Tukey HSD tests revealed significant differences between 2h and 6h and between 4h and 6h respectively. No significant differences for the interaction term (treatment × time) were detected for any of the layers.

The nematode community was dominated by the *Chromadora* / *Ptycholaimellus* group, having an overall average relative abundance of 28.8 % (± 1.6 % SE). The second most abundant group was *Sabatieria* (24.5 % ± 1.4 % SE) followed by *Daptonema* (14.9 % ± 0.8 % SE) and the lowest relative abundances were of the groups *Terschellingia* (9.8 % ± 1.4 % SE) and *Metachromadora* (7.3 % ± 0.6 % SE) (Fig. 6.3). Other nematodes not belonging to these groups averaged 15 % (± 0,9 % SE) of the total nematode community.

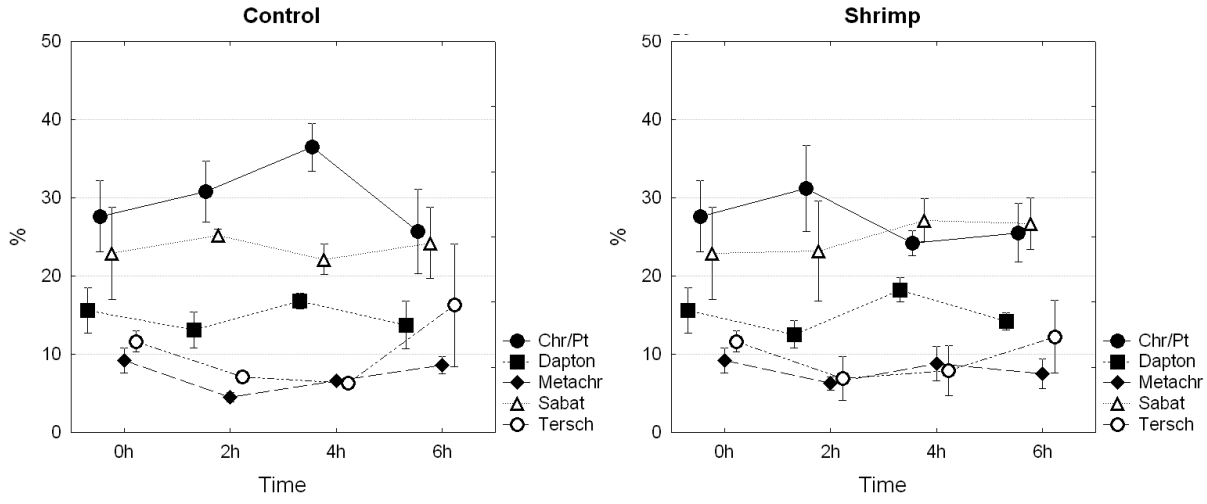


Figure 6.3: Relative abundance (%) of the dominant nematode groups (Chr/Pt: *Chromadora* and *Ptycholaimellus*; Dapton: *Daptonema*; Metach: *Metachromadora*; Sabat: *Sabatieria*; Tersch: *Terschellingia*) in the Reference cores (T 0h) and in the Control and Shrimp cores at times 2, 4 and 6h considering the whole core (sum of all layers). Vertical bars represent the standard error.

A two-way ANOVA for each nematode group on the relative abundance per core (integrating all layers) revealed no significant differences between the treatments, times or the interaction treatment × time.

Vertical distribution

In the reference situation (0 hours) and in the control cores the *Chromadora* / *Ptycholaimellus* group was highly dominant at the 0.0-0.5 cm layer throughout the experiment, with relative densities fluctuating around 60 and 70 % (Fig. 6.4). Deeper down this dominance was shared first with the *Daptonema* group, at 0.5-1.0 cm, with relative densities of about 40 % each, and also with the *Sabatieria* and *Metachromadora* groups, at 1.0-1.5 cm, where each group represented about 20 % of the community. At 1.5-2.0 cm deep *Sabatieria* became the dominant nematode group, with relative densities fluctuating between 30 and 40 % of the nematode community. In the deeper layers *Terschellingia* also became dominant and at the bottom layer (3.0-4.0 cm) dominance was shared between *Sabatieria* and *Terschellingia*, with relative densities fluctuating between 25 and 45 % each. The other nematode groups showed low relative abundances below 5 %.

Adding decomposing shrimp to the surface of sediment cores clearly affected the nematode community. At the surface layer significant changes in dominance were mostly observed among *Chromadora* / *Ptycholaimellus* and *Sabatieria* nematode groups (Table 6.1). There was a decrease in relative densities of the *Chromadora* / *Ptycholaimellus* group from values around 70% (control cores T 6h) to values around 40 % (shrimp cores T 6h). *Sabatieria* relative densities on the shrimp cores reached almost 30 % of the nematode community at the end of the experiment, while in the control cores it was almost absent in this layer throughout the experiment.

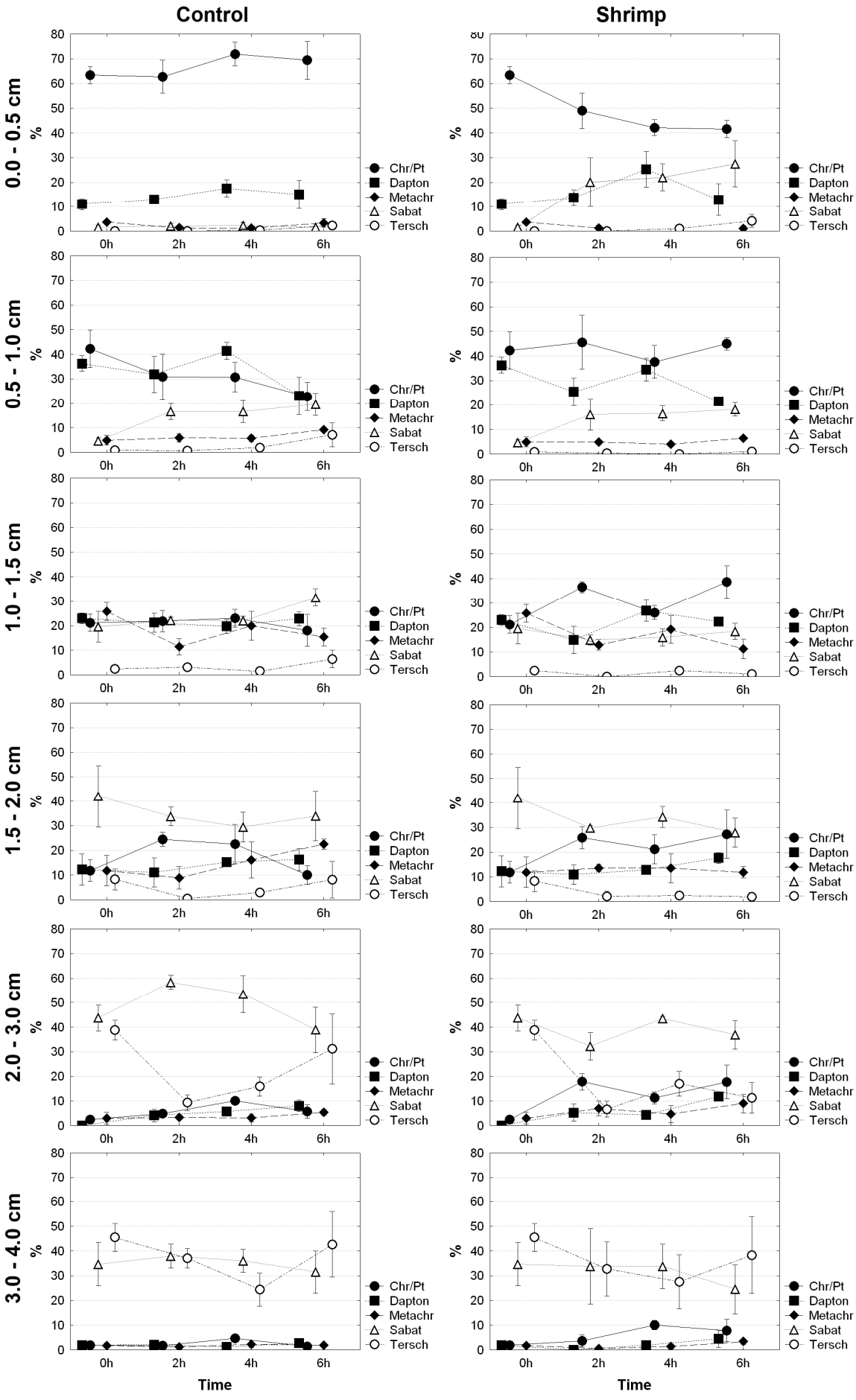

Control
Shrimp
0.0 - 0.5 cm
0.5 - 1.0 cm
1.0 - 1.5 cm
1.5 - 2.0 cm
2.0 - 3.0 cm
3.0 - 4.0 cm
%
0h
2h
4h
6h
Time
Chr/Pt
Dapton
Metachr
Sabat
Tersch

Figure 6.4: Relative abundance (%) of the dominant nematode groups (Chr/Pt: *Chromadora* and *Ptycholaimellus*; Dapton: *Daptonema*; Metach: *Metachromadora*; Sabat: *Sabatieria*; Tersch: *Terschellingia*) in the Reference cores (T 0h) and in the Control and Shrimp cores at times 2, 4 and 6h for each sediment layer (0.0-0.5, 0.5-1.0, 1.0-1.5, 1.5-2.0, 2.0-3.0 and 3.0-4.0 cm). Vertical bars represent the standard error.

The *Chromadora* / *Ptycholaimellus* group also showed significant differences between treatments for layers 0.5-0.1, 0.1-1.5 and 2.0-3.0 cm due to higher relative densities in the shrimp cores at these layers. Significant differences for *Sabatieria* at layers 1.0-1.5 and 2.0-3.0 cm were due to lower relative densities at the shrimp cores. Significant differences between treatments were found for the *Metachromadora* and *Terschellingia* groups at the 0.5-1.0 cm layer as well.

Table 6.1: Results from the two-way ANOVA's performed on each layer on the relative dominance of each nematode group (*Chr. / Pt.*: *Chromadora* and *Ptycholaimellus*, *Dapton.*: *Daptonema*, *Metach.*: *Metachromadora*; *Sabat.*: *Sabatieria*; *Tersch.*: *Terschellingia*). Tr: treatment (control and shrimp cores); Ti: Time (2h, 4h and 6h); Tr×Ti: Treatment × Time interaction. * Significant differences ($p < 0,05$); ns: non significant. Results in Italics refer to Kruskal-Wallis tests.

Depth	*Chr. / Pt.*			*Dapton.*			*Metachr.*			*Sabat.*			*Tersch.*		
(cm)	Tr	Ti	Tr×Ti	Tr	Ti	Tr×Ti	Tr	Ti	Tr×Ti	Tr	Ti	Tr×Ti	Tr	Ti	Tr×Ti
0.0-0.5	*	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
0.5-1.0	*	ns	ns	ns	*	ns	*	*	ns	ns	ns	ns	*	ns	ns
1.0-1.5	*	ns	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns	ns
1.5-2.0	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	***ns***	***ns***	-
2.0-3.0	*	ns	ns	ns	ns	ns	***ns***	***ns***	-	*	ns	ns	ns	ns	ns
3.0-4.0	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

The effect of time was only noticed at the 0.5-1.0 cm layer for the *Daptonema* and *Metachromadora* nematode groups, with significant differences between 4 and 6h for both nematode groups. No significant differences for the interaction effect treatment × time were observed.

Discussion

Visual observations

When removing the shrimps from the sediment surface, black sediment spots were observed at the sediment surface, indicating reduced sediment. In a microcosm experiment with decaying macrofauna, Ólafsson (1992) also observed black spots when removing the dead macrofauna from the sediment surface. The colour of the sediment gives an indication of the redox state in the sediment, with the darker colours reflecting a more negative redox potential (Rosenberg *et al.*, 2001; Diaz & Trefry, 2006). Under decaying organisms oxygen is consumed due to bacterial activity, creating anoxic sediment patches. Natural oxygen concentrations in muddy sediments of the Tagus estuary salt marshes reach already extremely low values in the first millimetre, and become undetectable at 14 mm deep (Cartaxana & Lloyd, 1999). Since the shrimps occupied almost all the core's sediment surface, oxygen stress at the surface layer was probably very high.

Nematode community

There were no significant differences in total nematode densities and relative densities between the reference, control and shrimp cores. This indicates that the experimental handling and the treatments had no significant effect on the nematode total densities within the studied sediment depths (0-4 cm) and that the relative densities of each nematode group remained the same. This implies that the differences observed in this study were due to vertical migration of the nematodes.

Heip *et al.* (1990) characterized muddy sediments as being dominated generally by a few dominant genera, including *Sabatieria*, *Terschellingia* and *Daptonema*. Chromadorids become more important with increasing mean grain size. In the Oosterschelde estuary (The Netherlands) a very similar community was found, dominated by the genera *Chromadora*, *Daptonema*, *Metachromadora*, *Sabatieria* and *Terschellingia* (Steyaert *et al.*, 2007). The nematode communities in the Tagus estuary are thus typical intertidal estuarine communities.

The nematodes were grouped according to genera except for the genera *Chromadora* and *Ptycholaimellus*, which were lumped. The nematodes in our samples belonging to the genera *Chromadora* and *Ptycholaimellus* were morphologically similar in terms of size and body shape. Their similar buccal cavities, together with previous feeding observations place them in the same feeding type, epigrowth feeders *sensu* Moens & Vincx (1997).

Nematodes from the same functional group or same genera can behave differently (e.g. Moens *et al.*, 1999b; De Mesel *et al.*, 2003). However the vertical distribution of the *Chromadora* / *Ptycholaimellus* group generally presented a unimodal distribution. They did not show several peaks of abundance as a result of different migration directions of closely related species. This was also true for the other groups. Therefore grouping of nematode species in genus groups allowed a straightforward analysis of the response of the nematode community to the shrimp addition.

The first and most obvious response was the general decrease in nematode densities at the surface layer of the shrimp cores (Fig. 6.2). In a microcosm experiment with decaying macrofauna, Ólafsson (1992) also observed lower numbers of nematodes in the patches with dead animals, although these differences were not statistically significant. In the referred experiment the microcosm contained a ca. 7 cm thick mud layer. There was no vertical slicing of the sediment so if the effect of decaying macrofauna was mostly felt at the surface, it

would be partly diluted when sampling together with deeper sediment. In fact we only found statistical differences in total densities at the surface layer.

The nematode communities normally concentrated at the surface layer (control cores) tried to escape probably sulphidic and therefore toxic conditions by migrating downwards. This is not an unknown behaviour, since nematodes are able to migrate actively over a wide depth range for instance to pursue oxidised sediment layers, irrespective of sediment depth (Steyaert *et al.*, 2005). In fact the nematode density peak kept on changing with time, being at the 0.5-1.0 cm layer after 2 h, at the 1.0-1.5 cm layer after 4 h and at the 1.5-2.0 cm layer after 6 h (fig 6.2). The diffusion of toxic compounds from the surface deeper into the sediment obliged nematodes more sensitive to these toxins to keep on migrating downwards.

The lower densities at the sediment surface and higher densities deeper down in the sediment at the shrimp cores were mostly due to a downward migration of *Chromadora* / *Ptycholaimellus*. Indeed dominance of this group at the 0.0-0.5 cm layer decreased, while at deeper layers their relative densities became higher (Fig. 6.4; Table 6.1).

Both *Chromadora* and *Ptycholaimellus* mostly occur naturally at the surface of muddy sediments (e.g. Steyaert *et al.*, 2003; Steyaert *et al.*, 2005; this study) where the highest oxygen concentrations are found. Steyaert *et al.* (2007) observed a total disappearing of *Chromadora macrolaima* after being subjected to two weeks of suboxic and anoxic conditions, coinciding with a high mortality of *Ptycholaimellus ponticus*. According to their buccal cavity and feeding type, these nematodes feed mostly on diatoms and other microalgae (Moens & Vincx, 1997), therefore they would not benefit from adding decaying shrimp to the sediment surface. Their migration deeper into the sediment therefore reflects their sensitivity to toxic conditions, often associated with anoxia. Nematode migration is a known behaviour as a reaction to oxygen deficiency and sulphide stress (e.g. Ott *et al.*, 1991; Hendelberg & Jensen, 1993; Ott *et al.*, 2004; Steyaert *et al.*, 2007).

The opposite behaviour could be observed in the *Sabatieria* group. *Sabatieria* relative abundance at the surface layer was significantly higher in the shrimp cores (Fig. 6.4; Table 6.1). The average density (times 2h, 4h and 6h) of *Sabatieria* at the surface layer in the control cores was 5.8 ind cm^{-3} (± 1.7 SE) while in the shrimp cores was 18.1 ind cm^{-3} (± 4.4 SE). Although the peak abundances were still located deeper down in the sediment, *Sabatieria* densities at the surface layer were comparatively higher in the shrimp cores. This shows that there was active migration of *Sabatieria* to the surface and the increase in dominance was not only due to the absence of *Chromadora* and *Ptycholaimellus*. Although *Sabatieria* is sensitive to anoxia in the long term, it can tolerate periods of oxygen depletion (Steyaert *et al.*, 2007).

Sabatieria is commonly classified as a deposit feeder (*sensu* Moens & Vincx, 1997) which has bacteria and microphytobenthos as main food sources. Nematodes migrate towards an "optimal" food source (Jensen, 1995; Santos *et al.*, 1995; Moens *et al.*, 1999a) and studies with stable isotopes and labelled food sources revealed opportunistic vertical migration of *Sabatieria* towards food sources (Franco *et al.*, in press b; Franco *et al.* in prep). *Sabatieria* species are adapted to survive under low concentrations of oxygen and high sulphide concentrations which are often reflected in its vertical distribution (Hendelberg & Jensen, 1993; Steyaert *et al.*, 1999; Steyaert *et al.*, subm.; Franco *et al.*, in press b; Franco *et al.*, in prep; this study). The intolerance of other nematodes to such conditions and also the ability to respond fast enabled *Sabatieria* to occupy and explore this niche.

To what extent *Sabatieria* migration behaviour is a direct response to higher food availability or, instead, the consequence of the absence of competitors at the sediment surface or presence of competitors at the sediment horizons generally domintade by *Sabatieria* or even the combination of both factors, is difficult to determine. Nematodes tend to be selective feeders, showing different feeding preferences, between even congeneric species, allowing them to partition the available food sources (Moens *et al.*, 1999b; De Mesel *et al.*, 2004). Species

interactions and different feeding strategies can be responsible by nematode vertical segregation (Steyaert *et al.*, 2003).

Daptonema, *Metachromadora* and *Terschellingia* were not highly affected by the experiment. In the study by Steyaert *et al.* (2007) *Daptonema* species demonstrated high sensitivity to oxygen stress conditions; however that experiment lasted for two weeks. The shorter observation time of our study might be responsible for the observed differences. Moreover identification to species level might help clarifying this case, since more than one species of *Daptonema* was present in our samples (pers. obs.).

Metachromadora vivipara has been reported previously as highly adapted to low oxygen and highly sulphidic conditions (Van Gaever *et al.*, 2006; Steyaert *et al.*, 2007). Therefore the fact that this genus was not affected was not surprising. The same is true for *Terschellingia* species, reported as tolerant to suboxic and anoxic conditions (Steyaert *et al.*, 2007). However the whole depth distribution of *Terschellingia* may not have been totally sampled in our experiment, since its vertical distribution can reach 10 cm deep, becoming more dominant in deeper sediment layers (Steyaert *et al.*, unpublished data). Even though some of these nematodes have been reported as tolerant to low oxygen and high sulphide concentrations conditions, they did not show an opportunistic behaviour as *Sabatieria* did.

Although significant differences between both treatments were noted at the 0.5-1.0 cm layer for the nematode groups *Metachromadora* and *Terschellingia*, these were probably influenced by the migration of the *Chromadora* / *Ptycholaimellus* group. In the *Metachromadora* case, higher densities of the *Chromadora* / *Ptycholaimellus* group at the 0.5-1.0 cm layer lowered their relative abundance in the shrimp cores (whose absolute densities were similar to the control cores). *Terschellingia* densities at the 0.5-1.0 cm layer were indeed higher in the control cores (after 4 and 6h), however, as mentioned before, at this depth we were probably sampling the very upper distribution limit of *Terschellingia* and, except for one case, all

subsamples contained 0 or 1 *Terschellingia* individuals. Due to calculations of densities and relative densities (also influenced by other nematode groups' densities), these differences became significant, without really indicating active migration of these nematodes or a treatment effect.

In muddy sediments excessive organic enrichment leads to characteristically altered nematode community (Schratzberger & Warwick, 1998). Nematode species are differently adapted to living in low oxygen conditions and once subjected to hypoxia and/or anoxia some species might even disappear after a short period of time (Steyaert *et al.*, 2005). An accumulation of dead discards at the sediment surface might therefore alter the nematode community or even have a permanent damage if remaining in the sediment for some time, favouring species well adapted to low oxygen conditions and high sulphide concentrations. However the most probable situation is that the rising tide will bring it all back to normal conditions, as they would be if deposition of dead discards had not occurred. Estuaries are by nature very dynamic systems and the organism living there must be well adapted to such conditions. This experiment revealed two surviving strategies to such changing environment. On one hand nematodes that cannot withstand certain conditions, like oxygen stress and high sulphide concentrations, can actively migrate away from the stressed areas and on the other hand, also through migration within the sediment, more tolerant and opportunistic nematodes can therefore exploit niches left empty by others.

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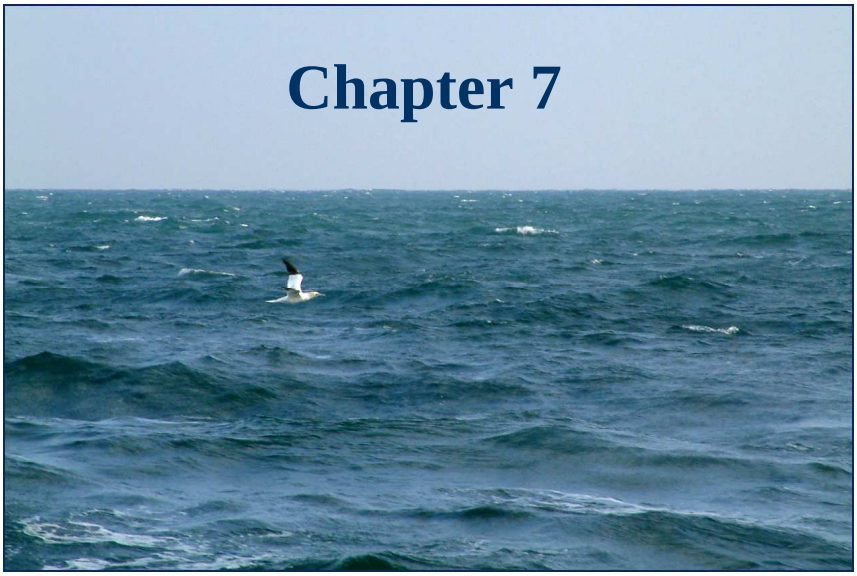
Chapter 7



General Discussion

Phaeocystis in the benthic system

As mentioned before, degradation of *Phaeocystis* was accepted to happen within the water column only (Rousseau, 2000). After observations of massive sedimentation of this alga to the seafloor the need for assessing the fate of *Phaeocystis*, both in the water column and marine sediments emerged. In the water column *Phaeocystis* is ingested by a wide array of marine organisms, like protozoans, bivalves, amphipods, euphausiids, copepods and fishes (Weisse *et al.*, 1994). In the benthic system our results indicate that *Phaeocystis* was incorporated in the nematode tissues at similar rates as diatoms. However the consumption of *Phaeocystis* by the nematode community is not easy to quantify and the carbon uptake we measured was not nearly enough to sustain the nematode community carbon demands. That was not uncommon and is present in other studies as well (e.g. Ólafsson *et al.*, 1999). In estuarine systems the main nematode food sources are microphytobenthos (50 %) and bacteria (39 %) (Van Oevelen *et al.*, 2006c). In the absence of microphytobenthos nematodes have to explore other food sources in subtidal sediments and the sedimenting phytoplankton might be an alternative food source. The path through which *Phaeocystis* derived labelled OM entered the nematodes is not yet clearly understood. Since nematodes have several food sources as bacteria, ciliates, diatoms and other algae, other nematodes or oligochaetes and other items like detritus or dissolved OM (Moen & Vincx, 1997; Moens *et al.*, 1999b) it is possible for *Phaeocystis* derived OM to enter the nematodes through several paths. Further investigation on this matter including more controlled experimentation using labelled *Phaeocystis* could help determining the path through which *Phaeocystis* derived OM does enter the nematodes tissues and to what extent it is important.

Bacterial community

The bacterial communities show a fast response to an input of OM which is only later transferred to higher trophic levels (Bühring *et al.*, 2006). As the first compartment to react to an input of OM to the sediment, the bacterial community revealed a sediment specific response, with higher diversity and more striking vertical and temporal changes in bacterial community composition in fine sediments.

Since bacteria are an important food source for nematodes (Moens & Vincx, 1997) the trophic interaction between these two compartments of the trophic web has long been investigated. Nematodes grazing on bacteria is well documented (e.g. Trotter & Webster, 1984; Montagna & Bauer, 1988; Epstein & Shiaris, 1992; Moens *et al.*, 1996; Moens & Vincx, 1997; Moens, *et al.*, 1999c; De Mesel *et al.*, 2004) and migration of nematodes towards specific food sources such as bacteria, has been observed experimentally (e.g. Andrew & Nicholas, 1976; Trotter & Webster, 1984; Grewal & Wright, 1992; Moens *et al.*, 1999c). Nematode attraction to bacteria varies not only with different bacterial strains (Andrew & Nicholas, 1976; Grewal & Wright, 1992; Moens *et al.*, 1999c; De Mesel *et al.*, 2004) but also with their density, leading to species specific nematode responses to certain bacterial densities (Moens *et al.*, 1996; Moens *et al.*, 1999c; Moens & Vincx, 2000). Changes in the nematode community may therefore be associated with changes on the bacterial community, but not due to changes in diversity, as hypothesized by Vanaverbeke *et al.* (2004b). Changes in bacterial community that might induce changes in the nematode community are then higher biomass and densities observed in spring compared to winter values at both stations and/or changes in the community composition, also present at both stations.

DGGE presents it self as an useful tool that can easily be used to assess changes in total bacterial community and can be applied on further research on this trophic interaction. The

use of DNA tools in free-living marine nematode investigation is also taking its first steps (e.g. Cook *et al.*, 2005; Derycke *et al.*, 2005, 2006).

Nematode community

The nematode communities of both stations on the BCS have been previously sampled in 1999. At station 115bis the nematode community was sampled monthly from March to July and again in October 1999 to a depth of 5 cm. Similarly to our results the abundance peak was registered not immediately after the deposition of the phytoplankton bloom, but later in July (ca 4000 ind 10 cm^{-2}) (Steyaert *et al.*, subm.). In 2003 it was even later with the highest values registered in September and October 2003. Densities were slightly higher in 2003 compared to the ones observed in 1999. This can be explained by the fact that we sampled 10 cm of sediment, whereas in 1999 only 5 cm was examined. However these differences become striking in October when densities were about 3 times higher in 2003. On this sampling occasion, 40 % of the nematode community was found below 5 cm, and therefore sampling the top 5 cm of the sediment would only retrieve about 60 % of the nematode community. This may be the reason why in October 1999 lower densities were observed when compared to July 1999 (Steyaert *et al.*, subm) and to October 2003 (present study). At station 330 the nematode community was sampled monthly from March to July 1999 to a depth of 10 cm. Densities followed the same seasonal trend as in our study for the same period of the year, with the highest densities in May (ca 600 ind 10 cm^{-2} in 1999) (Vanaverbeke *et al.*, 2004b). Densities were about twice as high in 2003 when compared to 1999; however interannual variability is generally higher than seasonal variability (Coull, 1985).

The first response of the nematode community following the deposition of a phytoplankton bloom in both types of sediments is a change in densities and dominance towards selective deposit-feeding nematodes (1A-nematodes, Wieser, 1953) (Vanaverbeke *et al.*, 2004b) and deposit-feeding nematodes, the 1B-nematodes (Wieser, 1953) (Steyaert *et al.*, subm.), both

having bacteria as an important food source (Wieser, 1953). As discussed before, bacterial community dynamics can be one of the factors inducing such changes. This first reaction on the nematode community in fine sediment was described to be initially among *Sabatieria celtica* and *S. punctata* species at the sediment surface and later among *Daptonema riemanni* and *D. fistulatum* species deeper down in the sediment (Steyaert *et al.*, subm). Indeed, a higher concentration of nematodes at the sediment surface was observed for fine sediments after deposition of the phytoplankton bloom (80 % of the nematode community on the upper 5 cm in April). Later, after burial of the OM, the nematode community concentrated deeper down in the sediment (40 % of the nematode community below 5 cm in October).

Natural stable isotopic signatures of nematodes at both stations indicated that changes on the nematode signatures were not coupled with changes in SPM or sediment POM, indicating that nematodes do not feed on bulk POM but rather selectively. Since $\delta^{13}C$ signatures of the meiobenthos at station 330 and *Sabatieria, Richtersia* and nematodes inhabiting the sediment surface at station 115bis were generally higher than the POM signatures, suggesting a selective feeding on recently deposited OM. However after experimentally adding labelled algae to the sediment surface, the total uptake derived from these food sources was rather low. Several methodological limitations could be behind these low results, as discussed previously. Rudnick (1989) in a mesocosm experiment which lasted for 6 months, during which labelled phytodetritus were being constantly produced in the water column, observed two different faunal groups, within meiobenthos, in regard to the proportion of carbon assimilation derived from the labelled phytodetritus. In one group, dominated by harpacticoid copepods, specific activities identical to that of the phytoplankton were reached with a lag of less than 2 months between detrital deposition and assimilation; the second group, which included most other meiobenthic taxa, still had remarkably low specific activities of 10 to 30 % of phytoplankton values after 5 months. Therefore these organisms were exploiting organic carbon that had

been produced before the starting of the experiment and stayed buried in the sediment, of silt-clay type (Rudnick, 1989). That could easily be the case in fine sediments on the BCS but hardly in permeable sediments, where advective transport pumps oxygen into the sediment (Ziebis *et al.*, 1996; Janssen *et al.*, 2005) while removing decomposition products (Huettel *et al.*, 1998) resulting in a fast mineralization of organic carbon and recycling of nutrients (Huettel & Rusch, 2000; Janssen *et al.*, 2005; Bühring *et al.*, 2006) preventing the build up of labile OM in the sediment. These processes might be responsible as well by the much higher variability observed on isotopic signatures of POM in permeable sediments when compared to fine ones.

Taken into account the carbon uptake from the experiments with a labelled food supply, and the estimated respiration for nematodes, only a very small percentage of the diary nematode needs were supported by the carbon uptake observed on the experiments. This also indicates the exploitation of other food sources either than carbon derived from the added algae.

Even though respiration estimations from nematode biomass can involve a certain error, major trends were distinguished. Respiration of nematodes can be directly measured in microchambers, individually, if the nematode is large enough, or in groups of nematodes (Moens & Vincx, 2000; Kim & Shirayama, 2001). However applying this technique and extrapolate individual respiration rates measured in a few individuals for short periods of time to community level would deviate from real values in the field, being this deviation difficult to quantify. A good comparison of both methods could be interesting to determine how accurate both are.

Stout nematodes in permeable sediments

The understanding of the dynamics of permeable sediment recently increased drastically. The biogeochemistry of these sediments strongly differs from finer sediments. Advective transport

has an important role in coarse sediments, responsible for many of the sediment characteristics. Relatively strong bottom water currents can prevent the deposition of sedimenting phytodetritus at the sediment surface of permeable sediments (Huettel & Rusch, 2000; Precht & Huettel, 2004) and due to advection, sedimenting phytoplankton cells can penetrate deeper in the sediment inducing subsurface peaks (Huettel & Rusch, 2000; Ehrenhauss & Huettel, 2004; Ehrenhauss *et al.*, 2004a). Advective transport of oxygen into the sediment (Ziebis *et al.*, 1996; Janssen *et al.*, 2005) and fast removal of decomposition products (Huettel *et al.*, 1998) accelerates POM degradation resulting in a fast mineralization of organic carbon and recycling of nutrients (Huettel & Rusch, 2000; Janssen *et al.*, 2005; Bühring *et al.*, 2006). These processes prevent a build up of labile OM and the establishment of clear vertical gradients.

The nematode community inhabiting these sediments must be adapted to such sediment characteristics. Investigations concerning these communities are still scarce and the role of the stout nematodes within these communities still have to be determined, since they can become dominant in certain periods of time and/or areas with permeable sediments and almost absent in others (Willems *et al.*, 1982; Vanaverbeke *et al.*, 2004a; Urban-Malinga *et al.*, 2006).

This body shape implies lower mobility which restrains these nematodes to the surface of the sediments where oxygen is available (Soetaert *et al.*, 2002). It is suggested that due to the fact that these nematodes are stouter and normally with strong and armoured cuticles, they are better protected against predation (Soetaert *et al.*, 2002). Also due to its lower mobility the probability of an encounter with a predator is lower (Moens *et al.*, 2000). However no information on the predation pressure in such sediments is available and therefore it is not possible to understand if a better protection against predation is an advantage or not in those sediments.

Nevertheless, their densities and relative abundance does increases after deposition of phytoplankton bloom (Vanaverbeke *et al.*, 2004a) with that specific nematode body shape being somehow favoured. However natural stable isotopic signatures as well as the results from the labelled food supply experiments revealed no clear differences in feeding strategies when compared with other nematodes. What life cycle feature makes these stout and short nematodes such successful colonizers is not yet known so further research within these nematode types may therefore also be interesting.

***Sabatieria* on the Belgian Continental Shelf and the Tagus estuary**

Nematodes from the genera *Sabatieria* displayed a quite interesting and consistent behaviour on both the BCS and the Tagus estuary, migrating towards a potential food source. *Sabatieria,* was among the first species to react upon arrival of OM in the fine sediment station on the BCS (Steyaert *et al.*, subm). The study sites on the BCS and the Tagus estuary differed considerably, the first being a marine subtidal station and the second an estuarine intertidal mud flat, with a much higher mud content. The common conditions were an input of OM which induced oxygen depletion in the sediment. On the BCS, *Sabatieria* revealed the same ability to migrate to the surface of the sediment to feed. On the Tagus estuary, *Sabatieria* migrated to the surface of the sediment towards a potential food source, even though the sediment conditions were toxic to other nematodes. The ability of *Sabatieria* to react fast and opportunistically migrate towards better conditions (e.g. of food or oxygen) coupled with its tolerance to low oxygen and/or high sulphidic conditions, enabled this genera to be widespread and generally present with relatively high abundances in fine sediments (e.g. Heip *et al.*, 1990; Vanreusel, 1990; Steyaert *et al.*, 1999; Vanhove *et al.*, 1999; Schratzberger *et al.*, 2000b; Schratzberger *et al.*, 2006; Steyaert *et al.*, subm) which easily become anoxic after the

settling of OM at the sediment surface (e.g. Ólafsson, 1992; Van Duyl *et al.*, 1992; Bickford, 1996; Kristensen, 2000; Steyaert *et al.*, subm).

Chemosynthetic food sources

The exploitation of a chemosynthetic derived food source by meiobenthos in fine sediments was clearly present in the copepods case and probably in some other deep-dwelling nematodes. Stable isotopic signatures revealing such a food source have been previously reported for meiobenthos however in a very contrasting environment: for one nematode species, dominant in sulphidic microbial mat sediments in a cold methane-venting seep and even more depleted $\delta^{13}C$ values for one copepod species indicated the exploitation of a methane-derived food source (Van Gaever *et al.*, 2006).

For a shallow subtidal system, stable isotopic signatures that clearly indicate the exploitation of a chemosynthetic derived food source have not been previously reported. Moreover copepods demonstrated a high selectivity towards this particular food source on a particular sampling event, probably due to a strong dominance by these copepods in that particular sampling event. Therefore, identifying which copepods are exploiting a chemosynthetic derived food source should not be difficult. It was not possible to detect a similar feeding strategy in deep-dwelling nematodes, since measurements on a bulk nematode sample contain many individuals reflecting many possible feeding strategies. The eventual signal of chemosynthetic derived food sources could be diluted by higher isotopic values of nematodes not feeding on these food sources.

Nematodes tend to be selective feeders, for instance preferring certain bacterial strains over others (Andrew & Nicholas, 1976; Grewal & Wright, 1992; Moens *et al.*, 1999c; De Mesel *et al.*, 2004) and/or specific bacterial densities (Moens *et al.*, 1996; Moens *et al.*, 1999c; Moens & Vincx, 2000). Our stable isotope results also indicate a certain degree of feeding selectivity

by nematodes, since the nematode isotopic signatures did not follow the changes observed in the POM in the sediment and remained relatively constant with time. Splitting the whole nematode community, for instance into genera level, to determine which nematodes exploit which food source is a difficult task since a biomass minimum is needed to perform a stable isotope analysis. However these nematodes may have a representative biomass within the nematode community since the stable isotopic signatures of the nematode community were deviated towards more depleted $\delta^{13}C$ values. To determine which nematodes exploit a chemosynthetic derived food source may therefore be also possible.

Symbioses between marine nematodes and sulphur-oxidizing chemoautotrophic bacteria have been reported for nematodes in two different cases: in the case of mouthless nematodes (genus *Astomonema*) which have endosymbionts living intracellularly or extracellularly in the gut of their host and in the Stilbonematinae, in which bacteria (sulphur-oxidizing chemolithoautotrophs) cover the outside of the body with stable isotope analysis suggesting that these nematodes depend on the bacteria for their nutrition (Ott *et al.*, 1991; Ott *et al.*, 2004 and references therein). Symbioses may therefore be a way through which nematodes can explore a chemosynthetic derived food source.

To determine which nematodes and/or copepods exploit a chemosynthetic derived food source and how is an interesting follow up for this matter.

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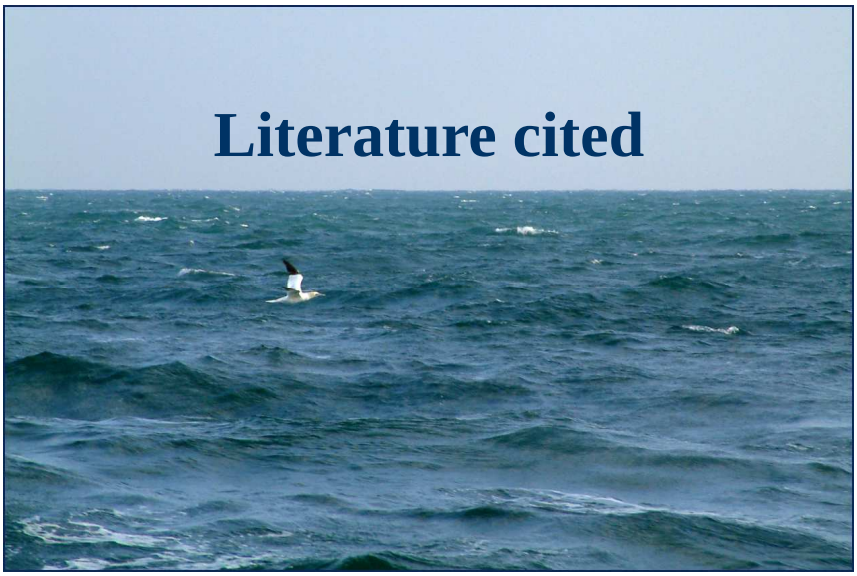

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