Biodiversity, distribution patterns and trophic position of meiobenthos associated with reduced environments at continental margins

Saskia Van Gaever

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BIODIVERSITY, DISTRIBUTION PATTERNS AND TROPHIC POSITION
OF MEIOBENTHOS ASSOCIATED WITH REDUCED ENVIRONMENTS
AT CONTINENTAL MARGINS.

BIODIVERSITEIT, DISTRIBUTIEPATRONEN EN TROFISCHE POSITIE
VAN MEIOBENTHOS GEASSOCIEERD MET GEREDUCEERDE MILIEUS
OP CONTINENTALE RANDEN.

by/door

SASKIA VAN GAEVER

Promotor: Prof. Dr. Ann Vanreusel

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Nature composes some of her loveliest poems

for the microscope and the telescope.

Theodore Roszak, *Where the Wasteland Ends*, 1972

MEMBERS OF THE READING COMMITTEE

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Saskia,

Oostakker, september 2008

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SUMMARY

The discovery of reduced[1] deep-sea environments and their remarkable communities of bacteria and metazoan organisms became one of the most important events in marine biology of last decades. The variable reduced environments share common features such as high content of reduced compounds (sulphides and hydrocarbons), often oxygen deficiency, high abundance and metabolic activity of bacterial populations, and production of autochthonous organic matter by bacteria using energy of chemical bindings (chemosynthesis). Metazoan macro- and megafaunal life in reduced environments is generally characterised by low diversity (which however may increase to the periphery of the biotope), often high degree of endemism and peculiar biological traits. Deep-sea cold seeps are one of the most impressive reduced environments and have been studied rather frequently since their discovery two decades ago. However, most biological studies of cold seeps have focused on the endosymbiont-bearing megafauna (siboglinid tube worms, mytilid mussels, vesicomyid clams), or on the microbiological processes (Levin 2005), while organisms of intermediate size class or so-called meiofauna (0.032–1 mm) are studied far less completely. Round worms or nematodes constitute the most important group of the meiofauna with respect to densities and biomass, followed by harpacticoid copepods, nauplii, polychaetes, tardigrades and other groups (Giere 1993). Because marine nematodes have a worldwide distribution, are the most prominent members of the smaller-sized animals and have a direct link with the sediment and with the processes that occur immediately above the sediment, this group can function as an interesting tool to describe habitat heterogeneity in the marine environment. In this thesis, nematodes are used as key group to explore the meiobenthic communities inhabiting different cold seeps associated with pockmarks[2] or mud volcanoes, and located in the North and South Atlantic Ocean. The following questions were assessed: (1) What factors have an important influence on the seep meiobenthos concerning structure, diversity and distribution at multiple scales? (2) To what extent does the meiobenthos take advantage from the typical seep chemosynthetic food sources? (3) Are the meiofaunal species morphologically or physiologically adapted to resist the often toxic geochemical conditions in seeps? (4) What is the specificity of the successful meiofaunal seep species? In order to obtain this overview, the nematofauna was characterised in terms of taxonomic composition, distribution patterns on different spatial scales, habitat preferences, vertical distribution within sediments, phylogeny, presence of symbionts, and nutritional sources.

The first study was carried out in the Darwin Mounds (59°N 7°W) and an adjacent field of seabed pockmarks in the bathyal North-East Atlantic at circa 1000 m water depth. CHAPTER 2 describes the meiobenthic community structure at different spatial scales within this area in order to study the influence of micro- and macro-scale habitat heterogeneity. Multivariate analysis of the generic relative abundances indicated no significant influence on the nematode distribution resulting from the large scale habitat heterogeneity across the carbonate mounds. The study of the small-scale biogenic structures, i.e. dead tests of the xenophyophore[3] *Syringammina fragilissima*, revealed a nematofauna composed of similar genera as the surrounding sediment, although a shift towards genera with larger body sizes was observed. The vertical nematode distribution patterns, however, showed some unusual features. Stilbonematid nematodes were identified in the sediment below 1 cm depth, together accounting for up to 20% of the total nematode community in these deeper layers, and probably reflecting the presence of anoxic, sulphide-enriched micro-environments. Nematodes of the subfamily Stilbonematinae have a conspicuous coat of ectosymbiotic, chemoautotrophic bacteria, and were previously only reported from shallow marine sands (Ott et al. 1991) and shallow hydrothermal vents (Kamenev et al. 1993, Thiermann et al. 1997). Thus this represents the first observation of three genera of stilbonematids (*Leptonemella*, *Eubostrichus*, *Catanema*) in deep-sea sediments.

The meiobenthos associated with a variety of habitats along the Congo Channel system and in the nearby cold seep REGAB in the Gulf of Guinea (Equatorial West African margin, at 5°S 9°E, 3150–4800 m) was investigated in CHAPTER 3. Different meiofaunal communities were distinguished, corresponding with a multiple-scale (from m's to 200 km) spatial variability. Sites located along the Congo Channel were clearly affected by the frequent turbidity events in this active submarine system. Extremely low meiobenthic densities and very low proportions in the most surficial sediment layers indicated a recent, drastic sediment disturbance. The nematofauna flourishing in the reduced seep sediments is dominated by two species (*Sabatieria mortenseni* Ditlevsen 1921 and *Desmodora* sp.), both present in relatively low numbers but by their large body size representing a strongly elevated total nematode biomass compared to non-seep background sediments. *Sabatieria mortenseni* is a cosmopolitan nematode well adapted to living in anoxic or suboxic sediments. In the REGAB seep, its proliferation and substantial biomass is likely to be related to the use of chemosynthetic food sources.

Through investigations of the nematofauna on the methane-seeping Håkon Mosby Mud Volcano (HMMV, Barents Sea, at 72°N 14°E, 1280 m), a better insight in the function, adaptations and origin of the dominant taxa is acquired. CHAPTER 4 describes the highly significant differences in diversity and density among the meiofaunal assemblages found in the distinct habitats at HMMV. The bare, muddy sediments from the active centre harboured the lowest meiobenthic densities. In this habitat, nematodes were strongly depleted, whereas copepods and nauplii became dominant with densities tenfold those of

the nematodes. In contrast, the highest meiobenthic densities were assigned to one single nematode species, *Halomonhystera disjuncta* Bastian 1865, thriving with extremely high numbers in the bacterial, sediment-covering *Beggiatoa* mats. Enhanced densities and dominance of selected species at the HMMV were controlled by both direct (abiotic) and indirect (bacteria) consequence of sediment geochemistry. Based on biomarker fatty acid and stable carbon isotope analyses of *H. disjuncta* in CHAPTER 5, it was concluded that this species benefits from chemosynthetic derived food sources, in particular from the dense sulphide-oxidising *Beggiatoa* bacteria. Additionally, the dominant copepod species from the volcano's centre was subjected to the same trophoecological analyses, which indicated the utilisation of a methanotrophic food source.

CHAPTER 6 discusses the spatial variation and distribution patterns of the meiobenthos observed at the HMMV and two pockmark sites offshore mid-Norway (64°N 5°E), i.e. the Storegga Slide and Nyegga pockmarks. Three main habitats were distinguished across the different sampling sites with respect to the biogeochemical sediment conditions and associated epifauna: (1) reduced sediment with suboxic conditions, with or without bacterial mats, (2) siboglinid tubeworm patches, and (3) sediment outside the seepage and devoid of chemosynthetic fauna. Background sediments and Siboglinidae fields supported comparable high nematode genus richness similar to other deep-sea sediments, whereas the reduced sediments yielded a genus poor nematode assemblage dominated by one or two successful species. Molecular analyses were applied to unravel the phylogenetic relationship of the dominant *H. disjuncta* from HMMV with the morphologically similar shallow-water species. The mono-specific population of *H. disjuncta* appeared to be strictly associated with bacterial mats at HMMV, and consisted of at least one new cryptic species of the *H. disjuncta* complex (Derycke et al. 2007) documented for intertidal habitats in the North Sea. The reduced sediments at the Nyegga pockmarks comprised high densities of *Terschellingia longicaudata* de Man 1907, another cosmopolitan nematode species described from organic rich, oxygen poor, shallow marine environments. Both ecological and molecular observations point to a past or recent connection between continental margins and shallow-water habitats.

The ecological results from CHAPTER 4 and 6 were combined to examine the nematofauna at micro-, macro- and mega-scale across the different seepage sites along the Norwegian continental margin (730–1280 m, including HMMV, Storegga Slide and Nyegga pockmarks). The major aim of CHAPTER 7 was to determine to what extent habitat heterogeneity and geographical distance contribute to the nematode diversity at different spatial scales. Furthermore, also the importance of different habitats within a single cold seep on the diversity of the corresponding meiobenthos was verified. This study suggested a considerable effect of habitat heterogeneity on the spatial structure and diversity patterns of nematode communities at the macro-scale within a single seep. Some of the characterised assemblages were strongly restricted to a specific seep habitat. These results demonstrated that the

persistence of multiple seepage sites and of distinct habitat types within each seep is necessary to preserve the high diversity of nematode communities occurring in the Norwegian deep sea.

Summarizing, this thesis provides previously unavailable data on the meiobenthic life, and in particular on the nematofauna, inhabiting reduced sediments in the deep sea. The innovative character lays in the comparison of nematode assemblages on genus and species level across several seepage related sites in the North and South Atlantic, completed with the first trophoecological and molecular analyses carried out on a deep-sea seep nematode species (*Halomonhystera disjuncta*). Different habitats distinguished by their typical biogeochemical conditions revealed to be a key factor in shaping the density and diversity of seep nematode assemblages. Molecular analyses provided a strong indication of a dispersal route between the shallow-water habitats and reduced environments in the deep sea. Nevertheless, more molecular analyses should be employed to examine this connection. The unusual success of a single nematode species with an ovoviviparous reproduction strategy in the bacterial mats at HMMV was explained by the trophic relationship with the abundant sulphide-oxidising *Beggiatoa* bacteria. These results point to a very important role of nematode communities in the transfer of methane-derived carbon from primary, microbial producers to higher trophic levels within the reduced environment. Therefore nematodes can be considered as a fundamental biological and trophic part of the cold seep ecosystem in the deep sea.

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[1] *Reduced marine environment*: Marine sediments characterised by the presence of reduced chemical compounds and where energy is supplied mainly through chemosynthetic production. Reduced environments comprise for example hydrocarbon and brine seeps, hydrothermal vents, whale falls, some hypoxic settings, sewage outfalls and seagrass beds (Giere 1993, Levin and Michener 2002).

[2] *Pockmark*: Small-scale, craterlike depression at the seafloor from which the origin is linked to the emission of reduced gasses and fluids from the deeper bottom layers. The name refers to the resemblance with the scar of the chicken pox.

[3] *Xenophyophores*: Marine, giant protozoans wich are encountered in all oceans but reach their highest densities on the abyssal plains. They were originally described in 1889 as sponges. A recent genetic study suggested that they form a specialised group within the Foraminifera. There are about 42 known species, and *Syringammina fragillisima* represents one of the largest species with a maximal diameter of 20 cm.

De ontdekking van gereduceerde[4] milieus in de diepzee en hun merkwaardige gemeenschappen van bacteriën en meercellige organismen was één van de belangrijkste gebeurtenissen in de mariene biologie van de laatste decennia. De verscheidene gereduceerde milieus delen gemeenschappelijke kenmerken zoals een hoog gehalte aan gereduceerde verbindingen (sulfides en hydrocarbonaten), een vaak voorkomend zuurstoftekort, een hoge abundantie en metabolische activiteit van bacteriële populaties, en productie van autochtoon, organisch materiaal door bacteriën, gebruikmakende van energie van chemische verbindingen (chemosynthese). Het meercellig macro- en megafaunaal leven in gereduceerde milieus is in het algemeen gekarakteriseerd door een lage diversiteit (die echter wel kan toenemen naar de periferie van het biotoop), met vaak een hoge mate aan endemisme en bijzondere, biologische kenmerken. Koude bronnen (verder aangeduid met 'cold seeps') in de diepzee zijn één van de meest indrukwekkende gereduceerde milieus en zijn tamelijk frequent bestudeerd sinds hun ontdekking twee decennia geleden. Het merendeel van de biologische studies in cold seeps was echter toegespitst op de megafauna met endosymbionten (Siboglinidae kokerwormen, Mytilidae mossels, Vesicomyidae bivalven), of op de microbiologische processen (Levin 2005). Organismen van intermediaire grootteklasse of de zogenaamde meiofauna (0.032–1 mm) zijn evenwel veel minder intens bestudeerd. Rondwormen of nematoden vormen de meest belangrijke groep van de meiofauna zowel wat betreft densiteiten als biomassa, met daaropvolgend harpacticoide copepoden, nauplii, polychaeten, tardigraden en andere groepen (Giere 1993). Mariene nematoden kunnen functioneren als een nuttig instrument voor het beschrijven van habitat heterogeneïteit in het mariene milieu aangezien zij wereldwijd verspreid zijn, de belangrijkste vertegenwoordigers van de meiofauna vormen en een directe verbinding hebben met het sediment en de processen die plaatsvinden net boven het sediment. In deze thesis worden nematoden als sleutelgroep gebruikt voor het bestuderen van de meiobenthische gemeenschappen die voorkomen in verschillende koude bronnen geassocieerd met 'pockmarks'[5] of moddervulkanen, gelocaliseerd in de Noord- en Zuid-Atlantische Oceaan. De volgende vragen werden behandeld: (1) Welke factoren hebben een belangrijke invloed op de meiobenthosgemeenschap van seeps wat betreft de structuur, diversiteit en distributie over meerdere ruimtelijke schalen? (2) In welke mate benut het meiobenthos de chemosynthetische voedingsbronnen typisch voor seeps? (3) Vertonen meiofaunasoorten morfologische of fysiologische

aangepassingen aan de vaak toxische geochemische condities in seeps? (4) Wat is de specificiteit van de meiofaunasoorten in seeps? Om dit overzicht te verwerven werd de nematofauna gekarakteriseerd in termen van taxonomische samenstelling, distributiepatronen op verschillende ruimtelijke schalen, habitat preferenties, verticale verdeling in het sediment, fylogenie, aanwezigheid van symbionten en voedingsbronnen.

De eerste studie werd uitgevoerd in de Darwin Mounds (59°N 7°W) en een aangrenzend veld van pockmarks in de bathyale Noord-Oost Atlantische Oceaan op ongeveer 1000 m waterdiepte. HOOFSTUK 2 beschrijft de structuur van de meiobenthische gemeenschap op verschillende ruimtelijke schalen in dit gebied. Multivariate analyses van de relatieve abundanties van de genera wezen op geen significante invloed van de grootschalige habitatheterogeneïteit op de verspreiding van de nematoden over de 'carbonate' heuvels. De studie van de kleinschalige biogene structuren, i.e. het dode omhulsel van de 'xenophyophore'[6] *Syringammina fragilissima*, bracht een nematofauna samengesteld uit gelijkaardige genera als die van het omgevende sediment aan het licht, alhoewel een verschuiving naar genera met groter lichaamsvolume werd geobserveerd. De verticale verdeling van de nematoden toonde echter sommige ongewone karakteristieken. Nematoden behorende tot de subfamilie Stilbonematinae werden geïdentificeerd in het sediment vanaf 1 cm diepte en maakten samen tot 20% van de totale nematodengemeenschap in de diepere lagen uit. Deze bevindingen reflecteren waarschijnlijk de aanwezigheid van anoxische, sulfide-aangerijkte micro-milieus in de diepere sedimentlagen. Stilbonematinae nematoden zijn bedekt met ectosymbiotische, chemoautotrofe bacteriën, en waren voordien enkel beschreven van ondiepe, mariene zanden (Ott et al. 1991) en ondiepe hydrothermale bronnen (Kamenev et al. 1993, Thiermann et al. 1997). Dit vertegenwoordigt dus de eerste observatie van drie Stilbonematinae genera (*Leptonemella*, *Eubostrichus*, *Catanema*) in diepzeesedimenten.

Het meiobenthos geassocieerd met een variëteit aan habitats langsheen het Congo kanaalsysteem en in de nabijgelegen cold seep REGAB in de Golf van Guinea (Equatoriaal West-Afrikaanse rand, op 5°S 9°E, 3150–4800 m) werd onderzocht in HOOFDSTUK 3. Verschillende meiofaunale gemeenschappen werden onderscheiden, gecorreleerd met een ruimtelijke variabiliteit op meerdere schalen (van m's tot 200 km). Staalnameplaatsen gelegen langsheen het Congo kanaalsysteem waren duidelijk beïnvloed door de regelmatig voorkomende sterke turbiditeit in dit actief submarien systeem. Extreem lage meiobenthische densiteiten en erg lage proporties in de meest oppervlakkige sedimentlagen waren een indicatie van een recente, ingrijpende verstoring van het sediment. De nematofauna in de gereduceerde seep sedimenten werd gedomineerd door twee soorten (*Sabatieria mortenseni* Ditlevsen 1921 en *Desmodora* sp.); beide aanwezig in relatief lage aantallen, maar hun groot individueel lichaamsvolume resulteerde in een sterk toegenomen totale nematodenbiomassa in vergelijking tot de omgevende sedimenten buiten de seep. *Sabatieria mortenseni* is een kosmopolitische nematode die goed is

aangepast aan het leven in anoxische en suboxische sedimenten. Het succes en de hoge biomassa van deze soort in de REGAB seep is waarschijnlijk gerelateerd aan het gebruik van chemosynthetische voedingsbronnen.

Aan de hand van de studies van de nematofauna op de actieve Håkon Mosby Mud Volcano (HMMV, Barents Zee, op 72°N 14°E, 1280 m), werd een beter inzicht verkregen in de functie, adaptaties en oorsprong van de dominante taxa. HOOFDSTUK 4 beschrijft de sterk significante verschillen in diversiteit en densiteit tussen de meiofaunale gemeenschappen aangetroffen in de aparte habitats op de HMMV. De naakte, modderige sedimenten van het actief centrum herbergden de laagste meiobenthische densiteiten. In dit habitat ontbraken nematoden bijna volledig, terwijl copepoden en naupliuslarven er dominant waren met densiteiten tien maal zo hoog als die van de nematoden. De hoogste meiobenthische densiteiten werden daarentegen toegewezen aan één enkele nematodensoort, *Halomonhystera disjuncta* Bastian 1865, die in extreem hoge aantallen in de bacteriële, sedimentbedekkende *Beggiatoa* matten gedijde. Verhoogde densiteiten en dominantie van geselecteerde soorten op de HMMV werden beïnvloed door zowel directe (abiotische) als indirecte (bacteriën) consequenties van de geochemische sedimentcondities. Gebaseerd op de analyses van vetzuren en stabiele koolstofisotopen van *H. disjuncta* in HOOFDSTUK 5, werd geconcludeerd dat deze soort profiteert van chemosynthetisch afgeleide voedingsbronnen, met name van de abundante sulfide-oxiderende *Beggiatoa* bacteriën. Daarnaast werd de dominante copepodensoort van het centrum van de vulkaan onderworpen aan dezelfde trofo-ecologische analyses; hieruit bleek dat de copepode gebruik maakt van een methanotrofe voedingsbron.

HOOFDSTUK 6 bediscussieert de ruimtelijke variatie en distributiepatronen van het meiobenthos geobserveerd op de HMMV en twee pockmarks op de continentale rand van Noorwegen (64°N 5°E), i.e. de Storegga Slide en Nyegga pockmarks. Op basis van de biogeochemische sedimentcondities en de geassocieerde epifauna werden drie belangrijke habitats onderscheiden: (1) gereduceerd sediment met suboxische condities, met of zonder bacteriële matten, (2) velden van Siboglinidae kokerwormen en (3) sediment buiten de invloed van de seep en zonder chemosynthetische fauna. De omgevende diepzeesedimenten en de Siboglinidae velden hadden een hoge rijkdom aan nematodengenera, gelijkaardig aan die van andere diepzeesedimenten. In tegenstelling hiermee herbergden de gereduceerde sedimenten de meest genusarme nematodengemeenschappen, telkens gedomineerd door één of twee succesvolle soorten. Moleculaire analyses werden toegepast om de fylogenetische relatie tussen de dominante *H. disjuncta* van HMMV met de morfologisch gelijkaardige soort van ondiepe habitats te ontrafelen. De monospecifieke populatie van *H. disjuncta* was schijnbaar uitsluitend geassocieerd met de bacteriële matten op HMMV, en bestond uit minstens één nieuwe cryptische soort van het *H. disjuncta*-complex (Derycke et al. 2007), gedocumenteerd voor de intertidale habitats in de

Noordzee. De gereduceerde sedimenten in de Nyegga pockmarks bevatten hoge densiteiten van *Terschellingia longicaudata* de Man 1907, een andere kosmopolitische nematodensoort die beschreven werd in organisch-rijke, zuurstofarme, ondiepe mariene milieus. Zowel de ecologische als moleculaire observaties wijzen op een vroegere of recente connectie tussen continentale randen en ondiepe mariene habitats.

De ecologische resultaten van Hoofdstuk 4 en 6 werden samengevoegd om vervolgens de nematofauna op micro-, macro- en mega-schaal over de verschilende seep-gerelateerde milieus langsheen de Noorse continentale rand (730–1280 m, inclusief HMMV, Storegga Slide en de Nyegga pockmarks) te onderzoeken. HOOFDSTUK 7 richt zich op het determineren van de mate waarin habitatheterogeneïteit en geografische afstand bijdragen tot de nematodendiversiteit op verschillende ruimtelijke schalen. Daarnaast werd eveneens het belang van verschillende habitats binnen eenzelfde seep op de diversiteit van het aanwezige meiobenthos nagegaan. Deze studie suggereerde een aanzienlijk effect van habitatheterogeneïteit op de ruimtelijke structuur en diversiteitspatronen van nematodengemeenschappen op een macro-schaal binnen één enkele seep. Sommige van de gekarakteriseerde gemeenschappen werden aangetroffen in één enkel specifiek habitat binnen de cold seep. Deze resultaten toonden aan dat het bestaan van talrijke cold seeps en van verschillende habitattypes binnen elke afzonderlijk seep noodzakelijk zijn voor het behoud van een hoge diversiteit van nematoden in de Noorse diepzee.

Samenvatttend verstrekt deze thesis voordien onbeschikbare data over het meiobenthisch leven, in het bijzonder over de nematofauna, die voorkomt in gereduceerde sedimenten in de diepzee. Een vernieuwend element is het vergelijken van nematodengemeenschappen op genus- en soortsniveau over meerdere cold seeps in de Noord- en Zuid-Atlantische Oceaan, aangevuld met de eerste trofo-ecologische en moleculaire analyses uitgevoerd op een diepzeenematodensoort uit cold seeps (*Halomonhystera disjuncta*). Verschillende habitats, onderscheiden door typische biogeochemische condities, bleken een sleutelfactor voor het bepalen van de densiteit en diversiteit van nematodengemeenschappen in seeps. Moleculaire analyses verstrekten een sterke indicatie van een dispersieroute tussen ondiepe mariene habitats en gereduceerde milieus in de diepzee. Aanvullende moleculaire analyses zijn echter noodzakelijk om deze connectie te onderzoeken. Het ongewone succes van één enkele nematodensoort met een ovovivipare reproductiestrategie in de bacteriële matten van HMMV werd verklaard door de trofische relatie met de abundante sulfide-oxiderende *Beggiatoa* bacteriën. Deze resultaten wijzen op een heel belangrijke rol van nematodengemeenschappen in de overdracht van methaan-afgeleide koolstof van de primaire, microbiële producenten tot de hogere trofische niveaus in het gereduceerd milieu. Nematoden kunnen

daarom beschouwd worden als een fundamenteel biologisch en trofisch onderdeel van het ecosysteem van koude bronnen in de diepzee.

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[4] *Gereduceerd marien milieu*: Mariene sedimenten gekarakteriseerd door de aanwezigheid van gereduceerde chemische componenten en waar de energie voornamelijk afhankelijk is van chemosynthetische productie. Gereduceerde milieus zijn onder andere koude bronnen, hydrothermale bronnen, walviskarkassen, sommige hypoxische milieus, lozingspunten van riolen en zeegrasvelden (Giere 1993, Levin en Michener 2002).

[5] *Pockmark*: Kleinschalige, kraterachtige depressie in de zeebodem, waarvan het ontstaan in verband gebracht wordt met het ontsnappen van gereduceerde gassen en vloeistoffen vanuit de diepere bodemlagen. De naam verwijst naar de gelijkenis met het litteken van de pokken.

[6] *Xenophyophores*: Mariene, reusachtige ééncellige organismen die aangetroffen worden in alle oceanen, maar in de grootste aantallen op de abyssale vlaktes. Zij werden oorspronkelijk in 1889 als sponzen beschreven. Een recente genetische studie suggereerde echter dat zij een gespecialiseerde groep binnen de Foraminifera vormen. Er zijn naar schatting 42 gekende soorten waarvan *Syringammina fragillisima* met een maximale diameter van 20 cm één van de grootste is.

CHAPTER 1
General introduction, aims and thesis outline

General introduction, aims and thesis outline

1. THE DEEP-SEA TOPOGRAPHY

Ninety per cent of the complete ocean floor lies beyond the shallow margins of the continents, and the largest fraction is located at more than 2 km water depth. Therefore, the deep-sea bottom can be considered as the most extensive, continuous environment on earth. Yet, it is one of the most remote and difficult habitats to observe and sample. Seafloor spreading and sedimentation of inorganic and organic particles structure the topography of the deep-sea bottom which starts at the shelf break at circa 200 m (Gage and Tyler 1991). Seaward of the continental shelf, the continental slope is characterised by evenly spaced and parallel isobaths, gradually increasing in water depth. The slope marks the underlying boundary between the oceanic and the continental crust, which consists of a dynamic system of plates. Crust is being formed at mid-ocean ridge spreading centres and is consumed by subduction at 'active' or seismic margins, often observed in the Pacific. 'Passive' or aseismic margins are typically found in the Atlantic Ocean. The continental slope may be interrupted by submarine canyons, recognised as irregular channels cutting down the slope and acting as conduits for transport to the deep ocean basin beyond. Positive topographic features (hundreds of m's to a few km's in diameter) encountered on the slope and rising up several tens of meters above the seafloor are for example carbonate mounds and mud volcanoes. Eventually, the seabed levels off and ends in a wide and relatively flat abyssal plain extending from 4 km to 6 km water depth.

In the last decades, a more intensive sampling of the continental margins using manned submersibles as well as ROV devices, has provided evidence for a high habitat heterogeneity with a variety of fascinating ecosystems such as cold water coral reefs (De Mol 2002, Freiwald 2002, Freiwald et al. 2004, Roberts et al. 2006) and cold seeps (Sibuet and Olu 1998, review in Levin 2005). An incredibly high diversity of unique ecosystems (e.g. open slopes, deep-water coral mounds, cold seeps, anoxic systems, canyons) is also found along the continental margins in Europe, comprising the research topics of the HERMES (Hotspot Ecosystem Research on the Margins of European Seas) project, an Integrated EU-FP6 project.

2. Hotspot Ecosystem Research on the Margins of European Seas (HERMES) [1]

The HERMES project which started in April 2005 was designed to gain insights into the biodiversity, structure, function and dynamics of the so-called "hotspot" ecosystems along the Europe's deep ocean margin. Hotspot ecosystems are discontinuous environments that are characterised by chemical, physical, topographic and geological factors, and that contain a wealth of species. Some study sites are located at open slopes where biological communities are affected by landslides and deep-ocean currents while other sites provide a habitat to ecosystems that are dependent on fluids escaping from the seabed, known as cold seeps. Cold-water coral mounds, canyon communities and microbial driven ecosystems in anoxic environments are also investigated by HERMES scientists. All of these ecosystems are of interest either because of their special biodiversity, their fragility and/or because of their economic importance.

The HERMES program is unique because it is the first significant attempt to study ecosystems and their environments along Europe's deep-ocean margin by using a multi-disciplinary approach. In order to understand these hotspot ecosystems, many scientists and researchers in the fields of biodiversity, geology, sedimentology, physical oceanography, microbiology and biogeochemistry, Geographic Information Systems (GIS), data management and socio-economics are involved.

This thesis will focus on a number of seep structures that encompass some of the HERMES hotspot ecosystems in the North Atlantic: the Håkon Mosby Mud Volcano in the Nordic Sea, pockmarks at the Storegga Slide and nearby Nyegga area off mid-Norway, and the carbonate mounds (Darwin Mounds) in the Rockall Trough and associated pockmarks. For each of these study areas, a detailed study of the metazoan meiofaunal assemblages was carried out in several habitat types with contrasting biogeochemical conditions. The group of meiofauna is in this study defined as the total of metazoan benthic organisms passing through a 1 mm sieve and retained on a 32 µm sieve. Meiofaunal communities are composed of two groups. Temporary meiofauna are those juveniles of the macrofauna that will eventually grow into larger organisms. Permanent meiofauna are those groups where adults are less than one mm in length, e.g., Nematoda, Copepoda, Gastrotricha, Turbellaria, Acari, Gnathostomulida, Kinoryncha, Tardigrada, Ostracoda, and some Nemertinea, Oligochaeta, and Polychaeta. The two standard meiofauna texts (Higgins and Thiel 1988, Giere 1993) were used in the identification of major taxonomic groups.

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[1] http://www.edu-hermes.org

3. BIOZAIRE PROJECT [2]

'BIOZAIRE' refers to the study of the benthic communities, and the physical and chemical environmental characteristics in the deep sea influenced by the Zaire canyon (now called Congo canyon). This program was developed in 2000 by IFREMER (Institut Français de Recherche pour l'Exploitation de la Mer) in the framework of investigating the West African margins in collaboration with the oil company Total.

The studied area was characterised by contrasting environmental conditions, mainly driven by the activities of one of the world's largest and very active submarine canyons, the Congo Canyon/Channel system that extends westward off the Congo-Angola margin for 760 km down to the abyssal plain at 4900 m depth. The Congo Channel has been shown to carry large quantities of particulate matter originating from the continent through intense turbidity currents that were recorded down to 4800 m depth in the channel.

Concerning the detritus-based ecosystems, the aim of BIOZAIRE was to understand the dynamic of the energy inputs, their origins and their consequences on the biodiversity of the benthic communities on 4 stations: two stations (1350 m and 4000 m depth) under the pelagic and continental input, one (4000 m) under the influence of the Congo Canyon, the last one (3500 m) in the vicinity of a cold seep area. Concerning the chemosynthetic based ecosystems, an area on the Gabon margin at 3500 m water depth was prospected.

The program mainly aimed to: (1) characterize the physical and chemical conditions of the environment, (2) determine the source and intensity of inputs of matter, as well as their variations over time, (3) complete the description of the various benthic communities encountered, and (4) understand the interactions between detritus-based and chemosynthesis-based ecosystems. Within this BIOZAIRE project, a detailed metazoan meiobenthic study was conducted at the stations affected by the Congo Channel, and the pockmark complex on the northern border of the Congo Canyon.

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[2] http://www.ifremer.fr/francais/produits/zaiango/biozaire.htm

4. REDUCED MARINE ENVIRONMENTS

Marine environments become reduced and contaminated with dissolved hydrogen sulphide in two general modes: (1) in marine soft bottoms, rich in organic matter, hydrogen sulphide is developed by the microbial reduction of oxidised sulphur species, mainly of sulphate; (2) volcanic, geothermal and other geological activities often lead to the venting of water and gasses rich in hydrogen sulphide, often combined with rich amounts of methane and ammonium. As manifold as the processes and origins are the areas where sulphide biotopes can be encountered: from deep-sea cold seeps and hydrothermal vents to the subsurface layers of tidal mudflats and mangroves, from marine basins like the depth of the Baltic Sea and the Black Sea ot meromictic lakes, from local local gas seeps connected to submarine oil fields to coastal muds polluted by sewage. All of these diverse biotopes have been found to harbour, at least temporarily, meiobenthic organisms (Giere 1993).

5. SEEP ENVIRONMENTS IN THE DEEP SEA

Recently conducted ocean margin research has provided evidence for a wide range of ecosystems associated with fluid, gas and mud escape structures. Habitats like gas emitting pockmarks and mud volcanoes are colonised by bacterial mats, chemosynthetic fauna and a variety of associated organisms. Cold seep systems bear a wide range of microhabitats such as surface and subsurface sediments, oxic and anoxic patches, mineral concretions and symbiotic megafauna such as tubeworm aggregations and bivalve beds. Systems like the mud volcanoes and pockmarks represent distinct geological structures on continental margins, and are excellent natural laboratories for investigation of chemosynthetic-driven ecosystems.

5.1. GLOBAL DISTRIBUTION AND GENERAL FEATURES

Seeps are among the most geologically diverse of the reducing environments explored to date, and new sites are still being reported every year (Figure 1). Since the first discovery of such a system 25 years ago on the Florida Escarpment in the Gulf of Mexico (Paull et al. 1984), cold seep communities have been identified in a broad range of tectonic settings. Active seeps have been reported from all oceans of the world, even in the Antarctic regions (Domack et al. 2005), on both passive and active margins at depths ranging between 15 and 7400 m (Sibuet and Olu-Le Roy 2002, Levin 2005 and

references therein). Indirect evidence suggests that seep communities may even occur in trenches at 9–10 km depth (Mironov et al. 2000).

Cold seeps occur where chemically modified fluids are released into the ocean without an appreciable temperature rise above the seafloor. Seep ecosystems may be fuelled by a variety of organic hydrocarbon sources, including methane, petroleum, other hydrocarbon gasses and gas hydrates, which are only stable below about 500 m (Sloan 1990) (Figure 2). All of these sources are ultimately of photosynthetic origin because they are generated from accumulations of marine or terrestrial organic matter.
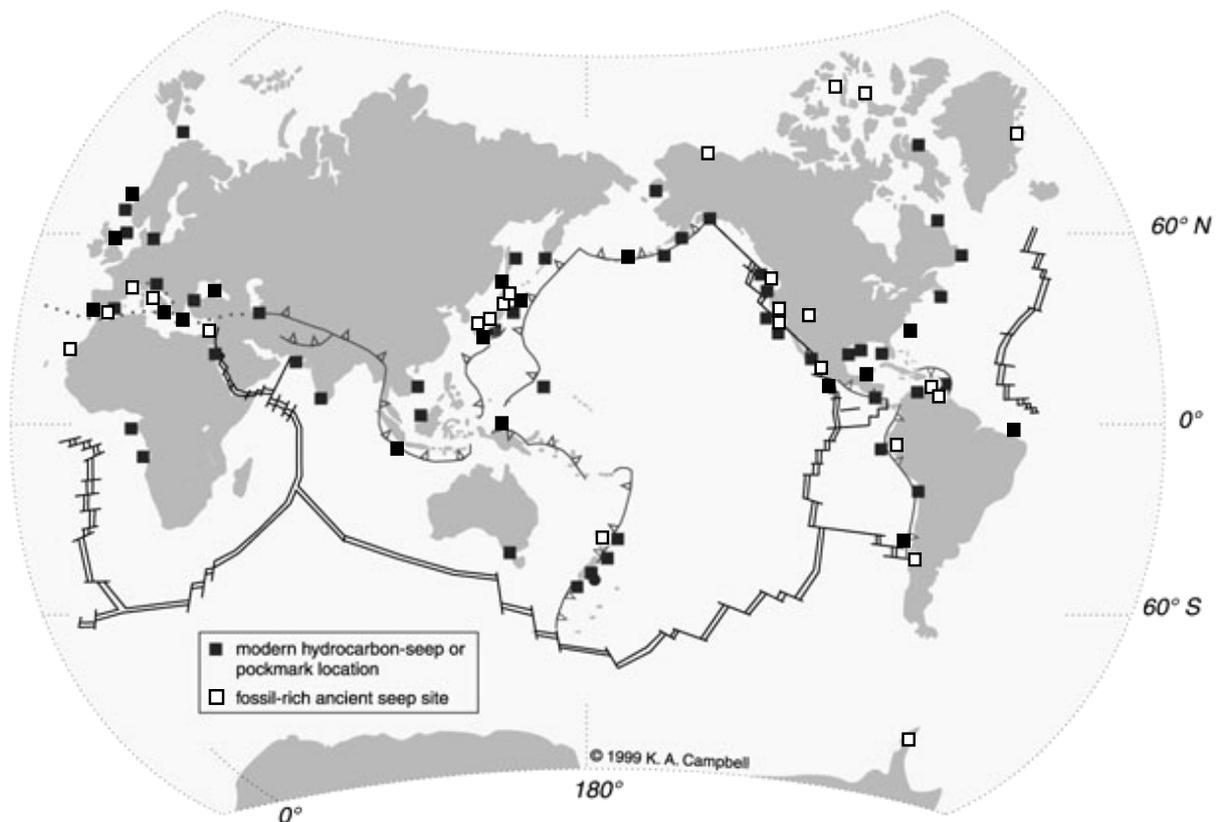
Figure 1: Disbribution map of modern and ancient hydrocarbon seeps. Seep distribution is from Hovland and Judd 1988, with additions from Corselli and Basso 1996, Acosta et al. 2003, Sibuet and Olu-Le Roy 2002, Domack et al. 2005, Levin 2005, Van Rensbergen et al. 2005, Cambell 2006 and references therein, Sergeeva and Gulin 2007.
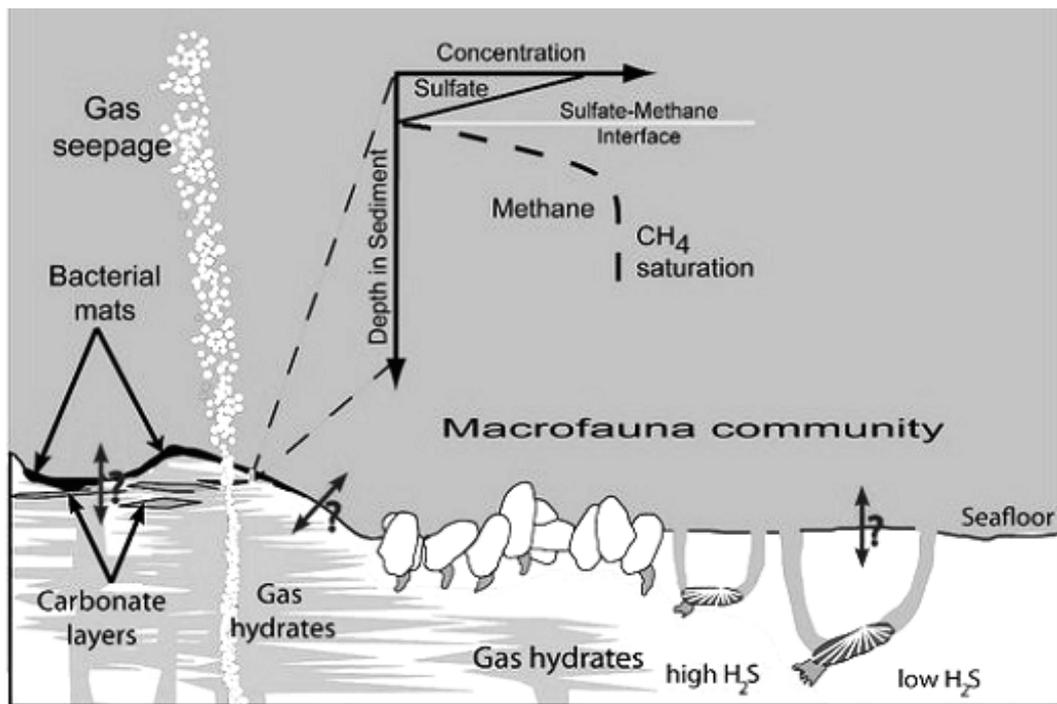
Figure 2: Scheme of biogeochemical relationships at seep locations illustrating the chemosynthetic megafauna and microbial mats, including a graph concerning sulphate and methane concentration in relation to sediment depth [3].

Another geochemical feature typical for cold seep environments is the presence of high concentrations of sulphide resulting from the reduction of sulphate and coupled to the anaerobic oxidation of methane (AOM, Boetius et al. 2000) (Figure 2). The AOM is carried out by several groups of Archaea, living in syntrophic consortia with sulphate reducing bacteria from the *Desulfosarcina/Desulfococcus* an *Desulfobulbus* groups (Orphan et al. 2002). The overall reaction involving methane oxidation and sulphate reduction can be formulated as:

$$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$$

High rates of this reaction generate a large microbial biomass that can provide a significant supply of methane-derived carbon to the microbial community. Heterotrophic bacteria play an important role in transferring this carbon to higher-order consumers, where it is recognised by light $\delta^{13}C$ ratios (Levin and Michener 2002). One of the typical features at cold seeps is the presence of dense microbial mats,

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[3] http://www.marum.de/English/Project_E3.html

sometimes covering an extensive surface of the seep sediments. These microbial mats, often very patchily distributed, usually comprise a mixture of taxa but are dominated by large filamentous sulphide oxidising bacteria (*Beggiatoa, Thioploca, Acrobacter, Thiotrix*). Mats are coloured in white, yellow, orange or grey. Some of these microbes, such as the filamentous *Beggiatoa* bacteria, have a gliding movement with which they position themselves within a steep gradient of oxygen and sulphide, tolerating only low amounts of both (Jørgensen and Revsbech 1983). Despite the harsh geochemical conditions that are typical for these microbial mats, they can support specialised metazoan communities, often dominated by a few adapted genera or species (Buck and Barry 1998, Bernhard et al. 2001, Levin et al. 2003, Robinson et al. 2004, Levin 2005).

5.2. GEOCHEMISTRY OF SEEPS

5.2.1. BRINE SEEPS

Brine seeps are recognised by the migration of warm, hypersaline fluids through fissures in the underlying sediment. Due to the high density of the brine, it is trapped at the sediment surface after cooling down to the ambient temperature. Within these concentrated brine pools, only extremophiles such as certain bacteria can live. Methane is present in high concentrations in these areas, providing energy by the process of chemosynthesis to organisms which live in the pool's vicinity. Dense communities of methanotrophic mussels are observed to cluster around the pool's edge.

One of the most discussed sites is the sulfurous brine seep at 72 m water depth at the East Flower Garden bank, northwest Gulf of Mexico, which produces conditions conducive to the growth of a luxuriant prokaryotic biota. Bright et al. (1980) and Brooks et al. (1979) reported on the geochemistry of this brine lake and confirmed that the source of the brine are the salt bodies that underlie the Gulf of Mexico shelf. The brine's salinity reaches nearly 200‰ leading to a sharp density difference that prevents much mixing at the interface between the brine pool and overlying water. The brine is anoxic and Brooks et al. (1979) found H_2S levels of 2200 µM inside the brine pool. The geochemical signature confirmed that the high levels of dissolved organic carbon and hydrocarbon gas (methane, ethane, and propane) are consistent with underlying salt deposits that trap oil and gas in the subsurface (Brooks et al. 1979).

Brines have been suggested as a possible setting for the Burgess Shale type preservation (Powell 2005). The pools of hypersaline, anoxic brine on the continental slope of the Gulf of Mexico promote the preservation of organic material to an exceptional degree. One type of brine pool, the brine-filled pockmark, maintains by the emission of nutritive methane dense beds of symbiont-containing mussels at the pool's edges. Fish, attracted by this abundant food supply, are subjected to a lethal concentration of H_2S, anoxia and osmotic stress. When the fish die and sink into the pool, they are preserved by the brine, and possibly buried by the collapsing walls of the pockmark. These features may lead to unusually fine preservation of soft anatomy, often referred to as Lagerstätte deposits (MacDonald 1992, Parsons-Hubbard et al. 2008).

5.2.2. HYDROCARBON SEEPS

The fluids discharged at the seafloor at marine hydrocarbon seeps consist of petroleum, methane or other hydrocarbon gasses. These seep fluids have either a biogenic source, i.e. bacterial production of gas, or a thermogenic source, i.e. relating to subsurface petroleum reservoirs that leak to the surface. Some seep gasses arise from the dissociation of methane hydrates. Most seeps release methane with trace gasses including n-alkanes (ethane, propane, etc.) and hydrogen sulfide. Carbon dioxide can be important or even dominant.

Natural petroleum seepage is estimated to contribute 10% of the total hydrocarbons entering the oceans annually (Wilson et al. 1973). The Isla Vista oil seep in the Coal Oil Point region of the Santa Barbara Channel was explored by Montagna and Spies (1985). The oil seep area is curious; *Beggiatoa* mats were observed directly over the active seepage where gas bubbles and oil droplets rise to the sediment surface. The petroleum is used by heterotrophic degraders as carbon source, while sulphide is the energy source via oxidizing bacteria. Meiofauna feeds on both these types of bacteria, and is itself consumed through macrobenthic predation and larval fish predation. In this way, the petroleum-based carbon gets eventually into the highest levels of the marine food web (Montagna and Spies 1985).

Methane seeps are broadly distributed along the tectonically active and passive margins (Levin 2005). Investigators have described highly specialised, symbiont-bearing megafauna and complex microbial assemblages. Spatial variability in the abundance and composition of seep fauna has been attributed to the magnitude of fluid flow and the correlated chemical depth profile (Barry et al. 1997, Sahling et al. 2002, Levin et al. 2003). Low net flow rates appear to provide sufficient methane from depth to fuel the

near-surface communities while still allowing downward transport and mixing of oxygen- and sulphate-rich seawater in the upper few centimeters of the sediment. More intense fluid flow is associated with altered porewater composition and elevated sulphide concentrations extending to the sediment surface and allowing the growth of microbial mats (Tryon and Brown 2001, Levin et al. 2003). The seepage of reduced fluids results in a broad range of geological and sedimentary constructs. The most conspicuous manifestation of seepage is bubbles escaping from the seabed, which can be visualised by film or video or observed as acoustical plumes through echo sounding. Topographic depressions (pockmarks) sometimes result from escaping gas but topographic highs (mounds, mud volcanoes, mud diapirs) may also be raised by seeping gas and are equally common.

5.3. GEOLOGICAL STRUCTURES FORMED BY SEEPS

5.3.1. MUD VOLCANOES

Mud volcanoes are geological structures where mud and fluid are seeping through the seafloor and which are located at tectonically active or inactive areas of continental margins. Mud volcanism is a phenomenon typically driven by overpressured sediment in subduction zones and by salt tectonism in passive margins. Large quantities of methane are often released at the surface of mud volcanoes (Olu-Le Roy et al. 2004). Similar to the process at cold methane seeps, archaea/bacteria consortia at mud volcanoes use this methane and produce sulphides in the sediment by chemoautotrophic processes (Boetius et al. 2000) whereas symbiotic bacteria use methane or sulphide to sustain high biomass production in large-sized invertebrates (Fiala-Médioni and Felbeck 1990). With regard to the global climate change, the study of these gas seeps is an important contribution to our understanding and quantification of the methane cycle. Also, knowledge of how life can be based on methane as energy source is important for our view on the evolution of the earth's atmosphere, which was rich in methane and poor in oxygen for the largest part of it's existence.

To date about 800 offshore located mud volcanoes have been recorded, and it was estimated that more than 10 000 would exist in deep marine waters (Jerosch et al. 2007, and references therein). However, up to now, the Håkon Mosby Mud Volcano (HMMV), located at the continental slope of the Barents Sea, is the only active mud volcano reported from high northern latitudes (Figure 3a). Estimations of the size of the different habitats present at HMMV have been made in a predictive study based on video-mosaic transects that partially covered the volcano in 2003 (Jerosch et al. 2007). Approximately 16% (115 165

m²) of the flat centre is nearly devoid of any benthic communities. This area is considered as a region of high methane discharge into the bottom water. *Beggiatoa* mats (Figure 3b) cover an area of 5% (38 244 m²) located in the south-eastern part, while the hummocky outer part is colonised dominantly by siboglinid tube worms (*Oligobrachia haakonmosbiensis* and *Sclerolinum contortum*) (37%, 276 121 m²) (Figure 3c) and only occasionally by *Beggiatoa*. The chemoautotrophic communities as *Beggiatoa* and Siboglinidae are indicators for a significant density of efficient "biofiltering" microbial organisms which limit the release of methane from the sediments to the water column. The present mud flow ascends close to the northern edge of the flat unit of HMMV, and is drained in a south-south-eastern direction (Jerosch et al. 2007). The meiobenthic communities at HMMV and its different habitats were explored in several chapters of this thesis.

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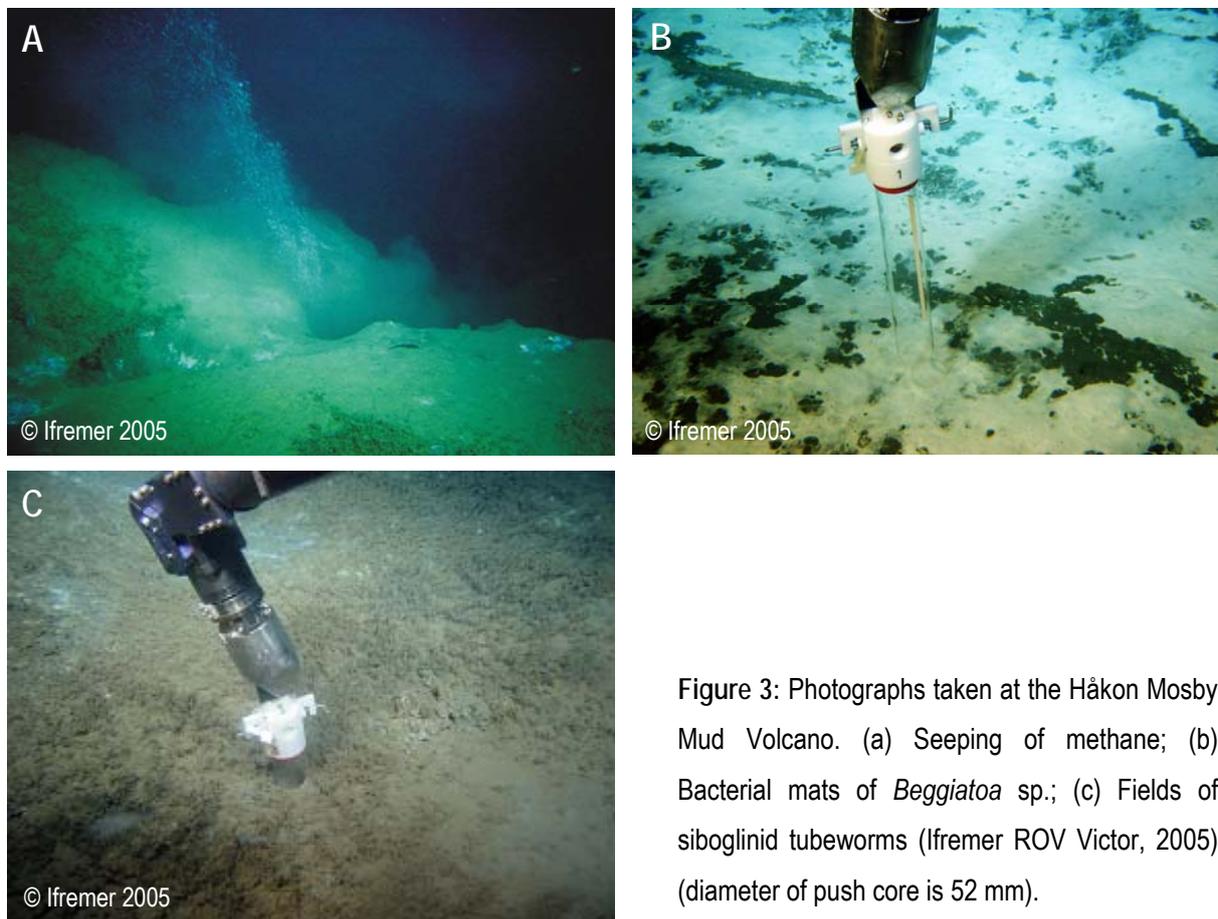
© Ifremer 2005

Figure 3: Photographs taken at the Håkon Mosby Mud Volcano. (a) Seeping of methane; (b) Bacterial mats of *Beggiatoa* sp.; (c) Fields of siboglinid tubeworms (Ifremer ROV Victor, 2005) (diameter of push core is 52 mm).

5.3.2. POCKMARKS

The most common seep-related features at continental margins are pockmarks, although mud volcanoes are also quite common (Acosta et al. 2003). Pockmarks are recognised as crater-like

depressions that commonly occur world-wide on muddy seabeds (Hovland et al. 2002). Such features are generally inferred to be associated with fluid expulsion, and imply overpressures in the subsurface, similar to mud volcanoes. Pockmarks occur as discrete features, or as aggregated structures forming extensive pockmark fields (up to 1000 km², Kelley et al. 1994), and are observed in a broad range of marine geological settings, including shallow bays, continental shelves, continental rises and slopes, and hydrocarbon provinces (Ussler III et al. 2003, and references therein).

Numerous pockmarks shape the morphology of the seafloor at the continental margin off Norway, which is marked by the Storegga Slide scar (Bugge 1983, Bünz 2003, Hovland et al. 2005). This slide scar was produced by a series of giant Holocene slope failures. The Storegga Slide area is estimated to have involved several thousand (>3000) cubic kilometers of material (Haflidason et al. 2004). A new type of pockmarks was recently discovered adjacent to the Storegga Slide in the so-called Nyegga area. They are circular in plane view and contain up to 10 m high ridges of methane-derived authigenic carbonate rock. Seabed surveys revealed the existence of an apparently pockmark-specific micro- and macrofauna, including bacterial mats, fields of small tubeworms (*Oligobrachia haakonmosbiensis* and *Sclerolinum contortum*), and large basket stars (Figures 4a, b). In addition to the typical discoloration of the seafloor at spots with bacterial mats, slimy and "fluffy" filaments were seen undulating in the currents set up by an ROV (Hovland et al. 2005). Based on a synthesis of visual, geophysical, geochemical, and mineralogical data, Hovland et al. (2005) concluded that the complex pockmarks at Nyegga have only had one violent episode, i.e. the one that formed the circular complex features. Subsequent seepage is expected to have a micro-seepage nature (Hovland et al. 2005). The Storegga Slide and Nyegga pockmarks were visited by the RV 'PourquoiPas?' during a multidisciplinary cruise, and were subsequently subjected to a thorough meiobenthic study within this thesis.

In addition, the meiofaunal communities associated with different stations along the Darwin Mounds and neighbouring pockmark depressions in the northern Rockall Trough (NE Atlantic) were examined. These mounds are a group of small, cold-water coral-topped mounds, often with 'tail-like' features which occur at 1000 m water depth. Sidescan sonar images revealed a field of circular seabed pockmarks, typically around 50 m in diameter, located immediately south of the Darwin mounds (Masson et al. 2003, Kiriakoulakis et al. 2004). Masson et al. (2003) concluded that both mounds and pockmarks were created by fluid escape from below the seafloor. Mounds would occur when fluid escape carries subsurface sand to the surface, where it accumulates in mounds because the prevailing bottom currents are not strong enough to disperse it. Pockmarks form where subsurface sand is largely absent and the muddy material is eroded by fluid escape but dispersed by bottom currents. The seafloor around the

pockmarks consists of uniform, heavily-burrowed, muddy sediments and no specific biological communities, nor any sedimentological or photographic evidence for active seepages, were observed. The sharpness of the distinct pits (< 5 m wide) that occur within the otherwise featureless pockmark depressions is the only evidence, albeit tentative, for possible present-day pockmark activity (Masson et al. 2003).
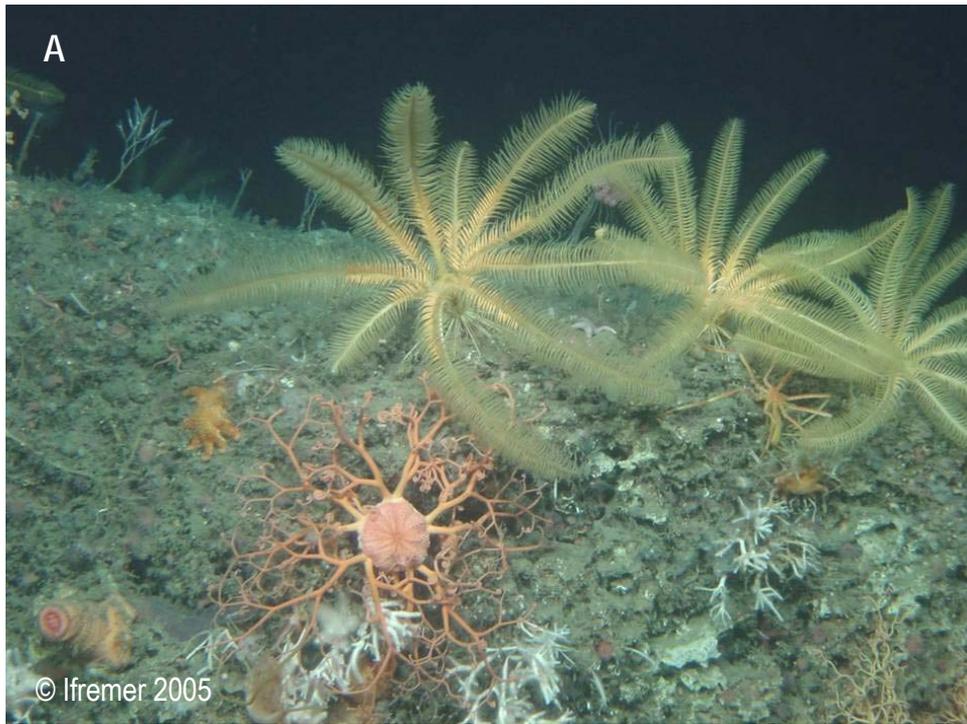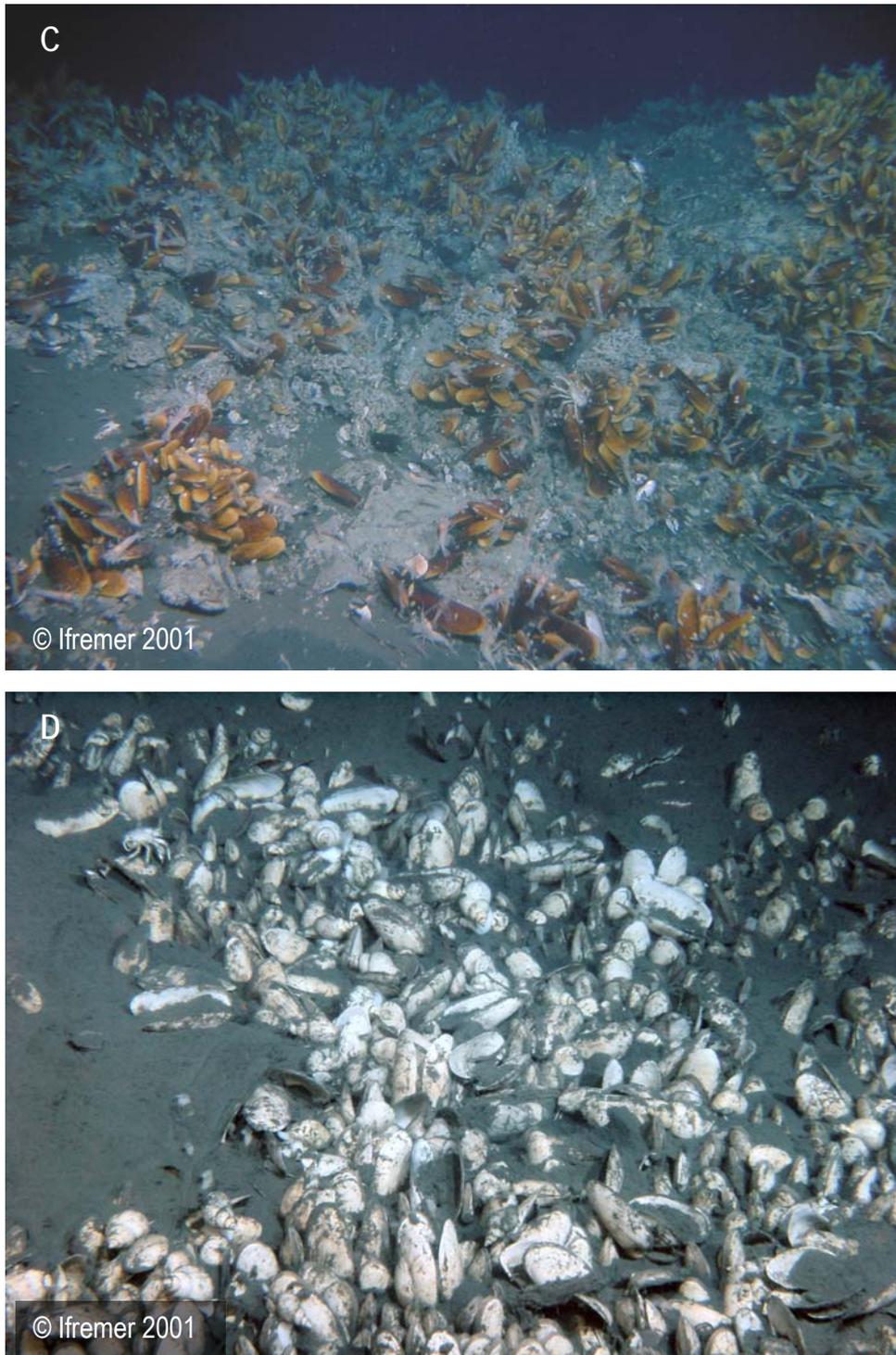
Figure 4: (a) and (b) Perched anemones, hydrozoans and other unidentified sessile organisms on the carbonate rocks of the Nyegga pockmarks (off Norway), accompagnied by numerous basket stars (ROV Victor, Vicking, chief scientist H. Nouzé); (c) Mytilidae site (M2) and (d) Vesicomyidae site (V1) in the pockmark REGAB in the Gulf of Guinea (ROV Victor, BIOZAIRE 2, chief scientist M. Sibuet).

Within the BIOZAIRE project, a giant pockmark complex in the Gulf of Guinea, and in addition also the nearby Congo Channel were sampled for meiobenthic analyses. This so-called REGAB pockmark has a depression of 15–20 m and is composed of several individual pockmarks < 100 m in diameter. Several types of macrofaunal assemblages, either dominated by bivalves of the families Mytilidae (Figure 4c) or Vesicomyidae (Figure 4d), or by siboglinid polychaetes (*Escarpia southwardae*) were mapped over the 800 m diameter pockmark area (Olu-Le Roy et al. 2007).

6. Chemosynthetic benthic communities

Complex ecosystems occur on active and passive continental margins. Their complexity is linked to the environment which favours either detritus-based communities, usually with a high biodiversity of mainly small-sized species, or chemosynthesis-based communities. Detritus-based communities depend indirectly on the photosynthetic production in the upper water layers of the ocean and may be coupled to seasonal variations of phyto-zooplankton cycles. Chemosynthesis-based communities on the other hand, depend on autochtonous, local chemical energy which is the basis for organic carbon production in large quantities (Sibuet and Olu-Le Roy 2002). Within the rather homogeneous deep-sea environment, these highly productive communities associated with patchy, energy-rich hydrothermal vents and cold seeps have drawn considerable interest due to their dependence on microbial chemoautotrophy. Among the most remarkable of the fauna exploiting the abundant chemical energy of vents and seeps are the symbiont-bearing species. These mostly sessile taxa, such as clams, mussels and siboglinid tubeworms, cluster in areas where fluids rich in reduced chemicals exit from the seafloor and mix with ambient seawater (Bergquist et al. 2003).

In the rather monotonous and nutrient-poor environment of the deep sea, scientists discovered in 1977 the first hydrothermal vent during the exploration of the Galápagos Rift, which is a spreading centre in the equatorial eastern Pacific (Van Dover et al. 2002). These clearly productive, but isolated, faunal 'oases' harbour dense colonies of bivalve mollusks and giant tubeworms, making up biomass concentrations ranging from 10 to 70 kg m^{-2} wet weight (Gage and Tyler 1991), which are many orders of magnitude greater than those supported by the deep-sea floor nearby. It was found that these vent and seep systems support biomass standing crops comparable to some of the most productive marine systems (e.g., Tunnicliffe 1991, Lutz and Kennish 1993, Dando et al. 1994, Sarrazin and Juniper 1999, Tunnicliffe et al. 2003). Furthermore, these unique ecosystems show no dependency on the input of organic matter from the overlying water column, but they rely on energy produced by chemoautotrophic microbes, either as free-living prokaryotes or living as symbionts within the body tissues of the aberrant

fauna. Since the first discovery, numerous chemosynthetic systems, associated with several forms of submarine seepage, have been explored around the globe (Van Dover et al. 2002). Ecosystems similar to those of hydrothermal vents were subsequently also found at cold seeps (Levin 2005).

The deep-sea hydrothermal and cold seep communities have been rather intensively studied since their discovery. Now we have more or less full awareness of their taxonomic composition and of their biochemistry and physiology. The bacterial activity as well as the feeding, respiration, growth and other characteristics of the megafauna as consumers and symbiotic partners of the bacteria have been examined. However, infaunal organisms of intermediate size or meiofauna have been studied to a lesser extent. The metazoan meiofauna includes important densities of free-living round worms or nematodes and in decreasing order of abundance also harpacticoid copepods, tiny polychaetes, ostracods, kinorhynchs, and many others. In other investigations, larger shelled protists (foraminifers) were sometimes counted in addition to the meiofaunal metazoans and even outnumbered many other groups. The meiobenthos plays a very important role everywhere on the sea-bottom, creating microstructure and micro-landscapes of sediments, consuming and stimulating the growth of bacterial populations, recycling of nutrients and serving as food for larger animals. However, there are few data obtained up to now on nematodes and meiofauna associated with deep-sea vent and seep ecosystems.

6.1. NUTRITIONAL STRATEGIES OF SEEP FAUNA

Since the discovery of chemosynthesis as a major nutritional pathway at hydrothermal vents, chemosynthetic production has been identified as the main energy source also in several cold seep environments (Paull et al. 1984). Methane, oil and brine seeps support filamentous chemoautotrophic bacterial mats and invertebrates with endosymbionts. The common requirement of seep communities is the presence of a reduced compound, typically hydrogen sulphide or methane, that can be oxidized by bacteria to release energy for the fixation of organic carbon from CO_2 or methane (Van Dover et al. 2002). Most attention has been paid to the trophic position of the megafaunal and macrofaunal taxa inhabiting these cold seeps. Because the carbon fixation through photosynthesis and chemosynthesis involves distinct isotope fractionation, stable isotopic signatures have been useful to investigate the nutritional modes of seep fauna (Levin and Michener 2002). Microbial processes such as bacterial sulphide oxidation, sulphate reduction and AOM are particularly important in seep sediments and generate isotopically light $\delta^{13}C$ signatures. Methane gas itself can have $\delta^{13}C$ values of –45‰ to –100‰ or less (Schoell 1988). Very negative tissue carbon signatures of $\leq$ –50‰ are known to be associated with methane-derived carbon (Levin and Michener 2002), while archaeal lipids may even reflect greater

fractionation of carbon reaching values of ≤ −100‰ (Elvert et al. 2000, Zhang et al. 2003). The most information concerning nutrition has been collected for the large epifauna hosting endosymbiotic chemoautotrophic bacteria. Both methane-based and sulphur-based symbioses appear to fuel the extraordinarily high biomass at seeps via tubeworms, mussels and clams.

At shallow-water seeps, the absence of a high chemosynthetic contribution to the nutrition of the seep infauna appears to be a widespread phenomenon (Dando et al. 1991, Jensen et al. 1992, Juhl and Taghon 1993, Levin et al. 2000). $\delta^{13}C$ signatures for the seep infauna indicated a largely plankton-derived diet. In contrast to these results from shallow water, macrofauna from bathyal and abyssal depths showed a highly variable dependence on chemosynthetic food sources. Some heterotrophic macrofaunal species at seeps clearly utilise photosynthesis-based food resources while others specialise on isotopically light food sources such as anaerobic methane-oxidising archaea (Levin 2005). With an average $\delta^{13}C$ signature of −55‰, the Florida escarpment mat sediments harbour the 'lightest' macro-invertebrate assemblage $\delta^{13}C$ known from any seep, with over 50% of the macrofaunal tissue C derived from methane (Levin and Mendoza 2007).

Almost no information is available about the nutrition of meiofauna living within the seep sediments. Nematode isotope analyses carried out by Van Dover et al. (2003) for the Blake Ridge seeps (2155 m) revealed very light $\delta^{13}C$ (−45 to −50‰) but relatively heavy $\delta^{15}N$ (9–11 ‰), indicating utilisation of chemosynthetically derived organic matter at a higher trophic level such as decomposers. Similar light $\delta^{13}C$ values have also been reported for the nematodes found in the Unimak clam beds (~3270 m) and in the oxygen minimum zone off Mexico at 800 m (Levin and Mendoza 2007). Different seepage and fluid flow regimes among different microhabitats (Tryon and Brown 2001) may be a key determinant of meiofaunal community structure and feeding modes at cold seeps.

6.2. METAZOAN SEEP COMMUNITY: A FOCUS ON MEIOFAUNA

Previous investigations of metazoan seep meiofauna covering a large variety of environments, water depths and geographic regions revealed no consistent response to the seep conditions in terms of densities. Enhancements of meiofaunal abundances have been observed at shallow hydrocarbon seeps (16 m, Montagna et al. 1987), eastern Pacific methane seeps in Monterey Bay (906 m, Buck and Barry 1998), in microbial mats in the Gulf of Mexico (2230 m) and on the Blake Ridge (2150 m, Robinson et al. 2004). Olu et al. (1997) documented extremely high meiofauna densities (>11300 ind. 10 cm^{-2}) at the mud volcano Atalante at 5000 m on the Barbados Trench. Little or no difference in densities compared

to control sediments was reported for hydrocarbon seeps off Santa Barbara, California (15 m, Montagna and Spies 1985), the Hatsuchima seep off Japan (1170 m, Shirayama and Ohta 1990), brine seeps in the Gulf of Mexico (70 m, Powell and Bright 1981, Powell et al. 1983) and methane seeps in the Black Sea (Sergeeva and Gulin 2007). In contrast, meiofaunal densities were significantly reduced at shallow methane seeps in the North Sea (150 m, Dando et al. 1991) and off Denmark (10 m, Jensen et al. 1992). Moreover, the increased habitat heterogeneity at seep areas often results in a higher variability of meiofaunal density within than outside seeps (Montagna and Spies 1985).

In most studies, meiofaunal densities were determined by the nematodes as they made up the largest fraction of the meiobenthos. However, at a shallow brine seep in the Gulf of Mexico (72 m, East Flower Garden) the meiofauna was dominated by gnathostomulids, with platyhelminths, nematodes and amphipods found in lower abundances (Powell and Bright 1981, Powell et al. 1983). On the Blake Ridge, nematodes inhabiting *Acrobacter* microbial mats and mussel beds exhibited similar relative proportions (~30–50%) as the harpacticoid copepods (Robinson et al. 2004). In general, most studies of metazoan meiofaunal seep communities describe a fauna comprised largely of annelids and nematodes, with reduced species richness and elevated dominance compared to more typical deep-sea sediment environments.

6.3. SEEP NEMATODE DIVERSITY

With about 27000 described species (Hugot et al. 2001) and estimations by various researchers of a total species number living on the planet ranging between 100 000 and 1 000 000 (Lambshead and Boucher 2003, Blumenthal and Davis 2004), nematodes represent a very diverse Phylum. Thanks to their cosmopolitanism of genera and species, nematodes represent a good indicator for the study of biodiversity in the benthic environment. Geochemical features are shown to result in a local increase in dominance of a particular species in the meiobenthic community. For instance, sulphide can be expected to reduce diversity and elevate dominance, as found in hydrothermal vent meiofauna (Vanreusel et al. 1997, Thiermann et al. 1997).

Nematode species dominating seep sediments were *Desmolaimoides thiobioticus* (39% of total community) in the brine seep in the Gulf of Mexico (Jensen 1986), *Astomonema southwardorum* (31% or 225 ind. 10 cm^{-2}) in the active methane seep in the North Sea (Dando et al. 1991), *Sabatieria punctata*, *Theristus anoxybioticus* and *Leptonemella aphanothecae* in the methane seeps of the northern Kattegat (total meiofaunal densities: 650 ind. 10 cm^{-2}, Jensen et al. 1992), 2 *Daptonema* spp. (20.2 and 12.9%) and a *Chromadorita* sp. (12.3%) in the bathyal cold seep off Japan (Shirayama and

Ohta 1990). Several of these nematode species developed specific adaptations to the toxic environmental conditions of seep sediments, such as endo- or ectosymbiotic bacteria or sulphur storage in intracellular inclusions, described more in detail in section 6.4. *Sabatieria punctata* (Comesomatidae) has a world-wide distribution and seems to be an opportunistic, coastal cosmopolitan that is able to survive and thrive in these reduced environments. The genus *Sabatieria* obtains its maximum densities at the shelf break but disappears in the deep sea at 3000 m water depth (e.g., Vincx 1986, Jensen 1987, Soetaert and Heip 1995, Vanaverbeke et al. 1997). It is known to be tolerant to hypoxic environments (Soetaert and Heip 1995, Modig and Ólafsson 1998, Boyd et al. 2000, Steyaert et al. 2007). Apparently, these restricted, sulphidic habitats the nematode species are colonizers which have high resistance to environmental stress.

6.4. ADAPTATIONS AND TOLERANCE OF NEMATOFAUNA

Seepage typically is associated with a sediment matrix exhibiting distinct geochemical conditions, including high alkalinity, hydrogen sulphide, methane and ammonium concentrations in pore fluids and limited oxygen availability (Gieskes et al. 2005). Such conditions are challenging to metazoan life forms, for which sulphide is toxic and oxygen is required. Reduced availability of oxygen, high sulphide concentrations, and food quality might be considered as possible reasons for an insignificant meiobenthic response towards the excess supply of organic carbon at the reduced seep sediments.

Inhabitants of anoxic and usually sulphide-rich systems were defined as the thiobios (Boaden and Platt 1971), whereas the oxybios comprises all inhabitants of the well oxygenated sediments (Powell et al. 1983). Considering the chemical conditions of their habitat, thiobiotic meiofauna is suspected to have developed specific metabolic or morphological modifications. For the thiobiotic nematodes from the Flower Garden brine seeps (Gulf of Mexico), Jensen (1986) reported a significant body elongation. A higher proportion of body surface area per unit body volume and a shorter body radius have been suggested to be adaptations to low oxygen pressure in the environment as well as adaptations to epidermal uptake of dissolved organic matter as additional nourishment of thiobiotic species.

Field and laboratory studies of the nematode *Theristus anoxybioticus* (Xyalidae), inhabiting muddy sediments at methane seeps in the Kattegat, Denmark, have revealed a remarkable life history and some special biological features (Jensen 1995). The reproductive adults and juveniles are clearly vertically separated within the sediments, with the adults living in the oxic, uppermost cm and the

juveniles in the deeper, anoxic and sulphidic layers. In contrast with the adults, the sensitivity of the juvenile nematodes to oxygen was striking, indicating an obligate anaerobic metabolism. In addition to the active downwards migration of newly hatched juveniles, and the upward migrations of the adult nematodes, also a shift from bacterial feeding during juvenile stages to diatom feeding during adulthood was observed (Jensen 1995). *Theristus anoxybioticus* succeeded successfully in adapting to the patchily distributed seep conditions, with juveniles that are regarded as obligate anaerobes.

Symbiont-bearing nematodes have been observed from several seep environments. *Astomonema southwardorum* (Chromadoridae) dominated the pockmark sediments in the North Sea (Dando et al. 1991). Detailed investigations on *A. southwardorum* through electron microscopy and fluorescent in situ hybridization, revealed the presence of thiotrophic bacteria completely filling up the gut lumen. In addition, the reduction of the digestive system and the lack of a mouth suggest that these endosymbionts serve as the main nutritional source for the nematode (Musat et al. 2007). Within the Nematoda, both endo- and ectosymbiotic associations have been described. Nematodes belonging to the subfamily Stilbonematinae (Desmodoridae) are known to harbour ectosymbiotic bacteria, attached to the nematode's cuticle in a species-specific pattern (Ott et al. 2004) (Figure 5). The form and size of the bacteria range from small (1–2 μm) cocci through 2- to 5-μm-long rods to nonseptate filaments of up to 100 μm in length (Ott et al. 2004). In most cases they cover the whole body, leaving only the anterior-most part (head) and the tip of the tail free. The nematodes possess a fully functional gut and appear to graze on their sulphide-oxidising symbionts (Ott et al. 1991, Polz et al. 1992, Ott et al. 2004). Experiments with stilbonematids revealed an extremely high resistance to sulphide. More than 80% of the nematodes was still alive after twelve hours of exposure to high concentrations of sulphide (500 μmol l^{-1}) (Ott 1995). The symbiont layer of the stilbonematids thus appears, in addition to its role in the nutrition of the nematode, as a defensive coat against high sulphide concentrations. Moreover, the bacteria are identified as sulphur-oxidisers, detoxifying the hydrogen sulphide and protecting the nematode against the toxic conditions.

Leptonemella aphanothecae is a stilbonematid nematode occurring in the sandy seep sediments of the Kattegat, Denmark, down to sediment depths of 22 cm and more (Jensen et al. 1992). The stilbonematids were previously only recorded from shallow water habitats, but are in this thesis also identified from deep-sea sediments (CHAPTER 2).
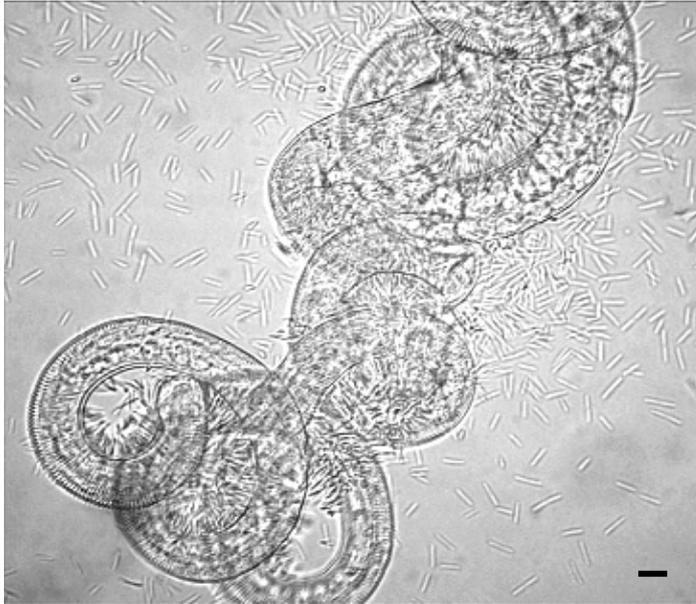
Figure 5: Photograph of a stilbonematid (*Leptonemella* sp.) covered by rod-like bacteria (original picture, scale bar: 10 µm).

Another example of an ectosymbiotic association has been reported from *Beggiatoa* mats at the Santa Barbara basin (~600 m) where the dominant nematode *Desmodora masira* (Desmodoridae) was observed to harbour rod-shaped bacteria under the nematode's annulated cuticle (Bernhard et al. 2000).

Finally, Thiermann et al. (2000) documented on the most abundant nematode *Oncholaimus campylocercoides* (Oncholaimidae) (up to 600 ind. 10 cm^{-2}) in a shallow-water hydrothermal vent off Milos (Greece). This species was able to survive elevated sulphide conditions (400 µmol l^{-1}) for four days by producing internal sulphur inclusions (S$_8$ polysulfur chains). According to Thiermann et al. (2000), these drop-like crystals may not be regarded as a proper detoxification system, but function as energetic deposits for later oxidation to thiosulphate, sulphide or sulphate under oxic conditions, because they reduce only temporarily the concentration and toxic effect of H$_2$S.

The greatest toxic influence is probably due to hydrogen sulphide, and it was supposed to play a major role in controlling the distribution of benthos in areas with methane seepages (Sergeeva and Gulin 2007). Seep organisms may experience exceptionally high sulphide levels of 1 mmol l^{-1} or higher, but only a few taxa are able to tolerate these concentrations. These high fluxes of sulphide are toxic for all aerobically living metazoans by blocking the cytochrome-c-oxidase of their respiratory chain (Bagarinao 1992), Some meiofaunal organisms have developed several strategies and detoxification mechanisms to withstand sulphidic conditions (Fenchel and Finlay 1995). However, some of them depend on minor oxygen concentrations, at least temporarily. From the nematode species *Oncholaimus*

campylocercoides living at shallow-water hydrothermal vent sites off the Greek Island Milos, Thiermann et al. (2000) reported that its sulphide tolerance under hypoxic conditions became greatly reduced when ambient sulphide levels reached 1 mmol l^{-1}. Depending on the ambient sulphide concentrations, diffusion of sulphide into the body tissue of small-sized organisms can be very fast, overriding all detoxification mechanisms. It can be suggested that in many seep sediments, the adaptive capability of nematodes is exhausted due to extremely high sulphide concentrations coupled with restricted (limited) oxygen availability.

There is still a lively debate on the effects of oxygen depletion on the distribution and structure of meiobenthic communities, and often they are difficult to separate from the effects of other environmental factors such as food availability and high-sulphide porewater levels. In shallow-water habitats characterised by long-term anoxia, meiobenthic organisms, in particular nematodes, have been found to be extremely tolerant to low oxygen concentrations. Respiration measurements and experiments on the tolerance to low suboxic conditions (Schiemer et al. 1990) demonstrated that even species found in anoxic sediments will use oxygen when it becomes available. Based on the theoretical considerations and oxygen consumption rates of different meiobenthic groups, Powell (1989) concluded that aerobic metabolism can be maintained at oxygen concentrations as low as 0.1 µmol l^{-1}, which is below the detection level of present methods for the determination of oxygen.

7. AIMS AND THESIS OUTLINE

The overall aim of this study was to increase the knowledge on the biology of deep-sea reducing biotopes, particularly on cold seeps located in the North and South Atlantic. Most preceding studies on cold seep communities have focused on the macrofauna. However, free-living nematodes are known to be the prevailing metazoan group in the majority of marine sediment ecosystems. Being in the meiofaunal size range, they are characterised by a direct benthic development, with a life-cycle closely coupled to the sediment and no specific dispersal phase (Somerfield et al. 2006). Therefore nematodes play an important role in the marine benthic ecosystem (Heip et al. 1985, Coull 1999), and are characterised by high species richness (Heip et al. 1985, Lambshead 1993). The few cold seep studies that dealt with the meiofauna were mostly restricted to higher taxon level (e.g., Buck and Barry 1998, Olu et al. 1997, Robinson et al. 2004, Soltwedel et al. 2005a, Sergeeva and Gulin 2007) and thus far only 3 studies - of which only one from the deep sea - were conducted at genus or species level (Shirayama and Ohta 1990, Jensen et al. 1992, Dando et al. 1991). This thesis, however, describes the

meiobenthic communities associated with different deep-sea cold seep environments, providing information on nematode genus or species level.

The following questions were assessed: (1) What are the factors shaping the meiofaunal community structure, diversity and distribution patterns at multiple scales at deep-sea cold seeps? (2) What is the role of chemosynthesis-based nutritional sources for the meiofauna at deep-sea cold seeps? (3) Do seep meiofaunal species show adaptations in order to withstand the often hostile geochemical conditions typical for these deep-sea sediments? (4) What is the specificity of the dominant meiofaunal species associated with deep-sea cold seep sediments?

Three chapters of this thesis have already been published in the international literature or were accepted for publication; the remaining chapters are submitted for publication. Each chapter is intended to be an autonomous part, which can be read separately from the other chapters. Consequently, an overlap concerning introductions and methodologies is unavoidable. The REFERENCES have been compiled in a single list at the end of the thesis. The total nematode genus list is given in APPENDIX.

CHAPTER 2 describes the meiobenthic community structure at different spatial scales on the Darwin Mounds and an adjacent field of seabed pockmarks in the bathyal north-east Atlantic at ~1000 m water depth. No substantial evidence of seepage was observed in the pockmark features. The micro-scale and macro-scale patterns of meiobenthic abundance and diversity were linked to the environmental conditions in the area, mainly differences in sediment organic carbon and presence of biogenic structures (xenophyophore). This chapter has been published as: VAN GAEVER S, VANREUSEL A, JA HUGHES, BJ BETT, KIRIAKOULAKIS K (2004) The macro- and micro-scale patchiness of meiobenthos associated with the Darwin Mounds (north-east Atlantic). *Journal of the Marine Biological Association of the UK 84: 547–556.*

CHAPTER 3 reports on a multiple-scale investigation of the meiobenthic communities carried out in the lower Congo Basin (Equatorial West African margin, 3150–4800 m). Estimation was made of the degree of spatial heterogeneity of the meiobenthos at a variety of habitats along the Congo Channel system and the nearby located active REGAB cold seep. The structure, density, vertical distribution patterns in the sediment and biomass of the benthos were presented and correlated to the biogeochemical environment conditions. This chapter has been accepted as: VAN GAEVER S, GALÉRON J, SIBUET M, VANREUSEL A. Deep-sea habitat heterogeneity influence on meiofaunal communities in the Gulf of Guinea. *Deep-Sea Research Part II.*

In CHAPTER 4 the meiobenthic communities inhabiting the methane-venting Håkon Mosby Mud Volcano (SW Barents Sea slope, ~1280m) were documented. This chapter aims to investigate the influence of different sub-habitats and the associated biogeochemical conditions on the inhabiting meiobenthos at the active mud volcano. Furthermore, it focused on the discovery of a successful parental-caring nematode which reached unexpectedly high densities in the sulphidic sediments at the mud volcano. This chapter has been published as: VAN GAEVER S, MOODLEY L, DE BEER D, VANREUSEL A (2006) Meiobenthos at the Arctic Håkon Mosby Mud Volcano, with a parental-caring nematode thriving in sulphide-rich sediments. *Marine Ecology Progress Series* 321: 143–155.

CHAPTER 5 deals with the trophoecology of two species dominating the metazoan meiofauna at the Håkon Mosby Mud Volcano. Analyses of fatty acids and their stable carbon isotopes have been used to determine the importance of chemosynthetic food sources for the dominant copepod species from the volcano's centre and the successful nematode species thriving in the surrounding microbial mats. This chapter has been submitted as: VAN GAEVER S, MOODLEY L, PASOTTI F, HOUTEKAMER M, MIDDELBURG JJ, DANOVARO R, VANREUSEL A. Trophic specialisation of metazoan meiofauna at the Håkon Mosby Mud Volcano: fatty acid biomarker isotope evidence. *Marine Biology.*

CHAPTER 6 focuses on the spatial variation and distribution patterns of the meiobenthos associated with different seepage related habitats (730–1280 m). Therefore, biological sampling was carried out at different fluid escape sites offshore Norway: the Håkon Mosby Mud Volcano (HMMV) at 72°N and the Storegga Slide and nearby Nyegga pockmarks at 64°N. Three main habitats were distinguished across the different sampling sites with respect to the biogeochemical sediment conditions and associated epifauna: (1) reduced sediment with suboxic conditions and sometimes covered by bacterial mats, (2) siboglinid tubeworm patches, and (3) sediment outside the influence of seepage and lacking any large chemosynthetic fauna. Finally, molecular analyses were used to examine the phylogenetic relationship of the dominant seep nematode species at HMMV with the morphologically similar shallow-water species from the North Sea. This chapter has been submitted as: VAN GAEVER S, PASOTTI F, OLU-LE ROY K, DERYCKE S, VANREUSEL A. Meiofaunal communities at cold seeps along the Norwegian margin: influence of habitat heterogeneity and evidence for connection with shallow-water habitats. *Deep-Sea Research Part I.*

CHAPTER 7 aimed at determining the relationship between the biodiversity patterns of the meiobenthos and different spatial scales at several seepage sites along the Norwegian continental margin (730–1280 m), including the HMMV, Storegga Slide and Nyegga pockmarks. Furthermore, the importance of

different habitats within a single seep on the diversity of the corresponding meiobenthos was examined. This chapter has been submitted as: VAN GAEVER S, RAES M, PASOTTI F, VANREUSEL A. Spatial scale and habitat-dependent diversity patterns in nematode communities in three seepage related sites along the Norwegian margin. *Marine Ecology.*

In the general discussion (CHAPTER 8), the main results of the preceding chapters are summarised in order to acquire an overview of the main contributions of this thesis to the knowledge of seep associated meiobenthos in the deep sea. In addition, research recommendations are formulated.

CHAPTER 2

The macro- and micro-scale patchiness of meiobenthos associated with the Darwin Mounds (north-east Atlantic)

The macro- and micro-scale patchiness of meiobenthos associated with the Darwin Mounds (north-east Atlantic)

ABSTRACT

Meiobenthic community structure was investigated at different spatial scales (from 100 m's to cm's) on and adjacent to a group of coral-topped sandy mounds in the bathyal NE Atlantic (Darwin Mounds, Rockall Trough) and related to the environmental conditions in the area, mainly differences in sediment organic carbon content and presence of biogenic structures. Meiobenthic abundances were similar to those observed in other deep-sea sites, with nematodes representing at least 94% of the total community. The dominant nematode genera were *Microlaimus*, followed by *Sabatieria*, *Richtersia*, *Rhynchonema* and *Trefusia*, together with typical deep-sea genera (e.g. *Halalaimus* and *Acantholaimus*). Multivariate analysis of nematode generic relative abundances at the different stations indicated that there was no significant influence on distribution resulting from large scale topographic and biogeochemical conditions around the mounds. The same genera were associated with dead tests of the xenophyophore *Syringammina fragilissima* and in the surrounding sediments. The vertical distribution of nematodes on and adjacent to the mound showed some unusual features, as the deeper layers of the sediments were inhabited by stilbonematids. These genera harbour ectosymbiotic, chemoautotrophic bacteria and have not previously been recorded from the deep sea. The occurrence of stilbonematids in notable numbers in the subsurface layers of the sediments in the vicinity of the Darwin Mounds provides evidence for the occurrence of anoxic microenvironments.

INTRODUCTION

During the last two decades, improved sampling methods and the expansion of investigations into a wide variety of habitats have dramatically increased our knowledge of ocean margin ecosystems. Once considered to be temporally constant and spatially uniform, we now know that deep-sea sediments form a dynamic, richly textured environment that is inextricably linked to the global biosphere. An important characteristic of the deep-sea benthos is that the species diversity of many faunal groups increases with depth below the continental shelf (>200 m) to a maximum at mid to lower bathyal depths, and then decreases again with increasing distance seaward on the abyssal plain (>4000 m) (e.g. Gage and Tyler 1991). The macrofauna is more diverse than the megafauna and reaches peak diversity at a greater depth (~2300–2800 m) (Levin et al. 2001). Meiofaunal assemblages also show parabolic patterns of diversity with peaks shifted to even greater depths (Boucher and Lambshead 1995).

The deep-sea, soft-sediment environment is highly complex and provides a habitat for a rich faunal community. Global, regional and small-scale dynamics are important in defining the distribution of species (Levin et al. 2001). A variety of processes are responsible for the maintenance of high local species diversity, particularly in relation to environmental gradients and habitat shifts. Transient biogenic structures, such as mounds, burrows, and the tests of large agglutinated rhizopods contribute more to niche diversification on the deep-sea floor than in shallow, unstable, soft sediments. The combination of regional-scale phenomena such as boundary currents, productivity gradients, sediment heterogeneity, oxygen availability, hydrodynamic regimes, catastrophic physical disturbance, spatial patchiness caused by biological activities and a patchy food supply, guarantee a strongly enriched, diversified deep-sea fauna (Gage 1996, Levin et al. 2001).

The Darwin Mounds (discovered in 1998, Bett 2001) occur in the northeast corner of the Rockall Trough in the Northeast Atlantic, immediately south of the Wyville Thomson Ridge in a depth range of 900 to 1060 m (Masson et al. 2003). Hydrographically, the mound area is dominated by the Continental Slope Current and the North Atlantic Current in surface waters. The net flow of these two currents is to the northeast. This north-easterly flow is partially blocked by the Wyville Thomson Ridge, which deflects some of the flow to the west. The Darwin Mounds lie within the deeper part of the westward-deflected flow. There are hundreds of small seabed mounds spread over an area of *c.* 100 km², on which colonies occur of the cold-water corals *Lophelia pertusa* and *Madrepora oculata* (Gubbay et al. 2002). These reef-building corals are often associated with seabed structures in the Northeast Atlantic (e.g. Rogers

1999). *Lophelia pertusa* is so abundant in the Porcupine Seabight that their skeletal remains have contributed to carbonate mound structures up to 300 m high in 700–1200 m water depth. In the Darwin Mounds, the coral cover is relatively sparse and consisting of discrete colonies that appear to be restricted to mound locations (Masson et al. 2003).

These comparatively small mounds (50–100 m diameter, 5 m elevation) with their associated coral growths create significant local-scale habitat heterogeneity to the background environment, a level-bottom, fine sand sediment drift (Masson et al. 2003). They therefore provide a suitable location to test the importance of small-scale heterogeneity on infaunal biodiversity. In addition, Darwin Mound 'tails', downstream features that can be identified by sidescan sonar (see Masson et al. 2003), support enhanced populations of the giant protozoan *Syringammina fragilissima* (Xenophyophorea) (Bett 2001). The xenophyophore tests may provide further habitat heterogeneity for the meiobenthos of the Darwin Mounds area.

The aim of this study was to investigate the importance of local-scale topographic features, such as mounds and pockmarks (located adjacent to the Darwin Mounds, Masson et al. 2003) and of small-scale biogenic structures, i.e. xenophyophore tests, in influencing the density and diversity of the associated meiobenthic communities. In particular, we examine how the presence of these structures influences the spatial distribution of nematodes.

Materials and methods

1. Study area

The investigation was carried out in the Darwin Mounds area (NE Atlantic), a cluster of seabed mounds in the northeast corner of the Rockall Trough at a depth of *c.* 1000 m (Figure 1). The numerous mounds in the area appear to be sub-circular topographic structures that are typically 50–100 m across and about 5 m high. The Darwin Mounds seem to be unique in possessing tail-like features up to 500 m long that are elongated in a south-westerly direction, parallel to the prevailing bottom current (Figure 2).
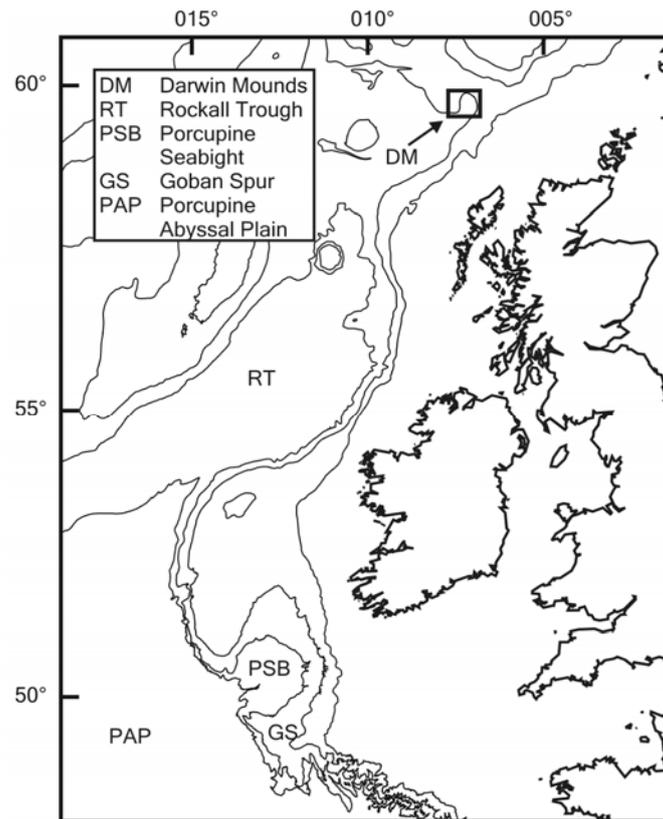
Figure 1: Location of the Darwin Mounds study area (picture courtesy of B. Bett).
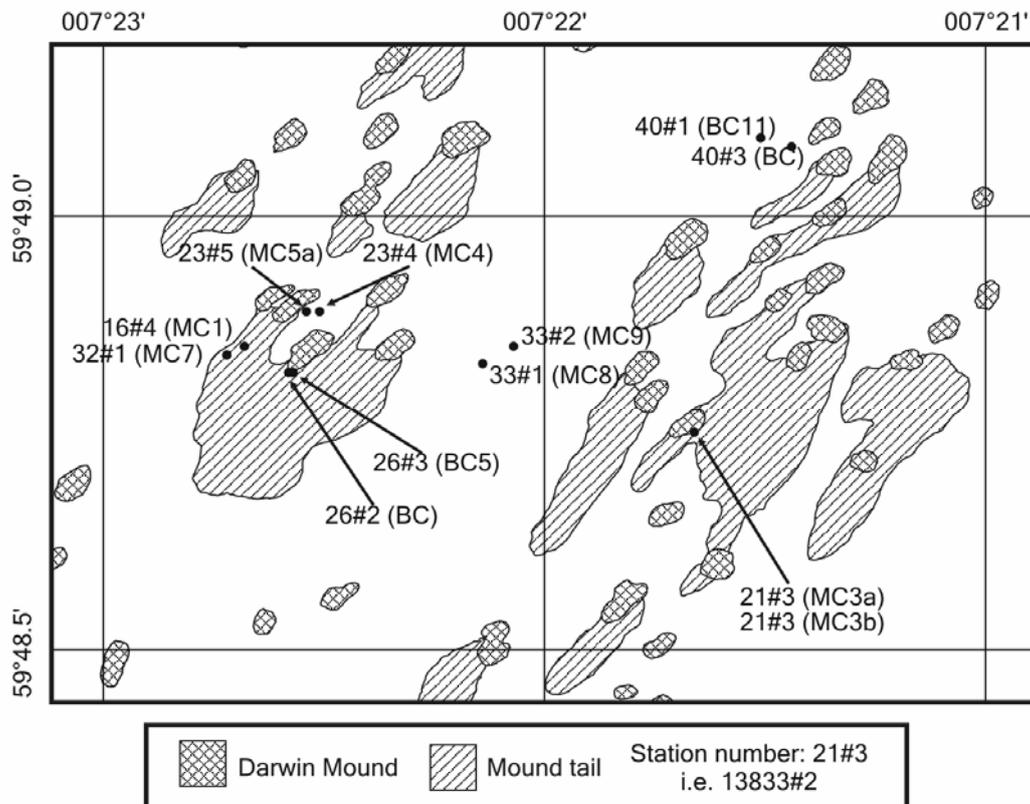
Figure 2: A detailed view of the Darwin Mounds sampling sites shown on a simplified interpretation of sidescan sonar imagery (adapted from Masson et al. 2003).

Geochemical analyses of cores collected on and around the mounds revealed higher concentrations and a deeper penetration of fresh organic material (OM) on the mounds than on the tail or background sediments. Observations with camera tows over tailed mounds showed the presence of coral colonies on the mounds. These 'thickets' of cold-water corals, mainly *Lophelia pertusa* together with the less abundant *Madrepora oculata,* provide a habitat for a large number of invertebrate organisms. It is suggested that megabenthos density is around 2–3 times higher on the mounds than in the surrounding background sediments (Bett 2001). The abundance of echiuran worms associated with coral growths is thought to be responsible for the rapid burial of labile organic matter in mound sediments.

Immediately to the southwest of the Darwin Mounds there is a very extensive field of seabed pockmarks, that are of similar dimension as the mounds (*c.* 50 m diameter) with a negative relief of one or two metres. The megafauna of the pockmark area appears to be broadly similar in composition and abundance to that of the background sediments in the Darwin Mounds area (B. Bett pers. observ.). It is generally accepted that seabed pockmark features are created by the escape of fluids from beneath the seabed. Masson et al. (2003) further suggest that pore water fluid escape may be the common origin of both the pockmarks and the Darwin Mounds; the different structures depending on the underlying sediments, the pockmarks forming in soft muds, the mounds forming where sands are present.

2. SAMPLING AND TREATMENT OF SAMPLES

Sediment samples were collected in August 2000 during RRS *Discovery cruise* 248. Replicate meiofaunal samples (i.e. obtained from separate deployments) were collected from the mounds, mound edge, pockmarks and background sediments (off mound) using a hydraulically-damped multiple corer (individual core surface area 25 cm^2). The samples taken from the mound tails were collected from the same deployment and so should be considered as pseudoreplicates.

Two box cores containing xenophyophores were recovered from the mound tails. From each of these the sediment beneath the test was sampled with cut off syringes (3.14 cm^2) and further samples were collected at 5, 10 and 25 cm horizontal distance from the xenophyophore. The numbers of the cores, subsamples and the corresponding station parameters are listed in Table 1. All sediment cores were horizontally sliced into 1 cm thick layers for the first 5 centimetres in order to determine the vertical distribution of the meiobenthos in the sediment. The samples from the box cores were divided into two layers: 0–2 cm and 2–5 cm. The overlying water was collected separately. All samples were fixed in 4% buffered formaldehyde. In the laboratory samples were sieved over a 1 mm sieve and collected on a 32

µm sieve. Metazoan meiofauna organisms were finally extracted from the 32 µm sediment residue by centrifugation with Ludox and stained with rose Bengal.

Table 1: Station parameters including multicore no., station number, sampling location, geographical position and water depth. Both samples collected each time on, off and along tail and edge were considered as replicate samples.

Multicore	Samples	Sampling location	Coordinates		Depth
MC1	13816#4	Tail of mound	59°48.85´N	07°22.68´W	947
MC7	13832#1	Tail of mound	59°48.84´N	07°22.72´W	946
MC3a	13821#3	Edge of mound	59°48.75´N	07°21.66´W	960
MC3b	13821#3	Edge of mound	59°48.75´N	07°21.66´W	960
MC4	13823#4	Top of mound	59°48.89´N	07°22.51´W	946
MC5a	13823#5	Top of mound	59°48.89´N	07°22.54´W	945
MC8	13833#1	Off mound off tail	59°48.83´N	07°22.14´W	954
MC9	13833#2	Off mound off tail	59°48.85´N	07°22.07´W	955
MC10	13835#1	Pockmark area	59°36.01´N	07°41.86´W	904
MC11	13835#2	Pockmark area	59°36.00´N	07°41.86´W	905
BC5	13826#3	Tail of mound	59°48.82´N	07°22.57´W	946
BC11 (protist)	13840#1	Tail of mound	59°49.09´N	07°21.51´W	958

Meiofaunal taxa (32 µm–1 mm) were enumerated and identified under a stereomicroscope. From each section at least 100 nematodes were picked out at random and mounted on glycerine slides. A formalin-ethanol-glycerol technique (Seinhorst 1959, Vincx 1996) was used to prevent dehydration of the specimens. Since typically more than 90% of the nematode species from bathyal sites are undescribed, nematodes were only identified to genus level. Although individual genera can be highly speciose in deep-sea samples, we believe that analysis at the generic level provides a tractable and internally consistent means of assessing taxonomic diversity across the range of habitats encountered in the present study.

Three specimens of the xenophyophore *Syringammina fragilissima* were obtained in box-cores taken from the tail-region of the mounds; Xenophyophore 1 (X 1) was retrieved from box core sample 13826#2, X 2 from box core sample 13840#1 and X 3 from box core sample 13840#3. All three specimens were devoid of stained cytoplasm and were assumed to be dead. Nematodes (>45 µm in X1

and >63 μm in X2 and X3) were obtained from these xenophyophores by gently breaking apart the tests, followed by wet-sorting the rose-Bengal stained sediment residues under a stereomicroscope.

Sediments from each location (top of mound, tail of mound, edge of mound, off mound, pockmark area) were subjected to granulometric analysis by means of a coulter counter LS 100. At four locations (mound top, tail, off mound and pockmarks) samples were collected and analysed for total organic carbon. Analyses of total organic carbon were performed according to the protocol in Kiriakoulakis et al. (2001). Data on the organic carbon from the edge of the mound are missing.

3. DATA ANALYSES

To estimate the biodiversity of the meiobenthos communities, generic diversity numbers, the rarefaction or expected number of genera based on 70 individuals (E$G_{(70)}$) according to Hurlbert (1971), and the Shannon-Wiener diversity index H' were calculated (Heip et al. 1998). To determine the percentage difference in species composition between samples, the dissimilarity index 100(1-[2c/(a+b)]) was used, where a= total number of species in one sample, b= total number of species in other sample, and c = number of species common to both samples. After testing the necessary assumptions, analysis of variance (ANOVA; using the STATSOFT Statistica program) was used to test the differences between total meiobenthos abundances among the different stations. A multivariate analysis (using PcOrd4) was used to examine the impact of the topographic and biogenic structures on the nematode communities of the different stations and subsamples. Based on a Detrended Correspondence Analysis (DCA), in order to determine the length of the gradient in the sample plot, we applied a Correspondence Analysis (CA) on arcsine transformed relative abundances assuming a unimodal response model.

RESULTS

1. VARIABILITY RELATED TO THE MOUNDS

1.1. SEDIMENT PROPERTIES

The sediments at the different locations were predominantly sands with a particle diameter between 150 and 400 μm, (mean particle diameter of 191.8 $\pm$ 30.0 μm), with the exception of the pockmark area where the sediments were 'muddier' (mean particle diameter of 58.6 μm). At this latter site, the silt-clay (<63 μm) and very fine sand (<125 μm) fractions comprised up to 63.8% of the sample, whereas at the other locations they accounted for only 7.2–18.4%.

1.2. HORIZONTAL DISTRIBUTION ON THE MOUND

Total meiofaunal densities ranged from 666 to 864 individuals 10 cm^{-2}, with the highest density in the pockmark area and the lowest on the tail of the mound. A total of 15 different meiofaunal taxa were found in the samples, but only two taxa were represented by more than 10 individuals 10 cm^{-2} (Table 2). In all samples nematodes were the numerically dominant taxon, representing at least 93.9% of the meiobenthos. Harpacticoid copepods (including nauplii) had a maximum relative abundance of 4.4% on the mound tails. Other meiofaunal taxa were found in low numbers or only occasionally (tardigrades, polychaetes, turbellarians, bivalves, ostracods, gastrotrichs, ophiuroids, amphipods, kinorhynchs, tanaids, aplacophorans and sipunculids). There were no significant differences ($p > 0.05$) in the relative abundances of the meiobenthos between stations.

Since no significant differences ($p > 0.05$) were detected in nematode densities between the mound, the mound tail, the pockmark and the background sediments, it was concluded that total nematode abundances are not strongly correlated with the organic carbon fraction in the surficial sediment (Figure 3).

Table 2: Densities of all metazoan meiofauna taxa and the relative abundance of the dominant taxa on the different stations. SD: standard deviation.

Station ind. 10 cm²	Mound Mean	SD	Tail Mean	SD	Edge Mean	SD	Off mound Mean	SD	Pockmark area Mean	SD
Amphipoda	0.2	0.2					0.2	0.2	0.2	0.2
Aplacophora	0.2	0.2							0.2	0.2
Bivalvia	0.4	0.0	0.8	0.8	1.4	0.6	0.6	0.6	1.4	1.4
Cladocera	0.2	0.2								
Copepoda	25.1	8.6	20.8	0.8	16.7	2.5	19.8	0.6	18.0	1.6
Gastrotricha	0.2	0.2			0.4	0.4	0.6	0.6		
Kinorhyncha					0.2	0.2	0.4	0.0		
Nauplii	7.2	3.7	8.4	6.1	6.7	0.4	9.6	3.7	11.8	3.1
Nematoda	723.6	70.7	625.2	58.2	735.5	25.9	780.0	229.0	820.1	11.8
Oligochaeta			0.2	0.2						
Ophiuroidea	0.6	0.2	0.2	0.2					0.2	0.2
Ostracoda	0.4	0.4	0.4	0.4	0.4	0.4				
Polychaeta	3.1	1.6	3.1	0.4	2.0	2.0	2.7	2.0	2.5	1.0
Sipunculida	0.2	0.2					0.2	0.2	0.2	0.2
Tanaidacea			0.2	0.2						
Tardigrada	4.3	0.8	4.7	3.5	4.3	0.4	3.1	1.6	8.6	5.1
Turbellaria	2.2	0.6	2.2	0.6	2.7	1.2	0.8	0.4	0.4	0.0
Total	767.8	87.6	666.1	71.3	770.2	33.9	818.0	238.8	863.6	24.7
Relative abundance (%)										
Nematoda	94.2		93.9		95.5		95.4		95.0	
Copepoda + nauplii	4.2		4.4		3.0		3.6		3.4	

In total 155 nematode genera were identified. The dominant genus was *Microlaimus* (Microlaimidae), accounting for 16.3% of the mound community, becoming less abundant in the tail sediments (13.3%) but representing 16.9% of nematode community associated with the background sediments (Table 3). *Sabatieria* (Comesomatidae) was subdominant in all samples from the mound and background sediments, reaching a maximum relative abundance of 11.1% in the mound edge samples. Other common and abundant genera included: *Richtersia* (Selachinematidae), *Halalaimus* (Oxystominidae), *Rhynchonema* (Xyalidae), *Trefusia* (Trefusiidae) and *Acantholaimus* (Chromadoridae). The nematode community associated with the pockmarks appeared to be somewhat different, with *Molgolaimus* (Desmodoridae) as the dominant genus (13.8%), whereas *Microlaimus* was the subdominant genus, accounting for 7.9% of all nematodes.

The generic diversity of the nematode communities showed no clear trend based on Hill numbers. The number of nematode genera in individual samples ranged from 91 in a sample from the mound to 72 in a pockmark sample. $EG_{(70)}$ was highest in a sample collected on the tail (34), but differences between stations are not significant.
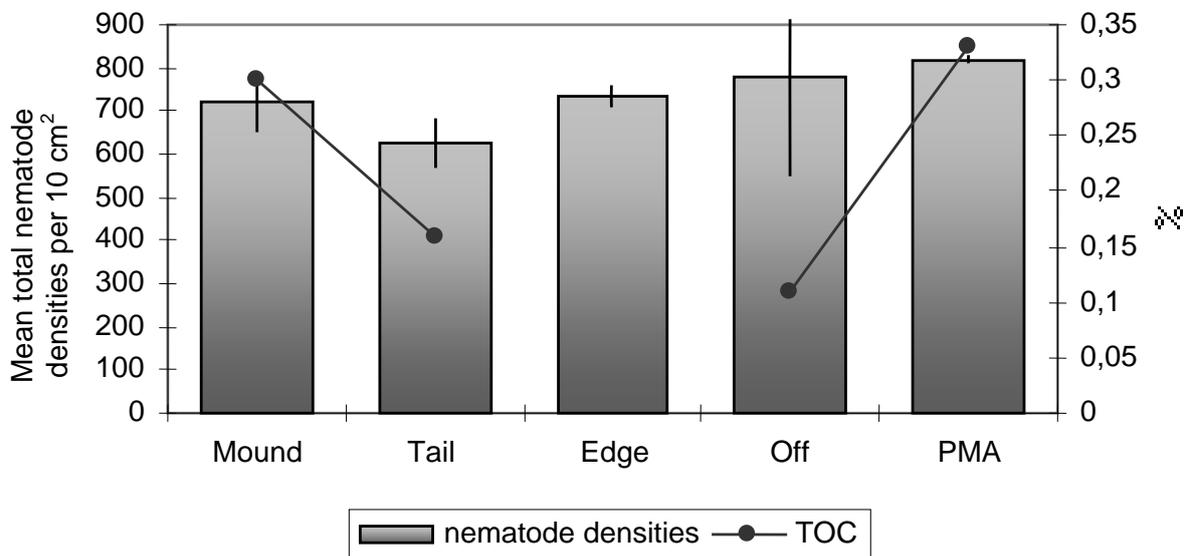
Figure 3: Mean and SD of total densities of nematodes on the five different locations (mound top, mound tail, mound edge, off mound, pockmark area) and percentage of total organic carbon (data from K. Kiriakoulakis, pers. comm.).

Table 3: Relative abundances of the ten most important nematode genera on the different stations.

Mound		Tail		Edge		Off mound		Pockmark area	
Genus	*%*	*Genus*	*%*	*Genus*	*%*	*Genus*	*%*	*Genus*	*%*
Microlaimus	16.3	*Microlaimus*	13.3	*Microlaimus*	16.8	*Microlaimus*	17.0	*Molgolaimus*	13.8
Sabatieria	8.4	*Sabatieria*	7.9	*Sabatieria*	11.1	*Sabatieria*	7.5	*Microlaimus*	7.9
Richtersia	5.1	*Halalaimus*	5.2	*Acantholaimus*	4.4	*Trefusia*	5.9	*Halalaimus*	6.7
Rhynchonema	4.0	*Richtersia*	5.0	*Trefusia*	3.8	*Halalaimus*	3.6	*Sabatieria*	6.2
Trefusia	3.4	*Leptolaimus*	2.9	*Rhynchonema*	3.5	*Pomponema*	3.5	*Leptolaimus*	4.0
Latronema	2.9	*Daptonema*	2.8	*Halalaimus*	3.4	*Molgolaimus*	3.4	*Trefusia*	3.9
Halalaimus	2.6	*Trefusia*	2.8	*Molgolaimus*	3.2	*Xyala*	3.2	*Syringolaimus*	3.8
Desmodora	2.5	*Leptonemella*	2.5	*Bathynox*	2.8	*Rhynchonema*	2.9	*Acantholaimus*	3.5
Pomponema	2.2	*Acantholaimus*	2.5	*Daptonema*	2.5	*Oxystomina*	2.9	*Tricoma*	3.4
Acantholaimus	2.1	*Psammonema*	2.4	*Monoposthia*	2.3	*Acantholaimus*	2.5	*Monhystera*	3.3

1.3. VERTICAL DISTRIBUTION WITHIN THE SEDIMENT

The vertical distribution of nematode densities was different at the 5 stations (Figure 4). At the pockmark area the nematodes were highly concentrated in the upper centimetre of the sediment, which represented on average almost 50% of the total nematode density in the sediment. At the mound edge and in the background sediments, maximum nematode densities occurred between 1 and 2 cm sediment depth. The sediments on the mound showed a more even vertical profile.

The deeper sediment layers at all stations contained members of the subfamily Stilbonematinae, a special group of free-living nematodes with ectosymbiotic bacteria, (Ott et al. 1991). Three Stilbonematinae genera (*Leptonemella*, *Eubostrichus*, *Catanema*) were present at each station, except for *Leptonemella* that was not found in the pockmark sediments. *Leptonemella*, accounted for up to 4% of the total nematode community in one sample of the tail sediments and up to 20% of all nematodes present in the deeper sediment layers (below 1 cm sediment depth) on the mound. Stilbonematids were never found between 0 and 1 cm depth, and in all samples reached their maximum densities between 4 and 10 cm sediment depth.
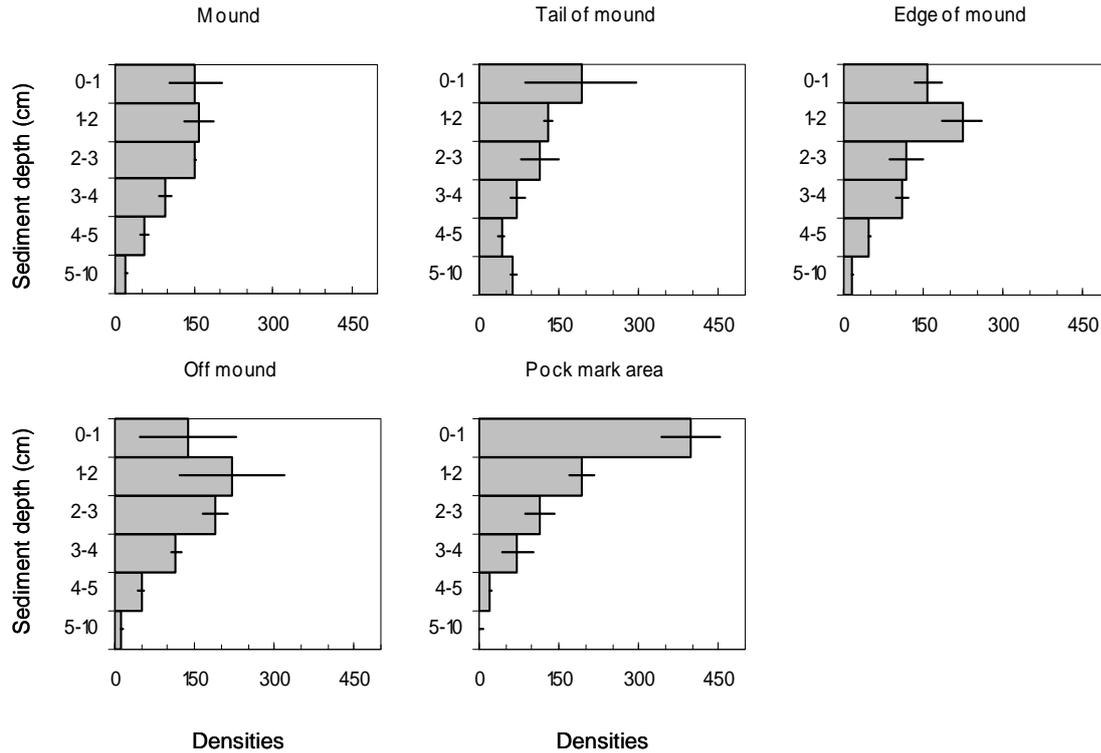
Figure 4: Vertical distribution of the Nematoda between 0–10 cm sediment depth (ind 10 cm^{-2}); mean and SD of densities.

1.4. MULTIVARIATE ANALYSES OF NEMATODE COMMUNITIES

Correspondence Analysis based on the relative abundances of nematode genera in the 10 samples (5 stations) revealed no obvious differences between the communities. In a second ordination, where each core section was considered separately, it was possible to compare the nematode communities in different sediment layers (Figure 5). This ordination shows a gradual change of the nematode community from the surface to the deeper sediment layers. The samples from the pockmark area (MC10 and MC11) had a somewhat different composition from the other sites. As the deepest sediment slices (5–10 cm) of the pockmark area have extremely low nematode densities, they seemed to be 'outliers' in the analysis and were excluded to enable the main trends to be visualised in more detail.

The surficial sediment layers of the pockmark area had a distinct nematode community, as there were some apparent shifts in the dominant nematode genera: *Molgolaimus* was the most dominant genus (13.8% and 1.9–3.4% in other stations), whereas *Microlaimus* was only half as abundant as in all other

stations. Also the nematode genera *Leptolaimus* (4.0%), *Syringolaimus* (3.8%), *Tricoma* (3.4%) and *Monhystera* (3.3%) were more abundant in the pockmarks than at the other stations (Figure 5).
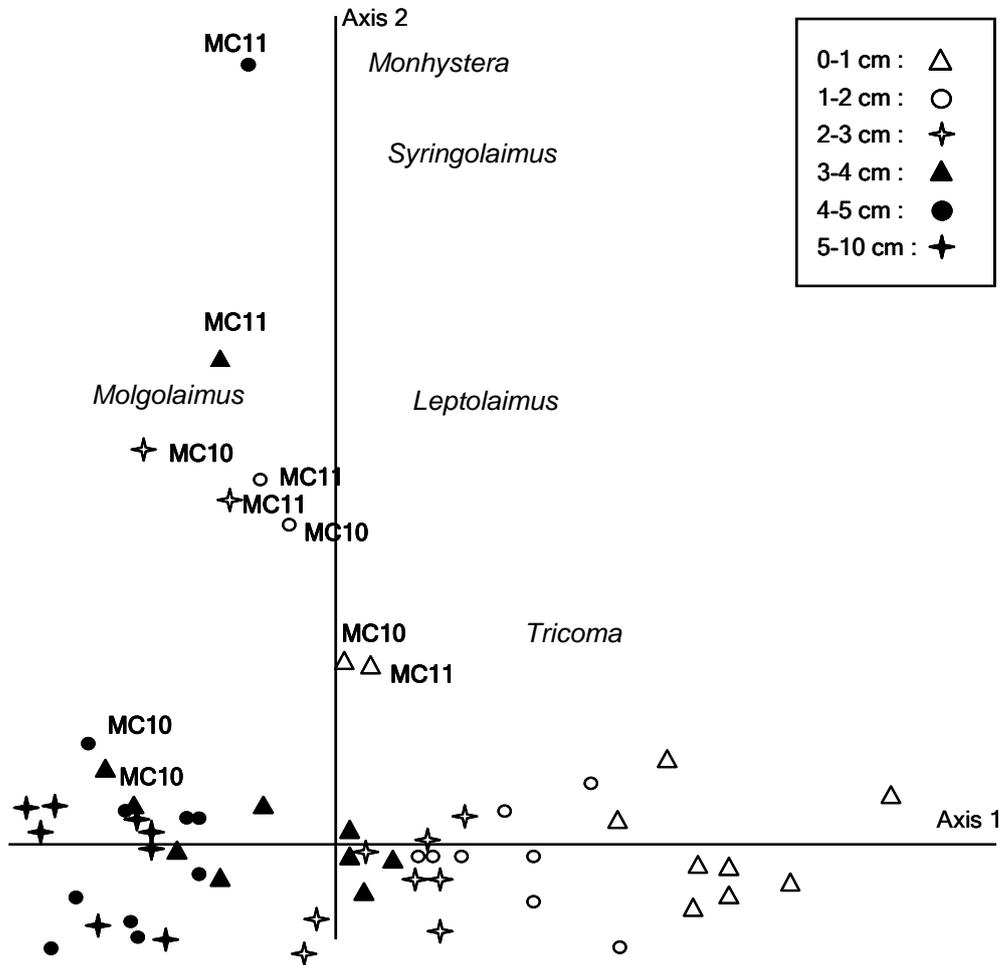
Figure 5: Downweighted multivariate correspondence analysis of the nematode relative abundances of all separate sediment slices of all stations. Eigenvalue axis 1 = 0.3010; eigenvalue axis 2 = 0.2020. Letter codes refer to samples (see Table 1).

2. VARIABILITY RELATED TO XENOPHYOPHORES

Since the xenophyophores were retrieved from the tails of the Darwin Mounds, the nematode densities in the sediments associated with xenophyophores were compared to those observed in the tail sediments, based on multicorer samples. On average, nematode densities from the boxcorer samples were 2 to 6 times lower than the densities in the tail sediments sampled by the multicorer (497–604 organisms 10 cm^{-2}). The vertical distribution of nematode densities was also rather different between

the multicorer and boxcorer samples. Only the vertical nematode distribution from underneath the xenophyophores was similar to those found in the multicore samples. It seems these densities were not influenced by any sampling artefacts. Therefore we compared nematode communities from within and beneath the xenophyophore tests (retrieved by boxcorer) with those of the tail region as sampled with the multicorer.

In addition, 711 nematodes were collected from on and within the three xenophyophore tests recovered during the cruise (ranging from 141–392 ind. per *Syringammina fragilissima* specimen). At the generic level, the overall biodiversity of nematode communities associated with the xenophyophores was lower (29–59 genera) than the number found in the tail sediments (73–81 genera). The expected number of genera and Shannon Wiener index calculated for the community in the tail sediments (E$G_{(70)}$ 30–34, H′ 3.6–3.7) were higher than those of the community associated with the xenophyophore tests (E$G_{(70)}$ 19–27, H′ 2.7–3.3).

Table 4: Percentage species difference between the different samples (two replicates of tail sediment, two replicates of sediment underneath a xenophyophore, three xenophyophores).

	MC1	MC7	BC5/Z	BC11/Z	X 1	X 2	X 3
MC1 (tail)	0 %						
MC7 (tail)	27 %	0 %					
BC5/Z (underneath protist)	46 %	42 %	0 %				
BC11/Z (underneath protist)	42 %	43 %	40 %	0 %			
X 1 (on/in protist)	47 %	45 %	47 %	56 %	0 %		
X 2 (on/in protist)	53 %	54 %	53 %	55 %	41 %	0 %	
X 3 (on/in protist)	55 %	55 %	45 %	45 %	57 %	53 %	0 %

Table 4 shows the dissimilarity indices between the nematode communities associated with 3 different xenophyophore specimens, from the tail station (two samples taken by multicorer) and from the sediment beneath a xenophyophore (two samples taken with cut off syringes). The two samples collected at the tail of the mound are relatively consistent in their composition (dissimilarity 27%). The generic composition of these tail sediments is quite distinct (dissimilarity 42%) from that found in the sediment beneath the xenophyophores. The nematode communities of the xenophyophore tests were also distinct (dissimilarity 45–56%) from those of both the tail sediments and the underlying sediments. These differences are also reflected in the changes in generic abundances (Tables 3, 5). *Microlaimus* is not the dominant genus in the xenophyophore test samples and larger nematodes such as

Syringolaimus, *Trefusia*, *Halalaimus* and *Acantholaimus* are more common. This may be partially explained by the different mesh sieves that were used. The communities associated with the three protists are very different to each other in terms of generic abundances (Tables 4, 5).

Table 5: Relative abundances of the ten most important nematode genera associated with three xenophyophores.

Genus	X 1 %		X 2 %		X 3 %
Syringolaimus	18.6	*Trefusia*	12.4	*Halalaimus*	20.6
Trefusia	9.9	*Microlaimus*	10.1	*Acantholaimus*	19.9
Viscosia	7.9	*Acantholaimus*	9.6	*Daptonema*	9.9
Paracanthonchus	7.4	*Syringolaimus*	7.3	*Actinonema*	8.5
Anticoma	5.9	*Viscosia*	6.7	*Trefusia*	5.0
Halalaimus	5.6	*Anticoma*	6.2	*Monhystera*	4.3
Microlaimus	4.1	*Choanolaimus*	5.6	*Sabatieria*	4.3
Oxystomina	4.1	*Halalaimus*	5.1	*Microlaimus*	2.8
Acantholaimus	3.3	*Thalassoalaimus*	4.5	*Syringolaimus*	2.8
Choanolaimus	3.1	*Camacolaimus*	3.4	*Gammanema*	2.1

DISCUSSION

1. MEIOBENTHIC COMMUNITY

There is a general tendency for metazoan meiobenthic densities to decrease with increasing bathymetric depth within limited geographical areas (Vincx et al. 1994, Soltwedel 2000). In this study all samples were collected in a narrow bathymetric range (904 to 960 m water depth), allowing the investigation of the relationship between the meiobenthos and variation in micro-habitats without undue influence from water depth or latitudinal variations. Further, the abundances observed (765 ± 99 ind. 10 cm^{-2}) are of the same order of magnitude as those found at comparable water depths on the Goban Spur (612 ± 54 ind. 10 cm^{-2} at 670 m), in the southern Rockall Trough (Vanaverbeke et al. 1997). The meiofaunal abundances observed here are also within the range predicted by Soltwedel (2000) for the temperate region of the European continental margin (178–2604 ind. 10 cm^{-2}). Densities reported from sites at 600 m (1410 ± 110 ind. 10 cm^{-2}) and 900–960 m (1523 ± 267 to 1593 ± 143 ind. 10 cm^{-2}) in the Porcupine Seabight were two times higher than the values reported here (Vincx et al. 1994, Vanreusel et al. 1995). The Porcupine Seabight is an area where significant deposition of phytodetritus occurs

(Vanaverbeke et al. 1997), which may induce temporal variability in nematode densities. Mass phytodetritus deposition has been observed in the Darwin Mounds area, but was not apparent during the period of the RRS *Discovery* cruise.

The meiobenthic communities around the Darwin Mounds were very diverse. We identified 15 higher meiofaunal phyla and 155 nematode genera, ranging from 72 to 91 genera per station. With nematodes (>94%) and copepods the two dominant metazoan taxa, the major taxonomic composition of the meiobenthos was broadly similar to that of other communities in the Northeast Atlantic (Vincx et al. 1994). Except for the more superficial profile at the pockmark area, the vertical distributions of nematodes in the sediments of the Darwin Mounds were similar to those of the Porcupine Seabight, where meiobenthic animals penetrate deep in the sediment and only some 35% inhabit the upper 1 cm at a depth of 900 m (Vanreusel et al. 1995). These patterns may reflect the penetration of fresh organic matter into the sediment resulting from enhanced bioturbation. Large echiuran worms are associated with coral growth on the Darwin Mounds, and may be significant in the rapid downward transport of fresh organic material into the sediments.

2. NEMATODE DIVERSITY

The nematode community of the Darwin Mounds was dominated by one genus: *Microlaimus*. The other nematode genera that subdominated the meiobenthic community in the sediments were *Sabatieria*, *Molgolaimus* (only more abundant at the pockmark area), *Richtersia*, *Halalaimus*, *Rhynchonema*, *Trefusia* and *Acantholaimus*. *Halalaimus* and *Acantholaimus* appear to be typical, dominant deep-sea genera. Also in the adjacent Goban Spur (Vanaverbeke et al. 1997) *Sabatieria* was the dominant nematode genus. This genus achieves maximum densities in the North Atlantic at bathyal depths and has a preference for low oxygen environments (Soetaert and Heip 1995). The reduced sediments of the Hatsushima cold seep (Sagami Bay, Central Japan, at 1100–1200 m) (Shirayama and Ohta 1990) were dominated by *Microlaimus*, although the relative abundance of this genus was higher (25%) at the well-oxygenated control area of that study. *Molgolaimus*, often common in reduced conditions, shared the dominance in the hydrothermal sediments of the North Fiji Basin (~1900 water depth, Vanreusel et al. 1997) with *Leptolaimus* and *Monhystera*, each representing 18.4–20.2% of the nematode community. The relative abundance of *Microlaimus* ranged between 0–1.6% in these hydrothermal sediments (Vanreusel et al. 1997). Vanhove et al. (1999) found the genera *Microlaimus* and *Molgolaimus* to be strongly represented in the deep-sea communities in the Weddell Sea (Antarctica). At the upper slope sites (600–1200 m) both genera had relative abundances ranging

between 5 and 15% of the total community. The dominance of *Microlaimus* increased with water depth, accounting for up to 20% of all nematodes at approximately 2000 m water depth. *Molgolaimus* was rare in the deepest areas investigated, but became more abundant than *Microlaimus* at shelf break sites in the Weddell Sea (Vanhove et al. 1999). It seems that nematode genera, like *Microlaimus* and *Molgolaimus,* behave as opportunistic genera which exploit and benefit from higher but often unpredictable organic enrichment, including aberrant environments such as mud mounds and cold seeps.

The Darwin Mounds have a rich nematode community at the generic level, with 155 different genera identified, ranging between 72–91 genera per sample. On the Goban Spur, 60 nematode genera were found per sample at 670 m (Vanaverbeke et al. 1997).

It is not yet clear what accounts for the presence of Stilbonematinae in the deeper sediment layers. Nematodes of the subfamily Stilbonematinae have a conspicuous coat of ectosymbiotic bacteria that provide nutrition for the host. The bacteria are sulphur-oxidizing chemoautotrophs that utilise sulphide and thiosulphate with oxygen, and probably also nitrate, as an electron acceptor. The stilbonematids garden their symbionts by migrating between the oxidized surface and the sulphidic deeper layers of the sediment (Ott et al. 1991). To date this group of nematodes has only been described from shallow marine sands, in particular tropical calcareous sands (Ott et al. 1991), and in shallow sublittoral gasohydrothermal vents (Kamenev et al. 1993, Thiermann et al. 1997). In this study, stilbonematids were for the first time identified from deep-water sediments, suggesting possible sulphide enrichment in the deeper sediment layers around the mounds.

3. IMPACT OF LARGE TOPOGRAPHIC STRUCTURE

Multivariate analysis showed no clear differences between the nematode communities at the different stations around the mounds. The potential environmental variations introduced by the presence of seabed mounds (e.g. hydrodynamic and/or organic matter supply) had little influence on bulk gross nematode community characteristics. There were, however, significant variations in the communities with varying depth into the sediment at all stations. These down-core variations showed some evidence of differential responses between the stations. The pockmark location appears to be notably different to the stations around the mounds, having a more superfical distribution of nematodes (Figure 4), a markedly different distribution of stilbonematid genera, and a clearly differentiated genera composition with depth (Figure 5). The comparative distinctiveness of the pockmark location may be explained by

sediment type, i.e. muddy in the pockmark area and sandy at all other study sites. There appears to be no evidence for local horizontal gradients in organic matter supply; the differences in the vertical distribution of meiofauna in the sediments are generally controlled by the availability of food and oxygen.

4. IMPACT OF SMALL BIOGENIC STRUCTURE

From the very low nematode densities found in the surface layers of the box core samples, we assume that there has been a loss of surficial sediment caused by a bow wave from the box corer. Displacement of the sediment surface can result in meiofaunal density estimates from box corer samples being half those from multiple corer samples (Bett et al. 1994, Danovaro et al. 2000). Previous studies have revealed that sediments beneath and adjacent to large xenophyophore tests contain significantly more metazoan macrofauna than surrounding sediments (Levin and Thomas 1988). This disturbance of the surface is well demonstrated in box core sample 5, as only the sediment sampled underneath the protist contained nematode densities (422–985 ind. 10 cm^{-2}) similar to those found in the tail sediments (567–683 ind. 10 cm^{-2}) as retrieved by the multicorer. Probably the xenophyophore test prevented sediment displacement. These results again demonstrate the difference in quality of samples recovered by the multicorer and the box corer. Considering this, we must be careful in interpreting trends in community structure, diversity and density based on box-core samples. Consequently, we have only compared nematode genera associated with the xenophyophore, from beneath the xenophyophore and from the tail sediments sampled by the multicorer.

There appear to be some minor shifts in the relative abundances of the dominant nematodes living within the xenophyophores. The generic composition of nematode communities in the sediment beneath the xenophyophores was similar to that of the communities from the open tail sediments. The assemblage found on the xenophyophore tests appeared to have a different generic composition. The more dominant genera (*Syringolaimus*, *Trefusia*, *Halalaimus* and *Acantholaimus*) found in association with the xenophyophores had body lengths two or three times longer than genera found on the mound and in the surrounding sediments (e.g., *Microlaimus*, *Sabatieria* and *Molgolaimus*). The open framework of the xenophyophore test contrasts with the surrounding fine sandy sediments, although anoxic microenvironments may be formed within dead xenophyophore tests through the passive trapping of organic matter together with the decomposition of the xenophyophore stercomata (Hughes and Gooday 2004).

CONCLUSION

The Darwin Mounds area provides a richly textured habitat for a meiobenthic deep-sea community with a high local biodiversity. Nematode abundances are similar to those previously reported from similar depths in the NE Atlantic, and are similar in terms of taxonomic composition. No significant differences were found in nematode density, diversity or community composition between the different stations on the mounds and in the background sediments. The horizontal topographic variations and gradients in OM supply at the Darwin Mounds appear to have only a minor influence on nematode abundances and generic diversity. Only in the muddier sediments of the pockmark area some changes in the relative abundances of nematode genera were observed. The study of small-scale biogenic structures on the seafloor – namely dead tests of the xenophyophore *Syringammina fragilissima* – revealed that the nematode fauna of these tests consisted of similar genera as in the surrounding sediment, although there was a shift towards genera with larger body sizes. The presence of the mounds and the xenophyophores did not significantly increase the spatial diversity of nematodes at generic level.

It appears that nematodes experience, and some are adapted to, anoxic conditions at shallow sediment depths in the Darwin Mounds area. Nematodes of the subfamily Stilbonematinae were found in the samples; their body surface is covered with chemoautotrophic, sulphur-oxidizing bacteria; these taxa have not previously been recorded from deep-sea environments. Stilbonematids occurred in notable numbers in the deeper layers of the sediments of the Darwin Mounds. The occurrence of stilbonematids probably reflects the presence of anoxic microenvironments in the deeper sediment layers, which may in turn result from the rapid burial of fresh organic matter at this site.

ACKNOWLEDGEMENTS

CHAPTER 3

Deep-sea habitat heterogeneity influence on meiofaunal communities in the Gulf of Guinea

Deep-sea habitat heterogeneity influence on meiofaunal communities in the Gulf of Guinea

ABSTRACT

To estimate the degree of spatial heterogeneity of benthic deep-sea communities, we carried out a multiple-scale (from m's to 200 km) investigation in the lower Congo Basin (Equatorial West African margin, 3150–4800 m) in which we examined the metazoan meiofauna at a variety of habitats along the Congo Channel system and in the associated cold seep. We investigated the structure, density, vertical distribution patterns in the sediment and biomass of meiofaunal communities in the Gulf of Guinea and how they are controlled by hydrologic and biogeochemical processes. The meiofaunal communities in the Gulf of Guinea were shaped by heterogeneous conditions on the margin, and reflect the multiple-scale spatial variability that corresponds with different identified habitats. The two control sites, located at >100 km away from the canyon, were inhabited by very dense and the most diverse meiobenthic communities. Similar meiobenthic communities inhabited the transition zone between the canyon and the cold seep. Sites located along the Congo Channel were obviously affected by the local high-velocity bottom currents and unstable sedimentary conditions in this active submarine system. Extremely low meiobenthic densities and very low proportions in the most surficial sediment layers provided evidence for recently highly disturbed sediments at these sites. The ROV *Victor 6000* provided images of the cold seep, showing a patchy distribution of several types of patchy distributed megafaunal communities dominated by three key symbiotic taxa (Mytilidae, Vesicomyidae and Siboglinidae). These cold seep sediments were colonised by a unique meiobenthic community, characterised by a high small-scale (m's) patchiness, low species richness and the prominent dominance of two large-sized nematode species: *Sabatieria mortenseni*, which is a cosmopolitan nematode known from littoral habitats, and an undescribed *Desmodora* species. The high individual body weight of *Sabatieria mortenseni* and its dominance at the cold seep site resulted in a significantly higher nematode biomass at the seep compared to the surrounding sites. In addition, the vertical nematode profiles, with maximum proportions in subsurficial layers, points to a chemosynthesis-based meiobenthic community in this cold seep, in contrast to the phytodetritus-based communities at the control sites and at the transition zone.

INTRODUCTION

Most studies documenting metazoan communities from deep-water cold seeps describe a fauna characterised by impoverished species richness, elevated dominance and high biomass compared to more common bathyal environments (Buck and Barry 1998, Olu et al. 1997, Robinson et al. 2004, Levin 2005). Mega- and macrofaunal seep communities are relatively well described from a wide number of seep locations from different margins (Sibuet and Olu 1998, Sibuet and Olu-Le Roy 2002, Levin 2005). Megafaunal communities consist of high densities of siboglinid tubeworms (Annelida), mytilid, vesicomyid and lucinid bivalves. It has been shown that the distribution of these species may be regulated by geology, including the geometry of seeps, pattern and intensity of fluid flows and the occurrence of hydrates (Olu-Le Roy et al. 2007). However, studies on seep meiofauna from the deep sea are few and have often been restricted to bulk measurement of abundance and biomass of major taxa (e.g. Powell et al. 1986, Olu et al. 1997, Robinson et al. 2004, Soltwedel et al. 2005a). Only four meiofaunal studies at seeps, including two from deeper water (Shirayama and Ohta 1990, Jensen et al. 1992, Dando et al. 1991, Van Gaever et al. 2006), also provided information at the genus or species level.

Active cold seeps occur on geologically active and passive continental margins in all oceans of the world, including the polar regions both Arctic and Antarctic (Domack et al. 2005, Levin 2005). The first seep community described in the South East Atlantic was discovered in 1998 on a giant pockmark along the Equatorial African margin at 3150 m water depth (Ondréas et al. 2005). Submarine pockmarks are crater-like depressions that are typically wide (10–800 m in diameter), shallow (up to 45 m deep) features, occurring in most seas, oceans and in many diverse geological settings (Hovland et al. 2002). Pockmarks occur as discrete features, or as coalescing structures forming extensive pockmark fields up to 1000 km² (Kelley et al. 1994). They occur wherever some form of fluid and/or gas discharge is focused. Biogenic pockmark fluids originate from the production of methanogenic bacteria (biogenic gas) inside the sediments, from where it migrates to the seafloor entrained in pore water (Kelley et al. 1994). Hydrocarbon pockmark gas is produced by the thermal alteration of sedimentary organic matter (Hovland and Judd 1988).

Deep-sea canyons are of particular interest for the study of biogeochemical processes as they often act as major pathways for particle-loaded, near-bottom currents transporting material from the continent to the open ocean. Enhanced particle transport along canyons implies an effective food supply to benthic

organisms, resulting in higher faunal densities and biomass (Rowe et al. 1982, Soltwedel et al. 2005b). However, some submarine canyons are very unstable environments due to tidal currents and periodic turbidity flows into the system (Puig et al. 2004). Benthic communities often reflect the unstable and organically enriched conditions of these canyon systems. Indeed, macrofaunal communities of canyons exhibit higher densities and biomasses and lower diversities than the communities inhabiting the adjacent abyssal plains, continental shelf and slopes (e.g., Gage et al. 1995, Vetter and Dayton 1998). The meiofauna of submarine canyons has been rarely studied, and densities are very variable. High fluctuations in the meiobenthic densities were observed in the Nazaré Canyon. This was not only explained by the impact of physical factors, but also by a very strong seasonality in fluvial input (Garciá et al. 2007).

During the multidisciplinary BIOZAIRE 3 cruise (December–January 2003–2004) to the Gulf of Guinea, samples were collected from the passive continental margin of West Africa in the Congo Channel system and adjacent sites. The Congo deep-sea fan is one of the world's largest active submarine canyons, extending 760 km from the river mouth to the abyssal plain (>5100 m), downslope from the Congo-Angola continental shelf and covering an estimated area of 330 000 km². One of the particular characteristics of the Congo Channel system is the permanent connection between the river mouth and the abyssal plain, and this explains why this fan is still active during current high sea level (Khripounoff et al. 2003). Dense chemosynthetic communities have been discovered in a deep isolated pockmark, located at less than 8 km north of the Congo Canyon. The large size of the pockmark, 800–1000 m in diameter, is due to the collapse of several individual pockmarks gathered in a pockmark cluster (Charlou et al. 2004, Olu-Le Roy et al. 2007). This giant pockmark, called REGAB, and its surroundings were explored in detail by the remote operated vehicle (ROV) *Victor 6000* as part of the ZAIANGO (1998–2000) and BIOZAIRE projects (2001–2003) carried out conjointly by the oil company TOTAL and a number of French research institutes.

The BIOZAIRE 3 cruise aimed at understanding the dynamics of the energy inputs, their origins and their consequences for the development of the benthic fauna in four different environments. The present study highlights the density, diversity and biomass of metazoan meiofauna in different sites and habitats along the Congo Channel system and within the REGAB cold seep in the Gulf of Guinea (Equatorial West African margin, 3150 m). The following questions were addressed: (1) Do the meiofaunal composition, density and biomass vary on a macroscale (>100 km) and mesoscale (5–20 km) in the Gulf of Guinea and is there biological patchiness on a microscale (m's) within the seep? (2) To what

extent are the communities similar to other communities from similar environments worldwide and can we relate the observed biological patchiness to particular habitat characteristics?

MATERIALS AND METHODS

1. STUDY AREA

The Congo River is the second largest river in terms of water discharge (40.8×1000 m^3 s^{-1}) and has a strong link with the deep-sea sediments. Frequent turbidity currents carry huge amounts of material down to the abyssal plain up to several tens of kilometres outside the submarine channel system and are suspected to sustain the particular ecosystem on the abyssal plains in the Gulf of Guinea. The finest particles of the upper part of the turbidity currents are even able to overflow the canyon flanks, but these phenomena might not occur at each turbidity event. The organic matter of the turbiditic overflow has a strong terrigenous signal, and its influence is visible in the total organic carbon content as well as in the *n*-alcohol composition (Treignier et al. 2006, Treignier et al. submitted).

The Congo Canyon represents a very active submarine system, as two major turbidity events with speeds of up to 97–120 cm s^{-1} at 150 m above the channel floor were reported from March 2001 and January 2004. The current speed close to the channel bottom would have been even higher as the flow had enough energy to transport a large quantity of sand and large plant remains. Smaller, local turbidity events without any speed accelerations are suspected to arrive frequently (Khripounoff et al. 2003, Vangriesheim et al. submitted).

The REGAB cold seep in the Gulf of Guinea is located at an average depth of 3150 m, on the oceanic crust <8 km north of the Congo Canyon, (Figure 1). This 800-m wide, 15–20 m deep pockmark appears to be formed by the association of several individual pockmarks, resulting in a large area covered by a dense chemosynthetic community. Photographs taken with the ROV *Victor 6000* illustrate seepages of methane, gas hydrates, carbonate crusts, and a very patchy distribution of faunal assemblages, either dominated by bivalves of the families Mytilidae (*Bathymodiolus* sp.) or Vesicomyidae (*Calyptogena* sp.), or by siboglinid tubeworms (*Escarpia southwardae*, Andersen et al. 2004). The distribution of these symbiont-bearing megafauna is controlled by the substrate and the present methane concentrations in

seawater. In this chemosynthetic environment, increased macrofaunal densities with higher spatial fluctuations were found in comparison to the background sediments (Olu-Le Roy et al. 2007).

2. SAMPLING STRATEGY

Meiofaunal sediment samples were collected in the Gulf of Guinea by the French RV Atalante during the cruises BIOZAIRE 2 in November–December 2001 and BIOZAIRE 3 in December–January 2003–2004. The cold seep REGAB which was directly under the effect of methane emission (Treignier et al. 2006, Treignier et al. submitted), was sampled with push cores of the ROV *Victor 6000* (surface of 21.2 cm²), while all other sites were sampled with a multiple corer (tube surface of 30.2 cm²) (Figure 1 and 2, Table 1).

Table 1: Dive and core numbers, latitudes (S), longitudes (E), depths (m), sampling devices, mean grain sizes (μm) and sampling habitat. R: sites sampled along a transect between the cold seep and the canyon; M: cold seep sites characterised by high mytilid densities; V: cold seep sites characterised by high vesicomyid densities.

Site	Dive number	Core number (replicates)	Latitude (°S)	Longitude (°E)	Water depth (m)	Sampling device	Mean grain size (μm)	Sampling habitat
A		MTB 22 (A, B)	07°20.80′	11°30.01′	1304	multicorer	4.398	control site
C		MTB 21 (A, B)	07°39.90′	10°00.41′	3994	multicorer	4.483	control site
D		MTB 20 (A, B)	05°50.80′	08°20.79′	3964	multicorer	6.031	levee (13 km south of Congo channel)
D′		MTB 19 (A, B)	05°43.13′	08°27.01′	4067	multicorer	6.097	bottom of channel
Lobe		MTB 33 (A, B)	06°28.29′	06°01.69′	4788	multicorer	4.061	lobe of channel
R3		MTB 26 (A, B)	05°48.67′	09°42.71′	3174	multicorer	7.629	transition zone
R4		MTB 27 (A, B)	05°48.15′	09°42.70′	3167	multicorer	5.926	transition zone
R6		MTB 28 (A, B)	05°48.00′	09°42.74′	3163	multicorer	5.803	transition zone
M1	PL146-9	CT2	05°47.85′	09°42.61′	3152	ROV push core		cold seep (mytilids)
M2	PL146-9	CT14	05°47.88′	09°42.68′	3154	ROV push core		cold seep (mytilids)
M3	PL147-10	CT13	05°47.84′	09°42.66′	3155	ROV push core		cold seep (mytilids)
V1	PL145-8	CT2	05°47.98′	09°42.47′	3151	ROV push core		cold seep (vesicomyids)
V3	PL147-10	CT10	05°47.80′	09°42.71′	3153	ROV push core		cold seep (vesicomyids)
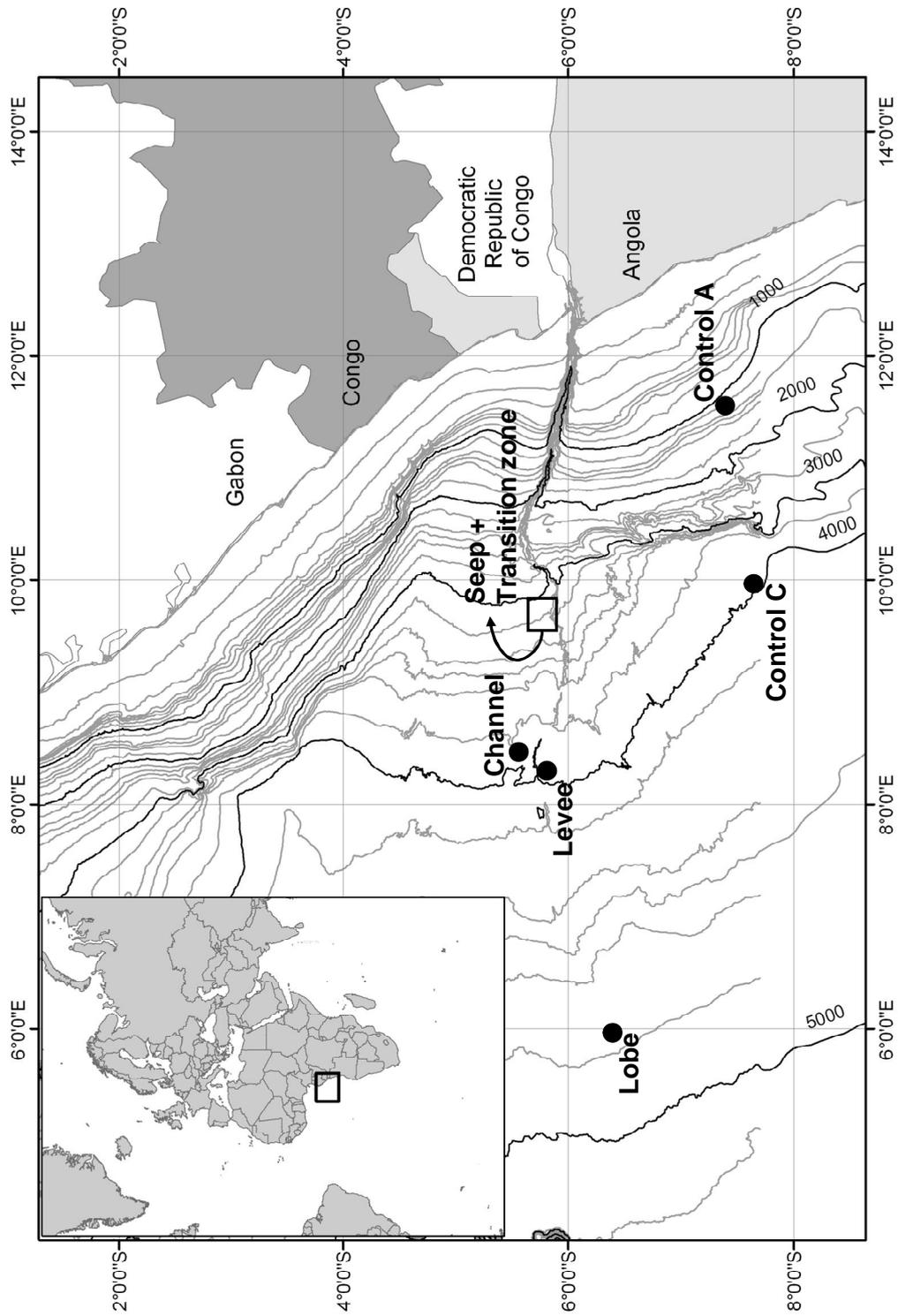
Figure 1: Map indicating the location of the Congo Channel system and sampling sites in the Gulf of Guinea, with 1000-m isobaths.
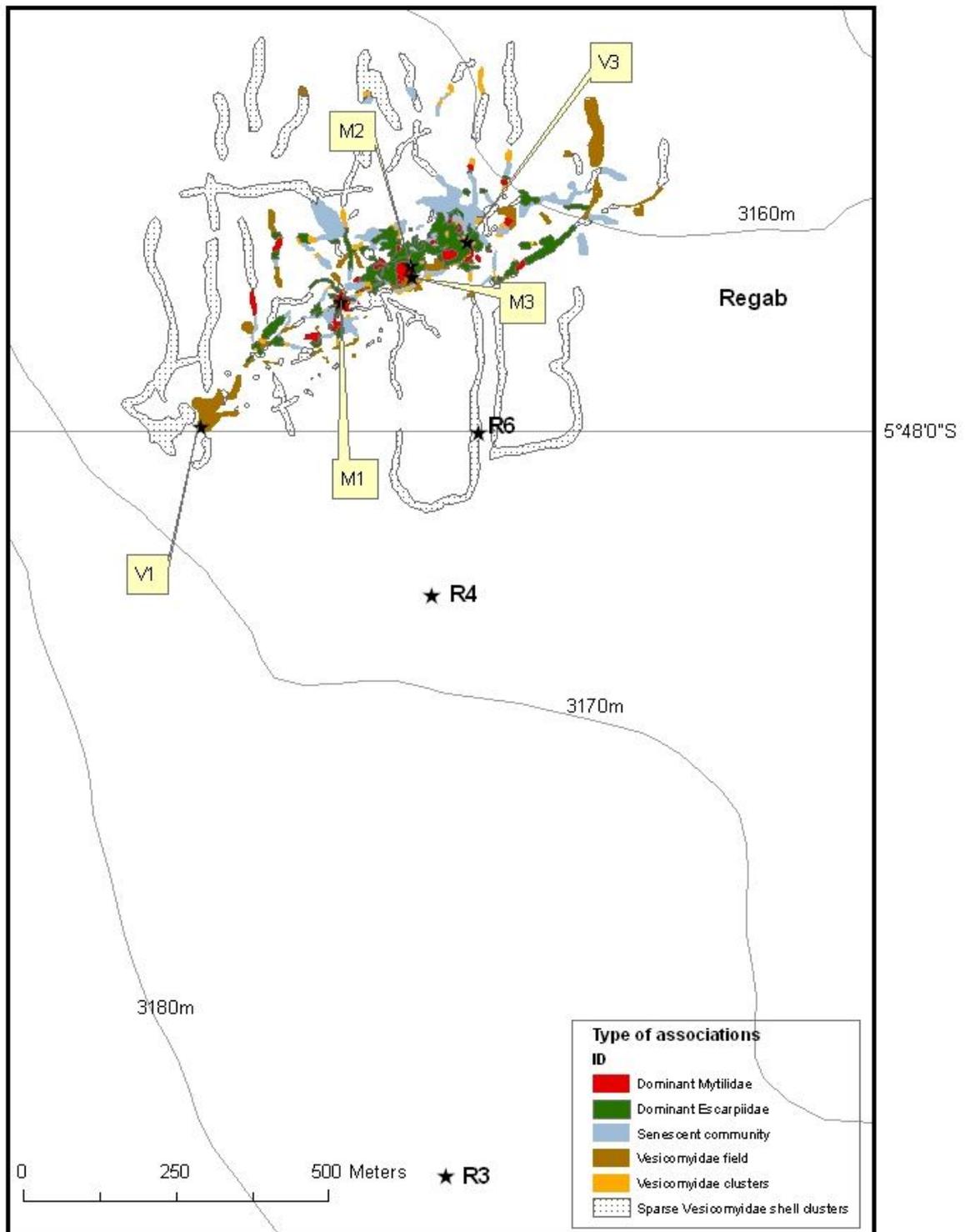
Figure 2: Detailed map of the REGAB cold seep. Points in the carbonate crust area indicate 5 different cold seep sites sampled with push cores of the ROV Victor. Points R3, R4, R6 indicate locations of the multicores in the transition zone between cold seep and Congo canyon (adapted from Olu-Le Roy et al. 2007).

Two samples (V1, V3) of the REGAB cold seep were taken at a patch densely colonised by clams (Vesicomyidae) and three samples (M1, M2, M3) at a patch dominated by mussels (Mytilidae) (Figure 2).

Furthermore three adjacent sites (R3 at 3174 m, R4 at 3167 m, R6 at 3163 m) were sampled at the transition zone located at the southern edge of this cold seep in a zone influenced by the Congo Channel system (Figure 2).

Along the Congo Channel system three sites were sampled: one at the abyssal plain (Lobe at 4788 m), one at the channel bottom (Channel at 4067 m) and one 13 km south of the canyon (Levee at 3964 m). Finally also two control sites (A at 1304 m, C at 3994 m) mainly dependent on plankton input, were sampled far away (150–200 km) from the canyon (Figure 1). At each site, two replicate cores were sampled.

The sediment of each core was sectioned horizontally in 1-cm layers down to 5 cm sediment depth. If collected, the following 5-cm slice was taken as one bulk sample. All sediment slices were fixed in 4% buffered formaldehyde and washed over a 32 μm-mesh sieve. Metazoan meiofaunal organisms were extracted from the sediment by centrifugation with Ludox, and stained with Rose Bengal. Afterwards the meiofauna was sorted out, enumerated and identified to major taxa under the stereomicroscope. From each slice, 100 nematodes were picked out at random, mounted in glycerine slides and identified to genus level.

Body length (L, μm) (excluding filiform tails) and maximal body width (W, μm) of 100 nematodes were measured in order to estimate the dry weight (DW). Individual biomasses were calculated from Andrassy's (1956) formula for wet body weight (WW). A specific nematode gravity of 1.13 g cm^{-3} and a dry-to-wet weight ratio of 0.25 were assumed (Wieser 1960).

Sediments from each site (except the cold seep) were subjected to granulometric analysis by means of a Coulter counter LS 100.

3. STATISTICS

The STATISTICA6 software was used to perform parametric and non-parametric analyses. Bartlett's and Cochran's test were used to verify the homogeneity of variances prior to the analysis. Parametric (one-way ANOVA) analysis on log-transformed data was used to compare total nematode and individual nematode biomass of the different sites along the Congo Channel. Non-parametric analysis (Kruskal-Wallis ANOVA by ranks) was carried out to describe differences in total meiobenthic densities.

Non-metric multidimensional scaling (MDS, PRIMER v5.0 statistical package from the Plymouth Marine Laboratory) was applied in order to visualize the dissimilarity among sites with respect to nematode genus composition. A first MDS was made using the data of all sites, while in the second MDS only the group of control, levee and transition sites was plotted. The stress value gives a measure for goodness-of-fit of the MDS ordination: a low stress value (<0.2) indicates a good ordination with no real prospect for a misleading interpretation (Clarke 1993). Both MDS plots had a stress value of 0.08, indicating that most of the variability present in the data was reduced into the two dimensions of the plot.

RESULTS

1. DENSITY AND DIVERSITY OF THE MEIOBENTHIC COMMUNITY

Average total meiofaunal densities differed significantly among sites (Kruskal-Wallis, p = 0.01) (Figure 3 and 4, Table 2). Highest densities were found at the control areas (1005 ± 78 ind. 10 cm^{-2} in control site C, 1320 ± 460 ind. 10 cm^{-2} in control site A). No strong decrease with water depth was present since meiofaunal densities at 4000 m were still around 1000 ind. 10 cm^{-2}. The sites along the Congo Channel were characterised by extremely low densities (<20 ind. 10 cm^{-2}); except the Levee site which showed intermediate densities (506 ± 100 ind. 10 cm^{-2}). The transition zone between seep and canyon also showed intermediate densities (677–796 ind. 10 cm^{-2}) whereas the seep centre showed a high variation in densities from about 870 to less than 20 ind. 10 cm^{-2}.

Diversity at meiobenthic phylum level was high at the control sites and the transition zone with a maximum of 14 different meiobenthic taxa in control site A. Lower numbers of meiofaunal taxa were

observed at the sites along the canyon and at the seep centre sites, with a minimum of only 2 taxa (nematodes and polychaetes) at the channel bottom. Nematodes were the dominant group at all sites, making up between 49.5–96.5% of the meiofauna, followed by the copepods and nauplii which reached together a maximum of 8.4% at control site A (Figure 3). The samples obtained from the cold seep had lower percentages (0.8–1.2%) of copepods and nauplii, and these did not occur at all at the Channel site. In contrast, some other meiobenthic taxa became more abundant at the cold seep: kinorhynchs were strongly concentrated at the sites with clams (up to 15.1%), while in one of the mussel bed sites (M2), polychaetes accounted for 26.0% and gastrotrichs for 16.5% of the associated meiobenthos. Other meiobenthic taxa, including ostracods, bivalves and tanaids, were observed frequently, but in low densities. Tardigrades were only present at the shallowest control site A.
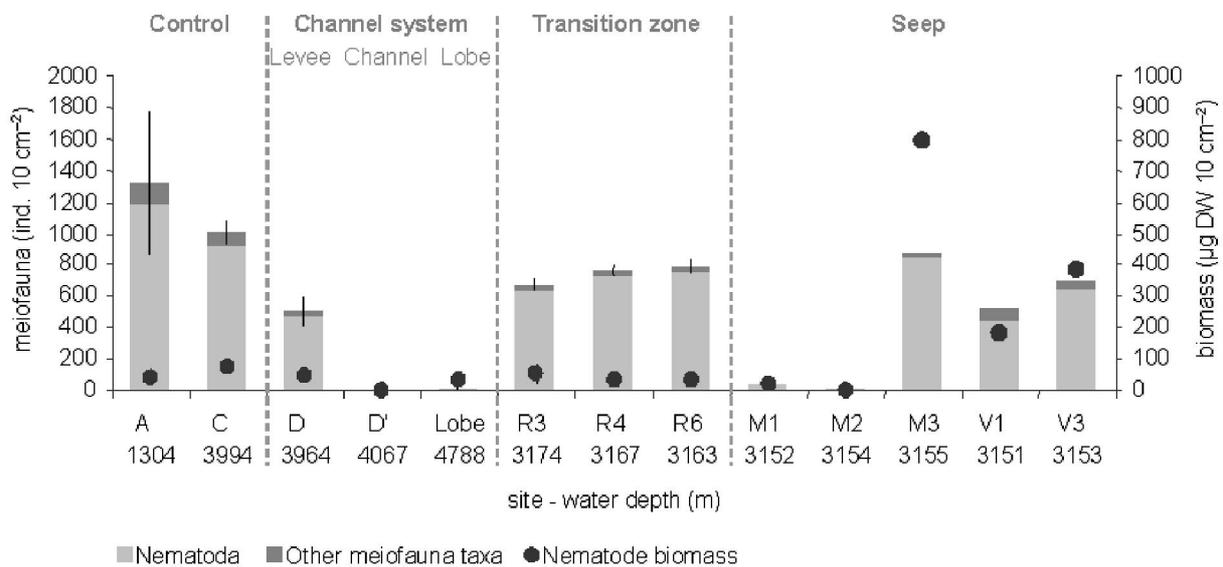
Figure 3: Meiofaunal densities with indication of nematode densities (ind. 10 cm^{-2}), and total nematode biomass (µg DW 10 cm^{-2}) (mean values ± 1 SD).

2. VERTICAL NEMATODE PROFILES IN THE SEDIMENTS

As nematodes made up the largest fraction of the meiobenthos at all sites, their densities followed the same pattern compared to the total meiobenthic densities, with highest values at the control sites and lowest at the channel bottom (Figure 3 and 4).

Table 2: Densities of all Gulf of Guinea metazoan meiofaunal taxa and relative abundance of Nematoda, Harpacticoida and nauplii (0 to 10 cm). SE: standard error.

	Control			Channel system		Transition zone			Seep				
Site	A	C	Levee	Channel	Lobe	R3	R4	R6	M1	M2	M3	V1	V3
Depth (m)	1304	3994	3964	4067	4788	3174	3167	3163	3152	3154	3155	3151	3153
	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)					
Density (ind./10 cm²)													
Amphipoda													
Bivalvia	1.5 (1.5)	0.2 (0.2)											
Gastrotricha	0.7 (0.7)					0.7 (0.3)	0.2 (0.2)	0.7 (0.7)	1.4				
Harpacticoida	50.7 (7.9)	58.3 (2.6)	17.4 (1.8)		0.5 (0.2)	27.5 (4.3)	26.7 (2.8)	20 (7.5)	0.5	1.4	6.6	2.4	3.3
Hydrozoa	0.7 (0.7)	1.2 (1.2)											
Isopoda	0.7 (0.7)					0.2 (0.2)							
Kinorhyncha	6.5 (0.5)	1 (0)	0.2 (0.2)			2 (1)	0.7 (0.3)	1.8 (0.5)			2.4	79.6	34.8
Nauplii	60.4 (27)	20 (9.8)	19.4 (7.1)		0.2 (0.2)	16.2 (3.3)	13.4 (3.8)	12.3 (4.3)			0.5	1.9	2.8
Nematoda	1181.6 (419)	916.6 (66.9)	466.4 (90.2)	2.5 (2.5)	13.7 (8.8)	625.3 (50.8)	724.8 (49.3)	757.9 (42.1)	35.3	9.9	842.7	442.6	650.2
Ostracoda	4 (1)	5.5 (1.8)	1.2 (0.2)			3 (0)	2.2 (1.2)	1.7 (0.1)					
Polychaeta	6.6 (0.3)	2.2 (0.5)	1.3 (0.7)	0.2 (0.2)	0.3 (0)	1.5 (0.2)	1.2 (0.5)	1.2 (0.2)	5.6	5.2	18.4		0.5
Tanaidacea	1.2 (0.5)		0.2 (0.2)										
Tantulocarida	0.5 (0.5)		0.2 (0.2)										
Tardigrada	4.5 (1.8)		0										
Turbellaria	0.5 (0.5)		0.3 (0)								1.4		0.9
Total	1320 (460)	1005 (78)	507 (100)	3 (3)	15 (9)	677 (45)	770 (40)	796 (55)	42.8	19.8	873.4	526.5	692.5
Relative abundance (%)													
Copepoda + nauplii	8.4	7.8	7.3	0.0	4.5	6.5	5.2	4.1	1.2	7.0	0.8	0.8	0.9
Nematoda	89.5	91.2	92.1	93.8	93.3	92.4	94.1	95.2	82.1	49.5	96.5	84.1	93.8

The control sites showed a gradual decrease in densities from the surface to the deeper layers (Figure 5). A similar pattern was observed for the canyon levee whereas along the channel system subsurface maxima up to 3 and 5 cm depth were observed. Also three samples from the seep centre (i.e., M3, V1 and V3) showed subsurface density maxima at 2 and 3 cm sediment depth. Away from the centre at the transition zone the nematodes showed a very even vertical distribution pattern down to 4 cm sediment depth. Only at the furthest transition site (R3) a clear surface maximum similar to the control sites was present.
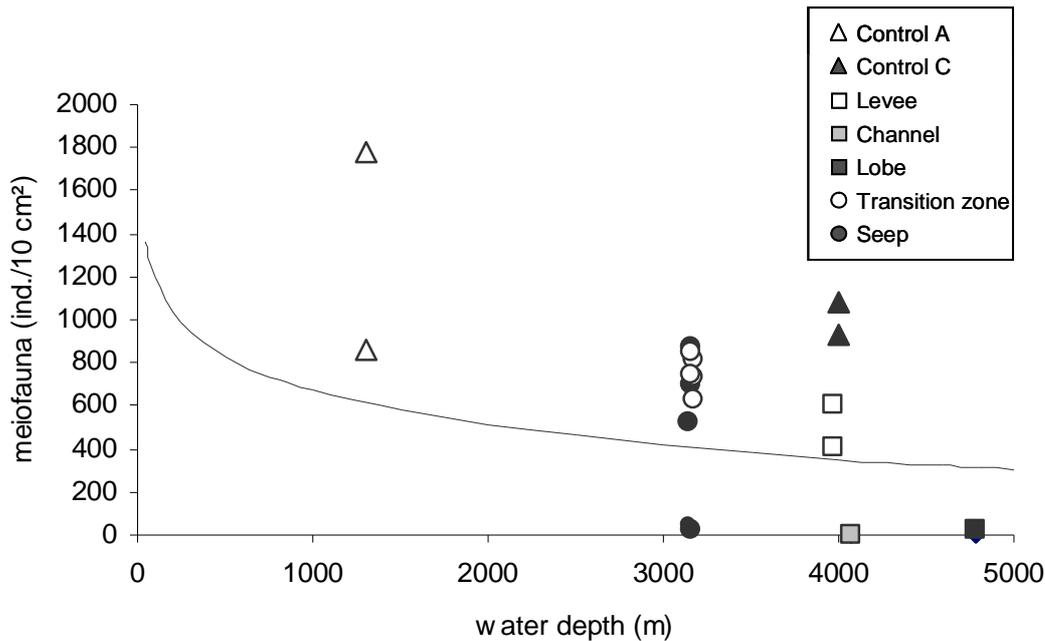
Figure 4: Average meiofaunal densities of the different sites in the Gulf of Guinea arranged according to water depth (m), compared with the best regression line [y = 2821.76 – 286.86*(ln depth)] from Soltwedel (1997) for the continental margins off the western African coast between Guinea (10°N) and Angola (18°S).

3. NEMATODE BIOMASS

There is a significant increase ($F_{6,14}$ = 10.25, p = 0.0002) in individual nematode biomass measured in the samples of the cold seep (0.32–0.94 μg DW); these had up to 9 times higher biomass than the average calculated for the individual nematodes of the other sites (0.03 $\pm$ 0.01 to 0.18 $\pm$ 0.20 μg DW). This results in significantly higher total nematode biomasses in the two samples from the clam beds (181.45–383.61 μg DW 10 cm^{-2}) and one sample from a mussel bed (M3 with 792.18 μg DW 10 cm^{-2}) compared to the nematode biomasses from the other sites (ranging between 0.20 $\pm$ 0.20 μg DW 10 cm^{-2} in Channel site and 74.97 $\pm$ 15.41 μg DW 10 cm^{-2} in control site C) ($F_{6,14}$ = 3.89, p = 0.017) (Figure 3).

Figure 5: Depth profiles of nematode densities for the different sites (ind. 10 cm^{-2}) (mean values ± 1 SD) (in seep profiles: white bars = *Sabatieria mortenseni* ; black bars = *Desmodora* sp. ; grey bars = remaining genera).

4. NEMATODE GENUS DIVERSITY

Nematode communities in both control sites were similar to eachother, although different proportions in the dominant genera were present; *Leptolaimus* had the highest relative abundance (13.2%) in site A whereas *Microlaimus* dominated the deeper site C with 33.6% (Table 3). These two genera were responsible for the high densities in both control sites. *Microlaimus* also made up 25.9–35.6% of the nematode densities at the deeper channel sites. The nematode communities found at the three transition zone sites on the southern border of the cold seep were dominated by the genera *Thalassomonhystera* (up to 17.5%), *Acantholaimus* (up to 11.9%), *Microlaimus* (up to 10.7%), *Leptolaimus* (up to 9.4%) and *Amphimonhystrella* (up to 9.0%). The samples taken at the Levee site revealed a similar nematode assemblage dominated by *Thalassomonhystera* (21.5%) and *Acantholaimus* (18.4%). Samples obtained from the cold seep showed a completely different nematode assemblage compared to the other sites. These communities consisted of a very low number of genera (4–14) compared to the control sites (65–82) (Table 4). All seep samples were strongly dominated by *Sabatieria mortenseni*, and/or by one undescribed *Desmodora* species. An illustration of the dominant nematode species or genera at the seep site, and at the non-seep sites is shown in Figure 6. As all photographs of the total nematodes were put on the same scale, the significant difference between the mean body size at REGAB seep and the other sites is obvious. The two additional pictures for each nematode are details of morphological structures (amphid, spicule, precloacal supplements) which are necessary to identify them to genus or species level (Figure 6).

Nematode genus diversity for all sites is shown in Table 4. The highest values were found at the shallowest control station at 1304 m, while the lowest nematode diversity was present at the cold seep samples and the deeper channel samples (Channel site and Lobe site). Diversity estimates were still high at the transition zone (site R3, R4 and R6), at the Levee site and the deeper control site C.

An MDS based on the nematode genus densities of all samples revealed a clear distinction of the seep and deeper canyon samples, attributed to the high dominance of different species at the seep and very low densities in the canyon (Figure 7). The upper MDS plot was based only on the group of control, levee and transition sites, and confirmed the gradual differences in nematode community structure among these sites.

Table 3: Mean relative abundances of the most abundant nematode genera per site in the Gulf of Guinea.

Site	Control		Channel system			Transition zone			Seep				
	A	C	Levee	Channel	Lobe	R3	R4	R6	M1	M2	M3	V1	V3
Depth (m)	1304	3994	3964	4067	4788	3174	3167	3163	3152	3154	3155	3151	3153
Sabatieria	3.3	0.1	0.5			1.0	0.3	0.3	17.0*		69.4*	87.0*	60.4*
Microlaimus	4.8	33.6*	1.5	35.6*	25.9*	10.7*	3.4	3.0					0.2
Thalassomonhystera	9.7*	7.5	21.5*	31.1*	1.1	11.8*	13.6*	17.5*					
Desmodora	0.9					0.1			80.1*	58.3*	25.8*	1.9	26.2*
Acantholaimus	5.8	14.1*	18.4*		5.0	7.1	11.9*	9.8*					
Halalaimus	7.4	1.7	4.2	20.0	3.3	3.5	7.6	5.2					
Leptolaimus	13.2*	0.5	1.0			9.4	6.2	8.7					0.2
Amphimonhystrella	3.9	0.6	3.5		8.9	8.2	8.4	9.0					
Theristus	3.3	6.9	7.8			2.3	2.8	1.1					
Tricoma	4.5	3.6	3.2			5.5	6.1	6.3					
Metadesmolaimus	0.6	1.9	0.4		7.8	4.5	0.4	2.6			0.4	5.7*	7.5
Sphaerolaimus	1.2		0.9		6.7	1.3	1.7	4.2					
Epsilonema					6.7					14.3*			
Syringolaimus	0.1	0.5	0.6		6.7			0.1		5.4			
Cervonema	0.7	0.7	6.4			1.0	0.9	0.5					0.1
Kraspedonema		0.1			9.1*		0.4					0.4	
Thalassoalaimus			0.1	13.3			0.1	0.1					
Paracanthonchus	0.8	5.3	0.3			0.2	0.1	0.1					0.1

* two most abundant nematode genera in site
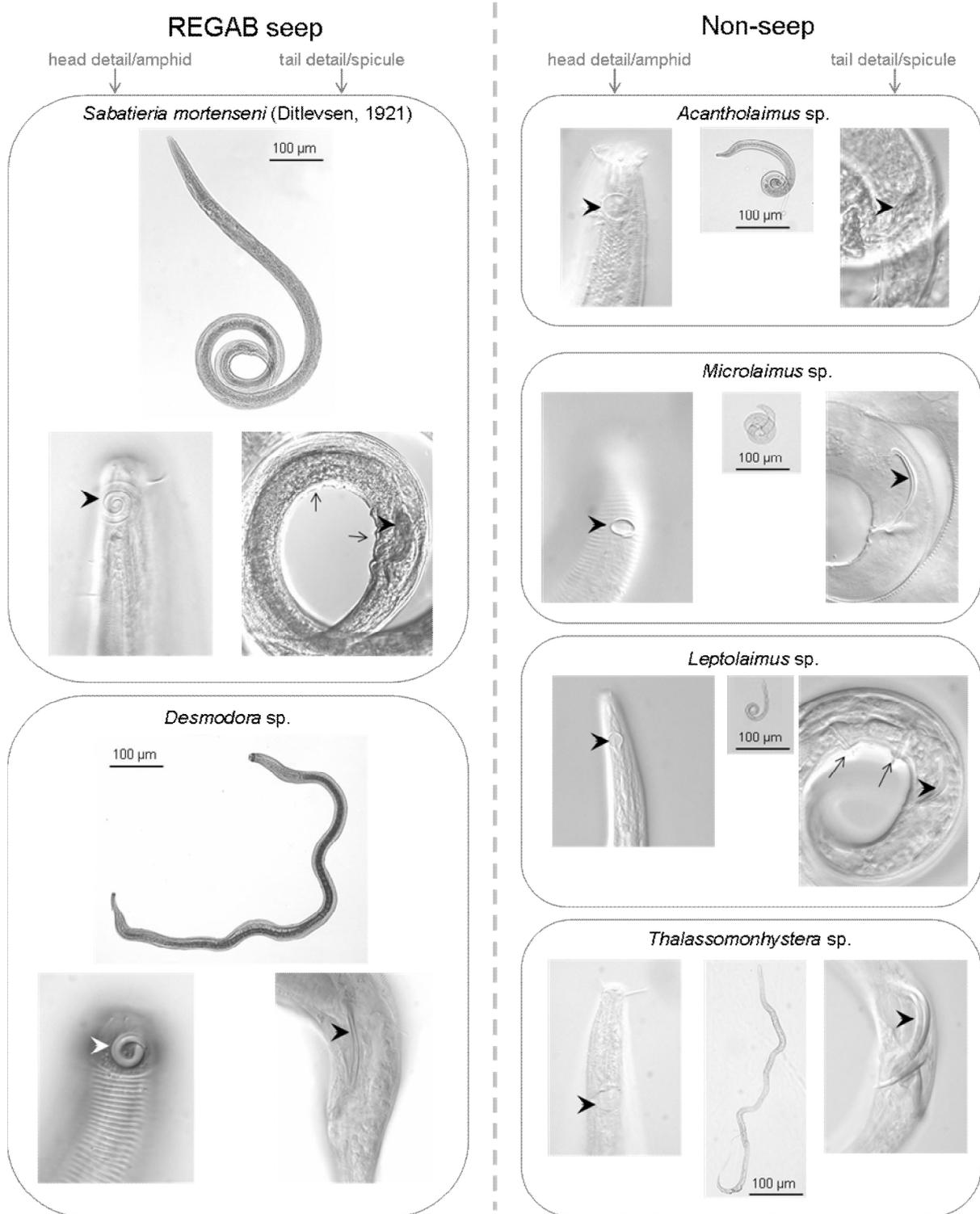
Figure 6: Overview of the dominant nematodes (males) in the seep site and in the non-seep sites in the Gulf of Guinea. Photographs on the left show the two dominant nematode species from REGAB, photographs on the right show representative species for the four most abundant nematode genera in the non-seep sites. Photographs of the nematode habitus are all placed on the same scale. Closed arrows point to the amphid at the anterior part or the spicule at the posterior part of the nematode, open arrows point to the precloacal supplements.

Table 4: Diversity indices at nematode genus level: Hill's diversity numbers of order 0, 1, 2 and ∞ (N_0, ..., N_∞) and the expected number of genera per 100 individuals ($EG(100)$)

	Control		Channel system			Transition zone			Seep				
Site	A	C	Levee	Channel	Lobe	R3	R4	R6	M1	M2	M3	V1	V3
Depth (m)	1304	3994	3964	4067	4788	3174	3167	3163	3152	3154	3155	3151	3153
N_0	82	65	69	4	19	73	72	62	4	7	8	8	13
N_1	29.4	13.2	17.9	3.7	7.6	23.0	22.1	19.8	1.8	4.0	2.2	1.8	3.0
N_2	18.5	6.6	9.9	3.6	6.1	16.0	14.7	12.7	1.5	2.7	1.8	1.3	2.3
N_∞	6.9	3.0	4.8	2.8	3.8	8.3	6.5	5.7	1.2	1.7	1.4	1.1	1.7
$EG(100)$	33	22	27	4	9	28	27	26	3	7	4	6	7
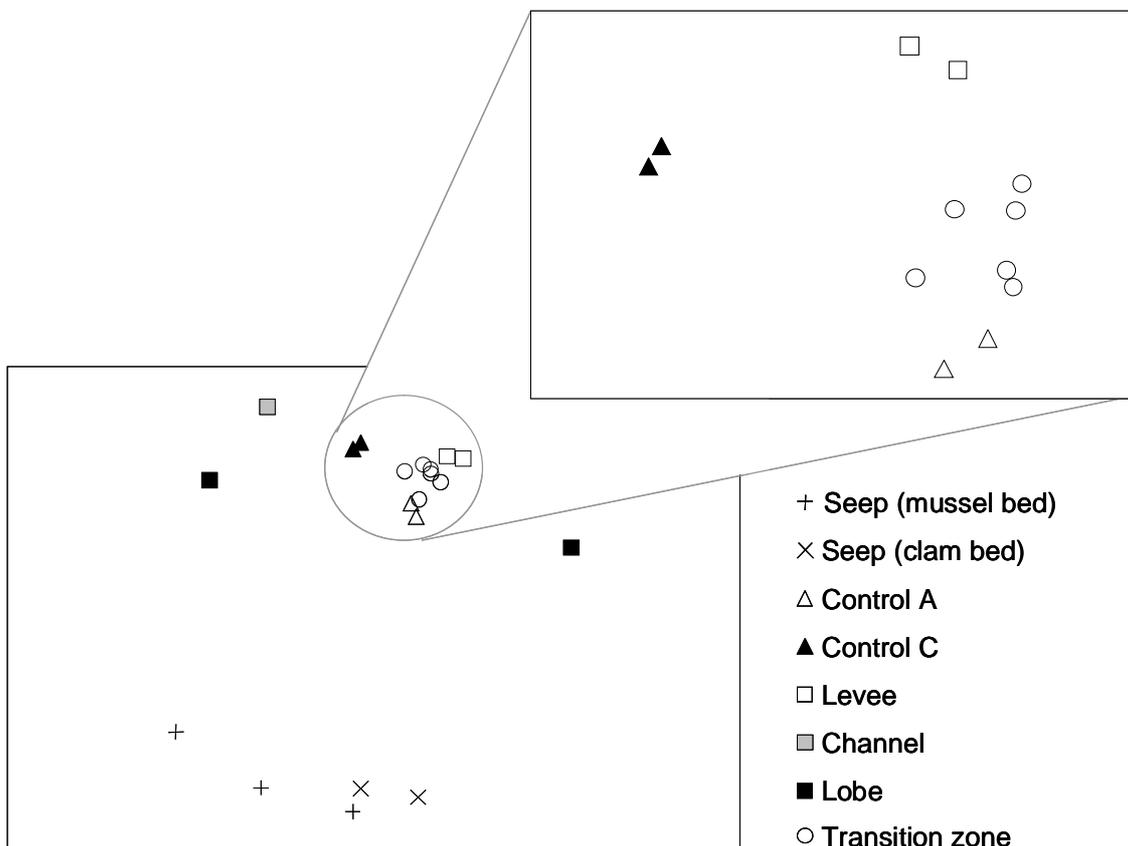
Figure 7: Nonmetric-multidimensional scaling plots based on the Gulf of Guinea nematode community (relative abundances of genera). Plot in background is based on all sites, plot on foreground shows only the control sites, levee site and transition zone.

DISCUSSION

1. DEEP-SEA CONTROL SITES IN THE GULF OF GUINEA

Meiofaunal abundances at the control sites were two or three times higher compared to the regression line of meiofaunal density against water depth based on the study from the continental margins off the western African coast between Guinea (10°N) and Angola (18°S) (Soltwedel 1997) (Figure 4). Soltwedel (1997) reported low meiofaunal densities at transects sampled off Gabon and the Congo estuary, compared to those at transects located more north and south of this area. The western South Atlantic is characterised by gradients in surface productivity due to spatially and seasonally varying coastal upwelling. It was hypothesised that the identified discrepancies between lower meiofaunal standing stocks and high pigment values may indicate a dislocation of (phyto-) detritus possibly caused by a coastal branch of the Benguela Current and the Congo River outflow (Soltwedel 1997). Another explanation could have been predator-prey interactions regulating the meiobenthic stock sizes (Soltwedel 1997), but this can be rejected as maximum macrobenthic densities (702.0 ± 121.4 ind. 0.25 cm^{-2}, Galéron et al. submitted) were found at the control site C characterised by a dense meiobenthic infauna. The sediment at both control sites was well oxygenated (>35 mm at site C) (J.C. Caprais pers. comm.). Particulate matter input originating from the water-column primary productivity (Rabouille et al. submitted) and concentrations of n-alcohols considered as biomarkers of pelagic and terrestrial origin of particles were measured at control site C (Treignier et al. 2006). This station revealed the highest short/long ratio (6.4) of n-alcohols, which is indicative of a major marine plankton input (Treignier et al. 2006, Treignier et al. submitted). However, the value also suggests that control site C is likely to receive terrestrial material. Previous studies indicated that the influence of the Congo River plume extends over an extensive area and even reaches the deepest control site. The high meiobenthic densities at the control sites of the Gulf of Guinea could be explained by the frequent input of food originating from pelagic resources (phytoplankton, zooplankton and bacteria) (Treignier et al. 2006). The sediments of the deepest control station C were characterised by exceptionally high relative abundances of *Microlaimus*. This nematode genus is known to be opportunistic, and is often found to be dominant or subdominant in areas with an increased organic input such as on the Darwin Mounds (Rockall Trough, north-east Atlantic) (Van Gaever et al. 2004) and the Siboglinidae fields of the Håkon Mosby Mud Volcano (SW Barents Sea slope) (Van Gaever et al. 2006). Vertical nematode distributions with highest relative abundances in the most surficial layers in both control sites pointed to a surface-derived food supply for the endobenthos.

2. TRANSECT IN TRANSITION ZONE BETWEEN CONGO CANYON AND COLD SEEP

In the transition zone at the southern edge of the REGAB seep, densities were slightly lower than at the control station C at a similar depth, but still twice as high as predicted by the regression for the continental margins off the western African coast (Soltwedel 1997). Nematode diversity appeared to be also slightly lower than at the control station C, and typical deep-sea genera dominated these communities: *Thalassomonhystera*, *Acantholaimus*, *Microlaimus*, *Leptolaimus* and *Amphimonhystrella*. The vertical profiles of the nematode abundance in the sediment showed a gradual change from the seep towards the canyon; there was a more even distribution with the highest proportion of nematodes in the layer 2–3 cm close to the seep to a more typical deep-sea vertical profile with the maximum nematode proportion in the surface layer further from the seep.

3. SITES ALONG AND INFLUENCED BY THE CONGO CHANNEL SYSTEM

Data on meiobenthic densities of submarine canyon channel systems are sparse but highly variable. Meiofaunal densities of ca. 1000 ind. 10 cm^{-2} were reported from the Ardencaple Canyon (Western Greenland Sea, Soltwedel et al. 2005b), in the Monterey Canyon off northern California (Carman et al. 2004) and in the Alaminos Canyon (Gulf of Mexico, Baguley et al. 2006), while Robinson et al. (2004) observed very low meiofaunal densities (<90 ind. 10 cm^{-2}) at seeps in Alaminos Canyon. Also the meiobenthic communities inhabiting the sediments of the Nazaré Canyon and adjacent open slope showed fluctuations in density (9.9–236.5 ind. 10 cm^{-2}) due to local high-velocity bottom currents and differences in organic matter input (Garciá et al. 2007).

Extremely low meiofaunal densities, diversity and biomass were found at the deeper sites along the Congo Channel system, and the highest nematode proportions were present in the subsurficial layers. The nematode genus *Microlaimus* made up a very important fraction of the community at these Channel sites. This genus was also dominant during the recolonisation of shallow Antarctic sediments after a catastrophic iceberg scouring and seems to be able to withstand great sedimentary disturbance. This scouring disturbance led to a certain degree of reduction in diversity and a notable decrease in meiofaunal abundance (>95%) which was outside the range of seasonal fluctuation, even considering the patchy distribution of nematodes as described in a previous study on temporal variation (Vanhove et al. 2000). The pioneering meiofaunal colonisers after the iceberg scouring were copepods and ostracods, followed by nematodes with a *Microlaimus* sp. as dominant species throughout the whole

period (Lee et al. 2001). The almost complete absence of a meiobenthic community and the high relative abundance of *Microlaimus* at the deeper Congo Channel sites from this study are possibly controlled by the current activity in the Congo submarine valley, and the additional very weak oxygen penetration of 7 mm at the Channel site and only 2–3 mm at the Lobe site (J.C. Caprais pers. comm.). All other sites were well oxygenated up to 23–39 mm in the sediment (J.C. Caprais pers. comm.). Periodic turbidity events occur frequently in the Congo Canyon (Khripounoff et al. 2003), and may cause resuspension and transport of the surface sediments down to the abyssal plain, leading to unstable sediment substrate. Moreover, high sedimentation rates may lead to fauna being buried by sediment. According to Treignier et al. (submitted), there is a high input of terrestrial, organic matter, but apparently large turbidity events prevent the establishment of meiofauna communities. Higher predation pressure on the nematodes could be another explanation for the extremely low meiobenthic densities and subsurface maxima in the Channel and Lobe site. Unfortunately, no macro- or megafaunal data of these sites were available to check this hypothesis. The sediments at the Levee site located at 13 km south from the Congo Canyon were oxygenated up to 39 mm sediment depth (J.C. Caprais pers. comm.) and likely to be less exposed to turbidity flows compared to the sites inside the channel system. Meiofaunal densities, nematode diversity, dominant nematode genera and vertical nematode distribution at the Levee site were very similar to those at the transition zone. Although an overflow onto the levees occurred in 2001, the increase of organic matter at the surface which was observed up to 9 months after the event, disappeared after two years (Khripounoff et al. 2003). The sedimentation of terrestrial organic matter in this site depends on the frequency of the overflows, which is linked to their thickness and expansion (Treignier et al. submitted).

4. Giant pockmark complex REGAB

A completely different meiobenthic community was present at the cold seep REGAB. Highest meiofaunal densities were similar to those at the transition zone, although the seep samples were characterised by a very high variability in density and exhibited low nematode diversity. Kinorhynchs and polychaetes also showed a very patchy distribution. A maximum of only 14 nematode genera was found in this site, and two dominant nematode species (*Sabatieria mortenseni* and *Desmodora* sp., Figure 6) made up on average more than 70% of the total nematode densities. The very large individual biomass of *Sabatieria mortenseni* resulted in a significantly higher biomass in the two samples from the clam bed and one sample from a mussel bed.

Sabatieria is one of the dominant genera in muddy, reduced sediments of shallow water sites all over the world (Warwick and Gee 1984, Vincx 1986, Jensen 1987, Ott et al. 1991, Polz et al. 1992, Soetaert and Heip 1995), and many of its species are considered eurytopic and tolerant of unstable, highly polluted environments (Jensen 1984, Vanreusel 1990, Steyaert et al. 1999). The genus can attain high proportions at oxygen minima along the slope but gradually disappears almost completely in sediments deeper than 2000 m (Soetaert and Heip 1995, Vanaverbeke et al. 1997). *Sabatieria mortenseni* is a cosmopolitan that has already been reported from littoral sediments with a high amount of clay and mud in Brazil, USA, Antarctica (Ditlevsen 1921, Allgén 1947, Allgén 1959, Pastor de Ward 1984) and in the Strait of Magellan (Chile) in a depth range of 8–550 m (Chen 1999). The observation of a dominant species in the reduced seep sediments known from shallow waters with a wide distribution is similar to the dominant nematode from the sulphide-rich sediments at Håkon Mosby Mud Volcano (*Halomonhystera disjuncta*) (Van Gaever et al. 2006).

The genus *Desmodora* is present in most of the assemblages studied in deep-sea sediments, but always at low densities (relative abundance is a maximum of 1% of the total community). Some species of *Desmodora* were described from hydrothermal vents of the Pacific (Verschelde et al. 1998). Bernhard et al. (2000) reported the presence of the ectosymbiont-harbouring *Desmodora masira* in the oxygen minimum zone of the Santa Barbara Basin (California margin, 475–600 m). The species observed at the REGAB seep has not been described.

Metazoan meiofaunal assemblages within seeps often show large variations in densities and highly patchy distributions. At a mud volcano in the northern Barbados prism densities ranged between 116 and 11364 ind. 10 cm^{-2} across different seep sub-habitats (Olu et al. 1997). The metazoan meiobenthic communities present at Håkon Mosby Mud Volcano (HMMV) in the Eastern Atlantic were characterised by similar highly significant differences (513–11292 ind. 10 cm^{-2}) among sub-habitats (Van Gaever et al. 2006). The most surprising result were the unusually high densities of a single nematode species, *Halomonhystera disjuncta*, associated with the sulphidic sediments underneath the microbial mats and thriving on chemosynthetically derived food sources (Van Gaever et al. 2006).

Meiofaunal abundances within the REGAB seep varied between 20 ind. 10 cm^{-2} and 873 ind. 10 cm^{-2}. The small-scale heterogeneity in methane levels is hypothesised to have an important influence both on the megafaunal and meiofaunal patchiness at this seep site. The meiofaunal assemblages at the seep were strongly dominated by two nematode species (*Sabatieria mortenseni* and *Desmodora* sp.) which disappeared completely in samples collected outside the seep. These species did not reach extremely

high densities, but were characterised by a very large individual biomass, resulting in a maximum total nematode biomass at the mussel bed at REGAB similar to the maximum total biomass made up by the abundant *Halomonhystera disjunta* at HMMV (Van Gaever et al. 2006).

The increased nematode biomass and subsurface distribution maxima suggest that this unique meiobenthic community at REGAB is based on chemosynthesis, in contrast to the phytodetritus-based assemblages found at the control sites and the transition zone. There was no clear evidence of ecto- or endosymbionts in the seep nematodes, but it is possible that these nematodes benefit from free-living chemoautotrophic bacteria as food source. Obviously, the diversity and density patterns of the meiofauna indicate that these cold seep communities are strongly controlled by the patchy biogeochemical sediment conditions such as variable levels of oxygen, sulphide and methane.

CONCLUSION

Distinct meiofaunal communities could be distinguished, reflecting a multiple-scale (from m′s to 200 km) spatial variability that corresponds with the different habitats in the Gulf of Guinea. Apparently, nematode abundances could be as different on a microscale (m′s) in the cold seep as they were on larger spatial scales (tens of km). Nematode assemblage composition exhibited more consistent patterns within a smaller area, but was also highly variable if different habitats on a mesoscale and macroscale were compared.

Studies of the total metazoan meiofauna community and on the nematode genus level can yield important information concerning habitat characteristics in the deep sea. Increasing nematode densities and biomass often point out the presence of an abundant food resource and/or high food quality. The use of chemosynthesis in reduced sediments can result in significantly higher nematode biomasses. The proliferation of some nematode 'indicator' genera helps to identify environmental conditions.

For instance, the genus *Sabatieria* is well adapted to living in fine and reduced sediments. This nematode is an indicator for anoxic to sub-oxic sediments and will be successful in those environments where a substantial fraction of organic matter becomes incorporated below the oxic zone in the sediment, or in sediments where it may benefit from chemosynthetic nutritional pathways. *Sabatieria mortenseni* was very dominant in the seep samples from this study. Apparently, dominant nematodes

from these isolated habitats are often cosmopolitan eurybathic species with very high tolerance levels. Similar trends were found for Foraminifera, where no seep endemics have been identified and most seep genera were also characteristic of other low oxygen, organic rich settings (Bernhard et al. 2001, Rathburn et al. 2000, 2003). The high resistance of these meiofaunal species permits them to successfully colonise a specific niche in these marine sediments, often with toxic conditions. However, molecular studies on nematodes indicate that the existence of cryptic species is likely to be substantial within marine free-living nematodes, as shown for the species *Halomonhystera disjuncta* (Derycke et al. 2007).

Microlaimus may be a successful opportunistic coloniser in recently disturbed and organically enriched sediments and can exploit different types of substrate. Frequently occurring turbidity events in the Congo Channel prevent the establishment of a stable meiofaunal community and result in extremely low densities in the channel system. The conditions in the canyon are harsh, and only opportunistic and good colonisers like *Microlaimus* species are found albeit in low densities.

In general, the physical (strong bottom currents in channel system) conditions, the fluctuating sedimentary biogeochemical conditions (in particular at the seep site), as well as the food supply from the continental shelf are reflected in the densities and diversity patterns of the meiobenthos at the different deep-sea habitats in the Gulf of Guinea. The extended knowledge of nematode diversity and density patterns in the deep sea provide essential information on habitat preference. Biogeochemical variables of the sediment (such as oxygen, methane, sulphide, pH, etc.) as well as environmental conditions of the surrounding area on a small scale (organic content, macrofaunal communities, etc.) and even on a larger scale (water depth, topographic submarine structures, water bottom currents) all have an impact on the community structure of marine nematodes. Moreover, because marine nematodes are distributed worldwide, are the most prominent members of the smaller-sized animals, and have a direct link with the sediment and with the processes that occur immediately above the sediments, this group can be utilised as an interesting tool to describe and identify habitat heterogeneity in the marine environment.

ACKNOWLEDGEMENTS

CHAPTER 4

Meiobenthos at the Arctic Håkon Mosby Mud Volcano,
with a parental-caring nematode thriving in the sulphide-rich sediments

Meiobenthos at the Arctic Håkon Mosby Mud Volcano
with a parental caring nematode thriving in sulphide-rich sediments

ABSTRACT

Håkon Mosby Mud Volcano (HMMV, SW Barents Sea slope, 1280 m) is one of the numerous cold methane-venting seeps existing along the continental margins. Analyses of video-guided core samples revealed extreme differences in the diversity and density of the metazoan meiobenthic communities associated with different sub-habitats (centre, microbial mats, Siboglinidae field, outer rim) of this mud volcano. Diversity was lowest in the sulphidic, microbial mat sediments that supported the highest standing stock with unusually high densities (11000 ind. 10 cm^{-2}) of one nematode species related to *Halomonhystera disjuncta.* Stable carbon isotope analyses revealed that this nematode species was thriving on chemosynthetically derived food sources in these sediments. Ovoviviparous reproduction has been identified as an important adaptation of parents securing the survival and development of their brood in this toxic environment. The proliferation of this single species in exclusive association with free-living, sulphide-oxidising bacteria (*Beggiatoa*) indicates that its dominance is strongly related to trophic specialisation, evidently uncommon among the meiofauna. This chemoautotrophic association was replaced by copepods in the bare, sulphide-free sediments of the volcano's centre, dominated by aerobic methane oxidation as chemosynthetic process. Copepods and nauplii reached maximum densities and dominance in the volcano's centre (500 ind. 10 cm^{-2}). Their strongly depleted carbon isotope signatures indicated a trophic link with methane-derived carbon. This proliferation of only selected meiobenthic species supported by chemosynthetically derived carbon suggest that in addition to the sediment geochemistry, the associated reduced meiobenthic diversity may equally be related to the trophic resource specificity in HMMV sub-habitats.

INTRODUCTION

Rapid changes in biodiversity are occurring globally and are calling for a better understanding of what controls the structure and functioning of communities inhabiting the seafloor (Solan et al. 2004), the largest habitat on earth. Extreme environments such as cold seeps and hydrothermal vents do not only reveal information on the boundaries of metazoan life but they may also unravel fundamental aspects of ecology. Sediments of cold seep ecosystems, ultimately driven by methane seeping from great depths, often harbour a consortium of archaea and bacteria that generates large amounts of sulphide (Boetius et al. 2000, Orphan et al. 2002), which is toxic for many forms of life (Bagarinao 1992). The subsequent microbial mediated oxidation of sulphide supplies significant amounts of organic carbon in the form of microbial biomass, and sulphide-oxidising bacteria like *Beggiatoa* form prominent white mats in many methane seeping environments (Pimenov et al. 2000, Levin 2005). Although being an unusual trophic resource, the high bacterial production at seeps may fuel the higher trophic levels but observations on sediment-dwelling infauna and their adaptation strategies are limited (Levin 2005). Studies are generally more abundant of macrobenthos and in microbial-mat covered seep sediments, high-density patches of macrofauna species consist often of aggregations of dorvilleids or hesionid polychaetes (Robinson et al. 2004, Levin 2005). In all studies of deep-sea cold seeps, dominance was high and species diversity was relatively low within the macrofauna, compared to the surrounding sediments (Levin et al. 2000). The limited number of species that proliferate in seeps seem to flourish on chemosynthetic derived organic matter (Levin and Michener 2002).

Meiofaunal studies from deep-sea cold seep areas are generally scarce. Some of these studies documented an increased meiofaunal density or biomass compared to nearby control sites (Buck and Barry 1998, Olu et al. 1997, Robinson et al. 2004). For the seep areas in the Gulf of Mexico, Robinson et al. (2004) recorded meiofaunal densities 10 times higher in a microbial mat zone (1934 m, ca. 6500 ind. 10 cm^{-3}) than in the nearby non-seep sediments (2238 m). Olu et al. (1997) documented one to two orders of magnitude greater meiofaunal densities on mud volcanoes than expected for non-seep sediments at a similar depth (5000 m). In contrast, little or no difference in density from control sites has been observed at other deep-sea cold seeps (Shirayama and Ohta 1990). In two shallow seeps (methane seeps of a North Sea pockmark and of the northern Kattegat), reduced meiofaunal densities occurred (Dando et al. 1991, Jensen et al. 1992). Overall, no univocal response of the metazoan meiofaunal abundance to seep conditions emerges from previous studies (Levin 2005). Moreover, increased habitat heterogeneity of seep areas often results in higher variability of meiofaunal density

within than outside seeps (Montagna and Spies 1985). Additionally, deep-sea seep meiofaunal studies are often restricted to family or higher taxon level but repeatedly reveal nematodes as the dominant taxon, similar to regular deep-sea sediments (Levin 2005).

Here we report on a study of the metazoan meiofauna from the deep-sea Håkon Mosby Mud Volcano (HMMV). Soltwedel et al. (2005a) presented the first numbers on the meiobenthic abundances at the HMMV and identified nematodes as the most abundant metazoan taxon at different sub-habitats. However, nematode diversity and trophoecology remain unknown. The present study examined the metazoan meiobenthos with special reference to nematode diversity and densities at four sub-habitats of HMMV differing strongly in sediment geochemistry (de Beer et al. 2006). Special attention was paid to microbial mat sediments which often support enhanced densities of fauna in otherwise food-limited, deep-sea sediments (Levin 2005, Soltwedel et al. 2005a). The importance of chemosynthetic nutrition was estimated through sediment bulk and labile organic matter carbon isotopes as well as dominant species natural carbon isotope signatures that reflect the dietary contribution or importance of photosynthetic- versus chemosynthetic-derived carbon (Van Dover et al. 2003, Levin 2005). Finally, the importance of symbiotic relationships for dominant microbial mat species was examined using light and electron microscopy.

MATERIALS AND METHODS

1. STUDY AREA

HMMV is a cold seep, located on the continental slope off northern Norway at a water depth of 1280 m (Figure 1). It has a relief of 8–10 m in height and a diameter of about 1.5 km. HMMV has a concentric structure with a mud-oozing centre which is constituted of highly gas-saturated sediments and has a very strong thermal gradient at the sediment–water interface (Ginsburg et al. 1999). Beyond this central plain, also referred to as the thermal "eye" of the volcano (Soltwedel et al. 2005a), the seafloor has a complex topography of hills and depressions, and is covered by extensive microbial mats (grey and white) of big chemoautotrophic bacteria. According to Pimenov et al. (2000), grey microbial mats are constituted by the genera *Beggiatoa*, *Thioploca* and the morphologically similar genera *Leucothrix* and *Thiothrix*. In contrast, the white mats are dominated by a single filamentous morphotype with a diameter of 10 μm and abundant sulphur inclusions. This big morphotype is referred to as

Beggiatoa (de Beer et al. 2006). The periphery and parts of the outer rim of HMMV are colonised by dense patches of Siboglinidae (Figure 1, 2) (Vogt et al. 1997a, Gebruk et al. 2003, de Beer et al. 2006). The different sub-habitats, each with typical environmental conditions, are displayed in Figure 2. However, this description of the sub-habitats at HMMV only provides a generalised view, as the small-scale (cm) patchiness is rather pronounced.

The macrobenthic chemosynthetic communities of HMMV are dominated by two species of small Siboglinidae, *Sclerolinum contortum* and *Oligobrachia haakonmosbiensis*, both harbouring symbiotic, autotrophic methane-oxidising bacteria and living in colonies covering hundreds of square meters of the seafloor (Gebruk et al. 2003, Soltwedel et al. 2005a). Other organisms which form associations with the siboglinids include diverse polychaetes, bivalves and amphipods, as well as gastropods, ophiuroids and tanaidaceans (Gebruk et al. 2003). Striking is the absence of typical seep bivalves, Vesicomyidae and Mytilidae (Milkov et al. 1999, Boetius et al. 2000).

2. SAMPLING AND SAMPLE PROCESSING

Sediment samples were collected during the cruise ARKTIS XIX/3b aboard the German RV Polarstern in June–July 2003. In total, 4 HMMV sub-habitats were sampled using a video-guided multiple corer (90 mm core diameter). At each sub-habitat (centre, microbial mats, Siboglinidae field, outer rim), 3 replicate tubes were taken from 2 or 3 different deployments. As a control, two sites located outside HMMV (10's kms) were sampled (Figure 1, 2; Table 1). For both the microbial mats and Siboglinidae field, replicates were selected based on the presence of respectively bacteria (*Beggiatoa*) or Siboglinidae, whereas the outer rim replicates did not contain either of them.

The overlying water from each sediment core was collected separately. The sediment was sectioned horizontally in 1-cm layers down to 5 cm sediment depth. The following 5-cm slice (5–10 cm) was taken as one bulk sample. After fixation with 4 % buffered formaldehyde, the samples were washed over a 32 µm-mesh sieve. Metazoan meiofaunal organisms were extracted from the residue by centrifugation with Ludox, and were stained with Rose Bengal. Organisms were sorted and identified to major taxon level under a stereoscopic microscope. From each sediment section, 100 nematodes were randomly picked out, mounted on glycerine slides and identified down to genus level.

Figure 1: Study area and sampling sites indicated on a bathymetric map of the Håkon Mosby Mud Volcano (HMMV, adapted from Edy et al. 2004).
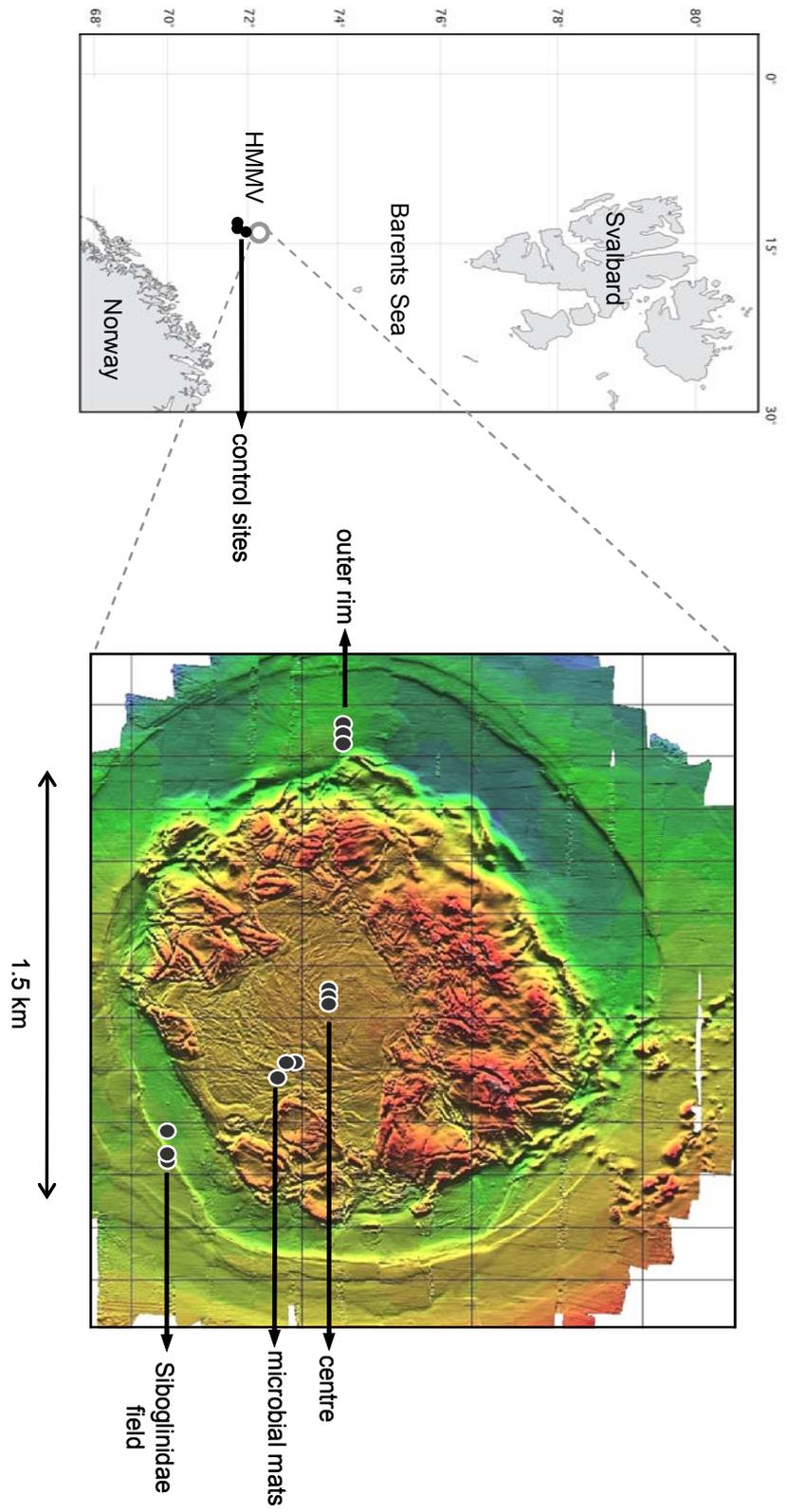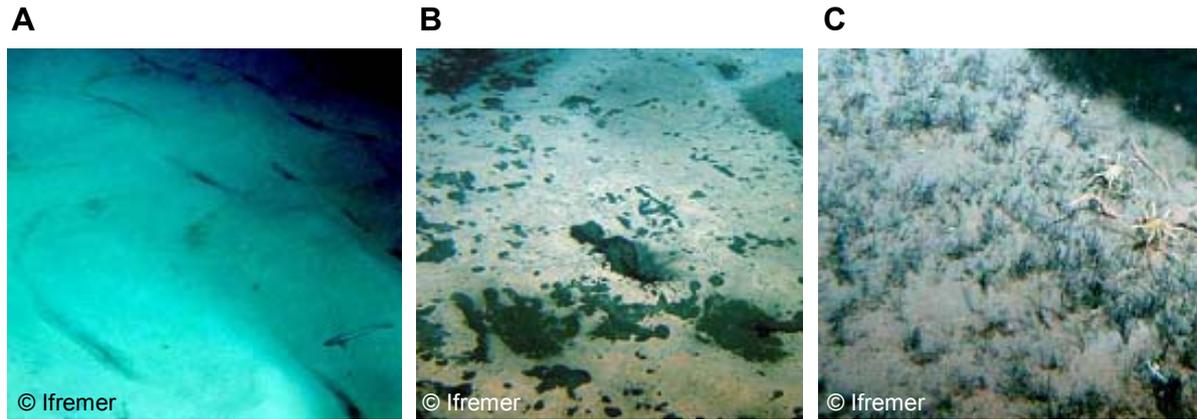
Figure 2: Photographs of HMMV sub-habitats: (A) centre, (B) microbial mats and (C) Siboglinidae field (Sauter et al. 2003).

Table 1: Station parameters including corresponding sub-habitat, geographical position, water depth and multicore sample number. Samples collected in a similar sub-habitat were taken as replicate samples.

Sampling site	Latitude	Longitude	Depth (m)	Multicorer sample
Centre	72°0.25'N	14°43.49'E	1286.0	PS 64/312 PS 64/313 PS 64/314
Microbial mats	72°0.18'N	14°43.81'E	1287.0	PS 64/321 PS 64/323 PS 64/324
Siboglinidae field	72°0.05'N	14°44.14'E	1288.2	PS 64/356 PS 64/357 PS 64/367
Outer rim	72°0.21'N	14°42.47'E	1289.4	PS 64/362 PS 64/363a PS 64/363b
Control site	71°41.96'N	13°26.10'E	1885.0	PS 64/390a PS 64/390b
Control site	71°58.97'N	14°45.41'E	1296.0	PS 64/395

From each replicate of the 5 different sub-habitats, body length (L, μm) and maximum body width (W, μm) of 100 nematodes were measured in order to estimate the dry weight (dwt). Individual biomasses were calculated from Andrassy's (1956) formula for wet body weight (μg wwt = L x W²/1600000). A specific nematode gravity of 1.13 g cm^{-3} and a dry weight to wet weight ratio of 0.25 were assumed (Wieser 1960). Dry weight was converted into carbon assuming a conversion factor of 0.5.

3. *IN SITU* MICROPROFILES

In situ profiles were made at the microbial mats, data from other sites are reported in de Beer et al. (2006). Micro-sensors for O_2 and H_2S were made and used as described in Revsbech (1989) and Jeroschewski et al. (1996). The tip diameters were ca. 20 µm, the response time less than 3 seconds. The sensors were calibrated after mounting on a free-falling deep-sea profiler (Wenzhofer and Glud 2002). After deployment, the profiler was inspected by the remotely operated vehicle. Secondly, the sub-habitat was inferred from sediments recovered by a benthic chamber, with a closing bottom, on the same lander (mounted ca. 75 cm from profiler). The profiler was pre-programmed to measure one or two vertical profiles, with a spatial resolution of 250 µm, over a depth of 17 cm. After each downward step, 4 seconds elapsed before a reading was made. Each reading was the average of 4 samplings over a period of 4 seconds. The profiling started ca. 6 hours after reaching the seafloor.

4. CARBON ISOTOPE MEASUREMENTS

Samples for sediment and faunal carbon isotope analysis were collected separately and intact sediment layers (0–1 and 1–2 cm) were stored frozen. After thawing in the laboratory, distinct bundles of filamentous bacteria were carefully picked with a thin needle from the surface of the first layer, transferred to Milli-Q water and put into pre-combusted silver boats. Similarly, 50–60 nematodes and 20–30 copepods were processed from sub-samples sieved over a 32 µm sieve. Samples were acidified with 2.5 % HCl and then dried overnight at 55°C. Sediment $\delta^{13}C_{org}$ measurements were done on ground freeze-dried sediments of both layers.

The carbon isotope signatures of labile carbon in the sediment were estimated through sediment–water slurry incubations. For that, 1 ml of thawed, homogenised surface sediment from the microbial mats was transferred into 30 ml glass incubation bottles, diluted with 30 ml 0.2 µm filtered Atlantic deep-sea water and sealed with screw caps fitted with rubber septa. Following 6 days dark incubation at room temperature, 2 ml of water was removed with a syringe and filtered into a 10 ml helium pre-flushed vial and then acidified with phosphoric acid. The $\delta^{13}C$ of ΣCO_2 produced reflects the $\delta^{13}C$ of the organic carbon remineralised (i.e. labile organic carbon) and was calculated from the difference in δ^{13}-ΣCO_2 at the start and end of the incubations: $\delta^{13}C_{org\text{-}labile} = ([\Sigma CO_{2\ end}] \times \delta^{13}\text{-}\Sigma CO_{2\ end}) - ([\Sigma CO_{2\ start}] \times \delta^{13}\text{-}\Sigma CO_{2\ start})/ ([\Sigma CO_{2\ end}] - \Sigma CO_{2\ start})$. The isotope signature of ΣCO_2 changed from $+0.4 \pm 0.1‰$ to $-15.6 \pm$

0.4‰, as a result of about a 177 % increase in ΣCO_2. The measurement of $\delta^{13}C$ and concentration of ΣCO_2 was done as described in Moodley et al. (2005). Values in the text are averages of 2 or 3 replicates ± standard errors.

5. SCANNING ELECTRON MICROSCOPY

Any ectosymbiotic relation in the nematodes was checked with scanning electron microscopy (SEM). Nematode specimens were fixed in hot 4 % formaldehyde and subsequently dehydrated using an ethanol series (25 %, 50 %, 75 %, 2 x 100 %). To prevent tissue damage and collapsing, the specimens were put into a box until fixing onto the stub, and were critical point-dried with CO_2 (Eisenback 1986). Afterwards, the dried nematodes were placed individually on a standard specimen stub, sputter-coated with gold and finally observed under a scan (Jeol JSM-840) at 15 kV.

6. STATISTICS

After testing the assumptions of normality, analysis of variance (ANOVA with confidence level of 99 %, program S-Plus) and Tukey's post hoc comparison tests were used to describe differences in meiobenthic densities among sites. Relative nematode genus densities were ordinated by non-metric Multi-Dimensional Scaling (MDS, PRIMER). A stress value < 0.1 is a measurement of the goodness-of-fit test, indicating a good representation with no prospect of a misinterpretation. To compare the diversities of the sampling sites, a cumulative dominance curve was constructed using the program PRIMER.

RESULTS

1. BIOCHEMICAL PROFILES

Bottom waters were fully oxic at all sites (260–290 µM, de Beer et al. 2006) but oxygen penetration depth in sediment differed strongly between sites. Oxygen penetration was limited to <1 mm in microbial mat sediment and 1–3 mm at the centre site. Deepest penetration was measured in the control sites (ca. 5 cm). Hydrogen sulphide (H_2S) reached a maximum concentration in the microbial

mats, it appeared at 5 mm depth in the sediment and increased with depth to concentrations up to 1 mM at 2 cm depth (Figure 3).
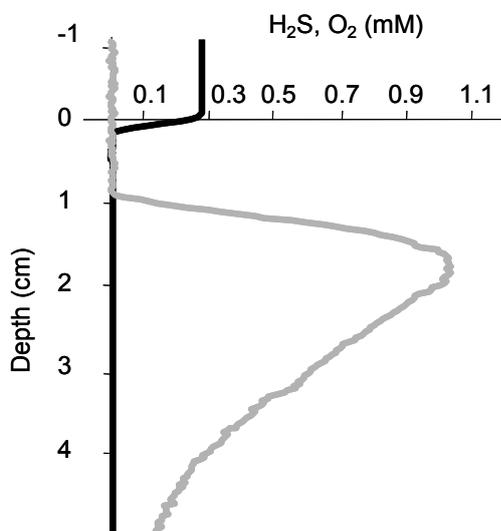
Figure 3: In situ microprofiles of oxygen (black) and hydrogen sulphide (grey) at the microbial mat site.

2. MEIOBENTHIC DENSITIES AND COMMUNITY COMPOSITION

Metazoan meiofaunal densities were significantly higher at the microbial mats (11292 ± 2256 ind. 10 cm^{-2} or 11 x 10^6 ind. m^{-2}) than those at the other HMMV and control sites (ranging from 513 ± 38 ind. 10 cm^{-2} at the centre to 2165 ± 759 ind. 10 cm^{-2} at the outer rim) ($F_{4,10}$ = 70.34, p < 0.001) (Figure 4, Table 2). Nematodes, primarily one species (see below), accounted for more than 98% of the extremely high densities at the microbial mats.

Sediments at the central HMMV site were inhabited by a community with a low number of taxa (5) similar to those at the microbial mat site, but with only very few nematodes (< 5%) and a strong dominance of copepods, predominantly a species morphologically very similar to *Tisbe wilsoni* (Tisbidae), and nauplius-larvae (sum 95%). Average density of copepods (220 ± 75 ind. 10 cm^{-2}) in the volcano's centre was twice that at the microbial mat site and was 4–8 times higher than densities at the other HMMV and control sites (Table 2).

The outer rim, Siboglinidae field and control sites harboured more meiofaunal taxa, including ostracods, tardigrades, turbellaria, bivalves, kinorhynchs, loriciferans, amphipods, tanaids, gastrotrichs, isopods, hydroids, tantulocarids and ophiuroids. Total meiobenthic densities increased from the control sites outside the mud volcano (968 ± 156 ind. 10 cm^{-2}) to the Siboglinidae field (1741 ± 578 ind. 10 cm^{-2})

and the outer rim (2165 ± 759 ind. 10 cm^{-2}). Polychaetes, mostly juveniles, were present in all sub-habitats, except for the centre of the mud volcano, and were strongly concentrated in one sample taken at the Siboglinidae field with a maximum of 80 ind. 10 cm^{-2}. In contrast, the sediment samples from the microbial mat contained a mere 3 polychaetes 10 cm^{-2}.

3. NEMATODE BIOMASS

Although mean individual nematode biomass (dwt) showed no significant differences ($F_{4,10}$ = 2.79, p = 0.09) among the HMMV and control sites, the total biomass differed significantly ($F_{4,10}$ = 70.96, p < 0.0001), with the lowest values at the volcano's centre (1.64 ± 0.42 µg dwt 10 cm^{-2}) and the highest at the microbial mats (611.01 ± 331.79 µg dwt 10 cm^{-2}) due to the extremely high nematode densities in these mats (Figure 4). Nematodes associated with the microbial mats generally had the lowest individual biomass (0.06 ± 0.03 µg dwt), while the highest average individual biomass was found at the outer rim (0.16 ± 0.05 µg dwt). The mean individual body length and width of the nematodes were not significantly different among the different sites (length: $F_{4,10}$ = 2.64, p = 0.1; width: $F_{4,10}$ = 1.18, p = 0.4).
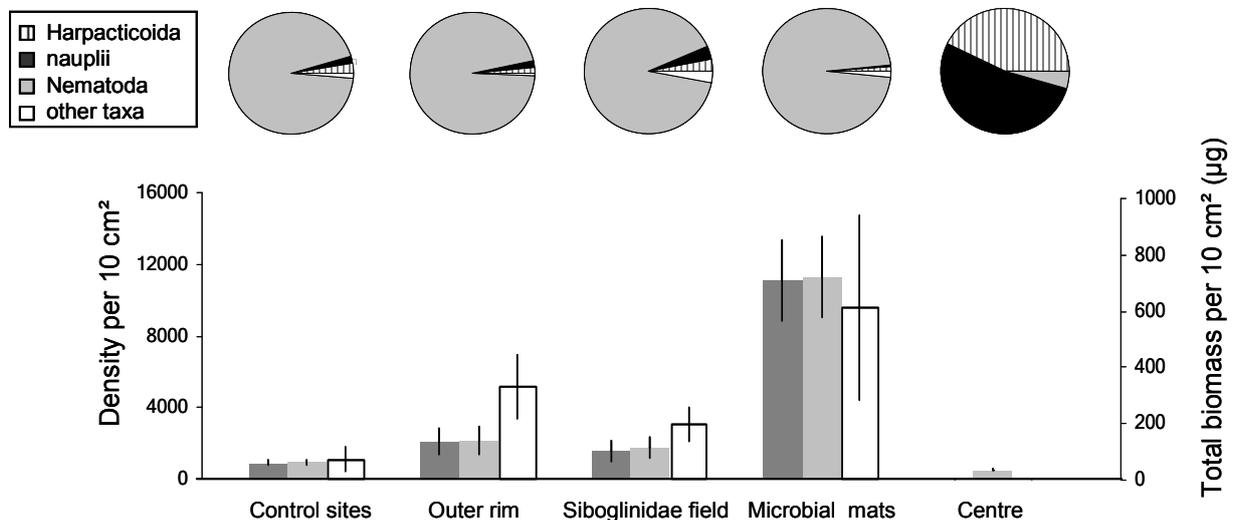
Figure 4: Proportional abundance of meiofaunal taxon densities (pies), meiofaunal densities (light grey bars), nematode densities (dark grey bars) and total nematode biomass (dry weight) (white bars) at different sub-habitats of HMMV and two control sites outside HMMV (mean ± 1 SD).

Table 2: Densities of all metazoan meiofaunal taxa and relative abundances of Nematoda, Harpacticoida and nauplii (0–10 cm). Significance was tested using ANOVA; * indicates significant values (99% probability level).

Taxon	Control sites		Outer rim		Siboglinidae field		*Beggiatoa* mats		Centre	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Density (ind. 10 cm^{-2})										
Acari					0.2	0.3				
Amphipoda			0.2	0.2	1.4	0.8				
Bivalvia	0.2	0.2	0.7	0.5	3.3	2.0				
Gastrotricha	0.4	0.5	0.3	0.1						
Harpacticoida	26.0	5.0	32.5	4.8	54.4	9.0	132.0	62.8	*219.6	74.6
Hydrozoa			0.5	0.7						
Isopoda	0.2	0.3	0.2	0.1	0.3	0.2				
Kinorhyncha	0.9	0.7	1.7	1.2	0.5	0.7				
Loricifera	0.3	0.3	0.9	0.8	0.4	0.4			0.1	0.1
nauplii	17.6	4.3	39.2	17.0	59.2	23.4	48.9	50.6	*270.8	37.3
Nematoda	911.8	160.8	2079.1	750.1	1575.4	564.6	*11109.3	2267.9	22.6	24.9
Ophiuroidea					0.3	0.5				
Ostracoda	1.1	1.4	2.8	4.0	1.6	1.2				
Polychaeta	4.1	1.4	5.3	3.2	41.4	39.9	1.8	1.1		
Tanaidacea	0.7	0.7	0.2	0.2	0.5	0.6				
Tantulocarida	0.2	0.2	0.1	0.1	0.1	0.1				
Tardigrada	3.8	3.3	0.3	0.1	0.4	0.3				
Turbellaria	1.0	1.4	1.3	0.2	2.0	2.3			0.1	0.2
Total	968.4	156.1	2165.1	759.3	1741.3	577.8	*11292.1	2256.2	513.2	38.4
Relative abundance (%)										
Harpacticoida	2.8	0.8	1.6	0.3	3.4	1.1	1.2	0.6	42.3	12.0
nauplii	1.9	0.8	1.9	0.9	3.5	0.1	0.5	0.5	53.0	8.2
Nematoda	95.3	1.5	96.5	1.0	93.1	1.0	98.3	1.2	4.7	5.4

4. NEMATODE AND COPEPOD VERTICAL DISTRIBUTIONS

Relative proportions of nematodes in the first centimetre showed the highest variability in the microbial mat site. A very steep gradient (with 70% and 93% in 0–1 cm) was found in two replicates. Vertical nematode distributions at the control sites and the Siboglinidae field had a similar pattern, with more than 50% of the nematodes in the uppermost sediment layer and more than 70% in the top 0–2 cm layer (Figure 5). The nematode community inhabiting the outer rim revealed an equal distribution down to 4 cm sediment depth (20% in each layer) in all replicates.
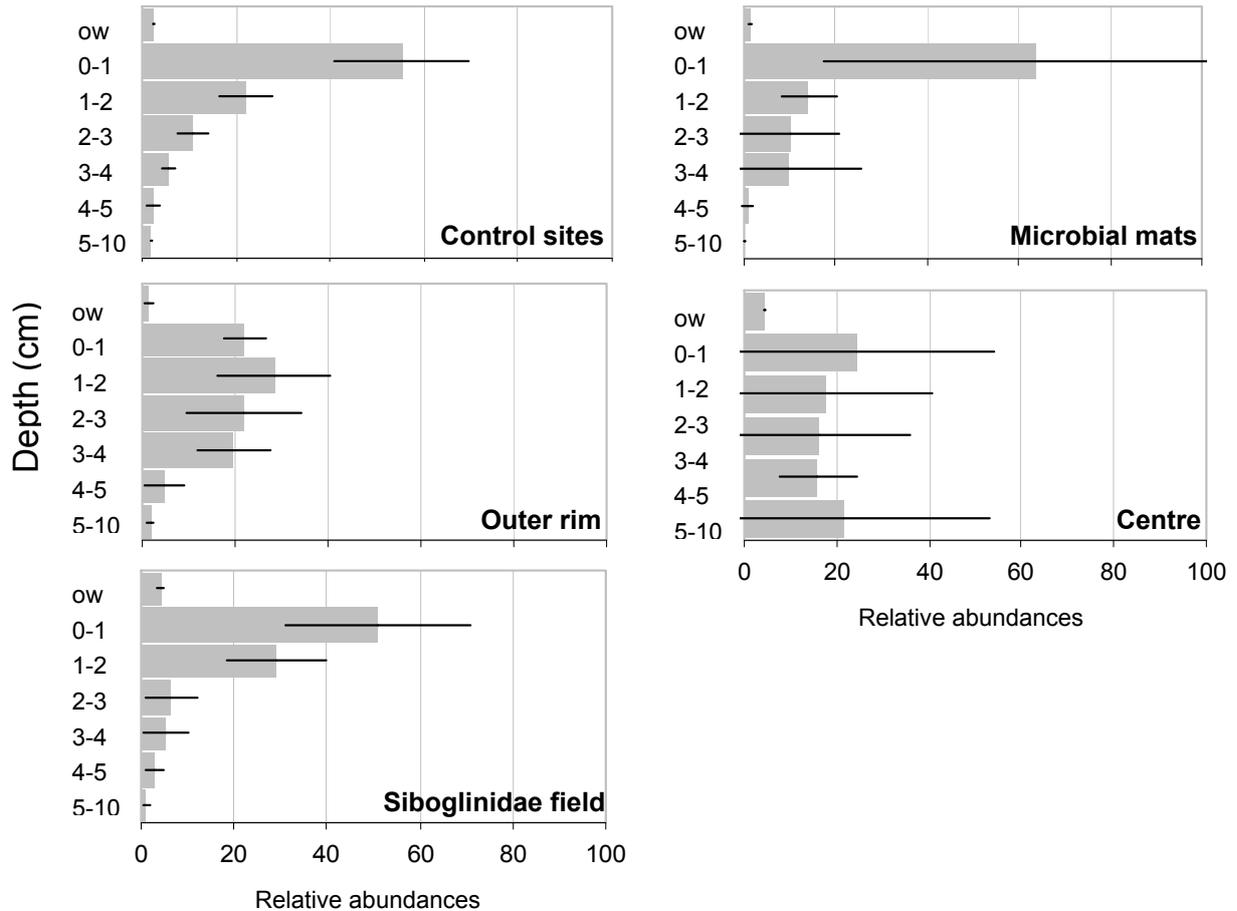
Figure 5: Proportional abundance of nematodes per cm sediment layer (10 cm^{-2}) at the different sites in and outside HMMV (ow: overlying water) (mean ± 1 SD).

Vertical copepod distributions were similar at the control sites, outer rim, microbial mats and Siboglinidae field, with the highest relative abundances in the most surficial layers. The first sediment layer in these sub-habitats contained on average more than 60% of the total copepod number and more than 80% was found in the 0–2 cm layer (Figure 6). These average densities were only slightly lower in the Siboglinidae field, due to one replicate in which 50% of the copepods were present beneath 4 cm sediment depth. However, the most striking copepod community inhabited the centre site, with the highest total copepod densities and 54% of this number present in the overlying water. Nevertheless, the copepods colonised the centre sediments down to 4 cm depth in relatively high proportions, the 4–5 cm layer contained only a few individuals.
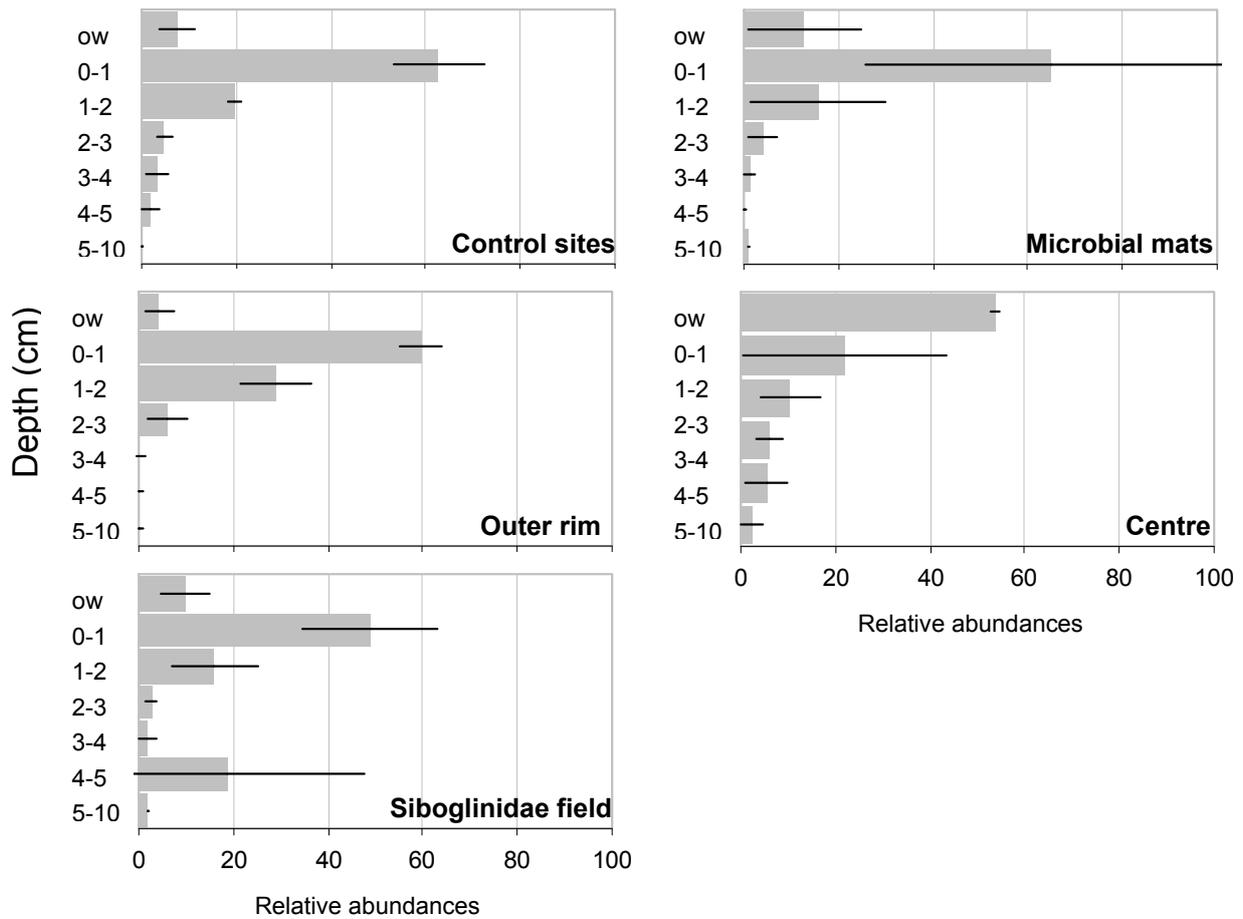
Figure 6: Proportional abundance of copepods per cm sediment layer (10 cm^{-2}) at the different sites in and outside HMMV (ow: overlying water) (mean ± 1 SD).
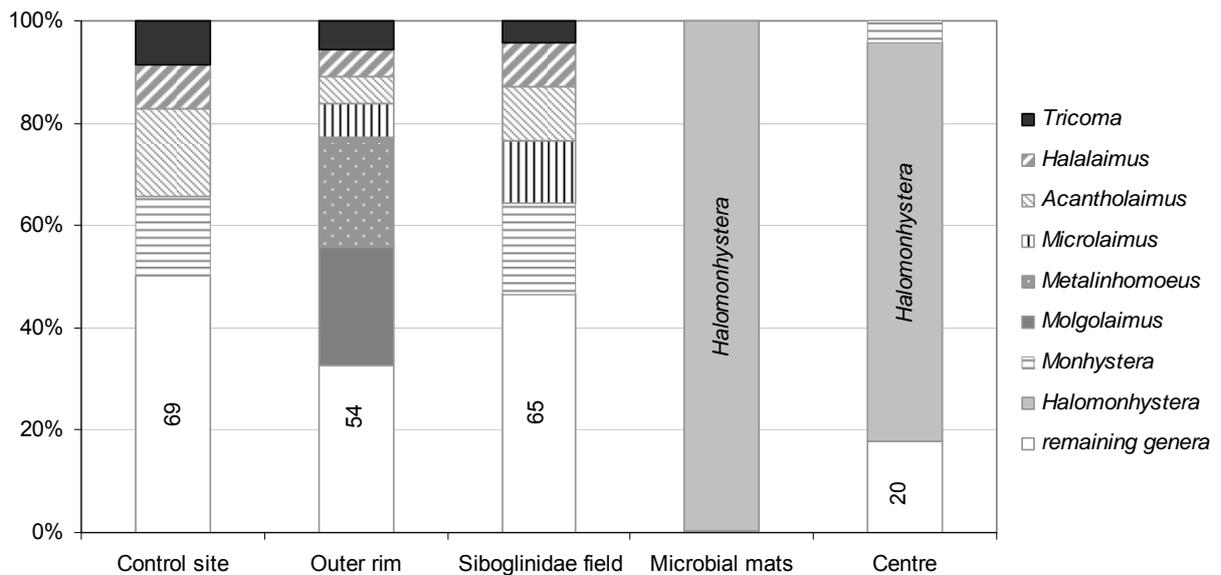
Figure 7: Proportional abundance of the most abundant nematode genera at the different sites inside and outside HMMV (numbers in white bars are number of remaining genera).

5. Nematode diversity

The microbial mat sediments, and to a lower extent, the central HMMV sediments, were colonised by one very dominant nematode species which was completely absent from the control sites, outer rim or Siboglinidae field (Figure 7). This species, *Halomonhystera disjuncta* (Monhysteridae), made up 98% (11069 ± 2269 ind. 10 cm^{-2}) of the total metazoan meiofaunal community and more than 99% of the nematode abundances in the microbial mats (Figure 8a). In the top 1-cm sediment layer at the microbial mat site, *Halomonhystera disjuncta* was the only occurring nematode species. From 1 cm downwards into the sediment, 23 other genera were also found, with *Sabatieria*, *Tricoma* and *Laimella* as most abundant but in very low numbers (max. 52 ind. 10 cm^{-2}) compared to *Halomonhystera*. The other HMMV sub-habitats were inhabited by a more diverse nematode community (Figures 7, 8a) (61–73 genera per sample). Nevertheless, there were some differences concerning proportions of the dominant genera: *Acantholaimus* (Chromadoridae), *Monhystera* (Monhysteridae), *Halalaimus* (Oxystominidae) and *Tricoma* (Desmoscolecidae) dominated the control sites, whereas *Molgolaimus* (Desmodoridae) and *Metalinhomoeus* (Linhomoeidae) were most abundant at the outer rim, and *Monhystera, Microlaimus, Acantholaimus* and *Halalaimus* dominated the nematode assemblage at the Siboglinidae field. An MDS plot based on the nematode genus densities (stress value 0.01) revealed a cluster of the control, outer rim and Siboglinidae samples, while the microbial mat and centre samples were clearly separated, reflecting the extremely different nematode communities of these two sub-habitats (Figure 8b). After exclusion of the microbial mat and centre samples, the analysis (with stress value of 0.08, Figure 8b) placed the replicate samples of the three other sites in three distinct clusters, suggesting important differences among the nematode assemblages of these three sites.

6. Stable isotopes

Both sediment total organic and labile organic carbon isotopes (–28.1 ± 0.6‰ and –36.8 ± 4.3‰, respectively) collected from the mud volcano clearly indicate the prevalence of chemoautotrophy in the microbial mat sediments, directly evidenced by the microbial mats (*Beggiatoa*) with a carbon isotope signature of –42.7 ± 0.2‰. Nematodes collected from the microbial mats, identified as *Halomonhystera disjuncta*, had δ^{13}C signatures of –41.6 ± 0.4‰. Nematodes (several species) and copepods obtained from the control sites had carbon isotope signatures of –20.0 ± 0.2‰ and –19.1‰, respectively. At this site, a δ^{13}C value of –22.1 ± 0.6‰ was found for sediment total organic carbon. Copepods from the central HMMV site had a carbon isotope signature of –51.2 ± 1.7‰.
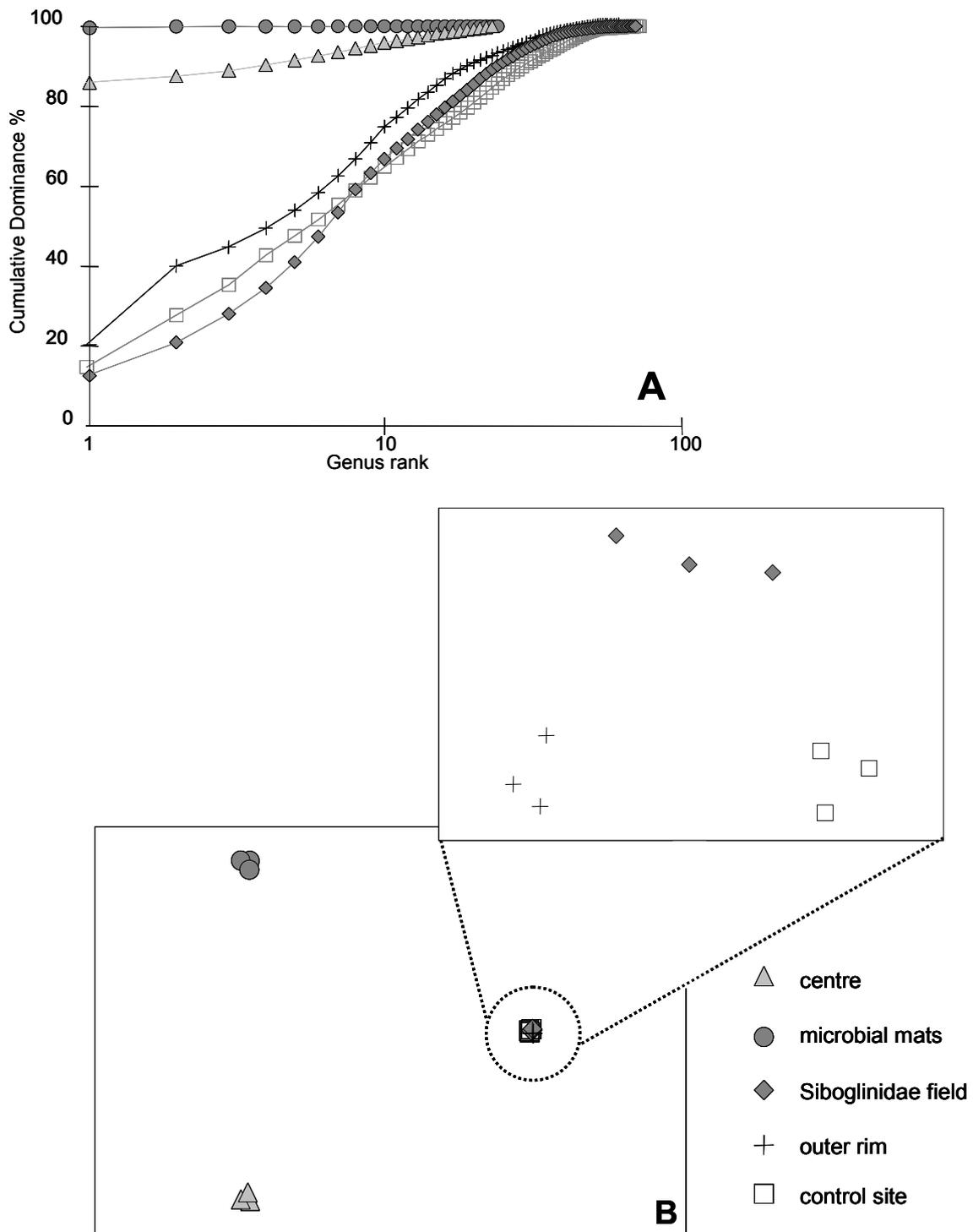
Figure 8: (a) *K*-dominance curve of all nematode genera found at the different sites and (b) nonmetric-multidimensional scaling plots based on nematode genus densities. Plot in background is based on all sites, plot on foreground displays only the control sites, outer rim sites and Siboglinidae sites.

7. LIGHT, SCANNING AND TRANSMISSION MICROSCOPY

Several adult individuals of the monhysterid nematode associated with the microbial mats were examined for the presence of symbionts. Detailed investigations of these specimens with light and scanning electron microscopy revealed absolutely no evidence of ectosymbionts (Figure 9). Initial results of TEM on formaline fixed specimens showed no evidence for endosymbionts. A high percentage (60%) of the females of *Halomonhystera disjuncta* found at HMMV carried several eggs and/or juveniles inside the uterus (up to 7 juveniles), indicating an ovoviviparous reproduction mode (Figure 9).
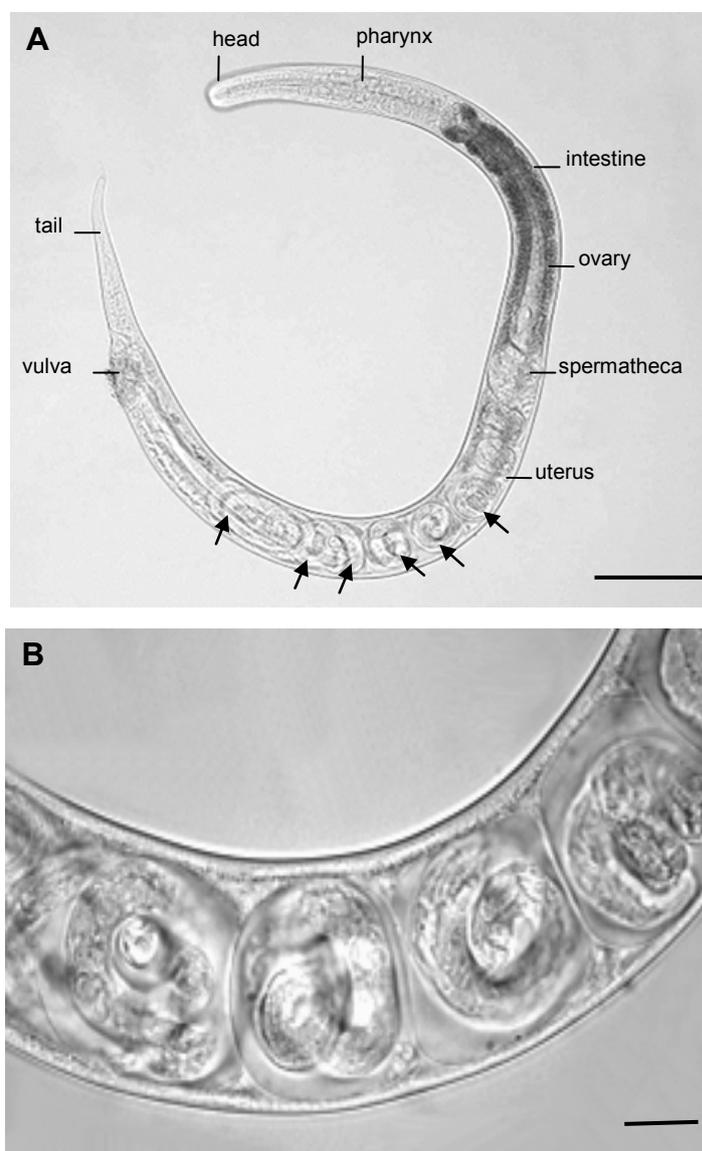
Figure 9: *Halomonhystera disjuncta* of HMMV: (A) light microscope photograph of female with arrows indicating the juveniles (scale bar: 100 µm) and (B) detail of uterus with juveniles (scale bar: 10 µm).

DISCUSSION

1. NEMATODE THRIVING IN SULPHIDIC HMMV SEDIMENTS

Free-living deep-sea nematode assemblages are generally characterised by high species diversity but low standing stock due to food limitation (Vanreusel et al. 1997). Whilst the Siboglinidae field and outer rim sediments at HMMV contained a "regular" deep-sea nematode community almost similar to the control sites in terms of standing stock ($\sim$0.1 g C m^{-2}) and composition, the microbial mat sediments harboured one specific taxon in very high numbers. A single nematode species thrived with unexpectedly high densities (11 x 10^6 ind. m^{-2}) and biomass ($\sim$0.3 g C m^{-2} in 0–5 cm). There are no previous reports of such strong dominance and high densities of a single marine nematode species. This species was absent outside the microbial mats (except for very low numbers in the centre) indicating a strong preference for sulphidic sediments and its associated trophic resource.

Compared to shallow seeps, deep-sea seep sediments receive limited and largely refractory organic matter from surface waters and are supported primarily by local organic carbon production. At the microbial mat site, both sediment total organic and labile organic carbon isotope signatures were depleted (–28.1 ± 0.6‰ and –36.8 ± 4.3‰, respectively), indicating that much of the metabolic activity is indeed driven by chemosynthetically fixed carbon. In contrast, sediment total organic carbon at the control sites had carbon isotope signatures (–22.1 ± 0.6‰) typical of phytoplankton-derived carbon (Levin and Michener 2002, Van Dover et al. 2003). The nematodes at the microbial mats had strongly negative carbon isotope values (–41.6 ± 0.4‰ in contrast to –20.0 ± 0.2‰ for nematodes from the control sites), clearly indicating the utilisation of chemosynthetically derived organic matter. Bacterial mats at HMMV, dominated by *Beggiatoa*, had much lighter carbon isotope signatures (–42.7 ± 0.2‰) than other values reported for '*Beggiatoa*-like' mats (usually –26 to –30‰). However, a similarly depleted δ^{13}C value (–40.35‰) was measured for filamentous sulphur bacteria at Pacific methane seeps (Levin and Michener 2002).

The successful nematode species of the microbial mats at HMMV was morphologically identified as *Halomonhystera disjuncta*, a cosmopolitan, bacterivorous nematode known for its high resistance to environmental stress, especially to heavy metals (Vranken et al. 1989). *Halomonhystera disjuncta* was

formerly known as *Geomonhystera disjuncta*, which was only very recently assigned to a new genus, *Halomonhystera*, by Andrássy (2006). Molecular analyses (Chapter 7) confirmed that this nematode belongs to the *H. disjuncta* complex, which consists of several cryptic species. *Halomonhystera disjuncta* seemed to have a high interspecific morphological variability and significant genetic differences within the different lineages of this species (Derycke et al. 2007). As this species is locally abundant, has a short life cycle and reproduces well under laboratory conditions, it was often chosen as test organism for ecotoxicological studies (Vranken et al. 1989). It is the first time that *Halomonhystera disjuncta* is reported in high abundances in deep-sea sediments.

2. ADAPTATION OF *HALOMONHYSTERA DISJUNCTA* TO TOXIC ENVIRONMENTAL CONDITIONS

Specimens of *Halomonhystera disjuncta* examined by light microscopy showed no association with ectosymbionts. Further detailed research for endosymbiotic bacteria is needed since only formalin-fixed specimens were available which may result in some leaching out of internal structures. Chemosynthetic ectosymbionts are easily recognised by their relatively large size and dense aggregations (Ott et al. 2004), and endosymbiotic relationships are often accompanied by a reduction of the digestive tract (Dubilier et al. 2001). The digestive tract is fully developed in this monhysterid species and so far there is no evidence for endosymbionts. Therefore a possible trophic link with free-living sulphide-oxidising bacteria is suggested; this is also supported by the low stable carbon isotope values. Earlier observations on nematodes, gastropods and polychaetes have already suggested possible trophic links with free-living sulphide-oxidising bacteria (Levin and Michener 2002, Van Dover et al. 2003). Bulk nematode isotope signatures presented by Van Dover et al. (2003) for another deep-sea seep (Blake Ridge, 2155 m) reveal very light $\delta^{13}C$ (–46 to –50‰) but relatively heavy $\delta^{15}N$ (9 to 11‰). These values suggest utilisation of chemosynthetically derived organic matter, but at a high trophic level, not expected if nutrition was linked to symbiotic associations.

Halomonhystera disjuncta from HMMV showed no extreme morphological modifications, but exhibited an ovoviviparous reproductive mode. This species has already been described to have an ovoviviparous reproduction strategy in toxic environmental conditions (Boffé 1985). Ovoviviparity is known for a number of marine nematode species. Walker and Tsui (1968) reported that ovoviviparity was induced in the soil nematode *Rhabditis* sp. by temporary (60–120 minutes) exposure to sulphur dioxide. Permanent sulphidic and also anoxic sediments at HMMV create harsh conditions, which

suggests that internal development of juveniles is an adaptation for securing the survival and growth of the vulnerable brood. Since brooding behaviour requires a substantial parental energy investment, it must provide strong benefits. Parental care and the release of free-living juveniles have substantial advantages. The immediate motility of the new recruits allows migration in and out of the anoxic sediment. It ensures the temporary availability of oxygen to both embryos and juveniles which is necessary for proper growth (Fernandez et al. 2000). These unique metazoans are evidently not restricted by high sulphide concentrations, and directly exploit the abundant new carbon resources ultimately driven by anaerobic methane oxidation. The sulphidic environment further protects against predation which may additionally account for the extremely high nematode densities.

3. MEIOBENTHIC COMMUNITY VARIABILITY IN HMMV SUB-HABITATS

An important change in community structure in this cold seep area was noticed not only in the microbial mat site but also in the centre sediments. Sulphide levels in the centre sediments were below detection, and oxygen penetrated only 1–3 mm (de Beer et al. 2006). These bare sediments harboured very few nematodes (<10 ind. 10 cm^{-2}), which supports the exclusive trophic relationship of *Halomonhystera disjuncta* found at HMMV with trophic resources driven by sulphide. In contrast, copepods and nauplii reached maximum densities and dominance in the volcano's centre and penetrated down to 5 cm in the anoxic sediments (Figure 5). Their strongly depleted δ^{13}C values (–51.2 ± 1.7‰) suggest a strong trophic link with methane-derived carbon, evidently unexploited by nematodes.

The sediments of the three other HMMV sub-habitats (Siboglinidae field, outer rim and control sites) were colonised by more diverse meiobenthic assemblages. Clearly, these sub-habitats support a less specialised meiobenthos, dominated by some typical deep-sea nematode genera (Figure 6). The higher number of nematode genera found in the Siboglinidae field, outer rim and control sites is probably due to the less toxic conditions and higher oxygen levels in the sediments. Intensive ventilation activities of the Siboglinidae through their 70-cm long chitin tubes create an oxygenated habitat down to 10 cm sediment depth by pumping methane and sulphide upwards and oxygen down into the sediment. Sediments in the Siboglinidae field had maximum hydrogen sulphide concentrations of <150 μM (de Beer et al. 2006). According to de Beer et al. (2006), the control sediments contained no methane or free sulphide, and oxygen penetration depth was ca. 5 cm. Biogeochemical data of the outer rim

sediments are not yet available, but the meiobenthic community had a structure similar to that of the Siboglinidae field. Although the number of nematode genera was comparable, the nematode densities in the control sites were only half of the densities in the Siboglinidae field and outer rim, of which the higher densities indicate increased food availability on the mud volcano.

4. COMPARISON WITH OTHER SEEP AND NON-SEEP SITES

Compared to meiofaunal densities reported in other studies from the deep Northeast Atlantic, the densities counted in the control sites (968 ± 156 ind. 10 cm^{-2}) adjacent to HMMV were in the same order of magnitude. The meiobenthos associated with the control sites in the present study was composed of the same taxa found in the other deep-sea areas (Vincx et al. 1994).

Our study at HMMV showed highly significant differences in meiobenthic densities between the microbial mat site and the adjacent control sites, and even between the different sub-habitats (centre, microbial mats, Siboglinidae field, outer rim) located on the mud volcano. A similar observation of extremely high meiofaunal abundance was made for a mud volcano in the northern Barbados prism (Western Atlantic), where densities over 11300 ind. 10 cm^{-2} were found (Olu et al. 1997). Species or genus diversity was not examined but the high density of mainly large nematodes was explained by a local organic enrichment (particularly due to bacterial activity) of the adjacent sediments, although the presence of symbiotic bacteria could not be discarded (Olu et al. 1997). As in the case of HMMV, large variation in densities (116–11364 ind. 10 cm^{-2}) across seep sub-habitats was also recorded by Olu et al. (1997). In contrast, Soltwedel et al. (2005a) found no significant differences in overall meiofaunal abundances among sub-habitats in samples taken at HMMV in 2002, with densities ranging roughly between 1600 and 3000 ind. 10 cm^{-2}.

5. NEMATODES ASSOCIATED WITH SEEP ENVIRONMENTS

There are only a few investigations of metazoan meiofauna at cold seeps and these seep studies rarely go beyond bulk measurements of abundance. Variability in meiobenthic densities is always caused by nematode abundances, as they are generally the most abundant meiobenthic taxon in cold seeps. An exception is the meiobenthos of the shallow brine seep in the Gulf of Mexico,

dominated by gnathostomulids (Powell et al. 1983). Only four cold seep studies, of which only one is from deeper water (Jensen 1986, Shirayama and Ohta 1990, Dando et al. 1991, Jensen et al. 1992) provide species-level information on nematodes. Dominant nematode species varied among the different seeps: *Desmolaimoides thiobioticus* at the brine seep in the Gulf of Mexico (Jensen 1986), *Astomonema southwardorum* in the active methane seep of a North Sea pockmark (Dando et al. 1991), *Sabatieria punctata*, *Theristus anoxybioticus* and *Leptonemella aphanothecae* in the methane seeps of the northern Kattegat (Jensen et al. 1992), two *Daptonema* spp. and a *Chromadorita* sp. in the bathyal cold seep off Japan (Shirayama and Ohta 1990). Moreover, no consistently dominant nematode family was found in the various reduced seeps across the oceans. Generally, there seems to be a high degree of endemism for nematodes, as each individual reducing ecosystem has its own more or less specialised nematode species. Some of these species have developed specific adaptations to their toxic environment: *A. southwardorum* is a mouthless and gutless nematode which harbours endosymbiotic bacteria (Giere et al. 1995), while the cuticle of *L. aphanothecae* is covered by sulphide-oxidising bacteria (Jensen et al. 1992), typical for the subfamily of Stilbonematinae (Ott et al. 2004). These tight symbiotic relations with chemosynthetic bacteria function as detoxification mechanisms for the high, toxic sulphide levels characteristic for these environments. *Oncholaimus campylocercoides*, which dominated the meiofauna at the shallow-water hydrothermal vents off Milos, is able to survive the sulphidic conditions by storing sulphur in intracellular inclusions (Thiermann et al. 2000).

Halomonhystera disjuncta from HMMV microbial mats succeeded in establishing a dense population at least 20 times larger than other abundant seep nematodes such as *Oncholaimus campylocercoides*, *Theristus anoxybioticus* or *Astomonema southwardorum* (Dando et al. 1991, Thiermann et al. 2000). However, no sulphide inclusions were found in the successful monhysterid nematode of HMMV. This might indicate that the proliferation of *Halomonhystera disjuncta* in these toxic sediments is the result of active adaptation and trophic specialisation.

CONCLUSION

The meiobenthic communities associated with sub-habitats of HMMV appeared to be highly variable, with the strongest contrasts associated with sediment geochemistry. The anoxic and sulphidic sediments at the microbial mats were colonised by a nematode community significantly different from those of the other sub-habitats. An ovoviviparous nematode species, belonging to the *Halomonhystera*

disjuncta complex (recently identified as a cosmopolitan group of morphologically similar but cryptic species), was thriving in the toxic environment underneath the microbial mats. This nematode species secured its population with parental investment in brood survival and development. Furthermore, the rather high number of copepods in the centre was due to one species morphologically very similar to *Tisbe wilsoni*, a copepod species typically found in littoral habitats. Overall, enhanced densities and dominance of selected species at HMMV are a product of both direct (abiotic) and indirect (bacterial resource specificity) consequence of sediment geochemistry, resulting in highly significant differences between the meiobenthic communities of the various sub-habitats in a single deep-sea cold seep area.

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CHAPTER 5

Trophic specialization of metazoan meiofauna at the Håkon Mosby Mud Volcano: fatty acid biomarker isotope evidence

Trophic specialisation of metazoan meiofauna
at the Håkon Mosby Mud Volcano: fatty acid biomarker isotope evidence

ABSTRACT

We report the results of a detailed investigation on the trophoecology of two meiofaunal species that dominate the metazoan meiofauna at the Håkon Mosby Mud Volcano (HMMV), a deep-sea cold methane-venting seep. Analyses of fatty acids (FAs) and their stable carbon isotopes have been used to determine the importance of chemosynthetic nutritional pathways for the dominant copepod species (morphologically very similar to *Tisbe wilsoni*) inhabiting the volcano's centre and the successful nematode *Halomonhystera disjuncta* which is thriving in the surrounding microbial mats. The strong dominance of bacterial biomarkers (16:1ω7c, 18:1ω7c and 16:1ω8c) coupled with their individual light carbon isotopes signatures (δ^{13}C ranging from −52 to −81‰) and the lack of symbiotic relationships with prokaryotes (as revealed by molecular analyses and fluorescent in situ hybridization) indicated that chemosynthetic derived carbon constitutes the main diet of both species. However, the copepod showed a stronger preference for the utilisation of methanotrophic bacteria and contained polyunsaturated FAs of bacterial origin (20:5ω3 and 22:6ω3 with isotope signatures δ^{13}C <−80‰). Therefore, HMMV can be regarded as a persistent deep-sea cold seep, allowing a chemosynthesis-based trophic specialisation by the dominant meiofaunal species inhabiting its sediments. The present investigation, through the determination of the fatty acid profiles, provides the first evidence for trophic specialisation of meiofauna associated with sub-habitats within a cold-seep.

INTRODUCTION

Unlike the majority of seafloor environments, hydrothermal vents and cold seeps represent marine ecosystems that may be largely independent from the export of the photosynthetic primary production as primary energy source (e.g., Levin 2005, Stewart et al. 2005, Saito and Osako 2007). In situ chemosynthetically produced microbial biomass, either as free-living prokaryotes or in symbiotic relationships with fauna, can be a major, sometimes the only, source of energy for resident benthic fauna. Unravelling the trophic relevance of the photosynthetic versus chemosynthetic organic carbon is of primary importance for improving our understanding of the functioning of these ecosystems. While a large amount of information has been acquired on the trophodynamics of the surface-dwelling megafauna (e.g., large tube worms, mussels and clams) that colonise these habitats, very limited information is available on the sediment-dwelling fauna (infauna). Data available from larger macrofauna inhabiting deep-sea seeps suggest a dependence on chemosynthetic derived carbon, typically stronger than that estimated for shallow-water seeps (Levin 2005). Furthermore, this nutritional link is primarily heterotrophic (feeding on free-living chemoauto- or heterotrophic bacteria) and it depends to a lesser extent on bacterial symbiotic relationships (Levin 2005). Trophoecological studies on the metazoan meiofauna are scarce, primarily due to the small size of the fauna and the inherent difficulty in obtaining sufficient biomass for subsequent analyses of the stable isotopes or fatty acids (FAs). Nematodes generally represent a major fraction of seep sediment infauna, and some of the highest marine nematode densities and biomasses have been observed in cold seep sediments (e.g., Olu et al. 1997, Van Gaever et al. 2006); bulk tissue stable carbon and nitrogen isotope signatures have revealed that some cold seep nematodes utilise chemosynthetic derived carbon (Van Dover et al. 2003, Van Gaever et al. 2006). At the seep addressed in this study, Van Gaever et al. (2006) reported the strong dominance and proliferation of a single nematode species, *Halomonhystera disjuncta*, in the microbial mat sub-habitat. The striking high nematode biomass could not be attributed to photosynthetic produced carbon because of the low organic matter input. Moreover, in the seep centre, the nematodes were replaced by a species of copepods (morphologically very similar to *Tisbe wilsoni*) with bulk tissue $\delta^{13}C$ values indicative of a strong trophic link with methane-derived carbon ($\delta^{13}C \sim -51‰$). This provided a hint at meiofauna diversity/distribution patterns related to trophic specialisation (Van Gaever et al. 2006) but remains unresolved. Meiobenthic (nematode) assemblages in non-seep or shallow seep sediments are more diverse and may be related to the presence and utilisation of both photosynthetic and chemosynthetic derived carbon (Levin et al. 2000, Levin 2005). However, the extent to which these meiobenthic diversity and biomass patterns are related to trophic resources remains unclear just as the

carbon flow pathways (symbiosis versus feeding on free living prokaryotes, Levin et al. 2000, Levin and Mendoza 2007).

In this study, we report on a detailed investigation of metazoan meiofauna trophoecology at the Håkon Mosby Mud Volcano (HMMV). HMMV is located at 1280 m water depth on the Barents Sea and is the only mud volcano in a polar region that has been mapped in detail by photo and video camera observations and studied in detail for its biogeochemical habitats and microbial communities (Niemann et al. 2006, de Beer et al. 2006, Jerosch et al. 2007). Significantly different meiobenthic densities were found at the different sub-habitats (seep centre, microbial mats, Siboglinidae field, and outer rim); the anoxic and sulphidic sediments at the microbial mat habitat were inhabited by extremely high densities (>11000 ind. 10 cm^{-2}, 0–5 cm sediment depth) of *Halomonhystera disjuncta (*originally referred to and described as *Geomonhystera disjuncta*; Van Gaever et al. 2006). The central, seep part of the volcano was characterised by high concentrations of methane and the lack of sulphide (de Beer et al. 2006). Moreover, here nematodes were virtually absent while copepods were abundant. Different sediment geochemistry across seep sub-habitats supporting distinct microbial communities (Niemann et al. 2006) may strongly regulate and explain meiofaunal distribution patterns in this deep-sea cold seep (Van Gaever et al. 2006). We investigated the trophic preferences of the dominant meiofaunal species inhabiting this mud volcano using FA biomarkers analysis.

Fatty acid analyses have been repeatedly used as source-specific indicators of organic matter both in environmental and food web studies (e.g., Canuel et al. 1995, Kharlamenko et al. 1995, Phleger et al. 2005). This tool can be complemented by the analysis of stable carbon isotope signatures of the dominant FAs for further clarification of the food sources and trophic pathways (e.g., Abrajano et al. 1994, Pond et al. 1997). With methane at HMMV having a δ^{13}C of ~ –60‰ (Niemann et al. 2006) and microbial mat sediment porewater dissolved inorganic carbon δ^{13}C ~ –26‰ (mass balance estimate of sediment-water slurry incubations, Van Gaever et al. 2006), in situ chemosynthetically produced carbon is expected to be reflected in more negative δ^{13}C$_{org}$ values than that of surface water photosynthetic produced carbon (e.g., Levin and Michener 2002, MacAvoy et al. 2002); although this can sometimes be masked or magnified depending on the carbon fixation pathway (e.g., Robinson and Cavanaugh 1995, Stewart et al. 2005).

Bacterial symbionts (ecto- or endosymbionts) can be either a source and pathway of chemosynthetically derived organic matter entering the higher trophic levels or play a role of detoxification in fauna inhabiting sulphide rich sediments (e.g., Thiermann et al. 2000, Levin 2005). In order to clarify

prokaryotes-meiofauna relationships we also examined the presence of symbiotic bacteria in nematodes, using Transmission Electron Microscopy (TEM) photography coupled with Automated rRNA Intergenic Spacer Analysis (ARISA), Fluorescent In Situ Hybridisation (FISH) and Catalyzed Reporter Deposition Fluorescent In Situ Hybridisation (CARD-FISH).

MATERIALS AND METHODS

Sediment samples for meiofaunal analyses were collected at the HMMV during the VICKING cruise aboard the RV 'PourquoiPas?' in April–May 2006 (Table 1). The flat central zone of the volcano is characterised by grey mud flows with a high geothermal gradient (Pimenov et al. 2000). Beyond this central plain, the seafloor has a complex topography of hills and depressions, and is covered by extensive microbial mats of chemoautotrophic bacteria, dominated by giant sulphur-oxidising *Beggiatoa* bacteria (Pimenov et al. 2000, Niemann et al. 2006). Samples were retrieved at both the volcano's centre and the surrounding microbial mats. A multiple corer (liner's surface of 30.2 cm²) was deployed at the centre site, while the microbial mats were sampled with push cores of the ROV *Victor 6000* (liner's surface of 21.2 cm²).

Table 1: Sampling device, dive and core numbers, latitudes (N), longitudes (E) and depths (m) of sampling sites.

Sampling site	Water depth (m)	Latitude (°N)	Longitude (°E)	Sampling device	Dive number	Core number
Centre	1273	72° 0.28′	14° 43.56	multicorer		MT8
Microbial mat	1258	72° 00.16′	14° 43.95′	push corer	PL 276-06	CT19

The nematodes for TEM were picked out onboard, cleared of adhering sediment particles and immediately fixed in a Karnovsky solution diluted 50% with distilled water at room temperature. The nematodes were processed according to the protocol in Willems et al. (2005). Cross sections of 70 nm thick were made and post-stained with a Leica EM stain for 30 minutes in uranyl acetate at 40°C and 5 min in lead stain at 20°C. Electron microscopy was done using a TEM 1010 Jeol, operating at 80 kV.

The samples for FISH and CARD-FISH analyses were stored onboard in formaldehyde 4% and kept at room temperature. In the lab, nematode specimens were selected from frozen samples in order to perform ARISA. Specimens of *Halomonhystera disjuncta* have been selected for the over mentioned analyses.

At first, individuals for FISH analysis were washed in pure ethanol (96%). The samples have been incubated for 1 hour at 37°C in a solution of Lysozime (10 mg/ml) in 0.05 M EDTA (pH 8) and 0.1 M Tris-HCl (pH 7.5). Afterwards, the samples were washed in Milli-Q water and in pure ethanol (96%). Hybridization was carried out for 1.5–3 hours at 46°C in a dark thermostatic chamber, adding a solution 20:1 of Hybridization Buffer (0.9 M NaCl; 20 mM Tris-HCl (pH 7.4); 0.01% SDS; 35% Formamide) and probe (50 ng/µl). The added probe was the universal probe for Eubacteria (EUB338: 5′-GCT GCC TCC CGT AGG AGT-3') labeled in 5′ with CY3 (Ex 550nm e Em 570nm). Thereafter, the nematodes were washed in a Washing Buffer (20mM Tris-HCL (pH 7.4); 1mM NaCl; 0.01% SDS; 5mM EDTA) for 15-30 min in the dark in a thermostatic chamber at 48°C, and finally in Milli-Q water. The nematodes were mounted on microscope slides with 20 µl of PBS (a solution of PBS-glycerol-ascorbic acid) anti-fade per slide. Visualization was done using a light microscope (Zeiss Axioskop 2) equipped with epifluorescence (Amann et al. 1995).

In order to satisfy nematodes handling needs and to improve the CARD-FISH technique several steps were carried out before the protocol application.   Intact individuals have been washed in milli-Q water and in PBS:pure ethanol 1:1 (v/v) for 1 min. In order to improve the penetration of the probe into the animal, the experiments have been carried out both on intact specimens both on part of nematodes previously cut in 3 pieces (head, central body, tail).

To improve intact specimen cuticle permeability, an alternative experiment has been carried out. The experiment consisted in 4 treatments which have been done before the normal CARD-FISH procedure : (i) enzymatic digestion with incubation with 500 µl Proteinase K (375 mU/ml; 50 µg/ml) in PBS per slide for 15 min in a thermostatic chamber at 37°C (Ross et al. 1995); (ii) UV-C treatment for 3 and 10 min (nematodes were put into petri); (iii) sonication for 10 min at 4°C (Nematodes were put into eppendorf); (iv) sonication plus UV-C treatment (3 and 10 min). Each treatment was carried out on specimens previously washed as follows: (i) wash nematodes from Formaldehyde (4%) with PBS for 1 min; (ii) wash the sample in a solution of Glacial Acetic Acid and Milli-Q (1:1, v/v) for 30 min at room temperature in low agitation (Vandekerckhove et al. 2002); (iii) wash the sample in Milli-Q for 1 min; (iv) put the

sample in a solution of PBS + TWEEN 20 (0.1%) + SDS (0.2%) for 10 min; (v) wash the sample in PBS (Vandekerckhove et al. 2002).

Thereafter, the specimens have been put into a milli-Q water drop positioned at the center of the slide and the drop has been left to dry out into a thermostatic chamber at 37°C. The fixed specimens have been then treated ufollowing the protocol by Penthaler et al. 2002. About 20 µl per slide of LOW-GELLING-POINT (0.2%) has been added and let it dry off in a thermostatic chamber at 37°C. This passage have been repeated 3 times in order to fix the intact/pieces of nematodes to the microscope slides. The sample has been washed with pure ethanol (96%) for 1 min and left to dry out. To inactivate endogenous peroxidase, the samples have been incubated for 10 min at room temperature with 100 µl of HCl 0.01 M per slide. Afterwards, the samples have been washed in PBS and then in Milli-Q for 2 times. The samples have been left to dry out. For Eubacteria identification, the samples have been incubated in a thermostatic chamber at 37°C for 1 hour with 200 µl per slide of a solution of Lysozime (10mg/ml) and EDTA-TrisHCl. Attention has been paid not to let the samples dry out. The samples have been washed 3 times in Milli-Q water and then in pure ethanol (96%). The samples have been then left to dry out. The hybridization has been done adding to each slide 200 µl of HYBRIDIZATION BUFFER (0.9M NaCl; 20mM Tris-HCl pH 7.5; 10% dextran sulfate; 0.02% SDS; 55% Formamide: 1% Blocking Reagent) and 2 µl of probe with a final concentration of 50 ng/µl. The samples have been incubated in a thermostatic chamber at 35°C for 3 hours in low agitation, paying attention not to let the samples dry out. The same probe used for FISH (EUB338) has been used for CARD-FISH, but in this case the probe was labeled with the horseradish peroxidase enzyme (HRP) in 5′. The excess of probe has been washed off adding 200 µl of previously heated WASHING BUFFER (3mM NaCl; 5mM EDTA pH 8; ,20 mM Tris-HCl pH 7.5; , 0.01% SDS) to each slide and incubating the samples for 5-10 min in a thermostatic chamber at 37°C. The WASHING BUFFER has been then left slip away from over the slide.

To equilibrate the HRP-labeled probe, each slide has been incubated with 100 µl of PBS + Triton X-100 (0.05%) for 15 min at room temperature. The solution has then left slip away from the slide. About 30 µl per nematode of a mixture of TYR-Cy5 + AMPLIFICATION BUFFER (1:50) has been added to the samples. The incubation with the reporter has been done for 10-15 min in the dark into a thermostatic chamber at 37 °C.

The excess of mixture has been washed away adding 100 µl of PBS + Triton X-100 (0.05%) to each slide and incubating the samples at room temperature for 5-15 min in the dark. The samples have been

finally washed with Milli-Q and pure ethanol (96%) and let dry off. Per each slide 20 µl of PBS (a solution of PBS-glycerol-ascorbic acid) anti-fade has been added before putting the slide cover. The visualization has been done using a light microscope (Zeiss Axioskop 2) equipped with epifluorescence (blue light: Ex 450-490 nm and Em 515 nm; green light: Ex 546/12 nm and Em 590 nm) and with a Bio-Rad MRC-1024 confocal laser scanning system equipped with a krypton-argon laser (excitation filter wavelength, 647 nm; emission filter wavelength, 680 +/- 32 nm) for use with Cy5.

For the fingerprinting analysis ARISA, DNA has been extracted from *Halomonhystera disjuncta* specimens with the protocol used by Floyd et al. (2002). Before DNA extraction, nematodes were carefully washed to remove epibiontic bacteria and then cut to facilitate DNA extraction. The universal primer 16S-1392F (5′-GYACACACCGCCCGT-3′) and the bacterial primer 23S-125R (5′-GGGTTBCCCCATTCRG-3′) were used (Hewson and Fuhrman 2004). Negative and positive controls were used.

The sediment samples selected for fauna FAs and isotope analyses were frozen onboard. Given that FAs constitute a minor fraction of animal organic matter, a large number of these small-sized organisms would have to be isolated and cleared of adhering sediment particles for reliable analysis of meiofauna FA composition and compound specific stable isotope analysis. This can be very time-consuming or virtually impossible especially in cases of fauna impoverished deep-sea sediment. These restrictions were overcome by performing small volume extractions coupled with large volume injections (30 µl; GC analyses commonly employ 1–2 µl injections). Furthermore, to reduce contamination, loss of FAs and ensure maximum extraction of FAs one can choose to analyse total FAs using a one-step procedure of extraction and methylation that can provide composition profiles similar to those obtained with conventional methods involving multiple steps (Christie 2003, and references therein). Here we employed a one-step method according to Masood et al. (2005) that was first tested for our application by comparing with results obtained with a modified large volume multi-step Bligh and Dyer (1959) extraction method of total fatty acids (Boschker and Middelburg 2002). We compared laboratory cultured bacteria and pelagic diatoms and their blank extractions. The one-step method produced similar or superior FA yields (ratio of total [FA] in multi-step:one-step in diatom and bacteria extraction equalled 1.03 and 0.67 respectively) and in accordance with Masood et al (2005), with no evidence of polyunsaturated fatty acid (PUFA) loss (e.g. FA 20:5ω3 had an occurrence of 6 and 9 % in the multi- and one-step method respectively). Additionally, there was very limited contamination in the small volume one-step method because only traces of FA 16:0 and 18:0 equivalent to <1% of FA were found. The addition of the antioxidant (BHT) did not alter PUFA yield and was therefore not further applied.

In this study three replicates of the meiofauna (160 nematodes and 30 copepods each) and strands of *Beggiatoa* were handpicked with a needle, cleared of adhering particles and rinsed twice in 0.2 µm filtered Milli Q before being transferred to 2.5 ml GC vials that were frozen at –80ºC and subsequently freeze dried. Fatty acid extraction and preparation of methyl esters (FAME) were carried out according to Masood et al. (2005) with reagent volumes adapted for use in this 2.5 ml GC-vials using FAME C19:0 as internal standard to calculate concentration of FAs. These individual samples were analysed separately for their FA compositions employing a Large Volume Splitless injection method on a Thermo Finnigan Trace GC Ultra GC. Main items of this method: large volume liner with glass wool, pre-column deactivated silica 5m x 0.53µm and analytical column SGE BPX-70 50m x 0.32mm x 0.25 µm. After these individual runs, the three replicates of each taxa were combined and evaporated to obtain sufficient material for analysis of FA carbon isotope signatures. The carbon isotopic composition of the dominant FAME was determined with a gas-chromatograph combustion interface isotope-ratio mass spectrometer (GC-c-IRMS) consisting of a HP G1530 GC (Hewlett Packard, USA) connected to a Delta-plus IRMS via a type III combustion interface from Thermo Finnigan (Bremen, Germany). In this case a HP 5MS analytical column (60m x 0.32mm x 0.25µm) was employed. The identification of FAMES was based on comparison of retention times with authentic commercially available reference material and standards. The stable carbon isotope ratios (δ^{13}C) of individual FAs were calculated from FAME data by correcting for the one carbon atom in the methyl group that was added during derivatisation (Boschker and Middelburg 2002).

Fatty acid source designation was achieved using data on distinctive FAs for bacteria, algal carbon and higher organisms (e.g., Parrish et al. 2000, Boschker and Middelburg 2002, Volkman 2006). Although individual FAs biomarkers are rarely produced by a single class or organism, interpretations can be verified using FAs combinations or spectra (Dalsgaard et al. 2003), even more so when complemented with FA δ^{13}C signatures (see above). Multivariate analyses of FAs composition profiles (non-transformed percentage abundance) were performed using the program PRIMER Version 5 (Plymouth Routines in Multivariate Ecological Research). Similarities between profiles were investigated using the SIMPER (Similarity of Percentages) function and statistical differences were determined using the ANOSIM (Analysis of Similarities) function.

RESULTS AND DISCUSSION

The FAs composition profiles of each component (*Beggiatoa*, nematodes and copepods) showed a very high similarity among the three replicates (SIMPER, similarity >96%). In accordance with FA profiles reported for *Beggiatoa*, *Thioploca*, and other sulphide-oxidising bacteria (Zhang et al. 2005, and references therein), a few FAs dominated the compositional pattern of the free-living sulphide-oxidising bacteria that typify the microbial white mat sub-habitat of the HMMV cold seep (Fig. 1). The monounsaturated FAs (MUFAs) 16:1ω7 (69%) and 18:1ω7 (21%) and the saturated FA 16:0 (6%) together contributed ~96% of the FAs composition of the *Beggiatoa* (Fig. 1). The corresponding light stable carbon isotope signatures ($\delta^{13}C \sim -50$‰, Table 2) can be attributed to the chemoautotrophic synthesis of these FAs (Conway and Capuzzo 1991, Pranal et al. 1996, Zhang et al. 2005). Conform earlier conclusions (e.g. Zhang et al. 2005), these results indicate that 16:1ω7 and 18:1ω7 can be used as signature biomarkers for sulphide-oxidising bacteria in hydrogen sulphide-rich sediments such as the HMMV mat sub-habitat.
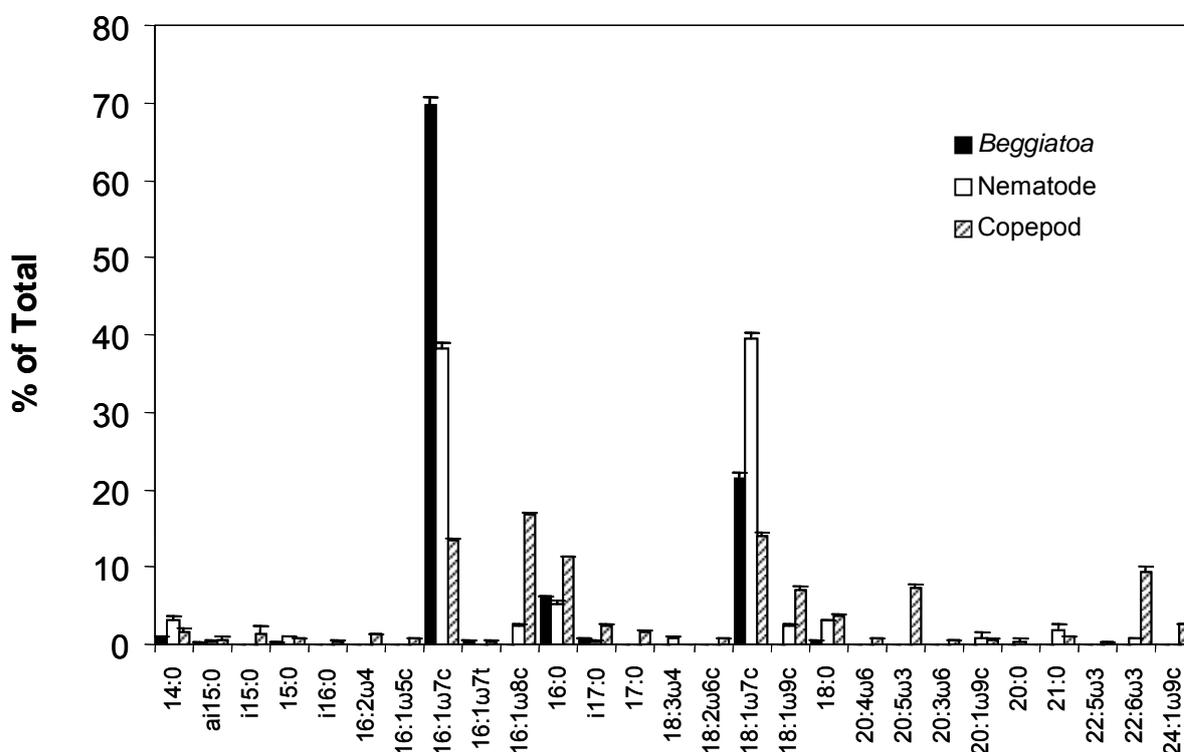
Figure 1: Fatty acid composition of *Beggiatoa*, the microbial mat nematode and the seep centre copepod, collected at the Håkon Mosby Mud Volcano. Average % of total (n = 3) ± 1 SD.

Table 2: $\delta^{13}C_{org}$ (‰) of the dominant fatty acids and bulk tissue. – : denotes absent (i.e. not detected in LVGC and GC-IRMS) and nd: denotes present but not detected on the GC-IRMS due to low concentration. nm = not measured

	Microbial Mat	Microbial Mat	Seep Centre
	Beggiatoa	Nematode	Copepod
16:0	−46.0	−34.1	−69.9
16:1ω7c	−49.8	−52.6	−78.8
16:1ω8c	–	nd	−81.6
18:0	nd	−41.7	−69.1
18:1ω7c	−49.5	−53.8	−72.7
18:1ω9c	–	−40.8	−76.5
20:5ω3	–	–	−79.7
22:6ω3	–	nd	−86.1
Bulk tissue			
Year 2003	−42.7 (0.2)	−41.6 (0.4)	−51.2 (1.7)
Year 2006	nm	−50.2 (0.8)	nm

The successful meiobenthic species in this sub-habitat, the nematode *Halomonhystera disjuncta*, was characterised by a FAs profile indicative of a bacterial diet (Fig. 1): strong dominance of light $\delta^{13}C$ MUFAs (16:1ω7 and 18:1ω7 together account for 80% of FAs carbon with corresponding $\delta^{13}C$ of – 54‰, Table 2) and absence of ω3 and ω6 long-chain polyunsaturated FAs (PUFAs, commonly originating from photosynthetic plankton). Although MUFAs such as 16:1ω7 and 18:1ω7 can be derived from diatoms (e.g., Dijkman and Kromkamp 2006, Volkman 2006), their light $\delta^{13}C$ values confirmed utilisation of microbial mediated chemosynthetically derived carbon. As discussed above, this suggests that sulphide-oxidising bacteria constituted the main diet of *Halomonhystera disjuncta*.

TEM sections revealed well-developed intestines and ovaries in this nematode but no evidence of symbiontic cells. The conclusion that bacteria are used as a food source and not hosted as symbionts is supported by the lack of bacteria in the nematode tissues as revealed either by FISH, CARD-FISH or ARISA (data not shown). Other observations of nematodes feeding on *Beggiatoa* bacteria were

previously recorded for *Diplogaster bernenesis* (Sudhaus and Rehfeld 1990), a well known species from freshwater, and *Diplolaimelloides deconincki* in a harbour site in Denmark (Bernard and Fenchel 1995). In the latter study a large number of aerobic and anaerobic ciliate species were found to ingest filamentous sulphur bacteria. Another close relationship between nematodes and *Beggiatoa* was described in a study on the dynamics of nematodes in wastewater treatment processes (Salvadó et al. 2004). It had been suggested that nematodes play an important role in wastewater bio-oxidation by their grazing activity on the bacterial community (Woombs et al. 1986). The most significant biotic relationship found by Salvadó et al. (2004) was between nematodes and filamentous bacteria (specifically the genus *Beggiatoa*), and to a lesser extent, nematodes with ciliates. Based on the biofilm thickness, they concluded that the nematodes also could have a positive influence on the microbial communities. The mechanical effect of nematodes on the biofilm structure would allow a better oxygen penetration and nutrient diffusion into the biofilm and thus, stimulating its growth. *Beggiatoa* appeared also to be attached on rock samples retrieved from a hydrothermal vent field located in the Azores archipelago (North Altantic, Cardigos et al. 2005). Two species, the nematode *Enoplus meridonalis* (116 specimens) and the oligochaete *Lumbricillus* sp. (59 specimens) were abundant in the thick *Beggiatoa* mats although no direct trophic relationship with the bacteria was shown (Cardigos et al. 2005). It can be concluded that nematodes are often associated with *Beggiatoa* bacteria in sulphidic conditions in both freshwater as marine habitats. Their close relationship may be due to environmental factors or trophic factors. At the HMMV, the high biomass and standing stock of *Halomonhystera disjuncta* can be attributed to feeding on the free-living sulphide-oxidising bacteria, a trophic specialisation previously not seen for any other deep-sea meiofaunal seep species (Van Gaever et al. 2006).

Although the copepod from the seep centre also had a strong contribution of bacteria synthesised FAs (Fig. 1), the FAs profiles were significantly different among the three groups examined (Fig 2; ANOSIM, Global R = 1, p < 0.05). The difference between *Beggiatoa* and the nematode were caused by a higher percentage abundance of 16:1ω7 in *Beggiatoa* and relatively higher percentage contributions of 18:1ω7 and 18:0 in the nematode (SIMPER, Fig. 1). The difference between the nematode and copepod is not only an overall more diverse set of FAs but also the presence and dominance of ω3 long-chain PUFAs in the copepod (Fig. 1). More specifically, SIMPER analysis revealed that the primary differences are the relatively higher percentage abundance of 18:1ω7 and 16:1ω7 in the nematode (39 and 38% versus 14 and 13% respectively) but higher percentage abundances of 16:1ω8, 22:6ω3 and 20:5ω3 in the copepod (17, 9 and 7% versus 2, 0.7 and 0 %; respectively Fig. 1). Although photosynthetic plankton is normally the predominant source of 22:6ω3 and 20:5ω3 in the marine environment, long-

chain PUFAs can also be synthesized by some bacteria (e.g., Delong and Yayanos 1986, Nichols 2003). This assertion of bacterial origin is supported by the extremely light $\delta^{13}C$ of all dominant FAs including the PUFAs ($\delta^{13}C < -69‰$; Table 2) indicating clearly a greater dependence on methane in the seep centre sediments as a result of prevailing higher methane fluxes compared to the microbial mat sediments (Niemann et al. 2006). Methane in HMMV has a $\delta^{13}C$ of ~ $-60‰$ (Niemann et al. 2006). This is further supported by the dominance of the methanotroph type I specific bacterial biomarker $16:1\omega8c$ (e.g., Pranal et al. 1996, Kiyashko et al. 2004, see Fig.1) with very light carbon isotope signatures ($-81‰$ $\delta^{13}C$; Table 2). In corroboration, this FA was also found to be dominant in the seep centre surface sediment and with a very light carbon isotope signature ($\delta^{13}C$ $-80‰$; Niemann et al. 2006). Large difference in the nematode bulk tissue isotope values measured in 2003 (Van Gaever et al. 2006) and in 2006 (this study; Table 2) indicate that heterogeneity can be high. Unfortunately, copepod bulk tissue was measured only in 2003 (Table 2) but could have similarly been much lighter in 2006. This may explain the seemingly larger difference between FA and bulk tissue isotope signature compared to nematodes, otherwise generally in the order of 3–5‰ (e.g., Boschker and Middelburg 2002).
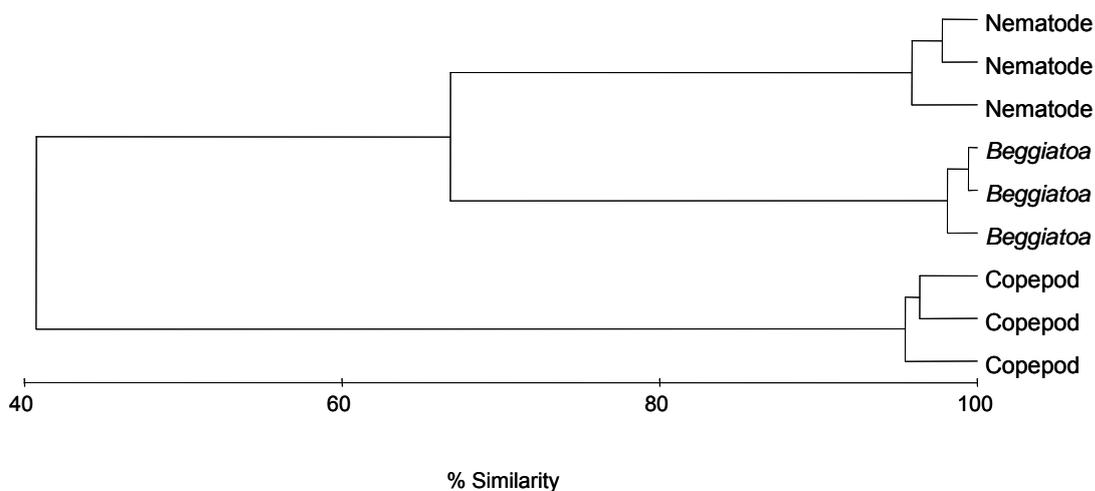
Figure 2: Hierarchical cluster analysis of fatty acid composition profiles of *Beggiatoa*, the microbial mat nematode and the seep centre copepod.

Overall, the results indicate that both these meiobenthic species proliferate on chemosynthetically derived carbon, but also that their segregation is the result of their spatial and trophic specialisation. The high biomass and abundance reached by the nematode thriving on sulphide-oxidising bacteria in the microbial mat sub-habitat suggest a certain level of trophic preference, which is different from the one found for the seep centre copepod, which was feeding on methanotrophic biomass. Different microbial activities are associated with differences related to habitat-specific characteristics. For example, in the

seep centre, rapidly rising sulphate-free fluids prevent sulphide formation and thus oxidation, but support methanotrophic habitats, whereas high sulphide concentrations in the microbial mat habitat are translated into elevated chemosynthetically derived microbial biomass (de Beer et al. 2006, Niemann et al. 2006). The utilisation of chemosynthetic food sources for meiofauna may vary among sub-habitats; this appears of major importance for clarifying the drivers of diversity distribution, community structure and ecosystem functioning in chemoautotrophic systems.

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CHAPTER 6

Meiofaunal communities at cold seeps along the Norwegian margin: influence of habitat heterogeneity and evidence for connection with shallow-water habitats

Meiofaunal communities at cold seeps along the Norwegian margin: influence of habitat heterogeneity and evidence for connection with shallow-water habitats

ABSTRACT

Cold-seep environments and their associated symbiont-bearing megafaunal communities create islands of primary production to macro- and meiofauna in the otherwise relative monotonous and nutrient-poor deep-sea environment. To examine the spatial variation and distribution patterns of meiobenthos in different seepage related habitats, biological sampling was carried out at two regions offshore Norway: the Storegga Slide with several pockmarks (740 m) and nearby Nyegga pockmark area (730 m) at 64°N, and west of the Barents Sea at the active, methane-venting Håkon Mosby Mud Volcano (HMMV) (1280 m) at 72°N. Based on the biogeochemical sediment conditions and associated epifauna, three different habitat types were distinguished across the two regions: (1) reduced sediment with suboxic conditions and sometimes covered by bacterial mats, (2) sediment colonised by chemosynthetic, siboglinid tubeworms, and (3) sediment outside the influence of seepage and without large chemosynthetic fauna. Meiofaunal communities varied strongly in terms of genus diversity and dominance among the different habitat types. Control sites and Siboglinidae polychaete fields supported comparable high nematode genus richness similar to other deep-sea sediments, whereas the reduced sediments yielded a genus poor nematode community dominated by one or two successful species. Meiofaunal densities at the different habitats were clearly affected by the prevailing macrobenthos since their densities correlated negatively. An extremely dense (>11000 ind. 10 cm^{-2}), mono-specific population of the nematode *Halomonhystera disjuncta* appeared to be strictly associated with the bacterial mats at HMMV, and consisted of a new cryptic species of the *H. disjuncta* complex described from intertidal habitats in the North Sea. The reduced seep sediments at Nyegga did not comprise *H. disjuncta* but were dominated by *Terschellingia longicaudata*, another cosmopolitan nematode species known to be abundant in organic rich, oxygen poor, shallow-water environments. These observations point to a past or recent connection between margins and shallow-water habitats.

INTRODUCTION

In the last decades, manned submersibles as well as ROV devices have enabled observation and investigation of the habitat heterogeneity at a large number of fluid escape zones on the deep-sea floor. In contrast to mid oceanic ridge fluids which are characterised by high temperature and high flow rates, fluids at margins tend to have lower temperatures and flow rates, and are therefore called cold seeps. In general, cold seeps are related to geological structures identified according to their seafloor expression: pockmarks, mud domes or mud volcanoes (Sibuet and Olu 1998, Levin 2005). Biological assemblages associated with cold seeps are typically dominated by large, symbiont-bearing megafauna (vesicomyid clams, mytilid mussels, siboglinid tubeworms) or by sediment-covering microbial mats (Levin and Mendoza 2007). These biochemical but also biologically generated structures create distinct local 'habitats' for the infauna within seep sediments. The complexity of the biogenic structure, geochemical conditions and bacterial processes in the sediments are determining factors for the composition and diversity of these macro- and meiofaunal communities. The macrofaunal communities associated with large biogenic structures such as mussel beds and tube-worm bushes seem to include numerous seep endemic species (e.g., Cordes et al. 2003, Turnipseed et al. 2003, 2004, Bergquist et al. 2003, 2005, Levin 2005). In contrast, information at the species or genus level is currently scarce for meiofaunal communities since most studies have been restricted to higher taxon level (e.g., Buck and Barry 1998, Olu et al. 1997, Robinson et al. 2004, Soltwedel et al. 2005a, Sergeeva and Gulin 2007). Moreover, contrasting density patterns have been observed for meiofauna: they are sometimes enhanced, depleted or unchanged in the seep site compared to the nearby background sediments (reviewed in Levin 2005, Van Gaever et al. 2006, Sergeeva and Gulin 2007).

Here, we investigated the meiobenthos from two regions along the Norwegian continental margin where evidence of fluid escapes was encountered. The first region was the active methane seeping Håkon Mosby Mud Volcano (HMMV) situated west of the Barents Sea at 72°N 14°E. This mud volcano has already been examined for meiofauna (Soltwedel et al. 2005a, Van Gaever et al. 2006) and was revisited by the ROV Victor 6000 during the Vicking cruise aboard the RV "PourquoiPas?" in 2006. Additionally, the Storegga Slide (Bugge 1983, Bünz et al. 2003) and nearby Nyegga area (Hovland et al. 2005, Hovland and Svensen 2006) at 64°N 05°E offshore mid-Norway, characterised by the presence of a large complex of pockmarks, was sampled during the same cruise for the first time to describe the biological communities linked to cold-seeps.

The Håkon Mosby Mud Volcano is the only mud volcano in a polar region that has been mapped in detail by photo and video camera observations. Three methane bubbling sites were identified in 2003 (Sauter et al. 2006) and very high mud expulsion rates have been estimated in the inner crater from thermal gradients (up to 40°C m^{-1}), resulting in high temperature (up to 25°C) in the upper layers of sediment (Kaul et al. 2006). The concentric structure of the mud volcano can be divided in several sub-habitats characterised by significantly different biogeochemical sediment conditions (de Beer et al. 2006): (1) the flat central zone, corresponding to the mud flow expulsion area from the inner crater, lacking epifauna or bacterial mats, (2) the extensive white mats of tangled *Beggiatoa* filaments (Pimenov et al. 2000), and smaller, grey mats containing a complex sulphur oxidising community (*Beggiatoa* of various thickness, *Thiotrix* and *Thiomargarita*; Niemann et al. 2006), (3) the dense methane- or sulphide-depending chemosynthetic macrofaunal communities dominated by Siboglinidae fields (Vogt et al. 1997b, Gebruk et al. 2003). A predictive habitat map from video mosaic analysis confirmed this rather concentric distribution (Jerosh et al. 2007). The HMMV community is lacking any large seep specific symbiont-bearing mussels or clams (only small Thyasiridae bivalves mainly associated with Siboglinidae fields), but instead is dominated by two species of siboglinid tubeworms: *Sclerolinum contortum* and *Oligobrachia haakonmosbiensis*, both strictly restricted to the crater zone and present in a remarkably high biomass (Pimenov et al. 2000, Gebruk et al. 2003). Previous studies on the meiofauna of this mud volcano (Soltwedel et al. 2005a, Van Gaever et al. 2006) revealed variable but often extraordinarily high densities up to 11000 ind. 10 cm^{-2} of nematodes thriving in the sediments underneath the *Beggiatoa* mats. Moreover, this dense community consisted mainly of one successful nematode, *Halomonhystera disjuncta*, which appeared to be absent from the adjacent siboglinid dominated sediments and the surrounding control deep-sea sediments.

Halomonhystera disjuncta, formerly described as *Geomonhystera disjuncta* (Bastian 1865) (Monhysteridae), is a cosmopolitan marine species, reported from different shallow-water habitats such as the detached kelp (mostly *Laminaria saccharina*) accumulations in Velikaya Salma Strait (15–18 m water depth, White Sea, Mokievsky et al. 2005), on macroalgal detritus along the coast of Belgium and the south-western part of The Netherlands (Derycke et al. 2007) and in various marine sediments (Heip et al. 1985). *H. disjuncta* from Belgium and the Netherlands consists of at least five cryptic species (Derycke et al. 2007) with subtile morphological differences (Fonseca et al. 2008).

To examine whether similar meiofaunal distribution patterns and dominance of a single nematode (*Halomonhystera disjuncta*) seen at the HMMV are also present in other seepage sites along the Norwegian margin, we sampled different habitats at the recently discovered pockmarks on the Storegga

Slide and Nyegga area off mid-Norway. A new type of pockmark was observed along the northeastern flank of the Storegga Slide in the so-called Nyegga area during detailed seabed surveys with remote operated vehicles in 2003 and 2004. These pockmarks are morphologically more complex than 'normal' seabed pockmarks (Hovland and Judd 1988), and occur as near-circular, up to 15 m deep and 320 m wide depressions. They contain up to 10 m high ridges of methane-derived authogenic carbonate rock and harbour pockmark-specific micro- and macrofauna, which includes bacterial mats, fields of small tube-worms (*Sclerolinum contortum* and *Oligobrachia haakonmosbiensis*) and large pycnogonids (Hovland and Svensen 2006).

In this study, we identified three distinct habitats (control sediment, reduced sediment, and Siboglinidae field) across both seepage regions (Håkon Mosby Mud Volcano at 72°N 14°E; Storegga Slide/Nyegga at 64°N 05°E) along the Norwegian margin. Control sediments were visually normal deep-sea sediments outside the influence of any seepage, while the Siboglinidae habitat was defined as sediments with dense aggregations (or fields) of chemosynthetic, siboglinid tubeworms. The third habitat, called 'reduced sediments', were sites with blackish, anoxic sediments, sometimes covered by bacterial mats. This last habitat includes the samples obtained at the black, reduced sediments at Nyegga or Storrega, sometimes colonised by white bacterial filaments, as well as the grey bacterial and white *Beggiatoa* mats at HMMV (Lichtschlag et al. 2006). To analyse the data obtained from Storegga/Nyegga in comparison to those previously documented for HMMV, we added the meiofaunal data of the control site, Siboglinidae site and *Beggiatoa* site at HMMV from Van Gaever et al. (2006) in addition to newly collected samples at the HMMV.

With this unique given, we focussed on the following key questions: (1) To what extent do similar habitats at Nyegga and Storegga harbour similar meiofaunal communities (isocummunities as described by Thorson 1957) compared to those at Håkon Mosby Mud Volcano? (2) Do infaunal macrofauna densities have any effect on the associated infaunal meiofauna communities? (3) What is the distribution range of the abundant HMMV nematode *Halomonhystera disjuncta* at other seepage sites along the Norwegian margin and what is the phylogenetic position of this morphospecies with respect to the *H. disjuncta* species complex (Derycke et al. 2007) from shallow-water habitats from the North Sea and The Netherlands?

MATERIALS AND METHODS

1. STUDY AREA

The Storegga area, situated offshore mid-Norway, is well known for its giant Holocene slide, one of the largest ever mapped on continental margins (Figure 1) (Paull et al. 2008). On the northeastern flank of this Storegga Slide, complex pockmarks are located in the so-called Nyegga area at 740 m water depth. The deepest and most spectacular pockmark, called 'G11', was sampled in this study and measures about 220 m in diameter and is approximately 12 m deeper than the surrounding seafloor. Up to 30 cm tall stalked crinoids, abundant large ophiuroids (basket stars, up to 1 m diameter), together with hydrozoa and numerous other unidentified sessile organisms were perched at specific sites on the large carbonate rocks of the ridges (Hovland et al. 2005). Hovland et al. (2005, Hovland and Svensen 2006) inferred that the primary trophic stage within these pockmarks partly consists of a chemosynthetic micro-organic community, fuelled by advecting hydrocarbon-associated fluids, much in analogy with the HMMV-environment, even though there is no visual seepage at the Nyegga complex pockmarks. It is assumed that the Nyegga pockmarks were formed by a single sudden ('catastrophic') fluid flow and that subsequent micro-seepage is still active (Hovland et al. 2005).

The Håkon Mosby Mud Volcano (HMMV) is located on the Norwegian continental slope west of the Barents Sea, at an average water depth of 1280 m and at a distance of about 950 km north from the Storegga Slide (Figure 1). This concentric mud volcano (1.5 km diameter) was first observed in 1989 during a side scan sonar survey (Vogt et al. 1997b). An expedition in 1995 recovered siboglinid tubeworms, indicating active chemosynthesis, measured very high temperature gradients in the sediments, and recovered methane hydrate from 2 m subbottom depth (Vogt et al. 1997b). Apart from the Siboglinidae and *Beggiatoa*, the zoarcid fishes *Lycodes* spp. constituted the most abundant visible fauna at HMMV (Gebruk et al. 2003). First identifications of other members of the HMMV benthic community (polychaetes, caprellid amphipods, pycnogonids, etc.) include common Norwegian-Greenland Sea species, which were also found in the nearby background sediments although in less dense aggregations as those observed on the volcano (Gebruk et al. 2003). The discovery of the HMMV was the first indication of an active cold seep and of shallow hydrate in the Nordic Seas. Consequently, the mud volcano was subjected to more sampling campaigns and extensive studies investigating the gas hydrates, porewater chemistry, sea-floor geology, seep biota and heat flow.
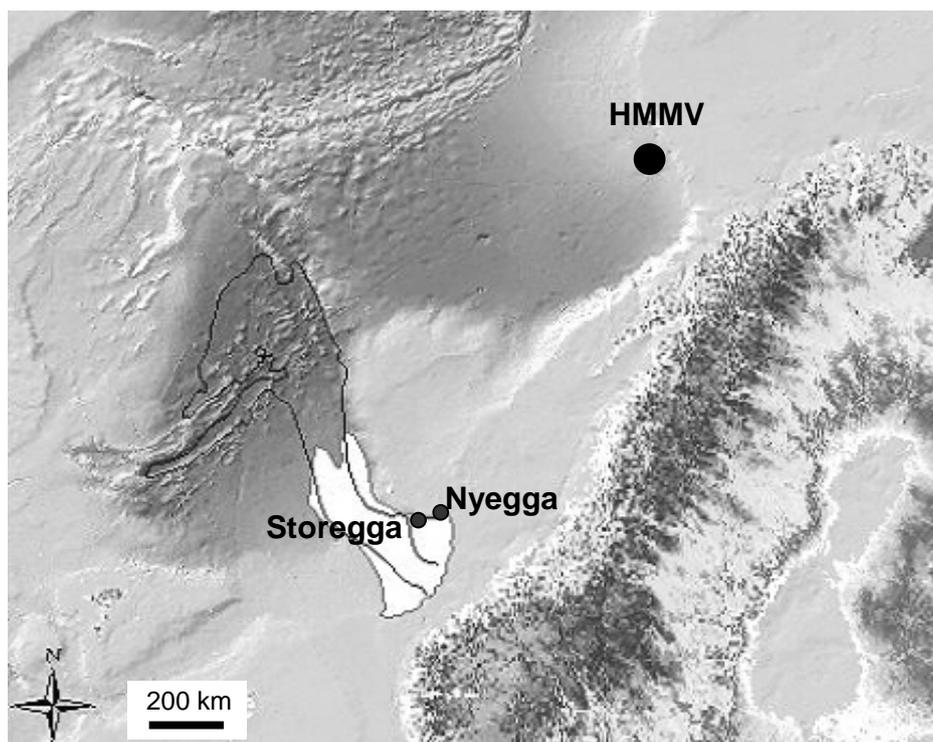
Figure 1: Map of the Norwegian margin with indication of the three sampling locations. The northern is the Håkon Mosby Mud Volcano, the southern locations are the Storegga Slide and Nyegga area. (HERMES Project, GIS Workpackage deliverables, Nordic Margins GIS webserver *)

2. SAMPLING STRATEGY AND HABITATS

Meiofaunal and macrofaunal sediment samples were collected at the Storegga Slide, the Nyegga area and the Håkon Mosby Mud Volcano (HMMV) during the Vicking cruise aboard the RV "PourquoiPas?" in May–June 2006 (Figure 1, Table 1). Samples for both meio- and macrofauna were retrieved at a Siboglinidae field and control sediment at both the Storegga Slide and the Nyegga area. Additional meiofaunal samples were also collected at the blackish sediments at Nyegga, and at grey bacterial mats at HMMV, both regarded as reduced sediments. Additional macrofaunal samples were collected at HMMV at a Siboglinidae patch and at the *Beggiatoa* mats. At each habitat in each location, 2 or more replicate cores for meiofauna and/or macrofauna were taken (Table 1). Meiofaunal samples at control sites were sampled with a multiple corer (1 deployment per control site, tube surface of 30.2 cm²), while the other sites were sampled with push cores of the ROV *Victor 6000* (surface of 21.2 cm²). Macrofaunal samples were collected as sub-samples from an USNEL boxcorer or blade cores of the

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* http://gis-web.jacobs-university.de

ROV. Macrofaunal samples were stored as bulk samples, and sieved over a 0.5 mm-mesh sieve. All organisms were sorted out and counted. Detailed study of macrofaunal community structure will be published separately (Decker et al. pers. comm.).

One meiofaunal sample (PL277-07 CT9) taken at the grey bacterial mat site at HMMV, was collected as bulk sample, fixed in acetone and stored for later molecular analyses on the nematodes. The sediment of the other meiofaunal samples was fixed in 4% buffered formaldehyde and washed over a 32 μm-mesh sieve. Metazoan meiofauna extraction was done by density gradient centrifugation, using Ludox (a colloidal silica polymer; specific gravity 1.18) as a flotation medium (Heip et al. 1985). All material was fixed with 4% buffered formalin and stained with Rose Bengal. The metazoan meiofauna was sorted out, enumerated and identified down to major taxa under the stereomicroscope. From each sample, 100 nematodes were picked out randomly, mounted onto glycerine slides using the formalin-ethanol-glycerol technique of Seinhorst (1959) to prevent dehydration, and identified up to genus level according to Warwick et al. (1998).

3. MOLECULAR ANALYSIS

The *Beggiatoa* and grey mats at the Håkon Mosby Mud Volcano were dominated by one (morpho) species identified as *Halomonhystera disjuncta*. To confirm this morphological identification, we extracted DNA from 12 specimens from the HMMV grey mats that had been preserved on aceton. The DNA extraction protocol from Derycke et al. (2005) was slightly modified because we were unable to retrieve PCR product from other gene fragments than the 18S fragment: instead of cutting the nematodes individually, 3 and 9 specimens were pooled together and put directly in WLB without cutting them to increase the amount of template DNA. After DNA preparation, ca. 790 bp of the ribosomal 18S gene were amplified with primers G18S4 (5'-GCT TGT CTC AAA GAT TAA GCC-3') and 4R (5'- GTA TCT GAT CGC CKT CGA WC-3'). In addition, 307 bp of the ribosomal D2D3 region of the 28S gene were amplified with primers D2/F1 (5'-TTCGACCCGTCT TGAAACACG-3') and D3b (5'- TCC TCG AAA GGA ACC AGC TAC TA-3') and the internal transcribed spacer region (ITS) was amplified with primers Vrain 2F (5'-CTT TGT ACA CAC CGC CCG TCG CT-3') and Vrain 2R (5'-TTT CAC TCG CCG TTA CTA AGG GAA TC-3'). For ITS, we were unable to retrieve any PCR product. Next to the nuclear fragments, we also amplified 422 bp of the mitochondrial COI gene with the primers JB2 (5'-ATGTTTTGATTTTACCWGCWTTYGGTGT-3') and JB5GED (5'-AGCACCTAAACTTAAAACATARTGRAARTG-3') from Derycke et al. (2007). PCR conditions were as in

Derycke et al. (2005), except for the annealing temperature, which was 50°C for COI, 54°C for D2D3 and 52°C for 18S. The extension time was 30s for COI, 45s for D2D3 and 90s for 18S. For COI and D2D3, 35 cycles were run, while 40 cycles were used to amplify the 18S fragment. Sequencing was performed as described in Derycke et al. (2008a) and all sequences have been deposited to Genbank under accession numbers x (18S), y (D2D3) and z (COI). All sequences were aligned in ClustalX v 1.81 (Thompson et al. 1997) using default alignment parameters (gap opening/gap extension costs for 15/6.66). To ensure that our specimens belong to *Halomonhystera disjuncta*, a maximum parsimony analysis was performed with Paup*4.0 beta 10 (Swofford 1998) using the 18S sequences from a broad range of genera across the phylum, including all marine genera from Meldal et al. (2007). In addition, we compared the D2D3 and COI sequences with published sequences from the *Halomonhystera disjuncta* species complex (Derycke et al. 2007) and with sequences from four other closely related monhysterid nematode species from shallow waters: *Diplolaimelloides meyli* (AM748756), *D. oschei* (AM748757), *D. deleyi* (AM748758) and *Diplolaimella dievengatensis* (AM748759). A neighbour-joining tree based on p-distances was constructed using MEGA v3.1 (Kumar et al. 2004).

4. STATISTICAL ANALYSIS

Non-metric multidimensional scaling (MDS) ordination using the Bray-Curtis similarity measure was applied to visualize the (dis)similarity between habitats from Nyegga, Storegga and HMMV with respect to the nematode genus composition. The stress value gives a measure for goodness-of-fit for the MDS ordination: a low stress value (<0.2) indicates a good ordination with no real prospect for a misleading interpretation (Clarke 1993). One-way Analysis of similarities (ANOSIM) was carried out to test for significant differences in the community structure between different habitats or regions. Similarity of Percentages (SIMPER) was used to investigate the degree of (dis)similarity between habitats in terms of nematode genus composition. Due to differences in sample size (multicorer - ROV push corer), relative abundance data were used. All analyses mentioned above were performed with the PRIMER5 software (Plymouth Marine Laboratory, Clarke and Gorley 2001). The STATISTICA6 software was used to calculate the product-moment correlation between meiofaunal and macrofaunal densities.

Table 1: Sampling locations, water depth (m), latitudes (N) and longitudes (E) of sampling sites, sample device, dive and core numbers, fraction of fauna sampled, and number of replicates. * denotes data published in Van Gaever et al. (2006).

Sampling location	water depth (m)	latitude (°N)	longitude (°E)	sampling device	dive number	core number	fauna	no. of replicates
Nyegga								
Control site	720	64° 40.16'	05° 17.38'	boxcorer		BC3	macro	4
Control site	719	64° 40.17'	05° 17.39'	multicorer		MT3	meio	2
Siboglinidae patch	732	64° 39.99'	05° 17.35'	blade corer	PL272-02	CL7+8	macro	2
Siboglinidae patch on G11 depression	733	64° 40.00'	05° 17.34'	push corer	PL272-02	CT14+18	meio	2
Reduced sediment (black spot)	733	64° 39.79'	05° 17.29'	push corer	PL272-02	CT1+8	meio	2
Storegga Nord Est								
Control site	742	64° 45.28'	05° 6.22'	boxcorer		BC1	macro	3
Control site	720	64° 45.28'	05° 6.21'	multicorer		MT2	meio	2
Siboglinidae patch	745	64° 45.27'	04° 58.87'	blade corer	PL275-05	CL5+7	macro	2
Siboglinidae patch	746	64° 45.27'	04° 58.87'	push corer	PL275-05	CT5+6	meio	2
Håkon Mosby Mud Volcano								
Control site *	1296	71° 58.97'	14° 45.41'	multicorer		PS 64/395	meio	1
Control site *	1885	71° 41.96'	13° 26.10'	multicorer		PS 64/390	meio	2
Siboglinidae patch	1261	72° 00.34'	14° 42.76'	blade corer	PL277-07	CL7+8	macro	2
Siboglinidae patch *	1288	72° 0.05'	14° 44.14'	multicorer		PS 64/356-357-367	meio	3
Beggiatoa mats	1258	72° 00.17'	14° 43.94'	blade corer	PL276-06	CL6+7+8	macro	3
Beggiatoa mats *	1287	72° 0.18'	14° 43.81'	multicorer		PS 64/321-323-324	meio	3
grey bacterial mats	1255	72° 00.32'	14° 43.38'	push corer	PL277-07	CT2+5+9	meio	2

RESULTS

1. METAZOAN MEIOFAUNAL COMMUNITIES ASSOCIATED WITH SIMILAR HABITATS AT HÅKON MOSBY MUD VOLCANO, STOREGGA SLIDE AND NYEGGA AREA

The most abundant meiofaunal taxon in all samples was Nematoda, accounting for 86–98% of the total meiofauna (Figure 2, Table 2), while adult copepods and their naupliar larvae always constituted the second most abundant groups. Meiofaunal sized polychaetes were present at every site. Amphipods, bivalves, kinorhynchs, loriciferans, ostracods, tanaids, tardigrades and turbellarians exhibited lower relative abundances and were not found at all sites. Total meiofaunal densities were highly variable among the different sites, and also within the same habitat type (Figure 2). For instance, the Siboglinidae habitat harboured maximum meiofaunal densities at the Nyegga area (mean ± 1 SD: 7028 ± 1279 ind. 10 cm^{-2}) and minimum densities at the Storegga Slide (41 ± 22 ind. 10 cm^{-2}). However, meiofaunal densities at the control sites also varied strongly, with values at Nyegga (1878 ± 524 ind. 10 cm^{-2}) and Storegga (3643 ± 473 ind. 10 cm^{-2}) being 2 to 4 times as high as those from HMMV (968 ±156 ind. 10 cm^{-2}).

The (dis)similarities in meiofaunal communities among all sites were illustrated in an ordination plot based on the relative abundance of the nematode genera. The reduced sediments were clearly separated from the Siboglinidae habitat (SIMPER dissimilarity 93.9%) and the control sediments (SIMPER dissimilarity 95.6%) (Figure 3). The nematode communities observed in the reduced sediments habitat were characterised by a low diversity in terms of genera number (19 ± 6), and the very high dominance of only one or two nematode species (Table 3). The white and grey bacterial mat sites from the Håkon Mosby Mud Volcano were grouped together in the MDS plot (Figure 3), due to the high relative abundances of *Halomonhystera disjuncta* in the samples, while this species was absent from all other habitats. The reduced sediments at Nyegga were plotted separately from the bacterial mat sites (Figure 3) because of their totally different nematode community composition (SIMPER dissimilarity = 97.6%), made up by two equally dominant species: *Thalassomonhystera* sp. (40%) and *Terschellingia longicaudata* (36%) (Figure 4, Table 3).
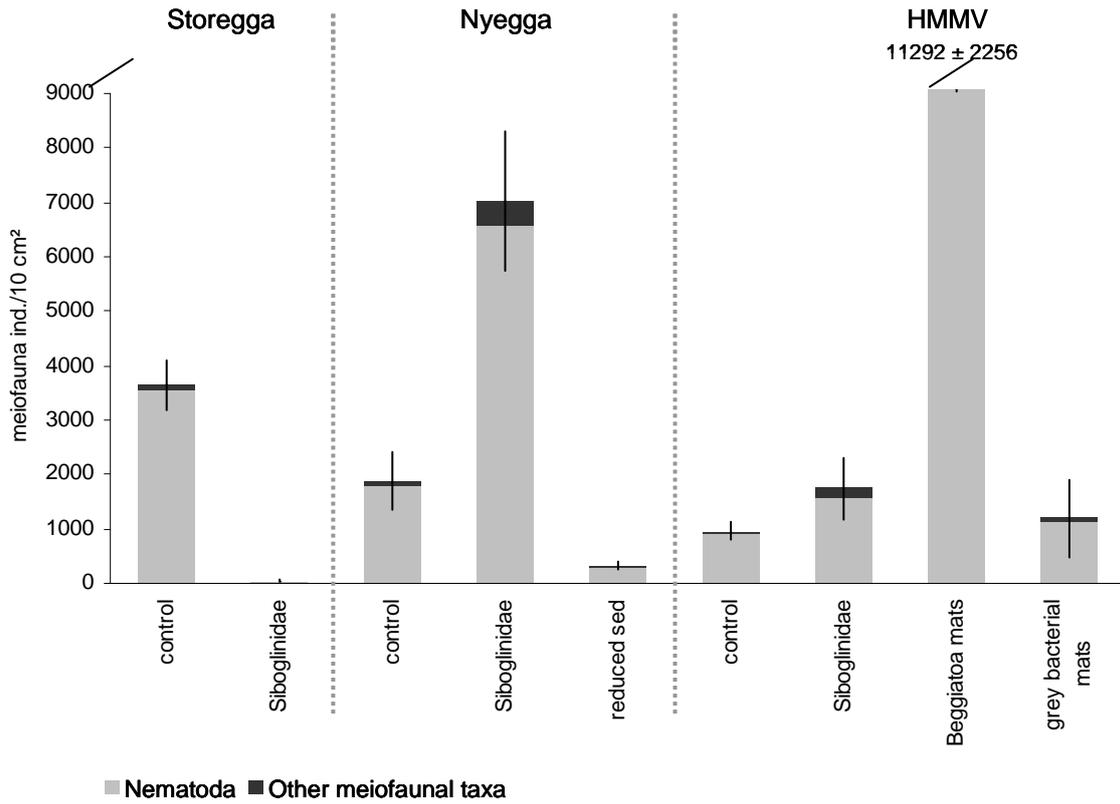
Figure 2: Mean total densities of meiofauna in the different habitats at Storegga Slide (740 m), Nyegga area (730 m) and Håkon Mosby Mud Volcano (1280 m) (mean ± 1 SD).
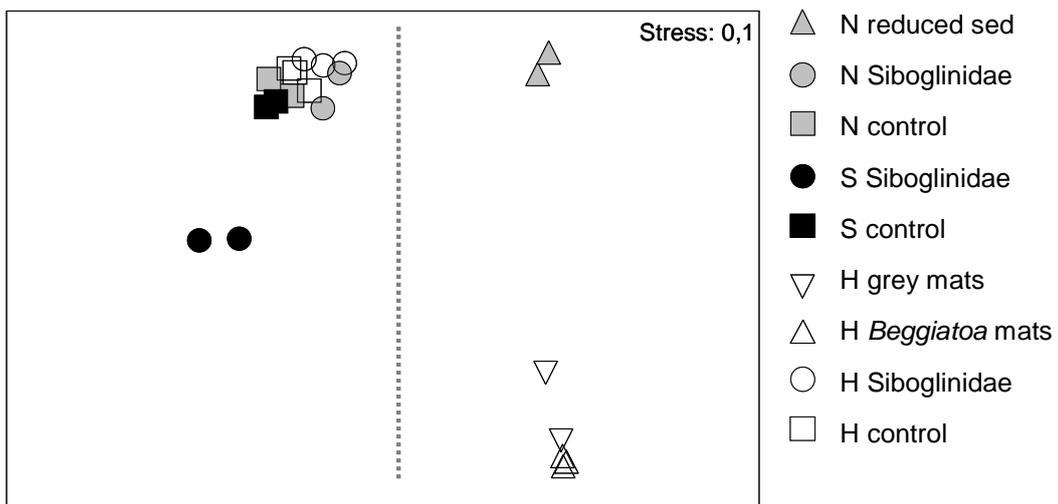
Figure 3: MDS illustrating similarity of nematode genera communities in the different habitats at the two regions (Storegga: S and Nyegga: N, Håkon Mosby Mud Volcano: H). All replicate samples were plotted.

Table 2: Densities of all metazoan meiofaunal taxa and relative abundances of Nematoda, Harpacticoida and nauplii (0 to 10 cm) for the different sites. SD = standard deviation

	Nyegga			Storegga		HMMV
Subhabitat	reduced sed mean (SD)	Siboglinidae mean (SD)	control mean (SD)	Siboglinidae mean (SD)	control mean (SD)	grey mats mean (SD)
Density (ind./10 cm²)						
Acari		0.2 (0.3)				
Amphipoda		3.5 (1.7)			0.7 (0.0)	
Bivalvia	0.2 (0.3)	12.5 (3.0)	1.8 (1.2)		2.2 (0.7)	
Gastropoda					0.2 (0.2)	
Gastrotricha		0.5 (0.7)				
Harpacticoida	8.5 (5.3)	211.2 (105.5)	32.3 (5.4)	0.9 (1.3)	39.7 (6.1)	45.4 (35.0)
Hydrozoa			0.7 (0.9)			
Isopoda					0.2 (0.2)	
Kinorhyncha		0.9 (1.3)	0.2 (0.2)		0.2 (0.2)	
Loricifera		0.5 (0.7)	0.8 (1.2)		0.5 (0.2)	
Nauplii	30.4 (29.0)	169.7 (56.3)	27.8 (3.7)	0.2 (0.3)	41.1 (14.5)	14.1 (9.3)
Nematoda	287.7 (26.6)	6590.6 (1098.9)	1784.3 (511.6)	39.1 (20.6)	3536.1 (454.7)	1137.0 (693.8)
Ophiuroidea		0.2 (0.3)				
Ostracoda		11.1 (11.7)	5.6 (1.4)		4.6 (0.5)	0.7 (1.0)
Polychaeta	5.2 (6.0)	20.2 (6.0)	13.7 (0.7)	0.5 (0.7)	14.2 (2.8)	0.2 (0.3)
Sipuncula			0.3 (0.5)			
Tanaidacea	0.9 (1.3)	7.3 (6.3)			0.5 (0.7)	
Tardigrada			9.9 (2.3)		2.6 (0.9)	
Turbellaria			0.7 (0.9)			0.7 (1.0)
Total	333 (69)	7028 (1279)	1878 (524)	41 (22)	3643 (473)	1198 (717)
Relative abundance (%) Copepoda + nauplii	11.7%	5.4%	3.2%	2.9%	2.2%	5.0%
Nematoda	86.4%	93.8%	95.0%	96%	97.1%	94.9%

The control sediments were inhabited by a typical, genus-rich (66 ± 6) deep-sea nematode assemblage, with high relative abundances of *Acantholaimus* (Chromadoridae), *Thalassomonhystera* (Monhysteridae), *Tricoma* (Desmoscolecidae) and *Leptolaimus* (Leptolaimidae) (Table 3). The Siboglinidae fields at HMMV and Nyegga harboured a similar genus-rich (64 ± 9) nematode assemblage, but exhibited a shift in dominant genera compared to the control sediments: *Aponema* (Microlaimidae), *Terschellingia* (Linhomoeidae), *Sabatieria* (Comesomatidae) and *Metalinhomoeus* (Linhomoeidae) (Table 3). A deviating nematode community was detected in the Siboglinidae habitat at Storegga, which harboured an unusually low number of nematode genera (20), extremely low nematode densities (39 ± 21 ind. 10 cm^{-2}), and the highest relative abundances of *Sabatieria* (41.0%) and *Rhabdocoma* (15.4%). Consequently, the two samples from this site were plotted at some distance from the other Siboglinidae and control samples in the MDS plot (Figure 3).

To clear up the (dis)similarities within the cluster of the remaining Siboglinidae and control samples, a one-way ANOSIM analysis was performed, indicating a significant effect (R = 0.606; p = 0.003) of the habitat type on the nematode community composition.

2. COMPARISON BETWEEN MACRO- AND MEIOFAUNAL DENSITIES

Average total macrofaunal densities counted in the control sediments (5081 ± 39 ind. m^{-2}) were of the same order of magnitude as the average densities found in the Siboglinidae fields (6778 ± 747 ind. m^{-2}) at Nyegga and Håkon Mosby Mud Volcano. In contrast, the Siboglinidae site at Storegga exhibited average macrofaunal densities (12083 ± 7582 ind. m^{-2}) which were twice as high as those found at Nyegga and HMMV, and made up by polychaetes for 67%. Furthermore, the sediments underneath *Beggiatoa* mats at HMMV contained very low average macrofaunal densities (1463 ± 1544 ind. m^{-2}), almost completely consisting of polychaetes from the family Capitellidae (M. Morineaux pers. comm.). When mean meiofaunal densities of the different sites were plotted against the corresponding mean macrofaunal densities (Figure 5), a negative linear correlation (R = –0.79) was detected although there were some very high standard deviations for the macrofauna.
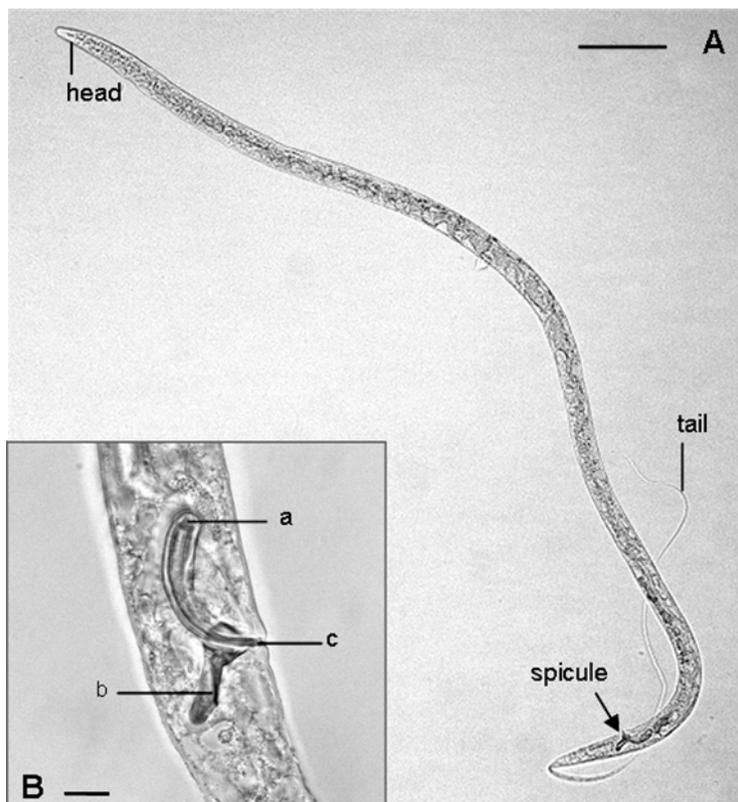
Figure 4: *Terschellingia longicaudata* from the Nyegga reduced sediments: (A) light microscopy photograph of male (scale bar: 100 μm) and (B) detail of the male copulating apparatus with indication of the spicule (a), gubernacular apophysis (b) and cloacal opening (c) (scale bar: 10 μm)
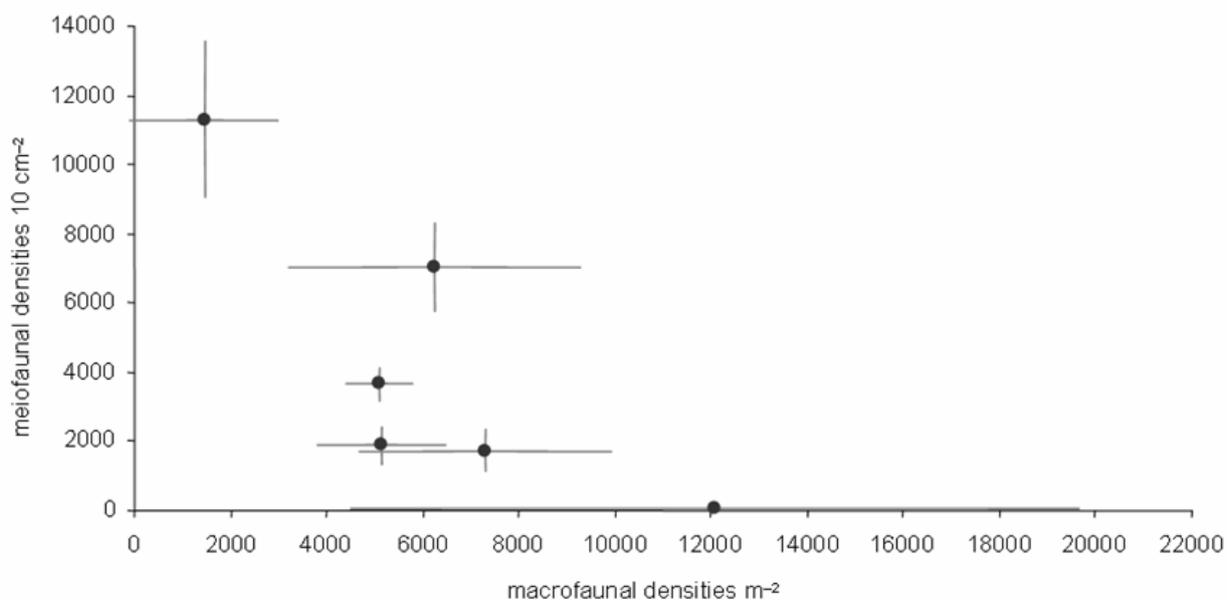
Figure 5: Meiofaunal densities in relation to macrofaunal densities in the control and S-boglinidae sites at Storegga and Nyegga, and the Siboglinidae and *Beggiatoa* sites at HMMV (mean values ± standard deviation).

Table 3 : Relative densities of the ten most dominant nematode genera in the different habitats at the three different locations.

Nyegga

Reduced sed Genus	%	Siboglinidae Genus	%	Control Genus	%
Monhystera	40.1	Aponema	17.1	Acantholaimus	12.6
Terschellingia	35.9	Terschellingia	11.8	Thalassomonhystera	12
Aponema	7.2	Sabatieria	8.5	Tricoma	10.3
Spilophorella	3.2	Tricoma	7.9	Greeffiella	7.3
Prochromadora	2.5	Metalinhomoeus	6.1	Halalaimus	6.8
Sabatieria	2.3	Halalaimus	5.9	Leptolaimus	6.3
Thalassomonhystera	1.9	Acantholaimus	4.1	Amphimonhystrella	4.8
Syringolaimus	1	Amphimonhystrella	3.6	Microlaimus	3.3
Halalaimus	0.9	Desmoscolex	3.4	Sabatieria	3
Linhomoeus	0.9	Thalassomonhystera	3	Metadesmolaimus	2.7

Storegga

Siboglinidae Genus	%	Control Genus	%
Sabatieria	39.7	Leptolaimus	21.5
Rhabdocoma	13.1	Richtersia	8.4
Leptolaimus	7.3	Acantholaimus	8.1
Rhabdodemania	7.2	Thalassomonhystera	6.6
Neochromadora	6.0	Metalinhomoeus	5.8
Paramonhystera	6.0	Tricoma	5.2
Thalassomonhystera	3.7	Rhabdodemania	4.2
Syringolaimus	2.5	Halalaimus	3.8
Monhystera	2.4	Desmoscolex	3.4
Halalaimus	2.1	Microlaimus	3.4

HMMV

Beggiatoa mats Genus	%	Grey mats Genus	%	Siboglinidae Genus	%	Control Genus	%
Halomonhystera	99.6	Halomonhystera	91.2	Monhystera	12.7	Acantholaimus	14.3
Sabatieria	0.2	Prochromadora	3.5	Microlaimus	8.9	Monhystera	13.4
Laimella	<0.1	Sabatieria	2.1	Acantholaimus	7.4	Halalaimus	7.3
Tricoma	<0.1	Thalassomonhystera	1.3	Comesa	7.1	Tricoma	7.2
Neochromadora	<0.1	Draconematidae	1.1	Daptonema	6.6	Linhystera	4.9
Theristus	<0.1	Syringolaimus	0.6	Halalaimus	6.1	Desmoscolex	3.9
Linhystera	<0.1	Desmoscolex	0.1	Amphimonhystrella	5.9	Neochromadora	3.6
Monhystera	<0.1	Amphimonhystrella	0.1	Theristus	5.1	Monhystrella	3.5
Daptonema	<0.1	Tricoma	0.1	Monhystrella	5.1	Desmoscolex	2.5
Viscosia	<0.1	Manganonema	<0.1	Leptolaimus	4	Sabatieria	2.4
		Halalaimus	<0.1	Sabatieria	3.1		

3. Distribution range of the nematode *Halomonhystera disjuncta* along the Norwegian margin

Extremely high densities (11069 ± 2269 ind. 10 cm^{-2}) of the monhysterid *Halomonhystera disjuncta* were found to be associated with the *Beggiatoa* mats at Håkon Mosby Mud Volcano (HMMV). This species accounted for 98% of the total nematode community in the *Beggiatoa* mats, and only some very few individuals of other species (e.g., *Sabatieria* sp., *Tricoma* sp.) were found in the harsh conditions of these sediments (Van Gaever et al. 2006). *Halomonhystera disjuncta* was also observed with a similar high dominance (95%) in the samples from the grey bacterial mats at HMMV, though in much lower densities (1075 ± 757 ind. 10 cm^{-2}). The species disappeared in the other HMMV habitats outside the bacterial mats site (except from some very few individuals in the methane rich volcano's centre), and not a single individual was found in the samples from the different habitats at Storegga and Nyegga.

4. Phylogenetic relationship of *Halomonhystera disjuncta* from HMMV with the *H. disjuncta* species complex from shallow-water habitats in the North Sea

Molecular analyses were performed on the dominant nematode species collected at the grey bacterial mats at Håkon Mosby Mud Volcano (HMMV) to confirm the morphological identification to species level. The 18S sequences obtained from 2 specimens were identical to each other and clustered within the Monhysteridae clade shown in Meldal et al. (2007). The sequences were most similar to the *Halomonhystera disjuncta* (formerly known as *Geomonhystera disjuncta*) sequence from Meldal et al. (2007) and was 2.3% different from the 18S sequences obtained from specimens belonging to one of the five cryptic species of the *H. disjuncta* complex from Belgium and The Netherlands (data not shown). For the D2D3 region, the HMMV sequence differed in 1 to 6.1% from those of the five cryptic *H. disjuncta* species, and was most similar to the species Gd1 and Gd4 as named in Derycke et al. (2007). The phylogenetic tree of the COI sequences confirmed the divergence patterns found in the nuclear fragments (Figure 6): the HMMV sequence was closer related to the *H. disjuncta* sequences than to the other monhysterid species. Divergence levels of COI between the HMMV sequence and the *H. disjuncta* sequences from the species complex range between 20.4–27.0% and are of the same magnitude as those observed between the cryptic *H. disjuncta* species. In view of these results in three molecular markers (18S, D2D3, COI), the specimens from the HMMV grey mats most likely represent a different cryptic species within the *H. disjuncta* species complex.
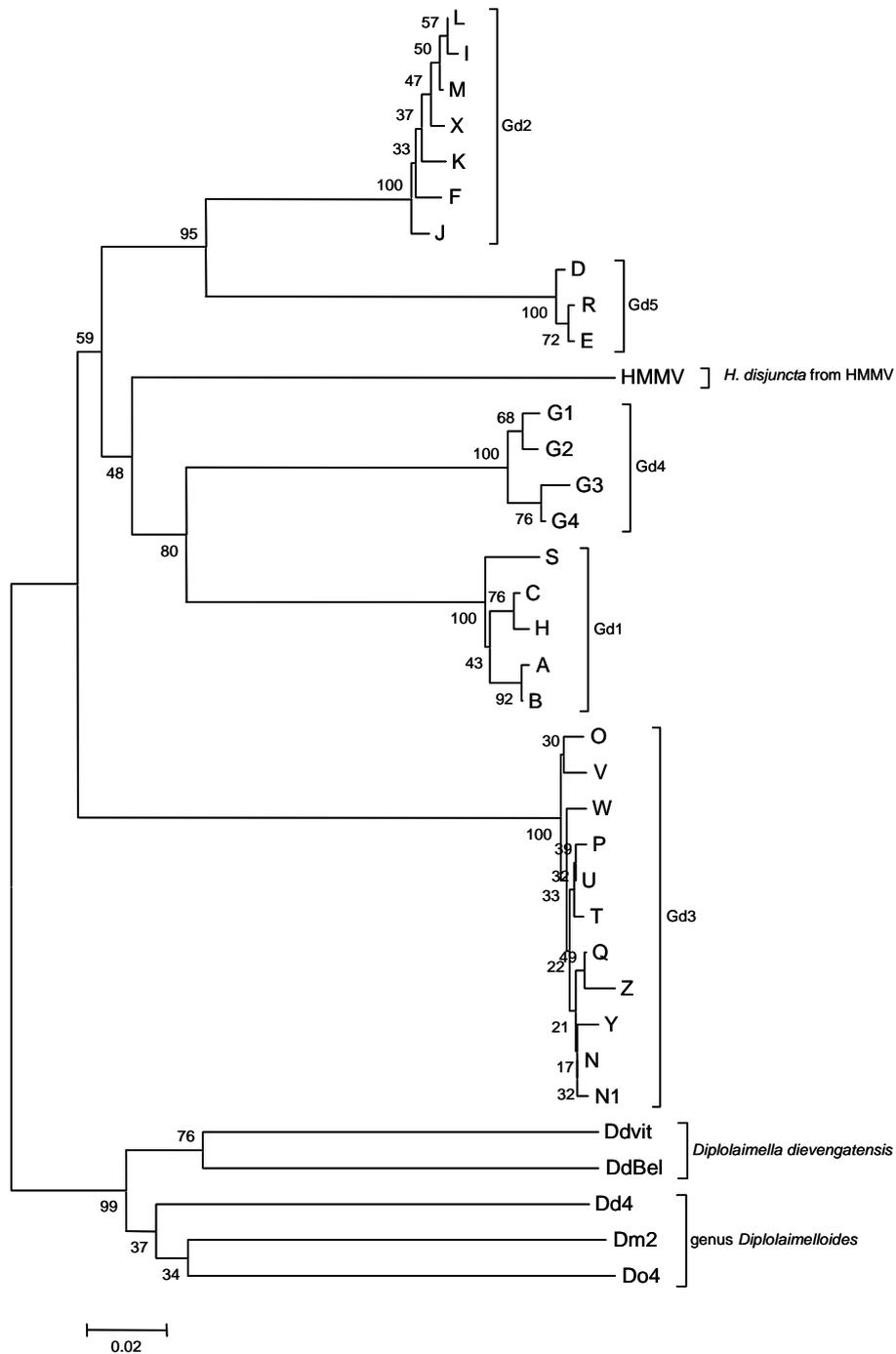
Figure 6: Phylogenetic position of HMMV among cryptic species of the *Halomonhystera disjuncta* species complex from Derycke et al. (2007). The closely related monhysterids *Diplolaimelloides meyli*, *D. oschei*, *D. deleyi* and *Diplolaimella dievengatensis* were used as outgroup. NJ tree of COI haplotypes with branch lengths calculated on the basis of P-distances. Bootstrap values are based on 1000 replicates.

DISCUSSION

1. METAZOAN MEIOFAUNAL COMMUNITIES ASSOCIATED WITH SIMILAR HABITATS AT HÅKON MOSBY MUD VOLCANO, STOREGGA SLIDE AND NYEGGA AREA

In general, meiobenthic densities counted in the control sites at the Storegga Slide and Nyegga area (1878–3643 ind. 10 cm^{-2}) were clearly enhanced compared to the expected densities for similar water depth in the Eastern Atlantic (about 1000 ind. 10 cm^{-2}; Soltwedel 2000). The high meiofaunal densities in the background sediments can be attributed to an increase in primary production at the time of sampling (23 May–6 June) in this area. Seasonal fluctuations in primary production were measured in the Norwegian Sea, with a spring bloom driven by the light regime and starting already in mid April (Irigoien et al. 1998) although further investigations are needed to confirm this link.

Habitat heterogeneity generated by biogenic structures is also known to influence the distribution and abundance of species in many marine environments (Bergquist et al. 2003 and references therein). Organisms increase habitat heterogeneity by various processes, with the supply of physical structures representing the most widespread and important process. Here we examined the structuring effect of different habitat types on meiobenthic communities in two fluid escape areas along the Norwegian margin. Habitat type has proven to be a significant factor determining the meiobenthos in terms of nematode genera composition. Siboglinidae fields and control sediments yielded comparable highly diverse nematode assemblages, but a shift in dominant families and genera was detected. In contrast, reduced sediments always supported a very unique and species poor nematode community, dominated by one or two nematode genera belonging to the Monhysteridae and Linhomoeidae families.

The reduced sediments at HMMV, covered by white or grey bacterial mats, were inhabited by *Halomonhystera disjuncta* in highly variable densities. The blackish, anoxic sediments at the Nyegga area, lacking bacterial mats, exhibited a completely different nematode community with high relative abundances of the species *Terschellingia longicaudata* (Linhomoeidae) (Figure 4). Similar to *H. disjuncta*, this nematode species is also a common inhabitant of intertidal habitats with an extensive geographical range including the estuarine sediments in the North Sea (Heip et al. 1985, Vranken et al. 1988, 1989), the mangrove mudflats in north-eastern Australia (Alongi 1987, Nicholas et al. 1991, Fisher and Sheaves 2003) and in the southeast coast of India (Chinnadurai and Fernando 2007), at the Atlantic coast of France, the Black Sea, the USA (Gulf of Mexico), China (Qingdao province), New

Zealand and the Solomon Islands (Sergeeva 1991, Zhang and Ji 1994, Burgess et al. 2005). Tolerance experiments in the laboratory confirmed that *Terschellingia* sp. succeeds well in withstanding reduced oxygen conditions and sulphide presence (Steyaert et al. 2007). A molecular study carried out on individuals of *Terschellingia longicaudata* collected from a broad range of localities (East and West Atlantic, Bahrain, Malaysia) revealed that the majority of the morphologically defined specimens belong to a single taxon distributed from the British Isles to Malaysia. 18S rRNA analysis also revealed two additional sequence types which may point to the existence of a species complex for *T. longicaudata* (Bhadury et al. 2008). Another example of a cosmopolitan nematode species colonising cold seep sediments is *Sabatieria mortenseni*. This species was recently observed in the cold seep adjacent to the Congo canyon in the Gulf of Guinea (South Atlantic), but was already reported from coastal sediments in Brazil, USA, Antarctica and in the Strait of Magellan (Chile) (Van Gaever et al. accepted, and references therein).

It can be concluded that seep sediments which are strongly affected by reduced fluids, generate a habitat which is difficult to explore by most of the typical deep-sea nematode species. In our study, only species, or cryptic species from a species complex, with a wide geographical distribution range were able to colonise these anoxic, sulphidic environments. Macro-algae which were observed at the HMMV deep-sea floor may function as a possible transport medium for nematode species if the exchange took place from the shallow to the deep-sea habitats. There are also examples of macrofaunal taxa although not at species level confirming this hypothesis, particularly at the shallowest seeps. The eastern Mediterranean cold-seep fauna is dominated by several bivalves belonging to families (Lucinidae) and genera (the Modiolinae *Idas*) mostly represented in organically rich mud in coastal waters or organic remains such as sunken wood (Olu-Le Roy et al. 2004). Nevertheless, the structure of the nematode community associated with an isolated reduced habitat within a cold deep-sea will certainly depend on several factors, for instance the presence of food resources (bacteria), tolerance levels to environmental stress, and dispersal capacities.

2. RELATIONSHIP WITH MACROFAUNAL DENSITIES

We observed a negative correlation between the macrobenthic densities and the corresponding meiobenthic densities. A clear example was found in the Siboglinidae patch at Storegga, where the very abundant macrobenthos (12083 ± 7582 ind. m^{-2}), strongly driven by a dense polychaete assemblage, was accompanied by extremely low meiofaunal densities (41 ± 22 ind. 10 cm^{-2}). Several studies have indicated the importance of infaunal polychaetes as structuring agent of meiofaunal assemblages, either

by predation or by sediment disturbance (e.g., Ronn et al. 1988, Tita et al. 2000). The omnivorous, opportunistic polychaete *Nereis diversicolor* was shown to have a significant negative impact on meiofaunal abundance in soft-bottom intertidal sediments (Reise 1979). In addition to the direct predation effect, the sediment disturbance caused by *Nereis* was possibly related to an intensive 'ploughing' of surface sediment during its food-searching activity. Based on the results from a microcosm experiment, Tita et al. (2000) suggested that this sediment disturbance by *Nereis* was an even more influential process on nematode assemblages than predation. Obviously, the highy abundant non-siboglinid polychaetes inhabiting the Siboglinidae patch at Storegga, will cause an intensive reworking of the sediment and therefore may have a strong negative effect on the infaunal nematode assemblage.

On the other hand, the lowest macrofaunal densities counted in the HMMV *Beggiatoa* mats were associated with the maximum meiofaunal densities found in this study. Probably, the harsh, almost lethal, geochemical sediment conditions have a severe impact on the colonisation success of macrofaunal species, subsequently resulting in a less abundant macrobenthos and a lower predation pressure and disturbance effect on the thriving *Halomonhystera disjuncta* population.

3. The ecology of *Halomonhystera disjuncta* from HMMV and its phylogenetic relationship with the *H. disjuncta* species complex from shallow-water habitats in the North Sea

Species of the nematode family Monhysteridae, which is known as a common deep-sea family, seem to be opportunists that inhabit extreme environments such as vents and seeps (Van Gaever et al. 2006, Zekely et al. 2006, Copley et al. 2007, Gollner et al. 2007). At the Håkon Mosby Mud Volcano, a remarkably dense population of the monhysterid *Halomonhystera disjuncta* was found associated with the bacterial mats habitat, with densities significantly exceeding those found at the control sites (Van Gaever et al. 2006). This species was absent outside the bacterial mats, except for very low numbers in the central part of the mud volcano, indicating a strong preference for the *Beggiatoa* dominated mats. *Halomonhystera disjuncta* was also completely absent from the different habitats sampled in seepage sites at Storegga and Nyegga, including the reduced sediments.

Apart from few meiofaunal polychaetes, no other metazoan meio- or macrofaunal species were present in these anoxic, sulphidic sediments at the *Beggiatoa* mats. Ecotoxicological experiments with *H.*

disjuncta from intertidal habitats provided evidence of a high resistance to environmental stress, especially to heavy metals (Vranken et al. 1989). The individuals obtained from the HMMV showed no striking morphological modifications compared to the shallow-water species. Even the ovoviviparous reproduction mode was observed in the shallow-water populations although facultatively and especially expressed after nickel contamination (A. Boffé unpublished data). The internal development of juveniles is most likely an adaptation securing the survival and growth of the vulnerable brood within the permanently sulphidic and anoxic sediments (Van Gaever et al. 2006). In addition, *H. disjuncta* developed a trophic specialisation within the HMMV bacterial mats. Recently performed analyses of the fatty acids and related stable isotopic signatures of individuals of *H. disjuncta* (see CHAPTER 5), indicated that this species proliferates on chemosynthetically derived carbon, in particular on the highly abundant sulphide-oxidising bacteria.

Halomonhystera disjuncta has proven to be a strong coloniser in shallow waters, successfully exploiting different substrata, such as marine sediments as well as decomposing algal thalli. Moreover, observations were made of nematodes rafting on algae (Derycke et al. 2007), pointing to substantial dispersal capacities of nematodes despite the lack of pelagic larvae within the phylum Nematoda. Figure 7 illustrates the observation of a seaweed clump within a field of siboglinid tubeworms at HMMV. It is likely that *H. disjuncta* also occurs along the shallow Norwegian coasts, as the species was reported from both the North Sea south of Norway (Vranken et al. 1988) and the White Sea north-east of Norway (Mokievsky et al. 2005). Consequently, the more offshore located HMMV may have been colonised by *H. disjuncta* through floating seaweeds originating from nearby intertidal habitats. Van Dover et al. (2002) hypothesised previously that some seep invertebrate species may be derived from shallow-water species. However, our observation does not exclude an alternative route for exchange from the deep-sea to shallow-water, and further investigation of the nematode communities at the nearby intertidal habitats is required.

The fact that this cryptic *H. disjuncta* species is able to flourish in such high densities at the HMMV bacterial mats could have two possible explanations, which are not mutually exclusive. Derycke et al. (2007) suggested that *H. disjuncta* may exhibit priority effects, i.e. the first colonising individuals have such a strong population development that they reduce the settlement of new species. This could be a first explanation for the high abundance of *H. disjuncta*, almost without any co-occurring species. The second explanation may be that the geochemical conditions within these sediment, i.e. high sulphide levels and oxygen depletion beneath 2 mm sediment depth (de Beer et al. 2006, Van Gaever et al. 2006), create a lethal environment for almost any metazoan life. The nematode *H. disjuncta*, previously

documented to prevail in sulphidic, shallow marine sediments (Heip et al. 1985), can be considered as pre-adapted to these toxic conditions and therefore resistant enough to colonise and exploit this extreme deep-sea habitat at the HMMV bacterial mats.

Figure 7: Photo of seaweed clump (*Fucus* sp.) surrounded by Siboglinidae patches (white arrows) on the seafloor at HMMV (ROV *Victor 6000*, IFREMER, 2006).

The molecular analyses on *Halomonhystera* specimens from the bacterial mats at Håkon Mosby Mud Volcano (HMMV) provided evidence to identify it as *Halomonhystera disjuncta*. When the 18S sequence was compared to 18S sequences from a broad range of marine nematode taxa, it was most similar to that of *Halomonhystera disjuncta.* A comprehensive genetic study carried out on *H. disjuncta* specimens collected at estuaries and coastal zones of Belgium and the southwestern part of The Netherlands revealed high levels of molecular diversity within this species: five co-occurring distinct lineages in the mitochondrial COI gene were found on a small spatial scale, showing low divergence levels within lineages (< 3%) and high divergence levels between lineages (> 13%) (Derycke et al. 2007). The specimens from HMMV most likely represent another cryptic species of the *H. disjuncta* species complex, as the patterns in the two sets of independently evolving gene fragments (nuclear versus mitochondrial) were concordant and the divergence levels were comparable with those found between known nematode species (Derycke et al. 2007). Several recent studies have shown that some

meiobenthic 'species' are indeed complexes of cryptic species and that putatively cosmopolitan species have a much more restricted geographical distribution (Schmidt and Westheide 2000, Rocha-Olivares et al. 2001, Bhadury et al. 2008, Derycke et al. 2008b).

CONCLUSION

In the rather homogeneous and oligotrophic environment of the deep-sea floor, seep environments can be considered as nutrient-rich islands with patchily distributed sub-habitats, each harbouring its specific associated epi- and infauna. Seep sediments which are strongly affected by reduced fluids and recognised by typical geochemical features (such as oxygen depletion, toxic sulphide levels, exclusion of most metazoan life, often high bacterial biomass), represent a unique niche for some very stress-resistant nematode species. Molecular analyses of *Halomonhystera disjuncta* specimens from the reduced HMMV sediments provided evidence that these nematodes may belong to (intertidal and deep-sea) species complexes of cryptic species. The reduced sediments at Nyegga are dominated by another cosmopolitan shallow-water nematode species *Terschellingia longicaudata*, known from marsh and mangrove anoxic mudflats. These observations point to an exchange of nematode species between the shallow-water and deep-sea seep habitats. The siboglinid tubeworm habitats occurring at the different seeps along the Norwegian margin generally host a similar high number of meiobenthic nematode genera. However, total densities of macrofauna were negatively correlated with the infaunal meiobenthic densities, in particular with the nematode densities. Especially at sites colonised by extremely dense populations of non-siboglinid polychaetes, nematode communities were significantly impoverished with respect to nematode genus diversity and nematode abundance. In conclusion, the seepage affected regions with multiple habitat types scattered along the Norwegian margin support several significantly different meiobenthic communities, and therefore contribute significantly to the diversification and persistence of the resident deep-sea meiofauna.

ACKNOWLEDGEMENTS

CHAPTER 7

Spatial scale and habitat-dependent diversity patterns in nematode communities in three seepage related sites along the Norwegian margin

Spatial scale and habitat-dependent diversity patterns in nematode communities in three seepage related sites along the Norwegian margin

ABSTRACT

Assessing the relative contribution of local diversity components to regional biodiversity may be the key to understand large-scale and even global patterns in species diversity. Here, the contribution of three spatial levels [micro-scale (m's), macro-scale (tens to hundreds of m's), and mega-scale (tens to hundreds of km's)] to the total nematode biodiversity (genus level) at three seepage sites along the Norwegian continental margin is evaluated. By calculating the additive partitioning of the overall (gamma) diversity in alpha and beta components, we also explored the influence of distinct habitat types, each with its unique geochemical conditions, on nematode genus composition and diversity.

The spatial pattern in beta diversity changed with the scale of observation. Apparently, nematode communities associated with different habitats at the Håkon Mosby Mud Volcano (HMMV) were much better defined through sampling at the macro-scale than at the micro-scale. Siboglinidae fields and background sediments outside any seep influence yielded comparably high levels of nematode genus richness, and exhibited low turnover rates within and across the different seep sites. In contrast, the bacterial mats at HMMV and the anoxic sediments at the Nyegga pockmarks harboured genus-poor nematode communities with an equally high dominance of one or two species, which were different for each seep. Different habitats, in particular at the HMMV, contributed significantly to the seep nematode richness. However, also the multiple seep sites along the Norwegian continental margin accounted for a high proportion of the regional nematode richness. This study demonstrates that the persistence of distinct habitat types within each seep and within multiple seepage sites is necessary to preserve the high diversity of nematode communities inhabiting the seeps in the Norwegian deep sea.

INTRODUCTION

Historically, the relationship between spatial scale, patterns of species diversity and ecological processes has constituted a major topic in ecological research (Whittaker 1960, Whittaker 1972, Legendre et al. 1997, Crawley and Harral 2001, Perry et al. 2002, Takashi 2004). Species diversity is often supposed to vary with spatial scale, in the sense that an increase in scale could offer more resources to species and consequently promote an increase in diversity (Crawley and Harral 2001). A large body of literature exists on the relationship between diversity patterns and multiple spatial scales in terrestrial systems (Chown and Gaston 2000, Godfray and Lawton 2001, Hill and Hamer 2004). Similar ecological studies in marine systems are more difficult to execute because these are open ecosystems, which harbour many highly mobile species not constrained by the habitat boundaries ubiquitous in terrestrial systems. Nevertheless, some marine environments, for instance the rocky intertidal, coral reefs, hydrothermal vents and cold seeps, create a naturally occurring hierarchical patch structure for the associated benthos. They are therefore amenable to explore questions of diversity patterns related to scale in marine systems. However, the effect of spatial scale on nematode communities in the deep sea has, to date, been only poorly studied.

In the present study, diversity patterns of the smaller-sized benthos (meiobenthos) inhabiting several cold-seep sites along the Norwegian continental margin were examined in relation to different spatial scales. The spatial scales of observation included here ranges between meters (micro-scale), tens to hundreds of meters (macro-scale) and tens to hundreds of kilometres (mega-scale). These scales were examined for the three deep-sea seep sites studied (Håkon Mosby Mud Volcano, Storegga Slide, Nyegga pockmarks). Hereby we define a seep as the complex of all habitats affected at least to some degree by seepage activity, while a (geographical) area is considered here to be the total of the seep area including the adjacent background sediments outside the immediate seep influence.

Another important aspect in marine ecology relates to the understanding of patterns in diversity and distribution of benthos by reference to the different habitats available to them in the environment. There is abundant evidence that the habitat structure has important effects on the spatial distributions of benthic populations in seep environments, both for the macrofauna (Bergquist et al. 2003, Levin et al. 2003, Levin 2005, Olu-Le Roy et al. 2007) and the meiofauna (Van Gaever et al. 2006, Van Gaever et al. accepted, Van Gaever et al. submitted). Macrofaunal species diversity at California methane seeps exhibited a habitat-related heterogeneity, consistent with sulphide inhibition of species richness and

evenness (Levin et al. 2003). Meiofaunal communities, in particular the nematode assemblages, also experienced a significant structuring effect by different habitats and their characteristic geochemical conditions in the pockmark seep in the Gulf of Guinea (Van Gaever et al. accepted).

The meiofaunal communities at the Håkon Mosby Mud Volcano (HMMV, Barents Sea, ~1280 m) were previously described at six distinct habitats: (1) the bare, muddy central zone devoid of macrofauna or visible bacterial mats; (2) the extensive mats of the filamentous bacterium *Beggiatoa*; (3) the smaller and more fragmented grey bacterial mats; (4) the fields of Siboglinidae polychaetes (chemosynthetic tubeworms); (5) the outer rim, a depression surrounding all former habitats; (6) the background sediments beyond the influence of the seepage (de Beer et al. 2006, Van Gaever et al. 2006, see Chapter 6). The well-oxygenated surface sediments at the Siboglinidae fields (de Beer et al., 2006), the outer rim and the background sediments yielded the most diverse nematode communities, while the mud-flowing centre harboured only few individuals. Unexpectedly high nematode densities (>11000 ind. 10 cm^{-2}) were observed in the sulphidic, anoxic sediments underneath the *Beggiatoa* mats, with more than 99% of the nematodes belonging to the species *Halomonhystera disjuncta* Bastian, 1865 (Van Gaever et al. 2006). This species dominated also the smaller, more isolated grey bacterial mats at HMMV, although densities were tenfold lower (~1000 ind. 10 cm^{-2}). Another reduced, suboxic habitat was identified at the Nyegga seep off mid-Norway (~740 m): blackish sediments without a sediment surface-covering bacterial mat. The nematode community identified in this black sediment was low in diversity, and strongly dominated by *Terschellingia longicaudata* de Man, 1907 (see Chapter 6). Additionally, a Siboglinidae patch and background sediments at both the Nyegga seep and the nearby Storegga seep (~730 m) were investigated for meiobenthos. Nematode diversity here was comparable to the corresponding Siboglinidae and background habitats at HMMV (see Chapter 6).

The objective of this study is to examine to what extent different spatial scales and different habitats within a seep contribute to the genus diversity of the associated nematode communities at three seeps (HMMV, Storegga Slide, Nyegga pockmarks) along the Norwegian continental margin. The two habitat types which were compared across the three spatial scales were the reduced sediments (covered by bacterial mats at HMMV) and Siboglinidae patches. Both *Beggiatoa* bacteria as well as the siboglinid tubeworms inhabit reducing sediments with variable levels of sulphide, but a difference is found in the sulphide depth profiles of both habitats (de Beer et al. 2006). The sulphide-oxidising *Beggiatoa* bacteria require a significant sulphide flux up to the surficial sediment layers. In contrast, Siboglinidae fields can establish in sediments where sulphide is only observed in the deeper sediment layers, because they use their subsurface part of the tube (root) to take up sulphide from the subsurficial depths. On the other

hand, oxygen is pumped through the anterior tube end reaching into the water column, and subsequently a steady source of sulfide and oxygen is transported via the highly adapted blood circulation system of the worm towards the symbiotic chemoautotrophic bacteria (Schulze and Halanych 2003). Several authors have proposed that the worm roots are not only important in sulphide uptake, but generally in geochemical engineering of the sediments in the direct environment (Julian et al. 1999, Bergquist et al. 2002), which could have a significant impact on the flourishing nematode asssemblages.

Biodiversity over the different spatial scales was measured at the three levels introduced by Whittaker (1972): alpha, beta, and gamma diversity. Thereby, alpha diversity refers to the diversity within a particular area or habitat, and is usually expressed by the number of species (i.e., species richness) in that ecosystem and the number of individuals per species (evenness). The degree of change in species diversity within and between habitats is reflected in beta diversity. For the beta diversity analyses, we will be refer to the corresponding diversity level as listed in Table 1. Accurate measurement of beta diversity is important in at least three ways: (1) it indicates the degree to which habitats have been partitioned by taxa; (2) values of beta diversity can be used to compare the habitat diversity of different ecosystems; (3) beta diversity and alpha diversity together measure the overall diversity or biotic heterogeneity of an area, the gamma diversity (Wilson and Shmida 1984). Here, we additively partitioned the gamma diversity for each seep and for the three seepage sites together in an alpha and several beta components in order to investigate the relationship between local (between habitats) and regional (between areas) nematode biodiversity. Additive partitioning of genus diversity in alpha and beta components has the advantage of allowing comparison among different seep sites.

Table 1: Schematic representation of the application of different beta diversity levels for three cold-seep environments along the Norwegian continental margin, with indication of the scale of observation, distance among samples, code for the diversity level, site, habitat type and sample type.

Scale	Distance	Code		Seep site	Habitat type	Samples
Micro	m's	1A	within habitat in single dive transect	HMMV	*Beggiatoa*, Siboglinidae	ROV
		1B	between habitats in single dive transect	HMMV	*Beggiatoa*, Siboglinidae	ROV
Macro	tens to hundreds of m's	2A	within habitat in single seep	HMMV	*Beggiatoa*, Siboglinidae	ROV + MUC
		2B	between habitats in single seep	HMMV	*Beggiatoa*, Siboglinidae	ROV + MUC
		2C	between habitats in single geographical area	HMMV	centre, *Beggiatoa*, grey mats, Siboglinidae, outer rim, background	MUC
Mega	tens to hundreds of km's	3A	within habitat between geographical areas	HMMV, Nyegga, Storegga	reduced sediments, Siboglinidae, background sediments	ROV + MUC
		3B	between seeps	HMMV, Nyegga, Storegga	*Beggiatoa*, Siboglinidae	ROV + MUC
		3C	between total geographical areas	HMMV, Nyegga, Storegga	all habitats	ROV + MUC

MATERIALS AND METHODS

1. STUDY AREA

The Håkon Mosby Mud Volcano (HMMV, ~1280 m water depth) is located at the Norwegian-Barents-Spitsbergen continental margin at 72°N, an area characterised by major submarine slides and smaller seafloor features (Vogt et al. 1997b) (Figure 1). This mud volcano is about 1.5 km in diameter and rises up to 10 m above the seafloor. It presents a concentric distribution of different habitats: the centre, microbial mats, and Siboglinidae fields (Figs. 2, 3). These three habitats are surrounded by a fourth habitat, referred to as the outer rim (Pimenov et al. 2000, Gebruk et al. 2003). Several locations

of bubble and fluid discharge have been observed with the ROV "Victor 6000" in the northern part of the mud volcano ('active site').
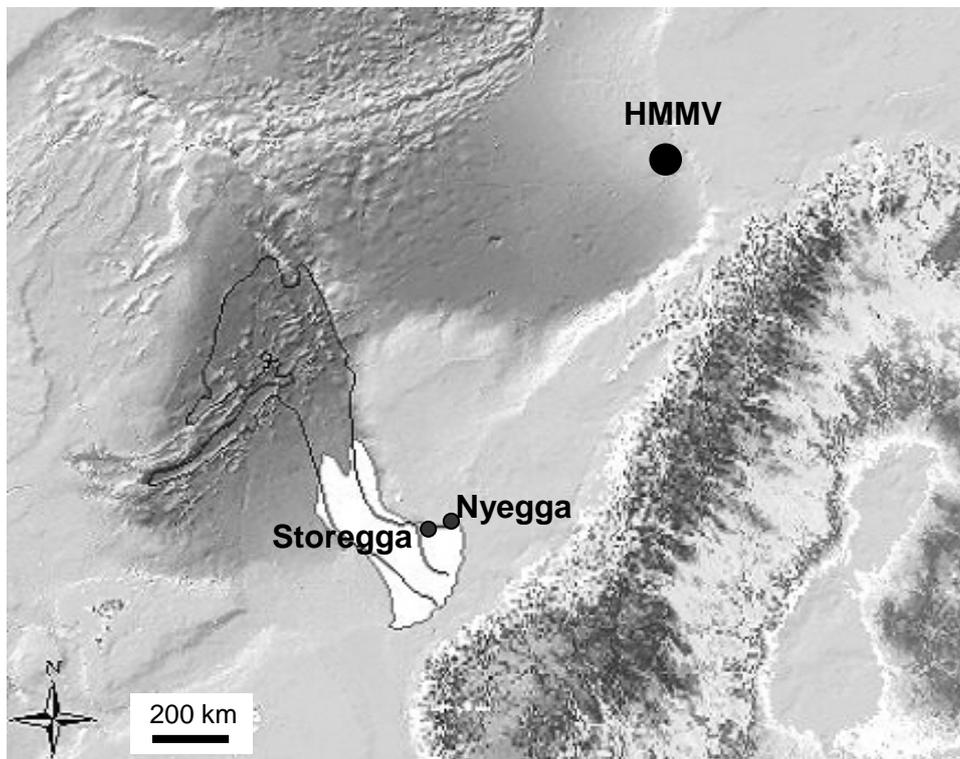
Figure 1: Map of the Norwegian margin with indication of the three sampling locations. The Håkon Mosby Mud Volcano (HMMV) is situated at about 950 km north of the southern seeps at the Storegga slide and Nyegga area. (HERMES Project, GIS Workpackage deliverables, Nordic Margins GIS webserver [*]).

The morphology of the continental margin off north-west Norway at 64°N is marked by the Storegga Slide scar, produced by a series of giant Holocene slope failures. The most recent failure occurred about 8200 years ago and was the last megaslide in this region (Bryn et al. 2005, Paull et al. 2008) (Figure 1). Seafloor pockmarks and subsurface chimney structures are common on the Norwegian continental margin north of the Storegga Slide scar (Paull et al. 2008). The Nyegga area, located on the northeastern flank of this Storegga Slide, is characterised by a cluster of complex pockmarks at 740 m water depth. These pockmarks are circular in plane view and possess up to 190 m long ridges of wide carbonate rocks. Other key features of the complex pockmarks include a distinct fauna with local bacterial mats, small tubeworms (siboglinids), stalked crinoids, and pycnogonids. Light hydrocarbon gases are present in the clay-rich sediments, although there is no visual seepage at the Nyegga complex pockmarks. It is assumed that the Nyegga pockmarks were formed by a single sudden ('catastrophic') fluid flow and that micro-seepage is still active (Hovland et al. 2005).

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[*] http://gis-web.jacobs-university.de
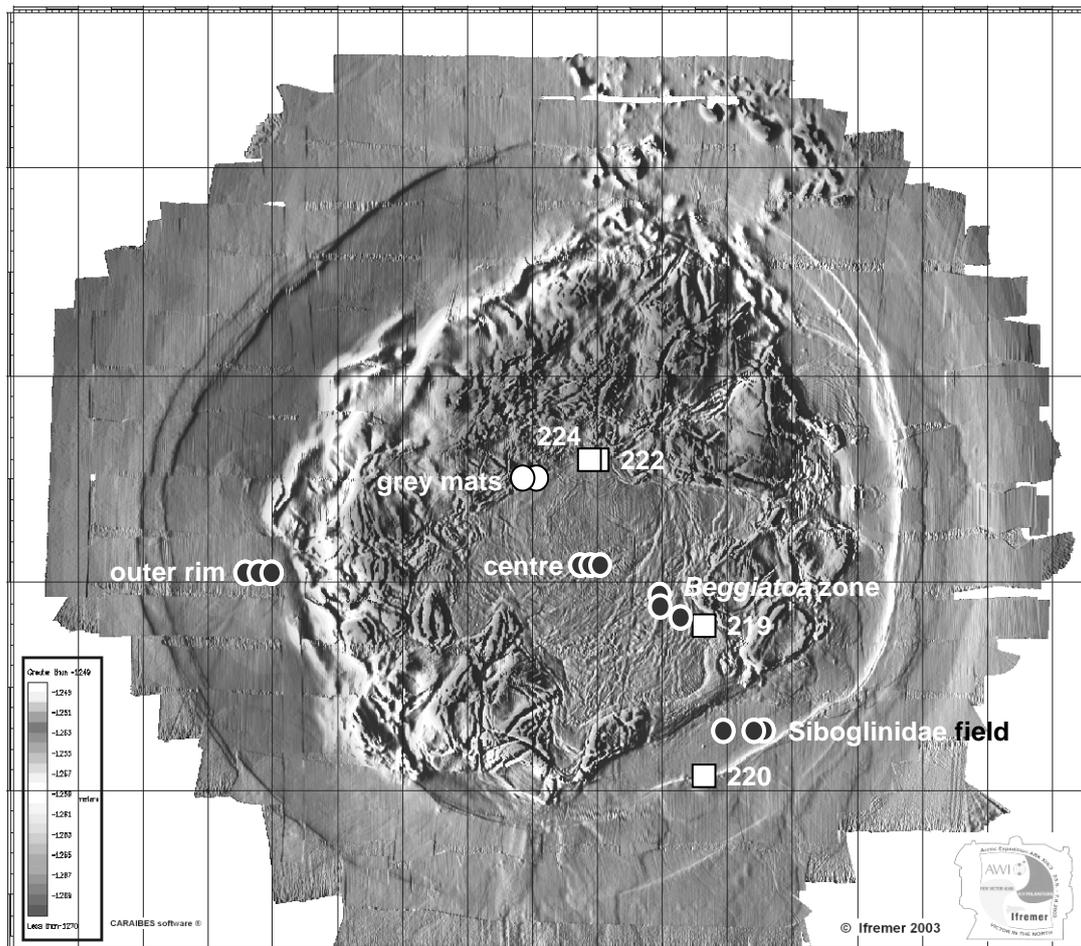
Figure 2: Bathymetric map of the Håkon Mosby Mud Volcano with indication of the 4 dive transects (white squares: 219, 220, 222, 224), the multicore replicates (black circles) and push core samples (white circles) collected from the different habitats (adapted from Edy et al. 2004).
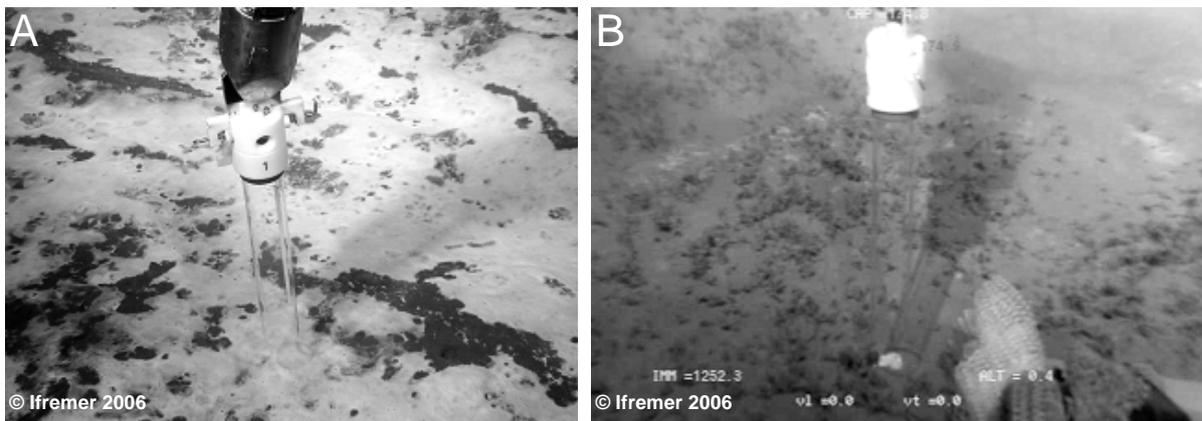
Figure 3: Pictures of *Beggiatoa* cores taken at the extensive *Beggiatoa* zone (a) and at small-sized patches within the Siboglinidae field (b).

2. SAMPLING AT MICRO-SCALE (M'S)

Sediment samples were collected at micro-scale at the HMMV during the ARKTIS XIX/3b cruise aboard the RV 'Polarstern' in June–July 2003. The ROV *Victor 6000* collected 2 to 4 cores (12 in total) of 21.2 cm^2 along 4 transects during 4 different dives (Figure 2, Table 2); 6 cores from 4 different dives were taken at *Beggiatoa* patches (Figure 3) and 6 cores from 3 different dives at Siboglinidae patches. In the zone with only bacterial mats, the sampling was within a small area (maximum 4 m^2), where each time large patches completely covered by *Beggiatoa* were selected (dive 219) (Figure 3a). At the Siboglinidae area (dive 220) and the northern active site with patchy appearance (dives 222, 224), a gradient was sampled within maximum 5 m's distance from inside a dense Siboglinidae patch to a less covered area with mixed patches of *Beggiatoa* and Siboglinidae (Figures 2, 3b). For further analyses micro-scale patterns were only considered within a single dive.

3. SAMPLING AT MACRO-SCALE (HUNDREDS OF M'S) AND MEGA-SCALE (TENS TO HUNDREDS OF KM'S)

During the cruise ARKTIS XIX/3b, 4 HMMV habitats were sampled at a macro-scale using a video-guided multiple corer (90 mm core diameter) (Table 2). At each habitat (centre, microbial mats, Siboglinidae field, outer rim), 3 replicate tubes were taken from 2 or 3 different deployments. As a control, two sites located outside HMMV (tens of km's) were sampled. For both the microbial mats and Siboglinidae field, replicates were selected based on the presence of bacteria (*Beggiatoa*) or Siboglinidae, whereas the outer rim replicates did not contain either. Detailed data on the composition of the meiofauna and nematode communities at the different habitats are published in Van Gaever et al. (2006).

During the VICKING cruise aboard the RV 'PourquoiPas?' in May–June 2006, additional sediment samples were collected at the Storegga Slide, the Nyegga area and the grey bacterial mats at HMMV (Table 2). Samples were taken at a Siboglinidae field and at background sediment both at Storegga and Nyegga. Meiofauna samples were also collected at a patch with blackish, anoxic sediments at Nyegga. In each habitat at each location, 2 or more replicate cores were taken. Samples at background sites were sampled with a multiple corer (1 deployment per background site, tube surface of 30.2 cm²), while the other sites were sampled with push cores of the ROV *Victor 6000* (surface of 21.2 cm²). Detailed data on the composition of the meiofauna and nematode communities are reported in Chapter 6.

Table 2: Coordinates, sampling device, water depth (m) and habitat type for all samples.

ROV = push core; MUC = multicorer.

Seep site	Campaign	Core sample	Latitude (N)	Longitude (E)	Sampling device	Depth (m)	Habitat type
HMMV	ARKTIS XIX/3b	Dive 219 / A	72° 0.16'	14° 43.88'	ROV	–	*Beggiatoa*
		Dive 219 / B	"	"	"	–	*Beggiatoa*
		Dive 219 / C	"	"	"	–	*Beggiatoa*
	ARKTIS XIX/3b	Dive 220 / 3	72° 0.00'	14° 43. 88'	ROV	–	Siboglinidae
		Dive 220 / 4	"	"	"	–	*Beggiatoa*
		Dive 220 / 5	"	"	"	–	Siboglinidae
		Dive 220 / 6	"	"	"	–	Siboglinidae
	ARKTIS XIX/3b	Dive 222 / 10	72° 0.36'	14° 43.56'	ROV	–	*Beggiatoa*
		Dive 222 / 12	"	"	"	–	Siboglinidae
	ARKTIS XIX/3b	Dive 224 / 22	72° 0.36'	14° 43.55'	ROV	–	*Beggiatoa*
		Dive 224 / 23	"	"	"	–	Siboglinidae
		Dive 224 / 28	"	"	"	–	Siboglinidae
	ARKTIS XIX/3b	PS 64 / 312	72° 0.25'	14° 43.49'	MUC	1286	centre
		PS 64 / 312	"	"	"	1286	centre
		PS 64 / 313	"	"	"	1286	centre
	ARKTIS XIX/3b	PS 64 / 321	72° 0.18'	14° 43.81'	MUC	1287	*Beggiatoa*
		PS 64 / 323	"	"	"	1287	*Beggiatoa*
		PS 64 / 324	"	"	"	1287	*Beggiatoa*
	VICKING	Dive 277-07 / 2	72° 0.32'	14° 43.38'	ROV	1255	grey bacterial mats
		Dive 277-07 / 5	"	"	"	1255	grey bacterial mats
	ARKTIS XIX/3b	PS 64 / 356	72° 0.05'	14° 44.14'	MUC	1288	Siboglinidae
		PS 64 / 357	"	"	"	1288	Siboglinidae
		PS 64 / 367	"	"	"	1288	Siboglinidae
	ARKTIS XIX/3b	PS 64/362	72° 0.21'	14° 42.47'	MUC	1290	outer rim
		PS 64 / 363a	"	"	"	1290	outer rim
		PS 64 / 363b	"	"	"	1290	outer rim
	ARKTIS XIX/3b	PS 64 / 390a	71° 41.96'	13° 26.10'	MUC	1885	background site
		PS 64 / 390b	"	"	"	1885	background site
		PS 64/395	71° 58.97'	14° 45.41'	"	1296	background site
Nyegga	VICKING	MT3 A	64° 40.17'	05° 17.39'	MUC	719	background site
		MT3 B	"	"	"	719	background site
	VICKING	Dive 272-02 / 14	64° 40.00'	05° 17.34'	ROV	733	Siboglinidae
		Dive 272-02 / 18	"	"	"	733	Siboglinidae
	VICKING	Dive 272-02 / 1	64° 39.79'	05° 17.29'	ROV	733	reduced sediments
		Dive 272-02 / 8	"	"	"	733	reduced sediments

Seep site	Campaign	Core sample	Latitude (N)	Longitude (E)	Sampling device	Depth (m)	Habitat type
Storegga	VICKING	MT2 A	64° 45.28'	05° 6.21'	MUC	720	background site
		MT2 B	"	"	"	720	background site
	VICKING	Dive 275-05 / 5	64° 45.27'	04° 58.87'	ROV	746	Siboglinidae
		Dive 275-05 / 6	"	"	"	746	Siboglinidae

4. SAMPLE TREATMENT

All samples were fixed with 4% formaldehyde at sea prior to further processing. After sieving over a 32 μm-mesh sieve, meiofauna extraction was done by density gradient centrifugation, using Ludox (a colloidal silica polymer; specific gravity 1.18) as a flotation medium (Heip et al. 1985, Vincx 1996). The meiofauna was fixed with 4% buffered formaldehyde again and stained with Rose Bengal. The meiofauna was sorted, counted and identified at higher taxon level under the stereomicroscope. Nematodes (100) were picked out randomly, mounted onto glycerine slides using the formalin-ethanol-glycerol technique of Seinhorst (1959) and Vincx (1996) to prevent dehydration, and identified down to genus level using Lorenzen (1994) and Warwick et al. (1998).

5. STATISTICAL ANALYSES

Differences in nematode assemblage structure between the different cores of the dive transects at HMMV were assessed by non-metric multidimensional scaling (nMDS) ordination based on Bray-Curtis similarity measures, with log-transformed relative abundance data. The stress value gives a measure for goodness-of-fit of the MDS ordination: a stress value below 0.2 gives a potentially useful 2-dimensional picture (Clarke and Warwick 2001). The significance level was calculated with the ANOSIM permutation test (Clarke and Green 1988).

The STATISTICA6 software was used for parametric (t-test) and non-parametric (Mann-Whitney U test or Kruskal-Wallis test) univariate analyses of variance to compare total nematode densities and genera turnover rates among different habitats. Bartlett's and Cochran's test were used to verify the homogeneity of variances prior to further analysis.

Alpha diversity was calculated at different spatial scales by means of different indices. Genus richness (N_0) was used to describe the alpha diversity within the different habitats. Rarefaction curves were

constructed from values of the Expected number of Genera for a theoretical sample of 600 individuals EG(600) (Hurlbert 1971). EG(100), EG(600), the Shannon-Wiener index H' (log based 2), and Pielou's eveness J' were used to compare alpha diversity levels at different spatial scales. These indices of alpha diversity were calculated using the PRIMER5 software (Plymouth Marine Laboratory, Clarke and Gorley 2001).

According to Wilson and Shmida (1984), Whittaker's (1960) original measure of beta diversity (β_W) is perhaps the most widely used measure of beta diversity, and is always the measure of choice when samples cannot be arranged along a single gradient (Wilson and Shmida 1984). In this study, beta diversity was measured as $\beta_W = (\gamma/\alpha) - 1$ (Whittaker 1972) where γ is the total number of genera at the coarse scale, and α is the average number of genera at the fine scale. As a second measure of beta diversity, Bray-Curtis similarity (β_S) was calculated for all different diversity levels summarised in Table 1, using the PRIMER5 software. Finally, beta diversity was also reflected by the turnover of genera (β_T) for each diversity level (Table 1), based on the formula of Koleff et al. (2003):

$$\beta_T = \frac{\min(b,c)}{\min(b,c) + a}$$

where the minimum (min) number of genera restricted to a pair of samples is divided by itself plus the number of shared genera (a) between samples b and c. The data were transformed into percentages, i.e. the sum of the components a', b' and c' was 100%. The matching components (a', b' and c') were plotted in a ternary graph together with the results of the turnover of genera (ranging from 0 to 1) within a habitat (1A) and between habitats (1B) (micro-scale at HMMV). Codes 1A to 3C are explained in Table 1. Differences between both groups of turnover values were subsequently also investigated by a t-test.

Lande's (1996) generally accepted formula for the alpha-beta-gamma relationship is $\gamma = \alpha + \beta$, where α is the local richness of sites within the region and β a measure of the variation in species among sites. Additive partitioning methods (Gering and Crist 2002) can be applied to assess the relative contribution of alpha and beta diversity to gamma diversity. Using the additive partitioning approach, total nematode diversity (γ diversity) at HMMV was calculated as the sum of alpha diversity (α) within each core sample, beta diversity (β_1) within a habitat (2A) and beta diversity (β_2) between different habitats (2C):

$$\gamma \text{ (total diversity)} = \alpha \text{ (within cores)} + \beta_1 \text{ (between cores)} + \beta_2 \text{ (between habitats)}$$

In a next step, gamma diversity of the three areas pooled together was additively partitioned as follows (3C):

$$\gamma_{\text{ (total diversity)}} = \alpha_{\text{ (within cores)}} + \beta_{1\text{ (between cores)}} + \beta_{2\text{ (between habitats)}} + \beta_{3\text{ (between areas)}}$$

The PARTITION software (Veech and Crist 2007) was used to perform all calculations of additive partitioning.

RESULTS

1. MICRO-SCALE DIVERSITY PATTERNS AT HMMV

The number of nematode genera per sample collected during ROV dives ranged from 1 to 27. On average, a more genus-rich community was observed in the cores from Siboglinidae patches (19 ± 6 genera) compared to those from *Beggiatoa* patches (6 ± 5 genera). Six of the 28 genera found in the set of 6 *Beggiatoa* cores were not observed in the Siboglinidae cores, while 21 genera were only present in the Siboglinidae cores (total of 45 genera). The alpha diversity calculated for the pooled data from the Siboglinidae patches [EG(100) = 25, H' = 4.22, J' = 0.77] was much higher than that of the *Beggiatoa* patches [EG(100) = 5, H' = 0.60, J' = 0.12] (Table 3).

The MDS graph depicted in Figure 4 visualises the (dis)similarities between the samples based on the nematode genera composition. Cores collected at the large zone with dense *Beggiatoa* mats (dive transect 219) were clearly separated from all other samples (ANOSIM: R = 0.998, p = 0.005). The 6 *Beggiatoa* cores yielded two completely different but both species poor nematode communities: the first community was dominated by *Halomonhystera disjuncta* (96–100% and 1–4 genera), a species that was present only in cores from dive transect 219 (Figure 3a). The second community (in the other *Beggiatoa* cores from the mixed habitats, Figure 3b) was dominated by *Microlaimus* (53–70% dominance and 6–18 genera in total), and also revealed high relative abundances of the genera *Metalinhomoeus*, *Aponema* and *Dichromadora*, but no *H. disjuncta* was present. The Siboglinidae cores harboured high relative abundances of *Sabatieria*, *Molgolaimus*, *Metalinhomoeus* and *Dichromadora*.

Total nematode densities per sample appeared to be highly variable at this micro-scale, ranging between 615 ind. 10 cm^{-2} in a Siboglinidae patch and 16203 ind. 10 cm^{-2} in a *Beggiatoa* patch.

Siboglinidae patches (1597 ± 1161 ind. 10 cm^{-2}) yielded lower average total nematode densities compared to *Beggiatoa* patches (4436 ± 6017 ind. 10 cm^{-2}), although the differences were not significant (Mann-Whitney U: p = 0.52). Within the *Beggiatoa* habitat, the cores inhabited by *Halomonhystera disjuncta* exhibited total densities (7849 ± 7436 ind. 10 cm^{-2}) seven times higher than those from the cores with the *Microlaimus*-dominated community (1024 ± 543 ind. 10 cm^{-2}).

The turnover rates of genera (β_T) between samples from the same habitat (1A) within a dive transect, and between samples from different habitats (1B) within a dive transect are visualised in a ternary plot (Figure 5, Table 3). Even though a higher mean beta turnover between samples from different habitats was observed, the 'between habitats' (β_T = 0.4 ± 0.2, β_S = 19.3 ± 6.8) turnover values did not differ significantly from the 'within habitat' values (average for both habitats: β_T = 0.2 ± 0.2, β_S = 65.0 ± 31.9) (t_{14} = 3.99, p = 0.07). The substitution of genera observed within the *Beggiatoa* habitat was minimal and always equalled 0 (β_S = 97.3 ± 2.4). The turnover within the Siboglinidae habitat was higher and ranged between 0.3–0.5 depending on the dive, resulting in a significant difference in genera turnover rate between 'within Siboglinidae' and 'within *Beggiatoa*' (t_5 = 40.55, p = 0.001).

Figure 4: Multidimensional scaling plot based on the nematode genus composition of the push cores from the micro-scale sampling at HMMV (with indication of habitat type and dive number).

Table 3: Diversity measures at the different spatial scales. Alpha diversity is expressed in genus richness (N_0) per habitat, expected number of genera for 100 and 600 individuals, Shannon-Wiener index (H'), and Pielou's eveness (J'). Beta diversity is expressed in Bray-Curtis similarity (β_S) between samples and beta turnover rate of genera (β_T) between samples.

Scale	Code		N_0	EG(100)	EG(600)	H'	J'	β_S mean (SD)	β_T mean (SD)
Micro	1A	within *Beggiatoa*	28	5	11	0.60	0.12	97.3 (2.4)	0 (0)
		within Siboglinidae	45	25	38	4.22	0.77	40.7 (14.4)	0.4 (0.1)
	1B	between *Beggiatoa*-Siboglinidae						19.3 (6.8)	0.4 (0.2)
Macro	2A	within *Beggiatoa*	41	4	11	0.43	0.08	36.0 (45.5)	0.5 (0.4)
		within Siboglinidae	86	31	52	4.80	0.75	28.0 (11.7)	0.3 (0.1)
	2B	between *Beggiatoa*-Siboglinidae						4.6 (7.1)	0.5 (0.3)
	2C	among all HMMV habitats						33.0 (40.7)	0.3 (0.1)
Mega	3A	within reduced sediments	40	5	11	0.22	0.04	2.0 (3.3)	0.6 (0.1)
		within Siboglinidae	90	32	57	4.85	0.75	25.9 (10.2)	0.3 (0.1)
		within background sediments	109	33	65	4.94	0.73	46.3 (6.1)	0.4 (0.0)
	3B	between seeps						20.1 (6.9)	0.2 (0.1)
	3C	between geographical areas						32.7 (18.8)	0.2 (0.0)

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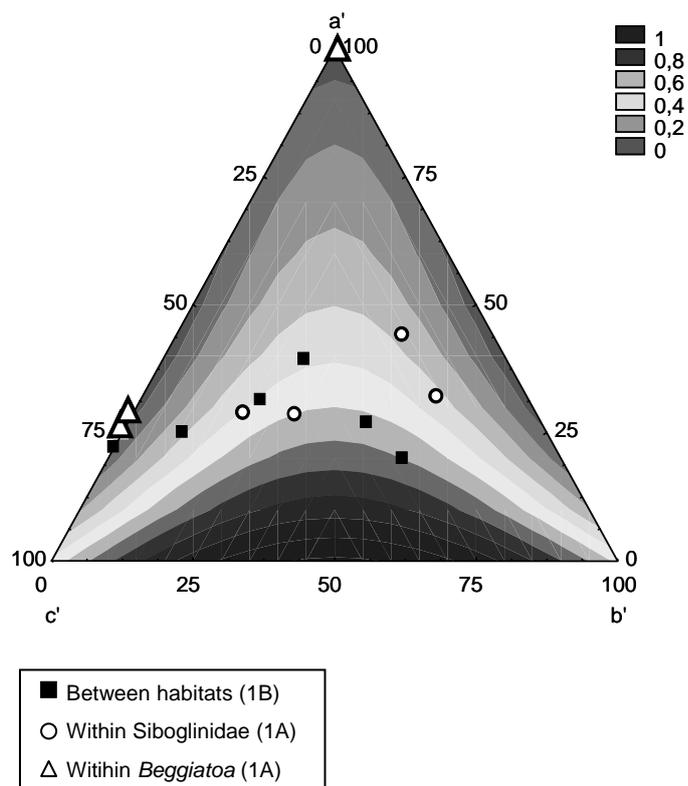
Figure 5: Ternary plot of the turnover of nematode genera at Norwegian seeps at the micro-scale. Turnover rates rang from 0 to 1, where 1 represents the complete turnover of genera.

- ■ Between habitats (1B)
- ○ Within Siboglinidae (1A)
- △ Witihin *Beggiatoa* (1A)

2. MACRO-SCALE DIVERSITY PATTERNS

The reduced seep sediments yielded significantly lower alpha diversity levels in comparison to all other habitats (N_0: t_9 = 8.37, p = 0.02, EG(100): t_9 = 13.15, p = 0.006, H': t_9 = 12.55, p = 0.006, J': t_9 = 11.22, p = 0.009). Background sediments and Siboglinidae patches harboured the most diverse nematode communities number (Table 4), except for one Siboglinidae patch at the Storegga Slide.

Indices for beta diversity at macro-scale were calculated for the nematode communities at HMMV. Only in this seep area a sufficiently high number of replicate samples was obtained within each habitat. Whittaker's beta diversity index for the habitats reached maximum values in the *Beggiatoa* mats and the central, active part of HMMV (Table 4). The pairwise combinations of all samples (ROV and MUC samples) within the same habitat resulted in a high but variable similarity value for the nematode assemblages in the *Beggiatoa* mats (2A: β_S = 36.0 ± 45.5) at HMMV. An extremely low similarity (2B: β_S = 4.6 ± 7.1) was found between the nematode communities from the *Beggiatoa* and Siboglinidae habitat. When all habitats within the geographical area were in the analysis, the average similarity among all HMMV habitats equalled 33 ± 40.7 (2C).

The average turnover rate (β_T) for the 'between *Beggiatoa*-Siboglinidae' comparison (2C: $\beta_T = 0.3 \pm 0.1$) equalled the turnover value for 'within Siboglinidae habitat' (2A: $\beta_T = 0.3 \pm 0.1$). In contrast the genera turnover within the *Beggiatoa* habitat was higher (2A: $\beta_T = 0.5 \pm 0.4$), but again very variable. However, when also the other HMMV habitats such as the centre, grey bacterial mats, outer rim and background sediments are included, a different pattern is observed. Additive partitioning of genus richness (α, β, γ) showed that the beta component due to the difference between habitats, was the most important contributor (70%) to the gamma (total) diversity at HMMV (Figure 6). In contrast, when additive partitioning was based on the Shannon-Wiener index (H') (Figure 6), the alpha diversity (54%) became more important. All observed beta components were significantly larger than the expected value for a random sampling.

Table 4: Diversity measures at the macroscale (2A). Alpha diversity: genus richness (N_0) per habitat, expected number of genera for 100 and 600 individuals, Shannon-Wiener index (H'), Pielou's eveness (J'), and the percentage of unique genera occurring in the habitat. Beta diversity: β_W, according to Whittaker (1960).

Seep	Habitat	N_0	EG(100)	EG(600)	H'	J'	Unique genera % (no.)	β_W
HMMV	Centre	23	7	-	1.17	0.26	0	82.6
	Beggiatoa mats	24	1	2	0.04	0.01	4 (1)	83.3
	Grey mats	9	4	8	0.44	0.12	0	55.6
	Siboglinidae	72	29	47	4.69	0.76	21 (15)	36.1
	Outer rim	61	25	45	4.20	0.71	12 (7)	24.6
	Background	73	33	55	4.80	0.78	23 (17)	39.7
Nyegga	Reduced	20	12	-	2.35	0.54	30 (6)	60.0
	Siboglinidae	61	26	45	4.47	0.75	25 (15)	54.1
	Background	63	29	51	4.58	0.77	32 (20)	47.6
Storegga	Siboglinidae	20	19	-	3.02	0.70	10 (2)	70.0
	Background	69	29	53	4.53	0.74	73 (51)	42.0

Figure 6: Stacked columns depicting the composition of the gamma diversity and the proportions of the alpha diversity and different beta diversity levels (between replicate cores, between habitats) in the Håkon Mosby Mud Volcano. Diversity was measured as genus richness based on presence-absence data, and the Shannon-Wiener index based on abundance data.

3. MEGA-SCALE DIVERSITY PATTERNS

The pooled nematode communities from the same habitat (3A: reduced sediments including *Beggiatoa* mats, Siboglinidae, background sediments) were compared across the three seeps. For this comparison only MUC samples were used, and ROV samples at micro-scale were excluded (Table 3). Changes in nematode genus richness within a similar habitat at the mega-scale were significantly different with the highest difference for the turnover rate between the within-reduced sediments and the within-Siboglinidae habitat (Kruskal-Wallis: $p < 0.0001$, Figure 7a), and the highest difference for the beta similarity between the within-reduced sediments and the within-background sediments (Kruskal-Wallis: $p < 0.0001$, Figure 7b) (Table 3).

The rarefaction curves for the total community associated with the reduced sediments, Siboglinidae, and background sediments (pooled data over all seepage sites) show that the expected number of genera for reduced sediments falls far below that of the Siboglinidae and the background sediments which are most diverse (Figure 8). The largest increase in EG is noticed within the Siboglinidae by expanding the scale from micro to macro. If the different habitats were pooled per geographical area (Figure 9), it can be concluded that the Nyegga pockmarks harbour the most genus rich community, and HMMV the least diverse nematode community.
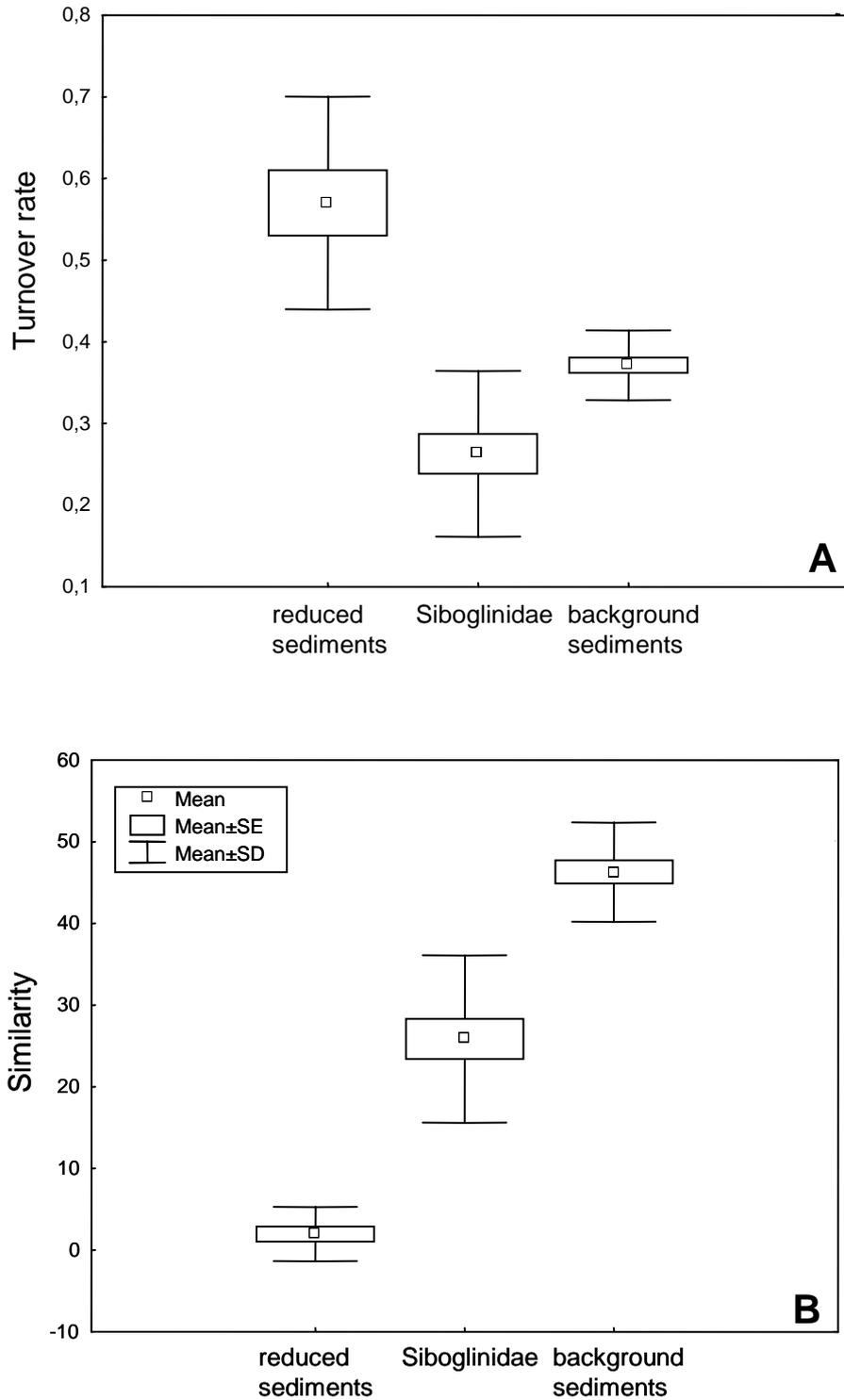
Figure 7: Boxplots visualising the variation in (dis)similarity in terms of nematode genera composition among samples within a similar habitat at mega-scale. Turnover rates (a) and similarity percentage (b) are shown for the habitat 'reduced sediments', 'Siboglinidae patches' and 'background sediments'.
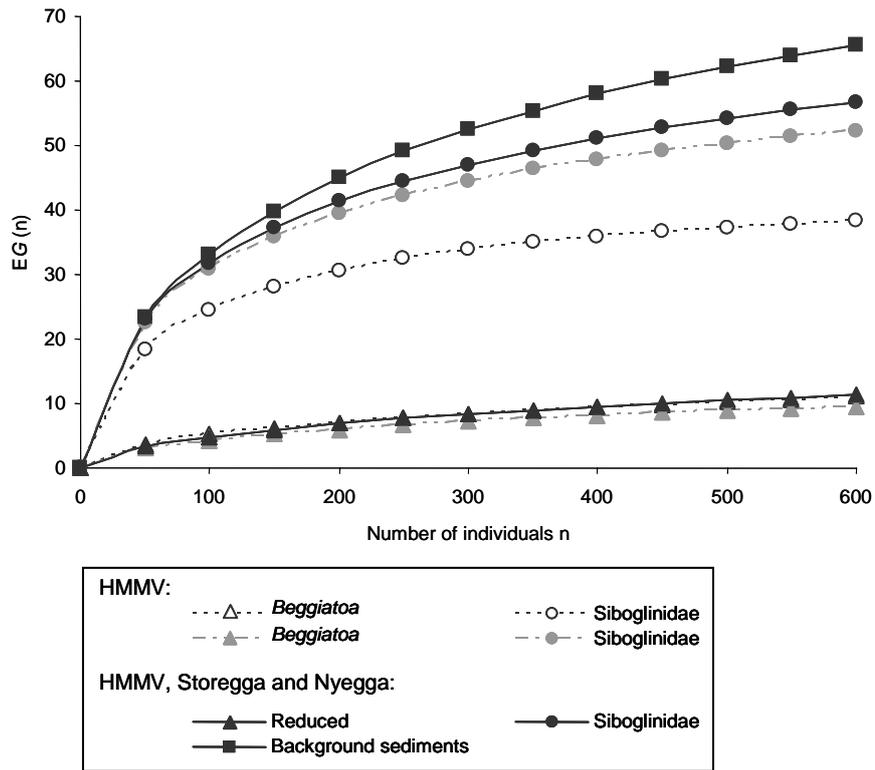
Figure 8: Rarefaction curves for the pooled data per habitat at three different spatial scales. White symbols represent the micro-scale (only at Håkon Mosby Mud Volcano, HMMV), grey symbols represent the macro-scale (only at HMMV), and black symbols represent the mega-scale (HMMV, Storegga Slide, Nyegga pockmarks).
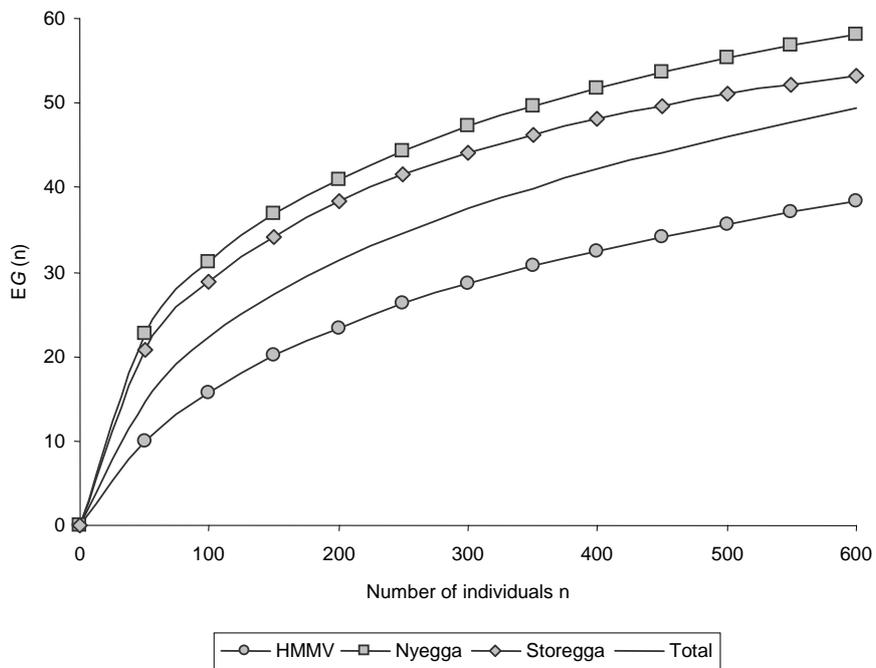
Figure 9: Rarefaction curves for the pooled data per sampling area at the Norwegian continental margin, and for the complete region.

Additive partitioning for the total dataset showed that the beta component (between habitats, 2B) contributed most (40%) to the total genus richness, although alpha diversity or sample core (22%) and seep beta diversity (28%, 3C) also accounted for a considerable proportion (Figure 10). In contrast, additive partitioning based on the Shannon-Wiener index demonstrated a very high contribution of the alpha diversity (63%) to the total 'Norwegian seeps' diversity.
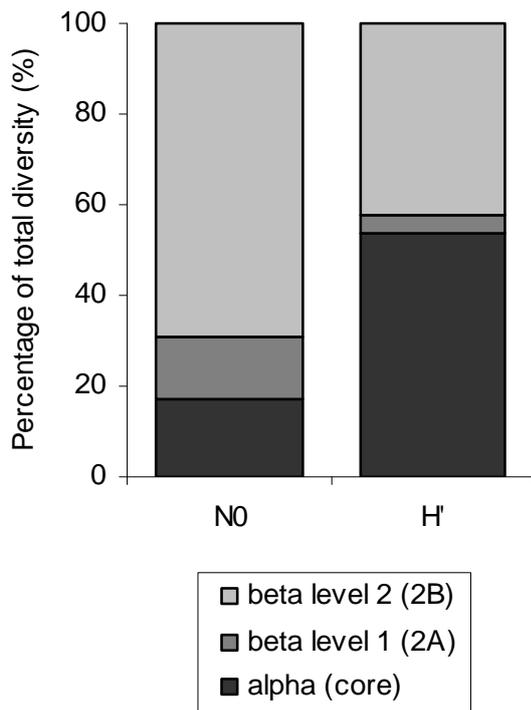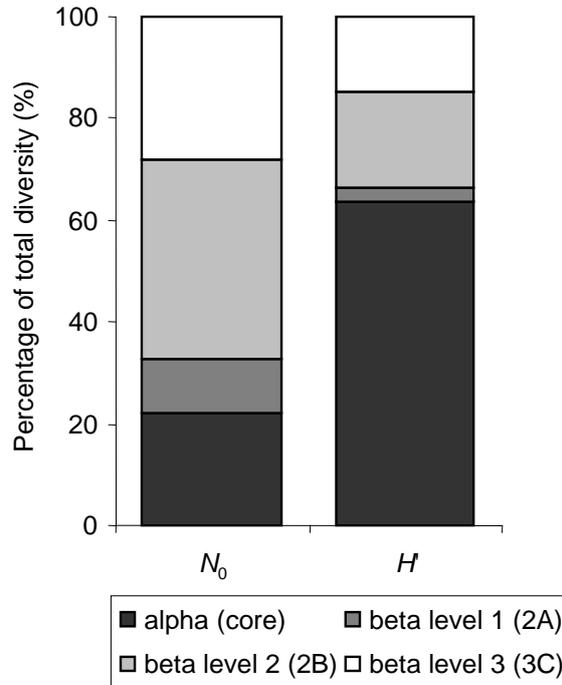

Figure 10: Stacked columns depicting the composition of the gamma diversity and the proportions of the alpha diversity and different beta diversity levels (between replicate cores, between habitats, between areas) for the total nematode community along the Norwegian margin.

DISCUSSION

1. MICRO-SCALE DIVERSITY PATTERNS AT HMMV

The different dive transects at HMMV showed a very high patchiness in habitat type and associated nematode assemblage, even on a meter scale. Thereby, nematode communities were strongly influenced by the neighbouring habitat patches. In particular the *Beggiatoa* mats revealed a high variation in the nematode community composition. The first community was dominated by *Halomonhystera disjuncta* (see also Van Gaever et al. 2006), while additionally a more diverse but less dense, *Microlaimus*-dominated community was defined in the present micro-scale study. The latter community was observed in the small-sized (~25 cm^2) *Beggiatoa* patches (Figure 3b) which were surrounded by Siboglinidae patches within the northern active site and the larger Siboglinidae field. In the MDS plot, these three *Beggiatoa* cores were plotted adjacent to the cores from Siboglinidae

patches, indicating an overlap in terms of nematode community composition. The close proximity of the Siboglinidae patches possibly generates a more intensive migration rate of nematodes towards the *Beggiatoa* patches. As Hodda (1990) stated, species most easily available to colonise a certain patch are those from the surrounding patches. In contrast, microbial mats within the extensive area (38244 m², Jerosch et al. 2007) densely covered by *Beggiatoa* mats (Figure 3a) were probably less colonised by nematodes from the more southern located Siboglinidae fields. Moreover, de Beer et al. (2006) measured a strong depletion of oxygen in the large *Beggiatoa* area, combined with sulphide levels toxic to most metazoan life, resulting in the absolute dominance of the single nematode species *Halomonhystera disjuncta*. It could be expected that the sediments underneath the small *Beggiatoa* patches yield lower sulphide concentrations compared to the large *Beggiatoa* area. Nevertheless, the presence of this bacterium suggests a sulphide flux reaching up to the sediment surface.

Small-scale laboratory experiments with defaunated sediments demonstrated that the colonisation of new niches via lateral interstitial migration is a species-specific process (Schratzberger et al. 2004). *Microlaimus* was probably the most successful migrant of the small, isolated bacterial patches. This opportunistic nematode genus has been recognised as a good coloniser in diverse marine habitats such as disturbed Antarctic sediments (Vanhove et al. 2000, Lee et al. 2001) and canyon sediments exposed to frequent turbidity events (Congo canyon, Van Gaever et al. accepted). Ullberg and Olafsson (2003) carried out a laboratory experiment to assess whether nematodes are able to choose settling spots when descending from the water column. Nematodes were extracted from sediment collected from a shallow sandy bay and allowed to settle in a water column of 1 m to the bottom, which was seeded with 4 small containers with different contents. The settling choice appeared to be species specific, where *Microlaimus* actively selected and colonised the benthic algae treatment in high numbers although this species was not recorded in the subsamples from the source.

2. MACRO-SCALE DIVERSITY PATTERNS

Sampling the different seep habitats with multiple replicates at a macro scale increased the number of nematode genera identified at the Siboglinidae and *Beggiatoa* habitat. The expected number of genera EG(600) for the Siboglinidae habitat at micro-scale increased with 27% if the habitat was examined at macro-scale. However, EG(600) did not change for the *Beggiatoa* habitat from micro- to macro-scale because of the very high dominance levels and low genus diversity in combination with very high numbers. The Whittaker's diversity index reached maximum values in the *Beggiatoa* mats, implying that this habitat was very poor in terms of nematode genera compared to the total HMMV

nematode community. The presence of this habitat even lowers the expected number of genera for the entire HMMV, resulting in a nematode genus community at HMMV which is less diverse in comparison to the Storegga Slide and Nyegga pockmarks (Figures 8, 9).

The genus richness generated by the different habitats at HMMV contributed to a large proportion (70%) to the overall genus diversity. On the other hand, the local (sample core) genus richness (alpha diversity) reached high percentages at HMMV when the Shannon-Wiener index was considered. This high alpha diversity (H') can be explained by the typical, common deep-sea genera (Vincx et al. 1994, Soetaert and Heip 1995, Vanaverbeke et al. 1997) regularly distributed across the different cores within a habitat: e.g., *Acantholaimus*, *Halalaimus*, *Microlaimus*, *Metalinhomoeus*, *Thalassomonhystera*, *Tricoma,* hence high evenness. All observed alpha diversity values were within the expected range (5%) after the randomization procedure, confirming the relevance of our sampling intensity. On the other hand, the high number of unique genera with restricted distributions might explain the significantly higher than expected beta diversity between habitats. Raes and Vanreusel (2006) reported significantly different nematode assemblages associated with different microhabitats (dead coral fragments, dead sponge skeletons, and underlying sediment) in a cold-water coral degradation zone in the Porcupine Seabight (NE Atlantic). In comparison to the HMMV, however, the partitioning of the diversity for the Porcupine Seabight showed a much lower additive value of the beta diversity attributed to the different microhabitats (in case of N_0: 21%, in case of H': 5%). A similar composition pattern of the gamma diversity between both studies was observed when the nematode genus abundance data were considered, with the highest proportion for the alpha (or within core) diversity (M. Raes unpubl. data).

The present investigation of the nematode genus diversity at the Norwegian seeps showed a disparity between the importance of different habitats (beta diversity) at the micro- and macro-scale observation. Interestingly, the beta turnover rate between the *Beggiatoa* and Siboglinidae habitat was lower at the micro-scale than at the macro-scale. This pattern can be assigned to the patchy occurrence of nematode assemblages, even within a single habitat type. In particular the *Beggiatoa* habitat harbours two completely different nematode communities depending on the surrounding habitats and the geochemical sediment conditions. We can conclude here that nematode communities associated with different seep habitats were much better sampled and better defined on the macro-scale than on the micro-scale. Similar patterns were observed in different ecological studies covering a wide range of environments from marine to terrestrial habitats, including research on rocky intertidal sessile assemblages along the Northwestern Pacific coast, British birds, mountain species, tree species in the Mediterranean environment and Malaysian tropical rain forests (e.g., Lennon et al. 2001, He et al. 2002,

Okuda et al. 2004, Zhang et al. 2006, Kallimanis et al. 2008). In general these studies indicated that by using scales of observation that differ only by one order of magnitude, the spatial pattern of alpha diversity could be considerably different. Areas of lower species richness at micro-scale might appear high in species richness at the macro- or mega-scale. Overall, the comparison between the micro- and macro-scale sampling of a seep demonstrated that only small-scale sampling at a seep environment is insufficient to cover the total nematode diversity on genus level in the different habitats, especially in genus-rich habitats.

3. MEGA-SCALE DIVERSITY PATTERNS

An increase in EG(600) from micro- to mega-scale was observed for nematode communities associated with the Siboglinidae habitat (38 → 52 → 57, Figure 8) which can be explained by a high degree of small-scale variation resulting from highly patchy occurrences of individuals (Hodda 1990). Additionally, the average Bray-Curtis similarity within a habitat decreased from micro- to mega-scale for both the *Beggiatoa* mats and Siboglinidae fields. Given their small body size and low mobility, nematodes are expected to be very susceptible to within-habitat physical changes. This, coupled with a limitation to long-distance dispersal and likely restrictions in gene flow (Derycke et al. 2007), resulted also in a significant decrease in nematode community similarity with distance (i.e. within 0.1-23 km) at two offshore muddy habitats in the Celtic Deep and the NW Irish Sea off the west coast of the United Kingdom (Schratzberger et al. 2008).

The additive partitioning for the total dataset revealed an important contribution of beta diversity to the overall regional nematode genus richness (gamma-level). This is a logical consequence of the fact that broadening the spatial scale simply increases the chance to encounter more rare species (Gering et al. 2003). The nematode community at a single seep is composed of a number of very patchily distributed and significantly different nematode assemblages, ranging from extremely low diverse communities in reduced sediments to highly diverse communities associated with siboglinid tubeworm fields. The HMMV Siboglinidae fields (H' (log based e) = 3.03) and background sediments (H' (log based e) = 3.05) investigated here exhibited nematode diversities comparable to those reported for deep-sea sediments at a comparable depth in the Hausgarten site (Greenland Sea) (H' (log based e) = 3.29 ± 0.02) (E. Hoste unpubl. data), and slightly higher than those documented from the Central Arctic (H' (log based e)= 2.08 ± 0.16 at 1000–3890 m, H' = 2.42 ± 0.16 at 1020–2150 m) (Renaud et al. 2006). The nematode communities inhabiting these siboglinid fields were constituted of nematode genera similar to

those of soft-sediment deep-sea benthos at comparable depths (Vanaverbeke et al. 1997, Soltwedel 2000, Vanreusel et al. 2000, Renaud et al. 2006).

CONCLUSION

Despite the many inherent difficulties in evaluating diversity patterns and species richness in deep-sea ecosystems, it has been proven worthwile to investigate the nematode diversity patterns in extreme environments such as cold seeps. The cold-seep environments, consisting of different habitats along the Norwegian margin, maintain a highly diverse nematode community, with some broadly distributed common deep-sea genera and a high number of rare genera with restricted habitat range. This study suggests a considerable effect of habitat heterogeneity on the spatial structure and diversity patterns of nematode communities at the macro-scale within a seep. Some of the observed nematode assemblages were strongly restricted to a specific seep habitat. To assure the long-term survival of diverse deep-sea nematode assemblages a certain diversity of suitable habitats is needed. The cold seeps located along the Norwegian margin offer a sufficiently large number of different habitats for nematodes in the otherwise rather monotonous deep-sea environment. Consequently, the authors are convinced that conservation programs should focus on patchily distributed seep sites on a regional scale along the European continental margins in order to maintain this type of biodiversity hot spots of nematodes, taking into account the necessity of dispersal possibility between the fragmented seeps.

ACKNOWLEDGEMENTS

CHAPTER 8

General discussion:

New insights in cold-seep associated meiobenthos in the deep sea

General discussion:

New insights in cold-seep associated meiobenthos in the deep sea

This thesis deals with different aspects of the meiofaunal communities inhabiting cold seeps located on the North and South Atlantic deep-sea floor. Rarely did previous studies on seep meiofauna go beyond bulk measurements of abundance, biomass or major taxa to describe distribution and diversity patterns (CHAPTER 1). In this study, however, meiofaunal communities were defined down to genus and species level to address different ecological issues. This chapter can be divided in four main parts discussing (1) the factors influencing the composition, density and distribution pattern of meiofaunal communities at deep-sea cold seeps; (2) the nutritional sources of meiofauna within the seep food web; (3) the morphological or physiological adaptations of meiobenthic seep species to the harsh environmental conditions; (4) the specificity of the dominant meiobenthic seep species.

1. STRUCTURING FACTORS FOR THE SEEP MEIOFAUNAL COMMUNITY COMPOSITION

1.1. MEIOBENTHOS

From the existing literature, no consistent response of metazoan meiofaunal abundance to seep conditions emerged (reviewed in Levin 2005). In this thesis, similar high fluctuations were observed in the densities of meiofauna influenced by seepage in the investigated mud volcano and pockmarks. Little or no density differences compared to background sites were found for the meiofauna from the pockmarks at the Darwin Mounds' tails (Rockall Trough), from the grey bacterial mats at Håkon Mosby Mud Volcano (HMMV) (Barents Sea), and from the samples obtained at the Vesicomyidae patches at the REGAB seep (Gulf of Guinea). In contrast, strongly reduced meiofaunal densities (<50 ind. 10 cm^{-2}) occurred at two samples from the Mytilidae patches at the REGAB seep, whereas extremely enhanced meiofaunal densities (up to 16000 ind. 10 cm^{-2}) were present in the *Beggiatoa* dominated mats at HMMV. This number represents one of the highest densities ever recorded for marine meiobenthos,

both from deep-sea and shallow-water habitats (Vincx et al. 1994, Raes 2006, Urban-Malinga et al. 2006), and exceeded the maximum densities (up to 13042 ind. 10 cm^{-2}, Luyten 1999 *in* Ingels et al. 2006) reported for the meiobenthos from Antarctic sediments. However, records were made of nematode densities (*Haliplectus*) reaching up to 45000 ind. 10 cm^{-2} in the temperate grassland of Saeftinghe (The Netherlands) (T. Bongers pers. comm.)

In total 23 different meiofaunal taxa belonging to 13 phyla were identified in the investigated study areas. The phylum Arthropoda was represented by the highest number of taxa and the phylum Nematoda by the highest densities. Nematodes and harpacticoid copepods occurred in each sample, although with variable relative abundances. Only the site at the bottom of the Congo canyon (Gulf of Guinea) was devoid of copepods. Reduced seep sediments lacking any chemosynthetic megafauna harboured a low number of taxa, ranging between 4 (*Beggiatoa* mats at HMMV) and 6 (Nyegga pockmark, grey bacterial mats at HMMV) taxa. An exception on this pattern was found at the Darwin Mounds, where the adjacent pockmark sediments yielded 11 taxa. However, these pockmark sediments may have comparable geochemical sediment conditions as the surrounding background because no clear evidence of substantial seepage activity was observed in this area (Masson et al. 2003). Generally, the nematofauna in the reduced seep sediments accounted always for the largest proportion of the meiobenthos, i.e. more than 86%. Occasionally, enhanced densities from some less dominant groups, such as kinorhynchs, polychaetes and tardigrades, were observed. The meiobenthic communities identified in association with aggregations of symbiont-bearing mussels and clams at the REGAB seep (Gulf of Guinea) were equally low diverse in terms of meiofaunal taxa (ranging between 4–7 taxa). In contrast, the sediments colonised by siboglinid polychaetes (only observed at the Norwegian margin), provided shelter to the most diverse meiobenthic assemblages composed of 13 to 16 different taxa. Only the Siboglinidae patches at the Storegga Slide were unexpectedly poor, yielding only nematodes, copepods, nauplii, and polychaetes.

In concordance with the general pattern in the deep sea, nematodes constituted the largest fraction of the seep meiofauna in this thesis. They accounted for 82–99% of the meiobenthos at all seep sites, except in one particular Mytilidae patch at the REGAB seep (50%) and in the muddy sediments at the active HMMV's centre (5%). In these latter two habitats, harpacticoid copepods became the most abundant group. Overall, the nematode community can be considered as a representative for the meiobenthos at deep-sea seeps and will be further documented in the part hereunder.

1.2. NEMATOFAUNA

The research at hand indicates a principal distinction for the seeps along the Norwegian margin between the nematode communities of seep sediments colonised by siboglinids on one hand, and the communities of reduced, anoxic seep sediments lacking symbiont-bearing megafauna (CHAPTER 4, 6, 7). Not only is the latter type of seep sediments associated with very genus-poor nematode assemblages, the results of this thesis also demonstrate that these assemblages are often strongly dominated by only one or two nematode genera. Oxygen availability limited to the first mm of the sediment, combined with very toxic sulphide concentrations (1 mM) at 2 cm depth, create a hostile environment to most metazoan meiofaunal species. Nevertheless, bacteria often reach high concentrations in these sediments, and despite the harsh geochemical conditions, some stress-resistant nematode species are able to benefit from this bacterial food source and thrive within this habitat. From the different investigated seep sites, it became clear that Siboglinidae tubeworm fields harbour the most diverse seep nematofauna (CHAPTER 6). The hypothesis has been put forward that the siboglinids strongly affect the geochemical conditions in the tube-surrounding sediment by their intensive ventilation acitivity (Julian et al. 1999, Bergquist et al. 2002). Consequently, a well-oxygenated sediment up to 5 cm depth is created (de Beer et al. 2006), providing a suitable habitat for a wide range of nematode species.

The study carried out at the seep site in the Gulf of Guinea revealed the presence of low diverse nematode communities in highly variable densities at the sediments between the mussel (Mytilidae) and clam (Vesicomyidae) beds (CHAPTER 3). The chemical measurements in this site were made close to the symbiont-bearing species and indicated that methane is an important factor in the distribution of the megafaunal assemblages (Olu-Le Roy et al. 2007). Methane concentration decreased with megafaunal density in mussel beds from M2 to M3 and to the least dense M1 site. The low methane concentrations at M1 could be explained by temporal variability in methane emissions. Nematode abundances proved to be influenced by the variable methane seepage intensity in the different sites. In addition, it appeared that the oxygenated zone in the surficial sediments was very thin (1.5–3.5 mm) in the areas of fluid seepage. The strong depletion in nematode densities (<40 ind. 10 cm^{-2}) in the mytilid beds M1 and M2 can be regarded as the consequence of the higher methane levels in combination with the low oxygen penetration.

Although the most dominant seep nematode species did not always show increased abundances compared to background communities, their success was in each case expressed in a significantly

elevated total biomass across the different seep sites in the North and South Atlantic. This higher biomass standing crop was the result of different investment strategies. The nematode *Halomonhystera disjuncta* invested in a substantial survival of the offspring through an ovoviviparous reproduction strategy, which secured the vulnerable brood against the sublethal sediment conditions at the bacterial mats on HMMV and resulted in an unexpectedly dense population (CHAPTER 4). The remarkable total nematode biomass attributed to the species *Sabatieria mortenseni* in the REGAB seep in the Gulf of Guinea (CHAPTER 3), on the other hand, was explained by the investment in a high individual body mass. These observations confirm the general trend that seep systems support larger faunal biomass standing crops in comparison with the neighbouring non-seep deep-sea floor (e.g., Sibuet and Olu-Le Roy 2002, Levin 2005).

At the Darwin Mounds in the Rockall Trough (CHAPTER 2), habitat variability in nematode communities on a horizontal scale was less pronounced, but here the vertical distribution of nematode genera within the sediment showed some special features. Nematodes of the subfamily Stilbonematinae were found only in notable numbers in the deeper sediment layers of the Darwin Mounds, probably indicating the presence of anoxic microenvironments in the deeper sediment layers. Stilbonematinae were already reported to occur in sulphidic shallow-water sediments in the micro-oxic zone just above the sulphide maximum (Schiemer et al. 1990). At pockmarks in the North Sea (115 m), the symbiont-bearing nematode *Astomonema southwardorum* exhibited a similar distribution pattern, with maximum densities at 5–8 cm depth. This density maximum corresponded with the peak of elemental sulphur content occurring just above the zone of maximum sulphate reduction and sulphide concentration (Dando et al. 1991). Both the distribution patterns of *Astomonema southwardorum* in the North Sea pockmarks and the Stilbonematinae suggest a tight link with the sediment geochemistry. While seep habitats are often visually recognised by the megafaunal feature (clam or mussel beds, Siboglinidae fields, bacterial mats) on top of the sediment, it are the geochemical characteristics and the fluid flow intensity within the sediment that are responsible for distinct infaunal assemblages (Sahling et al. 2002, Levin et al. 2003).

In comparison to the Siboglinidae dominated seep habitat, background sediments across the different sampling sites harboured an even more diverse nematofauna (CHAPTER 6), dominated by typical deep-sea genera such as *Acantholaimums*, *Halalaimus*, *Sabatieria*, *Tricoma*, *Monhystera* and *Thalassomonhystera* (Vincx et al. 1994, Soetaert and Heip 1995, Vanaverbeke et al. 1997). A similar conclusion was drawn for the meiobenthos at different habitats in cold-water coral reefs, where the underlying sediment was recognised as the most diverse microhabitat for nematodes (on genus level) in both the tropical and cold-water coral reefs (Raes and Vanreusel 2006, Raes et al. 2006). Several

investigations of the biodiversity of the deep-sea floor already highlighted the fact that this part of the ocean harbours a substantial fraction of the Earth's species pool (e.g., Hessler and Sanders 1967, Grassle 1989, Gage 1996, Smith et al. 1998, Lambshead and Boucher 2003). High species diversity in samples of macrobenthos of deep-sea sediments is now well established (Gage 1996). The muddy, bathyal slopes and abyssal plains may contain at least 250 species of macro- and meiobenthic invertebrates within a single square meter of sediment (Smith et al. 1998). Although the deep-sea fauna is made up of organisms similar to those found in coastal sediments, such as polychaete worms, pericarid crustaceans and various molluscs, the important difference is that there are many more species present. Besides species richness, eveness or equitability is another important component of the concept of diversity. In the deep sea, the relatively high eveness in distribution of species' abundances and consequently large fraction of species occurring as singletons or in low abundance, make the determination of the likely number of species present at even very local scales very uncertain. Furthermore, the total area of deep-sea bottom sampled for macrofauna is still minimal, and this is even more the case for the undersampled and often ignored meiofaunal fraction. Therefore the biodiversity of the nematofauna living in the deep-sea sediments is likely to be largely underestimated (Lambshead 1993, Gage 1996).

To obtain an idea of the importance of different spatial scales on the nematode diversity in deep-sea ecosystems, the meiobenthos was sampled at a micro-, macro- and mega-scale in the different seepage sites along the Norwegian margin (CHAPTER 7). The overall nematode diversity, including the background sediments, was divided into alpha and beta diversity to investigate the influence of different seep habitats within a geographical area on the nematode genus diversity. Sample diversity was the major contributor to the overall diversity when the Shannon-Wiener diversity index was considered, confirming the high level of small-scale patchiness of nematode genera (Heip et al. 1985). Notwithstanding this, the different habitat types also added an important number of rare genera to the overall nematode diversity. Distinct nematode communities could be identified, reflecting a multiple-scale (from m's to hundreds of km) spatial variability corresponding with the different habitats at the Norwegian seeps (CHAPTER 4, 6, 7) and in the Congo Channel system and adjacent pockmark in the Gulf of Guinea (CHAPTER 3). Nematode distribution patterns across different cold seeps in the Atlantic eventually seem to be strongly regulated by both the fluctuating, habitat-correlated sedimentary geochemical conditions (oxygen, sulphide and methane concentrations), biological acitivities (such as root penetrations, burrowing, pumping and sulphide extraction), as well as the food supply (for instance bacterial concentrations).

To conclude, in the rather homogeneous and oligotrophic environment of the deep-sea floor, cold seeps consist of a large number of patchily distributed habitats, each maintaining its specific nematode community. This variability of suitable habitats is of high importance to assure the persistence and diversification of meiobenthic cold seep communities. In addition, the presence of a sufficient number of separated reduced environments is essential for dispersal possibilities along the continental margins. These cold seep ecosystems merit a proper conservation, because they are hot spots of nematode diversity turnover and consequently contribute to the overall meiobenthic deep-sea biodiversity.

2. NUTRITION OF SEEP MEIOFAUNA

The most detailed information of nutrition at seeps has been reported for the large megafauna that host endosymbiotic chemoautotrophic bacteria. Much of the biomass of tubeworms, mussels and clams is fueled by both methane-based and sulphur-based symbioses. Little information is available about the diets of smaller invertebrates that live within seep sediments, largely because the effort required to obtain sufficient amounts of organic matter for analyses is prohibitory. Symbiotic chemosynthetic bacteria are a possible nutritional source in meiobenthic taxa, as ectosymbiosis was documented in seep nematodes (Dando et al. 1991, Jensen et al. 1992) and in euglenoids and ciliates (Buck and Barry 1998). Van Dover et al. (2003) reported isotopic signatures for nematodes from the Blake Ridge seeps (2155 m) and suggested utilisation of chemosynthetically derived organic matter at a higher trophic level, possibly as decomposers.

The main energy source available to the infauna inhabiting bathyal reducing environments is carbon fixed chemosynthetically in the seabed, made available through the activities of free-living or symbiotic bacteria. Sulphur oxidation and methane oxidation (methanotrophy) are the primary chemosynthetic pathways that support carbon fixation of significance to animals (Conway et al. 1994). Later work has suggested that free-living or symbiotic sulphate reducers that interact directly with other chemoautotrophs in consortia may also be important (Boetius et al. 2000, Dubilier et al. 2001). Seep fauna may obtain chemosynthetically fixed organic matter by (1) translocation from sulphide- or methane-oxidising symbionts, (2) feeding on free-living chemoautotrophic bacteria, or (3) consuming another animal that obtains nutrition through (1) or (2). Symbiosis-based nutrition appears to be rare among seep macrofauna. In contrast most of these species revealed to be heterotrophic, although light carbon and nitrogen isotope values provided evidence for chemoautotrophic symbioses in selected taxa. The study of MacAvoy et al. (2005) supported the hypothesis that fauna tightly associated with

tube worm aggregations at cold seeps in the Gulf of Mexico obtains the bulk of its nutrition from local source of primary production. The importance of chemosynthetic trophic pathways, however, varies regionally and among microhabitats, taxonomic groups, and feeding guilds (Levin and Michener 2002). Moreover, Levin et al. (2000) predicted that macrofauna of abyssal and rise seeps should exhibit much greater trophic specialisation (involving chemosynthesis) than the upper slope and shelf faunas, because the supply of food from the surface is more limited. It was indeed demonstrated for macrofauna that the contribution of chemosynthetically fixed carbon differs between deep-sea and shallow-water seeps. Seep macrofauna at Pacific methane seeps (520–4443 m) exhibited lighter $\delta^{13}C$ and $\delta^{15}N$ values than those in non-seep sediments. The carbon isotopic signatures were consistent with consumption of methane-oxidising archaea by some dorvilleid polychaetes ($\delta^{13}C$, –90.6‰ and –73.8‰, Levin and Michener 2002) and with grazing on filamentous sulphur bacteria by gastropods and polychaetes from the Oregon and California seeps. Other deep-sea studies reported a high variation in isotopic signatures of seep megafauna ranging from –28.6‰ to –76.4‰ (Paull et al. 1992, Suess et al. 1998). Contrastingly, studies of shallow seeps in the North Sea (150 m, Dando et al. 1991) and on the Eel River Shelf (35–55 m, Levin et al. 2000) indicated that most macrobenthos feed heterotrophically on photosynthetically produced carbon. North Sea macrofauna exhibited signatures even heavier than those from the Eel River slope, with $\delta^{13}C$ between –16‰ and –20‰ (Dando et al. 1991). However, methane seeps in the Skagerrak (280–360 m) supported dense aggregations of siboglinids (*Siboglinum poseidoni*) and bivalves (*Thyasira sarsi*) known to rely on chemoautotrophic bacteria (Dando et al. 1994). None of these studies examined the nutritional mode of the seep meiobenthos.

Investigations on the food web structure of cold seeps have so far not discussed the possible meiobenthic contribution as food sources for higher trophic levels. In other marine and freshwater habitats, meiofauna are known to increase food web complexity (Schmid-Araya et al. 2002) and meiobenthic invertebrates are often tightly linked to higher trophic levels such as macrofauna (Ólafsson 2003 and references therein) and fish (Limén et al. 2007 and references therein). Hereafter, the trophic position of seep associated meiobenthos will be discussed within the food web structure at the Håkon Mosby Mud Volcano (HMMV).

The diversity of the primary producers at HMMV was correlated with a high methane availability and different fluid flow regimes (Lösekann et al. 2007). In the active volcano center, the main methane-consuming process was bacterial aerobic oxidation, carried out by three bacterial clades. In sediments below the *Beggiatoa* mats encircling the HMMV centre, methanotropic archaea of the ANME-3 clade dominated the zone of anaerobic methane oxidation. ANME-3 archaea form cell aggregates mostly

associated with sulphate-reducing bacteria of the *Desulfobulbus* (DBB) branch. These ANME-3/DBB aggregates were highly abundant and accounted for up to 94% of the total microbial biomass at 2 to 3 cm below the sediment surface (Lösekann et al. 2007). In the volcano centre and the *Beggiatoa* site, carbon sources other than methane are not available. Only one phylogenetic group strongly dominated, suggesting that the limited carbon substrate spectrum appears to limit the diversity of the microbial community. A similar pattern was observed in the meiobenthic communities of both habitats: whereas the centre harboured a dominant copepod species (strongly resembling *Tisbe wilsoni*), the *Beggiatoa* mats exhibited extremely high densities of the nematode species *Halomonhystera disjuncta* (CHAPTER 4). Fatty acid biomarkers detected in the copepod species were characteristic for methanotrophic bacteria (CHAPTER 5). In contrast, similar analyses of *H. disjuncta* suggested a heterotrophic dependence on the sulphide-oxidising bacteria in the *Beggiatoa* mats. Macrofaunal communities were lacking in the former habitats, except for some infaunal polychaetes associated with the *Beggiatoa* mats.

The microbial and meiobenthic diversity increased considerably in zones outside of highest fluid flow which were populated by siboglinid tubeworms. In this zone, aerobic and anaerobic methane oxidizers were less abundant in the top 10 cm of the sediment and the total microbial biomass in the uppermost three centimeters was only about 9% of that at the *Beggiatoa* site (Lösekann et al. 2007). The presence of the tubeworms likely enhanced availability of organic carbon other than methane, e.g., worm exudates and sediment particles trapped by the worm tubes, and increased the diversity of the free-living bacteria. Additional primary production in the methane seep communities is also produced by bacteria living in symbioses with invertebrate hosts. In one of the siboglinid species living at the HMMV, *Oligobrachia haakonmosbiensis*, carbon isotope data (δ^{13}C from –51.1 to –56.1‰) suggested an important contribution of methane carbon to its nutrition (Gebruk et al. 2003). At the HMMV the methane seeps affect large areas on the seafloor and as a result there is a substantial increase in the biomass of symbiotrophic fauna relying on methane-oxidising bacteria (first of all, the siboglinid *O. haakonmosbiensis*). The fauna associated with the siboglinids obtain a portion of methane carbon in their diet. Carbon skeletons were especially depleted in ^{13}C in carnivorous caprellid amphipods associated with *O. haakonmosbiensis* (Gebruk et al. 2003). The zoarcid fish *Lycodes squamiventer*, which was present throughout the crater, has a diverse diet (various benthic organisms) with siboglinids playing only a minor role (Gebruk et al. 2003). It can be assumed that the species-rich nematode communities (CHAPTER 4) observed in these siboglinid tubeworm assemblages will represent an important trophic link between the primary producers and the diverse macro- and megabenthic community. Limén et al. (2007) found at a deep-sea hydrothermal vent that meiofauna could be

categorised as bacterial grazers, detritus feeders, predators and scavengers, and occupy several feeding guilds and trophic levels. Moreover, several nematode species show opportunistic feeding and can change feeding behaviour depending on the availability of food sources (Moens and Vincx 1997). In general the methane carbon at deep-sea seeps will be incorporated through the meiobenthos at different trophic levels (first and second order consumer) into the local seep biota.

The fatty acid and stable carbon isotope analyses of *H. disjuncta* and copepod species at HMMV provided the first insight into the trophic relations of meiofauna within these chemosynthetic-based communities in the deep sea. Alternative or additional approaches and methods may be valuable to elucidate the food web at seeps. These methods might include additional $\delta^{13}C$ in complement with $\delta^{15}N$ analyses, and more fatty acid analyses of tissues including the stable isotopic signatures of the separate fatty acids. Used together, $\delta^{13}C$ and $\delta^{15}N$ signatures of organisms can provide an indication of the carbon and nitrogen sources, fixation pathways, trophic level, and potential occurrence of chemoautotrophic endosymbiosis. However, other methods such as molecular sequencing of gut contents, use of inorganic isotopic tracers such as $^{14}CO_2$, $^{13}CO_2$, $^{35}SO_4$, as well as more traditional feeding experiments and gut content observations could be recommended. Full understanding of the total food web within these extreme environments is likely to come from detailed exploration of the relationships among the different invertebrate feeding guilds, (incorporating the micro-, meio- and macrofauna), their geochemical environment and the present microbial processes.

3. ADAPTATIONS AND TOLERANCES OF SEEP MEIOFAUNA

Studies of the pore water chemistry of deep-sea cold seeps indicate that considerable variation exists in the concentrations of sulphide, methane, and other chemical constituents. Methane concentrations in the upper sediment layers vary with organic content of the underlying deep sediment, the nature and magnitude of upward flow and the transport of methane-loaded pore water. Concentrations range widely from micromolar to millimolar concentrations, with values up to 10 mM recorded in sediments from the Florida Escarpment (3300 m water depth, Chanton et al. 1991) and up to ~20 mM in Eel River (c. 500 m, northern Californian margin, Levin 2005) and Hydrate Ridge (600–900 m, offshore Oregon, Levin 2005) sediments. Methane concentrations also vary among microhabitats (Treude et al. 2003). Typically methane is rapidly oxidised. Anaerobic methane oxidation is apparently coupled to sulphate reduction in some areas yielding high concentrations of hydrogen sulphide. Total hydrogen sulphide concentrations of up to 20–26 mM have been documented at upper

slope seeps on the Oregon and California margins (c. 2500 m, Sahling et al. 2002, Levin et al. 2003). Decay of organic matter on the deep-sea floor can also yield high sulphide concentrations, thus similar sulphide profiles may occur around whale or wood falls (Smith and Baco 2003).

In comparison, cold seeps at shallow depths display similarly high and variable concentrations of sulphide and methane. The brine of the coastal seep in the East Flower Garden (72 m water depth, NW Gulf of Mexico) is much denser (up to 196‰) than seawater, anoxic and contains exceptionally high levels of dissolved hydrocarbon gasses (e.g., methane: 121 mM) and hydrogen sulphide (2.2 mM) (Brooks et al. 1979). Also the North Sea pockmark (150 m) sediments were characterised by high total suphide concentrations at 10 cm sediment depth, as compared to surrounding areas (Dando et al. 1991). Samples obtained from the 'bubbling reefs' of the Kattegat, Denmark (10–12 m) diffused a strong hydrogen sulphide odour (2.1–3.3 mM, Jensen et al. 1992). A high methane anomaly and sharp local variation in the bottom seawater (Sakai et al. 1987) was recorded in the cold seep off Hatsushima, Central Japan. Overall, the millimolar sulphide concentrations found in seep sediments are much higher than the low micromolar concentrations characteristic of non-seep sediments. Sulphide levels measured in other reduced shallow-water habitats were of the same order of magnitude as in the seep sediments. Rinke et al. (2007) analysed the pore water of debris accumulations which is inhabited by the thiotrophic, ectosymbiont-bearing ciliate *Zoothamnium niveum* habitat in the Bay of Calvi (Mediterranean Sea). The sulphide concentrations in this habitat reached values of up to 1.2 mM and proved that the debris accumulations are a suitable habitat to sustain bacterial chemoautotrophy. Exceptionally high sulphide levels (18 mM, Neira and Rackemann 1996) were measured in intertidal sediments with decaying macroalgae (*Enteromorpha*). Finally also estuaries are known as environments where abiotic conditions fluctuate widely, with sometimes severe hypoxia and sulphide accumulation (1.2 mM in Gulf of Gdańsk, Poland, Jahn et al. 1997; 1 mM in Tay estuary, Scotland, Schiedek et al. 1997).

The mechanisms that enable organisms to survive in reducing environments have been a fertile area for the discovery of novel physiological and biochemical capabilities. Most of them considered the adaptations to sulphide because seep organisms may experience exceptionally high sulphide concentrations within the sediment. It was suggested that the sulphide biome would contain at least some primary (faunal) elements of the oldest biosystem on earth (Fenchel and Riedl 1970). Sulphides are highly toxic, and consequently organisms have adopted several mechanisms of avoidance. The mechanisms to avoid sulphide toxicity can be of behavioural, physiological and/or biochemical character. The response of benthic organisms and their detoxification mechanisms have been reviewed

by Powell et al. (1979), Grieshaber and Völkel (1998), Levin (2005): (1) the removal of sulphide at the body wall through a layer of sulphide-oxidising bacteria (e.g., stilbonematid nematodes, some solemyid, lucinid, vesicomyid, and mytilid bivalves), and/or enzymatic sulphide oxidation (e.g., priapulid *Halicrypus spinulosus*, lugworm *Arenicola marina*, isopod *Saduria entomon*), (2) sulphide insensitive haemoglobin, (3) reversible sulphide binding to blood components (polychaetes *Nereis diversicolor* and *Arenicola marina*, clams *Calyptogena magnifica* and *Lucinoma annulata*), (4) mitochondrial sulphide oxidation to less toxic compounds (e.g., thiosulphate) with ATP synthesis (clam *Solemya reidi*) and (5) reliance on anaerobic respiration at higher sulphide levels (polychaete *Marenzelleria* cf*. wireni*, Schiedek et al. 1997).

Symbiotic associations between marine invertebrates and sulphur-oxidising bacteria are a common feature in biota from sulphide-rich environments, such as those flourishing in the vicinity of hydrothermal vents, cold seeps and reduced shallow-water habitats (Musat et al. 2007). The bacterial partner can occur as an intracellular or extracellular endosymbiont inside the organism, or as an ectosymbiont attached to the host's body (Musat et al. 2007, and references therein). The large sizes of the siboglinid tubeworms (up to 2 m), mussels (up to 36 cm) and clams (up to 18.6 cm) at seeps are a result of symbiont-supported chemoautotrophic nutrition. Each of the species hosts either sulphide-oxidising symbionts, methanotrophic symbionts or both (Levin 2005 and references therein). They typically have a reduced gut and exhibit little reliance on photosynthetically fixed organic matter from the surface. Symbiont sulphide oxidation is a widespread adaptation present in siboglinid annelids, bivalves and annelids occurring at methane seeps (Childress and Fisher 1992, Levin 2005) and is also described for nematodes of the subfamily Stilbonematinae (Ott et al. 2004). For organisms that rely on oxidation of reduced compounds by symbiotic bacteria, acquisition of oxygen and sulphide (or methane) presents a challenge. Adaptations may include separating the acquisistion of oxygen and sulphide in space (by extending the body between environments) or in time (by moving between environments). Siboglinid tubeworms obtain oxygen from the anterior end and sulphide through diffusion into a 'root'-like posterior extension that penetrates deeply into the sediment (Sasayama et al. 2007). Vesicomyid clams also acquire oxygen and sulphide from different places, albeit over shorter distances (Heyl et al. 2007). Smaller taxa such as oligochaetes and nematodes have to migrate vertically between the oxygen and sulphide sources in the sediment, storing one or the other. Even large vacuole-bearing sulphur bacteria (e.g., *Thioploca*, *Beggiatoa*) are able to glide vertically in sheaths (Levin 2005).

Ectosymbiont-bearing nematodes belonging to the subfamily Stilbonematinae were encountered in the deeper sediment layers of the Darwin Mounds, Rockall Trough (CHAPTER 2). Stilbonematids are known

for their highly species-specific association with prokaryotic microorganisms. Originally thought to be parts of the worm, fungal spores or epibiotic cyanobacteria, the ectosymbionts have been finally identified as sulphide-oxidising chemoautotrophic bacteria that coat the nematode surface (Ott et al. 1991, Ott et al. 2004 and references therein). Stable isotope analyses suggested that the nematodes depend on the bacteria for their nutrition (Ott et al. 2004). In turn, they provide the microorganisms with sulphide and oxygen by repeatedly migrating through the redox boundary layer in marine sediments. Stilbonematids probably grow slowly and have long intermoult periods, high life expectancy and few offspring. The slow and sluggish lifestyle fits with a basal metabolism that is among the lowest ever measured in nematodes (Schiemer et al. 1990). This group of nematodes was previously only reported from intertidal and subtidal porous sands (Ott et al. 1991, Riemann et al. 2003, Kamenev et al. 1193, Thiermann et al. 1997) but was in this thesis also identified in the reduced deep-sea environment of the Darwin Mounds (circa 1000 m).

In this thesis, different techniques were conducted to screen the nematode species *Halomonhystera disjuncta* from HMMV for endosymbiotic or ectosymbiotic bacteria (CHAPTER 4, 5). For the first time such a dense (up to 16×10^6 ind. m^{-2}) infaunal mono-specific population of a metazoan was observed in highly sulphidic and anoxic marine sediments, significantly exceeding non-toxic background sites in terms of densities. Nematode specimens scrutinized by light, scanning and transmission electron microscopy, and subjected to fluorescence in situ hybridization showed no association with endo- or ectosymbionts. Moreover, this species has a fully developed gut, whereas the digestive system of endosymbiont-bearing nematodes is often reduced or non-functional (Giere et al. 1995, Ott et al. 2004). This species further showed no striking morphological modifications, but exhibited an ovoviviparous reproduction mode. A high percentage of the females was carrying several juveniles ready to emerge from their body. In the harsh sediment conditions underneath the *Beggiatoa* mats, the mobility of newly hatched juveniles can be considered as an important advantage for the survival of the brood, as they can migrate immediately to the most favorable geochemical sediment conditions.

The other dominant seep nematodes, such as *Sabatieria mortenseni* and *Desmodora* sp. at the REGAB seep (CHAPTER 3) as well as *Terschellingia longicaudata* and *Thalassomonhysera* sp. at the Nyegga pockmarks (CHAPTER 6), remain to be examined for morphological or physiological adaptations to the reduced sediment conditions. Yet, many questions remain unanswered concerning the geochemical controls on meiofaunal standing stocks at deep-sea cold seeps, and the physiological, enzymatic, metabolic, reproductive and molecular adaptations that permit meiobenthic organisms to live in these extreme environments. Laboratory exposure experiments designed to assess the resilience of seep

nematodes to abiotic stressors in different degrees could apply important information on this issue. Ex situ and in situ experiments could also test the behavioural response of nematodes to reduced seep conditions, and therefore also the potential capability of nematodes to migrate laterally towards less lethal sediment conditions. Although cultures of shallow-water nematode species have been established world-wide, it still remains a huge challenge to imitate the environmental conditions at the deep-sea floor in a laboratory and to successfully culture the first nematode species retrieved from deep-sea sediments.

4. SPECIFICITY OF SEEP MEIOFAUNAL SPECIES

The active dispersal of nematodes is mainly through sinusoidal, active movements through the sediment, and therefore likely restricted to a small area. Nevertheless, marine nematodes include a number of apparently cosmopolitan taxa that exhibit broad biogeographic ranges despite lacking any pelagic propagation stages or active swimming modes. This phenomenon is commonly referred to as the so-called 'meiofauna paradox' (Giere 1993). Interestingly, molecular tools have demonstrated that many cosmopolitan distributions of invertebrates in fact represent different cryptic[1] species (e.g., Todaro et al. 1996, Westheide and Schmidt 2003). The heterogeneity of marine environments was illustrated in the high amount of cryptic species that have been identified in all major marine taxa (Knowlton 1993). Recent molecular research has also shown that some nematode 'species' are indeed complexes of cryptic species with a much more restricted geographical distribution than previously excepted (Bhadury et al. 2008, Derycke et al. 2008b).

The reduced, anoxic sediments of the several cold seeps investigated in this thesis were each inhabited by one or two dominant nematode species. At least three of these species, i.e. *Halomonhystera disjuncta* Bastian 1865*, Terschellingia longicaudata* de Man 1907*,* and *Sabatieria mortenseni* Ditlevsen 1921, were already described as common inhabitants of intertidal habitats with an extensive geographical range. However, the existence of multiple shallow-water cryptic species in the species complex of *Halomonhystera disjuncta* was revealed by a detailed population genetic survey carried out along the Belgian coast and the southwestern part of The Netherlands (Derycke et al. 2007). Bhadury et al. (2008) collected specimens of the species *Terschellingia longicaudata* that had been identified by their morphological features from a range of localities (East and West Atlantic, Bahrain, Malaysia) and

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[1] Cryptic species are defined as species that are indistinguishable based on morphological characteristics but that show substantial gene differences. All cryptic species are commonly designated as a species complex within the morphologically defined (super) species.

habitats (estuarine, intertidal, subtidal). Using molecular approaches based on the amplification and sequencing of the small subunit ribosomal RNA (18S), they detected two sequence types that may possibly represent cryptic species (Bhadury et al. 2008). Nevertheless, some of the cryptic haplotypes from both *H. disjuncta* and *T. longicaudata* appeared to have a cosmopolitan distribution.

The phylogenetic relationship of the extremely abundant *Halomonhystera disjuncta* from HMMV with the shallow-water species complex of *H. disjuncta* was investigated through molecular analyses (CHAPTER 6). Genetic variation was screened using the mitochondrial cytochrome oxidase c subunit 1 (COI) gene, and complementary nucleotide sequencing of the small (18S) subunit gene of the ribosome and the expansion segment (D2-D3) situated at the 5' end of the large subunit gene (28S). Apparently, the dense *H. disjuncta* population recorded from the bacterial mats at HMMV consisted of (at least) one new cryptic species that was not yet detected in the North Sea. These results may point to an exchange route between the shallow-water and deep-sea habitats for nematode species. Given the limited oxygen and highly sulphidic nature of seep sediments, only shallow-water species which are tolerant enough to environmental stress will be able to explore this deep-sea niche.

It can be hypothesised that *H. disjuncta* also will occur along the shallow Norwegian coasts and colonised the more offshore located HMMV by making advantage of floating seaweeds. To determine how long ago this possible colonisation occurred, further investigation of the nematode communities at the nearby intertidal habitats is necessary. Marine speciation is thought to be rather quick and may occur at small spatial scales (Rocha-Olivares et al. 2001). Based on thousands of orthologous[2] groups of genes, Cutter (2008) proposed divergence times of <24 million years for the nematode genus *Caenorhabditis*. Therefore, if the cryptic species of *H. disjuncta* found at HMMV is not present in the Norwegian intertidal habitats but diverged from an ancestral coloniser, the colonisation should have taken place hundred thousands or even millions of years ago. On the contrary, if the similar cryptic species is also present along the Norwegian coasts, the colonisation could have happened more recently as nematodes can reproduce very efficiently (generation time of 8 days for *H. disjuncta,* Vranken et al. 1988).

It is obvious that the biodiversity of marine nematodes may often be underestimated due to the presence of morphologically cryptic species complexes. High-throughput techniques such as DNA barcoding may be a helpful tool to explore and screen biological diversity for cosmopolitan nematode

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[2] Homologous sequences are orthologous if they were separated by a speciation event. Orthologous genes are genes in different species that are similar to each other because they originated from a common ancestor.

species which are morphologically identical. Molecular analyses should therefore ideally be integrated with morphological and taxonomical identifications during biodiversity surveys in the world-wide distributed environments.

At the same time, also the dispersal capabilities of nematodes from coastal areas are likely to be higher than expected. In contrast with the general trend of typical, dominant seep-associated megafaunal groups (siboglinids, vesicomyid clams, mytilid mussels), the meiofauna shows no specific adapted seep species but seems to have a strong connection with coastal habitats. Moreover, in this thesis, each deep-sea seep site appeared to support completely different, dominant nematode species. Considering these dominant meiofaunal species, seep sites in the deep sea exhibit a higher dissimilarity among each other than the dissimilarity between deep-sea and shallow-water reduced environments. Overall, the meiobenthos observed in reduced environments can be considered as isocommunities (Thorson 1957, Por 1964); geochemical conditions (e.g., oxygen and sulphide concentration) function as the important key factor in structuring and characterising the composition and diversity of the associated meiobenthic communities. Consequently, marine sulphide rich ecosystems will often harbour similar worldwide distributed nematode species.

5. RELEVANCE AND FUTURE RESEARCH RECOMMENDATIONS

This thesis is the first comprehensive study dealing with meiofaunal and nematode communities associated with different cold seeps in the Atlantic deep sea. Different habitats distinguished by distinct biogeochemical conditions revealed to be a key factor in structuring the densities and diversity of the seep nematode assemblages. Much research on cold seep ecosystems is focused on productivity by symbioses between prokaryotes and eukaryotes. From the bacterial mats at HMMV an equally close relationship is described between free-living chemosynthetic bacteria and nematodes, explaining the unusual success of this single metazoan species secured with parental investment in brood survival and development. Fatty acid and stable carbon isotope analyses of two dominant metazoan meiofaunal species provided important information concerning the trophic relations and specialisation within these chemosynthesis-based communities. Molecular analyses of the nematode *Halomonhystera disjuncta* unraveled the phylogenetic relationship with the shallow-water *H. disjuncta* species complex, and indicated the connection with shallow-water habitats.

Cold seep ecosystems play an important role in regulating the natural environment by acting as filters for methane and sulphide. It is important that the diverse seep sites scattered along the deep ocean

margins are studied together with the potential damage to them due to human exploitation, pollution and global change. Also, a significant part of this deep-ocean area lies within economic zones. The deep-ocean margins are of direct interest for the exploitation of biological, energy and mineral resources. Already, the exploitation of these areas has caused significant damage to the fragile ecosystems such as to the deep-sea corals, as the results of trawling by the fishing industry, oil and gas exploration and the dumping of pollutants. Finally, despite the many inherent difficulties in evaluating diversity patterns and species richness in deep-sea ecosystems, there is clearly great interest in producing accurate diversity estimates and in understanding processes that regulate diversity in the deep sea. Therefore, one of the main outcomes of the HERMES project will be to use the new scientific knowledge obtained about cold seep systems in order to produce new biodiversity maps for deep ocean environments.

As a general conclusion of this thesis it can be stated that within the rather uniform deep-sea environment, cold seeps function as important sources of small-scale habitat patchiness that significantly influence the diversity and density of meiobenthic communities. Therefore, these reduced deep-sea ecosystems merit conservation, because they sustain a high variability of different meiobenthic, and in particular nematode, communities and subsequently adding a high beta-diversity value to the regional deep-sea diversity. Whereas our knowledge on reducing ecosystems in the deep sea is increasing, yet, there are still many unanswered questions to be resolved which need further investigation, new strategies of in situ work and further interdisciplinary research.

Although this thesis provided new and detailed information about the meiobenthic communities associated with deep-sea cold seeps in the Atlantic, it has also revealed the need for investigating other deep-sea seeps to evaluate the hypothesis that each isolated seep harbours a unique meiobenthic assemblage strongly dominated by only one or two species. Investigating also additional shallow-water seeps would yield information on the generality of our results for the meiobenthic diversity and density patterns at seeps. Examining the meiofauna inhabiting other reduced marine habitats (e.g., hydrothermal vents, whale falls) could be particularly relevant for understanding the composition, diversity and adaptation of the 'thiobios' in the sulphide biome. Furthermore intensive morphological studies combined with molecular research would give insight in the phylogenetic and phylogeographic relationships of the thiobiotic meiofauna in both shallow-water as deep-sea habitats.

Environmental factors that cause changes in deep-sea ecosystems operate over a wide range of spatial scales from km's (e.g., topographic submarine structures, water bottom currents) to cm's (e.g., organic content, macrofaunal communities). In deep-sea cold seeps, in particular the small-scale (cm's)

variation in biogeochemical conditions is very pronounced and has proven to significantly structure the metazoan meiobenthic communities. These fine-scale patterns may be better detected if samples for both biological and geochemical (pore water profiles of oxygen, sulphide and methane) analyses were taken simultaneously by a video-guided ROV. Interdisciplinary investigations including micro-, meio- and macrobiological studies combined with geochemical measurements seem essential to gain more understanding of the impact of biotic and abiotic variables on the meiofaunal seep assemblages.

It has been frequently been concluded that thiobiotic meiofauna directly or indirectly utilises the rich stock of sulphur bacteria which develops preferably in the oxic/sulphidic chemocline (Giere 1993). Several species, such as demonstrated for *Halomonhystera disjuncta* at HMMV (CHAPTER 5), probably graze on the bacterial mats of their environment. Other thiobiotic species evolved symbioses with bacteria. All these organisms take advantage of the microbial potential to metabolise reduced substances. However, we currently know little about the physiological or morphological adaptations of the seep dominant nematodes to resist to the unusually high levels of reduced compounds. It is therefore advisable to collect specimens from seep sites and try to cultivate a population in the laboratory. If we succeed in cultivating deep-sea seep nematodes, they could be subjected to rigorously developed tolerance experiments to test the nematode's resilience to different levels and combinations of hypoxic and sulphidic conditions.

To date little is known about the importance of meiofauna in transferring the chemosynthetic derived carbon to higher trophic levels at cold seeps. The application of stable isotope (carbon and nitrogen) and lipid analyses of dominant meiobenthic species at different seep sites may provide a better idea of the trophic position of this group in reduced habitats. At the same time, it would be interesting to perform similar analyses of the associated macro- and megafauna to evaluate the potential grazing pressure on the meiofauna. Additional techniques such as molecular sequencing of gut contents, gut content observations and the use of inorganic isotopic tracers ($^{14}CO_2$, $^{13}CO_2$, $^{35}SO_4$) could be employed to further examine the food web structure in these ecosystems. Finally, in situ feeding experiments could be conducted to follow the uptake of labelled potential food sources by the meiobenthos.

Marine nematodes are worldwide distributed and represent the most prominent members of the smaller-sized animals. Because of their infaunal life they have a direct link with the sediment and with the processes that occur immediately above the sediments. Moreover, this group may function as an important link in the carbon flow from the primary producers to the higher trophic groups. Increasing nematode densities and biomass often point out the presence of an abundant food resource and/or high

food quality. The use of chemosynthesis in reduced sediments can result in significant higher nematode biomasses. Nematodes can therefore be a useful tool to describe and compare diversity, density, dependency on chemoautotrophic pathways, and specificity of faunal communities in the reduced environments at continental margins.

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APPENDIX I
NEMATODE GENUS LIST

Classification up to family level after De Ley & Blaxter (2003); below family level after Lorenzen (1994).

Indication of study area:
[1]: Darwin Mounds (Chapter 2)
[2]: Gulf of Guinea (Chapter 3)
[3]: Håkon Mosby Mud Volcano (Chapter 4, 6, 7)
[4]: Nyegga (Chapter 6, 7)
[5]: Storegga (Chapter 6, 7)

PHYLUM NEMATODA POTTS 1932
CLASS ENOPLEA INGLIS 1983
SUBCLASS ENOPLIA PEARSE 1942
ORDER ENOPLIDA FILIPJEV 1929
Suborder Enoplina Chitwood and Chitwood 1937
Superfamily Enoploidea Dujardin 1845
Family Enoplidae Dujardin 1845
Enoplus Dujardin 1845 [1,3,4,5]
Family Thoracostomopsidae Filipjev 1927
Subfamily Trileptiinae Gerlach and Riemann 1974
Trileptium Cobb 1933 [4]
Subfamily Enoplolaiminae de Coninck 1965
Enoploides Ssaweljev 1912 [1,2,3,4]
Enoplolaimus de Man 1893 [3,4,5]
Fenestrolaimus Filipjev 1927 [1,2,3,4,5]
Mesacanthion Filipjev 1927 [2,4]
Mesacanthoides Wieser 1953 [1,3,4]
Paramesacanthion Wieser 1953 [2,3,4]
Family Anoplostomatidae Gerlach and Riemann 1974
Subfamily Anoplostomatinae Gerlach and Riemann 1974
Anoplostoma Bütschli 1874 [1,2,3,4,5]
Subfamily Chaetonematinae Gerlach and Riemann 1974
Chaetonema Filipjev 1927 [1,4]
Family Phanodermatidae Filipjev 1927
Subfamily Crenopharyngidae Platonova 1976
Crenopharynx Filipjev 1934 [1,2,4,5]
Micoletzkyia Ditlevsen 1926 [2,4]
Phanodermopsis Ditlevsen 1926 [2,3,4]
Family Anticomidae Filipjev 1918
Anticoma Bastian 1865 [1,2,3,4]
Ododanticoma Platonova 1976 [3,4,5]
Paranticoma Micoletzky 1930 [1,4]
Suborder Trefusiina Siddiqi 1983

Superfamily Trefusioidea Gerlach 1966
Family Trefusiidae Gerlach 1966
Subfamily Trefusiinae Gerlach 1966
Rhabdocoma Cobb 1920 [1,2,3,4,5]
Trefusia de Man 1893 [1,4]
Trefusialaimus Riemann 1974 [1,2,3,4]
Suborder Oncholaimina de Coninck 1965
Superfamily Oncholaimoidea Filipjev 1916
Family Oncholaimidae Filipjev 1916
Subfamily Adoncholaimininae
Adoncholaimus Filipjev 1918 [3,4]
Subfamily Oncholaimellinae de Coninck 1965
Oncholaimellus de Man 1890 [2,4]
Viscosia de Man 1890 [1,2,3,4,5]
Subfamily Oncholaiminae Filipjev 1916
Prooncholaimus Micoletzky 1924 [1,4]
Subfamily Pontonematinae Gerlach and Riemann 1974
Pontonema Leidy 1855 [1,4]
Family Enchelidiidae Filipjev 1918
Bathyeurystomina Lambshead and Platt 1979 [3,4]
Belbolla Andrássy 1973 [4]
Eurystomina Filipjev 1921 [2,4]
Pareurystomina Micoletzky 1930 [1,4]
Symplocostoma Bastian 1865 [3,4]
Suborder Ironina Siddiqi 1983
Superfamily Ironoidea de Man 1876
Family Ironidae de Man 1876
Subfamily Thalassironinae Andrássy 1976
Dolicholaimus de Man 1888 [2,4]
Syringolaimus de Man 1888 [1,2,3,4,5]
Thalassironus de Man 1889 [1,2,3,4]
Trissonchulus Cobb 1920 [2,4]
Famila Leptosomatidae Filipjev 1916
Subfamily Platycominae Platonova 1976
Platycoma Cobb 1894 [1,2,4]
Subfamily Leptosomatinae Filipjev 1916
Leptosomatum Bastian 1865 [1,3,4]
Subfamily Synonchinae Platonova 1970
Synonchus Cobb 1894 [1,4]
Subfamily Thoracostomatinae de Coninck 1965
Pseudocella Filipjev 1927 [1,3,4]
Subfamily Cylicolaiminae Platonova 1970
Cylicolaimus de Man 1889 [1,4]
Family Oxystominidae Chitwood 1935
Subfamily Oxystomininae Chitwood 1935
Litinium Cobb 1920 [1,2,3,4,5]
Nemanema Cobb 1920 [1,2,3,4]
Oxystomina Filipjev 1921 [1,2,3,4,5]
Thalassoalaimus de Man 1893 [1,2,3,4]
Wieseria Gerlach 1956 [1,2,3,4,5]

Subfamily Halalaiminae de Coninck 1965
Halalaimus de Man 1888 [1,2,3,4,5]
Suborder Tripyloidina de Coninck 1965
Superfamily Tripyloidoidea Filipjev 1928
Family Tripyloididae Filipjev 1928
Gairleanema Warwick and Platt 1973 [1,2,4]
Tripyloides de Man 1886 [1,2,4]
Order Tripylonchida Cobb 1920
Suborder Tobrilina Tsalolichin 1976
Superfamily Tobriloidea de Coninck 1965
Family Rhabdodemaniidae Filipjev 1934
Rhabdodemania Baylis and Daubney 1926 [1,2,3,4,5]
Family Pandolaimidae Belogurov 1980
Pandolaimus Allgén 1929 [2,4]

CLASS CHROMADOREA INGLIS 1983
SUBCLASS CHROMADORIA PEARSE 1942
ORDER DESMOSCOLECIDA FILIPJEV 1929
Suborder Desmoscolecina Filipjev 1934
Superfamily Desmoscolecoidea Shipley 1896
Family Desmoscolecidae Shipley 1896
Subfamily Desmoscolecinae Shipley 1896
Desmoscolex Claparède 1863 [1,2,3,4,5]
Greeffiella Cobb 1922 [1,2,3,4]
Subfamily Meyliinae de Coninck 1965
Gerlachius Andrássy 1976 [1,2,3,4]
Noffsingeria Decraemer and Jensen 1982 [1,4]
Subfamily Tricominae Lorenzen 1969
Paratricoma Gerlach 1956 [1,4]
Tricoma Cobb 1894 [1,2,3,4,5]
Family Cyartonematidae Tchesunov 1990
Cyartonema Cobb 1920 [1,2,3,4,5]
ORDER CHROMADORIDA FILIPJEV 1929
Suborder Chromadorina Filipjev 1929
Superfamily Chromadoroidea Filipjev 1917
Family Chromadoridae Filipjev 1917
Subfamily Spilipherinae Filipjev 1918
Acantholaimus Allgén 1933 [1,2,3,4,5]
Spiliphera Bastian 1865 [1,4]
Subfamily Chromadorinae Filipjev 1917
Atrochromadora Wieser 1959 [3,4]
Chromadorella Filipjev 1918 [1,4]
Chromadorina Filipjev 1918 [1,3,4,5]
Prochromadora Filipjev 1922 [1,3,4]
Prochromadorella Micoletzky 1924 [3,4]
Subfamily Euchromadorinae Gerlach and Riemann 1973
Actinonema Cobb 1920 [1,2,3,4,5]
Endeolophos Boucher 1976 [1,4]
Rhips Cobb 1920 [1,4]
Steineridora Inglis 1969 [1,4]

Subfamily Hypodontolaiminae de Coninck 1965

Chromadorita Filipjev 1922 [1,2,3,4,5]

Dichromadora Kreis 1929 [1,2,3,4,5]

Innocuonema Inglis 1969 [1,2,3,4]

Neochromadora Micoletzky 1924 [1,2,3,4,5]

Parachromadorita Blome 1974 [3,4]

Spilophorella Filipjev 1917 [1,3,4,5]

Family Neotonchidae Wieser and Hopper 1966

Comesa Gerlach 1956 [1,2,3,4,5]

Filitonchus Platt 1982 [1,2,3,4]

Gomphionchus Platt 1982 [1,2,4]

Neotonchus Cobb 1933 [1,2,3,4,5]

Family Cyatholaimidae Filipjev 1918

Subfamily Pomponematinae Gerlach and Riemann 1973

Kraspedonema Gerlach 1954 [2,3,4]

Minolaimus Vitiello 1970 [1,2,4]

Nannolaimoides Ott 1972 [1,4]

Nannolaimus Cobb 1920 [2,4]

Pomponema Cobb 1971 [1,2,3,4,5]

Subfamily Paracanthonchinae de Coninck 1965

Acanthonchus Cobb 1920 [1,2,4]

Paracanthonchus Micoletzky 1924 [1,2,3,4,5]

Paracyatholaimoides Gerlach 1953 [1,2,3,4]

Paracyatholaimus Micoletzky 1922 [3,4,5]

Subfamily Cyatholaiminae Filipjev 1918

Cyatholaimus Bastian 1865 [1,4]

Longicyatholaimus Micoletzky 1924 [1,2,3,4,5]

Marylynnia Hopper 1977 [1,2,3,4,5]

Metacyatholaimus Stekhoven 1942 [1,2,3,4]

Paralongicyatholaimus Stekhoven 1950 [1,2,3,4]

Praeacanthonchus Micoletzky 1924 [1,2,3,4,5]

Family Selachinematidae Cobb 1915

Choanolaimus de Man 1880 [1,2,3,4]

Demonema Cobb 1894 [1,3,4]

Gammanema Cobb 1920 [1,2,4]

Halichoanolaimus de Man 1886 [1,2,3,4,5]

Latronema Wieser 1954 [1,4]

Richtersia Steiner 1916 [1,2,4,5]

Synonchiella Cobb 1933 [1,4]

ORDER DESMODORIDA DE CONINCK 1965

Suborder Desmodorina de Coninck 1965

Superfamily Desmodoroidea Filipjev 1922

Family Desmodoridae Filipjev 1922

Subfamily Desmodorinae Filipjev 1922

Desmodora de Man 1889 [1,2,3,4]

Paradesmodora Stekhoven 1950 [1,2,4]

Psammonema Verschelde and Vincx 1995 [1,2,3,4]

Subfamily Spiriniinae Gerlach and Murphy 1965

Chromaspirinia Filipjev 1918 [1,2,3,4]

Parallelocoilas Boucher 1975 [1,2,4]

Polysigma Cobb 1920 [1,4]
Spirinia Gerlach 1963
    Subgen. *Spirina* Gerlach 1963 [1,2,3,4,5]
    Subgen. *Perspiria* Wieser and Hopper 1967 [1,2,3,4]
Subfamily Stilbonematinae Cobb 1936
Catanema Cobb 1920 [1,4]
Eubostrichus Greeff 1869 [1,4]
Leptonemella Cobb 1920 [1,2,4]
Stygodesmodora Blome 1982 [2,4,5]
Subfamily Molgolaiminae Jensen 1978
Molgolaimus Ditlevsen 1921 [1,2,3,4,5]
Family Epsilonematidae Steiner 1927
Subfamily Epsilonematinae Steiner 1927
Epsilonema Steiner 1927 [1,2,4]
Metepsilonema Steiner 1927 [3,4]
Family Draconematidae Filipjev 1918
Draconematidae gen. [1,3,4]
Superfamily Microlaimoidea Micoletzky 1922
Family Microlaimidae Micoletzky 1922
Aponema Jensen 1978 [3,4]
Bathynox Bussau and Vopel 1999 [1,2,4]
Calomicrolaimus Lorenzen 1976 [1,4]
Microlaimus de Man 1980 [1,2,3,4,5]
Spirobolbolaimus Soetaert and Vincx 1988 [1,2,3,4,5]
Family Aponchiidae Gerlach 1963
Synonema Cobb 1920 [1,4]
Family Monoposthiidae Filipjev 1934
Monoposthia de Man 1889 [1,4]
ORDER MONHYSTERIDA FILIPJEV 1929
  Suborder Monhysterina de Coninck and Schuurmans Stekhoven 1933
    Superfamily Monhysteroidea de Man 1876
    Family Monhysteridae de Man 1876
Diplolaimelloides Meyl 1954 [1,4]
Halomonhystera Andrássy 2006 [1,3,4,5]
Monhystera Bastian 1865 [1,2,3,4,5]
Monhystrella Cobb 1918 [2,3,4,5]
Paramonhystera Wieser 1956 [1,2,3,4,5]
Thalassomonhystera Jacobs 1987 [1,2,3,4,5]
Superfamily Sphaerolaimoidea Filipjev 1918
Family Xyalidae Chitwood 1951
Ammotheristus Lorenzen 1977 [1,2,3,4]
Amphimonhystera Allgén 1929 [1,2,3,4]
Amphimonhystrella Timm 1961 [2,3,4,5]
Cobbia de Man 1907 [1,2,3,4]
Daptonema Cobb 1920 [1,2,3,4,5]
Elzalia Gerlach 1957 [1,2,4]
Gnomoxyala Lorenzen 1977 [1,2,4]
Gonionchus Cobb 1920 [1,4]
Linhystera Juario 1974 [3,4]
Manganonema Bussau 1993 [2,3,4]

Metadesmolaimus Stekhoven 1935 [1,2,3,4,5]
Omicronema Cobb 1920 [1,4]
Promonhystera Wieser 1956 [1,2,3,4]
Retrotheristus Lorenzen 1977 [1,2,3,4]
Rhynchonema Cobb 1920 [1,2,3,4]
Scaptrella Cobb 1917 [2,4]
Stylotheristus Lorenzen 1977 [1,2,3,4]
Theristus Bastian 1865 [2,3,4,5]
Trichotheristus Wieser 1956 [1,4]
Valvaelaimus Lorenzen 1977 [1,2,4]
Xenolaimus Cobb 1920 [2,4]
Xyala Cobb 1920 [1,4]
Family Sphaerolaimidae Filipjev 1918
    Subfamily Sphaerolaiminae Filipjev 1918
        Doliolaimus Lorenzen 1966 [1,2,3,4]
        Sphaerolaimus Bastian 1865 [1,2,3,4,5]
    Subfamily Parasphaerolaiminae Lorenzen 1978
        Parasphaerolaimus Ditlevsen 1918 [1,4,5]
    Subfamily Metasphaerolaiminae Gourbault and Foucher 1982
        Metasphaerolaimus Gourbault and Foucher 1982 [3,4]
Suborder Linhomoeina Andrássy 1974
    Superfamily Siphonolaimoidea Filipjev 1918
        Family Siphonolaimidae Filipjev 1918
            Siphonolaimus de Man 1893 [1,4]
        Family Linhomoeidae Filipjev 1922
            Subfamily Desmolaiminae Schneider 1926
                Anticyathus Cobb 1920 [2,3,4]
                Desmolaimus de Man 1880 [2,3,4]
                Megadesmolaimus Wieser 1954 [1,2,4]
                Metalinhomoeus de Man 1888 [1,2,3,4,5]
                Terschellingia de Man 1888 [1,2,3,4,5]
            Subfamily Eleutherolaiminae Gerlach and Riemann 1973
                Eleutherolaimus Filipjev 1922 [1,4]
                Eumorpholaimus Schulz 1932 [3,4]
            Subfamily Linhomoeinae Filipjev 1922
                Didelta Cobb 1920 [2,3,4]
                Disconema Filipjev 1918 [1,2,3,4]
                Linhomoeus Bastian 1865 [2,3,4]
                Paralinhomoeus de Man 1907 [1,2,3,4,5]
ORDER ARAEOLAIMIDA DE CONINCK AND SCHUURMANS STEKHOVEN 1933
    Superfamily Axonolaimoidea Filipjev 1918
        Family Axonolaimidae Filipjev 1918
            Ascolaimus Ditlevsen 1919 [1,2,4]
            Axonolaimus de Man 1889 [1,2,3,4]
            Odontophora Bütschli 1874 [1,4,5]
            Parodontophora Timm 1963 [2,4]
        Family Comesomatidae Filipjev 1918
            Subfamily Comesomatinae Filipjev 1918
                Comesoma Bastian 1865 [3,4]
                Comesomoides Gourbault 1980 [1,2,4]

Paracomesoma Hope and Murphy 1972 [1,4]

Subfamily Sabatieriinae Filipjev 1934

Cervonema Wieser 1954 [1,2,3,4,5]

Laimella Cobb 1920 [1,2,3,4,5]

Pierrickia Vitiello 1970 [1,2,3,4,5]

Sabatieria Rouville 1903 [1,2,3,4,5]

Setosabatieria Platt 1985 [1,2,4]

Subfamily Dorylaimopsinae de Coninck 1965

Dorylaimopsis de Coninck 1965 [1,2,3,4]

Paramesonchium Hopper 1967 [1,3,4,5]

Vasostoma Wieser 1954 [1,4]

Family Diplopeltidae Filipjev 1918

Subfamily Diplopeltinae Filipjev 1918

Araeolaimus de Man 1888 [1,2,4,5]

Campylaimus Cobb 1920 [1,2,3,4,5]

Diplopeltis Cobb in Styles and Hassal 1905 [1,4]

Diplopeltula Gerlach 1950 [1,2,3,4,5]

Morlaixia Vincx and Gourbault 1989 [1,2,4]

Southerniella Allgén 1932 [1,2,3,4,5]

Family Coninckiidae Lorenzen 1981

Coninckia Gerlach 1956 [1,2,3,4]

ORDER PLECTIDA MALAKHOV 1982

Superfamily Leptolaimoidea Örley 1980

Family Leptolaimidae Örley 1980

Subfamily Leptolaiminae Örley 1980

Alaimella Cobb 1920 [1,2,4]

Anomonema Hopper 1963 [1,4]

Antomicron Cobb 1920 [1,2,4]

Cricolaimus Southern 1914 [1,4]

Dagda Southern 1914 [1,2,4,5]

Diodontolaimus Southern 1914 [3,4]

Leptolaimoides Vitiello 1971 [2,3,4,5]

Leptolaimus de Man 1876 [1,2,3,4,5]

Stephanolaimus Ditlevsen 1918 [1,4]

Subfamily Camacolaiminae Micoletzky 1924

Camacolaimus de Man 1889 [1,2,3,4,5]

Procamacolaimus Gerlach 1954 [1,4]

Family Aegialoalaimidae Lorenzen 1981

Aegialoalaimus de Man 1907 [1,2,3,4,5]

Diplopeltoides Gerlach 1962 [1,2,3,4,5]

Family Paramicrolaimidae Lorenzen 1981

Paramicrolaimus Wieser 1954 [1,4]

Superfamily Ceramonematoidea Cobb 1933

Family Tarvaiidae Lorenzen 1981

Tarvaia Allgén 1934 [1,2,3,4]

Family Ceramonematidae Cobb 1933

Ceramonema Cobb 1920 [1,2,4]

Dasynemoides Chitwood 1936 [1,4]

Metadasynemella de Coninck 1942 [1,2,4]

Metadasynemoides Haspeslagh 1973 [1,4]

Pselionema Cobb 1933 [1,2,3,4,5]
Pterygonema Gerlach 1954 [1,2,3,4,5]
Family Tubolaimoididae Lorenzen 1981
Chitwoodia Gerlach 1956 [1,4]
Superfamily Haliplectoidea
Family Haliplectidae Chitwood 1951
Setoplectus Vitiello 1971 [2,3,4]

APPENDIX II

CURRICULUM VITAE Saskia Van Gaever

(as on 18 September 2008)

CURRICULUM VITAE

PERSONALIA

Name:	Saskia Van Gaever
Birth place and date:	Ghent – 07/09/1979
Nationality:	Belgian
Civil state:	married to Johan Van de Veire
Children:	Brent (30/09/2005)
	Jade (30/04/2007)

EDUCATION

1991–1997 Secondary education, Sciences-Mathematics, OLVO-Instituut, Oostakker

1997–2001 Licentiate in Biology, Ghent University, Biology Department
Undergraduate thesis: "Community analysis of macrofauna associated with cold-water coral reefs in the NE Atlantic" (in the framework of the international research program "Atlantic Coral Ecosystem Study")
Promotor: Prof. Dr. Ann Vanreusel, Ghent University, Marine Biology Section

2001–2006 Doctoral Training in Science (Biology), Ghent University, Biology Department

SCIENTIFIC CAREER

2001–2002 Doctoral research in framework of ACES-project: ecological study of the community structure of meiobenthos, in particular Nematoda, associated with sediments of the Darwin Mounds (NE Atlantic).
2002–2008 Assistant Prof. Dr. Ann Vanreusel, Marine Biology Section, Biology Department, Ghent University

SCIENTIFIC ACTIVITIES

A. Publications

Van Gaever S, Vanreusel A, Hughes JA, Bett BJ (2004) The macro- and micro-scale patchiness of meiobenthos associated with the Darwin Mounds (north-east Atlantic). *Journal of the Marine Biological Association of the UK* 84: 547–556.

Van Gaever S, Moodley L, de Beer D, Vanreusel A (2006) Meiobenthos at the Arctic Håkon Mosby Mud Volcano with a parental-caring nematode thriving in sulphide-rich sediments. *Marine Ecology Progress Series* 321: 143–155.

Sarrazin J, Walter C, Sarradin P-M, Brind'Amour A, Desbruyères D, Briand P, Fabri M-C, Van Gaever S, Vanreusel A, Bachraty C, Thiébaut E (2006) Community structure and temperature dynamics within a mussel assemblage on the Southern East Pacific Rise. *Cahiers de Biologie Marine* 47: 483–490.

Van Gaever S, Galéron J, Sibuet M, Vanreusel A (accepted) Deep-sea habitat heterogeneity influence on meiofaunal communities in the Gulf of Guinea. *Deep-Sea Research II*.

Van Gaever S, Moodley L, Pasotti F, Houtekamer M, Middelburg JJ, Danovaro R, Vanreusel A (submitted) Trophic specialisation of metazoan meiofauna at the Håkon Mosby Mud Volcano: fatty acid biomarker isotope evidence. *Marine Biology*.

Van Gaever S, Pasotti F, Olu K, Derycke S, Vanreusel A (submitted) Meiofaunal communities at cold seeps along the Norwegian margin: influence of habitat heterogeneity and evidence for connection with shallow-water habitats. *Deep-Sea Research I*.

Van Gaever S, Raes M, Pasotti F, Vanreusel A (submitted) Spatial scale and habitat-dependent diversity patterns in nematode communities in three seepage related sites along the Norwegian margin. *Marine Ecology*.

B. Workshops

Participation to 2nd ACES Workshop, 17–18 March 2001, Dunstaffnage, Scotland.

C. Congresses/Symposia

Oral Presentations

Van Gaever S, Vanreusel A. Meiobenthos associated with mud mounds (Darwin Mounds) in the NE Atlantic. 9th Benelux Congress of Zoology: Adaptation and Constraint, 8–9 November 2002, Antwerp University, Belgium. Book of Abstracts p. 219.

Van Gaever S, Vanreusel A, Moodley L. Meiobenthos colonising extreme environments at the active seeping Håkon Mosby Mud Volcano (1250 m, Barents Sea). Twelfth International Meiofauna Conference (TWIMCO), 11–16 July 2004, Ravenna, Italy. Book of Abstracts p. 78.

Van Gaever S, Vanreusel A, Moodley L, de Beer D, Galéron J. Meiobenthos at deep-sea cold seeps: diversity, adaptation and trophic position. 11th International Deep-Sea Biology Symposium, 9–14 July 2006, Southampton, UK. Book of Abstracts p. 83.

Van Gaever S, Moodley L, Pasotti F, Willems A, Vanreusel A. Meiofauna communities associated with cold seeps: understanding their function, adaptation and origin. European Geoscience Union: General Assembly, 14–18 April 2008, Vienna, Austria. CD Rom with Geophysical Research Abstracts.

Poster Presentations

Van Gaever S, Vanreusel A. Community analysis of macrofauna associated with cold water corals in the NE Atlantic. 8th Benelux Congress of Zoology: The animal in zoology: Tool or Target, 23–24 November 2001, Nijmegen, The Netherlands. Book of Abstracts p. 81.

Van Gaever S, Vanreusel A. Community analysis of macrofauna associated with cold water corals in the NE Atlantic. 2de VLIZ Jongerendag, 13 March 2002, Bruges, Belgium.

De Backer A, Vanreusel A, Van Gaever S. The biodiversity of the macrofauna associated with the cold water coral *Lophelia* in the Porcupine Seabight. 9th Benelux Congress of Zoology: Adaptation and Constraint, 8–9 November 2002, Antwerp University, Belgium. Book of Abstracts p. 67.

Van Gaever S, Vanreusel A, Moodley L. Mass aggregations of a new viviparous nematode species colonizing sulfidic sediments at the Håkon Mosby Mud Volcano. Doctoraatssymposium Faculteit Wetenschappen, 3 May 2005, Ghent, Belgium. Book of Abstracts p. 117.
Award Best Biology Poster

D. Sampling campaigns

Campaign RV Belgica 02/11 Cruise (22 April–1 May 2002, Meriadzek Terrace, northern Gulf of Biscay): "Spatial distribution and biodiversity patterns of the hyperbenthos along NE Atlantic continental margins"

Campaign RV Belgica CADIPOR 02/12 Cruise (17–28 May 2002, Gulf of Cadiz – El Arrach mud volcano field, Atlantic margin of Morocco): "Meiobenthos associated with mud volcanoes off Morocco"

Campaign RV Atalante BIOZAIRE 3 (12 December 2003–9 January 2004, Atlantic margin of Gabon, Congo, Angola): "The meiobenthic communities associated with the Congo Channel system and surrounding habitats". Rapport de campagne BIOZAIRE 3 pp. 81–82.

Campaign RV PourquoiPas? VICKING (20 May–11 June 2006, HMMV, Barentszee): "Meiobenthic communities and environmental conditions at cold seeps of the Norwegian margin". Vicking cruise report pp. 185–187.

EDUCATIONAL ACTIVITIES

A. Assistence Practicals

2001–2002 Practical Vertebrata – 2nd Candidature Biology (Prof. Dr. D. Adriaens) (8h)

2001–2002 Practical Invertebrates – 1ste Licence Biology (Prof. Dr. Magda Vincx) (4h)

2002–2005 Practical Biology – 1ste Candidature Veterinary (Prof. Dr. Gaetan Borgonie) (24h)

2002–2006 Main responsible Training Course "Invertebrates" Wimereux (France) – 1st Licence Biology (Prof. Dr. Ann Vanreusel) (7 days)

2002–2007 Main responsible Practical Statistics – 2nd Licence Biology (Prof. Dr. Ann Vanreusel) (40h)

2003–2006 Practical Invertebrates – 2nd Licence Biology (Prof. Dr. Magda Vincx) (4h)

2005–2007 Practical Biodiversity of Invertebrates – 2nd Bachelor Biology (Prof. Dr. Magda Vincx) (12h)

2006–2007 Main responsible Practical Biostatistics – Master Nematology (Prof. Dr. Ann Vanreusel) (12h)

2006–2008 Main responsible Practical Biostatistics – 3rd Bachelor Biology (Prof. Dr. Ann Vanreusel) (40h)

B. Assistance Undergraduate and Master Theses

2004–2005 Agata Gogul. Meiobenthic communities colonising a seep in the South Atlantic Ocean. Master thesis (Marine and Lacustrine Sciences)

2006–2007 Francesca Pasotti. Håkon Mosby Mud Volcano meiofauna: micro-scale distribution and possible endosymbiosis. Master thesis.