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

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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 the different sub-habitats (centre, microbial mats, Pogonophora 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 (11 000 ind. 10 cm^{-2}) of 1 nematode species related to *Geomonhystera 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 the 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 suggests 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.

KEY WORDS: Deep-sea cold methane seep · Sulphidic environment · Meiobenthos · Nematodes · *Geomonhystera disjuncta* · Ovoviviparity · Trophoecology · Carbon isotopes

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 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 macrofaunal species often consist 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 numbers of species that proliferate in seeps seem to flourish on chemosynthetically derived organic matter (Levin & Michener 2002).

Meiofaunal studies from deep-sea cold-seep areas are generally scarce. Some of these studies documented an increased meiofaunal density or volume compared to nearby control sites (Buck & 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 1 to 2 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 & Ohta 1990). In 2 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 unequivocal 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 & 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. (2005) presented the first numbers on the meiobenthic abundances at the HMMV and identified nematodes as the most abundant metazoan taxon in 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 in 4 sub-habitats of HMMV differing strongly in sediment geochemistry (de Beer et al. 2006). Special attention was paid to microbial mat sediments that often support enhanced densities of fauna in otherwise food-limited, deep-sea sediments (Levin 2005, Soltwedel et al. 2005). The importance of chemosynthetic nutrition was estimated through sediment bulk and labile organic matter carbon isotopes, as well as through the natural carbon isotope signatures of the dominant species, which 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 the dominant species of microbial mats was examined using light and electron microscopy.

MATERIALS AND METHODS

Study area. HMMV is a cold seep, located on the continental slope off northern Norway at a water depth of 1280 m (Fig. 1). It has a relief of 8 to 10 m in height and a diameter of about 1.5 km. HMMV has a concentric structure with a mud-oozing centre that 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. 2005), the seafloor has a complex topography of hills and depressions, and is covered by extensive microbial mats (grey and white) of large chemoautotrophic bacteria. According to Pimenov et al. (2000), grey microbial mats are constituted of 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 large 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 Pogonophora (Figs. 1 & 2) (Vogt et al. 1997, Gebruk et al. 2003, de Beer et al. 2006). The different sub-habitats, each with typical environmental conditions, are displayed in Fig. 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 2 species of small Pogonophora, *Sclerolinum contortum* and *Oligobrachia haakonmosbiensis*, both harbouring symbiotic, autotrophic, methane-oxidising bacteria and living in colonies covering 100s of square metres of the seafloor (Gebruk et al. 2003, Soltwedel et al. 2005). Other organisms which form associations with the pogono-

phorans 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).

Sampling and sample processing. Sediment samples were collected during the cruise ARKTIS XIX/3b aboard the German RV 'Polarstern' from June to 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, Pogonophora field, outer rim), 3 replicate tubes were taken from 2 or 3 different deployments. As a control, 2 sites located outside HMMV (10s of kilometres) were sampled (Figs. 1 & 2, Table 1). For both the microbial mats and the Pogonophora field, replicates were selected based on the presence, respectively, of bacteria (*Beggiatoa*) or Pogonophora, whereas the outer rim replicates did not contain either of these.

The overlying water from each sediment core was collected separately. The sediment was sectioned horizontally in 1 cm layers down to a 5 cm sediment depth. The following 5 cm slice (5 to 10 cm) was taken as 1 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.
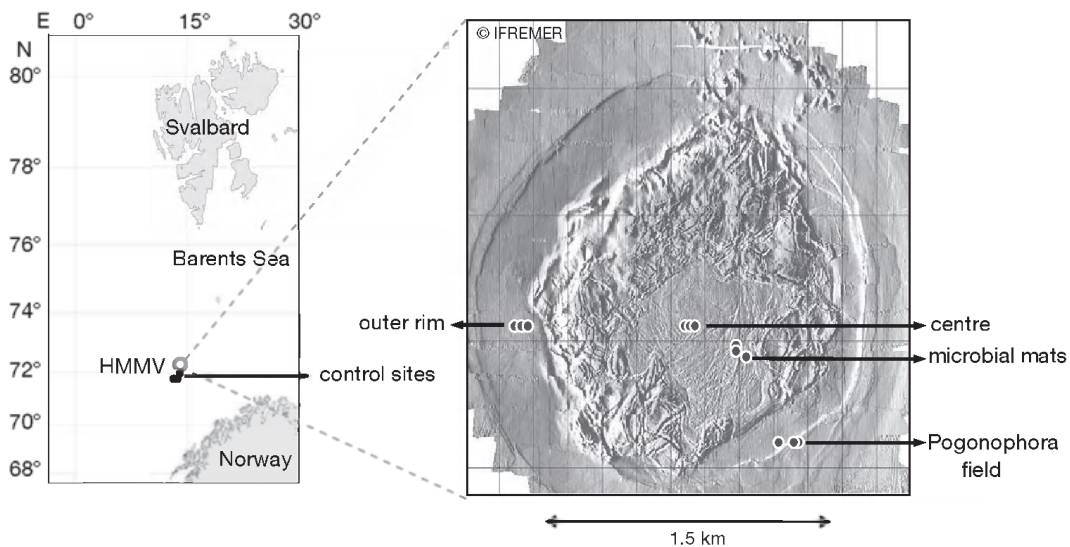

Fig. 1. Study area and sampling sites indicated on a bathymetric map of the Håkon Mosby Mud Volcano (HMMV; adapted from Edy et al. 2004)
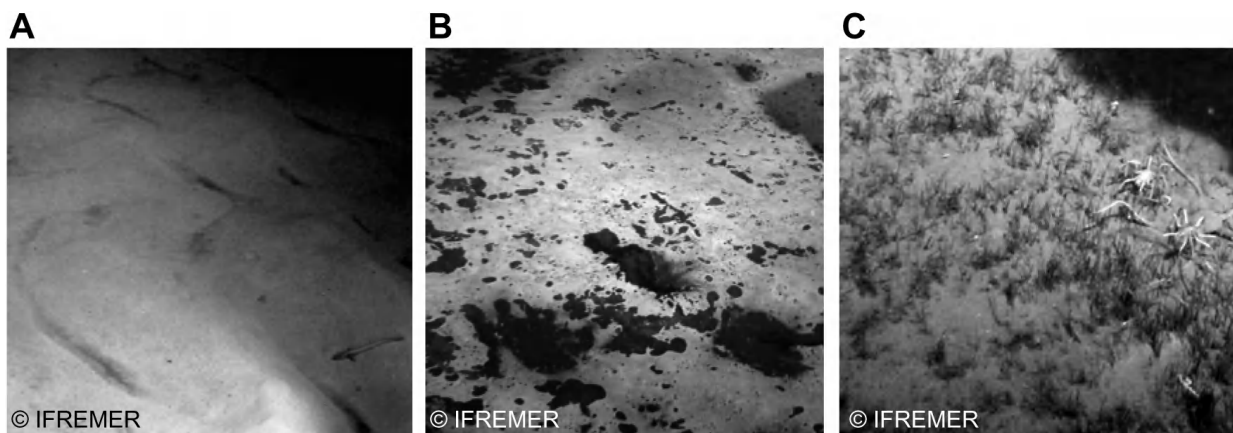

Fig. 2. Photographs of HMMV sub-habitats: (A) centre, (B) microbial mats and (C) Pogonophora field (Sauter et al. 2003)

Table 1. Station parameters including multicore sample number, geographical position, water depth and corresponding sub-habitat. 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
Pogonophora 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 dry weight (DW). Individual biomasses were calculated from Andrassy's (1956) formula for wet body weight (WW) (μg WW = $L \times W^2/1\,600\,000$). A specific nematode gravity of 1.13 g cm^{-3} and a DW:WW ratio of 0.25 was assumed (Wieser 1960). Dry weight was converted into carbon assuming a conversion factor of 0.5.

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 <3 s. The sensors were calibrated after mounting on a free-falling deep-sea profiler (Wenzhofer & 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 1 or 2 vertical profiles, with a spatial resolution of 250 μm, over a depth of 17 cm. After each downward step, 4 s elapsed before a reading was made. Each reading was the average of 4 samplings over a period of 4 s. The profiling started ca. 6 h after reaching the seafloor.

Carbon isotope measurements. Samples for sediment and faunal carbon isotope analysis were collected separately, and intact sediment layers (0 to 1 and 1 to 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 to 60 nematodes and 20 to 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 of 0.2 μm filtered Atlantic deep-sea water and sealed with screw caps fitted with rubber septa. Following 6 d 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 the 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: $\delta^{13} C_{org\text{-}labile} = [(\Sigma CO_{2\ end}) \times \delta^{13}$-$\Sigma CO_{2\ end}] - [(\Sigma CO_{2\ start}) \times \delta^{13}$-$\Sigma CO_{2\ start}]/[(\Sigma CO_{2\ end}) - \Sigma CO_{2\ start}]$. The isotope signature of ΣCO_2 changed from $+0.4 \pm 0.1$ to -15.6 ± 0.4‰, as a result of 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 ($\pm$SE).

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%, 2×100%). 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. 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.

Statistics. After testing the assumptions of normality, analysis of variance (ANOVA with a confidence level of 99%, program S-Plus) and Tukey's post hoc comparison tests were used to describe differences in meiobenthic densities among sites. Nematode genus densities were ordinated by non-metric multi-dimen-

sional scaling (MDS, PRIMER). A stress value of <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

Biochemical profiles

Bottom waters were fully oxic at all sites (260 to 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 to 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 depth in the sediment and increased with depth to concentrations of up to 1 mM at 2 cm depth (Fig. 3).

Meiobenthic densities and community composition

Metazoan meiofaunal densities were significantly higher at the microbial mats (11 292 ± 2256 ind. 10 cm^{-2} or 11 × 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) ($F_{4,3}$ = 50.27, p < 0.001) (Fig. 4, Table 2). Nematodes, pri-
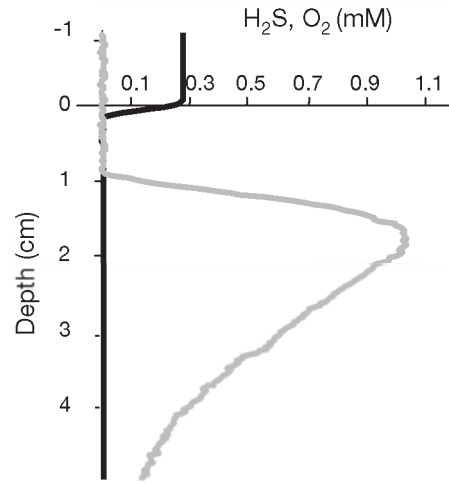

Fig. 3. *In situ* microprofiles of oxygen (black) and hydrogen sulphide (grey) at the microbial mat site

marily 1 species (see below), accounted for >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 to 8 times higher than densities at the other HMMV and control sites (Table 2).
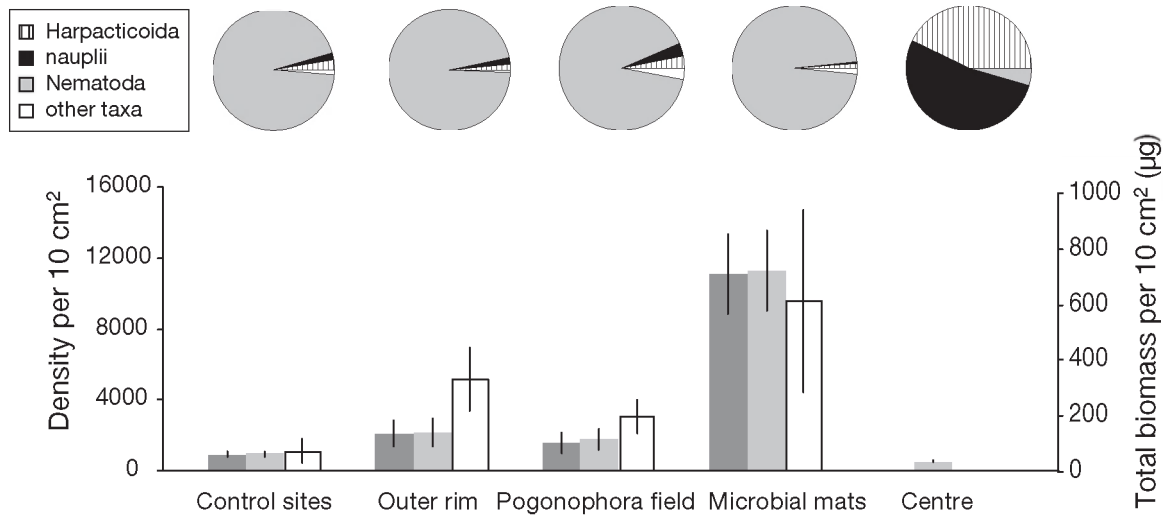

Fig. 4. Proportional abundance of meiofaunal taxon densities (pies), meiofaunal densities (light grey bars), nematode densities (dark grey bars) and total biomass (dry weight) of nematodes (white bars) at different sub-habitats of HMMV and 2 control sites outside HMMV (mean ± 1 SD)

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

Taxon	Control sites		Outer rim		Pogonophora 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	2 079.1	750.1	1 575.4	564.6	11 109.3*	2 267.9	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	2 165.1	759.3	1 741.3	577.8	11 292.1*	2 256.2	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

The outer rim, Pogonophora 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 Pogonophora 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 1 sample taken at the Pogonophora 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}.

Nematode biomass

Although mean individual nematode biomass (DW) showed no significant differences ($F_{4,3} = 3.49$, p = 0.05) among the HMMV and control sites, the total biomass differed significantly ($F_{4,3} = 6.81$, p = 0.007), with the lowest values at the volcano's centre (1.64 ± 0.42 µg DW 10 cm^{-2}) and the highest at the microbial mats (611.01 ± 331.79 µg DW 10 cm^{-2}), due to the extremely high nematode densities in the these mats (Fig. 4).

Nematodes associated with the microbial mats generally had the lowest individual biomass (0.06 ± 0.03 µg DW), while the highest average individual biomass was found at the outer rim (0.16 ± 0.05 µg DW). The mean individual body lengths and widths of the nematodes were not significantly different among the different sites (length: $F_{4,3} = 2.64$, p = 0.1; width: $F_{4,3} = 1.18$, p = 0.4).

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 to 1 cm) was found in 2 replicates. Vertical nematode distributions at the control sites and in the Pogonophora field had a similar pattern, with >50% of the nematodes in the uppermost sediment layer and >70% in the top 0 to 2 cm layer (Fig. 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.

Vertical copepod distributions were similar at the control sites, outer rim, microbial mats and in the Pogonophora field, with the highest relative abundances in the most surficial layers. The first sediment
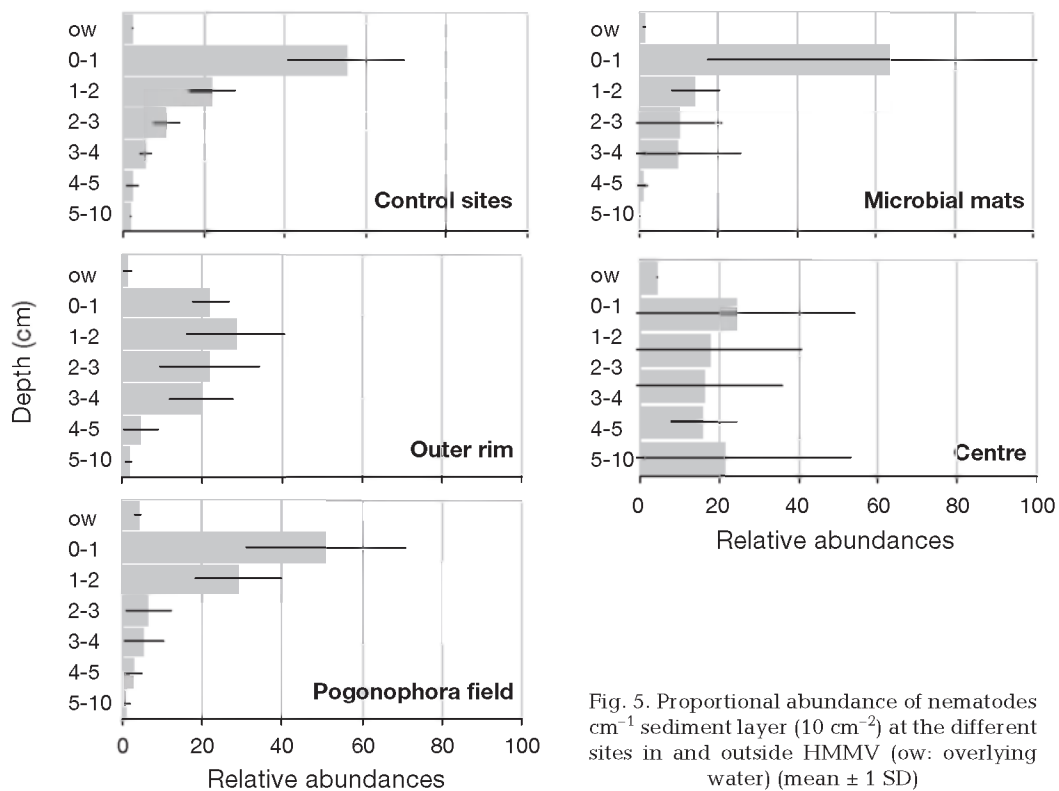
Fig. 5. Proportional abundance of nematodes cm^{-1} sediment layer (10 cm^{-2}) at the different sites in and outside HMMV (ow: overlying water) (mean ± 1 SD)

layer in these sub-habitats contained, on average, >60% of the total copepod number, and >80% was found in the 0 to 2 cm layer (Fig. 6). These average densities were only slightly lower in the Pogonophora field, due to 1 replicate in which 50% of the copepods was 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 was present in the overlying water. Nevertheless, the copepods colonised the centre sediments down to 4 cm depth in relatively high proportions; the 4 to 5 cm layer contained only a few individuals.

Nematode diversity

The microbial mat sediments, and to a much lower extent, the central HMMV sediments, were colonised by 1 very dominant nematode species that was completely absent at the control sites, outer rim and in the Pogonophora field (Fig. 7). This *Geomonhystera* sp. (Monhysteridae) made up 98% (11 069 ± 2269 ind. 10 cm^{-2}) of the total metazoan meiofaunal community and >99% of the nematode abundances in the microbial mats (Fig. 8A). In the top 1 cm sediment layer at the microbial mat site, *Geomonhystera* sp. 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 *Geomonhystera*. The other HMMV sub-habitats were inhabited by a more diverse nematode community (Figs. 7 & 8A) (61 to 72 genera sample^{-1}). 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 in the Pogonophora field. An MDS plot based on the nematode genus densities (stress value = 0.01) revealed a cluster of the control, outer rim and Pogonophora samples, while the microbial mat and centre samples were clearly separated, reflecting the extremely different nematode communities of these 2 sub-habitats (Fig. 8B). After exclusion of the microbial mat and centre samples, the analysis (with a stress value of 0.08) placed the replicate samples of the 3 other sites in 3 distinct clusters, suggesting important differences among the nematode assemblages of these 3 sites.

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 *Geomonhystera* sp., had $\delta^{13}C$ 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 $\delta^{13}C$ 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‰.

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 investiga-
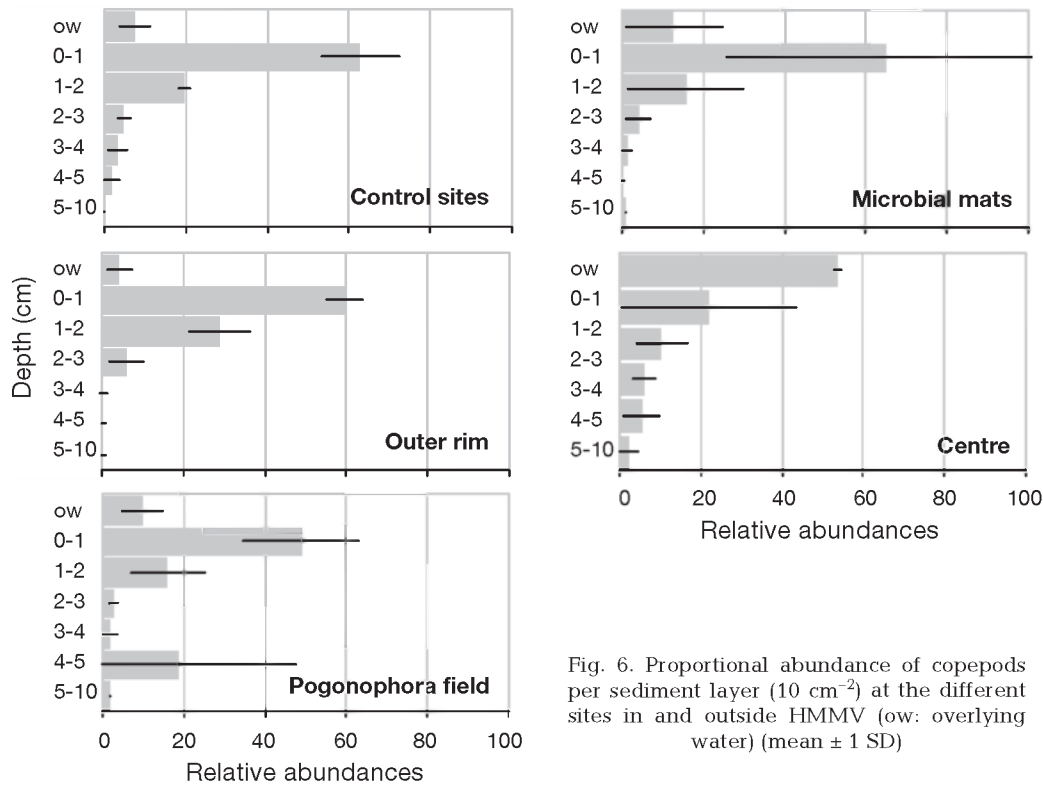

Fig. 6. Proportional abundance of copepods per sediment layer (10 cm^{-2}) at the different sites in and outside HMMV (ow: overlying water) (mean ± 1 SD)
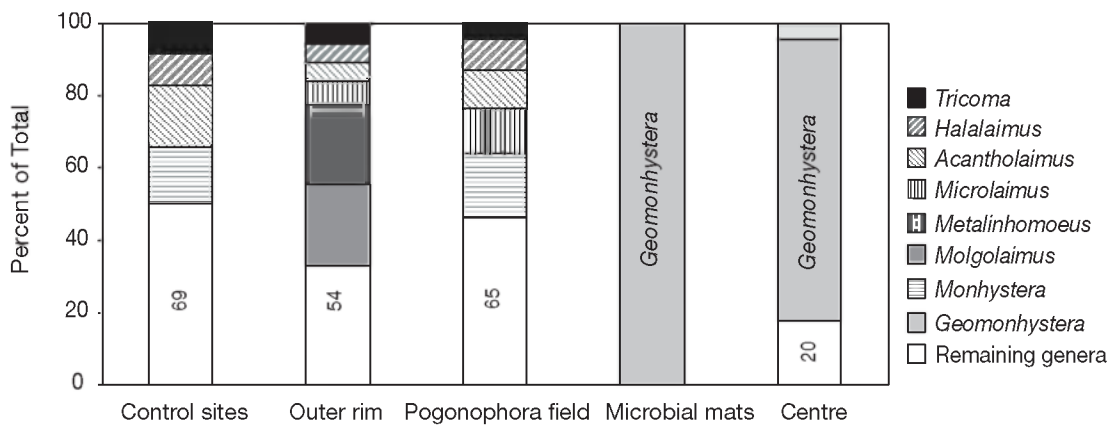

Fig. 7. Proportional abundance of the most abundant nematode genera at the different sites inside and outside HMMV (numbers in white bars are frequency of 'remaining' genera)
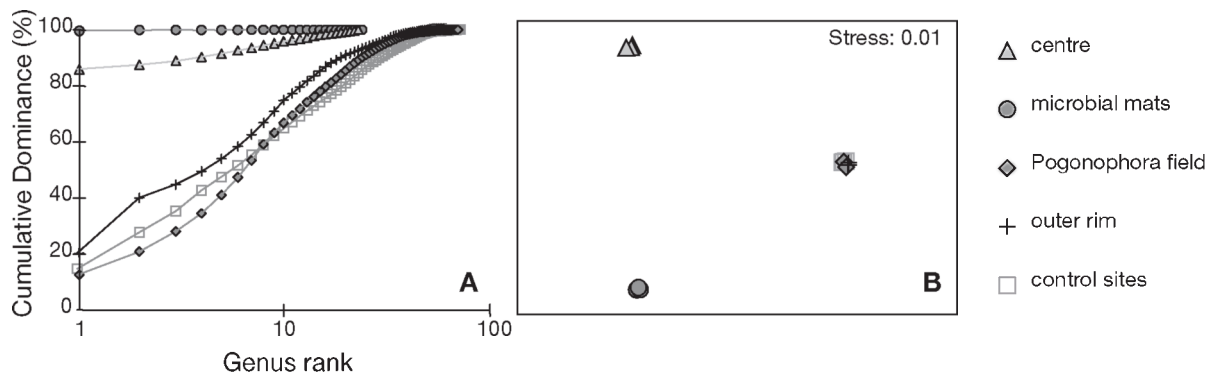
Fig. 8. (A) *K*-dominance curve of all nematode genera found at the different sites and (B) multi-dimensional scaling based on nematode genus densities

tions of these specimens with light and scanning electron microscopy revealed absolutely no evidence of ectosymbionts (Fig. 9). Initial results of TEM on formalin-fixed specimens showed no evidence for endosymbionts. A high percentage of the females of *Geomonhystera* sp. found at HMMV carried several eggs and juveniles inside the uterus (up to 7 juveniles), indicating an ovoviviparous reproduction mode (Fig. 9).

DISCUSSION

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 Pogonophora 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 (~0.1 g C m^{-2}) and composition, the microbial mat sediments harboured 1 specific taxon in very high numbers. A single nematode species thrived with unexpectedly high densities (11 × 10^6 ind. m^{-2}) and biomass (~0.3 g C m^{-2} in 0 to 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
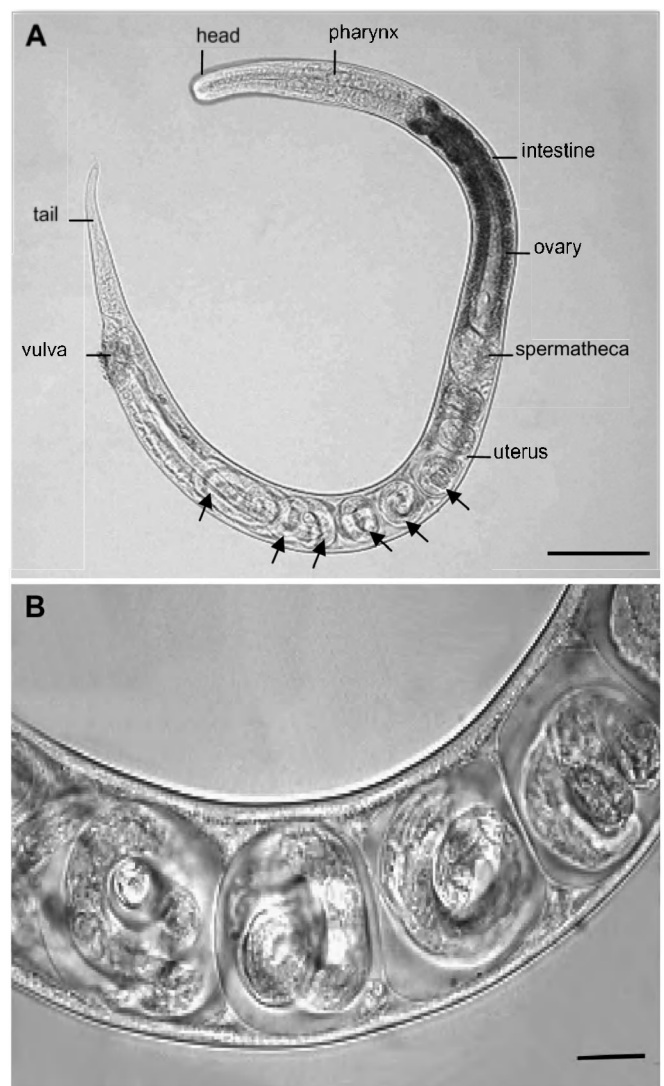

Fig. 9. *Geomonhystera* sp. of HMMV: (A) light microscope photograph of female with arrows indicating the juveniles (scale bar: 100 µm) and (B) detail of uterus with juveniles (scale bar: 10 µm)

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 & 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 $\delta^{13}C$ value (−40.35‰) was measured for filamentous sulphur bacteria at Pacific methane seeps (Levin & Michener 2002).

The successful nematode species of the microbial mats at HMMV was morphologically very similar to *Geomonhystera disjuncta*, a cosmopolitan, bacterivorous nematode known for its high resistance to environmental stress, especially to heavy metals (Vranken et al. 1989). Additionally, molecular analyses (Van Gaever et al. unpubl. data) confirmed that this nematode belongs to the *G. disjuncta* complex, which consists of several cryptic species. *G. disjuncta* seemed to have a high interspecific morphological variability and significant genetic differences within the different lineages of this species (Derycke et al. unpubl. data). 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 *G. disjuncta* is reported in high abundances in deep-sea sediments.

Adaptation of *Geomonhystera* sp. to toxic environmental conditions

Specimens of *Geomonhystera* sp. 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 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 sup-

ported 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 & 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.

Geomonhystera sp. from HMMV showed no extreme morphological modifications, except for its ovoviviparous reproductive mode. *G. disjuncta* has already been described to have an ovoviviparous reproduction strategy in toxic environmental conditions. Ovoviviparity is only known for a few marine nematode species. Walker & Tsui (1968) reported that ovoviviparity was induced in the soil nematode *Rhabditis* sp. by temporary (60 to 120 min) 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.

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 to 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 *Geomonhystera* sp. found at HMMV with trophic resources driven by sulphide. In contrast, copepods and nauplii reached maximum den-

sites and dominance in the volcano's centre and penetrated down to 5 cm in the anoxic sediments (Fig. 5). Their strongly depleted $\delta^{13}C$ values ($-51.2 \pm 1.7\permil$) suggest a strong trophic link with methane-derived carbon, evidently unexploited by nematodes.

The sediments of the 3 other HMMV sub-habitats (Pogonophora 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 (Fig. 6). The higher number of nematode genera found in the Pogonophora 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 Pogonophora 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 Pogonophora 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 Pogonophora field. Although the number of nematode genera was comparable, the nematode densities in the control sites were only half of the densities in the Pogonophora field and outer rim, from which the higher densities indicate increased food availability on the mud volcano.

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 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, Pogonophora 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 11 300 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 to 11 364 ind. 10 cm^{-2}) across seep sub-habitats was also recorded by Olu et al. (1997). In contrast, Soltwedel et al. (2005) 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}.

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 4 cold seep studies, of which only one is from deeper water (Jensen 1986, Shirayama & 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), 2 *Daptonema* spp. and a *Chromadorita* sp. in the bathyal cold seep off Japan (Shirayama & 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).

Geomonhystera sp. 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 *Geomonhystera* sp. in these toxic sediments is the result of active adaptation and trophic specialisation.

Conclusions

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 *Geomonhystera disjuncta* complex (recently identified as a cosmopolitan group of morphologically similar but cryptic species), was found 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 1 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.

LITERATURE CITED

Andrassy I (1956) The determination of volume and weight of nematodes. Acta Zool Hung 2:1–15

Bagarinao T (1992) Sulfide as an environmental factor and toxicant: tolerance and adaptations in aquatic organisms. Aquat Toxicol 24:21–62

Boetius A, Ravenschlag K, Schubert CJ, Rickert D and 6 others (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature 407: 623–626

Buck KR, Barry JP (1998) Monterey Bay cold seep infauna: quantitative comparison of bacterial mat meiofauna with non-seep control sites. Cah Biol Mar 39:333–335

Dando PR, Austen MC, Burke RA, Kendall MA and 6 others (1991) Ecology of a North Sea pockmark with an active methane seep. Mar Ecol Prog Ser 70:49–63

de Beer D, Sauter E, Niemann H, Kaul N, Foucher JP, Witte U, Schlüter M, Boetius A (2006) *In situ* fluxes and zonation of microbial activity in surface sediments of the Håkon Mosby Mud Volcano. Limnol Oceanogr 51(3):1315–1331

Dubilier N, Mulders C, Ferdelman T, de Beer D and 8 others (2001) Endosymbiotic sulphate-reducing and sulphide-oxidizing bacteria in an oligochaete worm. Nature 411: 298–302

Edy C, Bisquay H, shipboard scientific team (2004) Microbathymetry on ROV 'Victor 6000'. In: Klages M, Thiede JK, Foucher JP (eds) The expedition ARKTIS XIX/3 of the research vessel Polarstern in 2003. Reports of Legs 3a, 3b and 3c. Rep Polar Res 488:158–163

Fernandez M, Bock C, Pörtner HO (2000) The cost of being a caring mother: the ignored factor in the reproduction of marine invertebrates. Ecol Lett 3:487–494

Gebruk AV, Krylova EM, Lein AY, Vinogradov GM, Anderson E, Pimenov NV, Cherkashev GA, Crane K (2003) Methane seep community of the Håkon Mosby Mud Volcano (the Norwegian Sea): composition and trophic aspects. Sarsia 88:394–403

Giere O, Windoffer R, Southward EC (1995) The bacterial endosymbiosis of the gutless nematode, *Astomonema southwardorum*: ultrastructural aspects. J Mar Biol Assoc UK 75:153–164

Ginsburg GD, Milkov AV, Soloviev VA, Egorov AV and 5 others (1999) Gas hydrate accumulation at the Håkon Mosby Mud Volcano. Geo-Mar Lett 19:57–67

Jensen P (1986) Nematode fauna in the sulphide-rich brine seep and adjacent bottoms of the East Flower Garden, NW Gulf of Mexico. Mar Biol 92:489–503

Jensen P, Aagaard I, Burke RA, Dando P and 5 others (1992) 'Bubbling reefs' on the Kattegat: submarine landscapes of carbonate-cemented rocks support a diverse ecosystem at methane seeps. Mar Ecol Prog Ser 83:103–112

Jeroschewski P, Steuckart C, Kuhl M (1996) An amperometric microsensor for the determination of H_2S in aquatic environments. Anal Chem 68:4351–4357

Levin LA (2005) Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. Oceanogr Mar Biol Annu Rev 43:1–46

Levin LA, Michener RH (2002) Isotopic evidence for chemosynthesis-based nutrition of macrobenthos: the lightness of being at Pacific methane seeps. Limnol Oceanogr 47:1336–1345

Levin LA, James DW, Martin CM, Rathburn AE, Harris LH, Michener RH (2000) Do methane seeps support distinct macrofaunal assemblages? Observations on community structure and nutrition from the northern California slope and shelf. Mar Ecol Prog Ser 208:21–39

Milkov A, Vogt P, Cherkashev G, Ginsburg G, Chernova N, Andriashev A (1999) Sea-floor terrains of Haakon Mosby Mud Volcano as surveyed by deep-tow video and still photography. Geo-Mar Lett 19:38–47

Montagna PA, Spies RB (1985) Meiofauna and chlorophyll associated with *Beggiatoa* mats of a natural submarine petroleum seep. Mar Environ Res 16:231–242

Moodley L, Middelburg JJ, Soetaert K, Boschker HTS, Herman PMJ, Heip CHR (2005) Similar rapid response to phytodetritus deposition in shallow and deep-sea sediments. J Mar Res 63:457–469

Olu K, Lance S, Sibuet M, Hendry P, Fiala-Dedioni A, Dinet A (1997) Cold seep communities as indicators of fluid expulsion patterns through mud volcanoes seaward of the Barbados Accretionary Prism. Deep-Sea Res I 44:811–841

Orphan VJ, House CH, Hinrichs KU, McKeegan KD, DeLong EF (2002) Methane-consuming Archaea revealed by directly coupled isotopic and phylogenetic analysis. Science 293:484–487

Ott J, Bright M, Bulgheresi S (2004) Symbioses between marine nematodes and sulfur-oxidizing chemoautotrophic bacteria. Symbiosis 36:103–126

Pimenov NV, Savvichev AS, Rusanov II, Lein AY, Ivanov MV (2000) Microbiological processes of the carbon and sulfur cycles at cold methane seeps of the North Atlantic. Microbiology 69:709–720

Powell EN, Bright TJ, Woods A, Gittings S (1983) Meiofauna and the thiobios in the East Flower Garden Brine Seep. Mar Biol 73:269–283

Revsbech NP (1989) An oxygen microelectrode with a guard cathode. Limnol Oceanogr 34:474–478

Robinson CA, Bernhard JM, Levin LA, Mendoza GF, Blanks JK (2004) Surficial hydrocarbon seep infauna from the Blake Ridge (Atlantic Ocean, 2150 m) and the Gulf of Mexico (690–2240 m). PSZN I: Mar Ecol 25(4):313–336

Sauter E, Baumann L, Harms C, Hohmann C and 6 others (2003) Geochemical and biological investigations at Håkon Mosby Mud Volcano. In: Lemke P (ed) The expedition ARKTIS XIII/1 a,b of the research vessel Polarstern in 2002. Rep Polar Res 446:75–80

Shirayama Y, Ohta S (1990) Meiofauna in a cold-seep community off Hatsushima, Central Japan. J Oceanogr Soc Jpn 46:118–124

Solan M, Cardinale BJ, Downing AL, Engelhardt KAM, Ruesink JL, Srivastava DS (2004) Extinction and ecosystem function in the marine benthos. Science 306:1177–1180

Soltwedel T, Portnova D, Kolar I, Mokievsky V, Schewe I (2005) The small-sized benthic biota of the Håkon Mosby Mud Volcano (SW Barents Sea slope). J Mar Syst 55(3–4):271–290

Thiermann F, Vismann B, Giere O (2000) Sulphide tolerance of the marine nematode *Oncholaimus campylocercoides*—A result of internal sulphur formation? Mar Ecol Prog Ser 193:251–259

Van Dover CL, Aharon P, Bernhard JM, Caylor E and 15 others (2003) Blake Ridge methane seeps: characterization of a soft-sediment, chemo-synthetically based ecosystem. Deep-Sea Res I 50:281–300

Vanreusel A, Van den Bossche I, Thiermann F (1997) Free-living marine nematodes from hydrothermal sediments: similarities with communities from diverse reduced habitats. Mar Ecol Prog Ser 157:207–219

Vincx M, Bett BJ, Dinet A, Ferrero T and 5 others (1994) Meiobenthos of the deep Northeast Atlantic. Adv Mar Biol 30:1–88

Vogt PR, Cherkashev GA, Ginsburg GD, Ivanov G and 6 others (1997) Haakon Mosby Mud Volcano provides an unusual example of venting. EOS Trans Am Geophys Union 78(549):556–577

Vranken G, Tiré C, Heip C (1989) Effect of temperature and food on hexavalent chromium toxicity to the marine nematode *Monhystera disjuncta*. Mar Environ Res 27:127–136

Walker JT, Tsui RK (1968) Induction of ovoviviparity in *Rhabditis* by sulfur dioxide. Nematologica 14:148

Wenzhofer F, Glud RN (2002) Benthic carbon mineralization in the Atlantic: a synthesis based on *in situ* data from the last decade. Deep-Sea Res I 49:1255–1279

Wieser W (1960) Benthic studies in Buzzards Bay. II. The meiofauna. Limnol Oceanogr 5:121–137