Community structure and feeding preference of nematodes associated with methane seepage at the Darwin mud volcano (Gulf of Cádiz)

Ellen Pape^{1,*}, Tania Nara Bezerra¹, Heleen Vanneste², Katja Heeschen²,
Leon Moodley³, Frederic Leroux⁴, Peter van Breugel³, Ann Vanreusel¹

¹Marine Biology Section, Biology Department, Ghent University, Krijgslaan 281/S8, 9000 Gent, Belgium

²National Oceanography Centre Southampton, University of Southampton, Waterfront Campus, European Way, Southampton SO14 3ZH, UK

³Netherlands Institute of Ecology, Centre for Estuarine and Marine Ecology (NIOO-CEME), Workgroup of Ecosystem Studies, Korrिंगaweg 7, 4401 NT Yerseke, The Netherlands

⁴Electron Microscopy for Materials Science (EMAT), University of Antwerp, Groenenborgerlaan 171, 2020 Antwerpen, Belgium

ABSTRACT: We sampled the Darwin mud volcano (MV) for meiofaunal community and trophic structure in relation to pore-water geochemistry along a 10 m transect from a seep site on the rim of the crater towards the MV slope. Pore-water profiles indicated considerable variation in upward methane (CH₄) flow among sediment cores taken along the transect, with highest flux in the seep sediment core, gradually decreasing along the transect, to no CH₄ flux in the core taken at a 5 m distance. Low sulphate concentrations and high levels of total alkalinity and sulphide (H₂S) suggested that anaerobic oxidation of methane (AOM) occurred close to the sediment surface in the seep sediment core. High H₂S levels had a genus- and species-specific impact on meiofaunal densities. Nematode genus composition varied gradually between sediment cores, with the genus *Sabatieria* dominating almost all sediment cores. However, genus diversity increased with increasing distance from the seep site. These limited data suggest that the community structure of seep meiofauna is highly dependent on local (a)biotic habitat characteristics, and a typical seep meiofaunal community cannot be delineated. Stable isotope values suggested the nematode diet up to 10 m from the seep site included thiotrophic carbon. The thicker hemipelagic sediment layer (photosynthetic carbon), the increased trophic diversity, and the heavier nematode $\delta^{13}\text{C}$ farther from the seep site suggest a decrease in thiotrophy and an increase in photosynthetic carbon in the nematode diet.

KEY WORDS: Cold seep · Diversity · Stable isotope · Nematode · Diet

— Resale or republication not permitted without written consent of the publisher —

INTRODUCTION

Mud volcanoes (MVs), geological structures driven by fluid flow, are characterized by a high patchiness of biochemical and physical characteristics. Fluid flow rates, pore-water concentrations of hydrogen sulphide (H₂S) and methane (CH₄), and the thickness of the hemipelagic sediment veneer, on top of the reduced sediments can change rapidly over short

distances (metres to centimetres) (Levin et al. 2003). The heterogeneity in these properties is the main parameter driving the distribution of macro- and megafauna at seeps (Levin 2005), resulting in patches of tubeworm clusters, mussels or clams, and bacterial mats or bare reduced sediments. Meiofauna can also vary on a scale of metres in taxonomic composition and biodiversity in relation to sediment biogeochemistry (Van Gaeve et al. 2009c).

*Email: ellen.pape@ugent.be

There is no consistent meiofaunal response to seep conditions. Meiofaunal densities at different deep-sea seeps are higher (Olu et al. 1997, Van Gaever et al. 2006) or similar (Shirayama & Ohta 1990) compared to non-seep sediments. In seep environments, nematodes usually are the predominant metazoans, although sometimes copepods dominate (Van Gaever et al. 2006). Generally, deep-sea nematodes are characterized by high local diversity (Lambshead & Boucher 2003). Cold seeps, however, exhibit substantially reduced species diversity, harbouring only a few dominant species (Levin 2005, Vanreusel et al. 2010). The low diversity in these habitats has been attributed to the harsh abiotic conditions, created by the high H_2S and low oxygen levels (Levin 2005).

Besides high biogeochemical and physical heterogeneity, seeps differ from most deep-sea environments in the local production of organic matter through chemosynthesis. Consequently, possible food sources for seep fauna, including meiobenthos are: (1) organic matter derived from symbiotic chemoautotrophic bacteria and (2) free-living chemoautotrophic bacteria, in addition to (3) the photosynthetic organic matter delivered to all deep-sea habitats. Studies on the diet of seep meiofauna are few. Both Van Gaever et al. (2006, 2009b) and Spies & Des-Marais (1983) found seep nematodes to be feeding on free-living sulphur-oxidising bacteria. To date, there is no evidence of symbioses between nematodes and chemosynthetic bacteria at deep-sea seeps (Vanreusel et al. 2010), and observations of symbionts associated with seep nematodes are restricted to shallow waters (Dando et al. 1991, Ott et al. 2004).

The present study examined the community structure and feeding ecology of the meiofauna, with a focus on nematodes, at a MV in the Gulf of Cádiz, which we then related to geochemical gradients along a 10 m transect from a seep site towards nearby hemipelagic surface sediments, and 2 sites farther away from seep influence. Our study differs from previous analyses on seep meiofauna, because it concerns isolated seep sediments on a low-activity MV. We addressed the following questions:

–Does pore-water composition influence horizontal and vertical distribution of meiofauna on a small scale?

–Are the seep sediments colonized by a specialized community that differs from the hemipelagic sediments in density, biomass and taxonomic composition (genera and species)?

–What is the nematode diet inferred from stable isotope analyses and buccal morphology? Do seep conditions influence nematode trophic diversity?

MATERIALS AND METHODS

Study area

The Gulf of Cádiz (34 to 37° 15' N, 9 to 6° 45' W) is a tectonically active region west of the Strait of Gibraltar, encompassing the boundary between the European and African plates. It is one of the largest cold-seep areas on the European margin with >30 MVs between 200 and 4000 m deep (Pinheiro et al. 2003, Somoza et al. 2003, Van Rensbergen et al. 2005).

The summit (1100 m depth) of the Darwin MV (35° 23.51' N, 7° 11.48' W; Fig. 1A) is covered with a large, fractured carbonate crust. At the time of sampling, countless, but mostly empty, *Bathymodiolus mauritanicus* shells covered the MV top (Genio et al. 2008). Living specimens were only present in small clumps along cracks in the crust. When disturbed by the remotely operating vehicle (ROV) temperature probe, a small area of dark-coloured sediment ($\pm 100\text{ cm}^2$), from here on referred to as the 'seep site', emitted gas. Small carbonate blocks and white sediment, indicative of bacterial activity, which covered hard substrate, surrounded the seep site (Vanreusel et al. 2009). No dense aggregations of living chemosynthetic megafauna were associated with the seep sediments or 2 m away. At the MV centre, non-chemosynthetic megafauna comprised scavenging crabs, corals and stylasterine corals, attached to the carbonate crust.

Sampling strategy

Sediment cores were collected during the JC10 expedition to the Gulf of Cádiz in May 2007 onboard the RRS 'James Cook' (Table 1). We were unable to collect replicate samples because of the high heterogeneity of the habitat and the small size of the seep site. However, the present study is the first to identify potential interactions between seep meiofauna and pore-water geochemistry measured at such a small spatial scale. Furthermore, no meiofauna data were previously available from the Gulf of Cádiz, although it forms an important faunal cross road between the Mediterranean and the Atlantic.

Using ROV 'Isis', we collected 2 push cores (PUCs, 25.5 cm^2) at each of the 4 sites along a 10 m transect between the seep site and an area with a considerably thicker hemipelagic sediment layer (Fig. 1, Table 1). One PUC was taken with a core-liner, with openings every 2 cm, to extract pore-water using Rhizons (Seeberg-Elverfeldt et al. 2005). These pore-waters were sub-sampled on board for nutrient and

Fig. 1. Darwin mud volcano (MV). (A) Bathymetric map with the core locations on the MV indicated (courtesy of the National Oceanography Centre Southampton) (B) Schematic representation of the sampling strategy. PUC1: Push Core 1, sampled for pore-water geochemistry and meiofaunal community analyses; PUC2: Push Core 2, sampled for pore-water CH_4 concentration, porosity and nematode stable isotope signatures; MC: megacorer sample for meiofaunal community analyses

anion analyses. Subsequently, we sliced the top 10 cm of this core into 1 cm sections and fixed them in 4% formaldehyde for meiofaunal community analysis. The second PUC was sub-sampled for CH_4 and porosity analyses, and we stored the remaining sediment in 2 cm slices at -30°C for stable isotope analysis. Besides the 10 m transect, we sampled 2 sites at ~ 100 (on the MV) and ~ 1100 m from the seep site (off the MV) with a megacorer (75.4 cm^2). These samples were exclusively analyzed for community structure.

Pore-water geochemical analyses

Total alkalinity (TA) and hydrogen sulphide (H_2S) were measured immediately after pore-water extraction: TA by titrating against 0.05 M HCl while bubbling nitrogen through the sample (Ivanenkov & Lyakhin 1978) and H_2S using standard photometric procedures (Grasshoff et al. 1999) adapted for pore-waters with high H_2S levels (magnitude of mM). Concentrations of all other species were analyzed at

Table 1. Sediment cores in relation to distance from the Darwin mud volcano seep site. PUC: push core, MC: megacore. 0 = seep sediment. PUC1: meiofaunal community structure and porewater geochemistry. PUC2: porosity, porewater CH₄ concentration and nematode stable isotopes. MC1: Meiofaunal community structure

Distance from seep site (m)	Position	Height hemipelagic layer (% core length)	Gear	No. of cores	Analysis
0	35°23.539'N, 7°11.508'W	0	PUC	2	PUC1 PUC2
2	35°23.543'N, 7°11.506'W	22–33	PUC	2	PUC1 PUC2
5	35°23.543'N, 7°11.509'W	53–63	PUC	2	PUC1 PUC2
10	35°23.547'N, 7°11.511'W	71	PUC	2	PUC1 PUC2
100	35°23.537'N, 7°11.454'W	100	MC	1	MC1
1100	35°23.965'N, 7°11.121'W	100	MC	1	MC1

the National Oceanography Centre Southampton (NOCS). Sulphate (SO₄²⁻) was measured by ion chromatography (Dionex ICS2500), with reproducibility >1.5% (determined by repeat analysis of a seawater standard as well as single anion standards). We measured dissolved CH₄ in sediment samples taken immediately after opening the cores using the headspace vial method (Reeburgh 2007). An aliquot of sediment (~3 cm³) was withdrawn, placed in a glass vial, and 5 ml of 1 M NaOH was added to prevent further microbial activity (Hoehler et al. 2000). The vial was crimped shut, and the sample shaken vigorously to release the gases. CH₄ concentration in the headspace was determined by gas chromatography (Agilent 6850) at the NOCS. These headspace CH₄ measurements were then converted to dissolved CH₄ concentrations following Hoehler et al. (2000). Depressurization and warming of the cores during sediment retrieval is likely to have led to degassing, so concentrations of CH₄ (which is generally oversaturated in pore-waters) and H₂S represent minimum values. Therefore, profiles were compared relative to one another, rather than to measurements in other studies.

Meiofaunal community analysis

We washed the formaldehyde-fixed samples over a 32 µm mesh sieve and extracted the meiofauna from the sediment by Ludox centrifugation (Heip et al. 1985). Meiofauna was then sorted, enumerated and identified at coarse taxonomic level. From each

slice, ca. 100 nematodes were identified to genus level. *Sabatieria*, the dominant genus in all cores but one, was identified to species. Additionally, we measured length (L; µm) and maximal width (W; µm) for each nematode from the top 0 to 5 cm, to estimate individual biomass using Andrassy's formula (Andrassy 1956) for body wet weight (WW), adjusted for the specific gravity of marine nematodes (i.e. 1.13 g cm⁻³; µg WW = L × W²/1 500 000). C weight was calculated as 12.4 % of wet weight (Jensen 1984).

Stable isotope analysis

Nematodes from the top 6 cm of each core were hand-picked for δ¹³C and δ¹⁵N analysis. *Desmodora* (n = 50) and *Sabatieria* (n = 50) were picked separately, and the remaining genera were pooled to determine the 'Mix' isotope value (n = 100). When not sufficiently abundant, *Desmodora* and/or *Sabatieria* were included in the 'Mix' sample. Nematodes were rinsed with 2 µm filtered Milli-Q water, and then transferred to Milli-Q water in pre-combusted (550°C, 3 h) silver cups. After elutriation, nematodes were dried overnight at 60°C. Subsequently, we acidified samples and blanks in a desiccator containing 5% HCl. Isotope signatures were measured on an EA-IRMS, a Flash EA 1112 coupled to a DeltaV advantage IRMS (Thermo Electron Instruments) with a single low volume oxidation/reduction reactor (Carman & Fry 2002). Samples were calibrated against VPDB and N₂-Air with standards USGS40 and USGS41 (Qi et al. 2003), and all measurements were corrected for blanks. Isotope values were expressed in δ notation with respect to VPDB (δ¹³C) and air (δ¹⁵N): δX (‰) = [(R_{sample} / R_{standard}) - 1] × 10³, where X is ¹³C or ¹⁵N and R is the isotope ratio (Post 2002).

Transmission electron microscopy (TEM) and scanning transmission electron microscopy energy dispersive x-ray (STEM-EDX) analysis

Sabatieria and *Desmodora*, from the seep sediment core, were imaged with TEM to check for symbionts or visible S detoxification structures. Subsequently, we conducted STEM-EDX analysis to determine the chemical composition of internal structures. Nematodes were handled following Van Gaeve et al. (2009b).

Data analysis

Individual nematode size measurements (length, width, length/width and biomass) were compared among cores using Kruskal-Wallis tests, followed by nonparametric pairwise comparisons using Behrens-Fisher tests with the R package npmc (Munzel & Hothorn 2001, Helms & Munzel 2008). Nematode size measurements were averaged per core and depth layer as geometric means corrected for data skewness (Middelburg et al. 1997, Soetaert et al. 2009). We performed multi-dimensional scaling (MDS) analysis on standardized nematode genus abundances to compare genus composition between cores. Diversity indices were $\ln(\log_e)$ -transformed to highlight differences. We examined feeding preferences based on: (1) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of *Desmodora*, *Sabatieria* and 'Mix' and (2) buccal morphology of all genera following the classification of Wieser (1952), which assigns genera to 1 of 4 feeding types—selective deposit-feeder (1A), non-selective deposit-feeder (1B), epistrate feeder (2A) and predator/scavenger (2B). Isotope signatures were compared between *Sabatieria*, *Desmodora* and 'Mix' using 1-way ANOVA, followed by post hoc Tukey honestly significant difference tests. Trophic diversity was computed as the reciprocal of the trophic index (Heip et al. 1985). Spearman rank correlations were computed between distance from the seep site and: (1) genus diversity indices, (2) trophic diversity and (3) stable isotope signatures. We performed univariate statistical analyses using R (R Development Core Team 2010), and multivariate analyses and computation of diversity indices in Primer v6 (Clarke & Gorley 2006).

RESULTS

Pore-water geochemistry

Fig. 2 shows concentration–depth profiles for H_2S , SO_4^{2-} , CH_4 and TA in pore-water from all cores. From the 4 cores, only the seep sediment core showed a clear decrease in SO_4^{2-} with depth, accompanied by a peak in H_2S (up to 22 mM) and an increase in CH_4 and TA as high as 1000 μM and 33.7 meq l^{-1} , respectively. We observed very little change in SO_4^{2-} in the core taken 2 m from the seep site. However, H_2S , CH_4 and TA were enriched in the deeper sediment layers, relative to seawater. Concentrations of all of these species in cores collected 5 and 10 m from the seep site were similar to seawater concentrations and varied little with depth.

Meiofaunal community structure

Meiofaunal densities were highest in the core taken 2 m from the seep site (3229 ind. 10 cm^{-2}). Although densities in the seep sediment core (795 ind. 10 cm^{-2}) and in the core taken at 5 m distance (825 ind. 10 cm^{-2}) were considerably lower, they were still elevated compared to those collected 10 (228 ind. 10 cm^{-2}), 100 (436 ind. 10 cm^{-2}) and 1100 m (424 ind. 10 cm^{-2}) from the seep site (Fig. 3). Nematodes were the most abundant taxon (88.7 to 94.5 %) in all cores (Table 2). Meiofaunal densities in the seep sediment core below 1 cm decreased sharply (Fig. 2). In comparison, densities in the core taken 2 m from the seep site decreased more gradually with depth. Meiofauna in the 2 cores retrieved farthest from seep influence penetrated deepest in the sediment.

Nematode size

Overall, total nematode biomass in the top 5 cm of the seep sediment core was $\sim 10\times$ higher than that in the core taken 1100 m away (Fig. 3). Individual nematode size measurements (i.e. length, width and biomass) differed significantly among cores ($p < 0.001$; Fig. 4) and peaked in the seep sediment core. Also nematode length:width ratios varied significantly among cores ($p < 0.001$), with lowest ratios in the seep sediment core (Fig. 4C). *Sabatieria vasicola* and *S. punctata*, which dominated the seep sediment core (Table 4), were 2417 ± 397 (mean $\pm$ SD; $n = 42$) and 1131 ± 463 ($n = 33$) μm long, respectively.

Nematode community structure

Nematode genus composition varied little among cores (Fig. 5, Table 3). *Sabatieria* dominated all samples, except at 2 m from the seep site, where *Rhabdocomma* (23 %) prevailed. All diversity indices correlated positively with distance from the seep site (Fig. 6), but these correlations were significant only for N_0 and EG(100) (N_0 : $r = 0.93$, $p = 0.008$ and EG(100): $r = 0.81$, $p = 0.049$). *Desmodora* was only abundant (i.e. $\geq 1\%$ of total) in cores within 5 m off the seep (Table 3). The *Sabatieria* species composition of all sediment cores is presented in Table 4.

Nematode feeding preference

$\delta^{13}\text{C}$ ranged between -40.7 and -21.3‰ , and $\delta^{15}\text{N}$ between 0.9 and 15.3‰ (Fig. 7). $\delta^{13}\text{C}$ ($r = 0.34$, $p =$

Fig. 2. Vertical pore-water profiles of H_2S , SO_4^{2-} , CH_4 and total alkalinity (TA), and densities of nematodes and other meio-faunal taxa in relation to distance from the Darwin mud volcano seep site. Vertical arrows on x-axes indicate seawater values. Note the different scales on the x-axes

0.14) and $\delta^{15}\text{N}$ ($r = 0.24$, $p = 0.28$) became heavier with increasing distance from the seep site, though the correlations were not significant. No clear pattern emerged when plotting stable isotope signatures (mean $\pm$ SD) versus sediment depth (Fig. 7). 'Mix' ($\delta^{13}\text{C}$: $-31.2 \pm 4.9\text{‰}$; $\delta^{15}\text{N}$: $7.11 \pm 3.9\text{‰}$) was significantly more enriched in ^{13}C ($p = 0.02$) and ^{15}N ($p = 0.02$) than *Desmodora* ($\delta^{13}\text{C}$: $-38.5 \pm 2.0\text{‰}$; $\delta^{15}\text{N}$: $4.6 \pm$

2.2‰). *Sabatieria* ($\delta^{13}\text{C}$: $-36.3 \pm 2.4\text{‰}$; $\delta^{15}\text{N}$: $6.9 \pm 1.5\text{‰}$) and *Desmodora* displayed similar isotope values ($\delta^{13}\text{C}$: $p = 0.67$; $\delta^{15}\text{N}$: $p = 0.43$). Based on buccal morphology, deposit-feeders (1A + 1B) dominated all cores (data not shown), although trophic diversity increased with increasing distance from the seep site ($r = 0.70$, $p = 0.12$) and levelled off at 10 m distance (Fig. 8).

Fig. 3. Total nematode densities and biomass in the top 5 cm of the sediment cores in relation to distance from the Darwin mud volcano seep site (0 = seep sediment)

TEM and STEM-EDX

TEM revealed several, but mostly disintegrated bacteria bordering the cuticle of *Desmodora* (Fig. 9). Additionally, electron-lucent structures were observed near the cuticle (Fig. 9B). STEM-EDX analysis showed these contained trace amounts of sulphur. We observed no symbionts or detoxification structures in *Sabatieria*.

Table 2. Meiofaunal densities (ind. 10 cm⁻²) in the top 5 cm of the sediment cores in relation to distance from the Darwin mud volcano seep site (0 = seep sediment)

Taxon	Distance from seep site (m)					
	0	2	5	10	100	1100
Amphipoda	–	0.8	–	–	–	–
Bivalvia	2.0	–	1.2	–	0.1	–
Cladocera	–	–	–	0.4	–	–
Cnidaria	0.4	4.3	–	0.8	–	0.3
Copepoda						
Adults	7.8	122	12.5	3.9	16.9	7.7
Nauplii	9.8	155	9.4	4.7	11.1	2.5
Cumacea	–	–	–	0.4	0.3	0.1
Gastrotricha	0.4	–	1.6	–	–	–
Halacaroida	4.7	2.7	0.4	–	–	–
Holothuroidea	–	1.2	–	–	7.7	0.4
Isopoda	–	3.9	0.4	0.4	0.9	0.1
Kinorhyncha	0.8	–	–	0.4	–	–
Nematoda	725	2860	780	228	388	405
Oligochaeta	0.8	9.8	5.1	–	–	0.1
Ostracoda	–	0.8	1.6	–	1.1	0.4
Polychaeta	42.7	54.5	7.1	9.8	8.7	5.9
Tanaidacea	–	3.5	0.8	0.4	–	–
Tardigrada	–	8.6	5.9	–	1.5	1.2
Total	794	3227	825	248	436	424

Fig. 4. Mean nematode (A) length, (B) width, (C) length: width and (D) biomass as a function of sediment depth (cm), in relation to distance from the Darwin mud volcano seep site. 0 = seep sediment

Fig. 5. Multi-dimensional scaling plot of standardized genus-abundance data in relation to distance from the Darwin mud volcano seep site. Numbers = sediment depth (cm). 0 = seep sediment. Contour plots for the cores collected at 100 and 1100 m from the seep site overlap and are not shown

DISCUSSION

CH₄ seepage and spatial variability in pore-water geochemistry

Elevated pore-water CH₄ levels in the Darwin MV seep sediment core indicated a CH₄ flux from below the sediment surface, corroborating the gas escape from the seep sediments during sampling. However, CH₄ concentrations dropped from 1 mM down to <0.001 mM over a 10 m distance, suggesting focused flow. Pore-fluid analyses indicated some anaerobic consumption by microbes (i.e. anaerobic oxidation of methane, AOM); SO₄²⁻ decreased rapidly with depth in the seep site pore-fluids, accompanied by an increase in TA and elevated H₂S concentrations (Reeburgh 1976, Boetius et al. 2000, Knittel & Boetius 2009). The relatively small enrichments in H₂S, CH₄ and TA in the core taken 2 m from the seep site suggest AOM presence here as well, but likely concentrated at depths exceeding the core length. The constancy in the concentrations of SO₄²⁻, TA, H₂S and CH₄ with depth in the cores taken at 5 and 10 m distance suggest an absence of AOM. The high spatial variability in CH₄ flow at the Darwin MV illustrates the difficulty in taking replicate samples for pore-water geochemistry and associated fauna at seeps.

Table 3. Relative abundance (%) of the most abundant nematode genera (≥1%) in the top 5 cm of the sediment cores in relation to distance (Dist., m) from the Darwin mud volcano seep site (0 = seep sediment)

Dist.: Genus	0	2	5	10	100	1100
<i>Sabatieria</i>	44	24	39	14	30	24
<i>Desmodora</i>	19	19	8	11	9	10
<i>Ethmolaimidae n.gen.</i>	8	14	6	6	6	7
<i>Desmoscolex</i>	6	8	4	6	5	6
<i>Tricoma</i>	3	6	4	6	3	6
<i>Amphimonhystrella</i>	3	5	4	4	3	4
<i>Linhomoeus</i>	2	4	4	4	3	4
<i>Comesa</i>	2	4	4	4	3	4
<i>Thalassomonhystera</i>	1	2	2	3	2	3
<i>Cyartoneura</i>	1	2	2	3	2	3
<i>Rhabdocoma</i>	1	2	2	3	2	2
<i>Amphimonhystrella</i>	1	2	2	3	2	2
<i>Thalassomonhystera</i>	1	2	2	3	2	2
<i>Theristus</i>	1	2	2	3	2	2
<i>Microgaster</i>	1	2	2	3	2	2
<i>Leptolaimoides</i>	1	2	2	3	2	2
<i>Sabatieria</i>	1	2	2	3	2	2
<i>Thalassomonhystera</i>	1	2	2	3	2	2
<i>Acantholaimus</i>	1	2	2	3	2	2
<i>Theristus</i>	1	2	2	3	2	2
<i>Halalaimus</i>	1	2	2	3	2	2
<i>Hopperia</i>	1	2	2	3	2	2
<i>Molgolaimus</i>	1	2	2	3	2	2
<i>Leptolaimus</i>	1	2	2	3	2	2
<i>Amphimonhystrella</i>	1	2	2	3	2	2
<i>Greiffelia</i>	1	2	2	3	2	2
<i>Diplopeltula</i>	1	2	2	3	2	2
<i>Halalaimus</i>	1	2	2	3	2	2
<i>Leptolaimus</i>	1	2	2	3	2	2
<i>Amphimonhystrella</i>	1	2	2	3	2	2
<i>Microgaster</i>	1	2	2	3	2	2
<i>Theristus</i>	1	2	2	3	2	2
<i>Desmoscolex</i>	1	2	2	3	2	2
<i>Halichoanulaimus</i>	1	2	2	3	2	2
<i>Cervonema</i>	1	2	2	3	2	2
<i>Neochromadora</i>	1	2	2	3	2	2
<i>Oxystomina</i>	1	2	2	3	2	2
<i>Aegialolaimus</i>	1	2	2	3	2	2
<i>Monhystrella</i>	1	2	2	3	2	2
<i>Oxystomina</i>	1	2	2	3	2	2
<i>Campylolaimus</i>	1	2	2	3	2	2
<i>Linhystera</i>	1	2	2	3	2	2
<i>Leptolaimoides</i>	1	2	2	3	2	2
<i>Hopperia</i>	1	2	2	3	2	2
<i>Prototricoma</i>	1	2	2	3	2	2
<i>Metadesmolaimus</i>	1	2	2	3	2	2
<i>Linhomoeus</i>	1	2	2	3	2	2
<i>Sabatieria</i>	1	2	2	3	2	2
<i>Thalassomonhystera</i>	1	2	2	3	2	2
<i>Acantholaimus</i>	1	2	2	3	2	2
<i>Theristus</i>	1	2	2	3	2	2
<i>Halalaimus</i>	1	2	2	3	2	2
<i>Hopperia</i>	1	2	2	3	2	2
<i>Molgolaimus</i>	1	2	2	3	2	2
<i>Leptolaimus</i>	1	2	2	3	2	2
<i>Amphimonhystrella</i>	1	2	2	3	2	2
<i>Greiffelia</i>	1	2	2	3	2	2
<i>Diplopeltula</i>	1	2	2	3	2	2
<i>Halalaimus</i>	1	2	2	3	2	2
<i>Leptolaimus</i>	1	2	2	3	2	2
<i>Amphimonhystrella</i>	1	2	2	3	2	2
<i>Microgaster</i>	1	2	2	3	2	2
<i>Theristus</i>	1	2	2	3	2	2
<i>Desmoscolex</i>	1	2	2	3	2	2
<i>Halichoanulaimus</i>	1	2	2	3	2	2
<i>Cervonema</i>	1	2	2	3	2	2
<i>Neochromadora</i>	1	2	2	3	2	2
<i>Oxystomina</i>	1	2	2	3	2	2
<i>Aegialolaimus</i>	1	2	2	3	2	2
<i>Monhystrella</i>	1	2	2	3	2	2
<i>Oxystomina</i>	1	2	2	3	2	2
<i>Campylolaimus</i>	1	2	2	3	2	2
<i>Linhystera</i>	1	2	2	3	2	2
<i>Leptolaimoides</i>	1	2	2	3	2	2
<i>Hopperia</i>	1	2	2	3	2	2
<i>Prototricoma</i>	1	2	2	3	2	2
<i>Metadesmolaimus</i>	1	2	2	3	2	2
<i>Linhomoeus</i>	1	2	2	3	2	2
<i>Sabatieria</i>	1	2	2	3	2	2
<i>Thalassomonhystera</i>	1	2	2	3	2	2
<i>Acantholaimus</i>	1	2	2	3	2	2
<i>Theristus</i>	1	2	2	3	2	2
<i>Halalaimus</i>	1	2	2	3	2	2
<i>Hopperia</i>	1	2	2	3	2	2
<i>Molgolaimus</i>	1	2	2	3	2	2
<i>Leptolaimus</i>	1	2	2	3	2	2
<i>Amphimonhystrella</i>	1	2	2	3	2	2
<i>Greiffelia</i>	1	2	2	3	2	2
<i>Diplopeltula</i>	1	2	2	3	2	2
<i>Halalaimus</i>	1	2	2	3	2	2
<i>Leptolaimus</i>	1	2	2	3	2	2
<i>Amphimonhystrella</i>	1	2	2	3	2	2
<i>Microgaster</i>	1	2	2	3	2	2
<i>Theristus</i>	1	2	2	3	2	2
<i>Desmoscolex</i>	1	2	2	3	2	2
<i>Halichoanulaimus</i>	1	2	2	3	2	2
<i>Cervonema</i>	1	2	2	3	2	2
<i>Neochromadora</i>	1	2	2	3	2	2
<i>Oxystomina</i>	1	2	2	3	2	2
<i>Aegialolaimus</i>	1	2	2	3	2	2
<i>Monhystrella</i>	1	2	2	3	2	2
<i>Oxystomina</i>	1	2	2	3	2	2
<i>Campylolaimus</i>	1	2	2	3	2	2
<i>Linhystera</i>	1	2	2	3	2	2
<i>Leptolaimoides</i>	1	2	2	3	2	2
<i>Hopperia</i>	1	2	2	3	2	2
<i>Prototricoma</i>	1	2	2	3	2	2
<i>Metadesmolaimus</i>	1	2	2	3	2	2
<i>Linhomoeus</i>	1	2	2	3	2	2
<i>Sabatieria</i>	1	2	2	3	2	2
<i>Thalassomonhystera</i>	1	2	2	3	2	2
<i>Acantholaimus</i>	1	2	2	3	2	2
<i>Theristus</i>	1	2	2	3	2	2
<i>Halalaimus</i>	1	2	2	3	2	2
<i>Hopperia</i>	1	2	2	3	2	2
<i>Molgolaimus</i>	1	2	2	3	2	2
<i>Leptolaimus</i>	1	2	2	3	2	2
<i>Amphimonhystrella</i>	1	2	2	3	2	2
<i>Greiffelia</i>	1	2	2	3	2	2
<i>Diplopeltula</i>	1	2	2	3	2	2
<i>Halalaimus</i>	1	2	2	3	2	2
<i>Leptolaimus</i>	1	2	2	3	2	2
<i>Amphimonhystrella</i>	1	2	2	3	2	2
<i>Microgaster</i>	1	2	2	3	2	2
<i>Theristus</i>	1	2	2	3	2	2
<i>Desmoscolex</i>	1	2	2	3	2	2
<i>Halichoanulaimus</i>	1	2	2	3	2	2
<i>Cervonema</i>	1	2	2	3	2	2
<i>Neochromadora</i>	1	2	2	3	2	2
<i>Oxystomina</i>	1	2	2	3	2	2
<i>Aegialolaimus</i>	1	2	2	3	2	2
<i>Monhystrella</i>	1	2	2	3	2	2
<i>Oxystomina</i>	1	2	2	3	2	2
<i>Campylolaimus</i>	1	2	2	3	2	2
<i>Linhystera</i>	1	2	2	3	2	2
<i>Leptolaimoides</i>	1	2	2	3	2	2
<i>Hopperia</i>	1	2	2	3	2	2
<i>Prototricoma</i>	1	2	2	3	2	2
<i>Metadesmolaimus</i>	1	2	2	3	2	2
<i>Linhomoeus</i>	1	2	2	3	2	2

Table 4. Relative abundance of *Sabatieria* species (%) in the top 5 cm of the sediment cores in relation to distance (Dist.; m) from the Darwin mud volcano seep site (0 = seep sediment)

Dist.: 0		2		5		10		100		1100	
Species	%	Species	%	Species	%	Species	%	Species	%	Species	%
<i>S. vasicola</i>	29	<i>S. bitumen</i>	38	<i>S. bitumen</i>	41	<i>S. bitumen</i>	40	<i>S. ornata</i>	11	<i>S. stekhoveni</i>	52
<i>S. punctata</i>	21	<i>S. ornata</i>	35	<i>S. aff. breviseta</i>	16	<i>S. stekhoveni</i>	20	<i>S. bitumen</i>	10	<i>S. aff. breviseta</i>	10
<i>S. stekhoveni</i>	18	<i>S. propisinna</i>	18	<i>S. propisinna</i>	16	<i>S. ornata</i>	16	<i>S. demani</i>	7	<i>S. propisinna</i>	8
<i>S. ornata</i>	10	<i>S. stekhoveni</i>	10	<i>S. demani</i>	12	<i>S. propisinna</i>	13	<i>S. stekhoveni</i>	5	<i>S. conicauda</i>	7
<i>S. aff. breviseta</i>	8			<i>S. stekhoveni</i>	12	<i>S. demani</i>	12	<i>S. aff. breviseta</i>	3	<i>S. demani</i>	6
<i>S. conicauda</i>	5			<i>S. ornata</i>	3			<i>S. conicauda</i>	3	<i>S. lawsi</i>	6
<i>S. demani</i>	5							<i>S. punctata</i>	2	<i>S. ornata</i>	6
										<i>S. punctata</i>	4
										<i>S. vasicola</i>	3

Effect of pore-water geochemistry on meiofaunal distribution and tolerance to high H₂S levels

At the Darwin MV, meiofaunal densities were much higher in and immediately near the seep site (2 m) compared to at the sites showing no sign of seep influence in terms of pore-water geochemistry. Accordingly, Vanreusel et al. (2010) showed elevated meiofaunal standing stock in seep compared to non-seep sediments for several other seep systems. The seep sediment core had high H₂S content (up to 22 mM), as shown for several other seeps (Barry et al. 1997, Sahling et al. 2002, Levin et al. 2003). These high H₂S levels affected the vertical distribution in the sediment, in that the proportion of meiofauna confined to the sediment surface was highest in this core. Tolerance of high H₂S levels was genus (and species) specific. *Sabatieria* and *Desmodora*, which dominated the seep sediment core, were more tolerant to high H₂S than genera absent from this core.

In sulphidic environments, bacterial symbionts can help to detoxify H₂S (Ott et al. 2004). In bathyal oxygen minimum zone sediments, *Desmodora masira* had ectosymbionts (Bernhard et al. 2000). Although in our study, TEM cross-sections paralleled the annuli (in contrast to Bernhard et al. 2000), the low and irregular bacterial appearance implies that *Desmodora* from the seep sediment core did not harbour ectosymbionts. TEM showed electron-lucent structures near the cuticle resembling the sulphur inclusions described by Thiermann et al. (2000), but STEM-EDX analysis only detected traces of sulphur. However, elemental sulphur is known to leach from vesicles during the chemical fixation, dehydration and resin infiltration of biological samples, which may explain the low sulphur content (Lechlaire et al. 2006). Increased body length is another adaptation to sulphidic conditions, and enables a fast migration between anoxic, sulphidic and oxic H₂S-free sedi-

ments (Schratzberger et al. 2004, Levin 2005). Accordingly, *Sabatieria punctata* and *S. vasicola*, which dominated the seep sediment core, were amongst the longest nematodes in the present study.

Meiofaunal and nematode community structure

Meiofaunal density patterns were mainly driven by the dominant taxon, i.e. the nematodes. Nematodes dominate most seep habitats (Shirayama & Ohta 1990, Robinson et al. 2004, Van Gaever et al. 2009a, Van Gaever et al. 2009c), although some habitats are

Fig. 6. ln-transformed diversity indices based on abundances of nematode genera in relation to distance from the Darwin mud volcano seep site. N_0 , N_1 , N_{inf} : Hill's numbers; H' : Shannon-Wiener diversity index; J' : Pielou's evenness number; EG(100): expected number of genera for $n = 100$. 0 = seep sediment

Fig. 7. *Desmodora* (Des), *Sabatieria* (Sab) and 'Mix' (A) carbon and (B) nitrogen isotope signatures in relation to distance from the Darwin mud volcano seep site. 0 = seep sediment. At 10 m distance, no $\delta^{13}\text{C}$ was available for 0 to 2 cm due to the low amount of C, making the isotope value unreliable. Sediment layers: ○ = 0 to 2 cm; ● = 2 to 4 cm; ● = 4 to 6 cm

Fig. 8. Nematode trophic diversity in the top 5 cm of the sediment cores in relation to distance from the Darwin mud volcano seep site (0 = seep sediment)

dominated by copepods (Van Gaever et al. 2006). In the Darwin MV seep sediment core, meiofauna-sized polychaetes were subdominant, similar to the mussel beds at the REGAB seep (Van Gaever et al. 2009a).

Nematode genus composition clearly differed between cores with and without CH_4 flow. Thus, CH_4 flow affected not only densities and biomass, but also composition. Genus diversity was lowest in the seep sediment core and increased in cores farther from the seep site, as shown in previous studies (Van Gaever et al. 2009a,c). *Desmodora* and *Sabatieria* also dominated the REGAB seep in the Gulf of Guinea (Van Gaever et al. 2009a), although in association with different habitats: the REGAB samples originated

Fig. 9. Transmission electron microscope micrograph of a cross-section of *Desmodora* from the Darwin mud volcano seep sediment core: (A) overview and (B) detailed depiction showing bacteria associated with the cuticle. The white arrow points to electronluculent structures, possibly containing sulphur prior to ethanol dehydration. Ba: bacterial cell

from clam and mussel fields with low H_2S content ($<0.1 \mu\text{M}$; Olu et al. 2009). *S. vasicola* and *S. punctata*, which dominated the Darwin MV seep sediment core, also occur in shallow waters (Vitiello 1970, Jensen et al. 1992, Franco et al. 2008). Accordingly, the dominant species at the REGAB seep (*S. mortenseni*), the Arctic Håkon Mosby MV (*Halomonhystera disjuncta*) (Van Gaever et al. 2006) and the Nordic Nyegga seep (*Terschellingia longicaudata*) (Van Gaever et al. 2009c) inhabit shallow waters as well. The presence of these species in both shallow waters and at a deep-sea seep suggests a possible connection between these habitats, rather than between deep-sea seeps (Van Gaever et al. 2009c).

Nematode feeding preferences

$\delta^{13}\text{C}$ values suggest thiotrophic C is part of the nematode diet up to 10 m from the seep site. Except for 'Mix' in the 4 to 6 cm sediment layer, which displays a $\delta^{13}\text{C}$ of -21.3‰ , all $\delta^{13}\text{C}$ were less than -28‰ . Organic matter produced through sulphur-oxidation has an average $\delta^{13}\text{C}$ of -30‰ (RubisCO I) or -11‰ (RubisCO II), depending on the Rubisco enzyme involved (Robinson & Cavanaugh 1995). In comparison, photosynthetic C is characterized by a $\delta^{13}\text{C}$ of between -18 and -28‰ (Stewart et al. 2005), and CH_4 -derived C is more depleted in ^{13}C ($\delta^{13}\text{C} < -50\text{‰}$) (Levin & Michener 2002). Since we did not sample potential C sources for stable isotope analysis, we cannot estimate their relative contribution to the nematode diet. Nonetheless, a decrease in thiotrophic (RubisCO I) and an increase in photosynthetic C in the nematode diet farther from the seep site are implied by: (1) the thicker hemipelagic sediment veneer on top of the cores, suggesting a higher availability of photosynthetic C, (2) an increase in trophic diversity, and (3) heavier $\delta^{13}\text{C}$.

Spies & DesMarais (1983) and Van Gaever et al. (2006, 2009b) reported direct nematode consumption of sulphur-oxidisers. These bacteria live at the interface between oxic and anoxic sediments, where H_2S levels are $\leq 1 \mu\text{M}$ (Robertson & Kuenen 2006, Preisler et al. 2007). We doubt these bacteria inhabited the seep sediments given the absence of bacterial mats and the high H_2S levels at greater depth. In the core collected 2 m from the seep site, we observed no net SO_4^{2-} production, expected in the presence of sulphur-oxidisers. Nematodes can indirectly consume sulphur-oxidisers by assimilating dissolved organic matter (DOM) released upon bacterial lysis. Jensen (1987) suggested thiobiotic nematodes feed, at least

partially, on DOM. However, further evidence is needed to support this hypothesis. Stable isotope signatures suggest different feeding strategies for *Sabatieria* and *Desmodora* compared with the bulk nematode community, which may explain their success. As with other seeps (Vanreusel et al. 2010), deposit-feeders dominated. Finally, although this exploratory study hints at how meiofauna interact with the seep environment, much more, high-resolution research is required to understand their tolerance of sulphide, trophic interactions and dispersal capacities.

Acknowledgements. The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under the HERMIONE project, grant agreement no. 226354, and was supported by the FWO 'cold seeps' project No. G034607. The authors are greatly indebted to the crew and captain of the RRS 'James Cook' and the pilots of ROV 'Isis' for their expertise and professionalism. We also gratefully acknowledge M. Claeys for her assistance and the preparation of the TEM photographs. Finally, yet importantly, we greatly appreciated the comments of the editor, the reviewers and Lois Maignien.

LITERATURE CITED

- Andrassy I (1956) The determination of volume and weight of nematodes. *Acta Zool Hung* 2:1–15
- Barry JP, Kochevar RE, Baxter CH (1997) The influence of pore-water chemistry and physiology on the distribution of vesicomyid clams at cold seeps in Monterey Bay: implications for patterns of chemosynthetic community organization. *Limnol Oceanogr* 42:318–328
- Bernhard JM, Buck KR, Farmer MA, Bowser SS (2000) The Santa Barbara Basin is a symbiosis oasis. *Nature* 403: 77–80
- Boetius A, Ravensschlag K, Schubert CJ, Rickert D and others (2000) A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* 407:623–626
- Carman KR, Fry B (2002) Small-sample methods for delta $\delta^{13}\text{C}$ and delta $\delta^{15}\text{N}$ analysis of the diets of marsh meiofaunal species using natural-abundance and tracer-addition isotope techniques. *Mar Ecol Prog Ser* 240:85–92
- Clarke K, Gorley R (2006) PRIMER v6: user manual/tutorial. Primer-E Ltd, Plymouth
- Dando PR, Austen MC, Burke RA, Kendall MA and others (1991) Ecology of a North Sea pockmark with an active methane seep. *Mar Ecol Prog Ser* 70:49–63
- Franco MA, Soetaert K, Van Oevelen D, Van Gansbeke D, Costa MJ, Vincx M, Vanaverbeke J (2008) Density, vertical distribution and trophic responses of metazoan meiobenthos to phytoplankton deposition in contrasting sediment types. *Mar Ecol Prog Ser* 358:51–62
- Genio L, Johnson SB, Vrijenhoek RC, Cunha MR, Tyler PA, Kiel S, Little CTS (2008) New record of '*Bathymodiolus mauritanicus*' Cosel, 2002 from the Gulf of Cadiz (NE Atlantic) mud volcanoes. *J Shellfish Res* 27:53–61
- Grasshoff K, Ehrhardt M, Kremling K, Anderson L (1999) Methods of seawater analysis. Wiley-VCH, Weinheim

- Heip C, Vincx M, Vranken G (1985) The ecology of marine nematodes. *Oceanogr Mar Biol Annu Rev* 23:399–489
- Helms J, Munzel U (2008) NPMC: nonparametric multiple comparisons. R package version 10-7, accessed 17 April 2009
- Hoehler T, Borowski W, Alperin M, Rodriguez N, Paull C (2000) Model, stable isotope and radiotracer characterization of anaerobic methane oxidation in gas hydrate-bearing sediments of the Blake ridge. *Proc Ocean Drill Program, Sci Results* 164:79–85
- Ivanenkov V, Lyakhin Y (1978) Determination of total alkalinity in seawater. In: *Methods of hydrochemical investigations in the ocean*. Nauka Publ. House, Moscow, p 110–114
- Jensen P (1984) Measuring carbon content in nematodes. *Helgol Meeresunters* 38:83–86
- Jensen P (1987) Feeding ecology of free-living aquatic nematodes. *Mar Ecol Prog Ser* 35:187–196
- Jensen P, Aagaard I, Burke RA, Dando PR and others (1992) 'Bubbling Reefs' in the Kattegat: submarine landscapes of carbonate-cemented rocks support a diverse ecosystem at methane seeps. *Mar Ecol Prog Ser* 83:103–112
- Knittel K, Boetius A (2009) Anaerobic oxidation of methane: progress with an unknown process. *Annu Rev Microbiol* 63:311–334
- Lambshead PJD, Boucher G (2003) Marine nematode deep-sea biodiversity—Hyperdiverse or hype? *J Biogeogr* 30: 475–485
- Lechère JP, Frébourg G, Gaill F, Gros O (2006) *In situ* localization of sulphur in the thioautotrophic symbiotic model *Lucina pectinata* (Gmelin, 1791) by cryo-EFTEM microanalysis. *Biol Cell* 98:163–170
- 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, Ziebis W, Mendoza GF, Growney VA and others (2003) Spatial heterogeneity of macrofauna at northern California methane seeps: influence of sulfide concentration and fluid flow. *Mar Ecol Prog Ser* 265:123–139
- Middelburg JJ, Soetaert K, Herman PMJ (1997) Empirical relationships for use in global diagenetic models. *Deep-Sea Res I* 44:327–344
- Munzel U, Hothorn LA (2001) A unified approach to simultaneous rank test procedures in the unbalanced one-way layout. *Biom J* 43:553–569
- Olu Roy K, Lance S, Sibuet M, Henry P, Fiala-Medioni A, Dinot 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
- Olu Roy K, Caprais JC, Galéron J, Causse R and others (2009) Influence of seep emission on the non-symbiont-bearing fauna and vagrant species at an active giant pockmark in the Gulf of Guinea (Congo–Angola margin). *Deep-Sea Res II* 56:2380–2393
- Ott J, Bright M, Bulgheresi S (2004) Symbioses between marine nematodes and sulfur-oxidizing chemoautotrophic bacteria. *Symbiosis* 36:103–126
- Pinheiro LM, Ivanov MK, Sautkin A, Akhmanov G and others (2003) Mud volcanism in the Gulf of Cadiz: results from the TTR-10 cruise. *Mar Geol* 195:131–151
- Post DM (2002) Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83: 703–718
- Preisler A, de Beer D, Lichtschlag A, Lavik G, Boetius A, Jørgensen BB (2007) Biological and chemical sulfide oxidation in a *Beggiatoa* inhabited marine sediment. *ISME J* 1:341–353
- Qi H, Coplen TB, Geilmann H, Brand WA, Bohlke JK (2003) Two new organic reference materials $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements and a new value for the $\delta^{13}\text{C}$ of NBS 22 oil. *Rapid Commun Mass Spectrom* 17:2483–2487
- R Development Core Team (2010) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available at www.R-project.org
- Reeburgh WS (1976) Methane consumption in Cariaco Trench waters and sediments. *Earth Planet Sci Lett* 28: 337–344
- Reeburgh WS (2007) Oceanic methane biogeochemistry. *Chem Rev* 107:486–513
- Robertson L, Kuenen J (2006) The colorless sulfur bacteria. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds) *The prokaryotes: ecophysiology and biochemistry*. Springer, New York, NY, p 985–1011
- Robinson JL, Cavanaugh CM (1995) Rubisco in chemoautotrophic symbioses: implications for the interpretation of stable carbon isotope values. *Limnol Oceanogr* 40: 1496–1502
- Robinson C, Bernhard J, Levin L, Mendoza G, Blanks J (2004) Surficial hydrocarbon seep infauna from the Blake Ridge (Atlantic Ocean, 2150 m) and the Gulf of Mexico (690–2240 m). *PSZNI: Mar Ecol* 25:313–336
- Sahling H, Rickert D, Lee RW, Linke P, Suess E (2002) Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin, NE Pacific. *Mar Ecol Prog Ser* 231:121–138
- Schratzberger M, Whomersley P, Warr K, Bolam SG, Rees HL (2004) Colonisation of various types of sediment by estuarine nematodes via lateral infaunal migration: a laboratory study. *Mar Biol* 145:69–78
- Seeberg-Elverfeldt J, Schlüter M, Feseker T, Kölling M (2005) Rhizon sampling of pore waters near the sediment/water interface of aquatic systems. *Limnol Oceanogr Methods* 3:361–371
- Shirayama Y, Ohta S (1990) Meiofauna in a cold-seep community off Hatsushima, Central Japan. *J Oceanogr* 46: 118–124
- Soetaert K, Franco M, Lampadariou N, Muthumbi A, Steyaert M, Vandepitte L, vanden Berghe E, Vanaverbeke J (2009) Factors affecting nematode biomass, length and width from the shelf to the deep sea. *Mar Ecol Prog Ser* 392:123–132
- Somoza L, Diaz-del-Rio V, Leon R, Ivanov M and others (2003) Seabed morphology and hydrocarbon seepage in the Gulf of Cadiz mud volcano area: acoustic imagery, multibeam and ultra-high resolution seismic data. *Mar Geol* 195:153–176
- Spies R, DesMarais D (1983) Natural isotope study of trophic enrichment of marine benthic communities by petroleum seepage. *Mar Biol* 73:67–71
- Stewart FJ, Newton ILG, Cavanaugh CM (2005) Chemosynthetic endosymbioses: adaptations to oxic–anoxic interfaces. *Trends Microbiol* 13:439–448
- Thiermann F, Vismann B, Giere O (2000) Sulphide tolerance of the marine nematode *Oncholaimus campylocer-*

- coides*—A result of internal sulphur formation? *Mar Ecol Prog Ser* 193:251–259
- Van Gaever S, Moodley L, de Beer D, Vanreusel A (2006) Meiobenthos at the Arctic Hakon Mosby Mud Volcano, with a parental-caring nematode thriving in sulphide-rich sediments. *Mar Ecol Prog Ser* 321:143–155
- Van Gaever S, Galéron J, Sibuet M, Vanreusel A (2009a) Deep-sea habitat heterogeneity influence on meiofaunal communities in the Gulf of Guinea. *Deep-Sea Res II* 56: 2259–2269
- Van Gaever S, Moodley L, Pasotti F, Houtekamer M, Middelburg JJ, Danovaro R, Vanreusel A (2009b) Trophic specialisation of metazoan meiofauna at the Hakon Mosby Mud Volcano: fatty acid biomarker isotope evidence. *Mar Biol* 156:1289–1296
- Van Gaever S, Olu-Le Roy K, Derycke S, Vanreusel A (2009c) Metazoan meiofaunal communities at cold seeps along the Norwegian margin: influence of habitat heterogeneity and evidence for connection with shallow-water habitats. *Deep-Sea Res I* 56:772–785
- Van Rensbergen P, Depreiter D, Pannemans B, Moerkerke G and others (2005) The El Arraiche mud volcano field at the Moroccan Atlantic slope, Gulf of Cadiz. *Mar Geol* 219:1–17
- Vanreusel A, Andersen AC, Boetius A, Connelly D and others (2009) Biodiversity of cold seep ecosystems along the European margins. *Oceanography (Wash DC)* 22: 110–127
- Vanreusel A, De Groote A, Gollner S, Bright M (2010) Ecology and biogeography of free-living nematodes associated with chemosynthetic environments in the deep sea: a review. *PLoS ONE* 5:e12449
- Vitiello P (1970) Nématodes libres marins des vases profondes du Golfe du Lion. II. Chromadorida. *Tethys* 2: 449–500
- Wieser W (1953) Die Beziehung zwischen Mundhöhlengestalt, Ernährungsweise und Vorkommen bei freilebenden marinen Nematoden. *Ark Zool* 2:439–484

*Editorial responsibility: Paul Snelgrove,
St. John's, Newfoundland, Canada*

*Submitted: June 17, 2010; Accepted: June 30, 2011
Proofs received from author(s): September 20, 2011*