

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

Saskia Van Gaever · Leon Moodley · Francesca Pasotti ·  
Marco Houtekamer · Jack J. Middelburg ·  
Roberto Danovaro · Ann Vanreusel

Received: 8 October 2008 / Accepted: 20 February 2009 / Published online: 12 March 2009  
© The Author(s) 2009. This article is published with open access at Springerlink.com

**Abstract** We report the results of a detailed investigation on the trophoecology of two dominant meiofaunal species at the Håkon Mosby Mud Volcano (HMMV), a deep-sea cold methane-venting seep. Analyses of fatty acids (FAs) and their stable carbon isotopes were used to determine the importance of chemosynthetic nutritional pathways for the dominant copepod species (morphologically very similar to *Tisbe wilsoni*) inhabiting the volcano's centre and the abundant nematode *Halomonhystera disjuncta* from the surrounding microbial mats. The strong dominance of bacterial biomarkers (16:1 $\omega$ 7c, 18:1 $\omega$ 7c and 16:1 $\omega$ 8c) coupled with their individual light carbon isotopes signatures ( $\delta^{13}\text{C}$  ranging from  $-52$  to  $-81\text{‰}$ ) and the lack of symbiotic relationships with prokaryotes (as revealed by molecular analyses and fluorescent in situ hybridisation) indicated that chemosynthetically derived carbon constitutes the main diet of both species. However, the copepod showed a

stronger reliance on the utilisation of methanotrophic bacteria and contained polyunsaturated FAs of bacterial origin (20:5 $\omega$ 3 and 22:6 $\omega$ 3 with isotope signatures  $\delta^{13}\text{C} < -80\text{‰}$ ). Instead, the FA profiles of *H. disjuncta* suggested that sulphide-oxidising bacteria constituted the main diet of this nematode. Therefore, HMMV can be regarded as a persistent deep-sea cold seep, allowing a chemosynthesis-based trophic specialisation by the dominant meiofaunal species inhabiting its sediments. The present investigation, through the determination of the fatty acid profiles, provides the first evidence for trophic specialisation of meiofauna associated with sub-habitats within a cold seep.

## Introduction

Unlike the majority of seafloor environments, hydrothermal vents and cold seeps represent marine ecosystems that may be largely independent of the input of photosynthetic primary production as its primary energy source (e.g. Levin 2005; Stewart et al. 2005; Saito and Osako 2007). In situ chemosynthetically produced microbial biomass, either as free-living prokaryotes or in symbiotic relationships with fauna, can be a major, sometimes the only, source of energy for resident benthic fauna. Unravelling the trophic relevance of the photosynthetic versus chemosynthetic organic carbon is of primary importance to improving our understanding of the functioning of these ecosystems. While a large amount of information was acquired on the trophodynamics of the surface-dwelling megafauna (e.g. large tube worms, mussels and clams) that colonise these habitats, very limited information is available on the sediment-dwelling fauna (infauna). Data available from larger macrofauna inhabiting deep-sea seeps suggest a dependence on chemosynthetic derived carbon, typically stronger than that

---

Communicated by M. Kühl.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00227-009-1170-9) contains supplementary material, which is available to authorized users.

---

S. Van Gaever (✉) · A. Vanreusel  
Marine Biology Section, Department of Biology,  
Ghent University, Krijgslaan 281/S8, 9000 Ghent, Belgium  
e-mail: Saskia.VanGaever@UGent.be

L. Moodley · M. Houtekamer · J. J. Middelburg  
Center for Estuarine and Marine Ecology,  
Netherlands Institute of Ecology (NIOO-KNAW),  
PO Box 140, 4400 AC Yerseke, The Netherlands

F. Pasotti · R. Danovaro  
Dipartimento Scienze del Mare,  
Università Politecnica delle Marche,  
Via Breccie Bianche, 60131 Ancona, Italy

estimated for shallow-water seeps (Levin 2005). Furthermore, this nutritional link is primarily heterotrophic (feeding on free-living chemoauto- or heterotrophic bacteria) and it depends to a lesser extent on bacterial symbiotic relationships (Levin 2005). Trophoecological studies on the metazoan meiofauna are scarce, primarily due to the small size of the fauna and the inherent difficulty in obtaining sufficient biomass for subsequent analyses of the stable isotopes or fatty acids (FAs). Nematodes generally represent a major fraction of seep sediment infauna, and some of the highest marine nematode densities and biomasses were observed in cold seep sediments (e.g. Olu et al. 1997; Van Gaever et al. 2006); bulk tissue stable carbon and nitrogen isotope signatures have revealed that some cold seep nematodes utilise chemosynthetically derived carbon (Van Dover et al. 2003; Van Gaever et al. 2006). At the seep addressed in this study, Van Gaever et al. (2006) reported the strong dominance and proliferation of a single nematode species, *Halomonhystera disjuncta*, in the microbial mat sub-habitat. The striking high nematode biomass could not be attributed to photosynthetically produced carbon because of the low organic matter input. Moreover, in the seep centre, the nematodes were replaced by a species of copepods (morphologically very similar to *Tisbe wilsoni*) with bulk tissue  $\delta^{13}\text{C}$  values indicative of a strong trophic link with methane-derived carbon ( $\delta^{13}\text{C} \sim -51\text{‰}$ ).

Meiobenthic (nematode) assemblages in non-seep or shallow seep sediments are more diverse and may be related to the presence and utilisation of both photosynthetically and chemosynthetically derived carbon (Levin et al. 2000; Levin 2005). However, the extent to which these meiobenthic diversity and biomass patterns are related to trophic resources remains unclear, just as the carbon flow pathways (symbiosis versus feeding on free living prokaryotes; Levin et al. 2000; Levin and Mendoza 2007).

In this study, we report on a detailed investigation of metazoan meiofauna trophoecology at the Håkon Mosby Mud Volcano (HMMV). HMMV is located at a water depth of 1,280 m on the Barents Sea and is the only mud volcano in a polar region that was mapped in detail by photo and video camera observations and studied in detail for its biogeochemical habitats and microbial communities (Niemann et al. 2006; de Beer et al. 2006; Jerosch et al. 2007). Significantly different meiobenthic densities were found at the different sub-habitats (seep centre, microbial mats, Siboglinidae field and outer rim); the anoxic and sulphidic sediments at the microbial mat habitat were inhabited by extremely high densities ( $>11,000$  ind.  $10\text{ cm}^{-2}$ , 0–5 cm sediment depth) of *H. disjuncta* (originally referred to and described as *Geomonhystera disjuncta*; Van Gaever et al. 2006). The central, seep part of the volcano was characterised by high concentrations of methane and the lack of sulphide (de Beer et al. 2006). Moreover, here nematodes were virtually

absent while copepods were abundant. Different sediment geochemistry across seep sub-habitats supporting distinct microbial communities (Niemann et al. 2006) may strongly regulate and explain meiofaunal distribution patterns in this deep-sea cold seep (Van Gaever et al. 2006). Moreover, the tolerance of the nematode to high sulphide levels could be the main reason for its dense, mono-specific population at the microbial mats, in contrast to the absent copepod species. However, these density distribution patterns as well as trophic relationships remain unresolved and in this study we attempt to unravel carbon sources as well as carbon flow pathways of the two dominant taxa using multiple molecular approaches and transmission electron microscopy (TEM).

Bacterial symbionts (ecto- or endosymbionts) can be either a source and pathway of chemosynthetically derived organic matter entering the higher trophic levels or play a role in detoxification in fauna inhabiting sulphide-rich sediments (e.g. Thiermann et al. 2000; Levin 2005). We attempted to locate and identify symbiotic bacteria in the nematode *H. disjuncta*, using TEM photography coupled with automated rRNA intergenic spacer analysis (ARISA), fluorescent in situ hybridisation (FISH) and catalysed reporter deposition fluorescent in situ hybridisation (CARD-FISH). Fatty acid (FA) analysis combined with stable isotope analysis was applied to unravel the origin of the diet of the dominant copepod from the volcano's centre and the dominant nematode from the microbial mats. Fatty acid analyses were repeatedly used as source-specific indicators of organic matter, both in environmental and food web studies (e.g. Canuel et al. 1995; Kharlamenko et al. 1995; Phleger et al. 2005). This tool can be complemented by the analysis of stable carbon isotope signatures of the dominant FAs for further clarification of the food sources and trophic pathways (e.g. Abrajano et al. 1994; Pond et al. 1997; Lösekann et al. 2008). With methane at HMMV having a  $\delta^{13}\text{C}$  of  $\sim -60\text{‰}$  (Lein et al. 1999) and microbial mat sediment porewater dissolved inorganic carbon  $\delta^{13}\text{C} \sim -26\text{‰}$  (mass balance estimate of sediment-water slurry incubations, Van Gaever et al. 2006), in situ chemosynthetically produced carbon is expected to be reflected in more negative  $\delta^{13}\text{C}_{\text{org}}$  values than that of surface water photosynthetically produced carbon (e.g. Levin and Michener 2002; MacAvoy et al. 2002), although this can sometimes be masked or magnified depending on the carbon fixation pathway (e.g. Robinson and Cavanaugh 1995; Stewart et al. 2005).

## Materials and methods

### Sampling strategy

Sediment samples for meiofaunal analyses were collected at the HMMV during the VICKING cruise aboard the RV

“PourquoiPas?” in April–May 2006. The flat central zone of the volcano is characterised by grey mud flows with a high geothermal gradient (Pimenov et al. 2000). Beyond this central plain, the seafloor has a complex topography of hills and depressions, and is covered by extensive microbial mats of chemoautotrophic bacteria, dominated by giant sulphur-oxidising *Beggiatoa* bacteria (Pimenov et al. 2000; Niemann et al. 2006). Samples were retrieved at both the volcano’s centre (72°0.28′N; 14°43.56′E; 1273 m) and the surrounding microbial mats (72°00.16′N; 14°43.95′E; 1258 m). A multiple corer (surface area of 30.2 cm<sup>2</sup>) was deployed at the centre site, while the microbial mats were sampled with push cores of the ROV Victor 6000 (surface area: 21.2 cm<sup>2</sup>).

#### Transmission electron microscopy and molecular techniques

Transmission electron microscopy and multiple molecular approaches were used to identify possible bacterial symbionts in the dominant nematode species. The nematodes from the microbial mat sediment for TEM were picked out directly onboard, cleared of adhering sediment particles and immediately fixed in a Karnovsky solution diluted 50% with distilled water at room temperature. Further processing in the laboratory was according to the protocol in Willems et al. (2005) and cross-sections of 70 nm thick were examined using a TEM 1010 Jeol, operating at 80 kV.

Bulk sediment samples from the microbial mat site for nematode FISH and CARD-FISH were stored onboard in 4% formaldehyde at room temperature. Details of FISH and CARD-FISH are given in the online appendix (see “Protocol FISH and CARD-FISH”). For FISH, isolated and cleaned nematodes were further processed in Petri dishes according to the methods described in Pernthaler et al. (2002) and Vandekerckhove et al. (2002), followed by epifluorescence microscopy (Amann et al. 1995) on a Zeiss Axioskop 2 microscope. The preparation of the specimens for CARD-FISH analysis was modified from Vandekerckhove et al. (2002) after initial extra cleaning and dissection of the nematodes to improve probe penetration (see details on-line). The nematodes were then fixed on glass slides adding low-gelling-point (0.2%) to avoid specimen loss and CARD-FISH applied following the protocol by Pernthaler et al. (2002). Visualisation of the sample was done using a light microscope (Zeiss Axioskop 2) equipped with epifluorescence and with a Bio-Rad MRC-1024 confocal laser scanning system equipped with a krypton–argon laser (Amann et al. 1995; Vandekerckhove et al. 2002).

For the fingerprinting analysis ARISA, nematode specimens were isolated from bulk sediment that was stored in a

frozen state. DNA was extracted from *H. disjuncta* specimens with the protocol used by Floyd et al. (2002). Before DNA extraction, the nematodes were carefully washed to remove possible epibiotic bacteria and then cut to facilitate DNA extraction. The universal primer 16S-1392F (5′-GYACACACCGCCCGT-3′) and the bacterial primer 23S-125R (5′-GGGTTBCCCCATTTCRG-3′) were used (Hewson and Fuhrman 2004). Negative and positive controls were used.

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

Three replicates of the meiofauna (160 nematodes and 30 copepods each) and strands of *Beggiatoa* were hand-picked with a needle, cleared of adhering particles and rinsed twice in 0.2 µm filtered Milli-Q before being transferred to 2.5 ml GC vials that were frozen at –80°C and subsequently freeze dried. Fatty acid extraction and preparation of methyl esters (FAME) were carried out according to Masood et al. (2005) with reagent volumes adapted for use in this 2.5 ml GC-vials using FAME C19:0 as internal standard to calculate the concentration of FAs. These individual samples were analysed separately for

their FA compositions employing a large volume splitless injection method on a Thermo Finnigan Trace Ultra GC with the following configuration: large volume liner with glass wool, pre-column deactivated silica 5 m × 0.53 μm and analytical column SGE BPX-70 50 m × 0.32 mm × 0.25 μm. After these individual runs, the three replicates of each taxa were combined and evaporated to obtain sufficient material for analysis of FA carbon isotope signatures. The carbon isotopic composition of the dominant FAME was determined with a gas-chromatograph combustion interface isotope-ratio mass spectrometer (GC-c-IRMS; Boschker and Middelburg 2002). The identification of FAMES was based on comparison of retention times with authentic commercially available reference material and standards, confirmed by GC-MS analysis (de Goeij et al. 2008).

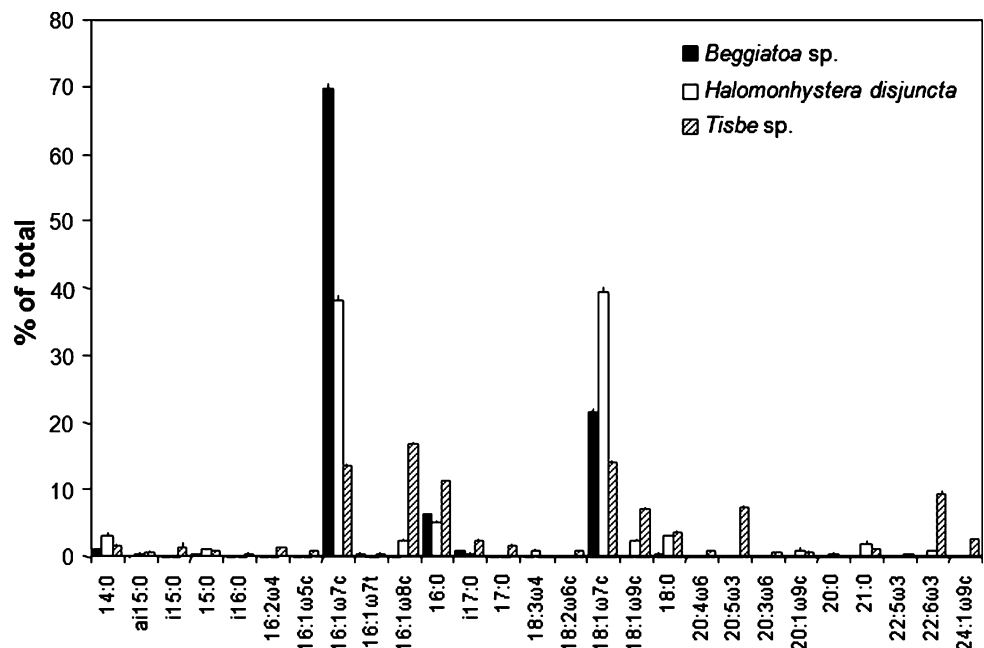
Fatty acid source designation was achieved using data on distinctive FAs for bacteria, algal carbon and higher organisms (e.g. Parrish et al. 2000; Boschker and Middelburg 2002; Volkman 2006). Although individual FAs biomarkers are rarely produced by a single class or organism, interpretations can be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

## Results

The FAs composition profiles of each component (*Beggiatoa*, nematodes and copepods) showed a very high similarity among the three replicates (SIMPER, similarity > 96%). In accordance with FA profiles reported for *Beggiatoa*, *Thioploca* and other sulphide-oxidising bacteria (Zhang et al. 2005; and references therein), a few FAs dominated the compositional pattern of the free-living sulphide-oxidising bacteria that typify the microbial white mat sub-habitat of the HMMV cold seep (Fig. 1). The monounsaturated FAs (MUFAs) 16:1 $\omega$ 7 (69%) and 18:1 $\omega$ 7 (21%) and the saturated FA 16:0 (6%) together contributed ~96% of the FAs composition of the *Beggiatoa* (Fig. 1). The corresponding light-stable carbon isotope signatures ( $\delta^{13}\text{C}$  ~-50‰; Table 1) can be attributed to the chemoautotrophic synthesis of these FAs (Conway and Capuzzo 1991; Pranal et al. 1996; Zhang et al. 2005). Conforming with earlier conclusions (e.g. Zhang et al. 2005), these results indicate that 16:1 $\omega$ 7 and 18:1 $\omega$ 7 can be used as signature biomarkers for sulphide-oxidising bacteria in hydrogen sulphide-rich sediments such as the HMMV mat sub-habitat.

Although the copepod from the seep centre also had a strong contribution of bacteria-synthesised FAs (Fig. 1), the FAs profiles were significantly different among the *Beggiatoa*, nematode and copepod (ANOSIM, global  $R = 1$ ,  $P < 0.05$ ). The difference between *Beggiatoa* and the nematode were caused by a higher percentage abundance of 16:1 $\omega$ 7 in *Beggiatoa* and relatively higher percentage contributions of 18:1 $\omega$ 7 and 18:0 in the nematode. The difference between the nematode and copepod is not only an overall more diverse set of FAs, but also the presence and

**Fig. 1** Fatty acid composition of *Beggiatoa* sp., the microbial mat nematode *Halomonhystera disjuncta* and the seep centre copepod *Tisbe* sp. collected at the Håkon Mosby Mud Volcano. Average percentage of total ( $n = 3$ )  $\pm$  1 SE





**Table 1**  $\delta^{13}\text{C}_{\text{org}}$  (‰) of the dominant fatty acids and bulk tissue

	Microbial mat		Seep centre
	<i>Beggiatoa</i> sp.	<i>Halomonhystera disjuncta</i>	<i>Tisbe</i> sp.
16:0	−46.0	−34.1	−69.9
16:1 $\omega$ 7c	−49.8	−52.6	−78.8
16:1 $\omega$ 8c	–	nd	−81.6
18:0	nd	−41.7	−69.1
18:1 $\omega$ 7c	−49.5	−53.8	−72.7
18:1 $\omega$ 9c	–	−40.8	−76.5
20:5 $\omega$ 3	–	–	−79.7
22:6 $\omega$ 3	–	nd	−86.1
Bulk tissue			
Year 2003	−42.7 (0.2)	−41.6 (0.4)	−51.2 (1.7)
Year 2006	nm	−50.2 (0.8)	nm

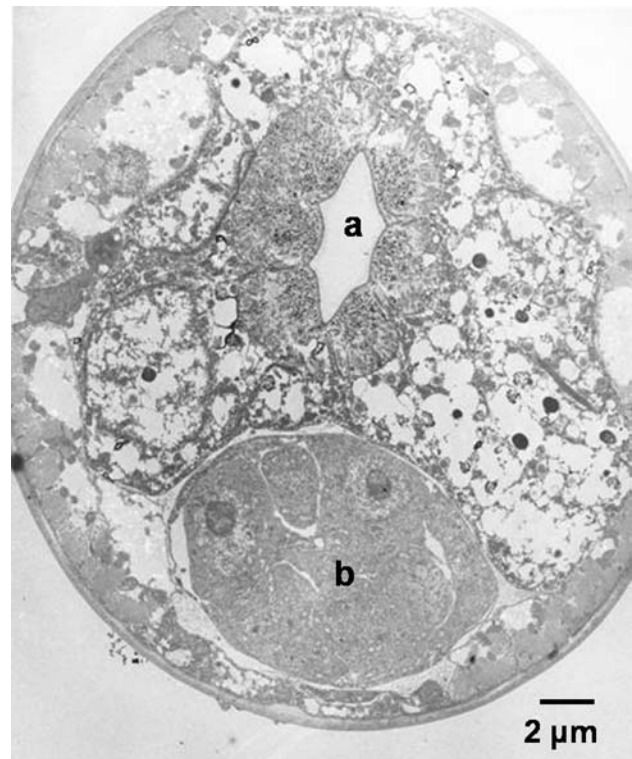
– Absent (i.e. not detected in LVGC and GC-IRMS), *nd* present, but not detected on the GC-IRMS due to low concentration, *nm* not measured

dominance of  $\omega$ 3 long-chain PUFAs in the copepod (Fig. 1). More specifically, SIMPER analysis revealed that the primary differences are the relatively higher percentage abundance of 18:1 $\omega$ 7 and 16:1 $\omega$ 7 in the nematode (39 and 38% versus 14 and 13%, respectively) compared to 16:1 $\omega$ 8, 22:6 $\omega$ 3 and 20:5 $\omega$ 3 in the copepod (17, 9 and 7% versus 2, 0.7 and 0%, respectively; Fig. 1).

TEM sections revealed well-developed intestines and ovaries in this nematode and clearly no evidence of symbiotic cells (Fig. 2). The conclusion that bacteria are used as a food source and not hosted as symbionts is supported by the lack of bacteria in the nematode tissues as revealed either by FISH, CARD-FISH or ARISA (data not shown).

## Discussion

The successful meiobenthic species in this sub-habitat, the nematode *H. disjuncta*, was characterised by a FAs profile indicative of a bacterial diet (Fig. 1): strong dominance of light  $\delta^{13}\text{C}$  MUFAs (16:1 $\omega$ 7 and 18:1 $\omega$ 7 together account for 80% of FAs carbon with a corresponding  $\delta^{13}\text{C}$  of −54‰; Table 1) and absence of  $\omega$ 3 and  $\omega$ 6 long-chain polyunsaturated FAs (PUFAs, commonly originating from photosynthetic plankton). Although MUFAs such as 16:1 $\omega$ 7 and 18:1 $\omega$ 7 can be derived from diatoms (e.g. Dijkman and Kromkamp 2006; Volkman 2006), their light  $\delta^{13}\text{C}$  values confirmed utilisation of microbial-mediated chemosynthetically derived carbon. As discussed above, this suggests that sulphide-oxidising bacteria constituted the main diet of *H. disjuncta*.



**Fig. 2** Transmission electron microscopy photograph of the cross-section of a female nematode *Halomonhystera disjuncta*. As in all cross-sections, there was no evidence of either ecto- or endosymbiotic bacteria. Indication on photograph of: **a** the intestine; **b** the ovary

Other observations of nematodes feeding on *Beggiatoa* bacteria were previously recorded for *Diplogaster bernensis* (Sudhaus and Rehfeld 1990), a well known species from freshwater, and *Diplolaimelloides deconincki* in a harbour site in Denmark (Bernhard and Fenchel 1995). In the latter study, a large number of aerobic and anaerobic ciliate species were found to ingest filamentous sulphur bacteria. Another close relationship between nematodes and *Beggiatoa* was described in wastewater treatment processes (Salvadó et al. 2004). It was suggested that nematodes play an important role in wastewater bio-oxidation by their grazing activity on the bacterial community (Woombs and Laybourn-Parry 1986). The most significant biotic relationship found by Salvadó et al. (2004) was between nematodes and filamentous bacteria (specifically the genus *Beggiatoa*), and to a lesser extent, nematodes with ciliates. Based on the biofilm thickness, they concluded that the nematodes also could have a positive influence on the microbial communities. The mechanical effect of nematodes on the biofilm structure would allow a better oxygen penetration and nutrient diffusion into the biofilm, thus, stimulating its growth. *Beggiatoa* appeared also to be attached to rock samples retrieved from a hydrothermal vent field located in the Azores archipelago (North Atlantic; Cardigos et al. 2005). Two species, the nematode *Enoplus meridionalis* and the

oligochaete *Lumbricillus* sp. were abundant in the thick *Beggiatoa* mats, although no direct trophic relationship with the bacteria was shown (Cardigos et al. 2005). It can be concluded that nematodes are often associated with *Beggiatoa* bacteria in sulphidic conditions in freshwater as well as marine habitats. Their close relationship may be due to environmental factors or trophic factors. At the HMMV, the high biomass and standing stock of *H. disjuncta* can be attributed to feeding on the free-living sulphide-oxidising bacteria, a trophic specialisation previously not seen for any other deep-sea meiofaunal seep species (Van Gaever et al. 2006).

Although photosynthetic plankton is normally the predominant source of 22:6 $\omega$ 3 and 20:5 $\omega$ 3 in the marine environment, long-chain PUFAs can also be synthesised by some bacteria (e.g. Delong and Yayanos 1986; Nichols 2003; Valentine and Valentine 2004; Lösekann et al. 2008). This assertion of bacterial origin is supported by the extremely light  $\delta^{13}\text{C}$  of all dominant FAs including the PUFAs normally attributed to phytoplankton (mean  $\delta^{13}\text{C} < -69\text{‰}$ ; Table 1). Methane in HMMV has a  $\delta^{13}\text{C}$  of  $\sim -60\text{‰}$  (Niemann et al. 2006). This clearly indicates a greater dependence on methane in the seep centre sediments as a result of prevailing higher methane fluxes compared to the microbial mat sediments (Niemann et al. 2006). The predominance of methanotrophic input is further supported by the dominance of the methanotroph type I specific bacterial biomarker 16:1 $\omega$ 8c (e.g. Pranal et al. 1996; Kiyashko et al. 2004, see Fig. 1) with very light carbon isotope signatures ( $-81\text{‰}$   $\delta^{13}\text{C}$ ; Table 1). In corroboration, this FA was also found to be dominant in the seep centre surface sediment and with a very light carbon isotope signature ( $\delta^{13}\text{C} -80\text{‰}$ ; Niemann et al. 2006). Large difference in the nematode bulk tissue isotope values measured in 2003 (Van Gaever et al. 2006) and in 2006 (this study; Table 1) indicate that heterogeneity can be high and confirm strong temporal variation in HMMV fauna carbon isotope signatures (Lösekann et al. 2008). Unfortunately, copepod bulk tissue was measured only in 2003 (Table 1), but could have similarly been much lighter in 2006. This may explain the seemingly larger difference between FA and bulk tissue isotope signature compared to nematodes, otherwise generally in the order of 3–5 $\text{‰}$  (e.g. Boschker and Middelburg 2002).

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

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

**Acknowledgments** The authors thank IFREMER (France) for providing sampling facilities on board of the RV “PourquoiPas?”, as well as the captain, crew and chief scientist, Hervé Nouzé, for their efforts during the VICKING campaign in 2006. We express our sincere gratitude to Lennart van IJzerloo, Ronald Rutgers and Pieter van Rijswijk for assistance with chemical analysis and to Massimiliano Molari, Elena Manini and Gian Marco Luna for support with the molecular analyses. We are also indebted to Prof. Dr. Gaetan Borgonie and Myriam Claeys for the transmission electron microscopy work on the nematodes. This research was supported by the GOA fund from Ghent University, the 6th FP HERMES, the FWO project “Cold Seeps” nr. G034607, MARBEF (Network of Excellence), and the Netherlands Organisation of Scientific Research. This publication is contribution number MPS-09004 of MarBEF (Marine Biodiversity and Ecosystem Functioning).

**Open Access** This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

## References

- Abrajano TA, Murphy DE, Fang J, Comet P, Brooks JM (1994)  $^{13}\text{C}/^{12}\text{C}$  ratios of individual fatty acids of marine mytilids with or without bacterial symbionts. *Org Geochem* 21:611–617. doi:10.1016/0146-6380(94)90007-8
- Amann R, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cell without cultivation. *Microbiol Rev* 59:143–169
- Bernhard C, Fenchel T (1995) Mats of colourless sulphur bacteria. II. Structure, composition of biota and successional patterns. *Mar Ecol Prog Ser* 128:171–179. doi:10.3354/meps128171
- Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37:911–917
- Boschker HTS, Middelburg JJ (2002) Stable isotopes and biomarkers in microbial ecology. *FEMS Microbiol Ecol* 40:85–95. doi:10.1111/j.1574-6941.2002.tb00940.x
- Canuel EA, Cloern JE, Ringelberg DB, Guckert JB, Rau GH (1995) Molecular and isotopic tracers used to examine sources of organic-matter and its incorporation into the food webs of San-Francisco Bay. *Limnol Oceanogr* 40:67–81
- Cardigos F, Colaço A, Dando PR, Avilla SP, Sarradin P-M, Tempera F, Conceição P, Pascoal A, Serrão Santos R (2005) Shallow water hydrothermal vent field fluids and communities of the D. João de

- Castro seamount (Azores). *Chem Geol* 224:153–168. doi:[10.1016/j.chemgeo.2005.07.019](https://doi.org/10.1016/j.chemgeo.2005.07.019)
- Conway N, Capuzzo JM (1991) Incorporation and utilization of bacterial lipids in the *Solemya*–velum symbiosis. *Mar Biol (Berl)* 108:277–291. doi:[10.1007/BF01344343](https://doi.org/10.1007/BF01344343)
- Dalsgaard J, St John M, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. *Adv Mar Biol* 46:225–340. doi:[10.1016/S0065-2881\(03\)46005-7](https://doi.org/10.1016/S0065-2881(03)46005-7)
- de Beer D, Sauter E, Niemann H, Kaul N, Foucher J-P, 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* 51:1315–1331
- de Goeij JM, Moodley L, Houtekamer M, Carballeira NM, van Duyl FC (2008) Tracing C-13-enriched dissolved and particulate organic carbon in the bacteria-containing coral reef sponge *Halisarca caerulea*: evidence for DOM feeding. *Limnol Oceanogr* 53:1376–1386
- Delong EF, Yayanos AA (1986) Biochemical function and ecological significance of novel bacterial lipids in deep-sea prokaryotes. *Appl Environ Microbiol* 51:730–737
- Dijkman NA, Kromkamp JC (2006) Photosynthetic characteristics of the phytoplankton in the Scheldt estuary: community and single-cell fluorescence measurements. *Eur J Phycol* 41:425–434. doi:[10.1080/09670260600937791](https://doi.org/10.1080/09670260600937791)
- Floyd R, Abebe E, Papert A, Blaxter M (2002) Molecular barcodes for soils nematode identification. *Mol Ecol* 11:839–850. doi:[10.1046/j.1365-294X.2002.01485.x](https://doi.org/10.1046/j.1365-294X.2002.01485.x)
- Hewson I, Fuhrman JA (2004) Richness and diversity of bacterioplankton species along an estuarine gradient in Moreton Bay, Australia. *Appl Environ Microbiol* 70:3425–3433. doi:[10.1128/AEM.70.6.3425-3433.2004](https://doi.org/10.1128/AEM.70.6.3425-3433.2004)
- Jerosch K, Schlüter M, Foucher J-P, Allais AG, Klages M, Edy C (2007) Spatial distribution of mud flows, chemoautotrophic communities, and biogeochemical habitats at Håkon Mosby Mud Volcano. *Mar Geol* 243:1–17. doi:[10.1016/j.margeo.2007.03.010](https://doi.org/10.1016/j.margeo.2007.03.010)
- Kharlamenko VI, Zhukova NV, Khotimchenko SV, Svetashev VI, Kamenev GM (1995) Fatty acids as markers of food sources in a shallow-water hydrothermal ecosystem (Kraternaya Bight, Yankich Island, Kurile Islands). *Mar Ecol Prog Ser* 120:231–241. doi:[10.3354/meps120231](https://doi.org/10.3354/meps120231)
- Kiyashko SI, Imbs AB, Narita T, Svetashev VI, Wada E (2004) Fatty acid composition of aquatic insect larvae *Stictochironomus pictulus* (Diptera: Chironomidae): evidence of feeding upon methanotrophic bacteria. *Comp Biochem Physiol B* 139:705–711. doi:[10.1016/j.cbpc.2004.08.013](https://doi.org/10.1016/j.cbpc.2004.08.013)
- Lein A, Vogt P, Crane K, Egorov A, Ivanov M (1999) Chemical and isotopic evidence for the nature of the fluid in CH<sub>4</sub>-containing sediments of the Haakon Mosby Mud Volcano, Barents Sea. *Appl Environ Microbiol* 73:3348–3362
- 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, Mendoza GF (2007) Community structure and nutrition of deep methane-seep macrobenthos from the North Pacific (Aleutian) margin and the Gulf of Mexico (Florida escarpment). *Mar Ecol Evol Persp* 28:131–151
- Levin LA, Michener R (2002) Isotopic evidence of chemosynthetic-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 RM (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. doi:[10.3354/meps208021](https://doi.org/10.3354/meps208021)
- Lösekan T, Robador A, Niemann H, Knittel K, Boetius A, Dubilier N (2008) Endosymbioses between bacteria and deep-sea siboglinid tube worms from an Arctic cold seep (Haakon Mosby Mud Volcano, Barents Sea). *Environ Microbiol* 10:3237–3254. doi:[10.1111/j.1462-2920.2008.01712.x](https://doi.org/10.1111/j.1462-2920.2008.01712.x)
- MacAvoy SE, Macko SA, Joye SB (2002) Fatty acid carbon isotope signatures in chemosynthetic mussels and tube worms from Gulf of Mexico hydrocarbon seep communities. *Chem Geol* 185:1–8. doi:[10.1016/S0009-2541\(01\)00394-1](https://doi.org/10.1016/S0009-2541(01)00394-1)
- Masood A, Stark KD, Salem N (2005) A simplified and efficient method for the analysis of fatty acid methyl esters suitable for large clinical studies. *J Lipid Res* 46:2299–2305. doi:[10.1194/jlr.D500022-JLR200](https://doi.org/10.1194/jlr.D500022-JLR200)
- Nichols DS (2003) Prokaryotes and the input of polyunsaturated fatty acids to the marine food web. *FEMS Microbiol Lett* 219:1–7. doi:[10.1016/S0378-1097\(02\)01200-4](https://doi.org/10.1016/S0378-1097(02)01200-4)
- Niemann H, Lösekann T, de Beer D, Elvert M, Nadalig T, Knittel K, Amann R, Sauter EJ, Schlüter M, Klages M, Foucher J-P, Boetius A (2006) Novel microbial communities of the Håkon Mosby mud volcano and their role as a methane sink. *Nature* 443:854–858. doi:[10.1038/nature05227](https://doi.org/10.1038/nature05227)
- Olu K, Lance S, Sibuet M, Hendry P, Fiala-Dedioni A, Dinat A (1997) Cold seep communities as indicators of fluid expulsion patterns through mud volcanoes seaward of the Barbados Accretionary Prism. *Deep Sea Res Part I Oceanogr Res Pap* 44:811–841. doi:[10.1016/S0967-0637\(96\)00123-9](https://doi.org/10.1016/S0967-0637(96)00123-9)
- Parrish CC, Abrajano TA, Budge SM, Helleur RJ, Hudson ED, Pulchan K, Ramos C (2000) Lipid and phenolic biomarkers in marine ecosystems: analysis and applications. In: Wangersky P (ed) *The handbook of environmental chemistry, vol 5, D. Marine chemistry*. Springer, Heidelberg, pp 193–223
- Pernthaler A, Pernthaler J, Amann R (2002) Fluorescence in situ hybridization and catalyzed reporter deposition for the identification of marine bacteria. *Appl Environ Microbiol* 68:3094–3101. doi:[10.1128/AEM.68.6.3094-3101.2002](https://doi.org/10.1128/AEM.68.6.3094-3101.2002)
- Phleger CF, Nelson MM, Groce AK, Cary SC, Coyne KJ, Nichols PD (2005) Lipid composition of deep-sea hydrothermal vent tube worm *Riftia packyptila*, crabs *Munidopsis subsquatnosa* and *Bythograea thermhydrion*, mussels *Bathymodiolus* sp. and limpets *Lepetodrilus* spp. *Comp Biochem Physiol B* 141:196–210. doi:[10.1016/j.cbpc.2005.03.001](https://doi.org/10.1016/j.cbpc.2005.03.001)
- Pimenov NV, Savvichev AV, 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. doi:[10.1023/A:1026666527034](https://doi.org/10.1023/A:1026666527034)
- Pond DW, Segonzac M, Bell MV, Dixon DR, Fallick AE, Sargent JR (1997) Lipid and lipid carbon stable isotope composition of the hydrothermal vent shrimp *Mirocaris fortunata*: evidence for nutritional dependence on photosynthetically fixed carbon. *Mar Ecol Prog Ser* 157:221–231. doi:[10.3354/meps157221](https://doi.org/10.3354/meps157221)
- Pranal V, FialaMedioni A, Guezennec J (1996) Fatty acid characteristics of stable isotope ratios. *Limnol Oceanogr* 40:1496–1502
- Saito H, Osako K (2007) Confirmation of a new food chain utilizing geothermal energy: unusual fatty acids of a deep-sea bivalve, *Calyptogena phaseoliformis*. *Limnol Oceanogr* 52:1910–1918
- Salvadó H, Palomo A, Mas M, Puigagut J, Gracia M (2004) Dynamics of nematodes in a high organic loading rotating biological contactors. *Water Res* 38:2571–2578. doi:[10.1016/j.watres.2004.03.007](https://doi.org/10.1016/j.watres.2004.03.007)

- Stewart FJ, Newton ILG, Cavanaugh CM (2005) Chemosynthetic endosymbioses: adaptations to oxic–anoxic interfaces. *Trends Microbiol* 13:439–448. doi:[10.1016/j.tim.2005.07.007](https://doi.org/10.1016/j.tim.2005.07.007)
- Sudhaus W, Rehfeld K (1990) *Diplogaster coprophilus* n. sp. and *D. affinis* n. sp. (Nematoda, Rhabditida) from cow pats and related species, with notes on distribution, ecology and phylogeny. *Rev Nematologie* 13:51–65
- Thiermann F, Vismann B, Giere O (2000) Sulphide tolerance of the marine nematode *Oncholaimus campyloceroides*: a result of internal sulphur formation? *Mar Ecol Prog Ser* 193:251–259. doi:[10.3354/meps193251](https://doi.org/10.3354/meps193251)
- Valentine RC, Valentine DL (2004) Omega-3 fatty acids in cellular membranes: a unified concept. *Prog Lipid Res* 43:383–402. doi:[10.1016/j.plipres.2004.05.004](https://doi.org/10.1016/j.plipres.2004.05.004)
- Van Dover CL, Aharon P, Bernhard JM, Caylor E, Doerries M, Flickinger W, Gilhooly W, Goffredi SK, Knick KE, Macko SA, Rapoport S, Raulfs EC, Ruppel C, Salerno JL, Seitz RD, Sen Gupta BK, Shank T, Turnipseed M, Vrijenhoek R (2003) Blake Ridge methane seeps: characterization of a soft-sediment, chemo synthetically based ecosystem. *Deep Sea Res Part I Oceanogr Res Pap* 50:281–300. doi:[10.1016/S0967-0637\(02\)00162-0](https://doi.org/10.1016/S0967-0637(02)00162-0)
- Van Gaever S, Moodley L, de Beer D, Vanreusel A (2006) Meiobenthos at the Arctic Håkon Mosby Mud Volcano, with a parental-caring nematode thriving in sulphide-rich sediments. *Mar Ecol Prog Ser* 321:143–155. doi:[10.3354/meps321143](https://doi.org/10.3354/meps321143)
- Vandekerckhove TTM, Coomans A, Cornelis K, Baert P, Gillis M (2002) Use of the *Verrucomicrobia*-specific probe EUB338-III and fluorescent in situ hybridization for detection of “*Candidatus Xiphinematobacter*” cells in nematode hosts. *Appl Environ Microbiol* 68:3121–3125. doi:[10.1128/AEM.68.6.3121-3125.2002](https://doi.org/10.1128/AEM.68.6.3121-3125.2002)
- Volkman JK (2006) Lipid biomarkers for marine organic matter. In: Volkman JK (ed) *The handbook of environmental chemistry*, vol 2, N. Springer, Heidelberg, pp 27–70
- Willems M, Houthoofd W, Claeys M, Couvreur M, Van Driessche R, Adriaens D, Jacobsen K, Borgonie G (2005) Unusual intestinal lamellae in the nematode *Rhabditophanes* sp. KR3021 (Nematoda: Alloinematidae). *J Morphol* 264:223–232. doi:[10.1002/jmor.10332](https://doi.org/10.1002/jmor.10332)
- Woombs M, Laybourn-Parry J (1986) The role of nematodes in low rate percolating filter sewage treatment works. *Water Res* 20:781–787. doi:[10.1016/0043-1354\(86\)90104-1](https://doi.org/10.1016/0043-1354(86)90104-1)
- Zhang CL, Huang Z, Cantu J, Pancost RD, Brigmon RL, Lyons TW, Sassen R (2005) Lipid biomarker and carbon isotope signatures of a microbial (*Beggiatoa*) mat associated with gas hydrates in the Gulf of Mexico. *Appl Environ Microbiol* 71:2106–2112. doi:[10.1128/AEM.71.4.2106-2112.2005](https://doi.org/10.1128/AEM.71.4.2106-2112.2005)