Vol. 359: 161–170, 2008
doi: 10.3354/meps07344

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published May 5

Trophic ecology of siphonostomatoid copepods at deep-sea hydrothermal vents in the northeast Pacific

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ABSTRACT: Siphonostomatoid copepods are often numerically important at deep-sea hydrothermal vents but their role in vent food webs has been little investigated. We examined food sources of 2 vent copepod species, *Stygiopontius quadrispinosus* and *Benthoxinus spiculifer*, and their potential role in the diet of paralvinellid worms, using a combination of complementary techniques: (1) stable carbon and nitrogen isotopes, (2) fatty acid composition and (3) morphological examination of copepod mouth structures using scanning electron microscopy. All 3 techniques revealed distinct differences between the 2 copepod species. Fatty acid composition identified bacteria as the main food source for both copepod species and indicated that *S. quadrispinosus* may be more specialised than *B. spiculifer*. Stable carbon and nitrogen isotopes provided further evidence that the 2 species partition food sources but feed at the same trophic level. The fatty acid composition and stable isotopes of both paralvinellid worms showed that they are generalists, with a varied diet. Further, in samples where *S. quadrispinosus* were highly abundant, both worms had stable carbon and nitrogen isotopic compositions that indicated that they were feeding on copepods. Although neither worm appeared anatomically equipped for seizing live copepod prey, we suggest that dead copepods may be consumed along with other particulate debris by the paralvinellid worms. The contribution of copepod remains to the detrital pool in these mineral substratum habitats remains to be quantified.

KEY WORDS: Hydrothermal vents · Copepoda · Polychaeta · Food webs · Stable isotopes · Fatty acids · Morphology · Northeast Pacific

INTRODUCTION

Copepods can be very abundant at deep-sea hydrothermal vents yet their food sources and links to higher trophic levels have been little investigated. Several studies of the distribution of vent-specific Siphonostomatoid copepods have hypothesised that their high densities are sustained by chemoautotrophic bacterial production (Dinet et al. 1988, Humes & Lutz 1994, Heptner & Ivanenko 2002). Mouthpart ultrastructure and observations of partly digested bacteria in the foregut of siphonostomatoid copepods (Dinet et al. 1988, Heptner & Ivanenko 2002) support this hypothesis. However, these speculations about a bacterivorous feeding mode for vent siphonostomatoid copepods are based largely on circumstantial evidence. Analysis of prey biomarkers in copepod tissue can provide more direct evidence of their dietary preferences, although their application to vent copepods can be technically challenging given the limited sample size usually obtained in submersible grab or suction samples.

Naturally occurring biomarkers are useful tools for determining the diet and trophic position of organisms from habitats such as hydrothermal vents, where sam-

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ples are difficult to obtain and where manipulative experiments are problematic. Stable isotopes (e.g. Van Dover & Fry 1989, 1994, Fisher et al. 1994, Colaço et al. 2002, Van Dover 2002, Levesque et al. 2003, 2006) and fatty acid biomarkers (e.g. Pranal et al. 1996, Pond et al. 1997, 2002, Phleger et al. 2005) have been used to study the feeding ecology of invertebrates in deep-sea hydrothermal vent communities. Stable carbon isotope ratios provide information about the diet of a consumer (Fry & Sherr 1984) while stable nitrogen isotopes indicate the trophic level at which organisms feed (Minagawa & Wada 1984). On average, consumers are similar or slightly enriched (+0.4‰) in $\delta^{13}C$ values compared to their food source, whereas $\delta^{15}N$ displays a stepwise increment of on average 3.4‰ between trophic levels (Post 2002). It is, however, difficult to identify specific dietary items of an organism with stable isotope measurements alone, especially when the organism uses a variety of food sources. In comparison, fatty acid biomarkers permit identification of specific dietary components. For example, different groups of microorganisms have characteristic fatty acid signatures (Dalsgaard et al. 2003), so that the relative abundance of these acids in the neutral lipid pool of consumers can provide information about the utilisation of specific prey (Lee et al. 1971). Also, lipid biomarkers can be useful in detecting endosymbiotic bacterial activity in organisms (Conway & Capuzzo 1991). However, lipid biomarkers cannot yield estimates of trophic level, whereas stable nitrogen isotopes can.

On sulphide chimneys at deep-sea hydrothermal vents in the Northeast Pacific Ocean, sulphide worms (*Paralvinella sulfincola*) share habitat, often exclusively, with sulphide copepods (*Stygiopontius quadrispinosus*, Humes 1987). These species thrive in environments characterised by high temperatures and vigorous vent fluid flows, where *S. quadrispinosus* abundances can reach 9 ind. cm^{-2} (Tunnicliffe et al. 1993, Sarrazin et al. 1997, 1999, Tsurumi et al. 2003). Extremely high densities of *S. quadrispinosus* with >15 000 specimens in 210 ml flocculent material at the Gorda Ridge have been reported (Humes 1990). Both sulphide worms (Tunnicliffe et al. 1985, 1993, Morineaux et al. 2002) and copepods may feed on chemoautotrophic bacteria, but the importance of bacteria in their diets remains uncertain. Previous studies suggest that *P. sulfincola* has a mixed diet typical of generalist feeding (Levesque et al. 2003). The palm worm *P. palmiformis* is also known to have a varied diet (Levesque et al. 2003), and can occur sympatrically with *P. sulfincola* under less severe hydrothermal conditions (Sarrazin et al. 1999). Another siphonostomatoid copepod species, *Benthoxinus spiculifer* (Humes 1984), is also found on sulphide chimneys in habitats dominated by less severe fluid flows, where *P. palmiformis* is present (Tsurumi et al. 2003).

The aims of the present study were to (1) determine the specific diet and trophic position of the siphonostomatoid copepods *Stygiopontius quadrispinosus* and *Benthoxinus spiculifer* and (2) evaluate the potential contribution of copepods to the diet of the paralvinellid worms *Paralvinella sulfincola* and *P. palmiformis* using stable carbon and nitrogen isotopes and fatty acid composition. As a complement to the biomarkers, we used scanning electron microscopy (SEM) to examine the mouthparts of the 2 copepod species in order to relate morphological characteristics to food selection.

MATERIALS AND METHODS

Field sampling. Five initial samples were collected at Northeast Pacific vents in July 2002: 1 on Axial Volcano, Juan de Fuca Ridge, and 4 on Explorer Ridge. All samples were taken on active sulphide edifice surfaces, in habitats subject to diffuse but vigorous hydrothermal discharge. Fauna and particulate organic matter were collected using the suction sampler on the remotely operated vehicle ROPOS. With the suction sampling device, water was pumped into 2 l acrylic jars fitted with 2 layers of 200 µm Nitex nylon mesh at the outflow to retain organisms. All samples were taken with a high flow rate in order to maximise the sampling efficiency. Small fish are caught with the same sampling technique (S. K. Juniper pers. obs.), leading us to assume a high sampling efficiency (i.e. few escapes, species captured at *in situ* proportions) for smaller organisms such as polychaetes and copepods. Between samples, the suction hose was flushed with bottom seawater to minimise cross-contamination of samples. After collection, samples were kept at ambient bottom temperature (~2°C) before being brought to the surface. On shipboard, samples were concentrated and frozen at –80°C until analysis.

In 2003, 2 additional samples were obtained from an area of diffuse venting on the Endeavour Segment of the Juan de Fuca Ridge. The first sample was taken on a sulphide flange where the flow intensity was high and the second on a plateau-like mound of sulphide within an assemblage dominated by *Paralvinella palmiformis* where flow intensities were moderate. The lack of *P. sulfincola*, which dominates high-flow vent sites (Sarrazin et al. 1997, 1999), the copepod assemblage, and visual observations, all indicated that this latter site was more typical of moderate hydrothermal flow conditions compared to the other 6 samples in our study. The five 2002 samples were analysed *in toto*, while the 2003 samples were shared with other researchers.

Stable isotope analysis. In the laboratory, metazoans were separated from the non-living particulate matter (hereinafter 'detritus') under a stereomicroscope. Invertebrates were identified and, depending on the size of the animal, specimens were prepared for stable isotope analysis either individually (polychaetes) or pooled to allow a sufficient number for the analysis (copepods) (see Table 1). Polychaetes were first dissected to remove the gut, then acidified with 0.1 N HCl in glass vials, rinsed once with Milli-Q water and dried at 55°C for 24 h. Dried worms were ground in the vials with a glass rod and a known amount transferred to a tin capsule (D1008, Elemental Microanalysis). Copepods were pooled and acidified in thick tin capsules ('smooth wall tin capsule' D4057, Elemental Microanalysis), resistant to damage by HCl, and dried at 55°C for 24 h. The particulate matter was dried, ground and prepared for stable isotope analysis similarly to the invertebrate samples.

Stable carbon and nitrogen isotopic composition were measured using a Micromass Isoprime isotope ratio mass spectrometer, in line with a Carlo Erba C/N element analyser. Copepods were analysed with a trap current of 600 µA and polychaetes, 200 µA. Stable isotopic compositions are reported relative to Vienna Pee-Dee Belemnite (carbon) and atmospheric nitrogen as:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000\ (‰)$$

where X is ^{13}C or ^{15}N, and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Lipid analysis. Individual specimens of paralvinellid worms were analysed for fatty acids, while specimens of the same species of copepods were pooled together in order to obtain sufficient biomass for the analysis (see Table 1).

Lyophilised samples of copepods and worms were finely ground in an agate mortar and poured into Kimax tubes. Lipids were extracted according to Barnes & Blackstock (1973), using a solvent ratio of 2:1:0.8 (chloroform:methanol:water). Tubes were shaken vigorously, sonicated for 30 min, centrifuged for 5 min at 125 × *g* and the organic layer collected. This procedure was repeated twice, and the 3 organic layers pooled. Neutral lipids were eluted on a deactivated silica gel slurry column (8 g, 700 mesh) using 7 ml 5% ethyl acetate/hexane, 7 ml 15% ethyl acetate/hexane and 7 ml 20% ethyl acetate/hexane. The neutral lipid fraction was saponified with 0.5 N KOH at 100°C for 2 h then extracted 3 times with 3 ml hexane. Neutral fatty acids were converted to fatty acid methyl esters (FAME) using BF_3-methanol (2 h, 100°C). FAMEs were then analysed by flame-ionisation detection on an Agilent Model 6890N gas chromatograph (GC) equipped with a DB-5 column (Agilent Technologies, 30 m × 0.32 mm × 0.25 µm [length × diameter × internal diameter]). Helium was the carrier gas and the column was programmed as follows: 100°C (hold 1 min), 214°C at 4°C min^{-1}, 216°C at 0.5°C min^{-1}, 219°C at 4°C min^{-1}, 223°C (hold 3 min) at 0.5°C min^{-1}, 270°C at 30°C min^{-1}, 315°C (hold 10 min) at 1.5°C min^{-1}. Fatty acids were identified following Ackman (1986), using the commercial standards 37-Component, Bacterial Acid Methyl Esters, and PUFA No. 1 (Supelco, Sigma-Aldrich), and by using a Varian 2000 GC-MS. We will use the term odd and/or branched fatty acids (OBFA) to describe those fatty acids that have odd-numbered carbon chains and/or iso (i-) and anteiso (ai-) branches.

Statistics. Because the suction samples were unequal in size, copepod abundance in each sample was normalised to the number of paralvinellid worms. Spearman's rank correlation was used to investigate the relationship between the copepod abundance (expressed as copepods per worm) and stable carbon and nitrogen isotopic enrichment in worms. Abundance data were log $x + 1$ transformed before using Spearman's rank correlation in order to reduce the importance of the sample with the highest number of copepods per worm.

SEM. Wet specimens were mounted on a cold stage and observed with a Hitachi 4300 SE/VP variable pressure SEM under 100 Pa partial vacuum. Mouthparts of at least 5 individuals of each species were examined.

RESULTS

Species composition and abundance

Three siphonostomatoid copepod species were found in the samples—*Stygiopontius quadrispinosus, Benthoxinus spiculifer* and *Aphotopontius forcipatus* (Humes 1987). The number of *S. quadrispinosus* per paralvinellid worm ranged from 4 to 1853 (Table 1). *S. quadrispinosus* was present in all 7 samples, while *B. spiculifer and A. forcipatus* were present in 2 samples each. *S. quadrispinosus* was always numerically dominant at sites with vigorous vent flows, representing 99 to 100% of the total copepod abundance. At the site with moderate vent flows, *B. spiculifer* and *S. quadrispinosus* occurred in similar abundance (Table 1). *A. forcipatus,* found in 2 samples, occurred in low abundance, 5 or fewer copepods per worm (Table 1).

Stable isotopes

The $\delta^{13}C$ of *Paralvinella sulfincola* ranged between –9.7 and –13.6‰ and $\delta^{15}N$ between 3.4 and 7.6‰ (Table 1). There was also substantial within-site variation in the $\delta^{13}C$ and $\delta^{15}N$ isotopic composition of indi-

Table 1. Number of copepods per paralvinellid worm and number of invertebrates used for analysis of stable carbon and nitrogen isotopes (SIA) and lipid composition (LA). Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic ratios of invertebrates (±SE) and detritus, and number of replicates (n) are also presented. Results from LA are in Table 2

Site	Vent flow	Year	Species	No. of copepods per worm	SIA	n	$\delta^{13}C$	SE	$\delta^{15}N$	SE	LA	n
Axial	High	2002	*Paralvinella sulfincola*		1	5	–9.7	0.2	6.2	0.2		
Axial	High	2002	*Stygiopontius quadrispinosus*	33.5	100	2	–13.2	0.3	4.7	0.2		
Axial	High	2002	Detritus				–16.9		4.6			
Explorer	High	2002	*Paralvinella sulfincola*		1	7	–13.4	0.7	6.9	0.4	1	1
Explorer	High	2002	*Stygiopontius quadrispinosus*	47.6	100	2	–14.8	0.1	4.4	0.3		
Explorer	High	2002	*Aphotopontius forcipatus*	0.2								
Explorer	High	2002	Detritus				–22.1		5.1			
Explorer	High	2002	*Paralvinella sulfincola*		1	3	–13.6	0.6	7.6	0.3		
Explorer	High	2002	*Stygiopontius quadrispinosus*	1852.5	100	4	–13.8	0.2	4.4	0.1		
Explorer	High	2002	*Aphotopontius forcipatus*	5.0								
Explorer	High	2002	*Benthoxinus scupilifer*	5.0								
Explorer	High	2002	Detritus				–22.2		5.7			
Explorer	High	2002	*Paralvinella sulfincola*		1	7	–12.7	0.2	6.3	0.5		
Explorer	High	2002	*Stygiopontius quadrispinosus*	140.3	100	3	–13.4	0.1	4.3	0.1		
Explorer	High	2002	Detritus				–16.9		2.8			
Explorer	High	2002	*Paralvinella sulfincola*		1	10	–11.2	0.3	5.4	0.2		
Explorer	High	2002	*Stygiopontius quadrispinosus*	3.9	50	2	–13.6	0.4	4.4	0.0		
Explorer	High	2002	Detritus				–14.1		3.2			
Endeavour	High	2003	*Paralvinella sulfincola*		1	4	–13.5	0.2	3.4	0.6		
Endeavour	High	2003	*Stygiopontius quadrispinosus*	18.1	127	1	–14.3		0.8			
Endeavour	High	2003	Detritus				–16.2		0.9			
Endeavour	Moderate	2003	*Paralvinella palmiformis*		1	3	–15.3	0.5	2.9	0.6	1	1
Endeavour	Moderate	2003	*Stygiopontius quadrispinosus*	250.0	100	3	–15.9	0.1	–0.2	0.1	1200	1
Endeavour	Moderate	2003	*Benthoxinus scupilifer* female		50	5	–14.8	0.1	–0.3	0.1		
Endeavour	Moderate	2003	*Benthoxinus scupilifer* male	170.0[a]	60	5	–13.1	0.3	1.2	0.3	1000[a]	1
Endeavour	Moderate	2003	Detritus				–19.7		[b]			

[a]Copepod abundance per worm calculated for both sexes together, SI analysis done on males and females separately
[b]Due to low nitrogen content in the particulate matter, no $\delta^{15}N$ could be obtained

viduals; on average these ranges were 1.7‰ for $\delta^{13}C$ and 2.3‰ for $\delta^{15}N$. The largest range in $\delta^{13}C$ and $\delta^{15}N$ among individual *P. sulfincola* within 1 site was 5.3 and 3.7‰, respectively.

The carbon and nitrogen isotopic signatures of *Stygiopontius quadrispinosus* also varied among sites, with $\delta^{13}C$ ranging from –13.2 to –15.9‰ and $\delta^{15}N$ from –0.2 to 4.7‰ (Table 1). At the site with moderate vent discharge, *Benthoxinus spiculifer* occurred in sufficient abundance (170 copepods per worm) to obtain samples for stable isotope analysis (Table 1, Fig. 1a). The female:male ratio was 1:1, enabling us to perform stable isotope analysis on both sexes. The carbon and nitrogen isotopic signatures of female and male *B. spiculifer* were different: males were more enriched in both ^{13}C and ^{15}N (Table 1, Fig. 1a). A large number of females carried eggs and 2 samples with females were analysed after removal of eggs to study the effect of eggs on the isotopic signatures. $\delta^{13}C$ for *B. spiculifer* with eggs measured –14.9‰ and without, –14.7‰, and $\delta^{15}N$ for *B. spiculifer* with and without eggs measured –0.4 and –0.2‰, respectively. As similar results were obtained, we pooled the isotopic data for female *B. spiculifer* with and without eggs. The carbon isotopic signature of female *S. quadrispinosus* differed from both male and female *B. spiculifer* by being more ^{13}C-depleted (Fig. 1a). The nitrogen isotopic signature of female *S. quadrispinosus* was, however, similar to *B. spiculifer* females (Fig. 1a). No measurements could be obtained for male *S. quadrispinosus,* since the sample contained almost exclusively females. $\delta^{13}C$ for the particulate matter ranged from –14.1 to –22.2‰, and $\delta^{15}N$ from 0.9 to 5.7‰ (Table 1).

There was a significant correlation between the relative abundance of *Stygiopontius quadrispinosus* (expressed as number per worm) and the fractionation of $\delta^{13}C$ and $\delta^{15}N$ between *S. quadrispinosus* and *Paralvinella sulfincola* ($\delta^{13}C$: $p < 0.02$, $R = -0.82$; $\delta^{15}N$: $p < 0.05$, $R = 0.75$). In the sample with the highest number of *S. quadrispinosus* per worm (1853), *P. sulfincola* was enriched by 0.2‰ in ^{13}C and 3.2‰ in ^{15}N, compared to *S. quadrispinosus* (Fig. 1b, Table 1). This compares

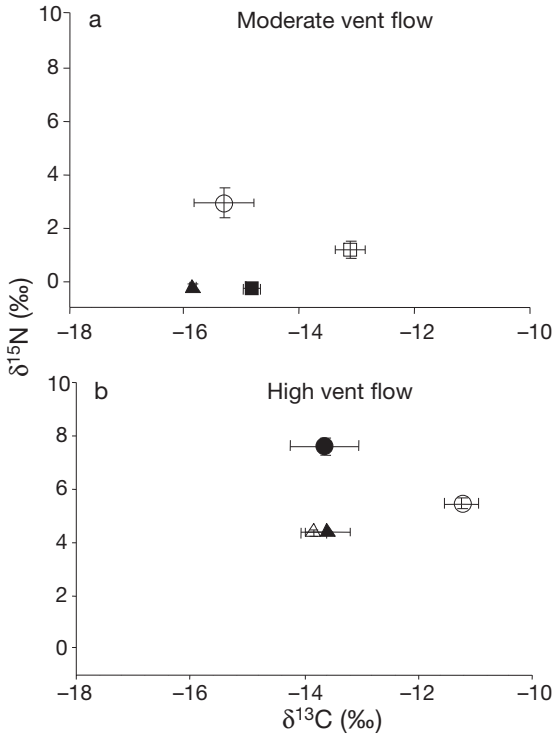

Fig. 1. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic composition of (a) *Paralvinella palmiformis* (○), *Stygiopontius quadrispinosus* (▲) and *Benthoxinus spiculifer* females (■) and males (□) from a site with moderate vent flow and (b) *P. sulfincola* and *S. quadrispinosus* from 2 sites with high vent flow. In (b), circles represent *P. sulfincola* from sites with high (●) and low (○) copepod densities. Triangles represent *S. quadrispinosus* at high (▲) and low (△) densities. Error bars are SE

with ^{13}C and ^{15}N enrichment (in *P. sulfincola*) of 2.4 and 1.2‰, respectively, relative to *S. quadrispinosus* in the sample containing the fewest *S. quadrispinosus* (4 per worm) (Fig. 1b, Table 1).

Morphology

SEM micrographs of *Benthoxinus spiculifer* and *Stygiopontius quadrispinosus* mouthparts showed a difference in size. The mouth opening of *B. spiculifer* was about 20 µm, roughly 4 times that of *S. quadrispinosus* (Fig. 2). In addition, *S. quadrispinosus* had plumose setules surrounding its mouth opening, whereas *B. spiculifer* did not (Fig 2).

Lipid composition

Saturated and monounsaturated fatty acids (SFA and MUFA) dominated the neutral lipid of all sampled organisms, constituting 80 to 89% of the total fatty acids in each (Table 2). Major SFA were 16:0 (11 to 22%) and 18:0 (5 to 6%). While the principal MUFA in all samples contained 16 and 18 carbons, the speciation and composition differed among species. The worms and copepods all contained significant amounts of 18:1ω7 (20 to 36%), and levels were conspicuously high in *Benthoxinus spiculifer* (36%). The fatty acid 16:1ω7 was also very abundant in all samples, ranging between 10 and 28% overall, with the highest proportions found in *Stygiopontius quadrispinosus* (28%). *Paralvinella palmiformis* and *P. sulfincola* contained higher proportions of 18:1ω9, 20:1ω11 and 22:1ω11 and lower proportions of 16:1ω5 and 22:1ω7, relative to the 2 copepods. Generally, the copepods contained higher MUFA (63 to 65%) and lower SFA (~24%) levels than the worms (MUFA: 48 to 50%; SFA: 32 to 33%).

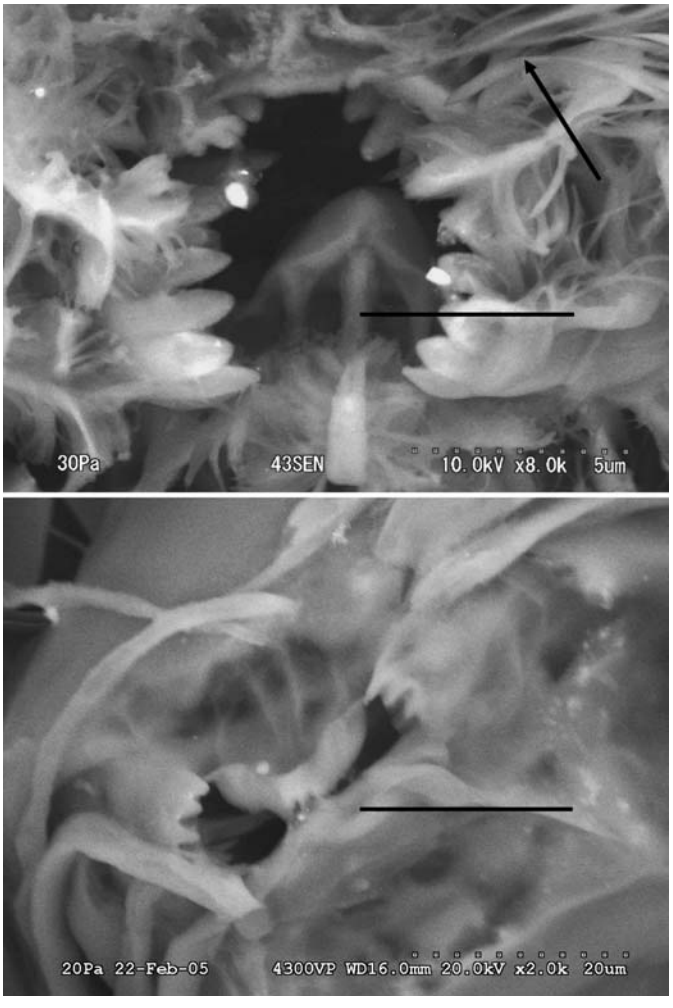

Fig. 2. SEM micrographs of mouthparts of (a) *Stygiopontius quadrispinosus* (scale bar = 5 µm; arrow indicates plumose setules) and (b) *Benthoxinus spiculifer* (scale bar = 20 µm)

Table 2. *Paralvinella palmiformis*, *P. sulfincola*, *Benthoxinus spiculifer* and *Stygiopontius quadrispinosus*. Neutral fatty acid composition. nd = not detected; numbered superscripts refer to numbers of isomers; OBFA = odd and/or branched fatty acids (underlined in 'Fatty acid' column); NMID = non-methylene interrupted dienes

Fatty acid[a]	*P. palmiformis* (%)	*P. sulfincola* (%)	*B. spiculifer* (%)	*S. quadrispinosus* (%)
Saturates				
14:0	0.98	1.54	4.14	2.25
i-15:0	0.13	0.09	1.15	0.47
ai-15:0	0.05	0.03	0.44	0.38
15:0	0.91	0.19	0.27	nd
i-16:0	0.25	0.21	0.32	0.37
16:0	22.41	20.13	11.07	15.03
i-17:0	1.01	1.61	0.87	0.22
ai-17:0	0.50	0.87	0.32	0.25
17:0	0.64	1.04	0.30	0.30
18:0	6.10	5.79	4.51	4.58
Total	33.38	31.74	24.12	23.85
Monounsaturates				
14:1[b]	0.19	0.46	0.74	nd
16:1ω7	14.58	9.96	18.68	28.07
16:1ω5	0.47	0.18	2.78	2.92
17:1ω7	0.46	0.92	0.02	nd
∑17:1[b]	0.88[3]	0.25[1]	0.39[2]	nd
18:1ω9	5.26	5.47	0.70	1.01
18:1ω7	19.86	23.43	35.75	25.07
18:1ω5	1.01	0.84	0.97	0.52
20:1ω11	4.11	1.95	0.35	0.53
20:1ω9	nd	2.03	nd	nd
20:1ω7	1.00	1.53	3.41	2.27
22:1ω11	0.28	1.21	0.02	nd
22:1ω9	1.09	nd	0.33	2.28
Total	49.71	48.45	64.59	63.25
Polyunsaturates				
18:2a[b]	0.17	0.22	3.30	3.31
18:2b[b]	1.80	0.68	0.25	0.40
18:2ω6	1.63	0.91	0.15	0.48
18:2ω4	3.86	4.09	3.22	6.61
20:4ω6	0.61	0.90	0.27	nd
20:5ω3	1.89	2.67	0.63	nd
20:3[b]	0.28	0.90	0.28	0.07
20:2ω6	0.39	1.11	nd	nd
20:2a (NMID)[b]	nd	2.54	nd	nd
20:2b (NMID)[b]	1.50	3.15	1.95	1.57
22:2a (NMID)[b]	0.82	0.48	nd	nd
22:2b (NMID)[b]	2.34	0.34	0.21	nd
22:4[b]	0.13	0.67	nd	nd
Total	16.91	19.82	11.30	12.90
Dietary indices				
∑OBFA	4.92	5.33	4.24	1.99
16:1ω7 to 16:0	0.65	0.49	1.69	1.87
18:1ω7 to 18:0	3.25	4.05	7.92	5.47

[a]Only those fatty acids >0.5% of total or those relevant to the Discussion included (<0.5% and not included in Table: 12:0, 13:0, i-14:0, ∇19:0, 19:0, 20:0, 21:0, 22:0, 23:0, 24:0, 14:1 [2 isomers], 16:1ω9, 22:1ω7, 24:1, 18:2 [2 isomers], 20:3ω6, 20:2 [3 isomers], 22:6ω3, 22:3, 22:2); totals include all detected fatty acids

[b]Double bond positions not discernible

Total polyunsaturated fatty acids (∑PUFA) were low in all samples, ranging between 11 and 20% overall (Table 2). PUFA levels were higher in the worms than the copepods, and a wider variety of compounds were detected. Worms and copepods differed in the distribution of 18:2 PUFA, notably 18:2a, 18:2b and 18:2ω6. Also, worms contained moderate levels of 20:5ω3 (2 to 3%), compared to the low or undetectable amounts found in the copepods. All samples contained notable amounts of 20:2 and 22:2 PUFA, some of which we have designated as non-methylene interrupted dienes (NMID). Total NMID levels were higher in the worms (5 to 7%) than the copepods (~2%), but no prevailing group- or species-specific trends emerged in individual NMIDs.

Several dietary indices were calculated that highlighted differences between the copepods and the worms (Table 2). Levels of OBFA were lowest in *Stygiopontius quadrispinosus* (2%); this species had lower levels of many OBFAs, compared to *Paralvinella palmiformis* and *P. sulfincola* (~5% of total) and some acids were not detected at all (i.e. 15:0, 17:1). OBFA composition and levels (4%) in *Benthoxinus spiculifer* were more similar to the worms than to *S. quadrispinosus*. Two ratios were computed to estimate bacterial input to the diet (Table 2). Both yielded higher (more bacteria) values for the copepods than for the worms. The greatest difference was for the 16:1ω7 to 16:0 ratios, which were roughly 3-fold higher in *S. quadrispinosus* (1.9) and *B. spiculifer* (1.7) than in *P. palmiformis* (0.7) and *P. sulfincola* (0.5). 18:1ω7 to 18:0 ratios were also higher in the copepods (5.5 to 7.9), as compared to the worms (3.3 to 4.1). Considering just the copepods, the fatty acid dietary ratios highlighted the fact that *S. quadrispinosus* contained comparatively more 16:1ω7 and *B. spiculifer* contained more 18:1ω7.

DISCUSSION

Most copepod species at vents belong to the order Siphonostomatoida (Humes 1987), copepods with the labrum and

labium fused together to form a siphon adapted for parasitic feeding. However, rather than being parasites, vent copepods have evolved to feed on fine-grained food, suggested to be composed mainly of bacteria (Heptner & Ivanenko 2002). Bacteria have a specific fatty acid composition that is often characterised by large amounts of ω7 and ω9 16- and 18-carbon MUFA, little or no PUFA, and elevated levels of odd and/or branched carbon chains (Dalsgaard et al. 2003). The high MUFA, low ∑PUFA and low ω3 PUFA levels of *Stygiopontius quadrispinosus* in the present study indicate a diet based on bacteria (cf. Pranal et al. 1996). Furthermore, few individual OBFAs together with low ∑OBFA levels in *S. quadrispinosus* point to a specialisation on specific bacteria strains (cf. Pranal et al. 1996). The lipid composition of *Benthoxinus spiculifer* also suggests that bacteria constitute a significant part of its diet. However, it may feed on different bacteria than *S. quadrispinosus* as evidenced by the 2 types of bacteria biomarkers, 16:1ω7 and 18:1ω7. The diet of *S. quadrispinosus* has a strong bacterial component, based on high levels of 16:1ω7, while *B. spiculifer* is more enriched in 18:1ω7. Also, *B. spiculifer* had higher ∑OBFA levels, indicating ingestion of a variety of bacterial strains (cf. Pranal et al. 1996). Samples of *S. quadrispinosus* and *B. spiculifer* analysed for fatty acids in the present study came from a single sample taken under moderate vent flow conditions. Consequently, the differences we observe between the 2 species in terms of the bacteria biomarkers, 16:1ω7 and 18:1ω7, cannot be attributed to between-site flow regime differences but rather to selective feeding on different bacteria strains and/or microscale heterogeneity in the distribution of the 2 copepods and their food sources within this habitat. Up to the limits of thermal tolerance, production of autotrophic bacteria will tend to be higher closer to more vent openings that supply reducing substances such as H_2S (Sievert et al. 2000). Since the highest densities of *S. quadrispinosus* occur adjacent to vigorous vent flows, it follows that their diet may be dominated by autotrophic bacteria. On the other hand, *B. spiculifer* occurs further away from vent openings where there may be a variety of autotrophic and heterotrophic bacteria available for ingestion.

Stable isotope signatures of the 2 copepod species differed, corroborating the lipid evidence for dietary differences. There were also differences in carbon and nitrogen isotopic composition between female and male *Benthoxinus spiculifer*, males being more enriched in both ^{13}C and ^{15}N. Several hypotheses can be envisaged to explain the isotopic differences between sexes. Females and males may have different spatial distribution and subsequently use different food sources. Food source heterogeneity can occur at small scales at hydrothermal vents (Limén et al. 2007). It is also possible that females and males share habitat but select for different diets. Alternatively, differences between the sexes in terms of lipid storage could explain some of the observed results. Copepod eggs are usually rich in lipids (Hirche & Kattner 1993); this could affect the carbon isotopic signature of ovigerous females, since lipids are depleted in ^{13}C (McConnaughey & McRoy 1979, Tieszen et al. 1983). However, females with and without eggs had similar stable carbon isotope signatures, although this similarity could be non-ovigerous females storing ^{13}C-depleted lipids for future investment in reproduction, as pelagic copepods do (Hagen & Schnack-Schiel 1996). Nevertheless, since nitrogen isotopic signatures also differed between sexes, we are obliged to conclude that the observed contrasts in stable isotope ratios between sexes were the result of food partitioning.

While the structure of mouthparts and buccal appendages in marine pelagic copepods is closely related to the type of food ingested (Anraku & Omori 1963, Michels & Schnack-Schiel 2005), the functional morphology of the feeding apparatus in vent copepods is not well known (Heptner & Ivanenko 2002, Tsurumi et al. 2003). *Aphotopontius mammillatus,* a vent copepod from the Pacific Ocean, has been described as an archetypical bacterial feeder, having a short and robust siphon with the ventral margin of the labium surrounded by plumose ornamentation (Heptner & Ivanenko 2002). The mouthparts of *Stygiopontius quadrispinosus* in the present study are similar to those of *A. mammillatus*; both have a short siphon and a small mouth opening surrounded by plumose setules. Based on morphology, Heptner & Ivanenko (2002) argued that the primary food source of *A. mammillatus* is bacteria and bacterial films, typically abundant at hydrothermal vents. We observed some notable differences in the buccal morphology between *S. quadrispinosus* and *Benthoxinus spiculifer* using SEM: *B. spiculifer* has a larger mouth opening than *S. quadrispinosus*, and lacks the plumose setules found around the mouth opening of the latter. Plumose setules may serve as a sensory organ (Heptner & Ivanenko 2002) to detect bacteria and bacterial films in the environment. The lipid composition of *S. quadrispinosus* indicates that it is specialised in its diet. Its smaller mouth and plumose setules may permit more selective feeding than the mouth of *B. spiculifer*.

Paralvinella sulfincola and *P. palmiformis* exhibited a wide range of isotopic signatures, both within and between sites, suggesting that they utilise several food sources and that utilisation varies between individuals. Other stable isotope studies have reported evidence for generalist feeding (Levesque et al. 2003) in these 2

species, as well as a case of apparently exclusive feeding on a chemoautotrophic microbial food source (Limén et al. 2007). Behavioural studies have shown *P. sulphincola* to limit its feeding activity to sulphide mineral surfaces around its tube opening and the tubes of neighbouring conspecifics (Morineaux et al. 2002, Grelon et al. 2006). Thus, even in situations where *P. sulphincola* are utilising several food sources, food items still have to be found on surfaces adjacent to its tube opening.

The lipid biomarker analyses confirm that *Paralvinella sulfincola* and *P. palmiformis* usually exploit multiple food sources. Similar to the copepods, the lipid composition of the 2 paralvinellid worms showed high levels of ω7 and ω9 16- and 18-carbon MUFA, while ∑PUFA and ω3 PUFA were relatively less abundant. However, the worm tissue bacterivory indices (16:1ω7 to 16:0 and 18:1ω7 to 18:0) were lower than in the copepods, and ∑OBFA was higher, indicating utilisation of a wider range of food items. Higher levels of 18:1ω9 detected in worms compared to copepods may reflect a larger proportion of detritus in the worms' diet. Pond et al. (2000) found detritus from mid-Atlantic Ridge vents to be dominated by 16:1ω9 and 18:1ω9. Elevated levels of 18:1ω9 have also been used to infer carnivory in pelagic invertebrates (Falk-Petersen et al. 1999). Here they could indicate substantial animal remains in particles being consumed by the worms. Some PUFA (i.e. 20:5ω3) found in the worms are markers for pelagic phytoplankton (Dalsgaard et al. 2003). Particulate matter from vents where these samples originate does contain small numbers of diatoms (Levesque et al. 2005), which may explain the 20:5ω3 detected in the worms. An alternative explanation for the detected PUFA is that they are produced within the worms from bacterial precursors. Such pathways of *in vivo* essential fatty acid (EFA; i.e. 20:5ω3 and 20:4ω6) formation have been suggested for the hydrothermal vent worms *Ridgeia piscesae*, *Protis hydrothermica* (Pond et al. 2002) and *Riftia pachytila* (Phleger et al. 2005). In environments where the available food contains low levels of PUFA, particularly ω3 PUFA, organisms modify dietary lipids to produce some of the lipids (e.g. NMID, EFA) needed to maintain membrane fluidity (Pranal et al. 1996). For example, 20:2 and 22:2 NMID are thought to be formed *in vivo* via elongation and desaturation of 16:1ω7 and 18:1ω7 (Zhukova 1991) and thus accumulate in certain hydrothermal vent invertebrates (e.g. Pranal et al. 1996).

Chemoautotrophic bacteria and detritus have been considered as the 2 main food sources for *Paralvinella sulfincola.* Our results indicate a third potential food source for the worm: the copepod species *Stygiopontius quadrispinosus*. *S. quadrispinosus* can be very abundant in *P. sulfincola* habitats (Humes 1990, Tsurumi et al. 2003). A comparison among sites we studied reveals a correlation between the number of *S. quadrispinosus* and the carbon and nitrogen isotopic signatures of *P. sulfincola.* Where *S. quadrispinosus* were most abundant (1852 ind. per worm), the $\delta^{13}C$ and $\delta^{15}N$ signatures of *P. sulfincola* suggest that copepods were a primary food source for the worm. Stable carbon isotopes ratios in *P. sulfincola* tissues were similar or slightly enriched in ^{13}C, compared to *S. quadrispinosus*, as would be expected between a consumer and its prey (e.g. Post 2002). While $\delta^{15}N$ of *S. quadrispinosus* was relatively constant among sites at Axial and Explorer Ridge (range 0.4‰), $\delta^{15}N$ values for *P. sulfincola* ranged widely (2.2‰). This could be interpreted as evidence that *S. quadrispinosus* mainly grazes on primary producers (as indicated by its lipid composition) while *P. sulfincola* alternates between grazing on bacteria (worms depleted in ^{15}N), and consuming copepods when the latter are present at high densities (worms enriched in ^{15}N). Grazing on detritus would give *P. sulfincola* a similarly enriched ^{15}N signature. However, $\delta^{13}C$ and $\delta^{15}N$ values for detritus (without copepods) show that it was not a main source of nutrition for *P. sulfincola* (Table 1). Isotopic signatures of *P. palmiformis* also suggest utilisation of copepods (especially *Benthoxinus spiculifer* females and *S. quadrispinosus*) as a food source, although a density comparison was not possible.

Are these Paralvinellids preying directly on copepods or ingesting them non-selectively? Neither worm has morphological characteristics typical of predatory polychaetes, such as an eversible pharynx and jaws (e.g. carnivorous species described from hydrothermal vents; Desbruyères et al. 1985). Instead, their mouths are surrounded by oral and branchial tentacles that can be used to capture and transport particles to the mouth. The digestive tracts of paralvinellid worms are also characteristic of non-selective deposit feeders (Desbruyères et al. 1985). Morphological and histological evidence (Desbruyères et al. 1985) as well as behavioural studies (Grelon et al. 2006) indicate that Paralvinellids feed on particles and possibly bacterial films. However, in habitats where *Stygiopontius quadrispinosus* densities are high (e.g. Humes 1990, Tsurumi et al. 2003), particulate matter collected from the substratum would also contain a substantial fraction of dead *S. quadrispinosus* and their remains. These copepods are within the size range (mean length 0.6 to 0.7 mm; Tsurumi et al. 2003) of food particles typically captured by *Paralvinella sulfincola* (~0.8 mm, Morineaux et al. 2002). Harsh physical conditions associated with intense vent flows where *S. quadrispinosus* thrive may generate high mortality rates. Vent crabs from shallow hydrothermal vents

scavenge on zooplankton that can be instantly killed by vent plumes (Jeng et al. 2004). Similarly, paralvinellid worms may benefit from copepod mortality close to intense vent discharge.

While *Stygiopontius quadrispinosus* is typically found in high-flow regimes, *Benthoxinus spiculifer* is more frequent under moderate flow conditions (Tsurumi et al. 2003). At 1 site sampled in the present study, the 2 copepods were found in similar numbers in moderate vent discharge along with *Paralvinella palmiformis*. The isotopic signature of *P. palmiformis* indicated that both *S. quadrispinosus* and female *B. spiculifer* could be dietary sources for the worm. Scavenging on dead copepods may thus occur in more than one paralvinellid species, and potentially involve several species of vent copepods, especially where the latter are present at high densities.

CONCLUSIONS

The combination of biomarkers, i.e. stable isotopes and fatty acids, provided information on trophic level (stable isotopes) and dietary composition (fatty acid composition) for 4 hydrothermal vent invertebrates. Bacteria are a major food source for the 2 copepod species, *Stygiopontius quadrispinosus* and *Benthoxinus spiculifer*. However, inter-specific differences were evident in SEM micrographs of mouthparts, in fatty acid composition and in stable isotope ratios. *S. quadrispinosus* appears to be more specialised in its food selection than *B. spiculifer*. The fatty acid composition and stable isotope signatures of both paralvinellid worms indicate that they are generalists with varied diets. Stable isotope signatures identify vent copepods as one of their dietary components, likely through scavenging, and at high copepod densities this may represent a significant food source for the worms. Further biomarker and microscopic work is required to quantify the contribution of copepod remains to the detrital pool in these vent habitats.

In other marine and freshwater systems, meiofauna (nematodes, copepods and others) can represent an important link to higher trophic levels (Smith & Coull 1987, Aarnio & Bonsdorff 1993, Ólafsson 2003 and references therein). Meiofaunal metabolism often exceeds that of macrofauna (Leguerrier et al. 2003 and references therein) and therefore has a large impact on the turnover of carbon. In addition, meiobenthic organisms comprise several functional groups and so contribute significantly to food-web complexity (Schmid-Araya et al. 2002). Meiobenthic species should be considered in future studies of carbon flow and food-web structure at deep-sea hydrothermal vents.

Acknowledgements. We thank J. Wells and C. Parrish for GC-MS analysis and valuable discussions, A. Adamovicz and J. McKay for stable isotope analysis, and A. Ouellet and Y. Gélinas for GC method development. The ROPOS team and the captain and crew of RV 'Thomas G. Thompson' are thanked for their collaboration at sea. NSERC Canada and FQRNT (Québec) supported this research through grants to S.K.J. and the GEOTOP Research Centre, and an NSERC postdoctoral fellowship to C.J.S.

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Editorial responsibility: Otto Kinne, Oldendorf/Luhe, Germany

Submitted: October 31, 2005; Accepted: November 19, 2007
Proofs received from author(s): April 7, 2008