



Evidence for a chemoautotrophically based food web at inactive hydrothermal vents (Manus Basin)

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ABSTRACT

Hydrothermal vents are ephemeral systems. When venting shuts down, sulfide-dependent taxa die off, and non-vent taxa can colonize the hard substrata. In Manus Basin (Papua New Guinea), where hydrothermally active and inactive sites are interspersed, hydroids, cladorhizid sponges, barnacles, bamboo corals, and other invertebrate types may occupy inactive sites. Carbon and nitrogen isotopic compositions of animals occupying inactive sites are consistent with nutritional dependence on either chemoautotrophically or photosynthetically produced organic material, but sulfur isotopic compositions of these animals point to a chemoautotrophic source of sulfur from dissolved sulfide in vent fluids rather than sulfur derived from seawater sulfate through photosynthesis. Given that suspension-feeding and micro-carnivorous invertebrates are the biomass dominants at inactive sites, the primary source of chemoautotrophic nutrition is likely suspended particulates and organisms delivered from nearby active vents.

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1. Introduction

Invertebrate communities associated with active deep-sea hydrothermal vents have been the focus of considerable research since the first report of the Galapagos hot springs in 1977 (Corliss et al., 1979). The life cycle of active hydrothermal vent communities is reasonably well understood for a number of sites that have been intermittently studied for years to decades, but little is known about the ecology of organisms colonizing inactive sulfide substrata or other nearby hard surfaces.

Vent formation begins as seawater seeps below the ocean seafloor, reacts with hot rock, and eventually builds up enough thermal buoyancy to rise through conduits to mix with cold, oxygen-rich water (e.g., Fornari and Embley, 1995; Parson et al., 1995; Tolstoy et al., 2008). Dissolved metals precipitate at the mixing zone to form sulfide chimneys and mounds (i.e., polymetallic sulfide deposits; Haymon, 1983). These polymetallic

sulfide deposits continue to build during the lifetime of an active vent, providing substratum for a variety of invertebrates and metabolically diverse microbes that nourish life in hydrothermal environments (e.g., Karl, 1995).

In time, hydrothermal activity terminates and sulfide chimneys often topple, leaving piles of metal-rich mounds on the seafloor and an environment that no longer delivers the fluid flux required by endemic vent organisms. Absence of noxious concentrations of dissolved hydrogen sulfide opens a hospitable new environment to a different suite of invertebrate species. While it is routine to observe megafaunal and macrofaunal invertebrates associated with inactive sulfide mounds along mid-ocean ridges and spreading centers of back-arc basins, little is known about the role of chemoautotrophic primary production in the nutrition of these animals.

Stable-isotope analysis is a valuable tool for generating or constraining hypotheses about trophic resources in systems where direct observations of feeding interactions and samples are difficult to obtain, such as at hydrothermal vents (Conway et al., 1994; Van Dover, 2000, 2007). Carbon and nitrogen stable-isotope compositions have been used to advance our understanding of food and energy flow in a variety of marine settings (e.g., Rau and Hedges, 1979; Rau et al., 1992; Van Dover et al., 1992; Wainright et al., 1993). Characterization of patterns of isotopic compositions among individuals and among species at vents permits development of hypotheses regarding trophic issues such as changes in diet during growth within species

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(Pond et al., 1997, 2000; Polz et al., 1998; Rieley et al., 1999), site-specific differences in diet within species (Trask and Van Dover, 1999; Colaço et al., 2002), and trophic interactions among species (Van Dover and Fry, 1989, 1994; Fisher et al., 1994; Southward et al., 1994; Vereshchaka et al., 2000; Colaço et al., 2002; Van Dover, 2002). Isotopic techniques also have been used to infer the importance of free-living chemoautotrophic microorganisms in the diet of heterotrophic invertebrates within vent communities (e.g., Van Dover and Fry, 1994). Sulfur isotopes are less frequently employed in trophic studies, but where organic sulfur may be derived from seawater sulfate or dissolved sulfide through autotrophic processes, sulfur isotopes can be useful dietary discriminators (Fry et al., 1983; Peterson and Fry, 1987; Vetter and Fry, 1998).

In this study, we report the results of stable-isotope analysis ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$) of dominant taxa at hydrothermally inactive sites at Solwara 1 (also known as the Suzette vent field) in Manus Basin, Papua New Guinea. Inactive sites were defined as massive sulfide (or, in one instance, volcanic rock) deposits without evident warm- or hot-water fluxes and by the absence of characteristic vent-endemic taxa for the region. To provide an isotopic context for biological samples (non-vent-endemic taxa) from inactive sites, we also sampled and analyzed dominant biota (vent-endemic taxa) from hydrothermally active sulfide mounds. Our objective was to provide insight regarding the primary source of nutrition for invertebrates at inactive sites at Solwara 1. At least three non-exclusive conditions were deemed possible:

- (1) Sinking photosynthetically derived particles provide all or nearly all of the nutrition for the invertebrates colonizing inactive sulfide mounds.
- (2) *In situ* production of organic carbon from acid-labile sulfides mobilized by microbial activity (or low-level, inconspicuous venting) may support chemoautotrophic primary production within the boundary layer overlying the substratum.
- (3) Proximity of inactive sites (sometimes within 10 s of meters) to active vent sites within Solwara 1 suggests that allochthonous, chemoautotrophically derived organic material may be readily available to suspension feeders.

2. Material and methods

A research cruise to the Solwara 1 hydrothermal vent field ($3^{\circ}47.45'\text{S}$, $152^{\circ}5.65'\text{E}$; $\sim 0.8\text{ km}^2$) was undertaken 31 Dec 2005 through 30 Jan 2006 on the M/V *DP Hunter* using the Perry Slingsby Remotely Operated Vehicle (ROV) *TST212*. Solwara 1 lies approximately 50 km northwest of Rabaul (East New Britain Province, Papua New Guinea), within Manus Basin. The crest of Solwara 1 is at $\sim 1460\text{ m}$ and is characterized by the presence of active and inactive massive sulfide chimneys. Active chimneys and inactive sites were a variety of shapes and sizes: from small deposits with $< 0.5\text{ m}$ relief above the seafloor, to slender, finger-like sulfide branches reaching $> 10\text{ m}$ above the seafloor, to extensive fields of chimneys with diameters of 3 m or more. Inactive sulfide chimneys ($\sim 2\text{ m}$ height, 0.5 m diameter) and clear diffuse vents dominated the Solwara 1 field, with interspersed black smokers ($116\text{--}296^{\circ}\text{C}$), grey smokers, and white smokers ($95\text{--}119^{\circ}\text{C}$). There were three main discrete sites of activity: Williamson (also referred to as 'Far West' and 'West' by Nautilus Minerals) to the west, Binns and Paine to the north (also referred to as 'Central'), and Kowalczyk to the east (also referred to as 'East'). In most areas where active hydrothermal venting was observed, there were also remnants of old sulfide chimneys (no longer actively venting).

'Inactive' and 'active' sampling sites with substantial invertebrate biomass were selected during reconnaissance video transects. Animals were collected from six inactive sites at areas known as Binns and Paine, Williamson, Fullagar Extended (also known as 'Far East'), Nat Nat, and 99, and from four active sites at Kowalczyk, Binns, Williamson, and Fullagar Extended (Fig. 1, Table 1). The substratum of the inactive sites was massive sulfide deposited by hydrothermal processes, except at 99, where the substratum was volcanic rock and not a sulfide deposit. Of the six inactive sites sampled, the westernmost part of Williamson and 99 supported the largest areas of inactive substratum and the greatest biomass and abundance of non-vent-endemic taxa (cladorhizid sponges, *Keratois* sp. bamboo corals, stalked barnacles, and hydroids; Table 1, Fig. 2). Fullagar Extended, Kowalczyk, and Binns were characterized by active sulfide deposits interspersed with inactive sulfide deposits. Paine, 99, and Nat Nat were characterized by large areas of sedimented seabed with discrete patches of inactive substratum (Table 1). At the time of sampling, Binns and Kowalczyk were the most active of the four active sites sampled and supported the highest biomass and abundance of vent-endemic taxa (Fig. 2). At Fullagar Extended there were only a few localized areas of active venting with high biomass and abundance of vent-endemic taxa.

Specimens were collected using a suction sampler, 'biological scoop', or the ROV manipulator claw. Invertebrates were preserved for laboratory identification in 10% buffered formalin (24 h) and stored in 70% ethanol. Consultants verified taxonomic identifications (see acknowledgements). Mode of feeding for each invertebrate taxon is inferred based on literature review and functional anatomy. Voucher specimens are archived at the Duke University Marine Laboratory (Beaufort, NC). Samples of sulfide rocks from hydrothermally active and inactive sites were collected for sulfur isotopic analysis of associated organic material and stored dry. Whole animals and tissues for isotope analysis were stored frozen, dried at 60°C , and processed in the laboratory.

2.1. Stable-isotope analysis

Muscle tissue was preferentially used for isotopic analysis. For smaller organisms, multiple individuals were homogenized using a mortar and pestle as a single sample. For certain small taxa, we analyzed pooled samples of individuals assumed to be in the same taxonomic and trophic category (e.g., hydroids, limpets, isopods, or amphipods). Animal tissues and the outer surface of sulfide samples were acidified to remove carbonate, and dried. Carbonate-free residues were weighed into tin capsules and converted to CO_2 , N_2 and SO_2 for isotope analysis using a Carlo Erba elemental analyzer coupled to an OPTIMA stable-isotope-ratio mass spectrometer (GV Micromass, Manchester, UK). Carbon and nitrogen isotopic compositions were determined simultaneously on each sample using a dual furnace system composed of an oxidation furnace at 1020°C and a reduction furnace at 650°C . Using the Carlo Erba elemental analyzer, samples for sulfur were separately pyrolyzed at 1050°C by means of a combination oxidation and reduction single furnace system. The resulting gases were chemically dried and directly injected into the source of the mass spectrometer. Isotopic composition of stable isotopes is based on the standard expression as a delta value (δ):

$$\delta^{\text{n}}\text{X} = (R_{\text{sample}}/R_{\text{standard}} - 1) * 1000\text{‰ (per mil)},$$

where X is the heavy isotope (^{13}C , ^{15}N , or ^{34}S), and R is the abundance ratio of the heavy to light isotopes ($^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, or $^{34}\text{S}/^{32}\text{S}$).

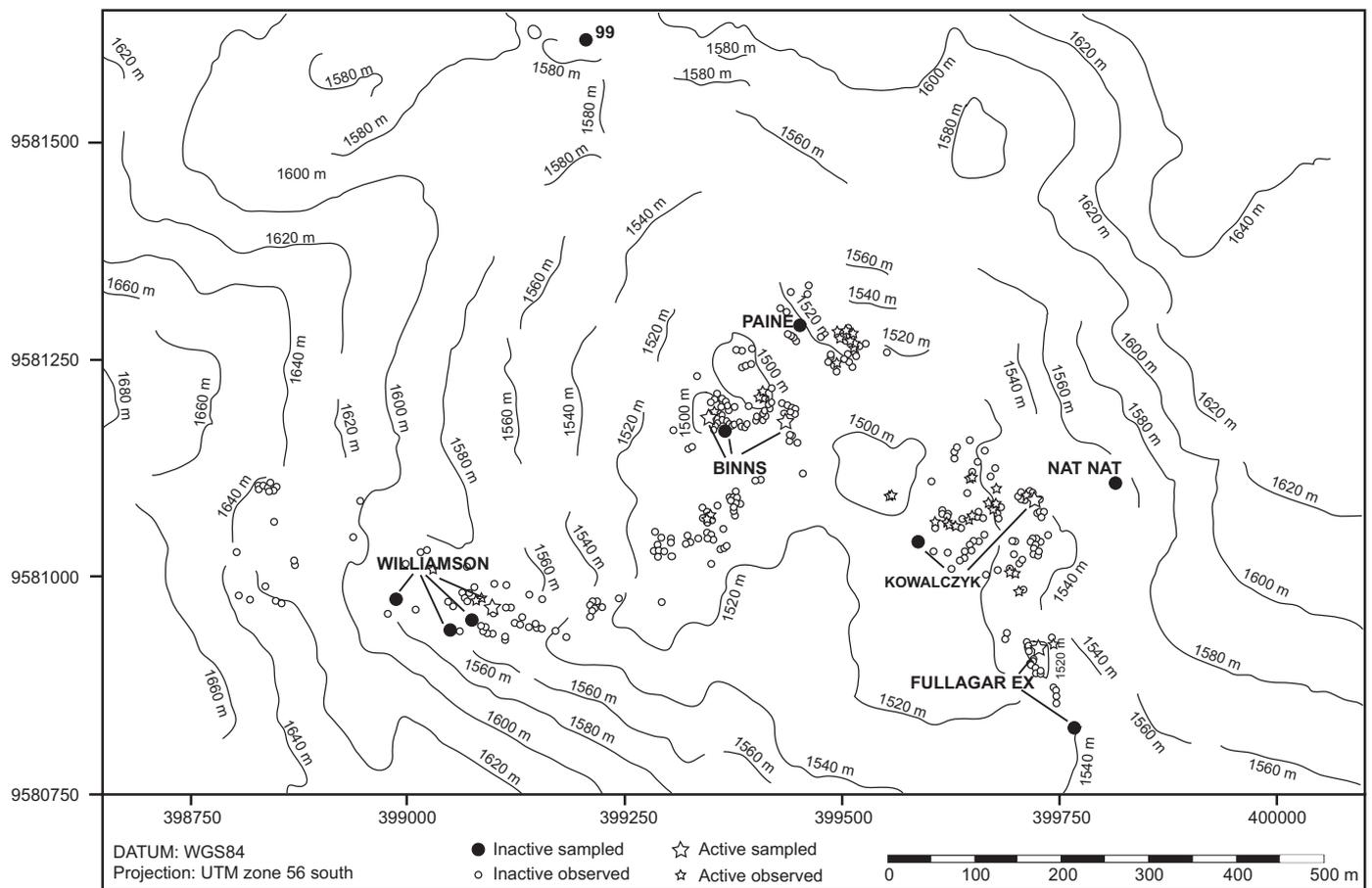


Fig. 1. Manus Basin: Solwara 1 vent field sample and site locations. Axes are scaled to the Universal Transverse Mercator (UTM) coordinate system. Latitude and longitude for sites are provided in Table 1.

Table 1
Site locations within the Solwara 1 vent field and their dominant fauna.

Site	Habitats sampled	Latitude (S)	Longitude (W)	Depth (m)	Inactive habitat biomass-dominant organisms	Active habitat biomass-dominant organisms
Binns	A, I	−3.788683	152.0943102	1500	<i>Vulcanolepas</i> cf. <i>parensis</i> , <i>Munidopsis</i> spp.	<i>Alviniconcha</i> sp. 1, <i>Ifremeria nautilei</i>
Fullagar Extended	A, I	−3.791047	152.0969462	1520	Hydroids, <i>Abyssocladia dominalba</i>	<i>Alviniconcha</i> sp. 1, <i>Ifremeria nautilei</i>
Kowalczyk	A	−3.789491	152.0968758	1550	<i>Vulcanolepas</i> cf. <i>parensis</i>	<i>Alviniconcha</i> sp. 1, <i>Ifremeria nautilei</i>
Nat Nat	I	−3.789329	152.0977315	1560	<i>Keratoisis</i> sp.	<i>Alviniconcha</i> sp. 1, <i>Ifremeria nautilei</i>
Painé	I	−3.788655	152.0935177	1500	Hydroids, <i>Munidopsis</i> spp.	<i>Alviniconcha</i> sp. 1, <i>Ifremeria nautilei</i>
Williamson	A, I	−3.790589	152.0912824	1570	<i>Keratoisis</i> sp.	<i>Alviniconcha</i> sp. 1, <i>Ifremeria nautilei</i>
99	I	−3.784692	152.0922521	1600	<i>Keratoisis</i> sp.	Not applicable

A: hydrothermally active habitat; I: hydrothermally inactive habitat.

Standard reference materials assigned $\delta^{13}\text{C}$ values of 0.0‰ are the fossil of *Belemnita americana* from the PeeDee formation in South Carolina for ^{13}C (PDB), atmospheric molecular N_2 for ^{15}N , and the troilite of the Canyon Diablo meteorite for ^{34}S (CDT). In the laboratory, samples were analyzed using carbon dioxide, nitrogen, and sulfur dioxide standard gases calibrated against NBS 22, atmospheric N_2 and NBS 127, respectively. Sulfur and carbon values were corrected for cross-mass overlap with isotopes of oxygen. Reproducibility of the measurement is typically better than $\pm 0.2\%$ for these elements using the continuous flow interface on the OPTIMA. Where multiple individuals were analyzed, isotopic data are reported as mean \pm standard error.

3. Results

Carbon isotopic compositions (Tables 2 and 3; Fig. 3) of organisms intimately associated with hydrothermal activity, either through endosymbionts, bacterial grazing, or other interpretation of strong fidelity to active hydrothermal systems [(e.g., hairy snails (*Alviniconcha* sp. 1), black snails (*Ifremeria nautilei*), predatory snails (*Eosipho* sp.), sessile barnacles (*Eochionelasmus ohtai*), shrimp (*Chorocaris vandoverae*), limpets], ranged from -29.7% to -19.9% . Carbon isotopic compositions of the mobile and omnivorous crab (*Austinograea alayseae*), and the stalked barnacle that occurs at both active and inactive sites (*Vulcanolepas* cf. *parensis*), also fell within this range. Organisms

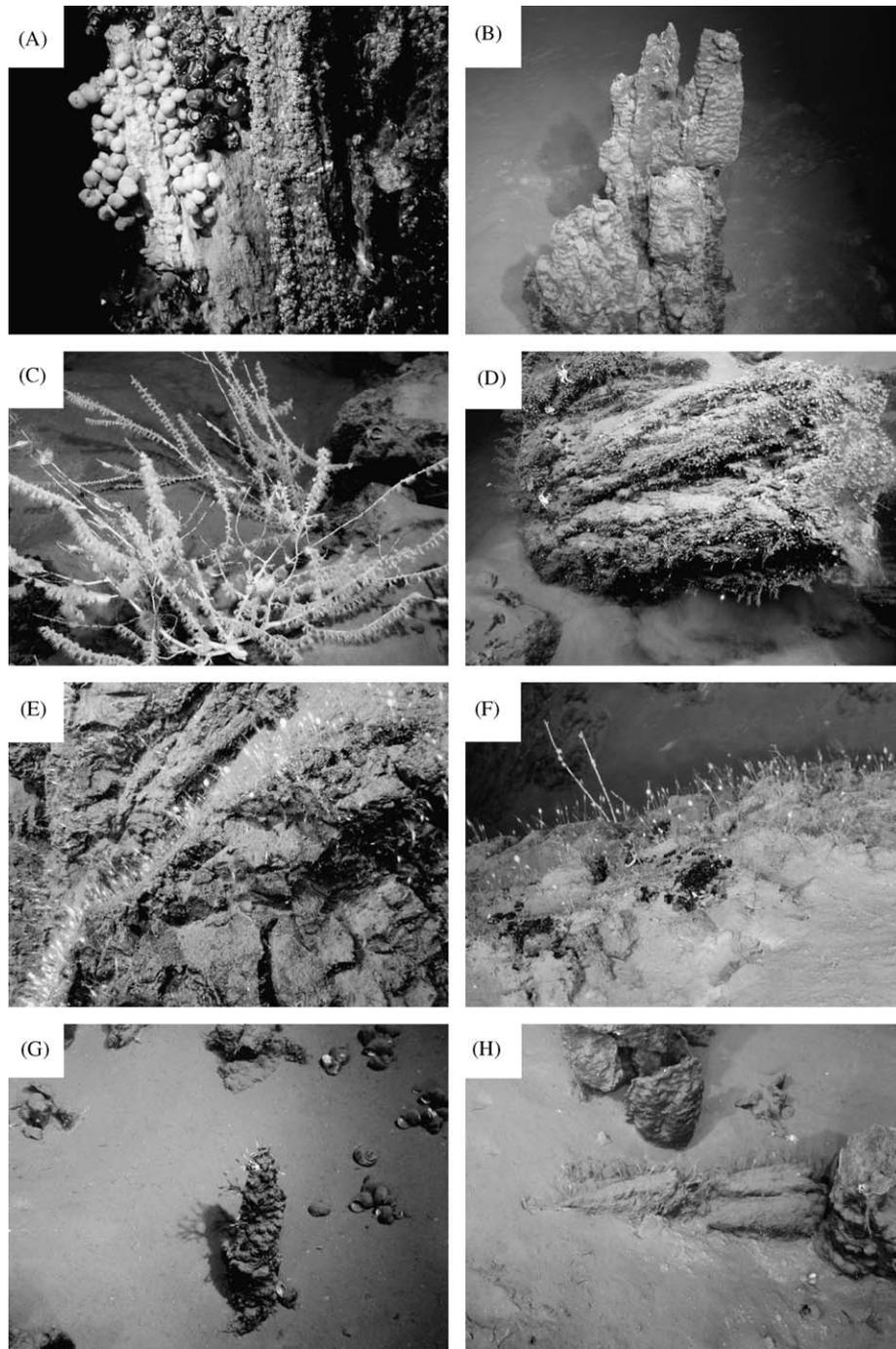


Fig. 2. *In situ* photographs from Manus Basin, illustrating typical habitats. (A) Active sulfide mound colonized by sessile barnacles (*Eochionelasmus ohtai*), black snails (*Ifremeria nautilei*), and hairy snails (*Alviniconcha* sp. 1); (B) Inactive sulfide mound with little associated biomass; (C) Inactive sulfide: *Keratoisis* sp. bamboo coral; (D) Inactive sulfide: anemones, hydroids and squat lobsters (*Munidopsis* sp.); (E and F) Inactive sulfide: cladorhizid sponges; (G and H) Inactive sulfide: stalked barnacles (*Vulcanolepas* cf. *parensis*). Photos provided by Nautilus Minerals Niugini Limited taken during the 2007 field campaign ("Wave Mercury 2007"; also referred to as "Luk Luk 07")

exclusively collected from inactive sites had carbon isotopic compositions ranging from -25.8‰ to -17.2‰ .

Whereas there was considerable overlap in carbon isotopic compositions of organisms at active and inactive sites, the trend was for the most ^{13}C -depleted values to be associated with *Alviniconcha* sp. 1 and *I. nautilei*, *Eosipho* sp. that likely feeds on these snails, and *E. ohtai*. Only five carbon isotope values of organisms collected from inactive sites fell outside the range of values observed in organisms from active sulfide mounds. Where samples for a given taxon from both active and inactive sites were

analyzed [squat lobsters (*Munidopsis* spp.), *C. vandoverae*, and *Vulcanolepas* cf. *parensis*], $\delta^{13}\text{C}$ values were overlapping.

Nitrogen isotopic compositions (Tables 2 and 3; Fig. 3) of organisms from active and inactive sites overlapped considerably. Taxa inferred to be primary consumers of symbiotic or free-living microbial autotrophs (*Alviniconcha* sp. 1, *I. nautilei*, and limpets) had the lowest $\delta^{15}\text{N}$ values at active sites (1.0–6.9‰). These nitrogen isotopic compositions were matched in tissues of deposit feeders and grazers at inactive sites [amphipods (*Ventrella* cf. *sulfuris*), sea cucumbers (*Chiridota* sp.), squat lobsters (*Munidopsis*

Table 2Carbon, nitrogen, and sulfur isotopic compositions ($\text{‰} \pm$ standard error) of tissues from active sites, Solwara 1 vent field, Manus Basin.

Organism	Site	Tissue type	Inferred primary feeding mode	n	Carbon (‰)	Nitrogen (‰)	Sulfur (‰)
Hydroids	Binns	Whole	Suspension	1	-21.8	8.3	-
Limpets	Fullagar Extended	Whole specimens pooled	Grazer	1	-21.8	4.8	2.1
Limpets	Binns	Whole specimens pooled	Grazer	4	-21.9 \pm 0.3	4.7 \pm 0.8	1.4 \pm 1.6
Limpets	Kowalczyk	Whole specimens pooled	Grazer	1	-21.6	5.9	1.2
				all	-21.8\pm0.2	4.9\pm0.6	2.2\pm1.3
Hairy Snail:							
<i>Alviniconcha</i> sp. 1	Fullagar Extended	Foot muscle	Endosymbionts	5	-26.2 \pm 0.5	4.4 \pm 0.2	2.8 \pm 1.6
<i>Alviniconcha</i> sp. 1	Binns	Foot muscle	Endosymbionts	8	-25.8 \pm 0.7	5.2 \pm 0.3	-0.2 \pm 0.6
<i>Alviniconcha</i> sp. 1	Williamson	Foot muscle	Endosymbionts	1	-26.9	6.7	-9.4
				all	-26.0\pm0.5	5.1\pm0.3	0.2\pm1.1
Black Snail:							
<i>Ifremeria nautiliei</i>	Fullagar Extended	Foot muscle	Endosymbionts	4	-25.5 \pm 0.4	4.9 \pm 0.4	4.2 \pm 0.9
<i>Ifremeria nautiliei</i>	Binns	Foot muscle	Endosymbionts	7	-25.5 \pm 0.6	3.2 \pm 0.7	0.3 \pm 1.5
<i>Ifremeria nautiliei</i>	Williamson	Foot muscle	Endosymbionts	2	-26.3	6.2	3.5
<i>Ifremeria nautiliei</i>	Kowalczyk	Foot muscle	Endosymbionts	1	-26.9	4	-2.4
				all	-25.8\pm0.4	4.2\pm0.5	1.7\pm1.0
Predatory Snail:							
<i>Eosipho</i> sp.	Williamson	Foot muscle	Predator	1	-28.3	9.1	-7.3
Sessile Barnacle:							
<i>Eochionelasmus ohtai</i> cf. <i>manusensis</i>	Fullagar Extended	Whole body	Suspension	4	-24.9 \pm 1.0	9.2 \pm 0.4	-0.3 \pm 1.7
<i>Eochionelasmus ohtai</i> cf. <i>manusensis</i>	Binns	Whole body	Suspension	3	-24.3 \pm 1.7	6.3 \pm 0.6	-0.2 \pm 1.3
				all	-24.7\pm0.9	8.0\pm0.7	-0.3\pm1.1
Stalked Barnacle:							
<i>Vulcanolepas</i> cf. <i>parensis</i>	Williamson	Whole specimen	Suspension	1	-22	11.9	-
Shrimp:							
<i>Chorocaris vandoverae</i>	Binns	Abdomen	Scavenger	1	-19.9	12/0	5.4
<i>Chorocaris vandoverae</i>	Williamson	Abdomen	Scavenger	8	-21.9 \pm 0.4	9.0 \pm 0.4	1.2 \pm 1.8
<i>Chorocaris vandoverae</i>	Kowalczyk	Abdomen	Scavenger	2	-23.0	7.0	-2.4
				all	-21.8\pm0.4	9.2\pm0.5	0.9\pm1.5
Squat Lobster:							
<i>Munidopsis lauensis</i>	Kowalczyk	Abdomen	Scavenger	1	-22.4	6.2	1.2
Brachyuran Crab:							
<i>Austinograea alayseae</i>	Binns	Muscle	Scavenger	5	-20.2 \pm 0.2	8.7 \pm 0.3	-1.8 \pm 0.9
<i>Austinograea alayseae</i>	Kowalczyk	Muscle	Scavenger	1	-19.8	8.5	-1.6
				all	-20.4\pm0.1	8.7\pm0.2	-1.8\pm0.5

n, number of independent samples; -, sample lost or too small.

sp.), and ophiuroids (*Ophiacantha* sp.)). The most ^{15}N -enriched $\delta^{15}\text{N}$ compositions ($>12\text{‰}$) were observed in *Vulcanolepas* cf. *parensis*, cladorhizid sponges (*Cladorhiza*, *Abyssocladia*), bamboo corals (*Keratoisis* sp.) and the anemone associated with *Keratoisis* sp. Where samples for a given taxon from both active and inactive sites were analyzed (*Munidopsis* spp., *C. vandoverae*, *Vulcanolepas* cf. *parensis*), $\delta^{15}\text{N}$ tended to be more depleted in the heavier isotope in individuals from the inactive sites.

Sulfur isotopic composition (Tables 2 and 3; Fig. 3) of organisms from active and inactive sites ranged between -10‰ and $+10\text{‰}$. Where samples for a given taxon from both active and inactive sites were analyzed (*Munidopsis* sp., *C. vandoverae*), $\delta^{34}\text{S}$ values were overlapping. Limitations of the sampling effort precluded statistical tests for differences in isotopic compositions between sites, except for *I. nautiliei*, *Alviniconcha* sp. 1, and *E. ohtai* between Binns and Fullagar Extended. Within these taxa, there was no significant difference in carbon, nitrogen, or sulfur isotopic compositions of individuals from Binns and Fullagar Extended, except for $\delta^{15}\text{N}$ values of *E. ohtai*, which were significantly (2-tailed *t*-test; $p < 0.5$) more enriched in ^{15}N at Fullagar Extended ($\delta^{15}\text{N}_{\text{ave}} = 9.3\text{‰}$) than at Binns ($\delta^{15}\text{N}_{\text{ave}} = 6.3\text{‰}$). There was no significant difference in the $\delta^{34}\text{S}$ values (Table 4; 2-tailed *t*-test, $p > 0.5$) for organic sulfur in surface layers of rocks from hydrothermally active ($\delta^{34}\text{S}_{\text{ave}} = -0.9\text{‰}$) and inactive sites ($\delta^{34}\text{S}_{\text{ave}} = 0.5\text{‰}$).

4. Discussion

Interpretation of nutritional resources within an ecosystem using isotopic methods is possible only if there are isotopically distinctive pools of available organic material. In this instance, the isotopic distinction between photosynthetically and chemosynthetically derived organic carbon and nitrogen is not unambiguous, but evidence presented here – particularly the sulfur isotopic data – indicates that microbial chemoautotrophic production may be a major supplement to the photosynthetically derived organic material available to invertebrates colonizing inactive sulfides and volcanic rock.

Galkin (1997) reported concentrations of non-vent suspension-feeding invertebrates (gorgonians, actinians, hydroids, etc.) at the periphery of Manus vent fields and proposed that at least some of their nutrition was derived from photosynthetic sources as well as from vent bacteria, but Galkin was not able to provide data in support of this hypothesis. In this present study, we show that carbon isotopic data alone does not allow one to accept or reject this hypothesis: tissues of non-vent invertebrates had $\delta^{13}\text{C}$ values within the range of values observed for sinking oceanic organic particulates in the northeast Pacific (i.e., -22‰ to -25.3‰ ; Wu et al., 1999) and overlapped $\delta^{13}\text{C}$ values observed in *Alviniconcha* sp. 1 (-23‰ to -30‰ ; this study), which has a high autotrophic capacity and is thought to depend primarily on endosymbiotic

Table 3
Carbon, nitrogen, and sulfur isotopic compositions (‰; mean ± standard error) of tissues from hydrothermally inactive sites, Solwara 1 vent field, Manus Basin.

Organism	Site location	Tissue type	Inferred feeding type	n	Carbon (‰)	Nitrogen (‰)	Sulfur (‰)
Cladorhizid (carnivorous) Sponge:							
<i>Abyssocladia dominalba</i>	Binns	Whole specimen	Predator	1	−21.0	9.9	0.5
<i>Abyssocladia dominalba</i>	Williamson	Sponge top	Predator	4	−20.0 ± 0.4	13.0 ± 0.5	−2.8 ± 0.2
<i>Abyssocladia dominalba</i>	Paine	Sponge top	Predator	1	−18.8	12.0	−0.7
				all	−20.0 ± 0.4	12.3 ± 0.6	−1.9 ± 0.6
Cladorhizid (carnivorous) Sponge:							
<i>Cladorhiza abyssicola</i>	Nat Nat	Whole specimen	Predator	1	−18.3	12.0	1.7
hydroids	Binns	Whole	Suspension	1	−21.2	8.9	4.2
hydroids	Williamson	Whole	Suspension	1	−21.7	8.2	−9.9
hydroids	Paine	Whole	Suspension	1	−	−	−8.0
hydroids	99	Whole	Suspension	1	−22.4	9.4	−4.8
hydroids*	Fullagar Extended	Whole	Suspension	1	−5.6	8.6	−
				all	−21.8 ± 0.3	8.8 ± 0.3	−4.6 ± 3.1
Bamboo Coral:							
<i>Keratoisis</i> sp.	Williamson	Polyps	Suspension	4	−20.2 ± 0.5	12.1 ± 0.2	−8.3 ± 0.7
<i>Keratoisis</i> sp.	99	Organic node	Suspension	2	−21.1	12.5	−2.4
				all	−20.5 ± 0.5	12.3 ± 0.2	−6.3 ± 1.2
Anemone associated with <i>Keratoisis</i> sp.							
anemone cf. <i>Hadalanthus</i> sp.	Williamson	Whole specimen	Suspension	2	−19.6	11.5	−9.0
anemone cf. <i>Hadalanthus</i> sp.	99	Whole specimen	Suspension	1	−19.2	16.9	−3.6
				all	−19.5 ± 0.3	13.3 ± 1.8	−7.2 ± 1.8
Amphipod:							
<i>Ventiella</i> cf. <i>sulfuris</i>	Fullagar Extended	Whole specimen	Scavenger	1	−23.7	1.4	−
Stalked Barnacle:							
<i>Vulcanolepas</i> cf. <i>parensis</i>	Binns	Whole body	Suspension	2	−20.6 ± 0.6	11.2 ± 1.2	2 ± 3.8
<i>Vulcanolepas</i> cf. <i>parensis</i>	99	Whole body	Suspension	1	−22.9	8.7	−6.6
<i>Vulcanolepas</i> cf. <i>parensis</i>	Fullagar Extended	Whole body	Suspension	1	−22.3	12.0	3.0
				all	−21.6 ± 0.6	10.8 ± 0.9	0.1 ± 2.7
Squat Lobster:							
<i>Munidopsis</i> sp.	Binns	Abdomen	Scavenger	4	−22.1 ± 0.2	5.5 ± 0.3	3.5 ± 0.8
<i>Munidopsis</i> sp.	Williamson	Abdomen	Scavenger	1	−17.2	7.0	−9.0
<i>Munidopsis</i> sp.	Paine	Abdomen	Scavenger	1	−21.8	6.4	3.7
<i>Munidopsis</i> sp.	Fullagar Extended	Abdomen	Scavenger	1	−21.2	6.2	2.4
				all	−21.2 ± 0.7	6.0 ± 0.3	1.6 ± 1.8
Shrimp:							
<i>Chorocaris vandoverae</i>	Binns	Abdomen	Scavenger	2	−21.6	9.3	2.3
Ophiuroid (Brittlestar):							
<i>Ophiacantha</i> sp.	99	Gonads	Deposit	1	−22.3	11.1	−5.5
Holothurian (Sea Cucumber):							
<i>Chiridota hydrothermica</i>	Fullagar Extended	Intestine	Deposit	2	−24.2	4.3	4.7

n, number of samples; −, sample lost or too small. *carbon outlier, excluded from calculation of means, standard error.

and free-living chemoautotrophic bacteria for its nutrition (Henry et al., 2008). The broad ranges of carbon, nitrogen, and sulfur isotopic compositions among individuals of *Alviniconcha* sp. 1 (and other Manus Basin species) suggest that diets are not narrowly constrained; determinants of isotopic variability within species remain topics for further research.

Interpretation of nitrogen isotope data is also subject to ambiguity with respect to photosynthetic or chemosynthetic sources of nutrition: $\delta^{15}\text{N}$ values for sinking oceanic organic particulates (1–7‰; Wu et al., 1999), together with the empirical observation of ~3‰ enrichment in ^{15}N with a single trophic step (Minagawa and Wada, 1984; Wada et al., 1987), suggest that invertebrates relying primarily on photosynthetically derived particulate organic material reaching the seabed might have $\delta^{15}\text{N}$ values on the order of 4–11‰ or higher. Observed $\delta^{15}\text{N}$ values in vent taxa (1–13‰) overlapped this range. Species inferred to be primary consumers (endosymbiont-bearing *Alviniconcha* sp. 1, *I. nautili* and grazing limpets) were relatively depleted in ^{15}N (1–7‰), consistent with their position just above the autotrophic base of the food web. The most ^{15}N -enriched individuals (> 1‰) belonged to the biomass dominant taxa at the

non-active sulfide mounds (*Abyssocladia dominalba*, *Cladorhiza abyssicola*, *Keratoisis* sp., anemone).

The sulfur isotopic composition of photosynthetically derived organic material ($\delta^{34}\text{S} = 16\text{--}19\text{‰}$; Fry 1988) closely matched that of seawater sulfate ($\delta^{34}\text{S} = 21\text{‰}$; Rees et al., 1978). Dissolved sulfide in end-member vent fluid is considerably more depleted in ^{34}S ($\delta^{34}\text{S} < 10\text{‰}$; Shanks, 2001) than seawater sulfate, as is the isotopic composition of inorganic sulfur in chimney sulfides ($\delta^{34}\text{S}$: 1.9–3‰; Hekinian et al., 1980) and of organic sulfur associated with chimney sulfides and volcanic rock ($\delta^{34}\text{S}$: −3.4‰ to 3.8‰; this study). Sulfur isotopic composition of specimens from active and inactive sites were all below 10‰, indicating incorporation of a significant amount of organic sulfur derived from sulfide rather than seawater sulfate and implicating chemoautotrophic production as a major nutritional resource for all organisms analyzed. Some of the most ^{34}S -depleted tissues (< 0‰) were consistently observed in organisms from inactive sites (e.g., *Keratoisis* sp., anemones, hydroids), indicating that most of their organic sulfur is ultimately derived from a non-seawater inorganic sulfur source. The overlap between $\delta^{34}\text{S}$ values of invertebrates from active and inactive sites provides further

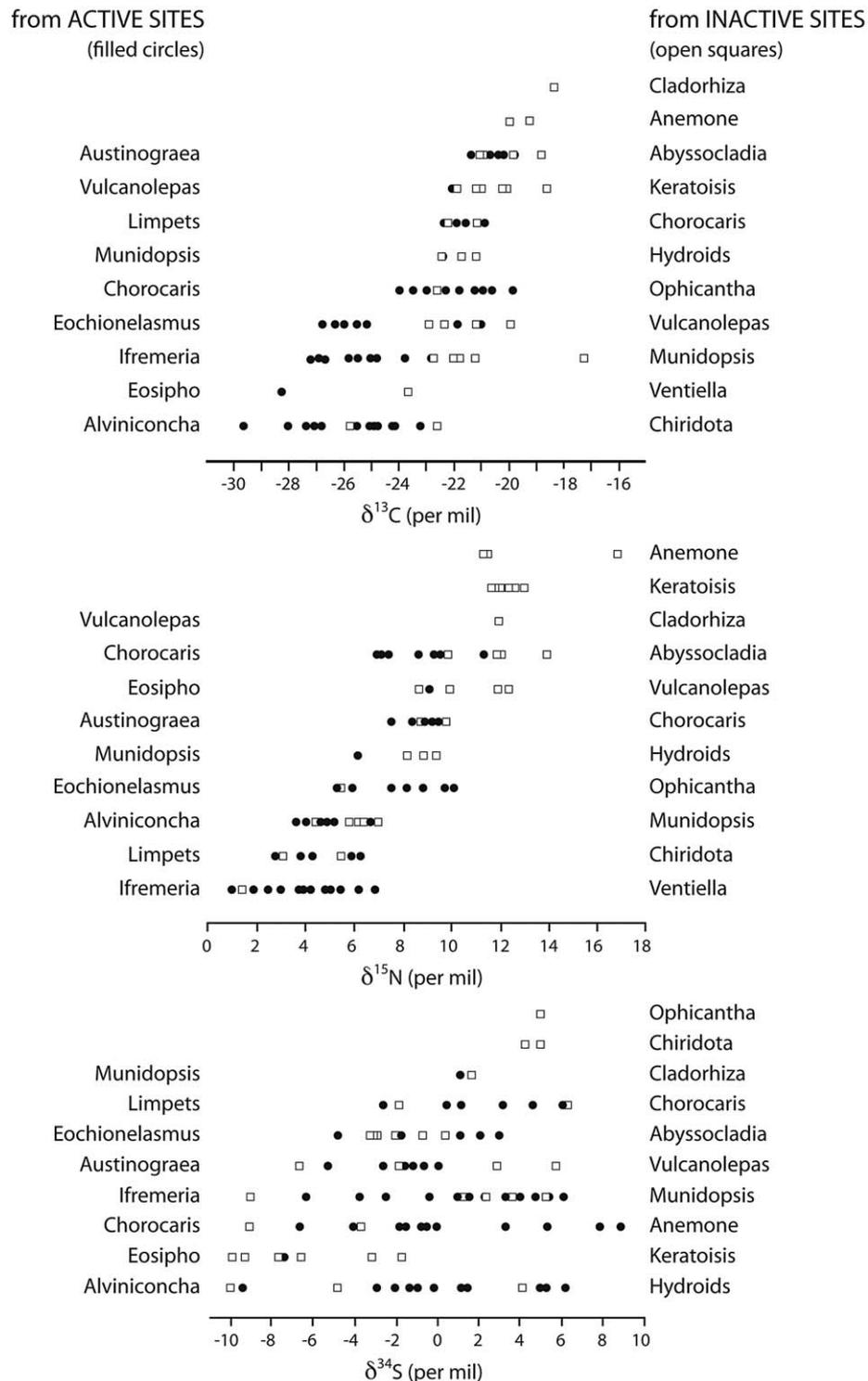


Fig. 3. Carbon ($\delta^{13}\text{C}$), nitrogen ($\delta^{15}\text{N}$), and sulfur ($\delta^{34}\text{S}$) isotopic compositions (‰) for invertebrates collected from active (filled circles) and inactive (open squares) sulfide mounds, Solwara 1 Field, Manus Basin. Taxa are ordered along y-axes according to increasing values within each category of sampling area (active or inactive).

support for the hypothesis that the base of the food web in both instances is predominantly chemoautotrophic.

From the discussion above, carbon and nitrogen isotopic composition of invertebrates from inactive sites are consistent with, but not proof of, nutrition predominantly derived from microbial chemoautotrophic production. The ^{34}S -depleted nature of the tissue samples allows us to reject the hypothesis that photosynthetically derived organic material provides all or nearly all of the nutrition for these animals. The conspicuous nature

(high biomass and abundance) of invertebrate assemblages at inactive sites seems likely to be supported by favorable food regimes and enhanced growth rates in proximity to active hydrothermal vents via local supply of chemoautotrophically produced organic particulates. Chemoautotrophic production by microorganisms that mobilize particulate sulfide (Eberhard et al., 1995; Suzuki et al., 2004) may also contribute to the secondary production, but the bulk isotope methods used here do not allow us to address this question. We emphasize that the dominant

Table 4

Sulfur isotopic compositions (‰; mean ± standard error) of organic material in mineral substrata from hydrothermally active and inactive sites, Solwara vent field, Manus Basin.

Site	Habitat type	n	Sulfur (‰)
Fullagar Extended	Active	1	1.1
Binns	Active	2	−2.3
Williamson	Active	1	−2.2
Kowalczyk	Active	1	1.1
		all	−0.9 ± 0.8
Fullagar Extended	Inactive	1	−0.6
Binns	Inactive	1	−3.4
Williamson	Inactive	2	−0.1
Paine	Inactive	1	3.8
Nat Nat	Inactive	1	2.6
Kowalczyk	Inactive	1	3.3
99	Inactive	1	−1.2
		all	0.5 ± 0.9

All samples were massive sulfides except the sample from the 99 site, which was volcanic (dacite or andesite).

biomass species at inactive sites are inferred to be suspension feeders (*Keratoisis* sp., *Vulcanolepas* cf. *parensis*) or 'micro-carnivores' (cladorhizid sponges, hydroids) that rely on micro-zooplankton or other particulate organic material for their nutrition, which is consistent with an allochthonous rather than autochthonous food source.

Other studies have cited a minor role of export of chemosynthetic production to the surrounding deep-sea environment, as in studies of seep sites in the Gulf of Mexico, where marauding fish may derive as much as 10% (MacAvoy et al., 2008) to nearly 100% (MacAvoy et al., 2002) of their nutrition from chemoautotrophic sources. This present report is, to our knowledge, the first to implicate active hydrothermal systems in the nutrition of a relatively high biomass of sessile, suspension-feeding, non-vent organisms colonizing inactive sulfide deposits in the region. A similarly high biomass of suspension-feeding invertebrates has been reported in association with inactive Gorda Ridge sulfide mounds (Van Dover et al., 1990), but the mode of nutrition of the Gorda Ridge invertebrates remains unstudied. It is possible that the animals that take advantage of exported organic material of chemoautotrophic organic material in Manus Basin – the corals, hydroids, cladorhizid sponges, barnacles, etc – are a select subset of the non-vent deep-sea fauna that can tolerate high levels of toxic compounds (e.g., heavy metals) emitted in vent fluids (Seewald et al., 2007) and accumulated in diet items through trophic magnification.

5. Conclusion

Carbon, nitrogen and sulfur isotopic compositions of organisms collected from hydrothermally active and inactive sites in Manus Basin are consistent with local export of organic material from active to inactive sites, where this export is inferred to supply a large percentage of the diet of non-vent endemic, sessile, suspension-feeding and micro-carnivorous invertebrates. Growth rates and reproduction of non-vent organisms with isotopic signatures of vent organic material are presumed to be greater than those of individuals belonging to the same species but remote from a 'supplementary' deep-sea source of chemoautotrophic primary production.

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References

- Colaco, A., Dehairs, F., Desbruyères, D., 2002. Nutritional relations of deep-sea hydrothermal fields at the Mid-Atlantic Ridge: a stable isotope approach. *Deep-Sea Research I* 49, 395–412.
- Conway, N.M., Kennicutt, M.C., Van Dover, C.L., 1994. Stable isotopes in the study of marine chemosynthetic-based ecosystems. In: Lajtha, K., Michener, R. (Eds.), *Stable Isotopes in Ecology and Environmental Sciences*. Blackwell Scientific Publications, Cambridge, MA, pp. 158–186.
- Corliss, J.B., Dymond, J., Gordon, L.I., Edmond, J.M., von Herten, R.P., Ballard, R.D., Green, K., Williams, D., Bainbridge, A., Crane, K., vanAndel, T.H., 1979. Submarine thermal springs on the Galapagos Rift. *Science* 203, 1073–1083.
- Eberhard, C., Wirsén, C.O., Jannasch, H.W., 1995. Oxidation of polymetallic sulfides by chemolithoautotrophic bacteria from deep-sea hydrothermal vents. *Geomicrobiology Journal* 13, 145–164.
- Fisher, C.R., Childress, J.J., Macko, S.A., Brooks, J.M., 1994. Nutritional interactions in Galapagos Rift hydrothermal vent communities: inferences from stable carbon and nitrogen analyses. *Marine Ecological Progress Series* 103, 45–55.
- Fornari, D.J., Embley, R.W., 1995. Tectonic and volcanic controls on hydrothermal processes at the mid-ocean ridge: an overview based on nearbottom and submersible studies. In: Humphris, S.E., Zierenberg, R.A., Mullineaux, L.S., Thomson, R.E. (Eds.), *Seafloor Hydrothermal Systems: Physical, Chemical, Biological and Geological Interactions*. Geophysical Monograph 91, American Geophysical Union, pp. 1–46.
- Fry, B., 1988. Food web structure on Georges Bank from stable C, N, and S isotopic compositions. *Limnology and Oceanography* 33, 1182–1190.
- Fry, B., Gest, H., Hayes, J.M., 1983. Sulfur isotopic compositions of deep-sea hydrothermal vent animals. *Nature* 306, 51–52.
- Galkin, S.V., 1997. Megafauna associated with hydrothermal vents in the Manus Back-Arc Basin (Bismarck Sea). *Marine Geology* 142, 197–206.
- Haymon, R.M., 1983. Growth history of black smoker hydrothermal chimneys. *Nature* 301, 695–698.
- Hekinian, R., Fevrier, M., Bischoff, J.L., Picot, P., Shanks, W.C., 1980. Sulfide deposits from the East Pacific Rise near 21°N. *Science* 207, 1433–1444.
- Henry, M.S., Childress, J.J., Figueroa, D., 2008. Metabolic rates and thermal tolerances of chemoautotrophic symbioses from Lau Basin hydrothermal vents and their implications for species distributions. *Deep-Sea Research I* 55, 679–695.
- Karl, D.M., 1995. Ecology of free-living, hydrothermal vent microbial communities. In: Karl, D.M. (Ed.), *The Microbiology of Deep-Sea Hydrothermal Vents*. CRC Press, New York, NY, pp. 35–124.
- MacAvoy, S.E., Carney, R.S., Fisher, C.R., Macko, S.A., 2002. Use of chemosynthetic biomass by large, mobile, benthic predators in the Gulf of Mexico. *Marine Ecology Progress Series* 225, 65–78.
- MacAvoy, S.E., Morgan, E., Carney, R.S., Macko, S.A., 2008. Chemoautotrophic production incorporated by heterotrophs in Gulf of Mexico hydrocarbon seeps: an examination of mobile benthic predators and seep residents. *Journal of Shellfish Research* 27, 153–161.
- Minagawa, M., Wada, E., 1984. Stepwise enrichment of ¹⁵N along food chains: Further evidence and the relation between stable N isotope composition and animal age. *Geochimica et Cosmochimica Acta* 48, 1135–1140.
- Parson, L.M., Walker, C.L., Dixon, D.R., 1995. *Hydrothermal Vents and Processes*. Geological Society Special Publication No. 87, The Geological Society, London.
- Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecological Systems* 18, 293–320.
- Polz, M.F., Robinson, J.J., Cavanaugh, C.M., Van Dover, C.L., 1998. Trophic ecology of massive shrimp aggregations at a Mid-Atlantic Ridge hydrothermal vent site. *Limnology and Oceanography* 43, 1631–1638.
- Pond, D.W., Segonzac, M., Bell, M.V., Dixon, D.R., Fallick, A.E., Sargent, J.R., 1997. Lipid and carbon stable isotope composition of the hydrothermal vent shrimp *Mirocaris fortunata*: evidence for nutritional dependence

- on photosynthetically fixed carbon. *Marine Ecology Progress Series* 157, 221–231.
- Pond, D.W., Gebruk, A., Southward, E., Southward, A., Fallick, A., Bell, M.V., Sargent, J., 2000. Unusual fatty-acid composition of storage lipids in the bresiliid shrimp *Rimicaris exoculata* couples the photic zone with MAR hydrothermal sites. *Marine Ecology Progress Series* 198, 171–179.
- Rau, G.H., Hedges, J.I., 1979. Carbon-13 depletion in a hydrothermal vent mussel: suggestions of a chemosynthetic food source. *Science* 203, 648–649.
- Rau, G.H., Ainley, D.G., Bengtson, J.L., Torres, J.J., Hopkins, T.L., 1992. $^{15}\text{N}/^{14}\text{N}$ and $^{13}\text{C}/^{12}\text{C}$ in Weddell Sea birds, seals, and fish: implications for diet and trophic structure. *Marine Ecology Progress Series* 84, 1–8.
- Rees, C.E., Jenkins, W.J., Monster, J., 1978. The sulphur isotopic composition of ocean water sulphate. *Geochimica et Cosmochimica Acta* 42, 377–381.
- Rieley, G., Van Dover, C.L., Hedrick, D.B., Eglinton, G., 1999. Trophic ecology of *Rimicaris exoculata*: a combined lipid abundance/stable isotope approach. *Marine Biology* 133, 495–499.
- Seewald, J., Reeves, E., Saccocia, P., Rouxel, O., Walsh, E., Craddock, P., Tivey, M.A., Bach, W., Tivey, M.K., 2007. Tracing styles of hydrothermal circulation in Manus Basin using vent fluid composition. *Geophysical Research Abstracts* 9, 10057.
- Shanks III, W.C., 2001. Stable isotopes in seafloor hydrothermal systems: vent fluids, hydrothermal deposits, hydrothermal alteration, and microbial processes. *Reviews in Mineralogy and Geochemistry* 43, 469–525.
- Suzuki, Y., Inagaki, F., Takai, K., Nealson, K.H., Horikoshi, K., 2004. Microbial diversity in inactive chimney structures from deep-sea hydrothermal systems. *Microbial Ecology* 47, 186–196.
- Southward, A.J., Southward, E.C., Spiro, B., Rau, G.H., Tunnicliffe, V., 1994. $^{13}\text{C}/^{12}\text{C}$ of organisms from Juan de Fuca Ridge hydrothermal vents: a guide to carbon and food sources. *Journal of the Marine Biological Association of the United Kingdom* 74, 265–278.
- Tolstoy, M., Waldhauser, F., Bohnenstiehl, D.R., Weekly, R.T., Kim, W.-Y., 2008. Seismic identification of along-axis hydrothermal flow on the East Pacific Rise. *Nature* 451, 726–729.
- Trask, J.L., Van Dover, C.L., 1999. Site-specific and ontogenetic variations in nutrition of mussels (*Bathymodiulus* sp.) from the Lucky Strike hydrothermal vent field, Mid-Atlantic Ridge. *Limnology and Oceanography* 44, 334–343.
- Van Dover, C., 2000. *The Ecology of Deep Sea Hydrothermal Vents*. Princeton University Press, Princeton, NJ.
- Van Dover, C., 2002. Trophic relationships among invertebrates at the Kairei hydrothermal vent field (Central Indian Ridge). *Marine Biology* 141, 761–772.
- Van Dover, C., 2007. Stable isotopes in marine chemoautotrophically based ecosystems: an update. In: Michener, R., Lajtha, K. (Eds.), *Stable Isotopes in Ecology and Environmental Science*. Blackwell Publishing, MA, pp. 202–237.
- Van Dover, C., Grassle, F., Boudrias, M., 1990. Hydrothermal vent fauna of Escanaba Trough (Gorda Ridge). In: McMurray, G.R. (Ed.), *Gorda Ridge: A Seafloor Spreading Center in the United States Exclusive Economic Zone*. Springer-Verlag, New York, pp. 286–287.
- Van Dover, C.L., Fry, B., 1989. Stable isotopic compositions of hydrothermal vent organisms. *Marine Biology* 102, 257–263.
- Van Dover, C.L., Fry, B., 1994. Microorganisms as food resources at deep-sea hydrothermal vents. *Limnology and Oceanography* 39, 51–57.
- Van Dover, C.L., Fry, B., Grassle, J.F., Garritt, R.H., Starczak, V., 1992. Stable isotopic evidence for entry of sewage-derived organic material into a deep-sea food web. *Nature* 360, 153–156.
- Vereshchaka, A.L., Vinogradov, G.M., Lein, A., Dalton, S., Dehairs, S., 2000. Carbon and nitrogen isotopic composition of the fauna from the Broken Spur hydrothermal vent field. *Marine Biology* 136, 11–17.
- Vetter, R.D., Fry, B., 1998. Sulfur contents and sulfur-isotope compositions of thiotrophic symbioses in bivalve molluscs and vestimentiferan worms. *Marine Biology* 132, 453–460.
- Wada, E., Terazaki, M., Kabaya, Y., Nemoto, T., 1987. N and C abundances in the Antarctic Ocean with emphasis on the biogeochemical structure of the food web. *Deep-Sea Research II* 34, 829–841.
- Wainright, S.C., Fogarty, M., Greenfield, R., Fry, B., 1993. Long-term changes in the Georges Bank food web: trends in stable isotopic compositions of fish scales. *Marine Biology* 115, 481–493.
- Wu, J., Calvert, S.E., Wong, C.S., Whitney, F.A., 1999. Carbon and nitrogen isotopic composition of sedimenting particulate material at Station Papa in the subarctic northeast Pacific. *Deep-Sea Research II* 46, 2793–2832.