Stable isotope signatures and methane use by New Zealand cold seep benthos

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ABSTRACT

The carbon isotopic composition of seep faunal tissue represents a time-integrated view of the interaction between biology and the biogeochemical gradients within the environment. Here we provide an initial description of carbon and nitrogen stable isotope signatures of dominant symbiont-bearing megafauna and heterotrophic mega- and macrofauna from 10 methane-seep sites on the continental margin of the North Island of New Zealand (662–1201 m water depth). Isotopic signatures suggest that sulfide oxidation supports symbiont-bearing taxa including solemyid and vesicomyid bivalves, and methanotrophic symbionts are present in the seep mussel *Bathymodiolus* sp. Multiple species of Frenulata (Siboglinidae) are present and have a range of isotopic values that are indicative of both thiotroph- and methanotroph-based nutrition. Isotopic composition of the tubeworm *Lamellibrachia* sp. varied by 23.3‰ among individuals although there was no consistent difference among sites. Variation in methane use by heterotrophic fauna appears to reflect the availability of hard vs. soft substrate; macrofauna on hard substrates had high δ^{13}C signatures, reflecting consumption of photosynthetic-derived organic matter. Two unique, biotic assemblages were discovered to be fueled largely by methane: a hard-substrate, multi-phyla sponge-associated community (inhabiting the sponge *Pseudosuberites* sp.) and a soft-sediment assemblage dominated by ampharetid polychaetes. Isotope signatures yield estimates of 38–100% and 6–100% methane-derived carbon in sponge associates and ampharetid-bed macrofauna, respectively. These estimates are comparable to those made for deeper methane seeps at the Florida Escarpment (3290 m) and Kodiak, Alaska seeps (4445 m). The overall high use of methane as a carbon source by both symbiont-bearing and heterotrophic fauna suggests that New Zealand methane seeps are an ideal model system to study the interaction among metazoans, bacteria, archaea, and their resulting effect on methane cycles.

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1. Introduction

Methane seeps are increasingly recognized as a patchy (Luff et al., 2004; Robinson et al., 2004) but widespread feature of the world's continental margins in both the past (Campbell, 2006) and present (Sibuet and Olu, 1998). The use of methane as a carbon source is one of the many unusual features that distinguishes methane-seep fauna from those in other systems. Methane use was first documented for bathymodiolin mussels harboring methanotrophic symbionts in the Gulf of Mexico (Childress et al., 1986). More recently, widespread incorporation of methane-derived carbon (MDC) has been documented for heterotrophic seep invertebrates (e.g., Levin and Michener, 2002; Gebruk et al., 2003; Levin and Mendoza, 2007) and for the surrounding non-seep fauna (MacAvoy et al., 2002, 2003, 2008). Methane-derived carbon may be obtained by seep metazoans in several ways. They can consume chemoautotrophic archaea (anaerobic methane oxidizers; Valentine, 2002), and aerobic methane-oxidizing bacteria (Ding and Valentine, 2008), as well as from sulfide-oxidizing or sulfate-reducing bacteria which can take up methane-derived carbon after it passes into the dissolved inorganic carbon (DIC) pool (Wegener et al., 2008).

Stable isotope signatures provide valuable insight into nutritional sources of seep fauna (Van Dover, 2007). Biogenic methane is isotopically depleted in ^{13}C (usually δ^{13}C of −50 to −110‰) thus it serves as its own biomarker (Whiticar, 1999). Carbon fixation by other metabolic processes, such as phytoplankton photosynthesis or autotrophy coupled to sulfide oxidation, also results in distinctive δ^{13}C signatures. δ^{15}N signatures provide information about local N fixation (often associated with symbiosis) or trophic level in heterotrophs. When novel seep communities are discovered, an initial characterization of trophic structure and key nutritional sources can be obtained from a survey of stable isotope signatures.

During late 2006 and early 2007, the eastern continental margin of New Zealand was intensively surveyed for the presence of methane seeps using a towed camera and acoustic tools to record methane bubble plumes and map the extent of seep-related seabed structures (Baco

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et al., 2009-this issue; Greinert et al., this issue; Jones et al., 2009-this issue; Klaucke et al., 2009-this issue). Here we discuss the isotopic signatures of invertebrate assemblages found at 10 seep sites located at bathyal depths of 662 to 1200 m. The sites are described in more detail in Greinert et al. (2009-this issue) and the general geology in Barnes et al. (2009-this issue). The symbiont-bearing seep megafauna present included siboglinid polychaetes, specifically the vestimentiferan, *Lamellibrachia* sp., and members of the Frenulata (previously known as pogonophorans; Southward et al., 2005), bathymodiolin mussels (*Bathymodiolus* sp.), vesicomyid (*Calyptogena* spp.) and solemyid clams (Baco et al., 2009-this issue). Ampharetid polychaetes formed dense beds in sulfidic sediments (Sommer et al., 2008, 2009-this issue) and small frenulates (*Siboglinum* spp.) formed beds in sediments at the periphery of seep sites. Many of the sites had widespread, blocky carbonates, some covered with sponges (*Pseudosuberites* sp. undescribed, Class Demospongiae: Order Hadromerida: Family Suberitidae)

or multiple species of deep-water coral (Baco et al., 2009-this issue). Sled, grab and multicore samples yielded many other invertebrate taxa which are being described elsewhere (e.g. Baco et al., 2009-this issue; Campbell et al., 2009-this issue).

Here we describe carbon and nitrogen stable isotope signatures of dominant mega- and macrofauna at methane seeps on the New Zealand continental margin. We focus on site-specific variability and address the following questions for the New Zealand seep fauna:

(1) What nutritional pathways are likely among the symbiont-bearing megafauna?
(2) What are the nutritional sources of heterotrophic macrobenthos and megabenthos and how variable are they within a seep patch and among seep sites?
(3) Which assemblages and taxa exhibit high concentrations of methane-derived C in their tissues?

Table 1
Sampling sites, dates, depths, and gear used for faunal collection.

Region	Site	Station	Date (mm/dd/yr)	Gear	Depth (m)	Latitude (S)	Longitude (E)
Builder's Pencil		21	11/5/06	Epibenthic sled	812	39°32.62′	178°19.72′
		34	11/6/06	Grab	802	39°32.62′	178°20.01′
		36	11/6/06	Multicorer	794	39°32.62′	l78°20.01′
		38	11/6/06	Epibenthic sled	815	39°32.65′	178°19.62′
		32	11/6/06	Epibenthic sled	817	39°32.68′	178°19.70′
		18	11/5/06	Epibenthic sled	780	39°32.83′	178°19.95′
		23	11/5/06	Grab	787	39°32.84	178°19.94′
		30	11/6/06	Epibenthic sled	790	39°33.01′	178°19.91′
		22	11/5/06	Grab	787	39°32.79′	178°19.97′
LM-3		238	3/6/07	TV-Grab	908	39°58.62′	178°14.16′
		216	3/4/07	TV-MUC	662	40°01.52′	178°09.65′
Rock Garden	Knoll	10	11/4/06	Epibenthic sled	760	40°02.22′	178°38.86′
		6	11/4/06	Epibenthic sled	730	40°02.31′	178°08.58′
		11	11/4/06	Grab	752	40°02.38′	178°08.59′
		7	11/4/06	Epibenthic sled	766	40°02.42′	l78°09.00′
		12	11/4/06	Epibenthic sled	749	40°02.65′	178°08.9l′
Omakere Ridge	LM-9	45	11/7/06	Epibenthic sled	1150	40°00.52′	177°51.38′
		50	11/8/06	Epibenthic sled	1140	40°00.93′	177°51.56′
		57	11/8/06	Multicorer	1143	40°01.04′	177°51.72′
		46	11/7/06	Multicorer	1145	40°01.08′	177°51.59′
		49	11/7/06	Multicorer	1140	40°01.09′	177°51.58′
		58	11/8/06	Grab	1144	40°01.09′	177°51.63′
		59	11/8/06	Grab	1144	40°01.10′	177°51.63′
		60	11/8/06	Grab	1145	40°01.13′	177°51.73′
		61	11/8/06	Grab	1144	40°01.15′	177°51.62′
		164	2/22/07	TV-Grab	1110	40°03.20′	177°49.11′
		174	2/23/07	TV-Grab	1104	40°03.19′	177°49.30′
	Kaka	261	3/9/07	TV-MUC	1165	40°02.12′	177°48.93′
		232	3/6/07	TV-MUC	1169	40°02.15′	177°48.96′
		242	3/7/07	TV-MUC	1172	40°02.15′	177°47.95′
	Bear's Paw	197	3/3/07	TV-MUC	1100	40°03.17′	177°49.17′
		198	3/3/07	TV-MUC	803	40°03.17′	177°49.16′
		186	2/28/07	TV-MUC	1201	40°03.14′	177°49.24′
Uruti Ridge	East	70	11/10/06	Epibenthic sled	720	41°17.18′	176°35.49′
	LM-10	68	11/10/06	Epibenthic sled	716	41°17.49′	176°33.12′
	Hihi	67	11/10/06	Epibenthic sled	740	41°17.65′	176°33.66′
Opouawe Bank	North Tower	122	11/18/06	Epibenthic sled	1040	41°46.77′	175°24.05′
		79	11/13/06	Epibenthic sled	1045	41°46.83′	175°24.25′
		290	3/12/07	TV-MUC	1061	41°46.98′	175°24.27′
		298	3/14/07	Bigo	1059	41°46.95′	175°24.18′
		112/113	11/17/06	Multicorer	1036	41°46.90′	175°24.08′
		277	3/11/07	FLUFO 6	1048	41°46.90′	175°24.11′
		86	11/13/06	Multicorer	1050	41°46.92′	175°24.12′
		84	11/13/06	Multicorer	1053	41°46.99′	175°24.04′
		83	11/13/06	Epibenthic sled	1040	41°46.88′	l75°24.14′
	South Tower	81	11/13/06	Epibenthic sled	1050	41°47.27′	175°24.53′
		116	11/18/06	Multicorer	1049	41°47.31′	175°24.45′
		118	11/18/06	Multicorer	1051	41°47.36′	175°24.43′
		82	11/13/06	Multicorer	1059	41°47.37′	175°24.26′
		123	11/18/06	Multicorer	1051	41°47.40′	175°24.45′
	Takahe	309	3/16/07	TV-MUC	1056	41°46.34′	175°24.68′

Latitude and longitude are given for the end of epibenthic sled deployments.

2. Regional settings

The Hikurangi margin is the southern extent of the Tonga–Kermadec–Hikurangi subduction zone where the thick, sediment-rich Hikurangi Plateau region of the Pacific plate is being subducted under the Indo-Australian plate (Lewis and Pettinga, 1993). The region is dominated by a wide (maximum of 80 km) and thick (maximum of 7 km) accretionary prism of sediment (Lewis and Pettinga, 1993), with the sites discussed here occurring on the ridge crests of thrust faults (Barnes et al., 2009-this issue). The geologic underpinnings of our sample locations are discussed in detail in Barnes et al. (2009-this issue).

3. Materials and methods

Sampling took place aboard the *RV TANGAROA* (Cruise — TAN0616) from 5 November to 18 November 2006 and aboard the *RV SONNE* (Cruise — SO191) from 22 February to 14 March 2007. Collection gear included an epibenthic sled (25 mm stretched mesh diameter), vanVeen grab (surface area 0.2 m^2 and 90l volume) and multicore (9.6 cm internal diameter tubes) on TAN0616 and a video-guided multicorer (10.0 cm internal diameter tubes), TV-guided hydraulic grab (1.8 m^2 opening), and benthic landers (sediment opportunistically collected from the chambers) on SO191. A total of 51 stations at 10 seep sites spread over 5 regions were sampled on the 2 cruises within a depth range of 662 to 1201 m (Table 1, Fig. 1). During the SO191 we focused on several faunal associations in particular: ampharetid beds and commensals from sponges as they occurred at locations of seepage. Plankton was collected on either a 63 μm or 150 μm sieve from the ship's unfiltered flow-through seawater supply to constrain the planktonic isotopic signature for the region.

3.1. Stable isotope analyses

Symbiont-bearing megafauna, heterotrophic invertebrates, and filamentous bacteria were collected by hand picking them from dredged material, or by sieving sediment samples through 0.3 mm mesh and sorting the retained material live under a dissecting microscope. Specimens were identified to family level (lower if possible) and allowed to clear gut contents overnight in filtered seawater, washed in milli-Q water and placed in pre-weighed tin boats or combusted vials (500 °C for 4 hours) and frozen at either −20 or −70 °C. In the laboratory, specimens were oven dried (60 °C), weighed and acidified with 1% $PtCl_2$ to remove inorganic C. Stable isotope measurements ($\delta^{13}C$, $\delta^{15}N$) were made on 0.2–1.0 mg of dry weight, usually from single individuals. Samples were analyzed either on a Eurovector elemental analyzer interfaced with a continuous flow Micromass Isoprime isotope ratio mass spectrometer at Washington State University or with a Thermo Finnigan Delta XP Plus with a Costech 4010 Elemental Analyzer at the Scripps Institution of Oceanography analytical facility.

Isotope ratios are expressed as $\delta^{13}C$ or $\delta^{15}N$ in units of per mil (‰). Standards were Pee Dee Belemnite for $\delta^{13}C$ and nitrogen gas for $\delta^{15}N$ (atmospheric). Estimates of the percentage of methane-derived carbon in the macrofaunal carbon pool of each region and habitat were generated using a 2-source, single isotope mixing model as in Fry and Sherr (1984). The formula used is

$$F_m = \left(\delta_t - \delta_{POC/SOB} \right) / \left(\delta_m / \delta_{POC} \right) \tag{1}$$

where F_m is the fraction of methane-derived carbon, δ_t, $\delta_{POC/SOB}$, and δ_m refer to the $\delta^{13}C$ signatures of sample tissue, particulate organic
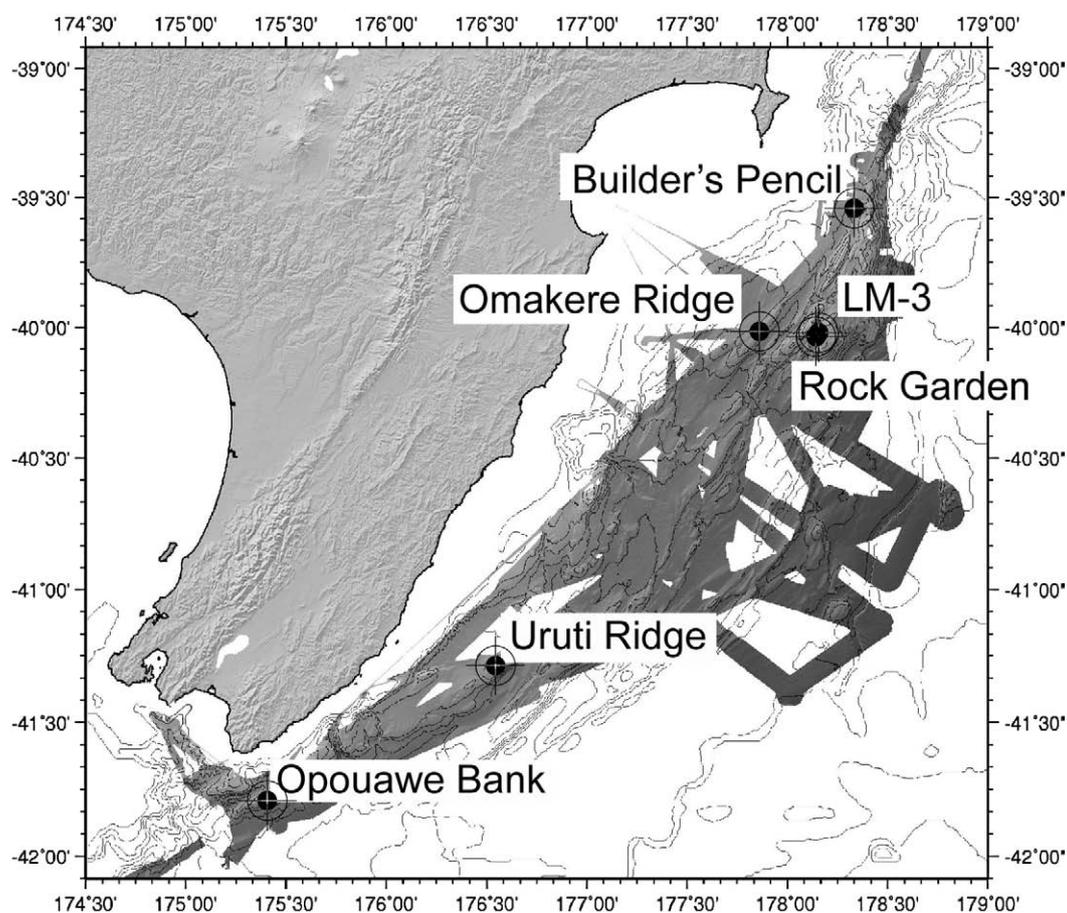

Fig. 1. Methane-seep regions sampled for symbiont-bearing megafauna and heterotrophic macrofauna. Omakere Ridge includes LM-9, Kaka, and Bear's Paw. Opouawe Bank includes North Tower, South Tower, and Takahe. For site maps see Greinert et al. (2009-this issue).

Table 2
Stable isotopic composition of known symbiont-bearing taxa at New Zealand methane seeps.

Taxa	Site	n	$\delta^{13}C$ Mean	SE	$\delta^{15}N$ Mean	SE	$\delta^{13}C$ Min	Max	$\delta^{15}N$ Min	Max
Mollusc										
Bathymodiolus	LM-3	5	−63.0	1.2	1.1	0.3	−65.8	−60.0	−0.1	2.5
Calyptogena	Builder's Pencil	1	−36.3		2.8					
	LM-9	2	−35.6		0.3		−36.5	−34.7	−0.8	1.3
Solemyidae	Takahe	1	−32.3		4.4					
	LM-9	3	−31.5	0.8	−0.1	0.6	−33.0	−30.2	−1.4	0.6
	North Tower	1	−30.7		−0.7					
Siboglinid polychaetes										
Lamellibrachia sp.	Builder's Pencil	11	−35.9	1.6	4.0	0.4	−43.1	−26.2	0.6	6.7
	LM-3	3	−28.5	2.9	2.2	0.8	−34.4	−22.5	0.8	4.7
	North Tower	4	−28.0	2.9	4.9	0.6	−33.4	−19.8	3.8	6.4
Frenulata	Bear's Paw	4	−43.7	2.0	2.6	1.5	−48.9	−40.1	−0.2	6.6
	Builder's Pencil	2	−66.6		5.6		−72.9	−60.3	5.4	5.8
	LM-9	13	−40.2	2.9	2.4	1.3	−51.8	−18.5	−4.4	14.8
	Takahe	3	−36.4	4.1	7.3	0.5	−44.6	−31.7	6.3	8.2
	Uruti Ridge	2	−43.1		6.0		−50.0	−36.3	1.1	10.9
	North Tower	4	−36.1	1.1	−4.4	1.5	−38.6	−33.5	−7.0	0.1

carbon or sulfide-oxidizing bacteria (POC/SOB) and methane, respectively. The $\delta^{13}C$ of either plankton or sulfide-oxidizing bacteria and the extremes in methane isotopic concentrations were used for $\delta_{POC/SOB}$ and δ_m, respectively, to make a robust, albeit wide ranging, estimate of MDC (see Levin and Michener, 2002 and the results for further explanation.) The $\delta^{13}C$ of methane varies both spatially and temporally (Ziebis and Haese, 2005), therefore extreme values of methane were used. This fine scale variability means measured end points, whether site-specific or not, may or may not reflect the $\delta^{13}C$ value of source methane in contact with the bacteria or archaea fixing carbon. In this instance, use of extremes reflects a realized range rather than a finite estimate based on estimated means. No trophic shift was included as this is negligible (<1‰ per trophic level) for $\delta^{13}C$ and end points for the model were chosen as the maximum values for filamentous free-living microbial sulfide oxidizers, as the isotopic concentration of non-filamentous, sulfide-oxidizing bacteria was not possible to constrain for this system.

3.2. Statistical analyses

Means were calculated for raw isotope data within each site, not pre-averaged within species. Values are given as mean ± 1 standard error unless indicated otherwise. When sampling with an epibenthic sled, species cannot be treated as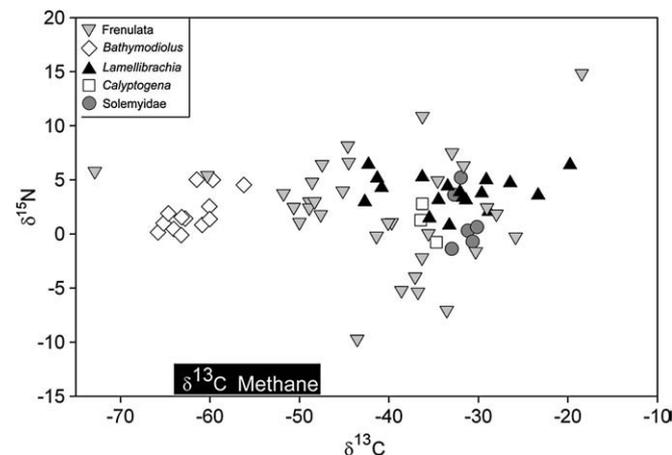 spatial replicates, as pre-averaging within species would imply. Global comparisons were completed with data averaged by species within a site, to be consistent with the literature. The $\delta^{13}C$ values of *Lamellibrachia* sp. vestimentum and trophosome were compared with a paired *t*-test to determine if the two tissues yield the same isotopic composition. Isotopic values of *Lamellibrachia* sp. were compared among sites using a one-way analysis of variance (ANOVA) using the means of all tissues sampled from within an individual. To elucidate sampling biases inherent to the gear used, the mean isotopic composition of sampling gear was compared with a one-way ANOVA with Tukey post-hoc test. All data met the underlying assumptions of the statistics employed without transformation.

4. Results

Possible invertebrate food sources exhibited a broad range of $\delta^{13}C$ and $\delta^{15}N$ values. The mean $\delta^{13}C$ value of surface water plankton was −22.2 ± 0.5‰ and the $\delta^{15}N$ value was 4.5 ± 0.5‰. The $\delta^{13}C$ of

Fig. 2. Stable isotopic composition of symbiont-bearing invertebrate fauna from New Zealand methane seeps. Range of methane isotopic concentration is from Sommer et al. (2009-this issue).
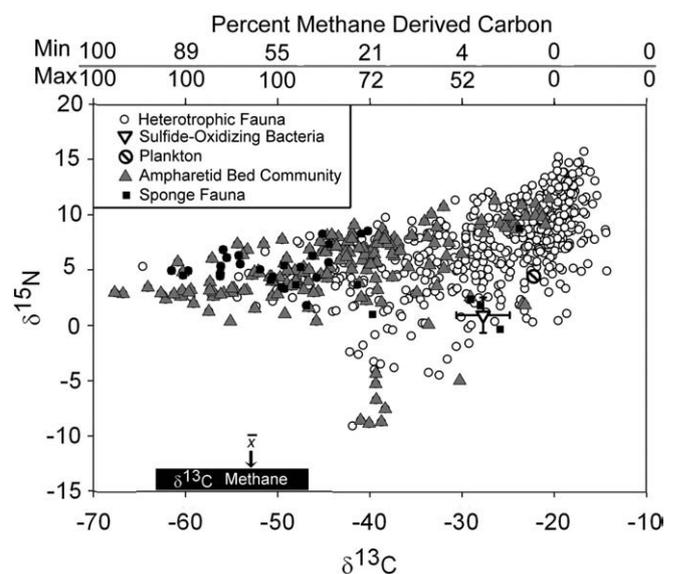
Fig. 3. Stable isotopic composition of heterotrophic invertebrate fauna, filamentous, sulfide-oxidizing bacteria, and plankton samples from New Zealand methane seeps. The range of estimated percent methane-derived carbon in animal tissues is given as a top x-axis (see text for model estimation methods). Range and mean of methane isotopic concentration are from Sommer et al. (2009-this issue).

Table 3

Mean, standard error, and range of stable isotope values of heterotrophic fauna collected from each methane-seep station on the New Zealand continental margin.

Region	Sites	Station	N	# of sp.	$\delta^{13}C$ Mean	SE	Min	Max	$\delta^{15}N$ Mean	SE	Min	Max	Seep fauna present	$\delta^{13}C$ values of most ^{13}C-depleted organisms present
Builder's	21		3	3	−20.1	0.8	−21.3	−18.6	9.2	1.7	6.2	12.0		Ophiuroidea (−21)
Pencil	34		19	16	−21.1	0.7	−26.6	−14.4	8.2	0.5	3.4	11.8		Glyceridae (−27), Amphipoda (−25.4), Ophiuroidea (−23)
	36		3	3	−23.4	0.7	−24.8	−22.5	9.5	1.0	8.3	11.5		Isopoda (−25), Cirratulidae (−23), Cumacea (−23)
	38		18	13	−23.3	1.2	−34.0	−18.4	9.0	1.0	0.4	14.8	L	Gastropoda (−34), Nematoda (−27), Isopoda (−25)
	32		5	5	−21.3	2.0	−29.0	−18.0	10.1	1.3	6.7	13.9	P, L	Gastropoda (−29), Isopoda (−22)
	18		20	15	−21.4	1.2	−44.0	−16.8	9.8	0.6	5.1	14.9		Sipuncula (−44), Cirratulidae (−23), Syllidae (−23)
	23		13	11	−29.3	2.2	−46.5	−14.4	7.0	0.7	3.1	11.4	B, F, SH	Oligochaeta (−47), Isopoda (−35), Amphipoda (−35)
	30		15	12	−19.6	0.4	−22.4	−17.3	9.7	0.5	6.8	12.7		Amphipoda (−22), scale worm (−22), Glyceridae (−21)
	22		9	8	−18.5	0.6	−21.4	−16.6	10.4	0.9	7.4	14.2		Amphipoda (−21), Phyllodocidae (−20), Onuphidae (−20)
	Mean				−22.2	0.5	−30.0	−17.5	9.0	0.3	5.3	13.0		
LM-3	238		28	15	−49.5	1.5	−61.5	−23.8	5.5	0.3	1.9	8.9	S, B	Scale worm (−62), Terebellidae (−60), Tanaidacea (−56)
	216		20	10	**−52.7**	**1.8**	**−62.1**	**−31.4**	**3.4**	**0.4**	**0.5**	**7.6**		Cumacea (−62), Ampharetidae (−60), Dorvilleidae (−57),
	Mean				−50.8	1.1	−61.8	−27.6	4.7	0.3	1.2	8.2		
Rock	Knoll	10	2	1	−20.5	3.3	−23.8	−17.2	9.8	3.2	6.6	13.1		Pholoidae (−24)
Garden		6	26	15	−19.7	0.4	−27.1	−16.8	10.0	0.6	4.0	15.8		Syllidae (−27), Glyceridae (−22), Amphipoda (−21)
		11	2	2	−19.6	0.0	−19.6	−19.6	10.3	0.9	9.4	11.2		Syllidae (−20), Lumbrineridae (−20)
		7	3	3	−20.8	0.8	−22.2	−19.3	9.7	0.7	8.4	10.8		Glyceridae (−22), scale worm (−21)
		12	7	6	−19.3	0.4	−20.8	−18.0	11.8	0.8	9.1	14.9		Scale worm (−21), Glyceridae (−20), Onuphidae (−20)
		Mean			−19.8	0.3	−22.7	−18.2	10.3	0.4	7.5	13.1		
Omakere	LM-9	45	5	5	−19.7	0.8	−21.7	−17.6	8.4	0.6	6.8	10.4	B, S, SP	Polychaeta (−22), Amphipoda (−22), Maxillopoda (−19)
Ridge		50	1	1	−19.7		−19.7	−19.7	10.3		10.3	10.3	F, SP	Ophiuroidea (−20)
		57	17	16	−31.0	2.1	−52.3	−14.3	5.7	0.8	−0.2	12.1		Cumacea (−52), Maldanidae (−43), Nematoda (−38)
		46	12	12	−25.7	1.2	−32.4	−20.3	4.4	0.8	0.7	10.0	F	Aplacophora (−32), Bivalvia (−31), Spionidae (−30)
		49	3	3	−23.8	1.6	−25.7	−20.6	4.7	1.9	2.4	8.5	F	Bivalvia (−26), Amphipoda (−25), Gastropoda (−21)
		58	22	15	−21.4	0.9	−37.4	−17.0	8.1	0.8	−3.7	12.8	B, F	Aplacophora (−37), Tanaidacea (−29), Paraonidae (−23)
		59	15	14	−20.0	0.4	−22.4	−16.6	8.3	1.0	0.4	12.7		Oligochaeta (−22), scale worm (−22), Terrebellidae (−22)
		60	19	19	−23.6	0.9	−35.5	−19.5	8.1	0.8	−0.9	15.3		Cumacea (−36), Cirratulidae (−29), Isopoda (−27)
		61	7	6	−19.5	1.3	−27.4	−17.3	10.5	1.6	1.3	13.9		Ophiuroidea (−27), Lumbrineridae (−19), Polychaeta (−19)
		Mean			−23.6	0.6	−30.5	−18.1	7.4	0.4	1.9	11.8		
	Kaka	261	25	19	**−36.7**	**2.0**	**−52.5**	**−21.3**	**7.4**	**0.5**	**2.9**	**10.9**		Ampharetidae (−52), Cumacea (−50), Orbiniidae (−49)
		232	14	10	**−33.0**	**1.8**	**−46.7**	**−23.3**	**7.3**	**0.5**	**3.7**	**9.9**	B	Cumacea (−47), Amphipoda (−42), Ampharetidae (−37)
		242	31	22	**−29.5**	**0.9**	**−41.9**	**−18.0**	**7.0**	**0.8**	**−9.0**	**13.9**		Spionidae (−42), Paraonidae (−37), Bivalvia (−37)
		Mean			−32.7	1.0	−47.0	−20.9	7.2	0.4	−0.8	11.6		
	Bear's Paw	197	7	3	**−44.9**	**3.5**	**−54.2**	**−26.1**	**6.6**	**0.7**	**4.5**	**9.7**		Ampharetidae (−54), Orbiniidae (−51), Dorvilleidae (−26)
		198	42	15	**−46.7**	**1.7**	**−64.0**	**−24.3**	**5.4**	**0.4**	**1.7**	**9.6**		Cumacea (−64), Orbiniidae (−61), Amphipoda (−61)
		186	10	9	**−35.4**	**1.7**	**−43.8**	**−27.9**	**6.0**	**0.8**	**2.5**	**9.8**	F	Isopoda (−44), Amphipoda (−42), Amphinomidae (−40)
		Mean			−44.6	1.4	−54.0	−26.1	5.7	0.3	2.9	9.7		
Uruti	East	70	17	12	−22.3	0.9	−28.7	−16.7	10.0	0.6	4.1	13.8		Lumbrineridae (−29), Chrysopetelidae (−29), scale worm (−27)
Ridge	LM-10	68	6	6	−20.4	0.5	−22.5	−19.3	11.2	1.4	5.3	14.6	F	Lumbrineridae (−23), Nematoda (−21), Maldanidae (−20)
	Hihi	67	6	14	−18.7	0.5	−22.0	−15.4	10.4	0.8	3.8	14.0		Ophiuroidea (−22), Paraonidae (−21), Glyceridae (−21)
		Mean			−20.6	0.5	−24.4	−17.1	10.4	0.4	4.4	14.1		
Opouawe	North	122	14	10	−20.1	0.7	−25.4	−15.8	10.1	0.5	4.4	11.4		Maldanidae (−25), Lumbrineridae (−25), Trichobranchidae (−22)
Bank	Tower	83	14	11	−30.4	2.2	−40.8	−19.9	7.5	1.1	−3.4	12.7		Nereididae (−41), Amphipoda (−41), Syllidae (−39)
		86	10	8	−24.9	1.4	−34.0	−19.3	7.3	1.3	0.1	12.4		Amphipoda (−34), Dorvilleidae (−29), Oligochaeta (−28)
		84	9	9	−19.4	0.6	−22.4	−16.3	7.7	0.9	1.2	10.8		Amphipoda (−22), Tanaidacea (−21), Spionidae (−20)
		79	18	15	−32.1	2.8	−50.1	−17.2	7.1	1.2	−1.8	23.2	F, S, L	Hesionidae (−50), Maldanidae (−44), Cirratulidae (−44)
		290	12	6	**−48.7**	**3.1**	**−67.7**	**−35.4**	**4.7**	**0.5**	**2.9**	**8.2**		Ampharetidae (−68), Spionidae (−45), Amphipoda (−40)
		298	32	23	**−32.7**	**1.2**	**−47.8**	**−21.2**	**6.2**	**0.5**	**−3.9**	**10.6**		Amphinomidae (−48), Polychaeta (−43), Capitellidae (−41)
		112/113	20	15	−19.8	0.5	−25.8	−15.6	8.8	0.7	3.8	14.0	F, SM	*Bathysiphon* (−26), Ophiuroidea (−23), Thyasiridae (−22)
		277	3	3	**−41.9**	**11.8**	**−64.6**	**−25.0**	**5.9**	**0.5**	**5.3**	**6.9**		Capitellidae (−65), Spionidae (−36), Dorvilleidae (−25)
		Mean			−29.3	0.9	−42.1	−20.6	7.0	0.3	0.9	12.2		
	South	81	54	23	**−34.7**	**1.2**	**−59.4**	**−19.6**	**4.4**	**0.5**	**−3.2**	**12.0**		Ampharetidae (−59), Lumbrineridae (−49), Spionidae (−47)
	Tower	116	11	11	−27.2	1.1	−36.2	−23.3	8.0	0.7	4.8	11.8	B	Amphipoda (−36), Bivalvia (−29), Maldaniae (−27)
		118	16	16	−23.8	0.7	−30.4	−20.9	6.9	0.6	1.8	10.6	B	Glyceridae (−30), Amphinomidae (−28), Isopoda (−27)
		82	5	5	−17.9	0.5	−19.7	−16.5	8.5	1.1	5.0	11.7		Amphipoda (−20), Cumacea (−18), Opheliidae (−18)
		123	15	14	−21.9	0.9	−33.6	−18.4	8.4	1.2	−4.2	12.6		Tanaidacea (−34), Protista (−23), Isopoda (−23)
		Mean			−25.1	0.4	−35.9	−19.8	7.6	0.2	0.8	11.7		
	Takahe	309	57	23	**−38.4**	**1.1**	**−53.7**	**−20.6**	**5.4**	**0.7**	**−8.8**	**11.5**		Ampharetidae (−54), Spionidae (−47), Capitellidae (−46)

N is the number of samples analyzed. # of species indicates the number of putative species analyzed from each station. Seep fauna include the chemoautotrophic taxa collected (L = *Lamellibrachia* sp., B = sheath-forming bacteria, F = frenulata, S = sponge, SH = *Calyptogena* spp. shell hash, SP = solemyid periostracum, SM = solemyid). Most ^{13}C-depleted values and ID indicate those taxa with the lowest $\delta^{13}C$ values (given in parentheses). Bold values are those from ampharetid beds. Underlined values are fauna collected from a *Pseudosuberites* sp. community.

methane ranged from −46.7 to −63.2‰ with a mean of −52.9‰ (Sommer et al., 2009-this issue). Filamentous, sulfide-oxidizing bacteria had a $\delta^{13}C$ of −33.6 to −22.0‰ (mean = −27.7 ± 2.9, $n = 4$) and a $\delta^{15}N$ of −4.7 to 2.5‰ (mean = 1.0 ± 1.6). Two sets of mixing model end points were used to account for variable inputs. Maximum estimates of methane-derived carbon (MDC) were based on a $\delta_{POC/SOB}$ of −22.2‰ (from the phytoplankton sample) and the least negative value of methane $\delta_m = -46.7$‰. Minimum MDC estimates used a $\delta_{POC/SOB}$ value of −33.6‰ (sulfide-oxidizing bacteria minimum) and the most depleted value of methane ($\delta_m = -63.2$‰) from Sommer et al. (2009-this issue). Use of all other possible mixing model end points yields intermediate estimates of MDC.

Table 4
Mean station carbon isotopic signatures for macrofauna obtained by each type of sampling gear used.

Region	Site	TV-MUC		MUC		TV-Grab		Grab		Sled		Chambers
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean
All		−40.7	2.8	−23.5	1.1	−49.5		−21.6	1.2	−22.5	1.1	−37.3
Builder's Pencil				−23.4				−23.0	3.3	−21.1	0.6	
LM-3		−52.7				−49.5						
Rock Garden	Knoll							−19.6		−20.1	0.3	
Omakere Ridge	LM-9			−26.8	2.1			−21.1	0.9	−19.7	0.0	
	Kaka	−33.1	2.1									
	Bear's Paw	−42.3	3.5									
Uruti Ridge										−20.5	1.0	
Opouawe Bank	North Tower	−48.7		−21.4	0.7					−28.2	1.5	−37.3
	South Tower			−24.6	0.5					−34.7	1.2	
	Takahe	−38.4										

TV-MUC = video-guided multicore; MUC = blind multicore; TV-Grab = video-guided hydraulic grab; Grab = blind vanVeen grab; Sled = epibenthic sled; Chambers = respiration chambers from benthic lander deployments. SE is not given if <3 samples were present.

4.1. Symbiont-bearing taxa

A total of 5 taxonomic groups of symbiont-bearing fauna, represented by 60 individuals from 7 sites were analyzed (Table 2, Fig. 2). Isotopic signatures indicated the use of two distinct metabolic pathways. The bathymodiolin mussel had a δ^{13}C isotopic signature (δ^{13}C of −60‰ to −65.8‰) clearly indicating methanotrophic endosymbiosis, whereas Vesicomyidae and Solemyidae (δ^{13}C of −36.5 to −30.2‰) almost certainly use the products of symbiont sulfide oxidation to fuel their biomass. A minimum of three frenulate species (Polychaeta, Siboglinidae) were present. These species exhibited a wide range of carbon isotopic signatures among individuals, suggesting the presence of methanotrophic symbionts (δ^{13}C = −72.9‰), sulfide-oxidizing symbionts (δ^{13}C = −33.5‰) and possibly uptake of dissolved inorganic carbon (DIC) from plankton sources (δ^{13}C = −18.5‰). *Lamellibrachia* sp. had δ^{13}C values ranging from −19.8 to −43.1‰, with no significant difference between the δ^{13}C of their trophosome and vestimentum (paired *t*-test; $t = 0.71$, df = 13, $p = 0.49$) nor among the sites where they were collected (1-way ANOVA: $F_{2,15} = 1.77$, $p = 0.20$).

4.2. Heterotrophic fauna

A total of 751 heterotrophic individuals were examined from 10 different seep locations. A complete list of the species collected and their respective isotopic values are available upon request from the first author. This assemblage exhibited a 53.4‰ range in δ^{13}C (from −67.7 to −14.3‰) and a 24.8‰ range in δ^{15}N (from −9.0 to +15.8‰; Fig. 3, Table 3), reflecting varying nutritional sources across taxa within a site and across sites within a taxon. Incorporation of MDC was estimated in sipunculids, arthropods (cumaceans, tanaids, and amphipods), molluscs (gastropods) and annelids (≥6 families), all had δ^{13}C values of <−40‰ indicating that 21% to 73% of their carbon was derived from methane.

A comparison of average isotopic values for entire samples, revealed that gear types were not equally effective at collecting seep fauna (Table 4). Assemblages collected with the video-guided multicore, which targeted ampharetid-bed communities, had a more negative carbon isotopic signature than epibenthic sleds, blind multicores, or blind grabs, which were not different from each other ($F_{3,42} = 30.62$, $p \ll 0.01$, Tukey post-hoc test). Insufficient replication did not allow inclusion of video-guided grabs and benthic landers in the analysis.

The Uruti Ridge, Rock Garden, and Builder's Pencil sites, sampled largely by epibenthic sled and blind grabs (Table 1), were covered by extensive carbonate precipitates (rocks, boulders and stones; Campbell et al., 2009-this issue; Greinert et al., 2009-this issue; Liebetrau et al., 2009-this issue; Naudts et al., 2009-this issue). Despite the presence of chemosynthetic, symbiont-bearing megafauna (*Lamellibrachia* sp., *Calyptogena* spp. and frenulates), the majority of heterotrophic taxa from these sites had isotopic signatures similar to photosynthesis-based organic matter. Only 5% of the individuals from Rock Garden, 21% at Uruti Ridge and 24% at Builder's Pencil had δ^{13}C signatures less than our plankton value of −22.2‰ (Fig. 4, Table 3), suggesting primarily photosynthetic food source utilization by these assemblages. At Builder's Pencil, half of the taxa that consumed chemosynthesis-based organic matter ($\delta^{13}C_{sample} < \delta^{13}C_{plankton}$) were from two samples collected by the blind grab. With the exception of one sipunculan, all of the fauna at Uruti Ridge and Builder's Pencil with evidence of MDC (δ^{13}C < −33.6‰) were collected by cores and grabs. These included two species of amphipod, one gastropod, an isopod, and an oligochaete (Table 3). No specimens collected at Rock Garden appeared to use MDC (i.e. had a minimal estimate of MDC>0%).

Macrobenthos collected from LM-3 (Rock Garden), Bear's Paw (Omakere Ridge) and Takahe (Opouawe Bank) sites exhibited the most negative carbon isotopic signatures (Fig. 4) with 96%, 83% and 80%, respectively, of all individuals analyzed using MDC. A diverse group of annelids including ampharetid and orbiniid polychaetes, as well as amphipods and cumaceans, had δ^{13}C values <−50‰. The ^{13}C-depleted average and δ^{15}N values (<6‰) suggest the use of chemosynthetic food sources. However, three or fewer gear deployments were made at these sites (Table 2), and they were selective in targeting ampharetid beds (with the TV-guided multicore) or the *Pseudosuberites* sp. sponge fauna (with the TV-guided grab).
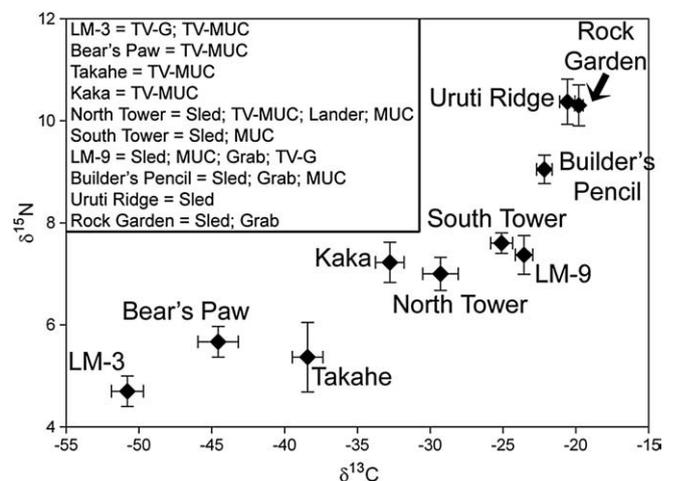

Fig. 4. Mean stable isotopic composition of heterotrophic invertebrate fauna at each site sampled on the New Zealand continental margin. Error bars are ±SE. Legend gives sampling equipment used at each location using the abbreviations: Sled = epibenthic sled; MUC = blind multicorer; Grab = vanVeen grab; TV-G = video-guided hydraulic grab; TV-MUC = video-guided multicorer; Lander = Bigo and Flufo benthic landers. For more details see Materials and methods and Table 1.

The remaining 4 study sites (LM-9 and Kaka both at Omakere Ridge, North and South Tower both at Opouawe) all had comparable mean $\delta^{15}N$ values (7.2 to 7.6‰) but a 9‰ range in mean $\delta^{13}C$ (-23.6 to -32.7‰). Site-averaged MDC estimates for heterotrophic macrobenthos ranged from limited (LM-9, 0–4% MDC) to higher (Rock Garden, 7–60%) values (Table 5). The most negative heterotroph $\delta^{13}C$ values in the study were from a cumacean at LM-3 (-62.1‰) and an ampharetid at North Tower (-67.7‰). Yet coexisting with these taxa were species with individual $\delta^{13}C$ values as high as -14.3 to -18‰. The most negative $\delta^{15}N$ value for macrobenthos at each of these 5 (LM-3, LM-9, North and South Tower, and Kaka) sites ranged from -9 to 0.5‰, indicating considerable chemosynthesis-derived nutrition. These taxa included a new genus of spionid polychaete ($\delta^{15}N = -5.4 \pm 0.8$‰, $n = 9$), a tanaid ($\delta^{15}N = -4.2$‰, $n = 1$), an aplacophoran ($\delta^{15}N = -3.4$‰, $n = 1$), a second species of spionid ($\delta^{15}N = -3.4$‰, $n = 1$), two species of dorvilleid polychaetes ($\delta^{15}N = -1.0 \pm 1$‰, $n = 3$), a nematode ($\delta^{15}N = -1.8$‰, $n = 1$), and an arborescent foraminiferan ($\delta^{15}N = 0.5$‰, $n = 1$).

There were two multi-species invertebrate assemblages with high levels of MDC: taxa living within the sponge *Pseudosuberites* sp. and the invertebrate assemblage inhabiting ampharetid beds. ^{13}C-depleted *Pseudosuberites* sp. were collected from LM-3 (Station 238; $\delta^{13}C = -56.6$‰, $n = 1$), LM-9 (Station 45; $\delta^{13}C = -57.1$‰, $n = 1$), and North Tower (Station 79; $\delta^{13}C = -50$‰, $n = 1$). At LM-3 the associated fauna, mostly living within the sponge, consisted of 15 species including capitellid, cirratulid, terebellid, amphinomid, syllid, glycerid, and scale worm polychaetes, and tanaid, isopod, and pycnogonid arthropods. The commensal invertebrates had a mean $\delta^{13}C$ of -49.5 ± 1.5‰ indicating 38 to 100% MDC. However, a sponge-associated amphinomid polychaete had a $\delta^{13}C$ signature of -23.8‰; this was the only species with a $\delta^{13}C$ higher than -40‰. At North Tower the sponge-commensal fauna had $\delta^{13}C$ values from -23.7 to -47.0‰. Here the assemblage included ^{13}C-depleted ($\delta^{13}C < -40$‰) maldanid polychaetes and limpets, intermediate ($-40 < \delta^{13}C < -30$‰) sipunculans and a holothurian in the family Psolidae and less ^{13}C-depleted ($\delta^{13}C > -25$‰) lumbrinerid and paraonid polychaetes.

Ampharetid beds contained dense assemblages of two species of Ampharetidae and a host of other invertebrate taxa. These assemblages had $\delta^{13}C$ values indicating high use of chemosynthesis-derived nutrition. At Bear's Paw, Kaka, Takahe, and LM-3, the ampharetid-bed macrofauna had a mean $\delta^{13}C$ value of -39.4 ± 0.7‰. The ampharetid species themselves had a $\delta^{13}C$ of -50.3 ± 1.1‰ or 56–100% MDC and $\delta^{15}N = 4.2 \pm 0.2$‰ ($n = 37$). Associated fauna included cumaceans (mean $\delta^{13}C$ of -52.1 ± 3.8‰, $\delta^{15}N = 3.7 \pm 0.3$‰, $n = 10$), eight species of amphipod (mean $\delta^{13}C$ of -38.7 ± 1.2‰, $\delta^{15}N = 6.8 \pm 0.3$‰, $n = 34$), an orbiniid polychaete ($\delta^{13}C = -49.1 \pm 2.5$‰, $\delta^{15}N = 7.2 \pm 0.3$‰, $n = 10$), at least seven species of dorvilleid polychaete ($\delta^{13}C = -29.7 \pm 1.3$‰, $\delta^{15}N = 6.9 \pm 0.5$‰, $n = 37$). The aforementioned species of spionid ($\delta^{13}C = -39.7 \pm 0.4$‰, $\delta^{15}N = -7.3 \pm 0.6$‰, $n = 8$) and another spionid species ($\delta^{13}C = -38.4 \pm 2.7$‰, $\delta^{15}N = 7.2 \pm 0.7$‰, $n = 10$) were also collected from this habitat.

5. Discussion

5.1. Symbiont-bearing taxa

The fauna of New Zealand methane seeps exhibit a broad range of isotope signatures indicative of multiple trophic pathways, but with a significant role for methane. Bathymodiolin mussels are known to have symbionts with multiple metabolic pathways and their use of methane has been reported frequently (Childress et al., 1986; Brooks et al., 1987; Cary et al., 1988; Van Dover et al., 2003). Sulfide oxidation is a well established source of energy for solemyid and vesicomyid bivalves (Fisher, 1990). *Lamellibrachia* spp. are reported to only have thiotrophic (sulfide-oxidizing) symbionts (Brooks et al., 1987; Fisher, 1990). The $\delta^{13}C$ values (-19.8 to -43.1‰) of the New Zealand *Lamellibrachia* sp. population, however, suggest incorporation of methane-derived carbon into their tissues. The carbon isotopic compositions of *Lamellibrachia* sp. from Builder's Pencil are equivalent to the most negative $\delta^{13}C$ values of *Lamellibrachia* spp. reported in the literature ($\delta^{13}C = -43.7$‰; Brooks et al., 1987). *Lamellibrachia* spp. commonly has a very heavy $\delta^{13}C$ composition compared to other

Table 5
Estimates of percent methane-derived carbon (MDC) in tissues of heterotrophic invertebrates in methane-seep sediments.

Geographic location	Water depth (m)	Habitat	Percent MDC			References
			Minimum	Maximum	Average	
Kodiak Seep, Gulf of Alaska	4413–4444	Frenulate field	32 ± 8	51 ± 6	44 ± 9	1, 2
	4413–35	*Calyptogena* beds	12 ± 8	40 ± 5	39 ± 6	1, 2
Unimak, Aleutian Islands	3283	Frenulate field			11 ± 6	2
	3267	*Calyptogena* beds			21 ± 4	2
Hydrate Ridge, OR	590	Microbial mats	20 ± 5	44 ± 4		1
	590	*Calyptogena* beds	0	27 ± 3		1
Eel River Seeps, CA	520	Microbial mats	0	5		1
	520	*Calyptogena* beds	0	22 ± 5		1
Florida Escarpment, FL	3290	Microbial mats			55 ± 8	2
	3234	Frenulate field			223 ± 7	2
	3290	*Calyptogena* beds			$31 + 6$	2
Builder's Pencil	780–817	Carbonates, shell hash	0	4 ± 3		3
LM-3	908	Sponge assemblage	53	100		3
	662	Ampharetid bed	51	100		3
Rock Garden	730–766	Mixed	0	0		3
Omakere Ridge — LM-9	1140–1150	Mixed	0	4 ± 3		3
Omakere Ridge — Kaka	1165–1172	Ampharetid beds	6 ± 6	49 ± 11		3
Omakere Ridge — Bear's Paw	803–1201	Ampharetid beds	18 ± 7	77 ± 12		3
Uruti Ridge	716–740	Carbonates, frenulate fields	0	1 ± 1		3
Opouawe Bank — North Tower	1036–1061	Ampharetid beds	22 ± 11	73 ± 15		3
		Mixed	0	15 ± 7		3
Opouawe Bank — South Tower	1040–1059	Mixed	0	14 ± 8		3
Opouawe Bank — Takahe	1056	Ampharetid beds	8	57		3

Data are averages across species for upper and lower estimates of MDC within a geographic location and habitat. Non-New Zealand sites indicate sediment-dwelling macrobenthos. MDC estimates are obtained from a 2-source mixing model (Fry and Sherr, 1984) using the $\delta^{13}C$ of methane and either surface plankton (to obtain a maximum or average estimate) or sulfide-oxidizing bacteria (to obtain a minimum estimate). "Mixed" are samples from more than one type of habitat and all samples collected via epibenthic sled.
1 = Levin and Michener (2002).
2 = Levin and Mendoza (2007).
3 = This study.

thiotrophic seep organisms. For example, $\delta^{13}C$ values are −20.1‰, −22.8‰ and −23.6 to −26.6‰ respectively for *Lamellibrachia luymesi* in the Gulf of Mexico (MacAvoy et al., 2005), for *Lamellibrachia* sp. at a Chilean seep (Sellanes et al., 2008), and for *Lamellibrachia* sp. at a Mediterranean mud volcano (Olu-Le Roy et al., 2004).

Many factors influence the $\delta^{13}C$ of vestimentiferans, including DIC source and size, growth rate and growth form of the individual (Fisher et al., 1990; MacAvoy et al., 2005). The DIC pool used may either be from the overlying water column, as it is in hydrothermal vent vestimentiferans (Fisher, 1990), or potentially sediment pore water DIC taken up through their tube from below the sediment surface (MacDonald et al., 1989; Fisher, 1990; Kennicutt et al., 1992; Dattagupta et al., 2006). A DIC pool that contains relatively high proportions of C recycled from methane would also impart a ^{13}C-depleted signature to faunal symbionts that are fixing carbon from this pool; $\Delta^{14}C$ analyses support uptake of methane-derived DIC by thiotrophic species (Brooks et al., 1987; Paull et al., 1989, 1992). Due to mass balance arguments, DIC limitation to the trophosome of a vestimentiferan results in reduced carbon fractionation (i.e. the realized enzymatic discrimination against ^{13}C) between the source DIC pool and its tissues. This limitation can result from a low respiratory surface to body size ratio, which decreases with increasing size/age (Fisher et al., 1990; Kennicutt et al., 1992). In addition, rapid growth or a high density of vestimentiferans can also impact availability of DIC and resultant carbon isotopic signatures (Kennicutt et al., 1992). These latter factors can help to explain increased $\delta^{13}C$ values in *Lamellibrachia* sp. The uncharacteristically ^{13}C-depleted $\delta^{13}C$ values of some of the *Lamellibrachia* sp. individuals found in New Zealand potentially mirror a DIC pool impacted by methane. The $\delta^{13}C$ signatures from foraminiferan tests and vesicomyid shells from New Zealand, also reflect DIC pools heavily influenced by methane oxidation (Martin et al., 2009-this issue).

The presence of discreet groups of frenulates with widely differing isotope signatures (Table 2) suggests that multiple methanotrophic and thiotrophic species are present off New Zealand. Frenulates with $\delta^{13}C$ values indicative of methanotrophy could result from symbionts which are methanotrophic or symbionts which fix carbon from a methane influenced or derived DIC source. Although vestimentiferans have never been reported to have methanotrophic symbionts, such symbionts have been unequivocally identified in one frenulate species, *Siboglinum poseidoni* (Schmaljohann and Flügel, 1987; Schmaljohann et al., 1990). Methanotrophic symbionts have been suggested in a variety of other frenulate species, yet evidence has either been inconclusive or has rejected this form of symbiosis (see discussion in Dando et al., 2008). *Oligobrachia haakonmobiensis*, a species present at the Håkon Mosby methane seep, is an example of a species with carbon isotopic values indicative of methane oxidization ($\delta^{13}C$ value of −66‰), but its symbionts did not possess characteristic genes indicative of methanotrophy (Lösekann et al., 2007).

The $\delta^{13}C$ values of siboglinid taxa identified Builder's Pencil as a location with a geochemical environment not present or not sampled at the other seep sites. Although the $\delta^{13}C$ values of the sub-population of *Lamellibrachia* sp. present at Builder's Pencil were not statistically different from the other sites, four individuals had $\delta^{13}C$ values 7.3‰ less than those at LM-3 and North Tower. In addition, a highly ^{13}C-depleted frenulate ($\delta^{13}C = −72.9‰$) was sampled at Builder's Pencil from a separate location. Both of these values are indicative of a DIC pool at Builder's Pencil which is impacted by methane oxidation and/ or high methane release.

5.2. Heterotrophic fauna

The macrofauna collected from the Bear's Paw ampharetid beds ($−44.6 ± 1.4‰$) was the most ^{13}C-depleted assemblage among the New Zealand seeps, and also among the most depleted globally. The New Zealand mean $\delta^{13}C$ values are comparable to those of macro-

benthos in microbial mats at the Florida Escarpment (−42.8‰; Levin and Mendoza, 2007) and Hydrate Ridge, Oregon (−43.8‰), in frenulate fields and clam beds of Kodiak, Alaska (−46.4‰ and −40.9‰, respectively; Levin and Michener, 2002), in mussel beds on the Blake Ridge (−40 to −50‰; Van Dover et al., 2003), and in a frenulate field/bacterial mat in the North Sea (a range of −17 to −44.9‰; Gebruk et al., 2003; Table 5). The ampharetid-bed fauna is more depleted in ^{13}C than macrofauna in vesicomyid clam beds off California (−25.1‰), Oregon (−33.4‰; Levin and Michener, 2002) and Unimak Island, Alaska (−26.5‰; Levin and Mendoza, 2007). These negative $\delta^{13}C$ values are likely the result of periodically high methane release (Linke et al., 2009-this issue; Naudts et al., 2009-this issue), high bottom-water methane concentration (Faure et al., 2009-this issue; Law et al., 2009-this issue) potentially impacting the DIC pool, and from consumption of methanotrophic-microbial biomass, which can have a −30‰ fractionation factor from methane (Summons et al., 1994).

The average $\delta^{15}N$ signatures for macrofauna at 7 of the 10 New Zealand sites studied fell between 4.7‰ and 7.6‰ (Fig. 4). These $\delta^{15}N$ values are consistent with those observed for macrofauna with comparable $\delta^{13}C$ signatures from other Pacific seep sites (Levin et al., 2003), but are 5–10‰ heavier than those observed at the Florida Escarpment (Levin and Mendoza, 2007) and exhibit a smaller range than those present in the Sea of Okhotsk (1.9–8.4‰; Sahling et al., 2003).

Although the mixing model presented here is used to identify methane input into the food web, the role of nitrogen stable isotopic values can also elucidate the contribution of chemosynthetic production to heterotrophic food webs (Van Dover et al., 2003). Twenty-two individuals collected in this study had $\delta^{15}N$ between 0 and −9.0‰. All of their $\delta^{13}C$ signatures were between −29 and −41.8‰, thus these taxa did not reflect the highest MDC. Fourteen of these individuals (~2/3) belong to an undescribed spionid polychaete species that lives deep in seep sediments and was collected from Kaka, Takahe, and North and South Tower. The remainder included 2 dorvilleid polychaetes, 2 nematodes, 1 amphipod, 1 tanaid and 1 cumacean, and an aplacophoran. All of these must be consuming chemosynthetic microbial producers or have microbial symbionts themselves. Negative $\delta^{15}N$ values in symbiont-bearing taxa have been hypothesized to be a function of N_2 fixation (Brooks et al., 1987; Fisher, 1990) and nitrate and ammonium assimilation, with experimental evidence supporting the latter two in both sulfide-oxidizing and methane-oxidizing fauna (Lee and Childress, 1994).

We hypothesize that the sponge *Pseudosuberites* sp. facilitates transfer of methane released from fissures in carbonate rocks into a metazoan food web while mitigating its release into the overlying water column. Studies of estimated MDC assimilation by metazoans have largely been limited to soft-sediment habitats (e.g. Levin and Michener, 2002; Levin et al., 2003; Levin and Mendoza, 2007). In the present study, areas with abundant hard substrate lacked heterotrophic taxa with a high estimated proportion of MDC. The commensal sponge fauna was an important exception. At present the only known methanotrophic sponges are members of the family Cladorhyzidae (Vacelet et al., 1995). These can form large bushes, 1.5–2 m in diameter and 0.4 m high, as observed at the Barbados accretionary prism (Olu et al., 1997). No commensal communities were reported from that site. As cladorhizids lack cavities and many have spicules adapted to catch zooplankton, their other food source (Vacelet et al., 1995), there may be a limited number of taxa that can live within its interstices. *Pseudosuberites* sp. possesses defined structures such as dendritic crevices suitable for commensal living, and is not known to be a carnivore. This sponge may play a novel role as an ecosystem engineer providing habitat and energy via its tissues or symbionts. This sponge community was present at three of the sites (LM-3, LM-9, and North Tower) and is further discussed in Baco et al. (2009-this issue). The function of encrusting sponges as a hard-substrate methane sink,

equivalent to the sediment filter in soft-sediment habitats (Treude et al., 2003; Sommer et al., 2006), is a fertile area for research. Sponges identified to use chemoautrophic food sources have also been observed in the Sea of Okhotsk (Sahling et al., 2003). In addition the interplay between commensal microbial communities within these sponges may add to the burgeoning field of microbial-sponge interactions (Taylor et al., 2007).

Mean $\delta^{13}C$ values of heterotrophic taxa may have been biased by the sampling method employed. Seep fauna (i.e. siboglinid polychaetes, symbiont-bearing clams, and sponges with $\delta^{13}C$ values of $< -50‰$) were found at all sampling sites (Baco et al., 2009-this issue), confirming areas of active seeping at each site. However, the absence of megafauna with chemosynthetic symbionts did not preclude the use of chemosynthetic nutritional sources by consumers. The sampling techniques ranged from epibenthic sleds that integrate large swaths of epifauna and minimal infaunal species to video-guided multicores that selectively sampled infaunal ampharetid-bed habitats. Large carbonate areas and shell hash, which were present at Builder's Pencil, could only be sampled by epibenthic sleds. This sampling method recovered few heterotrophic species which consume methane, with the exception of one sipunculid, a taxon whose high volume of sediment ingested makes it especially sensitive to seep input (Van Dover et al., 2003). At Builder's Pencil, the sampling that was done with the blind grab yielded the greatest abundance of heterotrophs with chemosynthesis-based diets.

Directed sampling with either the video-guided multicore or grab resulted in an average MDC that was much higher than for other sampling methods, largely because these samplers targeted ampharetid beds and a sponge assemblage. It is uncertain whether the fauna in areas that were covered with carbonate (Uruti Ridge and Builder's Pencil) and had to be sampled with sleds, actually had reduced MDC or whether the estimates of MDC reflect gear selectivity (i.e. sleds collected more filter feeders). In other regions, seep carbonates have high abundances of photosynthesis-based fauna that are not reliant on seepage-fueled microbes for nutrition and are taking advantage of the hard substrate (Sellanes et al., 2008). This may be the case at Builder's Pencil and Uruti Ridge, or we may have missed the heterotrophic fauna that used the chemosynthesis-based nutrition.

The New Zealand seeps represent a potential model system for quantifying the role that metazoans play in the oxidation of methane. Seep taxa can enhance conditions for associated organisms by modifying the in-situ chemical and microbial environment (Cordes et al., 2005a). Examples include bio-irrigation and sulfate transport of symbiont-bearing clams to fuel sulfide oxidation (Wallmann et al., 1997; Levin et al., 2003; Luff et al., 2004), or increasing sediment-sulfate concentrations by tubeworms (Cordes et al., 2005a; Dattagupta et al., 2006, 2008). This facilitation can drive community composition (Cordes et al., 2005b). We hypothesize that the sponge and ampharetids in New Zealand enhance the food supply for their associated fauna by funneling methane to oxidizing microbes, potentially increasing the proportion of aerobic methanotrophy within this system (as suggested in Sommer et al., 2009-this issue). Further studies of the interactions between organisms in these communities and the chemistry that fuels them are necessary to quantify the fate of methane and its release into the water column.

6. Conclusions

Methane emissions at seeps are complex and not constant over time (Tryon and Brown, 2001, 2004; Greinert, 2008). The presence and isotopic composition of seep fauna provide a time-integrated proxy for the chemistry and magnitude of the fluid released. This approach can be especially enlightening for hard substrates, where techniques to quantify methane emissions (e.g. benthic chambers) are not currently available. In this study, we compared isotopic composition of symbiont-bearing among sites and identified Builder's Pencil, dominated by carbonate and shell hash, as an area where methane release is potentially high enough to impact the DIC pool, with implications for faunal trophodynamics. Through a comparison of heterotrophic faunal isotopic signatures among different seep habitats, we identified two novel biotic associations which are largely fueled by methane; the presence of sponge cover and ampharetid beds may be useful indicators of high methane-emission areas. This approach identifies the important role for methane in the nutrition of New Zealand seep fauna.

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