Isotopic evidence for chemosynthesis-based nutrition of macrobenthos: The lightness of being at Pacific methane seeps

Lisa A. Levin

Integrative Oceanography Division, Scripps Institution of Oceanography, La Jolla, California 92093-0218

Robert H. Michener

Boston University Stable Isotope Laboratory, Department of Biology, 5 Cummington St., Boston, Massachusetts 02215

Abstract

   The importance of chemosynthetic nutritional pathways was examined for macrofaunal invertebrates ($>300 \ \mu$m) from methane seeps in the Gulf of Alaska (4,413–4,443 m), on the Oregon margin (590 m), and on the northern California slope [Eel River margin] (520 m) by use of natural abundance stable isotopic data. Seep macrofauna exhibited lighter δ^{13}C and δ^{15}N values than those in nonseep sediments, but isotopic signatures varied among seep sites. Macrofaunal isotopic signatures indicated chemosynthetically fixed carbon sources with a significant contribution from methane-derived carbon (MDC) in macrofauna from sediments of pogonophoran fields (average δ^{13}C, −46.44‰, 32%–51% MDC) and *Calyptogena phaseoliformis* beds (average δ^{13}C, −40.89‰, 12%–40% MDC) in the Gulf of Alaska and in microbial mat sediments on the Oregon margin (average δ^{13}C, −43.80‰, 20%–44% MDC). Lesser influence of MDC was noted in macrofauna from sediments of *Calyptogena pacifica* beds on the Oregon (average δ^{13}C, −33.38‰, 0%–27% MDC) and California (δ^{13}C, −25.10‰, 0%–22% MDC) margins and from California microbial mat sediments (δ^{13}C, −22.23‰, 0%–5% MDC). Although most macrofauna appeared to be heterotrophic, light δ^{15}N and δ^{13}C values together provided evidence for chemoautotrophic symbioses in selected taxa. Carbon isotopic signatures were consistent with consumption of methane-oxidizing archaea by some dorvilleid polychaetes (δ^{13}C, −90.62‰ and −73.80‰) and with grazing on filamentous sulfur bacteria by gastropods and polychaetes from the Oregon and California seeps. The importance of chemosynthetic trophic pathways varies regionally and among microhabitats, taxonomic groups, and feeding guilds.

   The potential for chemosynthetically fixed carbon to support large endosymbiont-bearing invertebrates and to fuel entire animal communities in the ocean was first realized about two decades ago (Felbeck et al. 1981; Cavanaugh 1983). Since the discovery of chemosynthesis as a major nutritional pathway at hydrothermal vents, other reducing systems have been found in which energy is supplied mainly through chemosynthetic production (Paull et al. 1985). Methane, hydrocarbon and brine seeps, whale falls, some hypoxic settings, sewage outfalls, and seagrass beds are now known to support filamentous chemoautotrophic bacterial mats and invertebrates with chemoautotrophic endosymbionts (Fisher 1990; Cavanaugh 1994; Conway et al. 1994; Sibuet and Olu 1998). Although most attention has been on

megafaunal or epifaunal taxa in reducing environments (typically mollusks and pogonophorans), sediments in these settings support a broad range of smaller infaunal invertebrates (macrofauna) whose nutritional mode has not been well studied. Many of these are deposit feeders or omnivores. Energy sources available to the infauna that inhabit bathyal reducing environments should include photosynthetically fixed material deposited from above, such as phytoplankton-derived organic matter, land-derived material transported across the shelf, or carbon fixed chemosynthetically in the seabed, made available through the activities of free-living or symbiotic bacteria. Sulfur oxidation and methane oxidation (methanotrophy) are the primary chemosynthetic pathways that support carbon fixation of significance to animals (Conway et al. 1994; Van Dover 2000). Recent work has suggested that free-living or symbiotic sulfate reducers that interact directly with other chemoautotrophs in consortia may also be important (Boetius et al. 2000; Dubilier et al. 2001).

   Because the different carbon fixation pathways described above involve distinct isotopic fractionation, stable isotopic signatures have been particularly useful for elucidating nutritional modes of organisms in vent and seep environments (Conway et al. 1994; Van Dover and Fry 1994). Very negative tissue carbon signatures (e.g., δ^{13}C $\leq$ −50‰) are typically associated with methane-derived carbon; methane gas itself can have δ^{13}C values of −60‰ to −70‰ or less (Whalen 1993; Van Dover 2000). Carbon fixation fueled by energy derived from sulfide oxidation that involves form I Rubisco often produces δ^{13}C values between −27‰ and

−37‰, but alternative pathways that involve form II Rubisco can yield much heavier $\delta^{13}C$ values (−9 to −16‰) (Brooks et al. 1987; Fisher 1990; Robinson and Cavanaugh 1995). Phytoplankton-derived organic matter typically has $\delta^{13}C$ signatures of −15‰ to −25‰ (Fry and Sherr 1984), with a value of −21.0‰ on the northern California margin (N. Blair and L. Leithold pers. comm.). Terrestrial flood debris in the form of particulate organic carbon that empties from the Eel River onto the northern California margin has an average signature of around −24.9‰ (Leithold and Hope 1999).

Nitrogen signatures ($\delta^{15}N$) can provide information about nitrogen sources and trophic level (Michener and Schell 1994). Animals in reduced settings that bear chemoautotrophic symbionts often exhibit light, sometimes negative $\delta^{15}N$ signatures if local inorganic nitrogen is assimilated or fixed (Conway et al. 1994). Unlike $\delta^{13}C$, which changes little as it is passed to consumers (~1 per mil trophic level^{-1}) (Gearing 1991), nitrogen signatures increase by ~3.4‰ with each successive trophic level (DeNiro and Epstein 1978, 1981; Minagawa and Wada 1984). Used together, $\delta^{13}C$ and $\delta^{15}N$ signatures of animals can provide an indication of the carbon and nitrogen sources, fixation pathways, trophic level, and potential occurrence of chemoautotrophic endosymbioses.

The primary objective of this study was to evaluate the importance of methane-derived carbon and of chemosynthesis in general to macrofaunal nutrition at methane seeps in three regions of the north Pacific. We compared $\delta^{13}C$ and $\delta^{15}N$ signatures of macrofauna from two upper slope settings, the Eel River margin off northern California (520 m) and northern Hydrate Ridge on the Oregon margin (590 m), and from abyssal seeps near Kodiak Seamount in the Gulf of Alaska (4,413–4,444 m). Comparisons are also drawn with macrofauna from nonseep sediments in each region. We hypothesized that chemosynthesis should be more important to macrofauna in the abyssal setting, where the flux of photosynthetically derived carbon is much lower than on the upper slope. We asked: (1) Does the contribution of chemosynthetically fixed carbon to macrofauna vary among (a) geographic regions, (b) different microhabitats within a region (i.e., clambeds, pogonophoran fields, or microbial mats), (c) major taxonomic groups, and (d) polychaete feeding groups? (2) Can we identify specific chemosynthetic food sources of macrofauna (e.g., symbioses, sulfide oxidizing bacteria, or methane-oxidizing archaea)? We also present preliminary isotopic data to address the nutritional association of mobile megafauna with methane seeps.

Materials and methods

Sampling at seeps was carried out during 5–12 August 1999 in the Gulf of Alaska near Kodiak Seamount (4,413–4,445 m; 58°55.6′N, 149°32.9′W) and during 16–17 August 1999 on the Oregon margin at Hydrate Ridge (590 m; 44°40.1′N, 125°05.8′W) with the submersible Alvin and the R/V *Atlantis*. Sampling on the northern California slope (520 m; 40°47.1′N, 124°35.7′W) was conducted during 12–16 October 2000 with the Remotely Operated Vehicle (ROV) Jason and the R/V *Thompson* (Table 1). Eel River flood de-

bris is deposited episodically within the northern California study area (Alexander and Simoneau 1999); hereafter, we refer to this site as the Eel River margin. Animals were collected from seep sediments with tube cores (8.3 cm diameter), Ekman box corers, or by scoop sampler. Nonseep samples were collected with a multiple corer (8.6 cm tube diameter) in the regions surrounding the seeps off Alaska (4,327–4,428 m) and Oregon (817–887 m). Nonseep macrofauna from the Eel River margin (500–525 m) were collected from areas with no visible seepage activity by tube core and scoop bag with the ROV Jason.

Once on board ship, core sediments were kept cold (~5°C) until they could be processed. Large animals were removed by hand and frozen at −70°C (Oregon and Gulf of Alaska) or −20° C (Eel River margin). Sediments were sieved on a 300-μm mesh screen, and the residue was sorted on board ship at 12× magnification under a dissecting microscope, to remove macrofaunal invertebrates and large filamentous bacteria. All specimens were handled with methanol-washed forceps. Invertebrates were placed in filtered seawater overnight to allow guts to clear. They were then rinsed in milli-Q water and placed in combusted vials (500°C overnight) or preweighed tin boats, then frozen for transport back to the laboratory. Mollusks were removed from their shells, large bivalves were separated into different tissue types (foot, gill, and mantle), pogonophorans and polychaetes were removed from tubes, and soft tissue was removed from decapods and urchins prior to freezing or drying. In the laboratory, all specimens were oven-dried, and larger specimens were powdered. Isotopic analyses were conducted on single individuals (or part of one) by use of a Finnigan Conflo II continuous flow system and a Fisons NA1500 elemental analyzer coupled to a Finnegan Delta-S isotope ratio mass spectrometer. Prior to combustion, all samples were acidified with 1% $PtCl_2$ to remove carbonates. A total of 149 seep and 34 nonseep macroinfauna were examined, along with selected mobile megafauna.

Isotopic compositions are expressed as $\delta^{13}C$ and $\delta^{15}N$ values in units of per mil (‰). These are defined as $\delta X = [R(\text{sample})/R(\text{standard})] - 1] \times 1000$, where X is ^{13}C or ^{15}N, R is $^{13}C:^{12}C$ or $^{15}N:^{14}N$, and standards are Pee Dee Belemnite carbon and nitrogen gas (atmospheric).

Comparisons of isotopic values across sites, microhabitats, and taxonomic groups were performed by use of one-way analysis of variance and a posteriori Tukey's Honestly Significant Difference (HSD) tests. Isotopic data are presented as mean ±1 standard error unless indicated otherwise.

We examined the contribution of methane-derived carbon (F_m) for macrofauna using single isotope, two–end member linear mixing models (Fry and Sherr 1984). To obtain an upper estimate, we used the model $F_m = (\delta_I - \delta_{POC})/(\delta_m - \delta_{POC})$, where δ_I, δ_m, and δ_{POC} are the carbon isotopic signatures of infauna, methane, and particulate organic carbon (POC), respectively. To obtain a conservative estimate, we replaced δ_{POC} with δ_{SOB}, where δ_{SOB} is the carbon isotopic signature of sulfide-oxidizing bacteria (SOB). F_m was calculated by use of $\delta^{13}C$ values for each macrofaunal individual sampled, then averaged to generate values for each microhabitat and region. Variation in F_m was determined by taking the standard deviation of individual estimates. We

Table 1. Location and date of faunal sampling.

Location	Date	Vehicle/sampler	Dive/drop no.	Water depth (m)	Latitude (°N)	Longitude (°W)	Microhabitats sampled
Gulf of Alaska	6–12 Aug 1999	RV *Atlantis*/ALVIN	3,444–3,449	4,413–4,447	56°55.6'	149°32.9'	*Calyptogena phaseoliformis* beds, pogonophoran fields
Gulf of Alaska	7–11 Aug 1999	RV *Atlantis*/Multicorer	Drops 1–9	4,327–4,428	56°55'–56°59'	149°31'–149°34'	nonseep, turbidite
Oregon Margin	16–17 Aug 1999	RV *Atlantis*/ALVIN	3,451–3,452	580–600	44°40.16'	125°05.8'	*Calyptogena pacifica* beds, microbial mats
Oregon Margin	17 Aug 1999	RV *Atlantis*/Multicorer	Drop 10	817	44°37.3'	125°06.1'	nonseep slope
Eel River Margin, CA	12–16 Oct 2000	RV *Thompson*/JASON	288–294	500–525	40°47.1'	124°35.7'	*Calyptogena pacifica* beds, microbial mat, nonseep

Table 2. Isotopic signatures of mat-forming, filamentous sulfur bacteria at Pacific methane seeps.

Seep	$\delta^{13}C$	$\delta^{15}N$
Oregon margin		
Orange mat	−40.35	2.51
Orange mat	−30.72	−3.58
White mat	−31.79	−3.43
Average	−34.28	−1.50
Standard error	3.05	2.00
Eel River margin		
White mat	−26.57	5.86
White mat	−27.92	1.80
White mat	−29.89	3.27
White mat	−33.37	5.67
White mat	−28.56	5.77
White mat	−26.57	9.76
Average	−28.81	5.35
Standard error	1.04	1.10

used measured methane $\delta^{13}C$ values of −65‰ for the Oregon margin (Suess and Whiticar 1989) and −60‰ and −47‰ for the Eel River microbial mats and clambeds, respectively (H. H. Richnow and W. Ziebis pers. comm.). The isotopic composition of methane has not been measured in the Gulf of Alaska seeps; we adopted −70‰ as an average value for unaltered biogenic methane (Whalen 1993; M. Whalen pers. comm.). The mean $\delta^{13}C$ for POC was taken to be the average value for nonseep animals in each region. The mean $\delta^{13}C$ for SOB in the Gulf of Alaska was estimated to be −37‰, a value typical for seep bivalves with sulfide-oxidizing symbionts (Conway et al. 1994; this study). For the Oregon and Eel River margins, the $\delta^{13}C$ for SOB was entered as the average value of microbial mat bacteria at each site (−34.3‰ and −28.8‰, respectively; Table 2). The assumption of three possible carbon sources (from methane oxidation, sulfide oxidation, and phytoplankton-derived carbon) is highly reasonable for the Gulf of Alaska and probably for the Oregon margin. It is less realistic for the northern California (Eel River) margin, where terrestrial flood debris represents a known carbon input to the system (Alexander and Simoneau 1999; Leithold and Hope 1999). Many of the macrofauna studied were too small to measure $\delta^{34}S$, which might have allowed further discrimination among nutritional sources.

Results

Isotopic signatures of microbes—Direct isotopic measurements of microbes were made for large, filamentous bacteria forming visible white or orange mats on the Oregon and Eel River (California) margins (Table 2). Average mat $\delta^{13}C$ signatures, −34.3‰ and −28.8‰ for the Oregon and Eel River margin mats, respectively, clearly reflected chemosynthetic carbon fixation. Average $\delta^{15}N$ signatures were −1.5‰ and 5.4‰ for Oregon and the Eel River mats, respectively. The Eel River microbial mats were isotopically heavier with respect to both carbon ($F_{1,8} = 4.74$, $P = 0.066$) and nitrogen ($F_{1,8} = 10.78$, $P = 0.013$). Analyses of 16 s rRNA sequences
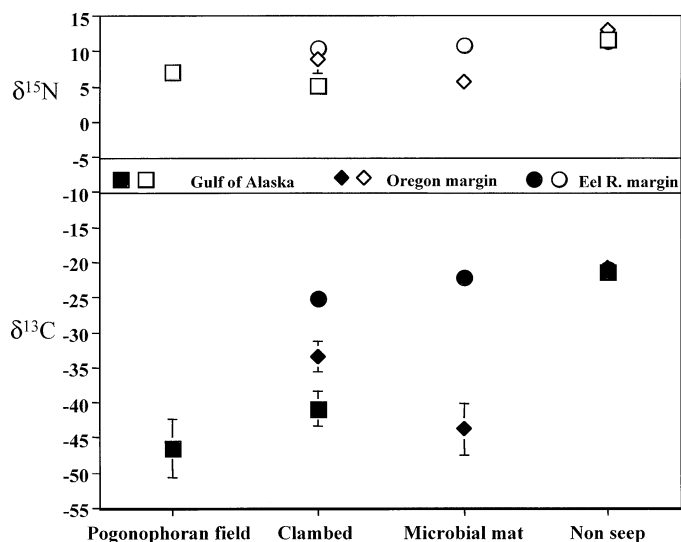
Fig. 1. Average (±1 SE) isotopic signatures for macrofaunal taxa (>300 μm), collected in nonseep sediments and in *Calyptogena* beds (clambed), pogonophoran fields, and microbial mat habitats from Pacific methane seeps in three regions. Samples are from 4,413–4,444 m in the Gulf of Alaska, 590–800 m on the Oregon margin, and 520–525 m on the Eel River margin.

from the Eel River and Oregon microbial mats indicated that they are composed of a diverse assemblage of sulfide-oxidizing forms (W. Ziebis and K. Zengler pers. comm.). No surficial microbial mats were observed at seeps in the Gulf of Alaska, although some bacterial filaments were observed in subsurface sediments.

Nonseep macrofaunal signatures—In all three regions, the nonseep macrofauna, which included polychaetes, mollusks, peracarid crustaceans, and echinoderms, exhibited consistent isotopic signatures indicative of a phytoplankton-based food web (Fig. 1, Web Appendices 1–3). Average $\delta^{13}C$ values were $-21.39‰ \pm 0.81‰$, $-20.78‰ \pm 0.92‰$, and $-20.53‰ \pm 1.09‰$ at seeps in the Gulf of Alaska, Oregon margin, and Eel River margin, respectively. Average $\delta^{15}N$ signatures were all relatively high ($11.68‰ \pm 0.61‰$, $12.95‰ \pm 0.87‰$, and $11.17‰ \pm 0.49‰$, respectively), which indicates the use of recycled nitrogen. Carbon and nitrogen isotopic signatures of nonseep macrofauna did not differ across regions ($F_{2,38} = 0.616$, $P = 0.546$ for $\delta^{13}C$ and $F_{2,37} = 1.341$, $P = 0.275$ for $\delta^{15}N$).

Two individuals encountered at the nonseep sites had isotopic signatures indicative of chemosynthetic food sources: a single tanaid in the Gulf of Alaska ($\delta^{13}C = -33.37‰$, $\delta^{15}N = 5.72‰$) and one specimen of *Mediomastus californiensis* ($\delta^{13}C = -30.56‰$, $\delta^{15}N = 9.37‰$) on the Eel River margin. These occurrences suggest the possibility of patchy, unrecognized sources of reduced compounds within the regions sampled as "nonseep," but they were rare and had little effect on average "nonseep" isotopic signatures for each region.

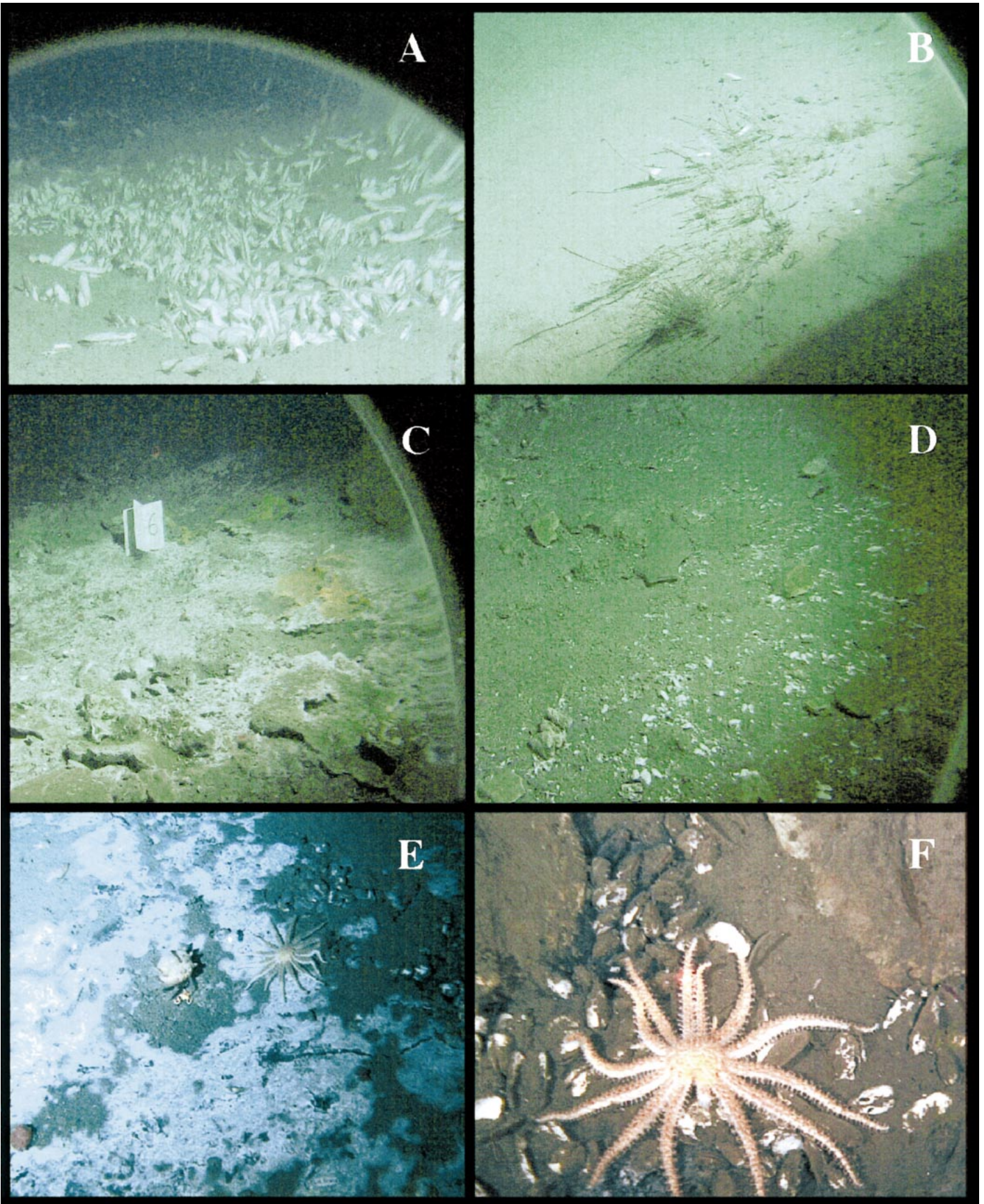*Gulf of Alaska seeps*—Two seep microhabitats were observed and sampled in the Gulf of Alaska, clambeds formed

by dense aggregations of *Calyptogena phaseoliformis* (Fig. 2A), and dense aggregations of Siboglinidae (Rouse 2001) (hereafter referred to as pogonophorans) in the genera *Polybrachia* and *Spirobrachia* (Fig. 2B). The clambed and pogonophoran settings occurred in close proximity, in patch sizes from 1 to 10s of square meters. Average macrofaunal $\delta^{13}C$ signatures were very light in both the pogonophoran fields ($-46.44‰ \pm 4.18‰$) and clambeds ($-40.89‰ \pm 2.52‰$). Average $\delta^{13}C$ values in each setting did not differ significantly from each other but were significantly lighter than nonseep values ($F_{2,56} = 20.686$, $P < 0.0001$) (Fig. 1). Within each habitat, there was considerable variation among individuals; some forms exhibited extremely light $\delta^{13}C$ values (Fig. 3A) (Web Appendix 1: http://www.aslo.org/lo/toc/vol_47/issue_5/1336a1.pdf). These included a dorvilleid polychaete ($-90.62‰$) and a nereid polychaete ($-62.07‰$) from the clambeds and pogonophorans ($-65.60‰$, $-60.96‰$), a gammarid amphipod ($-64.78‰$), and an anemone ($-62.56‰$) from the pogonophoran fields. Several other animals had $\delta^{13}C$ values of $-24‰$ or heavier (e.g., a syllid, trichobranchid polychaete, and sipunculan) (Fig. 3A), which suggests phytoplankton-based nutrition.

Nitrogen signatures were significantly lighter in the Gulf of Alaska seep than nonseep macrofauna ($F_{2,56} = 16.269$, $P < 0.0001$) (Fig. 3A). Average $\delta^{15}N$ values were $7.00 \pm 1.49‰$ in the pogonophoran fields and $5.02 \pm 0.62‰$ in the clambeds.

Three galatheid crabs collected from the pogonophoran fields and clambeds all had very light $\delta^{13}C$ signatures ($-47.3‰$, $-47.2‰$, and $-52.3‰$) and $\delta^{15}N$ signatures between 7.0‰ and 9.0‰ (Fig. 3A), which indicates that they were relying on chemosynthetically fixed carbon but were probably feeding at several trophic levels above the carbon fixers. The commensal polychaete *Nautilina calyptogenicola*, living within the mantle of *C. phaseoliformis*, had $\delta^{13}C$ values of $-38.1‰$ to $-38.8‰$ with a highly variable $\delta^{15}N$ ranging from 2.0‰ to 7.3‰. The *C. phaseoliformis* mantle had $\delta^{13}C$ and $\delta^{15}N$ values of $-36.4‰$ and 1.6‰, respectively. Foot, gill, and adductor muscle had $\delta^{13}C$ values between $-38‰$ and $-39‰$ and $\delta^{15}N$ values from $-1.9‰$ to $+1.5‰$. These values suggest that *N. calyptogenicola* and *C. phaseoliformis* share a primary carbon source but the polychaete may be consuming clam tissue.

Oregon margin seeps—Two distinct seep settings were present at Hydrate Ridge on the Oregon margin. Where methane bubbles were observed emanating from the seafloor, white and yellow microbial mats formed over the sediment surface (Fig. 2C). In distinct patches where no bubbling occurred, aggregations of the vesicomyid clam *Calyptogena pacifica* were evident (Fig. 2D).

Carbon isotopic signatures of macrofauna were significantly lighter in microbial mat–covered sediments ($\delta^{13}C = -43.80‰ \pm 3.63‰$) than in *Calyptogena* bed sediments ($\delta^{13}C = -33.38‰ \pm 2.17‰$); both were much lighter than the nonseep macrofauna ($F_{2,24} = 18.023$, $P < 0.0001$) (Figs. 1, 3B). The 10‰ difference between the two seep microhabitats may indicate greater incorporation of carbon derived from methane or sulfide oxidation by macrofauna in the mi-

crobial mat sediments, where seepage flow rates are higher (Tryon and Brown 2001).

In the case of $\delta^{15}N$ values, variation was high (Fig. 3B, Web Appendix 2: http://www.aslo.org/lo/toc/vol_47/issue_5/1336a2.pdf). Nitrogen isotopic signatures of macrofauna in microbial mats (5.66‰ ± 1.09‰) did not differ from those in clambed sediments (8.89‰ ± 2.06‰) but were significantly lighter than those in nonseep sediments (12.95 ± 0.87‰) ($F_{2,24} = 6.403$, $P = 0.006$) (Fig. 1).

As in the Gulf of Alaska, very negative $\delta^{13}C$ signatures were evident in several macrofaunal individuals from the microbial mat (Web Appendix 2), a dorvilleid polychaete (−73.8‰), and a cirratulid polychaete (−52.7‰), which suggests the incorporation of methane-derived carbon. Both specimens had $\delta^{15}N$ signatures near 6‰, which suggests that chemoautotrophic symbionts probably were not present. No macrofauna sampled from the Oregon seeps had isotopically heavy signatures similar to those in nonseep sediments or characteristic of sulfide oxidation with form II Rubisco (Web Appendix 2).

Mobile sea urchins (*Allocentrotus fragilis*) and tanner crabs (*Chionoecetes tanneri*) were collected from seep and nonseep patches. Single specimens collected on and off seeps had similar signatures (Fig. 3B). The crabs exhibited $\delta^{13}C$ signatures similar to those of average nonseep macrofauna and relatively high $\delta^{15}N$ values, which suggests carnivory on macrofauna. The sea urchins had slightly lighter $\delta^{13}C$ signatures than the average nonseep macrofauna but were 10‰ heavier than the Oregon seep macrofaunal average (Fig. 3B).

Eel River margin—Microbial mats (Fig. 2E) and *C. pacifica* beds (Fig. 2F) were present as distinct microhabitats on the Eel River margin. Methane bubbles were often observed emanating from sediments in the vicinity of microbial mats. Carbon isotopic signatures of clambed macrofauna (−25.10‰ ± 0.61‰) were significantly lighter than those of microbial mat (−22.23‰ ± 0.79‰) or nonseep sediments (−20.92‰ ± 1.29‰) (Fig. 3C) ($F_{2,106} = 9.400$, $P = 0.0002$, Web Appendix 3: http://www.aslo.org/lo/toc/vol_47/issue_5/1336a3.pdf). Mat and nonseep macrofaunal carbon signatures did not differ from each other (Figs. 1, 3C). This was true despite the likelihood that mat sediments have elevated sulfide fluxes (Sahling et al. 2002) and concentrations (W. Ziebis pers. comm.) relative to clambed or nonseep sediments. The lightest $\delta^{13}C$ values observed at the Eel River seeps were for tubificid oligochaetes (−39.7‰ and −34.8‰), the vesicomyid clam *C. pacifica* (−36.4‰–−36.7‰), a dorvilleid polychaete (−35.9‰), the gastropods *Astyris permodesta* (−33.3‰−−35.4‰) and *Neptunia* sp. (−32.0‰), a maldanid polychaete (−31.5‰), and the capitellid polychaete *M. californiensis* (−30.5‰).

No significant differences in $\delta^{15}N$ signatures were observed among microhabitats for Eel River macrofauna ($F_{3,131}$

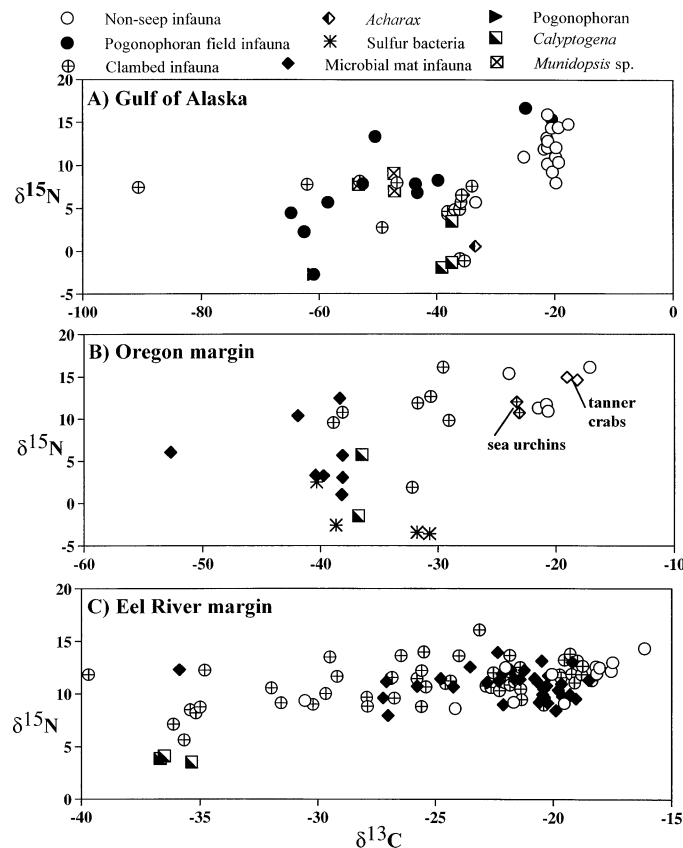Fig. 3. Dual isotope plot of macroinfauna (>300 μm) from Pacific methane seeps. Each point represents a distinct individual. Sulfur bacteria, known symbiont-bearing animals, and mobile taxa are indicated with separate symbols. (A) Macrofauna from clambed, pogonophoran field, and nonseep microhabitats in the Gulf of Alaska (4,413–4,444 m). (B) Macrofauna from clambed, microbial mat, and nonseep microhabitats on the Oregon margin (590 m for seeps and 800 m for nonseeps). (C) Macrofauna from clambed, microbial mat, and nonseep microhabitats on the Eel River margin (500–525 m).

= 2.45, $P = 0.067$); $\delta^{15}N$ values were 10.51‰ ± 0.28‰, 10.92‰ ± 0.36‰, and 11.48‰ ± 0.61‰ in clambed, microbial mat, and nonseep sediments, respectively (Web Appendix 3).

Across-site comparisons—Comparison of average isotopic signatures for seep macrofauna across regions indicates significantly heavier $\delta^{13}C$ ($F_{2,150} = 67.321$, $P < 0.0001$) and $\delta^{15}N$ ($F_{2,151} = 193.0$, $P \leq 0.0001$) values on the Eel River margin than at the two higher-latitude regions but no differences between the Gulf of Alaska and Oregon margin macrofauna. A slightly different result emerges when microhabitats are evaluated separately but compared across regions. Macrofauna $\delta^{13}C$ values in clambeds were lighter on average

←

Fig. 2. Pacific seep microhabitats. Gulf of Alaska (4,413–4,444 m): (A) *C. phaseoliformis* beds and (B) Pogonophoran (Siboglinidae) beds of *Spirobrachia* and *Polybrachia* spp. Oregon margin, hydrate ridge (590 m): (C) microbial mats and (D) *C. pacifica* beds. Eel River margin (520–525 m): (E) microbial mats and (F) *C. pacifica* beds.

in the Gulf of Alaska ($-42.04‰ \pm 2.68‰$) than on the Oregon ($-33.38‰ \pm 3.95‰$) or Eel River margins ($-25.91‰ \pm 5.88‰$); the Oregon and Eel River values did not differ from each other ($F_{2,90} = 29.813$, $P < 0.0001$) (Fig. 1). The same pattern was observed for $\delta^{15}N$; Gulf of Alaska clambed macrofauna ($5.2‰ \pm 0.60‰$) were lighter than Oregon margin ($8.89‰ \pm 1.10‰$) or Eel River ($10.65‰ \pm 0.42‰$) clambed macrofauna (Fig. 1). A comparison of microbial mat habitats indicated that Oregon margin mat macrofauna exhibited significantly lighter $\delta^{13}C$ and $\delta^{15}N$ signatures than the Eel River margin mat macrofauna ($\delta^{13}C$, $-43.80‰ \pm 1.88‰$ vs. $-21.93‰ \pm 0.98‰$; $\delta^{15}N$, $5.66‰ \pm 0.64‰$ vs. $10.93‰ \pm 0.32‰$) (Fig. 1).

Taxon differences—Among the major taxa found in seep sediments at all sites combined, pogonophorans had significantly lighter $\delta^{13}C$ values ($-55.24‰$) than the other taxa ($F_{5,150} = 3.418$, $P = 0.005$), but no differences were observed among echinoderms ($\delta^{13}C = -24.18‰$), mollusks ($-32.98‰$), other polychaetes ($-30.61‰$), oligochaetes ($-32.64‰$), and peracarid crustaceans ($-27.99‰$). Across-site comparisons of $\delta^{15}N$ for seep macrofaunal taxa revealed significantly lighter average values for mollusks ($6.24‰$) and pogonophorans ($0.59‰$) than for echinoderms ($11.00‰$), peracarid crustaceans ($10.74‰$), other polychaetes ($9.66‰$), and oligochaetes ($9.62‰$) ($F_{5,151} = 9.943$, $P < 0.0001$). When $\delta^{15}N$ signatures of different taxa were compared within a region, the pogonophorans ($0.60‰$) were lighter than the other groups ($3.51–7.86‰$) in the Gulf of Alaska ($F_{4,40} = 2.885$, $P = 0.036$). On the Eel River margin, mollusks exhibited significantly lighter $\delta^{13}C$ and $\delta^{15}N$ values than echinoderms, polychaetes, or peracarid crustaceans ($F_{4,106} = 8.750$, $P < 0.0001$ for $\delta^{13}C$; $F_{4,106} = 6.415$, $P = 0.0001$ for $\delta^{15}N$).

Annelid feeding modes—The relationship between annelid feeding mode at seeps and reliance on chemosynthetically based food resources was examined by comparing isotopic signatures of individuals classified as carnivores, omnivores, surface-deposit feeders, subsurface-deposit feeders, and commensals (on symbiont-bearing animals). The pogonophora, although they are now considered annelids (Rouse 2001), were excluded from this analysis because they are known to rely on chemoautotrophic symbionts (Schmaljohann et al. 1990). When data from all sites were combined, $\delta^{13}C$ was unrelated to feeding mode ($P = 0.236$), but, as expected, feeding mode was reflected in $\delta^{15}N$ values ($F_{5,80} = 3.41$, $P = 0.008$). Carnivores had heavier $\delta^{15}N$ values ($11.70‰$) than surface-deposit feeders ($8.31‰$) and commensals ($4.66‰$) but did not differ from omnivores ($9.34‰$) or subsurface-deposit feeders ($10.80‰$). Among the Eel River margin macrofauna, subsurface-deposit feeders had lighter average $\delta^{13}C$ values ($-27.0‰$) than carnivores ($-21.7‰$) and omnivores ($-22.7‰$) but did not differ from surface-deposit feeders ($-25.1‰$) ($F_{4,48} = 3.593$, $P = 0.013$).

Discussion

The role of different chemosynthesis-based nutritional sources for macrofauna at seeps—Seep macrofauna may ob-

tain chemosynthetically fixed organic matter by (1) translocation from sulfide- or methane-oxidizing symbionts, (2) feeding on free-living chemoautotrophic bacteria, or (3) consuming another animal that obtains nutrition through (1) or (2). Symbiosis-based nutrition appears to be rare among seep macrofauna. Light (often negative) $\delta^{15}N$ values occurring together with light $\delta^{13}C$ values suggest the presence of symbionts, because they are likely to use very local nitrogen sources. $\delta^{15}N$ values between $-2.73‰$ and $3.0‰$ were measured in the bivalves *Calyptogena* spp., *Acharax* sp., pogonophorans, ampharetid, polynoid and nereid polychaetes, and an anemone. These taxa had $\delta^{13}C$ values between $-60.96‰$ and $-32.20‰$ (Web Appendices 1–3). The bivalves and pogonophorans are known to support chemoautotrophic symbionts. The anemone and annelids merit further investigation in this regard.

Free-living, chemosynthetic bacteria are abundant at seeps. Filamentous, sulfide-oxidizing microbes form conspicuous mats on the sediment surface at the Oregon and Eel River seeps (Fig. 2C,E) but are also abundant in subsurface sediments (L. Levin unpubl. data). Although the filaments, which often occur many to a sheath, were as large or larger than some macrofauna, others were certainly small enough to be grazed by infaunal or epifaunal invertebrates. Selected ampharetid, dorvilleid, nereid, and trichobranchid polychaetes and the gastropods *A. permodesta* and *Provanna* sp. had $\delta^{13}C$ and $\delta^{15}N$ signatures consistent with consumption of mainly mat microbes (Web Appendices 2, 3). Some dorvilleid species were frequently observed to have microbial filaments inside their guts.

High rates of subsurface chemosynthesis by free-living, methane-oxidizing archaea, sulfate reducing bacteria, and consortia of these metabolic types recently have been documented at methane seeps (Boetius et al. 2000; Hinrichs et al. 2000; Orphan et al. 2001*a,b*). These microbes are known to produce lipids with extraordinarily light $\delta^{13}C$ signatures (e.g., $\delta^{13}C < -100‰$; Hinrichs et al. 2000; Thiel et al. 2001; Orphan et al. 2001*b*) and may represent an important food supply for the isotopically light polychaetes from the Gulf of Alaska and Oregon margin seeps.

When the macrofaunal carbon pool is derived largely from methane, then individuals should exhibit very light $\delta^{13}C$ signatures (e.g., $\ll -50‰$). However, most seep macrofauna probably use a variety of food sources, and their interpretation from isotopic signatures is complex. Combined use of photosynthetic- and methane-derived carbon could yield intermediate macrofaunal $\delta^{13}C$ signatures indistinguishable from those associated with form I Rubisco sulfide oxidation. Combined consumption of sulfide oxidizers having form I and II Rubisco could yield tissues with phytoplankton-like $\delta^{13}C$ signatures.

We can constrain the percentage of methane-derived carbon in macrofauna using two source-mixing models, in which either methane and phytoplankton or methane and sulfide oxidizers are the end members. These yield upper and lower estimates of methane-derived carbon in macrofaunal tissues. For the Gulf of Alaska, these estimates are 32% ($\pm8\%$) to 51% ($\pm6\%$) in the pogonophoran fields and 12% ($\pm8\%$) to 40% ($\pm5\%$) in the *C. phaseoliformis* beds. Estimates of methane-derived carbon in macrofauna from the
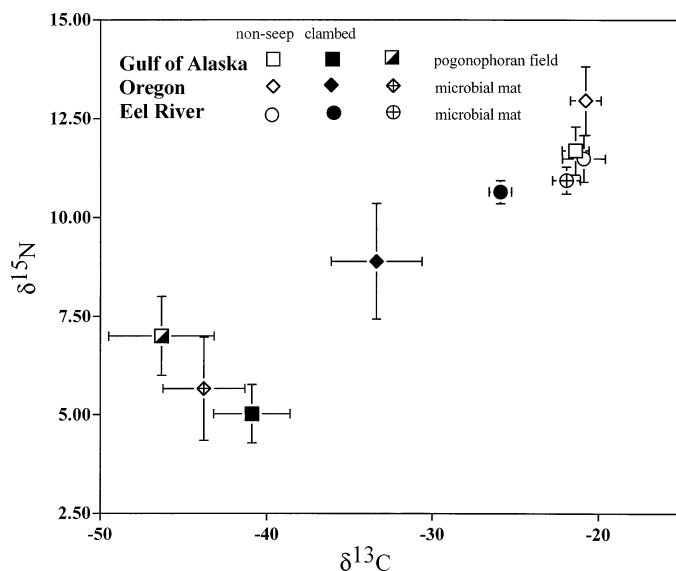
Fig. 4. Dual isotope plot summarizing mean (± 1 SE) δ^{13}C and δ^{15}N values for macrofauna in each microhabitat within each region studied.

Oregon margin are 20% ($\pm 5\%$) to 44% ($\pm 4\%$) in the microbial mat sediments and 0% to 27% ($\pm 3\%$) in *C. pacifica* beds. The Alaska seep sites and the Oregon microbial mat habitat may support, on average, the isotopically lightest infaunal assemblages known on earth, with respect to carbon and possibly nitrogen as well (Fig. 4). δ^{13}C signatures of macrofauna from seep microhabitats on the Eel River margin were significantly heavier (Fig. 4), which suggests less direct reliance on methane (0%–22% $\pm 5\%$ in clambeds and 0%– 5% in microbial mats). The interpretation of Eel River isotopic signatures is made more difficult by the fact that terrestrial carbon sources with average δ^{13}C signatures of around -25% are present on the Eel River slope (Leithold and Hope 1999). Over half of the organic carbon in finer Eel River slope sediments (<25 μm), the particles likely to be consumed by deposit feeders, consists of highly refractory kerogen and terrestrial plant material (N. Blair and L. Leithold pers. comm.) that are probably less readily assimilated than the marine carbon.

Analysis of ^{14}C may offer a useful alternative approach to the assessment of methane-derived carbon in animal tissues (Paull et al. 1985, 1992). Fossil methane carbon from deep within sediments is extremely depleted in ^{14}C and thus should retain a distinctive δ^{14}C signature whether it is fixed by methane oxidizers, sulfide oxidizers, or sulfate reducers. ^{14}C analyses of mussels and pogonophorans from the Florida Escarpment suggested that, despite exceptionally light δ^{13}C signatures, these animals do not obtain most of their carbon from fossil methane (Paull et al. 1985). A challenge before us is the availability of affordable, low-mass techniques and avoidance of blank contamination with modern carbon for ^{14}C analyses of the smaller macrofauna (typically <1 mg tissue dry wt) that are the focus of this study.

Comparison with other seep sites—Studies of shallow seeps in the North Sea (150 m, Dando et al. 1991) and on

the Eel River shelf (35–55 m, Levin et al. 2000) indicated that most infauna feed heterotrophically on photosynthetically produced carbon. North Sea macrofauna exhibited signatures even heavier than those from the Eel River slope, with δ^{13}C between -16% and -20% (Dando et al. 1991). However, methane seeps in the Skagerrak (280–360 m) supported dense aggregations of pogonophorans (*Siboglinum poseidoni*) and bivalves (*Thyasira sarsi*) known to rely on chemoautotrophic bacteria (Dando et al. 1994).

Suess et al. (1998) reported variation in isotopic signatures of seep megafauna from the Edge and Shumagin sectors ($>4,500$ m) in the Aleutian trench, near our study site in the Gulf of Alaska. Edge animals had δ^{13}C signatures from -28.6% to -64.3%, with the lightest value from a pogonophoran tube. Even lighter δ^{13}C signatures (-43% to -76.4%) were documented for megafaunal mussels, pogonophorans, corals, and anemones at Florida Escarpment seep sites (3,266 m) (Paull et al. 1992). None of these studies examined small infauna.

Seep habitats as a continuum—The results of this study indicate the nutrition of macrofauna at Pacific seeps varies and suggest that assemblages lie along a continuum from having mainly chemosynthetically produced diets to mainly photosynthesis-based diets (Fig. 4). Levin et al. (2000) predicted that macrofauna of abyssal and rise seeps should exhibit much greater trophic specialization (involving chemosynthesis) than the upper slope and shelf faunas, because the supply of food from the surface is more limited. Although the Gulf of Alaska assemblages support this idea, the variation between clambed and microbial mat assemblage signatures on the upper Oregon slope indicates that macrofaunal nutrition is more complicated and may be driven by local processes. Different seepage and fluid flow regimes among microhabitats (Tryon et al. 2000; Tryon and Brown 2001) may be a key determinant of macrofaunal community structure and feeding modes at methane seeps (Sahling et al. 2002; Levin et al. unpubl. data).

External organic matter inputs and the lability of photosynthetically derived carbon sources may affect regional patterns of macrofaunal nutrition. These factors are influenced by water depth, distance from shore, upwelling, latitude, horizontal fluxes, and other hydrographic properties. Reduced bottom-water oxygen concentrations may enhance preservation of organic matter from the surface (Cowie and Hedges 1992), thereby increasing the availability of phytodetrital food within sediments. The Eel River slope seeps are within the California oxygen minimum zone, where the bottom-water O_2 concentration ranges from 13 to 27 μM kg^{-1} (authors' unpubl. data).

Do seeps provide a significant food resource for nonseep taxa?—The possible export of seep production to nonseep settings has long intrigued scientists, but there are few data that address this issue. Kelly et al. (1998) noted that isotopic signatures in Gulf of Mexico sediments may reflect suspension and dispersal of seep bacteria into nonseep areas. We hypothesize that, as food becomes limiting, seep-related resources should comprise an increasingly larger component of the diet for nonseep vagrants (Carney 1994) that are pres-

ent in the vicinity of the seeps. Only at the deepest seeps, in the Gulf of Alaska, were the mobile consumers (*Munidopsis* sp.; $\delta^{13}C$ -47 to $-52‰$) isotopically similar to the seep infauna (Fig. 3A). On the Oregon margin, the isotopic signatures of mobile sea urchins and crabs more closely resembled those of nonseep rather than seep infauna (Fig. 3B). The same was true for a mobile asteroid, *Rathbunaster* sp., and sessile cnidarians (*Anthomastus ritteri* and a stalked coral) sampled near seeps on the Eel River margin (Levin et al. unpubl. data). Thus, the Oregon and Eel River megafauna exhibited minimal dietary contribution from seep production. Juhl and Taghon (1993) found similar trends for mobile fish and invertebrates at shallow (132 m) seeps on the Oregon margin. These data are too few to provide an adequate test of whether seeps supply significant nutrition to vagrant taxa. However, they do support the hypothesis that interaction of seep endemics, colonists, and vagrants may be greater in deeper, food-limited systems.

Examination of macrofaunal invertebrates from Pacific methane seeps at different water depths, latitudes, and microhabitats indicates that nutritional symbioses are rare. Most macroinfauna are heterotrophic and appear to consume free-living bacteria. This finding mirrors the results of Van Dover and Fry (1994) for smaller invertebrates in hydrothermal vent food webs. Both microorganism and macrofaunal invertebrate assemblages exhibit highly heterogeneous isotopic compositions, which indicates the consumption of food derived from multiple synthetic pathways. The importance of chemosynthetic food sources to macroinfauna varies geographically and among microhabitats, taxa, and feeding guilds but appears, on average, greatest at abyssal depths. Full understanding of macrofaunal nutrition within extreme environments such as methane seeps is likely to come from detailed exploration of the relationships among invertebrate feeding guilds, their geochemical environment, and microbial processes.

References

ALEXANDER, C. R., AND A. M. SIMONEAU. 1999. Spatial variability in sedimentary processes on the Eel continental slope. Mar. Geol. **154:** 243–254.

BOETIUS, A., AND OTHERS. 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. Nature **407:** 623–626.

BROOKS, J. M., AND OTHERS. 1987. Deep-sea hydrocarbon seep communities: Evidence for energy and nutritional carbon sources. Science **238:** 1138–1142.

CARNEY, R. S. 1994. Consideration of the oasis analogy for chemosynthetic communities at Gulf of Mexico hydrocarbon vents. Mar. Geol. Lett. **14:** 149–159.

CAVANAUGH, C. M. 1983. Symbiotic chemoautotrophic bacteria in marine invertebrates from sulfide-rich habitats. Nature **302:** 58–61.

———. 1994. Microbial symbiosis: Patterns of diversity in the marine environment. Am. Zool. **34:** 79–89.

CONWAY, N., M. KENNICUTT II, AND C. VAN DOVER. 1994. Stable isotopes in the study of marine chemosynthetic based ecosystems, p. 158–186. *In* K. Lajtha and R. Michener [eds.], Stable isotopes in ecology and environmental sciences. Blackwell Scientific.

COWIE, G. L., AND J. I. HEDGES. 1992. The role of anoxia in organic matter preservation in coastal sediments: Relative stabilities of the major biochemicals under oxic and anoxic depositional conditions. Org. Geochem. **19:** 229–234.

DANDO, P. R., AND OTHERS. 1991. Ecology of a North Sea pockmark with an active methane seep. Mar. Ecol. Progr. Ser. **70:** 49–63.

———, I. BUSSMANN, S. J. NIVEN, S. C. M. O'HARA, R. SCHMALJOHANN, AND L. J. TAYLOR. 1994. A methane seep area in the Skagerrak, the habitat of the pogonophore *Siboglinum poseidoni* and the bivalve mollusc *Thyasira sarsi.* Mar. Ecol. Prog. Ser. **107:** 157–167.

DENIRO, M. J., AND S. EPSTEIN. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta **42:** 495–506.

———, AND ———. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Acta **45:** 341–351.

DUBILIER, N., AND OTHERS. 2001. Endosymbiotic sulphate reducing and sulphide oxidizing bacteria in an oligochaete worm. Nature **411:** 298–302.

FISHER, C. R. 1990. Chemoautotrophic and methanotrophic symbioses in marine invertebrates. Rev. Aquat. Sci. **2:** 399–436.

FELBECK, H., J. J. CHILDRESS, AND G. N. SOMERO. 1981. Calvin-Benson cycle and sulphide oxidation enzymes in animals from sulphide-rich habitats. Nature **293:** 291–293.

FRY, B., AND E. B. SHERR. 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater systems. Contrib. Mar. Sci. **27:** 13–46.

GEARING, J. N. 1991. The study of diet and trophic relationships through natural abundance ^{13}C, p. 201–218. *In* D. C. Coleman and B. Fry [eds.], Carbon isotopic techniques. Academic.

HINRICHS, K. U., R. E. SUMMONS, V. ORPHAN, S. P. SYLVA, AND J. M. HAYES. 2000. Molecular and isotopic analysis of anaerobic methane-oxidizing communities in marine sediments. Org. Geochem. **31:** 1685–1701.

JUHL, A., AND G. TAGHON. 1993. Biology of an active methane seep on the Oregon continental shelf. Mar. Ecol. Prog. Ser. **102:** 287–294.

KELLEY, C. A., R. B. COFFIN, AND L. A. CIFUENTES. 1998. Stable isotope evidence for alternative bacterial carbon sources in the Gulf of Mexico. Limnol. Oceanogr. **43:** 1962–1969.

LEITHOLD, E. L., AND R. S. HOPE. 1999. Deposition and modification of a flood layer on the northern California shelf: Lessons from and about the fate of terrestrial particulate organic carbon. Mar. Geol. **154:** 183–195.

LEVIN, L. A., D. W. JAMES, C. M. MARTIN, A. E. RATHBURN, L. H. HARRIS, AND R. H. MICHENER. 2000. Do methane seeps support distinct macrofaunal assemblages? Observations on community structure and nutrition from the northern California slope and shelf. Mar. Ecol. Prog. Ser. **208:** 21–39.

MICHENER, R. H., AND D. SCHELL. 1994. Stable isotope ratios as tracers in marine aquatic food webs, p. 138–157. *In* K. Lajtha and R. Michener [eds.], Stable isotopes in ecology and environmental science. Blackwell Scientific.

MINAGAWA, M., AND E. WADA. 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between ^{15}N and animal age. Geochim. Cosmochim. Acta. **48:** 1135–1140.

ORPHAN, V. J., AND OTHERS. 2001*a.* Comparative analysis of methane-oxidizing archaea and sulfate-reducing bacteria in anoxic marine sediments. Appl. Environ. Microbiol. **64:** 1922–1934.

———, C. H. HOUSE, K. U. HINRICHS, K. D. MCKEEGAN, AND E. F. DELONG. 2001*b.* Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis. Science **293:** 484–487.

PAULL, C. K., J. P. CHANTON, A. C. NEUMANN, J. A. COSTON, C. S. MARTENS, AND W. S. SHOWERS. 1992. Indicators of meth-

ane-derived carbonates and chemosynthetic organic carbon deposits: Examples from the Florida Escarpment. Palaios **7:** 361–375.

————, A. J. T. JULL, L. J. TOLIN, AND T. LINICK. 1985. Stable isotope evidence for chemoautotrophy in an abyssal seep community. Nature **317:** 709–711.

ROBINSON, J. J., AND C. M. CAVANAUGH. 1995. Expression of form I and form II Rubisco in chemoautotrophic symbioses: Implications for the interpretation of stable carbon isotopic values. Limnol. Oceanogr. **40:** 1496–1502.

ROUSE, G. W. 2001. A claddistic analysis of Siboglinidae (Caullery, 1914 (Polychaeta, Annelida): Formerly the phyla Pogonophora and Vestimentifera. Zool. J. Linn. Soc. **132:** 55–80.

SAHLING, H., D. RICKERT, R. W. LEE, P. LINKE, AND E. SUESS. 2002. Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin. Mar. Ecol. Progr. Ser. **231:** 121–138.

SCHMALJOHANN, R., E. FABER, M. J. WHITICAR, AND P. R. DANDO. 1990. Co-existence of methane- and sulphur-based endosymbioses between bacteria and invertebrates at a site in the Skagerrak. Mar. Ecol. Prog. Ser. **61:** 119–124.

SIBUET, M., AND K. OLU. 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. Deep-Sea Res. II **45:** 517–567.

SUESS, E., G. BOHRMAN, R. VON HUENE, P. LINKE, K. WALLMANN, S. LAMMERS, AND H. SAHLING. 1998. Fluid venting in the eastern Aleutian subduction zone. J Geophys. Res. **103:** 2597–2614.

————, AND M. J. WHITICAR. 1989. Methane-derived CO_2 in pore water fluids expelled from the Oregon subduction zone. Palaeogeogr. Palaeoclimatol. Palaeoecol. **71:** 119–136.

THIEL, V., J. PECKMANN, H. H. RICHNOW, U. LUTH, J. REITNER, AND W. MICHAELIS. 2001. Molecular signals for anaerobic methane oxidation in Black Sea seep carbonates and a microbial mat. Mar. Chem. **73:** 97–112.

TRYON, M., AND K. M. BROWN. 2001. Complex flow patterns through Hydrate Ridge and their impact on seep biota. Geophys. Res. Lett. **28:** 2863–2866.

————, ————, M. TORRES, A. TREHU, J. MCMANUS, AND R. COLLIER. 2000. Measurement of transience and downward fluid flow near episodic methane gas vents, Hydrate Ridge, Cascadia. Geology **27:** 1075–1078.

VAN DOVER, C. L. 2000. The ecology of deep-sea hydrothermal vents. Princeton Univ. Press.

————, AND B. FRY. 1994. Microorganisms as food resources at deep-sea hydrothermal vents. Limnol. Oceanogr. **39:** 51–57.

WHALEN, M. 1993. The global methane cycle. Annu. Rev. Earth Planet. Sci. **21:** 407–26.