

Nutritional associations among fauna at hydrocarbon seep communities in the Gulf of Mexico

Stephen E. MacAvoy^{1,2,*} Charles R. Fisher³, Robert S. Carney⁴, Stephen A. Macko²

¹Biology Department, American University, Washington, DC 20016, USA

²Department of Environmental Sciences, University of Virginia, Charlottesville, Virginia 22903, USA

³Biology Department, Pennsylvania State University, University Park, Pennsylvania 16802, USA

⁴Coastal Studies Institute, Louisiana State University, Baton Rouge, Louisiana 70803, USA

ABSTRACT: The Gulf of Mexico supports dense aggregations of megafauna associated with hydrocarbon seeps on the Louisiana Slope. The visually dominant megafauna at the seeps — mussels and tube worms — derive their nutrition from symbiotic relationships with sulfide or methane-oxidizing bacteria. The structure of the tube worm aggregations provide biogenic habitat for numerous species of heterotrophic animals. Carbon, nitrogen and sulfur stable isotope analyses of heterotrophic fauna collected with tube worm aggregations in the Green Canyon Lease area (GC 185) indicate that most of these species derive the bulk of their nutrition from chemoautolithotrophic sources. The isotope analyses also indicate that although 2 species may be deriving significant nutritional input from the bivalves, none of the species analyzed were feeding directly on the tube worms. Grazing gastropods and deposit-feeding sipunculids were used to estimate the isotopic value of the free-living chemoautolithotrophic bacteria associated with the tube worms ($\delta^{13}\text{C}$ –32 to –20‰; $\delta^{15}\text{N}$ 0 to 7‰; $\delta^{34}\text{S}$ –14 to –1‰). The use of tissue $\delta^{34}\text{S}$ analyses in conjunction with tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ led to several insights into the trophic biology of the communities that would not have been evident from tissue stable C and N analyses alone.

KEY WORDS: Hydrocarbon seeps · Chemosynthesis · Sulfur isotopes

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INTRODUCTION

During trawls in the Green Canyon Lease area off the coast of Louisiana in 1985, previously unknown species of tube worms and bivalves were discovered near areas of seeping hydrocarbons and reduced sulfur gases (Kennicutt et al. 1985, Brooks et al. 1987). The Green Canyon tube worm and mussel species resembled the invertebrate species that had been discovered near hydrothermal vents in the late 1970s (Ballard 1977, Corliss & Ballard 1977). The hydrothermal vent invertebrates contained symbiotic chemoautotrophic bacteria that oxidized reduced sulfur species for energy (reviewed by Childress & Fisher 1992). Because the worms and bivalves found at the hydrocarbon seeps were related to the hydrothermal vent invertebrates and occurred at high densities around the seeps, it was postulated that they contained sym-

biotic bacteria similar to those in the hydrothermal system (Kennicutt et al. 1985). It was soon discovered that the tube worms (*Seepiophila jonesi* and *Lamellibrachia luymesii*) and clams (*Calyptogena ponderosa*, *Lucinoma atlantis* and *Vesicomya cordata*) harbored symbiotic sulfide-oxidizing chemoautolithotrophic bacteria and the dominant mussel (*Bathymodiolus childressii*) was symbiotic with methanotrophic bacteria (Childress et al. 1986, Brooks et al. 1987, Gardinar et al. 2001). Subsequently, another mussel was discovered (*Tamu fisheri*) that harbors chemoautolithotrophic sulfur-oxidizing symbionts (Fisher 1993). Free-living bacteria also abound in the seep environment, often forming large orange, yellow and white mats (MacDonald et al. 1989). It is generally believed that chemosynthetic primary production forms the base of the hydrocarbon seep community food web, although contributions from photosynthetically derived organic matter may

*Email: macavoy@american.edu

occur (Page et al. 1990, Kennicutt et al. 1992, Conway et al. 1994, Carney 1994, Fisher 1996, Pile & Young 1999, Levin & Michener 2002).

The methane and other reduced carbon compounds in the shallow sediments at this site largely derive from seepage from deeper source rocks (MacDonald et al. 1990, Sassen et al. 1999). The H_2S is apparently largely produced by anaerobic sulfate-reducing bacteria in the shallow sediments and is linked to methane oxidation and possibly the oxidation of higher molecular weight hydrocarbons (Formolo et al. 2004, Joye et al. 2004, Sassen et al. 2004), but some sulfides may also be associated with the oil reservoir seepage (MacDonald et al. 1989). The sediments may release some CH_4 , H_2S , $S_2O_3^{2-}$ and NH_4^+ into the water column (Conway et al. 1994), however, these compounds are only abundant at or below the sediment–water interface (Julian et al. 1999, Freytag et al. 2001). The carbon sources for the thiotrophic and methanotrophic bacteria are different. The carbon source for the thiotrophic bacteria is dissolved inorganic carbon (DIC), while the methanotrophic bacteria use CH_4 as both a carbon and energy source (Smith & Hoare 1977, Colby et al. 1979, Cary et al. 1988).

Stable isotope analyses have played an important role in understanding hydrothermal vent and hydrocarbon seep ecosystems. In fact, the first real evidence that the nutrition of vent animals was based on local primary production rather than photosynthetic production were stable C and N analyses (Rau & Hedges 1979, Rau 1981). Similarly, stable isotopes were important in determining the carbon and energy sources for symbiont-containing hydrocarbon seep fauna (Childress et al. 1986, Brooks et al. 1987, Fisher et al. 1993). Stable isotopes have also been a valuable tool for understanding trophic relationships among heterotrophic organisms within hydrothermal vent communities (Van Dover & Fry 1989, 1994, Fisher et al. 1994). Nitrogen isotopes are good indicators of food chain position (an approximately 3.4‰ enrichment per trophic level; Minagawa & Wada 1984), and carbon and sulfur isotopes have been shown to closely reflect an animal's food source (Fry & Sherr 1984, Peterson et al. 1985, Peterson & Howarth 1987, Hesslein et al. 1989, 1991, Kline et al. 1990, 1993, MacAvoy et al. 1998). The invertebrate and vertebrate taxa collected and observed among the chemoautolithotrophic animals at hydrocarbon seeps in the Gulf of Mexico are a fairly diverse group, including crabs, shrimp, isopods, gastropods, polychaete worms and fish (MacDonald et al. 1989, Carney 1994, Bergquist et al. 2003). To date, stable isotope analyses have only been reported for a limited number heterotrophic species from the Gulf of Mexico seeps (Brooks et al. 1987, Fisher 1996, MacAvoy

et al. 2002) and the nutritional relations among the seep fauna are not well understood.

In this study, tissue $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ analyses were used to investigate trophic linkages among the invertebrate fauna closely associated with tube worm aggregations located at hydrocarbon seeps off the coast of Louisiana (550 m depth). There were 4 primary objectives of this study: (1) To determine whether heterotrophic seep fauna collected with tube worms derived the bulk of their nutrition from chemoautolithotrophic or methanotrophic primary production. (2) to determine whether the symbiont-containing invertebrates were an important food source for heterotrophs closely associated with the vestimentiferans. (3) to determine if analyses of tissue $\delta^{34}S$ values provided additional insights into trophic relations among seep fauna, beyond what could be learned from analysis of $\delta^{13}C$ and $\delta^{15}N$. (4) To constrain the stable C, N, and S values of free-living seep bacteria consumed by heterotrophic primary consumers.

MATERIALS AND METHODS

The Green Canyon Lease Area occupies approximately 22 000 km² of seafloor lying between the 200 and 2400 m isobaths on the topographically and geochemically complex continental slope off the coast of Louisiana. For the management of oil and gas development, the area is subdivided into 4.8 × 4.8 km lease blocks. This study was conducted on communities from one of these lease blocks (GC 185; Bush Hill, located at 27° 46.96' N, 91° 30.46' W, 540 to 580 m b.s.l.). GC 185 is an active hydrocarbon seep area supporting chemoautolithotrophic communities that have been studied for various purposes for more than a decade (Brooks et al. 1987, Kennicutt et al. 1992, Fisher 1996, Sassen et al. 1999). Tube worms are the visually dominant symbiont-containing group at the site, although mussels symbiotic with thiotrophic or methanotrophic bacteria (*Tamu fisheri* and *Bathymodiolus childressi*, respectively) also occur here. Methane and oil are actively seeping from the sediments in several places on Bush Hill. Bacterial mats and carbonate outcrops are abundant, as are shallow gas hydrates (Nix et al. 1995, Sassen & Macdonald 1997, Sassen et al. 1999).

Isotope analysis. Two tube worm aggregations were collected intact with the DSV Johnson-Sea-Link II (Harbor Branch Oceanographic, Fort Pierce, FL) using a hydraulically activated device that encloses the tube worms and associated fauna, and removes the entire aggregation (Bushmaster Jr., Bergquist et al. 2000). One aggregation was dominated by larger individuals with no recent tube worm recruits present and is here-

after referred to as the older aggregation (BH-7 in Bergquist et al. 2002). The second younger aggregation (BH-1 in Bergquist et al. 2002) was dominated by small individuals, tube worm recruitment was recent or ongoing at the time of collection, and the mean tube worm age in the aggregation was calculated to be 3 yr (Bergquist et al. 2002). Tissue samples were dissected at sea from all animals and were frozen until shipment to the stable isotope laboratory. The tissue type sampled was muscle tissue for most invertebrates, exceptions being the tube worms and mussels (plume tissue and mantle tissue were used, respectively). Samples arriving at the laboratory were thawed, dried at 60°C for 3 d and homogenized by a mortar-and-pestle grinding tool. Approximately 5 to 6 mg of tissue was used for $\delta^{34}\text{S}$ measurements (1 to 2 mg was used for tube worm plumes owing to the high sulfur content) and 0.6 to 1.0 mg was used for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measurements. A Carlo Erba elemental analyzer coupled to a Micromass Optima isotope ratio mass spectrometer (Micromass) was used to obtain $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values (Fry et al. 1992, Giesemann et al. 1994). The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were determined concurrently and $\delta^{34}\text{S}$ was determined during separate analysis runs.

The isotope compositions are reported relative to standard material and follow the same procedure for all stable isotopic measurements as follows:

$$\delta^x\text{E} = [(\text{E}/\text{E})_{\text{sample}}/(\text{E}/\text{E})_{\text{standard}} - 1] \times 1000 \quad (1)$$

where E is the element analyzed (C, N or S), x is the molecular weight of the heavier isotope, and y the lighter isotope (x = 13, 15, 34 and y = 12, 14, 32 for C, N and S, respectively). The standard materials to which the samples are compared are PDB for carbon, air N_2 for nitrogen and CDT for sulfur. Reproducibility of all measurements was typically 0.3‰ for $\delta^{34}\text{S}$ and 0.2‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Approximately 217 analyses were conducted using 84 individuals from 24 different species.

Statistical analysis. Mann-Whitney *U*-tests were used for 2 group comparisons and Kruskal-Wallis tests were used for all multiple comparisons ($\alpha = 0.05$). The Dunn procedure was used to compare specific differences among groups if Kruskal-Wallis indicated a significant difference (Rosner 1990). The Dunn procedure reduces the risk of Type 1 error inherent in multiple comparison techniques by increasing the Z-score needed to reject the null hypothesis as the number of individual groups (treatments) increases. The statistical comparisons were only made when individual groups (treatments) had an

$N \geq 3$. Microsoft Excel 5.0 (Microsoft) and Statview SE + Graphics (Abacus Concepts) were used for individual statistical tests.

RESULTS

Chemoautolithotrophic symbiont-containing invertebrates

In 1998, 2 separate tube worm aggregations were collected at GC 185 (Bush Hill). Two tube worm species, *Lamellibrachia luymesii* and *Seepiophila jonesi*, were present in both collections, and the younger aggregation also included the mussels *Tamu fisheri* and *Bathymodiolus childressi*. The *B. childressi* mussel is symbiotic with methanotrophic bacteria; however, it retains a functional gut and is capable of filter feeding (Page et al. 1990, Pile & Young 1999). The other 3 symbiont-containing species harbor sulfur-oxidizing bacteria.

Tube worms were significantly ^{13}C -enriched relative to both mussel species (Table 1). *Bathymodiolus childressi* and *Seepiophila jonesi* $\delta^{15}\text{N}$ values were not significantly different from each other, but were significantly different from *Lamellibrachia luymesii* and *Tamu fisheri*. *L. luymesii* were ^{34}S -depleted relative to *B. childressi* (Table 1). *T. fisheri* $\delta^{34}\text{S}$ values fell between those of *L. luymesii* and *B. childressi*, however, *T. fisheri* were not significantly different from either group ($0.1 < p > 0.05$).

Heterotrophs

Likely consumers of primary production (chemosynthetic or photosynthetic) are grazing gastropods and deposit feeders. Most grazing gastropods, which were collected from the young tube worm aggregation, were depleted in ^{13}C relative to that typically seen in Gulf of Mexico (GOM) particulate organic matter

Table 1. Isotope values for invertebrates with symbionts collected at GC 185. Comparisons were made within the collection, not between the collections. Those with different letters are significantly different from each other ($p \leq 0.05$)

Invertebrate	$\delta^{13}\text{C} \pm \text{SD}$ (N)	$\delta^{15}\text{N} \pm \text{SD}$ (N)	$\delta^{34}\text{S} \pm \text{SD}$ (N)
Old tube worm bush			
<i>Lamellibrachia luymesii</i>	-20.1 \pm 1.2 (6)a	2.2 \pm 0.4 (6)a	-27.2 \pm 5.8 (4)a
<i>Seepiophila jonesi</i>	-22.0 \pm 0.4 (5)b	3.2 \pm 0.6 (5)b	-32.8 \pm 3.0 (3)a
Young tube worm bush			
<i>Lamellibrachia luymesii</i>	-21.5 \pm 1.2 (6)a	0.7 \pm 0.7 (6)a	-25.1 \pm 2.4 (5)a
<i>Seepiophila jonesi</i>	-22.2 \pm 2.3 (4)a	3.8 \pm 1.3 (4)b	
<i>Tamu fisheri</i>	-37.3 \pm 0.9 (3)b	-0.4 \pm 1.8 (3)a	-16.9 \pm 1.9 (3)a,b
<i>Bathymodiolus childressi</i>	-42.9 \pm 1.5 (6)b	6.1 \pm 1.2 (6)b	-5.3 \pm 1.4 (6)b

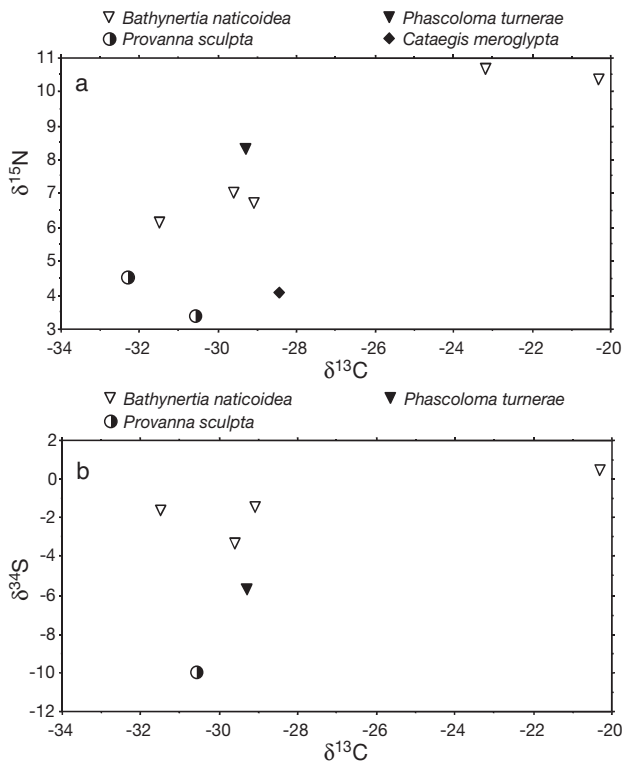


Fig. 1. $\delta^{13}\text{C}$ vs. (a) $\delta^{15}\text{N}$ and (b) $\delta^{34}\text{S}$ for grazing gastropods associated with a young tube worm bush at GC 185

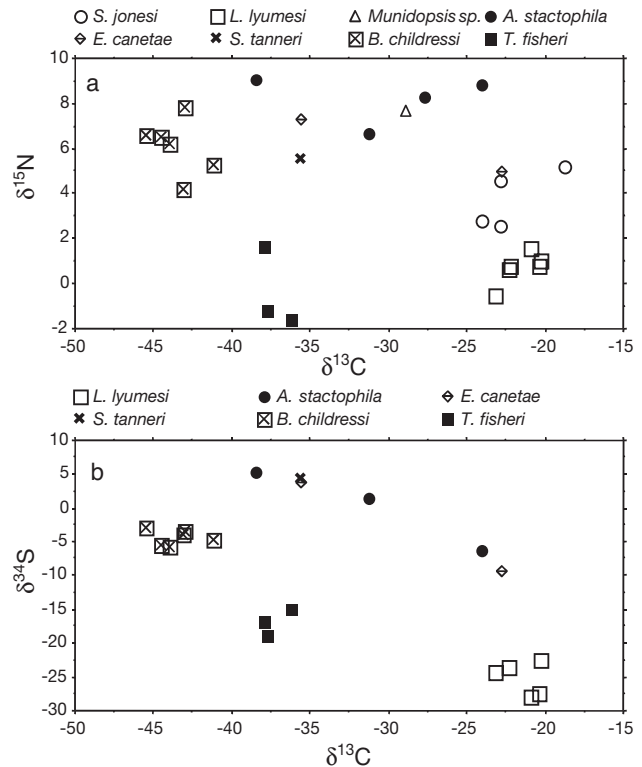


Fig. 3. $\delta^{13}\text{C}$ vs. (a) $\delta^{15}\text{N}$ and (b) $\delta^{34}\text{S}$ for a subset of invertebrates closely associated with a young tube worm bush at GC 185

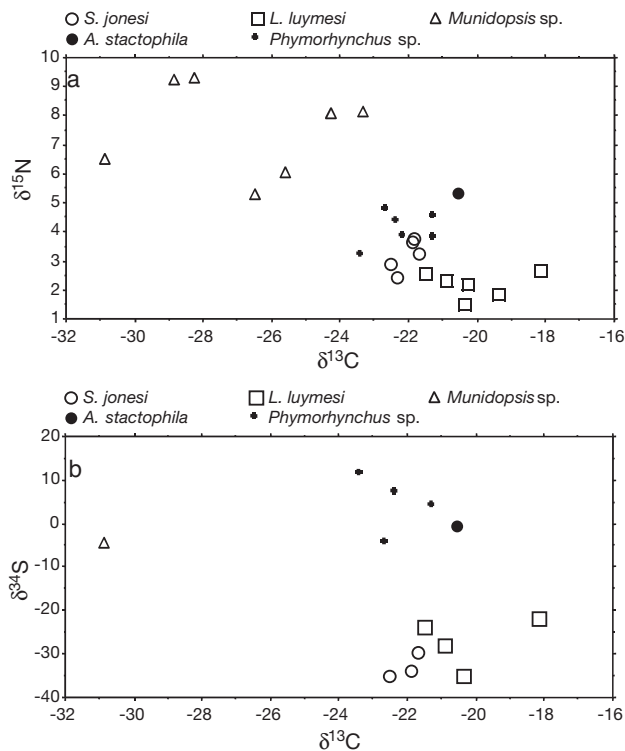


Fig. 2. $\delta^{13}\text{C}$ vs. (a) $\delta^{15}\text{N}$ and (b) $\delta^{34}\text{S}$ for a subset of invertebrates closely associated with an old tube worm bush at GC 185

(POM, $-21.4 \pm 1.4\text{‰}$; Fry 1983, Macko et al. 1984, although the tissue $\delta^{13}\text{C}$ values for 2 individuals were in the range of values reported for POM (Fig. 1). The $\delta^{15}\text{N}$ values of the grazers ranged from 2 to 10‰, which tended to be somewhat lower than particulate organic matter ($7.5 \pm 0.8\text{‰}$) and zooplankton (8.9 ± 0.9) in the NW GOM (Fry 1983, Macko et al. 1984). Sulfur in grazers as a group was highly ^{34}S -depleted relative to POM (marine POM $\delta^{34}\text{S} = 18$ to 20‰ ; overlying GOM bottom waters $\delta^{34}\text{S} = 20.3\text{‰}$; Aharon & Fu 2003), however, there was a 10‰ range among the 6 individuals analyzed (-10 to 0‰) (Fig. 1). The deposit-feeding, primary-consumer sipunculid *Phascolosoma turnerae* lives in the sediment at the base of the tube worm aggregation. *P. turnerae* in both tube worm collections were depleted in ^{13}C and ^{34}S relative to values expected for photosynthetic POM. *P. turnerae* isotope values were similar to those seen in grazing gastropods (Fig. 1, Table 2).

Most other invertebrate species captured within the tube worm aggregations were also ^{34}S -depleted relative to GOM POM (Table 2, Figs. 2 & 3). Nine of the other 14 species were ^{13}C -depleted. The only individual not depleted in either ^{13}C or ^{34}S relative to the other heterotrophs was a hagfish, *Eptatretus* sp. (Table 2). The 2 other vertebrates captured were a conger eel

Table 2. Isotope values (means \pm standard deviation [N]) for hydrocarbon seep organisms captured in Bushmaster collections at GC 185

Organism	$\delta^{13}\text{C} \pm \text{SD}$ (N)	$\delta^{15}\text{N} \pm \text{SD}$ (N)	$\delta^{34}\text{S} \pm \text{SD}$ (N)
Old tube worm bush			
Gastropods			
<i>Phymorhynchus</i> sp.	-22.2 ± 0.8 (6)	4.1 ± 0.6 (6)	4.6 ± 6.6 (4)
Limpet	-30.2 ± 2.5 (2)	9.6 ± 0.6 (2)	4.9 (1)
Worm-like taxa			
Nemertean	-23.1 (1)	7.5 (1)	-6.1 (1)
Crustacea			
<i>Munidopsis</i> sp.	-26.8 ± 2.7 (7)	7.5 ± 1.6 (7)	-4.4 (1)
<i>Alvinocaris stactophila</i>	-20.6 (1)	5.4 (1)	-0.3 (1)
Ayuielae shrimp	-33.2 (1)	2.6 (1)	
Atelecyliidae crab	-24.8 (1)	9.7 (1)	
Fish			
<i>Eptatretus</i> sp.	-20.9 (1)	11.5 (1)	12.2 (1)
<i>Dysommia rugosa</i>	-30.4 (1)	7.4 (1)	
<i>Ophichthus cruentifer</i>	-33.4 (1)	7.4 (1)	-13.3 (1)
Other			
<i>Phascolosoma turnerae</i>	-28.0 ± 0.5 (6)	6.3 ± 1.7 (6)	-7.6 ± 3.8 (4)
<i>Sclerasterias tanneri</i>	-32.9 ± 0.5 (2)	5.5 ± 0.1 (2)	
Younger tube worm bush			
Gastropods			
<i>Provanna sculpta</i>	-31.5 ± 1.2 (2)	4.0 ± 0.8 (2)	-9.9 (1)
<i>Cataegis meroglypta</i>	-28.5 (1)	4.1 (1)	
<i>Eosipho canetae</i>	-29.2 ± 9.1 (2)	6.2 ± 1.6 (2)	-4.5 ± 6.3 (4)
<i>Bathyerita naticoidea</i>	-26.7 ± 4.8 (5)	8.2 ± 2.2 (5)	-1.5 ± 1.4 (4)
Worm-like taxa			
Red nemertean	-22.2 (1)	-5.5 (1)	-9.6 (1)
Polynoid, <i>Harmothoe</i> sp.	-23.6 ± 0.1 (2)	5.7 ± 1.9 (2)	-8.9 ± 2.4 (2)
Polychaete (Amphinomidae)	-40.9 ± 0.9 (3)	8.1 ± 2.0 (3)	-9.7 ± 2.5 (3)
Maldanidae (<i>Nicomache</i> sp.)	-28.5 (1)	7.5 (1)	-2.9 (1)
Crustacea			
<i>Munidopsis</i> sp.	-28.7 (1)	7.7 (1)	-7.5 (1)
<i>Alvinocaris stactophila</i>	-30.4 ± 6.2 (4)	8.2 ± 1.1 (4)	0.9 ± 5.3 (4)
Other			
<i>Phascolosoma turnerae</i>	-29.3 (1)	8.3 (1)	-3.2 ± 3.5 (2)
<i>Sclerasterias tanneri</i>	-35.6 (1)	5.5 (1)	4.2 (1)

(*Ophichthus cruentifer*) and a cut throat eel (*Dysommia rugosa*). The 2 eels had tissue stable isotope values similar to many of the invertebrate heterotrophs collected with the same tube worm aggregations. However, the hagfish was the most ^{13}C -, ^{15}N - and ^{34}S -enriched of any animal captured (Table 2).

DISCUSSION

Very little is known about the feeding biology of most deep-sea animals, particularly the relatively newly discovered fauna of hydrocarbon seeps and hydrothermal vents. Much can be learned from behavioral observations and stomach content analyses, but these approaches are of limited use in these environments because of the difficulty of obtaining the former

and the fact that stomach contents of animals that eat bacteria are almost impossible to characterize, especially hours after the animals are collected. Additionally, captured consumers often have a chance to eat damaged animals in the collection containers, so the most recent meal may not be indicative of their normal food source (authors' pers. obs.).

Stable isotopes cannot tell you what an animal has been eating; however they can constrain the possibilities, and sometimes tell you what an animal has not been eating, as well as provide general information on food sources. Because of the remoteness of the communities we are studying, stable isotopes are an excellent way to start, particularly with the addition of $\delta^{34}\text{S}$ to the more commonly reported $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. This study provides basic information about what fuels the hydrocarbon seep communities in the Gulf of Mexico. However, our results must be interpreted within the context of what is known about the life history and feeding biology of the taxa, and tested by further research.

Chemoautolithotrophic symbiont-containing invertebrates

The tube worms in the collections were on the ^{13}C -enriched side of the range of values that have been reported for these species on the

Louisiana Slope (Brooks et al. 1987, Kennicutt et al. 1992). The fact that the tube worm species are ^{13}C -enriched relative to the thiotrophic-symbiont-containing bivalve (*Tamu fisheri*) collected at the same site is consistent with patterns seen in hydrothermal vent communities. As with the hydrothermal vent tube worms and mussels, this difference is likely due to (1) the fact that the symbionts in the 2 groups use different forms of RibuloseBisPhosphate carboxylase/oxygenase (that fractionate to different extents), and (2) differences in morphology and physiology resulting in differential dissolved inorganic carbon (DIC) supply and exchange (Fisher et al. 1990, Robinson & Cavanaugh 1995, Robinson et al. 1998, 2003). It is also possible that the 2 groups are taking up DIC that is quite different in $\delta^{13}\text{C}$. In addition to the DIC in the seawater, inorganic CO_2 can be produced from methane oxidation and

degradation of organic material in the sediments or at the sediment–water interface. As a result, $\delta^{13}\text{C}$ values for DIC in shallow sediments as low as -27.3‰ have been reported for this site (Sassen et al. 1999). *T. fisheri* is a relatively small species that lives partially buried with its siphon very close to the sediment–water interface, where DIC $\delta^{13}\text{C}$ values would reflect input from both interstitial and seawater sources. The 2 tube worm species could take up DIC either across their plumes (a well-vascularized gill-like gas exchange organ) or across their posterior ends (roots), which can be deeply buried in the sediment (Julian et al. 1999, Freytag et al. 2001). In the case of *Seepiophila jonesi* the plumes are located at the sediment–water interface while *Lamellibrachia luymesii* grows with its plume well above the sediment, up to a meter or more above the sediment in older aggregations (Bergquist et al. 2003). *L. luymesii* tissue carbon was significantly enriched in ^{13}C compared to *S. jonesi* in the older tube worm aggregation (Table 1). This is consistent with the plume being the primary DIC uptake organ and a relatively ^{13}C -depleted DIC pool at the sediment surface interface as a result of methanotrophic activity in the sediments. The difference in $\delta^{13}\text{C}$ values between species in the younger tube worm aggregation was not significant, but showed the same trend. The other mussel collected with one of the aggregations, *Bathymodiolus childressii*, harbors methanotrophic symbionts and its tissue $\delta^{13}\text{C}$ values reflect the $\delta^{13}\text{C}$ of methane at this site (-44 to -46‰ , Sassen et al. 1999).

As for carbon, differences between the tissue stable N isotope values in the 2 species of tube worms suggest that they are either utilizing different chemical species of N, tapping different pools of N, or discriminating differently after acquisition of their N source. We cannot distinguish between these possibilities at this time, but note that the different growth forms and resultant plume positions with respect to the sediments, described above for the 2 species, may contribute to this difference.

There is thought to be little fractionation between sulfide and sulfate or organic sulfur as a result of sulfide oxidation by chemoautolithotrophic bacteria (reviewed in Canfield 2001). Therefore, the tissue $\delta^{34}\text{S}$ values of animals with chemoautolithotrophic sulfur-oxidizing symbionts probably reflect their reduced sulfur source. Although the $\delta^{34}\text{S}$ values of the 2 tube worm species were not significantly different, the averages were separated by 5‰ , suggesting that they may be tapping different sulfide sources. Further analyses are needed to verify this trend. The mussel *Tamu fisheri* appears to be enriched in ^{34}S compared to *Lamellibrachia luymesii* in the same aggregation, although the trend is not statistically significant. The trend towards

a difference suggests that *T. fisheri* and *L. luymesii* may be acquiring sulfide from different pools. *T. fisheri* sulfide acquisition would be limited to the sediment–water interface or at most a few centimeters deep into the sediments, and the trend of more ^{34}S depletion in tissues of *L. luymesii* supports previous reports of sulfide uptake by root tissue (Freytag et al. 2001). *Bathymodiolus childressii* from the same collection as *T. fisheri* were on average even more enriched in ^{34}S , which is consistent with sulfur acquisition via a depleted sulfate pool by these methanotrophic symbiont-containing animals (average $\delta^{34}\text{S} = -16.9$ and -5.3‰ for *T. fisheri* and *B. childressii*, respectively, $p = 0.02$; however, this was not significant using the Dunn procedure for individual comparisons within a Kruskal-Wallis test). It should be kept in mind, however, that *B. childressii* (and likely *T. fisheri*) are capable of filter feeding and so any of their tissue stable isotope values could be affected by input from other sources (Page et al. 1990, Pile & Young 1999).

Heterotrophs

When all 3 stable isotopes are considered together, it is clear that all invertebrates collected in close association with the tube worm aggregations for this study are most likely obtaining the bulk of their nutrition from chemosynthetic production, as are 2 of the 3 fishes (Tables 1 & 2). In the cases where the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values alone could not differentiate between input of photosynthetic and chemosynthetic material (*Alvinocaris stactophila* and *Phymorhynchus* sp. in the younger bush and a Nemertean and a polynoid worm in the older bush; Table 2), very depleted $\delta^{34}\text{S}$ values (relative to ambient bottom water sulfate [20.3‰]; Aharon & Fu 2003) clearly indicate the importance of local primary production to the nutrition of these species, although they are greatly ^{34}S -enriched relative to the tube worm tissues. These results are somewhat similar to those of Micheli et al. (2002) who found that at hydrothermal vents on the East Pacific Rise, predators closely associated with the tube worms derive a majority of their nutrition from chemosynthetic material. However, unlike some of the invertebrate heterotrophs examined in Micheli et al. (2002), which would consume tube worm tissue under their experimental conditions, the chemosynthetic production consumed by invertebrate heterotrophs at these cold seeps is unlikely to include tube worms. The $\delta^{34}\text{S}$ values of these organisms could indicate a mixing of ^{34}S -depleted chemosynthetic production and ^{34}S -enriched photosynthetic production, however, the $\delta^{34}\text{S}$ values are much too ^{34}S -enriched for tube worm tissue to be a primary source of nutrition. This is consistent with the

observations of Kicklighter et al. (2004) who found that hydrocarbon seep tube worms (*Seepiophila jonesi* and *Lamellibrachia luymesii*) were unpalatable to predators such as mummichogs and the lesser blue crab. They propose that these tube worms either have chemical compounds in their tissues that deterred feeding (extracts of trophosome were unpalatable to both predators) or that the toughness of the tubes deterred predation. Lack of predation on tubeworms is also consistent with the long lives and great ages achieved by tube worms at these seeps (Bergquist et al. 2000, Cordes et al. 2003). The only species collected along with the tube worm aggregations that is not likely to obtain the bulk of its nutrition from chemoautotrophic production is the hagfish *Eptatretus* sp. (Table 2). This is consistent with the results of previous studies that examined the role of seep nutrition in a hagfish and a variety of other mobile benthic consumers in the Gulf of Mexico (MacAvoy et al. 2002, 2003).

The combined use of all 3 isotopes is also critical in evaluation of the possible role of the symbiont-containing animals in the nutrition of the heterotrophic fauna. Based on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values alone, one might erroneously conclude that the predatory/scavenging gastropod *Phymorhynchus* sp., and perhaps some individuals of the predatory *Eosipho canetae* and the grazing *Bathynnerita naticoidea*, were feeding primarily on the tube worm-derived carbon and nitrogen. However, the very large difference between the tissue $\delta^{34}\text{S}$ values of the tube worms and these consumers indicate that this is very unlikely, although a mixed diet of tube worms and surface-derived material is consistent with the isotope data (Fig. 2a). Taken together, the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ data also suggest that the only species that are likely to be obtaining significant dietary carbon and sulfur directly from the symbiont-containing bivalve species are the starfish *Sclerasterias tanneri* and the Amphinomid polychaete (Table 2). Micheli et al. (2002) found that hydrothermal vent mussels were also palatable to some invertebrate predators, particularly crabs. *Munidopsis* sp. and an Atelecyliid crab in this study had high tissue $\delta^{15}\text{N}$ values, reflecting their relatively high trophic level, however, their elevated $\delta^{34}\text{S}$ relative to tube worms, and high $\delta^{13}\text{C}$ relative to mussels, indicate that neither of these chemoautotrophic-containing invertebrates was the dominant food item for these predators. This is further supported by Bergquist et al. (2003) who note that among the thousands of hydrocarbon seep tube worms examined in their study, none showed any signs of predation (unlike their observations in hydrothermal vent *Ridgeia piscesae* communities).

Based on what is known of isotopic discrimination between trophic levels, the feeding biology of some of the species analyzed, and the limited mobility of those

species, one can constrain the range of stable isotope values in the free-living bacteria present on the tube worm tubes and in the sediment beneath the aggregation. This analysis is based on 1‰ enrichment associated with carbon, no enrichment associated with sulfur and 3‰ enrichment associated with nitrogen per trophic level (Minagawa & Wada 1984, Peterson & Howarth 1987). The stable isotope values of the 3 grazing gastropods captured within the tube worm aggregation (*Bathynnerita naticoidea*, *Provanna sculpta* and *Cataegis meroglypta*) suggest an isotope range for the material consumed off of tube worm tubes of -20 to -32 ‰ C, 0 to 7 ‰ N, and -14 to -1 ‰ S (Table 1, Fig. 1). The elevated $\delta^{15}\text{N}$ of some *B. naticoidea* relative to *P. sculpta* and *C. meroglypta* may reflect *B. naticoidea*'s ability to consume small animals as well as bacteria as polychaete setae, crustacean legs and sponge spicules have been reported as gut contents for this species (Zande 1994, Zande & Carney 2001). Very little is known about the ecology of *P. sculpta* (Waren & Bouchet 2001) other than the fact that this small gastropod (usually 5 mm in length or less) is endemic to the Louisiana hydrocarbon seeps (Carney 1994) and that it probably grazes in a similar manner as *C. meroglypta* (Waren & Bouchet 1993). Interestingly the tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *P. sculpta* individuals grouped very tightly around -32 ‰ and 4 ‰ respectively, and the 1 individual analyzed for $\delta^{34}\text{S}$, was very highly depleted in ^{34}S . Either this species is feeding very selectively on a specific population of the free-living bacteria present, or perhaps it has its own symbionts. The tissue $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values of the deposit-feeding sipunculid *Phascolosoma turnerae* suggest that the range of values of organic material present in the shallow sediments directly below the tube worm aggregations are -30 to -28 ‰ C, 0 to 5 ‰ N and -14 to -2 ‰ S (Table 2). In addition to providing insights into the biology of some of the individual species present at the seeps, this study clearly demonstrates the power of using a third isotope (S) for trophic analysis of seep, and presumably hydrothermal vent, communities. Since the seep communities were discovered in 1985 (Kennicutt et al. 1985, Brooks et al. 1987) photographs and observations have indicated that some fauna were consistently associated with the seeps. The elevated biomass and presence suggested that the closely associated fauna would derive the bulk of their nutrition from local chemoautolithotrophic production, even though these communities are present in relatively shallow waters where input of photosynthetic material is likely to be significant. This study supports the hypothesis that fauna tightly associated with tube worm aggregations at cold seeps in the Gulf of Mexico obtains the bulk of its nutrition from local source of primary production. This study is consistent with previous

stable isotope studies of hydrothermal vent communities in implicating free-living bacteria with a wide range of stable isotope values in the nutrition of the community (Fisher et al. 1994, Van Dover & Fry 1994). Finally, this study suggests that the tube worms themselves are not an important source of nutrition for any of the species analyzed, a finding that is compatible with the very long lives of the seep vestimentiferans.

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