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Multiple trophic resources for a chemoautotrophic community at a cold water brine seep at the base of the Florida Escarpment

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Abstract

The biological community that surrounds the hypersaline cold water brine seeps at the base of the Florida Escarpment is dominated by two macrofaunal species: an undescribed bivalve of the family Mytilidae and a vestimentiferan worm, *Escarpia laminata*. These animals are apparently supported by the chemoautotrophic fixation of carbon via bacterial endosymbionts. Water column and sediment data indicate that high levels of both sulfide and methane are present in surface sediments around the animals but absent from overlying waters. Stable isotopic analyses of pore water indicate that there are two sources of sulfide: the first is geothermal sulfide carried in groundwater leaching from the base of the escarpment, and the second is microbial sulfide produced in situ. The vestimentiferan *E. laminata*, and the mytilid bivalve (seep mussel) live contiguously but rely on different substrates for chemoautotrophy. Enzyme assays, patterns of elemental sulfur storage and stable isotopic analyses indicate that *E. laminata* relies on sulfide oxidation and the seep mussel on methane oxidation for growth.

Introduction

Dense biological communities were recently discovered at the base of a near vertical escarpment (>3 000 m) which forms the western edge of the Northern Florida-Bahama Platform (Paull et al. 1984). While many of the animals in these communities are morphologically similar to symbiont-bearing species found at hydrothermal vents of the Eastern Pacific (Lonsdale 1977, Corliss et al. 1979, Spiess et al. 1980, Hessler 1984, Hecker 1985), the sources of energy for the Florida community are not fully known. Highly saline brines seep out at the base of the Florida escarpment in localized channels (Paull et al. 1984, Commeau et al. 1987). It has been suggested that the brines carry reduced substrates such as sulfide and methane which are formed deep within the platform. While sediment porewater samples clearly contain high levels of ammonia, methane and sulfide, the concentrations of reduced substrates in the undiluted brine have been difficult to measure and the contribution of local sediment microbial processes has not been considered.

Due to the density of the brines, the reduced compounds appear to be confined to the sediments with little measurable diffusion into the water column above. This differs dramatically from the hydrothermal vent systems where thermal advection drives geothermally produced hydrogen sulfide out of the substratum into the overlying water (Orr 1975). Although the geology of the Florida escarpment communities is completely different from that of the hydrothermal vents, in both systems the sustained availability of reduced compounds supports rapid growth of chemosynthetic bacteria and large macrofauna (Hecker 1985).

Marine invertebrates of many types contain chemoautotrophic bacteria as symbionts. Examples include bivalves (Felbeck et al. 1981, Felbeck 1983), vestimentiferans (Cavanaugh et al. 1981, Felbeck 1981), pogonophorans (Southward et al. 1981), oligochaetes (Felbeck et al. 1983) and, most recently, gastropods (Stein et al. 1988). The presence of both highly reduced chemical compounds and molecular oxygen is important in sustaining these symbioses. A common feature of these organisms is the occurrence of internal bacterial symbionts, which utilize energy gained from the oxidation of reduced compounds for the fixation of carbon dioxide and production of cell carbon. Reduced sulfur species have been identified as substrates for energy production by symbionts of several hydrothermal vent species, *Riftia pachyptila*, *Bathymodiolus thermophilus*, and *Calyptogena magnifica* (Powell and Somero 1986). More recently, the symbiont-containing gill tissue of an undescribed mussel species taken from the Louisiana hydrocarbon seep areas has been shown to consume methane (Childress et al. 1986, Fisher et al. 1988). These mussels can also grow with methane as the sole carbon and energy source (Cary et al. 1988). Microscopic, enzymatic, and isotopic analyses of mussels taken on an earlier cruise from the Florida escarpment com-

munities showed that the endosymbionts residing in the gills are Type 1 methylotrophs (Cavanaugh et al. 1987). The nutritional strategy of the vestimentiferan *Escarpia laminata* common at the Florida seeps, was not investigated.

In the present study we compare the metabolic strategies of the two dominant species, the mytilid bivalve (seep mussel) and the vestimentiferan tube worm *Escarpia laminata*. Biochemistry and physiology are matched with geochemistry to provide an understanding of the metabolic requirements of these organisms. Isotopic and enzymatic evidence confirms the presence of both sulfur and methane based symbioses within the same localized microhabitat. The biogeochemical sources of methane and sulfur are also examined.

Materials and methods

Field collections

The tube worms used in this study were collected by the DSRV "Alvin" during October 1986 in conjunction with a systematic geological survey of the dense biological communities found associated with brine seeps at the base of the Florida Escarpment (26°02'N, 84°55'W). At depth (3 300 m), living individuals of the tube worm *Escarpia laminata* and an undescribed mytilid were collected, often in conjunction with pore water and sediment samples. The animals were placed in a thermally insulated PVC bucket which was sealed to retain bottom water until the submersible reached the surface (2 to 3 h). Individuals were removed immediately and held on ice in a glove bag under a nitrogen atmosphere to prevent oxidation of reduced substrates. All samples were separated by dive site in order to correlate the biology with the environmental geochemistry.

The valves of the smaller mytilids had an unusually tight seal and would retain the mantle water on the ship for long periods. The valve edges of the larger individuals were often damaged or deformed allowing leakage. Every effort was made to collect the mantle water from the younger, more uniform individuals in order to determine a minimum estimate of reduced substrates which might be available to the mussels on the sea floor. Mantle water was extracted using a syringe prior to the sacrifice of each mussel. Bucket water was also sampled to compare with mantle water in order to determine if any exchange had taken place. In the glove bag, blood was extracted from the mussels by slicing the mantle and collecting the draining fluid. Tube worms were carefully removed intact from their protective tubes and the body wall cut in order to collect a clean blood sample. Portions of the *Escarpia laminata* blood were frozen in liquid nitrogen to be assayed for the presence of sulfide binding proteins. Samples of animal tissue with symbionts as well as animal tissues without symbionts were immediately removed from each specimen and frozen in liquid nitrogen for later enzymatic and stable isotopic analyses.

Pore water from sediments adjacent to the animals was collected using several techniques. An in situ pore water sampler (WIMPER) was used on most occasions to obtain replicate samples of the upper 3 to 4 cm of sediment over approximately a 0.25 m^2 area. Standard push cores were also taken by DSRV "Alvin" and later sectioned once on the surface. Porewater from these core samples was obtained by squeezing (Reeburgh 1967). Water from above the sediment was collected with modified Niskin bottles and Edmond water samplers.

The major objective of this study was to determine the metabolic strategies of the two dominant macrofaunal species in different microhabitats. Samples chosen for analysis were from dives that had a complete video recording of the collection site and collection procedure. Enzymatic and isotopic analyses were performed on the same documented individuals.

Enzyme assays

The frozen tissues were analyzed at 22 °C for the activity of several enzymes that characterize chemoautotrophic potential. For sulfur-based chemoautotrophy, two enzymes were assayed: ATP-sulfurylase, important in catalyzing the synthesis of ATP in systems where energy is obtained from the metabolism of sulfur compounds, and RUBP carboxylase, the diagnostic enzyme for net fixation of carbon dioxide via the Calvin-Benson cycle. The techniques used for both assays have been previously described by Felbeck (1981). Methanol dehydrogenase is a key enzyme in methylotrophs, used in the oxidation of methanol. Methanol dehydrogenase activity was assayed by the spectrophotometric method of Anthony and Zatman (1965).

The capacities of different tissues to oxidize sulfide was determined by the method of Powell (Powell and Somero 1985, Vetter et al. 1987). This assay follows spectrophotometrically the reduction of the artificial electron acceptor benzyl viologen by sulfide. A 1:9 homogenate of tissue in 20 m*M* Tris-HCl buffer, pH 8, with 0.1% Triton X-100 was used for all determinations. The reaction buffer was 40 m*M* glycine pH 9, with 5 m*M* sulfide and 2 m*M* benzyl viologen. Typically 20 μl of homogenate were added to a total volume of 1 ml of reaction buffer. The linear reduction of benzyl viologen was followed at 578 nm using an extinction coefficient of 8.65 absorbance units $mmol^{-1}\ cm^{-1}$ (McKellar and Sprott 1979). The increase in absorbance was equivalent to the rate of sulfide oxidation. All assays were carried out on tissues that had been frozen and maintained at −80 °C or less.

Blood and mantle water thiol determination

The concentrations of different inorganic reduced sulfur compounds such as sulfide, thiosulfate, and sulfite were determined for both blood and mantle water by fluorometric derivatization followed by HPLC (Vetter et al. 1987), which is a modification of the original technique of Fahey et al. (1987). Samples were derivatized with monobromobimane

on board and stored frozen. On return to the laboratory, the derivatized samples were separated by HPLC on a C-18 reversed phase column and a methanol-water gradient and detected and quantified by fluorescence.

Sulfur determinations

Elemental sulfur contents of animal tissues were determined by benzene extraction (Vetter 1985). Freeze-dried tissue samples were extracted two times in benzene for 24 h. The resulting supernatants were combined, evaporated to dryness with nitrogen and redissolved in a known volume of benzene. Aliquots of the extract were spotted onto strips of filter paper and assayed according to Schedel and Trüper (1980).

The total sulfur content of the different tissues was determined by oxidation (Vetter 1985). Briefly, bacterial symbiont containing tissues and animal tissues were separated and freeze-dried. The freeze-dried tissues were combusted in a Parr bomb calorimeter under 30 atm of oxygen. The resulting sulfate was precipitated as barium sulfate and the total sulfur content was determined gravimetrically. Resulting precipitates were decomposed to SO_2 for isotopic analysis (Fry et al. 1983). $\delta^{34}S$ values are reported relative to Canyon Diablo trolite, with a precision of $\pm 0.2‰$.

Stable carbon isotope composition

Portions of the frozen tissues were later freeze-dried and prepared for stable carbon isotope composition. Portions of the tissues (3 ± 2 mg) were combusted at 850 °C for 3 h in evacuated, sealed quartz ampules in the presence of copper, copper (II) oxide and silver as described by Stump and Frazer (1973) and Northfelt et al. (1981). Carbon dioxide was purified, measured volumetrically, and sealed in pyrex tubes for subsequent mass-spectrometric analysis of the stable isotope ratios. All carbon isotopic results are expressed relative to PDB with a precision of $\pm 0.1‰$.

Blood binding

The presence of sulfide-binding proteins in the blood of symbiont-containing invertebrates and the techniques for their analyses has been described previously (Arp et al. 1984, 1985, 1987, Arp and Childress 1983, Childress et al. 1984). The method of analysis described in this paper is a simple dialysis method using ^{35}S-radiolabeled sulfide and does not require a gas chromatograph. Blood samples and buffer controls were placed in 1 ml cylinders of dialysis tubing with a solid plug in the bottom and a plug at the top which allowed sampling *via* a Hamilton syringe. The dialysis bag was suspended in a thermally controlled stirring reservoir of 15 ml total volume. The outer volume was filled with a "Riftia" Ringer's solution pH 7.5 (Arp et al. 1987). Radiolabeled and cold sulfide was added to a concentration of 100 μM in the outer buffer. The rate of diffusion and ultimate equilibrium was determined by repeated sampling and scintillation counting of the ^{35}S radioactivity in the dialysis cylinder containing the same buffer. The binding capacity of the blood was determined by replicate sampling of the blood container. The binding capacity was the difference in counts between the buffer and blood.

Table 1. *Escarpia laminata* and seep mussel enzyme activities ($\pm$SD) and sulfur contents ($\pm$SD) of seep organisms. All enzyme activities[a] are expressed as units per gram fresh weight, and both total and elemental sulfur contents[b] are expressed as percent dry wt of each tissue. 0 = not detected

	Escarpia laminata		Seep mussel	
	Trophosome	Vestimentum	Mantle	Gill
ATP Sulfurylase[a]	14.05 ± 4.5	0	0	0
RUBP Carboxylase[a]	0.1 ± 0.026	0.01 ± 0.002	0	0
Methanol Dehydrogenase[a]	0	0	0	24.45 ± 6.43
Sulfide Oxidase[a]	6.15 ± 1.25	1.24 ± 0.16	0.05 ± 0.16	1.69 ± 0.42
Total Sulfur[b]	2.36 ± 0.73	2.41 ± 0.34	0.72 ± 0.06	0.97 ± 0.06
Elemental Sulfur[b]	1.18 ± 0.32	0	0	0
n	4	3	5	5

[a] Enzyme activity expressed as units X gFW^{-1}
[b] Expressed as percent of dry weight

Results

Measurements of enzyme activity

Methanol dehydrogenase, a common enzyme of the bacterial pathway for methane oxidation, was used as the diagnostic test for the presence of methylotrophic bacteria. Methanol dehydrogenase activity was 24.45 ± 6.49 μmoles min^{-1} g^{-1} fresh wt ($n = 4$) in mussel gill tissue. No activity could be detected in the mantle tissue of the same mussels or in either tissue of *Escarpia laminata* (Table 1). The presence of methanol dehydrogenase confirms the methylotrophic potential of the symbiont containing gill tissue.

RUBP carboxylase and ATP sulfurylase are the two diagnostic enzymes most often used to confirm sulfur based chemoautotrophy. The trophosome tissue of *Escarpia laminata* was the only tissue of those analyzed with significant activity of either of these enzymes (Table 1).

Hydrogen sulfide is a potent inhibitor of cellular respiration. The benzyl viologen assay of Powell and Somero (1985) gives a good estimate of total sulfide oxidizing potential of a tissue. It does not discriminate between detoxifying

and energy exploiting aspects of sulfide oxidation. In the tissues examined, sulfide oxidase activity was highest in *Escarpia laminata* trophosome where levels were over five times greater than in vestimentum tissue. Sulfide oxidase activity in the seep mussel gill was similar to that of *E. laminata* vestimentum.

Sulfur concentrations

Eucaryotic organisms are not known to contain elemental sulfur. Although not all chemoautotrophic sulfur bacteria store elemental sulfur, the presence of large elemental sulfur reserves in an animal tissue is a clear indication of the presence of sulfur oxidizing bacteria in that tissue. The trophosome tissue of *Escarpia laminata* contained 1.18% of the dry wt as elemental sulfur. This was the only tissue that contained elemental sulfur (Table 1). The total sulfur pool can contain normal sulfur containing amino acids, sulfolipids and mucopolysaccharides as well as elemental sulfur, bound sulfide, membrane bound polysulfides and sulfide oxidation products such as thiosulfate. The total sulfur contents of both animal (vestimentum) and bacterial (trophosome) tissues of *E. laminata* were high, reflecting the active sulfur metabolism in both compartments of the symbiosis. The sulfur content of the seep mussel was low in both gill and mantle tissue (Table 1).

Analysis of seep mussel mantle water and blood

Since the brines of the Florida seeps are denser than seawater, they retain most of the reduced compounds in or near the sediment surface. Blood and mantle water data aid in understanding the levels and variability of sulfide and thiosulfate in mussels lying on the surface of the rich sediments. Although the mantle water levels are at best a minimum estimate, due to the potential for flushing and metabolism during transport to the surface, the presence of sulfide or its oxidation product thiosulfate would be a clear indication that mussels are exposed to hydrogen sulfide. The blood and mantle water of selected mussels were analyzed for sulfide and thiosulfate. The sulfide level in mussel blood was extremely variable (range 2.8–45 μM with a mean of 17.9 ± 19.57, $n=13$) both between and within collection sites. Similarly, thiosulfate had a broad range (3.0–42 μM, with a mean concentration of 13.7 ± 16.77, $n=13$) in the blood from the same specimens. The mantle water data can only confirm that, in the mussels examined, the gill tissues are routinely exposed to sulfide and thiosulfate. In addition, gas chromatographic analyses of the mantle water revealed low levels of methane (2.2 μM, J. Chanton, personal communication).

Hydrogen sulfide cannot be measured quantitatively by the technique of monobromobimane derivatization in blood containing a strong sulfide binding protein (see below). The proper technique of acidification and subsequent gas chromatographic analysis (Arp and Childress 1983) was not available on board. Consequently, sulfide concentrations measured in freshly recovered *Escarpia laminata* could not be measured reliably.

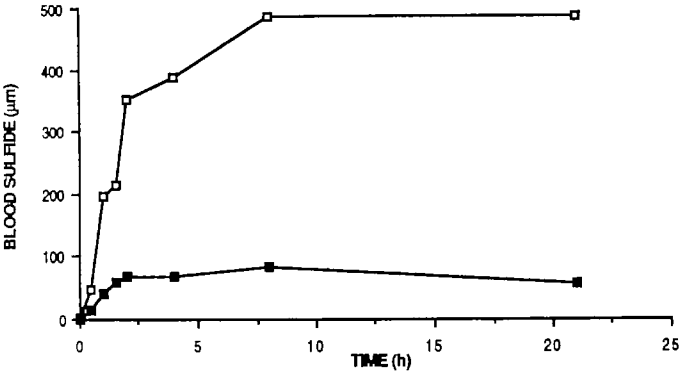

Fig. 1. *Escarpia laminata.* Sulfide binding capacity of blood (□) and "Riftia" saline control (■) as function of dialysis duration

Table 2. *Escarpia laminata* and the seep mussel stable isotope compositions. Mean values ($\pm$SD) for $\delta^{13}C$ (PDB) and $\delta^{34}S$ (Canyon Diablo troilite) are given for each tissue sampled. n=number of individuals analyzed

	Escarpia laminata		Seep mussel	
	Trophosome	Vestimentum	Mantle	Gill
$\delta^{13}C$	-47.2 ± 2.4	-44.8 ± 3.5	-73.0 ± 2.2	-72.9 ± 1.1
$\delta^{34}S$	-11.2 ± 2.4	-10.0 ± 0.6	8.2 ± 1.6	9.8 ± 1.6
n	4	3	5	5

Sulfide binding protein in *Escarpia laminata*

Frozen samples of seep mussel blood and *Escarpia laminata* blood were tested for the presence of sulfide binding protein via equilibrium dialysis against ^{35}S-radiolabeled sulfide. *E. laminata* contains a sulfide binding protein which concentrates sulfide six fold over the external concentration (Fig. 1). A similar experiment with the seep mussel showed no binding.

Stable isotopes

The measurement of stable isotopic composition can give insights into the source and pathway of CO_2 fixation and the geological or biological source of the reduced carbon and sulfur substrates utilized by different symbioses.

The carbon stable isotopic values for *Escarpia laminata* and the seep mussel (Table 2) were much lighter (depleted in ^{13}C) than -15 to $-20‰$ values of marine animals that obtain their carbon either directly or indirectly from photosynthetically-derived plant material (Fry and Sherr 1984). Both seep mussels and *E. laminata* exhibited $\delta^{13}C$

values significantly lower than these normal −15‰ to −20‰ values. *E. laminata* trophosome and vestimentum averaged −47.2±3.9 (SD), while seep mussel values were significantly lower at −73.0±2.2‰ and typical of methlylotrophic carbon metabolism.

Sulfur isotopic compositions also differed between the two dominant macrofaunal species. The *Escarpia laminata* vestimentum and the symbiont containing trophosome tissues had low $\delta^{34}S$ values (−11.2 and −10.0‰, respectively). In contrast, the seep mussels had higher $\delta^{34}S$ values of +9.9‰ for the gill and +8.2‰ for the mantle (Table 2).

An undescribed vesicomyid clam, *Calyptogena* sp., occurs in tan sediments separate from the mussel beds which occur on black sediments. These animals are not abundant and no enzyme or sulfur concentration data were obtained. Stable isotopic compositions of *Calyptogena* sp. ($\delta^{13}C = -36.7$‰, $n=3$; $\delta^{34}S = -16.0$‰, $n=1$) proved similar to those measured for *Escarpia laminata*.

Discussion

Metabolic comparison of the two species

Both sulfur and methane-based symbioses appear active at the Florida seep site. The results of enzyme assays, blood and mantle water measurements, and sulfur contents clearly indicate that *Escarpia laminata* utilizes hydrogen sulfide while methane is the dominant energy source for the seep mussel.

The levels of methanol dehydrogenase in seep mussel gill were much higher than those previously reported by Cavanaugh et al. (1987). A possible explanation for the difference is that their material was stored for over 1 yr prior to measurement, while the tissues in this study were transported in liquid nitrogen and assayed within three weeks of collection. No methanol dehydrogenase activity was detected in the non-bacterial mantle tissue or in either the trophosome or vestimentum of *Escarpia laminata*.

The levels of RUBP carboxylase and ATP sulfurylase reported here for the trophosome of *Escarpia laminata* are similar to those of the hydrothermal vent tube worm *Riftia pachyptila* (Felbeck 1981). *E. laminata* is morphologically similar to *R. pachyptila* where sulfur utilizing bacterial endosymbionts exist in the trophosome tissue (Cavanaugh et al. 1981). The slight RUBP carboxylase activity in the vestimentum of *E. laminata* may be the result of contaminating epibiotic bacteria or incomplete separation of the vestimentum from the trophosome during dissection. These key diagnostic enzymes for autotrophic CO_2 fixation based on sulfur oxidation were not detected in either of the mussel tissues tested. Methane oxidizing bacteria typically either fix carbon via the ribulose monophosphate or serine pathways.

Within a given tissue, sulfide oxidase activity represents the total capacity for oxidation of sulfide (Powell and Somero 1985). The highest levels of sulfide oxidase activity are in bacteria containing tissues that utilize sulfide directly for energy production. However, there is some sulfide oxidase activity in all tissues exposed to high environmental sulfide concentrations. This activity represents the detoxification requirements of aerobic tissues (Powell and Somero 1986, Vetter et al. 1987). The high sulfide oxidase levels in *Escarpia laminata* trophosome reflect a metabolism similar to *Riftia pachyptila* wherein most sulfide is delivered directly to the bacteria via a sulfide-binding protein. *E. laminata* has higher sulfide oxidase activity in the body wall than *R. pachyptila*. This may be because *E. laminata* lives partially buried in sulfide-rich sediments (>3.0 m*M*), whereas *R. pachyptila* grows attached to hard substrate and is exposed to much lower external sulfide concentrations (100 to 300 μM). The seep mussel has similar low sulfide oxidase levels in both gill and mantle tissue. This level of sulfide oxidase activity is typical of animal tissues from other high sulfide environments (Powell and Somero 1986, Vetter et al. 1987). The presence of sulfide in the mantle water, and its detoxification product (thiosulfate) in the blood, are clear indications that the seep mussel has the capacity to oxidize sulfide even though its symbionts metabolize methane.

Two other clear indications that *Escarpia laminata* relies upon a sulfur based bacterial endoymbiosis are the presence of large deposits of elemental sulfur in the trophosome and a circulating sulfide-binding protein in the blood. The presence of elemental sulfur is found only in procaryotes with a sulfur based metabolism. Sulfide-binding proteins have only been found in bivalves and vestimentiferans which harbor sulfur bacteria (Arp and Childress 1983, Arp et al. 1987). Neither of these traits is present in the seep mussel.

Stable isotopic comparison of the vestimentiferan and the seep mussel

The measurement of the stable isotopic compositions of both bacterial and animal compartments of the seep mussel and *Escarpia laminata* supplements the metabolic data by providing proof of long-term metabolic strategies of the bacteria and by showing the transfer of nutrients from bacterial symbiont to host. In addition, a comparison of the isotopic compositions of substrates in undiluted brines and sediment porewaters with the isotopic compositions of the bacterial tissues can indicate the ultimate sources of the methane and sulfide utilized by the symbionts.

The carbon isotopic composition of the bacteria containing tissues, the trophophosome in *Esparpia laminata* and the gill in the seep mussel, were not significantly different from the respective host tissue indicating a high degree of carbon flow from bacterial symbiont to host (Fig. 2). While the carbon isotope values of symbiont and host were very similar, the different isotopic composition of *E. laminata* and the seep mussel reflect their different microbial strategies of carbon fixation. The very low −72‰ mussel values are consistent with a methane-based nutrition (Paull et al. 1985), while the −44‰ to −47‰ values for *E. laminata* are near those expected for sulfur-based symbioses (Brooks et al. 1987).

While the Lousiana methane is petroleum associated, the Florida methane is substantially lighter and reflects a mi-

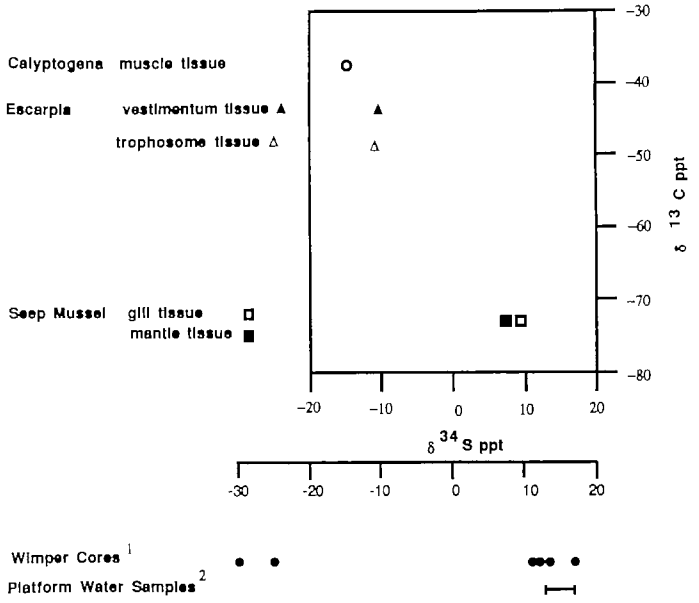

Fig. 2. *Escarpia laminata* and seep mussel. Double axis plot of stable carbon ($^{13}C/^{12}C$) and Sulfur ($^{34}S/^{32}S$) isotope ratios for host and symbiont containing tissues. Each symbol represents mean for a specific tissue. Open symbols = symbiont containing tissue and filled symbols = host tissue. Included is single measurement of seep Vesicomyid, *Calyptogena* sp. Lower plot represents distribution of sulfur isotope ratios in sediment[1] and platform pore water[2] (J. Chanton unpublished data, Claypool et al. 1980)

crobial origin. No oil seeps were observed at the Florida platform site. Previous studies of the methane utilizing mussels from the Louisiana hydrocarbon seep sites showed a very close similarity between animal and methane $\delta^{13}C$ values (−39‰ animal vs −41‰ methane; Brooks et al. 1987). At the Florida site, the seep mussel isotope values (−72‰) also resembled the values reported for the sediment methane (−80‰ to −90‰; J. Chanton, pers. comm.) but not to the extent of the Louisiana congeners. At least two possibilities could explain this discrepancy. First, microbial consumption of methane at the sediment-water interface could lead to ^{13}C enrichment of residual methane actually available to mussel symbionts (Zyakun 1981). Secondly, particulate material filtered by the mussel could contribute as an additional carbon source and could also conceivably cause an isotopic deviation from the main methane supply.

Brooks et al. (1987) have recently proposed that animals with sulfur-based chemoautotrophic symbioses have $\delta^{13}C$ values of −30 to −42‰, with lower values indicating reliance on methane. The −44 to −47‰ values found for *Escarpia laminata* in this study fall outside the −30 to −42‰ range proposed for sulfur symbioses even though the enzyme data clearly indicates sulfur based CO_2 fixation and not methylothrophy. On the basis of our results, we would extend the −30 to −42 range for sulfur-based symbioses to −47‰. In suggesting this extension, we parenthetically note that both isotopic fractionations and source isotopic compositions are important in determining these isotopic ranges, but that neither fractionations nor source isotopic compositions are well-known in most environmental surveys. As further studies of autotrophic bacteria determine fractionations expected for sulfide and methane oxidation, and better methods of sampling the water around organisms are developed, it should become possible to determine more precisely isotopic ranges expected from methane and sulfide-based nutrition.

In most aerobic zones of the ocean the source of reduced sulfur for organic compounds is the assimilatory reduction of seawater sulfate (28 m*M*) by marine phytoplankton and bacteria. Most organisms in the sea closely reflect the stable sulfur isotopic composition of seawater sulfate and have $\delta^{34}S$ values of +15‰ to +21‰ (Fry and Sherr 1984). Where sulfide is utilized by endosymbiont-bearing macrofauna surrounding hydrothermal vents, the animal tissue isotopic composition reflects the isotopic composition of the geothermally produced sulfide in the vent waters, rather than seawater sulfate (Fry et al. 1983). The stable sulfur isotopic composition of sulfide produced by microbial sulfate reduction is much lighter than either geothermally produced sulfide or seawater sulfate and can be traced in organisms that utilize this sulfur source.

Mesozoic anhydrite deposits taken from the Florida Platform at depths similar to the dive sites have $\delta^{34}S$ values ranging from +12.8 to +16.4‰ (Claypool et al. 1980) and it has been suggested that non-microbial reduction of sulfate dissolved out of anhydrites at elevated temperatures deep within the platform may produce sulfides isotopically similar to the original sulfate source (Orr 1975, Paull and Neumann 1987). Pore water samples taken from seep sediments indicate that in different areas of the seep field there is either an isotopic value indicative of microbially produced sulfide, $\delta^{34}S$ = −30 to −20‰ (Fig. 2), or that measured for sulfides in the brine aquifer, $\delta^{34}S$ = +10.9 to +16.7‰ (J. Chanton, unpublished data). Samples with sulfide isotopic compositions indicative of brine sulfide also had elevated chloride values typical of undiluted brine while porewater samples with a microbial sulfide isotopic signature have chloride concentrations similar to seawater (J. Chanton, unpublished data).

Sulfur isotopic compositions indicate that sulfides produced microbially in the sediments are likely an important sulfur source for *Escarpia laminata,* and the calyptogenid clam. Brine sulfur appears important for the seep mussel. The stable sulfur isotopic composition of *Escarpia laminata* is very light ($\delta^{34}S$ = −11.2‰). The sulfur isotopic composition of the calyptogenid clam from the Florida brine seeps was also light. Although we have no enzyme data for this species, congeners such as *Calyptogena magnifica* and *C. pacifica* are known sulfide utilizers (Childress and Mickel 1982, Arp et al. 1984). Given that the sulfur isotopic compositions of vestimentiferans and calyptogenid clams at hydrothermal vents reflect the isotopic composition of the sulfide source, we interpret the very light sulfur isotopic composition of the vestimentiferan worm and calyptogenid clam at the Florida brine seeps to indicate that the primary source of sulfide is microbial sulfate reduction and not geologically derived sulfide. In contrast, the seep mussel has a sulfur

isotopic composition that more closely resembles the geological sulfur from the brines, $\delta^{34}S = 9.85‰$ (Fig. 2).

The large isotopic separation that exists between *Escarpia laminata* and the seep mussel indicates that, although these two species live in close proximity to each other, they access completely different sources for their cellular sulfur. The +10‰ values of the mussels approximate those expected for animals that would be using brine sulfate and sulfides (+11 to +17‰), while the −10‰ values of *E. laminata* indicate a greater reliance on the microbial sulfides (−20 to −30‰).

In conclusion, our results show that a methane based symbiosis in the seep mussel and a sulfur based symbiosis in *Escarpia laminata* co-exist in close proximity within the Florida brine seep community. While the fact that these communities are clustered around brine seeps is evident, how the brines stimulate the growth of the community remains a mystery. Although the obvious explanation is that geologically produced sulfide and methane are carried out of the platform in the brines, the carbon and sulfide isotopic data indicate that much of the methane and sulfide is of recent microbial origin and in the case of carbon this in confirmed by the recent ^{14}C age of some of the methane (J. Chanton, personal communication). Sediments where microbial sulfide predominates may be areas where anaerobic oxidation of brine methane based on sulfate reduction occurs (Reeburgh 1983, Alperin and Reeburgh 1984). This would also generate the isotopically light CO_2 which is incorporated into *E. laminata*. The source of relatively recent microbially produced methane is more problematic.

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