The Pennsylvania State University
The Graduate School
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FOOD WEB ECOLOGY IN GULF OF MEXICO HYDROCARBON SEEP COMMUNITIES

A Dissertation in
Biology
by
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Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

August 2010
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ABSTRACT

Hydrocarbon seeps serve as oases of local primary production in the otherwise food-poor deep sea. Animals that have adapted to handle the toxic chemistry in these environments thrive and form dense high-biomass communities. In this thesis, stable isotopes were used to explore food web interactions in several seep-associated communities in the Gulf of Mexico. We used collection techniques that capture the entire local community associated with the target foundation species and subsampled from this community for stable isotope analysis. Tissue stable isotope values in the reef-building cold-water coral *Lophelia pertusa* refuted a long-standing hypothesis that cold-water corals rely on primary production from seeps around which they occur. They instead are probably taking advantage of the abundant carbonate rock substrate found at seeps. The communities associated with the corals also showed no indication of nutritional input from seeps.

Bathymodiolin mussel tissue $\delta^{13}C$ values showed that biogenic methane dominates the methane at lower-slope seeps and extremely low $\delta^{15}N$ values in one collection of *Bathymodiolus childressi* point toward an unusually abundant or isotopically depleted nitrogen source. Very low $\delta^{13}C$ values in the vestimentiferan tubeworms *Escarpa laminata* and *Lamellibrachia sp.* 1 support uptake of porewater dissolved inorganic carbon through their buried roots. Consistent differences in $\delta^{15}N$ between the co-occurring *E. laminata* and *Lamellibrachia sp.* 1 provide evidence for partitioning of inorganic nitrogen sources, a finding that has significant implications for the ecology and evolution of this taxonomic group. Extremely variable $\delta^{34}S$ values in vestimentiferans provide evidence that waste sulfate excreted from vestimentiferan roots is reduced to
sulfide by microbial consortia in the vicinity of the individual root, ensuring a sulfide supply to the vestimentiferans’ symbionts. Tissue $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values in heterotrophic animals collected with vestimentiferans, mussels, and vesicomyid clams showed that the majority of animals derive a significant portion of their nutrition from seep primary production.

The $\delta^{13}C$ values in the heterotrophs tracked the $\delta^{13}C$ of their associated symbiotic fauna, which varied substantially between collection locations. This indicates that most of the animals feed locally and that the $\delta^{13}C$ signature of the local hydrocarbon pool affect the $\delta^{13}C$ values of the entire community. The $\delta^{15}N$ values of heterotrophs collected with mussels were significantly more depleted than those collected with vestimentiferans both overall and within sites. This was consistent with an approximately 8‰ depletion in mussel tissue $\delta^{15}N$ values compared to vestimentiferan $\delta^{15}N$ values within the same sites. Together, these data indicate a difference in the isotope compositions of the inorganic nitrogen sources in the two microhabitats. The lack of isotope data for inorganic nitrogen at seeps prohibits further speculation into the underlying cause of this difference.
# TABLE OF CONTENTS

## LIST OF FIGURES

- vii

## LIST OF TABLES

- ix

## ACKNOWLEDGEMENTS

- x

## CHAPTER 1. Introduction to Gulf of Mexico cold seeps and stable isotopes in trophic studies

- 1
  - Gulf of Mexico Geological Setting: 1
  - Seep Ecology: 2
  - Stable isotopes in cold seep trophic studies: 3

## CHAPTER 2. Stable carbon and nitrogen isotope compositions of hydrocarbon seep bivalves on the Gulf of Mexico lower continental slope

- 9
  - Abstract: 9
  - Introduction: 10
  - Methods: 13
  - Results & Discussion: 15
  - Conclusions: 26

## CHAPTER 3. Stable isotopes provide new insights into seep vestimentiferan physiological ecology

- 31
  - Methods: 39

## CHAPTER 4. Trophic interactions in mussel, vestimentiferan, and clam communities at Gulf of Mexico lower-slope cold seeps

- 44
  - Abstract: 44
  - Introduction: 45
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methods</td>
<td>.................................................................................................</td>
<td>49</td>
</tr>
<tr>
<td>Results &amp; Discussion</td>
<td>.................................................................................................</td>
<td>54</td>
</tr>
<tr>
<td>Conclusions</td>
<td>.................................................................................................</td>
<td>68</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>.................................................................................................</td>
<td>69</td>
</tr>
<tr>
<td><strong>CHAPTER 5.</strong> Importance of seep primary production to <em>Lophelia pertusa</em> and associated fauna in the Gulf of Mexico</td>
<td>.................................................................................................</td>
<td>80</td>
</tr>
<tr>
<td>Abstract</td>
<td>.................................................................................................</td>
<td>80</td>
</tr>
<tr>
<td>Introduction</td>
<td>.................................................................................................</td>
<td>81</td>
</tr>
<tr>
<td>Methods</td>
<td>.................................................................................................</td>
<td>85</td>
</tr>
<tr>
<td>Results</td>
<td>.................................................................................................</td>
<td>91</td>
</tr>
<tr>
<td>Discussion</td>
<td>.................................................................................................</td>
<td>95</td>
</tr>
<tr>
<td>Conclusions</td>
<td>.................................................................................................</td>
<td>105</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>.................................................................................................</td>
<td>107</td>
</tr>
<tr>
<td><strong>CHAPTER 6.</strong> Trophic ecology in Gulf of Mexico cold seeps</td>
<td>.................................................................................................</td>
<td>116</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>.................................................................................................</td>
<td>126</td>
</tr>
<tr>
<td>APPENDIX A. Chapter 3 Vestimentiferan Geochemistry Schematic</td>
<td>.................................................................................................</td>
<td>139</td>
</tr>
<tr>
<td>APPENDIX B. Chapter 4 Individual Collection Figures</td>
<td>.................................................................................................</td>
<td>140</td>
</tr>
<tr>
<td>APPENDIX C. Chapter 5 Individual Collection Figures</td>
<td>.................................................................................................</td>
<td>156</td>
</tr>
</tbody>
</table>
LIST OF FIGURES

Figure 2-1: Map of Gulf of Mexico study sites from which bathymodiolin mussels and vesicomyid clams were collected..........................................................................................28

Figure 2-2: Mean and standard deviation of tissue (a) δ^{13}C and (b) δ^{15}N for all mussel collections ..........................................................................................................................29

Figure 2-3: δ^{15}N vs. δ^{13}C for (a) B. brooksi, (b) B. childressi, and (c) B. heckerae ........30

Figure 3-1: Map of Gulf of Mexico study sites from which vestimentiferans were collected .................................................................................................................................42

Figure 3-2: Average and standard deviation of tissue (a) δ^{13}C, (b) δ^{15}N, and (c) δ^{34}S for all vestimentiferan collections ........................................................................................................43

Figure 4-1 Map of Gulf of Mexico study sites from which seep communities associated with bathymodiolin mussels, vesicomyid clams, and vestimentiferan tubeworms were collected ........................................................................................................72

Figure 4-2: (a) δ^{15}N and (b) δ^{34}S vs. δ^{13}C for all heterotrophic animals associated with bathymodiolin mussels, vestimentiferan tubeworms, and vesicomyid clams ...............73

Figure 4-3: A close-up photo of the “cap worm” Protomystides sp. inhabiting its matrix of tubular casing atop the prostomium of E. laminata.........................................................74

Figure 4-4. Mean and standard deviation of tissue (a) δ^{13}C, (b) δ^{15}N, and (c) δ^{34}S values of associated fauna vs. the symbiotic fauna with which they were collected plotted with a 1:1 line .........................................................................................................................................75

Figure 4-5: (a) δ^{15}N and (b) δ^{34}S vs. δ^{13}C for the vesicomyid clams C. ponderosa and Calyptogena sp. nov. and their associated fauna ............................................................................76

Figure 4-6: (a) δ^{13}C, (b) δ^{15}N and (c) δ^{34}S in the “cap worm” Protomystides sp. vs. the paired E. laminata individual upon with the Protomystides sp. was found.............................77

Figure 4-7: δ^{13}C in paired samples of Protomystides sp. and E. laminata from a single collection from AT340................................................................................................................78

Figure 4-8: (a) δ^{13}C, (b) δ^{15}N, and (c) δ^{34}S in the commensal polynoid Branchipolynoe seepensis or Nautilliniellidae sp. vs. the paired B. heckerae, B. childressi, or C. ponderosa individual within whose gills the polynoid was found ..................................................79

Figure 5-1: Map of all Gulf of Mexico study sites from which corals were collected...110
Figure 5-2: Proportion of biomass and species richness comprised by each feeding guild when all seven coral collections are pooled ................................................................. 110

Figure 5-3: (a) δ^{34}S vs. δ^{13}C and (b) δ^{15}N vs. δ^{13}C for *L. pertusa* and associated fauna for all collections ..................................................................................................................... 111

Figure 5-4: (a) δ^{34}S vs. δ^{13}C and (b) δ^{15}N vs. δ^{13}C for all suspension-feeding organisms collected in this study, including hard and soft corals ................................................................. 112

Figure 5-5: Average δ^{34}S vs. δ^{13}C (a and c) and δ^{15}N vs. δ^{13}C (b and d) for Hydroidea spp, (a and b) and *Munidopsis* sp. 2 (c and d) samples grouped by site ............................................. 113

Figure 5-6: Average δ^{15}N vs. δ^{13}C for all *L. pertusa* tissue samples ........................................... 114

Figure 5-7: δ^{18}O vs. δ^{13}C in all authigenic carbonates and *L. pertusa* skeleton collected from the VK 826 and VL 862 study sites .................................................................................. 115

Figure 5-8: δ^{13}C, δ^{15}N and δ^{34}S values for taxa found across at least three of the four community types: juvenile, adult, and old vestimentiferan aggregations and coral aggregations .......................................................................................................................... 116
LIST OF TABLES

Table 2-1: Mean and standard deviations for tissue $\delta^{13}C$ and $\delta^{15}N$ compositions of seep mussels (*Bathymodiolus* spp.) and clams (*Calyptogena* spp.) on the lower Louisiana slope, Gulf of Mexico .................................................................27

Table 4-1: Average and standard deviation of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ compositions in all taxa collected with seep mussels, clams, and vestimentiferans .................................................70

Table 4-2: P-values for pairwise Mann-Whitney tests of isotope values of associated fauna between habitat types ..................................................................................................72

Table 5-1: Coordinates, depths, and dives associated with each site where we made coral collections ................................................................................................................107

Table 5-2: Average and standard deviation of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ compositions in all taxa collected with *Lophelia pertusa* ............................................................................108
ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor Chuck Fisher for his support and guidance throughout the time of my Ph.D. work. His patient discussion of ideas, no matter how ill-conceived, provided a supportive atmosphere for creative thought. I look forward to a continued friendship and collaboration as I move on in my career. I would also like to thank all the members of the Fisher Lab team, and in particular Elizabeth Podowski, who has been a wonderful friend, listener and travel partner, and Stephanie Lessard-Pilon, who started her Ph.D. work at the same time as I did and with whom I have shared so many of the ups and downs over the past 5 years of Ph.D. work. Erik Cordes has been my mentor from the beginning of this work and has helped immensely with learning the stable isotope techniques used throughout my thesis and continues to be a source of thoughtful career and life advice. Stéphane Hourdez has also been a huge help, especially on cruises with sorting and identifying animals and providing thoughtful discussion and is a wonderful travel partner. Bob Carney has been a great resource for discussion of science, career, art, and life. Both Ray Lee and Steve Macko have been a huge asset in running stable isotope samples and providing intellectual guidance as co-authors on manuscripts. I would also like to thank my committee members Kat Shea, Tracy Langkilde, and Doug Miller for their thoughtful discussion in planning the work in the Lau Basin that will be part of my post doctoral work, and for their advice on the studies contained in this thesis. There are a number of undergraduates without whose help, this work would have been very difficult to complete. There are also a number of people on the ship from other laboratories or government agencies, without whose help with collection processing, the large data sets in this paper would not have been possible. I, of course, have to thank the captains and crews of the RV Seward Johnson, the RV Gyre, the RV Atlantis, and the NOAA ship Ronald Brown and the submersible teams of the Johnson Sea Link, Alvin, and ROV Jason II for their hard work and patience in collecting the samples for this work. I had the extreme fortune of teaching an undergraduate field course with Chris Uhl. Through discussions in the course with students and outside of the course with Chris himself, I have learned so much about myself and about how to think of one’s work as art that fulfills the soul, in addition to providing income. I would like to thank Kurt Vandergrift for discussion of science and life and reading early, early versions of my first manuscript, which was 10 pages longer than it needed to be. Doug Fischer has been great support and a thoughtful listener in the later stages of this work as I look toward the next step, as well as a provider of welcome distraction. Finally, I have to thank my parents Bruce and Cindy Becker for their relentless support and caring, and my sisters Amber and Nicole, who are always willing to listen when I need someone to talk to.
CHAPTER 1

Introduction to Gulf of Mexico cold seeps and stable isotopes in trophic studies

Cold seeps, with high-biomass communities of endemic fauna, are proving to be widespread on continental margins the world over. In the Gulf of Mexico, there is particular interest in describing and understanding the biology of these communities, which are threatened by deep-water fishing and energy company activities. The purpose of this thesis is to describe aspects of the trophic ecology of several of the common seep community types found on the Gulf of Mexico continental slope. This chapter provides a brief review of the geological settings and existing knowledge on cold seep ecology, followed by some background on the use of stable isotopes in food web studies and an outline of this thesis.

Gulf of Mexico Geological Setting

The formation of hydrocarbon seeps of the Gulf of Mexico is governed by the underlying process of salt tectonics. Over the last 200 million years, the opening that now connects the Gulf to the Atlantic Ocean opened and closed several times (Pindell 1994). At times when the Gulf was closed off from the Atlantic, the seawater in the Gulf evaporated, leaving behind a layer of salt. Each time the Gulf again opened to the Atlantic, new seawater entered only to evaporate again during the next period of closure, leaving behind more salt. Upon the final opening, during the early Miocene, the water entering the Gulf covered the salt with a layer of sediment (Sassen et al. 1994). As sediment accumulated on the seafloor, the weight of the overlying water compacted this sediment into a thick layer of sandstone and shale (McGookey 1975). In some places
within this shale layer, organic matter had been trapped and developed into pockets of oil and natural gas (Kennicutt et al. 1992).

Some areas of the shale layer are more dense than others and thus provide differential downward force upon the incompressible salt layer. The underlying salt layer is more buoyant than the overlying shale; therefore, areas of the salt layer receiving less downward force rise up, forming salt domes (Humphris 1979). Occasionally, the upward force of the rising salt causes cracks in the overlying shale that act as conduits for hydrocarbons to migrate upward through the sediment layer of the seafloor (Kennicutt et al. 1988). The location where this material emerges from the sea floor is called a “cold seep”.

**Seep Ecology**

The seeping fluid provides an energy source for chemosynthetic primary production. Sulfide, methane, and some higher alkanes can act as electron donors to drive chemosynthesis in both free-living and symbiotic bacteria. Free-living chemosynthetic bacteria can form dense mats at cold seeps, attracting grazers and other higher-order consumers. There are a variety of symbiotic relationships at seeps as well. Vestimentiferan tubeworms lack a mouth or gut and instead contain a specialized organ, called a trophosome, containing sulfide-oxidizing chemoautotrophic bacteria from which the worms derive all of their nutrition. The vestimentiferans take up dissolved inorganic compounds across the epithelium of their plume and root and deliver them to the bacteria via the vascular system. These worms form dense aggregations and reach heights of about 1 to 2 meters from the seafloor. The chitinous tubes of the worms and the interstices between them add a degree of complexity to the otherwise monotonous
surrounding seafloor and provide habitat to a diverse assemblage of macrofauna.

Mussels, found at more active seep sites, form symbioses with methane-oxidizing bacteria or dual symbioses with both methane- and sulfide-oxidizing bacteria. The mussels can form small clumps to vast dense beds that, like the vestimentiferans, provide habitat for other animals. Vesicomyid clams are less abundant than vestimentiferans and mussels, but are also quite common at seeps. The clams contain sulfur-oxidizing symbionts in their gills and are usually found in habitats separate from vestimentiferans and mussels. Unlike mussels, the vesicomyid clams in the Gulf of Mexico typically do not form dense beds, but rather are found in small patches of scattered individuals, sometimes partially buried in the sediment. Communities of heterotrophic fauna inhabit the mud surrounding the clams or directly colonize the clam shells (e.g. anemones and hydroids).

Cold-water corals, and in particular the reef-forming scleractinian coral *Lophelia pertusa*, are major habitat-forming animals sometimes colonizing carbonate hardbottoms associated with seeps (Hovland and Thomsen 1997, Hovland and Risk 2003). Although not usually found directly in areas of active seepage, the coral has been found intermixed with old vestimentiferan aggregations (pers. obs., Cordes et al. 2008). It has been hypothesized that steady long-term nutritional input from seep primary production is the decisive factor allowing coral reefs to persist for 100’s to 1000’s of years (Hovland and Risk 2003).

**Stable isotopes in cold seep trophic studies**

In this thesis, I address questions of trophic relationships among species in Gulf of Mexico seep communities. Food web interactions are among the most fundamental of
concepts that describe how species in a community relate to one another. In terrestrial and shallow-water systems, one might use either simple observation (such as observing monarch caterpillars eating milkweed) or gut content analysis (which works best with larger animals, such as fish, that do not thoroughly chew or grind their food before swallowing). The macrofauna associated with seeps, generally ranging from 1 to 5mm, include polychaete worms, tiny brittle stars, anemones, and shrimp, which are generally small and fragile. A few larger species such as galatheid and brachyuran crabs and sea cucumbers also inhabit these communities, but these animals either consume detritus or grind their food so finely that no recognizable food items can be identified from gut contents.

To overcome these challenges, I use analysis of stable isotope compositions in animal tissues. A stable isotope is a naturally occurring, but less common, form of an element that has more or fewer neutrons than the most common form. For example, carbon-13 ($^{13}$C) contains one more neutron than the more common form, carbon-12. In food web studies, the key principle is the ratio of the heavy ($^{13}$C) to the light ($^{12}$C) isotope in an animal. Since the ratio in the animal has a predictable relationship to the ratio in that animal’s food source, carbon, nitrogen, and sulfur stable isotope ratios can be used to indicate food sources and trophic levels. For convenience, stable isotope values are expressed using δ (delta) notation and reported in units of permil (%o), where

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3,$$

X = $^{13}$C, $^{15}$N, or $^{34}$S and R = $^{13}$C/$^{12}$C, $^{15}$N/$^{14}$N, or $^{34}$S/$^{32}$S.

PDB (Pee Dee Belemnite) is used as the standard for carbon, air $N_2$ for nitrogen, and CDT (Canyon Diablo Triolite) for sulfur.
Two principles that are key to understanding how stable isotopes are used in food web studies are fractionation and mixing. Fractionation occurs during certain chemical or biological processes in which there are different reaction rates for molecules of slightly different mass. Fixation of inorganic carbon by autotrophs is often associated with a specific fractionation that depends on the mode of autotrophy. For example, dissolved carbon dioxide in seawater has a $\delta^{13}C$ composition of around 0‰. Marine phytoplankton fix carbon dioxide via photosynthesis, producing organic material with a $\delta^{13}C$ composition of about -22‰. Alternatively, chemoautotrophic bacteria that fix carbon dioxide via sulfide-oxidation produce organic matter with a $\delta^{13}C$ composition of about -33‰.

Among all of the biological and chemical reactions that take place in nutrient cycles, few of them cause significant fractionation, and therefore the isotope composition of a given material often represent a mixture of the source materials from which it was derived. An example of a mixture could be the tissue of a heterotrophic animal. In the case of carbon, there is very little isotopic fractionation associated with heterotrophic assimilation. Say we collected an animal close to a seep and wanted to know whether it was getting most of its nutrition locally from the seep (chemoautotrophic), or from surface-derived (photosynthetic) primary production. We would take a sample of muscle tissue from the animal, and analyze it for stable isotope content. If we found the $\delta^{13}C$ value of the tissue to be -31‰, we would conclude that the animal may be consuming a mixture of seep- and surface-derived nutrition but the majority is from seep primary production.
Both carbon and sulfur isotope ratios change very little during heterotrophic assimilation and therefore are similar to an animal’s food source (Rau et al. 1983, Hovland and Thomsen 1997, Post 2002). The nitrogen isotope ratio increases by a fairly consistent amount with each trophic level and is used to indicate trophic level, such as primary producer, consumer, and top predator (Minagawa and Wada 1984, McCutchan et al. 2003). When used for tissues of symbiotic fauna, stable isotope values can indicate mode of autotrophy, what inorganic compounds are assimilated, and the spatial variability in the isotope ratios of these inorganic compounds. Tissue stable isotope compositions can have implications ranging from organism-level physiology to interspecies interactions to landscape-wide geological processes.

In Chapter 2, tissue carbon and nitrogen isotope compositions were analyzed in mussels and clams from 14 seep sites spanning a depth range of 1000 to 2800 m along the lower Louisiana slope of the Gulf of Mexico. This study helped to illuminate processes (geological, geochemical, and microbial) that affect the chemical and isotope compositions of seeping fluids along the continental slope of the Gulf of Mexico as well as provide insight into the physiological ecology of the mussel species. The three species found on the lower slope, *Bathymodiolus childressi*, *B. brooksi*, and *B. heckerae*, showed site-specific differences in tissue $\delta^{13}C$, reflecting differences in the local methane pool. Mussels from sites on the lower slope sitting atop the contiguous salt sheet generally had tissue $\delta^{13}C$ values that reflected a stronger biogenic methane signal (-70.8 to -58.8‰) than mussels on the upper slope or seaward of the Sigsbee Escarpment (-67.3 to -40.4‰). Clams (*Calyptogena ponderosa* and *Calyptogena* sp. nov.) had a narrow range of $\delta^{13}C$ values between -37.0 and -34.4‰, indicating that their thiotrophic symbionts are fixing
primarily seawater dissolved organic carbon. The most depleted tissue $\delta^{15}\text{N}$ values yet reported for both mussels and clams are reported in this study at -23.7 and -9.2‰, respectively. These depleted values have implications for the assimilation of inorganic nitrogen by these symbioses and the concentrations of particular inorganic nitrogen sources in the local environment.

In Chapter 3, tissue $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and $\delta^{34}\text{S}$ compositions of vestimentiferan tubeworms were analyzed from seeps across the lower continental slope of the Gulf of Mexico. This large cohesive data set allowed us to address long-standing hypotheses about seep animal ecology, and vestimentiferan tubeworms in particular. The pattern of tissue $\delta^{13}\text{C}$ in the vestimentiferans provided strong evidence supporting the hypothesis that seep vestimentiferans take up porewater dissolved inorganic carbon (DIC) across their roots (Hovland and Risk 2003), and consistent differences in $\delta^{15}\text{N}$ suggested resource partitioning between co-occurring species. The high variability in $\delta^{34}\text{S}$ values among individuals of the same species collected <1m from one another indicated that isotope compositions of sulfur pools are highly variable on a very small spatial scale relative to carbon and nitrogen and supported hypotheses concerning sulfur cycling between vestimentiferan symbionts and consortia of sulfate-reducing archaea associated with vestimentiferan roots (Boetius et al. 2000, Dattagupta et al. 2006). These findings provide new directions for further study of seep ecology, vestimentiferan physiology and environmental carbon, nitrogen, and sulfur sources and cycling at seeps.

In Chapter 4, tissue $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ compositions were used to explore trophic interactions within the heterotrophic communities associated with vestimentiferans, mussels, and clams from seeps on the lower Gulf of Mexico continental
slope. The majority of animals collected in these habitats showed isotopic evidence for at least some incorporation of seep primary production. In general, the tissue $\delta^{13}C$ values of the heterotrophic fauna seemed to track those of their associated symbiotic fauna, indicating that both symbiotic and heterotrophic fauna tissue isotope values vary with some difference in the isotope composition of the endmember (i.e. inorganic) sources. Carbon isotopes supported the hypothesis of a trophic relationship between the cap worm Protomystides sp. and the vestimentiferan Escarpia laminata, but the $\delta^{15}N$ and $\delta^{34}S$ data were inconclusive. There was no isotopic evidence that commensal polynoids living inside the mantle cavities of mussels and clams are feeding on their hosts.

Chapter 5 investigates the importance of seep primary production to the nutrition of the cold-water coral Lophelia pertusa its associated community and examines local trophic interactions. L. pertusa commonly occurs around seeps in areas where seepage has apparently subsided but is often present within meters of seep fauna such as vestimentiferan tubeworms. A long-standing hypothesis for the occurrence and persistence of coral communities at seeps is that they rely on seep-derived primary production (Post 2002). Stable carbon, nitrogen, and sulfur compositions in seven quantitative L. pertusa community collections were analyzed and a significant seep signature was found in only one of the 35 species tested (Provanna sculpta, a common seep gastropod). The data suggest that it is the presence of authigenic carbonate substrate for settlement and growth as well as hydrodynamic processes that drive L. pertusa occurrence at seep sites in the Gulf of Mexico, not local primary production by seep microbes.
CHAPTER 2

Stable carbon and nitrogen isotope compositions of hydrocarbon seep bivalves on the Gulf of Mexico lower continental slope


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Abstract

Stable isotope compositions of cold seep bivalves can illuminate processes that affect the chemical and isotopic compositions of seeping fluids along the continental slope of the Gulf of Mexico as well as provide insight into the physiological ecology of these species. Carbon and nitrogen isotope compositions were analyzed in mussels and clams from 14 seep sites spanning a depth range of 1000 to 2800 m along the lower Louisiana slope of the Gulf of Mexico. Mussels of three species found on the lower slope, Bathymodiolus childressi, B. brooksi, and B. heckerae, showed site-specific differences in tissue δ¹³C, reflecting differences in the local methane pool. Mussels from sites on the lower slope sitting atop the contiguous salt sheet generally had tissue δ¹³C values that reflected a stronger biogenic methane signal (-70.8 to -58.8‰), than mussels on the upper slope or seaward of the Sigsbee Escarpment (-67.3 to -40.4‰). Clams (Calyptogena ponderosa and Calyptogena sp. nov.) had a narrow range of δ¹³C values between -37.0 and -34.4‰, indicating that their thiotrophic symbionts are fixing
primarily seawater dissolved organic carbon. The most depleted tissue $\delta^{15}\text{N}$ values yet reported for both mussels and clams are reported in this study at -23.7 and -9.2‰, respectively. These depleted values have implications for the assimilation of inorganic nitrogen by these symbioses and the concentrations of particular inorganic nitrogen sources in the local environment.

**Introduction**

Hydrocarbon seeps are a common deep-sea habitat on the Louisiana slope of the Gulf of Mexico. The seeping fluids provide a source of energy in the form of reduced chemicals, which supports a high biomass of specially adapted animals harboring methanotrophic and/or sulfur-oxidizing chemoautotrophic (hereafter “thiotrophic”) symbionts. We made 26 collections of mussels from 12 study sites that span 675 km east to west and 1000 to 2800m water depths. These sites encompassed a wide range of seep habitats with different abiotic (brine staining, brine pools, mud volcanoes, authigenic carbonates, etc.) and biotic (bacterial mats, vestimentiferans, etc.) characteristics (see Roberts *et al.*, in press for site descriptions). The size and extent of this data set has allowed us to describe the distribution of different carbon and nitrogen sources across this region as well as make some inferences about the physiological ecology of seep bivalves in the Gulf of Mexico.

Methane in seeping fluid is an important energy and carbon source for bathymodiolin mussels containing methanotrophic endosymbionts. The methane in Gulf of Mexico seep fluids originates from both geological processes deep within the crust (thermogenic) and from microbial degradation of seeping crude oil or anaerobic reduction of carbon dioxide or acetate (biogenic). Each of these processes produces
methane with a distinctive $\delta^{13}C$ composition. Several studies have directly measured the isotope composition of seep methane in sediments, and found that it ranges from at least -95 to -35‰ (Pohlman et al., 2009; Pohlman et al., 2005; Sassen et al., 1999; Tsunogai et al., 1998). Some common seep habitats, such as mussel beds, are simply not amenable to standard sampling techniques (e.g. push cores) because of the presence of the mussels themselves, which normally occur over beds of shell hash and/or authigenic carbonate. Stable carbon isotope compositions in mussels with methanotrophic endosymbionts have been shown to reflect the composition of the methane they consume as an energy and carbon source (Brooks et al., 1987) and change when mussels are moved to a location with a different methane source (Dattagupta et al., 2004). Thus, characterization of mussel tissue $\delta^{13}C$ provides an alternative method for determining the approximate stable isotope composition of their source methane and hence the relative contributions of biogenic and thermogenic methane to this pool. Vesicomyid clams, which we also characterized in this study, host thiotrophic endosymbionts. Unlike mussels, their $\delta^{13}C$ compositions are very consistent across numerous species from a range of vent and seep habitats (Childress et al., 1993; Fiala-Médioni et al., 1993; Fisher et al., 1988; Kennicutt et al., 1992).

Two of the species in the present study, namely the mussel Bathymodiolus childressi, which hosts methanotrophic endosymbionts, and the vesicomyid clam Calyptogena ponderosa, which hosts thiotrophic symbionts, are also found on the upper continental slope between about 500 and 700 meters, and their isotope compositions at some sites have been characterized (Kennicutt et al., 1992). Different subsurface geological processes affect the upper and lower slopes. The upper slope, at depths
between 300 and 700m is characterized by vertical migration of the Louann salt basement, forming salt pillars separated by areas of deep soft sediment, whereas the lower slope, below about 1000 m is characterized by a shallow contiguous salt sheet that is migrating laterally toward the Sigsbee Escarpment (Peel et al., 1995). These differences in underlying salt tectonics may influence the chemical and isotope composition of seeping fluids.

We also characterized isotope compositions of two mussel species for which very little prior isotope data exists. A few stable isotope values have previously been reported for the mussel *B. brooksi* (Fisher et al., 1993), the most common lower-slope mussel, and *B. heckerae* (Cavanaugh et al., 1987), found at depths >2180m on the lower slope (Cordes et al., 2009). *B. brooksi* harbors two phylotypes of symbiont, one is a methanotroph and the other a thiotroph (Duperron et al., 2009; Duperron et al., 2007; Fisher et al., 1993). *B. heckerae* harbors four phylotypes of symbionts, two that are apparently thiotrophic, one that groups with methanotrophs and another that groups with methylotrophs (Duperron et al., 2009; Duperron et al., 2007). Differential activities of these symbionts could have a wide range of effects on the tissue δ¹³C and δ¹⁵N compositions of their hosts. Here we show that the few values previously reported are not characteristic of the species, that both species have a much larger range in tissue δ¹³C and δ¹⁵N, and that some of the conclusions reached from interpretation of the previous data sets should be questioned.

Owing to the combination of extensive sampling from numerous locations and the small footprint of individual collections from each location (<0.5 m²), we were able to statistically test hypotheses in addition to making qualitative observations. To look for
evidence of resource partitioning between co-occurring species, differences in average isotope compositions between species from the same collections were tested. If two species collected from the same location have consistently and significantly different isotope values, they are either utilizing different source pools or different mechanisms for taking up and assimilating carbon or nitrogen. Within-species variation among sites was tested as well as among collections. Significant differences in tissue $\delta^{13}C$ values and the scale at which these differences occur provide valuable insights as to the processes underlying these differences, and the spatial scale at which they occur.

Methods

Sample collection

Twenty-six collections of mussels and 3 collections of clams were obtained using the mussel pot collection device (modified from the design of Van Dover (2002)) (Cordes et al., in press), a metal-ringed net, or by grabbing several individuals from within a 0.5 $m^2$ area using the submersible’s manipulator arm. Once onboard the ship, up to six (occasionally more) individuals of each species were sampled, per collection, for stable isotope analysis by dissecting a piece of mantle tissue. The tissue samples were rinsed with deionized water to remove any residual seawater and frozen at -70°C. Figure 2-1 shows the sites from which we made mussel and clam collections (also see Roberts et al., in press). Usually, three of the individual samples were processed and analyzed for carbon and nitrogen isotope content. Hereafter, the term “individual” refers to a mantle sample from one individual mussel or clam, “collection” refers to all individuals analyzed from a discrete mussel pot, net, or grab collection, and “site” refers to a seep area where the collection was made. These areas were discovered through examination of 3D
seismic data and are of variable extent (Roberts et al., in press). Our first hand knowledge of the extent of seep communities in an area is limited to the portions explored by submersible or ROV. In general, visual evidence of chemosynthetic communities within an area occurred over spatial scales of 100’s of meters to a kilometer or two. The site names follow the Minerals Management Service (MMS) lease block designation in which they occur (see Figure 2-1), and consist of a two-letter abbreviation, which stands for the region (AC=Alaminos Canyon, for example) followed by a three-digit number for the 3 x 3mi lease block. General descriptions and more information on the sites can be found in Roberts et al. (Roberts et al., in press).

**Stable isotope analysis**

In the laboratory, all samples were dried at 60°C, homogenized, and acidified to remove any inorganic carbonate. Samples were redried, and most subsamples were analyzed for stable carbon and nitrogen isotope composition at the Stable Isotope Facility at the University of California, Davis using an Integra elemental analyzer coupled with a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, United Kingdom). Additional samples were analyzed by RWL (School of Biological Sciences, Washington State University) or SAM (University of Virginia Stable Isotope Laboratory) using continuous-flow isotope ratio mass spectrometry involving Costech or a Carlo Erba elemental analyzer coupled to a Micromass Optima isotope ratio mass spectrometer (EA/IRMS). Data from each of the laboratories are calibrated to NIST (National Institute of Standards and Technology) reference materials. Samples analyzed by different laboratories did not represent distinct subsets of the data (such as individual collections or species), but are rather haphazardly distributed across sites and species.
Values are expressed using δ (delta) notation and reported in units of permil (%), where 
\[ \delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 10^3, \]

\( X = ^{13}\text{C} \) or \(^{15}\text{N} \) and \( R = ^{13}\text{C}/^{12}\text{C} \) or \(^{15}\text{N}/^{14}\text{N} \).

PDB (Pee Dee Belemnite) was used as the standard for carbon and air \( \text{N}_2 \) for nitrogen.

**Statistical analyses**

To assess the significance of differences in isotope values between collections or sites, one-way ANOVA and Tukey’s HSD (honestly significant differences) was used when assumptions for parametric tests were met. When these assumptions were not met, a nonparametric Kruskal-Wallis test was utilized. Tests were carried out only for collections that had three or more individuals. Where only two groups were being tested (two species within a collection, two collections within a site, etc.) a 2-sample t-test was used, when parametric assumptions were met and a Mann-Whitney test when they were not. All statistical analyses were carried out using Minitab 15® statistical software.

**Results & Discussion**

*Bathymodiolin mussel \( \delta^{13}\text{C} \)*

Bathymodiolin mussels on the lower continental slope had a wide range of \( \delta^{13}\text{C} \) values both overall and within species (*Table 2-1*). Overall, mussel tissue \( \delta^{13}\text{C} \) values ranged from about -72 to -40‰, which is comparable to ranges previously reported for seep mussels elsewhere in the Gulf of Mexico (Cary *et al.*, 1989; Kennicutt *et al.*, 1992). Even within each species, the range in \( \delta^{13}\text{C} \) values was at least 26‰ (*Table 2-1*).

*Within each* Bathymodiolus *species,* Kruskal-Wallis tests revealed significant differences in tissue \( \delta^{13}\text{C} \) values between sites (*p*≤0.02). There were also significant
differences in tissue δ¹³C values within mussel species between some collections within sites. Significant and sometimes very large differences between sites and collections indicate that a large component of the overall range in mussel tissue δ¹³C values is likely due to differences in the isotope composition of the endmember carbon sources. Within sites, there is no apparent correlation between the distance separating collections and similarity in their tissue δ¹³C values. Thus, methane isotope compositions could be very different across a small distance (30 m) or very similar over a large distance (700 m) within a site. Mussels were often collected from visually distinctive environments within a site—dense mussel beds vs. partially buried in briny sediment supporting bacterial mats, for example. Substantial alteration of the isotope composition of methane may occur relatively close to the sediment surface as a result of microbial activity, which can vary considerably over short distances (Joye et al., in press). A portion of the variability in tissue δ¹³C values will also be a function of differences in animal and symbiont activities between species and microhabitats.

Tissue δ¹³C values in B. childressi, which contains only methanotrophic bacterial endosymbionts, largely reflect the isotope composition of methane in the local environment (Brooks et al., 1987). B. brooksi harbors both intracellular methanotrophic and thiotrophic bacteria and can potentially utilize methane and/or reduced sulfur species as energy sources and methane and/or DIC as carbon sources (Duperron et al., 2009; Duperron et al., 2007; Fisher et al., 1993). This suggests that B. brooksi could have a wide range of tissue δ¹³C values resulting from differential symbiont activities in different microhabitats. However, when B. brooksi co-occurred with B. childressi, their tissue δ¹³C values differed by no more than 5.5‰. In fact, B. brooksi that co-occurred
with *B. childressi* had a tissue $\delta^{13}$C range of only $2\%$, while *B. childressi* had a range of 7 to $9\%$ in these collections (3 individuals of each species in both collections). Since the variation in tissue $\delta^{13}$C values of individuals of all species is much smaller within a collection than between collections (Figure 3-3) and there is no consistent substantial difference between species within collections, we assume that the tissue $\delta^{13}$C values of all species generally reflects the composition of the local methane source when making comparisons between sites with large differences in the tissue $\delta^{13}$C values.

Most methane on the upper Louisiana slope is of thermogenic origin (Orcutt *et al.*, 2005; Sassen *et al.*, 1999), except in areas influenced by brine seepage, where methane has a significant biogenic component (Joye *et al.*, 2009). At sites on the upper slope where methane release is mostly of thermogenic origin, seep mussels have tissue $\delta^{13}$C values around $-40\%$ (Brooks *et al.*, 1987; Dattagupta *et al.*, 2004), while at sites on the upper slope where methane has a larger biogenic component, seep mussels have tissue $\delta^{13}$C values between $-63$ and $-57\%$ (MacAvoy *et al.*, 2008). On the Florida Escarpment, where the methane is dominantly of biogenic origin (Martens *et al.*, 1991), mussels have tissue $\delta^{13}$C values as low as $-76\%$ (Paull *et al.*, 1985).

The average $\delta^{13}$C values of collections at AC818 ($-54.8$ to $-44.1\%$), AC645 ($-40.4\%$), and GB647 ($-48.7\%$) indicate that the methane at these sites is of primarily thermogenic origin (Figure 2-2). The majority of mussel collections in this study, however, had average $\delta^{13}$C compositions between $-70.8$ and $-58.8\%$, indicating that biogenic methane comprised a varying but significant portion of the methane at these sites (Bernard *et al.*, 1977; Joye *et al.*, in press; Schoell, 1983). At the GB647 and GB697 study sites (the shallowest in this study at 1000m), the underlying salt formations are the
isolated salt masses (pillars) that are characteristic of the rest of the upper Louisiana slope. Whereas GB647 had mussels with tissue δ¹³C values indicating methane of thermogenic origin, mussels at GB697 had tissue δ¹³C values more indicative of a significant biogenic component (mean δ¹³C = -67.3 to -64.9‰). This variability between two sites in the same region at the same depth has been noted on the upper slope (Dattagupta et al., 2004) and may in part reflect differences in the local habitat such as influence of brine seepage (Joye et al., 2009). Unlike the upper slope, the lower slope landward of the Sigsbee Escarpment is characterized by lateral migration of a shallow, solid salt sheet toward the Escarpment (Peel et al., 1995). Alaminos Canyon is a reentrant into the Sigsbee Escarpment (Figure 2-1; Roberts et al., in press) and as such has access to deeper thermogenic methane. The AC818 and AC645 study sites are within the canyon, and thus mussels at these sites reflect the use of thermogenic methane.

Nested within these large scale differences in the underlying geology and processes that affect the δ¹³C of the methane over large areas are processes such as methanogenesis that can greatly alter the δ¹³C of methane released from sediments on very small spatial scales. For example, the AC601 study site was the only site at which the range of mean δ¹³C values (-65.6 to -45.0‰) was indicative of methane that is of primarily thermogenic origin and of primarily biogenic origin in different collection locations within the same study site. Collection AC601-1 with mean δ¹³C = -65‰ was about 2.5 km north of the other two, while AC601-2 (mean δ¹³C = -56.1‰) and AC601-3 (mean δ¹³C = -45.0‰) collections (Figure 2-2) were obtained on the same dive 26 m from each other in the southern area of the site. Both the AC601-1 and AC601-2 collections, which had mean δ¹³C values indicative of biogenic methane, consisted of B.
*brooksi* in isolated patches close to the shores of two separate brine lakes, while the AC601-3 collection consisted of *B. childressi* collected from the middle of a large contiguous mussel bed with no current surface expression of brine. We consider the notably different habitats (which would sustain different subsurface microbiology and produce different methane $\delta^{13}C$ values) to be the most likely cause of the very different tissue isotope values, as opposed to differences in symbiont types between species, because *B. brooksi* $\delta^{13}C$ values are either quite similar or more positive than *B. childressi* in collections with both species (Figure 2-2, but see Fisher *et al.*, 1993).

The best evidence for large differences in methane $\delta^{13}C$ values over very small spatial scales is the large range in tissue $\delta^{13}C$ values in *B. childressi* within some individual collections (Figures 2-2a and 2-3b). *B. childressi* tissue $\delta^{13}C$ values spanned 7 and 9‰ in the MC853 and MC640 collections, respectively. This is not confounded by the possibility of differential activity of different types of symbionts, since *B. childressi* harbors a single type of methanotrophic symbiont (Duperron *et al.*, 2009). Additionally, all previous studies indicate that the mussel obtains the overwhelming bulk of its nutritional carbon from methane (Cary *et al.*, 1988; Page *et al.*, 1990) and have found very little variation between individuals within a collection and virtually no fractionation during assimilation (Dattagupta *et al.*, 2004). Although this data set provides strong evidence for very patchy methane $\delta^{13}C$ values on small spatial scales, one cannot discount the possibility that the differences among individuals within a collection reflect differential input from an outside source, such as filter feeding (Page *et al.*, 1990).

Although when this data set is viewed broadly it is clear that methane $\delta^{13}C$ values drive a major component of the variation in mussel tissue $\delta^{13}C$ values, a portion of the
variability in tissue δ^{13}C values will be a function of different animal and symbiont activities between species and microhabitats. In a previous study, Fisher et al. (1993) found that *B. brooksi* had on average 5‰ more depleted tissue δ^{13}C values than co-occurring *B. childressi*. Since *B. brooksi* harbors both methanotrophic and thiotrophic endosymbionts, this finding contradicted the expectation that the tissue δ^{13}C values of the mussels would reflect a mixture of organic carbon derived from methane (<=50‰) and seawater CO₂ fixed via sulfide oxidation (-47 to -23‰; Fisher, 1990). The authors concluded that the thiotrophic symbionts that can co-occur in the same host cells as the methanotrophic symbionts might be fixing, and further fractionating, isotopically light respired carbon derived from methane. In the present study, with a much larger data set, we found either no difference or the opposite trend for co-occurring *B. brooksi* and *B. childressi*, with *B. childressi* having very similar or more depleted δ^{13}C values than *B. brooksi* (Figure 2-2). Authigenic carbonates formed as a result of microbial methane oxidation and sulfate reduction reflect the δ^{13}C composition of pore water dissolved inorganic carbon (DIC) (Roberts and Aharon, 1994). Carbonates collected with mussels in this study ranged in δ^{13}C from -51.7 to -28.7‰ (Roberts and Feng, in press), indicating that significant fixation of pore water DIC by thiotrophic endosymbionts could also produce highly depleted tissue δ^{13}C values. Thus, incorporation of varying amounts of pore water DIC (which can be ^{13}C-depleted), methane, and seawater CO₂, as well as differences in δ^{13}C composition of the internal DIC pool and differential activity of a mixed pool of symbionts could potentially produce tissue δ^{13}C values in *B. brooksi* (or *B. heckerae*) that are the same as, more depleted than, or more enriched than co-occurring *B. childressi* (and the local methane pool). Clearly, much work remains to be done before
we will fully understand the relative roles of symbiont types, symbiont activities, and endmember concentrations and stable isotope values in determining the δ\textsuperscript{13}C values of the tissues of mussels with multiple symbionts.

*Bathymodiolin mussel δ\textsuperscript{15}N*

The overall ranges in tissue δ\textsuperscript{15}N in *B. brooksi* and *B. childressi* were quite large (16 and 27‰, respectively) (*Table 2-1*); however, the mean tissue δ\textsuperscript{15}N values in the majority of the collections were between -6.8 and 2.3‰ (*Figure 2-2b*), similar to the ranges of these species from the upper Louisiana slope and Florida Escarpment (Cary et al., 1989; Kennicutt et al., 1992). Co-occurring *B. brooksi* and *B. childressi* had very similar δ\textsuperscript{15}N compositions in three collections. In two collections from GC852, however, *B. childressi* mean tissue δ\textsuperscript{15}N values were significantly higher than those of *B. brooksi* (p<0.001). Where *B. brooksi* and *B. heckerae* co-occurred, *B. heckerae* had either very similar δ\textsuperscript{15}N compositions to, or was more enriched than, *B. brooksi* and this difference was significant for one of the two collections (p=0.01). Based on the differences in symbionts alone, one can conclude at least the potential for resource partitioning with respect to energy and carbon sources between co-occurring mussel species. These data also suggest resource partitioning with respect to nitrogen sources in at least some of the microhabitats where more than one mussel species occurs. Inherent in the capability for resource partitioning with respect to inorganic nitrogen acquisition is the suggestion of different capabilities and or processes for nitrogen uptake among the three species.

The most remarkable isotope values in mussels in this study were the highly depleted δ\textsuperscript{15}N values in *B. childressi* from the GB647 study site. Ranging from -23.7 to -21.7‰, these are the most depleted δ\textsuperscript{15}N values yet reported in any modern animal. The
next most depleted $\delta^{15}N$ values were found in *B. brooksi* and *B. childressi* from MC853 and MC640 (-15.6 to -10.9‰), which is in the range of the lowest values previously reported for *B. childressi* (Dattagupta *et al.*, 2004; Kennicutt *et al.*, 1992; MacAvoy *et al.*, 2008). Based on what is known about seep fluids and discrimination during nitrate reduction, utilization of environmental ammonium at high concentrations and/or with low $\delta^{15}N$ values seems the most probable explanation for the very negative values in seep mussels.

In laboratory studies, the capability to take up and assimilate ammonium, nitrate, free amino acids, and particulate matter have all been demonstrated for *B. childressi* (Lee and Childress, 1994; Lee *et al.*, 1992; Page *et al.*, 1990). It is quite unlikely that particulate materials or amino acids with sufficiently negative $\delta^{15}N$ values are present in high enough concentrations to account for the $^{15}N$-depleted tissue values in the mussels. Similarly, vesicomyid clams (with very limited ability to filter feed) from MC853 were also anomalously depleted in $^{15}N$.

All three study sites (GB647, MC640, and MC853) contained brine sediments and had elevated levels of ammonium (Joye *et al.*, in press; S. Joye, pers. comm.). GB647, in particular had elevated levels of both ammonium and nitrate compared to bottom water at shallow sediment depths (at 1.5 cm sediment depth, ammonium was 3.7 mM compared to 36$\mu$M in bottom water and nitrate was 181$\mu$M compared to 20$\mu$M in bottom water; S. Joye, pers. comm.). Interestingly, both nitrogen species increased in concentrations at deeper sediment depths (4 to 12.5 cm), suggesting a deep source of ammonium and nitrate that could potentially have a different $\delta^{15}N$ composition than is found in bottom water (S. Joye, pers. comm.). Ammonia can be produced via denitrification, and the
fractionation associated with dissimilatory nitrate reductase is generally in the range of 20 to 30‰ (Granger et al., 2008), resulting in ammonia that is 20 to 30‰ lighter than the source nitrate. It is also plausible that the elevated concentrations of ammonium resulting from seeping of hypersaline effluent allows for a high degree of isotope fractionation during uptake and/or assimilation by the mussels or their symbionts. Isotope fractionation of ammonia during assimilation can be as high as 27‰ in marine bacteria (Hoch et al., 1994) and 20‰ in marine algae (Waser et al., 1998).

In other seep environments where millimolar concentrations of ammonium have been measured, such as the GC233 “brine pool” on the upper Louisiana slope (Joye et al., 2005; Lee et al., 1992), B. childressi had tissue δ¹⁵N values around -15‰ (Dattagupta et al., 2004). No isotope data are available for nitrogen in seeping fluids, but we suggest that the most negative values in mussel tissues reflect usage of an abundant and/or depleted ammonia pool in the seeping fluids, with the possibility that some use of nitrate contributes to the more positive values and perhaps resource partitioning in some microhabitats.

There were significant differences in δ¹⁵N of mussel tissues between sites and between some collections within sites when the collections were well-separated. Differences in mussel δ¹⁵N values within a site appear to be related to distance between collections to a greater degree than was seen with δ¹³C; where within-site differences in δ¹⁵N values were significant, the collections were 0.5 to 1 km away from each other.

Thus, the nitrogen pool utilized by mussels is more constant in isotope composition over moderate to small scales than carbon pools. This suggests a deep pool of nitrogen, which is not significantly altered by sediment microbes in the shallow subsurface as is the case
with methane. Although explanation of the high NH$_4^+$ content in seep brines is lacking, Joye et al. (2004; in press) hypothesized that ammonium attached to sediment particles is desorbed by the hypersaline brine. In this scenario, ammonium concentrations and the consequent level of isotope discrimination during assimilation by mussel endosymbionts may be related to salinity of upward-migration fluid and sediment particle size, both of which do not vary on as small a spatial scale as microbial community composition and activity.

*Vesicomyid clams*

The vesicomyid clams had much narrower overall tissue δ$^{13}$C ranges than the mussels (Table 2-1). The ranges of both *C. ponderosa* and *Calyptogena* sp. nov. tissue δ$^{13}$C values (-37 to -34‰) were similar to *C. ponderosa* on the upper Louisiana slope (Kennicutt et al., 1992) and to other vesicomyid species found at seeps near Japan (Fiala-Médioni et al., 1993) and at hydrothermal vents (Childress et al., 1993; Fisher et al., 1988), indicating that this family probably uses the same inorganic carbon source at different locations and that the source has little isotope variation. *Calyptogena* species harbor thiotrophic endosymbionts, and therefore utilize dissolved inorganic carbon (DIC) rather than the more isotopically variable methane as a carbon source. This carbon source is likely DIC in epibenthic bottom water taken up by the clam’s incumbent siphon and pumped across its gills. DIC in seawater has a consistent δ$^{13}$C composition around 0‰ (Peterson and Fry, 1987) and use of this carbon source is also reflected in the δ$^{13}$C values of vesicomyid shells (reviewed in Childress and Fisher, 1992). On the other hand, the δ$^{13}$C values of DIC in pore fluids at seeps can be quite low and this is often reflected in the δ$^{13}$C values of authigenic carbonates at seeps (Roberts and Aharon, 1994). The
carbonates collected from the clam beds in this study had $\delta^{13}C$ values between -25 and -15 ‰ (Roberts et al. in press). The narrow range of tissue $\delta^{13}C$ values in seep vesicomyid clams suggests that any depleted DIC in seawater at the sediment water interface is effectively diluted by the pumping of overlying seawater through the clam’s mantle cavity.

The majority of clams of both species, *C. ponderosa* and *Calyptogena* sp. nov., had tissue $\delta^{15}N$ values between -1.0 and 4.4‰, which is within the range of nitrogen isotope values of *C. ponderosa* on the upper Louisiana slope (Kennicutt et al., 1992). The exception was *C. ponderosa* from MC853, which had tissue $\delta^{15}N$ values ranging from -9.2 to -3.9‰. These clams were collected very close to the mussel collection from this site and those mussels were also quite depleted in $^{15}N$. Although the clams were enriched in tissue $\delta^{15}N$ relative to the mussels, they were the most depleted $\delta^{15}N$ values for clams in this study, and the individual with $\delta^{15}N = -9.2\%$ is the most depleted value yet reported for any vesicomyid. In general, vesicomyid clam $\delta^{15}N$ values are depleted compared with non-seep animals, indicating that they utilize a local nitrogen source rather than particulate organic nitrogen. Very little is known about nitrogen uptake and utilization by this thiotrophic symbiosis, although ammonium assimilation enzyme activity has been found in the hydrothermal vent clam *Calyptogena magnifica* (Lee et al., 1999). Vesicomyids are known to take up sulfide from interstitial pools in the sediment, using sulfide-binding components present in their blood and their well-vascularized foot, which they can extend several body lengths into the sediment (reviewed by Childress and Fisher, 1992). The very depleted tissue $\delta^{15}N$ values in the collections from MC853
suggest that these clams are also capable of acquiring nitrogen from depleted or abundant interstitial nitrogen sources, likely ammonia, in the sediment beneath them.

**Conclusions**

Analysis of the stable isotope compositions of seep bivalves on the lower continental slope and comparison to the already existing data set from the upper slope has provided insights into the physiology and the local environment. Differences in bivalve isotope values, particularly in mussels, between the upper and lower slope provide us with clues about geological and microbial processes affecting the local carbon and nitrogen pools on large spatial scales. The underlying causes of the full range and variation in tissue $\delta^{13}C$ and $\delta^{15}N$ values of seep bivalves is, however, extremely complex. The variability in $\delta^{13}C$ of mussels is primarily affected by variability in isotope composition of methane. On a large scale, methane isotope composition is likely affected by geological processes (between sites or regions of the continental slope) and by both geochemical and microbial processes on a finer scale (between collections within a site). The substantial variation found within some collections indicates that the isotope composition of methane can also vary significantly on very small scales such as within a mussel bed. This in turn suggests that relatively high rates of microbial methanogenesis are patchily distributed in relatively shallow seep sediments. The large variation between collections in tissue $\delta^{15}N$ values of both clams and mussels are also likely driven by differences in their source pools, in this case most likely the concentration and $\delta^{15}N$ value of interstitial ammonia. Overlain on this are the individual physiological constraints of the animals and their symbionts related to inorganic nitrogen uptake and assimilation and the relative availability of all inorganic nitrogen species in their environment. In the case
of *B. brooksi* and *B. heckerae*, which harbor symbioses with multiple types of bacteria, tissue stable isotope compositions will also be affected by the relative abundance and activity of each symbiont type. Future research on the physiology of seep bivalves, particularly inorganic nitrogen assimilation by *C. ponderosa*, *B. brooksi*, and *B. heckerae* may help to elucidate some of the observed trends. Paired measurement of chemical and isotope compositions in seep sediment, overlying seawater, and seep animals would also provide valuable insight into the physiological ecology of these species.

**Table 2-1.** Means and standard deviations for tissue $\delta^{13}$C and $\delta^{15}$N compositions of seep mussels (*Bathymodiolus* spp.) and clams (*Calyptogena* spp.) on the lower Louisiana slope, Gulf of Mexico.

<table>
<thead>
<tr>
<th>species</th>
<th>N</th>
<th>$\delta^{13}$C (%)</th>
<th>$\delta^{15}$N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bathymodiolus brooksi</em></td>
<td>68</td>
<td>$-61.1 \pm 6.9$</td>
<td>$-4.6 \pm 3.2$</td>
</tr>
<tr>
<td><em>B. childressi</em></td>
<td>27</td>
<td>$-61.8 \pm 8.3$</td>
<td>$-5.3 \pm 8.5$</td>
</tr>
<tr>
<td><em>B. heckerae</em></td>
<td>11</td>
<td>$-51.7 \pm 10.7$</td>
<td>$-3.5 \pm 2.6$</td>
</tr>
<tr>
<td><em>Calyptogena ponderosa</em></td>
<td>9</td>
<td>$-35.8 \pm 0.8$</td>
<td>$-1.0 \pm 4.4$</td>
</tr>
<tr>
<td><em>Calyptogena sp. nov</em></td>
<td>3</td>
<td>$-34.7 \pm 0.6$</td>
<td>$1.2 \pm 1.2$</td>
</tr>
</tbody>
</table>
**Figure 2-1.** Map of study sites from which mussels and clams were collected. Yellow site markers signify sites where mussels were collected, red signifies sites were clams were collected, and markers that are half yellow/half red signify sites where both clams and mussels were collected.
Figure 2-2. Mean and standard deviation of tissue (a) $\delta^{13}$C and (b) $\delta^{15}$N for all mussel collections. Mean tissue stable isotope values of species within a single collection are presented separately using different symbols for each species. Data points without associated numbers represent the mean of three individuals, in other cases the number of individuals is indicated just above or below the mean data point. The standard deviation from the mean is indicated by error bars. When no error bars are visible they are within the area of the symbol. Sample sizes are the same for (a) and (b).
Figure 2-3. $\delta^{15}$N vs. $\delta^{13}$C for (a) *B. brooksi*, (b) *B. childressi*, and (c) *B. heckerae*. Different symbols represent different study sites and different colors represent different collections. Each point represents an individual mussel.
CHAPTER 3

Stable isotopes provide new insights into vestimentiferan physiological ecology at Gulf of Mexico cold seeps

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Abstract

On the otherwise food-deprived seafloor of the Gulf of Mexico continental slope, high-biomass communities of specially adapted animals thrive in the toxic, but energy-rich, habitat of natural oil and gas seeps. Fundamental to understanding any animal community is knowing how animals acquire nutrients and interact with one another in the food web; however, studying these interactions is challenging at over 1000 m below the sea surface. Stable isotope analysis, which can be performed on dried animal tissues, has provided an invaluable tool for studying nutrition in seep animals. Most existing stable isotope data sets from seeps, however, are limited in geographic scope and sample size, which can and has led to incomplete or faulty interpretation. Here we present a large, cohesive data set of $\delta^{13}$C, $\delta^{15}$N, and $\delta^{34}$S compositions of vestimentiferan tubeworms, a dominant seep taxon. Vestimentiferans, which contain sulfide-oxidizing bacterial endosymbionts, act as ecosystem engineers and provide habitat for 100’s of other species. Our sampling design allowed us to test several hypotheses about how vestimentiferans acquire nutrients over their long lifespan (200+ years) and contribute to nutrient cycling.
Tissue $\delta^{13}$C values provided strong support for the hypothesis that seep vestimentiferans take up dissolved inorganic carbon (DIC) from sediment porewater across their roots (Dattagupta et al., 2006). $\delta^{34}$S values were extremely variable among individuals of the same species within one location (<1m$^2$ area), indicating high variability in the inorganic sulfur pools on a very small spatial scale. This finding supports the hypothesis that vestimentiferans use their roots to cycle sulfate and sulfide between their symbionts and free-living consortia of sulfate-reducing archaea in the sediment (Boetius et al., 2000). Finally, consistent differences in $\delta^{15}$N between two co-occurring vestimentiferan species provided the first strong evidence for partitioning of inorganic resources, which could have significant implications for the ecology and evolution of this taxonomic group.

**Background**

Stable isotopes have led to some of the most important discoveries about the nutrition and unique adaptations of cold seep animals and related taxa that live at hydrothermal vents (Childress et al., 1986; Fry et al., 1983; Kulm et al., 1986; Rau, 1981). As molecular, physiological, and quantitative techniques expand our knowledge and generate new hypotheses, stable isotopes remain a powerful tool as an independent means to falsify or support hypotheses and to point research in new directions. Most earlier isotope studies consisted of a small number of samples from one or two locations (Dattagupta et al., 2006; MacAvoy et al., 2005), preventing tests of many hypotheses or assessment of spatial variability. At Gulf of Mexico cold seeps, vestimentiferan tubeworms are keystone species and understanding how these animals acquire nutrients and affect nutrient cycling at seeps is essential to understanding the community structure and functioning of the entire seep ecosystem. The vestimentiferans not only dominate
biomass, but also act as ecosystem engineers by both providing heterogeneous physical structure and ameliorating chemical conditions, making the seep environment habitable by a diverse assemblage of heterotrophic species (Cordes et al., 2009). In this study, we have employed a rigorous sampling approach, which allowed us to test hypotheses addressing vestimentiferan tubeworm physiological ecology and interspecies interactions, as well as assess spatial variability in vestimentiferan tissue isotope compositions. The spatial scale at which variability occurred provided insight into the underlying processes that affect carbon, nitrogen, and sulfur cycling at seeps.

Twenty discrete aggregations (<1m² area) of vestimentiferans were collected at varying distances from one another, overall spanning 6.3 degrees in longitude across the Gulf of Mexico and 1800 m water depth (Figure 3-1). δ¹³C, δ¹⁵N, and δ³⁴S compositions were obtained for dissected vestimentum (muscle) tissue from up to 6 individuals of each species per aggregation. The two vestimentiferan species in our collections were Escarpia laminata, described from the Florida Escarpment, and Lamellibrachia sp. 1, a potential new species that is not distinguishable from the shallower Gulf species Lamellibrachia luymesi using the COI or 16S gene, but has distinctive morphological characteristics (Miglietta et al., in press).

**Tissue δ¹³C supports root DIC uptake**

All vestimentiferans lack a digestive tract and therefore must obtain nutrition solely from dissolved compounds and from their chemoautotrophic bacterial endosymbionts. Both vestimentiferans and vesicomyid clams, which also occur at vents and seeps, contain sulfur-oxidizing symbionts that fix dissolved CO₂ using primarily form II RuBisCO (Elsaied and Naganuma, 2001; Newton et al., 2008; Robinson and
Vent vestimentiferans acquire dissolved CO$_2$ across their plumes (Childress et al., 1993; Goffredi et al., 1997) and tissue $\delta^{13}$C values are between about -25 and -9‰ (Fisher, 1995). The more enriched end of this range has been explained by carbon limitation at the site of assimilation (Fisher et al., 1990). Vesicomyid clams have a relatively narrow range of tissue $\delta^{13}$C values between about -40 and -31‰ across both vent and seep habitats (Kennicutt et al., 1992). The isotope values of both taxa are consistent with use of seawater CO$_2$ ($\delta^{13}$C=0‰; Peterson and Fry, 1987) fixed via sulfide oxidation.

Like vent vestimentiferans, seep vestimentiferans can acquire dissolved CO$_2$ across their plumes (Freytag et al., 2001). Previous modeling and physiological studies suggested that seep vestimentiferans might also take up porewater DIC through the roots as part of a sulfate/bicarbonate anion exchange (Cordes et al., 2005a; Dattagupta et al., 2006). Porewater bicarbonate can be derived from microbial sulfate reduction coupled to hydrocarbon oxidation (Boetius et al., 2000; Joye et al., 2004), part of the same process that produces hydrogen sulfide in marine sediments. The resulting bicarbonate isotopically reflects hydrocarbon source material. Methane is the most common hydrocarbon driving this reaction and ranges in $\delta^{13}$C from about -117‰ to -30‰ in the Gulf (Sassen et al., 2002). A previous study compiled isotope data from numerous trawl and submersible vestimentiferan collections that were analyzed by several groups over years of research cruises (Kennicutt et al. 1992). The authors attributed the wide range of isotope values in seep vestimentiferans to incorporation of porewater DIC; however, the most depleted $\delta^{13}$C value from a vestimentiferan collected by submersible was around -42‰, close to that of vesicomyid clams, and the sampling and preparation methodology
of most samples is unknown, so conclusions should be viewed with caution (Kennicutt et al. 1992). In the present study, where we used uniform collection and analysis techniques on muscle samples from live individuals, we can more confidently address the hypothesis of porewater DIC uptake.

If vestimentiferans assimilate porewater bicarbonate, we would expect their tissue to have more depleted $\delta^{13}C$ values than clams and vent vestimentiferans. Additionally, since worms within an aggregation share the same root space and $\delta^{13}C$ compositions of hydrocarbon pools vary by location across seep sites (Chapter 2; Joye et al., in press), we would expect to see little variation among individuals within collections and larger variation among collections from different locations.

Vestimentiferans in this study had a wide range of $\delta^{13}C$ compositions, including the most depleted $\delta^{13}C$ values yet reported for vestimentiferans ($\delta^{13}C = -65\%o$ for E. laminata and $\delta^{13}C = -55\%o$ for Lamellibrachia sp. 1; Figure 3-2a). Individuals from the same collections had very similar $\delta^{13}C$ compositions, while there were large differences among collections, indicating isotopic differences in the endmember carbon sources.

Vestimentiferans cannot take up methane directly nor would there be isotopically depleted DIC in any appreciable concentration around plumes, which were at least 25 cm above the sediment surface in these collections. Thus, the hypothesis that vestimentiferans take up porewater DIC across their roots is supported.

This hypothesis is also supported by the most enriched vestimentiferan tissue $\delta^{13}C$ values, which were from juvenile (<15cm) E. laminata collected with bathymodiolin mussels (the three juvenile E. laminata had $\delta^{13}C = -26.8, -25.2, and -20.5\%o$). The mussel Bathymodiolus brooksi in these collections had $\delta^{13}C$ values ranging from -65 to -
55‰, indicating that they are incorporating methane, isotopically depleted DIC, or both
(B. brooksi contains both methylotrophic and chemoautotrophic symbionts; Fisher et al.,
1993). Clearly, the juvenile tubeworms are using a different, more $^{13}$C-enriched source
from the mussels. The $\delta^{13}$C values in juvenile E. laminata are consistent with
chemoautotrophic fixation of dissolved seawater CO$_2$. We hypothesize that these small
juveniles are not utilizing porewater DIC because their roots are not yet developed
enough to access it (Freytag et al., 2001), and any isotopically light DIC present in the
seeping fluids is rapidly diluted by overlying benthic seawater DIC.

**Evidence of resource partitioning from tissue $\delta^{15}$N**

Vestimentiferans at seeps have a very patchy distribution, with dense clumps of
worms inhabiting discrete areas, sometimes associated with fissures in carbonate
pavements or between carbonate boulders and the sediment, and aggregations of small
(young) individuals are rare. This implies a limited amount of acceptable habitat where
vestimentiferans can settle, along with strong competition for this space. Most
aggregations we collected contained both E. laminata and Lamellibrachia sp. 1. Where
the two species co-occurred, there was no apparent zonation pattern, which led to
speculation that the two species coexist by partitioning one or more resources. If
resource partitioning were occurring, we would expect to see consistent differences in
isotope values between species. Comparison of tissue $\delta^{13}$C and $\delta^{34}$S between E. laminata
and Lamellibrachia sp. 1 within the same collections did not support resource
partitioning (Figure 3-2a,c).

For nitrogen, however, $\delta^{15}$N values in E. laminata were consistently more
enriched than Lamellibrachia sp. 1 by 2.6 ± 0.7‰, except one collection, WR 269, in
which *E. laminata* was 4.3‰ more enriched. In this collection, the average $\delta^{15}$N values were notably more depleted than other collections. Partitioning of nitrogen was previously suggested by a study in which two tubeworm aggregations of the upper slope species *Lamellibrachia luymesi* and *Seepiophila jonesi* were analyzed (*MacAvoy et al., 2005*). In this case, the two species have distinctive growth patterns: *S. jonesi*’s plume is normally within cm’s of the sediment surface while *L. luymesi*, like the deeper-living *Lamellibrachia* sp. 1 and *E. laminata*, grow erect with their plumes present up to meters above the sediment. We see no such obvious differences in *E. laminata* and *Lamellibrachia* sp. 1 growth patterns, and the similarity in $\delta^{13}$C and $\delta^{34}$S values indicates that the species share physical plume and root space. Given this, the possible mechanisms producing the observed difference in $\delta^{15}$N values are the assimilation of different nitrogen sources (nitrate vs. ammonium, for example), the acquisition of the same chemical species across different parts of the worm’s body, or a difference in the mechanism of uptake or assimilation from the same source pool. The first two mechanisms clearly support partitioning of inorganic nitrogen resources, and suggest that vestimentiferans are limited by nitrogen at some point in their life cycle. Further study into the nitrogen metabolism by vestimentiferans is very likely to provide significant insights into the physiological ecology and evolution of this group.

**Tissue $\delta^{34}$S supports cycling of sulfate and sulfide between vestimentiferans and sediment microbial consortia**

Vestimentiferan tissue $\delta^{34}$S reflects the isotope composition of the environmental sulfide their symbionts use for chemoautotrophy (*Childress and Fisher, 1992*). In seep sediments, the majority of sulfide is produced via microbial sulfate reduction, which can
lead to a sulfide pool as much as 50‰ more depleted in $^{34}$S than seawater sulfate ($\delta^{34}$S ≈ 20‰) (Chambers and Trudinger, 1970; Harrison and Thode, 1958). However, since seep sediments act as a semi-closed system, sulfate becomes increasingly limited with depth (Dattagupta et al., 2008) and sulfate reduction rate (Aharon and Fu, 2000), reducing fractionation potential. While seawater sulfate is consumed at shallow sediment depths, vestimentiferans release waste sulfate (produced by symbiont sulfide oxidation) via their roots deeper in the sediment (Dattagupta et al., 2008; Dattagupta et al., 2006). It has been hypothesized that this waste sulfate is then reduced by consortia of sediment microbes, thus ensuring a long-lived sulfide supply for the symbiosis (Cordes et al., 2005a; Dattagupta et al., 2008; Dattagupta et al., 2006). Because of rate limitations that would result from excessive diffusion distances, the sulfate could only be effectively recycled if it is reduced by microbes in the immediate vicinity of the animal’s single root.

$\delta^{34}$S values in vestimentiferans were highly variable with an overall range of -23.1 to 18.4‰ (Figure 3-2c). There was also large variability among individuals of the same species in the same collection (up to 20‰), reflecting high variability in the endmember sulfide pool on a very small spatial scale. These results are consistent with the reduction of excreted sulfate by microbes close to the vestimentiferan roots. The small $E. \text{ laminata}$ collected with mussels had some of the most enriched $\delta^{34}$S values ranging from 4.6 to 16.8‰. This may reflect their lack of root development and plume sulfide uptake (Freytag et al., 2001). Since this was an active gas seep microhabitat, sulfate reduction rates could be high, causing lower fractionation rates (Aharon and Fu, 2000) and isotopically light sulfate to be consumed at very shallow sediment depths. It is
also possible that isotopically heavy sulfide from deeper sediment layers is pushed upward by migration of seeping fluids.

**Conclusions**

The patterns of tissue carbon and sulfur isotopes in this study underscore the importance of microbial transformation of seeping fluids and elucidate the nature of interactions between vestimentiferans and sediment microbes and their respective roles in carbon and sulfur cycling at seeps. Conversely, the consistency of δ^{15}N in vestimentiferans across the Gulf, especially within sites (Figure 3-2b), indicates that nitrogen isotopes are less affected by small-scale microbial processes and are likely governed by regional-scale geologic processes. Isotope data for inorganic nitrogen sources at seeps are completely absent from the literature and should be targeted in future studies. Additionally, the interspecific differences in nitrogen isotopes strongly suggest that exploration into resource partitioning and nitrogen metabolism in vestimentiferans will be key in broadening our understanding of the physiological ecology and evolution of this group.

**Methods**

**Sample collection**

Vestimentiferan aggregations were obtained using the Bushmaster Jr. collection device mounted on the front of the deep submergence vehicle Alvin (in 2006 or the remotely operated vehicle Jason II (Woods Hole Oceanographic Institution) in 2007, or by grabbing from within a 1-m^2 area using the submersible’s manipulator arm. The Bushmaster Jr. is a hydraulically actuated net lined with a 63-μm mesh and an open
diameter of 60 cm designed to collect intact aggregations of vestimentiferans (Bergquist et al., 2003). Once onboard the ship, up to six individuals of each species were sampled for stable isotope analysis by dissecting a piece of vestimentum (muscle) tissue. The samples were rinsed with deionized water to remove any residual seawater and frozen at -70°C. The term “individual” refers to a vestimentum sample from one individual worm, “collection” refers to all individuals analyzed from a single Bushmaster collection or discrete aggregation collection, and “site” refers to a seep area where the collection was made. These areas were discovered through examination of 3D seismic data, are of variable extent, and our firsthand knowledge of the extent of seep communities in an area is limited to the portions explored by submersible or ROV. In general, visual evidence of chemosynthetic communities within an area occurred over spatial scales of 100’s of square meters to a square kilometer or two.

*Stable isotope analysis*

Vestimentum samples were dried at 60°C, homogenized, and acidified to remove any inorganic carbonate. Samples were redried and subsamples were analyzed for stable carbon and nitrogen isotopes at the Stable Isotope Facility at the University of California, Davis using an Integra elemental analyzer coupled with a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, United Kingdom) or by RWL (School of Biological Sciences, Washington State University) using continuous-flow isotope ratio mass spectrometry involving Costech elemental analyzer coupled to a Micromass Optima isotope ratio mass spectrometer (EA/IRMS). Data from each of the laboratories are calibrated to NIST (National Institute of Standards and Technology) reference materials. All stable sulfur isotope analysis was performed by SAM at the University of Virginia.
Stable Isotope Laboratory using continuous-flow isotope ratio mass spectrometry involving a Carlo Erba elemental analyzer coupled to a Micromass Optima isotope ratio mass spectrometer (EA/IRMS).

Values are expressed using δ (delta) notation and reported in units of permil (‰), where

\[ \delta X = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 10^3, \]

\[ X = ^{13}\text{C}, ^{15}\text{N}, ^{34}\text{S} \text{ and } R = ^{13}\text{C}/^{12}\text{C}, ^{15}\text{N}/^{14}\text{N}, \text{ or } ^{34}\text{S}/^{32}\text{S}. \]

PDB (Pee Dee Belemnite) was used as the standard for carbon, air N\textsubscript{2} for nitrogen, and CDT (Canyon Diablo Triolite) for sulfur.
Figure 3-1. Map showing the collection sites for this study. The site names are based on Minerals Management Service (MMS) lease block designations and consist of a two-letter abbreviation, which stands for the region (AC=Alaminos Canyon, for example) followed by a three-digit number. A “collection” consists of individuals obtained from within a 1-m² area. Sites range in size from about 0.01 to 2 km². Numbers in parentheses indicate the number of aggregations collected at each site.
Figure 3-2. Average and standard deviation of tissue (a) $\delta^{13}$C (b) $\delta^{15}$N and (c) $\delta^{34}$S for all vestimentiferan collections. Each symbol represents the mean isotope value for all *E. laminata* (●) or *Lamellibrachia* sp. 1 (○) in one collection. Numbers above and below symbols indicate sample size. Sample sizes are the same for $\delta^{13}$C and $\delta^{15}$N analyses and symbols that do not have numbers have a sample size of 3 individuals. Where error bars are not visible for collections with sample sizes greater than 1, the bars are hidden within the symbol.
CHAPTER 4

Trophic interactions in mussel, vestimentiferan, and clam communities at Gulf of Mexico lower-slope cold seeps

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Abstract

We analyzed $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S values in heterotrophic communities associated with vestimentiferan tubeworms (11 collections), bathymodiolin mussels (20 collections), and vesicomyid clams (4 collections) across the Gulf of Mexico lower slope at depths below 1000 m. This was the first thorough characterization of the deep slope sites, many of which were visited for the first time in this study. The tissue $\delta^{13}$C contents of many heterotrophs indicated incorporation of organic carbon derived from methane, including heterotrophs collected with vestimentiferan tubeworms, many of which had $\delta^{13}$C values between -65 and -50‰. This included suspension-feeding stolonifers that colonize the tops of vestimentiferan tubes, indicating that particulate organic matter and/or meiofauna that incorporate methane-derived carbon are carried upward from the sediment, sometimes more than a meter, to the sessile cnidarians. The $\delta^{13}$C values of mussels,
vestimentiferans, and clams varied little among individuals within collections and substantially between collections, even those collected only 10’s of meters apart. As a whole, the δ^{13}C of heterotrophs tracked the δ^{13}C compositions of their associated symbiotic species, indicating that most animals feed locally and that the δ^{13}C signature of the inorganic carbon pool affects the δ^{13}C signature of the entire local community. δ^{15}N values were significantly more depleted in mussel-associated heterotrophs than in vestimentiferan- or clam-associated heterotrophs, which follows an approximate 7-8‰ depletion in mussel tissue δ^{15}N relative to that of vestimentiferans in the same sites. This indicates a difference in the isotope or chemical composition of the inorganic nitrogen pool in mussel microhabitats compared with vestimentiferan and clam microhabitats. The overall ranges of δ^{34}S values in heterotrophs associated with mussels and vestimentiferans were quite similar (δ^{34}S = -18.5 to +21.1‰ for mussel associates and δ^{34}S = -16.8 to +19.1‰ for vestimentiferan associates), although statistically, vestimentiferan associates were significantly more δ^{34}S-depleted than mussel associates. Heterotrophs associated with Calyptogena sp. nov. from the Alaminos Canyon 601 collection were significantly more depleted in δ^{34}S than heterotrophs associated with Calyptogena ponderosa from the other three collection sites. The δ^{34}S values of the clams themselves showed a similar trend, indicating a difference in the δ^{34}S composition of the inorganic sulfur pool at the different collection sites.

**Introduction**

Hydrocarbon seeps are a common occurrence in deep water on the Gulf of Mexico continental slope. The oil, natural gas, and other reduced chemicals emitted from seeps provide energy for local primary production by chemosynthetic microbes. In the
deep sea, where there is no sunlight and very little nutrition reaching the seafloor, seeps act as oases of primary productivity and support high-biomass animal communities (Carney, 1994). Because of the toxicity associated with the seeping fluids, many of the species have specialized adaptations to thrive in this habitat. One adaptation is the formation of symbioses with chemoautotrophic or methanotrophic bacteria. Animals that form symbioses, particularly bathymodiolin mussels and vestimentiferan siboglinid tubeworms, dominate biomass at seeps and act as foundation fauna, providing habitat for communities of heterotrophic animals. The mussels and vestimentiferans rely almost entirely on their symbionts for nutrition and are functionally autotrophic.

Free-living chemoautotrophic and methanotrophic bacteria supply food for abundant heterotrophic communities of both seep-obligate and regionally occurring species. Many of the regional species are found at greater densities at seeps because of the abundance of food and the habitat complexity provided by the larger symbiotic species. In this study, we investigate trophic interactions of the communities associated with three of the dominant symbiotic taxa found at cold seeps on the lower (>1000m) slope of the Gulf of Mexico.

Vestimentiferans and bathymodiolin mussels are the most common and abundant symbiotic taxa at seeps. On the Gulf of Mexico lower slope, there are three vestimentiferan species: *Escarpia laminata* and *Lamellibrachia* sp. 1 are common and frequently co-occur in the same aggregations, and *Lamellibrachia* sp. 2 is very rare and occurs with the other two (Miglietta et al., in press). All vestimentiferans contain sulfide-oxidizing chemoautotrophic endosymbionts. The three bathymodiolin mussel species that occur on the lower slope are *Bathymodiolus childressi*, which is also common at
shallower depths (overall depth range 525 to 2284 m and collected between 1005 and 2284 m in this study), *B. brooksi* (collected between 1080 and 2745), and *B. heckerae* (collected between 2180 and 2745 m) (Cordes *et al.*, in press). Where the depth ranges of these species overlap, *B. brooksi* often co-occurs in the same aggregations with either *B. childressi* or *B. heckerae*. *B. childressi* has only methanotrophic symbionts (Cavanaugh *et al.*, 1987), *B. brooksi* forms a dual symbiosis with both chemoautotrophic and methanotrophic bacteria (Fisher *et al.*, 1993), and *B. heckerae* contains four different symbionts: a methanotroph, two chemoautotrophs, and one methylotroph-related phylotype (Duperron *et al.*, 2007). A third, less common, symbiotic taxon comprises the vesicomyid clams. The species we collected on the lower slope were *Calyptogena ponderosa* and an undescribed *Calyptogena* sp. nov. Vesicomyid clams contain sulfide-oxidizing chemoautotrophic endosymbionts (Fisher, 1990).

Each of the symbiotic fauna types provides a different habitat, which can affect the species composition of the associated heterotrophic community. Although there is substantial species overlap, vestimentiferans and mussels harbor significantly different associated communities (Cordes *et al.*, in press). The associated community is affected by a combination of the symbiotic species themselves and the abiotic microhabitat in which they occur. Since the mussels harbor symbionts in their gills and do not have binding proteins to transport sulfide or methane, they must live in areas with significant concentrations of reduced chemicals in the epibenthic water. As a result, mussels inhabit areas with active seepage where methane and sulfide are present in sufficient concentrations above the sediment surface. Depending on the abundance and flux of seeping fluids, mussels can be present in small clumps or vast beds many layers thick. As
juveniles, vestimentiferans similarly require sulfide in the epibenthic water at the time of settlement and early stages of growth. However, adult seep vestimentiferans, like those collected in this study, can mine sulfide from deep below the sediment surface using a buried portion of their body called the root, and thus can inhabit areas where surface expression of sulfide has subsided (Freytag et al., 2001). In addition to differences in water chemistry, the physical structure provided by the symbiotic fauna also can influence species composition and the type of nutrition available to heterotrophic animals. Mussels have smooth oval-shaped shells piled on top of one another, while vestimentiferans have slender vertical tubes that can extend meters upward into the water column and form aggregations that resemble a “bush”. This different type of structure provides habitat that is not available in a mussel bed. For example, the chemical environment at the tops of vestimentiferan tubes is primarily normal benthic seawater even when sulfide is detectable at the base of the same aggregation (Cordes et al., 2005b).

Vesicomyid clam associated communities have not been the target of community studies, nor were they heavily sampled for the present trophic study. The clams are typically found in habitats separate from vestimentiferans and mussels and rarely form dense beds in the Gulf of Mexico. They are more often found in patches of scattered individuals partially buried in the sediment, which, incidentally, makes them difficult to locate and sample. Vesicomyids burrow through the sediment leaving distinctive trails. It has been hypothesized that since they acquire sulfide through their foot, they must move around because there is not sufficient flux to replenish the sulfide in one location.
(Fisher, 1990). Animals associated with vesicomyid clams either inhabit the sediment surrounding the clams or colonize the exposed portion of the clam shells.

**Methods**

**Study sites**

The study sites are named according to the Minerals Management Service lease block designations. Each name includes a two-letter abbreviation for the region (e.g. GC for Green Canyon) followed by a three-digit number. The 13 sites in this study are located along the lower continental slope of the Gulf of Mexico from 225 km south of Texas near the Texas-Louisiana border to south of Alabama (**Figure 4-1**). Sites ranged in depth from 970 m to 2800 m.

In the Alaminos Canyon (AC) area we sampled at three sites. The AC 601 study site contains a large brine lake around which were many pelagic sea cucumbers and a few solitary vestimentiferans. To the south, near the top of a ridge, there were many isolated clumps of vestimentiferans and exposed carbonate. Many of the vestimentiferan clumps were heavily encrusted with attached fauna and appeared to be rather old, although occasional smaller younger-looking aggregations were seen. Mussels were also present near the carbonates.

At the AC 645 study site, monoliferans (*Sclerolinum* sp.) and frenulates (*Oligobrachia* sp.) were observed in the western area. About 150 m to the east was a mussel bed and a large vestimentiferan “field”. Mussels (*Bathymodiolus brooksi*) were collected at this location. The mussels here were notably coated in a white precipitate.

The AC 818 study site was the deepest of sites sampled in this study at 2750 m. This site contains a relatively small linear area (along a fault) with vestimentiferans
(Escarpa laminata), mussels (mostly B. brooksi and a few B. heckerae), and monoliferans (Sclerolinum sp.). In some areas, active seepage is evidenced by stained sediment, bacterial mats, and oil bubbling out of the sediment. Dead clam shells were also present.

Atwater Valley (AT) 340 was a relatively large site with extensive and varied chemosynthetic communities and abundant carbonate boulders. This site contained some extensive mussel beds (B. brooksi and B. heckerae), and vestimentiferans (E. laminata and Lamellibrachia sp. 1) were also very abundant.

The Garden Banks (GB) 697 study site contained various indicators of active seepage including bacterial mats, carbonates, vestimentiferans (E. laminata and Lamellibrachia sp. 1 and 2), mussels (B. childressi), and vesicomyid clams (Calyptogena ponderosa). There were two main areas separated by about 3 km. The southern area, where we made one vestimentiferan collection, was at about 1270 m depth and contained lush vestimentiferan communities. The northern section contained a very active mud volcano with a few vestimentiferans nearby. Vestimentiferans and mussels were collected from the same location about 1 km south of the mud volcano at 1000 m depth. The GB 829 study site contained a single high-relief mound. On the slope of this mound was a dense mussel bed (B. childressi).

In the Green Canyon (GC) region, we visited two sites. The GC 600 site contained two main areas separated by about 1.3 km. In the northwest, gas was seen bubbling out of cracks in the carbonate rock and vestimentiferans (Lamellibrachia sp. 1) and mussels (B. brooksi) were observed growing in these cracks. We collected Calyptogena ponderosa and a few associated polychaetes at the southeast area. There
were very few living communities in the southeast, but mussel and clam shells littered the area. Pockmarks with crude oil bubbling out were observed throughout the site. Carbonates colonized by gorgonian soft corals occasionally occupied the edges of these pockmarks.

The GC 852 study site contained extensive and varied communities, including an extensive hard and soft coral community. The coral and chemosynthetic communities are located on the southern part of a 2 km long ridge feature that runs north to south and rises from 1500 to 1395 m depth. The southern end is the highest elevation and corals were abundant on a mound that rises an additional 20 m from this ridge feature. The corals inhabited carbonate pillars located in the northern part of this mound and vestimentiferans (*E. laminata* and *Lamellibrachia* sp. 1 and 2) and mussels were collected about 350 m south of the corals on the flanks of the mound.

Clams (*C. ponderosa*) were collected from the Mississippi Canyon (MC) 462 study site in an area with bacterial mat. Oil bubbles, gas bubbles, and gas hydrates were observed after the mat was disturbed. There were no carbonates in the immediate vicinity of the clams but carbonates containing hard and soft corals were found about 420 m to the south.

The Walker Ridge (WR) 269 study site contains an extensive monoliferan “field” in the eastern part of the site. About 300 m to the west, there were vestimentiferan (*E. laminata* and *Lamellibrachia* sp. 1) and mussel (*B. brooki*) communities and carbonate rocks.
Community collections

Collections were obtained during two expeditions in 2006 and 2007 using the deep submergence vehicle Alvin and the remotely operated vehicle Jason II, respectively. Quantitative collections of vestimentiferan and mussel communities were obtained using the Bushmaster Jr. and mussel pot/mussel scoop collection devices, respectively (Cordes et al., in press). The Bushmaster Jr. is a hydraulically actuated collection device with an open diameter of 0.7 m and lined with a 63-µm mesh (Cordes et al., 2005b). The device is placed over a clump of vestimentiferans and then closed to capture the vestimentiferans and all animals associated with the vestimentiferan tubes and interstices. The mussel pot device was modified from the design of Van Dover (2002). This cylindrical aluminum pot is 25 cm in diameter, 30.5 cm in height and has an internal volume of 0.015 m³. The submersible or ROV’s manipulator places the device over a clump of mussels and turns a handle one full rotation to close a Vectran™ skirt. Another full rotation released an outer ring to allow evaluation of collection efficacy. The mussel scoop is a 63-µm mesh Nitex liner (similar to the liner of the Bushmaster) fitted to the inside of a coarse-mesh net. The manipulator of the submersible dragged the scoop through the mussel bed then placed the entire scoop into an insulated biobox and closed the lid. Vesicomyid clams and their associated communities were sampled using the mussel scoop (AC601 collection) or by grabbing with the submersible’s manipulator (other three collections). Only associated fauna attached to the clam shells were obtained from manipulator grabs.

Once onboard the ship, Bushmaster collections were emptied into a large plastic tub and the device and holster rinsed with cold, filtered seawater. The vestimentiferan tubes were rinsed to remove any additional associated fauna and placed into a bucket of
cold filtered seawater, which was transported directly to the laboratory or stored in a cold room until the collection was processed. Rinse water from the device, holster, and vestimentiferan tubes was sieved to 1mm. The sieved material was visually inspected and animals removed and sorted. Associated fauna was identified to the lowest possible taxonomic level. Specimens of animals that could not be identified onboard the ship were fixed in 4% formalin and transported back to the Pennsylvania State University for further identification or sent to taxonomic experts.

Up to three individuals of each taxon from each collection were sampled for stable isotope analysis. Stable isotope samples were obtained from associated fauna by dissecting a piece of muscle tissue from large animals or using whole individuals for smaller animals. The samples were rinsed with deionized water to remove any residual seawater and frozen at -70°C. For the vestimentiferans, vestimentum (muscle) tissue was sampled from up to six individuals of each species from each collection.

Mussel pot and mussel and clam scoop collections were handled similarly. All mussels and clams in these collections were opened to check for the commensal polychaetes Branchipolynoe seepensis and the nautilliniellid, and mantle tissue was sampled from up to six individuals of each species from each collection.

*Stable isotope analysis*

All samples were dried at 60°C and homogenized. A drop of 1 N hydrochloric acid (HCl) was added to the homogeneous powder to remove any inorganic carbonate. If the sample bubbled, indicating reaction with carbonate, additional drops were added until reaction ceased. Samples were redried and subsamples were analyzed for stable carbon, nitrogen, and sulfur isotope composition. Tissue subsamples were analyzed for $\delta^{13}$C and
δ^{15}N at the Stable Isotope Facility at the University of California (UC), Davis using an Integra elemental analyzer coupled with a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, United Kingdom). Tissue subsamples were analyzed for δ^{34}S at the University of Virginia Stable Isotope Laboratory using continuous-flow isotope ratio mass spectrometry involving a Carlo Erba elemental analyzer coupled to a Micromass Optima isotope ratio mass spectrometer (EA/IRMS). Values are expressed using δ (delta) notation and reported in units of permil (‰), where 

$$\delta X = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 10^3,$$

X = ^{13}\text{C}, ^{15}\text{N}, or ^{34}\text{S} and R = ^{13}\text{C}/^{12}\text{C}, ^{15}\text{N}/^{14}\text{N}, or ^{34}\text{S}/^{32}\text{S}.

PDB (Pee Dee Belemnite) was used as the standard for carbon, air \text{N}_2 for nitrogen, and CDT (Canyon Diablo Triolite) for sulfur.

Statistical Analysis

Pairwise comparisons were carried out using 2-sample t-tests where the data met the assumptions for parametric statistical methods and Mann-Whitney U-test where these assumptions were not met (Zar, 2010). When multiple comparisons were made, a Bonferroni correction factor was applied to determine the p-value at which differences were considered significant (Zar, 2010).

Results & Discussion

Overall trends

We obtained 20 mussel community collections from 9 study sites, 11 vestimentiferan community collections from 4 sites, and 4 clam collections from 4 sites. One of the clam collections was obtained with the scoop net and contained a variety of
associated animals, while the other 3 collections were grabs of individual clams with the manipulator, and therefore only contained associated fauna that were attached to the clam shells. The undescribed clam *Calyptogena* sp. nov. was collected only from AC601 (the scoop collection) and *Calyptogena ponderosa* from the other three sites (manipulator grabs). The only other species collected for this study that is known to contain sulfur-oxidizing symbionts is the frenulate siboglinid tubeworm *Oligobrachia* sp. collected with vestimentiferans.

The tissue isotope values of the majority of seep-associated heterotrophs were consistent with at least some use of seep primary production (*Table 4-1, Figure 4-2*). Most of the tissue δ^{13}C values were more depleted than δ^{13}C in organic matter produced by marine phytoplankton, which ranges from about -22 to -15‰ (Gearing *et al.*, 1984); more than half of the animals were more depleted in δ^{15}N than normal ocean particulate organic nitrogen (PON) (δ^{15}N > 6‰) (Saino and Hattori, 1987); and almost all animals were more depleted in δ^{34}S than Gulf of Mexico bottom water sulfate, which averages 20.3‰ (Aharon and Fu, 2003). This is consistent with other studies that have found most animals in seep habitats obtain substantial nutrition from seep primary production (*Cordes *et al.*, in press.; Levin and Mendoza, 2007; Levin and Michener, 2002; MacAvoy *et al.*, 2005).

The average tissue δ^{13}C values in heterotrophs associated with mussels were significantly more depleted than δ^{13}C values of heterotrophs associated with vestimentiferans (*Table 4-2*). This was expected because of the abundance of methane in mussel microhabitats. Somewhat unexpected was that the associated heterotrophs in many vestimentiferan collections had values between about -60 and -45‰, which also
indicates use of methane-derived carbon. In adult vestimentiferan habitats, seepage usually has slowed to the point that methane and sulfide are no longer detectable above the sediment surface (Cordes et al., 2005b). The depleted values in the vestimentiferans themselves (δ13C = -65 to -55‰) can be explained by the mining of methane-derived dissolved inorganic carbon (DIC) from porewaters in the sediment (Chapter 3; Dattagupta et al., 2006). The depleted values in heterotrophs indicate that methane-derived DIC is also available at the sediment surface.

The suspension-feeding stoloniferans, hydroids, and zoanthids that colonize the tops of vestimentiferan tubes up to a meter above the sediment surface had δ13C values around -50‰, similar to the sediment-dwellers in the same collections (Figure 4-3, Suppl. Figures 4-13 to 4-16; Appendix B). Stoloniferans, hydroids, and zoanthids are all sessile cnidarians and feed on particulate organic matter (POM) and small swimming animals that come in contact with their nematocysts. Since dissolved reduced chemicals from seep fluids are not present in sufficient concentrations at the plume level of vestimentiferan aggregations to fuel chemoautotrophy or influence the δ13C of DIC (Cordes et al., 2005b), organic matter must be produced via chemoautotrophy in the sediment and subsequently transported to the tube tops. We did not sample meiofauna (animals between 63 µm and 1 mm), but they are a likely link between microbial primary production and heterotrophic macrofauna. In addition, resuspension of isotopically depleted POM, including bacteria, from shallow sediments may contribute to the depleted tissue isotope signal in the cnidarians.

Tissue δ13C values in the vestimentiferans and mussels varied little among individuals within a collection but substantially between collections, even ones in the
same site only 10’s of meters apart. The average $\delta^{13}C$ values of heterotrophs were correlated with the average $\delta^{13}C$ values of the mussels or vestimentiferans with which they were collected (Figure 4-4), indicating that most animals feed locally and that the $\delta^{13}C$ of both symbiotic and free-living bacteria reflect the $\delta^{13}C$ of the same carbon pool.

Unlike $\delta^{13}C$, average $\delta^{15}N$ values showed very little spatial variability. For a given vestimentiferan or mussel species, $\delta^{15}N$ values were quite consistent overall, and especially within a site (Chapter 2, Chapter 3). However, average $\delta^{15}N$ values in mussels were approximately 7-8‰ more depleted than those of vestimentiferans within the same sites (Figure 4-4 shows overall), indicating a difference in the isotope composition of the inorganic nitrogen pool in the different microhabitats, or some difference in fractionation associated with nitrogen uptake by the different taxa. If the difference were in the inorganic nitrogen pool, we would expect to see a similar trend in the tissue $\delta^{15}N$ of heterotrophic fauna associated with the mussels and vestimentiferans.

Mussel-associated heterotrophs had significantly more depleted $\delta^{15}N$ values than heterotrophs collected with clams and vestimentiferans (Table 4-2, Figure 4-2a, 4-4b). Comparisons of individual heterotrophic taxa collected with both vestimentiferans and mussels did not show any consistent differences in tissue isotope values between microhabitats, but $\delta^{15}N$ was sometimes significantly more depleted in the same taxon collected with mussels than with vestimentiferans. Taxa that had significantly more depleted average $\delta^{15}N$ values when collected with mussels were the shrimp *Alvinocaris muricola* when only the GC852 collections were pooled and the sipunculid *Phascolosoma turnerae* when all mussel and vestimentiferan collections were pooled. More depleted $\delta^{15}N$ values in heterotrophs from mussel habitats suggest a more $\delta^{15}N$-
depleted food source in this environment than in vestimentiferan and clam habitats. Few data exist on ammonia concentrations in different seep animal microhabitats, but one study found 33-1,414 µM concentrations of ammonia in the sediment porewater underlying mussels (Lee and Childress, 1994), while porewater underlying vesicomyid clams and vestimentiferans had concentrations of about 90 to 180 µM (Joye et al., in press). B. childressi has been shown to take up and assimilate ammonium and nitrate from the environment (Lee and Childress, 1996; Lee et al., 1992), and the mussels themselves generally had lower δ¹⁵N values than all other animals in this study and almost always had lower δ¹⁵N than the heterotrophs in the same collections (Figure 4-4b, Suppl. Figures 4-1 to 4-10; Appendix B). The abundant ammonia could contribute to depleted tissue δ¹⁵N values in two ways. Either the ammonia itself is more ¹⁵N-depleted in mussel habitats than vestimentiferan and clam habitats, or the abundance of ammonia allows greater biological fractionation. The isotope signatures of inorganic nitrogen have not been measured in any seep habitats, and very few data exist on in situ concentrations of inorganic nitrogen species, so we cannot speculate further on the cause of this difference.

Mussels and vestimentiferans had wide ranges of tissue δ³⁴S values (mussel δ³⁴S= -10.9 to +21.1‰ and vestimentiferan δ³⁴S= -23.1 to +18.4‰), but several vestimentiferan individuals were substantially more depleted than the lowest mussel tissue δ³⁴S values (Figure 4-2b). Vestimentiferans rely exclusively on sulfide-oxidizing endosymbionts for nutrition, and adults can mine sulfide from below the sediment surface (Freytag et al., 2001), where the δ³⁴S composition of sulfide can be more depleted than in surface layers (Dattagupta et al., 2008). Average tissue δ³⁴S of vestimentiferan associates were
significantly more depleted than those of mussel associates, although the ranges in mussel and vestimentiferan associates were similar; mussel associates ranged from $\delta^{34}$S = -18.5 to +20.8‰ and vestimentiferan associates ranged from $\delta^{34}$S = -16.3 to +19.1‰ (Figure 4-2b). The similarity in ranges of heterotrophic animals indicates that the sulfur sources available to animals that dwell above the sediment surface have similar ranges in $\delta^{34}$S compositions in both microhabitats. The tendency for vestimentiferan associates to have more depleted $\delta^{34}$S values could indicate that organic sulfur from vestimentiferans is incorporated into the food web, that vestimentiferan activities (such as release of sulfate from sulfide oxidation) affect the inorganic sulfur pool, or that sulfide-derived organic sulfur is simply more abundant in vestimentiferan habitats, whereas mussel associates may incorporate more sulfate-derived organic sulfur.

In addition to being similar, the ranges of tissue $\delta^{34}$S in vestimentiferan- and mussel-associated fauna were quite large (>35‰). In heterotrophs, this could be due to a mixed diet of organic matter derived from any combination of methanotrophy, chemoautotrophy, or photosynthesis. Bathymodiolin mussels and vestimentiferan tubeworms, on the other hand, gain the majority of their nutrition from their symbionts, and still had extremely variable $\delta^{34}$S values. The large variability in tissue $\delta^{34}$S values reflects the complexity of the sulfur cycle in the seep ecosystem, and calls into question whether reliable conclusions can be drawn from examining tissue $\delta^{34}$S values. In the current data set, tissue $\delta^{34}$S values below about -15‰ were only observed in vestimentiferans, whose nutrition is known to be from chemoautotrophy and which mine sulfide from the sediment. Animals with tissue $\delta^{34}$S values greater than -15‰ but notably more depleted than seawater sulfate ($\delta^{34}$S= +20‰), can be said to incorporate
seep nutrition, but we cannot tell whether from chemoautotrophy or methanotrophy.

Finally, δ^{34}S values around +20‰ could indicate some incorporation of organic sulfur derived from seawater sulfate, but this is not necessarily the case. Some vestimentiferans had δ^{34}S values around 20‰, and since vestimentiferan symbionts oxidize sulfide to sulfate, which is excreted as waste, it is unlikely that vestimentiferans are taking up extra sulfate from seawater. Alternatively, the sulfide the worms acquired may have been enriched in δ^{34}S due to very little fractionation during the reduction of sulfate to sulfide, or some fractionation process during uptake and assimilation resulted in the enriched tissue δ^{34}S value. Anything more than these broad-stroke observations may be unreliable until we know more about the seep sulfur cycle and metabolic capabilities of seep organisms. Specifically, tissue δ^{34}S values in seep animals have very little utility in determining potential predator-prey interactions between species.

Clams had a narrower δ^{34}S range than vestimentiferans and mussels, but there were far fewer clam samples than mussel and vestimentiferan samples in this study. The one collection of Calyptogena sp. nov. from AC601 had markedly more depleted δ^{34}S compositions (δ^{34}S=-14.1 to -2.9‰) than C. ponderosa collected from the other three sites (δ^{34}S= 4.4 to 12.8‰), and were therefore examined separately (Figure 4-2b). The fauna associated with the Calyptogena sp. nov. collection had similarly more depleted δ^{34}S values than the fauna associated with the C. ponderosa collections (of which there were only three individuals, a polychaete Glycera sp., a commensal nautilliellid, and an anemone (Actinaria), with enough material for δ^{34}S analyses) (Figure 4-5). Even the attached anemones in the AC601 collection were more depleted (δ^{34}S= -4.0 to 1.9‰), so the difference is not simply a function of collecting more sediment-dwelling polychaetes
and nemerteans in the scoop net for the AC601 collection. The matching isotope difference in the heterotrophs and clams suggests that the $\delta^{34}$S composition of the endmember sulfur source underlies the difference in sulfur isotopes of the two clam species, rather than a difference in their physiology or symbionts.

**Implications for feeding biology of individual taxa**

A main goal of this study was to use tissue stable isotope values to gain clues about the feeding biology of individual taxa. With this large data set we often sampled up to 3 individuals of a species in a single collection and sometimes several individuals in each of several different collections. Very little variability among tissue stable isotope values of individuals in a collection could indicate a specialist feeding strategy, especially if this pattern is repeated in more than one collection. A few taxa that seemed to show a specialist feeding strategy upon a food source with little isotopic variability are the brittle star *Ophioctenella acies* (*Suppl. Figures 4-1a, 4-2ab, 4-3a, 4-4ab, 4-5cd, 4-6cd, 4-8cd; Appendix B*), the sipunculid *Phascolosoma turnerae* (*Suppl. Figures 4-1cd, 4-7a, 4-11a, 4-15a; Appendix B*), and the polynoid polychaete *Harmothoe* sp. (*Suppl. Figures 4-1ab, 4-5cd, 4-7ac, 4-8a, 4-9cd, 4-15ab; Appendix B*). There were many other examples like this for species only found in one collection. In fact, most species tended to group together within collections on plots of $\delta^{15}$N vs. $\delta^{13}$C and $\delta^{34}$S vs. $\delta^{13}$C (*Suppl. Figures 4-1 to 4-16; Appendix B*).

The shrimp *Alvinocaris muricola* was one of the most common and numerically abundant animals in both mussel and vestimentiferan habitats (Cordes *et al.*, in press). *A. muricola*’s tissue stable isotopes values were often variable within collections (*Suppl. Figures 4-1cd, 4-2a, 4-3acd, 4-5abcd, 4-6ab, 4-8cd, 4-10cd, 4-12c, 4-13ab, 4-14cd, 4-
14ab, 4-15ab; Appendix B). In vestimentiferan collections, *A. muricola* were often among the most depleted in δ\(^{15}\)N relative to other animals within collections, including the vestimentiferans (Suppl. Figures 4-11a, 4-12a, 4-13ac, 4-14ac, 4-15a, 4-16ac; Appendix B). In mussel collections, *A. muricola* were sometimes enriched and sometimes depleted in δ\(^{15}\)N relative to other animals, but were always enriched relative to the mussels (Suppl. Figures 4-1c, 4-2ac, 4-3ac, 4-4ac, 4-5ac, 4-6ac, 4-8ac, 4-9ac, 4-10ac; Appendix B). The variability in *A. muricola*’s tissue stable isotope values suggests a generalist feeding strategy. The depleted δ\(^{15}\)N values may reflect the shrimp grazing upon free-living bacteria that are fixing local inorganic nitrogen and the enriched values may reflect some feeding upon animals at higher trophic levels, such as small predatory meiofauna, or some consumption of surface-derived material. Some *A. muricola* individuals had δ\(^{15}\)N compositions >6‰, which is similar to the δ\(^{15}\)N composition of surface-derived particulate organic matter (POM) (Saino and Hattori, 1987), although these same individuals still had relatively depleted δ\(^{13}\)C and δ\(^{34}\)S compositions. The overall ranges for δ\(^{13}\)C (-63.7 to -20.8‰) and δ\(^{34}\)S (-18.5 to +19.1‰) in *A. muricola* also reflect a combination of seep- and surface-derived nutrition.

Previous work has shown that the methane ice worm *Hesiocaeca methanicola* is a bacterivore, and therefore its tissue isotope values reflect the isotope compositions of the free-living microbial community (Fisher *et al.*, 2000). In the previous study, the worms occupied divots in a sulfide-containing methane hydrate and the tissue δ\(^{13}\)C, δ\(^{15}\)N and δ\(^{34}\)S values in the worms strongly indicated the primary food source available on the methane ice was chemoautotrophic bacteria. In mussel habitats, *H. methanicola* δ\(^{13}\)C values ranged from -62.9 to -29.0‰, δ\(^{15}\)N ranged from -6.1 to +5.3, and δ\(^{34}\)S from 0.7 to
large variability in the isotope compositions of the microbial population. This variability could arise from different microbial species, differences in the activity levels of the microbes, or spatial variability in the inorganic endmember sources.

The sediment-dwelling sipunculid *P. turnerae* collected with vestimentiferans and mussels had enriched δ$^{15}$N values relative to other animals in the same collections, but had δ$^{13}$C and δ$^{34}$S values that were quite depleted (δ$^{13}$C = -58 to -30‰ and δ$^{34}$S = -18.2 to +14.3‰) relative to surface POM and seawater sulfate, respectively (Suppl. Figures 4-1cd, 4-2ab, 4-4cd, 4-5cd, 4-6ab, 4-7abcd, 4-10cd, 4-11ab, 4-16ab; Appendix B). In most of the collections, *P. turnerae* was enriched in δ$^{15}$N by approximately 6-8‰ relative to the mussels or vestimentiferans with which they were collected. Previous work on the related species *Phascolosoma vulgare* showed that these sediment feeders do show some specificity in the grain size of sediment they ingest and that fecal pellets account for a staggering 92% of this species’ caloric intake (Hansen, 1978). In a study of marine copepods, a major meiofaunal taxon in marine sediments, feces were enriched in δ$^{15}$N by about 8‰ and δ$^{13}$C by about 1‰ relative to their food source (Checkley and Entzeroth, 1985). Thus, a diet primarily of fecal pellets could result in tissue stable isotope values that are enriched in δ$^{15}$N while remaining relatively depleted in δ$^{13}$C and δ$^{34}$S. The sediment-feeding holothurian *Chiridota heheva* was occasionally more enriched in δ$^{15}$N than other animals in the same collections, including the predators, but not as consistently as *P. turnerae* (Suppl. Figures 4-2c, 4-3ac, 4-4ac, 4-5c, 4-6c, 4-12ac, 4-13a; Appendix B). *C. heheva*’s diet may consist of a more variable mixture of fecal pellets, meiofauna, and sediment microbes than that of *P. turnerae*. 
The most depleted $\delta^{13}C$ value of all species in all collections was found in the
deposit-feeding bristle worms *Notomastus* sp. (~80.3‰), which was more depleted than
the $\delta^{13}C$ values of the mussels with which it was collected (*Table 4-1, Suppl. Figure 4-
10a; Appendix B*). *Notomastus* species in other ecosystems are sub-surface deposit
feeders (Kikuchi and Wada, 1996). Since more isotopically depleted DIC is present
below the sediment surface than above, the overall $\delta^{13}C$ value of the food available to
*Notomastus* sp. would be more depleted than the food available to surface deposit
feeders.

*Protomystides “cap worms” on *E. laminata* hosts*

The phyllodocid polychaete *Protomystides* sp. was frequently found on top of *E.
laminata* prostomia (*Figure 4-3*). The worms build a matrix of tubular casing that affixes
them to the prostomium. This casing can house more than 20 individual worms on a
single *E. laminata* individual, many of which are tiny juveniles (pers. obs.). A blood-
sucking (hematophagous) way of life was previously suggested for the related
phyllodocid *Galyptomystides aristata*, which is found in the Galapagos Rift and East
Pacific Rise (Jenkins et al., 2002). The guts of the *Protomystides* sp. in our collections
contained a red substance, which, given the location of the polychaetes, we assume to be
vestimentiferan blood. If there is a parasitic relationship between *Protomystides* sp. and
*E. laminata*, we would expect to see a correlation between the tissue stable isotope values
of *Protomystides* sp. and the *E. laminata* individual upon which they were living.
Tissue $\delta^{13}C$ and $\delta^{15}N$ values in *Protomystides* sp. were similar those of their paired host
*E. laminata*, whereas the $\delta^{34}S$ values of *Protomystides* sp. were enriched by
approximately 12 to 20‰ relative to their paired *E. laminata* (*Figure 4-6*). When paired
Protomytides sp. and E. laminata were regressed, there was a strong linear relationship between δ¹³C of Protomytides sp. and their paired E. laminata (p<0.001; R²=0.80; Figure 4-6a), but this relationship was not significant for tissue δ¹⁵N (p=0.7; R²=0.08; Figure 4-6b) or δ³⁴S (p=0.07; R²=0.35; Figure 4-6c). Since δ¹³C values tended to vary by collection, it was possible the apparent correlation simply reflected the use of the same carbon pool by both Protomytides sp. and E. laminata. To examine whether there was in fact an individual-level correlation, we examined a single collection that contained 6 paired Protomytides sp. and E. laminata samples. The total range in E. laminata δ¹³C values in this collection was -52.6 to -42.7‰ and the regression revealed a strong and significant correlation between paired individuals (p=0.01; R²=0.83; Figure 4-7).

The lack of a strong correlation in δ¹⁵N could be due to the small total variation in δ¹⁵N (about 2‰) compared with δ¹³C (about 28‰). The sample size for δ³⁴S is smaller (n=10) than δ¹³C and δ¹⁵N (n=21), because there was not always enough Protomytides sp. tissue left to run the sulfur analysis. Although the correlation was not significant, the data show a strong trend even with only 10 paired samples. Tissue δ³⁴S values in vestimentiferans do vary widely among individuals within single aggregations (Chapter 3), so a larger sample size of paired δ³⁴S samples from the vestimentiferans and Protomytides sp. could be the best indicator of a trophic relationship between the two species. However, if a correlation truly exists, the enrichment of Protomytides sp. by 12 to 20‰ relative to their paired E. laminata is perplexing, as it contradicts the assumed 0‰ trophic enrichment in δ³⁴S between animals and their food source (McCutchan et al., 2003). The enrichment in δ³⁴S could indicate that the specific vestimentiferan product upon which polychaetes are feeding (e.g. blood) is enriched in δ³⁴S relative to bulk muscle.
(vestimentum) tissue or that the product upon which Protomytides sp. are feeding does not meet their sulfur requirements, so they must obtain it from a different source. In the latter case, no correlation between E. laminata and Protomytides sp. $\delta^{34}S$ values would be expected. More paired $\delta^{34}S$ samples would be beneficial in determining whether there is a true linear relationship.

Some Protomytides sp. individuals also inhabited the outsides of tubes or insides of dead vestimentiferan tubes. The isotope compositions of these individuals were within the same range as those found on top of the live vestimentiferans (Suppl. Figures 4-14, 4-5cd; Appendix B), and thus there was no apparent difference in feeding niche from their counterparts atop E. laminata prostomia. The individuals found on the outsides of tubes were attached via the same casing we found on the tops of E. laminata prostomia, but these casings contained only 1-3 larger individuals, and no juveniles. The detailed morphology of the digestive system in the related vent species G. aristata showed the presence of a “septum” that could facilitate the long-term storage of a blood meal (Jenkins et al., 2002). If these seep congeners share this adaptation, they feed on vestimentiferan blood for the early stages of their life and then disperse to other locations at later stages, either feeding on stored blood or ingesting other materials. Another possibility is that blood is most important for females during reproduction and, like female mosquitoes, they need a continuous supply of blood before laying eggs. This could account for the large number of juveniles present only in the prostomia casings.

Commensal polynoids inside mussel and clam hosts

Another relationship that was of a priori interest was that of polynoid polychaetes living commensally within the gills of mussel and clam hosts. In a study of the
commensal polynoid *Branchipolyne symmytilida* living inside the body cavity of the hydrothermal vent mussel *Bathymodiolus thermophilus*, there was a strong correlation between the tissue isotope compositions of individual polynoids and their hosts for both δ\(^{13}\)C and δ\(^{15}\)N (Fisher *et al.*, 1994). Furthermore, the average enrichment in *B. symmytilida* tissue δ\(^{15}\)N relative to the host mussel tissue was 3.2‰, which is what is expected for single trophic level enrichment (δ\(^{15}\)N enrichment of 3.0 to 3.4‰ in the consumer; Minagawa and Wada, 1984).

In the present study, we collected *Branchipolyne seepensis* and a species of nautiliniellid inside the mantle cavity of *B. heckerae* and the nautiliniellid was also found inside the both *Calyptogena* sp. nov. and *C. ponderosa*. Figure 4-8 shows the tissue δ\(^{13}\)C, δ\(^{15}\)N and δ\(^{34}\)S values of the polynoids vs. the isotope values of their paired host bivalves. Although the δ\(^{13}\)C overall seem to show a linear relationship, this is probably because both the polynoids and the mussels reflect the stable isotope composition of the inorganic carbon pool in a particular location. The difference between the δ\(^{13}\)C of *B. seepensis* and that of their paired host *B. heckerae* ranged from -9.1 to +7.1‰ and the difference between *B. seepensis* tissue δ\(^{15}\)N and the δ\(^{15}\)N of their paired *B. heckerae* host ranged from -3.5 to +0.3. Neither of the isotopes shows any evidence of a trophic relationship between *B. seepensis* and their host mussel.

The nautiliniellid tissue δ\(^{13}\)C values were 1.7 to 5.8‰ more enriched than that of their *B. heckerae* hosts and their tissue δ\(^{15}\)N values were 1.0 to 2.5‰ more enriched. When found in clams, the difference between nautiliniellid tissue isotope values and those of their *Calyptogena* spp. hosts were -0.9 to -0.1‰ for δ\(^{13}\)C, -1.3 to 2.9‰ for δ\(^{15}\)N, and -7.3 to 11.7‰ for δ\(^{34}\)S. The δ\(^{13}\)C and δ\(^{15}\)N show that a trophic relationship is
possible between the nautiliniellids and their bivalve hosts. The nature of this trophic
relationship could be partial predation or consumption of a product such as mucous,
gametes, or pseudofeces.

Conclusions

This study highlighted some of the factors that affect isotope values in
heterotrophic communities associated with the dominant symbiont-containing species at
Gulf of Mexico hydrocarbon seeps. The tracking of associated fauna $\delta^{13}C$ with their
symbiont-hosting foundation species indicates that the $\delta^{13}C$ of the inorganic carbon
source affects the $\delta^{13}C$ compositions of the entire local community and that most of the
animals in our collections do indeed feed locally on seep material. The $\delta^{13}C$ values in
many collections, including vestimentiferan-associated communities, indicate the
incorporation of methane-derived carbon, even in sessile species that live meters above
the sediment in which the organic carbon is produced. This finding indicates that
methane-derived carbon is more important in vestimentiferan microhabitats than
previously thought, but not necessarily that methanotrophic bacteria are an important
food source for primary consumers. The depleted values in the vestimentiferans
themselves show that chemoautotrophic bacteria also incorporate methane-derived
carbon (see Chapter 3). Thus, $\delta^{13}C$ values in consumers are not a reliable indicator of the
importance of methanotrophic vs. chemoautotrophic primary production.

$\delta^{15}N$ values indicated that mussel microhabitats contain a more isotopically
depleted or more abundant inorganic nitrogen source, such as ammonia or nitrate, than
clam or vestimentiferan habitats. Isotope compositions of inorganic nitrogen sources
remain undocumented at seeps and deserve attention in future studies. $\delta^{34}S$ values are the
least-used in seep community isotope studies and remain the most difficult to interpret. Although statistically, $\delta^{34}\text{S}$ of associated animals in vestimentiferan communities are more depleted than mussel communities, the overall ranges are quite similar and very large (>35‰). This could reflect the large variability in the inorganic sulfur sources themselves. Although sulfide can potentially be very negative, sulfate limitation and microbial sulfate reduction rates can affect fractionation, leading to isotopic differences in inorganic sulfur pools on a micro scale. Nonetheless, because of the importance of sulfide as both an energy and sulfur source, $\delta^{34}\text{S}$ values may continue to give us clues about processes affecting sulfur isotopes at seeps.

Acknowledgements

We thank the captains, crews, and scientists aboard the research vessel Atlantis and National Oceanographic and Atmospheric Association (NOAA) Ship Ronald H. Brown, as well as the pilots and engineers of the deep submergence vehicle Alvin and the remotely operated vehicle Jason II. We particularly thank Stéphane Hourdez for his help with sampling and identifying polychaetes from these collections. This work was funded by the Minerals Management Service contract #1435-01-05-CT-39187 and the NOAA Office of Ocean Exploration. The map of the Gulf of Mexico for Figure 4-1 was produced by Mary Lee Eggart, Department of Geography & Anthropology, Louisiana State University.
Table 4-1. Stable isotope data for all taxa in this study collected with seep mussels, clams, and vestimentiferans. The number outside of the parentheses is the average isotope value of all individuals. The first number in the parentheses is the number of samples, and the second number is the standard deviation. Where there was only one sample, the parentheses are omitted.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>$\delta^{13}$C</th>
<th>$\delta^{15}$N</th>
<th>$\delta^{34}$S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cnidaria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actinaria spp.</td>
<td>-39.0 (33, 9.9)</td>
<td>3.1 (33, 3.4)</td>
<td>7.6 (24, 5.7)</td>
</tr>
<tr>
<td>Hydriodea spp.</td>
<td>-42.3 (15, 6.8)</td>
<td>4.2 (15, 0.6)</td>
<td>7.8 (11, 5.0)</td>
</tr>
<tr>
<td>Stolonifera spp.</td>
<td>-44.8 (20, 5.0)</td>
<td>3.7 (20, 1.3)</td>
<td>11.1 (17, 3.0)</td>
</tr>
<tr>
<td>Zoanthidae sp.</td>
<td>-44.4 (3, 1.2)</td>
<td>6.1 (3, 0.3)</td>
<td>3.1 (3, 0.8)</td>
</tr>
<tr>
<td>Ciliophora (Protozoa)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>folliculinid ciliate (pooled)</td>
<td>-25.7</td>
<td>-1.9</td>
<td></td>
</tr>
<tr>
<td>Nematoda</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nematoda spp. (pooled)</td>
<td>-61.9 (2, 0.1)</td>
<td>0.4 (2, 0.1)</td>
<td></td>
</tr>
<tr>
<td>Nemertea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nemertea sp.</td>
<td>-45.0 (4, 8.8)</td>
<td>2.7 (4, 2.7)</td>
<td>-2.4 (3, 5.8)</td>
</tr>
<tr>
<td>Sipunculida</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phascolosoma turnerae Rice, 1985</td>
<td>-44.8 (21, 11.0)</td>
<td>5.8 (21, 3.3)</td>
<td>3.8 (21, 7.9)</td>
</tr>
<tr>
<td>Annelida</td>
<td></td>
<td></td>
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<tr>
<td>Polychaeta</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Branchinotogluma sp.</td>
<td>-50.5 (23, 9.6)</td>
<td>1.8 (23, 3.2)</td>
<td>7.4 (17, 8.4)</td>
</tr>
<tr>
<td>Branchipolyneae seepensis Pettibone, 1986</td>
<td>-52.0 (4, 7.9)</td>
<td>-1.2 (4, 1.2)</td>
<td>8.0 (5, 7.0)</td>
</tr>
<tr>
<td>Cirratilidae sp.</td>
<td>-63.3 (3, 0.6)</td>
<td>0.2 (3, 0.4)</td>
<td>4.2 (3, 5.4)</td>
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<td>Eurythoe sp.</td>
<td>-67.9 (13, 6.0)</td>
<td>1.4 (13, 1.1)</td>
<td>7.1 (13, 3.6)</td>
</tr>
<tr>
<td>Flabelligeridae sp.</td>
<td>-48.4 (18, 10.4)</td>
<td>-0.4 (18, 2.8)</td>
<td>7.0 (13, 2.3)</td>
</tr>
<tr>
<td>Glyceria tesselata Grube, 1863</td>
<td>-45.8 (2, 4.4)</td>
<td>6.3 (2, 2.3)</td>
<td>0.6 (2, 1.6)</td>
</tr>
<tr>
<td>Glyceria sp.</td>
<td>-39.3 (4, 15.6)</td>
<td>5.3 (4, 1.9)</td>
<td>0.0 (3, 1.6)</td>
</tr>
<tr>
<td>Harmothoe sp.</td>
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<td>4.4 (29, 2.7)</td>
<td>4.2 (21, 4.3)</td>
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<tr>
<td>Hesioecaecia methanicola Desbruyères &amp; Toulmond 1998</td>
<td>-47.6 (15, 10.2)</td>
<td>0.9 (15, 3.1)</td>
<td>9.6 (5, 7.2)</td>
</tr>
<tr>
<td>Heteromystides sp.</td>
<td>-37.9 (15, 7.5)</td>
<td>8.1 (15, 1.6)</td>
<td>2.5 (13, 2.9)</td>
</tr>
<tr>
<td>Lumbrineriz sp.</td>
<td>-52.5 (4, 11.4)</td>
<td>5.3 (4, 1.8)</td>
<td>3.8 (2, 7.3)</td>
</tr>
<tr>
<td>Methanoaricia dendrobranchiata Blake, 2000</td>
<td>-54.9 (6, 5.2)</td>
<td>4.2 (6, 1.7)</td>
<td>5.5 (4, 4.5)</td>
</tr>
<tr>
<td>Nautilinellidae sp.</td>
<td>-51.9 (12, 12.1)</td>
<td>0.9 (12, 2.2)</td>
<td>0.7 (6, 11.1)</td>
</tr>
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<td>Nicomache sp.</td>
<td>-53.1 (10, 13.6)</td>
<td>1.8 (10, 0.8)</td>
<td>0.2 (7, 2.8)</td>
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<td>Notomastus sp.</td>
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<td>8.9</td>
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<td>Phyllodocidae sp.</td>
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<td>7.8 (10, 1.1)</td>
<td>3.0 (5, 3.6)</td>
</tr>
<tr>
<td>Prionospio sp.</td>
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<td>-0.6 (7, 2.4)</td>
<td>-0.2 (2, 11.2)</td>
</tr>
<tr>
<td>Protomystides sp.</td>
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<td>7.2 (40, 2.1)</td>
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<tr>
<td>Siboglinidae</td>
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<td>Oligobranchia sp.</td>
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<td>2.6 (3, 0.2)</td>
<td></td>
</tr>
<tr>
<td>Taxon</td>
<td>(\delta^{13}C)</td>
<td>(\delta^{15}N)</td>
<td>(\delta^{34}S)</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>------------------</td>
<td>------------------</td>
<td>------------------</td>
</tr>
<tr>
<td><strong>Mollusca</strong></td>
<td></td>
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<tr>
<td>Polyplacophora</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ichnochiton</em> sp.</td>
<td>-50.8</td>
<td>3.5</td>
<td>9.1</td>
</tr>
<tr>
<td><strong>Gastropoda</strong></td>
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<td></td>
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</tr>
<tr>
<td><em>Buccinum</em> sp.</td>
<td>-33.8</td>
<td>1.1</td>
<td>-6.9</td>
</tr>
<tr>
<td><em>Cataegis meroglypta</em> McLean, 1987</td>
<td>-52.7</td>
<td>1.2</td>
<td>12.6</td>
</tr>
<tr>
<td><em>Fucaria</em> sp.</td>
<td>-52.6 (3, 1.3)</td>
<td>1.8 (3, 1.8)</td>
<td>-1.7 (2, 0.1)</td>
</tr>
<tr>
<td><em>Paraleptopsis</em> sp.</td>
<td>-44.4 (3, 7.9)</td>
<td>3.0 (3, 2.1)</td>
<td>6.4</td>
</tr>
<tr>
<td><em>Phymorrynchus</em> sp.</td>
<td>-41.3 (3, 2.9)</td>
<td>2.5 (3, 1.2)</td>
<td>6.9 (2, 2.5)</td>
</tr>
<tr>
<td><em>Provanna sculpta</em> Warén &amp; Ponder, 1991</td>
<td>-46.4 (5, 5.7)</td>
<td>3.0 (5, 1.0)</td>
<td>7.0 (5, 2.6)</td>
</tr>
<tr>
<td><em>Pyropelta</em> sp.</td>
<td>-49.4</td>
<td>-1.3</td>
<td>12.2</td>
</tr>
<tr>
<td><strong>Bivalvia</strong></td>
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<td></td>
</tr>
<tr>
<td><em>Cuspidaria</em> sp.</td>
<td>-39.3 (5, 3.8)</td>
<td>4.0 (9, 1.6)</td>
<td>-1.8 (4, 10.0)</td>
</tr>
<tr>
<td><strong>Arthropoda</strong></td>
<td></td>
<td></td>
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<tr>
<td>Crustacea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Alvinocaris</em> muricola Williams, 1988</td>
<td>-46.7 (80, 9.5)</td>
<td>1.4 (80, 2.6)</td>
<td>5.2 (71, 8.6)</td>
</tr>
<tr>
<td>Amphipoda spp.</td>
<td>-45.2 (6, 14.0)</td>
<td>2.7 (14, 2.8)</td>
<td>7.0 (4, 9.0)</td>
</tr>
<tr>
<td><em>Chaceon</em> sp.</td>
<td>-29.4</td>
<td>6.9</td>
<td>2.4</td>
</tr>
<tr>
<td>Isopoda sp.</td>
<td>-36.2 (3, 5.7)</td>
<td>4.4 (3, 0.8)</td>
<td>5.9</td>
</tr>
<tr>
<td><em>Munidopsis</em> spp.</td>
<td>-33.5 (7, 8.2)</td>
<td>7.0 (7, 1.6)</td>
<td>5.3 (7, 8.2)</td>
</tr>
<tr>
<td><em>Munidopsis</em> sp. 1</td>
<td>-48.0 (2, 4.1)</td>
<td>6.1 (2, 0.4)</td>
<td>8.5 (2, 1.0)</td>
</tr>
<tr>
<td>unid. brachyuran</td>
<td>-53.5</td>
<td>4.2</td>
<td>9.4</td>
</tr>
<tr>
<td><strong>Echinodermata</strong></td>
<td></td>
<td></td>
<td></td>
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<td>Asteroidea</td>
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<td></td>
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</tr>
<tr>
<td>unid. sea star</td>
<td>-32.3 (3, 1.2)</td>
<td>6.4 (3, 1.5)</td>
<td>12.3 (3, 6.2)</td>
</tr>
<tr>
<td><em>Ophioctenella acies</em> Tyler et al., 1995</td>
<td>-44.4 (28, 7.1)</td>
<td>0.7 (28, 2.2)</td>
<td>6.6 (23, 9.4)</td>
</tr>
<tr>
<td><em>Ophienigma</em> spinilimbatum Stöhr &amp; Seganzac, 2005</td>
<td>-37.0 (23, 10.7)</td>
<td>3.2 (23, 2.1)</td>
<td>9.5 (12, 6.5)</td>
</tr>
<tr>
<td><strong>Holothuroidea</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Chiridota</em> heheva Pawson &amp; Vance, 2004</td>
<td>-43.7 (29, 9.5)</td>
<td>2.3 (29, 2.1)</td>
<td>12.3 (27, 4.8)</td>
</tr>
</tbody>
</table>
Table 4-2. P-values for pairwise Mann-Whitney U tests of isotope values of associated fauna between habitat types. Bold italicized numbers indicate p-values that were significant given a Bonferroni corrected $\alpha$ value of 0.0056.

<table>
<thead>
<tr>
<th></th>
<th>mussel</th>
<th>vestimentiferan</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta^{13}$C</td>
<td>$&lt;0.001$</td>
<td>0.001</td>
</tr>
<tr>
<td>clam</td>
<td>mussel</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>$\delta^{15}$N</td>
<td>$&lt;0.001$</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>clam</td>
<td>mussel</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>$\delta^{34}$S</td>
<td>$&lt;0.001$</td>
<td>0.007</td>
</tr>
<tr>
<td>clam</td>
<td>mussel</td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>

Figure 4-1. Map of sites in the Gulf of Mexico from which seep communities were collected. The site names are based on Minerals Management Service (MMS) lease block designations and consist of a two-letter abbreviation, which stands for the region (AC=Alaminos Canyon, for example) followed by a three-digit number. Yellow circles represent mussel collections, half yellow/half red circles denote where both mussel and vestimentiferan communities were collected, and the yellow triangle represents a clam collection. Where more than one symbol pertains to a site, the circle is the actual location of the site and the other symbol appears to the right or left and a box surrounds all symbols.
Figure 4-2. (a) $\delta^{15}\text{N}$ and (b) $\delta^{34}\text{S}$ vs. $\delta^{13}\text{C}$ for all animals sampled in this study. The rectangles represent the range of values for foundation fauna, while the points represent the associated heterotrophic fauna. Each point is one individual stable isotope sample.
Figure 4-3. A close up photo of *Protomystides* sp. inhabiting its matrix of tubular casing atop the prostomium of *E. laminata*. 
Figure 4-4. Mean and standard deviation of tissue (a) $\delta^{13}C$, (b) $\delta^{15}N$, and (c) $\delta^{34}S$ values in vestimentiferan and mussel collections. Each symbol represents the mean tissue isotope values for one collection, with the symbiotic fauna on the x-axis and the associated heterotrophic fauna from the same collection on the y-axis.

- ○ vestimentiferans and associates
- ● mussels and associates
- --- 1:1 line
Figure 4-5. (a) $\delta^{15}\text{N}$ and (b) $\delta^{34}\text{S}$ vs. $\delta^{13}\text{C}$ for the clams *C. ponderosa* and *Calyptogena* sp. nov. and their associated fauna.

- \(\Delta\) *C. ponderosa*
- \(\blacktriangle\) *Calyptogena* sp. nov.
- \(\square\) *C. ponderosa* associates
- \(\bullet\) *C. sp. nov.* associates
Figure 4-6. (a) $\delta^{13}$C, (b) $\delta^{15}$N and (c) $\delta^{34}$S in the small polychaete *Protomystides* sp. vs. the paired *E. laminata* individual upon which the *Protomystides* sp. was found. The solid line denotes the best fit regression line and the dotted line denotes a theoretical line for the x- and y-axis values being equal (1:1 line).

(a) $y = 1.1x + 5.3 \quad R^2 = 0.80$

(b) $y = -0.2x + 7.8 \quad R^2 = 0.01$

(c) $y = 0.4x + 7.4 \quad R^2 = 0.35$
Figure 4-7. (a) $\delta^{13}$C in paired samples of Protomystides sp. and *E. laminata* from a single collection from AT340.

\[ y = 0.84x - 7.1 \]
\[ R^2 = 0.83 \]
Figure 4-8. (a) $\delta^{13}$C, (b) $\delta^{15}$N, and (c) $\delta^{34}$S in the commensal polynoid *Branchipolynoe seepensis* or Nautilliniellidae sp. vs. the paired *B. heckerae*, *B. childressi*, or *C. ponderosa* individual within whose gills the polynoid was found.

- **B. heckerae and B. seepensis**
- **Calypptogena spp.**
- **B. heckerae** and Nautilliniellidae sp.

- 1:1 line
- 1-trophic level enrichment (+3.4‰ $\delta^{15}$N)
CHAPTER 5

Importance of seep primary production to *Lophelia pertusa* and associated fauna in the Gulf of Mexico

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Abstract

To investigate the importance of seep primary production to the nutrition of *Lophelia pertusa* and associated communities and examine local trophic interactions, we analyzed stable carbon, nitrogen, and sulfur compositions in seven quantitative *L. pertusa* community collections. A significant seep signature was only detected in one of the 35 species tested (*Provanna sculpta*, a common seep gastropod) despite the presence of seep fauna at the three sample sites. A potential predator of *L. pertusa* was identified (*Coralliophila* sp.), and a variety of other trophic interactions among the fauna occupying the coral framework were suggested by the data, including the galatheid crab *Munidopsis* sp. 2 feeding upon hydroids and the polychaete *Eunice* sp. feeding upon the sabellid polychaete *Euratella* sp. Stable carbon abundances were also determined for different sections of *L. pertusa* skeleton representing different stages in the growth and life of the aggregation. There was no temporal trend detected in the skeleton isotope values, suggesting that *L. pertusa* settles in these areas only after seepage has largely subsided. Isotope values of individual taxa that were collected from both *L. pertusa* and
vestimentiferan habitats showed decreasing reliance upon seep primary production with average age of the vestimentiferan aggregation, and finally, no seep signature was detected in the coral collections. Together our data suggest that it is the presence of authigenic carbonate substrata, a product of past seep microbial activity, as well as hydrodynamic processes that drive *L. pertusa* occurrence at seep sites in the Gulf of Mexico, not nutritional dependence upon primary production by seep microbes.

**Introduction**

*Lophelia pertusa*, the most well-known species of cold-water reef-forming coral, has a cosmopolitan distribution. It is found in all the world’s oceans except in the polar regions (Cairns, 1994; Zibrowius, 1980). *Lophelia pertusa* reefs enrich local biodiversity (Fosså and Mortensen, 1998; Rogers, 1999) and have long been recognized as prime fishing locations for commercially important deep-water species (Fosså *et al.*, 2002).

*Lophelia pertusa* was first described by Linnaeus in 1758, but the factors determining the coral’s distribution have only been well-studied since the late 1970’s (e.g. Rogers, 1999; Wilson, 1979b). Although *L. pertusa* is found worldwide, its distribution is patchy even within a localized region. Understanding the factors that determine *L. pertusa* distribution and its relationship with other species will be instrumental in focusing future conservation efforts for these fragile and important deep-sea ecosystems that are, in some places, already threatened by deep-water fisheries (Fosså *et al.*, 2002) and energy company activities.

The basic factors that have been proposed to control the distribution patterns of *L. pertusa* include (i) a hard substrate on which to settle and grow, (ii) oceanic water between 4 and 12°C (Dons, 1944; Frederiksen *et al.*, 1992; Freiwald, 1998; Teichert,
1958), (iii) sufficient current to deliver food and prevent sedimentation without excessive shear force, and (iv) possible reliance upon local production associated with seepage of light hydrocarbons (Hovland and Thomsen, 1997) or other seep products including porewater charged with CO, Ca, or other chemical species derived from oxidation of hydrocarbons (Hovland, 2008). The first two factors have been well-documented, whereas the latter two have not.

Previous studies have documented an increase in density of soft cold-water corals (Genin et al., 1986) and *L. pertusa* (Masson et al., 2003) on top of or at the edges of seamounts where current is accelerated relative to the surrounding seafloor. Presumably, an elevated flow rate would increase the encounter rate of the polyps with food particles. Frederiksen et al. (1992) observed that *L. pertusa* occurrence is correlated with a critical slope and proposed that breaking internal waves increase the food supply to the corals by encouraging bottom mixing and concentrating nutrients.

Hovland and Thomsen (1997) proposed a relationship between the occurrence of *L. pertusa* and the seepage of light hydrocarbons from the seabed. *Lophelia pertusa* has been reported in association with hydrocarbon seeps in Norwegian fjords (Hovland and Risk, 2003; Hovland and Thomsen, 1997), on the inside rims of pockmarks at the Kristen hydrocarbon field (Hovland, 2005, 2008), near 70°N on the Norwegian Margin (Lindberg et al., 2007), in the Gulf of Cádiz (eastern central Atlantic) (León et al., 2007), and in the Gulf of Mexico (Schroeder, 2002). More recently, Hovland and Mortensen (1999) proposed a “hydraulic theory” that incorporates both local current flow and hydrocarbon seepage to explain the occurrence and persistence of the Norwegian deep coral reefs. This theory suggests that the turbulent fluid flow over uneven bottom topography acts to
concentrate nutrients, but the critical factor that allows for the long-term establishment of a coral reef is the sub-seafloor migration of hydrocarbons. According to this theory, the migrating hydrocarbons fuel carbon fixation by chemosynthetic microbes, which form the basis of portions of the *L. pertusa* community food web.

Circumstantial evidence for the hydraulic theory has accumulated over the past decade. Enhanced seismic reflectors (Hovland and Risk, 2003), adjacent pockmarks (Hovland and Risk, 2003; Lindberg *et al.*, 2007; Sumida *et al.*, 2004), coral rubble (*L. pertusa*, *Madrepora oculata*, *Desmophyllum* sp.) and isolated live corals in areas of active mud volcanoes (León *et al.*, 2007; Sumida *et al.*, 2004), corals colonizing hydrocarbon-derived authigenic carbonates (León *et al.*, 2007; Schroeder, 2002), elevated levels of light hydrocarbons in sediment adjacent to coral, and locally elevated seawater methane and sulfide in sediments at the base of the reef (Hovland and Risk, 2003) are all indications that seeping fluids are present near the corals. A few studies have questioned the nutritional reliance of *L. pertusa* communities upon seep primary production (Duineveld *et al.*, 2004; Kiriakoulakis *et al.*, 2005; Masson *et al.*, 2003) and found no evidence for this linkage.

In this study, we used stable isotopes of carbon, nitrogen, and sulfur to determine whether seep primary production is an important nutritional source for *L. pertusa* and fauna directly associated with the coral. If methanotrophy is a significant source of carbon for *L. pertusa* communities, the tissue of the fauna should have values depleted in $^{13}$C reflecting that of the methane in the environment (approx. -55 to -40‰ at seeps on the Upper Louisiana Slope (Brooks *et al.*, 1987; Roberts and Aharon, 1994) to -83.7 to -80‰ on the Florida Escarpment; Paull *et al.*, 1985). Determining whether
Chemoautotrophy is an important nutritional source, however, can be ambiguous using carbon stable isotopes alone. Dissolved carbon dioxide in seawater has a δ\(^{13}\)C composition close to 0‰ and carbon fixation by chemoautotrophic microbes via sulfide oxidation can produce organic carbon depleted by up to -25‰ (Ruby et al., 1987). In the Gulf of Mexico, even vestimentiferans and clams, which are known to rely upon their sulfur-oxidizing chemoautotrophic bacterial symbionts for nutrition, have δ\(^{13}\)C ranges of -43 to -18‰ and -39.8 to -30.9‰, respectively (Brooks et al., 1987; Kennicutt et al., 1992). There is a particularly large range of δ\(^{13}\)C values in vestimentiferans, which obtain all of their nutrition from their bacterial endosymbionts. At the more negative end of this range, the δ\(^{13}\)C values can be explained by seeping hydrocarbons creating an isotopically light dissolved inorganic carbon pool via chemical equilibrium processes or uptake of light porewater dissolved inorganic carbon by vestimentiferans across their plumes and through their roots (Aharon et al., 1992; Freytag et al., 2001). The more positive δ\(^{13}\)C value can be explained by lower levels of discrimination as is typical of hydrothermal vent vestimentiferans (Childress and Fisher, 1992). The story is further complicated for heterotrophic fauna which can feed on a mixture of sources, including methanotrophic (δ\(^{13}\)C < -40‰) and chemoautotrophic (ca. -27‰; Sassen et al., 1993) free-living bacteria and photosynthetic primary production by surface phytoplankton (-22 to -15‰; Gearing et al., 1984). Sulfur isotopes, however, produce a distinctly depleted δ\(^{34}\)S signature in animals that rely upon sulfur-oxidizing chemoautotrophic bacteria. Whereas seawater sulfate is the ultimate sulfur source for most marine organisms, the primary sulfur source for chemoautotrophs is pore water sulfide produced by microbial sulfate reduction and methane oxidation. The tissue δ\(^{34}\)S values reported for vestimentiferans in the Gulf of
Mexico range from -37 to -24‰ (MacAvoy et al., 2002; MacAvoy et al., 2005), which is highly depleted in $^{34}$S when compared with Gulf of Mexico bottom water sulfate, which averages 20.3‰ (Aharon and Fu, 2003). Thus, if chemoautotrophy is a significant source of nutrition for coral-associated animals, we would expect to see notably depleted tissue $\delta^{34}$S values. Although this study focuses mainly on L. pertusa communities from the Upper Louisiana Slope (310 to 634 m), we also analyzed tissue and skeleton from hard and soft corals from deeper sites in the Gulf between 960 and 1400 m to assess whether there is an increased reliance upon seep primary production at depth, where photosynthetic-sourced surface material is generally more scarce (e.g. Tietjen et al., 1989).

A second goal of this study was to gain insight into the food web structure within the coral communities and identify potential trophic relationships between individual species commonly associated with the corals. Predator-prey interactions are a potentially important part of the complex interplay of abiotic and biotic factors that structure communities.

Methods

Collection Methods

Stable isotope analyses were performed on seven quantitative L. pertusa community collections from three study sites on the Upper Louisiana Slope of the Gulf of Mexico. These collections were made during two cruises in July 2004 and September 2005 (Cordes et al., 2008) onboard the R/V Seward Johnson using the Johnson Sea Link submersible and the Bushmaster Jr. collection device. The Bushmaster Jr. is a hydraulically actuated net lined with a 63 µm mesh (Cordes et al., 2005b). Site names are
based upon Minerals Management Service lease block designations and include a region name (such as Green Canyon) and a three-digit number. Two of the sites were within the Green Canyon (GC) region off of the southern Louisiana coast between 507 and 525 m depth. The third site was within the Viosca Knoll (VK) region off of the Mississippi and Louisiana coasts between 459 and 470 m depth. Site coordinates, depths, associated dives, and collection types are shown in Table 5-1 and Figure 5-1. The GC 234 site is one of the most well-studied seep site in the Gulf of Mexico. This site contains extensive chemosynthetic communities and some smaller colonies of \textit{L. pertusa} and soft corals occupying low-lying carbonate boulders. At the GC 354 study site, \textit{L. pertusa} colonies form conical structures consisting mostly of dead coral skeleton on isolated carbonate boulders. The main area of the VK 826 site consists of large \textit{L. pertusa} colonies attached to carbonate along the crest and flanks of a feature that rises about 100 m from the surrounding seafloor (see Cordes \textit{et al.} 2008 for more extensive descriptions of these study sites and additional data on the collections).

Once onboard the ship, the contents of the Bushmaster Jr. were emptied into a plastic tub. The device and its holster were rinsed with cold filtered seawater to remove any remaining organisms. This water, containing animals and other debris, was sieved to 2mm and the associated fauna removed. Encrusting and cryptic fauna were also manually removed from the coral framework. All fauna were then sorted to the lowest possible taxonomic level based on morphological characters. Specimens that could not be identified at sea were preserved and transported to Penn State University where they were identified based upon taxonomic literature and voucher collections or sent to taxonomic experts. Stephen Cairns (National Museum of Natural History, Smithsonian Institution)
and Stephen Viada (Continental Shelf Associates International, Inc.) identified all corals, Sabine Stöhr (Swedish Museum of Natural History) identified ophiuroids, Anders Warén (Swedish Museum of Natural History) identified gastropods, Stéphane Hourdez (Station Biologique de Roscoff, France) identified polychaetes, and Joseph Goy (Harding University) identified shrimp.

Additional samples of *L. pertusa* and other scleractinian and soft coral species were obtained by breaking off pieces of coral using the manipulator arm of the submersible. These samples were placed in an insulated biobox for transport to the surface. Unidentified corals were photographed immediately after collection, DNA samples taken, and specimens preserved for further identification by taxonomic experts.

In addition to the sites already mentioned, *L. pertusa* was sampled at an additional site in the VK region and in the Mississippi Canyon (MC) region just south of the Mississippi River Delta between 626 to 643 m depth. The VK 862 site was the only site with no apparent seep megafauna but contained numerous small *L. pertusa* and soft coral colonies. The MC 885 study site contained large dense fields of the gorgonian *Callogorgia americana delta* with occasional small colonies of *L. pertusa*. There were small seep areas at this site containing small vestimentiferan aggregations and bacterial mats.

Hard and soft coral species were also sampled from 4 deeper sites during a 2007 cruise using the ROV (remotely operated vehicle) *Jason II*. One of the deep sites was in the MC region at about 960 m depth, two were within the Garden Banks (GB) region off of the southern Texas and Louisiana coasts at about 1000 m depth, and one was in the GC region at about 1400 m depth (*Table 5-1*). The MC 462 study site contained bacterial mats.
mats, gas hydrates and carbonate outcrops with gorgonians. No vestimentiferans or mussels were observed at this site, but *Calyptogena ponderosa*, a clam with chemoautotrophic symbionts also found on the Upper Louisiana Slope, was collected from this site. The GB 697 study site hosted bacterial mats, carbonates, vestimentiferans, and mussels. The GB 647 site contained outcrops of both carbonate and asphalt with gorgonians and small isolated vestimentiferans in oily sediment. The GC 852 site included an area with abundant scleractinian (*Enallopsammia rostrata*, *Sollenosmilia variabilis* and *Madrepora oculata*) gorgonian, and antipatharian corals situated atop carbonate boulders. Vestimentiferans and mussels were also present nearby at this site (Fisher et al., 2007; Roberts et al., 2007).

**Shipboard and Laboratory Methods**

Tissue samples from all collections were obtained for stable isotope analyses. For each collection, between three and six stable isotope samples for each species that could be identified at sea were frozen at -20°C in plastic cryovials upon collection. For large individuals, muscle tissue was dissected from the specimen. For smaller individuals, the entire specimen was frozen, and in some cases it was necessary to pool individuals to obtain sufficient material.

*L. pertusa* skeleton samples were obtained from Bushmaster and grab collections. To test whether there was a change in the amount of seep-derived inorganic carbon being incorporated into the coral skeleton over the life of the colony, skeleton samples were taken from different locations in the colony using two different methods. First, skeleton samples were collected from two or three locations along single coral branches and the distances between the most basal and the most distal fragments were measured. Second,
in Bushmaster collections, skeleton samples were taken from live portions of the skeleton by breaking apart the skeleton surrounding a polyp and from the oldest portions of the skeleton that were attached to the carbonate rock. Skeleton samples were also collected from non-Bushmaster *L. pertusa* and the lower-slope scleractinian corals. These additional skeleton samples were all taken from live portions of the coral colony.

In the laboratory, tissue samples were dried within their cryovials in a 60°C oven for 3-4 days and then homogenized using a glass rod. Two or three drops of 10% hydrochloric acid were added to remove inorganic carbonate, and the samples were subsequently redried for an additional five days.

Coral skeleton samples from 2005 and 2007 were homogenized by placing the sample between two clean layers of 0.5mm latex and crushed. Homogenized samples were placed in 2-mL plastic cryovials and pretreated with 0.5 mL of bleach for 24 h. The samples were then pelleted in a centrifuge, rinsed three times with deionized water, and dried at 60 ºC for 4 days.

In 2004 tissue samples from coral and vestimentiferan collections and skeleton samples were sent to the Marine Science Institute at the University of California Santa Barbara (UCSB) where they were acidified (for tissue samples) or bleached (for carbonate and coral skeleton samples) prior to analysis. At UCSB, isotope values were determined by continuous-flow isotope ratio mass spectrometry using a Thermo-Finnigan Delta Plus Advantage isotope ratio mass spectrometer coupled with a Costech Technologies ECS 4010 elemental analyzer (EA/IRMS).

Tissue samples from 2005 collections were analyzed at the University of Virginia Stable Isotope Laboratory for the stable isotope compositions using continuous-flow
isotope ratio mass spectrometry involving a Carlo Erba elemental analyzer coupled to a Micromass Optima isotope ratio mass spectrometer (EA/IRMS). Values are expressed using δ (delta) notation and reported in units of permil (%), where

$$\delta X = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 10^3,$$

X = $^{13}$C, $^{15}$N, or $^{34}$S and R = $^{13}$C/$^{12}$C, $^{15}$N/$^{14}$N, or $^{34}$S/$^{32}$S.

PDB (Pee Dee Belemnite) was used as the standard for carbon, air N$_2$ for nitrogen, and CDT (Canyon Diablo Triolite) for sulfur.

Deep Gulf of Mexico coral samples and 2005 and 2007 skeleton samples were analyzed at the Stable Isotope Facility at the University of California (UC), Davis. Tissue samples were analyzed for δ$^{13}$C and δ$^{15}$N using an Integra elemental analyzer coupled with a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, United Kingdom). Twenty to fifty mg of the skeleton samples were reacted in a common 100% phosphoric acid bath at 90ºC and analyzed in the directly coupled dual inlet of a GV Instruments Optima isotope ratio mass spectrometer. Isotope values were reported relative to V-PDB through the use of the working laboratory standard, Carrara Marble.

Most inferences were made by qualitative analysis based upon source and fractionation values reported in the literature. For the skeleton samples taken from basal and distal ends of the same branch, the Wilcoxon Signed Rank test, a non-parametric paired sample test, was used to test whether the median difference δ$^{13}$C$_{\text{distal}}$ − δ$^{13}$C$_{\text{basal}}$ is equal to or greater than 0. For food web analysis, we assumed a mean trophic level fractionation of 0.4 ± 1.3‰ for δ$^{13}$C (Post, 2002; Rau et al., 1983), 3.4 ± 1.1‰ for δ$^{15}$N (Minagawa and Wada, 1984; Post, 2002), and 0.5 ± 0.56‰ for δ$^{34}$S (McCutchan et al., 2003).
Results

Quantitative community characteristics

We made seven Bushmaster collections of *L. pertusa* and associated fauna at three sites on the Upper Louisiana Slope of the Gulf of Mexico: two from the GC 234 study site, one from GC 354, and four from VK 826. The total associated fauna biomass (ash-free dry weight, AFDW) per m$^2$ coral surface area in six of the collections were between 4.4 – 8.8 g m$^{-2}$, with one collection from the VK 826 study site having a much higher biomass per surface area of 24.4 g m$^{-2}$ due to the presence of two large predatory crabs (*Eumunida picta* and *Bathynectes longispina*) that accounted for two thirds of the biomass in this collection (leaving 8.4 g m$^{-2}$ without the two crabs). This is comparable to the range of biomass per surface area found in vestimentiferan aggregations: most fall between 1.04 and 6.45 g m$^{-2}$, with one having higher biomass of 206.2 g m$^{-2}$, due to the high biomass of the symbiont-containing mussel *Bathymodiolus childressi* associated with it (Cordes *et al.*, 2006).

At GC 234, the suspension-feeding polychaete *Euratella* sp. (302 individuals) dominated the biomass of the combined collections, followed by the fish *Bellottia robustus* (1 indiv.). At GC 354, *Euratella* sp. (23 indiv.) again dominated the biomass, followed by *Odontozona edwardsi* (4 indiv.), the unidentified galatheid (4 indiv.), and *Munidopsis* sp. 3 (1 indiv.). At VK 826, *Bathynectes longispina* (1 indiv.) dominated the biomass followed by *Munidopsis* sp. 2 (4 indiv.).

In all seven *L. pertusa* collections combined, predators dominated the biomass and were the most species-rich trophic guild (Figure 5-1). Predatory polychaetes accounted for 11 of the 24 species in this guild. The next most species-rich taxon within
the predator guild was the crustaceans with eight species. The second most species-rich trophic guild was the detritivores, which was also dominated by polychaetes (11 of 15 detritivore species).

*Stable Isotope measurements*

The *L. pertusa*-associated fauna, in general, had tissue stable isotope values that ranged from -25 to -13‰ δ¹³C, 6 to 14‰ δ¹⁵N, and 10 to 25‰ δ³⁴S (*Table 5-2, Figure 5-3*). Species lying outside of this range include the gastropod *Provanna sculpta* from GC 234 (-30.5‰ δ¹³C, 3.9‰ δ¹⁵N, -5.4‰ δ³⁴S), *Caryophyllia berteriana* from GC 234 (-27‰ δ¹³C, 0.76‰ δ¹⁵N, and 27‰ δ³⁴S), one *Odontozona edwardsi* individual from VK 826 (-27.7‰ δ³⁴S), and the hydroid sample from GC 354 (1.9‰ δ³⁴S). The isotope values of the *P. sculpta* sample are within the range exhibited by this species when it was collected with a young vestimentiferan aggregation at GC 234 (δ¹³C -35.3 to -21.6‰, δ¹⁵N 4.4 to 5.0‰ and δ³⁴S -6.1 to -4.3‰; Cordes *et al.*, in press.).

We did not directly sample particulate organic matter (POM), but we examined the isotope values of animals that feed upon particulate matter to indirectly assess the input of seep organics into the POM pool. The range for all suspension/filter feeders, passive predators (cnidarians), and detritivores in all collections was -25.2 to -14.2‰ for δ¹³C, 4.5 to 15.6‰ for δ¹⁵N, and 1.9 to 24.9‰ for δ³⁴S (*Figure 5-3*), falling mostly within the range expected for a photosynthetic source pool for carbon (δ¹³C of -22 to -15‰) (Gearing *et al.*, 1984) and particulate organic nitrogen (PON) (δ¹⁵N of >6‰) (Saino and Hattori, 1987). All POM feeders except for one hydroid sample were within the δ³⁴S range expected for a food web based on phytoplankton, (δ³⁴S ca. 10 to 20‰) (Fry *et al.*, 1983; Peterson and Fry, 1987). Suspension-feeding species that were slightly
lighter in $\delta^{13}$C included the anemone (order Actinaria), some $L.\ pertusa$ samples, the $Callogorgoria\ americana\ delta$ sample from MC 885 ($\delta^{13}$C ca. -23.9‰), 3 of the 11 $Euratella$ sp. samples from VK 826 ($\delta^{13}$C ca. -24.2 to -23.6‰), $Caryophyllia\ berteriana$ from GC 234 ($\delta^{13}$C ca. -27‰), several of the sponge samples, and some hydroid samples. The three anemone samples were from a single collection at VK 826 and the mean $\delta^{13}$C of -25.0‰ was just outside the expected range for photosynthetic carbon. The light $L.\ pertusa$ samples were from VK 826 (6 of 18 samples from this site; overall mean $\delta^{13}$C for this site -22.0±1.6‰) and MC 885 (1 of 2 samples from this site with $\delta^{13}$C ca. -23.9‰; mean for this site -22.0±2.6‰). Of the sponge samples, eight were isotopically light in carbon with $\delta^{13}$C ratios ranging from -24.5 to -23.2‰. All of these isotopically light sponge samples were the unidentified encrusting sponge collected during four dives at VK 826. The $\delta^{15}$N and $\delta^{34}$S values for these individuals, however, remain within the expected range for particulate organic nitrogen (PON) (Saino and Hattori, 1987) and seawater sulfate (Fry et al., 1983; Peterson and Fry, 1987).

Hydroidea spp., $Munidopsis$ sp. 2, and $L.\ pertusa$ samples were obtained from several collections and from different sites. Samples from all three taxa cover wide ranges of $\delta^{13}$C values and have overall means within the expected photosynthetic range (-20.8±2.0‰ for hydroids, -18.15±3.2‰ for $Munidopsis$ sp. 2, and -21.7±1.9‰ for $L.\ pertusa$). For hydroids and $Munidopsis$ sp. 2, Viosca Knoll samples tended to lie in the more depleted end of the $\delta^{13}$C range and Green Canyon samples at the more enriched end (Figure 5-5). One hydroid sample collected from GC 354 had an unusually low $\delta^{34}$S value of 1.9‰, but the corresponding $\delta^{13}$C and $\delta^{15}$N values (-19.3 and 9.8‰, respectively) were within the range for photosynthetic carbon and PON. There was no
apparent site-specific trend for *L. pertusa* $\delta^{13}$C and $\delta^{15}$N (Figure 5-6). The solitary coral *Caryophyllia berteriana* had unusually low tissue $\delta^{15}$N value of 0.8‰ from the GC 234 study site at 501 m and 0.2‰ from the MC 462 study site at 960 m.

The carbonate rock to which the corals were attached had $\delta^{13}$C and $\delta^{18}$O values consistent with the precipitation of carbonate due to the activity of seep related microbes (Roberts and Aharon, 1994) (Figure 5-7). The coral skeleton samples were generally more positive in $\delta^{13}$C and more depleted in $\delta^{18}$O than the authigenic carbonate and showed a linear relationship between $\delta^{13}$C and $\delta^{18}$O typical of biological carbonates, which are out of isotopic equilibrium with seawater (Spiro et al., 2000). Coral skeleton sampled from different locations along single branches of *L. pertusa* showed no significant difference between basal and distal fragments (Wilcoxon Signed Rank test for median difference=0 vs. median difference > 0; p = 0.58). Similarly, there was no apparent difference in skeleton $\delta^{13}$C between basal fragments and growing ends within aggregations.

Hard and soft corals from deeper sites (between 960 and 1400 m) had similar tissue isotope values to the more shallow water species (Table 5-2, Figure 5-4). There was also no notable difference in the skeleton stable isotope values between deep and shallow corals (Figure 5-7).

We would expect to find the top predators at the upper right of a plot of $\delta^{15}$N vs. $\delta^{13}$C. Indeed, we find *Coralliophila* sp., *Munidopsis* sp. 2, *Odontozona edwardsi*, *Eunice* sp., *Periclemenes* sp., *Chloeia* sp., *Bathynectes longispina*, and the unidentified galatheid in the upper right of these plots. There are, however, exceptions to this trend, where predators had a lower-than-expected $\delta^{15}$N value relative to other species in the collections
(e.g. *Nibilia* sp. and *Eumunida picta* Suppl. Figures 5-2d and 5-4d; Appendix C) or lower-trophic-level species show an unusually enriched signature (e.g. the white sponge Figure 5-2b).

**Discussion**

*Importance of seep primary production to Lophelia pertusa and associated fauna*

Our primary goal in this study was to use stable isotope analysis to directly address the question: do *L. pertusa* communities in the Gulf of Mexico rely on local chemoautotrophic primary production by seep-related microbes? Our data suggest that seep primary production provides, at most, a very minor amount of nutrition to *L. pertusa* and its associated community. *Lophelia pertusa* $\delta^{13}C$ values ranged from -24.7 to -17.5‰ (Figure 5-4). Seven of the 17 *L. pertusa* samples were slightly more negative than the lower limit of the range of -22 to -19‰ suggested by Gearing et al. (1984) for a food web based on phytoplankton, but these same samples had $\delta^{15}N$ and $\delta^{34}S$ values well within the range of a non-chemosynthetic food source. It is possible that methanotrophy contributes a small amount of the total input to the base of the food web as evidenced by the isotopically light *L. pertusa* polyps; however, marine POM values in this range are not uncommon (e.g. Benner et al., 1997; Hofmann et al., 2000). The corals collected from deeper sites between 960 and 1400 m showed no apparent increase in reliance upon seep primary production, despite the general trend of decreased quantity and quality of surface-derived nutrition with depth (Tietjen et al., 1989) (Figure 5-4). It could be that cold-water corals settle only in locations where they have a consistent supply of high-quality surface material, as has been observed at Darwin Mounds in the NE Pacific, where there is no evidence of hydrocarbon seepage (Kiriakoulakis et al., 2004).
Isotope data for *L. pertusa*-associated suspension and filter feeders similarly indicate a primarily surface-derived food source for this feeding guild (Figure 5-4). The majority of individuals were within the expected photosynthetic range, with a few individuals that were more depleted in $^{13}$C. The depleted samples were the anemone (Actinaria sp.) and the unidentified encrusting sponge. For both of these taxa, the majority of individuals had $\delta^{13}$C values between -24.5 and -22.5‰, suggesting that these animals may specialize on a slightly more depleted food source relative to other suspension and filter feeders. The relatively tight clustering of the sponge samples from different areas within the VK 826 study site suggests that this species may specialize upon a particular food source or size fraction of POM. A few studies have shown significantly lower $\delta^{13}$C values in smaller size classes of POM (Bishop *et al.*, 1977; Rau *et al.*, 1990) and specialization upon small particles has been demonstrated for two deep-sea demosponges (Witte *et al.*, 1997). A similar scenario may be true of the anemones. These three samples were from a single collection, and the isotope values clustered together but stood away from other suspension feeders in this collection (Suppl. Figure 5-1c,d; Appendix C). A second possibility for the sponges is a symbiotic relationship with bacteria. This type of relationship is common in shallow coral reef habitats and has also been found at seeps and a deep-water coral habitat similar to those in this study (Vacelet *et al.*, 1995; van Duyl *et al.*, 2008).

Our evidence that *L. pertusa* and other suspension feeding species rely mainly on surface-derived nutrition lends support to hydrodynamic models (Duineveld *et al.*, 2007; Frederiksen *et al.*, 1992; Genin *et al.*, 1986) that suggest accelerated fluid flow over locally elevated mounds and uneven bottom topography are more important than seep
production in providing nutrients to the coral community. Seeps in the Gulf of Mexico are often found on topographic highs as a result of uplift by salt domes and the upward pressure of migrating hydrocarbons (past or present). Current velocity may be locally accelerated over these topographic highs, which may increase the flux of suspended material to the coral polyps and other suspension feeders (Genin et al., 1986). On a more local scale, the carbonate boulders formed at seeps may accelerate current over their peaks, which increases flux of food particles. Boulders may also promote bottom mixing by disrupting the prevailing current (Frederiksen et al., 1992), providing increased nutrition in the form of resuspended particles from the seafloor.

The high biomass of suspension feeders inhabiting the coral framework also indicates that there is a favorable flow regime in coral habitats that is necessary for the prosperity of suspension feeding animals. Indeed, it has been suggested that suspension feeders could be indicators of accelerated fluid flow at deep hard-bottom sites (Genin et al. 1986). Alternatively, a recent study by Duineveld et al. (2007) has found that near-bottom current speed was lower on mounds inhabited by corals than non-coral habitats. It is yet unclear whether this reduced current is a result of deceleration by the coral framework itself or that the corals selectively colonize areas with intermediate current speed. The authors have speculated that either settlement or feeding may be hampered by high-speed currents. This may explain the abundance of apparently suitable but unoccupied hard substrate that has been observed in the Gulf of Mexico and the NE Atlantic (Hovland and Risk, 2003). Moreover, if the coral acts to reduce current velocity or increase turbulence, it may facilitate the settlement of other suspension-feeding
species, an effect that may be enhanced as a coral ‘patch’ grows and develops (Duineveld 
et al., 2007; Wilson, 1979a).

Detritivores also feed on POM, but this source pool may more closely reflect
organic matter associated with the sediment. We might expect that if hydrocarbons are
present in the sediment near the base of L. pertusa colonies as suggested by Hovland and
Risk (2003), detritivores such as the sipunculid Phascolosoma turnerae and the small
ophiuroid may show an indication of seep input. We did not, however, detect a
significant seep signature in any of the detritivore or scavenger species collected with the
coral (Figure 5-4).

Unlike suspension feeders which depend entirely on what material is available in
the water column, many mobile trophic guilds have the ability to move between seep and
coral habitats and utilize some of the local seep production while also taking advantage of
the complex structure of the coral framework. In particular, we might expect highly
mobile animals, such as shrimp and crabs, to supplement their diet with production from
nearby vestimentiferan habitats. We did not find a significant seep signature in any
mobile taxa with the exception of a single individual of the tiny snail Provanna sculptra.

Provanna sculptra was an unusual data point within the coral associated fauna, but
closely resembles the range of isotope values of P. sculptra collected with
vestimentiferans. MacAvoy et al. (2005) suggest that P. sculptra either feeds very
selectively upon a particular free-living bacterial population or it possesses its own
symbionts. Symbiosis with chemoautotrophic bacteria has been suggested for the
hydrothermal vent species Provanna variabilis based upon isotope evidence (Bergquist et
and has been shown in other members of the family Provannidae found at vents (Stein et al., 1988; Wittenberg and Stein, 1995).

Bergquist et al. (2003) proposed a model for seep succession in which vestimentiferan larvae colonize areas of active seepage where sufficient amounts of hydrogen sulfide are being released into the water column. These areas are often initially colonized by Bathymodiolus childressi, which contains methanotrophic symbionts that require significant concentrations of methane above the sediment to fuel carbon fixation. The young vestimentiferans are likely taking up sulfide from the water column through their plumes, which are still close to the sediment/seawater interface. As the vestimentiferans age and seepage slows, surface expression of methane and sulfide both begin to dwindle and mussels become less abundant. By this time seep vestimentiferans are primarily reliant on the posterior portion of their bodies that extends into the sediment to “mine” for sulfide (Freytag et al., 2001). The vestimentiferan aggregations found close to L. pertusa aggregations are old, sparse, and may be in a stage of senescence. Corals may be a later successional stage, colonizing carbonate outcrops after most surface expression of seepage has subsided (Cordes et al., 2008; León et al., 2007).

The carbonate to which the corals were attached in our collections had δ¹³C and δ¹⁸O values indicating that they are authigenic carbonates formed as a result of microbial processes (Figure 5-7). The large range of δ¹³C values suggests a variety of origins and processes that formed the carbonates, including methane oxidation coupled with sulfate reduction (<40‰), degradation of crude oil (ca. -35 to -25‰), and fermentation (ca. -10 to 0‰) (Roberts and Aharon, 1994) (Figure 5-7). There was no indication of a seep signature in the most basal portions of the coral skeleton, nor any notable difference
between basal and distal (live, growing tips) portions of skeleton, suggesting that *L. pertusa* settles after seepage has largely subsided and that the isotopic signature of the inorganic carbon pool has remained relatively constant from the time of settlement to the time of collection.

When considering this model in terms of trophic succession, it is useful to compare our results with those of Cordes *et al.* (in press), who characterized the stable isotopes of animals associated with three vestimentiferan tubeworm aggregations of different ages: young, adult, and old (age estimates were based on a population growth model (Cordes *et al.*, 2003). Overall, there was a shift in tissue stable isotope values of vestimentiferan and coral associated fauna from more negative values indicative of seep derived nutrition in the young vestimentiferan aggregation to almost no indication of seep input in the coral community (Figure 5-3).

Species composition in vestimentiferan communities also changes gradually from younger to older aggregations. Young aggregations contain mostly seep “endemics” that are well-adapted to tolerate the toxic chemical environment around active seeps. As the average age of the vestimentiferans increases and the influence of seepage decreases, the community begins to include more and more species from the regional species pool (Cordes *et al.*, 2005b; Cordes *et al.*, 2006). Although coral communities are significantly different from adjacent vestimentiferan communities, they show the most overlap with old vestimentiferan aggregations and may represent the climax community in seep succession (Cordes *et al.*, 2008).

Despite the differences in abiotic environment and community composition of vestimentiferan and coral habitats, there were several taxa that were shared across these
different communities (Figure 5-8). When these shared taxa were collected with young and adult vestimentiferan communities, their tissue stable isotope values reflected significant input of seep organic material (Figure 5-8). This seep signature becomes progressively less pronounced (δ-values become less negative) with age of the vestimentiferan community and is largely undetectable in the coral collections. This indicates that these species can take advantage of seep primary production when present, but there is enough food available within the coral habitat that significant consumption of seep production is not required.

Most of the taxa shared between corals and vestimentiferan aggregations are mobile predators that may be generalists or may specialize upon a prey species whose nutritional source has shifted. As discussed in section 4.3, Munidopsis sp. 2 and Eunice sp. may specialize upon hydroids and Euratella sp., respectively. Both of these prey species probably feed primarily upon suspended POM (hydroids may also capture and ingest small mobile prey items). Both the hydroids and Euratella sp. showed a pronounced shift in tissue stable isotope values from the adult vestimentiferan habitat to the coral habitat (Figure 5-8). This indicates a shift in the composition of the suspended POM pool from largely seep-derived to mostly surface-derived material. The hydroid tissue stable isotope values showed very little difference between the old vestimentiferan and coral habitats, indicating that most suspended POM is of photosynthetic origin even among old vestimentiferan aggregations. Likewise, the shift in the tissue stable isotope values of P. turneræ, a detritivore, indicates a shift in the sediment POM pool from mostly chemoaautotrophic origin in young and adult vestimentiferan habitats to primarily surface origin in coral habitats.
Suspension feeders, passive predators, and grazers

The *L. pertusa* tissue stable isotope values varied considerably, suggesting that it may utilize several food sources or a source that itself is variable. *Lophelia pertusa* tissue δ\(^{15}\)N values differed by as much as 4.4 to 4.6‰ in a single collection (Suppl. Figures 5-1c,d and 5-3c,d; Appendix C) indicating that *L. pertusa* may be feeding across approximately two trophic levels in these areas, whereas in some collections the total δ\(^{15}\)N range was very narrow (range of ca. 0.5‰; Suppl. Figures 5-1a,b and 5-4; Appendix C), suggesting that *L. pertusa* feeds at a single trophic level in these areas.

Prior studies have suggested that both suspended POM and living zooplankton are potentially important constituents of *L. pertusa*’s diet (Coles, 1969; Duineveld et al., 2004; Duineveld et al., 2007; Freiwald, 2002; Mortensen, 2001). The variability of *L. pertusa* isotope values within collections could be a result of different size fractions being available to different locations within the coral thicket. For example, on the interior of the thicket where reduced current velocity or increased turbulence can reduce the thickness of the diffusive boundary layer, polyps may have access to smaller particles than polyps on the outside of the thicket.

We did not sample zooplankton or POM in this study; however, one collection contained a potential prey species for *L. pertusa*: a small isopod (Suppl. Figure 5-1c,d; Appendix C). This potential relationship is based on three isopod and three *L. pertusa* samples from a single collection, but is supported by all three isotope values. Isopods have been observed inhabiting the live portions of coral, and thus may come into contact with the polyps (Mortensen, 2001). Although isopods are likely not the primary food
source for *L. pertusa*, their isotope values may be similar to that of planktonic crustaceans with similar feeding niches that were not captured with our sampling technique.

The most common and abundant suspension feeding taxa besides *L. pertusa* were the hydroids and *Euratella* sp. Both of these taxa grouped relatively tightly within collections for all three isotope values but varied in $\delta^{13}C$ and $\delta^{34}S$ among collections. *Euratella* sp. varied greatly in $\delta^{13}C$ even between collections within the same study site (*e.g.* the four collections from VK 826 have a maximum standard deviation within a collection of 1.2‰ but mean $\delta^{13}C$ ranges from -22.8 to -15.1‰ for the entire site). Both taxa likely feed primarily upon POM, although one study has shown that zooplankton can make up about 12% of the diet of one shallow-water benthic hydroid species (Coma *et al.*, 1995). The tissue stable isotope values suggest that these animals specialize upon a particular food source, or size fraction of POM, that is relatively uniform within a collection location, but varies between locations, even within a study site.

The unusually depleted $\delta^{15}N$ value of the *Caryophyllia* spp. from both the shallow and deep sites indicates that these solitary corals specialize upon $\delta^{15}N$-depleted food sources. Low $\delta^{15}N$ values indicate that the source of nitrogen is local rather than PON from the surface and has undergone little biological processing (*i.e.* trophic level enrichment). High concentrations of ammonium in seep pore waters have been documented and can favor relatively high fractionation rates during ammonium assimilation by nitrifying bacteria (Lee and Childress, 1994; Lee and Childress, 1996). Nitrate may also be abundant at seeps, but assimilation of nitrate has not yet been shown to be a significant process in the nitrogen cycles at seeps.
The samples that were most $^{15}$N-enriched were the white sponge and the vase hexactinellid sponge (Figures 5-3 and 5-4). Interestingly, this is not a unique result. Similar values have been found for a sponge at the Porcupine Abyssal Plain, a non-seep habitat in the Northeast Atlantic (Iken et al., 2001). This could be the result of a carnivorous feeding strategy, such as that exhibited by a cave sponge (Vacelet and Boury-Esnault, 1995) or a previously unidentified microbial symbiosis.

**Predators**

The most well-supported predator-prey relationship in this study was the galatheid crab *Munidopsis* sp. 2 feeding upon hydroids (Suppl. Figures 5-1a,b, 5-2, and 5-3c,d; Appendix C). This relationship is supported in all but one collection (Suppl. Figure 5-4; Appendix C), which could be due to variation in individual feeding behavior. This relationship is also shown when these two species were collected in vestimentiferan aggregations (Cordes et al., in press), although the values within each species differ significantly between habitats (Figure 5-8). The unidentified galatheid occupies similar isotope ranges to *Munidopsis* sp. 2 and may occupy a similar trophic niche (Suppl. Figure 5-1a,b; Appendix C).

The tube-dwelling polychaete *Eunice* sp. shows a narrow range for all three isotope values (Suppl. Figures 5-2c,d and 5-3c,d; Appendix C), suggesting it may be a specialist. All three tissue stable isotope values suggest it may be feeding on *Euratella* sp. (Suppl. Figure 5-2c,d; Appendix C). Again, this relationship is consistent with isotope data for these two species collected within a vestimentiferan community (Cordes et al., in press), although the values of the individual species differ between habitats (Figure 5-8).
Snails in the genus *Coralliophila* from shallow water environments are known corallivores that can do considerable damage to corals (Baums *et al*., 2003; Brawley and Adey, 1982; Miller, 1981). The average stable isotope range of *L. pertusa* for all collections (-25 to -18‰ δ¹³C, 6 to 11‰ δ¹⁵N, 12 to 20‰ δ³⁴S; Table 5-2, Figure 5-3), and the range of *Coralliophila* sp. (-21 to -17‰ δ¹³C, 8 to 13‰ δ¹⁵N, 15 to 16.5‰ δ³⁴S, Table 5-2, Figure 5-3) is consistent with the diet of *Coralliophila* sp. being primarily *L. pertusa*. *Coralliophila* sp. has also been documented via high-resolution photography occupying the live portions of *L. pertusa* skeleton (S. Lessard-Pilon, pers. comm.).

Most of the large mobile predators collected in this study showed no clear feeding relationship to coral-associated species. This could indicate that either these animals do not feed primarily upon the *L. pertusa*-associated fauna, but rather are background deep-sea fauna that simply take advantage of the added habitat complexity of the *L. pertusa* framework, or they do not specialize upon any one species and their isotope values reflect a mixture of prey species.

**Conclusions**

*L. pertusa* communities rival adjacent vestimentiferan associated communities in biomass and abundance of the associated fauna. We have long known that the high biomass assemblages of seep fauna are possible because of abundant local primary production fueled by migrating hydrocarbons in the otherwise nutrient-poor deep-sea environment. Although *L. pertusa* often colonized carbonate that was only meters away from vestimentiferan aggregations, the results of this study show that the occurrence of *L. pertusa* at seep sites in the Northern Gulf of Mexico is not primarily due to reliance upon seep-derived nutrition, as hypothesized in the hydraulic theory (Hovland and Mortensen,
Rather, seep-induced features such as carbonate occurrence and uneven bottom topography provide appropriate substrate and may drive other processes relating to provision of food, such as current acceleration, bottom mixing, and nutrient concentration.

The role of *L. pertusa* as a foundation species (a large or abundant species that has a positive effect on community inhabitants; Dayton, 1972) and the nature of its influence on associated community structure is still largely unknown, but there are some intriguing possibilities. The coral framework could simply provide stable substrate upon which animals with similar habitat requirements can thrive, the three-dimensional structure of anastomosing branches may provide greater habitat complexity, or its physical structure may create flow patterns that make otherwise inaccessible nutrients available by altering the thickness of the diffusive boundary layer across which particles must travel (Duineveld *et al.*, 2007). Finally, our study has pointed to some potentially important trophic interactions that may play an important role in the co-occurrence of particular taxa. Although there are very few species that are potential coral specialists, there is unquestionably a greater abundance of certain species within the *L. pertusa* habitat than is found in other biogenic or background continental slope habitats.

Corals that occur at seeps in the Gulf of Mexico contribute to unique communities with mixtures of seep and background fauna. In many areas of the Northeast Atlantic and the Gulf of Mexico, these fragile habitats are potentially threatened by the movement of deep-water fisheries and energy company activity into deeper and deeper waters. Responsible stewardship of these valuable deep-water habitats will require further
research and exploration to constrain the factors that control their distribution and better understand their relationships with other deep-water species.

**Acknowledgements**

We thank the captains, crews, and scientists aboard the research vessels Seward Johnson I and II and National Oceanographic and Atmospheric Association (NOAA) Ship Ronald H. Brown, as well as the pilots and engineers of the *Johnson Sea Link II* submersible and the remotely operated vehicle *Jason II*. We also thank Cheryl Morrison and Stephen Cairns for their help at sea in identifying corals from the upper and deep continental slope and Elizabeth Podowski, Stéphane Hourdez, and Mike McGinley for their help in collecting and processing samples onboard the Seward Johnson. This work was funded by the Minerals Management Service contracts #1435-01-03-CT-72323 and 1435-01-05-CT-39187 and the NOAA Office of Ocean Exploration.

**Table 5-1.** Coordinates, depths, and dives associated with each site where we made collections. JSL stands for the Johnson Sea-Link submersible and J2 stands for the Jason II remotely operated vehicle. The two-letter abbreviations at the beginning of the site names stand for MMS lease block names: GC = Green Canyon, VK = Viosca Knoll, MC = Mississippi Canyon, and GB = Garden Banks.

<table>
<thead>
<tr>
<th>Site</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Depth (m)</th>
<th>Dives</th>
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<td>VK862</td>
<td>29:06.4</td>
<td>-88:23.1</td>
<td>335</td>
<td>JSL4734, 4869</td>
</tr>
<tr>
<td>VK826</td>
<td>29:09.5</td>
<td>-88:01.1</td>
<td>470</td>
<td>JSL4736, 4737, 4867, 4868, 4871</td>
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<tr>
<td>GC234</td>
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<td>-91:13.4</td>
<td>500</td>
<td>JSL4728, 4740</td>
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<tr>
<td>GC354</td>
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<td>-91:49.6</td>
<td>525</td>
<td>JSL4741</td>
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<tr>
<td>MC885</td>
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<td>-89:42.6</td>
<td>635</td>
<td>JSL4738</td>
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<td>960</td>
<td>J2-271</td>
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<td>GB647</td>
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<td>-92:24.0</td>
<td>1000</td>
<td>J2-280</td>
</tr>
<tr>
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<td>-92:06.7</td>
<td>1000</td>
<td>J2-274</td>
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<td>-91.10.0</td>
<td>1400</td>
<td>J2-273, 278</td>
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Table 5-2. Stable isotope data for all taxa included in this study. The number outside of the parentheses is the average isotope value of all individuals. The first number in the parentheses is the number of samples, and the second number is the standard deviation. Where there was only one sample, the parentheses are omitted.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>δ¹³C</th>
<th>δ¹⁵N</th>
<th>δ³⁴S</th>
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<td>Porifera</td>
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<td>sponge sp. 1</td>
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<td>7.7</td>
<td>15.6</td>
</tr>
<tr>
<td>sponge sp. 2 (lacy)</td>
<td>-18.1</td>
<td>11.0</td>
<td>22.2</td>
</tr>
<tr>
<td>sponge sp. 3 (white)</td>
<td>-17.2</td>
<td>15.1</td>
<td>17.8</td>
</tr>
<tr>
<td>sponge sp. 4 (yellow)</td>
<td>-16.9</td>
<td>10.6</td>
<td>23.6</td>
</tr>
<tr>
<td>Cnidaria</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Actinaria sp.</td>
<td>-25.0</td>
<td>8.5</td>
<td>16.5</td>
</tr>
<tr>
<td>Hydriodea spp.</td>
<td>-20.8</td>
<td>7.9</td>
<td>12.6</td>
</tr>
<tr>
<td>Cnidaria: corals</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>Anthopodium rubens</td>
<td>-18.5</td>
<td>9.9</td>
<td></td>
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<tr>
<td>Anthothela tropicalis</td>
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<td>10.8</td>
<td>20.4</td>
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<td>27.4</td>
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<td>10.3</td>
<td>19.7</td>
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<td>Keratoisis flexibilis</td>
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<td>14.9</td>
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<td>10.0 (6, 0.3)</td>
<td>20.1 (6, 0.9)</td>
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<td>8.5 (32, 1.9)</td>
<td>17.1 (33, 2.8)</td>
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<td>Madrepora oculata</td>
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<td>7.0 (4, 2.3)</td>
<td>13.1</td>
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<td>16.1</td>
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<td>Paracalyptrophora carinata</td>
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<td>21.4</td>
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<td>Cnidaria: corals &gt;900m</td>
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Table 5-2 (cont.).

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<th>$\delta^{15}$N</th>
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<td>7.8</td>
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<td>16.7 (4, 0.8)</td>
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<td>14.7 (21, 3.1)</td>
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<td>18.1 (3, 1.6)</td>
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<td>17.8</td>
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<td>15.8 (4, 0.7)</td>
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<td>15.6</td>
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<td>-19.8</td>
<td>12.6</td>
<td>14.7</td>
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<td>11.1 (9, 1.5)</td>
<td>15.8 (9, 4.6)</td>
</tr>
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<td>9.2 (4, 0.1)</td>
<td>17.6 (4, 5.9)</td>
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<td>8.9 (3, 0.6)</td>
<td>20.1 (3, 4.2)</td>
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<tr>
<td><em>Periclemenes</em> sp.</td>
<td>-19.5 (5, 1.7)</td>
<td>9.9 (5, 1.7)</td>
<td>17.4 (4, 1.8)</td>
</tr>
<tr>
<td><strong>Echinodermata</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Crinoidea</em> sp.</td>
<td>-19.8 (4, 1.0)</td>
<td>9.1 (4, 1.3)</td>
<td>20.8 (4, 2.6)</td>
</tr>
<tr>
<td><em>Ophioneridae</em> sp.</td>
<td>-19.0 (4, 0.8)</td>
<td>9.9 (4, 0.6)</td>
<td>21.9 (4, 2.7)</td>
</tr>
<tr>
<td><em>Ophiotreta valenciennesi rufescens</em> Koehler, 1896</td>
<td>-17.4</td>
<td>10.9</td>
<td>20.9</td>
</tr>
<tr>
<td><em>Ophiuroidea</em> sp. 2</td>
<td>-18.9 (2, 1.0)</td>
<td>10.4 (2, 1.3)</td>
<td>21.0 (2, 0.2)</td>
</tr>
<tr>
<td><strong>Chordata</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bellottia</em> sp.</td>
<td>-19.8</td>
<td>11.2</td>
<td>16.0</td>
</tr>
</tbody>
</table>

* (= _Stenopus_ sp. in Cordes et al. 2008)
Figure 5-1. A map of all study sites in the Gulf of Mexico where collections were obtained for this study. The two-letter abbreviations preceding three-digit numbers stand for MMS lease block designations: GB = Garden Banks, GC = Green Canyon, MC = Mississippi Canyon, and VK = Viosca Knoll.

Figure 5-2. Proportion of biomass and species richness comprised by each feeding guild when all seven coral collections are pooled. The numbers at the top of the bars indicate the average biomass per surface area and the total number of species. Note that encrusting fauna such as sponges and hydroids were not weighed or enumerated.
Figure 5-3. (a) $\delta^{34}$S vs. $\delta^{13}$C and (b) $\delta^{15}$N vs. $\delta^{13}$C for L. pertusa and associated fauna for all collections (points). The boxes signify the range of values for 95% of the samples in each category. The boxes labeled young, adult, and old represent values for the associated fauna collected with vestimentiferan aggregations of young, intermediate, and old age (based upon visual observation and modeled age; Cordes et al., in press; Cordes et al., 2007). The heavy black rectangle labeled “mussels” represents the range of Bathymodiolus childressi isotope values collected with the young vestimentiferans (mussels were not present in other collections). The box labeled “vestimentiferans” represents the range of values for Seepiophila jonesi and Lamellibrachia luymesi from all three vestimentiferan collections. The mussels and vestimentiferans represent the methanotrophic and chemoautotrophic end members, respectively.
Figure 5-4. (a) $\delta^{34}$S vs. $\delta^{13}$C and (b) $\delta^{15}$N vs. $\delta^{13}$C for all suspension feeding organisms collected in this study, including hard and soft corals that were collected by grabbing with the submersible’s manipulator arm. Corals from deep sites (>900m) are also included.

(a) Porifera (filter feeders)
- Hexactinellidae sp.1
- Hexactinellidae sp. 2 (vase)
- sponge sp. 1
- sponge sp. 2 (lacy)
- sponge sp. 3 (white)
- sponge sp. 4 (yellow)

Cnidaria (passive predators)
- Anthopodium rubens
- Anthothela tropicalis
- Leiopathes sp.
- Callogorgia americana delta
- Paracalyphrophora carinata
- Caryophyllia berteriana
- Echinomuricea atlantica
- Keratoisis flexibilis
- Lophelia pertusa
- Madrepora oculata
- Muriceides hartus

(b) Actinaria sp.
- Hydroidea spp.

Sipuncula (detritivore)
- Phascolosoma turnerae

Annelida (suspension feeders)
- Euratella sp.
- Serpulidae sp.

Bivalvia (filter feeder)
- Area sp.

Crustacea (suspension feeder)
- Costatoverruca floridana

Echinoidea (detritivores)
- Crinoidea sp. (suspension feeder)
- Ophiomeridae sp.
- Ophiuroidea sp.
- Ophiuroidea sp. 2
Figure 5-5. Average $\delta^{34}$S vs. $\delta^{13}$C (top panels) and $\delta^{15}$N vs. $\delta^{13}$C (bottom panels) for Hydroidea spp. (a and b) and Munidopsis sp. 2 (c and d) samples grouped by site. Error bars represent +/- one standard deviation.

Figure 5-6. Average $\delta^{15}$N vs. $\delta^{13}$C for all *L. pertusa* tissue samples including both grabs and Bushmaster collections. $\delta^{34}$S values were only obtained for samples from Viosca Knoll, so analysis of site differences was not possible. Error bars represent +/- one standard deviation.
Figure 5-7. $\delta^{18}O$ vs. $\delta^{13}C$ in all authigenic carbonates and *L. pertusa* skeleton collected from VK 826 and VK 862. All skeleton samples from scleractinian corals from the deep (>900m) sites are also shown. The open triangles represent authigenic carbonates, the dashes represent *L. pertusa* skeleton, and the open circles represent skeleton of scleractinian corals from deep sites.
Figure 5-8. δ^{13}C, δ^{15}N, and δ^{34}S values for taxa found across at least three of the four community types: juvenile, adult and old vestimentiferan aggregations and coral aggregations. (Gt = Glycera tesselata, M2 = Munidopsis sp. 2, Pt = Phascolosoma turnerae, Eun. sp. = Eunice sp., P. sp. = Periclemenes sp., H. spp. = Hydroidea spp., and Eur. sp. = Euratella sp.). Error bars represent +/- one standard deviation.
CHAPTER 6
Trophic ecology in Gulf of Mexico cold seeps

Cold seeps, which support abundant communities of both endemic and non-endemic fauna, are common on continental margins around the world. Understanding the ecology of these unique communities is becoming increasingly important as human activities threaten their survival. In the Gulf of Mexico, the largest threat is oil and natural gas drilling, which is continually advancing into deeper and deeper waters. Deep-water fishing also threatens deep-sea communities, and in particular cold-water coral reefs, which provide important habitat for many commercial species.

Two advances came together to allow for the large data sets collected from the lower continental slope (>1000 m depth), which are presented in Chapters 2, 3, and 4 of this thesis. The first is the technology and funding to explore this deeper, relatively unstudied portion of the slope, and the second is that many commercial stable isotope laboratories can quickly run large numbers of samples relatively inexpensively. The size and spatial extend of the data sets provided both replication for statistical analysis and the ability to examine any regional or bathymetric patterns in isotope values.

One distinctive finding on the lower slope was the prevalence of biogenic methane compared with the upper slope, where methane is mostly of thermogenic origin (Chapter 2, 3, 4) (Joye et al., in press). The highly depleted δ¹³C signature of biogenic methane was present in mussel, vestimentiferan, and heterotrophic animal tissues. The δ¹³C values of mussels indicated methane of thermogenic origin only in the deepest sites at Alaminos Canyon and in the shallowest sites at Garden Banks (Chapter 2). Alaminos Canyon is a reentrant into the solid salt sheet covering most of the lower slope, which
could allow for access to a deeper thermogenic methane source. The salt layer underlying the shallower Garden Banks sites forms upward-migrating salt pillars characteristic of the upper slope. The remaining sites lie atop the solid laterally-migrating salt sheet, which could limit the availability of deeper thermogenic methane.

A methane signature in mussels was expected, because they contain methanotrophic symbionts. However, it was surprising to find a clear biogenic methane signature in vestimentiferan tubeworms, which cannot take up methane and which obtain all of their nutrition from chemoautotrophic symbionts (Chapter 3). This finding supported previous hypotheses that vestimentiferans take up porewater dissolved inorganic carbon (DIC) as part of an anion exchange. Dattagupta et al. (2006) found strong evidence that vestimentiferans take up bicarbonate ions across their roots in exchange for releasing sulfate ions, a waste product of sulfide oxidation by their symbionts. Bicarbonate is produced in seep porewater when methane is oxidized by microbial consortia, and therefore would reflect the isotope composition of the source methane. Since vestimentiferans lack a mouth, gut, and anus, they obtain all the raw materials for chemoautotrophy from dissolved compounds in benthic seawater taken up across their plumes or from pore fluids taken up across their roots. Surrounding ocean water would quickly dilute any seep fluids emitted from the sediment surface, so a seep signature would not be detectable around the plumes of adult vestimentiferans (Cordes et al., 2005b). This leaves uptake of porewater DIC as the most plausible explanation for the observed tissue δ¹³C values. An important lesson for the general use of carbon isotopes in seep systems is that carbon isotopes cannot reliably be used to determine whether carbon is derived from methanotrophy or chemoautotrophy.
Given that vestimentiferans acquire methane-derived DIC by virtue of their ability to mine it directly from the sediment, it was surprising to find a biogenic methane $\delta^{13}C$ signature in not only sediment-dwelling and mobile heterotrophic associated fauna, but also in sessile animals that colonize the tops of vestimentiferan tubes a meter or more above the sediment (Chapter 5). Although dissolved seep chemicals do not reach the vestimentiferan plumes in detectable concentrations, particulate organic matter (POM) from the sediment reaches suspension-feeders at the plume level. The vast majority of heterotrophic animals collected with clams, vestimentiferans, and mussels showed a seep signature in their tissue isotope values. The $\delta^{13}C$ of mussels and vestimentiferans varied substantially by location, even between collections 10’s of meters apart (Chapters 2 and 3). The $\delta^{13}C$ signatures of the associated heterotrophic fauna, for the most part, tracked the $\delta^{13}C$ of the symbiont-containing fauna, suggesting that most animals not only feed at seeps, but also feed very locally (Chapter 4). In Chapter 5, we examined individual taxa across different-aged vestimentiferan aggregations and coral communities. There were distinct differences in isotope values, particularly between coral and vestimentiferan habitats, suggesting that these taxa, even those we presumed to be more mobile such as galatheid crabs, mostly feed in a localized area.

In most trophic studies at seeps, including Chapters 3 and 5 of this thesis, tissue $\delta^{15}N$ values in seep animals are used only as an indication of local versus surface-derived nitrogen. The inorganic nitrogen sources at seeps could be interesting because seep environments are often characterized by abundant ammonia and nitrate. Unfortunately, there are no isotope data for inorganic nitrogen sources at seeps. Both the mussel and vestimentiferan data sets had intriguing $\delta^{15}N$ values, but interpretation was limited by the
lack information about inorganic nitrogen sources. One collection of Bathymodio\textit{lus} childressi had $\delta^{15}\text{N}$ values around -25‰, which is the lowest seen thus far in any animal (Chapter 3). Laboratory experiments have shown that $B. \text{childressi}$ can take up and assimilate ammonium and nitrate (Lee and Childress, 1996; Lee \textit{et al.}, 1992). Since we do not know the isotopic fractionation associated with this process nor the isotope values of the endmember nitrogen sources, it is difficult to guess which compound they might be assimilating or whether the nitrogen source is isotopically depleted, very abundant, or both.

The tissue $\delta^{15}\text{N}$ values of vestimentiferans showed consistent differences between the two widely co-occurring vestimentiferan species, which could indicate resource partitioning of inorganic nitrogen sources (Chapter 3). Again, since no isotope data exist on different nitrogen compounds or depth profiles of nitrogen isotopes in sediments, we could not rule out any of the three possibilities: the vestimentiferans are using different compounds (such as nitrate vs. ammonia), the worms obtain the nitrogen from spatially separate locations, or there is a difference in the uptake mechanism between the two species that causes consistently different degrees of fractionation.

In Chapters 2 and 3, it was noted that nitrogen isotopes in mussels and vestimentiferans did not vary overall as much as the other two elements. Where large differences did occur, the collections were at different sites separated by kilometers. There were, however, differences in mussel and vestimentiferan $\delta^{15}\text{N}$ values from the same site. At the three sites where we made several collections of both mussels and vestimentiferans, the mussels were on average 7‰ more depleted in $\delta^{15}\text{N}$ than the vestimentiferans. The heterotrophic animals associated with mussels were also
significantly more depleted than animals associated with vestimentiferans (Chapter 5). Thus, $\delta^{15}N$ is fairly consistent within symbiotic taxa over a regional scale, but can vary substantially by microhabitat at a smaller scale within sites.

Sulfur isotopes are far less-frequently used in food web studies than carbon and nitrogen due to the scarcity of analytical labs to perform the mass spectroscopy, the relatively large amount of material needed to perform the analysis (5 mg compared to 0.5 mg for carbon and nitrogen together), and the expense to do so. However, in seep environments, where hydrogen sulfide is an important energy and nutritional sulfur source, sulfur isotopes can be very informative. Surface POM $\delta^{34}S$ reflects the $\delta^{34}S$ of seawater sulfate ($\delta^{34}S = 20‰$) (Aharon and Fu, 2003). At seeps, most animals have more depleted $\delta^{34}S$ values, indicating the incorporation of organic sulfur derived from hydrogen sulfide. Hydrogen sulfide in seep sediments is formed by microbial sulfate reduction coupled to methane oxidation (Boetius et al., 2000; Joye et al., 2004). This process results in sulfide with $\delta^{34}S$ as low as -30‰ (Chanton et al., 1993). Extremely low tissue $\delta^{34}S$ values around -25‰ were only observed in the vestimentiferan tubeworms, whose symbionts rely on sulfide to perform chemoautotrophy (Chapter 3). Mussels were generally more enriched in $\delta^{34}S$ than vestimentiferans, but still quite depleted relative to seawater sulfate. This indicates some incorporation of organic sulfur derived from sulfide, even in mussels with only methanotrophic symbionts (Chapter 2).

$\delta^{34}S$ values tended to be the most variable of any isotope among individuals of the same species within collections (Chapters 2, 3, 4, 5). Even vestimentiferan tubeworms, which primarily incorporate organic sulfur derived from sulfide, had extremely variable $\delta^{34}S$ values among individuals within collections (Chapter 3). This probably reflects
variability in the δ³⁴S composition of the sulfide pool itself. The degree to which microbes fractionate sulfur isotopes during the reduction of sulfate depends on a number of factors, including sulfate reduction rate and sulfate availability, which can be a function of sediment depth. Dattagupta et al. (2008) found sediment porewater sulfide that ranged in δ³⁴S from -13.9 to +12.7‰ in a single peeper sample (a peeper captures discrete porewater samples from different depths; Dattagupta et al., 2007). Where vestimentiferans are involved, the picture becomes even more complex, because the worms use their buried roots to both take up porewater sulfide and release waste sulfate ions into deeper layers of the sediment (Dattagupta et al., 2006). Some vestimentiferans had δ³⁴S values close to that of seawater sulfate, which could mean the sulfide they assimilated was barely fractionated, due to sulfate limitation in the sediment or very high sulfate reduction rates. This is the simplest potential explanation, but in reality, a there are a number of processes occurring in the vestimentiferan sulfur-cycle that we know very little about, any of which could contribute to the observed δ³⁴S values in vestimentiferans. First, vestimentiferans acquire sulfide through their plumes as juveniles and through their roots as adults, but either way must transport the sulfide to their symbionts, which reside in the trophosome deep inside the worm’s body. To accomplish this, vestimentiferans have specialized hemoglobin molecules that bind both sulfide and oxygen simultaneously. The isotopic fractionation associated with this process, if any, is not known. Additionally, the process by which sulfide-derived sulfur becomes assimilated by vestimentiferan tissues, and any associated fractionation, is not known. It is likely that their symbionts that oxidize sulfide to sulfate for energy to perform chemoautotrophy are also responsible for fixing sulfur into an organic form that can be
utilized by the host vestimentiferan. Finally, sulfate ions are produced inside the
trophosome as a waste product of chemoautotrophy. If the symbionts fix this very sulfate
into an organic form, the concentration of the internal sulfate pool would affect any
associated fractionation. Excess sulfate that does not get assimilated needs to be excreted
by active transport across the plume or via an anion exchange across the root epithelium.
The latter is more energetically favorable and has been shown to be the primary
mechanism of sulfate excretion in the vestimentiferan *Lamellibrachia luymesi*
(Dattagupta *et al.*, 2006). Either excretion mechanism is another potential source for
isotopic fractionation. It was hypothesized from modeling and empirical studies that the
release of sulfate into deeper layers of the sediment is then reduced to sulfide again by
microbial consortia, ensuring sulfide supply to vestimentiferans over their long life span
(Cordes *et al.*, 2005a; Dattagupta *et al.*, 2008). As stated above, reduction of sulfate to
sulfide can be associated with substantial isotopic fractionation, so this re-fractionation of
waste sulfate ions could add yet another degree of variability to the sulfur isotopes in this
system.

Although the vestimentiferans present an exceptionally complex scenario, there
are some general rules that we can apply to sulfur isotopes in the seep ecosystem. Very
low $\delta^{34}$S values are a good indication of incorporation of seep-derived nutrition, and
values less than about -15‰ seem to most strongly indicate a chemoautotrophic source.
Animals that obtain nutrition from chemoautotrophy or methanotrophy could have tissue
$\delta^{34}$S values anywhere in the range of -15 to +20‰. Although the variability in $\delta^{34}$S
makes sulfur isotopes the most difficult to interpret in seep systems, the variability itself
can be informative, and, in Chapter 3, highlighted the importance of small-scale microbial processes.

*Lophelia pertusa*, the most common cold-water reef-forming coral, is often found around seeps, but tissue stable isotope compositions indicated that it derives little, if any, nutrition from seep primary production (Chapter 5). *L. pertusa*’s occurrence at seeps likely has more to do with availability of hard substrate in the form of authigenic carbonate, a rarity on the muddy Gulf of Mexico seafloor. The communities associated with corals, even mobile species that also occur in active seep areas, showed no appreciable incorporation of seep primary production. This suggests that enough food reaches the coral habitat from the surface to sustain this high-biomass community.

New investigations are finding that these corals colonize anthropogenic substrates such as oil rigs and shipwrecks, and grow more quickly than previously estimated. While this new information is encouraging for the recovery of reef that has already been destroyed, we must not underestimate the fragility of these important habitat-forming animals. The fact that the coral does not live in areas of active seepage at any stage in its life (indicated by skeleton δ¹³C content; Chapter 5) could indicate that these animals are quite sensitive to the presence of oil, natural gas, and other toxic chemicals found in seep fluid. Thus, drilling for oil in the vicinity of a coral reef could have negative impacts on the corals and their associated communities.

The potential threat of oil drilling on deep-water corals in the Gulf of Mexico could not be more relevant than it is now with the April 20, 2010 British Petroleum (BP) oil rig explosion in the Gulf of Mexico. To alleviate the impact of the oil spill on Gulf coast communities, the federal government has approved the use of chemical dispersants
to break the oil into tiny particles that sink to the bottom of the ocean. The dispersants are most effective if they are applied at depth where the oil is continuing to leak out by 10’s to 100’s of thousands of barrels (42 gallons to a barrel) per day. The dispersants themselves are a toxic chemical, although many claim not as bad as the oil itself, and will need to be continually added to the oil until the leak can be stopped. There is no question that causing millions of tiny oil particles to sink to the bottom of the Gulf will impact suspension feeders like *L. pertusa* and other deep-water corals. Besides the toxicity of the dispersant-oil mix when ingested by the coral polyps, corals are sensitive to sedimentation, and the particles could stick to tentacles lessening their ability to feed effectively. The slick that is already growing on the ocean’s surface undoubtedly affects surface primary production upon which the corals depend for food. Monitoring of the impacts upon the deep-water coral communities in the Gulf will be extremely important in the coming years if we are to have any hope of preserving these valuable deep-water reef habitats.

Together this thesis has highlighted some of the aspects of nutrition in seep communities on the lower slope and cold-water corals on the Gulf of Mexico upper continental slope. Along with food web related findings, the broad spatial scale and large sample sizes of these studies allowed us to gain insight into regional-scale geological processes, small-scale microbial processes, organism physiology, and interspecies interactions. Future work should focus on characterizing the isotopes of inorganic compounds from the different seep microhabitats, with particular focus on nitrogen. Physiological studies on nitrogen metabolism in *vestimentiferans* would greatly increase our understanding of the ecology and evolution of this taxonomic group. Finally, future
work should focus on how deep-water corals obtain sufficient surface-derived food to support the large reefs and abundant associated communities and how the catastrophic BP oil spill will affect the health of these ecosystems.
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APPENDIX A

Chapter 3 Vestimentiferan Geochemistry Schematic

Supplemental Figure 3-1. Schematic drawing depicting some of the key processes, sources, and isotope values for carbon and sulfur in seep vestimentiferan habitats.

Seawater DIC: 
$\delta^{13}C \approx 0\%$ 

Seawater Sulfate ($SO_4^{2-}$): 
$\delta^{34}S \approx 20\%$

Seep methane (CH$_4$): 
varyes by location 
$\delta^{13}C = -117$ to $-30\%$

Seawater sulfate diffused into the sediment

Waste sulfate excreted from vestimentiferan roots

Microbial sulfate reduction/hydrocarbon oxidation:
$^{34}S$ fractionation up to $-50\%$ and highly variable

$CH_4 + SO_4^{2-} + H^+ \rightarrow H_2S + HCO_3^- + H_2O$

sulfide bicarbonate
Supplemental Figure 4-1. (a), (c) $\delta^{15}N$ vs. $\delta^{13}C$ and (b), (c) $\delta^{34}S$ vs. $\delta^{13}C$ for mussels and associated fauna from two discrete collections from the AC601 study site. These are the same collections designated AC601m1 (a,b) and AC601m2 (c,d) in Cordes et al. (in press).
Supplemental Figure 4-2. (a), (c) $\delta^{15}$N vs. $\delta^{13}$C and (b), (c) $\delta^{34}$S vs. $\delta^{13}$C for mussels and associated fauna from two discrete collections from the AC645 (a,b) and AC818 (c,d) study sites. These are the same collections designated AC645m4 (a,b) and AC818m1 (c,d) in Cordes et al. (in press).
Supplemental Figure 4-3. (a), (c) δ^{15}N vs. δ^{13}C and (b), (c) δ^{34}S vs. δ^{13}C for mussels and associated fauna from two discrete collections from the AC818 study site. These are the same collections designated AC818m2 (a,b) and AC818m3 (c,d) in Cordes et al. (in press).
Supplemental Figure 4-4. (a), (c) $\delta^{15}$N vs. $\delta^{13}$C and (b), (c) $\delta^{34}$S vs. $\delta^{13}$C for mussels and associated fauna from two discrete collections from the AC818 study site. These are the same collections designated AC818m4 (a,b) and AC818m5 (c,d) in Cordes et al. (in press).
Supplemental Figure 4-5. (a), (c) $\delta^{15}$N vs. $\delta^{13}$C and (b), (c) $\delta^{34}$S vs. $\delta^{13}$C for mussels and associated fauna from two discrete collections from the AT340 study site. These are the same collections designated AT340m1 (a,b) and AT340m2,3,4 (c,d) in Cordes et al. (in press). The AT340m2, AT340m3, and AT340m4 collections were combined because they were collected from the same mussel bed.
Supplemental Figure 4-6. (a), (c) δ¹⁵N vs. δ¹³C and (b), (c) δ³⁴S vs. δ¹³C for mussels and associated fauna from two discrete collections from the AT340 study site. These collections are not presented in Cordes et al. (in press) because they were not quantitative.
Supplemental Figure 4-7. (a), (c) $\delta^{15}$N vs. $\delta^{13}$C and (b), (c) $\delta^{34}$S vs. $\delta^{13}$C for mussels and associated fauna from two discrete collections from the GB697 study site. These are the same collections designated GB697m1 (a,b) and GB697m2 (c,d) in Cordes et al. (in press).
Supplemental Figure 4-8. (a), (c) $\delta^{15}$N vs. $\delta^{13}$C and (b), (c) $\delta^{34}$S vs. $\delta^{13}$C for mussels and associated fauna from two discrete collections from the GB829 and GC852 study sites. These are the same collections designated GB829m1 (a,b) and GC852m1 (c,d) in Cordes et al. (in press).
Supplemental Figure 4-9. (a), (c) $\delta^{15}N$ vs. $\delta^{13}C$ and (b), (c) $\delta^{34}S$ vs. $\delta^{13}C$ for mussels and associated fauna from two discrete collections from the GC852 study site. These are the same collections designated GC852m5 (a,b) and GC852m6 (c,d) in Cordes et al. (in press).
Supplemental Figure 4-10. (a), (c) \( \delta^{15}N \) vs. \( \delta^{13}C \) and (b), (c) \( \delta^{34}S \) vs. \( \delta^{13}C \) for mussels and associated fauna from two discrete collections from the GC852 study site. These are the same collections designated WR269m2 (a,b) and WR269m3,4 (c,d) in Cordes et al. (in press). The WR269m3 and WR269m4 collections were combined because they were collected from the same mussel bed.
Supplemental Figure 4-11. (a) $\delta^{15}N$ vs. $\delta^{13}C$ and (b) $\delta^{34}S$ vs. $\delta^{13}C$ for vestimentiferans and associated fauna from one collection from the AC601 study site. This is the same collection designated AC601t1 in Cordes et al. (in press).
Supplemental Figure 4-12. (a), (c) $\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$ and (b), (c) $\delta^{34}\text{S}$ vs. $\delta^{13}\text{C}$ for vestimentiferans and associated fauna from two discrete collections from the AC818 study site. These are the same collections designated AC818t1 (a,b) and GC852t2 (c,d) in Cordes *et al.* (in press).
Supplemental Figure 4-13. (a), (c) $\delta^{15}$N vs. $\delta^{13}$C and (b), (c) $\delta^{34}$S vs. $\delta^{13}$C for vestimentiferans and associated fauna from two discrete collections from the AT340 study site. These are the same collections designated AT340t1 (a,b) and AT340t2 (c,d) in Cordes et al. (in press).
Supplemental Figure 4-14. (a), (c) $\delta^{15}N$ vs. $\delta^{13}C$ and (b), (c) $\delta^{34}S$ vs. $\delta^{13}C$ for vestimentiferans and associated fauna from two discrete collections from the AT340 study site. These are the same collections designated AT340t3 (a,b) and AT340t4 (c,d) in Cordes et al. (in press).
Supplemental Figure 4-15. (a), (c) $\delta^{15}$N vs. $\delta^{13}$C and (b), (c) $\delta^{34}$S vs. $\delta^{13}$C for vestimentiferans and associated fauna from two discrete collections from the AT340 study site. These are the same collections designated AT340t5 (a,b) and AT340t6 (c,d) in Cordes et al. (in press).
Supplemental Figure 4-16. (a), (c) $\delta^{15}$N vs. $\delta^{13}$C and (b), (c) $\delta^{34}$S vs. $\delta^{13}$C for vestimentiferans and associated fauna from two discrete collections from the GC852 study site. The collection in panels (a,b) is the same collection designated GC852t1 (a,b) and in Cordes et al. (in press).
Supplemental Figure 5-1. (a), (c) $\delta^{34}$S vs. $\delta^{13}$C and (b), (c) $\delta^{15}$N vs. $\delta^{13}$C for *Lophelia pertusa* and associated fauna from Bushmasters taken on dive 4868 (a), (b) and dive 4871 (c), (d) at the VK 826 study site. These are the same collections designated V1e and V1f in Cordes et al. (2008).
Supplemental Figure 5-2. (a), (c) δ^{34}S vs. δ^{13}C and (b), (c) δ^{15}N vs. δ^{13}C for *Lophelia pertusa* and associated fauna from Bushmasters taken on dive 4728 (a), (b) and dive 4740 (c), (d) at the GC 234 study site. These are the same collections designated G1c and G1d in Cordes *et al.* (2008).
Supplemental Figure 5-3. (a), (c) $\delta^{34}S$ vs. $\delta^{13}C$ and (b), (c) $\delta^{15}N$ vs. $\delta^{13}C$ for *Lophelia pertusa* and associated fauna from Bushmasters taken on dive 4737 (a), (b) and dive 4867 (c), (d) at the VK 826 study site. These are the same collections designated V1b and V1d in Cordes *et al.* (2008).
Supplemental Figure 5-4. (a) $\delta^{34}$S vs. $\delta^{13}$C and (b) $\delta^{15}$N vs. $\delta^{13}$C for *Lophelia pertusa* and associated fauna from Bushmasters taken on dive 4741 at the GC 354 study site. This same collection designated G2a in Cordes *et al.* (2008).
VITA

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EDUCATION

Ph.D. in Biology, Pennsylvania State University, August 2010
B.S. in Ecology and Evolution, certificate in German Language, University of Pittsburgh, May 2005

PROFESSIONAL AND TEACHING EXPERIENCE

Graduate Assistant (2006 to present) Fisher Laboratory, Pennsylvania State University.
   Food web ecology in Gulf of Mexico seeps and megafaunal distribution in Lau Basin vents.
   Research Cruises:
     2009 Lau Basin – R/V Thomas Thompson, ROV Jason II
     2007 Deep Gulf of Mexico 2 – NOAA ship Ron Brown, ROV Jason II
     2006 Lau Basin – R/V Melville, ROV Jason II
     2006 Deep Gulf of Mexico – R/V Atlantis, Alvin
     2006 Deep Gulf of Mexico Survey Cruise – R/V Gyre
     2005 Lophelia I - R/V Seward Johnson, Johnson Sea-Link


Laboratory and Field Assistant (Summer 2002-2004) Pennsylvania Department of Agriculture Plum Pox Virus Survey, Harrisburg, PA

Teaching Assistant Penn State University
   2008 and 2009 Fall – Experimental Field Biology
   2006 and 2007 Spring – Populations and Communities
   2005 Fall – Biology: Basic Concepts and Biodiversity

PRESENTATIONS, AWARDS AND OUTREACH

ChEss Travel Grant (2010) for International Stable Isotope Ecology Conference
Braddock Scholarship (2005, 2008)
Biology Dept. Grad Student Assoc. (2007-2009) – president, webmaster, secretary
Intl. Chemosynthesis-Based Ecosystems Conference (2009) – oral presentation
Ocean Sciences Meeting (2008) sponsored by ASLO and AGU – poster presentation
Phi Eta Sigma Freshman Honor Society (2002) – service chair, vice president
Member Beta Beta Beta Biology Honor Society (2004)
Member Golden Key Honor Society (2004)