Deepwater Program: Studies of Gulf of Mexico Lower Continental Slope Communities Related to Chemosynthetic and Hard Substrate Habitats

By Steve W. Ross, Amanda W.J. Demopoulos, Christina A. Kellogg, Cheryl L. Morrison, Martha S. Nizinski, Cheryl L. Ames, Tara L. Casazza, Daniel Gualtieri, Kaitlin Kovacs, Jennifer P. McClain, Andrea M. Quattrini, Adela Y. Roa-Varón, and Andew D. Thaler

Open-File Report 2012–1032

U.S. Department of the Interior
U.S. Geological Survey

U.S. Department of the Interior
KEN SALAZAR, Secretary

U.S. Geological Survey
Marcia K. McNutt, Director

U.S. Geological Survey, Reston, Virginia 2012

For product and ordering information:
World Wide Web: http://www.usgs.gov/pubprod
Telephone: 1-888-ASK-USGS

For more information on the USGS—the Federal source for science about the Earth,
its natural and living resources, natural hazards, and the environment:
World Wide Web: http://www.usgs.gov
Telephone: 1-888-ASK-USGS

Suggested citation:
Ross, S.W., Demopoulos, A.W.J., Kellogg, C.A., Morrison, C.L., Nizinski, M.S., Ames, C.L., Casazza, T.L., Gualtieri, D., Kovacs, K., McClain, J.P., Quattrini, A.M., Roa-Varón, A.Y., and Thaler, A.D., 2012, Deepwater Program: Studies of Gulf of Mexico lower continental slope communities related to chemosynthetic and hard substrate habitats: U.S. Geological Survey Open-File Report 2012-1032, 301 p.

Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Although this report is in the public domain, permission must be secured from the individual copyright owners to reproduce any copyrighted material contained within this report.

Contents

By Steve W. Ross, Amanda W.J. Demopoulos, Christina A. Kellogg, Cheryl L. Morrison, Martha S. Nizinski, Cheryl L. Ames, Tara L. Casazza, Daniel Gualtieri, Kaitlin Kovacs, Jennifer P. McClain, Andrea M. Quattrini, Adela Y. Roa-Varón, and Andew D. Thaler i

1. INTRODUCTION AND PROJECT OVERVIEW 1

2. ENUMERATION OF MICROBES IN CHEMOSYNTHETIC COMMUNITY SEDIMENTS 5

2.1 Introduction 5

2.2 Methods 7

2.2.1 Sampling sites 7

2.2.2 Sediment Characteristics 9

2.2.3 Enumeration of microbes 10

2.3 Results 10

2.3.1 Sediment characteristics 10

2.3.2 Microbial enumeration 11

2.4 Discussion 11

2.5 Acknowledgments 17

3. FOOD-WEB STRUCTURE OF SEEP SEDIMENT MACROBENTHOS FROM THE GULF OF MEXICO 18

3.1 Introduction 18

3.2 Materials and Methods 21

3.2.1 Collection Methods 21

3.2.2 Lab Processing 24

3.3.3 Statistical Analysis 24

3.3 Results 24

3.3.1 Sediment Characteristics 24

3.3.2 Isotopic composition of primary source pools 28

3.3.3 Sediment macrofaunal isotopic composition 29

3.3.3.1 Green Canyon-GC 852 29

3.3.3.2 Alaminos Canyon-AC 601 30

3.3.3.3 Atwater Valley-AT 340 30

3.4 Discussion 31

3.5. Acknowledgments 34

4. MACROFAUNAL COMMUNITY STRUCTURE AND SEDIMENT ENVIRONMENTAL CHARACTERISTICS IN THREE SEEP ECOSYSTEMS 35

4.1 Introduction 35

4.2 Methods 36

4.2.1 Sampling Sites 36

4.2.2 Lab Processing 37

4.3 Results 38

4.3.1 Sediment Properties 38

4.3.2 Macrofauna abundance and composition 40

4.4 Discussion 42

4.5 Acknowledgments 43

5. PHYLOGEOGRAPHIC AFFINITIES OF SQUAT LOBSTERS (DECAPODA: GALATHEOIDAE) FROM LOWER CONTINENTAL SLOPE COLD-SEEP HABITATS IN THE NORTHERN GULF OF MEXICO 45

5.1 Introduction 45

5.2 Methods 49

5.2.1 Sample Collections 49

5.2.2 DNA extraction, amplification, and sequencing 52

5.2.3 Mitochondrial DNA sequence analysis 53
5.3 Results 54
5.3.1 Diversity of Galatheoidea 54
5.3.2 *Munidopsis* species 55
5.3.3 *Munida* species 59
5.4 Discussion 62
5.5 Acknowledgments 64
6. PRELIMINARY MOLECULAR ASSESSMENT OF SCLERACTINIAN CORAL AND OCTOCORAL BIODIVERSITY FROM LOWER CONTINENTAL SLOPE HABITATS IN THE NORTHERN GULF OF MEXICO 66
6.1 Introduction 66
6.2 Methods 69
6.2.1 Sampling sites 69
6.2.2 Molecular Methods 71
6.2.3 Phylogenetic analyses 72
6.3 Results 73
6.3.1 Scleractinian coral phylogeny 73
6.3.2 Octocoral phylogeny 78
6.4 Discussion 83
6.5 Acknowledgments 85
7. COMMUNITY STRUCTURE OF MESOPELAGIC FISHES OVER THE SLOPE OF THE NORTH-CENTRAL GULF OF MEXICO 86
7.1 Introduction 86
7.2 Methods 89
7.2.1 Study Areas 91

7.2.2 Tucker trawl 96

7.2.3 Data Analysis 98

7.3 Results 102

7.3.1 Site Comparisons 118

7.3.1.1 Species Richness 118

7.3.1.2 Across-Site Comparisons 120

7.3.1.3 Within-Site Comparisons 123

7.3.2 Depth, Diel, and Size Distributions 126

7.4 Discussion 135

7.4.1 General 135

7.4.2 Site Comparisons 137

7.4.3 Depth, Diel, and Size Distributions 140

7.4.4 Conclusion 142

7.5 Acknowledgments 143

8. DIETS AND FEEDING BEHAVIOR OF MIDWATER FISHES OVER COLD- SEEP SITES IN THE NORTH-CENTRAL GULF OF MEXICO 144

8.1 Introduction 144

8.2 Methods 148

8.2.1 Study Area 148

8.2.2 Sample Collection 149

8.2.3 Diet Analyses 153

8.2.4 Stable Isotope Analysis (SIA) 154

8.2.5 Statistical Analyses 156

8.3 Results 156

8.3.1 Primary Producers 160
8.3.2 Invertebrates 160
8.3.3 Fishes 163
8.3.3.1 Gonostomatidae 163
8.3.3.2 Myctophidae 179
8.3.3.3 Phosichthyidae 196
8.3.3.4 Sternoptychidae 202
8.3.3.5 Stomiidae 210
8.4 Discussion 212
8.5 Conclusions 218
8.6 Acknowledgments 218
9. DISTRIBUTIONS AND COMPOSITION OF THE MESOPELAGIC INVERTEBRATE FAUNA OVER THE SLOPE OF THE NORTH-CENTRAL GULF OF MEXICO 220
9.1 Introduction 220
9.2 Methods 221
9.3 Results 223
9.3.1 Species composition and relative abundance among study sites 237
9.3.2 Species composition and relative abundance among zones 239
9.4 Discussion 256
9.5 Acknowledgements 260
10. ANALYSIS OF FUNGAL DIVERSITY IN SEDIMENT FROM A METHANE SEEP, GULF OF MEXICO 261
10.1 Introduction and Rationale 261
10.2 Methods 262
10.2.1 Recovery 262

10.2.2 Extraction and Amplification 263

10.2.3 Analysis 263

10.3 Results 264

10.4 Discussion 266

10.5 Acknowledgments 267

References Cited 268

Figures

Figure 1.1. Four major study areas sampled in the Gulf of Mexico during the Chemo III project. (VK, Viosca Knoll; AT, Atwater Canyon; GC, Green Canyon; AC, Alaminos Canyon. Insets for each area illustrate sampling stations by gear type: BC, box core; OT, otter trawl; NN, neuston net; OD, on deck; PN, plankton net followed by mesh size; Phyto, phytoplankton water sample; CTD, conductivity, temperature, depth profiler; TT, Tucker trawl.) 4

Figure 2.1. Viral lysis of bacteria converts cellular components into dissolved organic matter, transferring nutrients away from grazers and stimulating other heterotrophic prokaryotic growth. 7

Figure 3.1. Collection sites in the Gulf of Mexico. (Contour lines represent 1,000-, 2,000-, and 3,000- m bathymetry.) 22

Figure 3.2. Dual stable isotope plots of different primary sources (open rectangles), and macrofauna (>300 micrometers, μm) from Gulf of Mexico seeps: (A) GC852, (B) AC601, and (C) AT340. (Each point represents a distinct individual. Ranges in stable carbon and nitrogen isotope values are given for primary sources, including particulate organic matter (POM), suspended organic matter (SOM), *Beggiatoa* bacteria, and sediment.) 27

Figure 4.1. *A*, Macrofaunal (>300 μm) density (individuals per square meter), and *B*, taxonomic composition by major taxa. 41

Figure 5.1. Multibeam bathymetry map of the northern Gulf of Mexico continental slope. Included on map are galatheiod collection localities (yellow dots) and lease blocks established by the Bureau of Ocean Energy

Management, Regulation and Enforcement for oil and gas activity management. (Map created by Harry Roberts, Louisiana State University). 52

Figure 5.2. Phylogenetic hypothesis for *Munidopsis* resulting from Bayesian analysis of mitochondrial cytochrome oxidase (COI) DNA sequence data. A and B are clades, or groupings of taxa. 57

Figure 5.3. Phylogenetic hypothesis for *Munida* resulting from Bayesian analysis of mitochondrial cytochrome oxidase I (COI) DNA sequence data. 61

Figure 6.1. Sampling locations in the northern Gulf of Mexico. 70

Figure 6.2. Phylogenetic hypothesis for scleractinian corals based upon Bayesian analysis of mitochondrial 16S sequences. (Species names in bold represent samples unique to this study, whereas others originate from GenBank or Morrison and others (2008). Numbers at nodes represent Bayesian posterior probabilities of node support.) 78

Figure 6.3. Phylogenetic hypothesis for octocorals based upon Bayesian analysis of mitochondrial *msh*1 sequences. (Species names in bold represent samples unique to this study, whereas others originate from GenBank. Numbers at nodes represent Bayesian posterior probabilities of node support. Vertical bars indicate the following families: Ac, Acanthogorgiidae; Go, Gorgoniidae; St, Stenogorgiinae; Pl, Plexauridae; Ch, Chrysogorgiidae; Pr, Primnoidae; Is, Isididae; Co, Coralliidae.) 81

Figure 6.4. Phylogenetic hypothesis for octocorals based upon Bayesian analysis of mitochondrial *ND2* sequences. (Species names in bold represent samples unique to this study, whereas others originate from GenBank. Numbers at nodes represent Bayesian posterior probabilities of node support. Vertical bars indicate the following families: Ac, Acanthogorgiidae; St, Stenogorgiinae; Ch, Chrysogorgiidae; Pr, Primnoidae; Is, Isididae; Co, Coralliidae.) 83

Figure 7.1. Study area locations in the north-central Gulf of Mexico sampled August 9-29, 2007. Three sites (AC601, GC852, and AT340) were over cold-seep habitats, and VK826 was over a coral-carbonate and seep habitat. Vector lines represent Tucker trawl tows and boxes are conductivity, temperature, and depth profiler (CTD) stations. 91

Figure 7.2. Naval Research Lab Layered Ocean Model (high resolution) assimilating Sea Surface Height (SSH) for four dates during our study period. The anomaly SSH satellite maps show the difference in measured sea height from the calculated, mean sea level. A +17 cm SSH contour generally tracks the loop current (LC) and loop current rings. Reds and yellows represent higher SSH and warmer waters of the LC and LC eddies. Blue to purple colors are negative SSH anomalies representing cold-core cyclonic eddies. 93

Figure 7.3. Conductivity, temperature, depth (CTD) (SeaBird 911*plus*) casts at the four Gulf of Mexico study sites, illustrating dissolved oxygen (milliliters per liter), fluorescence, water temperature (degrees Celsius), and salinity profiles (practical salinity units). 96

Figure 7.4. Tucker trawl being deployed over the stern of the R/V *Cape Hatteras* on August 13, 2007. 97

Figure 7.5. Species accumulation curves for the four Gulf of Mexico study sites plotted using the Colwell and others (2004) analytical method. 119

Figure 7.6. Multdimensional scaling ordination of 115 stations based on the Bray-Curtis similarity matrix calculated from standardized, fourth-root transformed fish abundances (117 species) with superimposed (*A*) SIMPROF groups labeled by day (D) or night (N) and (*B)* mean depths (in meters) with SIMPROF labels. The embedded dendrogram in *A* is labeled with SIMPROF groups. The table includes sites, number of stations, and sampled mean depth ranges (in parentheses) of each SIMPROF group. 122

Figure 7.7. Multidimensional scaling ordinations for three stations based on Bray-Curtis similarity matrices calculated from standardized, fourth-root transformed fish abundances. Each plot, *(A)* GC852 (50 stations, 91 species), *(B)* AT340 (35 stations, 71 species), and *(C)* VK826 (25 stations, 51 species), has superimposed SIMPROF groups labeled by day (D) or night (N). The embedded dendrograms include SIMPROF groups, followed by number of stations and sampled mean depth ranges (in parentheses). 126

Figure 7.8. Overall (all stations combined) temporal depth distributions of dominant midwater fishes (*Cyclothone acclinidens, C. alba, C. braueri*) by abundance and size class collected in the Gulf of Mexico, August 9-29, 2007. SL mm, standard length in millimeters. 128

Figure 7.9. Overall (all stations combined) temporal depth distributions of dominant midwater fishes (*Cyclothone pallida, C. pseudopallida, Valenciennellus tripunctulatus*) by abundance and size class collected in the Gulf of Mexico, August 9-29, 2007. SL mm, standard length in millimeters..... 129

Figure 7.10. Overall (all stations combined) temporal depth distributions of dominant midwater fishes (*Vinciguerria poweriae, Benthosema suborblitale, Diaphus dumerilii*) by abundance and size class collected in the Gulf of Mexico, August 9-29, 2007..... 131

Figure 7.11. Overall (all stations combined) temporal depth distributions of dominant midwater fishes (*Lepidophanes guentheri, Notolychnus valdiviae*) by abundance and size class collected in the Gulf of Mexico, August 9-29, 2007. 132

Figure 8.1. North-central Gulf of Mexico illustrating sampling areas for midwater fauna, August 9-25, 2007, at three cold-seep sites (AC601, GC852, and AT340) located on the slope at depths >1,000 meters. Each dot represents one station. 149

Figure 8.2. Mean δ^{13}C and δ^{15}N ($\pm$ 1 SE) stable isotope values for all samples collected. Data from all three sites for each species were grouped together. *, Detritus values are not shown. 162

Figure 8.3. The relationships between stomach fullness and depth of capture for 18 midwater fishes. Data from all three sites were grouped together to detect diel vertical migrations. 167

Figure 8.4. The relationship of standard length (SL) to δ^{15}N values in midwater fishes from the families (*A*) Gonostomatidae – *Cyclothone* spp.; (*B*) Gonostomatidae – *Gonostoma elongatum;* (*C*) Myctophidae; (*D*) Phosichthyidae; (*E*) Sternoptychidae; and (*F*) Stomiidae collected at all three sites. 179

Figure 9.1. Map of study area in the north-central Gulf of Mexico showing location of sampling sites. Three sites (AC601, GC852, and AT340) were over cold-seep habitats and one site (VK826) was over a *Lophelia pertusa* coral habitat. 222

Figure 9.2. Overall (all sampling sites combined) species richness of invertebrates for each major taxon collected by zone (surface, midwater, benthic) at three cold-seep sites (AC601, GC852, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. 232

Figure 9.3. Overall (all sampling sites combined) species richness of all major invertebrate taxa collected at three cold-seep sites (AC601, GC852, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. 234

Figure 9.4. Overall (all sampling sites combined) species richness and abundance of major invertebrate taxa collected at three cold- seep sites (AC601, GC852, and AT340) and one Lophelia pertusa site (VK826) in the Gulf of Mexico, August 9-29, 2007. 235

Figure 9.5. Species richness (by site) of invertebrates collected at three cold-seep sites (AC601, GC852, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. 238

Figure 9.6. Species richness of all major invertebrate taxa collected using surface gear over three cold-seep sites (AC601, GC852, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. 242

Figure 9.7. Species richness of major invertebrate taxa collected using bottom gear at two cold-seep sites (GC852 and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. 247

Figure 9.8. Species richness of major invertebrate taxa collected using midwater gear over three cold-seep sites (AC601, GC852, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. 255

Figure 10.1. Primers used for amplification of fungal LSU and ITS regions. 263

Figure 10.2. ... Redox profile for the Alaminos Canyon methane seep boxcore, mV, millivolts; cm, centimeters.__Courtesy of A.W.J. Demopoulos, USGS. Shaded area is the region dominated by an unidentified ascomycete clade. 265

Figure 10.3. Eukaryotic phylotypes recovered from Alaminos Canyon methane seep, 0–2 cm and 2–4 cm. Greater depths are not shown due to few sequences recovered below the redox zone. 266

Tables

Table 2.1 Nine sediment cores were collected from four sites in the Gulf of Mexico between June 21 and July 2, 2007, utilizing the utilizing the R/V *Ronald H. Brown* and the remotely operated vehicle (ROV) *Jason.* [m, meter; C, degrees Celsius, ‰, parts per thousand] 9

Table 2.2. Microbial abundance numbers from previous studies of sediments below 1,000 m water depth. 14

Table 3.1. Box core locations and depths, northern Gulf of Mexico. 23

Table 3.2. Redox potential profiles (Eh, in millivolts) in box cores from Green Canyon (GC 852), Alaminos Canyon (AC601), and Atwater Valley (AT340). 27

Table 4.1. Average (± 1 S.E.) sediment particle size, percent organic carbon and nitrogen, $\delta^{13}C$, $\delta^{5}N$, and salinity from three seeps in the Gulf of Mexico. [%, percent; cm, centimeter; salinity measured in practical salinity units] 39

Table 5.1. Species list for galatheoid samples examined. 51

Table 5.2. Pairwise uncorrected *P*-distances between mitochondrial cytochrome oxidase I (COI) sequences for *Munidopsis* samples 58

Table 6.1. Collection and information for scleractinian coral samples. 74

Table 6.2. Collection and information for octocoral samples 78

Table 7.1. Relative abundance of species comprising ~90 percent of total catch at each site. 101

Table 7.2. Stations sampled at four sites (see fig. 7.1) in the Gulf of Mexico (August 9-29, 2007) 103

Table 7.3. Total numbers and standard length ranges (millimeters in parentheses) of juvenile and adult fishes collected by Tucker trawl over three cold-seep sites (AC601, GC852, AT340) and one deep-sea coral site (VK826) in the Gulf of Mexico (August 9-29, 2007). 110

Table 8.1. Surface stations sampled over three cold-seep sites (AC601, GC852, and AT340) (see fig. 8.1) in the Gulf of Mexico (August 9-25, 2007). 151

Table 8.2. Mean $\delta^{15}N$ and $\delta^{13}C$ (±1 SE) stable isotope values by species collected at three cold-seep sites (AC601, GC852, and AT340) in the Gulf of Mexico, August 9-25, 2007. 158

Table 8.3. Percent volume (%V) and percent frequency (%F) of prey consumed by *Cyclothone alba* collected from two sites (AT340, and GC852) in the Gulf of Mexico separated by day and night. Additionally, two *C. alba* were captured at site AC601, both at night with empty stomachs. 168

Table 8.4. Percent volume (%V) and percent frequency (%F) of prey consumed by *Cyclothone pallida* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night. 169

Table 8.5. Percent volume (%V) and percent frequency (%F) of prey consumed by *Cyclothone braueri* collected from two sites (AT340 and GC852) in the Gulf of Mexico separated by day and night. Additionally, two *C. braueri* were captured at site AC601, both at night with empty stomachs. 170

Table 8.6. Percent volume (%V) and percent frequency (%F) of prey consumed by *Cyclothone pseudopallida* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night. 171

Table 8.7. Percent volume (%V) and percent frequency (%F) of prey consumed by *Gonostoma elongatum* collected from two sites (AT340 and GC852) in the Gulf of Mexico separated by day and night. 173

Table 8.8. Percent volume (%V) and percent frequency (%F) of prey consumed by *Hygophum benoiti* collected from AT340 in the Gulf of Mexico separated by day and night. Additionally, six *H. benoiti* were collected at AC601 at night, all with empty stomachs. At site GC852, 33 *H. benoiti* were collected during the day and 37 during the night, all with empty stomachs. 181

Table 8.9. Percent volume (%V) and percent frequency (%F) of prey consumed by *Benthosema suborbitale* collected from three sites (AC601, AT340 and GC852) in the Gulf of Mexico separated by day and night. 182

Table 8.10. Percent volume (%V) and percent frequency (%F) of prey consumed by *Diaphus mollis* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night. 185

Table 8.11. Percent volume (%V) and percent frequency (%F) of prey consumed by *Notolychnus valdiviae* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night. 187

Table 8.12. Percent volume (%V) and percent frequency (%F) of prey consumed by *Ceratoscopelus warmingii* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night. 189

Table 8.13. Percent volume (%V) and percent frequency (%F) of prey consumed by *Lepidophanes guentheri* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night..... 192

Table 8.14. Percent volume (%V) and percent frequency (%F) of prey consumed by *Lampanyctus alatus* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night..... 194

Table 8.15. Percent volume (%V) and percent frequency (%F) of prey consumed by *Pollichthys mauli* collected from two sites (AT340 and GC852) in the Gulf of Mexico separated by day and night. 198

Table 8.16. Percent volume (%V) and percent frequency (%F) of prey consumed by *Vinciguerria poweriae* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night..... 199

Table 8.17. Percent volume (%V) and percent frequency (%F) of prey consumed by *Argyropelecus aculeatus* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night..... 204

Table 8.18. Percent volume (%V) and percent frequency (%F) of prey consumed by *Argyropelecus hemigymnus* collected from two sites (AT340 and GC852) in the Gulf of Mexico separated by day and night. 207

Table 8.19. Percent volume (%V) and percent frequency (%F) of prey consumed by *Valenciennellus tripunctulatus* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night..... 208

Table 8.20. Percent volume (%V) and percent frequency (%F) of prey consumed by *Chauliodus sloani* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night..... 211

Table 9.1. Overall diversity and abundance of invertebrates collected (all sampling methods combined) at three cold-seep sites (GC852, AC601, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. 224

Table 9.2. Invertebrate species and number of individuals collected at the surface over two cold-seep sites (GC852 and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007..... 241

Table 9.3. Invertebrate species and number of individuals collected on the bottom at two cold-seep sites (GC852 and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007..... 244

Table 9.4. Invertebrate species and number of individuals collected in midwater over three cold-seep sites (GC852, AC601, and AT340) and at one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. 249

Table 10.1. Total sequences recovered, fungal sequences recovered, and sequences recovered from unidentified ascomycete clade by 2-cm sediment depth intervals from the Alaminos Canyon methane seep (2,300 m). 264

Conversion Factors

Inch/Pound to SI

Multiply	By	To obtain
	Length	
inch (in.)	2.54	centimeter (cm)
inch (in.)	25.4	millimeter (mm)
foot (ft)	0.3048	meter (m)
mile (mi)	1.609	kilometer (km)
mile, nautical (nmi)	1.852	kilometer (km)
yard (yd)	0.9144	meter (m)
	Area	
acre	4,047	square meter (m^2)
square foot (ft^2)	0.09290	square meter (m^2)
	Volume	
ounce, fluid (fl. oz)	0.02957	liter (L)
pint (pt)	0.4732	liter (L)
quart (qt)	0.9464	liter (L)
gallon (gal)	3.785	liter (L)
	Mass	
ounce, avoirdupois (oz)	28.35	gram (g)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

°F=(1.8×°C)+32

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows:

°C=(°F-32)/1.8

NOTE TO USGS USERS: Use of hectare (ha) as an alternative name for square hectometer (hm^2) is restricted to the measurement of small land or water areas. Use of liter (L) as a special name for cubic decimeter (dm^3) is restricted to the measurement of liquids and gases. No prefix other than milli should be used with liter. Metric ton (t) as a name for megagram (Mg) should be restricted to commercial usage, and no prefixes should be used with it.

1. INTRODUCTION AND PROJECT OVERVIEW

By Steve W. Ross[1]

[1]University of North Carolina Wilmington, Center for Marine Science, Wilmington, NC

This report summarizes research funded by the U.S. Geological Survey (USGS) in collaboration with the University of North Carolina at Wilmington (UNCW) on the ecology of deep chemosynthetic communities in the Gulf of Mexico. The research was conducted at the request of the U.S. Bureau of Ocean Energy Management, Regulation and Enforcement (BOEMRE; formerly Minerals Management Service) to complement a BOEMRE-funded project titled "*Deepwater Program:* Investigations of Chemosynthetic Communities on the Lower Continental Slope of the Gulf of Mexico." The overall research partnership, known as "Chemo III," was initiated to increase understanding of the distribution, structure, function, and vulnerabilities of these poorly known associations of animals and microbes for water depths >1,000 meters (m) in the Gulf of Mexico. Chemosynthetic communities rely on carbon sources that are largely independent of sunlight and photosynthetic food webs. Despite recent research directed toward chemosynthetic and deep coral (for example, *Lophelia pertusa*) based ecosystems, these habitats are still poorly studied, especially at depths greater than 1,000 m. With the progression into deeper waters by fishing and energy industries, developing sufficient knowledge to manage these deep ecosystems is essential. Increased understanding of deep-sea communities will enable sound evaluations of potential impacts and appropriate mitigations.

Cruise reports and preliminary data for the BOEMRE-funded projects are included in reports prepared by TDI-Brooks International, Inc. (2006a, b; 2007), and a final report that

complements this USGS report is near completion. Some USGS objectives were addressed with participation in the BOEMRE-supported cruises, while others were addressed through a USGS cruise (Ross, 2007). This research is part of a larger, long-term goal of the USGS deep-sea studies group to develop, integrate, and synthesize information on deep-sea corals (and hardgrounds) and chemosynthetic communities and to compare community composition, fauna/habitat linkages, genetic structures, and energetics across latitudes, habitat types, depth zones, and regions. A special issue of peer-reviewed papers that stemmed from this Chemo III project was recently published in the journal Deep-Sea Research – Part II (Roberts, 2010), and some of the data contained in this USGS report are also within the Deep-Sea Research publication.

Deep water (aphotic) coral, hardground, and chemosynthetic systems are important habitats for macrofauna, repositories of data on ocean climate and productivity, and hotspots of increased biodiversity. Fishes and crustaceans are megafauna of particular interest to decision-makers, but their abundance, microhabitat affinities, and importance in structuring these biotic communities are poorly known. Understanding trophic (food web) linkages between the benthos and the overlying waters is fundamental ecological knowledge about how deep ecosystems function. Deciphering barriers to gene flow that may isolate populations has been problematic for marine species. Even so, some slope species exhibit unexpected population heterogeneity. Genetic techniques applied to organisms associated with coral, hardbottom, and chemosynthetic habitats from various locations provide insights into species biology, mechanisms regulating community structure (dispersal), and information about reproduction and recovery after various impacts. Microbes, specifically bacteria, are the foundation of deep-sea chemosynthetic communities and may provide vital functions to deep-sea corals. Viruses are the most numerous

organisms in the ocean, and the majority appears to infect marine bacteria. As such, viruses control microbial diversity and carbon cycling. Little is known about viruses in the deep sea, and to understand cold seep dynamics, the viral ecology of this ecosystem (water and sediments) must be addressed. Sponges, important as deep-reef habitat for macrofauna, are recognized as "microbial apartment buildings," containing a wide variety of commensal and symbiotic bacteria and archaea. As with coral-associated microbes, assessing these microorganisms allows a better understanding of the biology of the macrofauna they inhabit, increases understanding of microbial biodiversity, and reveals species interdependencies.

The USGS Chemo III effort was composed of five interrelated study components, each with separate chapters in this report: Part I, midwater macrofauna (fishes and mobile invertebrates) taxonomy and ecology (abundances, habitat associations, behaviors); Part II, midwater and benthic trophic connectivity; Part III, crustacean taxonomy and ecology; Part IV, microbial ecology (composition, distributions); and Part V, genetics (including community genetics, phylogeography). While the above lists the five study parts as funded, actual chapter titles in this report are different (see Contents). This research in ≥1,000-m depths expanded and supplemented prior projects that focused on *Lophelia pertusa* communities (300-900 m) and provides comparisons with datasets from different areas/ecosystems (reviewed in Brooke and Schroeder, 2007; Ross and Nizinski, 2007). The four study areas and sampling completed in each area are illustrated in figure 1.1. Other chapters below may use different figures to document their sampling.

This USGS final report has been prepared, in part, to address time-dependent information needs as requested by the BOEMRE. Additional analyses, interpretation, and syntheses of the available data are expected to lead to future journal publications.

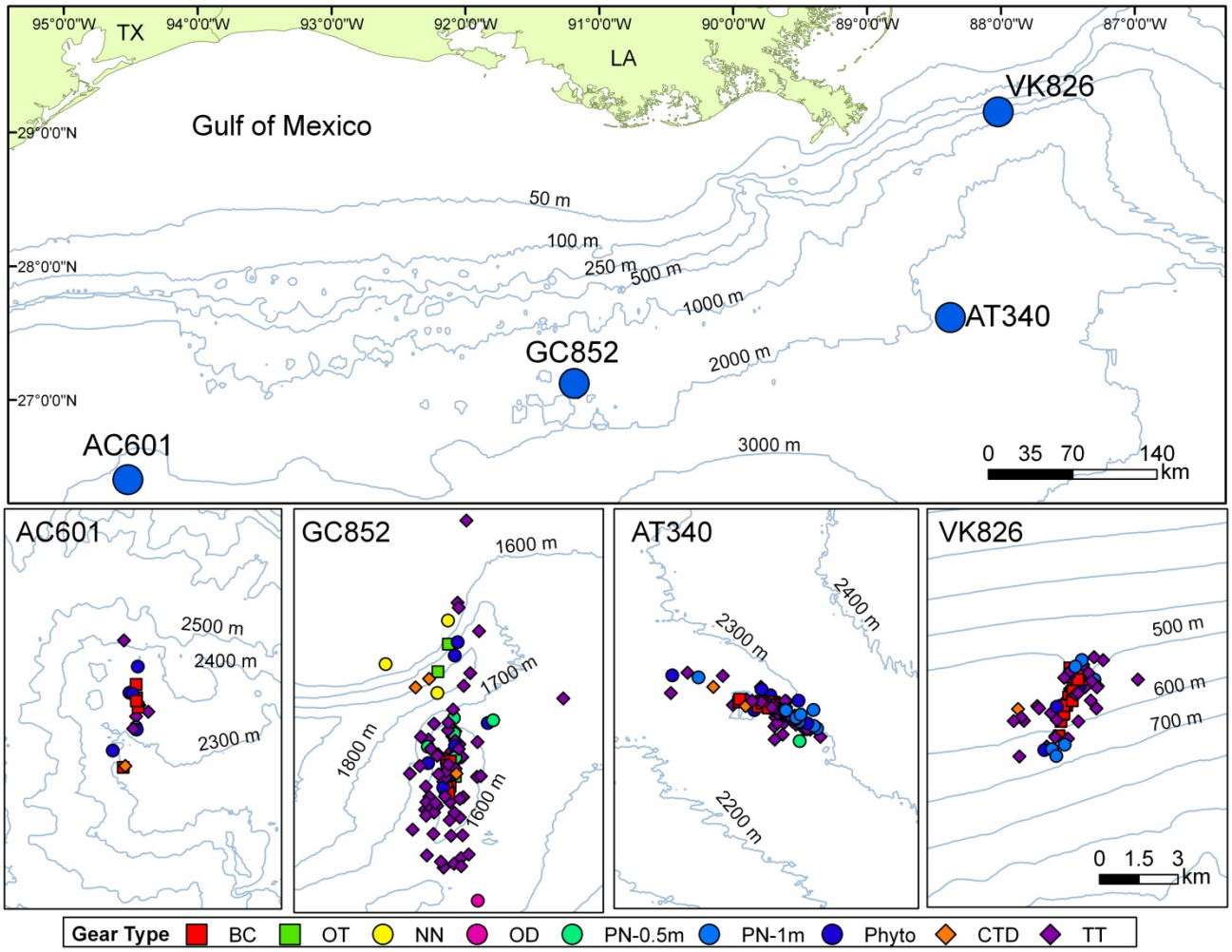


Figure 1.1. Four major study areas sampled in the Gulf of Mexico during the Chemo III project. (VK, Viosca Knoll; AT, Atwater Canyon; GC, Green Canyon; AC, Alaminos Canyon. Insets for each area illustrate sampling stations by gear type: BC, box core; OT, otter trawl; NN, neuston net; OD, on deck; PN, plankton net followed by mesh size; Phyto, phytoplankton water sample; CTD, conductivity, temperature, depth profiler; TT, Tucker trawl.)

2. ENUMERATION OF MICROBES IN CHEMOSYNTHETIC COMMUNITY SEDIMENTS

An expanded version of this paper was published as
Kellogg, C.A., 2010, Enumeration of viruses and prokaryotes in deep-sea sediments and cold seeps of the Gulf of Mexico, *in* Roberts, H.H., ed., Gulf of Mexico cold seeps: Deep-Sea Research II, v. 57, p. 2002-2007.

By Christina A. Kellogg

St. Petersburg Coastal and Marine Science Center, U.S. Geological Survey, St. Petersburg, FL

2.1 Introduction

Numerous cold seeps and their associated chemosynthetic communities have been documented and studied in the Gulf of Mexico over the past 25 years (for example, Kennicutt and others, 1985; Brooks and others, 1987; Cordes and others, 2007). Autotrophic bacteria, capable of utilizing hydrogen sulfides and methane, are the primary producers in these communities, much like the communities found in hydrothermal vents (Brooks and others, 1987). The microbiology of these cold seeps also includes heterotrophic bacteria and archaea (collectively referred to as prokaryotes) and viruses.

There are estimated to be 10^{30} marine viruses in the world oceans (Suttle, 2007), and typically the number in the underlying sediments exceeds the water-column abundance by 10 to 100 times (Middelboe and others, 2006). Bacteriophages, viruses that specifically infect bacteria, make up the majority (reviewed by Fuhrman, 2000). As the most numerous organisms, these viruses play a major role in the marine environment (Fuhrman, 1999; Wommack and Colwell, 2000; Suttle, 2005). Viruses affect bacterial diversity and succession; as a particular bacterial species achieves a competitive advantage and grows to higher numbers, the probability

of encountering a virus capable of lysing that specific strain of bacteria increases. Once a single cell is infected, the progeny viruses from that infection proceed to infect additional bacteria, until that bacterial 'bloom' is depleted. This clears the way for another bacterial species (succession) and maintains higher bacterial diversity than if a single species were to dominate. Viruses also affect productivity and the flow of carbon. Viral lysis of bacteria converts cellular components into dissolved organic matter, transferring nutrients away from grazers and stimulating other heterotrophic prokaryotic growth (fig. 2.1). In aquatic environments, mortality of bacteria due to viral infection is roughly equal to mortality by bacterivory (Bratbak and others, 1994). Additionally, viruses can themselves be a source of carbon; it has been shown that flagellates will feed on viral particles (Gonzalez and Suttle, 1993).

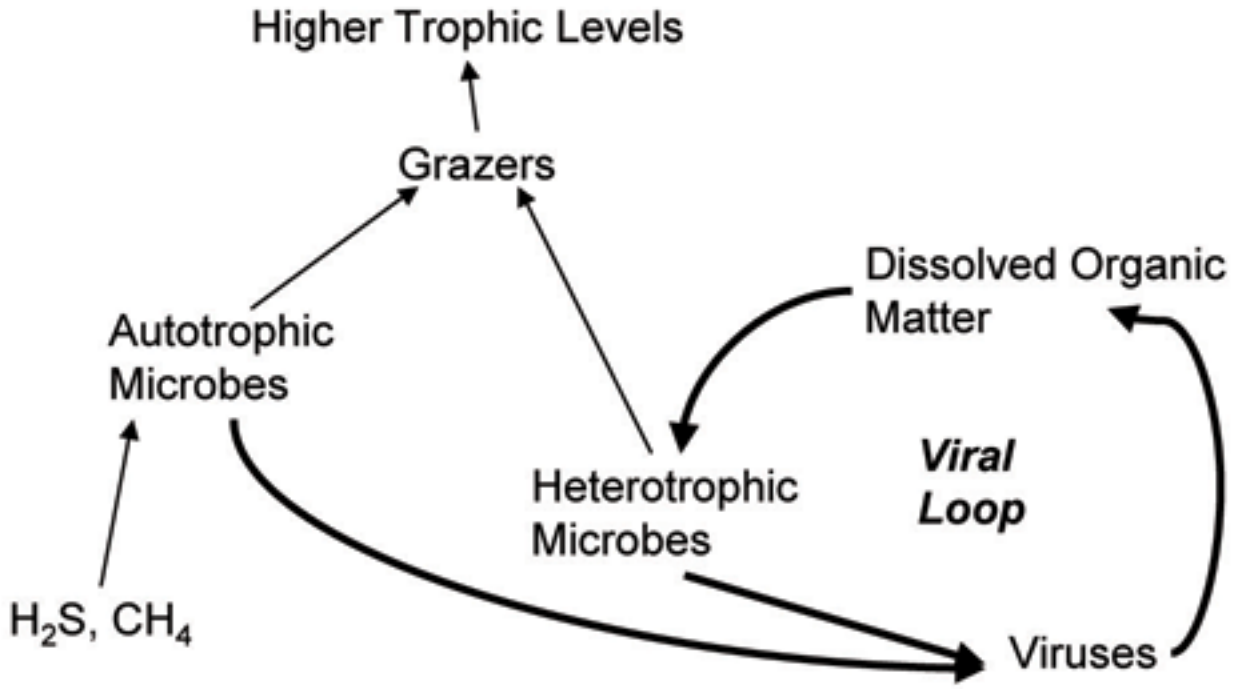


Figure 2.1. Viral lysis of bacteria converts cellular components into dissolved organic matter, transferring nutrients away from grazers and stimulating other heterotrophic prokaryotic growth.

High viral abundance in sediments has been shown to be an indicator of increased microbial activity (Middelboe and others, 2003; Glud and Middelboe, 2004). In spite of the fundamental role that microorganisms play in chemosynthetic communities, no investigation of the viral dynamics in the cold-seep environments of the Gulf of Mexico has been previously undertaken. This microbial enumeration component directly complements a bacterial biogeochemistry component of the BOEMRE-funded project titled "*Deepwater Program:* Investigations of Chemosynthetic Communities on the Lower Continental Slope of the Gulf of Mexico" (Chemo III), providing a more complete picture of the microbial dynamics that sustain a chemosynthetic community.

The objective of this study was to produce accurate baseline data on the abundance of prokaryotes and viruses in surface sediments below a water depth of 1,000 m. The sites chosen included a variety of cold-seep environments in the northern Gulf of Mexico to test the hypothesis that chemosynthetic communities are areas of increased viral production.

2.2 Methods

2.2.1 Sampling sites

Nine individual push cores of sediment were collected from four sites in the Gulf of Mexico (table 2.1) between June 21 and July 2, 2007, utilizing the R/V *Ronald H. Brown* and the remotely operated vehicle (ROV) *Jason*. Atwater Valley 340 is characterized by surface brine flows and populated by mussels (*Bathymodiolus brooksi*) and white bacterial mats (Roberts and others, 2007b; Lessard-Pilon and others, 2008). Four push cores were collected at this station:

AT340-2 was soft brown sediment in an active brine seep with salinity of 50 parts per thousand (‰); AT340-7 was black sediment in a less saline brine of 38 ‰; and both AT340-8 and AT340-9 were taken inside a white bacterial mat, also in a brine of 38 ‰. Green Canyon 852 was the shallowest site sampled. Two cores of fine-grained brown reference sediments (GC852-2 and GC852-4) were collected near authigenic carbonate boulders hosting azooxanthellate corals. However, numerous chemosynthetic communities including pogonophoran tubeworms and mussel beds also occur within 80-400 m of these reference sediments (Roberts and others, 2007b). Alaminos Canyon 645 contains tubeworms and mussels and has been the focus of multiple studies (Carney, 1994; Roberts and Aharon, 1994; Cordes and others, 2007). Two cores were collected in a pogonophoran tubeworm field at this site (AC645-2 and AC645-4); each was brown on the surface but gray underneath. Alaminos Canyon 818 is the deepest site and, unlike the other sites, does not occur on a mound-like bathymetric high. The AC818 has small clumps of chemosynthetic fauna along a narrow linear fault where seepage is occurring (Roberts and others, 2007b). The single core collected at this location (AC818-6) was cored through black sediment in an urchin field, away from the active chemosynthetic region of the site.

Table 2.1. Nine sediment cores were collected from four sites in the Gulf of Mexico between June 21 and July 2, 2007, utilizing the R/V *Ronald H. Brown* and the remotely operated vehicle (ROV) *Jason.* [m, meter; °C, degrees Celsius, ‰, parts per thousand]

Site	Sample ID	Description	Latitude / Longitude	Depth (m)	Temp (˚C)	Salinity (‰)
Atwater Valley 340	AT340-2	Seep site - brine	27˚25.169322'N 88˚21.770082'W	2,210	4.26	50
Atwater Valley 340	AT340-7	Seep site – brine, reduced sediment	27˚25.167810'N 88˚21.770286'W	2,211	4.26	38
Atwater Valley 340	AT340-8	Seep site – brine, microbial mat	27˚25.161312'N 88˚21.777882'W	2,209	4.26	38
Atwater Valley 340	AT340-9	Seep site – brine, microbial mat	27˚25.161390'N 88˚21.777966'W	2,209	4.26	38
Green Canyon 852	GC852-2	Reference sediments (non-seep)	27˚6.607194'N 91˚9.976008'W	1,400	4.27	35
Green Canyon 852	GC852-4	Reference sediments (non-seep)	27˚6.607176'N 91˚9.975996'W	1,400	4.27	35
Alaminos Canyon 645	AC645-2	Seep site – pogonophoran tubeworms	26˚21.267198'N 94˚29.899938'W	2,213	4.27	36
Alaminos Canyon 645	AC645-4	Seep site – pogonophoran tubeworms	26˚21.267186'N 94˚29.896944'W	2,213	4.27	36
Alaminos Canyon 818	AC818-6	Seep site – reduced sediments, urchin field	26˚10.867842'N 94˚37.362474'W	2,742	4.31	35

2.2.2 Sediment Characteristics

Immediately after reaching the ship's deck, sediment cores were transported to a cold room (10˚C) and surface sediments (the top 0-2 centimeters (cm) of the core) were collected. These sediment samples were transferred to whirlpak bags and frozen at -20˚C until analysis. To determine grain size, sediments were later thawed, treated with sodium hexametaphosphate, and examined using a Beckman Coulter LS 200 particle size analyzer. In addition to determining the average grain size, particle size data were then divided into percent sand and percent silt/clay based on size limits determined by the traditional dry sieve method.

2.2.3 Enumeration of microbes

Three replicate samples (0.5 cubic centimeters (cm^3) each) of surface sediment were collected from each sediment core using a sterile spatula for enumeration of prokaryotes and viruses. Samples for microscopy were processed immediately on board ship, using a modified version of Danovaro and others' (2001) method. Briefly, sediment was suspended in sterile, 0.02-micrometer (μm)-filtered Milli-Q water and sonicated to release particle-bound microbes. A low-speed centrifugation was used to pellet the sediment particles. The supernatant from this centrifugation was then filtered onto membranes, stained with a nucleic-acid specific dye (Sybr Gold), mounted on glass microscope slides, treated with an antifade solution, and frozen at -20˚C (Noble and Fuhrman, 1998). Sybr Gold stains the nucleic acids inside the bacteria and viruses, making the particles fluoresce when excited by blue light from the epifluorescence microscope. These slides were manually counted within 72 hours; a minimum of 10 fields of view and 400 particles was examined per slide. Samples were processed immediately without the addition of preservatives because it has been shown that rapid and variable viral decay occurs in aldehyde-fixed samples, resulting in underestimates of viral abundance (Wen and others, 2004).

2.3 Results

2.3.1 Sediment characteristics

Most sediment cores were dominated by smaller particles (>50 percent silt/clay). The average grain size ranged from 29.9 – 170.0 μm. Many of the cores were black or dark gray, indicating a reduced environment.

2.3.2 Microbial enumeration

Prokaryote counts were an order of magnitude lower in sediments directly in contact with megafauna (urchins, pogonophorans; Alaminos Canyon AC645 and AC818) compared to all other samples (10^7 versus 10^8 cells/gram (g) dry weight) and were highest in areas of elevated salinity (brine seeps; Atwater Valley AT340). Viral-like particle (VLP) counts were lowest in the reference sediments (Green Canyon 852) and pogonophoran cores (Alaminos Canyon AC645; 10^8 VLP/g dry weight), high in brine seeps (Atwater Valley AT340-2; 10^9 VLP/g dry weight), and highest in the microbial mats (Atwater Valley AT340-8, AT340-9; 10^{10} VLP/g dry weight). Virus-prokaryote ratios (VPR) ranged from <5 in the reference sediment to >30 in the microbial mats and >60 in the urchin field. Gulf of Mexico seep sites sampled in this study had higher VPRs than the reference sediments, indicating higher production of viruses in these environments. High viral numbers may be a result of increased burst sizes, reflecting higher rates of cellular activity (Middelboe, 2000), or may indicate a zone of virus accumulation (Suttle, 2007).

2.4 Discussion

The number of prokaryotes (bacteria and archaea) in a given sample can be affected by environmental factors such as oxygen levels, salinity, and nutrient levels. Higher levels of prokaryotes are expected in samples containing microbial mats, since the mats themselves are made up of prokaryotes. Higher levels of prokaryotes in brines may be due to the high salinity inhibiting protists and other grazers that typically feed on bacteria. Lower levels of prokaryotes in samples containing megafauna (urchins, tubeworms) may indicate that these animals (or smaller cryptic fauna associated with them) are feeding on bacteria.

At the time of sample collection, this was the first study to enumerate viruses in sediments below 1,000 m without preserving the samples in formaldehyde or glutaraldehyde. Viruses have been shown to decay rapidly in preserved samples, and the decay is not constant, confounding efforts to apply a conversion factor to previous counts (Danovaro and others, 2001; Wen and others, 2004). Currently, the most accurate methods to make estimates of viral abundance are processing the samples immediately to slides or flash-freezing the samples in liquid nitrogen (Wen and others, 2004). Viral abundances and VPRs from Gulf of Mexico cold-seep samples in this study are significantly greater than those reported from deep-sea sediments in the Mediterranean (Danovaro and Serresi, 2000; Danovaro and others, 2002; Danovaro and others, 2005).

The only published study to specifically enumerate microbes in cold-seep sediments examined cores from Sagami Bay, Japan (Middelboe and others, 2006). The Sagami Bay samples were preserved with gluaraldehyde and extracted without sonication, both of which are known to reduce viral counts (Danovaro and others, 2001; Wen and others, 2004). However, the environments sampled are similar to those described here from the Gulf of Mexico, so comparisons have been made in spite of these caveats. Prokaryote and viral abundances from non-cold-seep reference sediments were similar between the Gulf of Mexico and Sagami Bay at the same depth (~1,400 m). While prokaryote counts inside microbial mat samples were on par with microbial mat samples from Sagami Bay, Gulf of Mexico viral counts were two orders of magnitude higher, resulting in VPRs 10 times higher than in the Japanese samples. Comparing reduced (black) sediment samples from cold-seep sites in Sagami Bay and the Gulf of Mexico, the Gulf samples had one to two orders of magnitude more prokaryotes and three orders of

magnitude greater viral abundance. It is impossible to determine how much of this difference is due to the differences in methodology.

Table 2.2. Microbial abundance numbers from previous studies of sediments below 1,000 m water depth.

[Note that all studies outside the Gulf of Mexico employed some type of aldehyde fixative. The symbol ‡ indicates an average value; VPR, Virus-prokaryote ratio]

Geographic Area	Description	Sample ID	Lat/Long	Depth (meters)	Prokaryote abundance (cells per gram)	Viral abundance (viruses per gram)	VPR	Reference
Mediterranean	Deep-sea sediments	Sporades Basin	39°15.50'N 23°42.48'E	1,221	1.10×10^9‡	2.38×10^9‡	2.16	Danovaro and Serresi, 2000
		Cretan Sea	35°50.52'N 25°15.99'E	1,840	4.00×10^8‡	2.02×10^9‡	0.51	Danovaro and Serresi, 2000
		Ierapetra Trench	34°25.00'N 26°04.00'E	4,260	4.90×10^8‡	1.21×10^9‡	0.25	Danovaro and Serresi, 2000
		S1	35°46.29'N 28°43.15'E	3,870	3.50×10^8	4.19×10^7	0.12	Danovaro and others, 2002
		S2	33°39.34'N 33°18.34"E	2,100	4.20×10^8	7.91×10^7	0.19	Danovaro and others, 2002
		S3	33°23.18'N 28°19.04'E	3,055	2.77×10^8	3.57×10^7	0.13	Danovaro and others, 2002
		S4	34°52.91'N 22°31.97'E	2,950	6.53×10^8	6.92×10^7	0.11	Danovaro and others, 2002
		S5	35°42.38"N 20°08.80'E	3,200	5.66×10^8	6.62×10^7	0.12	Danovaro and others, 2002
		S6	35°37.18'N 17°23.27'E	4,000	1.84×10^8	8.58×10^7	0.47	Danovaro and others, 2002
		S7	36°36.66'N 12°14.77'E	1,290	6.45×10^8	7.09×10^7	0.11	Danovaro and others, 2002
		S8	38°24.05'N 06°53.72'E	2,850	6.54×10^8	1.20×10^8	0.18	Danovaro and others, 2002
		S10	40°33.99'N 04°57.14'E	2,755	2.53×10^8	5.11×10^7	0.2	Danovaro and others, 2002

Table 2.2. Microbial abundance numbers from previous studies of sediments below 1000 m water depth.—Continued

[Note that all studies outside the Gulf of Mexico employed some type of aldehyde fixative. The symbol ‡ indicates an average value; VPR, Virus-prokaryote ratio]

Geographic Area	Description	Sample ID	Lat/Long	Depth (meters)	Prokaryote abundance (cells per gram)	Viral abundance (viruses per gram)	VPR	Reference
		Calabrian Rise	38°30.62'N 17°58.87'E	2,255	1.60×10^{7}‡	5.00×10^{7}‡	3.13	Danovaro and others, 2005
		Topographic Highs	35°11.84'N 21°24.75'E	3,225	3.90×10^{7}‡	6.40×10^{7}‡	1.64	Danovaro and others, 2005
		Atalante Basin	35°18.20'N 21°23.33'E	3,363	4.60×10^{7}‡	5.20×10^{7}‡	1.13	Danovaro and others, 2005
Northeast Atlantic	Deep-sea sediments	Porcupine Abyssal Plain	48°50'N 16°20'W	4,800	-	1.46×10^{10}	-	Danovaro and others, 2001
Sagami Bay, Japan	Deep-sea sediments	OBB2	35°00.7'N 139°22.5'E	1,450	-	9.90×10^{8}‡	16.8‡	Middelboe and others, 2006
	Cold seep; inside microbial mat	Seep 3	35°00.1'N 139°13.6"E	1,200	2.18×10^{8}	6.80×10^{8}	3.12	Middelboe and others, 2006
	Cold seep; outside microbial mat	Seep 3	35°00.1'N 139°13.6"E	1,200	-	8.80×10^{8}	-	Middelboe and others, 2006
	Cold seep; near mussel bed	Seep 14	35°00.1'N 139°13.6"E	1,200	-	2.10×10^{8}	-	Middelboe and others, 2006
	Cold seep; black sediment	Seep 18	35°00.1'N 139°13.6"E	1,200	5.00×10^{6}	5.00×10^{6}	1	Middelboe and others, 2006
Gulf of Mexico	Deep-sea sediments (reference)	GC852-2	27°6.61'N 91°9.98'W	1,400	1.37×10^{8}	8.20×10^{8}	6.03	This study
	Deep-sea sediments (reference)	GC852-4	27°6.61'N 91°9.98'W	1,400	1.21×10^{8}	5.46×10^{8}	4.51	This study
	Cold seep; inside microbial mat	AT340-8	27°25.16'N 88°21.78'W	2,209	3.79×10^{8}	1.30×10^{10}	34.30	This study

Table 2.2. Microbial abundance numbers from previous studies of sediments below 1000 m water depth.—Continued

[Note that all studies outside the Gulf of Mexico employed some type of aldehyde fixative. The symbol ‡ indicates an average value; VPR, Virus-prokaryote ratio]

Geographic Area	Description	Sample ID	Lat/Long	Depth (meters)	Prokaryote abundance (cells per gram)	Viral abundance (viruses per gram)	VPR	Reference
	Cold seep; inside microbial mat	AT340-9	27˚25.16'N 88˚21.78'W	2,209	3.59×10^{8}	1.31×10^{10}	36.49	This study
	Cold seep; black sediment	AT340-7	27˚25.17'N 88˚21.77'W	2,211	2.59×10^{8}	3.49×10^{9}	13.47	This study
	Cold seep; black sediment	AC818-6	26˚10.87'N 94˚37.36'W	2,742	2.20×10^{7}	1.46×10^{9}	66.36	This study
	Cold seep; 50% brine	AT340-2	27˚25.17'N 88˚21.77'W	2,210	6.27×10^{8}	6.86×10^{9}	10.94	This study
	Cold seep; tube worm field	AC645-2	26˚21.27'N 94˚29.90'W	2,213	4.32×10^{7}	6.08×10^{8}	14.07	This study
	Cold seep; tube worm field	AC645-4	26˚21.27'N 94˚29.90'W	2,213	5.24×10^{7}	7.98×10^{8}	15.23	This study

Viral counts were lowest in the reference sediments (GC852-2, GC852-4) and pogonophoran tubeworm cores (AC645-2, AC645-4) and highest in the microbial mats (AT340-8, AT340-9). The highest viral abundances were at site AT340, which had salinities ranging from 38 parts per thousand to 50 parts per thousand. Danovaro and others (2005) have suggested that hypersaline conditions may preserve viruses or render them more resistant to decay. Additionally, two of the cores at AT340 contained microbial mats, where higher prokaryote host abundances contribute to higher viral abundances. Variation in viral abundances did not relate to water depth or salinity but was strongly correlated with grain size (viral adsorption to larger particles) and prokaryote abundance (host required for reproduction). Virus-prokaryote ratios (VPRs) are used as a measure of viral productivity in an environment.

The VLP and VPR ratios were all significantly greater than those reported from sediments in the deep Mediterranean Sea and in most cases were higher than ratios reported from recent data from a cold-seep site near Japan. This suggests that viral production is occurring at Gulf of Mexico cold-seep sites and that these viruses are mediating prokaryote mortality, thereby exerting control over the energy flow to higher trophic levels.

2.5 Acknowledgments

The author wishes to thank TDI-Brooks International, Inc., C. Fisher, and Bureau of Ocean Energy Management, Regulation and Enforcement for allowing her to participate in their Chemo III research cruise. She also thanks M. Bowles, K. Hunter, V. Samarkin, and K. Segarra for assistance in sample collection, as well as the crews of the R/V *Ronald H. Brown* and ROV *Jason*.

3. FOOD-WEB STRUCTURE OF SEEP SEDIMENT MACROBENTHOS FROM THE GULF OF MEXICO

An expanded version of this paper was published as
Demopoulos, A.W.J., Gualtieri, D., and Kovacs, K., 2010, Food-web structure of seep sediment macrobenthos from the Gulf of Mexico, in Roberts, H.H., ed., Gulf of Mexico cold seeps: Deep-Sea Research II, v. 57, p. 1972-1981.

By Amanda W.J. Demopoulos,[1] Daniel Gualtieri,[2] and Kaitlin Kovacs[1]

[1]Southeast Ecological Science Center, U.S. Geological Survey, Gainesville, FL
[2]Jacobs Technology, Southeast Ecological Science Center, U.S. Geological Survey, Gainesville, FL

3.1 Introduction

Chemosynthetic environments provide a haven for chemoautotrophic bacterial mats and megafaunal invertebrates that house endosymbiotic bacteria (Childress and others, 1986; MacDonald and others, 1989; Fisher and others, 1993; Sibuet and Olu, 1998). Research examining seep food webs has focused primarily on understanding the nutritional modes of the larger megafaunal or epifaunal taxa in seeps (Childress and others, 1986; Brooks and others, 1987; Fisher and others, 1993; Peek and others, 1998). Seeps located in the northern Gulf of Mexico slope environments contain tubeworms and clams that harbor symbiotic sulfide-oxidizing chemoautolithotrophic bacteria and mussels that contain chemoautolithotrophic sulfur-oxidizing symbionts (Brooks and others, 1987; Kennicutt and others, 1992).

Seep ecosystems also contain abundant heterotrophic species that do not house symbionts (MacAvoy and others, 2005), including sediment-associated macrofauna and meiofauna (Levin, 2005). Food resources for these smaller invertebrates have been examined in the shelf, upper, and lower slope in the northeastern Pacific Ocean, the Florida Escarpment (Levin and Mendoza,

2007), Blake Ridge methane seeps in the Atlantic (Van Dover and others, 2003), and the Hakon Mosby Mud Volcano in the southwestern Barents Sea (Van Gaever and others, 2006). Potential carbon sources (food) available to sediment infauna include photosynthetically fixed material raining from surface waters (for example, phytoplankton-derived organic matter), terrestrially derived material transported to deep waters, or organic carbon synthesized chemoautotrophically from dissolved inorganic carbon (DIC) either by symbiotic or free-living bacteria. Recent studies suggest that macrofaunal reliance on chemosynthetically derived material may increase with depth (Levin and Michener, 2002; Levin and Mendoza, 2007), because particulate flux from surface waters typically decreases with increasing water depth (Turley and others, 1995). The hypothesis that decreased particle flux leads to increased dependence on locally produced organic carbon has not been tested extensively because food-web studies have not been conducted at most deep seep environments (Levin and Mendoza, 2007).

Because of the remoteness of seep communities, it is challenging to conduct diet studies. Stable isotope analysis has proven useful in discerning nutritional modes and can provide basic information about primary carbon sources fueling seep communities (see reviews in Conway and others, 1994; Van Dover, 2007). Different carbon fixation pathways involve distinct carbon isotopic fractionation. Chemoautotrophic organic matter fixed at or near the sediment-seawater interface results in heavily depleted $\delta^{13}C$ values (-75 to -28 per mil, Paull and others, 1985; Brooks and others, 1987). Therefore, heterotrophic fauna with low $\delta^{13}C$ values (<-25 per mil) are likely dependent in some way on chemoautotrophic bacteria. In contrast, fauna that depend on photosynthetically fixed carbon would have tissue $\delta^{13}C$ values that are enriched relative to these other sources (for example, -25 to -15 per mil, Fry and Sherr, 1984).

Stable nitrogen isotope values ($\delta^{15}N$) can provide information on trophic position and sources of nitrogen because animals are expected to be ~3 per mil enriched relative to their diet (Minagawa and Wada, 1984; Post, 2002; McCutchan and others, 2003). If animals are using deep-sea particulate organic nitrogen (PON), then they should have values enriched in ^{15}N relative to deep-water PON sources (for example, 3-6 per mil, Paull and others, 1985). In contrast, animals that contain chemoautotrophic symbionts typically have very low, often negative, $\delta^{15}N$ values (Brooks and others, 1987). Therefore, combined use of $\delta^{13}C$ and $\delta^{15}N$ values of animals can yield information on nitrogen and carbon sources, trophic position, fixation pathways, and the possible presence of chemoautotrophic symbioses (Levin and Michener, 2002).

Stable isotope ratios have been used to infer type of nutrition and indicate presence of petroleum-derived carbon in the benthic food web (Spies and DesMarais, 1983; Paull and others, 1985; Kennicutt and others, 1992). Stable isotope analysis has revealed different food resources utilized, including chemoautotrophy from bacterial symbioses (Dando and others, 1991; Jensen and others, 1992) and local chemosynthetic production (Levin and Michener, 2002; Van Dover and others, 2003). For the Gulf of Mexico, results from upper and middle slope seeps indicate that heterotrophic megafauna and meiofauna rely on chemautotrophic production for nutrition (Powell and others, 1986; Brooks and others, 1987; Kennicutt and others, 1992; MacAvoy and others, 2002). However, nutritional relations for seep associates (macrofauna and meiofauna) in the lower slope of the northern Gulf of Mexico remain unknown.

This study investigated the importance of chemosynthesis to macro-infaunal nutrition in deep-slope cold seeps and wider regions in the northern Gulf of Mexico. We combined the use of $\delta^{13}C$ and $\delta^{15}N$ data to estimate food-web structure among consumer species. Our specific

question was: to what degree do seep-associated infaunal benthos derive their nutrition from chemosynthetic sources?

3.2 Materials and Methods

3.2.1 Collection Methods

Sampling was conducted at depths of 1,400 to 2,400 m during September 2007 aboard the R/V *Cape Hatteras*. Three seep sampling sites were selected: Atwater Valley (AT340), Green Canyon (GC852), and Alaminos Canyon (AC601) (fig. 3.1). At each of these sites, transects were established across known seep locations (table 3.1) based on information from Brooks and others (2008). Sediment samples for infaunal analysis were collected using an Ocean Instruments Mark III (50 cm x 50 cm) box core. Smaller tube cores (32 cm^2 x 10 cm depth) were inserted into the box cores to measure redox potential and collect sediments for sediment and infaunal isotope analysis. Vertical redox potential (Eh in millivolts, mV) profiles (at 0-1, 1-2, 2-3, 3-5, 5-7, and 7-10 cm depth) were obtained from undisturbed cores by inserting a combination redox electrode through the sediment surface; redox potential was then read on a portable pH-millivolt meter. To collect sediment macrofauna, sediment cores were sectioned (at 0-1, 1-2, 2-3, 3-5, 5-7, and 7-10 cm depth) after recovery. Sediment fractions were sieved through a 300-micrometer (μm) screen, and animals were sorted at sea to collect macrofauna for stable isotopic analysis. Living organisms were identified, placed into filtered seawater for 24 hours (h) to evacuate their guts, washed in Ultrapure water, then placed in preweighed tin boats or combusted vials, and frozen at -70°C.

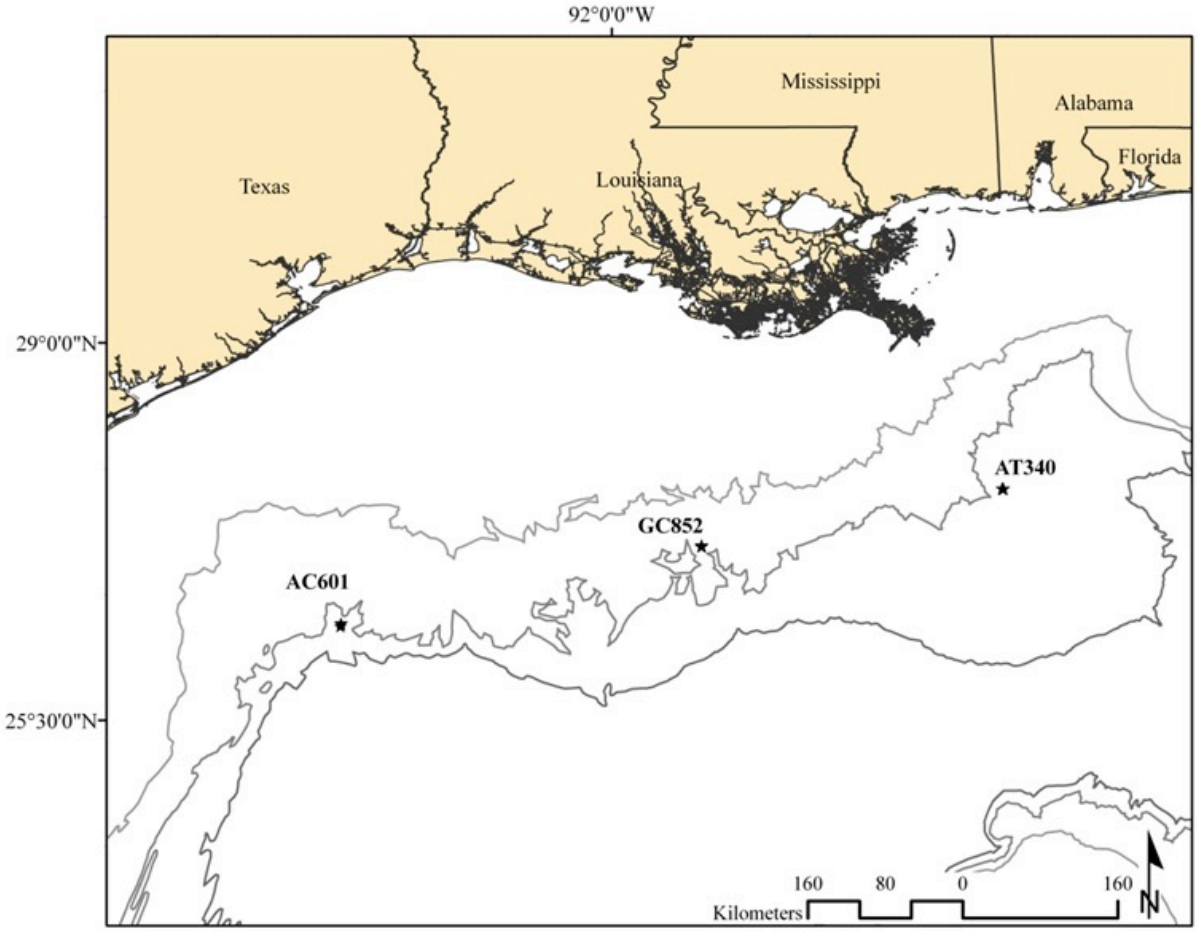


Figure 3.1. Collection sites in the Gulf of Mexico. (Contour lines represent 1,000-, 2,000-, and 3,000-m bathymetry.)

Table 3.1. Box core locations and depths, northern Gulf of Mexico.

Location	Date	Depth (meters)	Latitude	Longitude	Station #
Green Canyon (GC 852)					
	10-Aug-07	1,431	27° 06.996	91° 09.840	11
	10-Aug-07	1,426	27° 07.149	91° 09.961	13
	10-Aug-07	1,435	27° 06.978	91° 09.873	14
	10-Aug-07	1,449	27° 07.262	91° 09.997	15
	10-Aug-07	1,425	27° 07.198	91° 09.905	16
	13-Aug-07	1,431	27° 06.68	91° 09.92	55
	13-Aug-07	1,406	27° 06.654	91° 09.998	59
	13-Aug-07	1,400	27° 06.543	91° 09.953	60
	13-Aug-07	1,430	27° 07.112	91° 09.991	61
	13-Aug-07	1,425	27° 07.206	91° 09.907	62
Alaminos Canyon (AC 601)					
	18-Aug-07	2,391	26° 23.408	94° 30.798	130
Atwater Valley (AT 340)					
	24-Aug-07	2,239	27° 38.774	88° 21.808	193
	24-Aug-07	2,222	27° 38.791	88° 21.986	194
	24-Aug-07	2,222	27° 38.706	88° 21.686	196
	24-Aug-07	2,222	27° 38.679	88° 22.060	197
	25-Aug-07	2,227	27° 38.746	88° 21.966	207
	25-Aug-07	2,255	27° 38.646	88° 21.828	208
	25-Aug-07	2,222	27° 38.847	88° 22.483	209

Potential food resources, including suspended particulate organic matter (POM), sediments, and filamentous bacteria from surface sediments, also were processed for stable carbon and nitrogen isotopes. POM was collected by filtering 3 liters (L) of seawater from the surface from each site (5-15 stations per site) onto precombusted glass fiber filters (GFF). Suspended organic matter (SOM) was collected from box core surface water after push core subsamples were inserted. SOM was sieved through 300 μm screen to remove animals and then filtered onto pre-combusted GFF filters. SOM represents fine organic matter that was resuspended after box core recovery, with isotope values that may differ from bulk sediment organic matter. All GFF filters were dried and processed for stable isotope analysis.

3.2.2 Lab Processing

Dried sediments, POM, and SOM filters were acidified with 1 N HCl, dried, and then transferred into tin boats. Infaunal samples were dried at 60°C for 1-2 days to a constant weight, weighed in tin boats, and acidified with 1 percent $PtCl_2$ to remove inorganic carbon (Demopoulos and others, 2007; Levin and Mendoza, 2007). Stable isotope measurements of infauna were made on single individuals, parts of individuals, or several small specimens of the same species combined. Samples were analyzed for C and N compositions referenced to Vienna PeeDee Belemnite (VPDB) and nitrogen gas (atmospheric) (Peterson and Fry, 1987). Analyses were performed using a Costech (Valencia, Calif.) elemental analyzer interfaced to a GV instruments (Manchester, U.K.) Isoprime isotope ratio mass spectrometer. Reproducibility was monitored using several organic reference standards (Fry, 2007). Isotope ratios are expressed as $\delta^{13}C$ or $\delta^{15}N$ in units of per mil.

3.3.3 Statistical Analysis

All data are expressed as mean ± 1 standard error (SE) unless indicated otherwise. Data for multiple individuals of a single species were averaged within each site. Tests of site effects then used species as replicates to avoid overrepresentation of the most abundant taxa. All statistical analysis was performed using SPSS statistical software.

3.3 Results

3.3.1 Sediment Characteristics

Box core collections ranged from 15 to 1,000 m away from known seep locations. While sampling locations targeted areas of known seep assemblages, only one box core from Alaminos Canyon recovered a siboglinid tubeworm, a characteristic fauna from seep environments. The

remaining cores did not contain the typical seep megafauna (clams, mussels, tubeworms), nor large bacterial mats. However, bacterial filaments were present in many of the cores, both visible on the sediment surface and within the cores sorted for infauna.

Of the nine successful box cores collected at GC852, four had low redox profiles (below 0 mV, table 3.2), and six contained bacterial filaments indicative of chemosynthetic communities (Stations 13, 15, 55, 59, 61, and 62). The profiles with the lowest redox (Stations 13, 15, 16, and 62, fig. 3.2) had a shallow redox potential discontinuity (rpd) depth (~ 2 cm), and sediment color transitioned from brown on the surface to gray directly below; some of these cores had a noticeable sulfide odor. Sediment cores with high redox values (>0 mV, Stations 11, 14, 55, 59, and 60, table 3.2) were also brown on the surface and grey in subsurface sediments but generally contained a deeper rpd (4-5 cm).

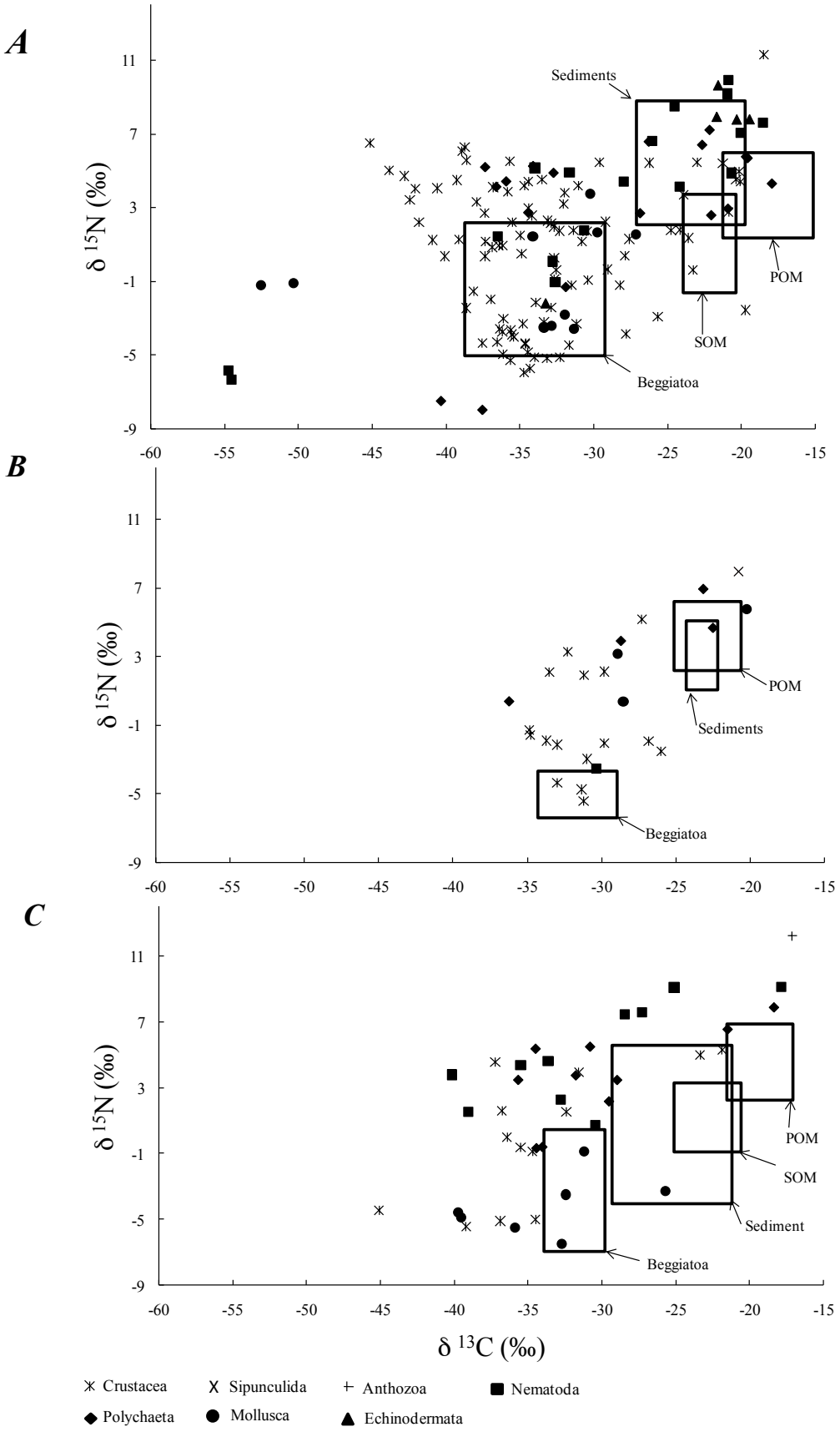

A
B
C
Sediments
POM
SOM
Beggiatoa
Sediment
δ 15N (‰)
δ 13C (‰)
Crustacea
Sipunculida
Anthozoa
Nematoda
Polychaeta
Mollusca
Echinodermata

Figure 3.2. Dual stable isotope plots of different primary sources (open rectangles), and macrofauna (>300 micrometers, μm) from Gulf of Mexico seeps: (A) GC852, (B) AC601, and (C) AT340. (Each point represents a distinct individual. Ranges in stable carbon and nitrogen isotope values are given for primary sources, including particulate organic matter (POM), suspended organic matter (SOM), *Beggiatoa* bacteria, and sediment.)

Table 3.2. Redox potential profiles (Eh, in millivolts) in box cores from Green Canyon (GC 852), Alaminos Canyon (AC601), and Atwater Valley (AT340).

		Fraction (centimeters)					
	Station	**0-1**	**1-2**	**2-3**	**3-5**	**5-7**	**7-10**
GC852							
	11	154.9	155.7	167.6	181.7	184.5	196.6
	13	-	35	-13.2	-152.8	-189.4	-471
	14	90	89.3	91.4	94.8	102.2	110
	15	-	-70.4	-303	-483	-497	-519
	16	38.6	-6.7	-86.1	-421	-447	-442
	55	44	47	49	51	35.4	-19.1
	59	19.1	25.5	33.6	39.2	48.5	49.2
	60	57.2	64.5	68.4	70.6	72.9	59.9
	62	24.4	-40.9	-443	-460	-466	-473
AC601							
	130	58	56	46	-2	-65.1	-120
AT340							
	193	-396	-397	-402	-409	-418	-425
	194	-220	-318	-390	-401	-411	-418
	195	76.2	76.6	78.9	78.6	74.5	63.7
	196	76.6	76.2	75.5	74.9	28.9	-3.1
	197	73	72.6	70.8	23.3	-31.5	-165.5

A single box core was collected at AC601 near to a known chemosynthetic ecosystem, approximately 15 m from a tubeworm assemblage (Brooks and others, 2008). This box core had oil visible on the water surface and bacteria were present on the sediment surface. The Eh

profile included a 0-2 cm oxidizing layer followed by reducing conditions below 2 cm (table 3.2).

Box cores collected at AT340 were approximately 75-260 m away from known seep environments. Of the seven cores collected, two were completely reducing throughout the sediment profile (Stations 193, 194, table 3.2). The remaining cores had either oxidizing conditions throughout the cores (table 3.2, Stations 195, 196, and 208) or had a 2-4 cm oxidizing layer, with reducing conditions below that depth (Stations 197 and 207). Five cores had *Beggiatoa* bacteria present both in the sorted sediments and visible on the sediment surface (Stations 193, 194, 196, 197, and 207).

3.3.2 Isotopic composition of primary source pools

Specific organic matter pools generally had similar isotope values at all three sites (fig. 3.2). There were no site differences in *Beggiatoa*, POM, SOM, or sediments between Green Canyon and Atwater Valley. This was determined using an analysis of variance (ANOVA) test, in which the F statistic is defined as the ratio of the variance calculated among the means to the variance within the samples, and the p value indicates the probability of obtaining a value for F that is as high as what would be observed by chance; that is, the lower the p value, the more significant the difference between the sites (*Beggiatoa*, ANOVA, F=2.835, p=0.078 for carbon; F=1.357, p=0.277 for nitrogen; SOM, Kruskal Wallis, $x^2 = 0.593$, p=0.441 for carbon, $x^2 = 2.370$, p=0.124 for nitrogen; and sediments ANOVA, F=0.181, p=0.672 for carbon; F=0.587, p=0.448 for nitrogen). Because only one box core was collected from Alaminos Canyon, this precluded among-site statistical comparisons of primary source pools. In addition, there was no significant difference in POM among the three sites (ANOVA, F=0.093, p=0.912 for carbon, F=1.232, p=0.308 for nitrogen). POM was the most enriched in $\delta^{13}C$ and $\delta^{15}N$, reflecting its

photosynthetic origin. In contrast, *Beggiatoa* was the most depleted in ^{13}C and ^{15}N across all sites; *Beggiatoa* $\delta^{13}C$ values ranged from -39.4 to -22.9 and $\delta^{15}N$ from -6.8 to 5.1 (fig. 3.2), reflecting chemosynthetic carbon fixation. SOM and sediment had intermediate isotope values, falling between POM and *Beggiatoa*.

3.3.3 Sediment macrofaunal isotopic composition

Invertebrates collected from each site included a variety of polychaete annelids, nematodes, crustaceans, molluscs, sipunculids, and echinoderms. A total of 240 infaunal samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ stable isotopes. Stable isotope values of macroinfauna across sites exhibited a large range (fig. 3.2, $\delta^{13}C$ from -54.7 to -17.9 per mil, $\delta^{15}N$ from -8 to 11.3 per mil), with the lightest values likely reflecting input from methane-derived carbon ($\delta^{13}C$), utilization of locally fixed nitrogen, or symbiosis ($\delta^{15}N$). There were no significant differences between carbon and nitrogen isotope values among sites (ANOVA, $F= 0.636$, $p = 0.533$ for $\delta^{13}C$, $F = 0.105$, $p =0.900$ for $\delta^{15}N$).

3.3.3.1 Green Canyon-GC 852

Stable isotope values of sediment macrofauna were the most variable at GC852; $\delta^{13}C$ ranged from -55 to -18 per mil and $\delta^{15}N$ ranged from -8 to 11 per mil (fig. 3.2). Animals that exhibited the lowest isotope values included nematodes ($\delta^{13}C = -54.6 \pm 0.1$ per mil, $\delta^{15}N = -6.1 \pm 0.2$ per mil) and one gastropod ($\delta^{13}C = -50.4$ per mil, $\delta^{15}N = -1.1$ per mil). They were depleted in ^{13}C and ^{15}N relative to measured primary sources, indicating that they may be assimilating methane oxidizing bacteria; these particular microorganisms typically have very negative carbon isotope values. Animals with isotope values consistent with *Beggiatoa*-derived diet included molluscs, nematodes, polychaetes, crustaceans, and ophiuroid echinoderms. Animals with

isotope values consistent with a phytoplankton-derived diet (that is, having $\delta^{13}C$ >-25 per mil and $\delta^{15}N$ >3 per mil) included ophiuroid and crinoid echinoderms, certain cumacean crustaceans, dorvilleid polychaetes, copepods, and an unidentified crustacean.

3.3.3.2 Alaminos Canyon-AC 601

Stable $\delta^{13}C$ values ranged from -36 to -20 per mil and $\delta^{15}N$ ranged from -6 to 8 per mil (fig. 3.2). Polychaete annelids, bivalve molluscs, and a sipunculid appeared to derive their nutrition from photosynthetically produced sources. The remaining fauna were depleted relative to POM, indicating that they rely on chemoautotrophically derived organic matter. Other fauna that had low $\delta^{13}C$ and $\delta^{15}N$ values included nematodes and Tanaid sp. A. Ostracods exhibited the largest range in ^{15}N values (-4.9 to 5.2 per mil), while Tanaid sp. A had the largest range in $\delta^{13}C$ (-34.8 to -26.0 per mil). Few taxa had isotope values consistent with a phytoplankton-derived diet, much like the other two sites.

3.3.3.3 Atwater Valley-AT 340

Infaunal stable $\delta^{13}C$ ranged from -45 to -17 per mil and $\delta^{15}N$ ranged from –7 to 12 per mil. Animals most enriched in ^{13}C and ^{15}N were an anemone, a maldanid polychaete, a cumacean, and a nematode. Infauna collected from the remaining stations were depleted relative to sources measured (fig. 3.2). Those most depleted in ^{15}N included other cumaceans, tanaids, and gastropod and aplacophoran molluscs. Low ^{15}N values may indicate presence of bacterial symbionts within these infauna, utilizing local nitrogen sources that are depleted in ^{15}N. Most of the infauna fell between two isotopic endmembers: phytoplankton and methane-derived carbon, including paranoid polychaetes, gammarid amphipods, certain nematodes, tanaids, and ostracods.

Intermediate isotope values may indicate that the fauna are relying on either a mixed diet of these two sources or on sulfur oxidizing bacteria.

3.4 Discussion

Stable isotope analysis indicated that infauna from Gulf of Mexico seep environments fell into three groups: (1) those that derived carbon from photosynthetic sources (most enriched in ^{13}C and ^{15}N), (2) consumers of chemosynthetically fixed carbon (possibly sulfide oxidizing bacteria), and (3) consumers depleted in^{13}C and ^{15}N relative to the measured bacterial sources, indicating possible assimilation of an unmeasured source (for example, methane-derived carbon) and (or) translocation from symbionts. Very light $\delta^{13}C$ and $\delta^{15}N$ values indicate the potential presence of symbionts. Light $\delta^{15}N$ values between -8 and 0 per mil were measured for multiple taxa, including several species of polychaetes, crustaceans, aplacophorans, and nematodes, indicating possible reliance on bacterial symbionts. Ectosymbioses have been documented in seep nematodes (Dando and others, 1991; Jensen and others, 1992). Light isotope values have also been found in polychaete annelids collected from the Gulf of Alaska seeps (Levin and Michener, 2002), although symbionts have not been isolated from these animals. Very depleted values of gastropods and nematodes at Green Canyon also may represent incorporation of methane-oxidizing archaea. Archael lipids in Florida sediments reflect large fractionation of carbon to yield $\delta^{13}C$ of <-100 per mil (Zhang and others, 2003). These taxa merit further study to examine food sources fueling these organisms and potential reliance on symbionts for nutrition.

In addition to bacterial symbionts, invertebrates utilize bacterial production through consumption of free-living chemoautotrophs. Chemosynthetic bacterial mats can be extensive in the Gulf of Mexico (MacDonald and others, 1989; Sassen and others, 1994) and may be a

significant source of nutrients to benthos in the northern Gulf of Mexico continental slope (Kelley and others, 1998). Free-living bacteria (both chemosynthetic and heterotrophic) on surfaces or suspended in the water column may be food for grazing and filter-feeding invertebrates, as has been suggested for hydrothermal vent fauna (Van Dover and Fry, 1989). Filaments present in box core collections were small enough to be grazed by infauna and epifauna. In addition, several taxa had $\delta^{13}C$ and $\delta^{15}N$ values consistent with assimilation of bacteria. Not all taxa found at these three seep sites target or assimilate *Beggiatoa*, but isotopic similarities suggest incorporation of chemoautotrophic production into their tissues. While sulfide oxidizing *Beggiatoa* represent one potentially important food source, other bacteria (for example, methane oxidizing archaea, methanotrophic bacteria), yet unmeasured isotopically, are likely important for infaunal seep associates.

Despite the large spatial separation among sites (fig. 3.1), there were remarkably similar isotopic compositions and ranges among primary producers and secondary consumers (fig. 3.2), suggesting possible isotopic uniformity in the diverse biogeochemical processes occurring at seeps (for example, Van Dover and Fry, 1994). Diverse patterns in isotope values have also been found in sedimented hydrothermal vents in the Gorda Ridge (Van Dover and Fry, 1994). Isotope diversity found in vents was attributed to the large diversity in microbial populations fueling these food webs (Van Dover and Fry, 1994). The wide range in both carbon and nitrogen isotopes found in our study indicates trophic complexity and possibly high microbial diversity both on small (within box core) and large (across sites) spatial scales.

Our results are consistent with isotope ranges published from Florida Escarpment and Atwater Valley and are depleted relative to background sediments in the northern Gulf of Mexico and Florida Escarpment (Levin, 2005; Levin and Mendoza, 2007; A.W.J. Demopoulos,

USGS, unpub. data, 2011). Seep infauna collected from Gulf of Alaska, Oregon, and Florida Escarpment seeps were considered the isotopically lightest known on Earth (Levin and Michener, 2002; Levin and Mendoza, 2007). Our data overlap theirs, although results from the Florida Escarpment represent the most depleted infauna from seep environments (Levin and Mendoza, 2007). Overall, most infauna near seeps apparently derive their nutrition from seep production, consistent with other slope studies (Levin and Mendoza, 2007).

These results represent new information on seep-associated infaunal nutrition. Many appear to be heterotrophic and consume free-living bacteria (Levin and Michener, 2002), similar to patterns found at hydrothermal vents (Van Dover and Fry, 1994). The diverse nature of our stable isotope data reflects upper slope work in the Gulf of Mexico (Brooks and others, 1987; Kennicutt and others, 1988; MacAvoy and others, 2002; 2005; 2008a), which has demonstrated that seeps are locations where microbial chemoautotrophic primary production can substitute photosynthesis as the primary source of ecosystem energy production (Brooks and others, 1987). Reliance on chemoautotrophic production fueled by hydrocarbon seepage rather than photosynthetically derived carbon may be dictated by differences in fluid flow rates and seepage (Van Dover, 2007), as thermogenic hydrocarbons are known to be widespread in the surface sediments throughout the continental slope of the Gulf of Mexico (MacDonald and others, 1989). Subsequent transport of biological productivity from seeps to surrounding benthos may contribute significantly to the ecology of the continental slope (MacDonald and others, 1989). However, the degree to which chemosynthetically derived carbon is transferred to surrounding ecosystems remains unknown, although transfer via larger, mobile fish and invertebrates has been suggested to be quite variable (MacAvoy and others, 2002; 2008b). Based on the great spatial separation among the sites sampled in this study and indication that several infauna from

these sites derive their nutrition from seep production, chemoautotrophic production may be quite diffuse and widespread over large areas of the Gulf of Mexico.

3.5. Acknowledgments

We thank the captain and crew of the R/V *Cape Hatteras*. We also thank the following individuals for their help at sea, site selection, and helpful suggestions: J. Gonzalez, Ross Lab, L. Levin. E. Cordes, and C. Fisher. Special thanks go to S. Ross for inviting us to participate in this cruise and for facilitating the box core sampling. We thank R. Lee (Washington State University) for conducting stable isotope analyses.

4. MACROFAUNAL COMMUNITY STRUCTURE AND SEDIMENT ENVIRONMENTAL CHARACTERISTICS IN THREE SEEP ECOSYSTEMS

By Amanda W.J. Demopoulos[1] Kaitlin Kovacs[1] and Daniel Gualtieri[2]

[1]Southeast Ecological Science Center, U.S. Geological Survey, Gainesville, FL
[2]Jacobs Technology, Southeast Ecological Science Center, U.S. Geological Survey, Gainesville, FL

4.1 Introduction

Descriptions of seep communities and their megafaunal associates have been studied extensively in the Gulf of Mexico (Bergquist and others, 2003a; Cordes and others, 2005; Cordes and others, 2007). Smaller macrofauna (>300 µm) associated with soft sediment environments within seeps in the Gulf of Mexico are not well known (Powell and others, 1986; Robinson and others, 2004; Levin and Mendoza, 2007), and few studies have examined these seep associates worldwide (see review by Levin, 2005).

Studies of meiofauna (<300 µm and >42 µm) from upper slope seep environments in the Gulf of Mexico (GOM) (Powell and Bright, 1981; Powell and others, 1983; Robinson and others, 2004) indicate no consistent patterns in meiofaunal densities. They can either be enhanced, depleted, or unchanged relative to background sediments (Levin, 2005). Sediment geochemical environment may play a role in controlling infaunal community composition and abundance. For example, enhanced densities of sediment infauna relative to background soft sediments were observed within the uppermost sediment layers, corresponding to the location of minimal porewater methane concentrations (Sergeeva and Gulin, 2007). In contrast, the presence of sulfide has been linked to increased abundance of meiofaunal taxa (Powell and

others, 1986), despite the toxicity associated with sulfide, indicating that certain meiofauna may be highly tolerant of toxic sediment environments. Thus, the abundance and composition of seep infauna may be strongly tied to sediment biogeochemistry, including sulfide concentration (Sahling and others, 2002).

Macro-infauna provide a variety of ecosystem services, including cycling of particulate organic matter and serving as food for fish and larger invertebrates for seep associates and transients (MacAvoy and others, 2008b; Demopoulos and others, Chapter 3), yet their relative abundance and general composition in GOM seeps is not understood, in particular in lower slope seep environments.

This chapter represents a preliminary assessment of the macrofaunal community composition and their associated sediment environment for three seep areas located on the deep slope of the GOM. We examined whether GOM seep communities were similar in terms of major taxonomic groups and how comparable these macrofauna were to seeps located outside of the GOM. The description of infaunal densities and composition adds to the existing knowledge of small macrofauna from seeps and provides information for future comparisons across biogeographical regions.

4.2 Methods

4.2.1 Sampling Sites

Investigations were conducted in three sites in the GOM: Atwater Valley (AT340), Green Canyon (GC852), and Alaminos Canyon (AC601). See Chapter 3 for more detailed site descriptions and for the site map. At each site, transects were established across known seep locations (Brooks and others, 2008). Sediment samples were collected using an Ocean

Instruments Mark III (50 cm x 50 cm) box core, which was then subsampled on deck using 2 tube cores (32 cm^2 x 10 cm depth), one each for macrofauna and sediment analysis. The top 10 cm of each core were sectioned horizontally (0-1, 1-2, 2-3, 3-5, 5-7, and 7-10 cm). Sediment for macrofaunal analysis was preserved in 10 percent formalin prior to sieving. Sediment cores intended for sediment analysis, for example, particle size and organic carbon and nitrogen content, were frozen at -20° C. Salinity was measured using a refractometer (± 2 practical salinity units, PSU) for porewater extracted from syringe samples taken at each sediment fraction.

4.2.2 Lab Processing

A portion of sediment for grain-size analysis was digested with hydrogen peroxide to remove organic material and subsequently wet sieved through 2000-µm (to separate rubble/large grain sizes) and 63-µm sieves. Size fractions (>2000 µm, 2000 - 63 µm, and <63µm) were dried at 60°C for 1-2 days (d), weighed, and percent rubble/shell (>2000 µm), sand (63 µm ≤ x <2000 µm), and silt/clay (<63 µm) calculated. Organic carbon and total nitrogen content of the sediments were determined using a Costech (Valencia, Calif.) elemental analyzer interfaced to a GV instruments (Manchester, U.K.) Isoprime isotope ratio mass spectrometer after carbonate material was dissolved using sulfurous acid.

In order to characterize sediment macrofauna, preserved sediment fractions were washed through a 300-µm sieve, and animals retained on the 300-µm sieve were sorted under a dissecting scope, identified to major taxonomic group, and transferred to 80 percent ethanol for storage.

4.3 Results

4.3.1 Sediment Properties

Results presented here are based on 10 replicate box cores collected from GC852 and 8 box cores collected from AT340. While only one core was collected from Alaminos Canyon, we included the results for general comparative purposes only. Porewater salinity ranged from 36.0 to 38.0 PSU. Sediment percent organic carbon and organic nitrogen were similar between GC852 and AT340 (table 4.1). However, at AC601, percent organic carbon was lower within the upper 0-3 cm (0.20-0.29 percent) and increased down core, ranging from 0.74 to 0.93 percent. Percent organic nitrogen exhibited the same pattern, increasing with depth from 0.02 percent (0-1 cm) to 0.09 percent (7-10 cm). No major differences in sediment $\delta^{13}C$ or $\delta^{15}N$ values were observed among the three seeps sites (table 4.1)

Table 4.1. Average (± 1 S.E.) sediment particle size, percent organic carbon and nitrogen, $\delta^{13}C$, $\delta^{5}N$, and salinity from three seeps in the Gulf of Mexico. [%, percent; cm, centimeter; salinity measured in practical salinity units]

Fraction	% Shell	% Sand	% Silt/Clay	% C	% N	$\delta^{13}C$	$\delta^{15}N$	Salinity
GC852 (N=10)								
0-1 cm	18.91 ± 18.8	21.8 ± 5.13	74.41 ± 7.94	0.55 ± 0.09	0.06 ± 0.01	-23.1 ± 0.6	4.4 ± 0.5	37.2 ± 0.1
1-2 cm	0.36 ± 0.08	29.64 ± 6.14	70.29 ± 6.18	0.55 ± 0.1	0.06 ± 0.01	-23 ± 0.7	2.9 ± 0.9	37.3 ± 0.3
2-3 cm	0.18 ± 0	26.31 ± 5.23	73.67 ± 5.23	0.49 ± 0.11	0.05 ± 0.01	-24.1 ± 0.6	4.3 ± 1.7	37.5 ± 0.5
3-5 cm	1.56 ± 1.42	37.34 ± 6.41	62.35 ± 6.57	0.46 ± 0.06	0.05 ± 0.01	-21.6 ± 0.4	3.3 ± 0.4	37.1 ± 0.1
5-7 cm	3.28 ± 3.19	31.54 ± 6.26	67.81 ± 6.39	0.41 ± 0.03	0.04 ± 0	-22.3 ± 0.6	2.6 ± 0.6	37.2 ± 0.2
7-10 cm	0.99 ± 0.85	24.48 ± 3.94	75.22 ± 3.89	0.58 ± 0.11	0.06 ± 0.01	-22.6 ± 0.4	1.6 ± 0.8	37.1 ± 0.2
AT340 (N=8)								
0-1 cm	0.69 ± 0.25	48 ± 3.97	51.09 ± 4.17	0.43 ± 0.1	0.04 ± 0.01	-22.6 ± 1.8	2.7 ± 0.9	37.6 ± 0.2
1-2 cm	0.71 ± 0.2	40.35 ± 6.19	58.77 ± 6.46	0.43 ± 0.09	0.05 ± 0.01	-23.7 ± 0.9	2.4 ± 1.5	37.3 ± 0.2
2-3 cm	1.31 ± 0.62	40.07 ± 9.93	58.94 ± 9.98	0.29 ± 0.09	0.03 ± 0.01	-23.7 ± 0.9	4.3 ± 1.3	37.5 ± 0.3
3-5 cm	11.49 ± 9.96	36.62 ± 8.23	53.33 ± 7.15	0.51 ± 0.11	0.05 ± 0.01	-23.3 ± 0.7	3.5 ± 0.4	37.4 ± 0.2
5-7 cm	0.98 ± 0.41	42.2 ± 9.46	62.33 ± 10.2	0.48 ± 0.1	0.04 ± 0.01	-23 ± 1.1	4.3 ± 1.7	37.4 ± 0.2
7-10 cm	2.36 ± 1.88	42.65 ± 8.15	55.29 ± 7.74	0.5 ± 0.11	0.04 ± 0.01	-23.3 ± 0.8	2.9 ± 0.8	36.7 ± 0.8
AC601[1] (N=1)								
0-1 cm	0.15 -	52.41 -	47.44 -	0.2 -	0.02 -	-22.8 -	4.9 -	37 -
1-2 cm	0.11 -	63.04 -	36.85 -	0.2 -	0.02 -	-24 -	1.3 -	38 -
2-3 cm	- -	47.54 -	52.46 -	0.29 -	0.03 -	-23.4 -	3.5 -	38 -
3-5 cm	- -	55.36 -	44.64 -	0.9 -	0.1 -	-21.8 -	3.7 -	36 -
5-7 cm	- -	54.33 -	45.67 -	0.74 -	0.08 -	-21.3 -	4 -	36 -
7-10 cm	- -	48.97 -	51.03 -	0.93 -	0.09 -	-22.5 -	4.2 -	37 -

[1]Only one core was collected from Alaminos Canyon.

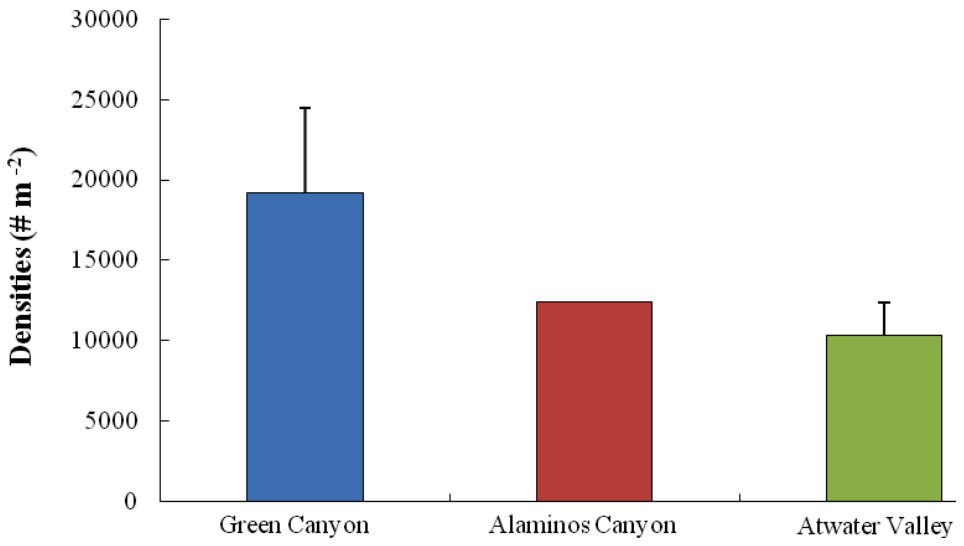
Particle size varied across sites. GC852 sediments were composed of generally finer material; more silt/clay particles were present (62.33-75.22 percent) relative to percent sand (21.80-37.34 percent) and shell (0.36-18.91 percent). In contrast, both AT340 and AC601 sediments had a sandier composition, ranging from 36.62 to 63.04 percent sand.

4.3.2 Macrofauna abundance and composition

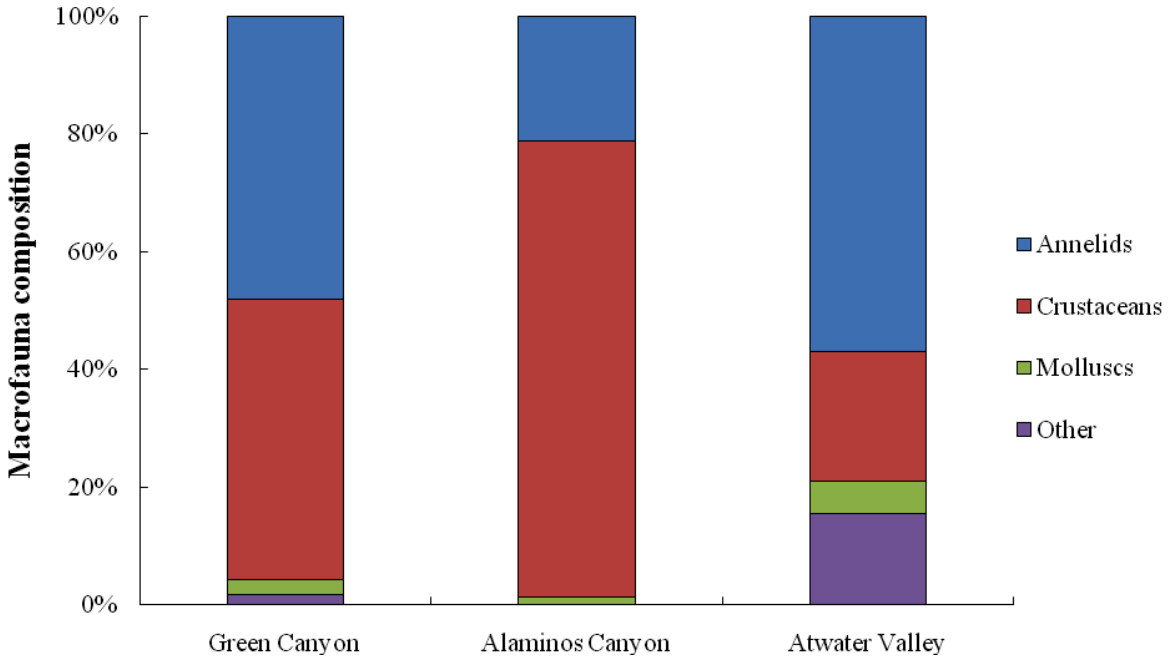
Total macrofaunal densities were the highest at GC852 (16,174 ± 4,357 individuals per square meter, ind m^{-2}), while AT340 and AC601 had similar densities (11,348 ± 2,532 ind m^{-2} and 12,131 ind m^{-2}) of infauna, respectively (fig. 4.1). Several phyla were represented in the sediments: annelids, crustaceans, molluscs and 'other,' which included hydroids, sipunculids, and porifera. Slight differences in macrofaunal composition were observed among the sites as follows. GC852 had an even mix of polychaete annelids (48 percent) and crustaceans (48 percent) with tanaids dominating the crustacean assemblage. Isopod and amphipod crustaceans were also present, in lower densities. In contrast, sediment macrofauna from AC601 was dominated by crustaceans (77 percent), mostly tanaids (64 percent), followed by polychaete annelids (21 percent). There was a notable absence of amphipod crustaceans from this single core. Lastly, AT340 sediments were composed of polychaetes (57 percent), followed by crustaceans (22 percent), mostly tanaids, and other taxa (15 percent) including hydrozoa. Other crustaceans observed at AT340 included cumaceans, isopods, and amphipods.

Figure 4.1. *A*, Macrofaunal (>300 μm) density (individuals per square meter), and *B*, taxonomic composition by major taxa.

A



B



Across the three sites, macrofauna were most abundant in the uppermost sediment fractions, with 72-80 percent found within the top 0-3 cm. Crustaceans declined dramatically with depth and were most abundant in the 0-1 cm fraction, while polychaetes had similar densities within each fraction from 0-3 cm, then declined with depth thereafter.

4.4 Discussion

Macrofaunal densities in deep-sea environments found outside the influence of seeps typically only reach a few thousand individuals per square meter (for example, 1,000-2,000 ind m^2); this pattern has been suggested to be a function of food availability (Levin and Gooday, 2003). In addition, the GOM is generally defined as a low productivity water mass, with low densities recorded for deep-sea benthic infauna (Tyler, 2003). For example, low infaunal densities have been observed from background sediments in the Florida Escarpment (254 ± 152 ind m^2, Levin and Mendoza, 2007), and infauna were composed of amphipod crustaceans followed by both polychaete and oligochaete annelids. In contrast, enhanced infaunal densities were recorded from microbial mat sediments in the Florida Escarpment (20,961 ± 11,618 ind m^2, Levin and Mendoza, 2007), comparable to those found within Green Canyon sediments in this study (19,160 ± 5288 ind m^{-2}), and higher than infaunal densities at Alaminos Canyon and Atwater Valley (fig. 4.1). Macrofaunal densities from the three GOM seeps also are comparable to densities found in shallower seeps off Eel River, Calif. (13,500 ± 4,770 ind m^{-2}, Levin and others, 2003), East Flower Garden brine seep, GOM (for polychaetes, 0-58,360 ind m^{-2}, Powell and others, 1986) and at Atwater Valley (13,906 ind m^{-2}, Robinson and others, 2004). In addition, seep infaunal communities are typically composed of polychaete and oligochaete annelids, gastropods, and gammarid amphipods (Powell and others, 1983; Levin, 2005). Our

results indicate that these major taxonomic groups were also present at the northern Gulf of Mexico seeps described here.

In general, sediment macrofauna from seeps worldwide exhibit high dominance and low diversity (Levin, 2005). For example, macrofauna from the Florida Escarpment seeps contained a high proportion of a single annelid taxa (Levin and Mendoza, 2007). Future species level identification will enable determination of whether northern GOM seeps exhibit the same patterns in species dominance observed in other seeps.

A vast majority of the fauna were found in the upper 3 cm of sediment. In microbial mat sediments, 91percent of the infauna occurred within the top 2 cm at Eel River Seeps (Levin and others, 2003), possibly a consequence of enhanced food availability near the sediment surface. Alternatively, avoidance of toxic sulfides can also affect the vertical distribution and composition of infauna (Levin and others, 2003).

This study represents the first preliminary analysis of seep sediment macrofauna from three deep-slope environments in the GOM. Because macrofaunal densities were comparable to microbial mat sediments found elsewhere in the GOM, the availability of bacteria as a food source may be promoting high densities of infauna (Powell and others, 1986). Food-web studies using stable isotopes indicate that sediment-associated bacteria may be fueling some of these organisms (Demopoulos and others, Chapter 3). Thus, enhanced densities of infauna may ultimately be a function of food supply and availability in these remote seep environments on the lower slope (>1,200 m).

4.5 Acknowledgments

We thank the captain and crew of the R/V *Cape Hatteras*. We also thank the following individuals for their help at sea, site selection, and helpful suggestions: J. Gonzalez, Ross Lab, L.

Levin. E. Cordes, and C. Fisher. Special thanks go to S. Ross for inviting us to participate in this cruise and for facilitating the box core sampling. We thank R. Lee (Washington State University) for conducting sediment organic carbon and nitrogen analyses.

5. PHYLOGEOGRAPHIC AFFINITIES OF SQUAT LOBSTERS (DECAPODA: GALATHEOIDAE) FROM LOWER CONTINENTAL SLOPE COLD-SEEP HABITATS IN THE NORTHERN GULF OF MEXICO

By Cheryl L. Morrison[1] and Martha S. Nizinski[2]

[1]Leetown Science Center, Aquatic Ecology Branch, U.S. Geological Survey, Kearneysville, WV
[2]NMFS National Systematics Laboratory, Smithsonian Institution, Washington, DC

5.1 Introduction

Squat lobsters (Chirostyloidea: Chirostylidae, Eumunididae, Kiwaidae; Galatheoidea: Galatheidae, Munididae, Munidopsidae) are a diverse group of crustaceans comprising over 870 recognized species worldwide (Baba and others, 2008), with several hundred additional species recognized by taxonomic experts as new to science. Collectively, the galatheoids and chirostyloids are considered to be ubiquitous members of the deep-sea fauna. Within the Galatheoidae, the genera *Munida* and *Munidopsis* are the most diverse, with 243 and 224 species, respectively. Most members of these genera occur in deep waters, with the majority of species of *Munida* found at shelf and slope depths and species of *Munidopsis* found mainly on continental slopes and abyssal plains (Baba and others, 2008). In the western North Atlantic, approximately 35 species of *Munida* are recognized (Baba and others, 2008), of which 20 have been reported from the Gulf of Mexico (Felder and others, 2009). Forty-eight species of *Munidopsis* also occur in the western North Atlantic (Baba and others, 2008), with 36 reported from the Gulf of Mexico (GOM; Felder and others, 2009).

Anomurans, which include the galatheoids, are particularly well represented at chemosynthetically based ecosystems (Chevaldonné and Olu, 1996; Martin and Haney, 2005). Anomurans in general, and galatheoids in particular, are the dominant top predator/scavengers found at these reducing habitats (Chevaldonné and Olu, 1996). Members of *Munidopsis* are frequently reported as part of faunal assemblages found associated with extreme environments, including hydrothermal vents and cold seeps (Martin and Haney, 2005). In fact, *Munidopsis* is the most speciose and widespread of all hydrothermal vent-associated taxa (Martin and Haney, 2005), and *Munidopsis* species have been reported from these habitats since their discovery in the late 1970s (Chevaldonné and Olu, 1996). Galatheoids were also recorded from cold-seep environments when these reducing habitats were first discovered in the GOM during the mid-1980s (Paull and others, 1984).

Although recent investigations conducted in the GOM involving quantitative surveys of mega- and macro-invertebrates (for example, Macdonald and others, 1989; Carney, 1994; Bergquist and others, 2003b; Cordes and others, 2006; 2007; 2009) report galatheids as being an important, and sometimes dominant, member of the seep-associated fauna, specific identifications of galatheids have been lacking. Galatheids are often collected opportunistically or photographed in lieu of samples. Many species and even genera of galatheids are morphologically similar, which makes identifications from photographs extremely problematic (Chevaldonné and Olu, 1996; C.L. Morrison, unpub. data, 2011). The lack of specific identification has led to vague general references to these decapods at vents and seeps (see Chevaldonné and Olu, 1996; Martin and Haney, 2005), thus hindering our ability to estimate galatheid species diversity at these sites. In situ photographs taken during recent expeditions to seep sites in the GOM illustrate live coloration; however, often no information from earlier

literature exists to compare with observations. The majority of galatheid species were described in the 18th and early 19th century. At this time, color patterns were often unknown or considered to be unimportant as diagnostic characters. Thus, the use of color characteristics to identify species potentially or to narrow the field of possible species is not practical currently. Additional uncertainty to our present level of knowledge regarding diversity estimates of galatheoids at cold-seep sites is caused by the lack of ecological information incorporated in taxonomic literature and the vague taxonomic accounts generally reported in ecological literature (Chevaldonné and Olu, 1996). As a result, it is difficult to assess and compare information from these two different but equally important references describing associated fauna found in and around cold-seep areas. Thus, the total diversity of galatheoids at cold-seep sites remains unresolved.

In recent studies (Chevaldonné and Olu, 1996; Martin and Haney, 2005; Macpherson and Segonzac, 2005) investigators surveyed published literature and examined additional material to assess the diversity of galatheoids associated with hydrothermal vents and cold seeps. The species list is not extensive for either of these reducing environments, yet confirmed identifications of galatheids at cold seeps are minimal. For example, Martin and Haney (2005) list a total of 2 species of *Munida* and 13 species of *Munidopsis* (counting *Munidopsis* sp. as one species) from known vent and seep sites, and of these, only *Munidopsis* sp. has been recorded from cold-seep sites in the GOM. Three unidentified species of *Munidopsis* along with *Munida microphthalma* are reported by Chevaldonné and Olu (1996) to occur in reducing environments in the western Atlantic, including the GOM. Macpherson and Segonzac (2005) record two species of *Munidopsis* occurring at Mid-Atlantic Ridge hydrothermal vent sites and five species at Atlantic seeps sites.

Molecular approaches, when used together with morphology data, have proven increasingly useful in species identifications. Genetic approaches provide an independent assessment of species diversity from that of morphology and may reveal underlying diversity not evident in taxa featuring morphological conservatism. For example, combined morphological and molecular approaches confirmed the existence of sibling species of *Mundia* from off New Caledonia (Macpherson and Machordom, 2005) and *Munidopsis* in the East Pacific (Jones and Macpherson, 2007). Macpherson and Machordom (2005) and Cabezas and others (2009) highlighted the fact that molecular data supported designation of new species when only subtle morphological differences were observed. In contrast, molecular analyses questioned the taxonomic validity of two sympatric species of *Munida* occurring off southern South America (Pérez-Barros and others, 2008). The authors of that study suggested that although morphological differences were apparent, the genetic divergence between the putative species was not substantial enough to warrant recognition of two species. Overall, species diversity estimates of the Pacific galatheoid fauna have increased with discovery of cryptic species due to increased sampling and better defined taxonomy (incorporating both morphological and molecular data). Application of similar approaches is expected to increase estimates of galatheoid diversity in the western Atlantic.

Phylogenetic relationships of squat lobsters are poorly understood, and there is still much to learn about the evolutionary history of these crustaceans. Recent work (Machordom and Macpherson, 2004; Macpherson and Machordom, 2005; Jones and Macpherson, 2007; Cabezas and others, 2009) has explored and attempted to clarify some of these relationships; however, molecular data are lacking for many species, particularly those in the western Atlantic, making construction of phylogenies difficult and incomplete. Machordom and Macpherson (2004) had

good support for most groups identified in their phylogenetic analyses; however, certain intrageneric relationships were not highly supported. They concluded that this low support was indicative of a rapid radiation event followed by stasis or constraints in morphological evolution of the taxa studied. Jones and Macpherson (2007) suggested that low-level genetic divergence observed among morphologically differentiated species of *Munidopsis* collected on the East Pacific Rise could suggest a recent and (or) rapid diversification. Cabezas and others (2009) also found minimal support for intrageneric relationships within their phylogeny of galatheoids from the South-West Pacific. Their results concur with those of previous works (Machordom and Macpherson, 2004; Jones and Macpherson, 2007) and thus they suggested that this observed pattern may be indicative of a rapid diversification and radiation events that appear to be fundamental to structuring the evolutionary history of this group.

Recent expeditions to cold-seep sites in the GOM collected several individuals of multiple species of galatheoids that were made available for study. Morphological and molecular analyses were conducted on these specimens to provide an initial assessment of the species composition of squat lobsters present at cold-seep habitats on the lower continental slope in the GOM and to examine species diversity in an evolutionary and phylogenetic context through comparisons with other species in the genera *Munidopsis* and *Munida*.

5.2 Methods

5.2.1 Sample Collections

Twenty-five galatheoid samples, some including multiple individuals (table 5.1), were collected from eight chemosynthetic and (or) deep-sea coral sites (AC818, AT340, GC234, GC354, GC600, GC852, VK826, WR269) between September 2003 and August 2007 from

depths between 1,188 and 2,746 m. Of these, 13 galatheoid samples, collected at 5 chemosynthetic sites (AT340, GC600, GC852, AC818, WR269) on the lower continental slope in the northern GOM (fig. 5.1), were used for molecular analysis. Additional information regarding sample sites can be found in Roberts and others (2007b). Samples were collected by manned submersible DSV *Alvin*, ROV *Jason2*, and otter trawl deployed near hydrocarbon seep sites (see table 5.1). After capture, specimens were sorted and preserved in 70 percent ETOH. A small portion of tissue (leg, abdomen, or egg) was placed in 95 percent ETOH for subsequent DNA analysis. In the laboratory, samples were examined and identified using authoritative keys, taxonomic literature, and comparative material housed at the National Museum of Natural History, Smithsonian Institution (USNM). Vouchers will be deposited at the USNM.

Table 5.1. Species list for chirostyloid and galatheoid samples examined.

Species	Site	Station[1]	Date	Depth (meters)	Collection Method	No. Ind.	Used in Phylogeny
Family Chirostylidae							
Gastroptychus affinis	GC852	AD-4185	5/21/2006	1,398	On bamboo coral	1	N
Family Eumunididae							
Eumunida picta	VK826	JSL-4733	7/22/2004	462	Bushmaster	1	N
Family Munididae							
Munida microphthalma	GC600	AD-4174	5/10/2006	1,188	Suction, carbonate	2	Y
	GC600	AD-4184	5/20/2006	1,200	Suction	3	Y
	GC852	AD-4186	5/22/2006	1,410	Suction, bushmaster scar	1	Y
	GC852	CH-07-027	8/11/2007	1,426-1,564	Otter Trawl	3	Y
	GC852	CH-07-042	8/12/2007	1,478-1,574	Otter Trawl	1	Y
Munida sanctipauli	VK826	CH-07-237	8/27/2007	550	Box Core	1	N
	VK826	CH-07-238	8/27/2007	482	Box Core	1	N
	VK826	CH-07-245	8/27/2007	476	Box Core	1	N
Family Munidopsidae							
Leiogalathea agassizii	GC354	JSL-4741	7/26/2004	563	Bushmaster	1	N
Munidopsis bermudezi	AC818	J2-282	7/01/2007	2,746	Bushmaster, *Escarpia*	2	Y
Munidopsis curvirostra	WR269	J2-275	6/18/2007	1,919	Suction, tubeworm	1	Y
	AT340	AD-4173	5/09/2006	1,139	Bushmaster, tubeworms	1	N
Munidopsis glabra	GC234	JSL-4587	9/05/2003	534	Bushmaster	26	N
Munidopsis livida	AC818	J2-282	7/01/2007	2,746	Bushmaster, *Escarpia*	1	Y
Munidopsis similis	AT340	J2-270	6/9/2007	2,192	Bushmaster, tubeworms	1	Y
	AT340	AD-4179	5/15/2006	2,184	Bushmaster	1	N
	GC852	CH-07-042	8/12/2007	1,478-1,574	Otter Trawl	1	Y
	AT340	AD-4180	5/16/2006	2,184	Bushmaster	1	N
	AT340	AD-4173	5/09/2006	1,139	Bushmaster, tubeworms	1	N
Munidopsis penescabra	VK826	CH-07-226	8/26/2007	477	Tucker Trawl	2	N
Munidopsis n. sp.	GC600	AD-4174	5/10/2006	1,188	Suction	1	Y
	GC234	JSL-4587	9/05/2003	534	Bushmaster	168	N
Munidopsis sp. A.	AC818	AD-4195	5/30/2006	2,744	Suction	1	Y
	AC818	J2-282	7/01/2007	2,746	Bushmaster, *Escarpia*	1	Y

[1]AD= DSV *Alvin*; J2= ROV *Jason2*; JSL= *Johnson-Sea-Link* submersible; CH= R/V *Cape Hatteras*, trawl.

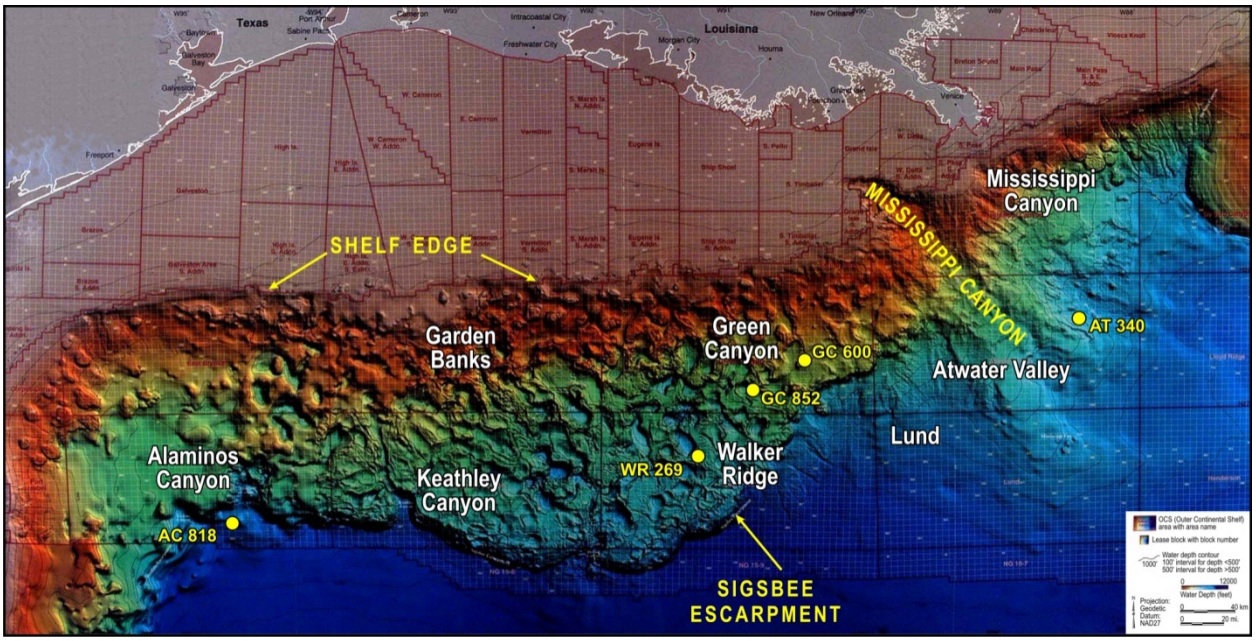


Figure 5.1. Multibeam bathymetry map of the northern Gulf of Mexico continental slope. Included on map are galatheiod collection localities (yellow dots) and lease blocks established by the Bureau of Ocean Energy Management, Regulation and Enforcement for oil and gas activity management. (Map created by Harry Roberts, Louisiana State University).

5.2.2 DNA extraction, amplification, and sequencing

Total DNA was isolated from ethanol-preserved tissue using the tissue protocol from the PureGene DNA extraction kit (Gentra Systems Inc., Minneapolis, Minn.). The following primers were used to amplify a 650 base pair (bp) portion of the mitochondrial cytochrome oxidase I gene (COI):

LCO1490 (5’ – GGTCAACAAATCATAAAGATATTGG – 3’) and HCO2198 (5’ - TAAACTTCAGGGTGACCAAAAAATCA) (Folmer and others, 1994); gala-COIF (5’ – CATCACTWAGWTTRATYATTCGAGCAGAA – 3’) and gala-COIR (5’ – GAAYAGGRTCTCCTCCTCCTAC – 3’) (Jones and Macpherson, 2007); CrustF2 (5’ –

GGTTCTTCTCCACCAACCACAARGAYATHGG – 3') (Costa and others, 2007) and COI-H (5' – TCAGGGTGACCAAAAAATCA – 3') (Machordom and Macpherson, 2004). Forward primers LCO1490 or gala-COIF paired with COI-H produced most of the sequences for this study. PCR reactions were carried out using 1X PCR buffer (10 millimoles (mM) Tris-HCl, pH 8.3, 20 mM KCl), 2 mM $MgCl_2$, 0.2 mM of dNTPs, 0.375 micromoles (µM) of each primer, 0.01 milligrams per milliliter (mg/mL) bovine serum albumin, 2.5 units of Taq polymerase, and approximately 100 nanograms (ng) of template DNA in a 20 microliter (µL) reaction volume. Thermal cycling was performed using an MJ-Research (Watertown, Mass.) PTC-200 thermocycler and the following cycle parameters: initial denaturing at 94°C for 2 min; followed by 35 cycles of 94 °C denaturing (1 min), 52–56 °C annealing (1 min), 72 °C extension (1 min), ending with a 5-min extension at 72 °C. PCR products were purified with Exonuclease I and Shrimp Alkaline Phosphatase (Promega Corp., Madison, Wisc.) and then used as templates in sequencing reactions with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Foster City, Calif.). Sequencing reactions were analyzed by capillary electrophoresis using the ABI PRISM 3100™ Genetic Analyzer and DNA Sequencing Analysis Software (Applied Biosystems, Foster City, Calif.).

5.2.3 Mitochondrial DNA sequence analysis

Forward and reverse sequences were assembled for each individual using Sequencher v. 4.0 (Gene Codes Corporation, Ann Arbor, Mich.). Multiple sequence alignments for *Munida* and *Munidopsis* species were carried out using Clustal X v. 1.4b (Thompson and others, 1997) with default gap opening/extension penalties. For both genera, most of the comparative sequence data available are from Pacific Ocean taxa. For the *Munida* analysis, comparative sequences from GenBank were included from the following studies: Machordom and

Macpherson (2004); Costa and others (2007), and Perez-Barros and others (2008). The majority of comparative *Munidopsis* sequences were from Jones and Macpherson (2007); several sequences from Cubelio and others (2007) were also included.

Phylogenetic trees were estimated via Bayesian analysis using MrBayes v. 3.1.2 (Huelsenbeck and Ronquist, 2001). For each dataset, two simultaneous Bayesian runs were completed for 8 X 10^6 generations with random starting trees, default priors, four Markov chains, and sampling every 100 generations. Stationarity of the Markov chain Monte Carlo (MCMC) analyses was determined by plotting negative log-likelihood values and parameter estimates against generation times. Trees from the burn-in (24,000 trees) were discarded before clade posterior probabilities were calculated from their frequencies.

5.3 Results

5.3.1 Diversity of Galatheoidea

Two hundred and twenty-six individuals were collected in 25 samples taken at 20 stations (table 5.1). Individuals in these samples represented two families (Chirostylidae, Galatheidae) and 13 species (2 chirostyliods, 11 galatheiods; table 5.1); two of these species are new to science. Seven species (*Eumunida picta*, *Munida sanctipauli*, *Munidopsis bermudezi*, *M. glabra*, *M. livida*, *M. penescabra*, *Munidopsis* n.sp.) have been previously reported from the GOM. Only three species (*Eumunida picta*, *Munida microphthalma* and *Munidopsis livida*) were recorded previously from cold seeps (Martin and Haney, 2005; Macpherson and Segonzac, 2005). Of these species, *E. picta* is the only species previously reported from cold seeps in the GOM (Martin and Haney, 2005).

At the majority of stations (N = 15) only one species was collected. Two species were collected at four stations, three of which were located at GC and one at AT. Three species were collected only once at a station located at AC818. Also of interest is that the combination of species collected at a station was never the same.

Most samples comprised one to a few individuals. However, two large collections were made at GC234, consisting of 26 individuals of *Munidopsis glabra* and 168 individuals of *Munidopsis* n. sp. These samples were collected using the *JSL* submersible and the Bushmaster tubeworm community collection device as part of a study of fauna associated with chemosynthetic communities (Cordes and others, 2005). These *Munidopsis* species, labeled *Munidopsis* sp. nov. 1 and 2 for *Munidopsis* n. sp. and *M. glabra*, respectively, were among the most abundant tubeworm-associated fauna in collections of *Lamellibrachia luymesi* at GC234 (Cordes and others, 2005). Although *Munidopsis* species are fairly inconspicuous in video data, collections of entire tubeworm communities allow a perspective on their abundances in these long-lived habitats.

Overall, *Munida microphthalma* and *Munidopsis similis* were collected most frequently; each species was captured in five samples. Four samples of *M. similis* were taken at AT340; one sample at GC852. *Munida microphthalma* was collected only at GC. With regard to species diversity by site, VK826 and AC818 were the most diverse with three species collected at each site. Two species were collected at AT340, GC234, GC600, and GC852, and one species at GC852, GC354, and WR269.

5.3.2 *Munidopsis* species

Eight individuals collected at four deep chemo sites in the northern GOM were included in the phylogenetic analysis. Six species of *Munidopsis* (*M. bermudezi*, *M. curvirostra*, *M.*

livida, *M. similis*, *Munidopsis* n. sp., and *Munidopsis* sp. A) were represented in the samples (table 5.1; fig. 5.2).

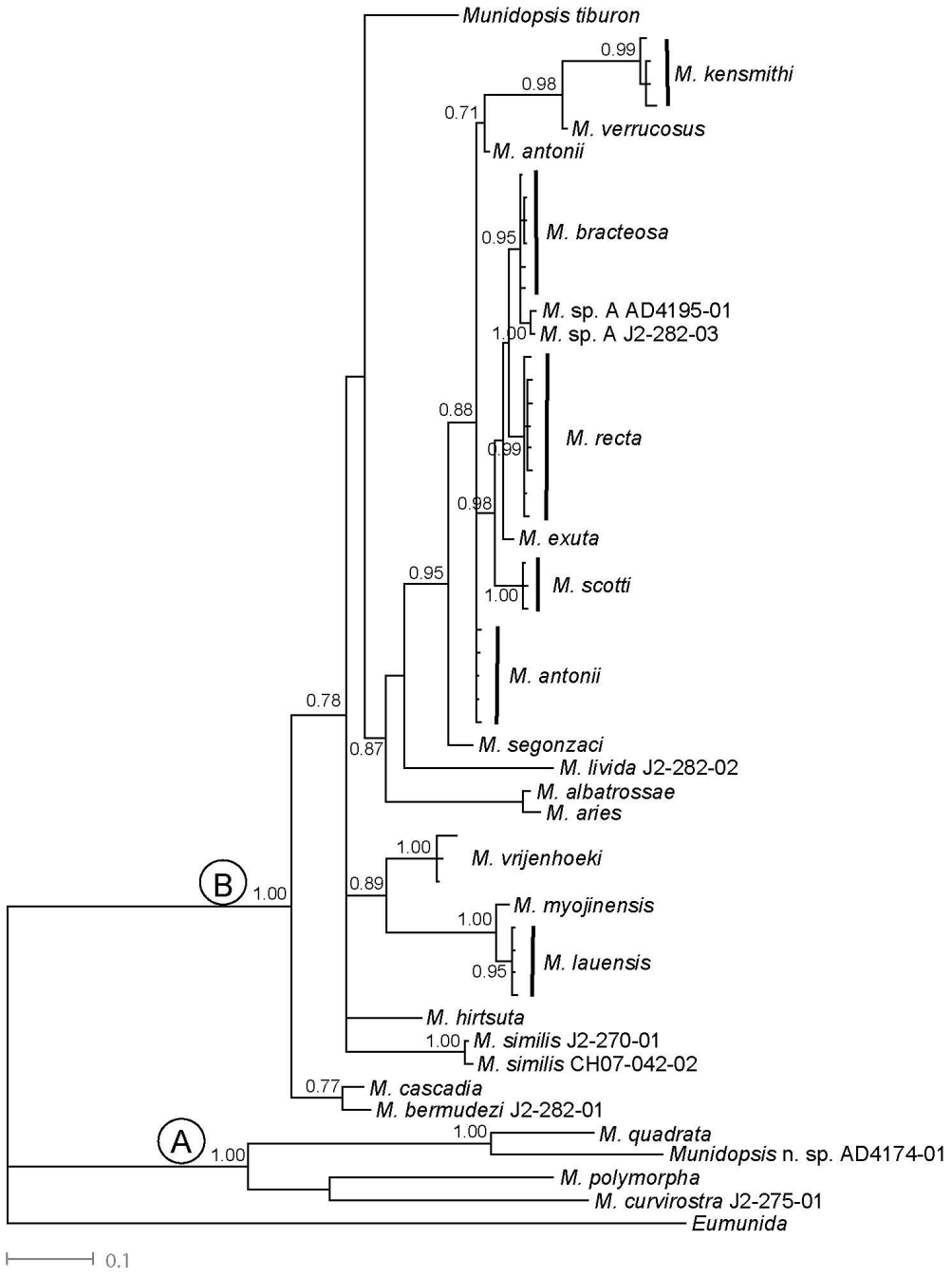


Figure 5.2. Phylogenetic hypothesis for *Munidopsis* resulting from Bayesian analysis of mitochondrial cytochrome oxidase (COI) DNA sequence data. A and B are clades, or groupings of taxa.

Partial mitochondrial COI sequences from the *Munidopsis* samples were determined and aligned with 45 additional sequences representing 18 *Munidopsis* taxa. *Eumunida sternomaculata*, a chirostyloid, was designated as an outgroup. The alignment contained 566 bp of sequence data; 233 sites were variable, 199 sites were parsimony-informative, and no gaps were present.

Results of the Bayesian phylogenetic analysis are shown in figure 5.2. *Munidopsis* samples from GOM cold-seep sites are taxonomically and phylogenetically diverse. Two distinct, well supported clades or groupings of taxa (clades A and B, fig. 5.2) were produced in the analysis. Cold-seep species collected in the GOM were not clustered together in the phylogeny. Rather, these species were placed in multiple locations throughout the phylogenetic tree with inclusion of some species from these samples in each of the major clades. Divergence between taxa from the two major clades was high, with an average uncorrected *P*-distance of approximately 20 percent (table 5.2 for *Munidopsis* n. sp. and *M. curvirostra*, other distances not shown).

Table 5.2. Pairwise uncorrected *P*-distances between mitochondrial cytochrome oxidase I (COI) sequences for *Munidopsis* samples.

Individual	*M.* sp. A. AD4195-01	*M.* sp. A. J2282-03	*livida* J2282-02	*similis* J2270-01	*similis* CH07-042-02	*bermudezi* J2282-01	*M.* n. sp. AD4174-01	*M. curvirostra* J2275-01
M. sp. A. AD4195-01	-							
M. sp. A. J2282-03	0.37	-						
M. livida J2282-02	10.64	10.78	-					
M. similis J2270-01	9.58	9.95	11.97	-				
M. similis CH07-042-02	9.91	10.25	12.72	0.55	-			
M. bermudezi J2282-01	11.01	11.31	12.01	11.6	12.54	-		
M. n. sp. AD4174-01	22.57	22.44	21.38	21.73	22.79	22.57	-	
M. curvirostra J2275-01	19.27	19.05	19.73	17.69	17.91	19.5	17.23	-

Clade A (fig. 5.2) included four species, two species from GOM seeps (*Munidopsis curvirostra* and *Munidopsis* n.sp.), one from the eastern Pacific (*M. quadrata*), and one from the eastern North Atlantic (*M. polymorpha*). Within this clade the GOM species grouped with species from other oceans or regions instead of together. Interestingly, *M. quadrata* is the sister taxon to *Munidopsis* n.sp.

The remaining four GOM *Munidopsis* species included in the phylogenetic analysis were placed in the distinct and well-supported Clade B (fig. 5.2). Concordant with morphology, sequences from multiple individuals of the same taxon grouped together in the phylogeny and were minimally divergent (*Munidopsis* sp. A, uncorrected *P*-distance=0.37 percent substitutions/site; *M. similis P-distance*=0.55 percent; fig. 5.2; table 5.2). *Munidopsis cascadia* (eastern Pacific) and *M. bermudezi* were sister taxa and basal to this clade (fig. 5.2). Corrected sequence divergence between these taxa was 3.9 percent. Of particular interest was the clade containing GOM *Munidopsis* sp. A individuals (this study) and *M. bracteosa* individuals from the eastern Pacific Ocean (Jones and Macpherson, 2007). These two species were approximately 1 percent divergent.

5.3.3 *Munida* species

Munida microphthalma was the only species of *Munida* from these collections included in this analysis. This species occurred at relatively high densities at two different Green Canyon sampling locations, GC852 (N=4) and GC600 (N=5).

Partial mitochondrial COI sequences from these nine *M. microphthalma* individuals were determined and aligned with 56 additional sequences representing 35 *Munida* taxa. *Eumunida sternomaculata*, a chirostyloid, was designated as an outgroup. The alignment contained 657 bp

of sequence data; 262 sites were variable, 246 sites were parsimony-informative, and no gaps were present.

Results of the Bayesian phylogenetic analysis are shown in figure 5.3. Multiple groupings of *Munida* taxa were supported by high posterior probabilities, yet resolution of relationships among these major groupings was poor. Sequences from all individuals of GOM *M. microphthalma* were included in one grouping. These sequences differed little from each other as illustrated by short branch lengths (fig. 5.3). In this analysis, the closest relative to GOM *M. microphthalma* was *M. tiresias* from New Caledonia. However, these species were quite differentiated with an average Tamura-Nei sequence divergence of 15 percent.

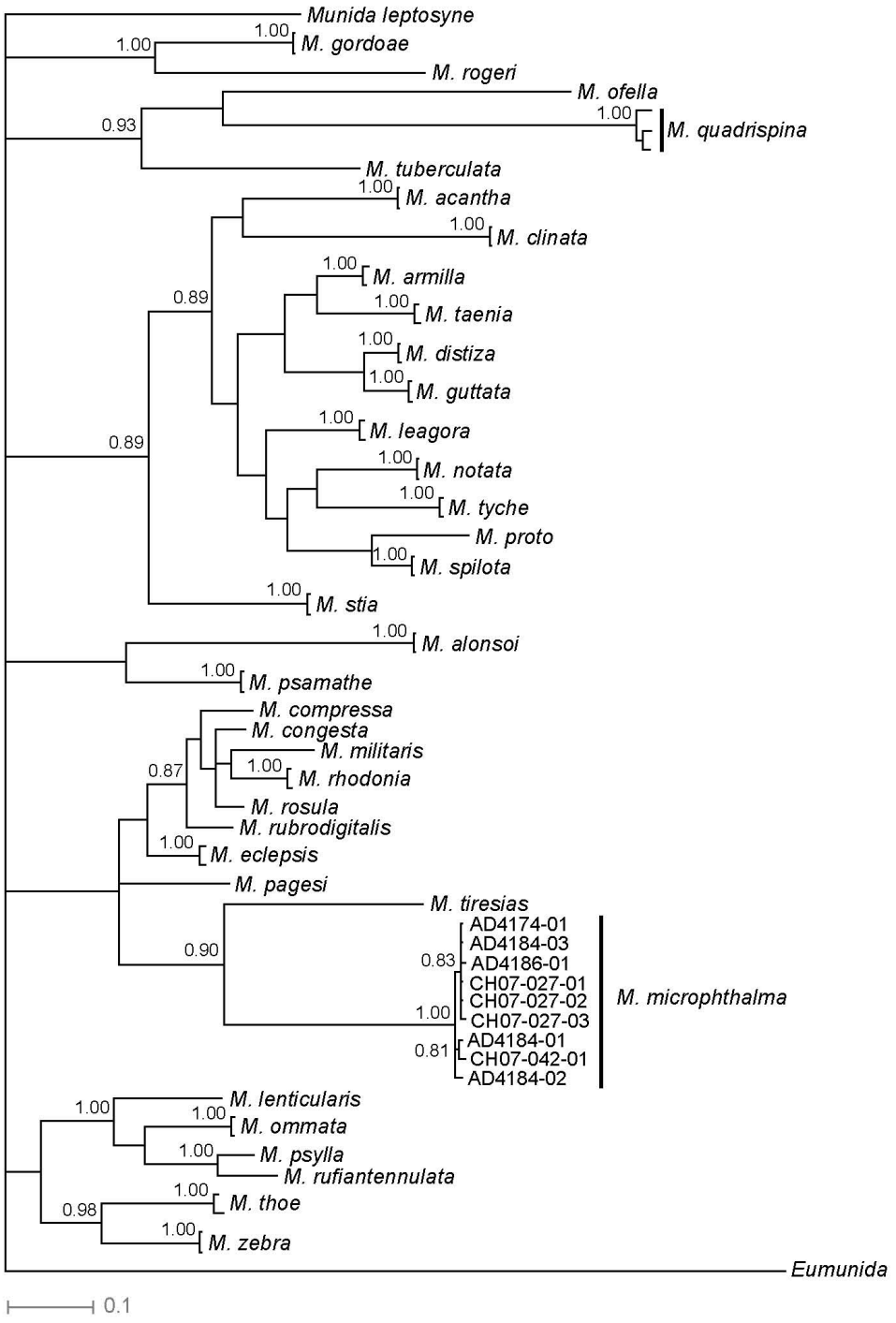


Figure 5.3. Phylogenetic hypothesis for *Munida* resulting from Bayesian analysis of mitochondrial cytochrome oxidase I (COI) DNA sequence data.

5.4 Discussion

Galatheoid crabs from the genera *Munida* and *Munidopsis* have been reported from cold-seep sites in the GOM, yet species accounts have been vague (Martin and Haney, 2005). Here we report eight species of *Munidopsis* and two species of *Munida* collected near chemosynthetic habitats in the northern Gulf of Mexico. Of these species, we examined the phylogenetic placement of six species of *Munidopsis* and one species of *Munida* relative to other species within each genus. Other species examined but not included in phylogenetic analysis were either not preserved correctly for DNA analysis or did not amplify for sequencing.

In this initial assessment of deep chemosynthetic sites in the northern GOM, high taxonomic diversity was found in the genus *Munidopsis*. This is not surprising given that this genus is extremely diverse, particularly in deep-sea environments. Additionally, species of *Munidopsis* are the most frequently encountered decapod crustaceans at hydrothermal vent and seep sites (Chevaldonné and Olu, 1996; Martin and Haney, 2005). The GOM species of *Munidopsis* included in our analysis represent divergent evolutionary lineages as illustrated by their placements throughout the phylogenetic tree. These species usually grouped with species from either the eastern Pacific or North Atlantic Oceans.

Two of the GOM seep species examined, *Munidopsis* n.sp. (GC600) and *M. curvirostra* (WR269), were members of a well-supported basal clade that also included *M. quadrata* (eastern Pacific Ocean) and *M. polymorpha* (eastern North Atlantic Ocean). *Munidopsis* species that fell into Clade A (fig. 5.2) were substantially differentiated from the other *Munidopsis* species examined.

At the base of the second major clade of *Munidopsis* taxa (Clade B, fig. 5.2), *Munidopsis cascadia* from the Pacific Ocean (off the coast of Oregon) formed a sister relationship with *M.*

bermudezi from the GOM. A molecular clock calibrated for other decapod crustacean taxa (sister species of snapping shrimps) separated by the Isthmus of Panama can be used to estimate the approximate timing of lineage divergence (1.5 percent, Morrison and others, 2004). Based on these calculations, divergence of these taxa is estimated to be slightly less than 3 million years ago, which is in accordance with the final closing of the Panamanian Seaway.

Our phylogenetic analysis corroborates the results presented by Jones and Macpherson (2007). *Munidopsis bracteosa* belongs to a species complex that includes *M. recta*, the most common species from the vast East Pacific Rise, *M. scotti* from the Juan de Fuca vent system, and *M. exuta* from the Mid-Atlantic Ridge. This species complex now also includes *Munidopsis* sp. A from the GOM. In fact, a close phylogenetic relationship was found between *Munidopsis* sp. A from GOM seeps and the recently described *M. bracteosa* (Jones and Macpherson, 2007) from the Juan de Fuca hydrothermal vent system and whalefall in Monterey Bay in the eastern Pacific Ocean. Sequence divergence of approximately 1 percent suggests that these individuals may have relatively recent faunal connections (less than a million years, applying the same rate calibration as above). These lineages may be in the process of speciating, since several distinctive morphological differences were identified.

Although the species belonging to the closely related clade mentioned above (*M. bracteosa*/ *M*. sp. A., *M. recta*, *M. scotti*, and *M. exuta*) can be identified morphologically, they differ by less than 2 percent mitochondrial sequence divergence. This value is close to the value of 1.6 percent suggested by Lefébure and others (2006) as the minimum amount of divergence needed between crustacean species to support recognition of distinct species. Jones and Macpherson (2007) hypothesize that species belonging to this clade have recently diverged or have undergone a rapid radiation. It is especially interesting that *M. exuta* and *Munidopsis* sp. A.

have such close molecular ties to the eastern Pacific species group, since they are distributed in different ocean basins and more recent pathways for larval dispersal between these ocean basins are not obvious. Additional sampling, as well as taxonomic and DNA sequence analyses, will be necessary to unravel this unexpected evolutionary pattern.

From these initial collections, it appears that the genus *Munida* is not as taxonomically diverse as *Munidopsis* at GOM deep chemosynthetic habitats. This is not surprising since only two *Munida* species have been reported from seep or vent habitats previously (Martin and Haney, 2005). The single species collected at GOM deep chemosynthetic sites included in the analysis, *Munida microphthalma*, is known from cold-seep sites in the Barbados Accretionary Prism, as well as throughout the North Atlantic Ocean (Martin and Haney, 2005).

The diversity of galatheoid crabs is high at these extreme deep-sea environments in the GOM. We are just beginning to recognize this diversity. High quality collections are key to our understanding of the morphology and taxonomy of this group. Most of these species cannot be identified from photographs or videos. Once we are able to positively identify the galatheoid fauna associated with cold-seep environments, we can utilize this information to make progress on the systematics, phylogenetics, phylogeography, and connectivity of the members of this group. We have made significant progress toward this goal, but more intensive sampling is needed to determine the total diversity and evolutionary relationships of the galtheoid crabs associated with GOM cold-seep habitats.

5.5 Acknowledgments

This investigation was financially supported by the USGS Outer Continental Shelf Ecosystem Program and was sponsored by the Bureau of Ocean Energy Management, Regulation and Enforcement. The USGS-BRD Leetown Science Center, Aquatic Ecology

Branch, provided partial funding. The following individuals kindly donated crab samples: E. Cordes and C. Fisher. The authors would like to thank TDI-Brooks International, Inc., C. Fisher, and H. Roberts for allowing C. Morrison to participate in a research cruise. Additionally, the authors thank the crew members of the R/V *Atlantis*, R/V *Ronald H. Brown*, and the R/V *Cape Hatteras*, as well as the crews for DSV *Alvin* and ROV *Jason2*, who made this research possible.

6. PRELIMINARY MOLECULAR ASSESSMENT OF SCLERACTINIAN CORAL AND OCTOCORAL BIODIVERSITY FROM LOWER CONTINENTAL SLOPE HABITATS IN THE NORTHERN GULF OF MEXICO

By Cheryl L. Morrison

Leetown Science Center, Aquatic Ecology Branch, U.S. Geological Survey, Kearneysville, WV

6.1 Introduction

Hydrocarbon seeps and their associated chemosynthetic communities are widespread in the Gulf of Mexico (GOM; Fisher and others, 2007). Over the past two decades, studies of the physiology of symbiont-containing megafauna and the ecology of the chemosynthetic communities occurring on the GOM upper continental slope have made these habitats model systems for the study of seep communities (reviewed by Fisher and others, 2007; Cordes and others, 2009). Typical seep chemosynthetic communities in the GOM are dominated by five species of bathymodiolin mussel species with methane- or sulfide-oxidizing symbionts, six vestimentiferan polychaete (tubeworm) species and (or) two species of vesicomyid clams that harbor sulfide-oxidizing symbionts (Cordes and others, 2009). For tubeworm species, a strong temporal component, or community succession, has been documented, where young, fluid-prone habitats consisting of seep endemics eventually transition as seepage subsides to older, mineral-prone habitats with lower biomass but higher non-endemic diversity (Bergquist and others, 2003a; Cordes and others, 2005; Fisher and others, 2007; Cordes and others, 2009). At this latter

stage in community succession, when fluid-gas expulsion has subsided, relict seeps are often represented by authigenic carbonates produced during active seepage. In some areas, these exposed carbonates may be colonized by deep-sea scleractinian corals, octocorals, and black corals, providing a direct link between cold seeps and coral communities in the deep GOM (Fisher and others, 2007; Cordes and others, 2009).

Azooxanthellate scleractinian corals are fairly well characterized and diverse in the GOM, with 94 species recorded, and a large percentage of these, 82 species, or 87 percent, occurring in deep water (>200 m; Cairns and others, 2009). Only 23 of these GOM scleractinian species occur at depths greater than 1,000 m (Cairns and others, 2009). The scleractinian coral *Lophelia pertusa* forms extensive reef structures on the upper continental slope (Schroeder and others, 2005). *Madrepora oculata* also occurs on the upper continental slope in the GOM (Cairns and others, 2009). The depth range of both of these species exceeds 1,000 m (Cairns, 1979; Cairns and others, 2009), yet there are no known reef areas at such depths in the GOM.

Scleractinian taxonomy and phylogeny are in a transition in which traditional skeletal characters are being supplemented with characters from microstructures, reproduction, and frequently, molecular genetics (Cairns and others, 2009). In particular, analyses of both mitochondrial and nuclear sequence data have established two ancient clades, or groupings, of scleractinian coral species, the "complex" and "robust" clades (Romano and Palumbi, 1996; see Romano and Cairns, 2000, and Kitahara and others, 2010a, for discussion). From 16S mitochondrial ribosomal RNA sequences, the two clades are easily distinguishable by sequence length: the "robust" corals have a short 16S sequence relative to the "complex" corals (Romano and Palumbi, 1997). Such molecular studies have also revealed that morphologically defined families often do not represent "natural" families that share a common ancestry (Fukami and

others, 2004; 2008). Based on a recent analysis including many deep-water, azooxanthellate species, and unlike shallow-water zooxanthellate species, the majority of azooxanthellate coral families do appear to be natural groupings, with the exception of the Caryophylliidae and the Oculiniidae (Kitahara and others, 2010a). In fact, the Caryophylliidae, which includes *Lophelia pertusa* and many solitary corals in the genus *Caryophyllia* plus other genera, is the least cohesive of extant coral families, with representatives falling into both the "complex" and "robust" sections of the scleractinian phylogeny (Le Goff-Vitry and others, 2004; Kitahara and others, 2010a).

Octocoral biodiversity is still poorly known in the GOM, yet diversity appears high, with 162 species recorded (Cairns and Bayer, 2009). About half of these GOM octocoral species (77 species) occur in deep water (>200 m), and 15 species have depth ranges greater than 1,000 m (Cairns and Bayer, 2009). Considering the entire GOM basin divided into four approximately equal quadrants at 90°W. latitude and 25°N. longitude for biogeographic comparisons (Felder and others, 2009), the highest biodiversity of both scleractinian and octocoral species has been recorded in the southeastern quadrant of the GOM, with numbers of species decreasing considering quadrants in a counterclockwise fashion (Cairns and others, 2009; Cairns and Bayer, 2009).

Although chemosynthetic communities occurring on the GOM upper continental slope (water depths <1,000 m) have been well characterized (for example, Kennicutt and others, 1985; Brooks and others, 1987; Cordes and others, 2007), few are known from the middle and lower continental slope, and they remain poorly studied. The objective of this study was to produce a preliminary genetic assessment of scleractinian coral and octocoral species diversity associated with chemosynthetic habitats at water depths of 1,000 m or greater in the northern GOM.

6.2 Methods

6.2.1 Sampling sites

Azooxanthellate scleractinian corals and octocorals associated with chemosynthetic habitats in the northern GOM were collected from five sites at depths ranging from 942 to 2,240 m (fig 6.1). The westernmost site, Alaminos Canyon 645 (AC645), was characterized by tubeworms and mussels and has been the focus of multiple studies (Carney, 1994; Roberts and Aharon, 1994; Cordes and others, 2007). The Alaminos Canyon site was also the deepest site where corals were collected for this study. Octocoral samples were obtained from two sites in the Garden Banks (GB), GB647 and GB697. Both of these sites were salt ridges with chemosynthetic communities and seep-associated carbonates where octocorals were found. Chemosynthetic communities including tubeworms and mussel beds occur near (80-400 m) to authigenic carbonate boulders hosting octocorals and scleractinian corals at Green Canyon 852 (GC852). This area is now referred to as the 'coral gardens' (Roberts and others, 2007a). Unlike other coral sites that were generally topographic highs, Mississippi Canyon 462 (MC462) was a depression in the continental slope and represents the most eastern coral collection site. Small carbonates at the bottom of the depression hosted scleractinian and octocorals.

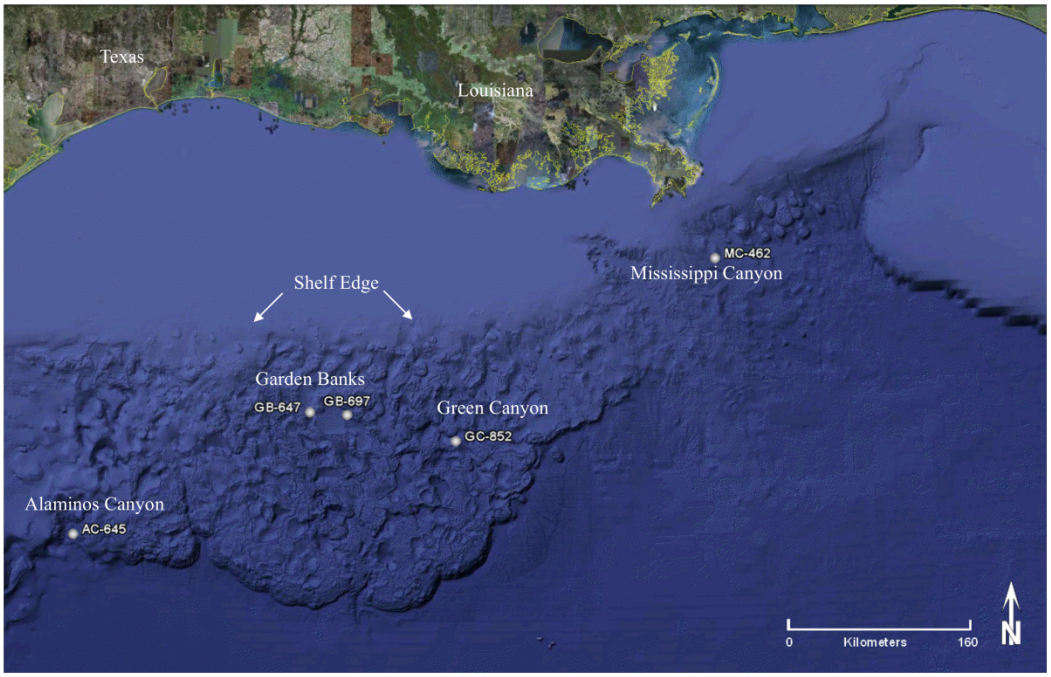


Figure 6.1. Sampling locations in the northern Gulf of Mexico.

Sampling occurred during May of 2006 aboard the R/V *Atlantis* using the Deep Submergence Vehicle (DSV) *Alvin* and June 2007 aboard the R/V *Ronald H. Brown* using the remotely operated vehicle (ROV) *Jason2*. Small pieces of scleractinian corals or octocorals were collected using the *Alvin* or *Jason2*. Once on board the vessel, a small piece of tissue was excised from each coral and preserved in 95-percent ETOH. Additionally, a larger piece of the skeleton was placed in 70-percent ETOH to serve as a voucher. Voucher specimens were identified by Dr. Stephen Cairns, Curator and Chair in the Department of Invertebrate Zoology and the National Museum of Natural History, Smithsonian Institution.

6.2.2 Molecular Methods

Total DNA was isolated from preserved coral tissue using the tissue protocol from the PureGene DNA extraction kit (Gentra Systems Inc., Minneapolis, Minn.). DNA concentrations were determined by fluorescence assay (Labarca and Paigen, 1980) and integrity of the DNA was visualized on 1-percent agarose gels (Sambrook and others, 1989).

For scleractinian corals, DNA sequences were obtained for a portion of the 16S mitochondrial DNA (mtDNA) gene region, allowing for comparisons with large number of scleractinian coral sequences available in GenBank from coral phylogenetic studies by Romano and Cairns (2000), Le Goff-Vitry and others (2004), and Kitahara and others (2010a; b). PCR primers for the 16S mtDNA gene region included universal primers 16Sar and 16Sbr of Palumbi and others (1991).

For octocorals, mitochondrial DNA sequences were obtained for portions of the *msh1* and ND2 genes, allowing for comparisons with GenBank sequence data from major works by Wirshing and others (2005), McFadden and others (2006), and France (2007). The 5' end of the *msh1* gene was amplified with the primers ND42599F (France and Hoover, 2001) and mut3458R (Sánchez and others, 2003). For bamboo corals (isidids), the forward primer msh5p8f (France, 2007) was paired with mut3458R. The primers 16S-647F and ND21418R (McFadden and others, 2006) were used to amplify the ND2 gene region.

PCR reactions were carried out using 1X PCR buffer (10 mM Tris-HCl, pH 8.3, 20 mM KCl), 2 mM $MgCl_2$, 0.2 mM of dNTPs, 0.375 µM of each primer, 0.01 mg/mL bovine serum albumin, 2.5 U of Taq polymerase, and approximately 100 ng of template DNA in a 20 µL reaction volume. Thermal cycling used an MJ-Research (Watertown, Mass.) PTC-200 thermocycler using the following cycle parameters: initial denaturing at 94°C for 2 min;

followed by 35 cycles of 94 °C denaturing (1 min), 52–56 °C annealing (1 min), 72 °C extension (1 min), ending with a 5-min extension at 72 °C. PCR products were purified with Exonuclease I and Shrimp Alkaline Phosphatase (Promega Corp., Madison, Wisc.) and then used as templates in sequencing reactions with the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit v3.1 (Applied Biosystems, Foster City, Calif.). Sequencing reactions were analyzed by capillary electrophoresis using the ABI PRISM 3100™ Genetic Analyzer and DNA Sequencing Analysis Software (Applied Biosystems, Foster City, Calif.). Sequence traces were edited, and forward and reverse sequences for each samples were assembled using Sequencher version 4.10 (Gene Codes Corporation, Ann Arbor, Mich.).

6.2.3 Phylogenetic analyses

New sequences were aligned with select sequences available in GenBank for representative species belonging to the families represented by samples from this study. Multiple sequence alignment was carried out using Clustal X v. 1.4b (Thompson and others, 1997), with default gap opening/extension penalties. The resulting alignment was checked by eye using MacClade v. 4.08 for OS X (Maddison and Maddison, 2005).

Phylogenetic analyses of DNA sequences were carried out using PAUP* 4.0b10 (Swofford, 2002) and MrBayes v. 3.04 (Bayesian analysis; Huelsenbeck and Ronquist, 2001). Pairwise genetic distances plus the neighbor-joining tree reconstruction method were calculated in PAUP, and nodal support was assessed using 1,000 bootstrap replicates (Felsenstein, 1985). The MrBayes analysis was run for 1,000,000 generations with random starting trees, default priors, two Markov chains, and sampling every 1,000 generations. Stationarity of the MCMC analyses was determined by plotting negative log likelihood values and parameter estimates

against generation times. Trees from the burn-in (300 trees) were discarded before posterior probabilities were calculated.

6.3 Results

6.3.1 Scleractinian coral phylogeny

Six scleractinian coral samples representing three families (Dendrophyliidae, Oculinidae and Caryophylliidae) were obtained from two sites (GC-852, northwest GOM, and MC-462, northeast GOM) for this study (table 6.1). Two of the species sampled at GC-852 were previously known only from the southeastern GOM: *Enallopsammia rostrata* and *Solenosmilia variabilis* (Cairns and others, 2009).

Table 6.1. Collection and information for scleractinian coral samples.

[Species identifications by Dr. Stephen Cairns, USNM]

Species Identification	USNM #	Dive #	ID #	Date	Site	Depth (m)	Family
Enallopsammia rostrata (Pourtalés, 1878)	1100985	AD-4190	01	25-May-06	GC-852	1,347	Dendrophyliidae
Madrepora oculata (Linnaeus, 1758)	1110233	J2-271	01	12-Jun-07	MC-462	954	Oculinidae
Caryophyllia polygona (Pourtalés, 1878)	1110967	J2-271	04	12-Jun-07	MC-462	954	Caryophylliidae
Solenosmilia variabilis (Duncan, 1873)	1110364	J2-278	10	23-Jun-07	GC-852	1,396	Caryophylliidae
Madrepora oculata (Linnaeus, 1758)	1110362	J2-278	13	23-Jun-07	GC-852	1,396	Oculinidae
Madrepora oculata (Linnaeus, 1758)	1110363	J2-278	14	23-Jun-07	GC-852	1,396	Oculinidae

The only "complex" coral sampled for this study, *Enallopsammia rostrata*, was included in an analysis in the *Lophelia* I final report (Morrison and others, 2008). The *E. rostrata* sample from GC-852 differed by one base from the 16S sequence for this species in GenBank (France and others, 1996), and differed by 2-3 bases from the congeneric species *E. profunda* from the northwestern Atlantic Ocean (fig. 4.5, Morrison and others, 2008).

Four of five "robust" scleractinian corals were successfully amplified at the mtDNA 16S gene and were aligned with other "robust" coral sequences from GenBank. The coral *Solenosmilia variabilis* collected from GC-852 did not amplify, nor did a comparative sample from the northwestern Atlantic Ocean. Two new sequences for *Madrepora oculata* samples J2-278-11 and J2-278-12 were identical, and only the latter was included in the phylogenetic analysis. The Bayesian analysis included 54 species belonging to 35 genera of "robust" corals. Based upon the well-established sister relationship between hexacorallians (scleractinians, anemones, zoanthids, and corallimorphs) and octocorallians (France and others, 1996; Berntson and others, 1999), two octocoral species, *Briareum* and *Pseudopterogorgia*, were designated as outgroups. Both the *Madrepora* sequences as well as the *Caryophyllia polygona* sequence fell within a well-supported clade containing species belonging to the families Oculinidae, Pocilloporidae, and Caryophylliidae (fig. 6.2, posterior probability 0.97). The Oculinidae clade comprised seven sequences of *Madrepora oculata* collected from the eastern and western North Atlantic Ocean and Gulf of Mexico. The two new samples from this study differed by 1 percent sequence divergence from an *M. oculata* sequence from the upper continental slope (*M. oculata* 4738-2B from MC-885, fig. 6.2). As in previous analyses (Le Goff-Vitry and others, 2004; Morrison and others, 2008), *Caryophyllia* solitary corals were closely allied with *Lophelia pertusa* (fig. 6.2). The *Caryophyllia polygona* sample from MC-461 was 3 percent divergent

from a sample of *C. polygona* from the upper continental slope (*C. polygona* 4738-04, fig. 6.2), and these sequences did not group together, yet both fell within the clade containing other *Caryophyllia* species and *L. pertusa*. A second major grouping was composed of mostly shallow-water species in the families Meandrinidae, Faviidae, Mussidae, Pectinidae, Merulinidae, Fungiidae, and Siderastreidae, and also several species belonging to the family Caryophylliidae (fig. 6.2, posterior probability 0.93). Most of the families within this grouping were not monophyletic, as has been seen in previous analyses (Fukami and others, 2004; 2008; Kitahara and others, 2010a).

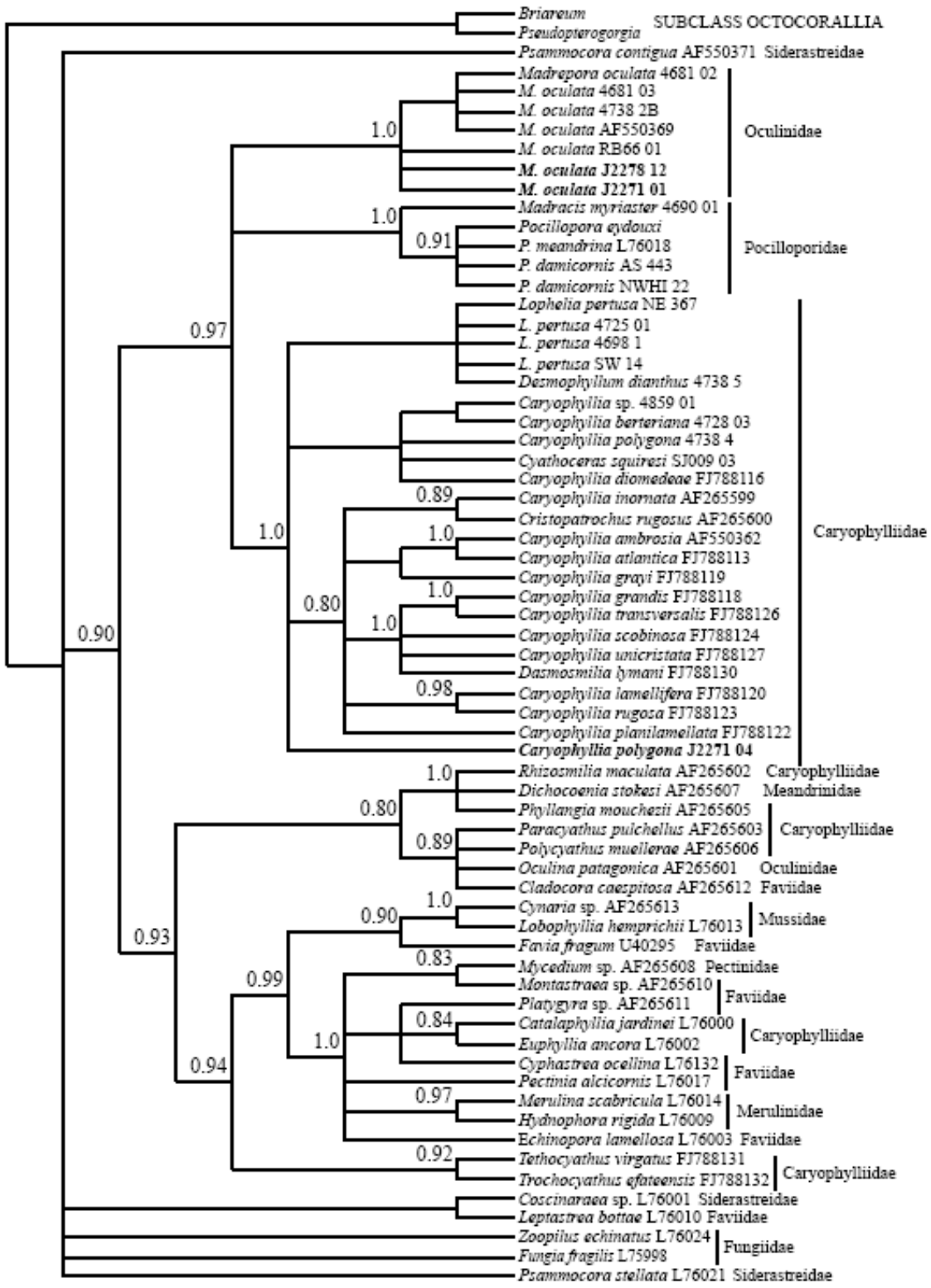

Briareum
Pseudopterogorgia
SUBCLASS OCTOCORALLIA
Psammocora contigua AF550371 Siderastreidae
Madrepora oculata 4681 02
M. oculata 4681 03
M. oculata 4738 2B
M. oculata AF550369
M. oculata RB66 01
M. oculata J2278 12
M. oculata J2271 01
Oculinidae
1.0
Madracis myriaster 4690 01
Pocillopora eydouxi
P. meandrina L76018
P. damicornis AS 443
P. damicornis NWHI 22
Pocilloporidae
1.0
0.91
Lophelia pertusa NE 367
L. pertusa 4725 01
L. pertusa 4698 1
L. pertusa SW 14
Desmophyllum dianthus 4738 5
Caryophyllia sp. 4859 01
Caryophyllia berteriana 4728 03
Caryophyllia polygona 4738 4
Cyathoceras squiresi SJ009 03
Caryophyllia diomedeae FJ788116
Caryophyllia inornata AF265599
Cristopatrochus rugosus AF265600
Caryophyllia ambrosia AF550362
Caryophyllia atlantica FJ788113
Caryophyllia grayi FJ788119
Caryophyllia grandis FJ788118
Caryophyllia transversalis FJ788126
Caryophyllia scobinosa FJ788124
Caryophyllia unicristata FJ788127
Dasmosmilia lymani FJ788130
Caryophyllia lamellifera FJ788120
Caryophyllia rugosa FJ788123
Caryophyllia planilamellata FJ788122
Caryophyllia polygona J2271 04
Caryophylliidae
0.97
1.0
0.89
1.0
1.0
0.80
1.0
1.0
0.98
0.90
Rhizosmilia maculata AF265602 Caryophylliidae
Dichocoenia stokesi AF265607 Meandrinidae
Phyllangia mouchezii AF265605
Paracyathus pulchellus AF265603
Polycyathus muellerae AF265606
Caryophylliidae
Oculina patagonica AF265601 Oculinidae
Cladocora caespitosa AF265612 Faviidae
1.0
0.80
0.89
Cynaria sp. AF265613
Lobophyllia hemprichii L76013
Mussidae
Favia fragum U40295 Faviidae
1.0
0.90
0.93
0.83
Mycedium sp. AF265608 Pectinidae
Montastraea sp. AF265610
Platygyra sp. AF265611
Faviidae
0.99
0.84
Catalaphyllia jardinei L76000
Euphyllia ancora L76002
Caryophylliidae
1.0
Cyphastrea ocellina L76132
Pectinia alcicornis L76017
Faviidae
0.94
0.97
Merulina scabricula L76014
Hydnophora rigida L76009
Merulinidae
Echinopora lamellosa L76003 Faviidae
0.92
Tethocyathus virgatus FJ788131
Trochocyathus efateensis FJ788132
Caryophylliidae
Coscinaraea sp. L76001 Siderastreidae
Leptastrea bottae L76010 Faviidae
Zoopilus echinatus L76024
Fungia fragilis L75998
Fungiidae
Psammocora stellata L76021 Siderastreidae

Figure 6.2. Phylogenetic hypothesis for scleractinian corals based upon Bayesian analysis of mitochondrial 16S sequences. (Species names in bold represent samples unique to this study, whereas others originate from GenBank or Morrison and others (2008). Numbers at nodes represent Bayesian posterior probabilities of node support.)

6.3.2 Octocoral phylogeny

Twelve octocoral samples were collected for this study (table 6.2). Samples were representative of three octocorallian sub-orders (Holaxonia, Calcaxonia, and Scleraxonia) and five families (Plexauridae, Isididae, Acanthogorgiidae, Chrysogorgiidae and Coralliidae; table 6.2).

Table 6.2. Collection and information for octocoral samples.

[Species identifications by Dr. Stephen Cairns, USNM]

Species Identification	USNM #	Dive #	ID #	Date	Site	Depth (meters)	Sub-order	Family
Paramuricea?	1100983	AD4190	02	25-May-06	GC-852	1,410	Holaxonia	Plexauridae
Bamboo coral	1099767	AD4194	01	29-May-06	AC-645	2,240	Calcaxonia	Isididae
Paramuricea?	1100984	AD4194	03	29-May-06	AC-645	2,240	Holaxonia	Plexauridae
Stenogorgiinae	1110234	J2-271	02	12-Jun-07	MC-462	954	Holaxonia	Plexauridae
Acanthogorgoria armata (Verrill, 1878)	1110361	J2-271	03	12-Jun-07	MC-462	954	Holaxonia	Acanthogoriidae
Keratoisis sp.	1110367	J2-273	05	14-Jun-07	GC-852	1,422	Calcaxonia	Isididae
Iridogorgia pourtalesii (Verrill, 1883)	1110366	J2-273	06	14-Jun-07	GC-852	1,422	Calcaxonia	Chrysogorgiidae
Paramuricea sp.	1110368	J2-273	07	14-Jun-07	GC-852	1,422	Holaxonia	Plexauridae
Chrysogorgia fewkesii (Verrill, 1883)	1110371	J2-274	08	17-Jun-07	GB-697	1,003	Calcaxonia	Chrysogorgiidae
Corallium medea (Bayer, 1964)	1110365	J2-278	09	23-Jun-07	GC-852	1,397	Scleraxonia	Coralliidae
Villogorgia sp.	1110369	J2-280	13	26-Jun-07	GB-647	942	Holaxonia	Plexauridae
cf. *Placogorgia* sp.	1110370	J2-280	14	26-Jun-07	GB-647	959	Holaxonia	Plexauridae

Six octocoral samples were successfully amplified and sequenced at the 5' *msh1* gene region. New sequences were aligned with others from GenBank for an alignment of 901 base pairs in length, including 69 ingroup taxa and *Telestula*, a stoloniferan, as the outgroup. Both Bayesian and neighbor-joining analyses produced trees with similar topologies, and the Bayesian cladogram is shown in figure 6.3. The three sub-orders represented in our samples (Holaxonia, Calaxonia, and Scleraxonia) were well supported in the analysis (fig. 6.3). *Corallium medea*, collected from GC-852, clustered with other *Corallium* species included in the analysis (*C. niobe* and *C. ducale;* sub-order Scleraxonia). Pairwise sequence divergence among these *Corallium* samples averaged 3.6 percent. Both *Keratoisis* sp. from GC852 and *Isidella* sp. from AC645 were members of a well-supported clade containing mostly *Isidella* and *Lepidisis* species from both the Atlantic and Pacific Oceans and corresponding to haplotype I1/2 of France (2007). The *Isidella* sp. from AC645 was the most divergent sequence of this isidid clade, averaging 2.8 percent sequence divergence, whereas others in the clade were minimally divergent, differing by less than 1 percent. *Iridogorgia pourtalesii* from GC852 clustered with other species belonging to the family Chrysogorgiidae, and differed from *Iridogorgia* sp. from GenBank by less than 1 percent.

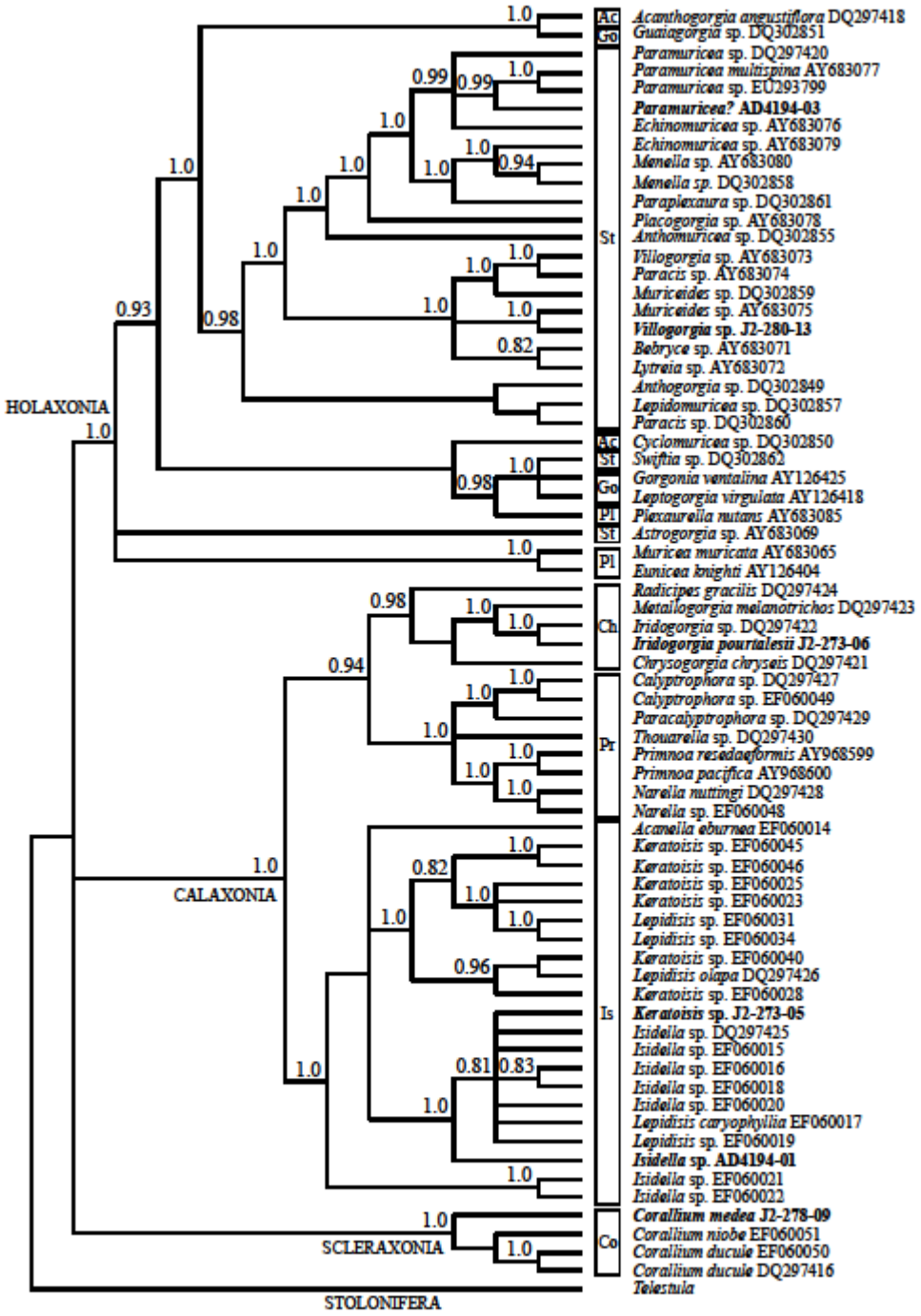

HOLAXONIA
CALAXONIA
SCLERAXONIA
STOLONIFERA
Ac
Go
St
Ac
St
Go
Pl
St
Pl
Ch
Pr
Is
Co
Acanthogorgia angustiflora DQ297418
Guaiagorgia sp. DQ302851
Paramuricea sp. DQ297420
Paramuricea multispina AY683077
Paramuricea sp. EU293799
Paramuricea? AD4194-03
Echinomuricea sp. AY683076
Echinomuricea sp. AY683079
Menella sp. AY683080
Menella sp. DQ302858
Paraplexaura sp. DQ302861
Placogorgia sp. AY683078
Anthomuricea sp. DQ302855
Villogorgia sp. AY683073
Paracis sp. AY683074
Muriceides sp. DQ302859
Muriceides sp. AY683075
Villogorgia sp. J2-280-13
Bebryce sp. AY683071
Lytreia sp. AY683072
Anthogorgia sp. DQ302849
Lepidomuricea sp. DQ302857
Paracis sp. DQ302860
Cyclomuricea sp. DQ302850
Swiftia sp. DQ302862
Gorgonia ventalina AY126425
Leptogorgia virgulata AY126418
Plexaurella nutans AY683085
Astrogorgia sp. AY683069
Muricea muricata AY683065
Eunicea knighti AY126404
Radicipes gracilis DQ297424
Metallogorgia melanotrichos DQ297423
Iridogorgia sp. DQ297422
Iridogorgia pourtalesii J2-273-06
Chrysogorgia chryseis DQ297421
Calyptrophora sp. DQ297427
Calyptrophora sp. EF060049
Paracalyptrophora sp. DQ297429
Thouarella sp. DQ297430
Primnoa resedaeformis AY968599
Primnoa pacifica AY968600
Narella nuttingi DQ297428
Narella sp. EF060048
Acanella eburnea EF060014
Keratoisis sp. EF060045
Keratoisis sp. EF060046
Keratoisis sp. EF060025
Keratoisis sp. EF060023
Lepidisis sp. EF060031
Lepidisis sp. EF060034
Keratoisis sp. EF060040
Lepidisis olapa DQ297426
Keratoisis sp. EF060028
Keratoisis sp. J2-273-05
Isidella sp. DQ297425
Isidella sp. EF060015
Isidella sp. EF060016
Isidella sp. EF060018
Isidella sp. EF060020
Lepidisis caryophyllia EF060017
Lepidisis sp. EF060019
Isidella sp. AD4194-01
Isidella sp. EF060021
Isidella sp. EF060022
Corallium medea J2-278-09
Corallium niobe EF060051
Corallium ducule EF060050
Corallium ducule DQ297416
Telestula

Figure 6.3. Phylogenetic hypothesis for octocorals based upon Bayesian analysis of mitochondrial *msh1* sequences. (Species names in bold represent samples unique to this study, whereas others originate from GenBank. Numbers at nodes represent Bayesian posterior probabilities of node support. Vertical bars indicate the following families: Ac, Acanthogorgiidae; Go, Gorgoniidae; St, Stenogorgiinae; Pl, Plexauridae; Ch, Chrysogorgiidae; Pr, Primnoidae; Is, Isididae; Co, Coralliidae.)

Six octocoral samples were successfully sequenced at the ND2 gene region. Although fewer ND2 gene region sequences were available in GenBank relative to the *msh1* gene region, representatives from each of the three sub-orders mentioned above were included. As with the analysis of the *msh1* gene region, the three sub-orders fell into separate, well-supported clades (fig. 6.4). Similar to the *msh1* results, *Corallium medea* clustered with *C. ducale*. The two *Corallium* sequences differed by 3.2 percent, whereas other sequences discussed below had closest matches that differed by less than 1 percent. *Iridogorgia pourtalesii* and another *Iridogorgia* sequence from GenBank fell within a clade containing members of the Chrysogorgiidae. *Isidella* sp. from AC645 clustered with a congener from GenBank and formed a well-supported clade with *Lepidisis*, also an isidid. *Acanthogorgia armata* from the MC462 clustered with an *Acanthogorgia* species, but *Acanthogorgia angustiflora* fell outside of this clade, as it did in the analysis of McFadden and others (2006). The Stenogorgiinae sample from MC462 clustered with *Paramuricea multispina*, and these sequences varied little (0.22 percent sequence divergence).

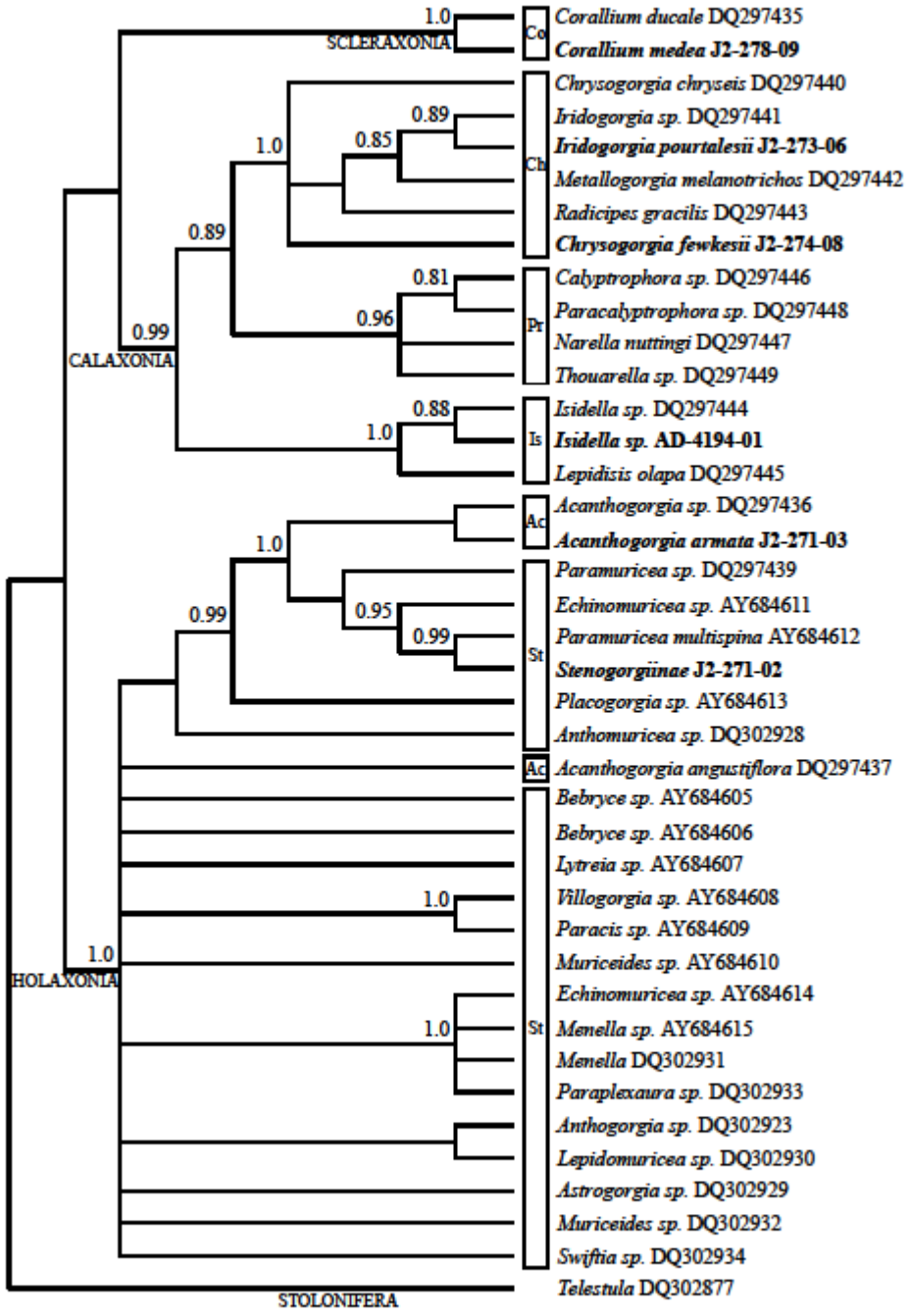

1.0
SCLERAXONIA
Co
Corallium ducale DQ297435
Corallium medea J2-278-09
Chrysogorgia chryseis DQ297440
Iridogorgia sp. DQ297441
Iridogorgia pourtalesii J2-273-06
Metallogorgia melanotrichos DQ297442
Radicipes gracilis DQ297443
Chrysogorgia fewkesii J2-274-08
Ch
0.89
0.85
1.0
0.89
0.81
0.96
Pr
Calyptrophora sp. DQ297446
Paracalyptrophora sp. DQ297448
Narella nuttingi DQ297447
Thouarella sp. DQ297449
0.99
CALAXONIA
0.88
1.0
Is
Isidella sp. DQ297444
Isidella sp. AD-4194-01
Lepidisis olapa DQ297445
Ac
Acanthogorgia sp. DQ297436
Acanthogorgia armata J2-271-03
1.0
Paramuricea sp. DQ297439
Echinomuricea sp. AY684611
Paramuricea multispina AY684612
Stenogorgiinae J2-271-02
Placogorgia sp. AY684613
Anthomuricea sp. DQ302928
St
0.99
0.95
0.99
Ac
Acanthogorgia angustiflora DQ297437
Bebryce sp. AY684605
Bebryce sp. AY684606
Lytreia sp. AY684607
Villogorgia sp. AY684608
Paracis sp. AY684609
Muriceides sp. AY684610
Echinomuricea sp. AY684614
Menella sp. AY684615
Menella DQ302931
Paraplexaura sp. DQ302933
Anthogorgia sp. DQ302923
Lepidomuricea sp. DQ302930
Astrogorgia sp. DQ302929
Muriceides sp. DQ302932
Swiftia sp. DQ302934
St
1.0
1.0
1.0
HOLAXONIA
Telestula DQ302877
STOLONIFERA

Figure 6.4. Phylogenetic hypothesis for octocorals based upon Bayesian analysis of mitochondrial *ND2* sequences. (Species names in bold represent samples unique to this study, whereas others originate from GenBank. Numbers at nodes represent Bayesian posterior probabilities of node support. Vertical bars indicate the following families: Ac, Acanthogorgiidae; St, Stenogorgiinae; Ch, Chrysogorgiidae; Pr, Primnoidae; Is, Isididae; Co, Coralliidae.)

6.4 Discussion

The deep sea has long been looked to for important clues to anthozoan evolution (Lindler and others, 2008; Kitahara and others, 2010a). Collections made as part of this project add to our knowledge of diversity of corals in the deep GOM and confirm the presence of deep corals in proximity to chemosynthetic sites. In many cases, the scleractinian and octocoral species collected were attached to exposed authigenic carbonates, concordant with the idea that such carbonates provide a direct link between cold seeps and coral communities (Fisher and others, 2007; Cordes and others, 2009).

Our analysis of the "robust" scleractinian corals included species belonging to families that typically do not form monophyletic groups: the Oculinidae and the Caryophylliidae (Romano and Cairns, 2000; Le Goff-Vitry and others, 2004; Kitahara and others, 2010a; b). Confirming previous phylogenetic analyses (Romano and Cairns, 2000; Le Goff-Vitry and others, 2004; Kitahara and others, 2010a; b), the Caryophyllidae appears to be an unnatural grouping given the scattered placement of species, even among the "robust" corals. However, results of phylogenetic analyses using different mitochondrial gene regions and different taxa sets support consistent grouping of *Caryophyllia* and *L. pertusa*, and several other species. Similar to findings of Kitahara and others (2010b), all *Caryophyllia* species appeared monophyletic, but this grouping was not exclusive, as *Lophelia pertusa*, *Desmophyllum*

dianthus, *Cyathoceras squiresi*, and *Dasmosmilia lymani* were included in this clade as well. Although members of this clade occur in both Atlantic and Pacific oceans, these taxa appear closely related according to the 16S phylogeny. As has been noted previously in the literature (for example, Shearer and others, 2002), scleractinian mitochondrial DNA evolves slowly. Therefore, the application of additional polymorphic markers is necessary for a better understanding of the relationships among the genera and species within this diverse clade of deep-sea corals.

Although similar types of species comprise chemosynthetic communities on the upper and lower continental slope in the GOM, little species overlap has been found (Cordes and others, 2007), with *Madrepora oculata* now being an exception to this observation. Similarly, it is intriguing that the two *Caryophyllia polygona* sequences representing samples from the upper continental slope and a deeper chemosynthetic site (MC-462) did not cluster together and were fairly divergent. This result was surprising, given the lack of species-level variability in coral mitochondrial DNA (Shearer and others, 2002), yet could be indicative of a cryptic species. However, *Madrepora oculata* from upper and lower continental slope sites differed little at the 16S gene and clustered together. Based upon the depth range of *L. pertusa* (146-1,200 m, Cairns and others, 2009), its presence was anticipated at depths investigated in this study, yet it was not found. Whether a species changeover occurs between upper and lower continental slope habitats for scleractinian coral species requires further investigation.

Octocorals from the GOM remain poorly known (Cairns and Bayer, 2009). The diverse octocoral collections made from five sites on the lower continental slope of the GOM add to our knowledge of this important faunal group. These collections contained at least 10 species from 3 alcyonacean sub-orders. Three of these octocoral samples have not been collected in the GOM

previously; *Acanthogorgia armata*, *Iridogorgia pourtalesii*, and *Corallium medea* (Cairns and Bayer, 2009). All of these species appeared closely related to congeners in our analysis, but the latter species, *C. medea*, was quite divergent from a congener *C. ducale*. Bamboo coral samples from the GOM were related to other corals in the family Isididae, and an *Isidella* sample from AC645 was the most genetically unique of the GOM bamboo corals. Molecular data confirmed the morphologically based taxonomic identifications and, as such, may be useful in guiding identification in the future when necessary. Although this survey increases our knowledge of octocoral biodiversity in the deep GOM, much taxonomic and systematic work remains before this GOM octocoral biodiversity inventory is complete.

6.5 Acknowledgments

The author wishes to thank TDI-Brooks International, Inc., C. Fisher, H. Roberts, the Bureau of Ocean Energy Management, Regulation and Enforcement (BOEMRE), and the National Oceanic and Atmospheric Administration (NOAA) for allowing her to participate in their DSV *Alvin* Chemo III research cruise. She also thanks E. Cordes and C. Kellogg for assistance in sample collection on the ROV *Jason2* research cruise, Dr. Stephen Cairns for specimen identification, R. Johnson for laboratory work, M. Springmann for creating sampling map, as well as the crews of the R/V *Atlantis*, the R/V *Ronald H. Brown*, the DSV *Alvin* and ROV *Jason2*.

7. COMMUNITY STRUCTURE OF MESOPELAGIC FISHES OVER THE SLOPE OF THE NORTH-CENTRAL GULF OF MEXICO

An expanded version of this paper was published as
Ross, S.W., Quattrini, A.M., Roa-Varón, A.Y., and McClain, J.P., 2010, Species composition and distributions of mesopelagic fishes over the slope of the north-central Gulf of Mexico, *in* Roberts, H.H., ed., Gulf of Mexico cold seeps: Deep-Sea Research II, v. 57, no. 21-23, p. 1926-1956.

By Steve W. Ross, Andrea M. Quattrini, Adela Y. Roa-Varón, and Jennifer P. McClain

University of North Carolina Wilmington, Center for Marine Science, Wilmington, NC

7.1 Introduction

The Gulf of Mexico (GOM) is a large, semi-enclosed, warm-temperate to subtropical marine system. Its maximum depth is around 3,700 m, and its bathymetry is quite complicated, exhibiting numerous canyons, escarpments, and topographic features (Bryant and others, 1990; Roberts and Aharon, 1994). GOM circulation is dominated by the Loop Current (LC), which originates at the Yucatan Channel, flows northward into the GOM, and exits via the Straits of Florida (Oey and others, 2005). Large, warm-core LC eddies periodically detach from the LC and propagate northwest or west, impinging on the slope (for example, Smith, 1986; Oey and others, 2005). Numerous cold-core eddies often form adjacent to the LC and warm-core LC eddies (Oey and others, 2005). The cyclonic and anti-cyclonic eddies may produce conditions that move nutrient-rich deep waters into the upper water column as they move along or across the slope (Biggs and others, 2005; Jochens and DiMarco, 2008). In addition to the complexities of the LC and its eddy fields, wind-induced currents play a role over deep GOM waters (Carnes and others, 2008), and riverine discharge can bring lower salinities and nutrients to deep areas (Morey and others, 2003; Hamilton and Lee, 2005). Variability in water properties is negligible

at depths greater than 800 m (Jochens and DiMarco, 2008). Thus, the complicated interactions between topography and currents probably impact species distributions and community composition of the slope mesopelagic fauna. In turn, the vertically migrating and possibly horizontally displaced mesopelagic fishes play a major role in moving energy through the water column, even from surface to bottom (Angel and Pugh, 2000; Gartner and others, 2008).

Since their recent discovery in the northern GOM (Paull and others, 1984; Kennicutt and others, 1985), the importance of cold seep (chemosynthetic) communities has been increasingly recognized. Benthic water chemistry at these sites is characterized by hydrocarbon (for example, methane) or sulfide seepage, presence of gas hydrates, and hypersaline water (Paull and others, 1984; Sibuet and Olu, 1998; Van Dover, 2000). This unusual water chemistry and associated substrata, including carbonate buildups (Roberts and Aharon, 1994), support unique biota dominated by sulfide-oxidizing or methanotrophic endosymbiotic organisms (Hecker, 1985; MacDonald and others, 1990). Various studies have concentrated on the chemosynthetic and associated seep or vent fauna (typically siboglinid tubeworms, mytilidid and vesicomyid bivalves, and hosionid polychaetes) (for example, Van Dover, 2000); however, data on the larger, surrounding fauna, especially the midwater fauna that occur over these areas, are usually lacking. Herring (1998; 2006) listed some of the micronekton, especially young bresiliid and alvinocaridid shrimps, captured over hydrothermal vents. At least in the GOM, hydrocarbon (liquid and gaseous) seepage reaches to the surface (MacDonald and others, 2002) and thus, has an opportunity to impact midwater fauna. Whether such deep benthic seeps or vents influence fishes or other fauna that occur above them is unknown.

Hydrocarbon seeps often co-occur with cold-water coral mounds, and the seeps have been implicated in coral mound formation (Hovland and others, 1998; Hovland and Risk, 2003).

However, there appears to be little evidence that seeps cause coral mound formation or attract *L. pertusa* (Duineveld and others, 2004; Williams and others, 2006). Nevertheless, there is usually massive carbonate deposition in cold-seep areas (Aharon, 1994) that, along with corals, results in two different types of carbonate substrata from different processes (Roberts and Aharon, 1994). Both habitats influence local communities by providing physical structure and trophic inputs. Cold-water corals are widely distributed on a variety of hard substrata in the GOM but are only densely developed at a few sites (Brooke and Schroeder, 2007).

Considering the size and importance of the GOM, research on the overall midwater fauna has been relatively restricted. The most expansive survey of GOM midwater fishes resulted from 35 stations (R/V *Alaminos*, 1965-1973), most over water depths >1,500 m, sampled from the surface to depth with nonclosing nets (Murdy and others, 1983). Murdy and others (1983) indicated that relatively few fish taxa dominated the GOM midwater fauna; however, one of their most abundant taxa (*Cyclothone* spp.) was not identified to species level. Bright and Pequegnat (1969) reviewed the Sternoptychidae from a portion of the Alaminos stations (see Murdy and others, 1983) and reported that the family was concentrated between 250 and 1,500 m. Backus and others (1970; 1977) did not provide explicit data for the GOM stations (all with nonclosing nets) used (along with other Atlantic data) to characterize Atlantic basin mesopelagic fish distribution patterns and myctophid zoogeography, respectively. Bangma and Haedrich (2008) reanalyzed 27 GOM stations (1961-1969) from the same archive used by Backus and others (1970; 1977), concluding that the GOM mesopelagic fish community contained more species than neighboring regions and defined a unique ecotone. Neither Murdy and others (1983), Backus and others (1970; 1977), nor Bangma and Haedrich (2008) presented diel or vertical distribution data for the GOM mesopelagic fishes. Most GOM studies on midwater fishes were

concentrated in the eastern Gulf of Mexico, especially around one station (off west-central Florida), and resulted in treatments of selected fish taxa (for example, gonostomatids: Lancraft and others, 1988; myctophids: Gartner and others, 1987; Hopkins and Baird, 1985a; sternoptychids: Hopkins and Baird, 1985b; stomiids: Sutton and Hopkins, 1996a). These studies mostly relied upon opening-closing Tucker trawls similar to the net used in our study, which allowed analyses of vertical distributions.

Most previous research on seep and deep-coral sites in the GOM was focused in depths <1,000 m. The first sampling of GOM chemosynthetic communities in waters >1,000 m revealed that the benthic invertebrate fauna differed from that of the shallower Louisiana upper slope (Cordes and others, 2007). No such depth-related comparisons are available for the overall GOM midwater fish fauna, and the only regional comparative treatment (Bangma and Haedrich, 2008) did not provide specific data on species composition or distributions. In collaboration with ongoing benthic studies (see Roberts and others, 2007a), we sampled the midwater fauna over three cold-seep habitats >1,000 m and a comparative area over a cold-water coral bank (<1,000 m) in the north-central GOM. Our goal was to intensively sample a few widely separated sites, covering most of the water column at each site over the 24-hour (h) period. Our objectives for this project were to (1) characterize the vertical distributions of the mesopelagic fishes, (2) describe diel migration patterns of the fishes, (3) describe size composition of the midwater fish fauna, and (4) compare ichthyofaunal composition and behavior among the three offshore, deeper sites and the inshore (coral bank) study site.

7.2 Methods

The study areas were over three lower slope cold-seep sites and one upper slope deep-sea coral site in the north-central to western GOM (fig. 7.1). As part of a larger multidisciplinary

project, these sites were also visited in companion study cruises and were sampled with a variety of methods (Roberts and others, 2007a; see other chapters in this report). Sampling for this study was accomplished August 9-29, 2007, using the R/V *Cape Hatteras* (Duke-UNC Oceanographic Consortium). New moon occurred on August 12, and full moon occurred on August 28.

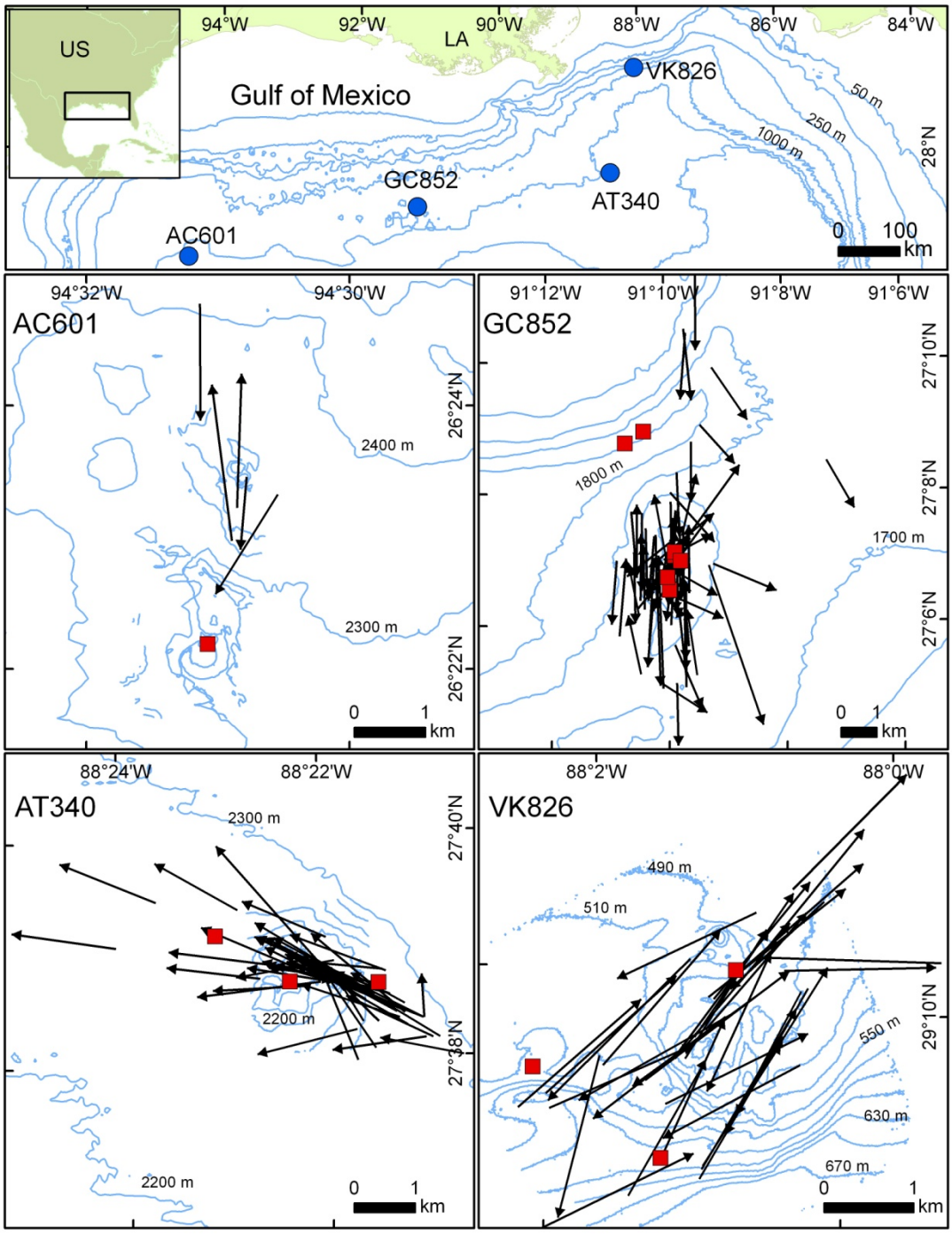


Figure 7.1. Study area locations in the north-central Gulf of Mexico sampled August 9-29, 2007. Three sites (AC601, GC852, and AT340) were over cold-seep habitats, and VK826 was over a coral-carbonate and seep habitat. Vector lines represent Tucker trawl tows and boxes are conductivity, temperature, and depth profiler (CTD) stations.

7.2.1 Study Areas

The three offshore seep sites were mapped, named, and described by Roberts and others (2007b). The easternmost study area in Atwater Valley Lease Block 340 (AT340) contained several mounds on a topographic high, and we sampled here over bottom depths of 2,001 to 2,397 m. The bottom in this area contained authigenic carbonate substrata, soft sediments, a brine seep, and chemosynthetic communities (Roberts and others, 2007b). The dominant bottom feature of the central study site (278 km west of AT340) in Green Canyon Lease Block 852 (GC852) was a north-south oriented ridge about 2 km long whose crest contained carbonate hard substrata, exhibited oil seepage, and was colonized by corals and chemosynthetic communities (Roberts and others, 2007b). The bottom around this ridge was composed of dense muddy sediments. Our samples at GC852 ranged over bottom depths of 1,301 to 1,785 m. The westernmost seep study area (328 km west of GC852) was located in Alaminos Canyon Lease Block 601 (AC601) and exhibited relatively low topography compared with the above sites. The bottom was characterized by a large brine lake, carbonate hard substrata, and chemosynthetic communities (Roberts and others, 2007b). This site had high methane concentrations in the water column. Our few samples in this area were over bottom depths of 2,306-2,523 m.

The inshore study area was over a well known deep-sea coral site named Vioska Knoll 826 (VK826, 156 km north of AT340). This site has two mound areas and represents the best developed *Lophelia pertusa* community in the GOM (Brooke and Schroeder, 2007). The bottom

was characterized by carbonate blocks, extensive scleractinian and antipatharian corals, hydrocarbon seeps, and chemosynthetic communities surrounded by soft substrata. Our sampling was conducted over bottom depths of 448 to 645 m.

During the study period the LC and associated rings may have impacted the midwater region of only one (AT340) of the four sampling sites (fig. 7.2). The Naval Research Laboratory Layered Ocean Model showed the LC extending to ~ 175 km south of AT340 and a large (~200 km diameter), warm-core LC ring to the west of AT340 that was present during the entire sampling period. In the beginning of the sampling period, the eastern edge of the LC ring overlaid AT340; the ring then moved west (up to 20 km) from AT340, but later reattached to the LC on August 23, 2008, as the LC extended ~100 km northward into the GOM. AT340 was between the edge of this LC ring and a cold core ring to the east during the time this site was sampled (August 20-25). Cyclonic cold-core eddies (~50-150 km diameter) present during this period were usually distant (~20 –100 km) from our sampling sites.

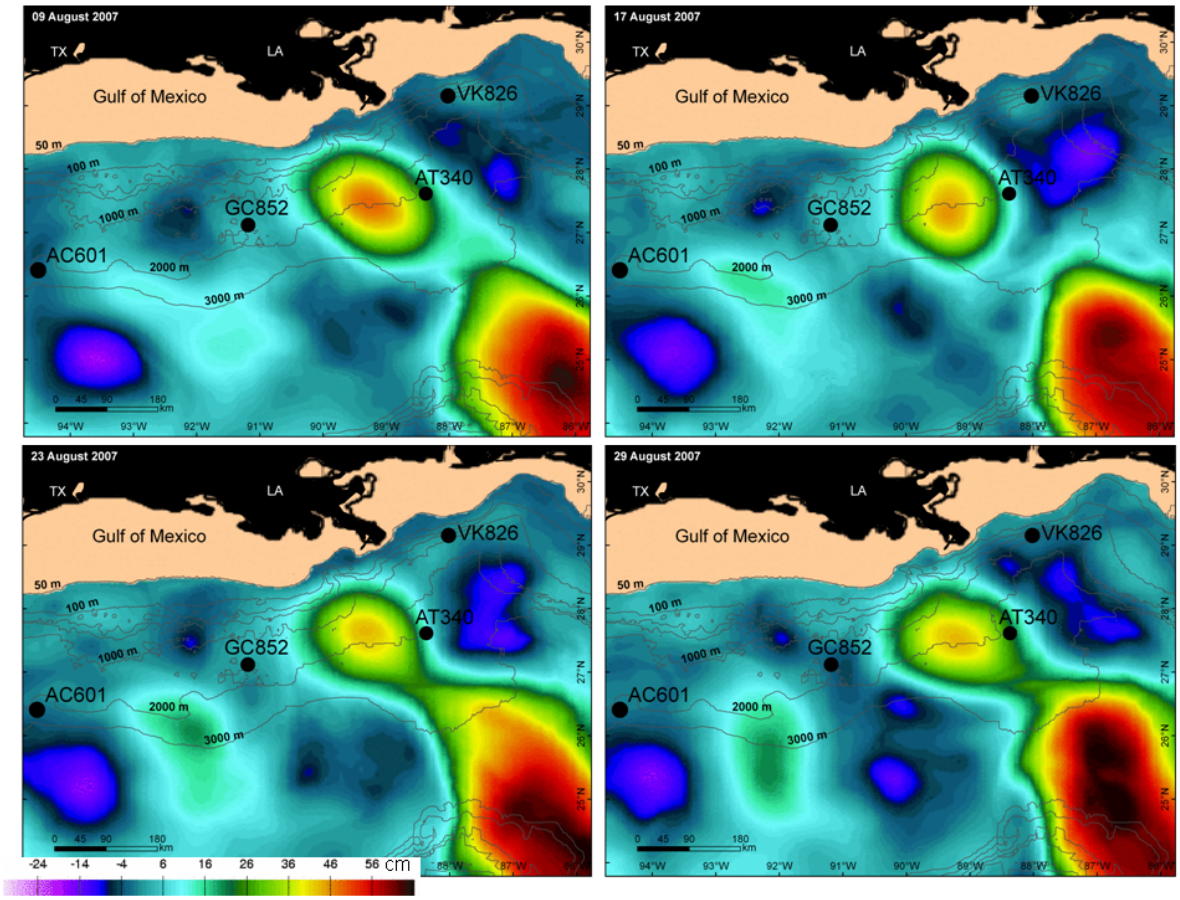


Figure 7.2. Naval Research Lab Layered Ocean Model (high resolution) assimilating Sea Surface Height (SSH) for four dates during our study period. The anomaly SSH satellite maps show the difference in measured sea height from the calculated, mean sea level. A +17 cm SSH contour generally tracks the loop current (LC) and loop current rings. Reds and yellows represent higher SSH and warmer waters of the LC and LC eddies. Blue to purple colors are negative SSH anomalies representing cold-core cyclonic eddies.

Periodically, one (AC601) to seven (GC852) conductivity, temperature, and depth (CTD) (SeaBird 911*plus*) casts were made in each study area to record profiles of temperature (ºC), salinity, dissolved oxygen (ml/L), and fluorescence (µg/L) through the water column (fig. 7.3). Fluorescence data exhibited typical similar patterns at all sites, with peak fluorescence levels in

the upper 120 m range. Higher fluorescence levels (up to 2.5 µg/L) in shallower waters (~35-65 m) were exhibited at the inshore VK826 site compared to the three offshore sites (up to 1.2 µg/L, ~80-120 m), suggesting higher primary productivity likely related to increased nutrient input from Mississippi River outflow. The AT340 site appeared most different in water column profiles compared with the other sites, perhaps due to influence of a nearby warm-core LC ring (fig. 7.2). While all sites exhibited the high salinity Subtropical Underwater signature (Jochens and DiMarco, 2008) in the upper 200 m (fig. 7.3), this indicator of LC water was strongest and occurred deeper at AT340. The oxygen minimum layer reached a similar minimum value at all sites; however, it was deeper (~160-720 m) and more variable at AT340, compared with AC601 (~100-600 m), GC852 (~120-680 m), and VK826 (~80-640 m). Warm water also persisted deeper at AT340, with a temperature of ~20º C at 200 m, versus ~13-16º C at 200 m at the other sites (fig. 7.3).

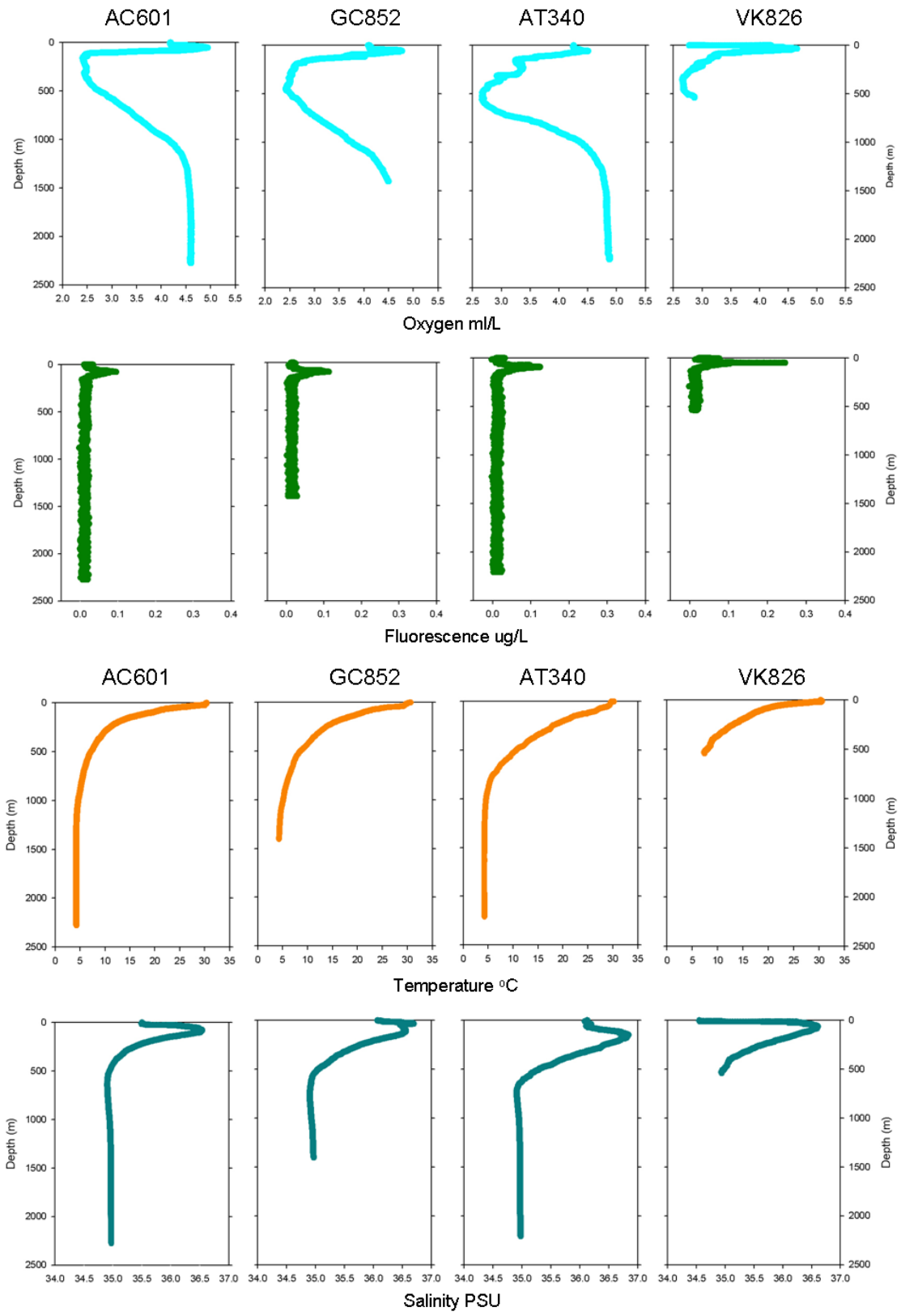

AC601
GC852
AT340
VK826
Depth (m)
Oxygen ml/L
Fluorescence ug/L
Temperature °C
Salinity PSU

Figure 7.3. Conductivity, temperature, depth (CTD) (SeaBird 911*plus*) casts at the four Gulf of Mexico study sites, illustrating dissolved oxygen (milliliters per liter), fluorescence, water temperature (degrees Celsius), and salinity profiles (practical salinity units).

7.2.2 Tucker trawl

Discrete depth Tucker trawling was conducted from the surface to 1,377 m. A plankton net (0.5 m diameter, 335 μm mesh) was suspended in the center mouth opening of the Tucker trawl (TT, 2 x 2 m, 1.59 mm mesh), providing two separate samples per deployment. Catches of juvenile and adult fishes from both of these nets were combined to give one sample per tow. The TT was deployed in an open position (fig. 7.4). Because of the rapid lowering, steep wire angle, and minimal forward movement, we assumed there was little fishing as the net was deployed. When it reached the target depth, it was towed for 30 min at about 2 km (3.71 km/h) ground speed, usually against the current. A weighted messenger was then sent down the ship's wire to trigger the net closed, after which it was retrieved. This was repeated as many times as possible throughout the day and night to cover most of the water column during the time the ship occupied the sampling area. A temperature-depth recorder (TDR, SeaBird SBE39) was attached to the upper net bar to record time, depth, and temperature every 5 seconds (s) during each tow. Actual fishing depth was determined after each trawl from the TDR data and was used to adjust fishing methods to achieve desired sampling depths.

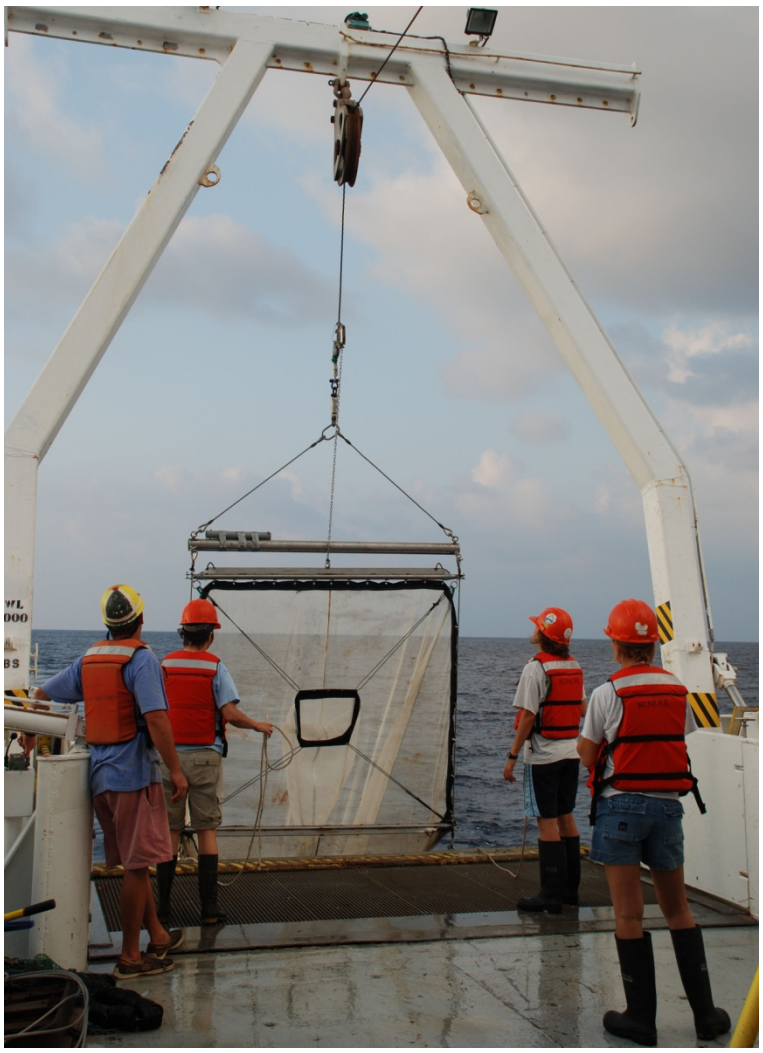


Figure 7.4. Tucker trawl being deployed over the stern of the R/V *Cape Hatteras* on August 13, 2007.

All fishes were fixed in 10-percent formalin seawater solution, and after the cruise were transferred to 50-percent isopropanol. Juvenile and adult specimens were identified to the lowest possible taxa and were measured to the nearest millimeter standard length (SL). Species captured (for example, monacanthids, carangids, exocoetids) that were exclusive to surface waters were eliminated from analyses.

7.2.3 Data Analysis

Data were analyzed to examine differences in species composition and distribution among sites, depths, and time of day. Time of day was divided into four categories: day (0730 to 1830 h CDT), night (2030 to 0530 h CDT), dawn (0530 to 0730 h CDT, 1 h on either side of average sunrise) and dusk (1830 to 2030 h CDT, 1 h on either side of average sunset). Mean fished depths per TT tow were calculated by averaging all depths recorded by the TDR from tow start to end time, and these were used in depth analyses and for depth ranges of each species. Samples from TTs which failed to close properly were excluded from most statistical analyses but were included in the overall species list and the relative abundance table. Because only a few night samples were taken at AC601, this site was excluded from univariate statistical analyses.

Multivariate analyses were used to examine differences in mesopelagic fish assemblages throughout the study area. All analyses were conducted in PRIMER 6.1 based on Clarke and Warwick (2001) and Clarke and Gorley (2006). Species' abundances were standardized per sample by dividing the number of individuals per species by the total number of fishes per sample. Next, standardized abundances were fourth-root transformed to down weight the common species relative to the rare species. Third, similarities between samples were calculated using a Bray-Curtis similarity coefficient. Hierarchical clustering with group average linking and nonmetric multidimensional scaling (MDS) analyses were then performed on the resulting Bray-Curtis similarity matrix; these analyses were performed on all samples combined (115 stations, 117 species) and then only for samples from each site (GC852=50 stations, 91 species; AT340=35 stations, 71 species; VK826=25 stations, 51 species). A one-way analysis of similarities (ANOSIM) was used to test if fish assemblages were significantly different (R=0 when groups are similar; R=1 when groups are different, Clarke and Warwick, 2001) among the

study sites. Similarity profile (SIMPROF) tests run during the hierarchical clustering analyses determined if there were significant groupings among the samples in each dendrogram, allowing us to determine patterns in mesopelagic fish assemblages among depths, time of day, and sites without assigning a priori factors to the dataset. Samples on the MDS plots were then coded for their respective SIMPROF groups assigned by PRIMER. Samples on each dendrogram were also labeled with SIMPROF codes and embedded within the respective MDS plot; the dendrograms were configured to save space by collapsing higher order clusters that were not significant into the level at which they were significant. In addition, mean depths of each sample were superimposed as bubbles onto the original MDS plots. If significant differences were found using ANOSIM or SIMPROF, the similarity percentages routine (SIMPER) was used to determine which species contributed to assemblage dissimilarities. SIMPER determines the contribution of particular species to the average dissimilarities among groups and to the average similarities of samples within each group. Because mesopelagic fishes often move vertically during dawn and dusk, including these data in the ANOSIM and SIMPROF analyses may obscure patterns. Thus, we omitted twilight stations (n=35) from these analyses.

Species richness and relative abundances were examined among sites. Species accumulation curves illustrated how thoroughly the mesopelagic fish fauna was sampled at each site and were used to compare species richness among sites. Curves were calculated with species collected in discrete depth TTs using the Colwell and others (2004) analytical method in EstimateS (ver. 8.0). Analysis of covariance (ANCOVA), followed by a Tukey post-hoc multiple comparison test (SPSS ver. 17) on log transformed species richness, was used to determine whether species accumulations were significantly different between GC852, AT340 and VK826. Relative abundances were calculated for most taxa (excluding genera groupings of

multiple species) collected at all sites (percent total catch per taxon) and at each site (percent total catch of each taxon per site). Frequencies of occurrence were calculated for each taxon collected at all sites (number of samples in which species occurred / total number of samples) and at each site (number of samples in which species occurred per site / total number of samples per site). Depth and temporal distributions of the dominant species (n = 11 species, bold in table 7.1) collected at GC852, AT340, and VK826 were examined in further detail.

Table 7.1. Relative abundance of species comprising ~90 percent of total catch at each site.

[Top 11 species in bold. Species are listed in order of overall percent relative abundance. Numbers in parentheses are frequencies of occurrence. n = number of stations, * = species presence <0.8 percent of total catch per site]

Species	AC601 n=5	GC852 n=69	AT340 n=49	VK826 n=36	Total n=159
Notolychnus valdiviae	*	20.0 (0.6)	11.5 (0.6)	5.0 (0.3)	15.4 (0.5)
Cyclothone pallida	18.2 (0.2)	16.1 (0.6)	6.0 (0.3)	*	11.5 (0.3)
Cyclothone pseudopallida	10.0 (0.2)	13.5 (0.6)	5.5 (0.3)	3.0 (0.2)	10.1 (0.4)
Cyclothone braueri	1.2 (0.4)	11.0 (0.5)	8.3 (0.4)	9.9 (0.3)	10.0 (0.4)
Cyclothone alba	1.2 (0.4)	10.7 (0.5)	6.2 (0.3)	12.4 (0.4)	9.6 (0.4)
Valenciennellus tripunctulatus	15.3 (0.6)	1.9 (0.4)	7.0 (0.6)	17.0 (0.5)	5.4 (0.5)
Vinciguerria poweriae	1.2 (0.4)	5.6 (0.5)	3.7 (0.5)	3.6 (0.4)	4.8 (0.5)
Lepidophanes guentheri	8.8 (0.4)	1.0 (0.4)	12.1 (0.7)	*	3.9 (0.4)
Benthosema suborbitale	9.4 (0.6)	1.7 (0.4)	7.6 (0.7)	2.6 (0.3)	3.5 (0.5)
Cyclothone acclinidens	*	3.3 (0.4)	0.8 (0.1)		2.2 (0.2)
Hygophum benoiti	3.5 (0.2)	1.5 (0.2)	1.8 (0.4)	3.8 (0.4)	1.9 (0.3)
Diaphus dumerilii	1.8 (0.2)	*	*	11.5 (0.6)	1.7 (0.2)
Myctophum affine	*	1.3 (0.4)	*	5.7 (0.4)	1.6 (0.3)
Pollichthys mauli		*	3.6 (0.4)	4.3 (0.3)	1.5 (0.2)
Gonostoma elongatum		*	3.0 (0.5)	*	1.3 (0.3)
Ceratoscopelus warmingii	4.1 (0.4)	*	2.5 (0.5)		1.2 (0.3)
Chauliodus sloani	1.2 (0.2)	1.1 (0.3)	*	1.2 (0.3)	1.0 (0.3)
Lampanyctus alatus	3.5 (0.6)	0.9 (0.3)	1.5 (0.3)		1.0 (0.3)
Vinciguerria nimbaria	*	*	1.2 (0.2)	1.8 (0.2)	0.9 (0.2)
Hygophum taaningi	*	*	2.0 (0.4)	2.1 (0.3)	0.8 (0.2)
Argyropelecus aculeatus	1.8 (0.4)	*	0.9 (0.2)	1.5 (0.3)	0.8 (0.2)
Diaphus mollis	*	*	0.8 (0.3)	1.3 (0.3)	0.6 (0.2)
Argyropelecus hemigymnus	1.2 (0.2)	*	0.8 (0.2)	*	0.6 (0.2)
Diaphus splendidus			2.2 (0.3)	*	0.6 (0.1)
Bregmaceros atlanticus		*	0.9 (0.2)	1.4 (0.2)	0.5 (0.2)
Vinciguerria attenuata	1.8 (0.2)	*	*	*	0.5 (0.2)
Melamphaes simus	4.1 (0.6)	*	*	*	0.4 (0.2)
Diogenichthys atlanticus	4.1 (0.2)	*	*		0.2 (0.1)
Synagrops spinosus		*		0.8 (0.1)	0.2 (0.1)
Bathophilus pawneei		*		1.0 (0.3)	0.1 (0.1)
Diaphus perspicillatus				0.8 (0.1)	0.1 (0.03)

7.3 Results

The 159 Tucker trawl stations (table 7.2, fig. 7.1) yielded 9,802 individuals, representing at least 126 species (30 families) of juvenile and adult midwater fishes (table 7.3). In terms of species richness, the collections at all sites were dominated by Myctophidae (lanternfishes, 38 species), Stomiidae (dragonfishes, 17 species), Gonostomatidae (lightfishes, 12 species), and Sternoptychidae (hatchetfishes, 10 species) (table 7.3). The Gonostomatidae dominated in overall relative abundance (56 percent of the total catch), followed by the Myctophidae (28 percent), Phosichthyidae (lightfishes, 6 percent), and Sternoptychidae (6 percent), all four families accounting for about 96 percent of the individuals collected.

One species, *Sphyraenops bairdianus*, a deepwater cardinalfish, Epigonidae (3 from VK826), appears to be a new record for the Gulf of Mexico. Two forms of *Cyclothone*, sp. 1 and sp. 2, did not match known species descriptions. Additional investigation may prove these to be undescribed species. Current data on the family Howellidae did not allow a generic or species level classification, and it is possible that we collected more than one species. One species, *Stemonosudis bullisi*, a barracudina, Paralepididae, considered to be endemic to the GOM, was collected at VK826 (table 7.3).

Table 7.2. Stations sampled at four sites (see fig. 7.1) in the Gulf of Mexico (August 9-29, 2007).

[D, day (0630 to 1920 h CDT); N, night (1920 to 0630 h CDT); N/D, samples covering both day and night; CTD, Seabird SBE 911+; TT, Tucker trawl; m, meters; *, net did not close; ^, net fished on bottom for portion of tow; NR, not recorded]

Station #	Gear	Date	Location	Time	Start Latitude	Start Longitude	End Latitude	End Longitude	Min Sample Depth (m)	Max Sample Depth (m)
CH-2007-001	CTD	9-Aug-07	GC852	N	27° 06.505	91° 09.964	27° 06.659	91° 09.893	Surface	1,406
CH-2007-002	TT	9-Aug-07	GC852	N	27° 07.200	91° 09.769	27° 06.278	91° 09.945	561	705
CH-2007-007*	TT	10-Aug-07	GC852	N	27° 07.318	91° 09.736	27° 08.244	91° 09.480	Surface	296
CH-2007-008	TT	10-Aug-07	GC852	N	27° 06.984	91° 09.844	27° 06.034	91° 10.025	272	39 8
CH-2007-010	CTD	10-Aug-07	GC852	N/D	27° 07.023	91° 09.863	27° 06.802	91° 09.058	Surface	1,551
CH-2007-017	TT	10-Aug-07	GC852	N	27° 07.407	91° 09.878	27° 06.436	91° 09.948	594	655
CH-2007-018*	TT	10-Aug-07	GC852	N	27° 06.354	91° 09.929	27° 07.386	91° 09.858	Surface	486
CH-2007-019	TT	10-Aug-07	GC852	N	27° 07.823	91° 09.780	27° 06.975	91° 09.917	392	426
CH-2007-020	TT	11-Aug-07	GC852	N	27° 05.979	91° 09.940	27° 07.053	91° 09.864	194	210
CH-2007-021	TT	11-Aug-07	GC852	N	27° 07.074	91° 09.893	27° 06.278	91° 09.982	232	292
CH-2007-022	TT	11-Aug-07	GC852	N	27° 06.550	91° 09.957	27° 07.604	91° 09.886	316	441
CH-2007-023*	TT	11-Aug-07	GC852	N	27° 07.715	91° 09.851	27° 06.680	91° 09.963	Surface	712
CH-2007-025	CTD	11-Aug-07	GC852	N/D	27° 07.080	91° 09.868	27° 06.878	91° 10.230	Surface	1,466
CH-2007-029	TT	11-Aug-07	GC852	D	27° 10.481	91° 09.684	27° 09.381	91° 09.547	630	836
CH-2007-030	TT	11-Aug-07	GC852	N	27° 07.591	91° 09.272	27° 06.815	91° 09.939	373	403
CH-2007-031*	TT	11-Aug-07	GC852	N	27° 06.280	91° 10.229	27° 07.036	91° 09.676	Surface	382
CH-2007-032	TT	11-Aug-07	GC852	N	27° 07.289	91° 09.857	27° 06.706	91° 10.685	468	558
CH-2007-033	TT	12-Aug-07	GC852	N	27° 06.204	91° 10.505	27° 07.071	91° 09.742	237	304
CH-2007-035	TT	12-Aug-07	GC852	N	27° 07.282	91° 09.630	27° 06.444	91° 10.342	146	181
CH-2007-036	TT	12-Aug-07	GC852	N	27° 06.467	91° 10.403	27° 07.270	91° 09.604	144	174
CH-2007-037	TT	12-Aug-07	GC852	N	27° 07.284	91° 09.637	27° 06.455	91° 10.320	172	212
CH-2007-038	TT	12-Aug-07	GC852	N	27° 06.779	91° 10.077	27° 08.391	91° 08.713	455	467
CH-2007-044	TT	12-Aug-07	GC852	D	27° 12.181	91° 09.454	27° 10.146	91° 09.474	745	895
CH-2007-045	TT	12-Aug-07	GC852	N	27° 08.756	91° 09.574	27° 07.816	91° 09.565	538	592

Table 7.2. Stations sampled at four sites (see fig. 7.1) in the Gulf of Mexico (9-29 August 2007).—Continued

[D, day (0630 to 1920 h CDT); N, night (1920 to 0630 h CDT); N/D, samples covering both day and night; CTD, Seabird SBE 911+; TT, Tucker trawl; m, meters; *, net did not close; ^, net fished on bottom for portion of tow; NR, not recorded]

Station #	Gear	Date	Location	Time	Start Latitude	Start Longitude	End Latitude	End Longitude	Min Sample Depth (m)	Max Sample Depth (m)
CH-2007-046*	TT	12-Aug-07	GC852	N	27° 06.034	91° 09.704	27° 05.290	91° 09.704	Surface	325
CH-2007-047	TT	12-Aug-07	GC852	N	27° 05.665	91° 09.647	27° 06.713	91° 09.744	245	361
CH-2007-048	TT	12-Aug-07	GC852	N	27° 07.203	91° 09.795	27° 06.338	91° 09.781	170	210
CH-2007-049	TT	13-Aug-07	GC852	N	27° 06.454	91° 09.621	27° 07.493	91° 09.626	425	537
CH-2007-050	TT	13-Aug-07	GC852	N	27° 07.626	91° 09.789	27° 06.726	91° 09.752	626	757
CH-2007-053	TT	13-Aug-07	GC852	N	27° 06.415	91° 09.700	27° 05.454	91° 09.695	466	539
CH-2007-054	TT	13-Aug-07	GC852	N	27° 08.270	91° 09.827	27° 07.304	91° 09.805	236	248
CH-2007-056	CTD	13-Aug-07	GC852	D	27° 06.697	91° 09.993	27° 06.559	91° 10.018	Surface	1,438
CH-2007-063	TT	13-Aug-07	GC852	N	27° 07.349	91° 10.193	27° 06.229	91° 10.163	388	447
CH-2007-064	TT	13-Aug-07	GC852	N	27° 05.002	91° 10.098	27° 06.993	91° 10.152	187	224
CH-2007-065	TT	13-Aug-07	GC852	N	27° 07.318	91° 10.205	27° 06.339	91° 10.338	146	178
CH-2007-066	TT	13-Aug-07	GC852	N	27° 06.364	91° 10.472	27° 07.361	91° 10.404	591	732
CH-2007-068	TT	14-Aug-07	GC852	N	27° 07.655	91° 10.406	27° 06.498	91° 10.396	608	739
CH-2007-070	TT	14-Aug-07	GC852	N	27° 06.208	91° 10.393	27° 07.194	91° 10.422	472	531
CH-2007-071	TT	14-Aug-07	GC852	N	27° 07.462	91° 10.356	27° 06.522	91° 10.354	187	267
CH-2007-072	TT	14-Aug-07	GC852	N	27° 06.686	91° 10.480	27° 07.828	91° 10.481	334	460
CH-2007-073	CTD	14-Aug-07	GC852	N/D	27° 08.739	91° 10.685	27° 09.180	91° 11.330	Surface	1,711
CH-2007-080	TT	14-Aug-07	GC852	D	27° 10.379	91° 09.659	27° 09.372	91° 09.736	847	1,027
CH-2007-082	TT	14-Aug-07	GC852	D	27° 07.841	91° 09.948	27° 06.897	91° 09.925	191	232
CH-2007-083	TT	14-Aug-07	GC852	D	27° 05.076	91° 09.857	27° 04.136	91° 09.852	737	842
CH-2007-084	TT	14-Aug-07	GC852	N	27° 05.194	91° 09.691	27° 06.258	91° 09.810	432	565
CH-2007-085	TT	14-Aug-07	GC852	N	27° 07.000	91° 09.830	27° 06.055	91° 09.802	332	391
CH-2007-086	TT	14-Aug-07	GC852	N	27° 06.133	91° 09.832	27° 07.162	91° 09.915	689	972
CH-2007-087	TT	15-Aug-07	GC852	N	27° 07.043	91° 09.791	27° 06.157	91° 09.875	948	1,017
CH-2007-088	TT	15-Aug-07	GC852	N	27° 05.024	91° 09.692	27° 06.632	91° 09.703	501	572

Table 7.2. Stations sampled at four sites (see fig. 7.1) in the Gulf of Mexico (9-29 August 2007).—Continued

[D, day (0630 to 1920 h CDT); N, night (1920 to 0630 h CDT); N/D, samples covering both day and night; CTD, Seabird SBE 911+; TT, Tucker trawl; m, meters; *, net did not close; ^, net fished on bottom for portion of tow; NR, not recorded]

Station #	Gear	Date	Location	Time	Start Latitude	Start Longitude	End Latitude	End Longitude	Min Sample Depth (m)	Max Sample Depth (m)
CH-2007-089	TT	15-Aug-07	GC852	N	27° 07.452	91° 09.710	27° 06.456	91° 09.731	150	150
CH-2007-090	TT	15-Aug-07	GC852	N	27° 06.872	91° 10.013	27° 07.949	91° 10.191	412	472
CH-2007-091	CTD	15-Aug-07	GC852	D	27° 08.915	91° 10.371	27° 08.768	91° 09.104	Surface	1,793
CH-2007-092	TT	15-Aug-07	GC852	D	27° 08.468	91° 07.273	27° 07.702	91° 06.813	951	1,044
CH-2007-093	TT	15-Aug-07	GC852	D	27° 09.883	91° 09.198	27° 09.065	91° 08.610	790	1,035
CH-2007-094	TT	15-Aug-07	GC852	D	27° 09.020	91° 09.419	27° 08.421	91° 08.831	NR	NR
CH-2007-095	TT	15-Aug-07	GC852	D	27° 08.001	91° 09.940	27° 07.224	91° 09.176	551	664
CH-2007-096*	TT	15-Aug-07	GC852	D	27° 06.985	91° 10.006	27° 07.324	91° 09.216	Surface	569
CH-2007-097	TT	15-Aug-07	GC852	D	27° 06.474	91° 10.045	27° 06.090	91° 09.078	289	401
CH-2007-098	TT	15-Aug-07	GC852	N	27° 05.122	91° 10.161	27° 04.635	91° 09.361	693	756
CH-2007-099	TT	15-Aug-07	GC852	N	27° 06.869	91° 09.295	27° 04.437	91° 08.386	561	635
CH-2007-101	TT	16-Aug-07	GC852	D	27° 06.882	91° 09.207	27° 06.447	91° 08.140	443	569
CH-2007-102	CTD	16-Aug-07	GC852	D	27° 06.944	91° 09.769	27° 11.322	91° 14.384	S	1,554
CH-2007-103	TT	16-Aug-07	GC852	D	27° 07.981	91° 10.268	27° 07.663	91° 09.187	520	625
CH-2007-104	TT	16-Aug-07	GC852	D	27° 06.933	91° 10.182	27° 06.401	91° 09.166	NR	NR
CH-2007-105	TT	16-Aug-07	GC852	D	27° 06.146	91° 10.299	27° 05.025	91° 10.148	886	1,016
CH-2007-106	TT	16-Aug-07	GC852	D	27° 05.257	91° 10.480	27° 06.145	91° 10.673	1,004	1,144
CH-2007-107	TT	16-Aug-07	GC852	D	27° 07.714	91° 10.575	27° 06.697	91° 10.493	291	319
CH-2007-108	TT	16-Aug-07	GC852	N	27° 05.657	91° 09.916	27° 04.715	91° 09.464	130	149
CH-2007-110	TT	16-Aug-07	GC852	N	27° 05.265	91° 09.515	27° 06.212	91° 09.680	446	532
CH-2007-111	TT	16-Aug-07	GC852	N	27° 07.135	91° 09.955	27° 06.190	91° 10.241	81	110
CH-2007-112	TT	16-Aug-07	GC852	N	27° 06.082	91° 10.482	27° 06.929	91° 10.661	136	145
CH-2007-113	TT	17-Aug-07	GC852	N	27° 06.958	91° 10.845	27° 06.001	91° 10.946	53	87
CH-2007-114	TT	17-Aug-07	GC852	N	27° 05.795	91° 10.799	27° 06.978	91° 10.690	49	58
CH-2007-115	TT	17-Aug-07	GC852	N	27° 07.113	91° 10.611	27° 06.010	91° 10.542	77	86

Table 7.2. Stations sampled at four sites (see fig. 7.1) in the Gulf of Mexico (9-29 August 2007).—Continued

[D, day (0630 to 1920 h CDT); N, night (1920 to 0630 h CDT); N/D, samples covering both day and night; CTD, Seabird SBE 911+; TT, Tucker trawl; m, meters; *, net did not close; ^, net fished on bottom for portion of tow; NR, not recorded]

Station #	Gear	Date	Location	Time	Start Latitude	Start Longitude	End Latitude	End Longitude	Min Sample Depth (m)	Max Sample Depth (m)
CH-2007-116	TT	17-Aug-07	GC852	N	27° 05.701	91° 10.320	27° 07.004	91° 10.227	18	30
CH-2007-117	TT	17-Aug-07	GC852	N	27° 06.340	91° 10.268	27° 05.345	91° 10.336	516	611
CH-2007-118	TT	17-Aug-07	AC601	N	26° 23.314	94° 30.559	26° 22.580	94° 31.020	244	268
CH-2007-119	TT	18-Aug-07	AC601	N	26° 23.215	94° 30.841	26° 24.247	94° 30.814	165	178
CH-2007-122	TT	18-Aug-07	AC601	N	26° 23.450	94° 30.771	26° 22.905	94° 30.832	518	700
CH-2007-124	TT	18-Aug-07	AC601	N	26° 22.962	94° 30.903	26° 24.152	94° 31.038	311	372
CH-2007-126	TT	18-Aug-07	AC601	N	26° 24.786	94° 31.143	26° 23.872	94° 31.135	38	57
CH-2007-134	CTD	18-Aug-07	AC601	D	26° 22.185	94° 31.077	26° 21.821	94° 30.335	Surface	2,278
CH-2007-136	TT	20-Aug-07	AT340	N	27° 38.175	88° 21.014	27° 38.083	88° 21.956	317	465
CH-2007-137	TT	20-Aug-07	AT340	N	27° 38.258	88° 21.699	27° 38.066	88° 22.702	255	352
CH-2007-138	TT	20-Aug-07	AT340	N	27° 38.876	88° 21.910	27° 38.752	88° 22.889	206	228
CH-2007-139	TT	21-Aug-07	AT340	N	27° 38.685	88° 22.052	27° 38.593	88° 23.286	113	126
CH-2007-140*	TT	21-Aug-07	AT340	N	27° 38.659	88° 22.195	27° 38.642	88° 23.122	Surface	425
CH-2007-141*	TT	21-Aug-07	AT340	N	27° 38.733	88° 22.647	27° 38.862	88° 23.578	Surface	586
CH-2007-143	CTD	21-Aug-07	AT340	N/D	27° 39.122	88° 23.084	27° 38.277	88° 20.382	Surface	2,198
CH-2007-151	TT	21-Aug-07	AT340	N	27° 38.083	88° 21.521	27° 38.751	88° 21.994	169	301
CH-2007-152	TT	22-Aug-07	AT340	D	27° 38.319	88° 21.485	27° 38.875	88° 22.066	407	763
CH-2007-153	CTD	22-Aug-07	AT340	D	27° 38.663	88° 21.469	27° 37.855	88° 19.026	Surface	2,304
CH-2007-154	TT	22-Aug-07	AT340	D	27° 37.987	88° 21.708	27° 38.762	88° 22.018	481	666
CH-2007-156	TT	22-Aug-07	AT340	D	27° 38.326	88° 21.637	27° 38.811	88° 21.935	818	935
CH-2007-157	TT	22-Aug-07	AT340	D	27° 38.400	88° 21.294	27° 38.740	88° 21.934	938	1,059
CH-2007-159	TT	22-Aug-07	AT340	D	27° 38.516	88° 21.610	27° 38.970	88° 22.381	310	437
CH-2007-160*	TT	22-Aug-07	AT340	N	27° 38.589	88° 21.721	27° 39.007	88° 22.591	Surface	627
CH-2007-161	TT	22-Aug-07	AT340	N	27° 38.343	88° 21.277	27° 38.667	88° 22.243	187	403
CH-2007-163	TT	22-Aug-07	AT340	N	27° 38.353	88° 21.046	27° 38.676	88° 21.893	135	301

Table 7.2. Stations sampled at four sites (see fig. 7.1) in the Gulf of Mexico (9-29 August 2007).—Continued

[D, day (0630 to 1920 h CDT); N, night (1920 to 0630 h CDT); N/D, samples covering both day and night; CTD, Seabird SBE 911+; TT, Tucker trawl; m, meters; *, net did not close; ^, net fished on bottom for portion of tow; NR, not recorded]

Station #	Gear	Date	Location	Time	Start Latitude	Start Longitude	End Latitude	End Longitude	Min Sample Depth (m)	Max Sample Depth (m)
CH-2007-164*	TT	22-Aug-07	AT340	N	27° 38.392	88° 21.217	27° 39.118	88° 22.124	Surface	421
CH-2007-165	TT	23-Aug-07	AT340	N	27° 38.166	88° 20.877	27° 38.715	88° 21.805	168	217
CH-2007-166	TT	23-Aug-07	AT340	N	27° 38.339	88° 21.034	27° 38.768	88° 21.906	131	220
CH-2007-168	TT	23-Aug-07	AT340	N	27° 38.544	88° 21.363	27° 38.880	88° 22.181	59	89
CH-2007-169	TT	23-Aug-07	AT340	N	27° 38.481	88° 21.438	27° 38.924	88° 22.252	152	212
CH-2007-171	TT	23-Aug-07	AT340	D	27° 39.344	88° 22.858	27° 39.795	88° 23.663	120	164
CH-2007-172	TT	23-Aug-07	AT340	D	27° 38.466	88° 21.212	27° 38.755	88° 21.748	279	494
CH-2007-173	TT	23-Aug-07	AT340	D	27° 39.085	88° 21.980	27° 39.430	88° 22.802	302	702
CH-2007-175	TT	23-Aug-07	AT340	D	27° 38.006	88° 20.645	27° 38.178	88° 21.475	359	827
CH-2007-176	TT	23-Aug-07	AT340	D	27° 38.657	88° 21.842	27° 38.890	88° 22.623	232	311
CH-2007-178	TT	23-Aug-07	AT340	D	27° 38.400	88° 21.216	27° 38.765	88° 22.082	297	391
CH-2007-180	TT	23-Aug-07	AT340	D	27° 39.436	88° 23.669	27° 39.813	88° 24.614	403	602
CH-2007-182	TT	23-Aug-07	AT340	D	27° 38.522	88° 21.466	27° 38.879	88° 22.379	533	892
CH-2007-183	TT	23-Aug-07	AT340	N	27° 38.343	88° 21.041	27° 38.728	88° 21.036	124	183
CH-2007-184	TT	23-Aug-07	AT340	N	27° 38.169	88° 20.947	27° 38.559	88° 21.804	473	959
CH-2007-186	TT	23-Aug-07	AT340	N	27° 38.280	88° 21.203	27° 38.819	88° 22.169	330	478
CH-2007-188	TT	24-Aug-07	AT340	N	27° 38.566	88° 21.511	27° 38.941	88° 22.385	275	300
CH-2007-189	TT	24-Aug-07	AT340	N	27° 38.460	88° 21.314	27° 38.848	88° 22.197	264	328
CH-2007-191	TT	24-Aug-07	AT340	N	27° 38.463	88° 21.357	27° 38.837	88° 22.285	251	297
CH-2007-192	TT	24-Aug-07	AT340	N	27° 38.832	88° 22.322	27° 39.206	88° 23.222	232	349
CH-2007-198	TT	24-Aug-07	AT340	N	27° 38.767	88° 21.397	27° 39.120	88° 22.339	277	344
CH-2007-199	TT	24-Aug-07	AT340	N	27° 38.718	88° 21.795	27° 39.237	88° 22.684	104	146
CH-2007-200	TT	24-Aug-07	AT340	N	27° 38.611	88° 21.584	27° 39.101	88° 22.504	465	750
CH-2007-202	TT	24-Aug-07	AT340	N	27° 38.640	88° 21.675	27° 39.095	88° 22.586	379	481

Table 7.2. Stations sampled at four sites (see fig. 7.1) in the Gulf of Mexico (9-29 August 2007).—Continued

[D, day (0630 to 1920 h CDT); N, night (1920 to 0630 h CDT); N/D, samples covering both day and night; CTD, Seabird SBE 911+; TT, Tucker trawl; m, meters; *, net did not close; ^, net fished on bottom for portion of tow; NR, not recorded]

Station #	Gear	Date	Location	Time	Start Latitude	Start Longitude	End Latitude	End Longitude	Min Sample Depth (m)	Max Sample Depth (m)
CH-2007-204	TT	24-Aug-07	AT340	N	27° 38.644	88° 21.662	27° 39.110	88° 22.691	499	704
CH-2007-205	TT	24-Aug-07	AT340	N	27° 38.706	88° 21.715	27° 39.100	88° 22.662	367	467
CH-2007-210*	TT	25-Aug-07	AT340	D	27° 38.651	88° 21.414	27° 38.828	88° 22.558	Surface	142
CH-2007-211	TT	25-Aug-07	AT340	D	27° 39.026	88° 24.074	27° 39.195	88° 25.116	743	1,025
CH-2007-212	CTD	25-Aug-07	AT340	D	27° 38.699	88° 22.354	27° 38.517	88° 20.747	S	2,212
CH-2007-213	TT	25-Aug-07	AT340	D	27° 38.782	88° 21.433	27° 38.881	88° 22.279	329	370
CH-2007-214	TT	25-Aug-07	AT340	N	27° 38.600	88° 21.459	27° 38.857	88° 22.587	290	360
CH-2007-216	TT	25-Aug-07	AT340	N	27° 38.573	88° 21.399	27° 38.848	88° 22.497	452	708
CH-2007-218	TT	25-Aug-07	AT340	N	27° 38.649	88° 21.746	27° 38.834	88° 22.774	540	605
CH-2007-219	TT	25-Aug-07	AT340	N	27° 38.777	88° 21.981	27° 39.923	88° 23.041	74	94
CH-2007-220	TT	25-Aug-07	AT340	N	27° 38.802	88° 22.198	27° 38.998	88° 23.552	1,019	1,377
CH-2007-222	CTD	26-Aug-07	VK826	D	29° 08.974	88° 01.244	29° 08.508	88° 01.634	Surface	631
CH-2007-223	TT	26-Aug-07	VK826	D	29° 10.204	88° 00.750	29° 11.049	88° 00.118	296	440
CH-2007-224*	TT	26-Aug-07	VK826	D	29° 09.957	88° 01.033	29° 10.688	88° 00.197	Surface	446
CH-2007-225	TT	26-Aug-07	VK826	N	29° 10.140	88° 00.823	29° 10.843	88° 00.101	360	462
CH-2007-226^	TT	26-Aug-07	VK826	N	29° 09.703	88° 01.112	29° 10.621	88° 00.548	350	477
CH-2007-227	TT	26-Aug-07	VK826	N	29° 09.583	88° 01.141	29° 10.396	88° 00.700	295	364
CH-2007-228	TT	27-Aug-07	VK826	N	29° 09.557	88° 01.221	29° 10.393	88° 00.692	88	135
CH-2007-229	TT	27-Aug-07	VK826	N	29° 09.424	88° 01.405	29° 10.158	88° 00.528	200	278
CH-2007-230	TT	27-Aug-07	VK826	N	29° 10.025	88° 00.689	29° 09.383	88° 01.487	238	324
CH-2007-232	TT	27-Aug-07	VK826	N	29° 09.581	88° 01.224	29° 10.452	88° 00.548	310	419
CH-2007-233	TT	27-Aug-07	VK826	N	29° 10.613	88° 00.346	29° 10.027	88° 00.909	222	267
CH-2007-234	TT	27-Aug-07	VK826	N	29° 09.781	88° 01.025	29° 10.514	88° 00.440	121	139
CH-2007-246	CTD	27-Aug-07	VK826	D	29° 10.135	88° 00.879	29° 10.080	88° 00.863	Surface	494
CH-2007-249	TT	27-Aug-07	VK826	D	29° 09.532	88° 01.743	29° 08.554	88° 01.883	361	442

Table 7.2. Stations sampled at four sites (see fig. 7.1) in the Gulf of Mexico (9-29 August 2007).—Continued

[D, day (0630 to 1920 h CDT); N, night (1920 to 0630 h CDT); N/D, samples covering both day and night; CTD, Seabird SBE 911+; TT, Tucker trawl; m, meters; *, net did not close; ^, net fished on bottom for portion of tow; NR, not recorded]

Station #	Gear	Date	Location	Time	Start Latitude	Start Longitude	End Latitude	End Longitude	Min Sample Depth (m)	Max Sample Depth (m)
CH-2007-250	TT	27-Aug-07	VK826	N	29° 08.738	88° 01.418	29° 09.589	88° 01.027	201	233
CH-2007-252	TT	27-Aug-07	VK826	N	29° 09.940	88° 01.004	29° 10.705	88° 00.452	116	156
CH-2007-253	TT	27-Aug-07	VK826	N	29° 10.321	88° 00.641	29° 09.399	88° 00.981	366	465
CH-2007-254	TT	27-Aug-07	VK826	N	29° 08.944	88° 01.243	29° 09.837	88° 00.928	222	239
CH-2007-256*^	TT	28-Aug-07	VK826	N	29° 09.769	88° 00.524	29° 08.884	88° 00.979	Surface	488
CH-2007-257	TT	28-Aug-07	VK826	N	29° 08.936	88° 00.968	29° 09.939	88° 00.433	269	361
CH-2007-259	TT	28-Aug-07	VK826	N	29° 10.075	88° 00.401	29° 09.280	88° 00.769	198	260
CH-2007-260	TT	28-Aug-07	VK826	N	29° 08.947	88° 00.949	29° 09.729	88° 00.502	139	166
CH-2007-262	TT	28-Aug-07	VK826	N	29° 10.048	88° 00.419	29° 09.152	88° 00.821	119	165
CH-2007-263	TT	28-Aug-07	VK826	N	29° 09.318	88° 00.754	29° 10.219	88° 00.266	306	332
CH-2007-264	TT	28-Aug-07	VK826	D	29° 10.480	88° 00.789	29° 10.008	88° 01.672	297	405
CH-2007-265	CTD	28-Aug-07	VK826	D	29° 09.430	88° 02.174	29° 09.340	88° 02.284	Surface	541
CH-2007-266	TT	28-Aug-07	VK826	D	29° 09.296	88° 01.252	29° 09.809	88° 00.348	359	442
CH-2007-267	TT	28-Aug-07	VK826	D	29° 09.623	88° 00.373	29° 09.095	88° 01.242	446	507
CH-2007-268	TT	28-Aug-07	VK826	D	29° 08.479	88° 02.019	29° 09.024	88° 01.021	369	534
CH-2007-276	TT	28-Aug-07	VK826	D	29° 10.164	88° 00.554	29° 10.283	87° 59.531	185	228
CH-2007-277	TT	28-Aug-07	VK826	N	29° 10.303	87° 59.495	29° 10.230	88° 00.712	151	203
CH-2007-278	TT	28-Aug-07	VK826	N	29° 09.857	88° 00.894	29° 09.160	88° 01.725	273	370
CH-2007-279	TT	28-Aug-07	VK826	N	29° 09.206	88° 02.009	29° 09.668	88° 01.113	221	287
CH-2007-281	TT	28-Aug-07	VK826	N	29° 09.890	88° 00.836	29° 09.266	88° 01.844	83	138
CH-2007-282	TT	28-Aug-07	VK826	N	29° 09.495	88° 01.692	29° 10.360	88° 01.012	301	370
CH-2007-286	TT	29-Aug-07	VK826	N	29° 09.268	88° 02.083	29° 10.060	88° 01.281	95	120
CH-2007-287	TT	29-Aug-07	VK826	N	29° 10.169	88° 01.191	29° 09.253	88° 02.043	342	516
CH-2007-289	TT	29-Aug-07	VK826	N	29° 09.181	88° 02.242	29° 09.884	88° 01.503	314	392
CH-2007-290	TT	29-Aug-07	VK826	N	29° 10.653	88° 00.560	29° 11.412	87° 59.879	26	47

Table 7.3. Total numbers and standard length ranges (millimeters in parentheses) of juvenile and adult fishes collected by Tucker trawl over three cold-seep sites (AC601, GC852, AT340) and one deep-sea coral site (VK826) in the Gulf of Mexico (August 9-29, 2007).

[Depth ranges are minimum and maximum depths from properly working Tucker trawls; *, no depth because net did not close; n, number of stations sampled; D, day; N, night; n/a, damaged specimens not measured; m, meters]

	Depth	AC601	GC852		AT340		VK826	
	Range	N	D	N	D	N	D	N
Species	(m)	n=5	n=17	n=52	n=16	n=33	n=8	n=28
Microstomatidae								
Dolicholagus longirostris	113-1377		4 (26-50)	3 (22-23)		2 (29, 40)		
Gonostomatidae								
Bonapartia pedaliota	222-460			1 (40)		3 (21-32)		3 (18-25)
Cyclothone acclinidens	359-1144	1 (21)	103 (14-52)	47 (12-46)	8 (31-34)			
Cyclothone alba	150-1144	2 (26, 27)	66 (13-27)	426 (10-29)	59 (13-27)	52 (11-28)	58 (12-28)	67 (11-28)
Cyclothone braueri	121-1144	2 (16.5, 24)	96 (12.5-27)	409 (11-28)	62 (12-26)	100 (13-25)	47 (12-25)	53 (11-25)
Cyclothone obscura	1019-1377					9 (16-55)		
Cyclothone pallida	150-1377	31 (14-49)	173 (15-50)	564 (13-51)	71 (12-50)	46 (15-49)		2 (19)
Cyclothone pseudopallida	150-1377	17 (20-36)	139 (12-43)	48 (11-45)	51 (15-40)	40 (14-30)	20 (13-29)	10 (11-28)
Cyclothone sp. 1	369-935		5 (15-18)		2 (15-17)		6 (16-17)	
Cyclothone sp. 2	443-836		3 (19-32)	5 (12-36)				
Cyclothone spp.	131-1144	23	503 (12-42)	1064 (12-35)	140 (12-53)	156 (12-38)	51 (12-20)	37 (12-20)
Gonostoma atlanticum	53-507			12 (21-27)	1 (27)	3 (25-30)	1 (44)	1 (22)
Gonostoma elongatum	49-1377		2 (28, 90)	31 (21-180)	4 (21-38)	54 (21-158)	3 (25-27)	4 (21-34)
Margrethia obtusirostra	144-239			6 (16-28)		3 (14-18)		1 (14)
Sternoptychidae								
Argyropelecus aculeatus	170-935	3 (13-30)		22 (15-45)	6 (13-42)	12 (13-43)	6 (n/a)	9 (16-50)
Argyropelecus affinis	*			1 (27)				
Argyropelecus gigas	372-757	1 (40)	1 (27)	5 (17-78)	1 (n/a)	1 (26)		2 (16, 17)

Table 7.3. Total numbers and standard length ranges (mm in parenthesis) of juvenile and adult fishes collected by Tucker trawl over three cold seep sites (AC601, GC852, AT340) and one deep-sea coral site (VK826) in the Gulf of Mexico (9-29 Aug 2007). —Continued

[Depth ranges are minimum and maximum depths from properly working Tucker trawls; *, no depth because net did not close; n, number of stations sampled; D, day; N, night; n/a, damaged specimens not measured; m, meters]

Species	Depth Range (m)	AC601	GC852		AT340		VK826	
		N	D	N	D	N	D	N
		n=5	n=17	n=52	n=16	n=33	n=8	n=28
Argyropelecus spp.	432-531			4 (10-12)				
Maurolicus weitzmani	1019-1377					1 (15)		
Polyipnus clarus	295-1144		1 (25)	1 (27)				3 (26-34)
Polyipnus spp.	329-1377			1 (17)	1 (19)	1 (n/a)	1 (19)	
Sternoptyx diaphana	302-1027	1 (18)	10 (13-23)	5 (13-35)	2 (15)	1 (17)		
Sternoptyx pseudobscura	737-1044		9 (13-45)	1 (44)	1 (30)			
Sternoptyx spp.	745-865		3 (11)					
Valenciennellus tripunctulatus	165-1035	26 (13-30)	13 (14-27)	72 (13-30)	19 (17-30)	117 (13-30)	29 (14-29)	142 (13-30)
Undetermined	187-267			1 (16)				
Phosichthyidae								
Ichthyococcus ovatus	306-625		1 (16)	3 (12-14)	1 (22)	1 (15)		4 (11-24)
Pollichthys mauli	59-739			3 (33-37)	19 (15-32)	51 (14-51)	1 (19)	42 (17-39)
Vinciguerria attenuata	165-625	3 (14-18)	3 (14-22)	18 (13-20)	4 (14-18)	4 (16-38)		4 (13.5-23)
Vinciguerria nimbaria	49-666	1 (16)	2 (18, 22)	26 (12-30)	13 (12-22)	11 (12-26)	2 (13, 25)	16 (17-39)
Vinciguerria poweriae	49-1377	2 (18, 29)	21 (13-20)	237 (12-35)	10 (12-24)	62 (12-30)	9 (12-18)	27 (12-29)
Vinciguerria spp.	113-605	2 (14, 15)		3 (13)		8 (12-17)		
Stomiidae								
Aristostomias tittmanni	626-757			1 (35)				
Astronesthes macropogon	206-228	1 (34)				1 (21)		
Astronesthes niger	373-403			1 (27)				
Astronesthes similus	77-625		1 (21)	1 (20)		1 (31)	1 (26)	

Table 7.3. Total numbers and standard length ranges (mm in parenthesis) of juvenile and adult fishes collected by Tucker trawl over three cold seep sites (AC601, GC852, AT340) and one deep-sea coral site (VK826) in the Gulf of Mexico (9-29 Aug 2007). —Continued

[Depth ranges are minimum and maximum depths from properly working Tucker trawls; *, no depth because net did not close; n, number of stations sampled; D, day; N, night; n/a, damaged specimens not measured; m, meters]

Species	Depth Range (m)	AC601	GC852		AT340		VK826	
		N	D	N	D	N	D	N
		n=5	n=17	n=52	n=16	n=33	n=8	n=28
Bathophilus longipinnis	53-149			2 (41-65)				
Bathophilus nigerrimus	*						1 (20)	
Bathophilus pawneei	121-442			1 (31)			4 (29-39)	6 (28-57)
Chauliodus sloani	53-1377	2 (23, 24)	9 (21-132)	40 (20-80)	5 (20-41)	10 (20-210)	4 (23-56)	8 (23-170)
Chauliodus spp.	551-757		1 (30)	2 (30, 32)				
Eustomias lipochirus	144-174			1 (80)				
Eustomias schmidti	144-217			1 (82)		1 (80)		
Leptostomias bilobulatus	*					1 (105)		
Melanostomias biseriatus	172-212			1 (40)				
Melanostomias valdiviae	236-248			1 (31)				
Photonectes margarita	255-352					1 (260)		
Photonectes sp.	540-605					1 (32)		
Photostomias guernei	136-895		2 (104, 120)	5 (49-75)		1 (100)		
Stomias affinis	77-836		2 (29,90)	3 (28-50)		9 (23-55)	2 (26,49)	1 (94)
Stomias longibarbatus	150			1 (86)				
Undetermined	168-531		1 (n/a)	1 (60)		1 (46)		
Chlorophthalmidae								
Chlorophthalmus agassizi	165-1377	1 (27)		1 (45)		1 (26)		
Notosudidae								
Scopelosaurus mauli	630-836		1 (65)					
Evermannellidae								
Coccorella atlantica	104-210			1 (48)		1 (31)		

Table 7.3. Total numbers and standard length ranges (mm in parenthesis) of juvenile and adult fishes collected by Tucker trawl over three cold seep sites (AC601, GC852, AT340) and one deep-sea coral site (VK826) in the Gulf of Mexico (9-29 Aug 2007). —Continued

[Depth ranges are minimum and maximum depths from properly working Tucker trawls; *, no depth because net did not close; n, number of stations sampled; D, day; N, night; n/a, damaged specimens not measured; m, meters]

Species	Depth Range (m)	AC601	GC852		AT340		VK826	
		N	D	N	D	N	D	N
		n=5	n=17	n=52	n=16	n=33	n=8	n=28
Alepisauridae-Omosudidae								
Omosudis lowii	847-1027		6 (20-30)					
Paralepididae								
Lestidiops affinis	135-664		2 (50, 54)			1 (55)		
Lestrolepis intermedia	95-516							5 (45-60)
Stemonosudis bullisi	88-135							2 (55, 57)
Sudis atrox	135-605			4 (24-46)		2 (24, 27)		
Uncisudis advena	119-203							2 (37, 40)
Myctophidae								
Benthosema suborbitale	38-1044	16 (10-31)	10 (10-27)	70 (10-29)	23 (11-23)	125 (10-30)	16 (12-16)	10 (11-16)
Bolinichthys photothorax	104-664		2 (14, 15)	4 (20-32)	1 (15)	6 (15-25)		3 (15-17)
Bolinichthys supralateralis	150-392			2 (14, 17)		3 (15-18)		3 (14-17)
Centrobranchus nigroocellatus	81-666			2 (14, 17)	1 (16.5)	1 (17)		2 (15, 24)
Ceratoscopelus warmingii	49-1144	7 (17-40)	8 (15-32)	31 (16-42)	6 (15-40)	43 (17-45)		
Diaphus brachycephalus	38-558	1 (13)		2 (38, 43)		6 (13-27)		
Diaphus dumerilii	38-739	3 (13-16)		3 (17-31)	2 (13, 17)	5 (12-19)	68 (12-27)	48 (12-23)
Diaphus fragilis	53-827			1 (16)	2 (13)			
Diaphus lucidus	104-447	1 (80)		2 (25, 65)		4 (19-70)		
Diaphus mollis	49-666	1 (30)	2 (12, 16)	17 (12-55)	3 (12-17)	13 (12-31)	3 (12-16)	10 (14-50)
Diaphus perspicillatus	121-405						5 (14-22)	3 (14-18)
Diaphus problematicus	49-1017		2 (12)	5 (12-70)	1 (43)	10 (14-66)	1 (15)	
Diaphus spp.	18-1025			4 (12-15)	2 (14)	2 (13, 15)		3 (16-18)
Diaphus splendidus	59-605				7 (12-35)	34 (12-32)		1 (20)

Table 7.3. Total numbers and standard length ranges (mm in parenthesis) of juvenile and adult fishes collected by Tucker trawl over three cold seep sites (AC601, GC852, AT340) and one deep-sea coral site (VK826) in the Gulf of Mexico (9-29 Aug 2007). —Continued

[Depth ranges are minimum and maximum depths from properly working Tucker trawls; *, no depth because net did not close; n, number of stations sampled; D, day; N, night; n/a, damaged specimens not measured; m, meters]

	Depth	AC601	GC852		AT340		VK826	
	Range	N	D	N	D		Range	N
Species	(m)	n=5	n=17	n=52	n=16	Species	(m)	n=5
Diaphus subtilis	144-370			3 (14-56)				1 (14)
Diaphus taaningi	144-398			2 (12, 32)				
Diaphus termophilus	359-827				1 (12)			
Diogenichthys atlanticus	38-1016	7 (16-18.5)	1 (n/a)	3 (16-18)	1 (18)	6 (14-17)		
Gonichthys cocco	74-895		3 (17-28)	2 (19, 22)		1 (20)		
Hygophum benoiti	83-1144	6 (12-14)	33 (12-18)	36 (12-16)	8 (12-22)	27 (12-19)	5 (12-13)	33 (12-18)
Hygophum macrochir	187-655			3 (12-15)		2 (12, 19)	1 (n/a)	
Hygophum reinhardtii	314-1027		2 (15)	1 (14)		1 (15)		1 (15)
Hygophum sp.	113-126					1 (n/a)		
Hygophum taaningi	59-1027	1 (12)	2 (17)	2 (12)	6 (12-25)	32 (12-40)	3 (12-14)	18 (12-17)
Lampadena luminosa	77-750			3 (20)		3 (22-46)		
Lampanyctus alatus	53-836	5 (15-45)	5 (14-40)	38 (14-45)	6 (19-30)	21 (17-55)		
Lampanyctus nobilis	113-301					3 (20-25)		
Lampanyctus spp.	77-220			1 (30)	1 (21.5)			
Lampanyctus tenuiformis	77-349			2 (24, 50)		2 (23, 28)		
Lepidophanes guentheri	18-1377	16 (16-24)	4 (15-27)	40 (15-57)	28 (15-40)	205 (14-65)	2 (17, 33)	
Lobianchia gemellarii	104-352			1 (18)		9 (16-26)		1 (15)
Myctophum affine	49-1377	1 (15)	10 (13-15)	48 (13-45)		4 (13-16)	14 (15-30)	43 (15-40)
Myctophum asperum	49-1017			1 (15)				3 (16-18)
Myctophum nitidulum	113-732			1 (16)		1 (18)		
Myctophum obtusirostre	95-625		1 (14)			1 (14)	2 (15, 20)	1 (18)
Myctophum selenops	130-150			2 (12, 14)				

Table 7.3. Total numbers and standard length ranges (mm in parenthesis) of juvenile and adult fishes collected by Tucker trawl over three cold seep sites (AC601, GC852, AT340) and one deep-sea coral site (VK826) in the Gulf of Mexico (9-29 Aug 2007). —Continued

[Depth ranges are minimum and maximum depths from properly working Tucker trawls; *, no depth because net did not close; n, number of stations sampled; D, day; N, night; n/a, damaged specimens not measured; m, meters]

Species	Depth Range (m)	AC601 N n=5	GC852 D n=17	GC852 N n=52	AT340 D n=16	AT340 Species	VK826 Range (m)	VK826 N n=5
Nannobrachium atrum	*			1 (65)				
Nannobrachium lineatum	272-1035		3 (66-85)	2 (28)		1 (26)		
Notolychnus valdiviae	49-1377	1 (14)	29 (11-21)	886 (11-22)	79 (11-19)	125 (16-21)	33 (11-20)	17 (11-18)
Notoscopelus resplendens	150-1035		2 (22, 45)	1 (56)				
Symbolophorus rufinus	886-1016		1 (21)					
Undetermined	49-1377	1 (n/a)	13 (12-31)	21 (12-24)	11 (12-22)	26 (13-28)	1 (n/a)	4 (12)
Polymixiidae								
Polymixia lowei	136-460		1 (25)	2 (25, 30)				
Bregmacerotidae								
Bregmaceros atlanticus	53-1059		1 (17)	9 (14-26)	3 (12-33)	14 (16-34)	1 (13)	13 (11-36)
Bregmaceros cantori	198-260							2 (29, 36)
Bregmaceros mcclellandii	49-328			2 (18, 20)		2 (17, 18)		
Bregmaceros spp.	77-447			3 (12)		1 (19)		
Moridae								
Physiculus fulvus	273-370							1 (42)
Melanonidae								
Melanonus zugmayeri	538-592		1 (55)	2 (63, 71)				
Ceratiidae								
Ceratias holboelli	382-426					1 (15)		
Ceratias uranoscopus	194-210			1 (35)				
Cryptopsaras couesii	146-935		1 (15)	2 (13, 15)	2 (8)			
Melamphaidae								
Melamphaes pumilus	136-1016	1 (16)	2 (22)	5 (12-17)		1 (13)		

Table 7.3. Total numbers and standard length ranges (mm in parenthesis) of juvenile and adult fishes collected by Tucker trawl over three cold seep sites (AC601, GC852, AT340) and one deep-sea coral site (VK826) in the Gulf of Mexico (9-29 Aug 2007). —Continued

[Depth ranges are minimum and maximum depths from properly working Tucker trawls; *, no depth because net did not close; n, number of stations sampled; D, day; N, night; n/a, damaged specimens not measured; m, meters]

Species	Depth Range (m)	AC601 N n=5	GC852 D n=17	GC852 N n=52	AT340 D n=16	AT340 Species	VK826 Range (m)	VK826 N n=5
Melamphaes simus	116-1044	7 (20-28)	4 (14-19)	12 (12-23)	4 (23-27)	4 (13-25)		3 (12-13)
Melamphaes typhlops	245-531			2 (22, 25)				
Scopeloberyx opisthopterus	146-1027		2 (19, 24)	4 (12-14)	1 (23)			
Scopeloberyx robustus	689-1377		1 (21)	2 (14, 15)		1 (21)		
Scopelogadus mizolepis	*					1 (23)		
Undetermined	311-372	1 (26)						
Cetomimidae								
Cetostoma regani	359-827				1 (74)			
Anoplogasteridae								
Anoplogaster cornuta	446-532			1 (85)				
Berycidae								
Beryx splendens	81-110			1 (18)				
Zenionidae								
Zenion hololepis	472-531			1 (21)				
Caproidae								
Antigonia combatia	*				2 (13, 14)			
Scorpaenidae								
Setarches guentheri	221-935				1 (19)			1 (15)
Peristediidae								
Peristedion sp.	144-174			1 (40)				
Acropomatidae								
Synagrops bellus	83-1377		1 (19)	3 (11-12)	3 (11.5-21)	12 (12-22)		2 (11, 13)
Synagrops sp.	53-87			1 (12)				

Table 7.3. Total numbers and standard length ranges (mm in parenthesis) of juvenile and adult fishes collected by Tucker trawl over three cold seep sites (AC601, GC852, AT340) and one deep-sea coral site (VK826) in the Gulf of Mexico (9-29 Aug 2007). —Continued

[Depth ranges are minimum and maximum depths from properly working Tucker trawls; *, no depth because net did not close; n, number of stations sampled; D, day; N, night; n/a, damaged specimens not measured; m, meters]

Species	Depth Range (m)	AC601 N n=5	GC852 D n=17	GC852 N n=52	AT340 D n=16	AT340 Species	VK826 Range (m)	VK826 N n=5
Synagrops spinosus	81-739			7 (19-23)			1 (20)	7 (12-24)
Synagrops trispinosus	245-1027			3 (11-18)				
Howellidae								
Undetermined	77-248		1 (48)	8 (9-25)		5 (13-20)		
Epigonidae								
Epigonus pectinifer	198-260							1 (21)
Sphyraenops bairdianus	121-364							3 (16-17)
Chiasmodontidae								
Pseudoscopelus altipinnis	472-531			1 (95)				
Pseudoscopelus scriptus	412-472			2 (49, 62)				
Percophidae								
Bembrops sp.	361-442						1 (17)	
Gempylidae								
Diplospinus multistriatus	222-447			1 (95)				1 (66)
Gempylus serpens	*			1 (30)				
Nesiarchus nasutus	130-149			1 (77)				
Total	18-1377	197	1334	4860	693	1544	404	696

7.3.1 Site Comparisons

7.3.1.1 Species Richness

Mesopelagic fish assemblages were generally comparable among sites. As a result of adverse weather, the AC601 site was only sampled on one night (5 stations). Thus, this station was represented by the least number of individuals (n=197) and fish species (n=31), none of which were unique to this site (table 7.3). The species-accumulation curve at this site did not approach an asymptote (fig. 7.5). The 69 tows at GC852 and the 49 tows at AT340 produced 99 species (6,193 individuals) and 80 species (2,312 individuals), respectively (table 7.3). Thirty-six Tucker trawl tows at the inshore station, VK826, yielded 58 species and 1,100 individuals (table 7.3). All three species accumulation curves (GC852, AT340, VK826, fig. 7.5) approached an asymptote, and species accumulation at each of the two offshore sites was significantly greater than that at VK826 (ANCOVA, $p<0.01$). Thirty fish species were collected only at GC852, while 10 species each were unique to AT340 and VK826. For the top four families (Myctophidae, Stomiidae, Gonostomatidae, Sternoptychidae), the offshore sites GC852 and AT340 yielded comparable numbers of species; however, except for the Gonostomatidae, families collected at VK826 were represented by fewer species than at the other two sites (table 7.3).

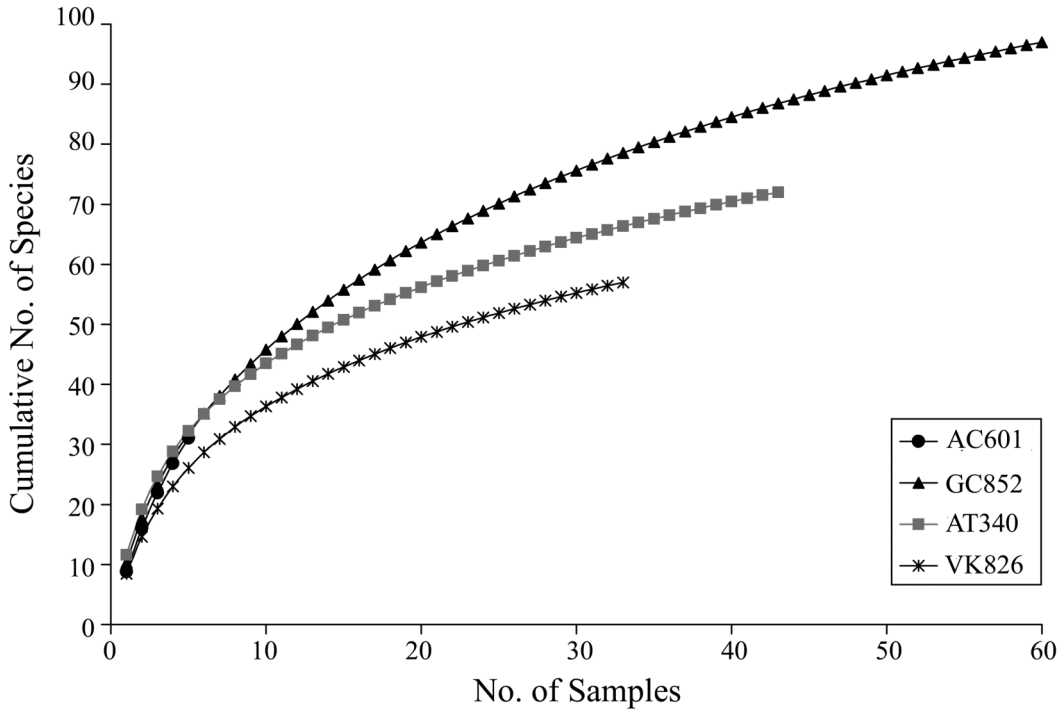
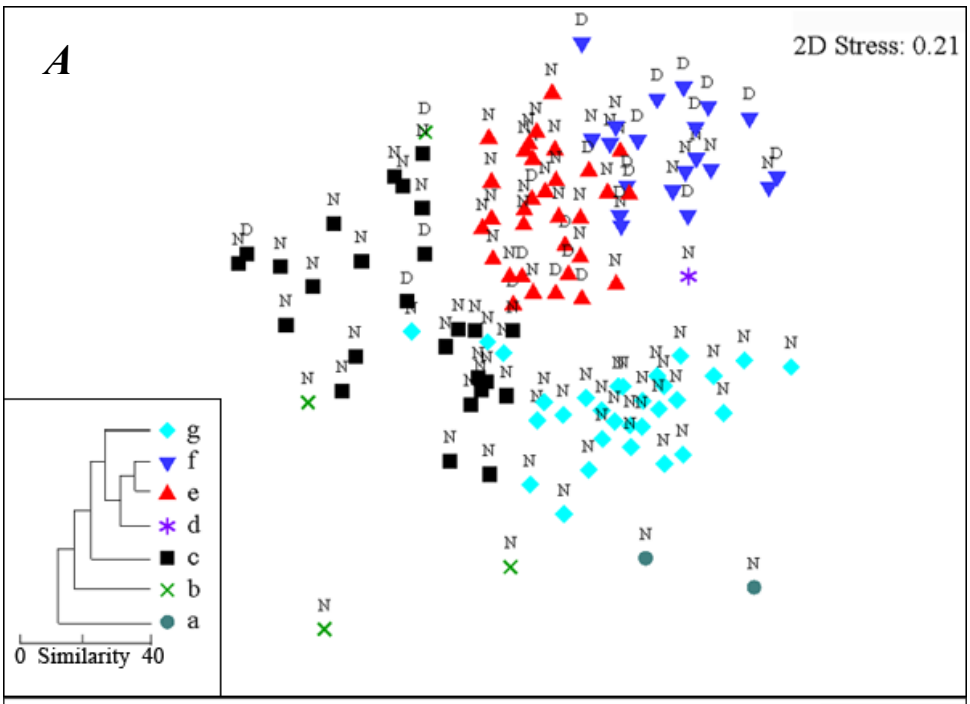


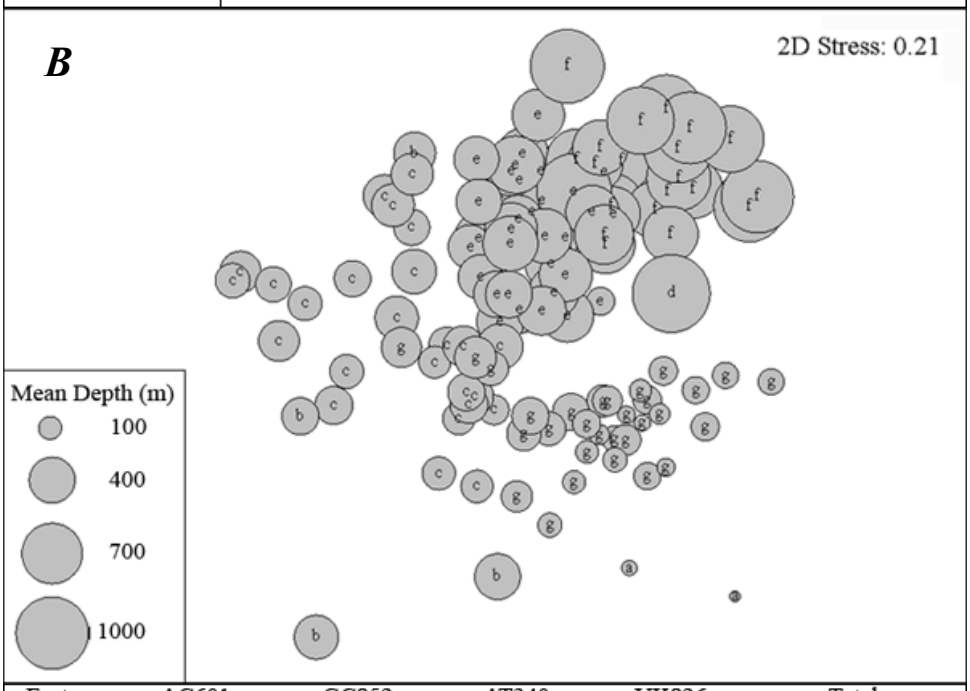
Figure 7.5. Species accumulation curves for the four Gulf of Mexico study sites plotted using the Colwell and others (2004) analytical method.

Thirty-one species (8 families) comprised about 90 percent of the overall abundance of fishes across the four sites (table 7.1). Eighteen species composed 92.4 percent of the fish fauna at AC601, dominated by *Cyclothone pallida*, *C. pseudopallida*, *Valenciennellus tripunctulatus*, *Lepidophanes guentheri*, and *Benthosema suborbitale*. At GC852, 14 species accounted for 89.6 percent of the fauna, and the dominant fishes were mostly gonostomatids (*C. pallida*, *C. pseudopallida*, *C. braueri*, *C. alba*) and a myctophid, *Notolychnus valdiviae*. AT340 yielded 22 species that accounted for 89.9 percent of its fauna, dominated by *N. valdiviae*, *C. braueri*, and *L. guentheri*. At the inshore VK826, 20 species composed 90.7 percent of the fauna, and the most abundant species were *C. braueri*, *C. alba*, *V. tripunctulatus*, and *Diaphus dumerilii*. The most abundant fishes were also generally the most frequently collected (table 7.1).

7.3.1.2 Across-Site Comparisons

Geographic and temporal patterns in mesopelagic fish assemblages were also not evident in multivariate analyses of the 115 day and night stations from the four study sites (ANOSIM, Global R = 0.20, p=0.1 percent, pairwise R values <0.3). There were significant station groupings at moderate levels of similarity (SIMPROF, p <1.5 percent fig. 7.6), but these exhibited little fidelity to geographic locations or time of day. Instead, station groupings appeared to be more related to similarities in sampled depths (see fig. 7.6*B*). Overall, the average similarity percentages within each SIMPROF group were fairly low (30-47 percent average similarity per group), revealing the variability in mesopelagic sampling and the diversity in the catches.





Factor	AC601	GC852	AT340	VK826	Total
a	1 (45)	1 (21)			2 (21, 45)
b				4 (262 - 398)	4 (262 - 398)
c	3 (171 - 318)	9 (195 - 350)	6 (267 - 395)	8 (218 - 377)	26 (171 - 395)
d			1 (1149)		1 (1149)
e		16 (150 - 1067)	12 (382 - 633)	4 (391 - 461)	32 (150 - 1067)
f	1 (584)	15 (586 - 1035)	4 (669 - 984)		20 (584 - 1035)
g		9 (51 - 185)	12 (63 - 264)	9 (98 - 333)	30 (51 - 333)

Figure 7.6. Multdimensional scaling ordination of 115 stations based on the Bray-Curtis similarity matrix calculated from standardized, fourth-root transformed fish abundances (117 species) with superimposed (*A*) SIMPROF groups labeled by day (D) or night (N) and (*B)* mean depths (in meters) with SIMPROF labels. The embedded dendrogram in *A* is labeled with SIMPROF groups. The table includes sites, number of stations, and sampled mean depth ranges (in parentheses) of each SIMPROF group.

Upper water column stations (<400 m) grouped together. SIMPROF groups a, b, c, and g (fig. 7.6*A*) were composed of stations with a mean depth of 214 m. Group a consisted of the two shallowest stations (21 and 45 m, both night) sampled in this study, and *L. guentheri* typified this group. Group b included four stations from VK826 with a mean depth of 343 m, and *Diaphus dumerilii* and *Vinciguerria nimbaria* typified this group and distinguished it from other groups. *Valenciennellus tripunctulatus* and *Argyropelecus aculeatus* typified group c (mean depth = 275 m) and distinguished this group from others. Species that dominated group g (mean depth = 156 m) included *N. valdiviae, V. poweriae, B. suborbitale, L. guentheri, Gonostoma elongatum,* and *Hygophum taaningi.*

Deeper stations also grouped together (station groups d, e, and f, fig. 7.6*A*) at a similarity level of 28 percent, and, except for one station at 150 m, generally these groups were composed of deep water stations >300 m (mean depth of the three groups = 609 m). Species that typified (SIMPER) group e (mean depth = 486 m) and distinguished it from others included (in order of percent contribution to average similarity within group) *Cyclothone alba, C. braueri, C. pseudopallida, C. pallida*, and *V. tripunctulatus.* Species that typified group f (mean depth = 778 m), distinguishing this group from others, included *C. pallida, C. acclinidens, C. pseudopallida,*

and *Hygophum benoiti.* Group d consisted of only one night station (mean depth=1,149 m) where *C. obscura* and *C. pallida* were the most abundant species collected.

7.3.1.3 Within-Site Comparisons

Although strong geographic differences among mesopelagic fish assemblages were lacking, depth patterns were apparent (see above). Therefore, we examined station relationships within each of the three well-sampled study areas (fig. 7.7), where, despite overlap, there were general depth-related station groupings (SIMPROF, p <5 percent). At the two deeper, offshore sites (fig. 7.7*A, B*), the species typifying the shallow station groupings were similar within sites. At GC852, shallow station group a (2 stations, 21 and 273 m) was typified by *Lepidophanes guentheri,* while *Notolychnus valdiviae, V. poweriae, B. suborbitale,* and *L. guentheri* distinguished group b (12 stations, mean depth = 139 m). The shallower stations at AT340 were within groups b (mean depth = 370 m) and c (mean depth = 340 m). Group b was represented by a diversity of taxa, but *L. guentheri, N. valdiviae,* and *B. suborbitale* were the main discriminating species. The two stations of AT340 group c were dominated by *V. tripunctulatus*. Low percent average similarities (27-37 percent) in the shallow station groupings at both offshore sites reflected the high diversity in the catches. The species dominating the deeper station groups were also comparable at the two offshore sites. At GC852, the mostly deeper station groups c through h (mean depths per group ranged from 227 m to 1,002 m) were typified by *Cyclothone acclinidens* (groups c, d, and e)*, C. alba* (groups f, g, and h)*, C. braueri* (groups f, g, and h), *C. pallida* (groups c, d, e, f, and g)*,* and *C. pseudopallida* (groups c, d, e, and g). In addition, *Sternoptyx pseudobscura, H. benoiti*, and *V. tripunctulatus* distinguished SIMPROF GC852 groups c, d, and h from others, respectively. Although *Cyclothone* spp. were the most abundant species in group f, additional taxa distinguished this group, including *Synagrops*

spinosus, Ceratoscopelus warmingii, and *Gonostoma elongatum.* Three deep stations comprised group a (mean depth = 898 m) at AT340, and *C. pallida, C. acclinidens, C. warmingii,* and *C. pseudopallida* dominated this station group. Somewhat higher values of percent average similarity (37-58 percent) within these deeper groups indicated more consistency in the catches.

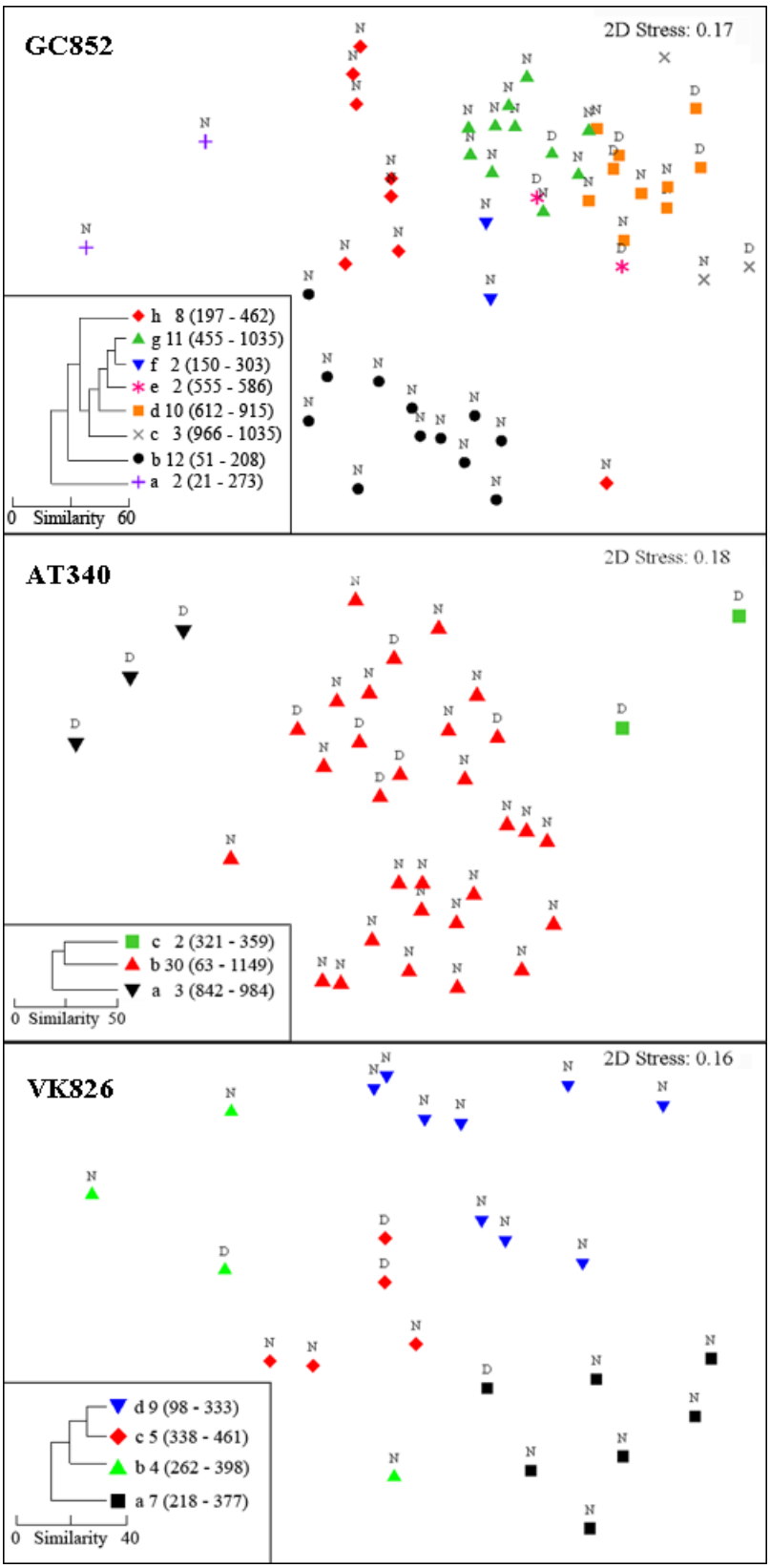

GC852
2D Stress: 0.17
h 8 (197 - 462)
g 11 (455 - 1035)
f 2 (150 - 303)
e 2 (555 - 586)
d 10 (612 - 915)
c 3 (966 - 1035)
b 12 (51 - 208)
a 2 (21 - 273)
0 Similarity 60
AT340
2D Stress: 0.18
c 2 (321 - 359)
b 30 (63 - 1149)
a 3 (842 - 984)
0 Similarity 50
VK826
2D Stress: 0.16
d 9 (98 - 333)
c 5 (338 - 461)
b 4 (262 - 398)
a 7 (218 - 377)
0 Similarity 40

Figure 7.7. Multidimensional scaling ordinations for three stations based on Bray-Curtis similarity matrices calculated from standardized, fourth-root transformed fish abundances. Each plot, *(A)* GC852 (50 stations, 91 species), *(B)* AT340 (35 stations, 71 species), and *(C)* VK826 (25 stations, 51 species), has superimposed SIMPROF groups labeled by day (D) or night (N). The embedded dendrograms include SIMPROF groups, followed by number of stations and sampled mean depth ranges (in parentheses).

SIMPROF station groups at VK826 were more variable in depth-related patterns (fig. 7.7*C*). Group a (mean depth = 271 m) was most distinct from the other groups and was distinguished by *V. tripunctulatus* and *A. aculeatus*. Group b (mean depth = 343 m) was typified by *D. dumerilii* and *V. nimbaria.* Although group d consisted of mostly shallow water stations (mean depth = 176 m) sampled at night, it was most similar to group c (mean depth = 402 m) (fig. 7.7*C*). The abundance of *V. poweriae* in both groups c and d probably influenced their similarity. *Hygophum benoiti*, *D. dumerilii,* and *Mycophum affine* were also distinguishing species in group d, whereas *C. alba, C. braueri,* and *C. pseudopallida* typified group c. VK826 station groups exhibited moderate percent average similarities (30-43 percent).

7.3.2 Depth, Diel, and Size Distributions

Depth, size, and time of capture distributions revealed a variety of patterns for the dominant species. Details of depth distributions by size and general time of sampling are provided for each study site (except AC601) for 11 of the most abundant species (figs. 7.8-7.11).

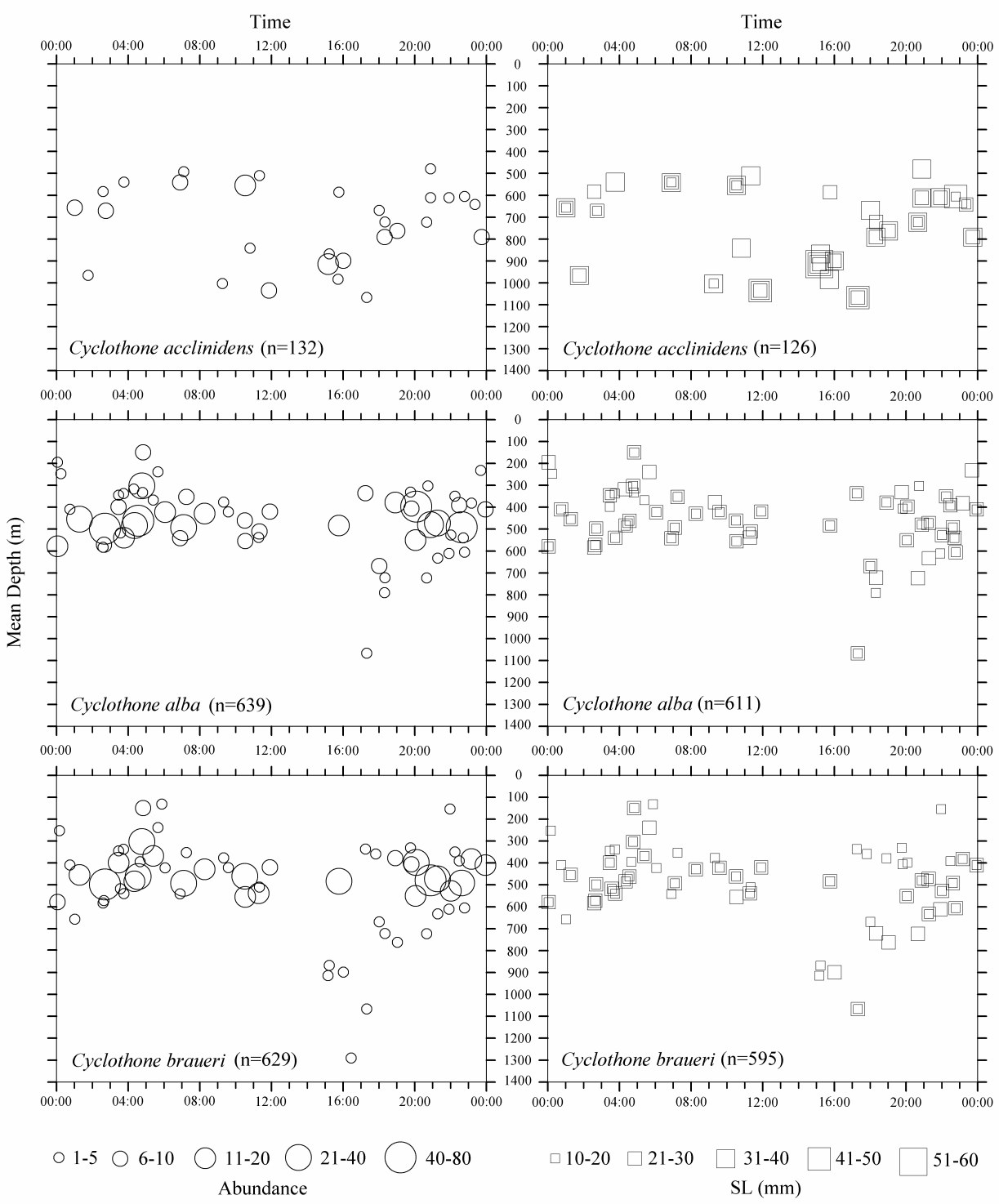

Time
Time
Mean Depth (m)
Cyclothone acclinidens (n=132)
Cyclothone acclinidens (n=126)
Cyclothone alba (n=639)
Cyclothone alba (n=611)
Cyclothone braueri (n=629)
Cyclothone braueri (n=595)
1-5
6-10
11-20
21-40
40-80
Abundance
10-20
21-30
31-40
41-50
51-60
SL (mm)

Figure 7.8. Overall (all stations combined) temporal depth distributions of dominant midwater fishes (*Cyclothone acclinidens, C. alba, C. braueri*) by abundance and size class collected in the Gulf of Mexico, August 9-29, 2007. SL mm, standard length in millimeters.

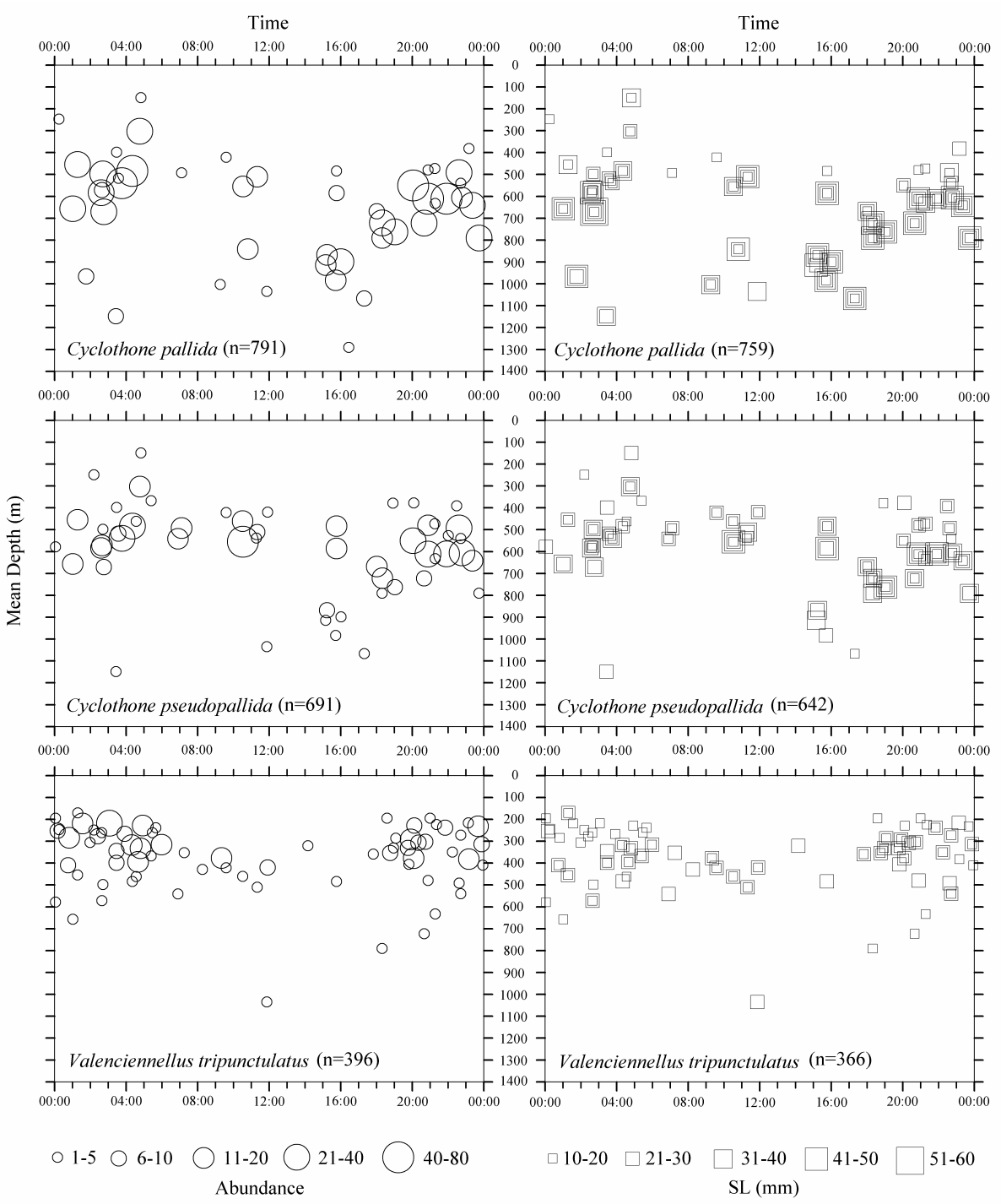


Figure 7.9. Overall (all stations combined) temporal depth distributions of dominant midwater fishes (*Cyclothone pallida, C. pseudopallida, Valenciennellus tripunctulatus*) by abundance and size class collected in the Gulf of Mexico, August 9-29, 2007. SL mm, standard length in millimeters.

Cyclothone spp., abundant at all sites except VK826 (figs. 7.8, 7.9), usually occurred in deeper waters (>130 m), especially during the day (>335 m). Most individuals were captured at night at GC852, with catches being more evenly distributed across times at AT340 and VK826. The two most abundant *Cyclothone* spp., *C. pallida* and *C. pseudopallida*, had the largest overall depth ranges (150-1,377 m, fig. 7.9) and exhibited similar diel distribution trends at the two offshore sites, remaining around 400-1,050 m during the day and shifting to 150-1,100 m (most around 450-750 m) at night. The few dawn samples of these species were toward the shallower depths of their ranges, while dusk samples tended toward the center of the ranges. *Cyclothone alba* and *C. braueri* did not exhibit any clear size related trends with depth or time (fig. 7.8). Both species were spread over a similar large depth range (150-1,144 m and 121-1,144 m, respectively) and were most abundant in about 300-600 m at all times. Although the bulk of these species did not exhibit much difference in diel depth distribution, a few individuals were captured in shallower depths (as shallow as 150 m) at night at GC852. *Cyclothone acclinidens* was only captured at the offshore sites, mostly at GC852 during the day. This species ranged from 480 to 1,067 m (fig. 7.8) but were most common at depths >500 m at all times.

Most *Valenciennellus tripunctulatus* were captured at night, and they tended to have larger mean sizes in the deeper parts of the range (fig. 7.9). This species did not exhibit strong diel patterns of vertical distribution. Night and day depth ranges covered about 150-450 m versus 750 and 1,000 m, respectively, at GC852, 200-600 m (most at 250-400 m) versus 300-500 m at AT340, and 200-350 m versus 350-450 m at VK826. Shallower depths were occupied at night

by some individuals at all sites, and substantial captures during dusk were in the upper depths (fig. 7.9).

Nearly all *Vinciguerria poweriae* were caught at night but did not exhibit a clear trend of size with depth (fig. 7.10). Where diel comparisons were possible, *V. poweriae* occupied a wider depth range at night, and most were in the upper 200 m at night. More individuals were caught during dusk, most in the upper 300 m, compared with dawn samples.

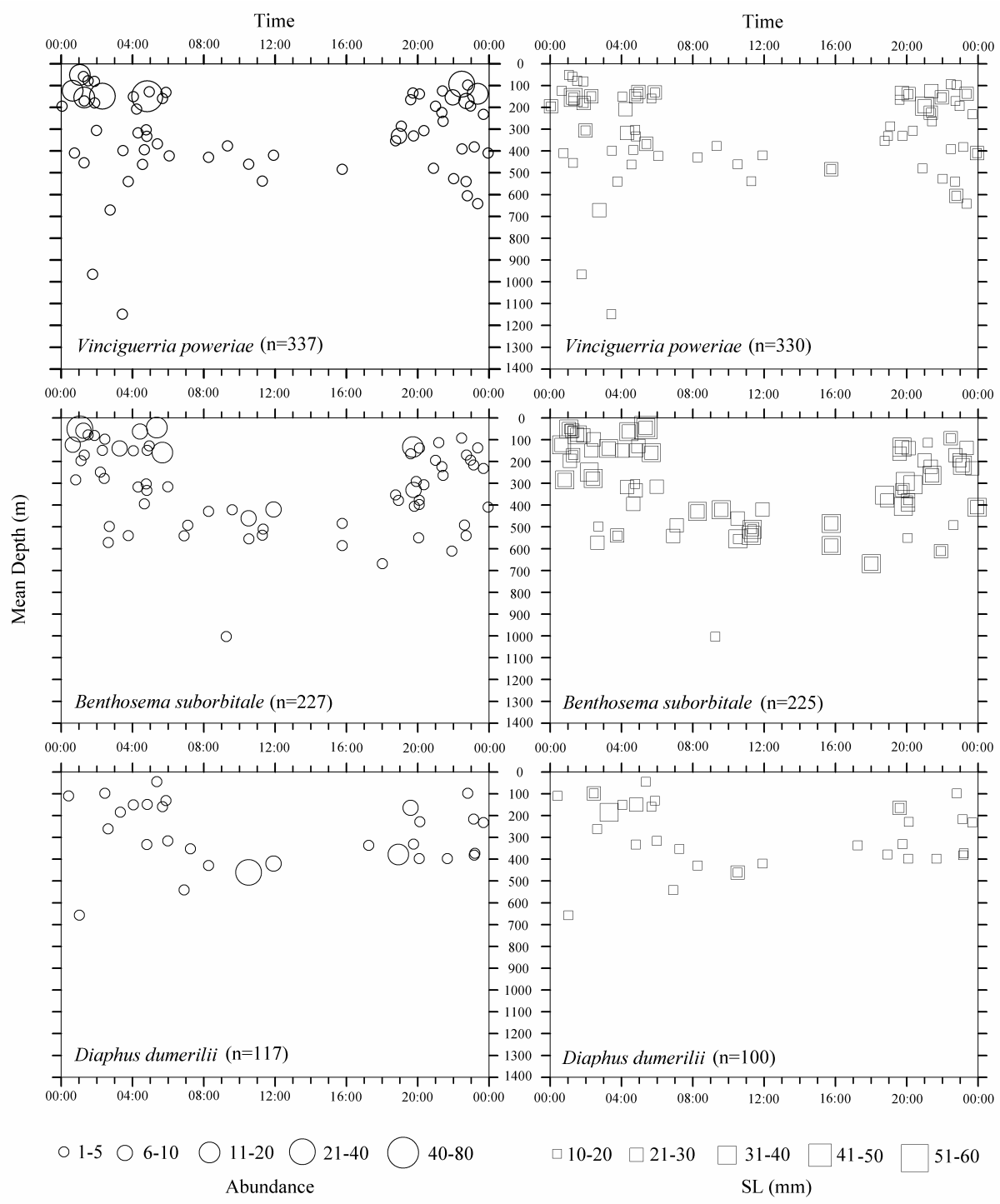


Figure 7.10. Overall (all stations combined) temporal depth distributions of dominant midwater fishes (*Vinciguerria poweriae, Benthosema suborblitale, Diaphus dumerilii*) by abundance and size class collected in the Gulf of Mexico, August 9-29, 2007.

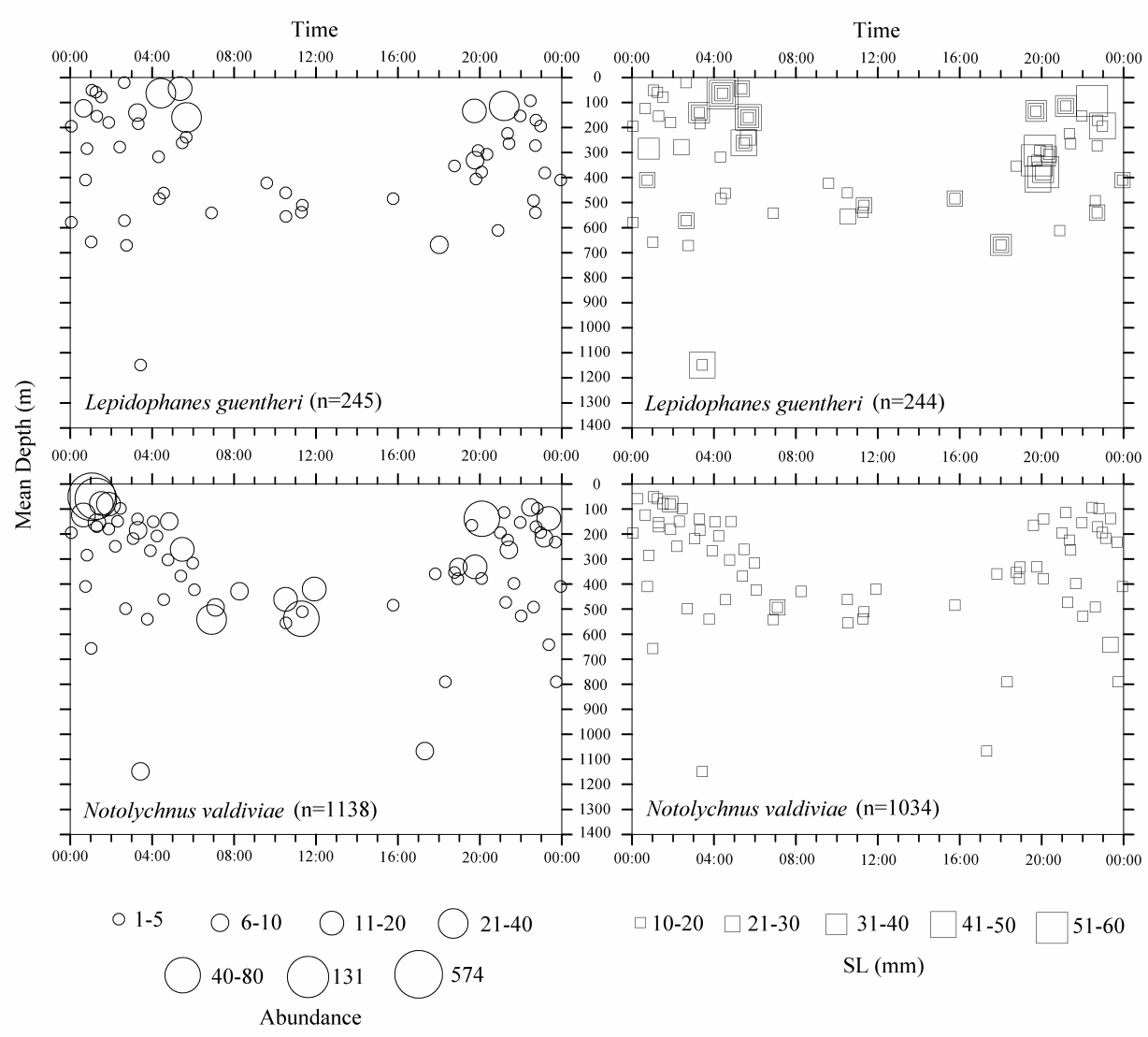


Figure 7.11. Overall (all stations combined) temporal depth distributions of dominant midwater fishes (*Lepidophanes guentheri, Notolychnus valdiviae*) by abundance and size class collected in the Gulf of Mexico, August 9-29, 2007.

Most myctophids, the strongest diel migrators we collected, were captured at night and at the offshore stations (figs. 7.9, 7.10). The dominant myctophid, *Notolychnus valdiviae*, was most abundant at GC852, where it occupied 550, 750, and 1,050 m depths by day and 50-750 m (most <100 m) at night (fig. 7.11). At AT340, *N. valdiviae* was most common in the upper 200

m (50-500 m) at night and occurred from 350 to 500 m during the day (fig. 7.11), but diel movement seemed less distinct than at GC852. This species occurred around 400-450 m during the day and around 50-200 m at night. No size related trends with depth were apparent for *N. valdiviae*. *Lepidophanes guentheri* were common only at the offshore sites. At GC852, this species was widely scattered in the water column at night (near surface to 650 m) with no clear trend, but at AT340 they were most common above 150 m at night and occurred in 400-650 m during the day. Most individuals collected during twilight were between 100 and 150 m with another small group between 250 and 350 m (fig. 7.11). Size differences with depth were not apparent for *L. guentheri*. *Benthosema suborbitale* was least abundant at VK826 and occurred in similar abundances at the two offshore sites. The relatively few individuals captured during the day at GC852 and AT340 occurred at 550 and 1,000 m and 400-650 m, respectively (fig. 7.10). At both of these sites, most *B. suborbitale* occurred above 150 m at night, although a few remained deep, >400 m. Dawn and dusk captures at AT340 were generally <150 m, while at GC852 they were more widely spread through the water column. At VK826, *B. suborbitale* individuals occurred around 400-450 m during the day and around 50-200 m (few at 300 m) at night. *Diaphus dumerilii* were most common at the inshore VK826 site (table 7.3). At VK826, they were captured during the day around 400-450 m, but most were above 200 m at night, and dawn/dusk captures were in the upper part of the depth range (fig. 7.10). Too few *D. dumerilii* were captured at GC852 or AT340 to discern any clear diel pattern, and they ranged over a mean depth of 150 to 657 m (table 7.3). No size trends with depth were observed for *D. dumerilii*. Other important but less abundant species also exhibited diel variability by site. *Gonostoma elongatum* was most abundant at the offshore sites and was mostly collected at night at all sites where it often occurred at shallower depths than during the day (table 7.3). The two most

common sternoptychids (*Argyropelecus aculeatus* and *A. hemigymnus*), after *V. tripunctulatus*, exhibited a wide depth range (185-842 m and 150-1,149 m, respectively), were also mostly collected at night, and occurred in shallower water at night (table 7.3). These two species, especially *A. hemigymnus*, occupied deeper depths at night at VK826 and AT340 compared with GC852. Diel comparisons were possible for the phosichthyids *Pollichthys mauli* and *Vinciguerria nimbaria* only at AT340 and VK826, where they occupied an overall depth range of 98-511 m and 98-539 m, respectively (table 7.3). Some *P. mauli* and *V. nimbaria* occurred in the upper 100 m at night. *Chauliodus sloani* was the most abundant stomiid, occurring at all sites, and exhibited an expanded depth range at night (table 7.3). Although all myctophid individuals did not exhibit diel vertical movements, many moved from lower depths into the upper 100 m at night (table 7.3). *Hygophum benoiti*, common at all sites, remained in deep water (>586 m) at all times at GC852, while at AT340 and VK826 it ranged into shallower depths at night than during the day. *Myctophum affine* was also common in the upper 100 m at night and occurred below 500 m at the only site (GC852) where it was collected during the day (table 7.3). *Ceratoscopelus warmingii* and *Lampanyctus alatus* (only collected at offshore sites) exhibited larger depth ranges at night, were common above 100-170 m at night, but were deeper than 500 m during the day (table 7.3). *H. taaningi* occurred at all sites but was only common at AT340 and VK826, where it moved into the upper 100 m at night and was deeper than 400 m during the day (table 7.3). *Diaphus mollis* and *D. splendidus* also exhibited expanded depth ranges at night and occurred in the upper 100 m at night but were all deeper than 350 m during the day (table 7.3).

7.4 Discussion

7.4.1 General

In most large scale analyses of mesopelagic fishes, the GOM stands out as a discrete region. Backus and others (1970) defined the GOM as 1 of 10 pelagic regions in the Western North Atlantic Ocean, dominated by generally tropical to subtropically distributed fishes. The GOM persisted as a discrete region in a broader analysis of the Myctophidae over the whole Atlantic Ocean (Backus and others, 1977). Similar results were obtained by Bangma and Haedrich (2008), who reported the GOM mesopelagic fishes constituted an ecotone between Sargasso Sea and Caribbean Sea faunas. We note that the above three studies dealt in different ways with essentially the same Woods Hole Oceanographic Institution database.

Despite high species richness, the Gulf of Mexico is no exception to the rule that mesopelagic fish assemblages are generally dominated by relatively few families and species. Numerically, the top two families in our samples, Gonostomatidae (principally *Cyclothone* spp.) and Myctophidae, also dominated the collections of Murdy and others (1983) and Hopkins and Lancraft (1984). While the Sternoptychidae were important in the above studies and ranked fourth in abundance in our study, the Phosichthyidae were more abundant in our samples than in those of Murdy and others (1983) or Hopkins and Lancraft (1984). This general mesopelagic ichthyofaunal structure was similar in the North Atlantic, where the most abundant fishes were consistently in the families Gonostomatidae, Myctophidae, Sternoptychidae and Melamphaidae (Backus and others, 1969; Badcock and Merrett, 1976; Howell and Krueger, 1987; Keene and others, 1987). The lack of consistency in sampling methods (notably discrete depth versus oblique sampling) and data treatment (omission of certain taxa) across studies impedes more detailed comparisons.

There is considerable similarity in dominant mesopelagic fishes among the GOM studies that treated the whole midwater ichthyofauna; however, there were also major differences. Aside from the overwhelming abundance of *Cyclothone* spp., the most abundant species varied somewhat among studies. While our top five non-*Cyclothone* species were, in decreasing order, *Notolychnus valdiviae, Valenciennellus tripunctulatus, Vinciguerria poweriae, Lepidophanes guentheri,* and *Benthosema suborbitale*, the most abundant non-*Cyclothone* in Murdy and others (1983) were *Sternoptyx pseudobscura, L. guentheri, Gonostoma elongatum, Ceratoscopelus warmingii,* and *B. suborbitale*. Hopkins and Lancraft (1984) reported *V. tripunctulatus, C. warmingii, Diaphus dumerilii, Lampanyctus alatus,* and *L. guentheri* as the dominant non-*Cyclothone* species in the eastern GOM. In a much greater sampling effort in the same area as that of Hopkins and Lancraft (1984), Hopkins and others (1996) reported the myctophids *N. valdiviae, D. dumerilii, L. guentheri, B. suborbitale*, and *L. alatus* to be most abundant (excluding *Cyclothone*). Backus and others (1970), also excluding *Cyclothone*, reported *N. valdiviae, L. alatus, B. suborbitale, V. poweriae,* and *Melamphaes pumilus* to be the dominant species in the upper 200 m at night in the GOM. The fifth most abundant species (*Melamphaes pumilus*) of Backus and others (1970) was not common in our samples, and several species (for example, *V. tripunctulatus, L. guentheri, Pollichthys mauli*) that ranked highly in our samples were uncommon or missing in Backus and others (1970). Treatments of only the myctophid fauna across the GOM (Backus and others, 1977) and in the eastern GOM (Gartner and others, 1987) confirmed the persistence of *N. valdiviae, C. warmingii, L. guentheri, L. alatus*, and *B. suborbitale* as abundant species. The most abundant stomiid fishes were the same three species (*Photostomias guernei*, *Chauliodus sloani*, *Stomias affinis*) in our samples and in the eastern GOM (Sutton and Hopkins, 1996a). The general similarities of dominant mesopelagic fishes

across the above studies, including our study, is evidence that this fauna is spatially and temporally stable, considering that the above collections were widely separated in time (1961 to 2007), that sampling and analysis methods varied, and that the samples were from different areas of the GOM.

The *Cyclothone* spp. are the most abundant member of the mesopelagic micronekton worldwide (Maynard and others, 1975; Badcock and Merrett, 1976; Miya and Nemoto, 1991; Gartner and others, 2008). Most of the species of this small-sized, generally nonmigrating genus are characteristic of the deeper depths (Maynard and others, 1975; De Forest and Drazen, 2009). This genus (at least 6 spp.) alone represented 50 percent of the numerical abundance in our overall fish catches, compared with 65 percent of all fishes in stations across the GOM (Murdy and others, 1983) and 34.1 percent of the overall micronekton (fishes and invertebrates) at an eastern GOM study area (Hopkins and Lancraft, 1984). In a longer term sampling of the same area as Hopkins and Lancraft (1984), Hopkins and others (1996) also reported that *Cyclothone* spp. dominated fish abundance. As in the eastern GOM (Hopkins and Lancraft, 1984; Hopkins and others, 1996) and off the Bahamas (McClain and others, 2001), *C. pallida* was the most abundant species (of all fishes) in our offshore GOM sites, closely followed by *C. pseudopallida*, *C. braueri*, and *C. alba*. The tendency for *Cyclothone* spp. to occupy deeper depths probably explained its rarity at the shallower VK826 site.

7.4.2 Site Comparisons

Our data suggested a fairly homogeneous mesopelagic fish fauna across the GOM with no clear geographic station groupings. Although our inshore study area yielded noticeable differences in fish composition compared with the offshore study areas, these were not statistically significant. Transitions from offshore to inshore were reported for the eastern GOM

with an abrupt change between bottom depths of 275-400 m (Hopkins and others, 1981), but VK826 (450-650 m) was well within the zone of typical deep-sea fishes. The lack of horizontal station groupings in the GOM supports the paradigm of faunal patterns being determined by large-scale water mass characteristics (Badcock and Merrett, 1976; Jahn and Backus, 1976; Sutton and others, 2008); thus, small-scale mesopelagic fish communities would not be expected. However, this lack of pattern in our statistical analyses was also influenced by the high catch variability, which is common in midwater samples. Although Backus and others (1970; 1977) and Bangma and Haedrich (2008) considered the GOM as a separate mesopelagic faunal region, we caution that this hypothesis was based on analysis of few samples that did not represent the whole GOM and analysis of only one fish family in one study (Backus and others, 1977). Most GOM studies did not sample the mesopelagic fauna in relation to the various water masses, which could influence geographic community patterns. The eastern and western basins of the GOM deeper than 900 m may be isolated (Weatherly and others, 2005), and a permanent cyclonic circulation in the upper 800 m of the Bay of Campeche may isolate that basin (Vázquez and others, 2005). Until more ubiquitous and synoptic data that better represent the upper 1,000 m of the GOM are available, existing data argue for a singular mesopelagic, tropical to subtropical ichthyofauna in the GOM.

Deep-sea bottom topography can influence distribution patterns of midwater pelagic fauna, perhaps indirectly at times through its influence on hydrography (Fock and others, 2004; Sutton and others, 2008; De Forest and Drazen, 2009). We are not aware of any data suggesting that the offshore ridges and seep sites over which we sampled modified local currents or other upper water-column parameters. Also, our sampling was usually well above the bottom. Pusch and others (2004) noted reduced species richness and abundances and truncated migration depth

ranges of mesopelagic fishes over North Atlantic seamounts. We also observed fewer species and lower abundances at the VK826 site; however, this is more likely related to the winnowing of oceanic species as one moves inshore (Hopkins and others, 1981). While the Vioska Knoll mounds are significant topographic highs, they are relatively small in area, and our Tucker trawl tows were not concentrated just over the high mounds but represented a larger area surrounding the mounds. Because of its shallower depth, mesopelagic fishes at VK826 by necessity had a truncated depth range available. Even though less water column was available at VK826, the fishes did not seem to take full advantage of the available range, since they were rarely seen on or near the bottom at VK826 (Sulak and others, 2007; S.W. Ross, UNCW, unpub. data, 2011). Some species may remain elevated off bottom over VK826 because of increased benthic predation around the coral and carbonate block habitats, as suggested for seamounts (Pusch and others, 2004). Although we have observed benthic predators eating mesopelagic fauna, this does not seem a likely explanation since midwater fishes were commonly observed all the way to the bottom at similar sites in similar depths off North Carolina (Gartner and others, 2008).

The strongest station cohesion was related to depth. Fishes composed a shallower group, including many diel vertical migrators, and a deeper group, including many species that did not migrate, with the boundary between these around 300-400 m depth. Baird and Wilson (1977) noted a persistent deep scattering layer (around 400-500 m) in the eastern GOM, containing largely nonmigrating species. Data are lacking from across the GOM to explore whether depth was consistently a major structuring factor in groups of mesopelagic fishes. Over the Mid-Atlantic Ridge, depth was also the major factor determining assemblage composition of deep-pelagic fishes (Sutton and others, 2008).

The potential impact of seasonal differences in interpreting GOM mesopelagic fish occurrence patterns is unclear. Large seasonal changes in micronekton fauna, probably in response to changing upper water column primary productivity, have been reported in other places (Maynard and others, 1975). But, most midwater fish datasets from the GOM resulted from sampling concentrated in the summer to fall months, and data were not examined for temporal patterns. Lancraft and others (1988) speculated that seasonal changes in the GOM (supposedly in the midwater community) might be small or nonexistent and that these might be masked by the patchy nature of the data. Just as more widespread sampling would help elucidate mesopelagic community structure (see above), synoptic sampling across all seasons is also needed to determine how mesopelagic fauna respond to seasonally changing oceanographic conditions.

7.4.3 Depth, Diel, and Size Distributions

Overall, our data compared with other studies illustrate considerable diversity in depth distributions and migratory behavior expressed by individual mesopelagic species. Most species of the genus *Cyclothone* were consistently reported not to undergo diel migrations (Badcock and Merrett, 1977; Miya and Nemoto, 1991; Kashkin, 1995; McClain and others, 2001) and were characterized as lethargic swimmers (McKelvie, 1989). While our two most abundant species in this genus, *C. pallida* and *C. pseudopallida*, exhibited the trend reported for the larger individuals to occur in deeper depths (Badcock and Merrett, 1976; Miya and Nemoto, 1991; McClain and others, 2001), diel depth distributions and overall ranges of our GOM samples deviated considerably, mostly at GC852, from those reported elsewhere. Although all individuals do not migrate, a substantial number of both species at GC852 shifted upward by 300 to 400 m at night. In most locations, *C. pallida* and *C. pseudopallida* usually occupied depths of

400-1,500 m and 400-800 m, respectively, at all times (Badcock and Merrett, 1976; Hopkins and others, 1981; Miya and Nemoto, 1986; Kashkin, 1995). Our data document the shallowest captures for these fishes as well as the first occurrences of what seem to be diel migrations. Diel migration was less clear for *C. alba* and *C. braueri*; however, both species exhibited upward shifts at night at GC852 that appear to be a diel migration. Although we are not aware of literature indicating a lunar effect on *Cyclothone*, GC852 was sampled during the new moon, and some mesopelagic fauna do move closer to the surface during the new moon (Clarke, 1973; Horning and Trillmich, 1999). We did not detect the layering of C. *braueri*, *C. alba*, and *C. pallida* (in descending depth order) as reported by Miya and Nemoto (1987), these species being comparable in depth ranges in our samples. In keeping with other reports (DeWitt, 1972; Hopkins and others, 1981; Kashkin, 1995), *C. acclinidens* occurred deeper than other *Cyclothone* at all times.

As reported elsewhere (Badcock and Merrett, 1976), most *Valenciennellus tripunctulatus* were captured at night. Lack of diel migration seems to be consistent behavior for this species (Badcock and Merrett, 1976; Hopkins and Baird, 1981); however, we captured it over a larger depth range than usually reported. Badcock and Merrett (1976) hypothesized that the difference in day/night captures was related to net avoidance during the day, when this species was most active.

The dominant Myctophidae in our samples exhibited typical diel migrations, but these varied with location and varied in some respects from other studies. The day and night depth ranges for *Notolychnus valdiviae* were similar to those reported elsewhere and suggested a definite upward migration at night (Badcock and Merrett, 1976; Gartner and others, 1987). Although Gartner and others (1987) indicated that the whole population of *N. valdiviae* at his

eastern GOM study area migrated, our data agreed more with Clarke (1973) and Badcock and Merrett (1976), indicating that some individuals remained at depth at night. We did not observe a clear trend of smaller individuals of *N. valdiviae* being shallower (Clarke, 1973; Gartner and others, 1987), nor did there seem to be a depression of the migrants to lower depths during the full moon (station VK826), as noted by Clarke (1973). Diel patterns of *Lepidophanes guentheri* in our GOM stations generally agreed with those from the eastern GOM (Gartner and others, 1987), except our depth ranges were somewhat shallower. Although all individuals do not migrate, many move all the way to the surface at night, and during the new moon they may occupy the shallowest parts of their range (Gartner and others, 1987). *Benthosema suborbitale* may exhibit ontogenetic diel movements, with juveniles (≤ approximately12 mm) reportedly not migrating (Clarke, 1973; Gartner and others, 1987). Juveniles of this species ≤12 mm SL occupied both deep and shallow zones at night in our collections. Their movements may be more complex, being tied more to coloration changes than absolute sizes (Badcock and Merrett, 1976). *Diaphus dumerilii* seems to be more affiliated with the upper slope to outer shelf interface (Hopkins and others, 1981; this study) and exhibits a shallower depth range than other myctophids (Gartner and others, 1987). While all individuals did not appear to exhibit diel migration, a substantial number moved toward the surface at night.

7.4.4 Conclusion

Our general conclusions are that (1) the mesopelagic realm of the GOM is represented by a species-rich fauna whose basic structure varies little over large distances and is perhaps similar over the whole GOM, (2) abundance and distribution patterns are related to depth, (3) a significant component of the fishes exhibit diurnal migrations and these may vary among different locations, and (4) a number of fishes expand their ranges at night but typically remain

in deeper depths at all times. These conclusions agree with most of the literature available for the GOM (see above). However, despite decades of sampling the midwater GOM fauna and a large number of papers published on this topic, this water body is still poorly known for deep-water fauna, and only one relatively small area in the eastern GOM is well studied for mesopelagic fauna. As noted above, many of the historical datasets did not have large sample sizes, were used in several different analyses from the GOM, and did not contain depth-discrete sampling. We propose that a better understanding of GOM midwater communities would be achieved by intense depth-discrete sampling (as in the eastern GOM) in strategic locations designed to represent different hydrographic and depth regimes, as well as different seasons.

7.5 Acknowledgments

Funding for this project was largely provided by the Department of the Interior U.S. Geological Survey under Cooperative Agreement No. 05HQAG0009, subagreement 05099HS004, and we especially thank Dr. Gary Brewer (USGS) for his support of our work. We are grateful to the crew of the R/V *Cape Hatteras* and all scientific staff for helping with fishing operations and sample processing. S. Artabane assisted with sampling and identification of fishes and C. Ames assisted with various aspects of the cruise. H. Roberts and C. Fisher provided multibeam maps of the seep sites to assist sample location. We thank G. Boland (BOEMRE), S.D. Brooke (MCI), and J. Sinclair (BOEMRE) for helpful comments on this chapter and J.H. Caruso for examining *Ceratias* spp. specimens.

8. DIETS AND FEEDING BEHAVIOR OF MIDWATER FISHES OVER COLD-SEEP SITES IN THE NORTH-CENTRAL GULF OF MEXICO

An expanded version of this paper was completed as a master's thesis:
McClain-Counts, J.P., 2010, Trophic structure of midwater fishes over cold seeps in the north-central Gulf of Mexico, M.S. thesis, University of North Carolina Wilmington.

By Jennifer P. McClain, Adela Y. Roa-Varón, Steve W. Ross, and Tara L. Casazza

University of North Carolina Wilmington, Center for Marine Science, Wilmington, NC

8.1 Introduction

Midwater fishes are an important component of the pelagic food web due to their high abundance and global distribution (Gjøsaeter and Kawaguchi, 1980; Cornejo and Koppelmann, 2006). These unique fishes inhabit the mesopelagic zone (200 to 1,000 m), and although midwater fishes are consumed by a variety of marine fauna, such as penguins (Adams and others, 2004), tuna (Potier and others, 2007), and grenadiers (Laptikhovsky, 2005), they are also important consumers of zooplankton. In low-latitude midwater fish assemblages, midwater fishes consumed 5-10 percent of the zooplankton production in the epipelagic zone (<200 m), with myctophids, sternoptychids, and stomiids consuming 31, 27, and 20 percent, respectively, of the zooplankton biomass per day (Hopkins and others, 1996). The ability of midwater fishes to affect both surface and benthic communities results from diel vertical migrations (DVMs).

DVMs are migratory patterns documented in many midwater fishes. During DVMs, midwater fishes migrate from the mesopelagic zone to the epipelagic zone (surface to 200 m) at night, primarily at sunset, and return to the mesopelagic zone at approximately sunrise. Dietz (1962) suggested DVMs occurred for feeding purposes due to the correlation between migrations

of mesopelagic fishes and their zooplankton prey (Merrett and Roe, 1974; Kinzer, 1977; Beamish and others, 1999; Yatsu and others, 2005). Additionally, increased densities of zooplankton and increased stomach fullness of midwater fishes collected in the epipelagic zone at night indicated midwater fishes primarily fed at night in the epipelagic zone (Backus and others, 1969; Kinzer, 1977; Williams and others, 2001; Pusch and others, 2004). Through DVMs, midwater fishes, particularly myctophids (Kinzer, 1977; Hidaka and others, 2001; Cornejo and Koppelmann, 2006), contributed significantly to the vertical transportation of organic matter from the epipelagic zone to the mesopelagic zone (Ashjian and others, 2002; Brodeur and Yamamura, 2005), thus impacting the trophic structure of the entire water column.

Previous dietary studies on midwater fishes utilized gut content analyses (GCA) to determine prey composition and trophic relationships. Midwater fishes were classified into three major feeding guilds: zooplanktivores, which consume planktonic organisms such as copepods, amphipods, and euphausiids; micronektonivores, which consume fishes and cephalopods; and generalists, which consumed a variety of unrelated taxa (Gartner and others, 1997). Placement into these guilds may be variable, with changes in feeding strategies possibly related to food availability (Kawaguchi and Mauchline, 1982). Variations within the diets of midwater fishes also occurred with changing seasons (Kawaguchi and Mauchline, 1982) and predator ontogeny (Kawaguchi and Mauchline, 1982; Young and Blaber, 1986; Hopkins and others, 1996; Beamish and others, 1999; Williams and others, 2001; Butler and others, 2001). Although GCA provides detailed diet data, this method produces little data when stomachs are empty, a common occurrence in midwater fishes. Additionally, these GCA only represent a short time frame (<24 h), and the cumulative feeding habits of the midwater fauna cannot be determined except through intensive temporal sampling.

Stable isotope analyses (SIA) can complement GCA and are becoming increasingly more important in ecological and biological fields. Although SIA cannot provide detailed diet data (for example, species-level identifications of prey), general information on the cumulative feeding habits of an organism (Fry, 2006) and trophic positions within a food web (Paradis and others, 2008) can be determined using SIA. Trophic studies utilizing SIA examined carbon and nitrogen signatures to elucidate relationships between predator and prey. The separation of heavy and light isotopes (termed fractionation) occurs during biological processes, such as photosynthesis, respiration, and digestion, because lighter isotopes require less energy to create or break bonds compared to heavier isotopes (Van Dover, 2000; Fry, 2006). Trophic fractionation, the shift in isotope ratios due to a change in trophic levels, can range from 1 to 5 per mil for nitrogen and 0 to 1 per mil for carbon (Lajtha and Michener, 1994); however, the majority of studies apply a shift of 3.4 per mil for nitrogen and 1 per mil for carbon (Minagawa and Wada, 1984; MacAvoy and others, 2002). Nitrogen isotopes are ideal for determining trophic relations in a food web, due to the high fractionation between trophic levels (Fry, 1988; Thomas and Cahoon, 1993; Van Dover, 2000; Hobson and others, 2002; Behringer and Butler, 2006; Fry, 2006), whereas carbon isotopes, with low trophic fractionation, are useful in determining carbon sources because fractionation during carbon fixation varies between primary producers (Lajtha and Michener, 1994; Fry, 2006). These differences in carbon fixation result in distinct ratios for different producers: -22 to -16 per mil for marine phytoplankton, -18 to -15 per mil for Sargassum spp., -16 to -5 per mil for turtlegrass, and –75 to -28 per mil for chemosynthetic material (Kennicutt and others, 1992; Hemminga and Mateo, 1996; Aharon and Fu, 2000; Post, 2002; Fry, 2006; Rooker and others, 2006).

In the Gulf of Mexico (GOM), the trophic structure of midwater fishes may be influenced by the presence of chemosynthetic cold seeps, but the extent of these effects (if any) is unknown. While a chemosynthetic trophic pathway provides a nutritional basis to support components of benthic cold-seep communities (Brooks and others, 1987), recent studies documented higher abundances of nonseep, benthic species in the vicinity of seeps (Levin, 2005) and some benthic species consuming chemosynthetic material (MacAvoy and others, 2002; 2008b). However, whether the trophic structure of midwater fishes is influenced by chemosynthetic energetic pathways, either in the water column above the seep or in the benthic communities through interaction, has not been examined.

Understanding the trophic relationships of midwater fishes provides necessary information regarding energy flow through the water column, particularly as midwater fishes that undergo DVMs can provide a link between surface and benthic communities. While previous dietary studies on midwater fish in the GOM only focused on a few species from the eastern GOM (Hopkins and Baird, 1985a; 1985b; Lancraft and others, 1988; Sutton and Hopkins, 1996b) and did not utilize SIA, this study incorporated multiple midwater fish species collected over three cold-seep habitats (>1,000 m) in the north-central GOM. The objectives were to utilize GCA and SIA to (1) determine basic feeding patterns of the dominant midwater fish species collected, (2) document feeding changes, if any, that occurred due to spatial, temporal, or size variations, (3) examine the relationship between feeding and DVM in midwater fishes, (4) document differences in feeding based on GCA and SIA, and (5) determine to what extent, if any, the midwater community utilized chemosynthetic energy sources from cold seeps in the GOM.

8.2 Methods

8.2.1 Study Area

Three cold-seep sites in the GOM were selected for sampling, based on previous data collected by TDI-Brooks International, Inc.: Green Canyon 852 (GC852), Atwater Valley 340 (AT340), and Alaminos Canyon 601 (AC601). The sites were located on the middle to lower slope in the north-central GOM, and each contained benthic chemosynthetic communities (fig. 8.1). AT340 (2,216 m) contained a topographic high topped with multiple mounds. The area had extensive carbonate substrata, large mussel beds, clumps of tubeworms, and a few soft corals. GC852 (1,450 m) was characterized by an elongated mound approximately 2 km long running north to south, with vast amounts of carbonate substrata and numerous corals on the crest. This area also contained tubeworms and mussel beds. AC601 (2,340 m) differed from the other two sites, having low relief and a large brine pool. Some carbonate substrata and a few isolated aggregations of tubeworms were present, none of which were near the brine pool. High methane concentrations were recorded in the water column over this site. See Ross and others (Chapter 7, this volume) for additional details of these sites.

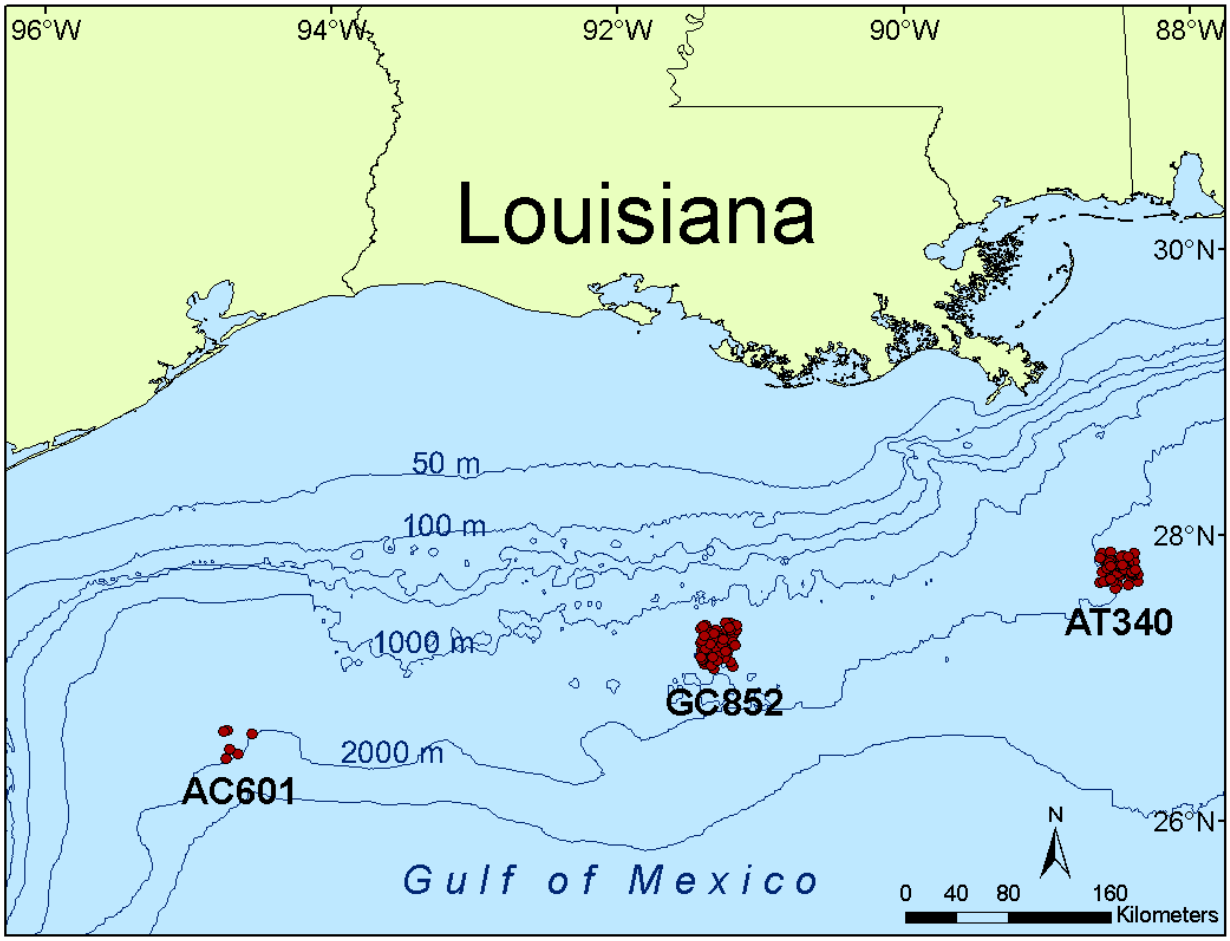


Figure 8.1. North-central Gulf of Mexico illustrating sampling areas for midwater fauna, August 9-25, 2007, at three cold-seep sites (AC601, GC852, and AT340) located on the slope at depths >1,000 meters. Each dot represents one station.

8.2.2 Sample Collection

Sampling was conducted during 24-h operations in the GOM August 9-25, 2007; however, due to inclement weather, only minimal night sampling was conducted at AC601. A total of 173 stations (45 day, 108 night, and 20 twilight) were sampled (table 8.1). Midwater fauna were collected using a discrete-depth Tucker trawl (2 x 2 m) with a plankton net (0.5 m diameter, 335 µm mesh) placed inside the Tucker trawl to simultaneously sample the smaller

components of the midwater community. The trawl was deployed open, and it was assumed no significant fishing occurred during deployment due to the rapid lowering, steep wire angle, and minimal forward movement of the vessel (Gartner and others, 2008; Ross and others, Chapter 7, this volume). Upon reaching the designated depth, the trawl fished for approximately 30 min at a 2-knot ground speed and was triggered closed using a double trip mechanism. All trawls were equipped with a Sea-Bird SBE39 temperature-depth recorder (TDR) attached to the upper frame bar to record time, depth, and temperature during deployment. Actual time and depth fished for each trawl were determined post-trawl using data from the TDR, and these data were used throughout the cruise to adjust fishing strategies to achieve desired sampling depths.

Table 8.1. Surface stations sampled over three cold-seep sites (AC601, GC852, and AT340) (see fig. 8.1) in the Gulf of Mexico (August 9-25, 2007).

[PN, plankton net; NN, Neuston net; 5 GB, 5-gallon bucket for POM samples; D, day (0630 to 1930 h CDT); N, night (1930 to 0630 h CDT)]

Station #	Gear	Date	Location	Time	Start Latitude	Start Longitude	End Latitude	End Longitude
CH-2007-004	5 GB	10-Aug-07	GC852	N	27° 07.979	91° 09.028	27° 07.979	91° 09.028
CH-2007-006	5 GB	10-Aug-07	GC852	N	27° 07.288	91° 09.771	27° 07.288	91° 09.771
CH-2007-009	PN - 0.5m	10-Aug-07	GC852	N	27° 07.042	91° 09.829	27° 06.048	91° 10.025
CH-2007-024	PN - 0.5m	11-Aug-07	GC852	N	27° 08.093	91° 09.807	27° 07.078	91° 09.925
CH-2007-028	PN - 0.5m	11-Aug-07	GC852	D	27° 07.769	91° 09.810	27° 08.269	91° 09.817
CH-2007-034	5 GB	12-Aug-07	GC852	N	27° 06.928	91° 09.881	27° 06.928	91° 09.881
CH-2007-039	PN - 0.5m	12-Aug-07	GC852	N	27° 08.036	91° 08.901	27° 08.138	91° 08.817
CH-2007-041	PN - 1m	12-Aug-07	GC852	D	27° 02.139	91° 09.618	27° 01.057	91° 09.738
CH-2007-043	NN	12-Aug-07	GC852	D	27° 10.116	91° 09.910	27° 10.824	91° 09.867
CH-2007-051	5 GB	13-Aug-07	GC852	N	27° 07.516	91° 09.790	27° 07.516	91° 09.790
CH-2007-052	PN - 0.5m	13-Aug-07	GC852	N	27° 07.274	91° 09.786	27° 06.794	91° 09.760
CH-2007-067	5 GB	13-Aug-07	GC852	N	27° 07.166	91° 10.418	27° 07.166	91° 10.418
CH-2007-069	PN - 0.5m	14-Aug-07	GC852	N	27° 07.505	91° 10.418	27° 07.015	91° 10.406
CH-2007-075	NN	14-Aug-07	GC852	D	27° 08.616	91° 10.180	27° 08.149	91° 10.169
CH-2007-076	5 GB	14-Aug-07	GC852	D	27° 06.656	91° 10.079	27° 06.656	91° 10.079
CH-2007-079	5 GB	14-Aug-07	GC852	D	27° 09.388	91° 09.765	27° 09.388	91° 09.765
CH-2007-081	5 GB	14-Aug-07	GC852	D	27° 09.660	91° 09.695	27° 09.660	91° 09.695
CH-2007-120	PN - 0.5m	18-Aug-07	AC601	N	26° 22.991	94° 30.838	26° 23.584	94° 30.851
CH-2007-121	5 GB	18-Aug-07	AC601	N	26° 24.247	94° 30.814	26° 24.247	94° 30.814
CH-2007-123	5 GB	18-Aug-07	AC601	N	26° 22.941	94° 30.823	26° 22.941	94° 30.823
CH-2007-125	5 GB	18-Aug-07	AC601	N	26° 23.705	94° 30.996	26° 23.705	94° 30.996
CH-2007-129	5 GB	18-Aug-07	AC601	D	26° 23.683	94° 30.923	26° 23.683	94° 30.923
CH-2007-133	5 GB	18-Aug-07	AC601	D	26° 22.504	94° 31.365	26° 22.504	94° 31.365
CH-2007-142	5 GB	21-Aug-07	AT340	N	27° 39.388	88° 24.022	27° 39.388	88° 24.022
CH-2007-146	5 GB	21-Aug-07	AT340	D	27° 38.812	88° 21.766	27° 38.812	88° 21.766
CH-2007-148	5 GB	21-Aug-07	AT340	D	27° 38.905	88° 21.766	27° 38.905	88° 21.766

Table 8.1. Surface stations sampled over three cold seep sites (AC601, GC852, and AT340) (see fig. 8.1) in the Gulf of Mexico (9-25 August 2007).—Continued

[PN, plankton net; NN, Neuston net; 5 GB, 5-gallon bucket for POM samples; D, day (0630 to 1930 h CDT); N, night (1930 to 0630 h CDT)]

Station #	Gear	Date	Location	Time	Start Latitude	Start Longitude	End Latitude	End Longitude
CH-2007-150	5 GB	21-Aug-07	AT340	N	27° 38.770	88° 21.118	27° 38.770	88° 21.118
CH-2007-155	PN - 0.5m	22-Aug-07	AT340	D	27° 37.933	88° 21.129	27° 37.921	88° 21.422
CH-2007-158	PN - 0.5m	22-Aug-07	AT340	D	27° 38.470	88° 21.414	27° 38.460	88° 21.402
CH-2007-162	5 GB	22-Aug-07	AT340	N	27° 38.607	88° 22.151	27° 38.607	88° 22.151
CH-2007-167	5 GB	23-Aug-07	AT340	N	27° 38.412	88° 21.200	27° 38.412	88° 21.200
CH-2007-170	PN - 0.5m	23-Aug-07	AT340	N	27° 38.396	88° 21.268	27° 38.578	88° 21.641
CH-2007-174	5 GB	23-Aug-07	AT340	D	27° 39.034	88° 21.969	27° 39.034	88° 21.969
CH-2007-177	PN - 1m	23-Aug-07	AT340	D	27° 38.640	88° 21.781	27° 38.872	88° 22.582
CH-2007-179	5 GB	23-Aug-07	AT340	D	27° 38.383	88° 21.177	27° 38.383	88° 21.177
CH-2007-181	PN - 1m	23-Aug-07	AT340	D	27° 39.326	88° 23.419	27° 39.653	88° 24.196
CH-2007-185	PN - 1m	23-Aug-07	AT340	N	27° 38.183	88° 20.712	27° 38.226	88° 20.129
CH-2007-187	PN - 1m	23-Aug-07	AT340	N	27° 38.235	88° 20.784	27° 38.434	88° 21.485
CH-2007-190	5 GB	24-Aug-07	AT340	N	27° 38.291	88° 20.933	27° 38.291	88° 20.933
CH-2007-201	PN - 1m	24-Aug-07	AT340	N	27° 38.335	88° 21.133	27° 38.665	88° 21.757
CH-2007-203	PN - 1m	24-Aug-07	AT340	N	27° 38.387	88° 21.184	27° 38.757	88° 21.929
CH-2007-206	PN - 1m	24-Aug-07	AT340	N	27° 38.415	88° 21.190	27° 38.819	88° 21.964
CH-2007-215	PN - 1m	25-Aug-07	AT340	N	27° 38.479	88° 21.082	27° 38.785	88° 22.031
CH-2007-217	PN - 1m	25-Aug-07	AT340	N	27° 38.577	88° 21.421	27° 38.838	88° 22.485
CH-2007-221	PN - 1m	25-Aug-07	AT340	N	27° 38.565	88° 20.778	27° 38.698	88° 21.596

Additional sampling was conducted to collect potential zooplankton and phytoplankton for stable isotope analysis (SIA, table 8.1). A 1.1 x 2.4-m Neuston net (6.4-mm mesh body and 3.2-mm tail bag) and plankton nets (335 µm and 505 µm mesh) were deployed at the surface and towed for 15-30 min to obtain zooplankton samples. Particulate organic matter (POM) was collected by filtering seawater through a 125-µm precombusted glass filter, and it was assumed that the majority of POM was phytoplankton (Kling and others, 1992). The POM and zooplankton samples provided a trophic baseline for SIA.

All fishes collected were preserved in 10-percent seawater-formalin solution and later transferred to 50-percent isopropyl for storage until dietary analyses. Invertebrates were preserved in 70-percent ethanol, with the exception of jelly and salp specimens that were preserved in 10-percent seawater-formalin solution. All specimens were sorted, identified to the lowest possible taxa, and measured to the nearest millimeter standard length (SL, fishes) or total length (TL, invertebrates). The life history stage of fishes was also recorded based on the presence or absence of gonads. A fish was classified as juvenile when either no gonads or immature gonads were documented.

8.2.3 Diet Analyses

Gut content analysis (GCA) was conducted for the 18 most abundant species using methods outlined in Ross and Moser (1995). Fish specimens were subsampled from each site to include all sizes ranges and depths sampled. Fishes were dissected and the stomachs were removed for GCA. Stomach fullness was estimated as 0, 5, 25, 50, 75, or 100 percent. Empty stomachs were documented and the percent number of empty stomachs was calculated for day and night samples at all sites; however, data on empty stomachs were not included in most analyses. Stomach contents were placed on a Petri dish and identified to the lowest possible

taxa. Similar prey items were then piled together on a grid of 1 mm squares and flattened to a uniform height, which was measured. The height multiplied by the number of squares occupied by the food item yielded volume in cubic millimeters. The sum of all prey volumes equaled the total volume of the stomach, and the volume of each prey item was converted to a percentage of the total prey volume (%V). The frequency of occurrence for a prey item equaled the number of times a prey item occurred in the fish species examined divided by the total number of stomachs analyzed for that species.

The relationship between DVMs and stomach fullness was examined by plotting stomach fullness as a function of time of day and mean depth sampled for each Tucker trawl. Time of day was divided into three categories: day (0730 to 1830 CDT), night (2030 to 0530 CDT), and twilight (0530 to 0730 and 1830 to 2030 CDT, 1 h on either side of the average sunrise and sunset). The mean depth for each Tucker trawl tow was calculated by averaging all depths recorded by the TDR from the start to the end of each tow, similar to Ross and others (Chapter 7, this volume). Trawls with no mean sampling depth were excluded.

8.2.4 Stable Isotope Analysis (SIA)

Before specimens were preserved in formalin or ethanol, samples of white muscle tissue were dissected from fishes and invertebrates and frozen for later SIA. For consistency, tissue was taken from similar areas based on the type of specimen (that is, muscle tissue was removed from the dorsal region of fishes, the caudal region of shrimps, the legs for crabs, and the mantle for mollusks). When specimens were too small to dissect tissue from, the entire specimen was utilized. Contamination from other tissue types was minimized by removing the head, tail, entrails, scales, and photophores prior to obtaining the tissue sample. For specimens taken whole, identification occurred either prior to sampling or a similar specimen was saved as a

reference for future identification. Collected isotope samples were dried and crushed into a powder. The majority of samples were dried to a constant weight in a convection oven at 50-60˚C. Additional samples were frozen at -80˚C for a minimum of 24 h and freeze dried in a VirTis Benchtop 3.3 Vac-Freeze. It was assumed no significant differences occurred in isotopic ratios as a result of different drying techniques (Bosley and Wainright, 1999).

Tissue samples were analyzed for carbon and nitrogen isotope ratios. For each sample, approximately 500 µg were placed into a tin capsule and combusted in an Elemental Combustion System Model 4010 coupled to a Delta V Plus Isotope Ratio Mass Spectrometer (IRMS) via Conflo II interface at the University of North Carolina Wilmington (UNCW). POM samples (provided by A. Demopoulos, USGS) were analyzed at Washington State University (WSU) using a Costech (Valencia, Calif.) elemental analyzer interfaced to a GV instruments (Manchester, U.K.) Isoprime IRMS. Precision of the IRMS was verified by repeated analysis of standards USGS 40 and USGS 41, which were incorporated into each sample run. Raw delta values were corrected for linearity and normalized to known reference materials USGS 40 and USGS 41. A similar procedure was utilized at WSU using egg albumin powder calibrated against National Institute of Standards reference materials. Reproducibility was monitored using several organic reference standards (Fry, 2007).

Isotope ratios were expressed in the standard delta (δ) notation as parts per thousand (‰) according to the following equation:

$$\delta X = \frac{(R_{sample} - R_{standard})}{R_{standard}} * 1000 \qquad (1)$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The global standards for $\delta^{13}C$ and $\delta^{15}N$ are Vienna PeeDee Belemnite and atmospheric nitrogen (air). A minimum of

five samples were analyzed per fish species, with the exception of *Photostomias guernei* (n = 4). In order to increase sample size, *Diaphus mollis, D. lucidus,* and *D. problematicus* were grouped together and referred to as *Diaphus* spp., and *Sternoptyx diaphana* and *S. pseudobscura* were grouped together and referred to as *Sternoptyx* spp.

8.2.5 Statistical Analyses

Statistical analyses were conducted on isotope ratios using SigmaStat 3.4. Data were analyzed for normality and homogeneity of variance using Kolmogorov-Smirnov and Levene Median tests. One way analysis of variance (ANOVA) was used to determine significant differences in isotopic values for primary producers, invertebrates, and fishes. A post-hoc Tukey test was used for pairwise comparisons of statistical differences among groups. Data that failed normality or equal variance tests were analyzed with ANOVA on the Ranks and the post-hoc Dunn's test. Species with low sample sizes (n <5) were not analyzed statistically. Species comparisons between sites AT340 and GC852 were analyzed using a two-way ANOVA and post-hoc Holm-Sidak test. Regressions of $\delta^{15}N$ against fish SL were conducted to determine whether ontogenetic shifts in diet occurred. Statistical significance was determined when p was <0.05. Isotope data were reported with the mean ±1 standard error.

8.3 Results

Tucker trawling resulted in a total of 123 tows (33 day and 90 night) from three sites (AC601, GC852, and AT340); however, AC601 consisted of only minimal sampling at night (n = 5). The mean sampled depth ranges for each site were 63 to 1,503 m for AT340, 21 to 1,067 m for GC852, and 45 to 584 m for AC601. A total of 8,716 fishes (30 families) were collected, but 97.7 percent of these fishes were from five families, Gonostomatidae (58.8 percent),

Myctophidae (27.4 percent), Phosichthyidae (5.8 percent), Sternoptychidae (4.4 percent) and Stomiidae (1.3 percent) (see Ross and others, Chapter 7, this volume).

The GCA was conducted on the 18 most abundant species within these 5 families (in order of decreasing abundance): *Notolychnus valdiviae, Cyclothone pallida, C. pseudopallida, C. braueri, C. alba, Valenciennellus tripunctulatus, Vinciguerria poweriae, Lepidophanes guentheri, Benthosema suborbitale, Hygophum benoiti, Pollichthys mauli, Gonostoma elongatum, Ceratoscopelus warmingii, Chauliodus sloani, Lampanyctus alatus, Argyropelecus aculeatus, Diaphus mollis*, and *A. hemigymnus*. Gut contents were analyzed for 2,925 stomachs, of which 1,636 (56 percent) were empty. A total of 119 food items (# species, # families) were identified and grouped into 16 general prey categories: Amphipoda, Annelida, Cephalopoda, Chaetognatha, Cnidaria, Copepoda, Crustacea, Decapoda, Euphausiacea, Fish, Gastropoda, Mollusca, Nematoda, Ostracoda, Salpida, and Other. Copepods were the dominant prey, documented in the stomachs of all midwater fish species except *C. sloani*, which was a piscivore.

SIA was conducted on 246 samples, collected from the Neuston net (n = 2), plankton nets (n =21), Tucker trawl (n = 123), other gear, and filtered seawater (n= 22). These samples represented 19 fish species (5 families), 15 invertebrate taxa, and three potential carbon sources (detritus, *Sargassum* sp., and POM, table 8.2).

Table 8.2. Mean $\delta^{15}N$ and $\delta^{13}C$ (±1 SE) stable isotope values by species collected at three cold-seep sites (AC601, GC852, and AT340) in the Gulf of Mexico, August 9-25, 2007.

[(n), the number of samples averaged for each taxon; SE, standard error; POM, particulate organic matter]

Species	mean $\delta^{15}N$ ±1 SE (n)			mean $\delta^{13}C$ ±1 SE (n)		
	AC601	AT340	GC852	AC601	AT340	GC852
Fish						
Argyropelecus aculeatus		8.68 ± 0.24 (5)	7.90 ± 0.44 (5)		-17.94 ± 0.41 (5)	-18.83 ± 0.10 (5)
Ceratoscopelus warmingii	6.36 ± 0.29 (3)	6.43 ± 0.63 (4)	7.21 ± 0.33 (5)	-19.6 ± 0.26 (3)	-18.98 ± 0.35 (4)	-19.34 ± 0.21 (5)
Chauliodus sloani			8.03 ± 0.24 (5)			-19.95 ± 0.54 (5)
Diaphus spp.			8.84 ± 0.27 (6)			-19.32 ± 0.23 (6)
Cyclothone acclinidens		9.52 (1)			-18.45 (1)	
Cyclothone alba			7.32 ± 0.32 (5)			-19.53 ± 0.12 (5)
Cyclothone pallida	8.44 ± 0.28 (5)	8.56 ± 0.63 (4)		-19.22 ± 0.12 (5)	-18.82 ± 0.13 (4)	
Gonostoma elongatum		7.28 ± 0.27 (5)	8.90 ± 0.32 (5)		-18.90 ± 0.11 (5)	-18.87 ± 0.39 (5)
Lampanyctus alatus	7.91 ± 0.22 (3)	7.49 ± 0.29 (5)	8.47 ± 0.27 (5)	-19.52 ± 0.22 (3)	-18.95 ± 0.19 (5)	-19.55 ± 0.18 (5)
Lepidophanes guentheri		6.75 ± 0.28 (5)	7.94 ± 0.25 (4)		-18.42 ± 0.05 (5)	-19.22 ± 0.10 (4)
Myctophum affine			5.63 ± 0.28 (5)			-21.52 ± 0.27 (5)
Photostomias guernei			8.96 ± 0.40 (4)			-18.49 ± 0.14 (4)
Pollichthys mauli		6.67 ± 0.25 (5)			-18.50 ± 0.13 (5)	
Sternoptyx diaphana			8.52 ± 0.07 (3)			-19.48 ± 0.14 (3)
Sternoptyx pseudobscura			8.67 ± 0.17 (4)			-19.96 ± 0.10 (4)
Valenciennellus tripunctulatus	9.15 ± 0.09 (5)	8.57 ± 0.28 (5)	9.06 ± 0.21 (5)	-19.97 ± 0.14 (5)	-18.76 ± 0.41 (5)	-19.73 ± 0.16 (5)
Vinciguerria poweriae		7.59 ± 0.32 (5)	7.94 ± 0.10 (10)		-18.98 ± 0.09 (5)	-19.60 ± 0.09 (10)
Invertebrate						
Acanthephyra purpurea		6.98 (1)	8.85 ± 0.24 (2)		-18.41 (1)	-17.41 ± 0.09 (2)
Ancistrocheirus lesuerii			6.19 (1)			-19.25 (1)
Chaetognatha		8.84 ± 1.24 (5)			-19.98 ± 0.19 (5)	
Copepoda		7.00 ± 1.53 (5)	6.21 ± 1.56 (5)		-19.77 ± 0.43 (5)	-19.91 ± 0.61 (5)
Euphausiacea	5.97 ± 0.66 (4)	4.98 ± 0.23 (5)	6.65 ± 0.25 (2)	-19.57 ± 0.31 (4)	-19.33 ± 0.08 (5)	-19.67 ± 0.02 (2)
Gennadas valens	6.83 ± 0.12 (4)	6.49 ± 0.10 (4)	7.50 ± 0.32 (6)	-19.16 ± 0.39 (4)	-18.26 ± 0.14 (4)	-18.47 ± 0.31 (6)

Table 8.2. Mean $\delta^{15}N$ and $\delta^{13}C$ (±1 SE) stable isotope values by species collected at three cold-seep sites (AC601, GC852, and AT340) in the Gulf of Mexico, August 9-25, 2007.

[(n), the number of samples averaged for each taxon; SE, standard error; POM, particulate organic matter]

	mean $\delta^{15}N$ ± 1 SE (n)			mean $\delta^{13}C$ ±1 SE (n)		
Species	**AC601**	**AT340**	**GC852**	**AC601**	**AT340**	**GC852**
Japetella diaphana			5.67 (1)			-19.55 (1)
Nematoscelis megalops			6.60 ± 0.33 (3)			-19.89 ± 0.08 (3)
Salpida		0.95 ± 0.14 (5)			-17.92 ± 0.27 (5)	
Sergia sp.			7.79 (1)			-19.10 (1)
Stigmatoteuthis arcturi			10.39 ± 1.04 (3)			-20.71 ± 0.10 (3)
Systellaspis debilis		5.93 ± 0.29 (6)	6.30 (1)		-17.84 ± 0.07 (6)	-17.17 (1)
Zooplankton	5.96 ± 2.17 (2)	5.69 ± 0.16 (3)	6.92 ± 1.09 (5)	-19.67 ± 0.41 (2)	-24.65 ± 4.65 (3)	-20.37 ± 0.93 (5)
Plant						
Detritus		3.60 ± 2.08 (5)			-9.95 ± 0.92 (5)	
POM	3.82 ± 0.57 (5)	5.25 ± 0.35 (8)	2.13 ± 0.64 (9)	-20.01 ± 0.78 (5)	-19.36 ± 0.69 (8)	-19.64 ± 0.74 (9)
Sargassum spp.	1.40 ± 0.33 (3)	0.84 (1)	1.88 ± 1.00 (5)	-17.71 ± 0.51 (3)	-18.48 ± 0.29 (1)	-18.33 ± 1.11 (5)

8.3.1 Primary Producers

Spatial variations in $\delta^{13}C$ and $\delta^{15}N$ were examined in the three potential carbon sources (detritus, *Sargassum* sp., and POM, table 8.2). No statistical comparisons were conducted on detritus (only collected at site AT340), or *Sargassum* spp. (n <5 at AT340 and AC601). Statistical comparisons conducted on POM documented no significant differences in $\delta^{13}C$ among sites; however, POM samples collected at GC852 were depleted in ^{15}N compared to samples collected at AT340 (post-hoc Tukey test, $p = 0.003$).

Species variations in $\delta^{13}C$ and $\delta^{15}N$ were also examined among the three potential carbon sources by combining data from all sites. Although a chemosynthetic signature was not detected in isotope samples for any carbon source, there was a clear distinction in $\delta^{13}C$ for each carbon source. Detritus was significantly enriched in ^{13}C compared to *Sargassum* spp. (Tukey, $p < 0.001$) and POM (Tukey, $p < 0.001$), while *Sargassum* spp. was significantly enriched in ^{13}C compared to POM (Tukey, $p = 0.031$, table 8.2). There were no significant differences in $\delta^{15}N$ between detritus and POM or detritus and *Sargassum* spp.; however, *Sargassum* spp. was significantly depleted in ^{15}N compared to POM (Dunn's, $p < 0.05$).

8.3.2 Invertebrates

Although no GCA was conducted on invertebrate specimens, SIA was conducted on 15 invertebrate species (7 orders) from all 3 sites combined (table 8.2). Due to the small sample sizes, spatial variations among invertebrate taxa were only conducted for Copepoda collected at GC852 and AT340 (table 8.2). There were no significant differences in $\delta^{13}C$ or $\delta^{15}N$ for Copepoda between GC852 and AT340. Small sample sizes also prevented statistical species comparisons for all invertebrates except Decapoda. Comparisons between two decapod species,

Gennadas valens and *Systellaspis debilis*, documented significantly depleted ^{13}C but significantly enriched ^{15}N in *G. valens* compared to *S. debilis* (post-hoc Tukey, $p = 0.034$, $p = 0.007$). Although no chemosynthetic signature was detected in any invertebrates, a trophic shift of approximately 2 per mil in nitrogen was documented in invertebrate specimens with increasing trophic levels at all three sites (fig. 8.2).

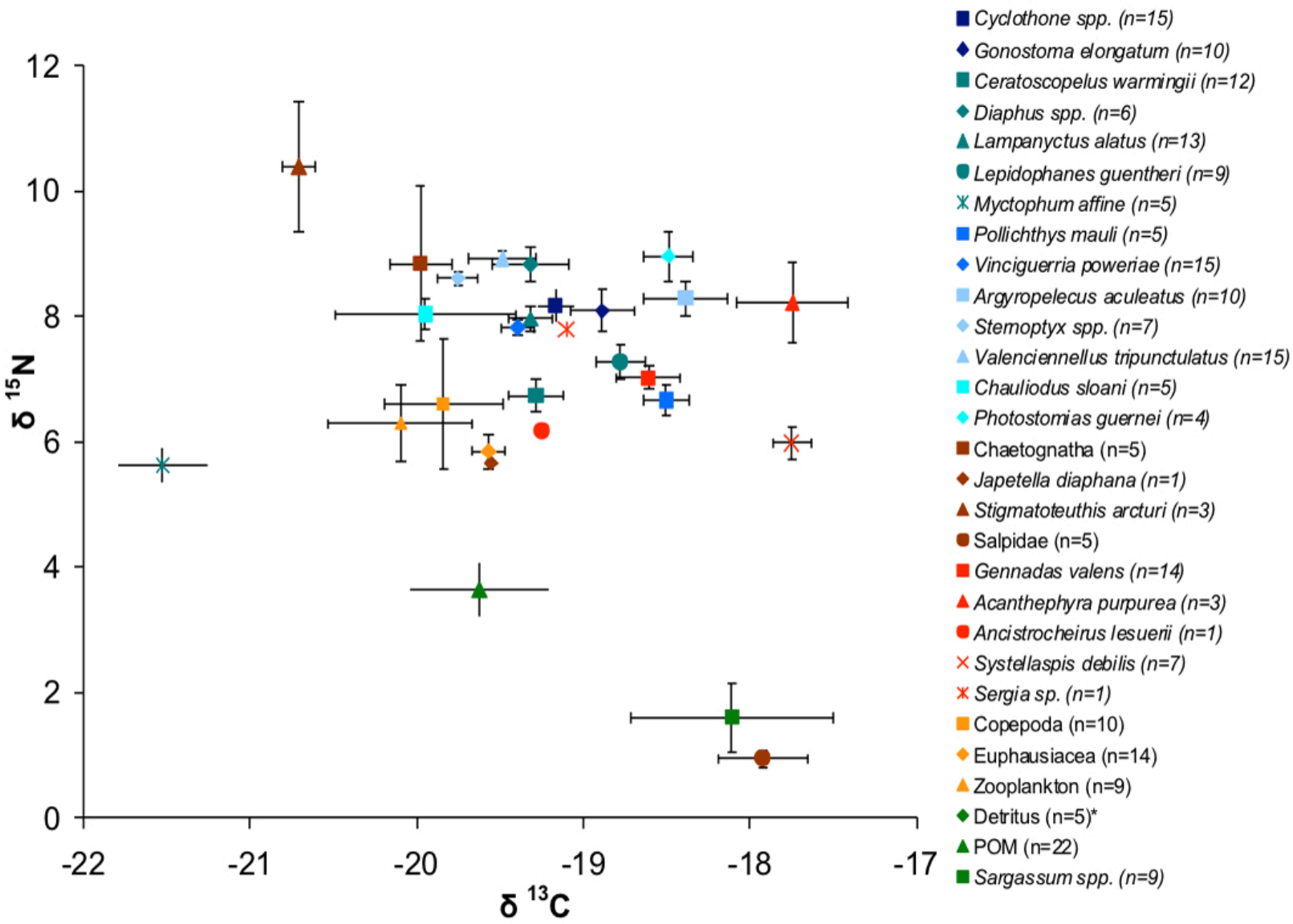


Figure 8.2. Mean $\delta^{13}C$ and $\delta^{15}N$ (± 1 SE) stable isotope values for all samples collected. Data from all three sites for each species were grouped together. *, Detritus values are not shown.

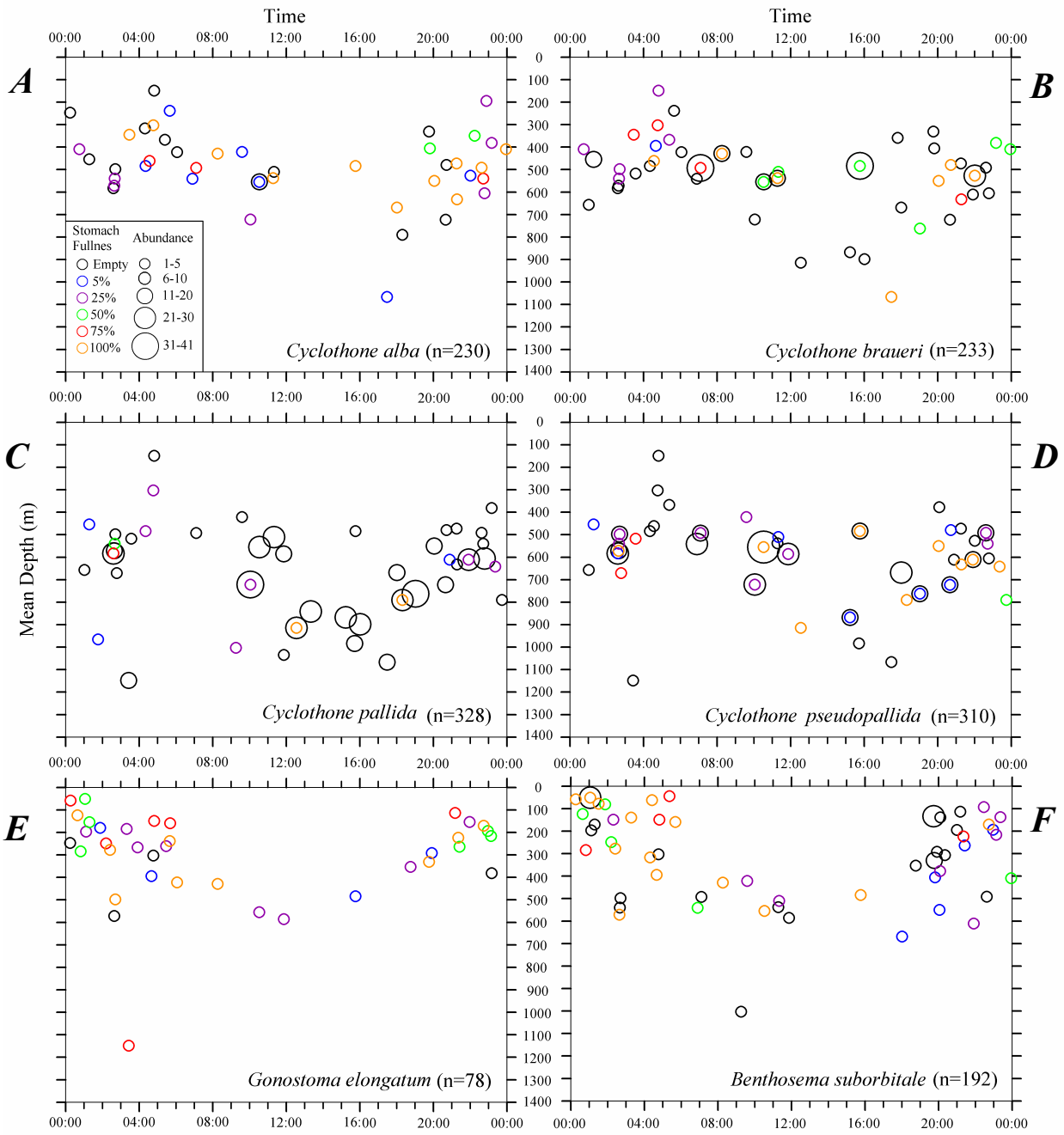
8.3.3 Fishes

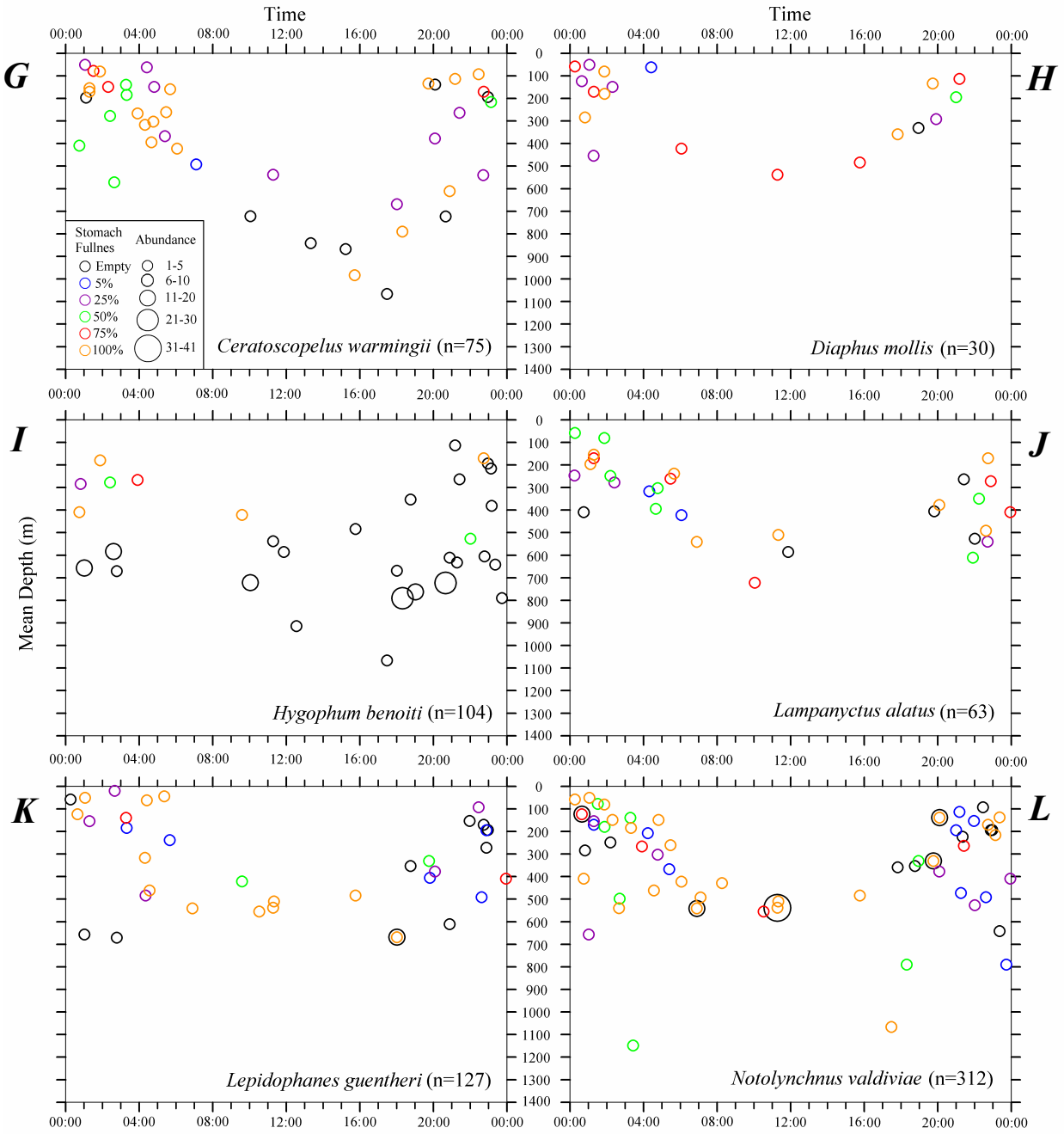
8.3.3.1 Gonostomatidae

GCA were conducted on five gonostomatid species: *C. alba, C. braueri, C. pallida, C. pseudopallida,* and *G. elongatum*. A high percentage of empty stomachs (>65 percent at all sites) occurred in all *Cyclothone* spp., with empty stomachs occurring more frequently in specimens collected during the day. In contrast, empty stomachs occurred less frequently in *G. elongatum* than *Cyclothone* spp. (22 percent at AT340 and 48 percent at GC852); however, empty stomachs only occurred in specimens collected at night. Migration patterns were examined to document general trends in feeding. No DVMs were apparent for *C. alba* and *C. braueri* (fig. 8.3*A, B*), with more full stomachs documented at night. Slight DVMs were evident for *C. pallida* and *C. pseudopallida*, with more full stomachs documented during the day for *C. pallida* (fig. 8.3*C*) and full stomachs occurring throughout a 24-h period for *C. pseudopallida* (fig. 8.3*D*). In contrast, *G. elongatum* exhibited a strong DVM pattern associated with more full stomachs in the epipelagic at night (fig. 8.3*E*).

Diet comparisons were examined to document differences among gonostomatid species. A similar diet composition was documented in all *Cyclothone* spp., with Copepoda and Ostracoda documented as the most important prey in both overall percent volume and percent frequency (tables 8.3-8.6). Despite these diet similarities, the species of Copepoda consumed varied among *Cyclothone* spp. *Pleuromamma* spp. was the dominant copepod consumed by *C. alba* (table 8.3), whereas *Aegisthus mucronatus* was more important in the diets of *C. braueri* (table 8.5). *Valdiviella minor* was volumetrically more important in the stomachs of *C. pseudopallida,* though *Lubbockia* sp. occurred more frequently (table 8.6). This diet

composition remained similar at all sites with one exception. At GC852, Amphipoda was volumetrically more important than Copepoda in the diet of *C. pallida* collected at night (table 8.4). Compared with *Cyclothone* spp., *Gonostoma elongatum* consumed a higher diversity of prey items, which included non-crustacean prey items such as Chaetognatha (table 8.7). Euphausiids and decapods were volumetrically more important in *G. elongatum*, whereas copepods occurred more frequently in the stomachs. Examination of the diets on a spatial scale revealed euphausiids were more important volumetrically for *G. elongatum* at GC852, whereas decapods were more important volumetrically at AT340 (table 8.7).


Time
Time
A
B
C
D
E
F
Mean Depth (m)
Stomach Fullnes
Empty
5%
25%
50%
75%
100%
Abundance
1-5
6-10
11-20
21-30
31-41
Cyclothone alba (n=230)
Cyclothone braueri (n=233)
Cyclothone pallida (n=328)
Cyclothone pseudopallida (n=310)
Gonostoma elongatum (n=78)
Benthosema suborbitale (n=192)


G
H
I
J
K
L
Time
Mean Depth (m)
Stomach Fullnes
Empty
5%
25%
50%
75%
100%
Abundance
1-5
6-10
11-20
21-30
31-41
Ceratoscopelus warmingii (n=75)
Diaphus mollis (n=30)
Hygophum benoiti (n=104)
Lampanyctus alatus (n=63)
Lepidophanes guentheri (n=127)
Notolynchnus valdiviae (n=312)

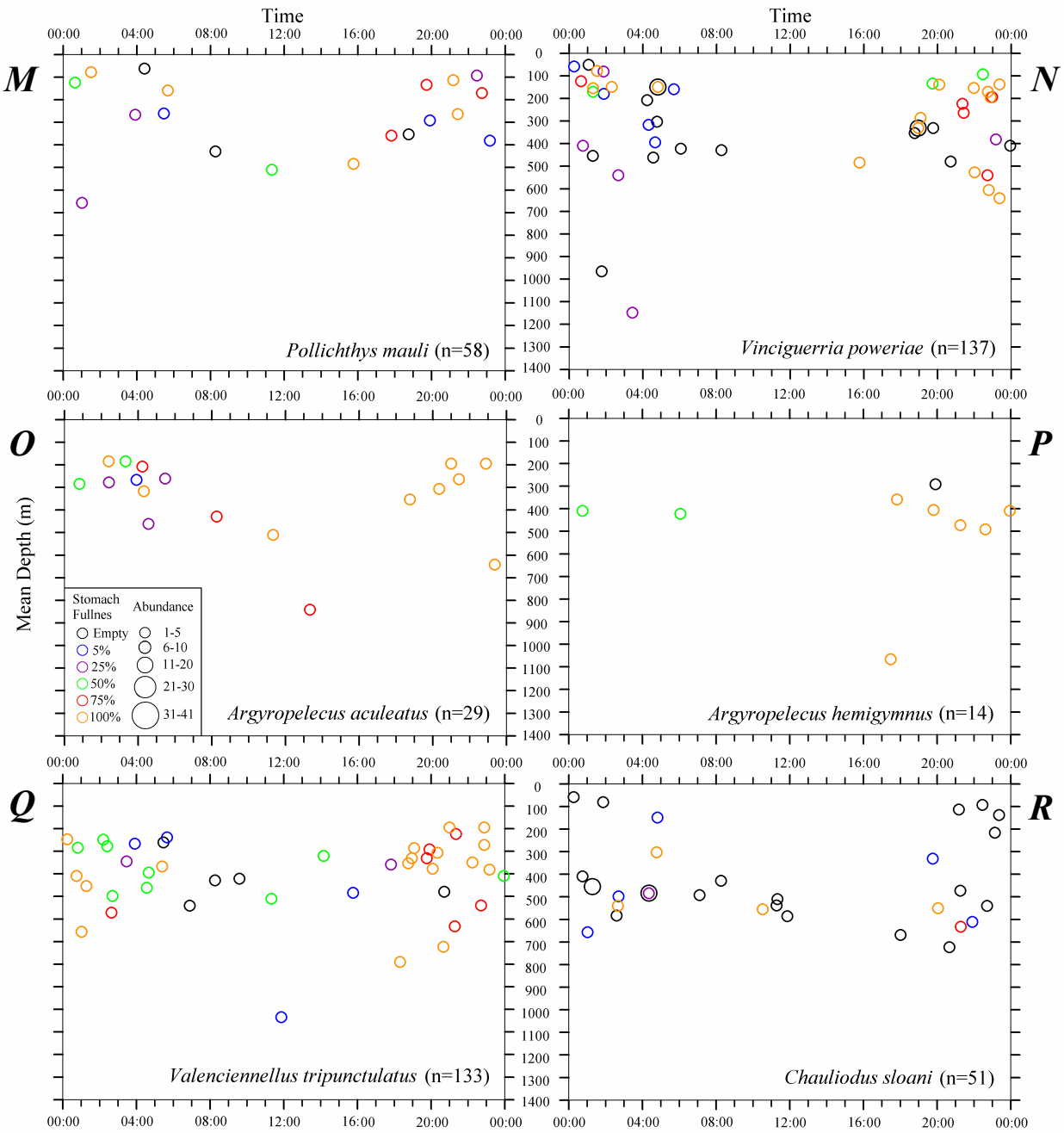


Figure 8.3. The relationships between stomach fullness and depth of capture for 18 midwater fishes. Data from all three sites were grouped together to detect diel vertical migrations.

Table 8.3. Percent volume (%V) and percent frequency (%F) of prey consumed by *Cyclothone alba* collected from two sites (AT340, and GC852) in the Gulf of Mexico separated by day and night. Additionally, two *C. alba* were captured at site AC601, both at night with empty stomachs.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AT340				GC852			
	Day (*n*=21) (E=46)		Night (*n*=16) (E=30)		Day (*n*=19) (E=43)		Night (*n*=36) (E=77)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
OSTRACODA	–	–	–	–	3.6	5.3	0.2	2.8
Halocyprididae	–	–	–	–	3.6	5.3	–	–
Myodocopida	–	–	–	–	–	–	0.2	2.8
COPEPODA	95	66.7	78.5	50	29.9	36.8	75.4	47.2
Aetideidae	–	–	1.3	6.3	–	–	–	–
Calanoida	20.9	14.3	15.7	18.8	5.4	5.3	29.5	22.2
Copepoda	0.2	4.8	–	–	–	–	–	–
Cyclopoida	–	–	–	–	0.1	5.3	–	–
Euchirella curticauda	43.5	9.5	–	–	–	–	–	–
Euchirella sp.	–	–	–	–	–	–	9.2	2.8
Heterorhabdidae	–	–	–	–	–	–	3.9	2.8
Lubbockia aculeata	1.5	4.8	–	–	–	–	–	–
Pleuromamma robusta	6.7	4.8	–	–	–	–	–	–
Pleuromamma sp.	–	–	4	6.3	–	–	14.7	5.6
Pleuromamma xiphias	17.3	9.5	53.1	6.3	18.6	5.3	14.7	5.6
Poecilostomatoida	–	–	–	–	0.8	15.8	–	–
Unidentified copepod parts	4.9	19	4.4	18.8	5.1	10.5	3.2	11.1
CRUSTACEA	2.3	19	2.2	12.5	60.6	42.1	14	27.8
Unidentified crustacean parts	2.3	19	2.2	12.5	60.6	42.1	14	27.8
DECAPODA	–	–	–	–	–	–	7.4	2.8
Decapoda	–	–	–	–	–	–	7.4	2.8
OTHER	2.8	19	19.3	43.8	5.9	36.8	3	25
Organic material	2.8	19	9.2	37.5	5.9	31.6	3	22.2
Unidentified animal parts	–	–	10.1	37.5	<0.1	5.3	<0.1	5.6

Table 8.4. Percent volume (%V) and percent frequency (%F) of prey consumed by *Cyclothone pallida* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=3) (E=15)		Day (*n*=0) (E=58)		Night (*n*=1) (E=43)		Day (*n*=6) (E=134)		Night (*n*=11) (E=91)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
OSTRACODA	23.1	33.3	–	–	–	–	9.4	33.3	24.9	18.2
Conchoecinae	–	–	–	–	–	–	5.7	16.7	–	–
Halocyprididae	23.1	33.3	–	–	–	–	–	–	–	–
Halocypridinae	–	–	–	–	–	–	3.8	16.7	–	–
Myodocopida	–	–	–	–	–	–	–	–	10	9.1
Unidentified ostracod parts	–	–	–	–	–	–	–	–	14.9	9.1
COPEPODA	68.4	33.3	–	–	–	–	71.6	16.7	3.1	9.1
Aetideidae	–	–	–	–	–	–	22.6	16.7	–	–
Haloptilus oxycephalus	–	–	–	–	–	–	–	–	3.1	9.1
Unidentified copepod parts	68.4	33.3	–	–	–	–	49	16.7	–	–
AMPHIPODA	–	–	–	–	–	–	–	–	29.9	9.1
Unidentified amphipod parts	–	–	–	–	–	–	–	–	29.9	9.1
CRUSTACEA	–	–	–	–	–	–	9.4	16.7	–	–
Unidentified crustacean parts	–	–	–	–	–	–	9.4	16.7	–	–
OTHER	8.5	33.3	–	–	100	100	9.5	33.3	42.1	63.6
Organic material	8.5	33.3	–	–	100	100	9.5	33.3	42.1	63.6

Table 8.5. Percent volume (%V) and percent frequency (%F) of prey consumed by *Cyclothone braueri* collected from two sites (AT340 and GC852) in the Gulf of Mexico separated by day and night. Additionally, two *C. braueri* were captured at site AC601, both at night with empty stomachs.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AT340				GC852			
	Day (*n*=8) (E=48)		Night (*n*=19) (E=75)		Day (*n*=18) (E=40)		Night (*n*=24) (E=85)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
OSTRACODA	57.6	25	–	–	23.7	16.7	1.2	4.2
Conchoecinae	54.2	12.5	–	–	–	–	–	–
Ostracoda	3.4	12.5	–	–	23.7	16.7	–	–
Unidentified ostracod parts	–	–	–	–	–	–	1.2	4.2
COPEPODA	42.4	87.5	68.1	52.6	68.4	72.2	64.4	41.7
Aegisthus mucronatus	16.2	25	–	–	32.5	16.7	–	–
Calanoida	5.3	25	36.1	21.1	1.8	5.6	10.9	8.3
Copepoda	–	–	–	–	–	–	<0.1	4.2
Corycaeus sp.	–	–	–	–	1.2	5.6	–	–
Cyclopoida	–	–	0.4	5.3	–	–	0.2	4.2
Lubbockia aculeata	–	–	–	–	1.8	5.6	–	–
Miracia efferata	1.2	12.5	–	–	–	–	–	–
Pleuromamma sp.	–	–	–	–	–	–	34.9	8.3
Unidentified copepod parts	19.7	25	31.6	26.3	31.1	72.2	18.3	16.7
CRUSTACEA	–	–	27.1	52.6	–	–	22.9	41.7
Unidentified crustacean parts	–	–	27.1	52.6	–	–	22.9	41.7
OTHER	–	–	4.8	31.6	7.9	16.7	11.5	37.5
Organic material	–	–	4.8	31.6	5.1	11.1	11.5	37.5
Unidentified animal parts	–	–	–	–	2.8	5.6	–	–

Table 8.6. Percent volume (%V) and percent frequency (%F) of prey consumed by *Cyclothone pseudopallida* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=1) (E=11)		Day (*n*=9) (E=54)		Night (*n*=13) (E=25)		Day (*n*=24) (E=90)		Night (*n*=29) (E=76)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
OSTRACODA	–	–	8.7	11.1	5.3	7.7	3.9	8.3	0.1	3.4
Conchoecinae	–	–	–	–	5.3	7.7	0.8	4.2	–	–
Myodocopida	–	–	–	–	–	–	3.2	4.2	–	–
Ostracoda	–	–	8.7	11.1	–	–	–	–	0.1	3.4
COPEPODA	100	100	86.3	77.8	92.8	76.9	85.7	54.2	60.8	48.3
Aegisthus mucronatus	–	–	9.1	11.1	–	–	–	–	0.5	3.4
Aetideidae	–	–	–	–	3	7.7	–	–	–	–
Calanoida	–	–	77.3	66.7	49.7	38.5	12.3	29.2	9.5	17.2
cf. *Mormonilla phasma*	–	–	–	–	–	–	20.5	4.2	–	–
Chiridus sp.	–	–	–	–	–	–	1.7	4.2	–	–
Copepoda	–	–	–	–	–	–	31.6	4.2	0.6	3.4
Harpacticoida	–	–	–	–	0.3	7.7	–	–	–	–
Lubbockia aculeata	–	–	–	–	–	–	0.8	4.2	–	–
Lubbockia sp.	–	–	–	–	–	–	0.2	4.2	–	–
Lubbockia squillimana	–	–	–	–	–	–	–	–	0.9	3.4
Lucicutia sp.	–	–	–	–	–	–	–	–	<0.1	3.4
Oithona sp.	–	–	–	–	–	–	0.3	4.2	–	–
Pleuromamma sp.	–	–	–	–	–	–	–	–	17	3.4
Pleuromamma xiphias	–	–	–	–	–	–	15.2	4.2	–	–
Rhincalanus sp.	–	–	–	–	–	–	–	–	0.4	3.4
Valdiviella minor	–	–	–	–	24.8	7.7	–	–	–	–
Unidentified copepod parts	100	100	–	–	15.1	23.1	3	8.3	31.9	13.8

Table 8.6. Percent volume (%V) and percent frequency (%F) of prey consumed by *Cyclothone pseudopallida* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night. —Continued

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=1) (E=11)		Day (*n*=9) (E=54)		Night (*n*=13) (E=25)		Day (*n*=24) (E=90)		Night (*n*=29) (E=76)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
CRUSTACEA	–	–	–	–	1.8	15.4	7.3	12.5	12.6	24.1
Unidentified crustacean parts	–	–	–	–	1.8	15.4	7.3	12.5	12.6	24.1
OTHER	–	–	5	22.2	<0.1	7.7	3	37.5	26.4	27.6
Animalia	–	–	2.7	11.1	–	–	–	–	–	–
Organic material	–	–	2.3	11.1	<0.1	7.7	3	29.2	26.3	24.1
Unidentified animal parts	–	–		–	–	–	<0.1	8.3	0.1	3.4

Table 8.7. Percent volume (%V) and percent frequency (%F) of prey consumed by *Gonostoma elongatum* collected from two sites (AT340 and GC852) in the Gulf of Mexico separated by day and night.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

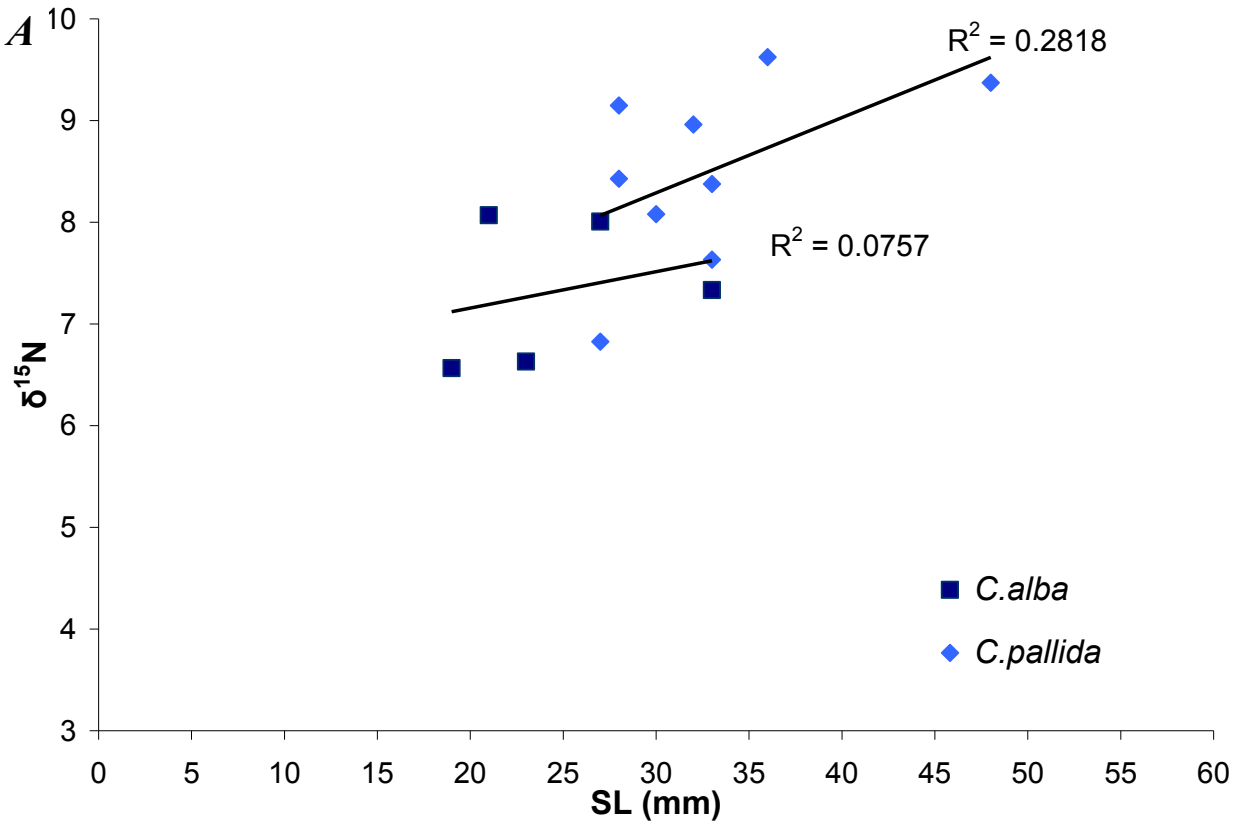
	AT340				GC852			
	Day (*n*=4) (E=0)		Night (*n*=41) (E=10)		Day (*n*=3) (E=0)		Night (*n*=20) (E=11)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
CNIDARIA	–	–	–	–	–	–	0.2	5
Cnidaria	–	–	–	–	–	–	0.2	5
OSTRACODA	–	–	0.1	4.9	–	–	<0.1	15
Halocyprididae	–	–	<0.1	2.4	–	–	<0.1	10
Myodocopida	–	–	0.1	2.4	–	–		
Ostracoda	–	–	–	–	–	–	<0.1	5
COPEPODA	–	–	1.7	31.7	–	–	1.3	35
Aetideus acutus	–	–			–	–	<0.1	5
Calanoida	–	–	0.1	2.4	–	–	0.2	15
Candacia longimana	–	–	0.1	2.4	–	–		
Copepoda	–	–	0.3	9.8	–	–	0.1	10
Corycaeus (Urocorycaeus) furcifer	–	–	–	–	–	–	<0.1	5
Corycaeus sp.	–	–	<0.1	2.4	–	–	<0.1	5
Eucalanidae	–	–	<0.1	2.4	–	–		
Gaetanus pileatus	–	–	0.4	2.4	–	–		
Haloptilus sp.	–	–	–	–	–	–	0.3	5
Pareucalanus attenuatus	–	–	0.1	2.4	–	–		
Pleuromamma xiphias	–	–	0.4	2.4	–	–	0.3	10
Rhincalanus cornutus	–	–	–	–	–	–	0.2	5
Temora stylifera	–	–	–	–	–	–	<0.1	5
Unidentified copepod parts	–	–	0.3	9.8	–	–	0.1	10
AMPHIPODA	–	–	0.3	2.4	–	–	0.4	10

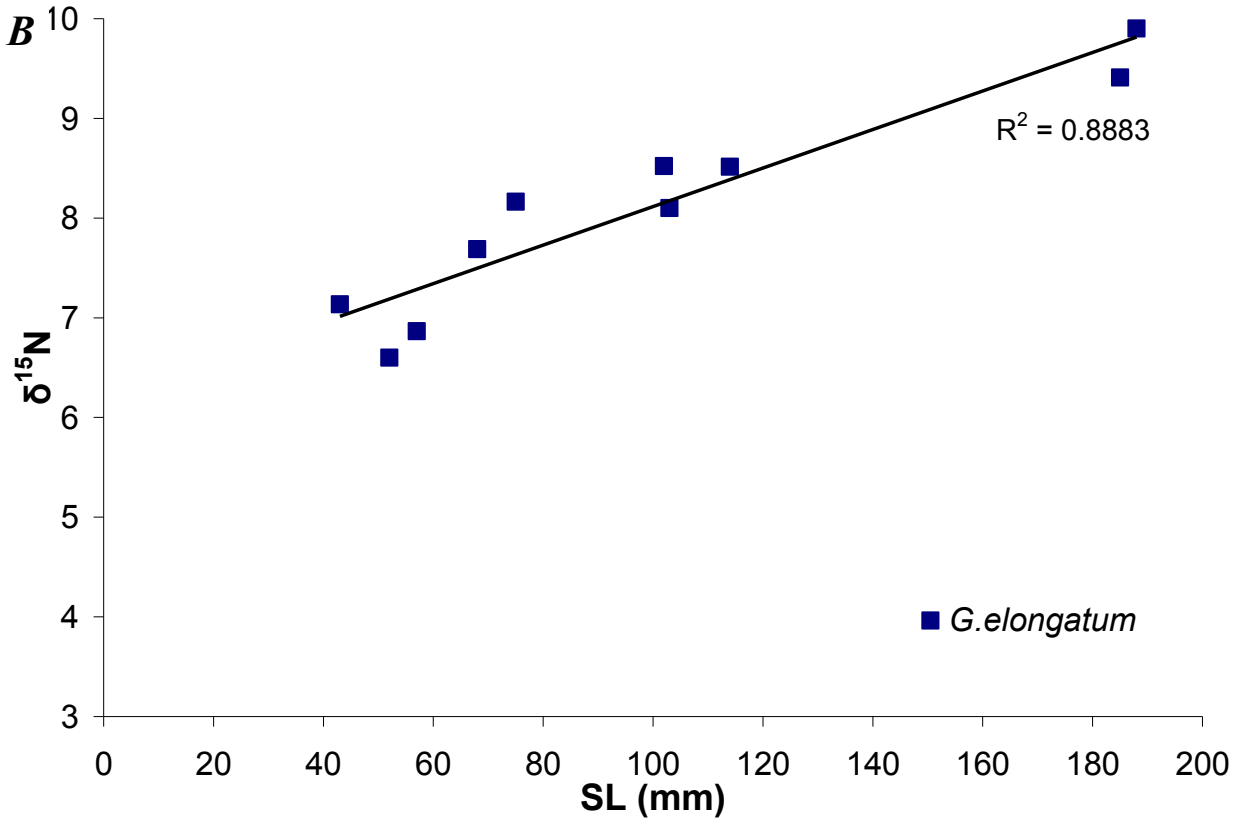
Table 8.7. Percent volume (%V) and percent frequency (%F) of prey consumed by *Gonostoma elongatum* collected from two sites (AT340 and GC852) in the Gulf of Mexico separated by day and night. —Continued

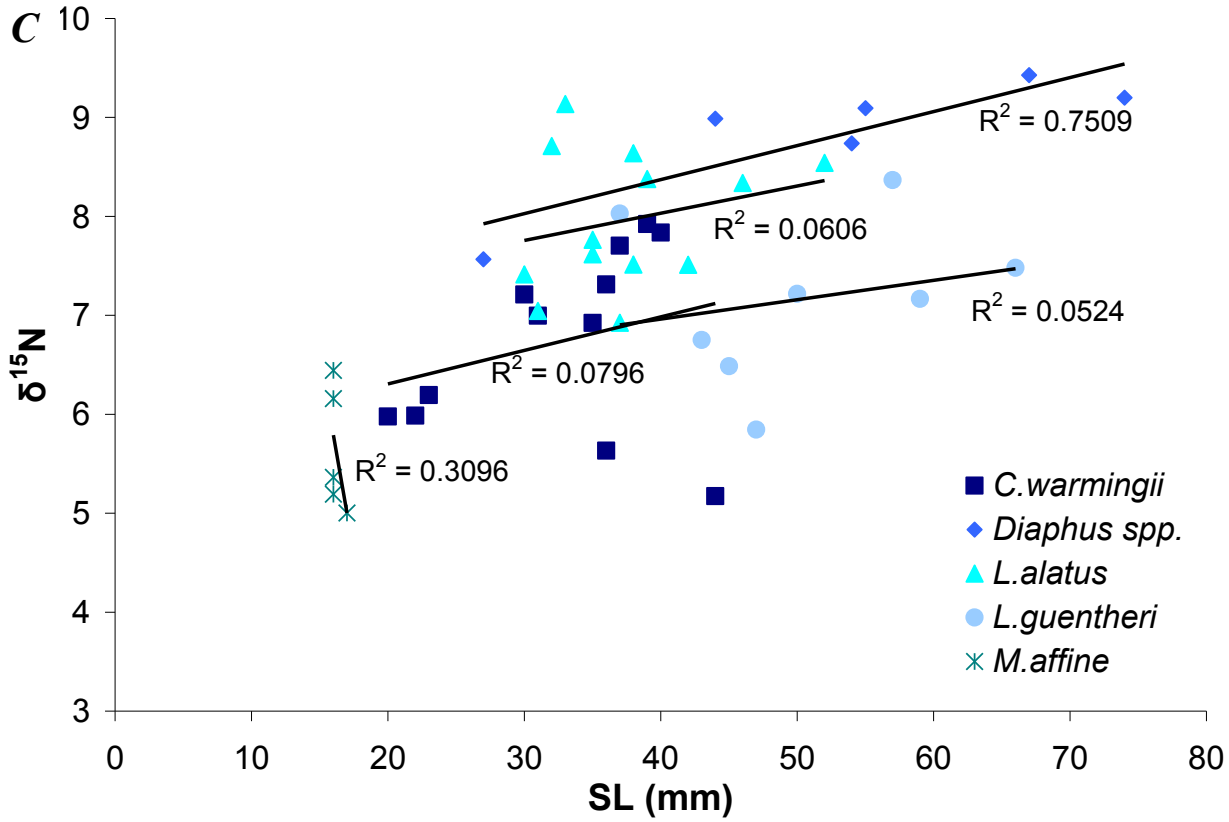
[n, number of full stomachs; E, number of empty stomachs; –, no value]

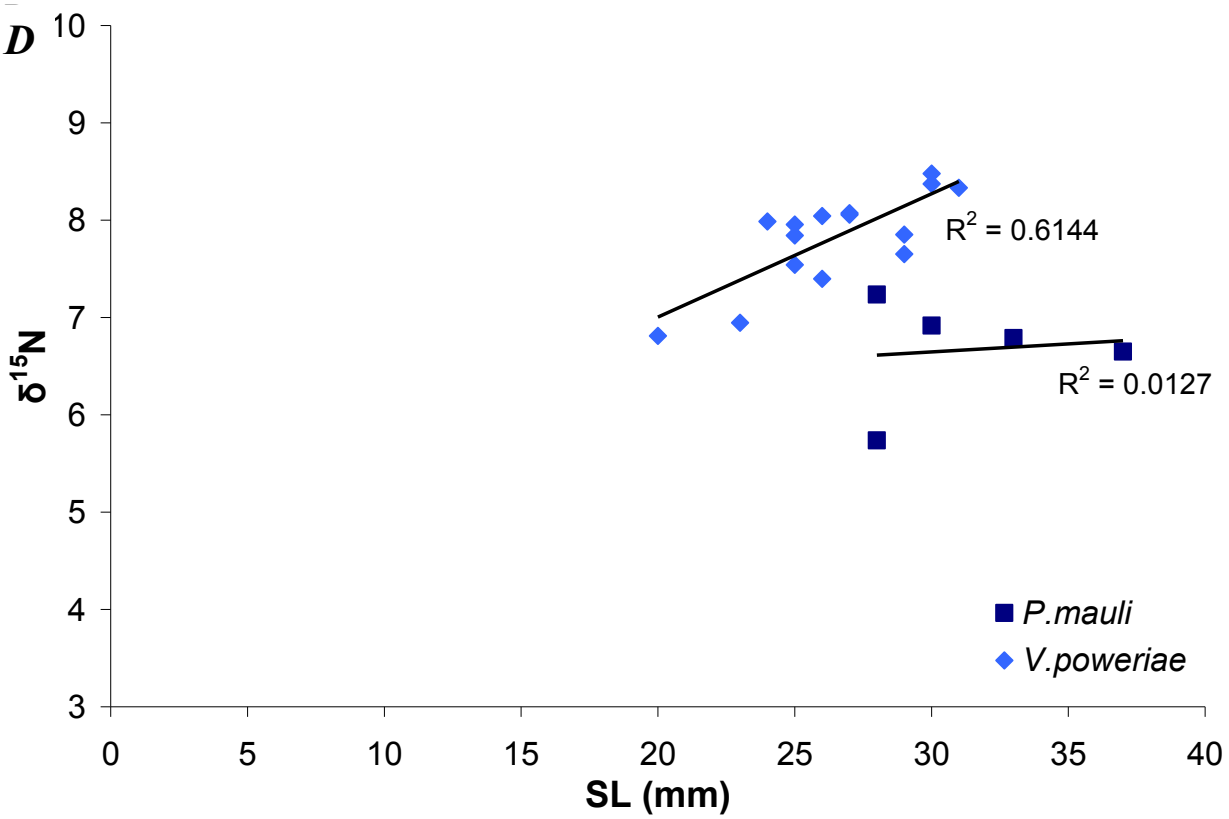
	AT340				GC852			
	Day (*n*=4) (E=0)		Night (*n*=41) (E=10)		Day (*n*=3) (E=0)		Night (*n*=20) (E=11)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
Amphipoda	–	–	–	–	–	–	0.4	10
Scina pusilla	–	–	0.3	2.4	–	–	–	–
EUPHAUSIACEA	–	–	3.8	7.3	1.7	33.3	40.4	15
Euphausiidae	–	–	2.1	7.3	–	–	16.2	15
Thysanoessa sp.	–	–	1.7	2.4	–	–	–	–
Thysanopoda sp.	–	–	–	–	–	–	24.2	5
Unidentified euphausiid parts	–	–	–	–	1.7	33.3	–	–
CRUSTACEA	45.8	50	3.5	43.9	13.3	33.3	10.7	50
Unidentified crustacean parts	45.8	50	3.5	43.9	13.3	33.3	10.7	50
DECAPODA	–	–	86.2	4.9	–	–	1.3	15
Decapoda	–	–	86.2	4.9	–	–	0.3	5
Lophogastridae	–	–	–	–	–	–	1	10
CHAETOGNATHA	–	–	–	–	–	–	1.6	5
Heterokrohnia sp.	–	–	–	–	–	–	1.6	5
OTHER	54.2	75	4.4	70.7	85	100	44	70
Organic material	54.2	75	3.9	70.7	85	100	42.9	60
Unidentified animal parts	–	–	0.5	2.4	–	–	1.1	10

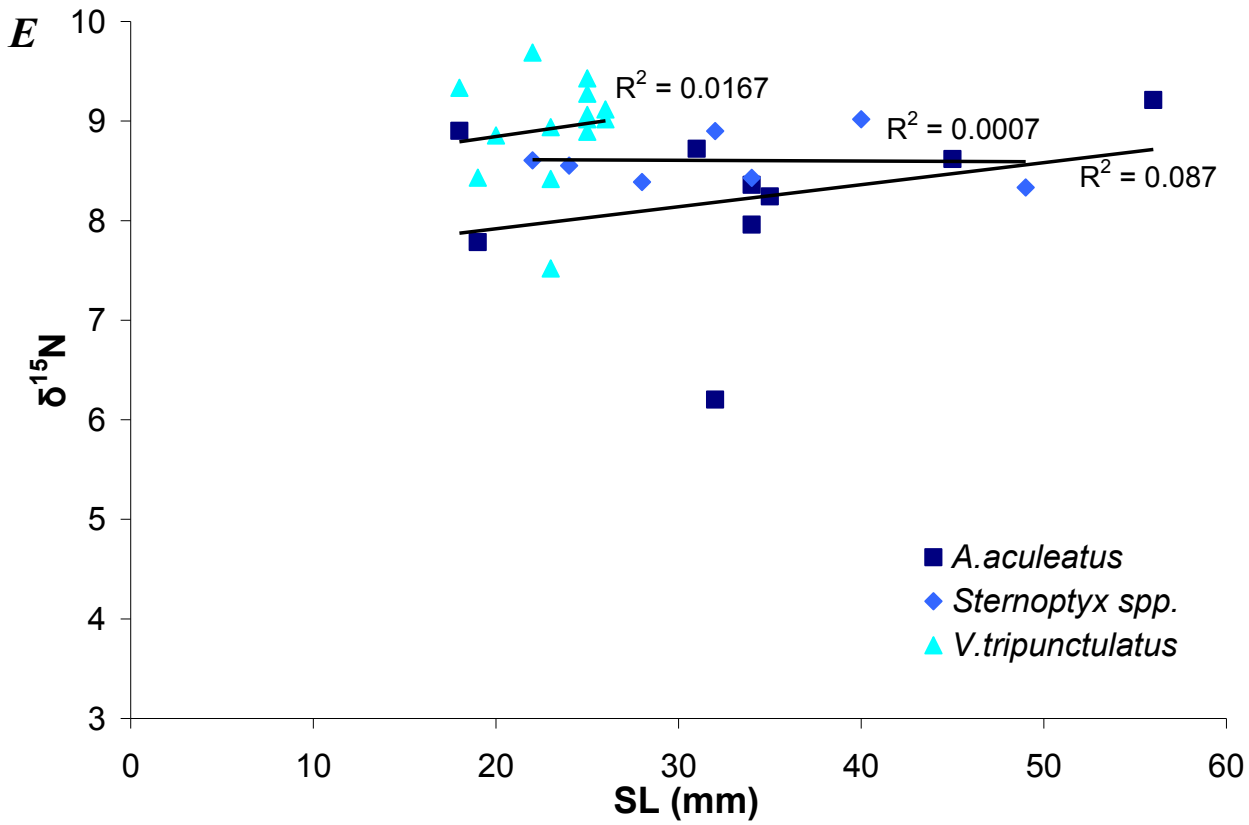
SIA was conducted on *C. alba, C. acclinidens, C. pallida* and *G. elongatum.* *Cyclothone acclinidens* was excluded from statistical analyses due to the small sample size (n = 1). *Cyclothone alba* was significantly depleted in ^{15}N compared to *C. pallida* (Dunn's, p = 0.021), but no differences in $\delta^{13}C$ values were documented. Additionally, no significant differences were documented in $\delta^{13}C$ or $\delta^{15}N$ between *Cyclothone* spp. and *G. elongatum*. No dietary ontogenetic shifts were documented for *Cyclothone* spp. (fig. 8.4*A*); however, a significant positive relationship between SL and $\delta^{15}N$ was indentified for *G. elongatum* (R^2=0.888, p <0.001, fig. 8.4*B*). No site comparisons were conducted between *Cyclothone* spp.; however, *G. elongatum* was significantly enriched in ^{15}N values at GC852 compared to AT340 (Dunn's, p = 0.006). Although no chemosynthetic signature was detected in any gonostomatid, a trophic shift of approximately 2 per mil in nitrogen and <1 per mil in carbon was documented with increasing trophic levels (fig. 8.2).


A
10
9
8
7
6
5
4
3
δ15N
R2 = 0.2818
R2 = 0.0757
C.alba
C.pallida
0
5
10
15
20
25
30
35
40
45
50
55
60
SL (mm)


B
10
9
8
7
6
5
4
3
δ15N
R2 = 0.8883
G.elongatum
0
20
40
60
80
100
120
140
160
180
200
SL (mm)


C
10
9
8
7
6
5
4
3
δ15N
0
10
20
30
40
50
60
70
80
SL (mm)
R2 = 0.7509
R2 = 0.0606
R2 = 0.0524
R2 = 0.0796
R2 = 0.3096
C.warmingii
Diaphus spp.
L.alatus
L.guentheri
M.affine


D
10
9
8
7
6
5
4
3
δ15N
0
5
10
15
20
25
30
35
40
SL (mm)
R2 = 0.6144
R2 = 0.0127
P.mauli
V.poweriae


E
10
9
8
7
6
5
4
3
δ15N
0
10
20
30
40
50
60
SL (mm)
R2 = 0.0167
R2 = 0.0007
R2 = 0.087
A.aculeatus
Sternoptyx spp.
V.tripunctulatus

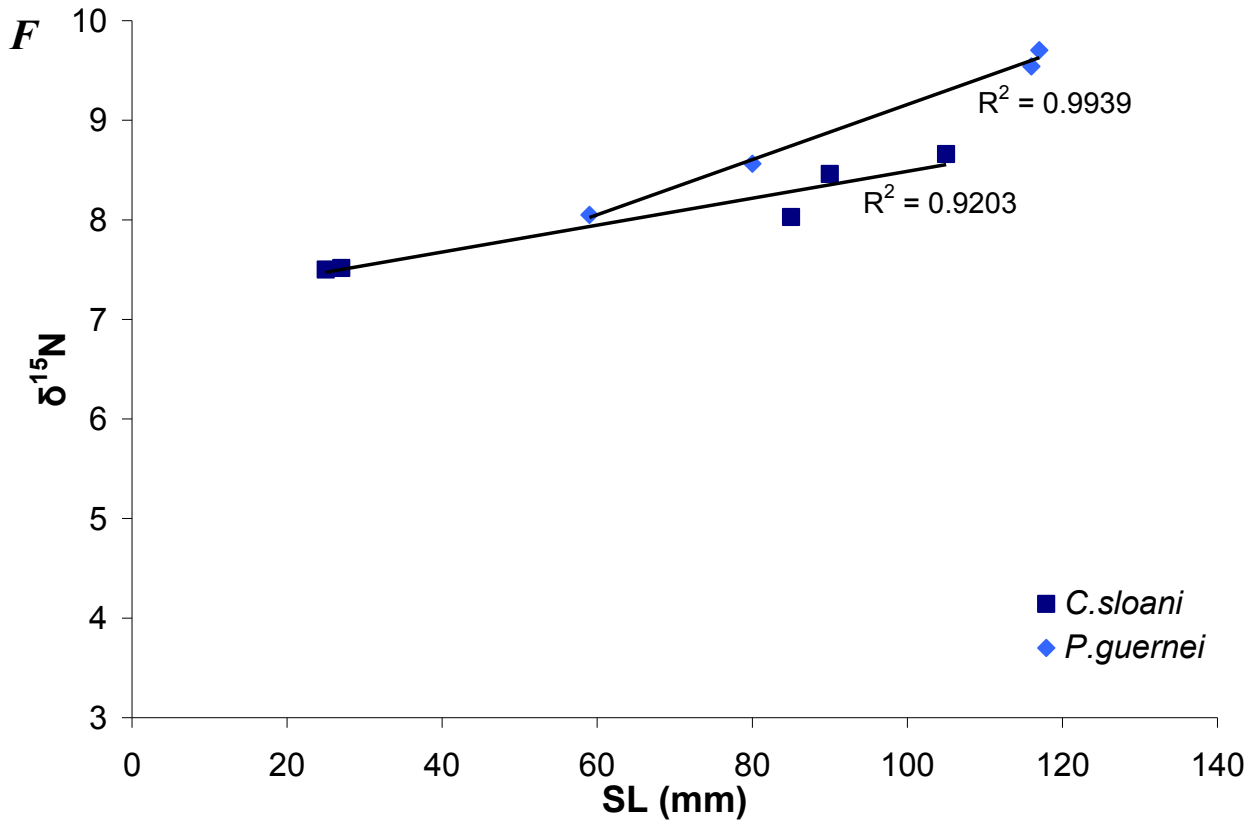

F
10
9
8
7
6
5
4
3
δ15N
0
20
40
60
80
100
120
140
SL (mm)
R2 = 0.9939
R2 = 0.9203
C.sloani
P.guernei

Figure 8.4. The relationship of standard length (SL) to $\delta^{15}N$ values in midwater fishes from the families (*A*) Gonostomatidae – *Cyclothone* spp.; (*B*) Gonostomatidae – *Gonostoma elongatum;* (*C*) Myctophidae; (*D*) Phosichthyidae; (*E*) Sternoptychidae; and (*F*) Stomiidae collected at all three sites.

8.3.3.2 Myctophidae

Seven species of Myctophidae (*B. suborbitale, C. warmingii, D. mollis, H. benoiti, L. alatus, L. guentheri,* and *N. valdiviae*) were analyzed for diet composition from the three sites. Empty stomachs occurred more frequently in *H. benoiti* than any other species collected (table 8.8). Generally, empty stomachs occurred more frequently during the day than at night and more frequently at GC852 than any other site for myctophids (tables 8.8-8.14). Additionally, DVMs were evident for all myctophids, with more full stomachs documented in specimens collected in the epipelagic zone at night (fig. 8.3*F-L*).

Diets of myctophids were diverse, ranging from 7 prey categories (*H. benoiti* and *N. valdiviae*) to 15 prey categories (*C. warmingii*). Crustaceans were the dominant prey in the diets of all myctophids; however, noncrustacean prey were documented in the stomachs of all myctophid species. *Pleuromamma* spp. (Copepoda) was the most abundant species identified in stomachs, occurring in all myctophid species, except *C. warmingii* and *H. benoiti,* and accounted for 44 percent of all identified copepod species. Prey preferences were variable in Myctophidae based on time of day and sites. Copepoda were the dominant prey for *H. benoiti* (table 8.8)*, B. suborbitale* (table 8.9)*, D. mollis* (table 8.10), and *N. valdiviae* (table 8.11) regardless of time or sites. Although copepods occurred more frequently in the diets of *C. warmingii* (table 8.12), *L. guentheri* (table 8.13), and *L. alatus* (table 8.14), other prey items were more important volumetrically. In *C. warmingii*, amphipods were volumetrically more in the diets of specimens collected during the night at AC601 and GC852, while fish were volumetrically more important

in specimens collected during the day samples at AT340. In *L. guentheri*, copepods were volumetrically more important in specimens collected during the night at AC601 and AT340, whereas euphausiids were volumetrically more important in specimens collected during the day at AT340, ostracods in specimens collected during the day at GC852, and amphipods in specimens collected during the night at GC852 (table 8.13). In *L. alatus,* copepods were volumetrically more important in specimens collected during the night at AC601, and during the day at AT340 and GC852, whereas amphipods were more important in specimens collected during the night at GC852, and euphausiids in specimens collected during the night at AT340 (table 8.14).

Table 8.8. Percent volume (%V) and percent frequency (%F) of prey consumed by *Hygophum benoiti* collected from AT340 in the Gulf of Mexico separated by day and night. Additionally, six *H. benoiti* were collected at AC601 at night, all with empty stomachs. At site GC852, 33 *H. benoiti* were collected during the day and 37 during the night, all with empty stomachs.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AT340			
	Day (*n*=1) (E=7)		Night (*n*=16) (E=11)	
Food Item	% V	% F	% V	% F
MOLLUSCA	–	–	0.2	18.8
Bivalvia	–	–	0.2	18.8
OSTRACODA	–	–	0.6	12.5
Myodocopida	–	–	0.1	6.3
Ostracoda	–	–	0.5	6.3
COPEPODA	–	–	52	81.3
Calanoida	–	–	15.7	50
Candacia curta	–	–	1.4	6.3
Candacia pachydactyla	–	–	2.8	6.3
Cyclopoida	–	–	5.8	62.5
Farranula gracilis	–	–	0.9	25
Unidentified copepod parts	–	–	25.4	68.8
AMPHIPODA	–	–	0.2	6.3
Amphipoda	–	–	0.2	6.3
CRUSTACEA	100	100	9.2	25
Unidentified crustacean parts	100	100	9.2	25
DECAPODA	–	–	3.8	6.3
Decapoda	–	–	3.8	6.3
OTHER	–	–	34	75
Organic material	–	–	34	75

Table 8.9. Percent volume (%V) and percent frequency (%F) of prey consumed by *Benthosema suborbitale* collected from three sites (AC601, AT340 and GC852) in the Gulf of Mexico separated by day and night.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=9) (E=4)		Day (*n*=13) (E=9)		Night (*n*=71) (E=48)		Day (*n*=2) (E=8)		Night (*n*=34) (E=36)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
MOLLUSCA	–	–	–	–	<0.1	1.4	–	–	–	–
Unidentified mollusk parts	–	–	–	–	<0.1	1.4	–	–	–	–
OSTRACODA	2.7	33.3	3	15.4	4.4	18.3	–	–	7	23.5
Archiconchoecinae	–	–	–	–	1.8	1.4	–	–	–	–
Conchoecinae	–	–	–	–			–	–	2	5.9
Halocyprididae	–	–	–	–			–	–	1.7	2.9
Halocypris sp.	–	–	–	–			–	–	1.7	2.9
Myodocopida	1.8	22.2	–	–	0.3	4.2	–	–	0.8	8.8
Ostracoda	0.9	11.1	3	15.4	0.4	4.2	–	–	–	–
Unidentified ostracod parts	–	–	–	–	1.9	11.3	–	–	0.8	5.9
COPEPODA	51.9	77.8	76.1	38.5	24.5	50.7	100	100	30.2	44.1
Calanoida	13.1	44.4	–	–	0.2	5.6	44.4	50	8.1	11.8
Candacia bipinnata	–	–	–	–	1.4	1.4	–	–	–	–
Candaciidae	–	–	–	–	0.4	1.4	–	–	–	–
Copepoda	0.4	22.2	51.9	7.7	6	22.5	–	–	2.7	26.5
Corycaeus (Urocorycaeus) furcifer	–	–	–	–	0.2	1.4		–	–	–
Corycaeus sp.	11	22.2	–	–	0.9	4.2	–	–	0.8	2.9
Cyclopoida	0.1	22.2	–	–	–	–	–	–		
Euchaetidae	–	–	–	–	–	–	–	–	6.8	2.9
Harpacticoida	0.3	11.1	–	–	<0.1	2.8	–	–	<0.1	2.9
Labidocera sp.	–	–	–	–	0.1	1.4	–	–	–	–
Pleuromamma abdominalis	2.6	11.1	–	–	–	–	–	–	2.5	2.9

Table 8.9. Percent volume (%V) and percent frequency (%F) of prey consumed by *Benthosema suborbitale* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night. —Continued

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=9) (E=4)		Day (*n*=13) (E=9)		Night (*n*=71) (E=48)		Day (*n*=2) (E=8)		Night (*n*=34) (E=36)	
Food Item	% V	% F	% V	% F	% V	% F	% V	% F	% V	% F
Pleuromamma borealis	–	–	–	–	2.9	2.8	–	–	–	–
Pleuromamma piseki	10.3	22.2	–	–	–	–	–	–	–	–
Pleuromamma sp.	–	–	–	–	2.4	2.8	55.6	50	6.8	2.9
Sapphirina metallina	2.1	11.1	–	–	–	–	–	–	–	–
Temora stylifera	3.4	11.1	–	–	–	–	–	–	–	–
Unidentified copepod parts	8.6	33.3	24.1	30.8	9.9	31	–	–	2.4	14.7
AMPHIPODA	5.8	11.1	–	–	4.7	5.6	–	–	4.4	2.9
Anchylomera blossevillei	–	–	–	–	1.8	1.4	–	–	–	–
Hyperiidea	5.8	11.1	–	–	1.8	1.4	–	–	4.4	2.9
Platysceloidea	–	–	–	–	0.9	1.4	–	–	–	–
Unidentified amphipod parts	–	–	–	–	0.2	1.4	–	–	–	–
EUPHAUSIACEA	–	–	–	–	12.8	5.6	–	–	1.8	5.9
Euphausiidae	–	–	–	–	10	2.8	–	–	0.1	2.9
Unidentified euphausiid parts	–	–	–	–	2.7	2.8	–	–	1.7	2.9
CRUSTACEA	24.3	44.4	11.1	7.7	18	46.5	–	–	17.5	41.2
Crustacea	–	–	–	–	–	–	–	–	0.2	2.9
Unidentified crustacean parts	24.3	44.1	11.1	7.7	18	46.5	–	–	17.3	41.2
DECAPODA	–	–	–	–	4.4	4.2	–	–	20.9	2.9
Decapoda	–	–	–	–	2.4	1.4	–	–	20.9	2.9
Unidentified decapod parts	–	–	–	–	2	2.8	–	–	–	–
ANNELIDA	–	–	–	–	2	2.8	–	–	–	–
Polychaeta	–	–	–	–	2	2.8	–	–	–	–

Table 8.9. Percent volume (%V) and percent frequency (%F) of prey consumed by *Benthosema suborbitale* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night. —Continued

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=9) (E=4)		Day (*n*=13) (E=9)		Night (*n*=71) (E=48)		Day (*n*=2) (E=8)		Night (*n*=34) (E=36)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
CHAETOGNATHA	–	–	1.1	7.7	–	–	–	–	–	–
Unidentified chaetognath parts	–	–	1.1	7.7	–	–	–	–	–	–
FISH	–	–	–	–	7.3	1.4	–	–	–	–
Myctophidae	–	–	–	–	7.3	1.4	–	–	–	–
OTHER	15.4	55.6	8.7	30.8	22	66.2	–	–	18.2	47.1
Organic material	15.4	55.6	7.8	23.1	22	64.8	–	–	18.2	47.1
Unidentified animal parts	–	–	0.9	7.7	<0.1	1.4	–	–	<0.1	2.9

Table 8.10. Percent volume (%V) and percent frequency (%F) of prey consumed by *Diaphus mollis* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=1) (E=0)		Day (*n*=4) (E=0)		Night (*n*=11) (E=1)		Day (*n*=0) (E=2)		Night (*n*=15) (E=0)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
GASTROPODA	–	–	–	–	<0.1	9.1	–	–	–	–
Gastropoda	–	–	–	–	<0.1	9.1	–	–	–	–
OSTRACODA	–	–	–	–	1	18.2	–	–	1.5	53.3
Conchoecinae	–	–	–	–	1	9.1	–	–		
Myodocopida	–	–	–	–	–	–	–	–	0.2	20
Ostracoda	–	–	–	–	–	–	–	–	0.4	13.3
Unidentified ostracod parts	–	–	–	–	<0.1	9.1	–	–	0.9	26.7
COPEPODA	63.3	100	12.5	25	26.4	81.8	–	–	15.2	60
Calanoida	–	–	12.5	25	<0.1	9.1	–	–	–	–
Copepoda	0.5	100	–	–	1.3	18.2	–	–	1	20
Cyclopoida	0.5	100	–	–	0.2	27.3	–	–	1.2	6.7
Farranula gracilis	–	–	–	–	0.1	9.1	–	–	–	–
Pleuromamma sp.	62.3	100	–	–	18.1	27.3	–	–	9	6.7
Unidentified copepod parts	–	–	–	–	6.7	45.5	–	–	4	26.7
EUPHAUSIACEA	–	–	34.4	25	<0.1	9.1	–	–	<0.1	13.3
Euphausiidae	–	–	34.4	25	–	–	–	–	<0.1	13.3
Unidentified euphausiid parts	–	–	–	–	<0.1	9.1	–	–	–	–
CRUSTACEA	36.6	100	–	–	16.3	54.5	–	–	5	53.3
Unidentified crustacean parts	36.6	100	–	–	16.3	54.5	–	–	5	53.3
DECAPODA	–	–	–	–	0.1	9.1	–	–	<0.1	6.7
Decapoda	–	–	–	–	0.1	9.1	–	–	<0.1	6.7
	–	–	–	–	–	–	–	–	–	–

Table 8.10. Percent volume (%V) and percent frequency (%F) of prey consumed by *Diaphus mollis* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night. —Continued

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=1) (E=0)		Day (*n*=4) (E=0)		Night (*n*=11) (E=1)		Day (*n*=0) (E=2)		Night (*n*=15) (E=0)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
CHAETOGNATHA	–	–	28.1	25	7.6	9.1	–	–	–	–
Sagittoidea	–	–	28.1	25	7.6	9.1	–	–	–	–
Unidentified chaetognath parts	–	–	–	–	–	–	–	–	–	–
FISH	–	–	–	–	–	–	–	–	<0.1	20
Unidentified fish parts	–	–	–	–	–	–	–	–	<0.1	20
OTHER	<0.1	100	25	25	48.4	81.8	–	–	78.1	86.7
Organic material	–	–	25	25	48.4	81.8	–	–	78.1	86.7
Unidentified animal parts	<0.1	100	–	–	<0.1	9.1	–	–	<0.1	33.3

Table 8.11. Percent volume (%V) and percent frequency (%F) of prey consumed by *Notolychnus valdiviae* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=1) (E=0)		Day (*n*=52) (E=44)		Night (*n*=66) (E=44)		Day (*n*=22) (E=7)		Night (*n*=77) (E=31)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
OSTRACODA	100	100	4.6	5.8	7.7	25.8	1.2	18.2	4.1	24.7
Conchoecinae	–	–	–	–	0.1	1.5	0.1	4.5	0.5	2.6
Halocyprididae	–	–	–	–	5.2	6.1	<0.1	4.5	1.5	3.9
Halocypridinae	–	–	0.4	1.9	–	–	–	–	<0.1	1.3
Myodocopida	–	–	–	–	0.6	6.1	1	9.1	0.7	2.6
Myodocopina	–	–	0.4	1.9	0.4	1.5	–	–	<0.1	2.6
Ostracoda	100	100	3.5	1.9	0.9	7.6	–	–	0.9	7.8
Unidentified ostracod parts	–	–	0.3	1.9	0.4	4.5	–	–	0.5	5.2
COPEPODA	–	–	68.9	63.5	55.3	63.6	16.6	31.8	40.3	51.9
Calanoida	–	–	3.4	7.7	7.1	13.6	1.6	9.1	16.9	18.2
Candaciidae	–	–	1.2	1.9	–	–	–	–	0.1	1.3
Copepoda	–	–	3.3	1.9	–	–	0.1	4.5	–	–
Corycaeidae	–	–	–	–	0.1	3	–	–	–	–
Cyclopoida	–	–	–	–	1.1	16.7	–	–	<0.1	5.2
Euchaeta sp.	–	–	–	–	–	–	9	4.5	–	–
Harpacticoida	–	–	<0.1	1.9	–	–	–	–	–	–
Lubbockia squillimana	–	–	–	–	–	–	–	–	<0.1	1.3
Oncaea sp.	–	–	0.2	1.9	0.2	3	–	–	0.1	2.6
Paracandacia simplex	–	–	2.1	1.9	–	–	–	–	–	–
Pleuromamma gracilis	–	–	–	–	–	–	–	–	0.4	1.3
Pleuromamma piseki	–	–	0.7	1.9	–	–	–	–	2.8	1.3
Pleuromamma sp.	–	–	39	13.5	4.7	4.5	–	–	0.8	2.6

Table 8.11. Percent volume (%V) and percent frequency (%F) of prey consumed by *Notolychnus valdiviae* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night. —Continued

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=1) (E=0)		Day (*n*=52) (E=44)		Night (*n*=66) (E=44)		Day (*n*=22) (E=7)		Night (*n*=77) (E=31)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
Pleuromamma xiphias	–	–	4.3	1.9	15.9	4.5	–	–	11.7	3.9
Poecilostomatoida	–	–	–	–	0.5	1.5	0.3	4.5	–	–
Unidentified copepod parts	–	–	14.7	30.8	25.6	22.7	5.8	13.6	7.4	26
EUPHAUSIACEA	–	–	–	–	8.4	1.5	16.2	13.6	22	6.5
Euphausiidae	–	–	–	–	8.4	1.5	–	–	21.9	5.2
Unidentified euphausiid parts	–	–	–	–	–	–	16.2	13.6	0.1	1.3
CRUSTACEA	–	–	0.1	1.9	22.9	37.9	48.5	68.2	19.7	41.6
Unidentified crustacean parts	–	–	0.1	1.9	22.9	37.9	48.5	68.2	19.7	41.6
DECAPODA	–	–	–	–	0.1	3	–	–	<0.1	1.3
Unidentified decapod parts	–	–	–	–	0.1	3	–	–	<0.1	1.3
CHAETOGNATHA	–	–	–	–	–	–	<0.1	4.5	–	–
Unidentified chaetognath parts	–	–	–	–	–	–	<0.1	4.5	–	–
OTHER	–	–	26.4	40.4	5.5	12.1	17.5	18.2	13.8	27.3
Organic material	–	–	26.2	34.6	5.5	10.6	17.5	18.2	13.8	27.3
Unidentified animal parts	–	–	0.2	5.8	<0.1	3	–	–	–	1.3

Table 8.12. Percent volume (%V) and percent frequency (%F) of prey consumed by *Ceratoscopelus warmingii* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=4) (E=1)		Day (*n*=3) (E=3)		Night (*n*=41) (E=2)		Day (*n*=3) (E=4)		Night (*n*=21) (E=6)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
CNIDARIA	–	–	–	–	1.7	2.4	–	–	–	–
Hydrozoa	–	–	–	–	1.7	2.4	–	–	–	–
MOLLUSCA	0.1	25	–	–	0.5	12.2	–	–	0.4	9.5
Bivalvia	–	–	–	–	0.1	2.4	–	–	–	–
Mollusca	–	–	–	–	–	–	–	–	0.4	4.8
Unidentified mollusk parts	0.1	25	–	–	0.4	9.8	–	–	<0.1	4.8
CEPHALOPODA	–	–	–	–	–	–	–	–	6.2	4.8
Cephalopoda	–	–	–	–	–	–	–	–	6.2	4.8
GASTROPODA	–	–	–	–	4.4	9.8	–	–	–	–
Gastropoda	–	–	–	–	4.4	9.8	–	–	–	–
OSTRACODA	1.4	50	–	–	2.3	31.7	–	–	4	33.3
Conchoecinae	–	–	–	–	0.3	2.4	–	–	–	–
Halocyprididae	–	–	–	–	0.2	2.4	–	–	–	–
Myodocopida	1.4	50	–	–	0.6	14.6	–	–	0.7	23.8
Ostracoda	–	–	–	–	0.9	9.8	–	–	0.1	9.5
Platycopida	–	–	–	–	0.1	2.4	–	–	–	–
Unidentified ostracod parts	–	–	–	–	0.2	7.3	–	–	3.1	9.5
COPEPODA	8.4	100	13.4	100	9.1	65.9	1.2	33.3	3.5	47.6
Calanoida	0.6	25	0.7	33.3	1	4.9	1.2	33.3	2.5	4.8
Candacia sp.	–	–	3.5	33.3	–	–	–	–	–	–
Copepoda	3.1	75	–	–	2.7	56.1	–	–	0.8	28.6
Corycaeidae	–	–	0.6	33.3	–	–	–	–	–	–

Table 8.12. Percent volume (%V) and percent frequency (%F) of prey consumed by *Ceratoscopelus warmingii* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night. —Continued

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=4) (E=1)		Day (*n*=3) (E=3)		Night (*n*=41) (E=2)		Day (*n*=3) (E=4)		Night (*n*=21) (E=6)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
Corycaeus sp.	–	–	–	–	0.2	4.9	–	–	0.2	4.8
Cyclopoida	–	–	–	–	<0.1	2.4	–	–	<0.1	4.8
Harpacticoida	–	–	–	–	<0.1	2.4	–	–	–	–
Microsetella rosea	–	–	–	–	–	–	–	–	<0.1	4.8
Miracia efferata	–	–	1.1	33.3	–	–	–	–	–	–
Miraciidae	–	–	–	–	<0.1	2.4	–	–	–	–
Temora stylifera	–	–	0.4	33.3	–	–	–	–	–	–
Unidentified copepod parts	4.7	25	7.1	33.3	5.1	19.5	–	–	<0.1	9.5
AMPHIPODA	13.2	50	0.4	33.3	0.8	12.2	–	–	8.4	28.6
Amphipoda	13.2	50	–	–	0.6	12.2	–	–	0.7	9.5
Hyperiidea	–	–	0.4	33.3	0.2	2.4	–	–	1.7	9.5
Phronima stebbingii	–	–	–	–	–	–	–	–	4.6	4.8
Unidentified amphipod parts	–	–	–	–	–	–	–	–	1.4	14.3
EUPHAUSIACEA	1.2	25	–	–	0.4	4.9	–	–	–	–
Euphausiidae	1.2	25	–	–	0.4	4.9	–	–	–	–
CRUSTACEA	13.5	50	8.4	33.3	5.1	34.1	44.4	33.3	4.2	42.9
Crustacea	–	–	–	–	0.1	2.4	–	–	0.3	4.8
Unidentified crustacean parts	13.5	50	8.4	33.3	5	31.7	44.4	33.3	3.9	38.1
DECAPODA	–	–	–	–	0.5	7.3	–	–	–	–
Caridea	–	–	–	–	0.1	2.4	–	–	–	–
Decapoda	–	–	–	–	0.2	2.4	–	–	–	–
Unidentified decapod parts	–	–	–	–	0.2	2.4	–	–	–	–

Table 8.12. Percent volume (%V) and percent frequency (%F) of prey consumed by *Ceratoscopelus warmingii* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night. —Continued

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=4) (E=1)		Day (*n*=3) (E=3)		Night (*n*=41) (E=2)		Day (*n*=3) (E=3)		Night (*n*=41) (E=2)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
ANNELIDA	–	–	–	–	1.4	7.3	–	–	1.6	19
Unidentified polychaete parts	–	–	–	–	1.4	7.3	–	–	1.6	19
CHAETOGNATHA	–	–	–	–	–	–	–	–	<0.1	4.8
Unidentified chaetognath parts	–	–	–	–	–	–	–	–	<0.1	4.8
FISH	–	–	77	33.3	0.2	4.9	–	–	0.1	4.8
Fish	–	–	77	33.3	–	–	–	–	–	–
Unidentified fish parts	–	–	–	–	0.2	4.9	–	–	0.1	4.8
SALPIDA	–	–	–	–	–	–	–	–	3.1	4.8
Salpidae	–	–	–	–	–	–	–	–	3.1	4.8
OTHER	62.3	75	0.9	33.3	73.7	92.7	54.3	66.7	68.6	81
Organic material	43.4	75	0.9	33.3	68.5	87.8	54.3	66.7	65.5	76.2
Unidentified animal parts	18.9	25	–	–	5.5	12.2	–	–	3.1	9.5

Table 8.13. Percent volume (%V) and percent frequency (%F) of prey consumed by *Lepidophanes guentheri* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=15) (E=0)		Day (*n*=20) (E=12)		Night (*n*=52) (E=12)		Day (*n*=3) (E=1)		Night (*n*=30) (E=13)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
GASTROPODA	–	–	–	–	0.8	1.9	–	–	–	–
Gastropoda	–	–	–	–	0.8	1.9	–	–	–	–
OSTRACODA	8.3	60	6.6	5	1.6	19.2	71.1	33.3	8.6	30
Conchoecinae	0.2	13.3	–	–	0.5	1.9	71.1	33.3	6.8	13.3
Halocyprididae	2.4	6.7	–	–	–	–	–	–	–	–
Myodocopida	0.5	20	6.6	5	–	–	–	–	1.1	10
Ostracoda	–	–	–	–	0.1	7.7	–	–	0.2	3.3
Unidentified ostracod parts	5.2	40	–	–	1	9.6	–	–	0.5	10
COPEPODA	75.9	86.7	11.9	50	21.6	59.6	12.1	66.7	25.6	66.7
Calanoida	27.6	20	2	10	5.8	17.3	3.7	33.3	9	10
Candacia curta	–	–	–	–	0.5	1.9	–	–	–	–
Candacia sp.	–	–	–	–	–	–	–	–	3.5	3.3
Copepoda	2.2	26.7	–	–	0.8	11.5	–	–	0.3	10
Corycaeus sp.	–	–	–	–	–	–	–	–	0.2	3.3
Cyclopoida	0.2	33.3	–	–	<0.1	5.8	–	–	0.5	23.3
Farranula gracilis	–	–	–	–	<0.1	1.9	–	–		
Harpacticoida	–	–	0.5	5	–	–	–	–	0.1	3.3
Pleuromamma gracilis	–	–	–	–	–	–	–	–	0.3	3.3
Pleuromamma piseki	14.2	6.7	–	–	8.1	11.5	–	–	2.9	3.3
Pleuromamma sp.	7.3	26.7	–	–	5.4	9.6	–	–	–	–
Unidentified copepod parts	24.4	53.3	9.4	35	1	15.4	8.5	33.3	8.8	33.3

Table 8.13. Percent volume (%V) and percent frequency (%F) of prey consumed by *Lepidophanes guentheri* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night. —Continued

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=15) (E=0)		Day (*n*=20) (E=12)		Night (*n*=52) (E=12)		Day (*n*=3) (E=1)		Night (*n*=30) (E=13)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
AMPHIPODA	–	–	9.9	5	0.2	1.9	–	–	35.3	6.7
Hyperiidea	–	–	–	–	–	–	–	–	5.9	3.3
Phronima sp.	–	–	–	–	–	–	–	–	29.4	3.3
Unidentified amphipod parts	–	–	9.9	5	0.2	1.9	–	–	–	–
EUPHAUSIACEA	–	–	25.7	10	20.2	13.5	14.9	33.3	0.1	3.3
Euphausiidae	–	–	25.7	10	8.1	1.9	14.9	33.3	–	–
Thysanopoda sp.	–	–	–	–	1.5	1.9	–	–	–	–
Unidentified euphausiid parts	–	–	–	–	10.5	9.6	–	–	0.1	3.3
CRUSTACEA	6.2	13.3	33.2	15	48.4	46.2	–	–	23.9	30
Unidentified crustacean parts	6.2	13.3	33.2	15	48.4	46.2	–	–	23.9	30
DECAPODA	–	–	–	–	0.3	9.6	–	–	0.2	3.3
Decapoda	–	–	–	–	–	–	–	–	0.2	3.3
Unidentified decapod parts	–	–	–	–	0.3	9.6	–	–	–	–
FISH	–	–	–	–	<0.1	1.9	–	–	<0.1	3.3
Unidentified fish parts	–	–	–	–	<0.1	1.9	–	–	<0.1	3.3
OTHER	9.6	53.3	12.8	35	7	36.5	1.9	33.3	6.2	53.3
Inorganic material	–	–	–	–	–	–	–	–	<0.1	3.3
Organic material	9.6	46.7	12.8	35	7	36.5	1.9	33.3	6.2	43.3
Unidentified animal parts	<0.1	6.7	–	–	–	–	–	–	<0.1	13.3

Table 8.14. Percent volume (%V) and percent frequency (%F) of prey consumed by *Lampanyctus alatus* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=6) (E=0)		Day (*n*=7) (E=0)		Night (*n*=12) (E=4)		Day (*n*=2) (E=3)		Night (*n*=29) (E=8)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
OSTRACODA	7.6	33.3	–	–	1.3	25	–	–	4.3	34.5
Conchoecinae	6.8	16.7	–	–	–	–	–	–	3.1	6.9
Halocyprididae	–	–	–	–	–	–	–	–	0.3	3.4
Myodocopida	0.8	33.3	–	–	1.2	16.7	–	–	<0.1	3.4
Ostracoda	–	–	–	–	–	–	–	–	0.4	6.9
Unidentified ostracod parts	–	–	–	–	<0.1	8.3	–	–	0.6	13.8
COPEPODA	34.9	83.3	45.4	71.4	21.9	66.7	28.3	100	18.4	48.3
Calanoida	10.9	33.3	42.7	57.1	0.5	16.7	26.1	100	0.6	6.9
Copepoda	–	–	–	–	7.9	33.3	–	–	0.5	17.2
Corycaeus sp.	–	–	–	–	–	–	–	–	0.4	3.4
Eucalanus sp.	–	–	–	–	–	–	–	–	5	3.4
Oncaea sp.	–	–	–	–	–	–	2.2	50	–	–
Paracandacia simplex	–	–	2.7	14.3	–	–	–	–	–	–
Pleuromamma piseki	–	–	–	–	8.2	8.3	–	–	–	–
Pleuromamma sp.	6.2	16.7	–	–	2.6	8.3	–	–	0.7	6.9
Unidentified copepod parts	17.8	50	–	–	2.6	28.6	–	–	11.1	27.6
AMPHIPODA	30.4	33.3	–	–	–	–	–	–	21.3	20.7
Amphipoda	–	–	–	–	–	–	–	–	1.6	10.3
Gammaridea	–	–	–	–	–	–	–	–	0.1	3.4
Hyperiidea	27.2	16.7	–	–	–	–	–	–	–	–
Lestrigonus sp.	–	–	–	–	–	–	–	–	2.8	3.4
Unidentified amphipod parts	3.1	16.7	–	–	–	–	–	–	16.9	6.9

Table 8.14. Percent volume (%V) and percent frequency (%F) of prey consumed by *Lampanyctus alatus* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night. —Continued

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=6) (E=0)		Day (*n*=7) (E=0)		Night (*n*=12) (E=4)		Day (*n*=2) (E=3)		Night (*n*=29) (E=8)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
EUPHAUSIACEA	–	–	–	–	23.9	16.7	–	–	5	6.9
Euphausiidae	–	–	–	–	21.3	8.3	–	–	0.1	3.4
Nematoscelis sp.	–	–	–	–	–	–	–	–	4.9	3.4
Unidentified euphausiid parts	–	–	–	–	2.6	8.3	–	–	–	–
CRUSTACEA	22.4	33.3	25.5	14.3	27.7	83.3	60.9	50	43.1	48.3
Crustacea	–	–	–	–	–	–	–	–	0.1	3.4
Unidentified crustacean parts	22.4	33.3	25.5	14.3	27.7	83.3	60.9	50	43	44.8
DECAPODA	–	–	–	–	–	–	–	–	3.9	3.4
Decapoda	–	–	–	–	–	–	–	–	3.9	3.4
FISH	–	–	–	–	–	–	–	–	<0.1	3.4
Unidentified fish parts	–	–	–	–	–	–	–	–	<0.1	3.4
SALPIDA	–	–	–	–	–	–	10.9	50	–	–
Salpidae	–	–	–	–	–	–	10.9	50	–	–
OTHER	4.7	16.7	29.1	28.6	25.2	50	–	–	4	31
Organic material	4.7	16.7	29.1	28.6	25.2	50	–	–	3.7	27.6
Unidentified animal parts	–	–	–	–	–	–	–	–	0.3	3.4

SIA was conducted on five species of Myctophidae, *C. warmingii, D. lucidus, D. mollis, D. problematicus, L. alatus, L. guentheri,* and *Myctophum affine.* Significant spatial variations for $\delta^{13}C$ and $\delta^{15}N$ were not documented for any myctophid; however, significant differences were documented among myctophid species. *Myctophum affine* was significantly depleted in ^{13}C and ^{15}N compared to *Diaphus* spp. (Tukey, $p < 0.001$), *L. alatus* (Tukey, $p < 0.001$), and *L. guentheri* (Tukey, $p < 0.001$, $p = 0.001$). *Myctophum affine* was also depleted in ^{13}C compared to *C. warmingii* (Tukey, $p < 0.001$). *Diaphus* spp. was significantly enriched in ^{15}N compared to *C. warmingii* (Tukey, $p < 0.001$) and *L. guentheri* (Tukey, $p = 0.001$). *Lampanyctus alatus* was significantly enriched in ^{15}N compared to *C. warmingii* (Tukey, $p < 0.001$). A dietary ontogenetic shift was evident in all myctophid species; however, a significant positive relationship between ^{15}N and SL was only documented for *Diaphus* spp. ($R^2=0.751$, $p = 0.03$, fig. 8.4*C*). An enrichment in ^{15}N was also documented with increasing trophic levels but was less evident in ^{13}C (fig. 8.2). Although no chemosynthetic signature was reported for any myctophid, *M. affine* was depleted in ^{13}C compared to POM samples (fig. 8.2) but was within the range of previously reported values for photosynthetic material.

8.3.3.3 Phosichthyidae

Two species of Phosichthyidae, *P. mauli* and *V. poweriae,* were analyzed for dietary composition at the three sites. Empty stomachs occurred less frequently in *P. mauli* (maximum of 44 percent) compared to *V. poweriae* (maximum of 72 percent), and more frequently in specimens collected during the day than at night (tables 8.15 and 8.16). DVMs were evident for *P. mauli* and *V. poweriae*, with full stomachs occurring more frequently in specimens collected in the epipelagic at night (fig. 8.3*M, N*).

Diet comparisons were conducted on both phosichthyid species. Overall, the diets of *P. mauli* were less diverse (seven prey categories; table 8.15) than for *V. poweriae*, (ten categories; table 8.16) and did not include any noncrustacean prey. Spatial variations were also evident for both species. At GC852, *P. mauli* primarily consumed amphipods; however, at AT340, euphausiids were volumetrically more important for *P. mauli,* though copepods occurred more frequently (table 8.15). In contrast, for *V. poweriae* collected at GC852*,* myctophids were the most important prey item in terms of volume and frequency, whereas at AT340 ostracods, particularly halocyprids, were volumetrically more important in specimens collected during the day and copepods for specimens collected at night (table 8.16).

Table 8.15. Percent volume (%V) and percent frequency (%F) of prey consumed by *Pollichthys mauli* collected from two sites (AT340 and GC852) in the Gulf of Mexico separated by day and night.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AT340				GC852	
	Day (*n*=9) (E=7)		Night (*n*=37) (E=8)		Night (*n*=2) (E=0)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
OSTRACODA	17.9	11.1	21.3	32.4	27.8	50
Conchoecinae	–	–	14.1	13.5	11.9	50
Halocyprididae	–	–	2.1	5.4	–	–
Myodocopida	–	–	<0.1	5.4	–	–
Ostracoda	17.9	11.1	3.4	16.2	–	–
Unidentified ostracod parts	–	–	1.5	13.5	15.9	50
COPEPODA	79.8	77.8	14.2	37.8	8.7	50
Calanoida	40.4	11.1	1.6	5.4	–	–
Copepoda	–	–	0.7	8.1	0.8	50
Corycaeus sp.	–	–	0.5	2.7	–	–
Cyclopoida	–	–	2.7	5.4	–	–
Harpacticoida	–	–	0.2	2.7	–	–
Pleuromamma borealis	–	–	3.3	2.7	–	–
Pleuromamma piseki	–	–	0.8	2.7	–	–
Pleuromamma sp.	–	–	3.8	10.8	–	–
Unidentified copepod parts	39.5	66.7	0.6	10.8	7.9	50
AMPHIPODA	–	–	4.4	2.7	63.5	50
Amphipoda	–	–	4.4	2.7	–	–
Unidentified amphipod parts	–	–	–	–	63.5	50
EUPHAUSIACEA	–	–	44.9	8.1	–	–
Euphausiidae	–	–	0.1	2.7	–	–
Nyctiphanes capensis	–	–	17.5	2.7	–	–
Stylocheiron sp.	–	–	6.6	2.7	–	–
Thysanopoda sp.	–	–	20.7	2.7	–	–
CRUSTACEA	–	–	11.4	56.8	–	–
Unidentified crustacean parts	–	–	11.4	56.8	–	–
DECAPODA	–	–	<0.1	2.7	–	–
Unidentified decapod parts	–	–	<0.1	2.7	–	–
OTHER	2.2	11.1	3.9	18.9	–	–
Organic material	2.2	11.1	3.9	18.9	–	–

Table 8.16. Percent volume (%V) and percent frequency (%F) of prey consumed by *Vinciguerria poweriae* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=2) (E=0)		Day (*n*=3) (E=4)		Night (*n*=39) (E=9)		Day (*n*=6) (E=15)		Night (*n*=51) (E=24)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
MOLLUSCA	–	–	–	–	<0.1	5.1	–	–	<0.1	5.9
Unidentified mollusk parts	–	–	–	–	<0.1	5.1	–	–	<0.1	5.9
GASTROPODA	–	–	–	–	–	–	–	–	<0.1	2
Gastropoda	–	–	–	–	–	–	–	–	<0.1	2
OSTRACODA	–	–	93.7	66.7	16.8	30.8	8.8	33	12.2	43.1
Archiconchoecinae	–	–	–	–	1.6	5.1	2.4	16.7	0.7	2
Conchoecinae	–	–	10	33.3	6.8	7.7	6.4	16.7	6.5	15.7
Halocyprididae	–	–	83.7	33.3	2.2	7.7	–	–	1.2	3.9
Halocypridinae	–	–	–	–	–	–	–	–	0.6	5.9
Halocypris sp.	–	–	–	–	–	–	–	–	0.6	3.9
Myodocopida	–	–	–	–	0.4	7.7	–	–	0.8	11.8
Myodocopina	–	–	–	–	1	5.1	–	–	–	–
Sarsiellidae	–	–	–	–	0.2	2.6	–	–	–	–
Unidentified ostracod parts	–	–	–	–	4.6	20.5	–	–	1.8	13.7
COPEPODA	–	–	6.3	33.3	19.3	64.1	10.9	50	12.3	39.2
Calanoida	–	–	–	–	2.9	15.4	–	–	4.4	11.8
Candacia bipinnata	–	–	–	–	0.4	2.6	–	–	–	–
Candacia varicans	–	–	–	–	0.4	2.6	–	–	–	–
Candaciidae	–	–	–	–			4	16.7	–	–
Copepoda	–	–	–	–	0.1	2.6	3	16.7	0.5	9.8
Corycaeus sp.	–	–	–	–	3.8	15.4	4	16.7	0.3	9.8
Cyclopoida	–	–	–	–	0.7	20.5	–	–	0.5	7.8
Farranula gracilis	–	–	–	–	0.2	2.6	–	–	–	–

Table 8.16. Percent volume (%V) and percent frequency (%F) of prey consumed by *Vinciguerria poweriae* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night.—Continued

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=2) (E=0)		Day (*n*=3) (E=4)		Night (*n*=39) (E=9)		Day (*n*=6) (E=15)		Night (*n*=51) (E=24)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
Lubbockia sp.	–	–	–	–	–	–	–	–	<0.1	2
Paracandacia bispinosa	–	–	–	–	–	–	–	–	2.2	2
Paracandacia simplex	–	–	–	–	0.5	2.6	–	–	–	–
Pleuromamma sp.	–	–	–	–	–	–	–	–	0.5	2
Sapphirina sp.	–	–	–	–	–	–	–	–	0.1	2
Temora sp.	–	–	–	–	<0.1	2.6	–	–	–	–
Undinula vulgaris	–	–	–	–	0.5	2.6	–	–	–	–
Unidentified copepod parts	–	–	6.3	33.3	9.9	51.3	–	–	3.8	21.6
AMPHIPODA	–	–	–	–	1.6	5.1	–	–	12.8	17.6
Brachyscelus crusculum	–	–	–	–	–	–	–	–	4.2	2
Brachyscelus sp.	–	–	–	–	–	–	–	–	0.2	2
Eupronoe armata	–	–	–	–	–	–	–	–	1.5	2
Hyperiidea	–	–	–	–	–	–	–	–	1	5.9
Primno latreillei	–	–	–	–	–	–	–	–	2	2
Themistella fusca	–	–	–	–	1.2	2.6	–	–	–	–
Tryphana malmi	–	–	–	–	0.4	2.6	–	–	–	–
Unidentified amphipod parts	–	–	–	–	–	–	–	–	4	11.8
EUPHAUSIACEA	–	–	–	–	10	7.7	–	–	–	–
Euphausiidae	–	–	–	–	5.1	5.1	–	–	–	–
Unidentified euphausiid parts	–	–	–	–	5	2.6	–	–	–	–
CRUSTACEA	100	100	–	–	43.7	69.2	–	–	20.2	60.8
Unidentified crustacean parts	100	100	–	–	43.7	69.2	–	–	20.2	60.8

Table 8.16. Percent volume (%V) and percent frequency (%F) of prey consumed by *Vinciguerria poweriae* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night.—Continued

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=2) (E=0)		Day (*n*=3) (E=4)		Night (*n*=39) (E=9)		Day (*n*=6) (E=15)		Night (*n*=51) (E=24)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
DECAPODA	–	–	–	–	0.6	2.6	–	–	11.3	2
Decapoda	–	–	–	–			–	–	11.3	2
Unidentified decapod parts	–	–	–	–	0.6	2.6	–	–	–	–
FISH	–	–	–	–	0.1	2.6	72.3	50	17.8	5.9
Myctophidae	–	–	–	–	–	–	72.3	50	17.7	3.9
Unidentified fish parts	–	–	–	–	0.1	2.6	–	–	0.1	2
OTHER	–	–	–	–	7.9	23.1	8	16.7	13.3	33.3
Organic material	–	–	–	–	7.9	20.5	8	16.7	13.3	31.4
Unidentified animal parts	–	–	–	–	<0.1	2.6	–	–	<0.1	7.8

SIA was conducted on *P. mauli* and *V. poweriae* (table 8.2). No spatial comparisons were conducted on *P. mauli,* and no significant differences were documented in $\delta^{13}C$ or $\delta^{15}N$ for *V. poweriae.* Despite no spatial variation, *P. mauli* was significantly enriched in ^{13}C (t-test, $p < 0.001$) but depleted in ^{15}N (t-test, $p = 0.002$) compared to *V. poweriae*. Additionally, a positive relationship between SL and $\delta^{15}N$ was identified for *P. mauli* and *V. poweriae*; however, this relationship was only significant for the latter ($R^2=0.614$, $p<0.01$, fig. 8.4*D*). A trophic shift of approximately 2 per mil in nitrogen and <1 per mil in carbon was documented for both phosichthyids, although no chemosynthetic signature was detected in either species (fig. 8.2).

8.3.3.4 Sternoptychidae

Three species of Sternoptychidae, *A. aculeatus, A. hemigymnus*, and *V. tripunctulatus*, were analyzed for diet composition. Empty stomachs occurred less frequently in *V. tripunctulatus* (maximum of 30 percent) compared to *A. aculeatus* (maximum of 32 percent) or *A. hemigymnus* (maximum of 89 percent), with empty stomachs occurring more frequently during the day than night. DVMs were evident for all sternoptychids. Although more full stomachs were documented at night in the epipelagic for *A. aculeatus* (fig. 8.3*O*), both *A. hemigymnus* and *V. tripunctulatus* had more full stomachs documented at night in the mesopelagic (fig. 8.3*P, Q*).

Diet comparisons were conducted on *A. aculeatus, A. hemigymnus,* and *V. tripunctulatus.* Copepoda and Ostracoda, particularly Conchoecinae, were the dominant prey items for *Argyropelecus* spp.; however, spatial variations were documented. Euphausiacea was volumetrically important in the stomachs of *Argyropelecus* spp. collected at GC852, although ostracods occurred more frequently in the stomachs of *A. aculeatus* (table 8.17) and copepods in the stomachs of *A. hemigymnus* (table 8.18). Ostracods were the most important prey in terms of

volume and frequency for *A. aculeatus* collected at AT340 and AC601, whereas copepods were more important for *A. hemigymnus*. In contrast, no spatial differences were documented for *V. tripunctulatus*, and Copepoda, particularly *Pleuromamma* spp., was the most important prey consumed in terms of volume and frequency (table 8.19).

Table 8.17. Percent volume (%V) and percent frequency (%F) of prey consumed by *Argyropelecus aculeatus* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852	
	Night (*n*=3) (E=0)		Day (*n*=5) (E=2)		Night (*n*=7) (E=2)		Night (*n*=15) (E=7)	
Food Item	% V	% F	% V	% F	% V	% F	% V	% F
MOLLUSCA	–	–	–	–	0.3	14.3	–	–
Unidentified mollusk parts	–	–	–	–	0.3	14.3	–	–
CEPHALOPODA	–	–	9.7	20	–	–	–	–
Unidentified cephalopod parts	–	–	9.7	20	–	–	–	–
GASTROPODA	–	–	1.9	40	3.6	14.3	0.1	6.7
Gastropoda	–	–	1.9	40	3.6	14.3	0.1	6.7
OSTRACODA	42.1	100	23.3	80	26.4	57.1	9.4	53.3
Archiconchoecinae	–	–	–	–	–	–	0.2	6.7
Conchoecinae	25.1	66.7	12.6	60	18.6	28.6	3.5	20
Halocypridinae	–	–	6.8	20	–	–	–	–
Halocypris sp.	4.6	33.3	–	–	–	–	–	–
Myodocopida	12.4	33.3	3.4	20	7.1	28.6	2.4	26.7
Ostracoda	–	–	–	–	–	–	2.7	6.7
Unidentified ostracod parts	–	–	0.5	20	0.7	14.3	0.6	20
COPEPODA	31.1	100	10.8	80	14.4	42.9	2.6	46.7
Calanoida	9.4	33.3	3.3	40	2.3	14.3	0.5	6.7
Copepoda	10.6	33.3	1.5	20	5.7	42.9	1.1	33.3
Corycaeus sp.	0.2	33.3	–	–	–	–	–	–
Cyclopoida	1.5	66.7	0.2	20	–	–		
Lubbockia aculeata	–	–	–	–	0.1	14.3	<0.1	6.7
Paracalanidae	9.4	33.3	–	–	–	–	–	–
Paracalanus aculeatus	–	–	–	–	–	–	0.5	6.7
Pleuromamma robusta	–	–	1.9	20	–	–	–	–

Table 8.17. Percent volume (%V) and percent frequency (%F) of prey consumed by *Argyropelecus aculeatus* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night.—Continued

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852	
	Night (*n*=3) (E=0)		Day (*n*=5) (E=2)		Night (*n*=7) (E=2)		Night (*n*=15) (E=7)	
Food Item	% V	% F	% V	% F	% V	% F	% V	% F
Pleuromamma xiphias	–	–	–	–	6.3	14.3	–	–
Poecilostomatoida	–	–	1.5	20	–	–	0.3	6.7
Unidentified copepod parts	–	–	2.4	40	–	–	0.1	13.3
AMPHIPODA	0.4	33.3	24.8	60	6	42.9	7.2	26.7
Amphipoda	0.4	33.3	2.9	20	0.1	14.3	1.5	6.7
Anchylomera blossevillei	–	–	–	–	–	–	3.2	6.7
Eusiridae	–	–	–	–	–	–	0.5	13.3
Gammaridea	–	–	–	–	–	–	0.7	6.7
Hyperiidea	–	–	–	–	0.2	14.3	1.4	6.7
Phronima sp.	–	–	5.8	20	–	–	–	–
Primno evansi	–	–	8.8	20	–	–	–	–
Primno sp.	–	–	–	–	5.7	14.3	–	–
Scina oedicarpus	–	–	1.5	20	–	–	–	–
Unidentified amphipod parts	–	–	5.8	20	–	–	–	–
EUPHAUSIACEA	–	–	–	–	–	–	14.2	13.3
Euphausiidae	–	–	–	–	–	–	14.2	13.3
DECAPODA	–	–	–	–	–	–	0.1	6.7
Unidentified decapod parts	–	–	–	–	–	–	0.1	6.7
CRUSTACEA	1.8	33.3	17.7	60	20.6	85.7	33.4	80
Crustacea	–	–	–	–	0.2	14.3	1.9	13.3
Unidentified crustacean parts	1.8	33.3	17.7	60	20.4	85.7	31.5	80
CHAETOGNATHA	–	–	–	–	–	–	1.6	20
Sagittoidea	–	–	–	–	–	–	1.6	20

Table 8.17. Percent volume (%V) and percent frequency (%F) of prey consumed by *Argyropelecus aculeatus* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night.—Continued

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852	
	Night (*n*=3) (E=0)		Day (*n*=5) (E=2)		Night (*n*=7) (E=2)		Night (*n*=15) (E=7)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
OTHER	24.7	33.3	11.7	40	28.8	85.7	31.5	60
Organic material	24.7	33.3	11.7	40	28.6	85.7	25.6	60
Unidentified animal parts	–	–	–	–	0.2	14.3	5.9	6.7

Table 8.18. Percent volume (%V) and percent frequency (%F) of prey consumed by *Argyropelecus hemigymnus* collected from two sites (AT340 and GC852) in the Gulf of Mexico separated by day and night.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AT340				GC852			
	Day (*n*=2) (E=2)		Night (*n*=1) (E=8)		Day (*n*=2) (E=1)		Night (*n*=6) (E=8)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
OSTRACODA	1.2	100	–	–	47.7	50	8.6	33.3
Conchoecinae	–	–	–	–	47.7	50	–	–
Myodocopida	–	–	–	–	–	–	5.7	16.7
Ostracoda	0.6	50	–	–	–	–	1.9	16.7
Unidentified ostracod parts	0.6	50	–	–	–	–	1	33.3
COPEPODA	52.3	100	25.9	100	38.8	100	23.5	100
Calanoida	3.3	100	18.5	100	11.9	50	20.8	66.7
Copepoda	48.6	50	–	–	26.8	50	0.2	50
Lubbockia aculeata	–	–	4.9	100	–	–	–	–
Lubbockia sp.	–	–	–	–	–	–	0.1	16.7
Pleuromamma abdominalis	–	–	–	–	–	–	0.9	16.7
Unidentified copepod parts	0.3	50	2.5	100	–	–	1.4	16.7
AMPHIPODA	0.9	50	–	–	–	–	–	–
Unidentified amphipod parts	0.9	50	–	–	–	–	–	–
EUPHAUSIACEA	–	–	–	–	–	–	47.3	16.7
Nematoscelis microps	–	–	–	–	–	–	47.3	16.7
CRUSTACEA	3	50	–	–	0.1	50	4.9	66.7
Crustacea	–	–	–	–	0.1	50	<0.1	16.7
Unidentified crustacean parts	3	50	–	–	–	–	4.9	66.7
OTHER	42.6	100	74.1	100	13.4	100	15.7	100
Organic material	42.6	100	74.1	100	13.4	100	15.7	100

Table 8.19. Percent volume (%V) and percent frequency (%F) of prey consumed by *Valenciennellus tripunctulatus* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=1) (E=0)		Day (*n*=12) (E=5)		Night (*n*=50) (E=13)		Day (*n*=13) (E=0)		Night (*n*=44) (E=7)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
OSTRACODA	–	–	1	25	1.4	16	0.6	38.5	1.7	20.5
Archiconchoecinae	–	–	–	–	–	–	–	–	0.4	2.3
Conchoecinae	–	–	0.9	16.7	0.6	4	0.5	30.8	0.2	4.5
Halocyprididae	–	–	–	–	–	–	0.1	7.7	–	–
Myodocopida	–	–	–	–	0.1	2	–	–	0.2	2.3
Myodocopina	–	–	–	–	0.1	2	–	–	–	–
Ostracoda	–	–	–	–	0.2	4	–	–	<0.1	2.3
Unidentified ostracod parts	–	–	0.1	16.7	0.3	6	–	–	1	11.4
COPEPODA	1.2	100	72.9	91.7	46.4	68	48.1	84.6	45.6	56.8
Aegisthus mucronatus	–	–	–	–	–	–	–	–	0.1	2.3
Aetideidae	–	–	–	–	0.1	2	0.4	7.7	–	–
Calanoida	–	–	12	41.7	7	18	28.8	76.9	3.2	9.1
Candacia curta	–	–	–	–	0.3	2	–	–	–	–
Copepoda	–	–	6.8	33.3	0.5	2	–	–	2.1	9.1
Corycaeus sp.	–	–	0.3	8.3	–	–	–	–	–	–
Cyclopoida	1.2	100	0.8	16.7	0.7	8	<0.1	15.4	0.9	9.1
Euchaeta sp.	–	–	–	–	–	–	–	–	1.5	2.3
Lubbockia aculeata	–	–	–	–	–	–	–	–	0.4	6.8
Lubbockia sp.	–	–	0.1	8.3	0.7	8	–	–	–	–
Lubbockia squillimana	–	–	0.3	8.3	0.2	2	–	–	–	–
Oithonidae	–	–	0.1	8.3	–	–	–	–	–	–
Pleuromamma abdominalis	–	–	3.3	16.7	–	–	–	–	0.3	2.3
Pleuromamma piseki	–	–	–	–	–	–	–	–	6.8	4.5

Table 8.19. Percent volume (%V) and percent frequency (%F) of prey consumed by *Valenciennellus tripunctulatus* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night.—Continued

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=1) (E=0)		Day (*n*=12) (E=5)		Night (*n*=50) (E=13)		Day (*n*=13) (E=0)		Night (*n*=44) (E=7)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
Pleuromamma robusta	–	–	–	–	–	–	–	–	1.5	2.3
Pleuromamma sp.	–	–	11.7	58.3	6	14	6.4	23.1	3.4	6.8
Pleuromamma xiphias	–	–	12.2	33.3	–	–	6.3	15.4	–	–
Poecilostomatoida	–	–	–	–	–	–	0.7	15.4	–	–
Rhincalanus cornutus	–	–	6.4	33.3	4.1	8	–	–	0.4	4.5
Rhincalanus sp.	–	–	1.2	8.3	0.8	2	–	–	–	–
Unidentified copepod parts	–	–	17.7	41.7	26	44	5.4	15.4	25.2	47.7
AMPHIPODA	–	–	–	–	–	–	<0.1	7.7	0.3	2.3
Amphipoda	–	–	–	–	–	–	<0.1	7.7	–	–
Unidentified amphipod parts	–	–	–	–	–	–	–	–	0.3	2.3
EUPHAUSIACEA	–	–	–	–	–	–	12.2	7.7	<0.1	2.3
Euphausiidae	–	–	–	–	–	–	12.2	7.7	–	–
Unidentified euphausiid parts	–	–	–	–	–	–	–	–	<0.1	2.3
CRUSTACEA	74.1	100	9.2	41.7	31.3	62	22.1	61.5	49.7	79.5
Unidentified crustacean parts	74.1	100	9.2	41.7	31.3	62	22.1	61.5	49.7	79.5
DECAPODA	–	–	–	–	–	–	–	–	–	–
Unidentified decapod parts	–	–	–	–	–	–	–	–	–	–
OTHER	24.7	100	16.8	50	21	50	17	53.8	2.7	31.8
Organic material	24.7	100	16.8	50	20.9	46	17	53.8	2.6	25
Unidentified animal parts	–	–	–	–	0.1	4	–	–	0.1	9.1

SIA was conducted on four sternoptychids, *A. aculeatus, Sternoptyx diaphana, S. pseudobscura,* and *V. tripunctulatus* (table 8.2). Although *A. aculeatus* was significantly enriched in $\delta^{13}C$ compared to *Sternoptyx* spp. (Tukey, $p = 0.001$) and *V. tripunctulatus* (Tukey, $p = 0.002$), there was no significant difference in $\delta^{15}N$. Spatial variations were not conducted for *Sternoptyx* spp. and no variations were documented in $\delta^{13}C$ or $\delta^{15}N$ for *A. aculeatus.* However, *V. tripunctulatus* was significantly depleted in ^{13}C at AC601 compared to AT340 ($p = 0.018$), but no differences were detected in $\delta^{15}N$. Although no dietary ontogenetic shifts were documented for Sternoptychidae (fig. 8.4*E*), there was trophic shift of approximately 2 per mil in nitrogen and <1 per mil in carbon with increasing trophic levels for sternoptychids (fig. 8.2), and no chemosynthetic signature was documented.

8.3.3.5 Stomiidae

Chauliodus sloani was the only stomiid species analyzed for diet composition. A high frequency (>75 percent) of empty stomachs occurred in *C. sloani*, with empty stomachs occurring more frequently during the day. A DVM was also documented for *C. sloani*, with more full stomachs documented at night in the mesopelagic (fig. 8.3*R*). Using GCA, fish, particularly *Bregmaceros* spp. and Myctophidae, were documented as the dominant prey in volume and frequency (table 8.20) and no spatial variations were recorded.

Table 8.20. Percent volume (%V) and percent frequency (%F) of prey consumed by *Chauliodus sloani* collected from three sites (AC601, AT340, and GC852) in the Gulf of Mexico separated by day and night.

[n, number of full stomachs; E, number of empty stomachs; –, no value]

	AC601		AT340				GC852			
	Night (*n*=0) (E=2)		Day (*n*=0) (E=5)		Night (*n*=2) (E=7)		Day (*n*=1) (E=8)		Night (*n*=8) (E=30)	
Food Item	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**	**% V**	**% F**
CRUSTACEA	–	–	–	–	–	–	–	–	<0.1	12.5
Unidentified crustacean parts	–	–	–	–	–	–	–	–	<0.1	12.5
FISH	–	–	–	–	99.7	50	100	100	87.5	37.5
Bregmaceros sp.	–	–	–	–	–	–	100	100	–	–
Myctophidae	–	–	–	–	–	–	–	–	62.2	12.5
Unidentified fish parts	–	–	–	–	99.7	50	–	–	25.3	25
OTHER	–	–	–	–	0.3	50	–	–	12.5	62.5
Organic material	–	–	–	–	0.3	50	–	–	12	50
Unidentified animal parts	–	–	–	–	–	–	–	–	0.5	12.5

SIA was conducted on two stomiid species, *C. sloani* and *Photostomias guernei* (table 8.2). Although *C. sloani* was depleted in ^{13}C and ^{15}N compared to *P. guernei*, small sample sizes prevented statistical comparisons. Spatial variations were also not statistically analyzed due to small sample sizes. The stomiids exhibited enrichment in $\delta^{15}N$ values with increasing trophic levels; however, enrichment in $\delta^{13}C$ values was less evident (fig. 8.2). A significant positive relationship between $\delta^{15}N$ values and SL was identified for *C. sloani* (R^2=0.920, p = 0.01) and *P. guernei,* (R^2=0.994, p = 0.003, fig. 8.4*F*). In addition to dietary ontogenetic shifts for *C. sloani* and *P. guernei,* there was trophic shift of approximately 2 per mil in nitrogen and <1 per mil in carbon with increasing trophic levels and no chemosynthetic signature was documented.

8.4 Discussion

Zooplankton was documented as the dominant prey for midwater fishes using both GCA and SIA. Crustaceans, particularly the copepod *Pleuromamma*, were consumed by all midwater fishes except *C. sloani*. Additionally, GCA documented three general feeding guilds similar to the findings of Gartner and others (1997). Although trophic enrichment in ^{13}C and ^{15}N with increasing trophic levels was lower than the average 3-4 per mil documented in previous studies (Roelke and Cifuentes, 1997; Post, 2002; Rybczynski and others, 2008), the enrichment was within reported ranges and may result from variation in the nitrogen source and metabolic processes (Minagawa and Wada, 1984); however, further investigation was required. Additionally, chemosynthetically derived carbon did not significantly contribute to the diets of midwater fauna, as evident by the lack of depleted ^{13}C, suggesting photosynthetic material, such as POM, supported the base of the midwater food web.

POM was the main carbon source for both invertebrate and fish fauna. Spatial variations documented in POM may result from changes in productivity and species composition.

Trichodesmium, a cyanobacterium, had extensive blooms documented in the GOM, which supply new nitrogen to the surrounding area (Holl and others, 2007). Additionally, previous literature reported *Trichodesmium* depleting ^{15}N in POM (Montoya and others, 2002). The presence of different water masses can also account for spatial variations in POM. A warm core eddy was documented at AT340 during sampling and can encourage diatom production and alter the phytoplankton composition (Thompson and others, 2007).

General trophic relationships documented using SIA for invertebrate species supported previous literature based on GCA. Zooplankton, euphausiids, and copepods occupied one trophic level above POM, with previous GCA for copepods (Dagg and others, 1989; Olson and others, 2006) and euphausiids (Kinsey and Hopkins, 1994) documenting POM-based diets. Larger crustaceans, such as *S. debilis* and *A. lesuerii*, were reported as trophically similar to zooplankton; however, previous studies reported crustaceans, fishes, and chaetognaths as the dominant prey consumed (Hopkins and others, 1994). Previous diet studies on Chaetognatha (Kehayias and others, 1996), *Stigmatoteuthis arcturi* (Cherel and Hobson, 2005), *G. valens* (Hopkins and others, 1994), and *A. purpurea* (Hopkins and others, 1994) were also supported by SIA.

Species composition may also contribute to variations between decapod species. Although *G. valens* and *S. debilis* consumed similar prey (Hopkins and others, 1994; Hopkins and Sutton 1998), isotopic variations in prey species may alter the isotopic signatures of *G. valens* and *S. debilis*. Prey, such as the commonly consumed euphausiids, can have different feeding strategies, as documented in *Thysanopoda* spp., which consumed ostracods, and *Euphausia* spp., which consumed noncrustacean prey (Kinsey and Hopkins, 1994). Diet

variations in prey alter $\delta^{15}N$ (Fry, 1988; Post, 2002) and can be documented on higher trophic levels within the food web.

Cyclothone spp. were classified as zooplanktivores. Similar feeding was documented for all *Cyclothone* spp. using GCA and SIA, supporting previous studies (Hopkins and others, 1996; Sutton and others, 1998). Variations in the species of zooplankton prey consumed suggested low competition for prey among species, as the dominant copepods consumed by each *Cyclothone* species inhabited different vertical distributions. *Cyclothone braueri* consumed the copepod *Aegisthus mucronatus*, which was documented from 300 to 500 m (Padmavati and others, 1998), whereas *C. pseudopallida* consumed *Valdiviella minor*, a mesopelagic/bathypelagic copepod documented at depths >500 m (Owre and Foyo, 1964), suggesting *C. pseudopallida* fed at deeper depths than *C. braueri.* The relationship between stomach fullness and DVMs also supported limited competition, as *C. braueri* occupied shallower depths and did not migrate. Variations in the isotopic signatures of *Cyclothone* spp. also suggested that *Cyclothone* spp. feed on different, but trophically similar, prey.

Gonostoma elongatum was classified as a large crustacean consumer; however, zooplankton was frequently documented in GCA as well. This was similar to previous studies in the eastern GOM (Lancraft and others, 1988; Hopkins and others, 1996) and was supported by SIA. Preference for euphausiids may be evident in the DVM documented in *G. elongatum,* as euphausiids undergo similar migration patterns (Brinton, 1967). However, prey preferences in *G. elongatum* can be variable. Ontogenetic effects were previously documented for *G. elongatum*, with zooplankton consumed until *G. elongatum* could consume large crustaceans (Clarke, 1982). Ontogenetic diet shifts were documented with SIA, with larger specimens consuming more euphausiids and less copepods and ostracods (Lancraft and others, 1988).

Myctophids had the most diverse diets of all midwater fishes analyzed and were classified as zooplanktivores, using GCA and SIA. Copepods, amphipods, euphausiids, and ostracods were the dominant prey items, supporting previous literature (Hopkins and Baird, 1985b; Hopkins and others, 1996; Sutton and others, 1998; Pusch and others, 2004). Although previous studies documented euphausiids as the dominant prey for *L. alatus* and *L. guentheri* (Hopkins and Baird, 1985b, Hopkins and others, 1996), copepods were more important to the diet in this study. This diet variation may be attributed to ontogeny, as the majority of specimens analyzed were juveniles and larger myctophids consume larger crustacean prey (Hopkins and Baird, 1985b). Ontogeny may also contribute to variability in isotopic signatures. Although this study only documented ontogenetic diet shifts in *Diaphus* spp., previous studies have documented ontogenetic diet shifts in other myctophid species (Hopkins and Baird, 1985b; Hopkins and others, 1996; Sutton and others, 1998; Pusch and others, 2004). Comparisons between data from GCA and SIA also suggested variations in diet. Although not evident in GCA, depleted ^{15}N in *M. affine* and *C. warmingii* may result from omnivory, previously documented in *C. warmingii* (Robinson, 1984). Additionally, the consumption of nitrogen-depleted prey, such as salps, was previously documented in the stomachs of *C. warmingii* (Sutton and others, 1998) and may contribute to depleted ^{15}N in *M. affine* and *C. warmingii*.

Both phosichthyid species were also classified as zooplanktivores. *Pollichthys mauli* primarily consumed zooplankton and euphausiids; this finding was similar to previous reports (Sutton and others, 1998). In contrast, *V. poweriae* consumed fish in addition to zooplankton, which, though not previously reported for *V. poweriae* (Hopkins and others, 1996), was similar to previously dietary studies on *V. nimbaria* (Hopkins and others, 1996; Champalbert and others, 2008). As both *P. mauli* and *V. poweriae* exhibit similar DVM patterns, the incorporation of

non-zooplankton prey may reduce competition for zooplankton prey between these species. SIA supports GCA, with enriched ^{15}N documented in *V. poweriae* compared to *P. mauli*. It was also possible that *P.* mauli may have targeted ^{15}N-depleted prey items, such as salps, as evident by the depleted isotopic signature. Size may also be a contribution to trophic differences between these phosichthyids, as ontogenetic diet shifts were documented for *Vinciguerria* spp. (Champalbert and others, 2008; this study).

All sternoptychids were classified as zooplanktivores. *Valenciennellus tripunctulatus* fed almost exclusively on copepods, with ostracods, amphipods, and euphausiids also being important foods, supporting previous literature (Hopkins and Baird, 1981; Hopkins and others, 1996; Sutton and others, 1998). Although *Argyropelecus* spp. ate a mixture of copepods, ostracods, amphipods, and euphausiids, which also supported previous studies (Hopkins and Baird, 1981; Hopkins and Baird, 1985a; Sutton and others, 1998), *A. hemigymnus* consumed only crustaceans, while *A. aculeatus* incorporated noncrustacean prey, like mollusks, into its diet. Previous studies reported mollusks as a rare prey item for *A. aculeatus* (Hopkins and Baird, 1981; Hopkins and Baird, 1985a); however, enriched $\delta^{15}N$ documented in SIA for *A. aculeatus* implies mollusks, such as cephalopods, which are enriched in ^{15}N compared to zooplankton, may be more common prey than originally documented. The incorporation of noncrustacean prey suggests *A. aculeatus* may adapt a more generalist feeding strategy, which reduces its dependence on zooplankton and competition for zooplankton with other midwater fishes. Prey competition may also be reduced through the utilization of vertical space. While DVMs were documented for *Argyropelecus* spp., with feeding documented at night (Hopkins and Baird, 1985a; this study), *A. aculeatus* may be feeding at shallower depths than *A. hemigymnus*, based on the presence of more full stomachs in the epipelagic for *A. aculeatus.* Interestingly, the

relationship between DVMs and stomach fullness for *V. tripunctulatus* was similar to *A. hemigymnus,* which contradicts previous literature that documented *V. tripunctulatus* as a nonmigratory species feeding actively during the day (Hopkins and Baird, 1981). This contradiction in time of feeding may be due to size differences, with larger specimens not migrating (Hopkins and Sutton, 1998).

Chauliodus sloani was classified as a piscivore. The presence of fishes, particularly myctophids, was also previously documented in the literature (Hopkins and others, 1996). In contrast to GCA, results from SIA classified *C. sloani* at a trophic level similar to zooplanktivores, which suggests *C. sloani* consumed zooplankton more frequently than previously reported. DVMs of *C. sloani* reflected migrational patterns of zooplankton, with *C. sloani* migrating to the epipelagic at night. Previous literature reported asynchronous migrations in *C. sloani* (Sutton and Hopkins, 1996b), and variations in diet composition may result from ontogeny. Juvenile *C. sloani* may consume zooplankton prey and incorporate more fish prey with increasing size, as documented for *G. elongatum* (Clarke, 1982). Additional specimens were needed to further investigate ontogenetic effects on diet.

Although GCA were not performed on other stomiids, SIA provided data to support Stomiidae as a tertiary consumer. *Photostomias guernei* occupied a slightly higher trophic position than *C. sloani*, as evident by the enriched ^{15}N. Previous literature documented *P. guernei* as a large crustacean consumer, with the decapod *G. valens* reported as the dominant prey (Hopkins and others, 1996). *Gennadas valens* is a carnivorous decapod (Hopkins and others, 1994) and was documented on the same trophic level as zooplanktivorous fish, and consumption by *P. guernei* would enrich ^{15}N in *P. guernei*. Consumption of carnivorous prey, whether fish or decapod, places the family Stomiidae at the top of the midwater food web.

8.5 Conclusions

1. The 18 fish species analyzed formed 3 major feeding guilds: zooplanktivore (consuming copepods, amphipods, and ostracods), consumers (consuming decapods and euphausiids) of larger sized crustaceans, and piscivores.
2. Stomiidae were the dominant piscivores, with the majority of their prey being myctophids and bregmacerotids.
3. Diel vertical migrations were documented in the majority of midwater fishes, with species migrating to the epipelagic at night; however, feeding was not limited to the epipelagic or to the night-time period.
4. Significant shifts in diet composition with ontogeny were documented in stomiids, *G. elongatum, V. poweriae,* and *Diaphus* spp.
5. Generally, GCA and SIA complemented each other, and differences between data highlighted the importance of utilizing both methods to discern trophic structure.
6. Photosynthetic material provided the base for the midwater food; however, chemosynthetic cold seeps may have minor influences that went undetected using the above methodology.

8.6 Acknowledgments

This project was largely funded by the U.S. Geological Survey under Cooperative Agreement No. 05HQAG0009, subagreement 05099HS004. We thank the crew of the R/V *Cape Hatteras* and all scientific personal for assisting with fishing operations and sample processing. S. Artabane assisted with sampling and fish identification, and C. Ames assisted with various aspects of the cruise and invertebrate identifications. C. Tobias, K. Duernberger, and A.

Demopoulos provided guidance and support for stable isotope analysis. We thank G. Boland (BOEMRE), S.D. Brooke (MCI), and J. Sinclair (BOEMRE) for helpful comments on this chapter. We would also like to thank M. Rhode for his suggestions and edits to this manuscript.

9. DISTRIBUTIONS AND COMPOSITION OF THE MESOPELAGIC INVERTEBRATE FAUNA OVER THE SLOPE OF THE NORTH-CENTRAL GULF OF MEXICO

By Martha S. Nizinski[1] and Cheryl L. Ames[2]

[1]NOAA, National Marine Fisheries Service, National Systematics Laboratory, Smithsonian Institution, Washington, DC

[2]University of North Carolina Wilmington, Center for Marine Science, Wilmington, NC

9.1 Introduction

Cold-seep habitats in the Gulf of Mexico are numerous, widespread, and the most thoroughly explored and investigated of any cold-seep habitats in the world (Fisher and others, 2007). Recent explorations (Roberts and others, 2007b) to some of the deeper seep sites within this region have revealed new findings (for example, new species, new assemblages of organisms, a variety of new features such as mud volcanoes and brine pools, shifts in community structure with depth) and added to our knowledge of the species diversity, community composition, and ecology of these reducing environments. Thus, even though these are some of the best known chemosynthetic sites, much remains to be learned about these environments.

The epibenthic megafaunal invertebrate assemblages associated with these reducing environments have been studied extensively (for example, MacDonald and others, 1990; Bergquist and others, 2003b; Fisher and others, 2007; Cordes and others, 2007; 2009). However, relationships between midwater assemblages and the seep benthic assemblages remain unknown. A few studies examined the species composition and ecology of the mesopelagic invertebrate

assemblages, in particular, the crustaceans and cephalopods, in the eastern Gulf of Mexico (for example, Heffernan and Hopkins, 1981; Hopkins and others, 1989; Passarella and Hopkins, 1991; Flock and Hopkins, 1992; Hopkins and others, 1994). These previous studies were part of an extensive oceanic ecology project designed to characterize and examine the fauna typical of low latitude oligotrophic regions (Hopkins and others, 1989). Whether mesopelagic invertebrate assemblages are connected in some manner to benthic seep habitats has not been investigated.

In collaboration with the mesopelagic fish investigation described in Chapter 7 and in cooperation with ongoing benthic studies (see Roberts and others, 2007b), we sampled the midwater invertebrate fauna at a variety of depths over three cold-seep habitats >1,000 m and one deepwater coral bank (<1,000 m) in the north-central GOM. Our goals were to characterize the invertebrate fauna collected throughout the water column at each site. Secondly, we compared the composition of invertebrate faunal assemblages among the three offshore, deeper chemosynthetic sites with that from the inshore, shallower coral site. Thirdly, we compared the invertebrate assemblages collected in different zones (surface, midwater, benthic) at all sites sampled.

9.2 Methods

Samples were collected at 290 stations located over and around four study sites in the north-central to western GOM: three lower slope (>1,000 m) cold-seep sites (GC852, AT340, and AC601) and one upper slope (<1,000 m) deep-sea coral site (VK826; fig. 9.1). Sampling for this study was conducted August 9-29, 2007, using the R/V *Cape Hatteras* (Duke-UNC Oceanographic Consortium). The majority of invertebrate samples reported here were captured in conjunction with sampling for midwater fishes. See Chapter 7 for a more detailed description of study sites and sampling techniques using a Tucker trawl.

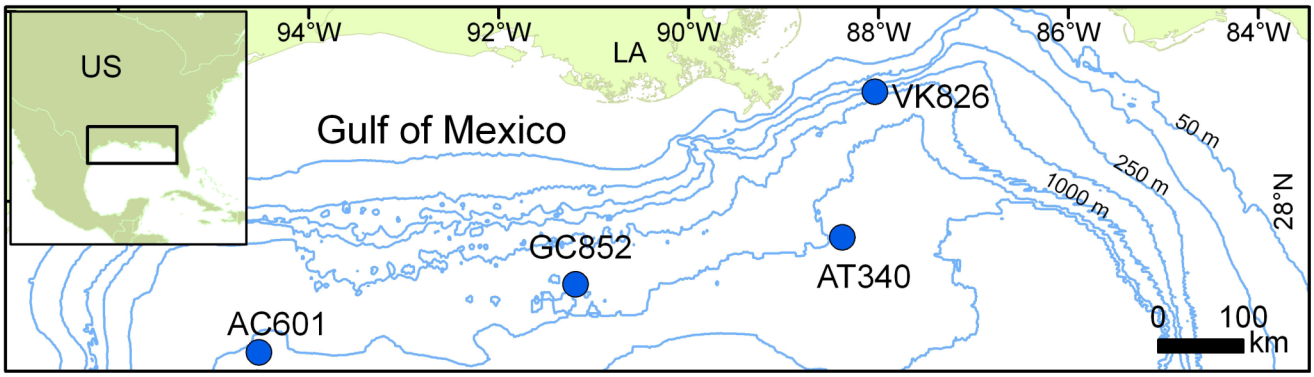


Figure 9.1. Map of study area in the north-central Gulf of Mexico showing location of sampling sites. Three sites (AC601, GC852, and AT340) were over cold-seep habitats, and one site (VK826) was over a *Lophelia pertusa* coral habitat.

Although the focus of this portion of the study was to characterize the mesopelagic invertebrate fauna, several individuals (n = 113) were collected in surface samples taken with 1-m diameter plankton nets (505-µm and 202-µm mesh) that were towed for 30 min on the surface in all study areas. Additionally, 160 epibenthic megafaunal invertebrates were captured with a variety of bottom gear (for example, otter trawl, box core, Tucker trawl). A 4.88-m otter trawl was towed on the seafloor for 30 min; box core sampling is described in Chapter 3. These data are reported here for completeness.

The majority of invertebrates were fixed and maintained in 75-percent ethanol. Cephalopods were fixed in a 10-percent formalin seawater solution and after the cruise were transferred to 50-percent isopropanol. Jellyfishes also were fixed in a 10-percent formalin seawater solution and maintained in formalin for long-term storage. In the laboratory, all collections were sorted and curated. Individuals were identified to the lowest possible taxon using historical literature, authoritative keys, and comparative material housed in the National

Museum of Natural History, Smithsonian Institution, Washington, D.C. Some material was either identified, or identifications were confirmed, by taxonomic experts at the Smithsonian Institution.

9.3 Results

Overall, 912 individuals representing at least 9 phyla, 23 classes, 86 families, and 188 species of larval, juvenile, and adult invertebrates were collected at 141 of the 290 stations sampled (fig. 9.1, table 9.1). These specimens were taken in 14 surface, 116 midwater, and 11 benthic collections. The majority of species were collected in midwater trawls. Among major taxa only four (Cnidaria, Mollusca, Crustacea, Chaetognatha) had species that were collected in all three zones sampled (fig. 9.2).

Table 9.1. Overall diversity and abundance of invertebrates collected (all sampling methods combined) at three cold-seep sites (GC852, AC601, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007.

[n, number of stations where invertebrate specimens were collected; – , no value]

Phylum	Class or Order	Family	Species	GC852 n=64	AC601 n=4	AT340 n=40	VK826 n=33	Total No. of Inds. n=141
Porifera	Demospongiae		Demospongiae spp. larvae (4)	4	–	6	4	14
Cnidaria	Anthozoa	cf. Hormathiidae	cf. Hormathiidae sp.	–	–	1	–	1
		Umbellulidae	*Umbellula* sp.	13	–	–	–	13
	Cubozoa	Alatinidae	*Alatina* sp.	–	–	1	1	2
		Tamoyidae	*Tamoya haplonema*	–	–	–	1	1
	Hydrozoa		Hydrozoa sp.	–	–	1	–	1
		Aequoreidae	*Aequorea macrodactyla*	–	–	6	–	6
			Aequorea sp.	–	–	2	–	2
			Zygocanna vagans	4	–	–	4	8
		Aglaopheniidae	*Aglaophenia* sp.	–	–	1	–	1
		Eirenidae	*Tima* sp.	1	–	–	–	1
		Geryoniidae	*Geryonia probascidalis*	–	–	–	1	1
		Halicreatidae	*Halicreas minimum*	1	–	–	–	1
		Hippopodiidae	*Hippopodius hippopus*	5	–	–	–	5
			Vogtia cf. *serrata*	1	–	–	–	1
			Vogtia spinosa	1	–	–	–	1
		Pandeidae	*Campaniclava clionis*(on *Clio recurva*)	4	–	2	2	8
	Scyphozoa		Scyphozoa sp.	–	–	–	1	1
		Atollidae	*Atolla* sp.	3	–	2	–	5
			Atolla vanhoeffeni	4	–	–	–	4
			Atolla wyvelli	5	–	–	–	5
		Nausithoidae	*Nausithoe* cf. *aurea*	1	–	–	–	1

Table 9.1. Overall diversity and abundance of invertebrates collected (all sampling methods combined) at three cold-seep sites (GC852, AC601, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. —Continued

[n, number of stations where invertebrate specimens were collected; – , no value]

Phylum	Class or Order	Family	Species	GC852 n=64	AC601 n=4	AT340 n=40	VK826 n=33	Total No. of Inds n=141
			Nausithoe punctata	–	–	–	1	1
			Nausithoe sp.	2	–	–	–	2
		Pelagiidae	*Pelagia noctiluca*	–	–	3	17	20
		Periphyllidae	*Periphylla periphylla*	11	–	2	–	13
		Rhopalonematidae	*Colobonema sericeum*	1	–	–	–	1
		Ulmaridae	*Aurelia aurita*	–	–	1	1	2
Ctenophora	Cydippida	Pleurobrachiidae	Pleurobrachiidae sp.	–	–	–	3	3
Mollusca	Bivalvia	Mytilidae	*Bathymodiolus brooksii*	2	–	–	–	2
	Cephalopoda	Chtenopterygidae	*Chtenopteryx sepioloides*	1	–	–	–	1
		Cranchiidae	*Bathothauma lyromma*	–	1	–	–	1
			Cranchia scabra	1	–	–	–	1
			Helicocranchia pfefferi	2	–	–	–	2
			Liguriella podophthalma	1	–	–	–	1
			Sandalops melancholius	–	–	1	–	1
		Cycloteuthidae	*Cycloteuthis serventyi*	1	–	–	–	1
		Enoploteuthidae	*Abralia redfieldi*	1	–	2	1	4
			Abraliopsis atlantica	1	–	2	–	3
			Abraliopsis sp.	1	–	–	–	1
			Ancistrocheirus lesueurii	3	–	–	–	3
			Enoploteuthis anapsi	1	–	–	–	1
			Enoploteuthis sp.	–	–	–	1	1
			Selenoteuthis scintillans	1	–	2	–	3
		Histioteuthidae	*Histioteuthidae* sp.	–	–	1	–	1
			Stigmatateuthis arcturi	17	1	–	–	18
		Lycoteuthidae	*Lycoteuthis springeri*	1	–	–	–	1

Table 9.1. Overall diversity and abundance of invertebrates collected (all sampling methods combined) at three cold-seep sites (GC852, AC601, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. —Continued

[n, number of stations where invertebrate specimens were collected; – , no value]

Phylum	Class or Order	Family	Species	GC852 n=64	AC601 n=4	AT340 n=40	VK826 n=33	Total No. of Inds n=141
			Selenoteuthis scintillans	1	–	–	–	1
		Neoteuthidae	*Neoteuthis thielei*	1	–	–	–	1
		Octopoteuthidae	*Octopoteuthis* cf. *megaptera*	1	–	–	1	2
		Ommastrephidae	*Ommastrephes bartramii*	–	–	–	1	1
		Onychoteuthidae	*Onykia carriboea*	1	–	1	–	2
		Pyroteuthidae	cf. *Pterygioteuthis giardi*	7	–	12	7	26
			Pyroteuthidae sp.	–	–	1	–	1
			Pyroteuthis magaritifera	–	1	2	1	4
		Sepiolidae	*Heteroteuthis dispar*	3	–	–	1	4
		Bolitaenidae	*Bolitaena pygmaea*	–	–	1	–	1
			Japetella diaphana	8	–	2	–	10
		Octopodidae	*Macrotritopus defilippi*	1	–	1	1	3
		Tremoctopodidae	*Tremoctopus violaceus*	–	–	8	–	8
		Vampyroteuthidae	*Vampyroteuthis infernalis*	2	–	–	–	2
	Gastropoda	Turridae	*Leucosyrinx verrilli*	1	–	–	–	1
	Gastropoda/ Heterobranchia	Atlantidae	cf. *Atlanta* sp.	4	–	–	–	4
		Carinariidae	*Carinaria lamarcki*	1	–	1	–	2
		Cavoliniidae	*Cavolinia tridentata*	1	–	4	–	5
			Cavolinia uncinata	1	–	–	–	1
			Clio recurva	6	–	2	2	10
			Cliopsis krohnii	3	–	–	1	4
			Diacavolinia cf. *elegans*	5	–	9	–	14
			Diacria cf. *rampali*	5	–	–	–	5
		Peraclididae	cf. *Peracle* sp.	7	–	–	–	7

Table 9.1. Overall diversity and abundance of invertebrates collected (all sampling methods combined) at three cold-seep sites (GC852, AC601, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. —Continued

[n, number of stations where invertebrate specimens were collected; – , no value]

Phylum	Class or Order	Family	Species	GC852 n=64	AC601 n=4	AT340 n=40	VK826 n=33	Total No. of Inds n=141
		Pterotracheidae	*Pterotrachea* cf. *coronata*	12	–	2	2	16
Annelida	Polychaeta	Alciopidae	Alciopidae sp.	1	–	–	–	1
Arthropoda/ Crustacea	Amphipoda	Cystisomatidae	*Cystisoma* cf. *latipes*	3	–	–	–	3
		Lanceolidae	*Lanceola* sp.	2	–	–	–	2
		Oxycephalidae	*Rhabdosoma whitei*	2	–	–	–	2
			Streetsia challengeri	–	–	–	1	1
		Phronimidae	*Phronima sedentaria*	10	–	6	–	16
		Phrosinidae	*Anchylomera blossevillei*	8	–	–	–	8
			Phrosina semilunata	2	–	–	–	2
		Platyscelidae	*Platyscelus* sp.	2	–	–	–	2
			Platysceloidea sp.	1	–	28	–	29
		Pronoidae	*Parapronoe* spp.	3	–	1	–	4
			Pronoidae sp.	1	–	–	–	1
		Vibiliidae	*Vibilia* sp.	2	–	–	–	2
	Copepoda	Megacalanidae	*Bathycalanus princeps*	6	–	1	–	7
	Decapoda/Anomura	Chirostylidae	*Eumunida picta*	–	–	–	3	3
		Galatheidae	*Munida microphthalma*	4	–	–	–	4
			Munida sanctipauli	–	–	–	3	3
			Munidopsis cf. *bermudezi* (molt)	1	–	–	–	1
			*Munidopsis n.*sp.	–	–	–	1	1
			Munidopsis penescabra	–	–	–	2	2
			Munidopsis similis	1	–	–	–	1
		Parapaguridae	*Parapagurus* sp.	8	–	–	–	8
	Decapoda/Astacidea	Nephropidae	*Nephropsis aculeata*	–	–	–	2	2
			Nephropsis agassizii	1	–	–	–	1

Table 9.1. Overall diversity and abundance of invertebrates collected (all sampling methods combined) at three cold-seep sites (GC852, AC601, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. —Continued

[n, number of stations where invertebrate specimens were collected; – , no value]

Phylum	Class or Order	Family	Species	GC852 n=64	AC601 n=4	AT340 n=40	VK826 n=33	Total No. of Inds n=141
	Decapoda/Brachyura		Brachyura sp.	–	–	1	–	1
		Calappidae	*Calappa* cf. *tortugae* megalopa	–	–	–	2	2
		Majidae	Majidae sp.	4	–	–	–	4
		Portunidae	*Portunus* sp.	–	–	2	10	12
	Decapoda/Caridea	Glyphocrangonidae	*Glyphocrangon aculeata*	3	–	–	–	3
			Glyphocrangon cf. *nobilis*	1	–	–	–	1
			Glyphocrangon longirostris	1	–	–	–	1
		Nematocarcinidae	*Nematocarcinus ensifer*	3	–	–	–	3
			Nematocarcinus rotundus	20	–	–	–	20
		Oplophoridae	*Acanthephyra* cf. *brevirostris*	2	–	–	–	2
			Acanthephyra curtirostris	2	–	1	–	3
			Acanthephyra eximia	4	–	–	–	4
			Acanthephyra pelagica	1	–	–	–	1
			Acanthephyra purpurea	15	–	1	–	16
			Janicella spinicauda	2	–	1	–	3
			Meningodora vesca	2	–	–	–	2
			Notostomus elegans	3	–	–	–	3
			Oplophorus gracilirostris	2	–	4	3	9
			Systellaspis debilis	8	–	7	–	15
			Systellaspis sp.	4	–	–	–	4
		Palaemonidae	*Leander tenuicornis*	1	–	–	–	1
		Pandalidae	*Heterocarpus oryx*	6	–	–	–	6
			Plesionika acanthonotus	–	–	–	1	1
			Stylopandalus richardi	18	–	–	–	18
		Pasiphaeidae	*Pasiphaea merriami*	7	–	–	3	10

Table 9.1. Overall diversity and abundance of invertebrates collected (all sampling methods combined) at three cold-seep sites (GC852, AC601, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. —Continued

[n, number of stations where invertebrate specimens were collected; – , no value]

Phylum	Class or Order	Family	Species	GC852 n=64	AC601 n=4	AT340 n=40	VK826 n=33	Total No. of Inds n=141
	Decapoda/ Dendrobranchiata	Aristeidae	*Hemipenaeus carpenteri*	3	–	–	–	3
			Hepomadus tener	2	–	–	–	2
		Benthesicymidae	*Gennadas elegans*	5	1	1	–	7
			Gennadas sp.	1	–	–	–	1
			Gennadas valens	46	2	8	–	56
		Penaeidae	*Funchalia villosa*	5	–	5	2	12
		Sergestidae	*Deoergestes curvatus*	4	1	–	–	5
			Deoergestes henseni	3	–	–	–	3
			Parasergestes armatus	4	–	1	–	5
			Sergestes atlanticus	1	–	–	–	1
			Sergestidae sp.	1	1	1	–	3
			Sergia grandis	1	–	–	–	1
			Sergia hansjacobi	1	–	–	3	4
			Sergia mollis	1	–	–	–	1
			Sergia regalis	3	–	–	–	3
			Sergia robusta	1	–	1	–	2
			Sergia splendens	13	–	–	–	13
			Sergia talismani	2	–	–	–	2
			Sergia wolffi	9	–	1	–	10
		Solenoceridae	*Pleoticus robustus*	1	–	–	–	1
	Decapoda/Palinura	Polychelidae	*Polycheles sculptus*	6	–	–	–	6
			Polycheles sculptus larva	1	–	–	–	1
		Scyllaridae	Scyllaridae sp. larva	1	–	1	–	2
	Euphausiacea	Euphausiidae	cf. *Thysanopoda sp.*	2	2	–	–	4
			Euphausia sp.	1	–	–	–	1

Table 9.1. Overall diversity and abundance of invertebrates collected (all sampling methods combined) at three cold-seep sites (GC852, AC601, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. —Continued

[n, number of stations where invertebrate specimens were collected; – , no value]

Phylum	Class or Order	Family	Species	GC852 n=64	AC601 n=4	AT340 n=40	VK826 n=33	Total No. of Inds n=141
			Euphausiidae sp.	5	–	1	–	6
			Nematoscelis megalops	15	–	–	–	15
			Nematoscelis sp.	6	–	–	–	6
			Stylocheiron abbreviatum	1	–	–	–	1
			Stylocheiron elongatum	1	–	–	–	1
			Thysanoessa spp.	9	–	–	–	9
			Thysanopoda tricuspida	–	–	1	–	1
	Paracarida	Eucopiidae	*Eucopia* cf. *grimaldii*	2	–	–	–	2
			Eucopia sp.	–	–	1	–	1
		Lophogastridae	*Gnathophausia zoea*	2	–	–	–	2
			Neognathophausia ingens	4	–	–	–	4
	Stomatopoda		Stomatopoda sp.larva	11	–	–	–	11
Chaetognatha	Chaetognatha		Chaetognatha spp.(4)	38	–	11	–	49
Echinodermata	Holothuroidea	Molpadiidae	*Molpadia blakei*	7	–	–	–	7
		Myriotrochidae	*Myriotrochus* cf. *rinkii*	1	–	–	–	1
			Myriotrochus n. sp.	1	–	–	–	1
		Psychropotidae	*Psychropotes depressa*	2	–	–	–	2
		Synallactidae	*Molpadiodemas involutus*	7	–	–	–	7
			Paroriza sp.	3	–	–	–	3
			Zygothuria lactea	1	–	–	–	1
		Synaptidae	*Protankyra brychia*	1	–	–	–	1
			Rynkatorpa felderi	1	–	–	–	1
	Ophiurida	Ophiodermatidae	*Bathypectinura heros*	2	–	–	–	2
		Ophioleucidae	*Ophioleuce* sp.	1	–	–	–	1

Table 9.1. Overall diversity and abundance of invertebrates collected (all sampling methods combined) at three cold-seep sites (GC852, AC601, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. —Continued

[n, number of stations where invertebrate specimens were collected; – , no value]

Phylum	Class or Order	Family	Species	GC852 n=64	AC601 n=4	AT340 n=40	VK826 n=33	Total No. of Inds n=141
		Ophiuridae	*Amphiophiura metabula*	6	–	–	–	6
			cf. *Ophiernus vallincola*	1	–	–	–	1
	Asteroidea	Astropectinidae	*Dytaster insignis*	1	–	–	–	1
			Plutonaster intermedius	1	–	–	–	1
		Goniasteridae	*Nymphaster arenatus*	1	–	–	–	1
Chordata/ Tunicata	Pyrosomatida	Pyrosomatidae	*Pyrosoma atlanticum*	27	1	3	2	33
	Salpida	Salpidae	*Iasis zonaria*	1	–	–	1	2
			Salpa cf. *maxima*	4	–	–	–	4
			Salpa cf. *fusiformis*	2	–	–	–	2
			Salpa cylindrica	2	–	14	–	16
			Salpa sp.	3	–	–	–	3
			Salpidae sp.	1	–	–	–	1
Total				**622**	**11**	**185**	**94**	**912**

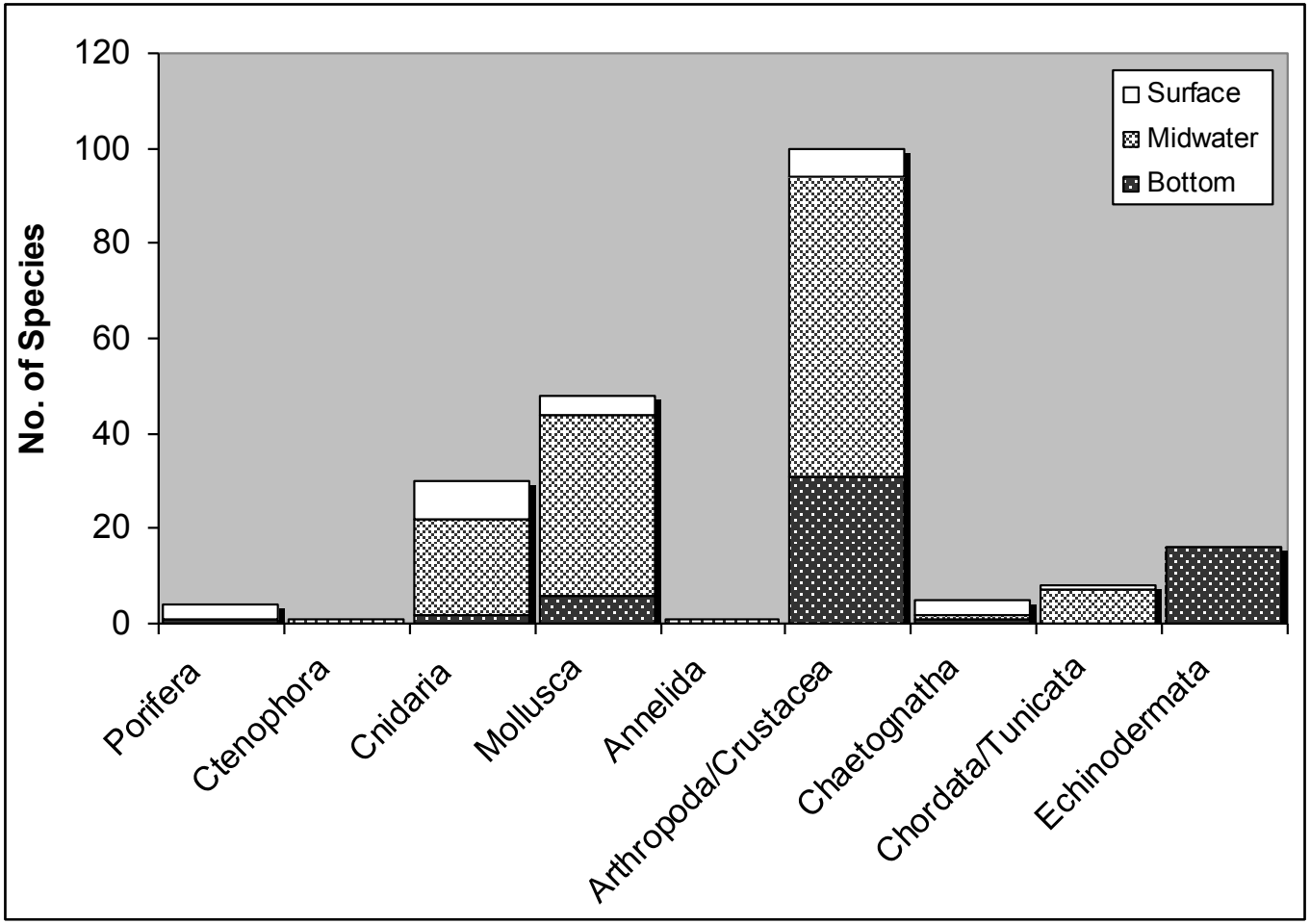


Figure 9.2. Overall (all sampling sites combined) species richness of invertebrates for each major taxon collected by zone (surface, midwater, benthic) at three cold-seep sites (AC601, GC852, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007.

Subphylum Crustacea (Phylum Arthropoda) was the dominant major taxon in both numbers of species and individuals, with a total of 85 species and 458 individuals represented in the samples (figs. 9.3, 9.4). Within the crustaceans, shrimps were the most speciose group with caridean and dendrobranchian shrimps constituting about half (41/85 species) of the total crustacean diversity. Carideans and dendrobranchians were equally diverse with 21 and 20 species, respectively. Dendrobranchians were slightly more abundant than the carideans (135 versus 126 individuals). Among the shrimps collected, *Sergia hansjacobi* represents a new record for the Gulf of Mexico fauna, having never been reported from this area before.

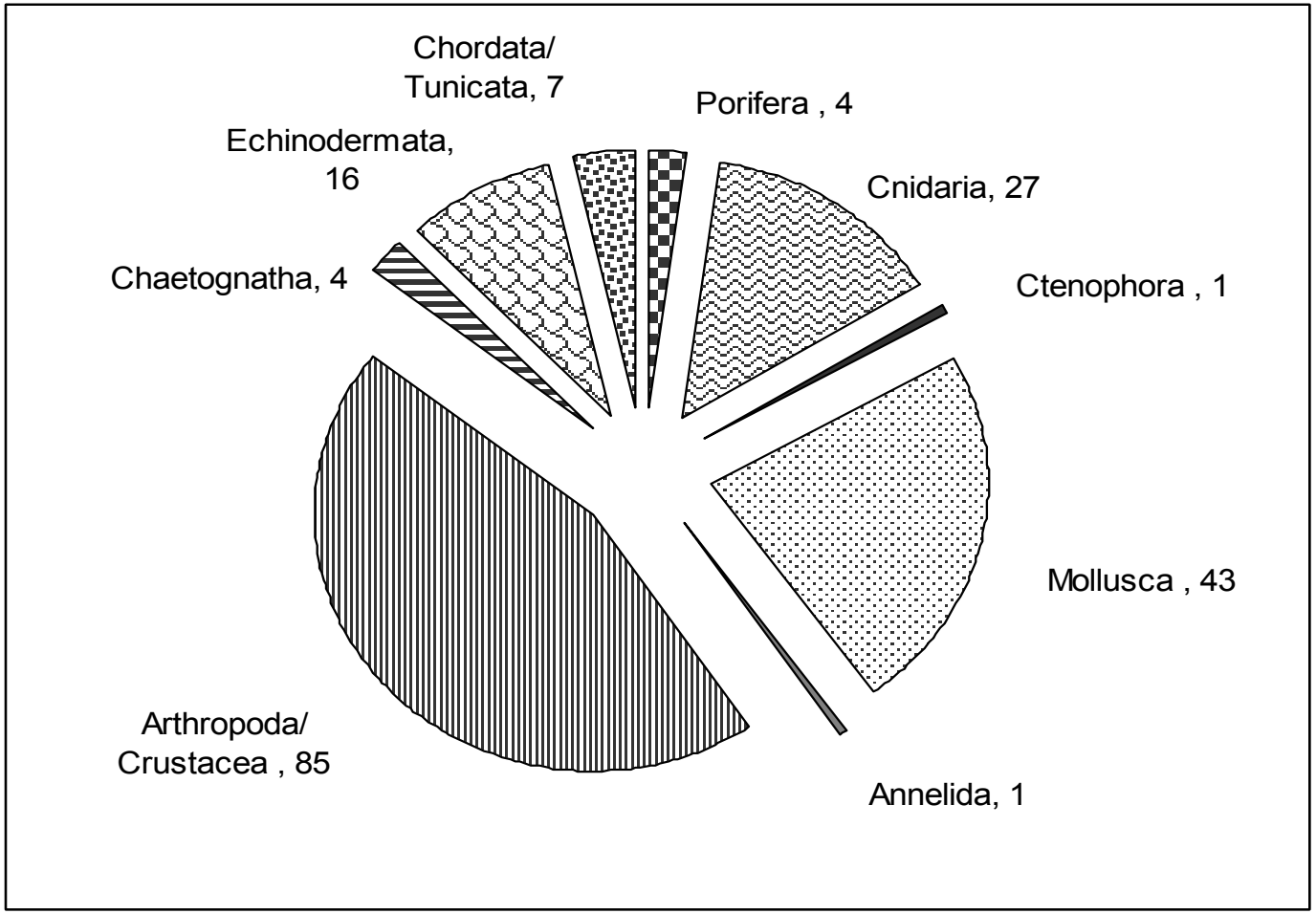


Figure 9.3. Overall (all sampling sites combined) species richness of all major invertebrate taxa collected at three cold-seep sites (AC601, GC852, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007.

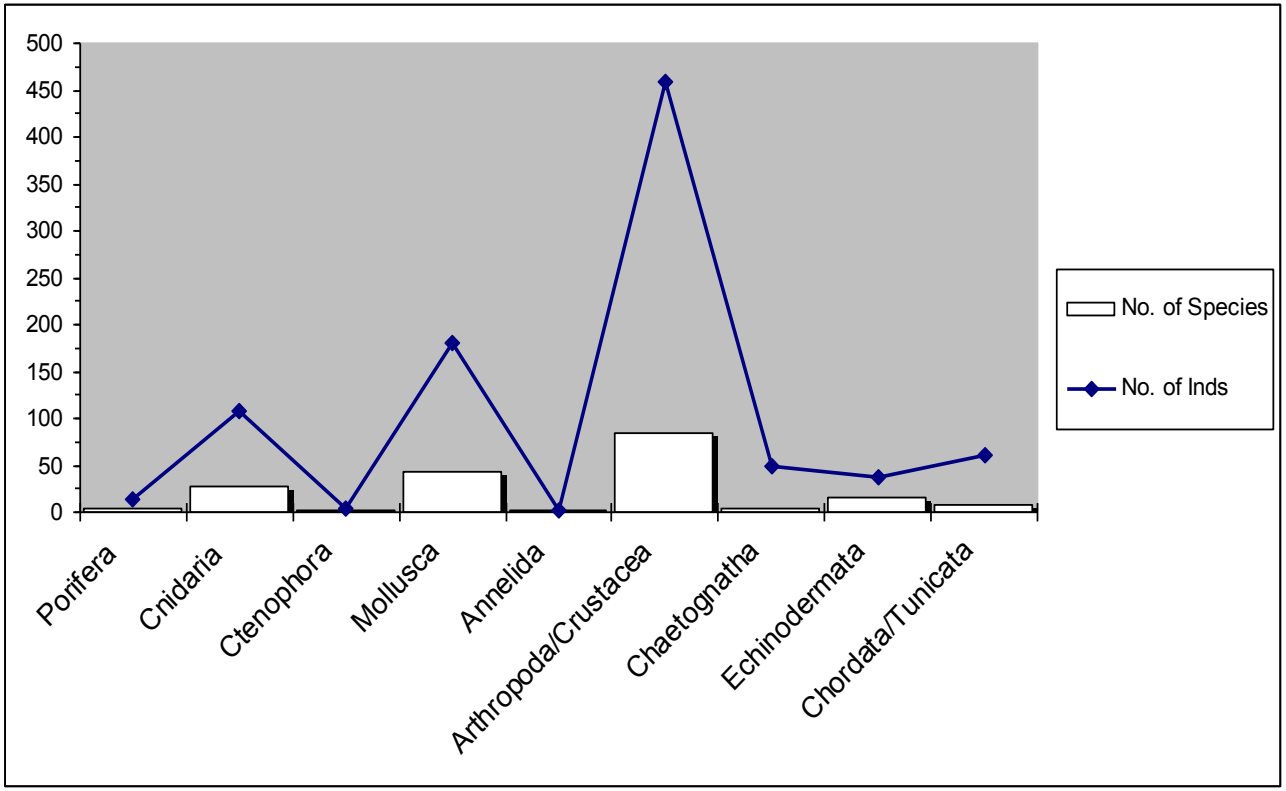


Figure 9.4. Overall (all sampling sites combined) species richness and abundance of major invertebrate taxa collected at three cold-seep sites (AC601, GC852, and AT340) and one Lophelia pertusa site (VK826) in the Gulf of Mexico, August 9-29, 2007.

Mollusca was the second most diverse phylum collected, and samples of these organisms consisted of 43 species and 181 individuals. Cephalopoda was the most diverse class of molluscs (15 families, 31 species). Gastropods and bivalves were also represented in the samples, including two individuals of *Bathymodiolus brooksii*, the mussel commonly found at deeper (1,000-3,300 m) chemosynthetic sites.

For the other groups of invertebrates, cnidarians (27 species, 108 individuals) were well represented in the samples with 25 species of jellies (Cubozoa, Scyphozoa, and Hydrozoa), two species of hydroids, and two species of anthozoans. All major taxa within the Echinodermata, except for crinoids, were represented in the relatively small collections of this group (16 species, 37 individuals). Samples consisted of nine species of sea cucumbers, four species of brittle stars, and three species of sea stars. Salps and pyrosomes (Urochordata), sponges (Porifera), chaetognaths (Chaetognatha), and polychaetes (Annelida) made up the remainder of the major taxa collected in these samples.

Among the invertebrates collected, the shrimp *Gennadas valens* (Dendrobranchiata: Benthesicymidae) was the most abundant species with 56 specimens collected, representing 6 percent of total individuals captured. The pyrosome *P. atlanticum* (n = 33) and chaetognaths (n = 49) were also relatively abundant in the samples. Sixty-seven of the species (36 percent) collected during this study were represented by single individuals, while another 30 species (16 percent) were each represented by only two individuals.

Comparing the distribution of species among study sites revealed that most species (129/188) were collected at only one of the four sites sampled. Forty-two species and 16 species were collected at two and three of the four sites, respectively. Only one species, *Pyrosoma atlanticum*, was collected at all study sites.

9.3.1 Species composition and relative abundance among study sites

Species diversity and relative abundance of invertebrates was highest at GC852 (table 9.1, fig. 9.5). Collections from the 64 stations sampled at this site yielded 622 individuals, consisting of at least 148 species and representing all phyla collected except Ctenophora and all class/orders identified in the study with exception of the Cubozoa. Crustaceans were the most speciose taxa with 73 species represented in collections. Crustaceans were also the numerical dominants of the GC852 megafaunal assemblage, where they constituted 55 percent of the total individuals collected at this site. Sixty-three percent of the crustacean catch taken here consisted of shrimps. Molluscs, echinoderms, and cnidarians contributed 34, 16, and 15 species, respectively, to the overall diversity at this site.

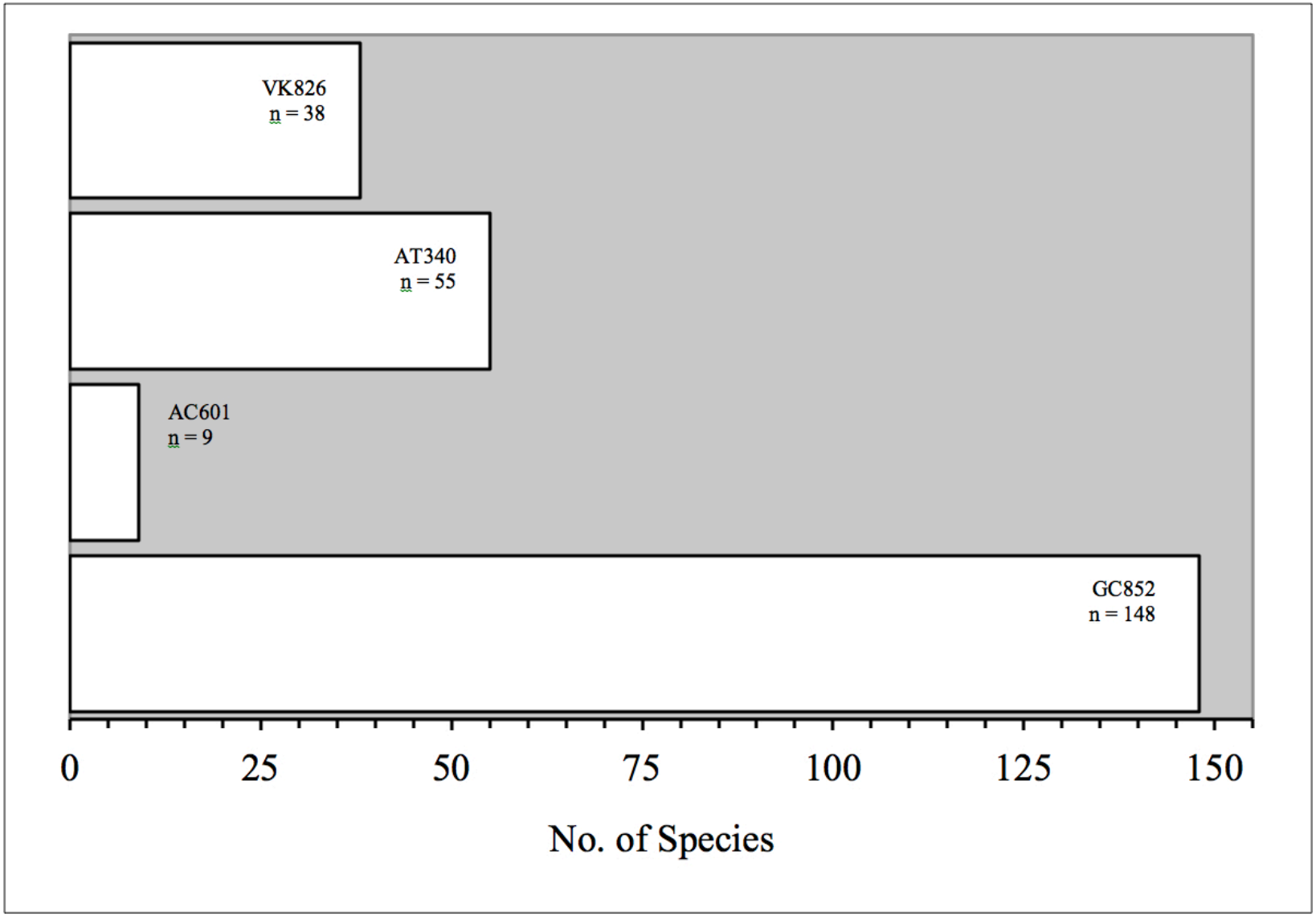


Figure 9.5. Species richness (by site) of invertebrates collected at three cold-seep sites (AC601, GC852, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007.

Although numbers of species and individuals were much less at AT340, species richness estimates were comparable proportionately (ratio of number of species to number of individuals) to that of GC852. Collections from the 40 stations sampled at AT340 yielded 185 individuals, consisting of at least 55 species, representing 6 of the 9 phyla collected in this study. No ctenophores, annelids, or echinoderms were collected in these samples. Crustaceans were the most speciose group with 22 species, followed by molluscs with 18 species and cnidarians with 9 species. Crustaceans were also the numerically dominant group at this site, constituting 41 percent of the total individuals collected. Amphipods, the most abundant crustacean taxon collected at this site, made up 47 percent of the crustacean catch.

Species richness (proportion of number of species to number of individuals) was much higher at VK826 than at the other sites. Collections from the 33 stations sampled here yielded 94 individuals, representing at least 38 species and 6 of the 9 phyla collected in this study. No annelids, chaetognaths, or echinoderms were collected in these samples. Crustaceans were the most speciose with 13 species, followed by molluscs with 11 species and cnidarians with 9 species. Again, crustaceans were the numerically dominant group among invertebrates sampled, and they accounted for 38 percent of the total individuals collected at this site. Cnidarians contributed 31 percent of the individuals and molluscs 20 percent to the total specimens caught at this site.

9.3.2 Species composition and relative abundance among zones

Invertebrates were collected in 14 of 58 stations where surface gear was deployed. No invertebrates were collected in the six surface tows made at AC601. Overall, 113 individuals representing 6 phyla, at least 18 families, and 26 species were collected in surface tows (table 9.2). Phylum Cnidaria was the most speciose group with eight species, followed by crustaceans

with seven species (fig. 9.6). Crustacea was the numerically dominant taxon, with 44 individuals (39 percent); amphipods made up 64 percent of the crustacean catch. Surface sampling was most productive at AT340, where 78 percent of the total number of individuals and 85 percent of the total species were collected.

Table 9.2. Invertebrate species and number of individuals collected at the surface over two cold-seep sites (GC852 and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007.

[n, number of stations where invertebrate specimens were collected; –, no value]

Phylum	Class or Order	Family	Species	GC852 n= 2	AT340 n= 8	VK826 n=4	No. of Inds n=14
Porifera	Demospongiae		Demospongiae sp. larvae (3)	1	6	4	11
Cnidaria	Cubozoa	Alatinidae	*Alatina* sp.	–	1	–	1
		Tamoyidae	*Tamoya haplonema*	–	–	1	1
	Hydrozoa		Hydrozoa sp.	–	1	–	1
		Aequoridae	*Aequorea macrodactyla*	–	6	–	6
		Aglaopheniidae	*Aglaophenia* sp.	–	1	–	1
	Scyphozoa		Scyphozoa sp.	–	–	1	1
		Pelagiidae	*Pelagia noctiluca*	–	3	–	3
		Ulmaridae	*Aurelia aurita*	–	–	1	1
Mollusca	Cephalopoda	Octopodidae	*Macrotritopus defilippi*	–	1	–	1
		Tremoctopoteuthidae	*Tremoctopus violaceus*	–	5	–	5
	Gastropoda/ Heterobranchia	Cavoliniidae	*Cavolinia tridentata*	–	4	–	4
			Diacavolinia cf. *elegans*	5	9	–	14
Arthropoda/ Crustacea	Amphipoda	Platyscelidae	*Platyscelidae* sp.	–	28	–	28
		Pronoidae	*Parapronoe* sp.2	–	1	–	1
	Copepoda	Megacalanidae	*Bathycalanus princeps*	–	1	–	1
	Decapoda/Brachyura	Calappidae	*Calappa* cf. *tortugae* megalopa	–	–	1	1
		Portunidae	*Portunus sayi*	–	1	10	11
	Decapoda/Caridea	Oplophoridae	*Oplophorus gracilirostris*	–	1	–	1
	Euphausiacea	Euphausiidae	*Thysanopoda tricuspida*	–	1	–	1
Chaetognatha	Chaetognatha	Chaetognatha	*Chaetognatha* spp. (3)	–	5	–	5
Chordata/ Tunicata	Salpida	Salpidae	*Salpa cylindrica*	–	14	–	14
Total				**6**	**89**	**18**	**113**

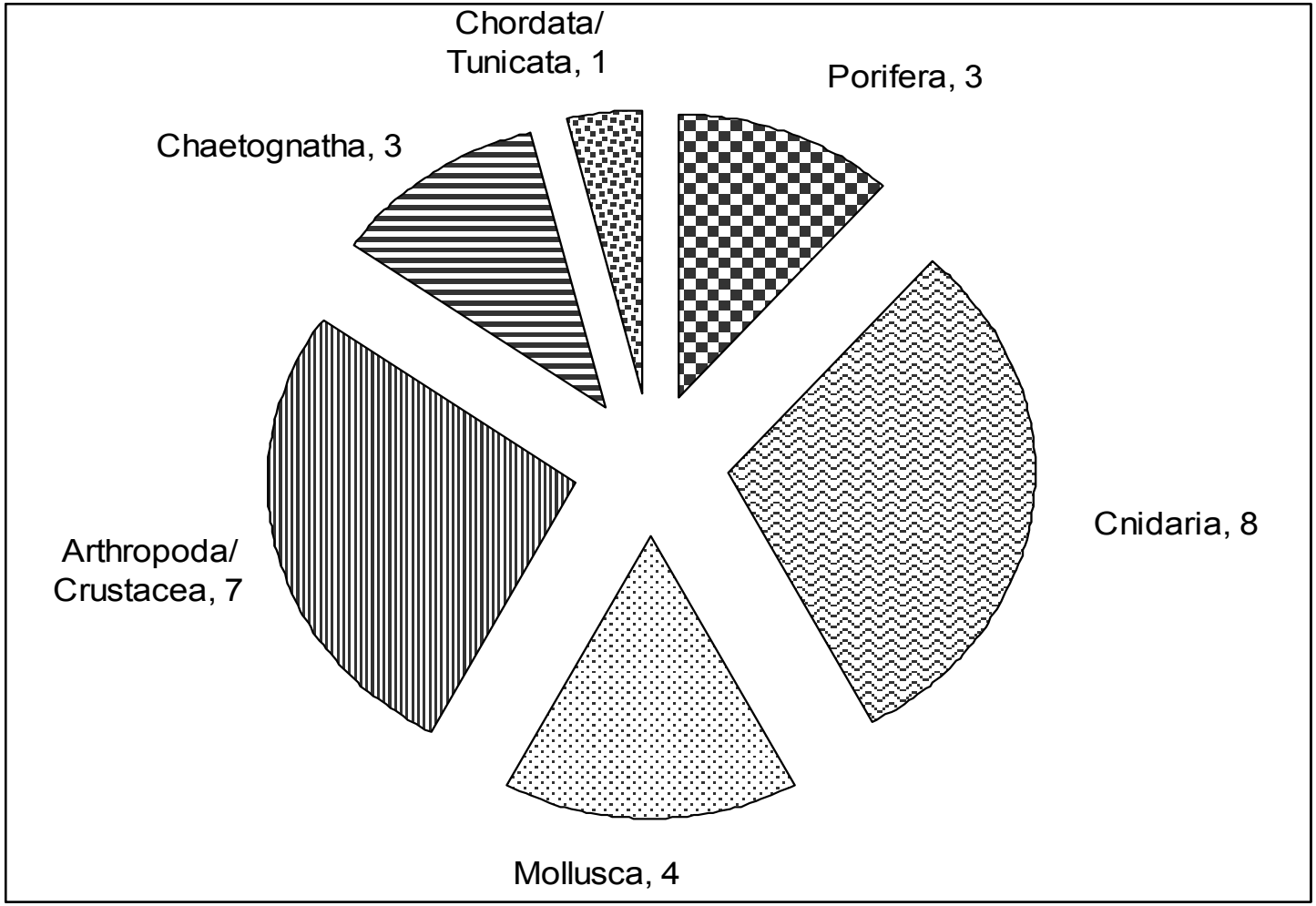


Figure 9.6. Species richness of all major invertebrate taxa collected using surface gear over three cold-seep sites (AC601, GC852, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007.

Benthic invertebrates were collected by a variety of gear. Four otter trawl samples were collected at GC852; otherwise, epibenthic mega-invertebrates were taken from box core samples (see Chapter 3 for composition of macro-invertebrate fauna collected with box cores) or from midwater Tucker trawls that hit the bottom. Overall, 160 epibenthic mega-invertebrates were collected at 11 stations. These represented 6 phyla and at least 35 families and 57 species (table 9.3). Crustacea was the most speciose taxon with 31 species, followed by echinoderms with 16 species (fig. 9.7). Crustaceans were also the numerically dominant taxon, constituting over half (58 percent) of the total individuals collected with benthic gear. Among the crustaceans, caridean shrimps were the most abundant group, making up 35 percent of the crustaceans sampled. Benthic sampling was most productive at GC852, where 91 percent of the total individuals and 86 percent of the total species were collected.

Table 9.3. Invertebrate species and number of individuals collected on the bottom at two cold-seep sites (GC852 and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007.

[n, number of stations where invertebrate specimens were collected; – , no value]

Phylum	Class or Order	Family	Species	GC852 n=5	AT340 n=1	VK826 n=5	No. of Inds n=11
Porifera	Demospongiae		Demospongiae sp.	2	–	–	2
Cnidaria	Pennatulacea	Umbellulidae	*Umbellula* sp.	13	–	–	13
	Anthozoa	cf. Hormathiidae	cf. Hormathiidae	–	1	–	1
Mollusca	Bivalvia	Mytilidae	*Bathymodiolus brooksii*	2	–	–	2
	Cephalopoda	Bolitaenidae	*Japetella diaphana*	1	–	–	1
		Lycoteuthidae	*Selenoteuthis scintillans*	1	–	–	1
		Octopodidae	*Macrotritopus defilippi*	–	–	1	1
	Gastropoda	Cavoliniidae	cf. *Cavolinia tridentata*	7	–	–	7
		Turridae	*Leucosyrinx verrilli*	1	–	–	1
Arthropoda/Crustacea	Amphipoda	Phronimidae	*Phronima sedentaria*	1	–	–	1
	Decapoda/Anomura	Chirostylidae	*Eumunida picta*	–	–	3	3
		Galatheidae	*Munida microphthalma*	4	–	–	4
			Munida sanctipauli	–	–	3	3
			Munidopsis cf. *bermudezi* molt	1	–	–	1
			*Munidopsis n.*sp.	–	–	1	1
			Munidopsis penescabra	–	–	2	2
			Munidopsis similis	1	–	–	1
		Parapaguridae	*Parapagurus* sp.	8	–	–	8
	Decapoda/Astacidea	Nephropidae	*Nephropsis aculeata*	–	–	2	2
			Nephropsis agassizii	1	–	–	1
	Decapoda/Brachyura	Majidae	Majidae sp.	4	–	–	4
	Decapoda/Caridea	Glyphocrangonidae	*Glyphocrangon aculeata*	3	–	–	3
			Glyphocrangon longirostris	1	–	–	1
			Glyphocrangon cf. *nobilis*	1	–	–	1
		Nematocarcinidae	*Nematocarcinus ensifer*	1	–	–	1

Table 9.3. Invertebrate species and number of individuals collected on the bottom at two cold-seep sites (GC852 and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. —Continued

[n, number of stations where invertebrate specimens were collected; – , no value]

Phylum	Class or Order	Family	Species	GC852 n=5	AT340 n=1	VK826 n=5	No. of Inds n=11
			Nematocarcinus rotundus	14	–	–	14
		Oplophoridae	*Acanthephyra eximia*	4	–	–	4
			Acanthephyra purpurea	1	–	–	1
		Pandalidae	*Heterocarpus oryx*	6	–	–	6
			Plesionika acanthonotus	–	–	1	1
			Stylopandalus richardi	1	–	–	1
	Decapoda/ Dendrobranchiata	Aristeidae	*Hemipenaeus carpenteri*	3	–	–	3
			Hepomadus tener	2	–	–	2
		Benthesicymidae	*Benthesicymus bartletti*	9	–	–	9
			Gennadas valens	4	–	–	4
		Sergestidae	*Sergia mollis*	1	–	–	1
			Sergia splendens	1	–	–	1
			Sergia wolffi	1	–	–	1
	Decapoda/Palinura	Polychelidae	*Polycheles sculptus*	6	–	–	6
	Stomatopoda		Stomatopoda sp. larva	2	–	–	2
Chaetognatha	Chaetognatha		Chaetognatha sp.	1	–	–	1
Echinodermata	Asteroidea	Astropectinidae	*Dytaster insignis*	1	–	–	1
			Plutonaster intermedius	1	–	–	1
		Goniasteridae	*Nymphaster arenatus*	1	–	–	1
	Holothuroidea	Molpadiidae	*Molpadia blakei*	7	–	–	7
		Myriotrochidae	*Myriotrochus* cf. *rinkii*	1	–	–	1
			Myriotrochus n. sp.	1	–	–	1
		Psychropotidae	*Psychropotes depressa*	2	–	–	2
		Synallactidae	*Molpadiodemas involutus*	7	–	–	7
			Paroriza sp.	3	–	–	3

Table 9.3. Invertebrate species and number of individuals collected on the bottom at two cold-seep sites (GC852 and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007.—Continued

[n, number of stations where invertebrate specimens were collected; – , no value]

Phylum	Class or Order	Family	Species	GC852 n=5	AT340 n=1	VK826 n=5	No. of Inds n=11
			Zygothuria lactea	1	–	–	1
		Synaptidae	*Protankyra brychia*	1	–	–	1
			Rynkatorpa felderi	1	–	–	1
	Ophiurida	Ophiodermatidae	*Bathypectinura heros*	2	–	–	2
		Ophioleucidae	*Ophioleuce* sp.	1	–	–	1
		Ophiuridae	*Amphiophiura metabula*	6	–	–	6
			cf. *Ophiernus vallincola*	1	–	–	1
Total				**146**	**1**	**13**	**160**

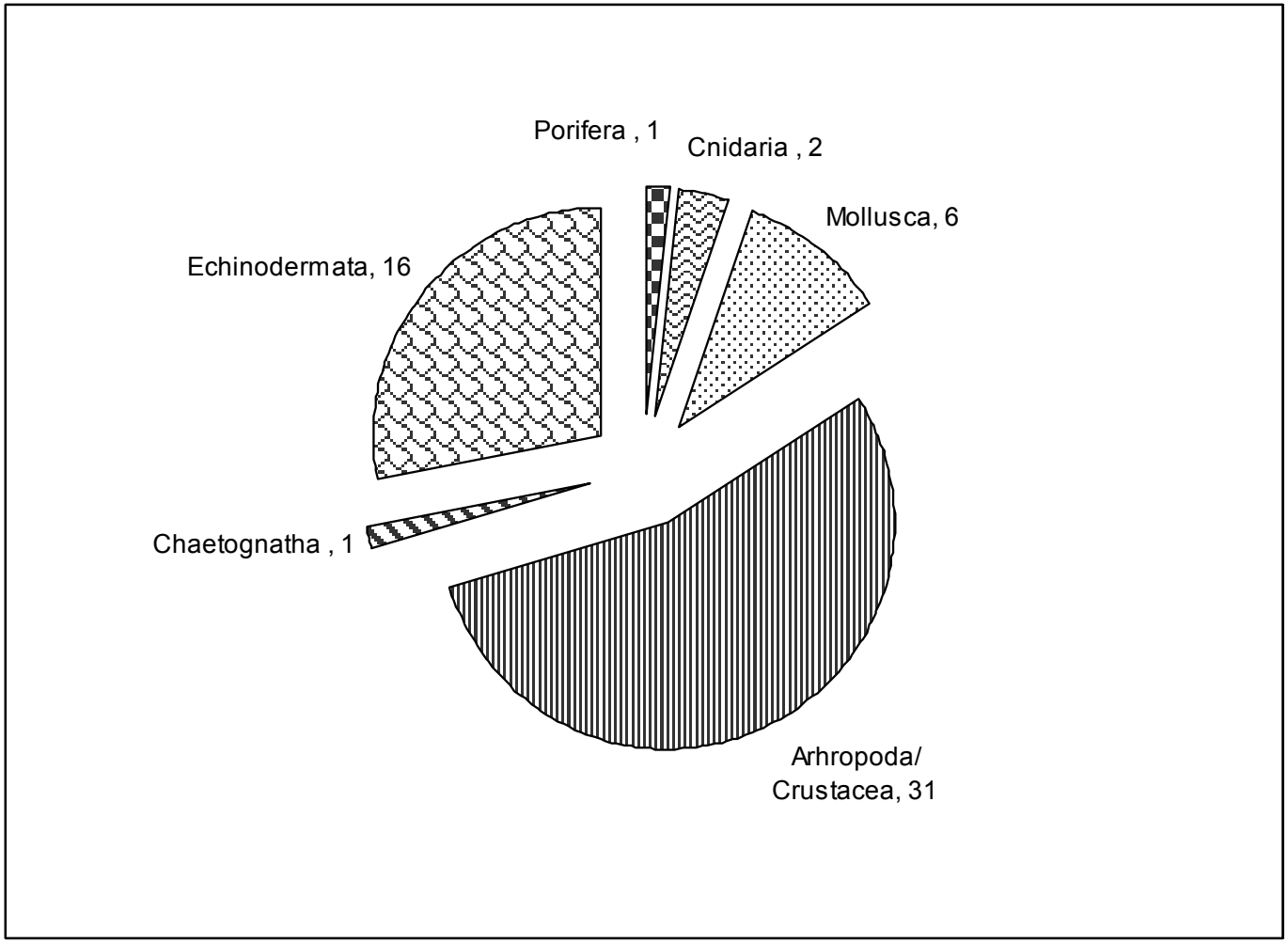


Figure 9.7. Species richness of major invertebrate taxa collected using bottom gear at two cold-seep sites (GC852 and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007.

Of the 159 midwater stations sampled, invertebrates were collected at 116 stations. Overall, 659 individuals representing 7 phyla, at least 62 families, and 132 species were taken in these samples (table 9.4). Crustaceans were again both the most taxonomically diverse and numerically dominant group, with 64 species and 332 individuals (fig. 9.8). Dendrobranchian shrimps were the most speciose and abundant group within the Crustacea, comprising 17 species and 123 individuals. Caridean shrimps were represented by 15 species and 92 individuals. Molluscs ranked second in both abundance (154 individuals) and diversity (38 species). Cephalopods were the dominant taxon within the molluscs, with 29 species and 100 individuals. No sponges or echinoderms were collected in the midwater samples. Midwater sampling was most productive at GC852, where 57 of 116 tows were conducted. Seventy-four percent of the total individuals and 83 percent of the total species were collected at this site.

Table 9.4. Invertebrate species and number of individuals collected in midwater over three cold-seep sites (GC852, AC601, and AT340) and at one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007.

[n, number of stations where invertebrate specimens were collected; – , no value]

Phylum	Class or Order	Family	Species	GC852 n= 57	AC601 n= 4	AT340 n= 31	VK826 n=24	No. of Inds n=116
Ctenophora	Cydippida	Pleurobrachiidae	Pleurobrachiidae sp.	–	–	–	3	3
Cnidaria	Cubozoa	Alatinidae	*Alatina* sp.	–	–	–	1	1
	Hydrozoa	Aequoreidae	*Aequorea* sp.	–	–	2	–	2
			Zygocanna vagans	4	–	–	4	8
		Eirenidae	*Tima* sp.	1	–	–	–	1
		Geryoniidae	*Geryonia probascidalis*	–	–	–	1	1
		Halicreatidae	*Halicreas minimum*	1	–	–	–	1
		Hippopodiidae	*Hippopodius hippopus*	5	–	–	–	5
			Vogtia cf. *serrata*	1	–	–	–	1
			Vogtia spinosa	1	–	–	–	1
		Pandeidae	*Campaniclava clionis* (on *Clio recurva*)	4	–	2	2	8
		Rhopalonematidae	*Colobonema sericeum*	1	–	–	–	1
	Scyphozoa	Atollidae	*Atolla* sp.	3	–	2	–	5
			Atolla vanhoeffeni	4	–	–	–	4
			Atolla wyvelli	5	–	–	–	5
		Nausithoidae	*Nausithoe* cf. *aurea*	1	–	–		1
			Nausithoe punctata	–	–	–	1	1
			Nausithoe sp.	2	–	–	–	2
		Pelagiidae	*Pelagia noctiluca*	–	–	–	17	17
		Periphyllidae	*Periphylla periphylla*	11	–	2	–	13
		Ulmaridae	*Aurelia aurita*	–	–	1	–	1
Mollusca	Cephalopoda	Bolitaenidae	*Bolitaena pygmaea*	–	–	1	–	1
			Japetella diaphana	7	–	2	–	9
		Chtenopterygidae	*Chtenopteryx sepioloides*	1	–	–	–	1

Table 9.4. Invertebrate species and number of individuals collected in midwater over three cold-seep sites (GC852, AC601, and AT340) and at one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. —Continued

[n, number of stations where invertebrate specimens were collected; – , no value]

Phylum	Class or Order	Family	Species	GC852 n= 57	AC601 n= 4	AT340 n= 31	VK826 n=24	No. of Inds n=116
		Cranchiidae	*Bathothauma lyromma*	–	1	–	–	1
			Cranchia scabra	1	–	–	–	1
			Helicocranchia pfefferi	2	–	–	–	2
			Liguriella podophthalma	1	–	–	–	1
			Sandalops melancholius	–	–	1	–	1
		Enoploteuthidae	*Abralia redfieldi*	1	–	2	1	4
			Abraliopsis atlantica	1	–	2	–	3
			Abraliopsis sp.	1	–	–	–	1
			Ancistrocheirus lesueurii	3	–	–	–	3
			Enoploteuthis anapsi	1	–	–	–	1
			Enoploteuthis sp.	–	–	–	1	1
			Selenoteuthis scintillans	1	–	2	–	3
		Histioteuthidae	*Histioteuthidae* sp.	–	–	1	–	1
			Stigmatateuthis arcturi	17	1	–	–	18
		Lycoteuthidae	*Lycoteuthis springeri*	1	–	–	–	1
		Neoteuthidae	*Neoteuthis thielei*	1	–	–	–	1
		Octopodidae	*Macrotritopus defilippi*	1	–	–	–	1
		Octopoteuthidae	*Octopoteuthis* cf. *megaptera*	1	–	–	1	2
		Ommastrephidae	*Ommastrephes bartramii*	–	–	–	1	1
		Onychoteuthidae	*Onykia carriboea*	1	–	1	–	2
		Pyroteuthidae	cf. *Pterygioteuthis giardi*	7	–	12	7	26
			Pyroteuthidae sp.	–	–	1	–	1
			Pyroteuthis magaritifera	–	1	2	1	4
		Sepiolidae	*Heteroteuthis dispar*	3	–	–	1	4
		Tremoctopodidae	*Tremoctopus violaceus*	–	–	3	–	3

Table 9.4. Invertebrate species and number of individuals collected in midwater over three cold-seep sites (GC852, AC601, and AT340) and at one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. —Continued

[n, number of stations where invertebrate specimens were collected; – , no value]

Phylum	Class or Order	Family	Species	GC852 n= 57	AC601 n= 4	AT340 n= 31	VK826 n=24	No. of Inds n=116
		Vampyroteuthidae	*Vampyroteuthis infernalis*	2	–	–	–	2
	Gastropoda/ Heterobranchia	Atlantidae	cf. *Atlanta sp.*	4	–	–	–	4
		Carinariidae	*Carinaria lamarcki*	1	–	1	–	2
		Cavoliniidae	*Cavolinia tridentata*	1	–	4	–	5
			Cavolinia uncinata	1	–	–	–	1
			Clio recurva	6	–	2	2	10
			Cliopsis cf. *krohnii*	3	–	–	1	4
			Diacria cf. *rampali*	5	–	–	–	5
		Peraclididae	cf. *Peracle sp.*	7	–	–	–	7
		Pterotracheidae	*Pterotrachea* cf. *coronata*	12	–	2	2	16
Annelida	Polycheata	Alciopidae	Alciopidae *sp.*	1	–	–	–	1
Arthropoda/ Crustacea	Amphipoda	Lanceolidae	*Lanceola sp.*	2	–	–	–	2
		Oxycephalidae	*Rhabdosoma whitei*	2	–	–	–	2
			Streetsia challengeri		–	–	1	1
		Phronimidae	*Phronima sedentaria*	9	–	6	–	15
		Phrosinidae	*Anchylomera blossevillei*	8	–	–	–	8
			Phrosina semilunata	2	–	–	–	2
		Platyscelidae	*Platyscelus sp.*	2	–	–	–	2
			Platysceloidea *sp.*	1	–	–	–	1
		Pronoidae	*Parapronoe sp.*	3	–	–	–	3
			Pronoidae *sp.*	1	–	–	–	1

Table 9.4. Invertebrate species and number of individuals collected in midwater over three cold-seep sites (GC852, AC601, and AT340) and at one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. —Continued

[n, number of stations where invertebrate specimens were collected; – , no value]

Phylum	Class or Order	Family	Species	GC852 n= 57	AC601 n= 4	AT340 n= 31	VK826 n=24	No. of Inds n=116
		Vibiliidae	*Vibilia* sp.	2	–	–	–	2
	Copepoda	Megacalanidae	*Bathycalanus princeps*	6	–	–	–	6
	Decapoda/Brachyura		Brachyura sp.	–	–	1	–	1
		Calappidae	*Calappa* cf. *tortugae* megalopa	–	–	–	–	1
		Portunidae	*Portunus* sp.	–	–	1	–	1
	Decapoda/Caridea	Nematocarcinidae	*Nematocarcinus rotundus*	6	–	–	–	6
			Nematocarcinus ensifer	2	–	–	–	2
		Oplophoridae	*Acanthephyra* cf. *brevirostris*	2	–	–	–	2
			Acanthephyra curtirostris	2	–	1	–	3
			Acanthephyra pelagica	1	–	–	–	1
			Acanthephyra purpurea	14	–	1	–	15
			Janicella spinicauda	2	–	1	–	3
			Meningodora vesca	2	–	–	–	2
			Notostomus elegans	3	–	–	–	3
			Oplophorus gracilirostris	2	–	3	3	8
			Systellaspis debilis	8	–	7	–	15
			Systellaspis sp.	4	–	–	–	4
		Palaemonidae	*Leander tenuicornis*	1	–	–	–	1
		Pandalidae	*Stylopandalus richardi*	17	–	–	–	17
		Pasiphaeidae	*Pasiphaea merriami*	7	–	–	3	10
	Decapoda/Dendrobranchiata	Benthesicymidae	*Gennadas valens*	42	2	8	–	52
			Gennadas elegans	5	1	1	–	7
			Gennadas sp.	1	–	–	–	1

Table 9.4. Invertebrate species and number of individuals collected in midwater over three cold-seep sites (GC852, AC601, and AT340) and at one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007). —Continued

[n, number of stations where invertebrate specimens were collected; – , no value]

Phylum	Class or Order	Family	Species	GC852 n= 57	AC601 n= 4	AT340 n= 31	VK826 n=24	No. of Inds n=116
		Penaeidae	*Funchalia villosa*	5	–	5	2	12
		Sergestidae	*Deoergestes curvatus*	4	1	–	–	5
			Deoergestes henseni	3	–	–	–	3
			Parasergestes armatus	4	–	1	–	5
			Sergestes atlanticus	1	–	–	–	1
			Sergestidae sp.	1	1	1	–	3
			Sergia grandis	1	–	–	–	1
			Sergia hansjacobi	1	–	–	3	4
			Sergia regalis	3	–	–	–	3
			Sergia robusta	1	–	1	–	2
			Sergia splendens	12	–	–	–	12
			Sergia talismani	2	–	–	–	2
			Sergia wolffi	8	–	1	–	9
		Solenoceridae	*Pleoticus robustus*	1	–	–	–	1
	Decapoda/Palinura	Polychelidae	*Polycheles sculptus* larva	1	–	–	–	1
		Scyllaridae	Scyllaridae sp. larva	1	–	1	–	2
	Euphausiacea	Euphausiidae	cf. *Thysanopoda* sp.	–	1	–	–	1
			Euphausia sp.	1	–	–	–	1
			Euphausiidae sp.	5	–	1	–	6
			Nematoscelis megalops	15	–	–	–	15
			Nematoscelis sp.	6	–	–	–	6
			Stylocheiron abbreviatum	1	–	–	–	1
			Stylocheiron elongatum	1	–	–	–	1
			Thysanoessa sp.	9	–	–	–	9
			Thysanopoda sp.	2	1	–	–	3

Table 9.4. Invertebrate species and number of individuals collected in midwater over three cold-seep sites (GC852, AC601, and AT340) and at one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007. —Continued

[n, number of stations where invertebrate specimens were collected; – , no value]

Phylum	Class or Order	Family	Species	GC852 n= 57	AC601 n= 4	AT340 n= 31	VK826 n=24	No. of Inds n=116
	Paracarida	Cystisomatidae	*Cystisoma* cf. *latipes*	3	–	–	–	3
		Eucopiidae	*Eucopia* cf. *grimaldii*	2	–	–	–	2
			Eucopia sp.		–	1	–	1
		Lophogastridae	*Gnathophausia zoea*	2	–	–	–	2
			Neognathophausia ingens	4	–	–	–	4
	Stomatopoda		Stomatopoda sp. larva	11	–	–	–	11
Chaetognatha	Chaetognatha		Chaetognatha sp.	37	–	6	–	43
Chordata/ Tunicata	Pyrosomatida	Pyrosomatidae	*Pyrosoma atlanticum*	27	1	3	2	33
	Salpida	Salpidae	*Iasis zonaria*	1	–	–	1	2
			Salpa cf. *fusiformis*	2	–	–	–	2
			Salpa cf. *maxima*	4	–	–	–	4
			Salpa cylindrica	2	–	–	–	2
			Salpa sp.	3	–	–	–	3
			Salpidae sp.	1	–	–	–	1
Total				**486**	**11**	**99**	**63**	**659**

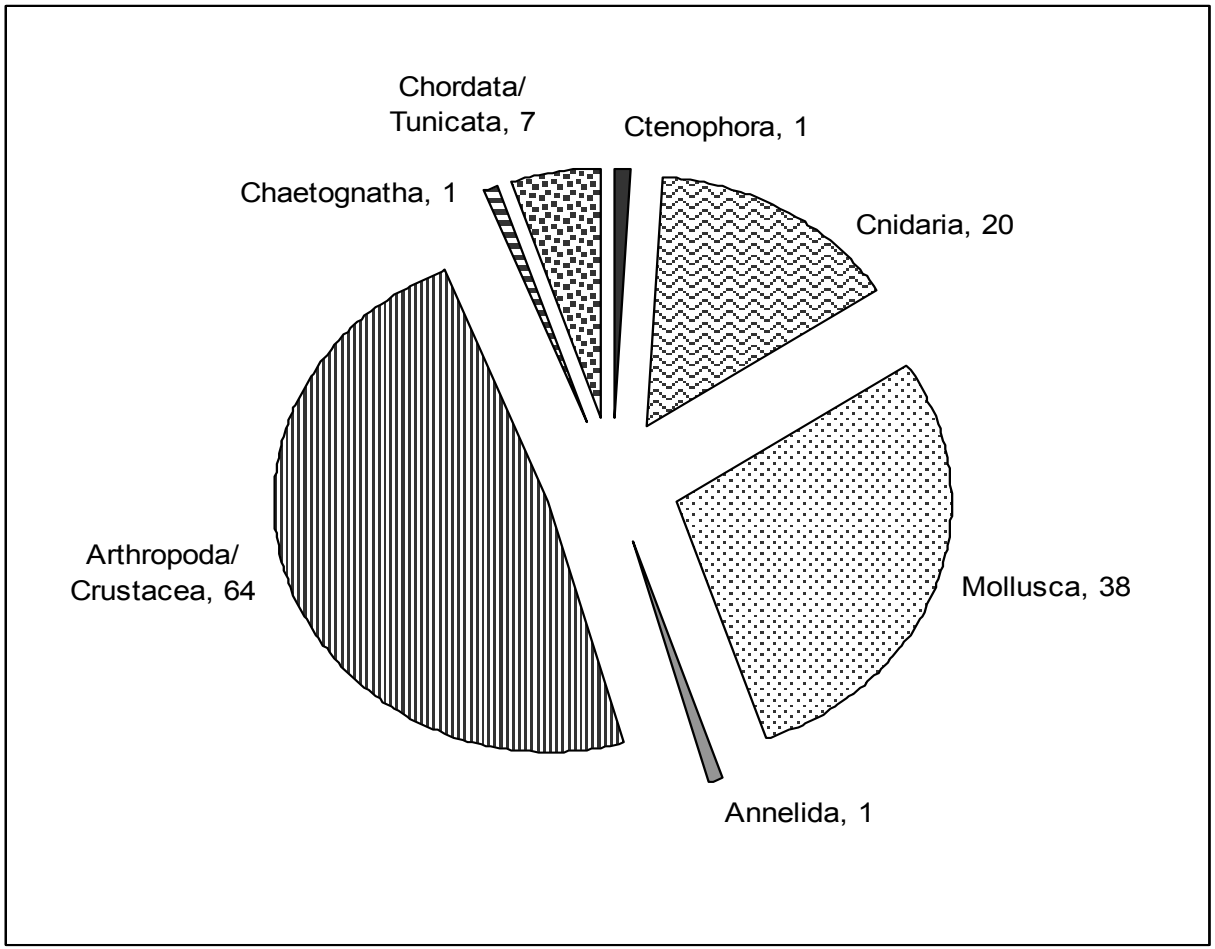


Figure 9.8. Species richness of major invertebrate taxa collected using midwater gear over three cold-seep sites (AC601, GC852, and AT340) and one *Lophelia pertusa* site (VK826) in the Gulf of Mexico, August 9-29, 2007.

9.4 Discussion

Throughout the Gulf of Mexico, over 90 cold-seep sites have been discovered; of these sites, at least 84 support seep-associated megafaunal communities (Fisher and others, 2007). Seep sites are described as oases because biomass and diversity of organisms associated with the seep are much greater than that of the surrounding deep-sea habitats (Carney, 1994). The characteristic chemoautotrophic invertebrates that inhabit seep sites, mostly epifaunal mussels and tubeworms, derive their nutrition from symbiotic relationships with sulfide- or methane-oxidizing bacteria (MacAvoy and others, 2002). The level of trophic interaction between the resident seep chemosynthetic organisms and those species found outside the direct influence of the cold seep has been the topic of recent investigations (MacAvoy and others, 2008a; b). Non-resident organisms that may benefit either directly or indirectly from the chemo-based food web at the seep site are considered to be background species more typical of the Gulf deep-sea community. Although the mobile benthic fauna occurring at several seep sites has been studied (MacAvoy and others, 2008b), it has not been determined whether the production from the seeps translates to assemblages occurring in the water column near the seeps. This study represents the first attempt to investigate the composition of the midwater invertebrate assemblage occurring over chemosynthetic environments in the Gulf of Mexico and to assess whether these assemblages are influenced by the enhanced productivity of nearby seep sites.

To address the question of whether the midwater invertebrate faunal assemblage benefits directly or indirectly from chemosynthetic based production, we must first know the faunal composition, relative abundances, and relative distributions of invertebrate species collected over the cold-seep sites. If production from cold seeps has an influence on the midwater invertebrate assemblage, one would expect an increase in species richness over seep sites, or an increase in

relative abundances of invertebrates over seep sites, and (or) the presence of unique species over seep sites (that is, species endemic to these areas). Based on data presented here, the midwater invertebrate assemblage collected over several seep sites does not appear to be influenced by the chemosynthetic production from these cold seeps. For example, shrimp, the most speciose taxon collected, are not unusually diverse when compared to results from other studies conducted at nonseep sites in the eastern Gulf region (Hopkins and others, 1989; 1994). Our study over the seep sites and other previous studies from the eastern Gulf indicate that the species collected represent a subset of the total diversity of species reported in the eastern Gulf of Mexico (Hopkins and others, 1989). Additionally, most species collected in this study are also reported from other nonseep habitats. For example, most decapod species collected over the seeps also appear in tallies of the bathypelagic decapod assemblage of the eastern Gulf of Mexico (Burghart and others, 2007) and are included among decapods found on continental slope and abyssal plain habitats in the Gulf (Wicksten and Packard, 2005). Based on previously published geographic distributions, the majority of species represented in our samples have widespread distributions. All but one species (*Sergia hansjacobi*) are listed in the most current checklist of Gulf of Mexico decapods (Felder and others, 2009). Thus, the species composition of the midwater assemblage collected in this study is representative of species previously reported at these depths from this region of the Gulf of Mexico.

Populations of species we collected are not likely enhanced by the chemosynthetic environment at the seeps, since the relative abundance of the midwater invertebrate faunal assemblage over these seep sites was not dramatically increased compared to that of nonseep sites. If increased productivity at the cold-seep environments was influencing the overlying water column, then high densities of organisms would be expected. Instead, the majority of

species were represented by only one or two individuals. Multiple individuals may not have been collected in a single sample due to temporal or spatial distributions of the organisms or possibly through gear avoidance. However, it is unlikely that observed patterns in abundances could be an artifact of sampling, as other mesopelagic collections made in the eastern Gulf of Mexico also yielded relatively low numbers of caridean shrimps (Hopkins and others, 1989) and cephalopods (Passarella and Hopkins, 1991). Low abundances of midwater invertebrates are characteristic of oligotrophic tropical-subtropical gyres (Hopkins and others, 1989; Passerella and Hopkins, 1991). Results presented here were consistent with those reported in these other studies where sampling, both in number of tows and extent of coverage, was more intensive. Possibly, similar oligotrophic conditions are present in the north-central Gulf region, as was suggested for the eastern Gulf. Further sampling, particularly of productivity parameters, is needed to confirm or refute this hypothesis.

No unique species were collected over the cold-seep sites. This is not an unexpected result since many benthic species collected in association with cold-seep habitats are not endemic to these habitats. Rather, species taken at cold-seep sites are either considered to be opportunistic species found in the vicinity of the seeps but not restricted to these habitats (that is, vagrants; Martin and Haney, 2005) or are species not usually found associated with seeps but are incidentally captured from habitats adjacent to seep sites (Carney, 1994).

Although no significant increases in species richness were observed for invertebrate samples taken in the water column over seep sites, species richness was relatively high for some taxa, suggesting that a large proportion of the background species was collected. This finding is significant, given that these data were collected with only a modest amount of sampling effort confined to a small region within the larger Gulf of Mexico ecosystem. Additionally,

preliminary examination of the data illustrated that the species accumulation curve for all midwater invertebrate taxa collected in this study had reached an asymptote. This suggests that our sampling, although limited on both spatial and temporal scales, adequately represented the midwater invertebrate faunal assemblage from this region of the Gulf of Mexico. Our findings are comparable with previous studies, which covered a greater area and included more sampling effort (for example, Hopkins and others, 1989). Fifteen species of caridean shrimps were captured in 116 trawls in the present study compared to the findings of Hopkins and others (1989), where 22 species were captured in 338 trawls. Of the species collected, nine were common to both studies. Species richness and abundances reported here may be characteristic of the midwater faunal assemblage reported throughout the entire Gulf. Many species collected in the present study are reported in annotated lists discussed by other researchers. Several species in common between the present study and others are wide-ranging species distributed throughout the entire Gulf of Mexico (Felder and others, 2009).

Our sampling regime produced collections of limited spatial and temporal coverage, and the species reported here are only a subset of a larger, more speciose assemblage. Catch composition may be dependent on the time of year the samples are collected, depth of capture, time of capture, and (or) the type of gear used. If midwater organisms in this region have patchy spatial distributions, our sampling locations and times when samples were taken would not adequately target the full spectrum of organisms constituting the total faunal assemblage. Our collections share some similarities to those described by other researchers at similar location and depths, but more extensive sampling will be needed to determine the overall diversity, abundance, and distribution of the midwater invertebrate faunal assemblage occurring in this region across seasons, discrete depths, and time of day.

Sampling throughout the water column provides a more complete picture of the faunal assemblages occurring over and near to chemosynthetic communities. However, conclusions from this study remain speculative. Given the sampling regime for this investigation, several data gaps prohibit more definitive conclusions. For example, the midwater sampling conducted over chemosynthetic sites did not necessarily sample the portion of the water column that is directly influenced by chemosynthetic processes. Therefore, further study is needed to address the transfer of energy from chemosynthetic sources through the midwater food chain. Vertically migrating organisms are a likely conduit. Isotope analysis could indicate utilization of chemo-based carbon by some midwater species. Samples were taken for some midwater invertebrate species, and these data are in the process of being analyzed (A.W.J. Demopoulos, unpub. data, 2011). But more precise and directed stratified sampling in conjunction with simultaneous collection of physical data from each site is needed to better assess the relationship between chemosynthetic and the overlying pelagic communities. Results from this study provide baseline and background information for the sites sampled. This analysis sets the stage for future work and to a better understanding of a potential link between chemosynthetic and pelagic systems.

9.5 Acknowledgements

We thank the captain and crew of the R/V *Cape Hatteras*. The science crew from the USGS Chemo III cruise is acknowledged for help with sample collecting, processing, and data requests. C. Ahearn, L. Cole, A. Collins, C. Mah, D. Pawson, E. Strong, D. Tippett, and M. Vecchione helped with species identifications. Figure 1 was used with permission by S.W. Ross. T. Munroe read earlier drafts and provided helpful suggestions.

10. ANALYSIS OF FUNGAL DIVERSITY IN SEDIMENT FROM A METHANE SEEP, GULF OF MEXICO

By Andrew D. Thaler

Duke University Marine Lab, Beaufort, NC

10.1 Introduction and Rationale

The extent of fungal diversity in marine ecosystems is poorly characterized, especially in the deep sea. A conservative estimate of global fungal biodiversity places the minimum total number of species at approximately 1.5 million (Hawksworth, 1991). This estimate was determined by calculating the number of fungal species that occur in close association with plants and does not account for fungi that occur in ecosystems whose primary production is not photosynthetically derived. Fungi in deep-sea chemosynthetic ecosystems may represent a reservoir of previously undiscovered biodiversity.

Marine-occurring fungi are found in mangroves, driftwood, shallow sediments, algae, coral (Hyde and others, 1998), and in deep-sea sediments (Damare and others, 2006). Fungi facilitate the decomposition of wood as well as lignin, chitin, and keratin (Reisert and Fuller, 1962; Kohlmeyer, 1972) and form marine lichens through symbiotic relationships with algae (Grube and Blaha, 2005). Until recently, only five species of fungi were reported from the deep sea (Kohlmeyer and Kohlmeyer, 1979; Hawksworth, 2001). Fungi have been found at hydrothermal vent (Gadanho and Sampaio, 2005) and methane seep ecosystems (Takishita and others, 2006; 2007). Pathogenic black yeasts were discovered infecting *Bathymodiolus* mussels

at a Fiji Basin hydrothermal vent field (Van Dover and others, 2007). With few exceptions the ecologic contribution of these fungi to deep-sea chemosynthetic ecosystems remains unknown.

The deep sea represents Earth's largest ecosystem. It is characterized by a pattern of high biodiversity and relatively low biomass, which reverses in areas of chemosynthetic activity – such as hydrothermal vents, whale falls, and methane seeps – where large aggregations of specially adapted organisms occur (Van Dover, 2000). Methane seeps are formed when hydrocarbons are released from gas hydrate reservoirs, providing an energy source for chemosynthetic communities (Van Dover and others, 2003). Many species of marine-occurring fungi can actively degrade hydrocarbons, and some common marine fungi thrive in hydrocarbon-rich environments (Ahearn and Meyers, 1976). *Cryptococcus curvatus*, a basidiomycete yeast, is the dominant eukaryotic microbe at methane seeps in Kurishima Knoll (Sea of Japan) (Takishita and others, 2006).

The Chemo III cruise provided an ideal opportunity to investigate fungal biodiversity at a seep site in the Gulf of Mexico and compare samples against the findings in Kurishima Knoll. In addition to examining the total biodiversity, chemical features of the seeps could also be measured, allowing for a finer scale assessment of fungi distribution at this methane seep ecosystem.

10.2 Methods

10.2.1 Recovery

A single box core (Ocean Institute Mark III [50x50 cm]) was collected from Alaminos Canyon lease block 601 during the Chemo III research cruise onboard the R/V *Cape Hatteras*. Twenty-two sub-cores were taken from that box core using sterile 40-mL serological pipettes (tip

removed; 1-cm diameter, 33-cm length). Sub-cores were sealed with parafilm and frozen (-5 ˚C topped with dry ice) for storage and transport. Reduction-oxidation potential sediment gradient was recorded by Amanda W.J. Demopoulos, USGS.

10.2.2 Extraction and Amplification

Sub-cores were sectioned in 1-cm increments by sediment depth (to 10 cm), and environmental DNA was extracted using a MoBio PowerSoil soil extraction kit. The first 900 base pairs of the large-subunit rDNA (LSU), an information-rich region that contains three divergent domains and is a standard barcode for the Fungal Tree of Life project, were amplified using primers (LR0R and LR5) that show an affinity for fungi. The resulting PCR product was cloned using Invitrogen TopoTA topo cloning kit and sequenced in the forward direction.

Primer	Region	Direction	Sequence
LROR	LSU	Forward	ACCCGCTGAACTTAAGC
LR3	LSU	Reverse	CCGTGTTTCAAGACGGG
LR5	LSU	Reverse	TCCTGAGGGAAACTTCG
LR7	LSU	Reverse	TACTACCACCAAGATCT
ITS1	ITS	Forward	TCCGTAGGTGAACCTGCGG
ITS4	ITS	Reverse	TCCTCCGCTTATTGATATGC

Figure 10.1. Primers used for amplification of fungal LSU and ITS (internal transcribed spacer) regions.

10.2.3 Analysis

Recovered sequences were compared against the National Center for Biotechnology Information (NCBI) database and aligned using ClustalW (Thompson and others, 1994). The program PAUP (phylogenetic analyis using parsimony) was used to generate maximum parsimony, maximum likelihood, and neighbor joining trees that were assembled and compared for consistency.

10.3 Results

Thirty-nine fungal sequences were recovered from the Alaminos Canyon methane seep (table 10.1). While many sequences aligned with known terrestrial fungal phylotypes, 25 belonged to an unknown Ascomycete clade with no known terrestrial members. These samples closely corrrelated with the reduction/oxidation boundary in the sediment (fig. 10.2). Phylotype accumulation curves indicate that total fungal diversity has not yet been completely sampled (fig. 10.3).

Table 10.1. Total sequences recovered, fungal sequences recovered, and sequences recovered from unidentified ascomycete clade by 2-cm sediment depth intervals from the Alaminos Canyon methane seep (2,300 m).

[Sediment becomes reduced at ~4 cm depth]

Depth (cm)	Total sequences recovered	Total fungal sequences recovered	Sequences recovered from unidentified ascomycete clade
0 – 2	27	11	4
2 – 4	27	23	18
4 – 6	7	5	3

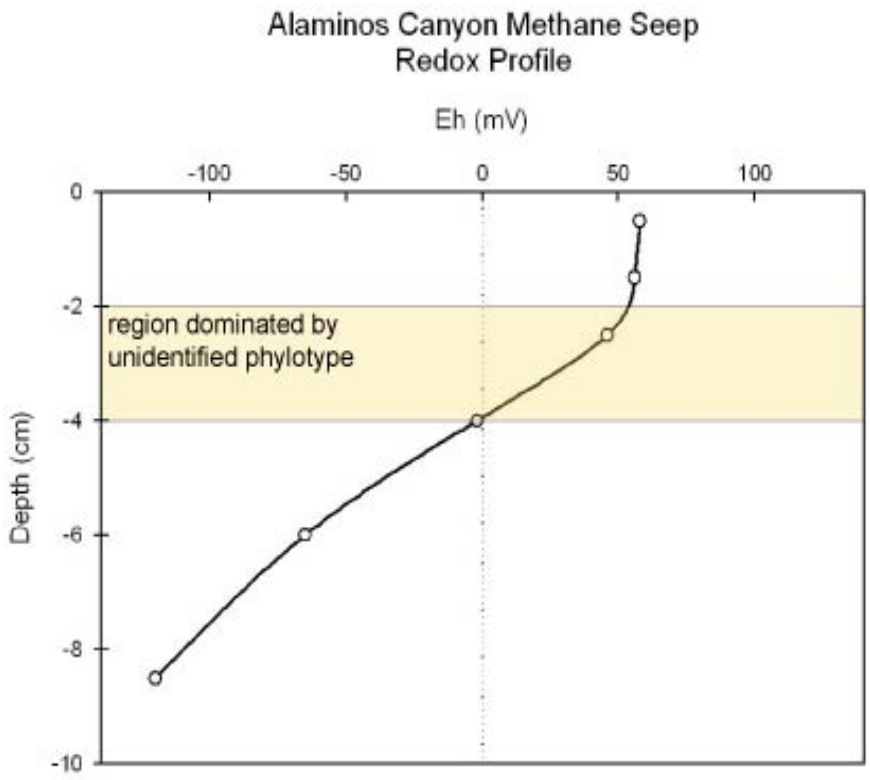


Figure 10.2. Redox profile for the Alaminos Canyon methane seep boxcore, mV, millivolts; cm, centimeters. Courtesy of A.W.J. Demopoulos, USGS. Shaded area is the region dominated by an unidentified ascomycete clade.

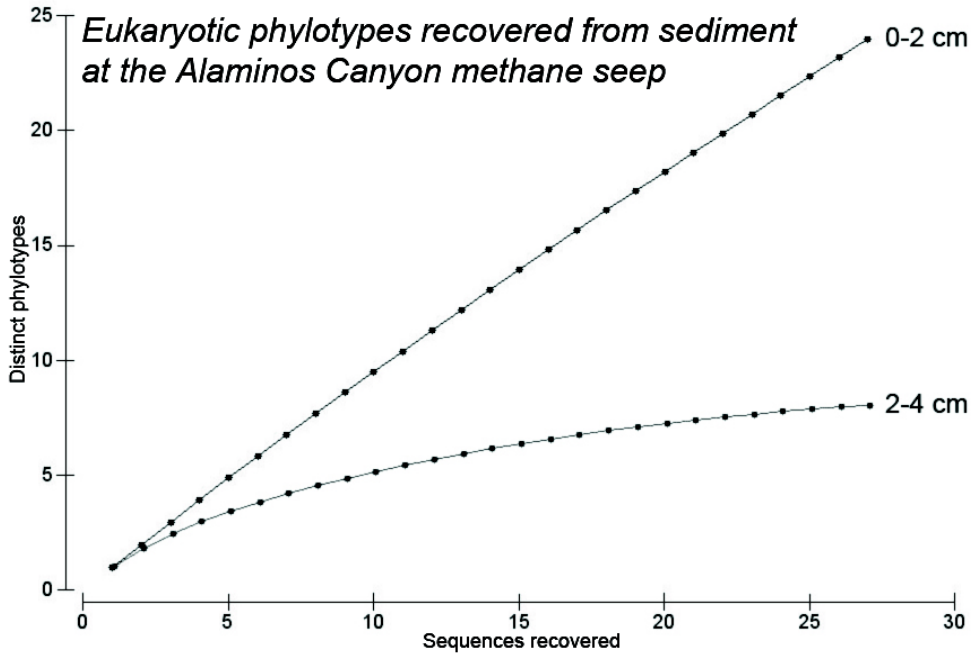


Figure 10.3. Eukaryotic phylotypes recovered from Alaminos Canyon methane seep, 0–2 cm and 2–4 cm. Greater depths are not shown due to few sequences recovered below the redox zone.

10.4 Discussion

The unidentified Alaminos Canyon ascomycete clade could represent a previously undescribed group of fungi that may be endemic to the deep sea. Previous studies by Takishita and others (2006) noted that the diversity of microbial organisms decreases steeply below the sediment surface and becomes dominated by a single fungal species, a trend similar to what we observe at Alaminos Canyon. Takashita and others did not connect sediment depth to the geochemical properties of the methane seep environment.

The Alaminos Canyon ascomycete clade is located immediately above the boundary where the redox potential transitions from oxidized to reduced. The Alaminos Canyon ascomycete clade is present in low numbers both above and below this region. The site specificity of this group indicates that it is not the product of contamination from terrestrial

spores and it may have an ecologic role in the seep environment. Cloning from PCR-amplified fragments of environmental samples does provide a good survey of diversity, but it cannot be used to estimate biomass. Since no recovered sequence was 100 percent identical to any other, we can assume that each sequence recovered represents an individual and thus that the occurrence of the Alaminos Canyon ascomycete clade increased 450 percent from the top 2 cm of sediment to 2 – 4 cm depth.

Other fungal species recovered were closely allied to terrestrial species. These could be opportunistic fungi that have taken hold in the deep sea, or spore-derived contaminants that, while present, do not contribute to the ecosystem.

10.5 Acknowledgments

Partial funding for this project was provided by NSF Grant DEB-0732984, Duke University, and the Marine Conservation Molecular Facility at the Duke University Marine Lab. Ship time was funded by the U.S. Geological Survey Chemo III Cruise. The author would like to thank Steve W. Ross of UNC Wilmington for graciously providing the opportunity to participate in this cruise, Amanda W.J. Demopoulos (USGS) for providing the redox profile for the Alaminos Canyon methane seep, and the crew of the R/V *Cape Hatteras* for their hard work and patience. The author would also like to thank Jason Jackson and Gregory Bonito for their guidance and expertise in working with fungal genetics and for their encouragement and willingness to help.

References Cited

Adams, N.J., Moloney, C., and Navarro, R., 2004, Estimated food consumption by penguins at the Prince Edward Islands: Antarctic Science, v. 5, p. 245-252.

Aharon, P., 1994, Geology and biology of modern and ancient submarine hydrocarbon seeps and vents: An introduction: Geo-Marine Letters, v. 14, p. 69-73.

Aharon, P., and Fu, B., 2000, Microbial sulfate reduction rates and sulfur and oxygen isotope fractionation at oil and gas seeps in deepwater Gulf of Mexico: Geochimica et Cosmochimica Acta, v. 64, p. 233-246.

Ahearn, D.G., and Meyers, S.P., 1976, Fungal degradation of oil in the marine environment *in* Jones, E.B.G., ed., Recent advances in aquatic mycology: New York, Halsted Press, p. 125-133.

Angel, M.V., and Pugh, P.R., 2000, Quantification of diel vertical migration by micronektonic taxa in the northeast Atlantic: Hydrobiologia, v. 440, p. 161-179.

Ashjian, C., Smith, S., Flagg, C., and Idrisi, N., 2002, Distribution, annual cycle, and vertical migration of acoustically derived biomass in the Arabian Sea during 1994-1995: Deep-Sea Research, v. 49, p. 2377-2402.

Baba, K., Macpherson, E., Poore, G.C.B., Ahyong, S.T., Bermudez, A., Cabezas, P., Lin, C.W., Nizinski, M., Rodrigues, C., and Schnabel, K., 2008, Catalogue of squat lobsters of the world (Crustacea: Decapoda: Anomura - families Chirostylidae, Galatheidae and Kiwaidae): Zootaxa, v. 1905, p. 1-220.

Backus, R.H., Craddock, J.E., Haedrich, R.L., and Robison, B.H., 1977, Atlantic mesopelagic

Zoogeography, *in* Nafpaktitis, B.G., Backus, R.H., Craddock, J.E., Haedrich, R.L., Robison, B.H., and Karnella, C., Fishes of the western North Atlantic Ocean, v. 7: New Haven, Conn., Memoir Sears Foundation for Marine Research, Yale University, p. 266-287.

Backus, R.H., Craddock, J.E., Haedrich, R.L., and Shores, D.L., 1969, Mesopelagic fishes and thermal fronts in the western Sargasso Sea: Marine Biology, v. 3, p. 87-106.

Backus, R.H., Craddock, J.E., Haedrich, R.L., and Shores, D.L., 1970, The distribution of mesopelagic fishes in the equatorial and Western North Atlantic Ocean: Journal of Marine Research, v. 28, p. 179-201.

Badcock, J., and Merrett, N.R., 1976, Midwater fishes in the eastern North Atlantic-I: Vertical distribution and associated biology in 30ºN, 23ºW, with developmental notes on certain myctophids: Progress in Oceanography, v. 7, p. 3-58.

Badcock, J., and Merrett, N.R., 1977, On the distribution of midwater fishes in the eastern North Atlantic, *in* Andersen, N.R., and Zahuranec, B.J., eds., Oceanic sound scattering prediction: New York, Plenum Press, p. 249-282.

Baird, R.C., and Wilson, D.F., 1977, Sound scattering and oceanic midwater fishes, *in* Andersen, N.R., and Zahuranec, B.J., eds., Oceanic sound scattering prediction: New York, Plenum Press, p. 549-563.

Bangma, J.L., and Haedrich, R.L., 2008, Distinctiveness of the mesopelagic fish fauna in the Gulf of Mexico: Deep-Sea Research II, v. 55, p. 2594-2596.

Beamish, R.J., Leask, K.D., Ivanov, O.A., Balanov, A.A., Orlov, A.M., and Sinclair, B., 1999, The ecology, distribution, and abundance of midwater fishes of the Subartic Pacific gyres: Progress in Oceanography, v. 43, p. 399-442.

Behringer, D.C., and Butler, M.J., IV, 2006, Stable isotope analysis of production and trophic relationships in a tropical marine hard-bottom community: Oecologia, v. 148, p. 334-341.

Bergquist, D.C., Andras, J.P., McNelis, T., Howlett, S., van Horn, M.J., and Fisher, C.R., 2003a, Succession in Gulf of Mexico cold seep communities: The importance of spatial variability: Marine Ecology, v. 24, p. 31–44.

Bergquist, D.C., Ward, T., Cordes, E.E., McNelis, T., Howlett, S., Kosoff, R., Hourdez, S., Carney, R., and Fisher, C.R., 2003b, Community structure of vestimentiferan-generated habitat islands from Gulf of Mexico cold seeps: Journal of Experimental Marine Biology and Ecology, v. 289, no. 2, p. 197-222.

Berntson, E.A., France, S.C., and Mullineaux, L.S., 1999, Phylogenetic relationships within the class Anthozoa (Phylum Cnidaria) based on nuclear 18S rDNA sequences: Molecular Phylogenetics and Evolution, v. 13, p. 417-433.

Biggs, D.C., Jochens, A.E., Howard, M.K., DiMarco, S.F., Mullin, K.D., Leben, R.R., Muller-Karger, F.E., and Hu, C., 2005, Eddy forced variations in on- and off-margin summertime circulation along the 1000-m isobath of the northern Gulf of Mexico, 2000-2003, and links with sperm whale distributions along the middle slope, *in* Sturges, W., and Lugo-Fernandez, A., eds., Circulation in the Gulf of Mexico: Observations and models: American Geophysical Union, Geophysical Monograph Series, v. 161, p. 71-85.

Bosley, K.L., and Wainright, S.C., 1999, Effects of preservatives and acidification on the stable isotope ratios (^{15}N: ^{14}N, ^{13}C: ^{12}C) of two marine animals: Canadian Journal of Fisheries and Aquatic Sciences, v. 56, p. 2181-2185.

Bratbak, G., Thingstad, F., and Heldal, M., 1994, Viruses and the microbial loop: Microbial Ecology, v. 28, p. 209-221.

Bright, T.J., and Pequegnat, W.E., 1969, Deep sea hatchet fishes of the Gulf of Mexico: Quarterly Journal of the Florida Academy of Sciences, v. 32, p. 26-36.

Brinton, E., 1967, Vertical migration and avoidance capability of euphausiids in the California current: Limnology and Oceanography, v. 1, p. 451-483.

Brodeur, Richard, and Yamamura, Orio, 2005, Micronekton of North Pacific: PICES Working Group 14 Final Report, PICES report No. 30, v. 11.

Brooke, S., and Schroeder, W.W., 2007, State of deep coral ecosystems in the Gulf of Mexico region: Texas to the Florida straits, *in* Lumsden, S.E., Hourigan, T.F., Bruckner, A.W., and Dorr, G., eds., The state of deep coral ecosystems of the United States: Silver Spring, Md., NOAA Technical Memo CRCP-3, v. 365, p. 271-306.

Brooks, J.M., Fisher, C.R., Roberts, H.H., Bernard, B., MacDonald, I.R., Carney, R., Joye, S., Cordes, E.E., Wolff, G., and Goehring, E., 2008, Investigations of chemosynthetic communities on the lower continental slope of the Gulf of Mexico: U.S. Department of the Interior, Bureau of Ocean Energy Management, Regulation and Enforcement, Gulf of Mexico OCS Region, prepared under BOEMRE Contract 1435-01-05-CT-39187 (M05PC00018), p. 383 [Interim Report 2].

Brooks, J.M., Kennicutt, M.C., II, Fisher, C.R., Macko, S.A., Cole, K., Childress, J.J., Bidigare, R.R., and Vetter, R.D., 1987, Deep-sea hydrocarbon seep communities: Evidence for energy and nutritional carbon sources: Science, v. 238, no. 4830, p. 1138-1142.

Bryant, W.R., Bryant, J.R., Feeley, M.H., and Simmons, G.R., 1990, Physiographic and bathymetric characteristics of the continental slope, northwest Gulf of Mexico: Geo-Marine Letters, v. 10, p. 182-199.

Burghart, S.E., Hopkins, T.L., and Torres, J.J., 2007, The bathypelagic Decapoda, Lophogastrida, and Mysida of the eastern Gulf of Mexico: Marine Biology, v. 152, p. 315-327.

Butler, M., Bollens, S.M., Burkhalter, B., Madin, L.P., and Horgan, E., 2001, Mesopelagic fishes of the Arabian Sea: Distribution, abundance and diet of *Chauliodus pammelas, Chauliodus sloani, Stomias affinis,* and *Stomias nebulosus*: Deep-Sea Research II, v. 48, p. 1369-1383.

Cabezas, P., Macpherson, E., and Machordom, A., 2009, Morphological and molecular description of new species of squat lobster (Crustacea: Decapoda: Galatheidae) from the Solomon and Fiji Islands (South-West Pacific): Zoological Journal of the Linnean Society, v. 156, p. 465-493.

Cairns, S. D., 1979, The deep-water Scleractinia of the Caribbean Sea and adjacent waters: Studies of the Fauna of Curaçao, v. 57, no. 180, 341 p.

Cairns, S.D., and Bayer F.M., 2005, A review of the genus Primnoa (Octocorallia: Gorgonacea: Primnoidae), with description of two new species: Bulletin Marine Science, v. 77, no. 2, p. 225-256.

Cairns, S.D. and Bayer, F.M., 2009, Chapter 13: Octocorallia (Cnidaria) of the Gulf of Mexico, *in* Tunnel, J.W., Felder, D., and Earle, S., eds., Gulf of Mexico origin, water, and biota: Biodiversity: College Station, Tex., Texas A&M University Press, p. 321-331.

Cairns, S.D., Jaap, W.C., and Lang, J.C., 2009, Chapter 14: Scleractinia (Cnidaria) of the Gulf of Mexico, *in* Tunnel, J.W., Felder, D., and Earle, S., eds., Gulf of Mexico origin, water, and biota: Biodiversity: College Station, Tex., Texas A&M University Press, p. 333-347.

Carnes, M.R., Teague, W.J., and Jarosz, E., 2008, Low-frequency current variability observed at the shelfbreak in the northeastern Gulf of Mexico: November 2004-May 2005: Continental Shelf Research, v. 28, p. 399-423.

Carney, R.S., 1994, Consideration of the oasis analogy for chemosynthetic communities at Gulf of Mexico hydrocarbon vents: Geo-Marine Letters, v. 14, p. 149-159.

Champalbert, G.A., Kouamé, B., Pagano, M., and Marchal, E., 2008, Feeding behavior of adult *Vinciguerria nimbaria* (Phosichthyidae), in the tropical Atlantic (0°-4°N, 15°W): Marine Biology, v. 156, p. 79-95

Cherel, Y., and Hobson, K.A., 2005, Stable isotopes, beaks and predators: A new tool to study the trophic ecology of cephalopods, including Giant and Colossal Squids: Proceedings of the Royal Society Biological Sciences, v. 272, p. 1601-1607.

Chevaldonné, P., and Olu, K., 1996, Occurrence of anomuran crabs (Crustacea: Decapoda) in hydrothermal vent and cold seep communities: A review: Proceedings of the Biological Society of Washington, v. 109, p. 286-298.

Childress, J.J., Fisher, C.R., Brooks, J.M., Kennicutt, M.C., Bidigare, R., and Anderson, A.E., 1986, A methanotrophic marine molluscan (Bivalvia, Mytilidae) symbiosis: Mussels fueled by gas: Science, v. 233, no. 4770, p. 1306-1308.

Clarke, K.R., and Gorley, R.N., 2006, PRIMER v6: User manual/tutorial: Plymouth, Mass., PRIMER-E, p. 186.

Clarke, K.R., and Warwick, R.M., 2001, Change in marine communities: An approach to statistical analysis and interpretation (2d ed.): Plymouth, Mass., PRIMER-E, p. 172.

Clarke, T.A., 1973, Some aspects of the ecology of lanternfishes (Myctophidae) in the Pacific Ocean near Hawaii: Fishery Bulletin, v. 71, p. 401-433.

Clarke, T.A., 1982, Feeding habits of stomiatoid fishes from Hawaiian waters: Fishery Bulletin, v. 80, p. 287-304.

Colwell, R.K., Mao, C.X., and Chang, J., 2004, Interpolating, extrapolating, and comparing incidence-based species accumulation curves: Ecology, v. 85, p. 2717–2727.

Conway, N., Kennicutt, M., and Van Dover, C.L., 1994, Stable isotopes in the study of marine chemosynthetic based ecosystems, *in* Lajtha, K., and Michener, R., eds., Stable isotopes in ecology and environmental sciences: London, Blackwell Scientific Publications, p. 158-186.

Cordes, E.E., Bergquist, D.C., and Fisher, C.R., 2009, Macro-ecology of Gulf of Mexico cold seeps: Annual Review of Marine Science, v. 1, p. 143-168.

Cordes, E.E., Bergquist, D.C., Predmore, B.L., Jones, C., Deines, P., Telesnicki, G., and Fisher, C.R., 2006, Alternate stable states: Convergent paths of succession in hydrocarbon-seep tubeworm-associated communities: Journal of Experimental Marine Biology and Ecology, v. 339, p. 159-176.

Cordes, E.E., Carney, S.L., Hourdez, S., Carney, R.S., Brooks, J.M., and Fisher, C.R., 2007, Cold seeps of the deep Gulf of Mexico: Community structure and biogeographic comparisons to Atlantic equatorial belt seep communities: Deep-Sea Research I, v. 54, p. 637-653.

Cordes, E.E., Hourdez, S., Predmore, B.L., Redding, M.L., and Fisher, C.R., 2005, Succession of hydrocarbon seep communities associated with the long-lived foundation species *Lamellibrachia luymesi*: Marine Ecology Progress Series, v. 305, p. 17–29.

Cornejo, R., and Koppelmann R., 2006, Distribution patterns of mesopelagic fishes with special reference to *Vinciguerria lucetia* Garman 1899 (Phosichthyidae: Pisces) in the Humbolt Current Region off Peru: Marine Biology, v. 149, p. 1519-1537.

Costa, F.O., deWaard, J.R., Boutillier, J., Ratnasingham, S., Dooh, R. T., Hajibabaei, M., and Hebert, P.D.N., 2007, Biological identifications through DNA barcodes: The case of the Crustacea: Canadian Journal of Fisheries and Aquaculture Science, v. 64, p. 272-295.

Cubelio, S.S., Tsuchida, S., Hendrickx, M.E., Kado, R., and Watanabe, S., 2007, A new species of vent associated Munidopsis (Crustacea: Decapoda: Anomura: Galatheidae) from the Western Pacific, with notes on its genetic identification: Zootaxa, v. 1435, p. 25-36.

Dagg, M.J., Frost, B.W., and Walser, W.E., Jr., 1989, Copepod diel migration, feeding and the vertical flux of pheopigments: Limnology and Oceanography, v. 34, p. 1062-1071.

Damare, S., Raghukumar, C., and Raghukumar, S., 2006, Fungi in deep-sea sediments of the Central Indian Basin: Deep-Sea Research I, v. 53, no. 1, p. 14-27.

Dando, P.R., Austen, M.C., Burke, R.A., Kendall, M.A., Kennicutt, M.C., Judd, A.C., Moore, D.C., O'Hara, S.C.M., Schmaljohann, R., and Southward, A.J., 1991, Ecology of a North Sea pockmark with an active methane seep: Marine Ecology Progress Series, v. 70, no. 1, p. 49-63.

Danovaro, R., Corinaldesi, C., Dell'anno, A., Fabiano, M., and Corselli, C., 2005, Viruses, prokaryotes and DNA in the sediments of a deep-hypersaline anoxic basin (DHAB) of the Mediterranean Sea: Environmental Microbiology, v. 7, no. 4, p. 586-592.

Danovaro, R., Dell'anno, A., Trucco, A., Serresi, M., and Vanucci, S., 2001, Determination of virus abundance in marine sediments: Applied and Environmental Microbiology, v. 67, no. 3, p. 1384-1387.

Danovaro, R., Manini, E., and Dell'Anno, A., 2002, Higher abundance of bacteria than of viruses in deep Mediterranean sediments: Applied and Environmental Microbiology, v. 68, no. 3, p. 1468-1472.

Danovaro, R., and Serresi, M., 2000, Viral density and virus-to-bacterium ratio in deep-sea sediments of the Eastern Mediterranean: Applied and Environmental Microbiology, v. 66, no. 5, p. 1857-1861.

De Forest, L., and Drazen, J., 2009, The influence of a Hawaiian seamount on mesopelagic micronekton: Deep-Sea Research I, v. 56, p. 232-250.

Demopoulos, A.W.J., Fry, B., and Smith, C.R., 2007, Food web structure in exotic and native mangroves: A Hawaii-Puerto Rico comparison: Oecologia, v. 153, no. 3, p. 675-686.

DeWitt, F.A., 1972, Bathymetric distributions of two common deep-sea fishes, *Cyclothone acclinidens* and *C. signata*, off southern California: Copeia, v. 1972, p. 88-96.

Dietz, R.S., 1962, The sea's deep scattering layer: Scientific American, August 1962, p. 1-8.

Duineveld, G.C.A., Lavaleye, M.S.S., and Berghuis, E.M., 2004, Particle flux and food supply to a seamount cold-water coral community (Galicia Bank, NW Spain): Marine Ecology Progress Series, v. 277, p. 13-23.

Felder, D.L, Álvarez, F., Goy, J.W., and Lemaitre, R., 2009, Decapoda (Crustacea) of the Gulf of Mexico, with comments on the Amphionidacea, *in* Felder, D.L., and Camp, D.K., eds., Gulf of Mexico origin, waters, and biota, v. 1, Biodiversity: College Station, Tex., Texas A&M University Press, p. 1019-1104.

Felder, D.L., Camp, D.K., and Tunnell, J.W., Jr., 2009, An introduction to Gulf of Mexico biodiversity assessment, *in* Felder, D.L., and Camp, D.K., eds., Gulf of Mexico origin, waters, and biota, v. 1, Biodiversity: College Station, Tex., Texas A&M University Press, p. 1-13.

Felsenstein, J., 1985, Confidence limits on phylogenies: An approach using the bootstrap: Evolution v. 39, p. 783-791.

Fisher, C.R., Brooks, J.M., Vodenichar, J.S., Zande, J.M., Childress, J.J., and Burke, R.A., Jr., 1993, The co-occurrence of methanotrophic and chemoautotrophic sulfur-oxidizing bacterial symbionts in a deep-sea mussel: Marine Ecology, v. 14, no. 4, p. 277-289.

Fisher, C., Roberts, H., Cordes, E., and Bernard, B., 2007, Cold seeps and associated communities of the Gulf of Mexico: Oceanography, v. 20, no. 4, p. 118-129.

Flock, M.E., and Hopkins, T.L., 1992, Species composition, vertical distribution, and food habits of the sergestid shrimp assemblage in the eastern Gulf of Mexico: Journal of Crustacean Biology, v. 12, p. 210-223.

Fock, H.O., Pusch, C., and Ehrich, S., 2004, Structure of deep-sea pelagic fish assemblages in relation to the Mid-Atlantic Ridge (45°-50°N): Deep-Sea Research I, v. 51, p. 953-978.

Folmer, O., Black, M., Hoeh, W., Lutz, R., and Vrijenhoek, R., 1994, DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates: Molecular Marine Biology and Biotechnology, v. 3, no. 5, p. 294-299.

France, S.C., 2007, Genetic analysis of bamboo corals (Cnidaria: Octocorallia: Isididae): Does lack of colony branching distinguish *Lepidisis* from *Keratoisis*?: Bulletin of Marine Science, v. 81, no. 3, p. 323-333.

France, S.C., and Hoover, L.L., 2001, Analysis of variation in mitochondrial DNA sequences (ND3, ND4L, MSH) among Octocorallia (=Alcyonaria) (Cnidaria: Anthozoa): Bulletin of the Biological Society of Washington, v. 10, p. 110-118.

France, S.C., Rosel, P.E., Agenbroad, J.E., Mullineaux, L.S., and Kocher, T.D., 1996, DNA sequence variation of mitochondrial large-subunit rDNA provides support for a two-subclass organization of the Anthozoa (Cnidaria): Molecular Marine Biology and Biotechnology, v. 5, p. 15-28.

Fry, B., 1988, Food web structure on Georges Bank from stable C, N, and S isotopic compositions: Limnology and Oceanography, v. 33, p. 1182-1190.

Fry, B., 2006, Stable isotope ecology: New York, N.Y., Springer.

Fry, B., 2007, Coupled N, C and S stable isotope measurements using a dual-column gas chromatography system: Rapid Communications in Mass Spectrometry, v. 21, p. 750-756.

Fry, B., and Sherr, E.B., 1984, $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems: Contributions in Marine Science, v. 27, p. 13-47.

Fuhrman, J.A., 1999, Marine viruses and their biogeochemical and ecological effects: Nature, v. 399, p. 541-548.

Fuhrman, J.A., 2000, Impact of viruses on bacterial processes, *in* Kirchman, D.L., ed., Microbial ecology of the oceans: New York, Wiley-Liss Inc., p. 327-350.

Fukami H., Budd, A.F., Paulay, G., Sole-Cava, A., Chen, C.A., Iwao, K., and Knowlton, N., 2004, Conventional taxonomy obscures deep divergence between Pacific and Atlantic corals: Nature, v. 427, p. 832-835.

Fukami, H., Chen, C.A., Budd, A.F., Collins, A., Wallace, C., Chuang, Y.-Y., Chen, C., Dai, C.-F., Iwao, K., Sheppard, C., and Knowlton, N., 2008, Mitochondrial and nuclear genes suggest that stony corals are monophyletic but most families of stony corals are not (Order Scleractinia, Class Anthozoa, Phylum Cnidaria): PLoSONE, v. 3, no. 9, p. e3222.

Gadanho, M., and Sampaio, J. P., 2005, Occurrence and diversity of yeasts in the mid-Atlantic ridge hydrothermal fields near the Azores archipelago: Microbial Ecology, v. 5, no. 3, p. 408-417.

Gartner, J.V., Jr., Crabtree, R.E., and Sulak, K.J., 1997, Feeding at depth, *in* Randall, D.J., and Farrel, A.P., eds., Deep sea fishes: New York, N.Y., Academic Press, p. 115-194.

Gartner, J.V., Jr., Hopkins, T.L., Baird, R.C., and Milliken, D.M., 1987, The lanternfishes (Pisces: Myctophidae) of the eastern Gulf of Mexico: Fishery Bulletin, v. 85, p. 81-98.

Gartner, J.V., Jr., Sulak, K.J., Ross, S.W., and Necaise, A.M., 2008, Persistent near-bottom aggregations of mesopelagic animals along the North Carolina and Virginia continental slopes: Marine Biology, v. 153, p. 825–841.

Gjøsaeter, J., and Kawaguchi, K., 1980, A review of the world resources of mesopelagic fish: FAO Fisheries Technical Paper, v. 193, p. 151.

Glud, R.N., and Middelboe, M., 2004, Virus and bacteria dynamics of a coastal sediment: Implications for benthic carbon cycling: Limnology and Oceanography, v. 49, p. 2073-2081.

Gonzalez, J.M., and Suttle, C.A., 1993, Grazing by marine nanoflagellates on viruses and virus-sized particles: Ingestion and digestion: Marine Ecology Progress Series, v. 94, p. 1-10.

Grube, Martin, and Blaha, Juliane, 2005, Halotolerance and lichen symbiosis, *in* Gunde-Cimerman, N., Oren, Aharon, and Plementiaš, Ana, eds., Adaptation to life at high salt concentrations in archaea, bacteria, and eukarya.: Netherlands, Springer, v. 9, p. 471-488.

Hamilton, P., and Lee, T.N., 2005, Eddies and jets over the slope of the northeastern Gulf of Mexico, *in* Sturges, W., and Lugo-Fernandez, A., eds., Circulation in the Gulf of Mexico: Observations and models: American Geophysical Union, Geophysical Monograph Series, v. 161, p. 123-142.

Hawksworth, D. L., 1991, The fungal dimension of biodiversity: magnitude, significance, and conservation: Mycological Research, v. 95, p. 641-655.

Hawksworth, D. L., 2001, The magnitude of fungal diversity: The 1.5 million species estimate revisited: Mycological Research, v. 105, no. 12, p. 1422-1432.

Hecker, B., 1985, Fauna from a cold sulfur seep in the Gulf of Mexico: Comparison with hydrothermal vent communities and evolutionary implications: Biological Society of Washington Bulletin, v. 6, p. 465-473.

Heffernan, J.J., and Hopkins, T.L., 1981, Vertical distribution and feeding of the shrimp genera *Gennadas* and *Bentheogennema* (Decapoda: Penaeidea) in the eastern Gulf of Mexico: Journal of Crustacean Biology, v. 1, no. 4, p. 461-473.

Hemminga, M.A., and Mateo, M.A., 1996, Stable carbon isotope in seagrasses: Variability in ratios and use in ecological studies: Marine Ecology Progress Series, v. 140, p. 285-298.

Herring, P., 1998, North Atlantic midwater distribution of the juvenile stages of hydrothermal vent shrimps (Decapoda: Bresiliidae): Cahiers Biologie Marine, v. 39, p. 387-390.

Herring, P., 2006, Presence of postlarval alvinocaridid shrimps over south-west Indian Ocean hydrothermal vents, with comparisons of the pelagic biomass at different vent sites: Journal of the Marine Biological Association of the United Kingdom, v. 86, p. 125-128.

Hidaka, K., Kawaguchi, K., Murakiami, M., and Takahashi, M., 2001, Downward transport of organic carbon by diel migratory micronekton in the western equatorial Pacific: Its quantitative and qualitative importance: Deep-Sea Research I, v. 48, p. 1923-1939.

Hobson, K.A., Fisk, A., Karnovsky, N., Holst, M., Gagnon, J.M., and Fortier, M., 2002, A stable isotope ($\delta^{13}C$, $\delta^{15}N$) model for the North Water food web: Implications for evaluating trophodynamics and the flow of energy and contaminants: Deep-Sea Research II, v. 49, p. 5131-5150.

Holl, C.M., Villareal, T.A., Payne, C.D., Clayton, T.D., Hart, C., and Montoya, J.P., 2007, *Trichodesmium* in the western Gulf of Mexico: $^{15}N_2$-fixation and natural abundance stable isotope evidence: Limnology and Oceanography, v. 52, p. 2249-2259.

Hopkins, T L., and Baird, R.C., 1981, Trophodynamics of the fish *Valenciennellus tripunctulatus*. I. Vertical distribution, diet and feeding chronology: Marine Ecology Progress Series, v. 5, p. 1-10.

Hopkins, T.L., and Baird, R.C., 1985a, Aspects of the trophic ecology of the mesopelagic fish *Lampanyctus alatus* (Family Myctophidae) in the eastern Gulf of Mexico: Biological Oceanography, v. 3, p. 285-313.

Hopkins, T.L., and Baird, R.C., 1985b, Feeding ecology of four hatchetfishes (Sternoptychidae) in the eastern Gulf of Mexico: Bulletin of Marine Science, v. 36, p. 160-277.

Hopkins, T.L., Flock, M.E., Gartner, J.V., Jr., and Torres, J.J., 1994, Structure and trophic ecology of a low latitude midwater decapod and mysid assemblage: Marine Ecology Progress Series, v. 109, p. 143-156.

Hopkins, T.L., Gartner, J.V., Jr., and Flock, M.E., 1989, The caridean shrimp (Decapoda: Natantia) assemblage in the mesopelagic zone of the eastern Gulf of Mexico: Bulletin of Marine Science, v. 45, no. 1, p. 1-14.

Hopkins, T.L., and Lancraft, T.M., 1984, The composition and standing stock of mesopelagic micronekton at 27°N 86°W in the eastern Gulf of Mexico: Contributions in Marine Science, v. 27, p. 143-158.

Hopkins, T.L., Milliken, D.M., Bell, L.M., McMichael, E.J., Heffernan, J.J., and Cano, R.V., 1981, The landward distribution of oceanic plankton and micronekton over the west Florida continental shelf as related to their vertical distributions: Journal of Plankton Research, v. 3, p. 645-658.

Hopkins, T.L., and Sutton, T.T., 1998, Midwater fishes and shrimps as competitors and resources partitioning in low latitude oligotrophic ecosystems: Marine Ecology Progress Series, v. 164, p. 37-45.

Hopkins, T.L., Sutton, T.T., and Lancraft, T.M., 1996, The trophic structure and predation impact of a low latitude midwater fish assemblage: Progress in Oceanography, v. 38, p. 205-239.

Horning, M., and Trillmich, F., 1999, Lunar cycles in diel prey migrations exert a stronger effect on the diving of juveniles than adult Galapagos fur seals: Proceedings of the Royal Society of London, v. 266, p. 1127-1132.

Hovland, M., Mortensen, P.B., Brattegard, T., Strass, P., and Rokoengen, K., 1998, Ahermatypic coral banks off mid-Norway: Evidence for a link with seepage of light hydrocarbons: Palaios, v. 13, p. 189-200.

Hovland, M., and Risk, M., 2003, Do Norwegian deep-water coral reefs rely on seeping fluids?: Marine Geology, v. 198, p. 83-96.

Howell, W.H., and Krueger, W.H., 1987, Family Sternoptychidae, marine hatchetfishes and related Species, *in* Gibbs, R.H., Jr., and Krueger, W.H., eds., Biology of midwater fishes of the Bermuda ocean acre: Washington, D.C., Smithsonian Institution Press, p. 32-50.

Huelsenbeck., J.P., and Ronquist, F., 2001, MRBAYES: Bayesian inference of phylogenetic trees: Bioinformatics, v. 17, p. 754-755.

Hyde, K.D., Jones, E.B.G., Leaño, E., Pointing, S.B., Poonyth, A.D., and Vrijmoed, L.L.P., 1998, Role of fungi in marine ecosystems: Biodiversity and Conservation, v. 7, p. 1147-1161.

Jahn, A.E., and Backus, R.H., 1976, On the mesopelagic fish faunas of slope water, Gulf Stream, and northern Sargasso Sea: Deep-Sea Research, v. 23, p. 223-234.

Jensen, P., Aagaard, I., Burke, R.A., Dando, P.R., Jorgensen, N.O., Kuijpers, A., Laier, T., O'Hara, S.C.M., and Schmaljohann, R., 1992, 'Bubbling reefs' in the Kattegat: Submarine

landscapes of carbonate-cemented rocks support a diverse ecosystem at methane seeps: Marine Ecology Progress Series, v. 83, no. 2-3, p. 103-112.

Jochens, A.E., and Dimarco, S.F., 2008, Physical oceanographic conditions in the deepwater Gulf of Mexico in summer 2000-2002: Deep-Sea Research II, v. 55, p. 2541-2554.

Jones, W.J., and Macpherson, E., 2007, Molecular phylogeny of the East Pacific squat lobsters of the genus Munidopsis (Decapoda: Galatheidae) with the descriptions of seven new species: Journal of Crustacean Biology, v. 27, p. 477-501.

Kashkin, N.I., 1995, Vertical distribution of *Cyclothone* (Gonostomatidae) in the Pacific Ocean (brief review): Journal of Ichthyology, v. 35, p. 53-60.

Kawaguchi, K., and Mauchline, J., 1982, Biology of myctophid fishes (Family Myctophidae) in the Rockall Trough, Northeastern Atlantic Ocean: Biological Oceanography, v. 1, p. 337-373.

Keene, M.J., Giggs, R.H., and Krueger, W.H., 1987, Family Melamphaidae, bigscales, *in* Gibbs, R.H., Jr., and Krueger, W.H., eds., Biology of midwater fishes of the Bermuda ocean acre: Washington, D.C., Smithsonian Institution Press, p. 169-185.

Kehayias, G., Lykakis, J., and Fragopoulu, N., 1996, The diets of the chaetognaths *Sagitta enflata, S. erratodentata atlantica* and *S. bipunctata* at difference seasons in Eastern Mediterranean coastal waters: ICES Journal of Marine Science, v. 53, p. 837-846.

Kelley, C.A., Coffin, R.B., and Cifuentes, L.A., 1998, Stable isotope evidence for alternative bacterial carbon sources in the Gulf of Mexico: Limnology and Oceanography, v. 43, no. 8, p. 1962-1969.

Kennicutt, M.C., Brooks, J.M., and Bidigare, R.R., 1988, Hydrocarbon seep communities: Four years of study: Oceanography, v. 2.

Kennicutt, M.C., Brooks, J.M., Bidigare, R.R., Fay, R.R., Wade, T.L., and McDonald, T.J., 1985, Vent-type taxa in a hydrocarbon seep region on the Louisiana slope: Nature, v. 317, p. 351-353.

Kennicutt, M.C., II, Burke, R.A., MacDonald, I.R., Brooks, J.M., Denoux, G.J., and Macko, S.A., 1992, Stable isotope partitioning in seep and vent organisms: Chemical and ecological significance: Chemical Geology, v. 101, p. 293-310.

Kinsey, S.T., and Hopkins, T.L., 1994, Trophic strategies of euphausiids in low latitude ecosystems: Marine Biology, v. 118, p. 651-661.

Kinzer, J., 1977, Observations on feeding habits of mesopelagic fish *Benthosema glaciale* (Myctophidae) off NW Africa, *in* Anderson, N.R., and Zahuranec, B.J., eds., Oceanic sound scattering prediction: New York, N.Y., Plenum Press, p. 381-392.

Kitahara, M.V., Cairns, S.D., Stolarski, J., Blair, D., and Miller, D.J., 2010a, A comprehensive phylogenetic analysis of the Scleractinia (Cnidaria, Anthozoa) based on mitochondrial CO1 sequence data: PLoSONE, v. 5, no. 7, p. e11490.

Kitahara, M.V., Cairns, S.D., and Miller, D.J., 2010b, Monophyletic origin of *Caryophyllia* (Scleractinia, Caryophylliidae), with descriptions of six new species: Systematics and Biodiversity, v. 8, no. 1, p. 91-118.

Kling, G.W., Fry, B., and O'Brien, J., 1992, Stable isotopes and planktonic trophic structure in Arctic lakes: Ecology, v. 73, p. 561-566.

Kohlmeyer, J., 1972, Marine fungi deteriorating chitin of hydrozoa and keratin-like annelid tubes: International Journal on Life in Oceans and Coastal Waters, v. 12, no. 4, p. 277-284.

Kohlmeyer, J., and Kohlmeyer, E., 1979, Marine mycology: The higher fungi: New York, N.Y., Academic Press.

Labarca, C., and Paigen, K., 1980, A simple, rapid, and sensitive DNA assay procedure: Analytical Biochemistry, v. 102, p. 344-352.

Lajtha, K., and Michener, R.H., eds., 1994, Stable isotopes in ecology and environmental science: Boston, Mass., Blackwell Scientific Publications.

Lancraft, T.M., Hopkins, T.L., and Torres, J.J., 1988, Aspects of the ecology of the mesopelagic fish *Gonostoma elongatum* (Gonosotmatidae, Stomiiformes) in the eastern Gulf of Mexico: Marine Ecology Progress Series, v. 49, p. 27-40.

Laptikhovsky, V.V., 2005, A trophic ecology of two grenadier species (Macrouridae, Pisces) in deep waters of the Southwest Atlantic: Deep-Sea Research I, v. 52, p. 1502-1514.

Le Goff-Vitry, M.C., Rogers, A.D., and Baglow, D., 2004, A deep-sea slant on the molecular phylogeny of the Scleractinia: Molecular Phylogenetics and Evolution, v. 30, p. 167-177.

Lefébure, T., Douady, C.J., Gouy, M., and Gibert, J., 2006, Relationship between morphological taxonomy and molecular divergence within Crustacea: DNA taxonomy proposal of a molecular threshold to help species delimitation: Molecular Phylogenetics and Evolution, v. 40, p. 435-447.

Lessard-Pilon, S.A., Porter, M.D., and Fisher, C., 2008, Changes at cold seep communities over the course of one year quantified using photomosaics and GIS, *in* Ocean Sciences Meeting, Orlando, Fla., 2008: American Society of Limnology and Oceanography.

Levin, L.A., 2005, Ecology of cold seep sediments: Interactions of fauna with flow, chemistry and microbes: Oceanography and Marine Biology: An Annual Review, v. 43, p. 1-46.

Levin, L., and Gooday, A.J., 2003, The deep Atlantic Ocean, *in* Tyler, P.A., ed., Ecosystems of the world volume 28: Ecosystems of the deep ocean: Amsterdam, Elsevier, p. 111-178.

Levin, L.A., and Mendoza, G.F., 2007, Community structure and nutrition of deep methane-seep macrobenthos from the North Pacific (Aleutian) margin and the Gulf of Mexico (Florida Escarpment): Marine Ecology, v. 28, p. 131-151.

Levin, L.A., and Michener, R.H., 2002, Isotopic evidence for chemosynthesis-based nutrition of macrobenthos: The lightness of being at Pacific methane seeps: Limnology Oceanography, v. 47, no. 5, p. 1336-1345.

Levin, L.A., Ziebis, W., Mendoza, G.F., Growney, V.A., Tryon, M.D., Brown, K.M., Mahn, C., Gieskes, J., and Rathburn, A.E., 2003, Spatial heterogeneity of macrofauna at northern California methane seeps: Influence of sulfide concentration and fluid flow: Marine Ecology Progress Series, v. 265, p. 123-139.

Lindler, A., Cairns, S.D., and Cunningham, C.W., 2008, From offshore to onshore: Multiple origins of shallow-water corals from deep sea ancestors: PLoSONE, v. 6, no. 6, p. e2429.

MacAvoy, S.E., Carney, R.S., Fisher, C.R., and Macko, S.A., 2002, Use of chemosynthetic biomass by large, mobile, benthic predators in the Gulf of Mexico: Marine Ecology Progress Series, v. 225, p. 65-78.

MacAvoy, S.E., Carney, R.S., Morgan, E., and Macko, S.A., 2008a, Stable isotope variation among the mussel *Bathymodiolus childressi* and associated heterotrophic fauna at four cold-seep communities in the Gulf of Mexico: Journal of Shellfish Research, v. 27, no. 1, p. 147-151.

MacAvoy, S.E., Fisher, C.R., Carney, R.S., and Macko, S.A., 2005, Nutritional associations among fauna at hydrocarbon seep communities in the Gulf of Mexico: Marine Ecology Progress Series, v. 292, p. 51-60.

MacAvoy, S.E., Morgan, E., Carney, R.S., and Macko, S.A., 2008b, Chemoautotrophic production incorporated by heterotrophs in Gulf of Mexico hydrocarbon seeps: An examination of mobile benthic predators and seep residents: Journal of Shellfish Research, v. 27, p. 153-161.

MacDonald, I.R., Boland, G.S., Baker, J.S., Brooks, J.M., Kennicutt, M.C., and Bidigare, R.R., 1989, Gulf of Mexico hydrocarbon seep communities: II. Spatial distribution of seep organisms and hydrocarbons at Bush Hill: Marine Biology, v. 101, p. 235-247.

MacDonald, I.R., Guinasso, N.L., Jr., Reilly, J.F., Brooks, J.M., Callender, W.R., and Gabrielle, S.G., 1990, Gulf of Mexico hydrocarbon seep communities: VI. Patterns in community structure and habitat: Geo-Marine Letters, v. 10, p. 244-252.

MacDonald, I.R., Leifer, I., Sassen, R., Stine, P., Mitchell, R., and Guinasso, N.L., Jr., 2002, Transfer of hydrocarbons from natural seeps to the water column and atmosphere: Geofluids, v. 5, p. 95-107.

Machordom, A., and Macpherson, E., 2004, Rapid radiation and cryptic speciation in squat lobsters of the genus Munida (Crustacea, Decapoda) and related genera in the South West Pacific: Molecular and morphological evidence: Molecular Phylogenetics and Evolution, v. 33, p. 259-279.

Macpherson, E., and Machordom, A., 2005, Use of morphological and molecular data to identify three new sibling species of the genus *Munida* Leach, 1820 (Crustacea, Decapoda, Galatheidae) from New Caldonia: Journal of Natural History, v. 39, p. 819-834.

Macpherson, E., and Segonzac, M., 2005, Species of the genus *Munidopsis* (Crustacea: Decapoda: Galatheidae) from the deep Atlantic Ocean, including cold-seep and hydrothermal vent areas: Zootaxa, v. 1095, p. 1-60.

Maddison, D.R., and Maddison, W.P., 2005, MacClade version 4: available for free download at http://macclade.org/download.html.

Martin, J.W., and Haney, T.A., 2005, Decapod crustaceans from hydrothermal vents and cold seeps: A review through 2005: Zoological Journal of the Linnean Society, v. 145, p. 445-522.

Maynard, S.D., Riggs, F.V., and Walters, J.F., 1975, Mesopelagic micronekton in Hawaiian waters: Faunal composition, standing stock, and diel vertical migration: Fishery Bulletin, v. 73, p. 726-736.

McClain, C.R., Fougerolle, M.F., Rex, M.A., and Welch, J., 2001, MOCNESS estimates of the size and abundance of a pelagic gonostomatid fish *Cyclothone pallida* off the Bahamas: Journal of the Marine Biological Association of the United Kingdom, v. 81, p. 869-871.

McCutchan, J.H., Jr., Lewis, W.M., Jr., Kendall, C., and McGrath, C.C., 2003, Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur: Oikos, v. 102, no. 2, p. 378-390.

McFadden, C.S., France, S.C., Sanchez, J.A., Alderslade, P., 2006, A molecular phylogenetic hypothesis of the Octocorallia (Cnidaria: Anthozoa) based on mitochondrial protein-coding sequences: Molecular Phylogenetics and Evolution, v. 41, p. 513-527.

McKelvie, D.S., 1989, Latitudinal variation in aspects of the biology of *Cyclothone braueri* and *C. microdon* (Pisces: Gonostomatidae) in the eastern North Atlantic Ocean: Marine Biology, v. 102, p. 413-424.

Merrett, N.R., and Roe, H.S.J., 1974, Patterns and selectivity in the feeding of certain mesopelagic fishes: Marine Biology, v. 28, p. 115-126.

Middelboe, M., 2000, Bacterial growth rate and marine virus-host dynamics: Microbial Ecology, v. 40, p. 114-124.

Middelboe, M., Glud, R.N., and Finster, K., 2003, Distribution of viruses and bacteria in relation to diagenetic activity in an estuarine sediment: Limnology and Oceanography, v. 48, p. 1447-1456.

Middelboe, M., Glud, R.N., Wenzhöfer, F., Oguri, K., and Kitazato, H., 2006, Spatial distribution and activity of viruses in the deep-sea sediments of Sagami Bay, Japan: Deep-Sea Research Part I-Oceanographic Research Papers, v. 53, p. 1-13.

Minagawa, M., and Wada, E., 1984, Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age: Geochimica et Cosmochimica Acta, v. 48, p. 1135-1140.

Miya, M., and Nemoto, T., 1986, Reproduction, growth, and vertical distribution of the mesopelagic fish *Cyclothone pseudopallida* (Gonostomatidae), *in* Ueno, T., and others, eds., Indo-Pacific fish biology: Proceedings of the second international conference on Indo-Pacific fishes, p. 830-837.

Miya, M., and Nemoto, T., 1987, Some aspects of the biology of the micronektonic fish *Cyclothone pallida* and *C. acclinidens* (Pisces: Gonostomatidae) in Sagami Bay, central Japan: Journal of the Oceanographical Society of Japan, v. 42, p. 473-480.

Miya, M., and Nemoto, T., 1991, Comparative life histories of the meso- and bathypelagic fishes of the genus *Cyclothone* (Pisces: Gonostomatidae) in Sagami Bay, central Japan: Deep-Sea Research, v. 38, p. 67-89.

Montoya, J.P., Carpenter, E.J., and Capone, D.G., 2002, Nitrogen-fixation and nitrogen isotope abundances in zooplankton of the oligotrophic North Atlantic: Limnology and Oceanography, v. 47, p. 1617-1628

Morey, S.L., Schroeder, W.W., O'Brien, J.J., and Zavala-Hidalgo, J., 2003, The annual cycle of riverine influence in the eastern Gulf of Mexico basin: Geophysical Research Letters, v. 30, no. 16, p. 1867.

Morrison, C.L., Johnson, R.L., King, T.L., Ross, S.W., and Nizinski, M.S., 2008, Molecular assessment of deep-sea scleractinian coral biodiversity and population structure of *lophelia pertusa* in the Gulf of Mexico, *in* Sulak, K.J., Randall, M.T., Luke, K.E., Norem, A.D., and Miller, J.M., eds., Characterization of northern Gulf of Mexico deepwater hard bottom communities with emphasis on *Lophelia* coral - *Lophelia* reef megafaunal community structure, biotopes, genetics, microbial ecology, and geology: U.S. Geological Survey Open-File Report 2008-1148; OCS Study BOEMRE 2008-015, 15 April 2008, 8 Chapters, p., 8 master appendices (incl. interactive dive track DVD).

Morrison, C.L., Rios, R., and Duffy, J.E., 2004, Phylogenetic evidence for an ancient rapid radiation of Caribbean sponge-dwelling snapping shrimps (*Synalpheus*): Molecular Phylogenetics and Evolution, v. 30, p. 563-581.

Murdy, E.O., Matheson, R.E., Jr., Fechhelm, J.D., and McCoid, M.J., 1983, Midwater fishes of the Gulf of Mexico collected from the R/V Alaminos, 1965-1973: Texas Journal of Science, v. 35, p. 109-127.

Noble, R.T., and Fuhrman, J.A., 1998, Use of SYBR Green I for rapid epifluorescence counts of marine viruses and bacteria: Aquatic Microbial Ecology, v. 14, p. 113-118.

Oey, L.-Y., Ezer, T., and Lee, H.C., 2005, Loop current, rings and related circulation in the Gulf of Mexico: Areview of numerical models and future challenges, *in* Sturges, W., and Lugo-Fernandez, A., eds., Circulation in the Gulf of Mexico: Observations and models: American Geophysical Union, Geophysical Monograph Series, v. 161, p. 31-56.

Olson, M.B., Lessard, E.J., Wong, C.H.J., and Bernhardt, M.J., 2006, Copepod feeding selectivity on microplankton, including the toxigenic diatoms *Pseudo-nitzschia* spp., in the coastal Pacific Northwest: Marine Ecology Progress Series, v. 326, p. 207-220.

Owre, H.B., and Foyo, M., 1964, Report on a collection of Copepoda from the Caribbean Sea: Bulletin of Marine Science of the Gulf and Caribbean, v. 14, p. 359-372.

Padmavati, G., Haridas, P., Nair, K.K.C., Gopalakrishnan, T.C., Shiney, P., and Madhupratap, M., 1998, Vertical distribution of mesozooplankton in the central and eastern Arabian Sea during the winter monsoon: Journal of Plankton Research, v. 20, p. 343-354.

Palumbi, S.R., Martin, A.P., Romano, S., McMillian, W.O., Stice, L., and Grabowski, G., 1991, The simple fool's guide to PCR: Honolulu, Hawaii, Special Publishing Department, Zoology, University of Hawaii.

Paradis, Y., Bertolo, A., and Magnan, P., 2008, What do the empty stomachs of northern pike (*Esox lucius*) reveal? Insights from the carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes: Environmental Biology of Fish, v. 83, p. 441-448.

Passarella, K.C., and Hopkins, T.L., 1991, Species composition and food habits of the micronektonic cephalopod assemblage in the eastern Gulf of Mexico: Bulletin of Marine Science, v. 49, no. 1-2, p. 638-659.

Paull, C.K., Hecker, B., Commeau, R., Feeman-Lynde, R.P., Neumann, C., Corso, W.P., Golubic, S., Hook, J.E., Sikes, E., and Curray, J., 1984, Biological communities at the Florida Escarpment resemble hydrothermal vent taxa: Science, v. 226, p. 965-967.

Paull, C.K., Jull, A.J.T., Toolin, L.J., and Linick, T., 1985, Stable isotope evidence for chemosynthesis in an abyssal seep community: Nature, v. 317, no. 6039, p. 709-711.

Peek, A.S., Feldman, R.A., Lutz, R.A., and Vrijenhoek, R.C., 1998, Cospeciation of chemoautotrophic bacteria and deep sea clams: Proceedings of the National Academy of Sciences, v. 95, p. 9962-9966.

Perez-Barros, P., D'Amato, M.E., Guzman, N.V., and Lovrich, G.A., 2008, Taxonomic status of two South American sympatric squat lobsters, *Munida gregaria* and *Munida subrugosa* (Crustacea: Decapoda: Galatheidae), challenged by DNA sequence information: Biological Journal of the Linnean Society, v. 94, p. 421-434.

Peterson, B.J., and Fry, B., 1987, Stable isotopes in ecosystem studies: Annual Review of Ecology, Evolution, and Systematics, v. 18, p. 293-320.

Post, D.M., 2002, Using stable isotopes to estimate trophic position: Models, methods and assumptions: Ecology, v. 73, p. 703-718.

Potier, M., Marsac, F., Cherel, Y., Lucas, V., Sabatié, R., Maury, O., and Ménard, F., 2007, Forage fauna in the diet of three large pelagic fishes (lancetfish, swordfish and yellowfin tuna) in the western equatorial Indian Ocean: Fisheries Research, v. 83, p. 60-72.

Powell, E.N., and Bright, M., 1981, A thiobios does exist--Gnathostomulid domination of the canyon community at the East Flower Garden Brine Seep: Internationale Revue der Gesamten Hydrobiologie, v. 66, p. 675-683.

Powell, E.N., Bright, T.J., and Brooks, J.M., 1986, The effect of sulfide and an increased food supply on the meiofauna and macrofauna at the East Flower Garden brine seep: Helgolander Meeresunters, v. 40, p. 57-82.

Powell, E.N., Bright, T.J., Woods, A., and Gittings, S., 1983, Meiofauna and the Thiobios in the east Flower Garden Brine Seep: Marine Biology, v. 73, p. 269-283.

Pusch, C., Beckmann, A., Porteiro, F.M., and von Westernhagen, H., 2004, The influence of seamounts on mesopelagic fish communities: Archive of Fishery Marine Research, v. 51, p. 165-186.

Reisert, P.S., and Fuller, M.S., 1962, Decomposition of chitin by Chytriomyces species: Mycologia, v. 54, no. 6, p. 647-657.

Roberts, H.H., ed., 2010, Gulf of Mexico cold seeps: Deep Sea Research Part II: Topical Studies in Oceanography, v. 57, issues 21-23, p. 1835-2060.

Roberts, H.H., and Aharon, P., 1994, Hydrocarbon-derived carbonate buildups of the northern Gulf of Mexico continental slope: A review of submersible investigations: Geo-Marine Letters, v. 14, no. 2-3, p. 135-148.

Roberts, H., Carney, R., Kupchik, K., Fisher, C., Nelson, K., Becker, E., Goehring, L., Lessard-Pilon, S., Telesnicki, G., Bernard, B., Brooks, J., Bright, M., Cordes, E., Hourdez, S., Hunt, Jr., J., Shedd, W., Boland, G., Joye, S., Samarkin, V., Bernier, M., Bowles, M., MacDonald, I., Niemann, H., Petersen, C., and Potter, J., 2007a, Alvin explores the deep northern Gulf of Mexico slope: EOS, v. 88, p. 341-342.

Roberts, H.H., Fisher, C.R., Brooks, J.M., Bernard, B., Carney, R.S., Cordes, E., Shedd, W., Hunt, Jr., J., Joye, S., MacDonald, I.R., and Morrison, C., 2007b, Exploration of the deep Gulf of Mexico slope using DSV Alvin: Site selection and geologic character: Gulf Coast Association of Geologists Society Transactions, v. 57, p. 647-659.

Robinson, B.H., 1984, Herbivory by the myctophid fish *Ceratoscopelus warmingii*: Marine Biology, v. 84, p. 119-123.

Robinson, C.A., Bernhard, J.M., Levin, L.A., Mendoza, G.F., and Blanks, J.K., 2004, Surficial hydrocarbon seep infauna from the Blake Ridge (Atlantic Ocean, 2150 m) and the Gulf of Mexico (690–2240 m): Marine Ecology, v. 25, no. 4, p. 313-336.

Roelke, L.A., and Cifuentes, L.A, 1997, Use of stable isotopes to assess groups of king mackerel, *Scomberomorus cavalla*, in the Gulf of Mexico and southeastern Florida: Fishery Bulletin, v. 95, p. 540-551.

Romano, S.L., and Cairns, S.D., 2000, Molecular phylogenetic hypotheses for the evolution of scleractinian corals: Bulletin of Marine Science, v. 67, no. 3, p. 1043-1068.

Romano, S.L., and Palumbi, S.R, 1996, Evolution of scleractinian corals inferred from molecular systematics: Science, v. 271, p. 640-642.

Romano, S.L., and Palumbi, S.R., 1997, Molecular evolution of a portion of the 16S ribosomal gene region in scleractinian corals: Journal of Molecular Evolution**,** v. 45, p. 397-411.

Rooker, J.R., Turner, J.P., and Holt, S.A., 2006, Trophic ecology of *Sargassum*-associated fishes in the Gulf of Mexico determine from stable isotopes and fatty acids: Marine Ecology Progress Series, v. 313, p. 249-259.

Ross, S.W., 2007, U.S. Geological Survey Deepwater Program: Investigations of chemosynthetic communities on the lower continental slope of the Gulf of Mexico cruise report for R/V Cape Hatteras: 8-29 August 2007 and semi-annual report for cooperative agreement no. 05HQAG0099, cooperative project award 05099HS004 to University of North Carolina, Wilmington, N.C., 34 p.

Ross, S.W., and Moser, M.L., 1995, Life history of juvenile gag, Mycteroperca microlepis, in North Carolina estuaries: Bulletin of Marine Science, v. 56, p. 222-237.

Ross, S.W., and Nizinski, M.S., 2007, State of the U.S. deep coral ecosystems in the southeastern United States region: Cape Hatteras to the Florida straits, *in* Lumsden, S.E., Hourigan, T.F., Bruckner, A., and Wand Dorr, G., eds., The state of deep coral ecosystems of the United States: Silver Spring, Md., NOAA Technical Memorandum CRCP-3, p. 233-270

Rybczynski, S.M., Walters, D.M., Fritz, K.M., and Johnson, B.R., 2008, Comparing trophic position of stream fishes using stable isotope and gut content analyses: Ecology of Freshwater Fish, v. 17, p. 199-206.

Sahling, H., Rickert, D., Lee, R.W., Linke, P., and Suess, E., 2002, Macrofaunal community structure and sulfide flux gas hydrate deposits from the Cascadia convergent margin, NE Pacific: Marine Ecology Progress Series, v. 231, p. 121-138.

Sambrook, J., Fritsch, E.F., and Maniatis, T., 1989, Molecular cloning: A laboratory manual, (2d ed.): New York, N.Y., Cold Spring Harbor Laboratory Press, 1659 p.

Sánchez, J.A., McFadden, C.S., France, S.C., and Lasker, H.R., 2003, Molecular phylogenetic analyses of shallow-water Caribbean octocorals: Marine Biology, v. 142, p. 975–987.

Sassen, R., MacDonald, I.R., Requejo, A.G., Guinasso, N.L., Kennicutt, M., Sweet, S.T., and Brooks, J.M., 1994, Organic geochemistry of sediments from chemosynthetic communities, Gulf of Mexico slope: Geo-Marine Letters, v. 14, p. 110-119.

Schroeder, W.W., Brooke, S.D., Olson, J.B., Phaneuf, B., McDonough, J.J., and Etnoyer, P., 2005, Occurrence of deepwater *Lophelia pertusa* and *Madrepora oculata* in the Gulf of Mexico, *in* Freiwald, A., and Roberts, J.M., eds., Cold-water corals and ecosystems: Berlin, Springer, p. 297–307.

Sergeeva, N.G., and Gulin, M.B., 2007, Meiobenthos from an active methane seepage area in the NW Black Sea: Marine Ecology, v. 28, p. 152-159.

Shearer, T.L., van Oppen, M.J.H., Romano, S.L., and Worheide, G., 2002, Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria): Molecular Ecology, v. 11, p. 2475-2487.

Sibuet, M., and Olu, K., 1998, Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins: Deep-Sea Research II, v. 45, p. 517-567.

Smith, D.C., IV, 1986, A numerical study of loop current eddy interaction with topography in the western Gulf of Mexico: Journal of Physical Oceanography, v. 16, p. 1260-1272.

Spies, R.B., and DesMarais, D.J., 1983, Natural isotope study of trophic enrichment of marine benthic communities by petroleum seepage: Marine Biology, v. 73, no. 1, p. 67-71.

Sulak, K.J., Brooks, R.A., Luke, K.E., Norem, A.D., Randall, M., Quaid, A.J., Yeargin, G.E., Miller, J.M., Harden, W.M., Caruso J.H., and Ross, S.W., 2007, Demersal fishes associated with *Lophelia pertusa* coral and hard-substrate biotopes on the continental slope, northern Gulf of Mexico, *in* George, R.Y., and Cairns, S.D., eds., Conservation and adaptive management of seamount and deep-sea coral ecosystems: University of Miami, Fla., p. 65-92.

Suttle, C.A., 2005, Viruses in the sea: Nature, v. 437, no. 7057, p. 356-361.

Suttle, C.A., 2007, Marine viruses–major players in the global ecosystem: Nature Reviews Microbiology, v. 5, p. 801-812.

Sutton, T.T., and Hopkins, T.L, 1996a, Species composition, abundance, and vertical distribution of the stomiid (Pisces: Stomiiformes) fish assemblage of the Gulf of Mexico: Bulletin of Marine Science, v. 59, p. 530-542.

Sutton, T.T., and Hopkins, T.L., 1996b, Trophic ecology of the stomiid (Pisces, Stomiidae) assemblage of the eastern Gulf of Mexico: strategies, selectivity and impact of a mesopelagic top predator group: Marine Biology, v. 127, p. 179-192.

Sutton, T.T., Hopkins, T.L., and Lancraft, T.M., 1998, Trophic diversity of a mesopelagic fish community, *in* Pierrot-Bults, A.C., and van der Spoel, S., eds., Pelagic Biogeography ICoPB II. Proceedings of the Second International Conference: Paris, France, United Nations Educational, Scientific and Cultural Organization IOC Workshop Report, no. 142, p. 353-357.

Sutton, T.T., Porteiro, F.M., Heino, M., Byrkjedal, I., Langhelle, G., Anderson, C.I.H., Horne, J., Søiland, H., Falkenhaug, T., Godø, O.R., Bergstad, O.A., 2008, Vertical structure, biomass and topographic association of deep-pelagic fishes in relation to a mid-ocean ridge system: Deep-Sea Research II, v. 55, p. 161-184.

Swofford, D.L., 2002, PAUP*. Phylogenetic analysis using parsimony (*and other methods) (ver. 4): Sutherland, Mass., Sinauer Associates.

Takishita, K., Tsuchiya, M., Reimer, J.D., and Maruyama, T.,2006, Molecular evidence demonstrating the basidiomycetous fungus *Cryptococcus curvatus* is the dominant microbial eukaryote in sediment at the Kuroshima Knoll methane seep: Extremophiles, v. 10, p. 165-169.

Takishita, K., Yubuki, N., Kakizoe, N., Inagaki, Y., and Maruyama, T., 2007, Diversity of microbial eukaryotes in sediment at a deep-sea methane cold seep: Surveys of ribosomal DNA libraries from raw sediment samples and two enrichment cultures: Extremophiles, v. 11, no. 4, p. 563-576.

TDI-Brooks International, Inc., 2006a, Reconnaissance cruise report R/V Gyre Cruise: 11 - 25 March 2006 for investigations of chemosynthetic communities on the lower continental slope of the Gulf of Mexico, BOEMRE Contract No.: 1435-01-05-39187, Technical Report No. 06-1706: College Station, Tex., TDI-Brooks International Inc., 52 p.

TDI-Brooks International, Inc., 2006b, Deep chemosynthetic community characterization cruise report 7 May – 2 June 2006 for investigations of chemosynthetic communities on the lower

continental slope of the Gulf of Mexico: College Station, Tex., TDI-Brooks International Inc., 217 p.

TDI-Brooks International, Inc., 2007, Deep chemosynthetic reconnaissance II cruise report 4 June – 6 July 2007 for investigations of chemosynthetic communities on the lower continental slope of the Gulf of Mexico: College Station, Tex., TDI-Brooks International Inc., 316 p.

Thomas, C.J., and Cahoon, L.B., 1993, Stable isotope analyses differentiate between trophic pathways supporting rocky-reef fishes: Marine Ecology Progress Series, v. 95, p. 19-24.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G, 1997, The CLUSTAL-X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools: Nucleic Acids Research, v. 25, no. 24, p. 4876-4882.

Thompson, J.D., Higgins, D.G., and Gibson, T.J., 1994, CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice: Nucleic Acids Research, v. 22, p. 4673-4680.

Thompson, P.A., Pesant, S., and Waite, A.M., 2007, Contrasting the vertical differences in the phytoplankton biology of a dipole pair of eddies in the south-eastern Indian Ocean: Deep-Sea Research II, v. 54, p. 1003-1028.

Turley, C.M., Lochte, K., and Lampitt, R.S., 1995, Transformations of biogenic particles during sedimentation in the northeastern Atlantic: Philosophical Transactions of the Royal Society of London Series B-Biological Sciences, v. 348, p. 179-189.

Tyler, P.A., 2003, The peripheral seas, *in* Tyler, P.A., ed., Ecosystems of the world: The deep sea: Amsterdam, Elsevier, p. 261-293.

Van Dover, C.L., 2000, The ecology of deep-sea hydrothermal vents: Princeton, N.J., Princeton University Press, 424 p.

Van Dover, C.L., 2007, Stable isotope studies in marine chemoautotrophically based ecosystems: An update, *in* Michener, R., and Lajtha, K., eds., Stable isotopes in ecology and environmental sciences (2d ed.): Cambridge, Mass., Blackwell Scientific Publications, Inc., p. 202-237.

Van Dover, C.L., Aharon, P., Bernhard, J.M., Caylor, E., Doerries, M., Flickinger, W., Gilhooly, W., Goffredi, S.K., Knick, K.E., Macko, S.A., Rapoport, S., Raulfs, E.C., Ruppel, C., Salerno, J.L., Seitz, R.D., Sen Gupta, B.K., Shank, T., Turnipseed, M., and Vrijenhoek, R., 2003, Blake Ridge methane seeps: Characterization of a soft-sediment, chemosynthetically based ecosystem: Deep Sea Research Part I: Oceanographic Research Papers, v. 50, no. 2, p. 281-300.

Van Dover, C.L., and Fry, B., 1989, Stable isotopic compositions of hydrothermal vent organisms: Marine Biology, v. 102, p. 257-263.

Van Dover, C.L., and Fry, B., 1994, Microorganisms as food resources at deep-sea hydrothermal vents: Limnology and Oceanography, v. 39, no. 1, p. 51-57.

Van Dover, C.L., Ward, M.E., Scott, J.L., Underdown, J., Anderson, B., Gustafson, C., Whalen, M. , and Carnegie, R.B., 2007, A fungal epizootic in mussels at a deep-sea hydrothermal vent: Marine Ecology, v. 28, p. 54-62.

Van Gaever, S., Moodley, L., de Beer, D., and Vanreusel, A., 2006, Meiobenthos at the Arctic Hakon Mosby Mud Volcano, with a parental-caring nematode thriving in sulphide-rich sediments: Marine Ecology-Progress Series, v. 321, p. 143-155.

Vázquez de la Cerda, A.M., Reid, R.O., DiMarco, S.F., and Jochens, A.E., 2005, Bay of Campeche circulation: An update, *in* Sturges, W., and Lugo-Fernandez, A., eds., Circulation in the Gulf of Mexico: Observations and models: American Geophysical Union Geophysical Monograph Series, v. 161, p. 279-293.

Weatherly, G.L., Wienders, N., and Romanou, A., 2005, Intermediate depth circulation in the Gulf of Mexico estimated from direct measurements, *in* Sturges, W., and Lugo-Fernandez, A., eds., Circulation in the Gulf of Mexico: Observations and models: American Geophysical Union Geophysical Monograph Series, v. 161, p. 315-324.

Wen, K., Ortmann, A.C., and Suttle, C.A., 2004, Accurate estimation of viral abundance by epifluorescence microscopy: Applied and Environmental Microbiology, v. 70, no. 7, p. 3862-3867.

Wicksten, M.K., and Packard, J.M., 2005, A qualitative zoogeographic analysis of decapod crustaceans of the continental slopes and abyssal plain of the Gulf of Mexico: Deep-Sea Research I, v. 52, p. 1745-1765.

Williams, A., Koslow, J.A., Terauds, A., and Haskard, K., 2001, Feeding ecology of five fishes from the mid-slope micronekton community off southern Tasmania, Australia: Marine Biology, v. 139, p. 1177-1192.

Williams, T., Kano, A., Ferdelman, T., Henriet, J-P., Abe, K., Andres, M., Bjerager, M., Browning, E.L., Cragg, B.A., De Mol, B., Dorschel, B., Foubert, A., Frank, T.D., Fuwa, Y., Gaillot, P., Gharib, J.J., Gregg, J.M., Huvenne, V.A.I., Leonide, P., Li, X., Mangelsdorf, K., Tanaka, A., Monteys, X., Novosel, I., Sakai, S., Samarkin, V.A., Sasaki, K., Spivak, A.J., Takashima, C., and Titschack, J., 2006, Cold-water coral mounds revealed: EOS: Transactions, v. 87, no. 47, p. 525-526.

Wirshing, H.H., Messing, C.G., Douady, C.J., Reed, J., Stanhope, M.J., and Shivji, M.S., 2005, Molecular evidence for multiple lineages in the gorgonian family Plexauridae (Anthozoa: Octocorallia): Marine Biology, v. 147, p. 497–508.

Wommack, K.E., and Colwell, R.R., 2000, Virioplankton: viruses in aquatic ecosystems: Microbiology and Molecular Biology Reviews, v. 64, no. 1, p. 69-114.

Yatsu, A., Sassa, C., Moku, M. and Kinoshita, T., 2005, Night-time vertical distribution and abundance of small epipelagic and mesopelagic fishes in the upper 100 m layer of the Kuroshio-Oyashio Transition Zone in Spring: Fisheries Science, v. 71, p. 1280-1286.

Young, J.W., and Blaber, S.J.M., 1986, Feeding ecology of three species of midwater fishes associated with the continental slope of eastern Tasmania, Australia: Marine Biology, v. 93, p. 147-156.

Zhang, C.L., Pancost, R.D., Sassen, R., Qian, Y., and Macko, S.A., 2003, Archaeal lipid biomarkers and isotopic evidence of anaerobic methane oxidation associated with gas hydrates in the Gulf of Mexico: Organic Geochemistry, v. 34, p. 827–836.