7-2-2015

Investigating Trophic Interactions of Deep-sea Animals (Sharks, Teleosts, and Mobile scavengers) in the Gulf of Mexico Using Stable Isotope Analysis

Diana A. Churchill

Florida International University, diana.churchill@gmail.com

DOI: 10.25148/etd.FIDC000085

Follow this and additional works at: <https://digitalcommons.fiu.edu/etd>

Part of the [Ecology and Evolutionary Biology Commons](#), and the [Marine Biology Commons](#)

Recommended Citation

Churchill, Diana A., "Investigating Trophic Interactions of Deep-sea Animals (Sharks, Teleosts, and Mobile scavengers) in the Gulf of Mexico Using Stable Isotope Analysis" (2015). *FIU Electronic Theses and Dissertations*. 2214.
<https://digitalcommons.fiu.edu/etd/2214>

This work is brought to you for free and open access by the University Graduate School at FIU Digital Commons. It has been accepted for inclusion in FIU Electronic Theses and Dissertations by an authorized administrator of FIU Digital Commons. For more information, please contact dcc@fiu.edu.

FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

INVESTIGATING TROPHIC INTERACTIONS OF DEEP-SEA ANIMALS (SHARKS,
TELEOSTS, AND MOBILE SCAVENGERS) IN THE GULF OF MEXICO USING
STABLE ISOTOPE ANALYSIS

A dissertation submitted in partial fulfillment of

the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

BIOLOGY

by

Diana Churchill

2015

To: Dean Michael R. Heithaus
College of Arts and Sciences

This dissertation, written by Diana Churchill, and entitled Investigating Trophic Interactions of Deep-Sea Animals (Sharks, Teleosts, and Mobile Scavengers) in the Gulf of Mexico Using Stable Isotope Analysis, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

William Anderson

Heather Bracken-Grissom

Kevin Boswell

Maureen Donnelly

Michael Heithaus, Major Professor

Date of Defense: July 2, 2015

The dissertation of Diana Churchill is approved.

Dean Michael R. Heithaus
College of Arts and Sciences

Dean Lakshmi N. Reddi
University Graduate School

Florida International University, 2015

DEDICATION

To my mother and father, Jackye and Steve Churchill, who encouraged my curiosity and have always supported my aspirations no matter how fanciful they have been. And to Adrienne Alpern, whose love and wisdom has kept me grounded.

ACKNOWLEDGMENTS

The work presented in this dissertation was not accomplished solely by the author. Many individuals assisted me throughout this process with mentorship and logistical support.

I would first like to thank Mike Heithaus, my major advisor, for taking a chance on an aquarist six years out of undergrad looking to do something more intellectual than scrubbing fish tanks. You have been an excellent role model for me, both professionally and personally. Your level of energy and excitement seem boundless and are often a needed reminder of what I love about science. You believed in my ideas and allowed me to run with them. Working under your mentorship has given me the resources and confidence to pursue my career aspirations.

I am grateful for all of the members of my dissertation committee: Bill, Anderson, Heather Bracken-Grissom, Kevin Boswell, and Mo Donnelly. I appreciate your generous contributions of expertise and time during my research. Special thanks to Craig Layman for his support during the early stages of this project when it was still a Master's thesis.

I am indebted to Dean Grubbs, Kirk Gastrich, and Chip Cotton for the considerable planning it took to successfully execute three deep-sea research cruises. Dean and Chip also provided invaluable expertise on deep-sea taxa identification. I would also like to thank Bill Chamberlain, John Harris, and the staff of the FIU Stable Isotope Laboratory for additional logistical support.

I am very thankful for the support and assistance provided by my current and former lab mates Cindy Bessey, Derek Burkholder, Camila Cáceres, Jeremy Kiszka, Phil Matich, Shomen Mukherjee, Rob Nowicki, Adam Rosenblatt, Robin Sarabia, Jordy

Thomson, Jeremy Vaudo, and Beth Whitman. Thank you to the multitude of volunteers who worked long, grueling hours aboard the R/V Weatherbird II and to all of the undergraduate volunteers who assisted in processing samples in the lab.

I especially want to thank my partner, Adrienne Alpern, for keeping me sane during graduate school. Your emotional support made the challenging times more bearable and your enthusiasm made Miami the best place to live. I'm so fortunate to have found you and I look forward to many more adventures.

Lastly, I would like to thank my parents. Even though you may not understand what I do professionally, you have provided me with unconditional love and support. I am eternally grateful for everything you have done for me. You both have encouraged my curiosity, creativity, love of nature and my drive to succeed.

During my research I was generously supported by Florida International University through a Teaching Assistantship and a University Graduate School Dissertation Year Fellowship. My research was primarily funded by two grants from BP/The Gulf of Mexico Research Initiative through the Florida Institute of Oceanography as well as a grant to the Deep-C Consortium with additional financial support from a Guy Harvey Scholarship Award. Permission to use Chapters II and V were provided by Elsevier Inc.

ABSTRACT OF THE DISSERTATION

INVESTIGATING TROPHIC INTERACTIONS OF DEEP-SEA ANIMALS (SHARKS,
TELEOSTS, AND MOBILE SCAVENGERS) IN THE GULF OF MEXICO USING
STABLE ISOTOPE ANALYSIS

by

Diana Churchill

Florida International University, 2015

Miami, Florida

Professor Michael Heithaus, Major Professor

The deep-sea is the largest habitat on earth, containing over 90 percent of the world's oceans and home to over 20,000 species. Deep-sea ecosystems are increasingly impacted by human activities including fishing and oil extraction. To understand potential impacts on deep-sea food webs, it is crucial to gather baseline data in these systems. I quantified the trophic interactions of three groups of deep-water animals across a range of trophic levels living in the northern and eastern Gulf of Mexico using stable isotope analysis. First, I propose methods for correcting $\delta^{15}\text{N}$ values for the presence of nitrogenous metabolic waste products (e.g., urea) in muscle tissue using chemical extractions and/or species-specific mathematical normalizations. Significant differences in $\delta^{15}\text{N}$, %N, and C:N values as a result of extractions were observed in eight of ten shark and all three hagfish species. The $\delta^{15}\text{N}$ values increased, but shifts in %N and C:N values were not unidirectional. Mathematical normalizations for $\delta^{15}\text{N}$ values were successfully created for four shark and two hagfish species. I then describe the trophic interactions of three consumer assemblages. Carbon isotopic values indicate a heavy

reliance on allochthonous nutrient inputs from surface waters. Nitrogen isotopic values reveal somewhat atypical taxa as top predators in the deep sea. Shark, teleost, and invertebrate species across a wide range of body sizes are feeding at a similar trophic level. This apparent lack of size structuring could be the result of a high degree of opportunistic scavenging or perhaps feeding at many trophic levels simultaneously in an oligotrophic system. There was a high degree of isotopic niche overlap among species within each consumer assemblage, perhaps the result of limited nutrient resources in the deep-sea. In general, individuals from the northern sampling stations displayed higher $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values than those from the eastern sites. With the exception of a few species, there were no strong relationships between body size and isotopic values. The present study is among the first characterizations of the trophic structure of deep-sea organisms in the Gulf of Mexico and establishes system baselines for future studies describing deep-water systems and investigating anthropogenic impacts.

TABLE OF CONTENTS

CHAPTER		PAGE
	PREFACE	1
I.	INTRODUCTION	2
	References.....	7
II.	EFFECTS OF LIPID AND UREA EXTRACTION ON $\delta^{15}\text{N}$ VALUES OF DEEP-SEA SHARKS AND HAGFISH: CAN MATHEMATICAL CORRECTION FACTORS BE GENERATED?	11
	Abstract	12
	Introduction.....	13
	Materials and Methods.....	14
	Results.....	17
	Discussion.....	18
	Acknowledgements.....	23
	References.....	23
III.	TROPHIC INTERACTIONS OF DEEP-SEA SCAVENGERS IN THE GULF OF MEXICO	34
	Abstract	35
	Introduction.....	36
	Materials and Methods.....	38
	Results.....	45
	Discussion.....	49
	Acknowledgements.....	56
	References.....	56
IV.	TROPHIC INTERACTIONS OF DEEP-SEA TELEOSTS ALONG THE NORTHERN AND WESTERN SLOPES OF THE GULF OF MEXICO	79
	Abstract	80
	Introduction.....	81
	Materials and Methods.....	83
	Results.....	88
	Discussion.....	91
	Acknowledgements.....	97
	References.....	97

V.	TROPHIC INTERACTIONS OF COMMON ELASMOBRANCHS IN DEEP-SEA COMMUNITIES OF THE GULF OF MEXICO REVEALED THROUGH STABLE ISOTOPE AND STOMACH CONTENT ANALYSIS	118
	Abstract.....	119
	Introduction.....	121
	Materials and Methods.....	124
	Results.....	130
	Discussion.....	133
	Acknowledgements.....	139
	References.....	140
VI.	CONCLUSION.....	158
	References.....	164
VII.	VITA.....	170

LIST OF TABLES

TABLE	PAGE
 CHAPTER II	
Table 1: Muscle samples used to test the effects of a chloroform/methanol extraction on $\delta^{15}\text{N}$ values of deep-water sharks and hagfish	26
Table 2: The results of Wilcoxon signed rank tests comparing chloroform/methanol extracted and whole muscle $\delta^{15}\text{N}$, %N and C:N values of deep-water sharks and hagfish.....	27
Table 3: Results for linear regression analysis of $\delta^{15}\text{N}$ -E by $\delta^{15}\text{N}$ -BULK, %N-BULK, and C:N-BULK for deep-water shark and hagfish species. Starred values indicate a significant relationship.....	29
Table 4: Results for paired t-tests, including the mean difference ($\pm$ SD), comparing extracted and mathematically normalized $\delta^{15}\text{N}$ values in deep-sea sharks and hagfish.....	30
 CHAPTER III	
Table 1: Summary of all muscle tissue samples collected from deep-sea scavengers in the eastern Gulf of Mexico	64
Table 2: Results for regression analysis of $\Delta\delta^{13}\text{C}$ by C:N-BULK using linear, Michaelis-Menten, and logistic models along with the corresponding AICc values	65
Table 3: Variation in stable isotope values by sampling time in deep-sea scavengers on the northern gulf slope near DeSoto Canyon (NGS-DC) and the Mississippi River (NGS-MR) and along the west Florida slope (WFS).....	66
Table 4: Results for linear regression analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by body size for six scavenger species	67
Table 5: Results for linear regression analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by depth for six scavenger species	68
Table 6: The percent of total area (TA) occupying unique isotopic niche space for each scavenger species.....	69
 CHAPTER IV	
Table 1: Summary of all muscle tissue samples collected from teleosts in the Gulf of Mexico	104

Table 2: Variation in stable isotope values by sampling time in teleosts on the northern gulf slope (NGS) and along the west Florida slope (WFS).....	105
---	-----

Table 3: Results for linear regression analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by size for five teleost species.....	106
--	-----

Table 4: Results for linear regression analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by depth for five teleost species.....	107
---	-----

Table 5: The percent of total area (TA) occupying unique isotopic niche space for each teleost species	108
--	-----

CHAPTER V

Table 1: The percent of total area (TA) and individuals occupying unique isotopic niche space for each shark species.....	147
---	-----

Table 2: Results for linear regression analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by size (PCL) for eight shark species	148
--	-----

Table 3: Results for linear regression analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by capture depth for six shark species.....	149
--	-----

CHAPTER VI

Table 1: The percent of total area (TA) and ellipse area occupying unique isotopic niche space for each consumer group	167
--	-----

LIST OF FIGURES

FIGURE	PAGE
 CHAPTER II	
Figure 1: Average differences in $\delta^{15}\text{N}$, %N, and C:N (at%) values between chloroform/methanol extracted muscle tissue and bulk muscle tissue of sharks (circles) and hagfish (squares)	31
Figure 2: Relationship between mean $\Delta\delta^{15}\text{N}$ and mean $\delta^{15}\text{N}$ -BULK (A), %N-BULK (B) or C:N-BULK (C) values among all shark species	32
Figure 3: Relationship between $\delta^{15}\text{N}$ -BULK and $\delta^{15}\text{N}$ -E in three species of sharks and two species of hagfish	33
 CHAPTER III	
Figure 1: Deep-sea scavengers were sampled at multiple sample stations located along the northern slope along the DeSoto Canyon (NGS-DC, circles) and south of the Mississippi River (NGS-MR, squares) and on the west Florida slope (WFS, triangles) of the Gulf of Mexico	70
Figure 2: Spatial and depth distributions of deep-sea scavengers collected along the northern and west Florida slopes of the Gulf of Mexico	71
Figure 3: Size distributions of deep-sea benthic scavengers in the Gulf of Mexico.....	72
Figure 4: C:N ratios of bulk and lipid extracted deep-water scavenger muscle tissue	73
Figure 5: Spatiotemporal variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for three deep-sea scavengers on the northern gulf slope and along the west Florida slope.....	74
Figure 6: Variation in stable isotope values with body size in <i>Bathynomus giganteus</i> , <i>Chaceon quinquedens</i> , <i>Eptatretus springeri</i> , and <i>Heterocarpus ensifer</i>	75
Figure 7: Relationship between mean body size and $\delta^{15}\text{N}$ values for scavengers	76
Figure 8: Temporal and spatial variation in stable isotopic values of deep-sea scavengers of the northeastern Gulf of Mexico and along the west Florida slope	77
Figure 9: Total area convex hulls of scavengers from the northern slope (DeSoto Canyon and south of the Mississippi River) and west Florida slope sampling regions	78

CHAPTER IV

Figure 1: Teleosts were sampled at multiple sample stations located along the northern slope (NGS, circles) and on the west Florida slope (WFS, triangles) of the Gulf of Mexico.....	109
Figure 2: The C:N ratios of bulk and lipid-extracted teleost muscle tissue.....	110
Figure 3: Spatial and depth distributions of teleosts collected along the northern and west Florida slopes of the Gulf of Mexico.....	111
Figure 4: Size distributions of teleosts collected along the northern and west Florida slopes of the Gulf of Mexico	112
Figure 5: Temporal variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for <i>Urophycis floridana</i> on the northern gulf slope and along the west Florida slope	113
Figure 6: Variation in stable isotope values (C and N) with body size	114
Figure 7: Temporal and spatial variation in stable isotopic values of teleosts captured along the northeastern Gulf of Mexico and along the west Florida slope	115
Figure 8: Relationship between the mean body size and $\delta^{15}\text{N}$ values for teleosts.....	116
Figure 9: Total area convex hulls and Bayesian ellipses of scavengers from the northern gulf slope and west Florida slope sampling regions.....	115

CHAPTER V

Figure 1: Deep-sea sharks were sampled at multiple sample stations (dots) located along the northern slope of the Gulf of Mexico (NGS) and waters of the west Florida slope (WFS)	150
Figure 2: Temporal variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for <i>Squalus cf. mitsukurii</i> on the northern gulf slope and along the west Florida slope	151
Figure 3: Temporal and spatial variation in stable isotopic values of sharks of the northeastern Gulf of Mexico (NGS) and along the west Florida slope (WFS)	152
Figure 4: Total area convex hulls of sharks from the NGS and WFS sampling regions.	153
Figure 5: Relationship between the mean size (PCL) and $\delta^{15}\text{N}$ for sharks.....	154

Figure 6: Variation in stable isotope values with body size in <i>Centrophorus</i> cf. <i>niaukang</i> ($\delta^{15}\text{N}$), <i>Mustelus canis</i> ($\delta^{15}\text{N}$), and <i>Squalus cubensis</i> ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$)	155
--	-----

Figure 7: Variation in $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) with length for <i>Squalus</i> cf. <i>mitsukurii</i> in the northeastern gulf and west Florida slope	156
---	-----

Figure 8: Sex differences in the relationship between size and $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) in <i>Squalus</i> cf. <i>mitsukurii</i> collected from NGS	157
---	-----

CHAPTER VI

Figure 1: The relationship between body size (mass) and $\delta^{15}\text{N}$ values along the northern gulf slope (NGS) and the west Florida shelf (WFS) among scavenger, teleost and shark taxa	168
---	-----

Figure 2: Total area convex hulls and Bayesian ellipses of scavengers, sharks and teleosts from the northern gulf slope (NGS) and west Florida shelf (WFS) sampling regions	169
---	-----

PREFACE

The following chapters have been published and have been formatted for those publications.

CHAPTER II

Churchill, Diana A., Heithaus, Michael R., & Dean Grubbs, R. (2015). Effects of lipid and urea extraction on $\delta^{15}\text{N}$ values of deep-sea sharks and hagfish: Can mathematical correction factors be generated? *Deep Sea Research Part II: Topical Studies in Oceanography*, 115(0), 103-108.

CHAPTER V

Churchill, Diana A., Heithaus, Michael R., Vaudo, Jeremy J., Grubbs, R. Dean, Gastrich, Kirk, & Castro, José I. (2015). Trophic interactions of common elasmobranchs in deep-sea communities of the Gulf of Mexico revealed through stable isotope and stomach content analysis. *Deep Sea Research Part II: Topical Studies in Oceanography*, 115(0), 92-102.

CHAPTER I
INTRODUCTION

The deep-sea is the largest habitat on earth. It makes up over 90 percent of the world's oceans, with an estimated volume of $1368 \times 10^6 \text{ km}^3$ and an average depth of 3800 meters (Ramirez-Llodra et al., 2011). The deep-sea is generally considered to start at approximately 200m depth, at the beginning of the continental shelf break, where the transition from shallow water to deep-water fauna occurs (Thistle, 2003). The prevailing scientific viewpoint 150 years ago was that the deep-sea possessed low biodiversity, no primary production, no seasonality, and a uniformly cold, dark, and nutrient-poor environment. This view of the deep-sea has changed substantially in the following decades. The famed cruise of the HMS *Challenger* (1872-1876) began the modern exploration of the ocean's deep-water environments, culminating in the *Galathea* expedition of 1950-1952, which showed that animals can be found even in the deepest parts of the oceans (Koslow, 2007). Hessler and Sanders (1967), demonstrated that the deep-sea was actually one of high diversity, collecting up to 365 individual species in a single macrofaunal sample using an epibenthic sled. In fact, the deep-sea may contain up to 10 million different species of small invertebrates, predominately crustaceans and molluscs (Grassle & Maciolek, 1992). In contrast, there are an estimated ~5 million species of arthropods in tropical rain forests (Novotny et al., 2002), an ecosystem that is considered to be one of the most diverse systems on the planet.

Despite the vast size and immense diversity, study of deep-sea ecosystems has lagged behind characterizations of shelf and coastal marine systems. The lack of knowledge represents an important gap as human exploitation of deep-sea resources expands. Deep-water trawl fisheries have experienced a 'boom-and-bust' pattern since the 1970's-1980's (Clark et al., 2008). In fact, there is a general trend of fisheries

moving into deep waters (Morato et al., 2006). Increased fish pressure is cause for concern and represents a management challenge because deep-sea fishes have biological traits that make them especially vulnerable to over fishing or disturbances in the environment including K-type life history traits, low fecundity and aggregation in restricted topographic areas (Haedrich, 1997; Koslow et al., 2000).

The Deepwater Horizon oil spill in April 2010 highlighted the potential impacts that deep water oil and gas exploration can have on deep-sea systems. The explosion released about five million barrels of crude oil into the water and response efforts discharged 1.84 million gallons of dispersant, which may have increased toxicity of spilled materials when they combine with crude oil (Rico-Martínez et al., 2013). The disaster resulted in the death of countless marine animals including dolphins (Williams et al., 2011), seabirds (Montevecchi et al., 2012), and deep-sea corals (White et al., 2012). In addition, there is likely to be damage to deep-sea environments and organisms that has yet to be documented. Even typical drilling activity in the Gulf of Mexico results in elevated total carbon, anoxic conditions and patchy zones of disturbed benthic communities (Continental Shelf Associates, 2006). Other anthropogenic impacts on the deep sea include disposal of litter and waste, mining for deep-sea mineral deposits, underwater cables, and climate change (reviewed in Ramirez-Llodra et al., 2011). To implement effective conservation measures and to better understand the human impacts in the deep-sea, baseline data are critical. The taxonomy of many deep-sea organisms is poorly resolved and there are likely many more new species in this environment, highlighting the need for basic taxonomic investigations. Among known species, often little is known about their trophic interactions and ecological roles in deep-water systems.

Diet and trophic ecology have traditionally been studied through stomach content analysis (Cortés, 1997; Hyslop, 1980). Analysis of gut contents allows for fine resolution of diets, but has limitations, particularly in the deep-sea. For example, the large sample sizes that are often required to fully characterize diets are difficult and expensive to obtain in deep-water studies because of ship costs, low population densities, and a high proportion of individuals are recovered with empty stomachs. In addition, deep-dwelling teleosts and elasmobranchs often regurgitate food as they are brought to the surface (Bowman, 1986) and prey items from deep-water communities often are fragile and difficult to identify (Cailliet et al., 1999; Drazen et al., 2001). Finally, stomach contents provide only a snapshot of the diet of an individual and may not reflect the proportions of prey groups ingested or assimilated because of variability in the digestibility of prey items and the potential for temporal variation in consumption patterns.

Stable isotope analysis, particularly of carbon and nitrogen, are becoming increasingly important in studies of trophic interactions (Fry, 2006; Layman et al., 2012) because it provides time-integrated information on assimilated, rather than ingested, biomass (Peterson & Fry, 1987; Vander Zanden & Rasmussen, 2001). The utility of stable isotope analysis derives from the fact that isotopic ratios in consumer tissues reflect those of their prey and shift relatively predictably with each trophic transfer (Bearhop et al., 2004). Values of $\delta^{15}\text{N}$ can be used to estimate relative trophic position because ^{15}N is generally enriched through each trophic transfer (reviewed in Caut et al., 2009; Post, 2002). Because carbon ratios are generally conserved through trophic transfers (Fry & Sherr, 1989) and vary among groups of primary producers or habitats (O'Leary, 1981; Smith & Epstein, 1971), $\delta^{13}\text{C}$ values can be used to elucidate foraging

locations and/or sources of primary production across many trophic levels. Because isotopes provide integrated views of diets and changes in stable isotope values of consumers reflect changes not only in their direct prey, but also in lower level trophic pathways, stable isotope analysis is attractive for assessing overall changes in communities. However, stable isotope analysis cannot identify specific prey items because multiple diet combinations can result in similar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of a consumer and discrimination factors can vary within and among taxa (Caut et al., 2009; del Rio et al., 2009; Post, 2002). Therefore, isotopic data must be interpreted with caution and, where possible, benefits from the insights provided by stomach content analysis.

The goal of this dissertation was to describe the trophic interactions of a diversity of deep-sea consumers in the Gulf of Mexico, to gain insights into the dynamics of the oligotrophic ecosystem, and provide a baseline against which potential changes associated with human impacts might be measured. I focused my work on three primary groups: large-bodied benthic scavengers (crustaceans, hagfish), mesopredators (primarily teleosts), and top predators (primarily elasmobranchs), all of which are likely important members of the benthic and/or demersal communities. In order to effectively employ stable isotopic analyses, I begin in Chapter II by quantifying the effects of a standard chloroform-methanol extraction on the $\delta^{15}\text{N}$ values from deep-water shark and hagfish muscle tissue. I present the observed changes in $\delta^{15}\text{N}$, %N and C:N values pre- and post-extraction, and develop taxa-specific mathematical correction equations for four shark and two hagfish species.

In Chapter III-V, I describe trophic interactions in the deep-sea environments in the northern and eastern Gulf of Mexico within benthic scavengers, teleosts and sharks, respectively. Stable isotope analysis was used investigate spatiotemporal variation in trophic interactions within and among numerically dominant species of each general guild and determine whether trophic interactions vary across body size and habitat depth. In each sampling region, I also describe the isotopic niches of common species and quantify the level of overlap among them. For three common species of sharks, data from stomach contents was integrated into the analyses.

In Chapter VI, I conclude by synthesizing insights from each of the different consumer groups in the deep waters of the Gulf of Mexico to understand trophic patterns at the community scale.

References

- Bearhop, S., Adams, C. E., Waldron, S., Fuller, R. A., & Macleod, H. (2004). Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology*, 73(5), 1007-1012.
- Bowman, R.E. (1986). Effect on regurgitation on stomach content of marine fishes. *Environmental Biology of Fishes*, 16, 171-181.
- Cailliet, G. M., Andrews, A. H., Wakefield, W. W., Moreno, G., & Rhodes, K. L. (1999). Fish faunal and habitat analyses using trawls, camera sleds and submersibles in benthic deep-sea habitats off central California. *Oceanologica Acta*, 22(6), 579-592.
- Caut, S., Angulo, E., & Courchamp, F. (2009). Variation in discrimination factors (Delta N-15 and Delta C-13): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology*, 46(2), 443-453.

- Clark, Malcolm R, Vinnichenko, Vladimir I, Gordon, John DM, Beck-Bulat, Georgy Z, Kukharev, Nikolai N, & Kakora, Alexander F. (2008). Large-scale distant-water trawl fisheries on seamounts *Seamounts: ecology, fisheries & conservation* (pp. 361).
- Continental Shelf Associates, Inc. (2006). *Effects of Oil and Gas Exploration and Development at Selected Continental Slope Sites in the Gulf of Mexico*. Gulf of Mexico OCS Region, New Orleans, LA.
- Cortés, E. (1997). A critical review of methods of studying fish feeding based on analysis of stomach contents: Application to elasmobranch fishes. *Canadian Journal of Fisheries and Aquatic Sciences*, 54(3), 726-738.
- del Rio, C. M., Wolf, N., Carleton, S. A., & Gannes, L. Z. (2009). Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews (Cambridge)*, 84(1), 91-111.
- Drazen, J. C., Buckley, T. W., & Hoff, G. R. (2001). The feeding habits of slope dwelling macrourid fishes in the eastern North Pacific. *Deep-Sea Research Part I Oceanographic Research Papers*, 48(3), 909-935.
- Fry, B. (2006). *Stable Isotope Ecology*. New York: Springer.
- Fry, B., & Sherr, E. B. (1989). $\delta^{13}\text{C}$ Measurements as Indicators of Carbon Flow in Marine and Freshwater Ecosystems. In P. W. Rundel, J. R. Ehleringer, & K. A. Nagy (Eds.), *Stable Isotopes in Ecological Research* (Vol. 68, pp. 196-229): Springer New York.
- Grassle, J Frederick, & Maciolek, Nancy J. (1992). Deep-sea species richness: regional and local diversity estimates from quantitative bottom samples. *American Naturalist*, 313-341.
- Haedrich, RL. (1997). *Deep-sea demersal fish and fisheries* (Vol. 23): Springer.
- Hessler, Robert R., & Sanders, Howard L. (1967). *Faunal diversity in the deep-sea*. Paper presented at the Deep Sea Research and Oceanographic Abstracts.

- Hyslop, E. J. (1980). Stomach content analysis - a review of methods and their application. *Journal of Fish Biology*, 17(4), 411-429.
- Koslow, JA, Boehlert, GW, Gordon, JDM, Haedrich, RL, Lorange, P, & Parin, N. (2000). Continental slope and deep-sea fisheries: implications for a fragile ecosystem. *ICES Journal of Marine Science: Journal du Conseil*, 57(3), 548-557.
- Koslow, T. (2007). *The Silent Deep*: University of Chicago Press Chicago, IL.
- Layman, C. A., Araujo, M. S., Boucek, R., Hammerschlag-Peyer, C. M., Harrison, E., Jud, Z. R., Matich, P., Rosenblatt, A. E., Vaudo, J. J., Yeager, L. A., Post, D. M., & Bearhop, S. (2012). Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biological Reviews (Cambridge)*, 87(3), 545-562.
- Montevecchi, W. A., Hedd, A., Tranquilla, L. M., Fifield, D. A., Burke, C. M., Regular, P. M., Davoren, G. K., Garthe, S., Robertson, G. J., & Phillips, R. A. (2012). Tracking seabirds to identify ecologically important and high risk marine areas in the western North Atlantic. *Biological Conservation*, 156, 62-71.
- Morato, T., Watson, R., Pitcher, T. J., & Pauly, D. (2006). Fishing down the deep. *Fish and Fisheries*, 7(1), 24-34.
- Novotny, Vojtech, Basset, Yves, Miller, Scott E, Weiblen, George D, Bremer, Birgitta, Cizek, Lukas, & Drozd, Pavel. (2002). Low host specificity of herbivorous insects in a tropical forest. *Nature*, 416(6883), 841-844.
- O'Leary, M.H. (1981). Carbon Isotope Fractionation in Plants. *Phytochem.*(20), 553-567.
- Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, 18, 293-320.
- Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, 83(3), 703-718.
- Ramirez-Llodra, Eva, Tyler, Paul A., Baker, Maria C., Bergstad, Odd Aksel, Clark, Malcolm R., Escobar, Elva, Levin, Lisa A., Menot, Lenaick, Rowden, Ashley A., Smith, Craig R., & Van Dover, Cindy L. (2011). Man and the Last Great Wilderness: Human Impact on the Deep Sea. *PLoS ONE*, 6(8), e22588.

- Rico-Martínez, Roberto, Snell, Terry W, & Shearer, Tonya L. (2013). Synergistic toxicity of Macondo crude oil and dispersant Corexit 9500A ® to the *Brachionus plicatilis* species complex (Rotifera). *Environmental Pollution*, 173, 5-10.
- Smith, B.N., & Epstein, S. (1971). Two Categories of $^{13}\text{C}/^{12}\text{C}$ ratios for higher plants. *Plant Physiology (Rockville)*(47), 380-384.
- Thistle, David. (2003). The deep-sea floor: an overview. *Ecosystems of the World*, 5-38.
- Vander Zanden, M. J., & Rasmussen, J. B. (2001). Variation in $\delta\text{N-15}$ and $\delta\text{C-13}$ trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, 46(8), 2061-2066.
- White, Helen K, Hsing, Pen-Yuan, Cho, Walter, Shank, Timothy M, Cordes, Erik E, Quattrini, Andrea M, Nelson, Robert K, Camilli, Richard, Demopoulos, Amanda WJ, & German, Christopher R. (2012). Impact of the Deepwater Horizon oil spill on a deep-water coral community in the Gulf of Mexico. *Proceedings of the National Academy of Sciences*, 109(50), 20303-20308.
- Williams, Rob, Gero, Shane, Bejder, Lars, Calambokidis, John, Kraus, Scott D., Lusseau, David, Read, Andrew J., & Robbins, Jooke. (2011). Underestimating the damage: interpreting cetacean carcass recoveries in the context of the Deepwater Horizon/BP incident. *Conservation Letters*, 4(3), 228-233.

CHAPTER II

EFFECTS OF LIPID AND UREA EXTRACTION ON $\delta^{15}\text{N}$ VALUES OF DEEP-SEA
SHARKS AND HAGFISH: CAN MATHEMATICAL CORRECTION FACTORS BE
GENERATED?

Abstract

Stable isotope analysis is broadly employed to investigate diverse ecological questions. In order to make appropriate comparisons among multiple taxa, however, it is necessary to standardize values to account for interspecific differences in factors that affect isotopic ratios. For example, varying concentrations of soluble nitrogen compounds, such as urea or trimethylamine oxide, can affect the analysis and interpretation of $\delta^{15}\text{N}$ values of sharks or hagfish. The goal of this study was to assess the effects of a standard chloroform/methanol extraction on the stable isotope values of muscle tissue obtained from 10 species of sharks and three species of hagfish collected from poorly-known deep-water (>200 meters) communities. We detected significant differences in $\delta^{15}\text{N}$, $\% \text{N}$, and C:N values as a result of extractions in 8 of 10 shark and all three hagfish species. We observed increased $\delta^{15}\text{N}$ values, but shifts in $\% \text{N}$ and C:N values were not unidirectional. Mathematical normalizations for $\delta^{15}\text{N}$ values were successfully created for four shark and two hagfish species. However, they were not successful for two shark species. Therefore, performing extractions of all samples is recommended.

Keywords: stable isotopes, sharks, hagfish, urea, TMAO, normalization

Introduction

Stable isotope analysis has become a valuable tool for investigating many important ecological questions. In general, these applications make use of the fact that there is natural variation in the isotopic ratios (e.g. $\delta^{15}\text{N}$, the ratio of ^{15}N to ^{14}N relative to a reference standard) that result from chemical or biological processes. These processes cause isotopic discrimination, defined as a change in the ratio of heavy to light isotopes in a compound after uptake, processing or transformation (Fry, 2006). Nitrogen is particularly important in the study of trophic ecology because it can be used to estimate relative trophic position as ^{15}N is enriched in relatively predictable ways through each trophic transfer (DeNiro & Epstein, 1981).

In order to make appropriate comparisons among multiple taxa, however, it is necessary to standardize stable isotope values to account for interspecific differences in physiological factors that affect isotopic uptake and fractionation. For example, elasmobranchs maintain high levels of the nitrogenous waste products urea and trimethylamine oxide (TMAO) in their tissues to assist with osmoregulation (Olson, 1999). Fisk et al. (2002) suggested these soluble nitrogenous compounds, which may have different nitrogen isotope dynamics than muscle tissue, can hinder comparison of $\delta^{15}\text{N}$ values for elasmobranch tissue samples among individuals or between taxa. Urea is a byproduct of metabolism and is expected to be depleted in ^{15}N (DeNiro & Epstein, 1981), creating lower $\delta^{15}\text{N}$ values and subsequently lower trophic level estimates for bulk tissue analyses that will vary depending on the amount of urea in a particular sample. Muscle tissue of hagfish can also contain TMAO at levels similar to those observed in marine elasmobranchs (reviewed in Currie & Edwards, 2010). In order to make direct

protein-to-protein comparisons, soluble nitrogenous compounds should be removed before the remaining tissue is analyzed for $\delta^{15}\text{N}$ values. Recent studies have indicated that the removal of urea can have little effect (Logan & Lutcavage, 2010; Reum, 2011), a mild effect (Kim & Koch, 2012), or a moderate effect (Hussey et al., 2012) on $\delta^{15}\text{N}$ values. Further work is needed to investigate the effects of soluble nitrogen compounds on the $\delta^{15}\text{N}$ values of elasmobranchs and hagfish. In addition, where costs of analyses are a limiting factor, it is important to determine if reliable mathematical corrections might be developed to reduce the need to analyze multiple samples. Normalizations have been proposed for a variety of aquatic animals (McConnaughey & McRoy, 1979; Post et al., 2007) and deep-sea fishes (Hoffman & Sutton, 2010) to account for the effects of variable amounts of lipids present in sampled tissues.

The goal of this study was to assess the effects of a standard chloroform/methanol extraction, which removes both lipids and soluble nitrogen compounds, on the stable isotope values of muscle tissue obtained from 10 species of sharks and three species of hagfish collected in deep-water (>200 meters) habitats of the Gulf of Mexico. Specifically, we were interested in examining the effect of extraction on $\delta^{15}\text{N}$, %N and C:N values and investigating whether it was possible to develop species-specific mathematical normalizations for non-extracted muscle $\delta^{15}\text{N}$ values.

Materials and methods

Sampling methods

The effect of chloroform/methanol extraction was tested on a total of 222 individuals from 10 species of sharks and three species of hagfish (Table 1). Sample

sizes among examined species ranged from 3 to 35 individuals; six species were represented by $n < 10$. Animals were collected along the continental slope of the Gulf of Mexico offshore of Louisiana to central Florida during research cruises in April 2005, April 2011, August 2011, and April 2012. Animals were sampled using demersal longlines with integrated traps. A standard longline set consisted of 550m of mainline on the bottom with 50 baited gangions with five hook sizes and three types of baited traps (Churchill et al., 2015). A small section of dorsal muscle tissue was removed from each individual and frozen onboard the research vessel for later processing.

An additional five individual sharks (*Carcharhinus falciformis* and *Galeocerdo cuvier*) were collected using pelagic longlines during a research cruise in October 2011. While these specific individuals were not collected in deep-water, both represented species that had been previously sampled using demersal longlines in waters > 300 m deep.

Stable Isotope Analysis

All muscle tissue was thawed and briefly (3-5 seconds) rinsed in deionized water to remove any debris and dried in a 60°C oven for a minimum of 48 hours. Samples were then homogenized by hand using a mortar and pestle. One portion of each sample was immediately prepared for bulk stable isotope analysis. A second portion was prepared for extraction to remove soluble nitrogen compounds using a modified Bligh and Dyer (1959) chloroform/methanol extraction. This process has been shown to remove both lipids and soluble nitrogen compounds (urea and TMAO) from elasmobranch muscle tissue (Hussey et al., 2012). Dried, homogenized muscle tissue

was immersed in a 2:1 mixture of chloroform and methanol with a solvent volume three to five times greater than the powdered muscle. Samples were vortexed for 30 seconds, left undisturbed for a minimum of 30 minutes, centrifuged for 10 minutes at 1318g and then decanted. This process was repeated at least three times or until the supernatant was clear. Samples were then re-dried overnight to remove excess solvent (Logan et al., 2008). No absolute measurements of amount of soluble nitrogen or lipid compounds were taken pre- or post-extraction. Between 0.4-0.7mg of paired tissue samples, extracted (E) and non-extracted (BULK) for each shark or hagfish were then weighed into tin capsules. Nitrogen isotope composition was measured on a ThermoFinnigan Delta C isotope ratio mass spectrometer (IRMS) coupled with a NA 1500 NE elemental analyzer at the Florida International University Stable Isotope Laboratory. The $^{15}\text{N}/^{14}\text{N}$ isotopic ratios are expressed in conventional delta notation in per mil (‰) relative to the levels of ^{15}N in atmospheric air. IRMS error based on the repeat analysis of an internal glycine standard (n=74) was less than 0.1‰. Approximately one sixth of the samples were run in duplicate. The mean absolute difference and standard deviation between duplicates was 0.1 ± 0.1 ‰ for $\delta^{15}\text{N}$. All reported C:N ratios are based on atomic mass.

Statistical analysis

The mean differences between extracted and BULK $\delta^{15}\text{N}$, %N, and C:N values ($\Delta\delta^{15}\text{N}$, $\Delta\%N$, and $\Delta\text{C:N}$) were calculated for each species. To examine if the observed differences were statistically significant, we used paired Student's t-tests (n>20) or nonparametric Wilcoxon signed rank tests for species with small sample sizes (n<20). We also examined the relationship between mean $\delta^{15}\text{N}$ -BULK, %N-BULK, or C:N-

BULK values and mean $\Delta\delta^{15}\text{N}$ values among all shark species using regression analysis. We were unable to include comparable analyses for hagfish species because of small sample sizes (n=3 species).

We conducted three linear regressions per species to generate a mathematical normalization of $\delta^{15}\text{N}$ -E values using $\delta^{15}\text{N}$ -BULK, C:N-BULK, or %N-BULK as predictors. This was performed on a random sample of half of all individuals from each species with at least 24 individuals. Using any relationships determined to be significant, the $\delta^{15}\text{N}$ -BULK values of remaining individuals were normalized using the model estimates and compared to the extracted values using paired t-tests.

Values reported throughout the manuscript are means $\pm$ SD. All data were normally distributed and equal in variance, therefore no data transformations were necessary. A criterion of $p < 0.05$ was used for all statistical tests. All analyses were completed in R (R Core Team, 2013).

Results

Chloroform/methanol extractions had species-specific effects on $\delta^{15}\text{N}$, %N and C:N values (Table 2; Figure 1). Six of the 10 shark species (*Centrophorus* cf. *granulosus*, *Centrophorus niaukang*, *Etmopterus bigelowi*, *Mustelus canis*, *Squalus cubensis* and *Squalus* cf. *mitsukurii*) - including all but two species with more than five individuals analyzed - and all of the hagfish species displayed significant, positive shifts in $\delta^{15}\text{N}$ values after extraction procedures. All of these species plus *Hexanchus griseus* and *G. cuvier* (which showed no significant shift in $\delta^{15}\text{N}$) displayed significant shifts in both %N and C:N values. Overall, sharks displayed an average shift of $0.7 \pm 0.5\text{‰}$ in $\delta^{15}\text{N}$

values, $-1.2 \pm 1.8\%$ in %N values and 0.3 ± 0.7 in C:N values among all species. Hagfish displayed an average shift of $1.4 \pm 0.4\%$ in $\delta^{15}\text{N}$ values, $3.7 \pm 2.51\%$ in %N values and -2.4 ± 2.3 in C:N values among all species. There were no significant relationships between mean $\Delta\delta^{15}\text{N}$ and mean $\delta^{15}\text{N}$ -BULK values ($t=-1.50$, $p=0.171$; Figure 2), %N-BULK ($t=1.69$, $p=0.13$) or C:N-BULK ($t=-1.95$, $p=0.09$; Figure 2).

Four species of deep-water sharks and two species of hagfish with sufficient sample sizes ($n \geq 24$) were used to generate predictions of $\delta^{15}\text{N}$ -E values using $\delta^{15}\text{N}$ -BULK, %N-BULK, or C:N-BULK (Table 3). Three shark (*C. cf. granulosus*, *S. cubensis* and *S. cf. mitsukurii*) and two hagfish species (*Myxine mcmillanae* and *Paramyxine springeri*) displayed significant relationships between $\delta^{15}\text{N}$ -E and $\delta^{15}\text{N}$ -BULK values (Figure 3). No significant relationships were found between $\delta^{15}\text{N}$ -E and %N-BULK or C:N-BULK values in any of the examined species. Using the 5 significant relationships, we created species-specific mathematical normalizations for $\delta^{15}\text{N}$ -BULK values (Table 4). The results of the paired t-tests indicate that the $\delta^{15}\text{N}$ values derived from mathematical normalizations were not statistically different from $\delta^{15}\text{N}$ -E values for all but one species (Table 4). For two species, however, p-values were low enough to suggest the potential for Type II error.

Discussion

Understanding the effects of chloroform/methanol extraction on the tissues of sharks and hagfish is important for accurately interpreting $\delta^{15}\text{N}$ values for ecological studies. We observed shifts in %N, C:N and $\delta^{15}\text{N}$ values as a result of the extractions (Figure 1). There were significant shifts in %N and C:N in eight shark and three hagfish

species (Table 2). Shifts in these values, however, were not unidirectional. Six shark species displayed reductions in %N and increases in C:N values; likely the result of the removal of nitrogenous compounds (i.e., urea and TMAO). Values of *H. griseus* and all hagfish species were affected in the opposite direction, displaying increases in %N and reductions in C:N values. For these species, it is likely that higher levels of lipids (i.e., large quantities of carbon) are present in the bulk muscle tissue and they are subsequently removed by the chloroform/methanol extraction. While no data exist on the fat content in the muscle tissue of these species, a large amount of oily fluid or residue remained present after standard drying times that was not observed for other species sampled.

Previous studies have reported small increases ($>1\%$) in $\delta^{15}\text{N}$ values in the muscle tissue of teleosts after chloroform/methanol lipid extractions (Logan & Lutcavage, 2008; Sotiropoulos et al., 2004; Sweeting et al., 2006). These observed changes have been attributed to the removal or alteration of some protein nitrogen or the removal of nitrogenous waste products (Logan & Lutcavage, 2008). Our observed changes in $\delta^{15}\text{N}$ values may have been a result of both the removal of urea and/or TMAO and protein-based nitrogen. Other extraction techniques, such as a deionized water rinse of homogenized tissue (Kim & Koch, 2012; Logan & Lutcavage, 2010), could have less effect on proteins. This method might not be suitable for individuals with high levels of lipids, as the oil present in the dried tissue could hinder the urea/TMAO extraction process.

There were no significant relationships between mean $\Delta\delta^{15}\text{N}$ and mean $\delta^{15}\text{N}$ -BULK, %N-BULK, or mean C:N-BULK values among shark species. If a single factor

(e.g. urea) were driving shifts in $\delta^{15}\text{N}$ values with extraction, then we would expect significant relationships between $\Delta\delta^{15}\text{N}$ values (affected by the removal of nitrogen compounds) and the %N and C:N values of bulk muscle tissues. This was not the case likely because %N and C:N are affected both by the amount of both nitrogenous compounds (i.e., urea and TMAO) and more carbon-rich compounds (e.g., lipids) that can vary considerably within and among taxa and do not necessarily covary. In the future, it may be beneficial to target individuals or species with naturally low lipid content for better insight into the effects of urea and/or TMAO removal on $\delta^{15}\text{N}$ values.

Given that both urea and TMAO are generally depleted in ^{15}N , it is unsurprising that the extractions resulted in positive shifts of $\delta^{15}\text{N}$ values, though these changes are variable and often species-specific. We found that extraction resulted in statistically significant and biologically relevant (see below) changes in six of the sampled shark species (*C. cf. granulosus*, *C. niaukang*, *E. bigelowi*, *M. canis*, *S. cubensis*, and *S. cf. mitsukurii*) and all of the sampled hagfish species. For sharks, $\delta^{15}\text{N}$ values increased as a result of chloroform-methanol extractions with a mean ($\pm\text{SD}$) of $0.7\pm0.5\%$ across all species. This was less than the $1.8\pm0.1\%$ reported by Kim and Koch (2012) for leopard sharks but similar to the $0.6\pm0.6\%$ reported by Hussey et al. (2012) for 21 species of elasmobranchs. Hussey et al. (2012) found that the greatest differences between extracted and bulk $\delta^{15}\text{N}$ values were observed in marine elasmobranchs (-0.1 to 1.4%), followed by laboratory-sampled (0.8 to -0.1%) and then estuarine (-0.1 to 0.4%) species. The differences observed in the deep-water sharks included in this study were comparable to the estuarine and some laboratory-sampled elasmobranchs, suggesting that the observed habitat gradient might not hold true for all shark species.

Our results support the conclusion that the removal of urea is important when interpreting $\delta^{15}\text{N}$ values of deep-sea sharks. Using a standard trophic discrimination factor of 3.4‰ (Wada et al., 1991), this represents approximately 0.2 trophic levels. However, trophic discrimination of $\delta^{15}\text{N}$ values may be much smaller at higher trophic levels. Using a scaled trophic framework, Hussey et al. (2014) reported discrimination factors of 3.4‰ and 5.1‰ for trophic level 3 consumers decreasing to 0.9‰ and 0.6‰ for trophic level 7. If this is true in our study system, the observed changes in $\delta^{15}\text{N}$ values represent a larger potential source of error in interpreting the trophic position of upper-level consumers such as sharks. Regardless of the accuracy of varying trophic level discrimination factors for sharks or hagfish, individual differences in the amount of soluble nitrogen compounds create an appearance of more trophic variation than actually exists. Accounting for the effects of urea and/or TMAO on $\delta^{15}\text{N}$ values must be done to obtain more ecologically meaningful values.

The observed mean ($\pm\text{SD}$) increases ($1.4\pm0.4\text{‰}$) in $\delta^{15}\text{N}$ across the three species of hagfish were greater than in all deep-water shark species. Using the standard discrimination factor, this represents approximately 0.4 trophic levels, twice that observed for deep-water sharks in this study. The removal of nitrogenous compounds from hagfish tissues, therefore, is crucial for making accurate ecological interpretations of $\delta^{15}\text{N}$ values. Previous studies of hagfish trophic ecology (e.g., McLeod & Wing, 2007; Zintzen et al., 2013) did not perform extraction of muscle tissue or account for the potential bias TMAO might introduce into the interpretation of $\delta^{15}\text{N}$ values. These results should be interpreted with caution since hagfish likely are feeding at higher

trophic levels than previously reported if the presence of TMAO decreased $\delta^{15}\text{N}$ values, as appears likely based on our results.

We were able to create species-specific mathematical correction factors for two of four deep-water shark and two hagfish species examined with the largest sample sizes ($n > 24$). In general, the differences between the predicted and observed $\delta^{15}\text{N}$ values were small in magnitude relative to analytical (IRMS) error, suggesting that using mathematically normalized $\delta^{15}\text{N}$ values should not affect the interpretation of patterns in stable isotope results of these species. This method, however, should be used with caution. One shark species (*C. niakang*) displayed no significant relationships between $\delta^{15}\text{N}$ -E and $\delta^{15}\text{N}$ -BULK, %N-BULK, or C:N-BULK values, making mathematical normalization impossible. Also, the normalized $\delta^{15}\text{N}$ values calculated for *S. cubensis* were significantly different from $\delta^{15}\text{N}$ -E values, rendering the normalization useless. Correction factors might not be suitable for every species, but when sufficient samples and clear relationships are present, they can reduce study costs if a subset of all samples collected (e.g. $n=30$) are extracted, rather than simply performing extractions of all samples collected. The general applicability of specific correction factors does need further work and would require samples from the same species from multiple populations and/or environments. It will also be critical to find a way to separate out the effects of lipids and nitrogen compounds on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Our results lay the foundation for such studies in the future. Although the exact correction factors presented in this study may not be broadly applicable, our results highlight the need to extract urea/TMAO from all individuals sampled, or to generate species-specific corrections for these deep-sea taxa. This information will be applicable to a wide variety of researchers.

Acknowledgements

This research was made possible by two grants from BP/The Gulf of Mexico Research Initiative through the Florida Institute of Oceanography as well as a grant to the Deep-C Consortium. The 2005 research cruise was funded by a Florida Institute of Oceanography Shiptime Grant and FIU's Department of Biological Sciences. We thank Chip Cotton and Ron Schatman for logistical support, the staff of the FIU Stable Isotope Laboratory, and all of the research cruise volunteers.

References

- Bligh, E.S., & Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911-917.
- Churchill, Diana A., Heithaus, Michael R., Vaudo, Jeremy J., Grubbs, R. Dean, Gastrich, Kirk, & Castro, José I. (2015). Trophic interactions of common elasmobranchs in deep-sea communities of the Gulf of Mexico revealed through stable isotope and stomach content analysis. *Deep Sea Research Part II: Topical Studies in Oceanography*, 115(0), 92-102.
- Currie, S., & Edwards, S. L. (2010). The curious case of the chemical composition of hagfish tissues-50 years on. *Comparative Biochemistry and Physiology Part A Molecular & Integrative Physiology*, 157(2), 111-115.
- DeNiro, Michael J., & Epstein, Samuel. (1981). Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, 45(3), 341-351.
- Fisk, A. T., Tittlemier, S. A., Pranschke, J. L., & Norstrom, R. J. (2002). Using anthropogenic contaminants and stable isotopes to assess the feeding ecology of greenland sharks. *Ecology*, 83(8), 2162-2172.
- Fry, B. (2006). *Stable Isotope Ecology*. New York: Springer.

- Hoffman, J. C., & Sutton, T. T. (2010). Lipid correction for carbon stable isotope analysis of deep-sea fishes. *Deep-Sea Research Part I-Oceanographic Research Papers*, 57(8), 956-964.
- Hussey, N. E., Olin, J. A., Kinney, M. J., McMeans, B. C., & Fisk, A. T. (2012). Lipid extraction effects on stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of elasmobranch muscle tissue. *Journal of Experimental Marine Biology and Ecology*, 434, 7-15.
- Hussey, Nigel E., MacNeil, M. Aaron, McMeans, Bailey C., Olin, Jill A., Dudley, Sheldon F. J., Cliff, Jeremy, Wintner, Sabine P., Fennessy, Sean T., & Fisk, Aaron T. (2014). Rescaling the trophic structure of marine food webs. *Ecology Letters*, 17(2), 239-250.
- Kim, S.L., & Koch, P.L. (2012). Methods to collect, preserve and prepare elasmobranch tissues for stable isotope analysis. *Environmental Biology of Fishes*, 95(1), 53-63.
- Logan, J. M., Jardine, T. D., Miller, T. J., Bunn, S. E., Cunjak, R. A., & Lutcavage, M. E. (2008). Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *Journal of Animal Ecology*, 77(4), 838-846.
- Logan, J. M., & Lutcavage, M. E. (2008). A comparison of carbon and nitrogen stable isotope ratios of fish tissues following lipid extractions with non-polar and traditional chloroform/methanol solvent systems. *Rapid Communications in Mass Spectrometry*, 22(7), 1081-1086.
- Logan, John M., & Lutcavage, Molly E. (2010). Stable isotope dynamics in elasmobranch fishes. *Hydrobiologia*, 644(1), 231-244.
- McConnaughey, T., & McRoy, C. P. (1979). Food-Web structure and the fractionation of Carbon isotopes in the bering sea. *Marine Biology*, 53(3), 257-262.
- McLeod, R. J., & Wing, S. R. (2007). Hagfish in the New Zealand fjords are supported by chemoautotrophy of forest carbon. *Ecology*, 88(4), 809-816.
- Olson, K.R. (1999). Rectal gland and volume homeostasis. In W. C. Hamlett (Ed.), *Sharks, Skates, and Rays: The Biology of Elasmobranch Fishes* (pp. 329-320). Baltimore, Maryland, USA: The John Hopkins University Press.

- Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., & Montana, C. G. (2007). Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, 152(1), 179-189.
- R Core Team. (2013). R: A language and environment for statistical computing (Version 2.15.2). Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <http://www.R-project.org/>
- Reum, J. C. P. (2011). Lipid correction model of carbon stable isotopes for a cosmopolitan predator, spiny dogfish *Squalus acanthias*. *Journal of Fish Biology*, 79(7), 2060-2066.
- Sotiropoulos, M. A., Tonn, W. M., & Wassenaar, L. I. (2004). Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissues: potential consequences for food web studies. *Ecology of Freshwater Fish*, 13(3), 155-160.
- Sweeting, C. J., Polunin, N. V. C., & Jennings, S. (2006). Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. *Rapid Communications in Mass Spectrometry*, 20(4), 595-601.
- Wada, E., Mizutani, H., & Minagawa, M. (1991). The use of stable isotopes for food web analysis. *Critical Reviews in Food Science and Nutrition*, 30(4), 361-371.
- Zintzen, V., Rogers, K. M., Roberts, C. D., Stewart, A. L., & Anderson, M. J. (2013). Hagfish feeding habits along a depth gradient inferred from stable isotopes. *Marine Ecology Progress Series*, 485, 223-U266.

Table 1 Muscle samples used to test the effects of a chloroform/methanol extraction on $\delta^{15}\text{N}$ values of deep-water sharks and hagfish.

Species	Common name	n
<i>Carcharhinus falciformis</i>	Silky shark	3
<i>Carcharhinus signatus</i>	Night shark	4
<i>Centrophorus</i> cf. <i>granulosus</i>	Gulper shark	30
<i>Centrophorus niaukang</i>	Taiwan gulper shark	35
<i>Etmopterus bigelowi</i>	Blurred lantern shark	10
<i>Galeocerdo cuvier</i>	Tiger shark	8
<i>Hexanchus griseus</i>	Bluntnose sixgill shark	9
<i>Mustelus canis</i>	Dusky smooth-hound	6
<i>Squalus cubensis</i>	Cuban dogfish	27
<i>Squalus</i> cf. <i>mitsukurii</i>	Shortspine spurdog	32
<i>Eptatretus minor</i>	None (Hagfish)	6
<i>Myxine mcmillanae</i>	Caribbean hagfish	24
<i>Paramyxine springeri</i>	Gulf hagfish	28

Table 2 The results of Wilcoxon signed rank tests comparing chloroform/methanol extracted and whole muscle $\delta^{15}\text{N}$, %N and C:N values of deep-water sharks and hagfish. Significant values ($p < 0.05$) are bold.

Species	n	$\delta^{15}\text{N}$				$\%N$			
		Mean $\delta^{15}\text{N}$ - BULK $\pm$ SD (‰)	Mean $\delta^{15}\text{N}$ - E $\pm$ SD (‰)	Test statistic	p	Mean $\%N$ - BULK $\pm$ SD	Mean $\%N$ - E $\pm$ SD	Test statistic	p
Sharks									
<i>Carcharhinus falciformis</i>	3	13.1 $\pm$ 1.2	13.8 $\pm$ 1.3	V=0	0.250	16.6 $\pm$ 1.0	15.6 $\pm$ 0.6	V=6	0.250
<i>Carcharhinus signatus</i>	4	11.0 $\pm$ 0.7	11.8 $\pm$ 0.1	V=1	0.250	16.6 $\pm$ 0.7	15.5 $\pm$ 0.6	V=10	0.125
<i>Centrophorus</i> cf. <i>granulosus</i>	30	14.5 $\pm$ 0.9	15.0 $\pm$ 0.9	t=-6.94	<0.001	16.5 $\pm$ 0.5	15.1 $\pm$ 0.6	t=9.69	<0.001
<i>Centrophorus niaukang</i>	35	12.4 $\pm$ 0.7	13.3 $\pm$ 0.8	t=-8.40	<0.001	16.6 $\pm$ 1.4	15.0 $\pm$ 0.5	t=5.92	<0.001
<i>Etmopterus bigelowi</i>	10	11.6 $\pm$ 0.8	12.3 $\pm$ 0.8	V=0	0.006	15.7 $\pm$ 1.0	14.7 $\pm$ 0.8	V=53	0.006
<i>Galeocerdo cuvier</i>	8	12.3 $\pm$ 0.7	12.5 $\pm$ 0.9	V=9	0.233	16.7 $\pm$ 0.7	15.2 $\pm$ 0.5	V=36	0.008
<i>Hexanchus griseus</i>	9	13.4 $\pm$ 0.4	13.8 $\pm$ 0.7	V=9	0.129	11.1 $\pm$ 2.6	14.7 $\pm$ 0.5	V=0	0.004
<i>Mustelus canis</i>	6	12.7 $\pm$ 1.3	13.4 $\pm$ 0.9	V=0	0.031	16.6 $\pm$ 0.5	15.4 $\pm$ 0.4	V=21	0.033
<i>Squalus cubensis</i>	27	12.3 $\pm$ 0.7	13.0 $\pm$ 0.6	t=-10.10	<0.001	17.2 $\pm$ 1.5	14.9 $\pm$ 1.0	t=7.97	<0.001
<i>Squalus</i> cf. <i>mitsukurii</i>	32	12.1 $\pm$ 0.6	12.8 $\pm$ 0.4	t=-10.00	<0.001	16.5 $\pm$ 0.9	15.1 $\pm$ 0.8	t=7.60	<0.001
Hagfish									
<i>Eptatretus minor</i>	6	11.8 $\pm$ 0.6	13.5 $\pm$ 1.0	V=0	0.031	7.9 $\pm$ 1.4	14.1 $\pm$ 0.4	V=0	0.031
<i>Myxine mcmillanae</i>	24	13.4 $\pm$ 0.9	14.7 $\pm$ 0.7	t=-12.66	<0.001	12.5 $\pm$ 0.5	14.0 $\pm$ 1.1	t=-6.62	<0.001
<i>Paramyxine springeri</i>	28	11.4 $\pm$ 1.0	12.9 $\pm$ 1.0	t=-21.92	<0.001	8.8 $\pm$ 2.1	13.9 $\pm$ 0.7	t=-13.72	<0.001

(Table 2 continued)

Species	C:N			
	Mean C:N-BULK±SD	Mean C:N-E±SD	Test statistic	<i>p</i>
Sharks				
<i>Carcharhinus falciformis</i>	2.8±0.2	3.1±0.1	V=1	0.500
<i>Carcharhinus signatus</i>	2.6±0.1	3.1±0.1	V=0	0.125
<i>Centrophorus</i> cf. <i>granulosus</i>	2.8±0.1	3.2±0.1	t=-13.64	<0.001
<i>Centrophorus niaukang</i>	2.7±0.1	3.2±0.1	t=-14.76	<0.001
<i>Etmopterus bigelowi</i>	2.8±0.2	3.2±0.1	V=0	0.006
<i>Galeocerdo cuvier</i>	2.8±0.2	3.1±0.1	V=0	0.008
<i>Hexanchus griseus</i>	5.1±1.5	3.2±0.1	V=45	0.004
<i>Mustelus canis</i>	2.7±0.1	3.1±0.1	V=0	0.036
<i>Squalus cubensis</i>	2.6±0.1	3.2±0.1	t=-21.66	<0.001
<i>Squalus</i> cf. <i>mitsukurii</i>	2.7±0.1	3.1±0.1	t=-21.16	<0.001
Hagfish				
<i>Eptatretus minor</i>	7.7±2.0	3.3±0.1	V=21	0.031
<i>Myxine mcmillanae</i>	3.9±0.2	3.2±0.1	t=13.18	<0.001
<i>Paramyxine springeri</i>	6.9±2.4	3.3±0.1	t=-13.72	<0.001

Table 3 Results for linear regression analysis of $\delta^{15}\text{N-E}$ by $\delta^{15}\text{N-BULK}$, %N-BULK, and C:N-BULK for deep-water shark and hagfish species. Starred values indicate a significant relationship. Significant values ($p < 0.05$) are bold.

Species	n	$\delta^{15}\text{N-BULK}$			%N-BULK			C:N-BULK		
		R^2	t	p	R^2	t	p	R^2	t	p
<i>Centrophorus cf. granulosus</i>	15	0.86	9.00	<0.001	0.22	-1.91	0.078	0.15	1.52	0.153
<i>Centrophorus niaukang</i>	18	<0.01	0.12	0.910	0.21	-1.91	0.078	0.10	-1.32	0.206
<i>Squalus cubensis</i>	14	0.76	6.23	<0.001	0.03	-0.65	0.526	0.11	1.23	0.242
<i>Squalus cf. mitsukurii</i>	16	0.34	2.71	0.017	<0.01	-0.14	0.887	0.06	1.43	0.357
<i>Myxine mcmillanae</i>	12	0.66	4.40	0.001	<0.01	-0.04	0.969	0.16	1.41	0.190
<i>Paramyxine springeri</i>	14	0.78	6.57	<0.001	0.13	1.32	0.210	0.07	-0.96	0.358

Table 4 Results for paired t-tests, including the mean difference ($\pm$ SD), comparing extracted and mathematically normalized $\delta^{15}\text{N}$ values in deep-sea sharks and hagfish. Significant values ($p < 0.05$) are bold. These represent situations where mathematical corrections performed poorly.

Species	Equation	n	Mean $\pm$ SD	<i>t</i>	<i>p</i>
<i>Centrophorus</i> cf. <i>granulosus</i>	$\delta^{15}\text{N-E} = 1.80 + 0.91 \times \delta^{15}\text{N-BULK}$	15	-0.16 $\pm$ 0.36	1.71	0.109
<i>Squalus cubensis</i>	$\delta^{15}\text{N-E} = 3.47 + 0.77 \times \delta^{15}\text{N-BULK}$	13	-0.14 $\pm$ 0.19	2.79	0.016
<i>Squalus</i> cf. <i>mitsukurii</i>	$\delta^{15}\text{N-E} = 7.67 + 0.42 \times \delta^{15}\text{N-BULK}$	16	0.05 $\pm$ 0.23	-0.83	0.418
<i>Myxine mcmillanae</i>	$\delta^{15}\text{N-E} = 6.36 + 0.62 \times \delta^{15}\text{N-BULK}$	12	0.14 $\pm$ 0.25	-1.86	0.089
<i>Paramyxine springeri</i>	$\delta^{15}\text{N-E} = 3.84 + 0.78 \times \delta^{15}\text{N-BULK}$	14	-0.36 $\pm$ 0.02	0.16	0.872

Figure 1 Average differences in $\delta^{15}\text{N}$, %N, and C:N (at%) values between chloroform/methanol extracted muscle tissue and bulk muscle tissue of sharks (circles) and hagfish (squares). Error bars represent standard deviation. Filled circles represent statistically significant differences (Table 2).

Figure 2 Relationship between mean $\Delta\delta^{15}\text{N}$ and mean $\delta^{15}\text{N}$ -BULK (A), %N-BULK (B) or C:N-BULK (C) values among all shark species.

Figure 3 Relationship between $\delta^{15}\text{N}$ -BULK and $\delta^{15}\text{N}$ -E in three species of sharks and two species of hagfish. Dotted lines indicate 95% confidence intervals for linear regressions.

CHAPTER III

TROPHIC INTERACTIONS OF DEEP-SEA SCAVENGERS IN THE GULF OF
MEXICO

Abstract

Scavengers often are important links in food webs and can be important to ecosystem dynamics through recycling energy and nutrients back into food webs quickly. The guild of scavengers is likely an important component of deep-sea food webs because these communities derive much of their energy resources via allochthonous inputs from surface waters in the form of photodetritus or discrete food-falls of marine carrion. Our aim was to examine the trophic interactions of mobile, benthic predator-scavengers in the deep-sea habitats of the northern and eastern Gulf of Mexico (GoM). We used stable isotope analysis (C and N) to investigate regional variation among common scavenger species, as well as intraspecific variation in trophic interactions. We were able to create taxa-specific mathematical corrections for $\delta^{13}\text{C}$ values to account for the high levels of lipids present in the sampled muscle tissue. We found significant spatiotemporal variation in $\delta^{13}\text{C}$ and/or $\delta^{15}\text{N}$ values within the most common species sampled (*Bathynomus giganteus*, *Chaceon quinquedens*, *Eptatretus springeri* and *Myxine mcmillanae*). There were no strong relationships between isotopic values and either depth or body size. We observed some variation in both carbon and nitrogen mean values among scavengers within each sampling region. However, there was also a moderate amount of isotopic niche overlap of individual species when comparing all individuals collected within each region. The present study provides an important characterization of the trophic interactions of deep-sea scavengers in the Gulf of Mexico and establishes system baselines for future investigations.

Introduction

Scavengers often are important links in food webs and can be important to ecosystem dynamics through recycling energy and nutrients back into food webs quickly. Large-bodied scavengers, which can form critical links in detrital pathways, vary from species that are largely obligate scavengers (e.g., vultures; Houston, 2001) to those that consume detritus opportunistically and primarily function as predators (e.g., wolves, lions, and hyenas; reviewed in Pereira et al., 2014). Much of this variation can be explained by the unpredictable nature of detritus, especially carrion, as a resource in space and time (Nowlin et al., 2008). For example, in the Serengeti, the availability of wildebeest carcasses, an important food source for scavengers, is highly variable, with the majority of deaths occurring during the 4-month dry season. Spatiotemporal variation in carrion or detritus availability could be an important force underlying the movement and spatial distributions of scavengers (Blazquez et al., 2009; Cortes-Avizanda et al., 2009). In both aquatic and terrestrial systems, scavenging can be involved in up to 45% of food web links, highlighting the potential for scavengers, and detrital food webs, to exert strong influences on ecosystem dynamics (Moore et al., 2004; Schmitz et al., 2008; Wilson & Wolkovich, 2011).

Scavengers are likely an important component of deep-sea food webs because these communities derive much of their energy resources via allochthonous inputs from surface waters (Meyers, 1997; Vetter & Dayton, 1999; Witte, 1999). These resources may be in the form of photodetritus (Billett et al., 1983), typically a mix of dead plankton, fecal pellets and bacteria (Gooday, 2002), or discrete food-falls of marine carrion (Smith 1985, Tyler et al. 1993). Bacteria and benthic invertebrates usually consume small particulate

matter (Drazen et al., 2008; Smith et al., 2002; Witte et al., 2003), while mobile benthic scavengers, such as crustaceans and hagfish, consume large food windfalls (Kemp et al., 2006; Witte, 1999). Because of the scarcity of food resources in deep-sea environments, many carnivores act as facultative scavengers, ingesting any organic material, living or dead, that is encountered (Thistle, 2003). Many large predators (e.g., sharks) in the deep-sea are likely to scavenge at least opportunistically (Compagno, 1984). The opposite is also true – species thought of as scavengers may also be active predators. For example, hagfish, traditionally thought to be scavengers, have been documented capturing teleosts (Zintzen et al., 2011). Geryonid crabs opportunistically feed on carcasses and small benthic organisms; larger crabs may also be capable of capturing demersal fish and squid (Domingos et al., 2008). The quick use of food resources by mobile benthic organisms facilitates the dispersal of nutrients throughout the deep-sea system and the incorporation of energy back into food webs.

Investigation of food webs in the deep-sea is challenging both in terms of sample collection and method of study. Low sample sizes, damaged samples, and gut eversion make traditional approaches, such as stomach content analysis, very difficult. An alternative approach to study trophic dynamics is stable isotope analysis. The stable isotope technique is becoming increasingly important in studies of trophic interactions (Fry, 2006). The method is able to provide information integrated over longer time frames on the basis of assimilated, rather than ingested, material (Peterson & Fry, 1987; Vander Zanden & Rasmussen, 2001). The method derives from the fact that isotopic ratios pass to consumers in predictable ways. Ratios of $^{15}\text{N}:$ ^{14}N relative to a standard ($\delta^{15}\text{N}$) can be used to estimate relative trophic position because ^{15}N is generally enriched

at each trophic transfer (Caut et al., 2009; Post, 2002) as a result of the preferential excretion of ^{14}N by organisms. Ratios of $^{13}\text{C}:^{12}\text{C}$ relative to a standard ($\delta^{13}\text{C}$) are related to the photosynthetic pathways of primary production in an individual's diet and foraging location (del Rio et al., 2009; Post, 2002). Since carbon ratios are generally conserved through trophic transfers (Fry & Sherr, 1989), $\delta^{13}\text{C}$ values can be used to elucidate sources of primary production across many trophic levels. However, three important drawbacks for stable isotope analysis are the inability to identify specific prey items, the possibility that multiple trophic pathways can arrive at the same $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, and the high degree of variability in discrimination factors among taxa (Kim et al. 2012).

The goal of the present study was to examine the trophic interactions of mobile, benthic scavengers in the deep-sea habitats of the northern and eastern Gulf of Mexico (GoM). We used stable isotope analysis (C and N) to investigate regional variation among common predator-scavenger species, as well as intraspecific variation in trophic interactions. In particular, we sought to provide baseline data, which may be used to understand variation in a deep-sea system and provide a benchmark for future comparisons.

Materials and methods

Study Site

Sampling took place along the continental slope off the south-central Gulf coast of Florida to Louisiana (Figure 1). The southern sampling sites were located on the West Florida Slope (WFS), approximately 270 km southeast of Tampa, Florida. Stations were concentrated on the continental slope in depths of approximately 250 to 2000 m, however

all animals used in this study were captured at depths shallower than 1600 m. The northern sites were spread across the northern slope of the Gulf (NGS) from an area approximately 25 km offshore of the Mississippi River (MR) delta through DeSoto Canyon (DC) and east onto the north Florida slope to an area 85 km southwest of Cape San Blas, Florida (Figure 1). These locations included sites on the continental slope to the east and west of DeSoto Canyon, along the canyon's eastern rim, and on the canyon floor. Depth ranges for these stations were approximately 200 to 2000 m. Sampling stations at the northern and southern sites were chosen to match depth and topography along a broad shelf and onto the slope. Samples from the NGS-MR region were ca. 35km from the site of the Deepwater Horizon oil spill, which occurred from April 20 to September 19, 2010.

Sampling Methods

Hagfish, isopods and crabs were collected as part of a larger fishery-independent survey during research cruises aboard the R/V *Weatherbird II* in April 2011, August 2011, and April 2012. Additional samples were obtained from an April 2005 cruise to the WFS. Each cruise in 2011-2012 included sampling in both the NGS (39 stations and 85 total sets) and the WFS (14 stations and 68 total sets). Sampling involved deploying two or three successive sets of demersal longlines with three integrated traps: a small box trap (60 cm x 60 cm x 38 cm with 2.5 cm mesh), a cylindrical trap (60 cm x 35 cm with 1.0 cm mesh) designed to capture hagfish and a small shrimp trap (40 cm x 20 cm x 15 cm with 1.0 cm mesh). All traps were baited with menhaden (*Brevoortia* sp.) or northern mackerel (*Scomber scombrus*). Each set was deployed within a 30 minute period and

retrieved following a soak of 4-8 hr. For additional sampling protocols, see Churchill et. al (Churchill et al., 2015b).

Once the animals were on board, each individual was identified to species, weighed and measured. We collected muscle from the dorsal region of the hagfish using clean scalpel blades. Samples were immediately frozen for later processing. All invertebrates were frozen whole for later processing.

Stable Isotope Analysis

Tissues used for stable isotope analyses were prepared using standard procedures. All hagfish muscle tissue was thawed and then rinsed briefly in deionized water. Invertebrate specimens were thawed and muscle tissue was removed from one or more legs (crabs) or plucked from the tail (shrimp). All tissue was then dried a minimum of 48 hours in a 60°C oven and ground to a fine powder. Homogenized samples were weighed out to the nearest microgram and packed into tin capsules. Samples were then analyzed on a ThermoFinnigan Delta C mass spectrometer at the Florida International University SERC Stable Isotope Laboratory to determine $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The error of the isotope-ratio mass spectrometer derived from repeat analyses of an internal glycine standard was less than 0.1‰ (n=80) during the present study. Approximately one-sixth of the samples were run in duplicate. The mean ($\pm$ standard deviation) absolute difference between duplicates was 0.1 ± 0.1 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. All reported C:N ratios use atomic mass values.

Many marine organisms, including crustaceans, have hard structural parts partially composed of inorganic carbonates. Inorganic carbon is generally enriched in

^{13}C (Fritz & Poplawski, 1974), leading to higher $\delta^{13}\text{C}$ values than might be observed from diet alone. Often, samples are washed or fumigated with hydrochloric acid prior to analysis to remove non-dietary inorganic carbon. Acidification may be avoided if the organism is sufficiently large to dissect out muscle tissue, allowing for carbonate-free samples (Mateo et al., 2008). Removal of muscle tissue was possible with all crustaceans included in this study. Consequently, no muscle tissue was acidified prior to carbon isotope measurements. Moreover, acidification can result in unpredictable variation in $\%C$ and $\delta^{13}\text{C}$ values (Brodie et al., 2011) and might have negatively impacted subsequent analyses.

Lipids tend to be depleted in ^{13}C (DeNiro & Epstein, 1977; Tieszen et al., 1983) relative to protein, and extractions are recommended for samples where the C:N ratio > 3.5 (Post et al., 2007). Therefore, a subset of muscle samples from each species was lipid extracted ($n=20$ or all collected samples with $C:N>3.5$ if $n<20$ per species). Samples were chosen to encompass the total range of C:N ratios of unextracted muscle tissue present in all sampled individuals within each species. Dried, powdered samples were immersed in a mixture of chloroform and methanol (2:1) with a solvent volume three to five times greater than that of the sample. Samples were then vortexed for 30 seconds, left undisturbed for at least 30 minutes and centrifuged for 10 min at 1318 g. The extraction process was repeated at least three times or until the supernatant was clear (Bligh & Dyer, 1959; Logan et al., 2008). Samples were then re-dried, weighed and analyzed for carbon isotopic composition.

For species where a large number of individuals ($n>20$) had high muscle lipid content, we created mathematical normalizations to correct $\delta^{13}\text{C}$ values. To make a

correction, we used three possible models to predict the change in $\delta^{13}\text{C}$ values after lipid extraction ($\Delta\delta^{13}\text{C}$) for whole muscle (BULK) C:N ratio.

(1) Linear: $y = a + bx$

(2) Michaelis-Menten: $y = \frac{ax}{1+bx}$

(3) Logarithmic: $y = b + a \ln(x)$

These models were selected because of previous use for lipid normalization of $\delta^{13}\text{C}$ values (Ehrich et al., 2011; Hoffman & Sutton, 2010; Post et al., 2007). We used Akaike's information criterion with a second order bias correction (AICc) to select the most parsimonious relationship among variables by selecting the model with the lowest AICc value. These species-specific equations were used to generate lipid-normalized $\delta^{13}\text{C}$ values for all individuals where C:N-BULK>3.5 and lipid extracted (LE) $\delta^{13}\text{C}$ values were not available. We also compared our mathematical normalizations to those presented by Post et al. (2007) and Hoffman and Sutton (2010) using either a Student's t-test or a Wilcoxon signed rank test, if sample sizes were small.

The muscle tissue of hagfish contains high levels of trimethylamine oxide (TMAO; reviewed in Currie & Edwards, 2010). The TMAO, a byproduct of metabolism, is expected to be depleted in ^{15}N , creating lower $\delta^{15}\text{N}$ values and subsequent lower trophic level estimates than in organisms that lack TMAO. We used chloroform/methanol extractions to remove TMAO from hagfish muscle tissue prior to analysis. For individual hagfish where extracted $\delta^{15}\text{N}$ values were not available, primarily because of logistical constraints of large sample sizes, we used species-specific mathematical normalizations developed for these species (Churchill et al., 2015a). We report and use normalized $\delta^{15}\text{N}$ values for all hagfish.

We used analysis of variance and Student's *t*-tests to examine intraspecific spatial and temporal variation in stable isotope values. Where necessary, because of small or unbalanced sample sizes, we used a Wilcoxon signed rank test (test statistic *V*) instead of a Student's *t*-test. Because of small sample sizes and variation in the abundance of common species, we were not able to complete full factorial analyses. For *Bathynomus giganteus* (Giant Isopod), *Chaceon quinquedens* (Red Deep-Sea Crab), *Eptatretus springeri* (Gulf Hagfish), and *Myxine mcmillanae* (Caribbean Hagfish) we were able to investigate variation between sampling regions. For *B. giganteus*, we were able to compare individuals from the NGS-DC and WFS regions across all 2011-2012 sampling times and from the NGS-MR region in August 2011 and April 2012. We were able to compare *C. quinquedens* individuals collected across all 2011-2012 sampling times in the NGS-DC, April 2012 in the NGS-MR and April 2011 and 2012 in the WFS. For *E. springeri*, we compared individuals from all 2011-2012 sampling times in NGS-DC, August 2011 and April 2011 in the NGS-MR, and April 2011 in the WFS. We were able to examine temporal differences of stable isotope values within specific sampling regions for *B. giganteus*, *Chaceon fenneri* (Golden Deep-Sea Crab), *C. quinquedens*, *E. springeri*, *Heterocarpus ensifer* (Armed Nylon Shrimp), and *M. mcmillanae*.

We used linear regression to investigate the effects of body size on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within species where sample size was ≥ 25 . We used the following measurements for size: total length (isopods), carapace width (crabs) and carapace length (shrimp). We also used linear regression to investigate the relationship between mean body size (mass) and mean $\delta^{15}\text{N}$ values across all deep-sea scavenger species collected as part of the present study. The analysis was done separately for each sampling region. Finally, linear

regression was used to assess the effects of capture depth on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within each species with adequate sample sizes ($n \geq 25$). For species with significant spatiotemporal variation in stable isotope values, we limited our analyses to specific sampling regions and/or times to reduce the effect of possible confounding variables.

For interspecific comparisons, we used one-way analysis of variance with a Tukey's *post-hoc* test for paired contrasts. Because a full factorial analysis was not possible, we pooled individuals of the same species from different times (April 2005, April 2011, August 2011, April 2012). Species with less than five captured individuals were not included in analyses. The analysis was performed separately for the NGS-MR, NGS-DC and WFS sampling regions.

We used metrics presented in Layman et al. (2007) to compare community-level trophic interactions in each sampling region (NGS-DC, NGS-MR, and WFS). Total area (TA) is an indication of the isotopic niche width of a population or community in isotopic bi-plot space. The $\delta^{13}\text{C}$ range is an estimate of the diversity of basal resources and the $\delta^{15}\text{N}$ range provides an estimate of diversity of trophic levels of the species when at the population scale. The TA metric is sensitive to differences in sample size. To account for this, we present the stable isotope Bayesian ellipses for each species (Jackson et al., 2011), which provides an estimate of the core isotopic niche width of a species. We also calculated the percent of TA and Bayesian ellipse that were unique to each species in each region. All stable isotope metrics were calculated using the SIAR package (Parnell & Jackson, 2013).

Unless otherwise noted, all data were normally distributed and equal in variance. All analyses were completed in R, version 3.0.2 (R Core Team, 2013).

Results

A total of 1263 individual scavengers from six species were captured at depths ranging from *ca.* 200 to 1100 meters (Figure 2). The size distributions of the six most common species are shown in Figure 3.

From these individuals, we collected and processed 519 muscle tissue samples (Table 1). All but one species (*Acanthephyra* sp.) had individuals with C:N-BULK>3.5, indicating the necessity for lipid extractions (Figure 4). A total of 109 muscle tissue samples were lipid-extracted and reanalyzed (*B. giganteus*: n=18, *C. fenneri*: n=3, *C. quinquedens*: n=7, *E. minor*: n=4, *E. springeri*: n=41, *H. ensifer*: n=8, and *M. mcmillanae*: n=28). The $\delta^{13}\text{C}$ -LE values were significantly different from values generated using the equation proposed by Post et al. (2007) in *C. quinquedens* ($V=0$, $p=0.016$), *E. springeri* ($t=3.18$, $p<0.01$), *H. ensifer* ($V=36$, $p<0.01$), and *M. mcmillanae* ($t=7.76$, $p<0.01$) but not in *B. giganteus* ($t=1.06$, $p=0.30$), *C. fenneri* ($V=5$, $p=0.5$), and *E. minor* ($V=3$, $p=1$). We also found significant differences between the $\delta^{13}\text{C}$ -LE values and those generated by the equation proposed by Hoffman and Sutton (2010) in *B. giganteus* ($t=6.64$, $p<0.01$), *H. ensifer* ($V=36$, $p<0.01$), and *M. mcmillanae* ($t=13.35$, $p<0.01$) but not in *C. fenneri* ($V=6$, $p=0.25$), *C. quinquedens* ($V=19$, $p=0.47$), *E. minor* ($V=6$, $p=0.25$), and *E. springeri* ($t=0.69$, $p=0.49$).

We were able to create lipid normalization models for *B. giganteus*, *E. springeri*, *H. ensifer*, and *M. mcmillanae* using linear, Michaelis-Menten, and Logarithmic models (Table 2). All models were statistically significant, but we chose the best on the basis of the lowest AICc value. For *B. giganteus*, this was the logarithmic model; for *H. ensifer* and *E. springeri*, the Michaelis-Menten model; and for *M. mcmillanae*, the linear model.

These species-specific models were used to generate lipid-normalized $\delta^{13}\text{C}$ values for any individuals where C:N-BULK > 3.5 and lipid extractions were not performed. For all individuals with a C:N ratio of less than 3.5, the $\delta^{13}\text{C}$ of bulk muscle tissue was used for all analyses.

We found significant intraspecific spatial and temporal variation in stable isotope values. *Bathynomus giganteus* differed between NGS-DC, NGS-MR and WFS sampling regions in $\delta^{13}\text{C}$ ($F=9.51$, $p<0.01$) and $\delta^{15}\text{N}$ values ($F=25.16$, $p<0.01$; Figure 5). Post-hoc analyses revealed that individuals from the NGS-MR possessed $\delta^{13}\text{C}$ values ca. 0.5‰ lower than in the NGS-DC and WFS. The NGS-MR had the highest $\delta^{15}\text{N}$ values (14.6‰), followed by NGS-DC and then WFS. Among *C. quinquedens* individuals in the NGS-DC and WFS regions, $\delta^{13}\text{C}$ values were lower in the NGS-DC (0.4‰, $W=314.5$, $p=0.009$) but $\delta^{15}\text{N}$ values were higher in the northern region (0.7‰, $W=320$, $p=0.01$). *Eptatretus springeri* caught in the NGS-MR possessed higher $\delta^{15}\text{N}$ values than those in the NGS-DC (0.9‰, $W=283$, $p<0.01$) but there were no observed differences in $\delta^{13}\text{C}$ values ($W=686.5$, $p=0.16$). *M. mcmillanae* from the NGS-MR also had higher $\delta^{15}\text{N}$ values than in the NGS-DC (0.8‰, $W=35.5$, $p=0.03$) but no difference was seen in $\delta^{13}\text{C}$ values ($W=97$, $p=0.68$). We detected significant temporal variation among three (*B. giganteus*, *C. quinquedens*, and *E. springeri*) out of six species of deep-sea scavengers (Table 3) but there was no consistent pattern across species from 2011 to 2012. We also found significant spatiotemporal variation in isotopic values when comparing multiple sampling times across all sampling regions in *B. giganteus* ($\delta^{13}\text{C}$: $F=14.18$, $p<0.01$; $\delta^{15}\text{N}$: $F=12.63$, $p<0.01$), *C. quinquedens* ($\delta^{13}\text{C}$: $F=7.85$, $p<0.01$; $\delta^{15}\text{N}$: $F=3.38$, $p=0.02$), and *E. springeri* ($\delta^{13}\text{C}$: $F=3.90$, $p=0.01$; $\delta^{15}\text{N}$: $F=11.42$, $p<0.01$; Figure 5).

We were able to examine the relationship between body size and isotopic values in six species of scavengers (Table 4; Figure 6). *Chaceon fenneri* and *M. mcmillanae* had no relationship between size and either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values. *Bathynomus giganteus* displayed a positive relationship between total length and $\delta^{13}\text{C}$ values, but a negative relationship to $\delta^{15}\text{N}$ values. *Eptatretus springeri* showed positive relationships between total length and both $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values. *Chaceon quinquedens* had a positive relationship between carapace width and $\delta^{13}\text{C}$ values, but no relationship for $\delta^{15}\text{N}$ values. *Heterocarpus ensifer* had a positive relationship between carapace length and $\delta^{15}\text{N}$ values, but not with $\delta^{13}\text{C}$ values. These relationships, though significant, were quite weak ($R^2 < 0.3$), indicating that much of the variation among individuals is not a result of ontogenetic shifts in diets or foraging locations.

We were able to examine the relationship between capture depth and isotopic values within six scavenger species (Table 5). *Bathynomus giganteus* displayed a weak (i.e., low R^2) but positive relationship between depth and $\delta^{13}\text{C}$ values, but no relationship with $\delta^{15}\text{N}$ values. *Heterocarpus ensifer* and *E. springeri* had weak but significant negative relationships between depth and $\delta^{15}\text{N}$ values, but no relationship with $\delta^{13}\text{C}$ values. *Chaceon fenneri*, *C. quinquedens*, and *M. mcmillanae* showed no relationship between depth and either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values.

There was significant variation among $\delta^{13}\text{C}$ values in the NGS-MR, NGS-DC and WFS sampling regions ($F=38.05$, $p<0.01$) with the NGS-MR displaying the highest $\delta^{13}\text{C}$ values, followed by the NGS-DC and then the WFS. We also observed significant differences among $\delta^{15}\text{N}$ values among the three sampling regions ($F=61.25$, $p<0.01$) than the WFS region. Similar to what was observed in individual species, the NGS-MR

possessed the highest $\delta^{15}\text{N}$ values, followed by the NGS-DC and then the WFS. Both the NGS-DC and WFS regions had similar $\delta^{13}\text{C}$ (NGS-DC: 3.8‰, WFS: 3.3‰) and $\delta^{15}\text{N}$ ranges (NGS-DC: 6.6‰, WFS: 6.9‰). However, the NGS-MR displayed reduced $\delta^{13}\text{C}$ (1.7‰) and $\delta^{15}\text{N}$ ranges (4.5‰) when compared to the other two regions.

At the community level, there was no significant relationship between mean body size and mean $\delta^{15}\text{N}$ values in either the NGS-DC ($t=1.16$, $p=0.27$; Figure 7b) or WFS sampling region ($t=0.11$, $p=0.92$; Figure 7c). However, there was a significant correlation in the NGS-MR region ($t=0.11$, $p=0.92$; Figure 7a)

We observed significant variation in isotopic values in all sampling regions. In the NGS-MR region, there were significant differences among species in $\delta^{15}\text{N}$ values ($F=9.97$, $p<0.001$) but not in $\delta^{13}\text{C}$ values ($F=1.45$, $p=0.25$; Figure 8a). *Myxine mcmillanae* had the highest $\delta^{15}\text{N}$ value in this region, but post-hoc analysis showed that there was no significant difference between this species and *B. giganteus*. In the NGS-DC region, there was significant variation among species in both $\delta^{13}\text{C}$ ($F=37.77$, $p<0.01$) and $\delta^{15}\text{N}$ values ($F=144.6$, $p<0.01$; Figure 8b). *Myxine mcmillanae* had the highest average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in this region. Of the species with enough individuals for inclusion in the analyses, *C. quinquedens* had the lowest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values; however, the single *Acanthephyra* sp. individual had the lowest overall isotopic values. In the WFS region, there was also significant variation among species in both $\delta^{13}\text{C}$ values ($F=87.7$, $p<0.01$) and $\delta^{15}\text{N}$ values ($F=376.2$, $p<0.01$; Figure 8c). Among species collected in the southern sampling region, *B. giganteus* had the highest average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, while *H. ensifer* had the lowest.

In agreement with the post-hoc analysis of the interspecific variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, we observed some overlap among the isotopic niche spaces of species within the NGS-DC, NGS-MR and WFS sampling regions, as defined by the TA and Bayesian ellipse metrics (Table 6; Figure 9). In the NGS-MR region, *B. giganteus* and *C. quinquedens* had >60% unique area, with 68% and 93% unique ellipse areas, respectively. *M. mcmillanae* only displayed 2.5% unique TA, but 47% unique ellipse area. In the NGS-DC region, *B. giganteus* and *M. mcmillanae* had >50% unique TA and >80% unique ellipse area. *Chaceon quinquedens* and *E. minor* displayed the highest amount of isotopic niche overlap with other species, with <10% unique TA, but 42% and 56% unique ellipse area, respectively. In the WFS region, *B. giganteus* and *H. ensifer* both had >90% unique TA and ellipse space. *E. springeri* and *C. fenneri* had <30% unique TA and <45% unique ellipse area, indicating a high amount of niche overlap with other species.

Discussion

$\delta^{13}\text{C}$ normalizations

Many deep-sea organisms possess high lipid content, and our results highlight the necessity of normalizing $\delta^{13}\text{C}$ values either by chemical extraction or mathematical correction in order to make suitable comparisons among multiple taxa. We observed differences between $\delta^{13}\text{C}$ -LE values and those generated using the Post et al. (2007) equation proposed for aquatic organisms in four out of six species (*C. quinquedens*, *E. springeri*, *H. ensifer*, and *M. mcmillanae*). We also observed difference between $\delta^{13}\text{C}$ -LE values and those generated by an equation for normalizing deep-sea fish values in three

species (*B. giganteus*, *H. ensifer*, and *M. mcmillanae*). Lipid content can be highly variable among taxa (Hoffman & Sutton, 2010; Post et al., 2007). While previous work supports the idea that C:N values of protein and $\Delta\delta^{13}\text{C}$ may be similar across aquatic organisms (Fry et al., 2003; Kiljunen et al., 2006; Schlechtriem et al., 2003; Sweeting et al., 2006), the broad level of taxa included in the present study warrants separate treatments, particularly for those species with high lipid content (C:N > 8). Our results suggest taxa-specific mathematical normalizations are required to correct $\delta^{13}\text{C}$ values of deep-sea organisms rather than employing previously published equations.

Intraspecific patterns

We found significant spatial variation in carbon and nitrogen stable isotope values among the most common scavenger species sampled in the present study. Individuals of *B. giganteus*, *C. quinquedens*, *E. springeri* and *M. mcmillanae* captured in the NGS-MR sampling region all had higher $\delta^{15}\text{N}$ values than those in the NGS-DC, likely because of baseline differences driven by riverine inputs. The Mississippi River delivers large amounts of organic material to the northern GoM, stimulating high levels of biological production (Dagg & Breed, 2003). The additional inputs to the system may increase nutrient cycling in the region, with subsequent increases in region-wide $\delta^{15}\text{N}$ values. We also observed differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between NGS-DC and WFS in *C. quinquedens*, which may be the result of trophic variation or baseline differences between sampling regions. *Bathynomus giganteus* individuals from the NGS-MR sampling region had slightly higher $\delta^{13}\text{C}$ values ($\sim 0.5\text{‰}$) than those observed in the NGS-DC or WFS. Higher $\delta^{13}\text{C}$ values are unlikely to be the result of riverine inputs,

which typically have lower $\delta^{13}\text{C}$ values (-23.8‰ to -26.8‰; Wang et al., 2004) than offshore GoM waters (-23.0‰ to -22.4‰; Wells & Rooker, 2009), but could be the result of *Sargassum* sp. (-16.6‰ to -16.2‰; Wells & Rooker, 2009) or outwelled coastal seagrass (-13.4‰ to -11‰; Moncreiff & Sullivan, 2001). We found very little evidence for isotopic variation associated with vertical distribution, inferred from capture depth. Only three species (*B. giganteus*, *H. ensifer* and *E. springeri*) of six displayed significant relationships between depth and $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values and all of these relationships were weak ($R^2 < 0.2$).

We observed temporal differences in stable isotope values in three species of deep-sea scavengers. A decline in $\delta^{15}\text{N}$ values over time (2005, 2011-2012) was only seen in *B. giganteus* found in the WFS sampling region, which may be representative of some changes in trophic position rather than baseline changes within the region as these differences were observed in no other species. Trophic position for all other sampled species in the NGS-MR, NGS-DC and WFS sampling regions appears to be fairly constant, as inferred through nitrogen stable isotope analyses. These results are similar to those observed in deep-sea sharks collected during the same sampling bouts (Churchill et al., 2015b). All but one species of shark (*Squalus* cf. *mitsukurii*) had similar $\delta^{15}\text{N}$ values between 2011 and 2012 (Churchill et al., 2015b). We recorded seasonal changes in $\delta^{13}\text{C}$ in *B. giganteus* in the WFS and *C. quinquedens* and *E. springeri* in the NGS-DC. Shifts in carbon values could be attributed to the seasonality associated with carbon inputs to the deep sea in the GoM (Rowe, 2013); however, the differences were usually small ($< 0.5\text{‰}$) and may not be biologically relevant. *Bathynomus giganteus* collected in the

WFS exhibited a decrease in $\delta^{13}\text{C}$ values over time, supporting the idea that this regional population may have experienced changes in trophic interactions over time.

There were no strong relationships between body size and either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values for any of the species collected in this study. Indeed, body size explained less than 25% of the variance in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The lack of strong relationships may be attributed to both feeding habits and age class at capture. Most of the collected species did not have a large range in size at capture, indicating that most or all of the individuals were part of the same age class. Any ontogenetic shift in diet observable through stable isotope analysis likely occurred in sizes smaller than were sampled in this study. In addition, most of these species are facultative scavengers (Barradas-Ortiz et al., 2003; Domingos et al., 2008; Martini, 1998) and are likely eating whatever food is available to them, creating variation determined by a suite of other factors beyond size, especially food availability.

Ecological Dynamics

Stable isotope analysis of this deep-sea community in the GoM revealed a relatively small range of $\delta^{13}\text{C}$ values (NGS-DC: 3.83‰; NGS-MR: 1.75‰; and WFS: 3.32‰) that likely represent a limited pool of basal resources. Possible carbon sources include plankton and detritus (-23.0‰ to -22.4‰; Wells & Rooker, 2009), *Sargassum* sp. (-16.6‰ to -16.2‰; Wells & Rooker, 2009), out-welled coastal seagrass (-13.4‰ to -11‰; Moncreiff & Sullivan, 2001), terrestrial sources via riverine inputs (-23.8‰ to -26.8‰; Wang et al., 2004), organic components in the sediment (-21.5‰ to -20‰; Gearing et al., 1977), whale falls (-32‰ to -20‰; Smith & Baco, 2003), and deep-sea

chemosynthetic communities (-65‰ to -25‰; MacAvoy et al., 2008). Given the very negative $\delta^{13}\text{C}$ values present in chemosynthetic systems, it seems likely that the deep-sea scavengers from the areas of the Gulf we sampled are reliant on allochthonous inputs from surface waters. The importance of photosynthetically derived inputs is supported by previous work that found both *B. giganteus* and *Eptatretus* species reliant on primarily photosynthetically derived nutrients using both $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ (MacAvoy et al., 2002). The $\delta^{13}\text{C}$ values observed in the scavengers (-18.8‰ to -14.5‰) compared to planktonic and detrital sources may reveal higher levels of nutrient recycling between surface production and benthic communities or large inputs of *Sargassum*.

There were larger ranges of $\delta^{15}\text{N}$ values across deep-sea scavenger species in the NGS-DC (6.57‰), NGS-MR (4.46‰) and WFS (6.91‰) sampling regions. On the basis of an average trophic enrichment factor of 2.75‰ (Caut et al., 2009), this group spans 1-2 trophic levels. However, using a crustacean-specific factor of 2.0‰ (Vanderklift & Ponsard, 2003) suggests that the span of trophic levels may be 2-3 levels. To our knowledge, no data are currently available on the trophic discrimination factors of hagfish. In the NGS region, two species of hagfish (*E. minor* and *M. mcmillanae*) had the most enriched $\delta^{15}\text{N}$ values (14.3‰ and 14.5‰, respectively). These values were higher than many of the elasmobranch species sampled in the same region, including two dogfish sharks in the genus *Squalus* (13.0‰ and 13.1‰); the gulper shark, *Centrophorus granulosus* (13.5‰); and the broadnose sixgill shark, *Hexanchus griseus* (14‰; Churchill et al., 2015b). Nitrogen isotopic values among deep-sea sharks and benthic scavengers suggest that these organisms are possibly consuming, either through predation or scavenging, similar food items, despite large differences in body size. Hagfish have

been documented capturing small teleosts (Zintzen et al., 2011), but we were unable to locate any additional information regarding the diets of these species. On the basis of $\delta^{15}\text{N}$ values and assuming a trophic enrichment factor of ca. 3.0‰, hagfish may also be utilizing carrion falls of larger animals including whales (11.2‰ to 13.5‰; Ruiz-Cooley et al., 2012), dolphins (12.5‰ to 14.0‰; Barros et al., 2010), or pelagic teleosts such as tuna or swordfish (12.1‰, unpublished data).

In the WFS region, *B. giganteus* had the highest $\delta^{15}\text{N}$ values (13.5‰). It was certainly the most numerically dominant scavenger in this region, accounting for ca. 80% of all invertebrates captured in traps, but it may also occupy the highest trophic position, on the basis of nitrogen isotopic values. Previous work on giant isopods in the southern GoM indicates that the most frequent diet components are fish, crustaceans, and cephalopods (Barradas-Ortiz et al., 2003). In both the NGS and WFS sampling regions, the shrimp, *Acantheephyra* sp. and *H. ensifer*, had the lowest $\delta^{15}\text{N}$ values, which suggests that these organisms are feeding at a somewhat lower trophic position than other benthic scavengers. While no dietary data exist for these specific species, *Acantheephyra* species often feed on macroplankton, such as euphausiids, cnidarians, and chaetognaths (Cartes et al., 2007), as well as small mesopelagic fish and bathypelagic crustaceans (Cartes, 1993). Congeners of *H. ensifer*, have been found to consume euphausiids, foraminiferans, sponges, detritus, other small benthic invertebrates and mesopelagic fishes (Rainer, 1992; Rajasree & Kurup, 2011). If the diets of the shrimp species collected in the deep waters of the GoM are similar, it may explain the small $\delta^{15}\text{N}$ values we observed.

Given the limited number of possible nutrient sources, it is not surprising that we detected some degree of interspecific overlap in isotopic values. Several species

occupied little unique isotopic niche space, despite some differences in mean isotopic values. Because of their similar body sizes it was surprising that, in the NGS-DC region, *B. giganteus* displayed no overlap in ellipse area (i.e., core isotopic niche width) with either hagfish species. *Bathynomus giganteus* and *E. springeri* share similar $\delta^{13}\text{C}$ values, but the giant isopods have higher $\delta^{15}\text{N}$ values than the hagfish, indicating that they typically feed at a higher trophic position but from food webs with similar basal resources. *Bathynomus giganteus* and *M. mcmillanae* share similar $\delta^{15}\text{N}$ values in ellipse area but display different $\delta^{13}\text{C}$ values. This overlap pattern suggests that these two species may be consuming items originating from different basal resources. The amount of unique area was somewhat higher in the WFS sampling region, which could indicate a higher level of resource partitioning by benthic scavengers in this region. Most notably, *H. ensifer* individuals from the WFS had the lowest $\delta^{13}\text{C}$ values in this study. It also appears that *H. ensifer* is also feeding at least one trophic level lower than most of the other deep-water scavengers in the GoM. The lower carbon values present in this species are likely the result of less trophic discrimination of carbon isotopes and not a different basal resource, assuming typical increase in carbon and nitrogen isotopic values of ca. 1‰ and 3‰, respectively, per trophic level.

Conclusions

Investigations of trophic interactions of organisms in deep-water environments have been limited by the difficulty of obtaining samples in a remote system. Here, using stable isotope analysis, we have been able to provide a preliminary characterization of the trophic structure of the benthic scavenger community in deep-sea habitats of the GoM.

Sampling of additional taxa, along with the application of additional biochemical markers, may reveal additional details regarding the sources of energy and nutrients input into the deep-water communities in the GoM and how these resources transfer to the deep-sea community. Such studies are of high priority given the increasing exploitation of deep-sea resources, the potential for anthropogenic impacts to these communities, and the still early stage of our understanding of these communities.

Acknowledgements

This research was made possible by two grants from BP/The Gulf of Mexico Research Initiative through the Florida Institute of Oceanography as well as a grant to the Deep-C Consortium. The 2005 research cruise was funded by a Florida Institute of Oceanography Shiptime Grant and FIU's Department of Biological Sciences. Additional financial support was provided by a FIU Graduate School Dissertation Year Fellowship. We thank Chip Cotton and Ron Schatman for logistical support, the staff of the FIU Stable Isotope Laboratory, and all of the research cruise volunteers.

References

- Barradas-Ortiz, Cecilia, Briones-Fourzán, Patricia, & Lozano-Álvarez, Enrique. (2003). Seasonal reproduction and feeding ecology of giant isopods *Bathynomus giganteus* from the continental slope of the Yucatan peninsula. *Deep Sea Research Part I: Oceanographic Research Papers*, 50(4), 495-513.
- Barros, Nélío B, Ostrom, PH, Stricker, CA, & Wells, RS. (2010). Stable isotopes differentiate bottlenose dolphins off west-central Florida. *Marine Mammal Science*, 26(2), 324-336.

- Billett, D. S. M., Lampitt, R. S., Rice, A. L., & Mantoura, R. F. C. (1983). Seasonal sedimentation of phytoplankton to the deep-sea benthos. *Nature*, 302(5908), 520-522.
- Blazquez, Miguel, Sanchez-Zapata, Jose A., Botella, Francisco, Carrete, Martina, & Eguia, Sergio. (2009). Spatio-temporal segregation of facultative avian scavengers at ungulate carcasses. *Acta Oecologica-International Journal of Ecology*, 35(5), 645-650.
- Bligh, E.S., & Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911-917.
- Brodie, Chris R, Leng, Melanie J, Casford, James SL, Kendrick, Christopher P, Lloyd, Jeremy M, Yongqiang, Zong, & Bird, Michael I. (2011). Evidence for bias in C and N concentrations and $\delta^{13}\text{C}$ composition of terrestrial and aquatic organic materials due to pre-analysis acid preparation methods. *Chemical Geology*, 282(3), 67-83.
- Cartes, J. E. (1993). Feeding habits of Oplophorid shrimps in the deep western Mediterranean. *Journal of the Marine Biological Association of the United Kingdom*, 73(1), 193-206.
- Cartes, J. E., Huguet, C., Parra, S., & Sanchez, F. (2007). Trophic relationships in deep-water decapods of Le Danois bank (Cantabrian Sea, NE Atlantic): Trends related with depth and seasonal changes in food quality and availability. *Deep-Sea Research Part I-Oceanographic Research Papers*, 54(7), 1091-1110.
- Caut, S., Angulo, E., & Courchamp, F. (2009). Variation in discrimination factors ($\Delta^{15}\text{N}$ and $\Delta^{13}\text{C}$): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology*, 46(2), 443-453.
- Churchill, Diana A., Heithaus, Michael R., & Dean Grubbs, R. (2015a). Effects of lipid and urea extraction on $\delta^{15}\text{N}$ values of deep-sea sharks and hagfish: Can mathematical correction factors be generated? *Deep Sea Research Part II: Topical Studies in Oceanography*, 115(0), 103-108.
- Churchill, Diana A., Heithaus, Michael R., Vaudo, Jeremy J., Grubbs, R. Dean, Gastrich, Kirk, & Castro, José I. (2015b). Trophic interactions of common elasmobranchs in deep-sea communities of the Gulf of Mexico revealed through stable isotope

and stomach content analysis. *Deep Sea Research Part II: Topical Studies in Oceanography*, 115(0), 92-102.

Compagno, L.J.V. (1984). *Sharks of the World: Hexanchiformes to Lamniformes*: United Nations Development Programme.

Cortes-Avizanda, Ainara, Selva, Nuria, Carrete, Martina, & Donazar, Jose A. (2009). Effects of carrion resources on herbivore spatial distribution are mediated by facultative scavengers. *Basic and Applied Ecology*, 10(3), 265-272.

Currie, S., & Edwards, S. L. (2010). The curious case of the chemical composition of hagfish tissues-50 years on. *Comparative Biochemistry and Physiology Part A Molecular & Integrative Physiology*, 157(2), 111-115.

Dagg, M. J., & Breed, G. A. (2003). Biological effects of Mississippi River nitrogen on the northern gulf of Mexico - a review and synthesis. *Journal of Marine Systems*, 43(3-4), 133-152.

del Rio, C. M., Wolf, N., Carleton, S. A., & Gannes, L. Z. (2009). Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews (Cambridge)*, 84(1), 91-111.

DeNiro, M.J., & Epstein, S. (1977). Mechanisms of carbon isotope fractionation associated with lipid synthesis. *Science*, 197, 261-263.

Domingos, S. S., Athie, A. A. R., & Rossi-Wongtschowski, Cldb. (2008). Diet of *Chaceon ramosae* (Decapoda, Brachyura) on the Southern Brazilian Exclusive Economic Zone. *Brazilian Journal of Oceanography*, 56(1), 59-63.

Drazen, J. C., Phleger, C. F., Guest, M. A., & Nichols, P. D. (2008). Lipid, sterols and fatty acid composition of abyssal holothurians and ophiuroids from the North-East Pacific Ocean: Food web implications. *Comparative Biochemistry and Physiology B-Biochemistry & Molecular Biology*, 151(1), 79-87.

Ehrich, Dorothée, Tarroux, Arnaud, Stien, Jenny, Lecomte, Nicolas, Killengreen, Siw, Berteaux, Dominique, & Yoccoz, Nigel G. (2011). Stable isotope analysis: modelling lipid normalization for muscle and eggs from arctic mammals and birds. *Methods in Ecology and Evolution*, 2(1), 66-76.

- Fritz, Peter, & Poplawski, S. (1974). 18 O and 13 C in the shells of freshwater molluscs and their environments. *Earth and Planetary Science Letters*, 24(1), 91-98.
- Fry, B. (2006). *Stable Isotope Ecology*. New York: Springer.
- Fry, B., Baltz, D. M., Benfield, M. C., Fleeger, J. W., Gace, A., Haas, H. L., & Quinones-Rivera, Z. J. (2003). Stable isotope indicators of movement and residency for brown shrimp (*Farfantepenaeus aztecus*) in coastal Louisiana marshscapes. *Estuaries*, 26(1), 82-97.
- Fry, B., & Sherr, E. B. (1989). $\delta^{13}\text{C}$ Measurements as Indicators of Carbon Flow in Marine and Freshwater Ecosystems. In P. W. Rundel, J. R. Ehleringer, & K. A. Nagy (Eds.), *Stable Isotopes in Ecological Research* (Vol. 68, pp. 196-229): Springer New York.
- Gearing, P., Plucker, F. E., & Parker, P. L. (1977). Organic carbon stable isotope ratios of continental margin sediments. *Marine Chemistry*(5), 251-266.
- Gooday, A. J. (2002). Biological responses to seasonally varying fluxes of organic matter to the ocean floor: A review. *Journal of Oceanography*, 58(2), 305-332.
- Hoffman, J. C., & Sutton, T. T. (2010). Lipid correction for carbon stable isotope analysis of deep-sea fishes. *Deep-Sea Research Part I-Oceanographic Research Papers*, 57(8), 956-964.
- Houston, D.C. (2001). *Condors and Vultures*: Voyageur Press.
- Jackson, A. L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, 80(3), 595-602.
- Kemp, K. M., Jamieson, A. J., Bagley, P. M., McGrath, H., Bailey, D. M., Collins, M. A., & Priede, I. G. (2006). Consumption of large bathyal food fall, a six month study in the NE Atlantic. *Marine Ecology Progress Series*, 310, 65-76.
- Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H., & Jones, R. I. (2006). A revised model for lipid-normalizing delta C-13 values from aquatic organisms,

- with implications for isotope mixing models. *Journal of Applied Ecology*, 43(6), 1213-1222.
- Layman, C. A., Arrington, D. A., Montana, C. G., & Post, D. M. (2007). Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology*, 88(1), 42-48.
- Logan, J. M., Jardine, T. D., Miller, T. J., Bunn, S. E., Cunjak, R. A., & Lutcavage, M. E. (2008). Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *Journal of Animal Ecology*, 77(4), 838-846.
- MacAvoy, S. E., Carney, R. S., Fisher, C. R., & Macko, S. A. (2002). Use of chemosynthetic biomass by large, mobile, benthic predators in the Gulf of Mexico. *Marine Ecology Progress Series*, 225, 65-78.
- MacAvoy, S. E., Morgan, E., Carney, R. S., & Macko, S. A. (2008). Chemoautotrophic production incorporated by heterotrophs in Gulf of Mexico hydrocarbon seeps: An examination of mobile benthic predators and seep residents. *Journal of Shellfish Research*, 27(1), 153-161.
- Martini, Frederic H. (1998). The Ecology of Hagfishes *The Biology of Hagfishes* (pp. 57-77): Springer.
- Mateo, Miguel A, Serrano, Oscar, Serrano, Laura, & Michener, Robert H. (2008). Effects of sample preparation on stable isotope ratios of carbon and nitrogen in marine invertebrates: implications for food web studies using stable isotopes. *Oecologia*, 157(1), 105-115.
- Meyers, P. A. (1997). Organic geochemical proxies of paleoceanographic, paleolimnologic, and paleoclimatic processes. *Organic Geochemistry*, 27(5-6), 213-250.
- Moncreiff, C. A., & Sullivan, M. J. (2001). Trophic importance of epiphytic algae in subtropical seagrass beds: evidence from multiple stable isotope analyses. *Marine Ecology Progress Series*, 215, 93-106.
- Moore, J. C., Berlow, E. L., Coleman, D. C., de Ruiter, P. C., Dong, Q., Hastings, A., Johnson, N. C., McCann, K. S., Melville, K., Morin, P. J., Nadelhoffer, K.,

- Rosemond, A. D., Post, D. M., Sabo, J. L., Scow, K. M., Vanni, M. J., & Wall, D. H. (2004). Detritus, trophic dynamics and biodiversity. *Ecology Letters*, 7(7), 584-600.
- Nowlin, Weston H., Vanni, Michael J., & Yang, Louie H. (2008). Comparing resource pulses in aquatic and terrestrial ecosystems. *Ecology*, 89(3), 647-659.
- Parnell, Andrew C, & Jackson, Andrew L. (2013). siar: Stable Isotope Analysis in R (Version R package version 4.2).
- Pereira, Laura M., Owen-Smith, Norman, & Moleon, Marcos. (2014). Facultative predation and scavenging by mammalian carnivores: seasonal, regional and intra-guild comparisons. *Mammal Review*, 44(1), 44-55.
- Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, 18, 293-320.
- Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, 83(3), 703-718.
- Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., & Montana, C. G. (2007). Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, 152(1), 179-189.
- R Core Team. (2013). R: A language and environment for statistical computing (Version 2.15.2). Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <http://www.R-project.org/>
- Rainer, S. F. (1992). Diet of prawns from the continental slope of north-western Australia. *Bulletin of Marine Science*, 50(2), 258-274.
- Rajasree, S. R. Radhka, & Kurup, B. Madhusoodana. (2011). Food and feeding habits of deepsea pandalid prawns *Heterocarpus gibbosus*, Bate 1888 and *Heterocarpus woodmasoni*, Alcock off Kerala, south India. *Indian Journal of Fisheries*, 58(3), 45-50.

- Rowe, Gilbert T. (2013). Seasonality In Deep-Sea Food Webs—A Tribute To The Early Works Of Paul Tyler. *Deep-Sea Research Part II Topical Studies in Oceanography*, 92, 9-17.
- Ruiz-Cooley, R. I., Engelhaupt, D. T., & Ortega-Ortiz, J. G. (2012). Contrasting C and N isotope ratios from sperm whale skin and squid between the Gulf of Mexico and Gulf of California: effect of habitat. *Marine Biology*, 159(1), 151-164.
- Schlechtriem, C., Focken, U., & Becker, K. (2003). Effect of different lipid extraction methods on delta C-13 of lipid and lipid-free fractions of fish and different fish feeds. *Isotopes in Environmental and Health Studies*, 39(2), 135-140.
- Schmitz, OJ, Jones, HP, & Barton, BT. (2008). Scavengers. *Encyclopedia of Ecology*, 3160-3164.
- Smith, C. R., & Baco, A. R. (2003). Ecology of whale falls at the deep-sea floor. *Oceanography and Marine Biology an Annual Review*, 41, 311-354.
- Smith, K. L., Baldwin, R. J., Karl, D. M., & Boetius, A. (2002). Benthic community responses to pulses in pelagic food supply: North Pacific Subtropical Gyre. *Deep-Sea Research Part I-Oceanographic Research Papers*, 49(6), 971-990.
- Sweeting, C. J., Polunin, N. V. C., & Jennings, S. (2006). Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. *Rapid Communications in Mass Spectrometry*, 20(4), 595-601.
- Thistle, David. (2003). The deep-sea floor: an overview. *Ecosystems of the World*, 5-38.
- Tieszen, L. L., Boutton, T. W., Tesdahl, K. G., & Slade, N. A. (1983). Fractionation and turnover of stable carbon isotopes in animal tissues: implication for $\delta^{13}\text{C}$ analysis of diet. *Oecologia*, 57, 32-37.
- Vander Zanden, M. J., & Rasmussen, J. B. (2001). Variation in delta N-15 and delta C-13 trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, 46(8), 2061-2066.
- Vanderklift, M. A., & Ponsard, S. (2003). Sources of variation in consumer-diet delta(15)N enrichment: a meta-analysis. *Oecologia*, 136(2), 169-182.

- Vetter, E. W., & Dayton, P. K. (1999). Organic enrichment by macrophyte detritus, and abundance patterns of megafaunal populations in submarine canyons. *Marine Ecology Progress Series*, 186, 137-148.
- Wang, X. C., Chen, R. F., & Gardner, G. B. (2004). Sources and transport of dissolved and particulate organic carbon in the Mississippi River estuary and adjacent coastal waters of the northern Gulf of Mexico. *Marine Chemistry*, 89(1-4), 241-256.
- Wells, R. J. D., & Rooker, J. R. (2009). Feeding ecology of pelagic fish larvae and juveniles in slope waters of the Gulf of Mexico. *Journal of Fish Biology*, 75(7), 1719-1732.
- Wilson, Erin E., & Wolkovich, Elizabeth M. (2011). Scavenging: how carnivores and carrion structure communities. *Trends in Ecology & Evolution*, 26(3), 129-135.
- Witte, U. (1999). Consumption of large carcasses by scavenger assemblages in the deep Arabian Sea: observations by baited camera. *Marine Ecology Progress Series*, 183, 139-147.
- Witte, U., Wenzhofer, F., Sommer, S., Boetius, A., Heinz, P., Aberle, N., Sand, M., Cremer, A., Abraham, W. R., Jorgensen, B. B., & Pfannkuche, O. (2003). In situ experimental evidence of the fate of a phytodetritus pulse at the abyssal sea floor. *Nature*, 424(6950), 763-766.
- Zintzen, V., Roberts, C. D., Anderson, M. J., Stewart, A. L., Struthers, C. D., & Harvey, E. S. (2011). Hagfish predatory behaviour and slime defence mechanism. *Scientific Reports*, 1.

Table 1 Summary of all muscle tissue samples collected from deep-sea scavengers in the eastern Gulf of Mexico.

Species	April 2005	April 2011			August 2011			April 2012			Total	Mean depth ± SD (m)
	WFS	NGS- MR	NGS- -DC	WFS	NGS- MR	NGS- DC	WFS	NGS- MR	NGS- DC	WFS	n	
<i>AcanthePHYra sp.</i>	0	0	0	0	0	1	0	0	0	1	2	536±122
<i>Bathynomus giganteus</i>	17	0	63	44	9	18	3	14	22	33	223	761±201
<i>Chaceon fenneri</i>	0	0	0	10	0	0	5	0	0	12	27	530±60
<i>Chaceon quinquedens</i>	0	0	8	9	0	12	0	4	10	5	48	831±390
<i>Eptatretus minor</i>	0	0	0	0	0	2	0	0	1	1	4	446±133
<i>Eptatretus springeri</i>	0	0	58	4	11	8	0	4	52	0	137	561±71
<i>Heterocarpus ensifer</i>	0	0	1	9	0	0	20	0	0	13	43	448±147
<i>Myxine mcmillanae</i>	0	0	17	0	4	3	0	2	9	0	35	702±179

Table 2 Results for regression analysis of $\Delta\delta^{13}\text{C}$ by C:N-BULK using linear, Michaelis-Menten, and logistic models along with the corresponding AICc values. Bold values are statistically significant.

Species	Linear: $y = a + bx$			Michaelis-Menten: $y = \frac{ax}{1+bx}$			Logarithmic: $y = b + a \ln(x)$		
	a (<i>t,p</i>)	b (<i>t,p</i>)	AICc	a (<i>t,p</i>)	b (<i>t,p</i>)	AICc	a (<i>t,p</i>)	b (<i>t,p</i>)	AICc
<i>Bathynomus giganteus</i>	-2.55 (-6.68, < 0.01)	0.82 (8.90, < 0.01)	26.6	-6.33 (-10.34, < 0.01)	3.36 (50.29, < 0.01)	25.3	4.42 (9.76, < 0.01)	-5.25 (-8.48, < 0.01)	23.8
<i>Heterocarpus ensifer</i>	-4.58 (-2.62, 0.04)	1.48 (2.88, 0.03)	3.91	-5.48 (-3.17, 0.02)	3.12 (33.72, < 0.01)	3.79	5.03 (2.90, 0.03)	-5.71 (-2.69, 0.04)	3.85
<i>Eptatretus springeri</i>	2.10 (7.84, < 0.01)	0.11 (2.95, 0.01)	57.4	-3.75 (-15.48, < 0.01)	1.57 (4.98, < 0.01)	52.6	0.85 (3.43, < 0.01)	1.22 (2.54, 0.02)	54.8
<i>Myxine mcmillanae</i>	-2.79 (-2.14, 0.04)	1.13 (3.39, < 0.01)	25.4	-5.94 (-4.48, < 0.01)	2.82 (11.95, < 0.01)	26.0	4.37 (3.33, < 0.01)	-4.31 (-2.42, 0.02)	25.7

Table 3 Variation in stable isotope values by sampling time in deep-sea scavengers on the northern gulf slope near Desoto Canyon (NGS-DC) and the Mississippi River (NGS-MR) and along the west Florida slope (WFS). Bold values indicate statistically significant differences.

Species	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
	Test statistic	<i>p</i> -value	Test statistic	<i>p</i> -value
NGS-MR				
<i>Bathynomus giganteus</i>	<i>t</i> =-0.42	0.68	<i>t</i> =0.03	0.97
NGS-DC				
<i>Bathynomus giganteus</i>	<i>F</i> =2.77	0.07	<i>F</i> =0.16	0.85
<i>Chaceon quinquedens</i>	<i>F</i> =8.17	<0.01	<i>F</i> =1.59	0.22
<i>Eptatretus springeri</i>	<i>F</i> =3.29	0.04	<i>F</i> =1.93	0.15
<i>Myxine mcmillanae</i>	<i>t</i> =1.15	0.26	<i>t</i> =0.03	0.97
WFS				
<i>Bathynomus giganteus</i>	<i>F</i> =21.13	<0.01	<i>F</i> =13.62	<0.01
<i>Chaceon fenneri</i>	<i>F</i> =0.32	0.73	<i>F</i> =0.06	0.94
<i>Chaceon quinquedens</i>	W=20.5	0.84	W=32	0.24
<i>Heterocarpus ensifer</i>	<i>F</i> =0.52	0.60	<i>F</i> =0.61	0.55

Table 4 Results for linear regression analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by body size (total length, TL; carapace width, CW; and carapace length, CL) for six scavenger species. Significant values are bold.

Species	Region	Cruise	Measurement	n	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
					R^2	t	p	R^2	t	p
<i>Bathynomus giganteus</i>	NGS	Apr 11/Apr 12	TL	99	0.06	2.41	0.02	0.04	-2.10	0.04
<i>Chaceon fenneri</i>	WFS	All	CW	27	<0.01	0.06	0.96	<0.01	-0.43	0.67
<i>Chaceon quinquedens</i>	NGS	Aug 11/Apr 12	CW	22	0.24	2.54	0.012	<0.01	-0.01	0.99
<i>Eptatretus springeri</i>	NGS	Apr 11/Apr 12	TL	110	0.16	4.57	<0.01	0.10	3.43	<0.01
<i>Heterocarpus ensifer</i>	WFS	All	CL	42	0.03	1.07	0.29	0.12	2.30	0.03
<i>Myxine mcmillanae</i>	NGS	All	TL	29	0.01	-0.53	0.60	<0.01	0.12	0.90

Table 5 Results for linear regression analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by depth for six scavenger species. Significant values are bold.

Species	n	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
		R^2	t	p	R^2	t	p
<i>Bathynomus giganteus</i>	206	0.05	3.30	0.01	0.01	1.69	0.09
<i>Chaceon fenneri</i>	27	0.06	-1.24	0.23	0.02	-0.76	0.46
<i>Chaceon quinquedens</i>	48	0.08	1.96	0.06	<0.01	0.64	0.53
<i>Heterocarpus ensifer</i>	42	<0.01	-0.07	0.94	0.13	2.43	0.02
<i>Myxine mcmillanae</i>	35	0.03	0.96	0.34	0.01	0.67	0.51
<i>Eptatretus springeri</i>	137	<0.01	-0.19	0.85	0.04	2.42	0.02

Table 6 The percent of total area (TA) occupying unique isotopic niche space for each scavenger species.

Species	n	% of TA unique	% of ellipse unique
NGS-MR			
<i>Bathynomus giganteus</i>	23	62.6	68.3
<i>Chaceon quinquedens</i>	4	62.0	93.4
<i>Eptatretus springeri</i>	15	33.1	68.9
<i>Myxine mcmillanae</i>	6	2.5	47.5
NGS-DC			
<i>Bathynomus giganteus</i>	103	58.2	85.0
<i>Chaceon quinquedens</i>	30	5.5	42.5
<i>Eptatretus minor</i>	3	0	56.1
<i>Eptatretus springeri</i>	118	34.8	20.1
<i>Myxine mcmillanae</i>	29	51.1	100
WFS			
<i>Bathynomus giganteus</i>	97	90.4	100
<i>Chaceon fenneri</i>	27	27.0	0
<i>Chaceon quinquedens</i>	14	37.6	44.0
<i>Eptatretus springeri</i>	4	0.9	42.3
<i>Heterocarpus ensifer</i>	42	100	100

Figure 1 Deep-sea scavengers were sampled at multiple sample stations located along the northern slope along the DeSoto Canyon (NGS-DC, circles) and south of the Mississippi River (NGS-MR, squares) and on the west Florida slope (WFS, triangles) of the Gulf of Mexico. Filled shapes indicate stations where scavengers were collected. The location of the Deepwater Horizon oil spill (DwH) is shown by a star.

Figure 2 Spatial and depth distributions of deep-sea scavengers collected along the northern and west Florida slopes of the Gulf of Mexico.

Figure 3 Size distributions of deep-sea benthic scavengers in the Gulf of Mexico.

Figure 4 The C:N ratios (at%) of bulk and lipid extracted deep-water scavenger muscle tissue.

Figure 5 Spatiotemporal variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for three deep-sea scavengers on the northern gulf slope and along the west Florida slope. Groupings with different letters in the inset table are significantly different based on post-hoc Tukey's tests; "-" denotes groups that were not included in pairwise comparisons.

Figure 6 Variation in stable isotope values with body size in *B. giganteus* ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), *C. quinquedens* ($\delta^{13}\text{C}$), *E. springeri* ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), and *H. ensifer* ($\delta^{15}\text{N}$).

Figure 7 Relationship between the mean body size and $\delta^{15}\text{N}$ for scavengers. Only significant relationships are shown. See Fig. 8 for species codes.

Scientific Name (Figure Code)	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
<i>Bathynomus giganteus</i> (Bg)	23	A	A
<i>Chaceon quinquedens</i> (Cq)	4	-	-
<i>Eptatretus springeri</i> (Es)	15	A	B
<i>Myxine mcmillanae</i> (Mm)	6	A	A

Scientific Name (Figure Code)	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
<i>Acantheephyra</i> sp. (Ac)	1	-	-
<i>Bathynomus giganteus</i> (Bg)	103	B	B
<i>Chaceon quinquedens</i> (Cq)	30	B	D
<i>Eptatretus minor</i> (Em)	3	-	-
<i>Eptatretus springeri</i> (Es)	118	B	C
<i>Heterocarpus ensifer</i> (He)	1	-	-
<i>Myxine mcmillanae</i> (Mm)	29	A	A

Scientific Name (Figure Code)	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
<i>Acantheephyra</i> sp. (Ac)	1	-	-
<i>Bathynomus giganteus</i> (Bg)	97	A	A B
<i>Chaceon fenneri</i> (Cf)	27	A B	B
<i>Chaceon quinquedens</i> (Cq)	14	B	B
<i>Eptatretus minor</i> (Em)	1	-	-
<i>Eptatretus springeri</i> (Es)	4	-	-
<i>Heterocarpus ensifer</i> (He)	42	C	C

Figure 8 Temporal and spatial variation in stable isotopic values of deep-sea scavengers of the northeastern Gulf of Mexico (NGS-MR and NGS-DC) and along the West Florida Slope (WFS). Error bars are $\pm$ SD. Species with different letters in inset tables are significantly different based on post-hoc Tukey's tests; "-" denotes species that were not included in pairwise comparisons.

Figure 9 Total area convex hulls of scavengers from the NGS-DC, NGS-MR, and WFS sampling regions. See Fig. 8 for species codes.

CHAPTER IV

TROPHIC INTERACTIONS OF DEEP-SEA TELEOSTS ALONG THE NORTHERN
AND WESTERN SLOPES OF THE GULF OF MEXICO

Abstract

The deep waters (>200 m) of the Gulf of Mexico (GoM) include a diverse demersal fish community; however, the status and ecological roles of many of these demersal fish species remains largely unknown. Here, I use stable isotope analysis (C and N) to investigate the trophic interactions of deep-water demersal teleosts in the northern and eastern GoM. Four hundred fifty-three muscle samples were collected from teleosts captured on benthic and mid-water longlines at depths from *ca.* 200 to 2000 m along the northern slope (NGS) and the west Florida slope (WFS) of the Gulf of Mexico during 2011 and 2012. There was significant intraspecific spatial and temporal variation in stable isotope values within several species, as well as evidence for ontogenetic shifts in trophic interactions of all five of the most commonly captured teleosts. The stable isotope analysis of deep-sea communities revealed relatively small ranges of $\delta^{13}\text{C}$ values (NGS: 2.62‰ and WFS: 2.21‰) that likely stem from a limited number of pathways carbon enters deep sea food webs in these locations. There was a larger range of $\delta^{15}\text{N}$ values in the NGS (6.2‰) than in the WFS (3.2‰) sampling region, suggesting that the NGS teleost community that was sampled spans at least two trophic levels, whereas the WFS community only spans approximately one level despite sampling similar species and body sizes in the two areas. We observed a high degree of isotopic niche overlap among species that is consistent with stomach contents analysis and is predicted for oligotrophic ecosystems. The present study provides an important characterization of the trophic interactions of deep-sea teleosts in the Gulf of Mexico and establishes system baselines for future investigations.

Introduction

Deep-sea fishes have biological traits that make them especially vulnerable to over fishing or disturbances in the environment including K-type life history traits, low fecundity and aggregation in restricted topographic areas (Haedrich, 1997; Koslow et al., 2000). The susceptibility to anthropogenic impacts is cause for concern and represents a management challenge because commercial exploitation of benthic resources has been shifting from the relatively shallow waters on the continental shelf into the waters over the continental slope (Morato et al., 2006). Therefore, investigations of the biology and ecology – including trophic interactions- of deep-sea teleosts is important for fisheries management and deep-sea conservation.

The deep waters (>200 m) of the Gulf of Mexico (GoM) include a diverse demersal fish community (Powell et al., 2003). Deep-sea species, including the Great Northern Tilefish (*Lopholatilus chamaeleonticeps*) and deep-water groupers, such as the Yellowedge (*Hyporthodus flavolimbatus*) and Warsaw (*Hyporthodus nigrilus*) have been exploited as part of directed commercial and/or recreational fisheries (Cass-Calay & Bahnick, 2002; Steimle, 1999). In addition, the King Snake Eel, *Ophichthus rex* (Clark, 2000), Gulf Hake, *Urophycis cirrata* (Perez et al., 2003), and Southern Hake, *Urophycis floridana* (Brown, 2007) have been reported as by-catch from other fisheries. Fisheries, however, are not the only threat to deep-sea fishes. For example, the northern GoM is rich in fossil fuels and wells have been drilled at least as deep as 2,450 m (Royal Dutch Shell, 2010) with the potential effects of such activities made apparent with the Deep-water Horizon oil spill in April, 2010. Even where no point source pollution exists, deep-

sea fish appear to be at risk for exposure to anthropogenic contaminants and the associated pathologies (Feist et al., 2015).

By examining the trophic interactions of the demersal fish community of the deep-waters of the GoM, we can better understand biological factors that could influence resource availability for deep-sea organisms. Traditional approaches to studying diets, including stomach contents analysis, are logistically difficult, owing to low sample sizes, damaged fauna, and gut eversion. An alternative approach that may have great value in deep-water systems is stable isotope analysis, which is commonly employed in studies of trophic interactions. The stable isotope method is able to provide information integrated over longer time frames on the basis of assimilated, rather than ingested, material (Peterson & Fry, 1987; Vander Zanden & Rasmussen, 2001). Carbon isotopes generally provide information on the sources of primary production supporting the diets of consumers because $\delta^{13}\text{C}$ (a standardized ratio of $^{13}\text{C}:^{12}\text{C}$) values generally only increases *ca.* 1‰ per trophic level (DeNiro & Epstein, 1978; Wada et al., 1991). For example, $\delta^{13}\text{C}$ has been used to distinguish between terrestrial vs. marine resources (Hobson, 1987), foraging in pelagic vs. benthic food webs (France, 1995), and between photosynthetic- and chemosynthetic-based communities in the deep-sea (MacAvoy et al., 2002). Nitrogen isotopes can be used to determine the relative trophic position of an organism because ^{15}N is enriched through each trophic transfer, generally with an increase of 2.5‰ to 3.4‰ in $\delta^{15}\text{N}$ values from food to consumer (Caut et al., 2009; Post, 2002).

The goal of the present study was to describe the trophic interactions of deep-water demersal teleosts in the northern and eastern GoM using stable isotope analysis (C

and N). In particular, we examined spatiotemporal variation in isotopic values among common species as well as intraspecific differences along size and depth gradients.

Materials and Methods

Study Site

Sampling took place along the continental slope off the south-central Gulf coast of Florida to Louisiana (Figure 1). The southern sampling sites were located on the West Florida Slope (WFS), approximately 270 km southeast of Tampa, Florida. Stations were concentrated on the continental slope in depths of approximately 250 to 2000 m and teleosts were collected from the full range of sampled depths. The northern sites were spread across the northern slope of the Gulf (NGS) from an area approximately 25 km offshore of the Mississippi River delta through DeSoto Canyon and east onto the north Florida slope to an area 85 km southwest of Cape San Blas, Florida (Figure 1). These locations included sites on the continental slope to the east and west of DeSoto Canyon, along the canyon's eastern rim, and on the canyon floor. Depth ranges for these stations were approximately 200 to 2000 m. Sampling stations at the northern and southern sites were chosen to match depth and topography along a broad shelf and onto the slope. The location of the Deep-water Horizon oil spill occurred near the center of our NGS sampling region, at the mouth of Desoto Canyon, approximately one year prior to our first sampling effort in April 2011.

Sampling Methods

Demersal teleosts were collected as part of a large fishery-independent survey. The majority of samples were collected during research cruises aboard the R/V *Weatherbird II* in April 2011, August 2011, and April 2012. Each cruise in 2011-2012 included sampling in both the NGS (39 stations and 85 total sets) and the WFS (14 stations and 68 total sets). Sampling involved deploying two or three successive sets of demersal longlines with three integrated traps: a small box trap (60 cm x 60 cm x 38 cm with 2.5 cm mesh), a cylindrical (60 cm x 35 cm with 1.0 cm mesh) trap and a small shrimp trap (40 cm x 20 cm x 15 cm with 1.0 cm mesh). All traps are baited with menhaden (*Brevoortia* sp.) or northern mackerel (*Scomber scombrus*). Each set was deployed in 20 to 30 minutes and was retrieved following a soak of 4-8 hr. Once the animals were on board, each individual was identified to species, weighed and measured. We collected muscle from the dorsal region using clean biopsy punches and froze for later processing. For additional sampling protocols, please see Churchill et al. (2015). Two additional species (Escolar, *Lepidocybium flavobrunneum* and Oilfish, *Ruvettus pretiosus*) were collected using pelagic longlines during a research cruise in October 2011. While these specific individuals were not collected in deep-water, both species are commonly found in this environment (Nakamura & Parin, 1993).

Stable Isotope Analysis

Tissues used for stable isotope analyses were prepared using standard procedures. All muscle tissue was thawed and then rinsed briefly in deionized water to remove any surface debris. All tissue was then dried a minimum of 48 hours in a 60°C oven and

ground to a fine powder. Homogenized samples were weighed out to the nearest microgram and packed into tin capsules. Samples were then analyzed on a ThermoFinnigan Delta C mass spectrometer at the Florida International University Stable Isotope Laboratory to determine $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The error of the isotope-ratio mass spectrometer determined by repeat analyses of an internal glycine standard was less than 0.1‰ (n=80) during the current study. Approximately one-sixth of the samples were run in duplicate. The mean absolute difference between duplicates was 0.1 ± 0.1 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. All reported C:N ratios used atomic mass.

Lipids tend to be depleted in ^{13}C (DeNiro & Epstein, 1977; Tieszen et al., 1983) relative to protein and extractions are recommended for samples where the C:N ratio > 3.5 (Post et al., 2007). Therefore, all whole muscle (BULK) samples that met this criterion underwent lipid extraction (LE; Goldface Tilefish, *Caulolatilus chrysops*: n=1; Escolar: n=5; Great Northern Tilefish: n=3; King Snake Eel: n=2; Oilfish: n=7; Shortdorsal Cutthroat Eel, *Synaphobranchus brevidorsalis*: n=1; Cutthroat Eel, *S. oregoni*; n=20; Gulf Hake: n=1; and Southern Hake: n=2; see Figure 2). Homogenized tissue samples were immersed in a mixture of chloroform and methanol (2:1) with a solvent volume three to five times greater than that of the powder. Samples were then vortexed for 30 seconds, left undisturbed for at least 30 minutes and centrifuged for 10 min at 1318 g. The extraction process was repeated at least three times or until the supernatant was clear (Bligh & Dyer, 1959; Logan et al., 2008). Samples were then re-dried, weighed and analyzed for carbon isotopic composition. We report and use $\delta^{13}\text{C}$ -LE values for all individuals with C:N-BULK>3.5 and $\delta^{13}\text{C}$ -BULK for all others.

We used analysis of variance and Student's t -tests to examine intraspecific spatial and temporal variation in stable isotope values in species with $n \geq 20$. Where necessary, because of small or unbalanced sample sizes, we used a Wilcoxon signed rank test instead of a Student's t -test. Because of small sample sizes and variation in the abundance of common species, we were only able to complete a full factorial analysis for the Southern Hake. We were able to investigate variation between sampling regions for Gulf Hake and Southern Hake. We were able to examine temporal differences of stable isotope values within specific sampling regions for Great Northern Tilefish; King Snake Eel; a Cutthroat Eel, *Synaphobranchus oregoni*; Gulf Hake and Southern Hake. For the King Snake Eels, we were only able to compare individuals from August 2011 and April 2012 and for Cutthroat Eel, *S. oregoni* we could only compare individuals collected in April 2011 and 2012.

Linear regression was used to investigate the effects of size on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within species where sample size was ≥ 20 . We used total length (TL) for snake and Cutthroat Eels and standard length (SL) for all other species. In addition, linear regression was used to investigate the relationship between mean body size and mean $\delta^{15}\text{N}$ values across all deep-sea teleost species collected as part of this study. Analyses were done separately for each sampling region. We also used linear regression to assess the effects of capture depth on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within each species where the sample size with accurate depth data was ≥ 20 . For species with significant spatiotemporal variation in stable isotope values, we limited our analyses to specific sampling regions and/or times to reduce the effect of possible confounding variables.

For interspecific comparisons, we used one-way analysis of variance with a Tukey's *post-hoc* test for paired contrasts. Because a full factorial analysis was not possible, we pooled individuals of the same species from different times (April 2011, August 2011, October 2011, and April 2012). Species with less than five captured individuals were not included in analyses. The analysis was performed separately for the NGS and WFS sampling regions.

We used metrics presented in Layman et al. (2007) to compare community-level trophic interactions in each sampling region (NGS and WFS) among species where $n \geq 5$. Total area (TA) is an indication of the isotopic niche width of a population or community in isotopic bi-plot space. The $\delta^{13}\text{C}$ range is an estimate of the diversity of basal resources and the $\delta^{15}\text{N}$ range provides an estimate of trophic length of the community. The TA metric is sensitive to differences in sample size. To account for sample size differences, we also present the stable isotope Bayesian ellipses for each species (Jackson et al., 2011), which provide an estimate of the core isotopic niche space. We also calculated the percent of TA and Bayesian ellipse that were unique to each species in each region. All stable isotope metrics were calculated using the SIAR package (Parnell & Jackson, 2013).

Unless otherwise noted, all data were normally distributed and equal in variance. The critical level of significance was $\alpha=0.05$ for all analyses/statistical tests. All analyses were completed in R, version 3.0.2 (R Core Team, 2013).

Results

A total of 571 individuals from 27 teleost species (5 species where $n > 20$) were captured at depths ranging from *ca.* 200 to 2000 meters (Figure 3). The size distributions of the five most common species are shown in Figure 4. From these individuals, we collected and processed 453 muscle tissue samples (Table 1).

Intraspecific patterns

There was significant intraspecific spatial and temporal variation in stable isotope values within several teleost species. Gulf Hake differed between the NGS and WFS sampling regions in $\delta^{15}\text{N}$ values ($W=755$, $p < 0.001$) but not in $\delta^{13}\text{C}$ values ($W=565.5$, $p=0.121$) while for Southern Hake both $\delta^{13}\text{C}$ ($W=3501$, $p < 0.001$) and $\delta^{15}\text{N}$ values ($W=4361.5$, $p < 0.001$) varied between regions. We also detected significant temporal variation among three (Great Northern Tilefish, Gulf Hake and Southern Hake) out of five species of teleosts (Table 2). We were able to complete full factorial analyses in Southern Hake comparing multiple sampling times across both regions (Figure 5). The $\delta^{13}\text{C}$ values were greater in the NGS ($F_1=33.1$, $p < 0.001$) but did not vary temporally in either region (season: $F_2=0.79$, $p=0.457$; season x region: $F_2=0.13$, $p=0.875$). Values of $\delta^{15}\text{N}$ were significantly higher in the NGS region ($F_1=190.5$, $p < 0.001$) and were lower during the April 2011 cruise than subsequent sampling times ($F_2=16.4$, $p < 0.001$). Although $\delta^{15}\text{N}$ values did not vary with the interaction of region and season ($F_2=2.6$, $p=0.08$), there is a possibility of Type II error because some sampling times or locations are represented by small sample sizes.

The relationship between body size and isotopic values was examined in five teleost species (Table 4). Great northern tilefish and Southern Hake both had significant, positive relationships between size and both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. King snake eels and Gulf Hake displayed a positive relationship between size and $\delta^{13}\text{C}$ values, but there was no relationship between size and $\delta^{15}\text{N}$ values. The Cutthroat Eel, *S. oregoni*, had a positive relationship between size and $\delta^{15}\text{N}$ values, but this was not evident for $\delta^{13}\text{C}$ values (Figure 6).

The relationship between capture depth and isotopic values was assessed within six species (Table 5). Great northern tilefish and Gulf Hake displayed a positive, but weak ($R^2 \leq 0.1$), relationship between depth and $\delta^{13}\text{C}$ values, but no relationship with $\delta^{15}\text{N}$ values. All other species showed no relationship between depth and either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values.

Community comparisons

Across all teleosts sampled, the NGS sampling region had higher $\delta^{13}\text{C}$ values (mean $\pm$ SD difference = $0.3 \pm 0.7\text{‰}$; $t=4.54$, $p<0.001$) and $\delta^{15}\text{N}$ values (mean $\pm$ SD difference = $1.6 \pm 1.1\text{‰}$; $t=18.67$, $p<0.001$) than the WFS region. Both regions had similar $\delta^{13}\text{C}$ ranges (NGS: 2.6‰, WFS: 2.2‰) but the NGS had almost twice the $\delta^{15}\text{N}$ range of the WFS (NGS: 6.2‰, WFS: 3.2‰).

We observed significant interspecific variation in isotopic values in both sampling regions. In the NGS region, there was significant variation among species in both $\delta^{13}\text{C}$ ($F_4=77.83$, $p<0.001$) and $\delta^{15}\text{N}$ values ($F_4=51.66$, $p<0.001$; Figure 7a). King Snake Eels had the highest average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in this region. Of the species with enough

individuals for inclusion in the analyses, the Cutthroat Eel, *S. oregoni*, had the lowest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values; however, the single Shortdorsal Cutthroat Eel had the lowest overall isotopic value. In the WFS region, there was also significant variation among species in both $\delta^{13}\text{C}$ values ($F_4=18.02$, $p<0.001$) and $\delta^{15}\text{N}$ values ($F_4=9.21$, $p<0.001$; Figure 7b). In the southern sampling region, the Spotted Hake, *Urophycis regia* displayed the lowest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The single Mexican Grenadier, *Coryphaenoides mexicanus*, had the highest $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Among those species included in analyses, Oilfish had the highest $\delta^{15}\text{N}$ value while Escolar had the highest $\delta^{13}\text{C}$ value. At the community level, there was no significant relationship between mean species body size and mean $\delta^{15}\text{N}$ values in either the NGS ($t=1.16$, $p=0.27$; 8a) or WFS sampling region ($t=0.11$, $p=0.92$; Figure 8b).

In agreement with the post-hoc analysis of the interspecific variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, we observed some overlap among the isotopic niche spaces of species within the NGS and WFS sampling regions, as defined by TAs and Bayesian ellipses (Table 6; Figure 9). In the NGS, both King Snake Eels and Cutthroat Eels, *S. oregoni*, had >80% unique TA and 100% unique ellipse area. The other three species (Great Northern Tilefish, Gulf Hake and Southern Hake) all displayed high amounts of overlap with each other. These three species had <10% unique TA and <20% unique ellipse area. In the WFS, Escolar and Southern Hake possessed the highest amount of unique isotopic niche space with >90% TA and 66% and 44% unique ellipse area, respectively. Oilfish had moderate levels of overlap with Gulf and Southern Hake. Both Gulf and Spotted Hake had the highest amount of overlap in this region with <10% unique TA but somewhat larger unique ellipse areas (54.5% and 34.4%, respectively).

Discussion

Intraspecific patterns

We found significant spatial variation in carbon and nitrogen isotope values within the two most common teleost species sampled in this study. Both Gulf and Southern Hake individuals collected in the NGS region had higher $\delta^{15}\text{N}$ values than those from the WFS. Individuals may be feeding at a slightly higher trophic position in the NGS or there may be isotopic baseline differences between the two areas. This general pattern in $\delta^{15}\text{N}$ values was also true for the shortspine spurdog shark, *Squalus cf. mitsukurii* (Churchill et al., 2015); giant isopod, *Bathynomus giganteus*; and red deep-sea crab, *Chaceon quinquedens* (Churchill et al., in prep), suggesting that there may be a difference in basal resources. One possible explanation for higher $\delta^{15}\text{N}$ values in the NGS is increased terrestrial inputs. The Mississippi River delivers large amounts of organic material to the northern GoM, stimulating high levels of biological production (Dagg & Breed, 2003). This may increase nutrient cycling in the region, with subsequent increases in region-wide $\delta^{15}\text{N}$ values. In addition, anthropogenic nitrogen sources in the river (e.g., septic system and animal waste) have more enriched $\delta^{15}\text{N}$ values (8‰ to 18‰) than atmospheric nitrogen (0‰ to 8‰; BryantMason et al., 2013), which could lead to higher $\delta^{15}\text{N}$ values within basal resources in the NGS.

Southern hake had slightly higher $\delta^{13}\text{C}$ values ($\sim 0.4\text{‰}$) in the NGS than those observed in the WFS. The variation in carbon is unlikely to be the result of riverine inputs, which typically have lower $\delta^{13}\text{C}$ values than offshore GoM waters (Wang et al., 2004). Among those species where analyses were possible, we found little evidence for isotopic variation associated with vertical distribution, inferred from capture depth. Only

two species (Great Northern Tilefish and Gulf Hake) of five displayed significant relationships between depth and $\delta^{13}\text{C}$ values and all of these relationships were weak ($R^2 < 0.2$). There were no significant relationships between depth and $\delta^{15}\text{N}$ values in any species, suggesting that there may be similar trophic interactions across the depth gradient at which each species is found.

We observed temporal differences in stable isotope values in three species of teleosts collected in deep-water. The $\delta^{15}\text{N}$ values of Great Northern Tilefish increased 0.7‰ between April 2011 and April 2012. Gulf and Southern Hake both experienced similar increases (0.3‰ and 0.9‰, respectively). *Squalus mitsukurii*, an abundant small shark species captured as part of the same sampling effort, also displayed increased $\delta^{15}\text{N}$ values between April 2011 and April 2012 (Churchill et al., 2015). The consistency in $\delta^{15}\text{N}$ shifts across three of the most common species collected in the NGS sampling region may be evidence of shifting baselines in the region, perhaps as a result of the Deepwater Horizon oil spill or increased contributions of anthropogenic nitrogen into the system. Continued sampling in the NGS region will allow for more detailed analyses of temporal shifts in $\delta^{15}\text{N}$ values.

We found evidence for ontogenetic shifts in trophic interactions of all five of the most commonly captured teleosts. Indeed, we detected a positive correlation between body size and $\delta^{15}\text{N}$ values, suggesting an increase in trophic level with increasing length within species. For some species, much of the observed variation in $\delta^{15}\text{N}$ values was explained by body size (e.g., ca. 50% in *S. oregoni*). In addition, several species displayed size-based shifts in $\delta^{13}\text{C}$ values, suggesting a change in the basal resources supporting their diets. The strongest relationships were seen in Great Northern Tilefish,

King Snake Eels and Southern Hake, with body size explaining approximately 30-40% of the variation in $\delta^{13}\text{C}$ values, The size of captured Great Northern Tilefish individuals ranged from juveniles (<42cm SL) to adults (>42cm SL; Steimle, 1999). Juvenile individuals feed primarily on benthic invertebrates, such as echinoderms, crustaceans and mollusks, while adults consume demersal fish species in addition to invertebrate taxa (Steimle, 1999). The consumption of higher trophic level prey (i.e., fishes) would lead to the observed increased $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values at larger sizes. While no stomach content data exist for Gulf Hake or Southern Hake, the congener *Urophycis tenuis* experiences a dietary shift from benthic invertebrates to herring at ~30cm SL (Hanson, 2011) and *Urophycis chuss* and *Urophycis regia* collected in the northeastern Atlantic also exhibited increasing piscivory with increasing size (Garrison & Link, 2000). The size range of both Gulf Hake and Southern Hake spanned the range at which this dietary shift occurs in other species, and on the basis of shifts in isotopic values it is highly likely that they make a similar switch.

Ecological dynamics

Stable isotope analysis of this deep-sea community in the GoM revealed a relatively small range of $\delta^{13}\text{C}$ values (NGS: 2.62‰ and WFS: 2.21‰) that likely represent a limited pool of basal resources. Possible carbon sources include plankton and detritus (-23.0‰ to -22.4‰; Wells & Rooker, 2009), *Sargassum* sp. (-16.6‰ to -16.2‰; Wells & Rooker, 2009), out-welled coastal seagrass (-13.4‰ to -11‰; Moncreiff & Sullivan, 2001), terrestrial sources via riverine inputs (-23.8‰ to -26.8‰; Wang et al., 2004), organic components in the sediment (-21.5‰ to -20‰; Gearing et al., 1977), whale falls

(-32‰ to -20‰; Smith & Baco, 2003), and deep-sea chemosynthetic communities (-65‰ to -25‰; MacAvoy et al., 2008). Given the more negative $\delta^{13}\text{C}$ values present in chemosynthetic systems, relative to photosynthetic, it seems likely that the teleosts from the deep-water areas of the GoM we sampled are reliant on allochthonous inputs from surface waters and incorporate little energy from chemosynthetic food webs. Indeed, the $\delta^{13}\text{C}$ values we observed in this study more closely resemble benthic predators reliant on photosynthetic carbon sources (-20‰ to -18‰) rather than organisms relying on chemoautotrophic carbon (-33‰ to -42‰) in the GoM (MacAvoy et al., 2002). The $\delta^{13}\text{C}$ values observed in the teleosts (-18.5‰ to -15.6‰) compared to planktonic and detrital sources may reveal higher levels of nutrient recycling between surface production and benthic communities or large inputs of *Sargassum*.

There was a large range of $\delta^{15}\text{N}$ values across deep-sea teleosts species in the NGS (6.2‰) than in the WFS (3.2‰) sampling region. Using an average trophic enrichment factor of 2.75‰ (Caut et al., 2009) would suggest that NGS teleost community spans at least two trophic levels, whereas the WFS teleost community only spans approximately one level. On average, the NGS had higher $\delta^{15}\text{N}$ values than the WFS. Increased nitrogen values may be the result of dietary differences or underlying baseline differences between the sampling regions. Further investigation is necessary to determine the underlying drivers of this variation in deep-sea communities. Among those teleost species with large sample sizes ($n \geq 20$), the range of mean $\delta^{15}\text{N}$ values (11.0‰ to 15.2‰) was similar to those observed in sharks (11.2‰ to 15.1‰; Churchill et al., 2015) and slightly higher than those seen in scavengers (9.2‰ to 14.6‰; Churchill et al., in prep) sampled during the same surveys, suggesting that all three groups of organisms

may reside at similar trophic positions. On the basis of nitrogen isotopic values, it would appear that many of the deep-water teleosts are top-level predators, similar to many of the sharks collected in the same region, despite being somewhat smaller-bodied than those elasmobranchs. In general, we found little evidence of larger species feeding at higher trophic levels, perhaps as a result of opportunistic scavenging that is likely occurring among all examined species. The lack of relationship between body size and apparent trophic position contrasts with patterns found in larger predators in coastal ecosystems where trophic levels (using inferences from $\delta^{15}\text{N}$ values) increase with taxa body sizes (Heithaus et al., 2013).

We observed high levels of isotopic niche overlap in some species and little to no overlap in others. In the NGS, those species with a high degree of overlap (TA and ellipse) were Great Northern Tilefish, Gulf Hake and Southern Hake. The similarity is not surprising because these species or their congeners have been found to consume a diet consisting of benthic invertebrates (e.g., crustaceans, polychaetes, mollusks) and fish. King snake eels and Cutthroat Eels, *S. oregoni*, displayed little overlap in TA and no overlap in ellipse area with any other species in the NGS. While no specific dietary information is available for *S. oregoni*, *Synaphobranchus kaupii*, a congener in the northeast Atlantic, was found to consume crustaceans, fish and cephalopods (Marques, 1998). Members of the *Synaphobranchus* genus are also considered to be facultative scavengers (Cousins et al., 2013; Yeh & Drazen, 2009). They may also be reliant on other scavenger species with better dentition than themselves for access to large food items. Jamieson et al. (2011) found that *Centroscymnus coelolepis*, the Portuguese Dogfish Shark, facilitated scavenging by *S. kaupii* by removing the exterior surface of

larger food items, in this case, bait. In the absence of the sharks, the Cutthroat Eels often waited more than two hours before feeding. In our study, *Synaphobranchus* species were collected at depths >1000m but small elasmobranch species were generally captured in depths of 300-700m (Churchill et al., 2015). The potential dependence on other scavengers may restrict *S. oregoni* from consuming larger food items, leading to lower $\delta^{15}\text{N}$ values than any of the other teleosts or any of the larger benthic scavengers found in the same region (Churchill et al., in prep). No dietary information is available for King Snake Eels. However, the higher $\delta^{15}\text{N}$ values may be explained by capture location. King Snake Eels were predominately found in the northwestern portion of the NGS region, closest to the Mississippi River outflow, which could result in higher nitrogen isotopic baseline values in this species. It is unknown if isotopic baseline or dietary differences underlie the enriched carbon values.

In the WFS, we found relatively high amounts of overlap between the three Hake species (Gulf, Southern and Spotted) as well as Oilfish. While Oilfish does not inhabit the same demersal habitats as the hake, they are known to have similar dietary preferences including fish, cephalopods, and crustaceans (Vasilakopoulos et al., 2011; Viana et al., 2012). Escolar shared no TA or ellipse area with any other species in the WFS. In the central North Pacific, Escolar has been found to consume cephalopods, crustaceans and fish. However, these results were from a small sample (>5 stomachs) and may not reflect the prey items consumed by individuals in the GoM. On the basis of $\delta^{15}\text{N}$ values, Escolar appears to have a similar trophic position as the hake species along the WFS but the $\delta^{13}\text{C}$ values indicate that there may be feeding from different food webs that could relate to habitat differences (e.g., demersal vs. pelagic) or diet.

Our study provides a stable isotopic characterization of the trophic interactions of teleost species collected along the continental slope in the GoM. While such studies do exist in other locations, there has been, to our knowledge, no such research done on the deep-water, demersal fish communities in the GoM. Our results provide a baseline to compare future changes in trophic interactions and investigate natural variation and potential anthropogenic impacts. The inclusion of additional biomarkers, such as the $\delta^{15}\text{N}$ values of proteinaceous amino acids, may reveal additional details regarding regional isotopic baselines or trophic position (see McClelland & Montoya, 2002 for additional details). Such studies are of high priority, given the increasing potential for anthropogenic impacts, such as increased fishing pressure or oil and gas exploration.

Acknowledgements

This research was made possible by two grants from BP/The Gulf of Mexico Research Initiative through the Florida Institute of Oceanography as well as a grant to the Deep-C Consortium. Additional financial support was provided by a FIU Graduate School Dissertation Year Fellowship. Access to pelagic samples was generously provided by Robert Heuter and Mote Marine Laboratory. We thank Chip Cotton, Kirk Gastrich, and Ron Schatman for logistical support; John Harris, Bill Anderson, and the staff of the FIU Stable Isotope Laboratory; and all of the research cruise volunteers.

References

Bligh, E.S., & Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911-917.

- Brown, K. (2007). Interstate fisheries management program implementation for North Carolina, Study II: documentation and reduction of bycatch in North Carolina Fisheries-Evaluation of the Estuarine Hook and Line Recreational Fishery in the Neuse River, North Carolina. *Morehead City (NC): North Carolina Division of Marine Fisheries. Completion Report, NOAA Award No. NA, 5.*
- BryantMason, April, Xu, Y. Jun, & Altabet, Mark. (2013). Isotopic signature of nitrate in river waters of the lower Mississippi and its distributary, the Atchafalaya. *Hydrological Processes*, 27(19), 2840-2850.
- Cass-Calay, Shannon L, & Bahnick, Melissa. (2002). Status of the yellowedge grouper fishery in the Gulf of Mexico. *NOAA, NMFS, SEFSC*, 75, 03-172.
- Caut, S., Angulo, E., & Courchamp, F. (2009). Variation in discrimination factors (Delta N-15 and Delta C-13): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology*, 46(2), 443-453.
- Churchill, D. A., Heithaus, M. R., & Grubbs, R. D. (in prep). Trophic interations of deep-sea scavengers in the Gulf of Mexico.
- Churchill, Diana A., Heithaus, Michael R., Grubbs, R. Dean, Vaudo, Jeremy J., & Castro, Jose I. (2015). Trophic interactions of common elasmobranchs in deep-sea communities of the Gulf of Mexico revealed through stable isotope and stomach contents analysis. *Deep-Sea Research Part II Topical Studies in Oceanography*.
- Clark, Steven T. (2000). Age, growth, and distributions of the giant snake eel, *Ophichthus rex*, in the Gulf of Mexico. *Bulletin of Marine Science*, 67(3), 911-922.
- Cousins, Nicola J., Linley, Thomas D., Jamieson, Alan J., Bagley, Philip M., Blades, Hannah, Box, Tetrienne, Chambers, Rosanna, Ford, Alexander, Shields, Mark A., & Priede, Imants G. (2013). Bathyal demersal fishes of Charlie-Gibbs Fracture Zone region (49-54 degrees N) of the Mid-Atlantic Ridge: II. Baited camera lander observations. *Deep-Sea Research Part Ii-Topical Studies in Oceanography*, 98, 397-406.
- Dagg, M. J., & Breed, G. A. (2003). Biological effects of Mississippi River nitrogen on the northern gulf of Mexico - a review and synthesis. *Journal of Marine Systems*, 43(3-4), 133-152.

- DeNiro, M.J., & Epstein, S. (1977). Mechanisms of carbon isotope fractionation associated with lipid synthesis. *Science*, 197, 261-263.
- DeNiro, Michael J., & Epstein, Samuel. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, 42(5), 495-506.
- Feist, S. W., Stentiford, G. D., Kent, M. L., Ribeiro Santos, A., & Lorange, P. (2015). Histopathological assessment of liver and gonad pathology in continental slope fish from the northeast Atlantic Ocean. *Marine Environmental Research*, 106(0), 42-50.
- France, RL. (1995). Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. *Marine ecology progress series. Oldendorf*, 124(1), 307-312.
- Garrison, L. P., & Link, J. S. (2000). Diets of five hake species in the northeast United States continental shelf ecosystem. *Marine Ecology Progress Series*, 204, 243-255.
- Gearing, P., Plucker, F. E., & Parker, P. L. (1977). Organic carbon stable isotope ratios of continental margin sediments. *Marine Chemistry*(5), 251-266.
- Haedrich, RL. (1997). *Deep-sea demersal fish and fisheries* (Vol. 23): Springer.
- Hanson, John Mark. (2011). Ontogenetic Diet Comparison of Atlantic Cod and White Hake Occurring at Historically Low Population Abundances. *Transactions of the American Fisheries Society*, 140(4), 1070-1077.
- Heithaus, M. R., Vaudo, J. J., Kreicker, S., Layman, C. A., Krutzen, M., Burkholder, D. A., Gastrich, K., Bessey, C., Sarabia, R., Cameron, K., Wirsing, A., Thomson, J. A., & Dunphy-Daly, M. M. (2013). Apparent resource partitioning and trophic structure of large-bodied marine predators in a relatively pristine seagrass ecosystem. *Marine Ecology Progress Series*, 481, 225-237.
- Hobson, Keith A. (1987). Use of stable-carbon isotope analysis to estimate marine and terrestrial protein content in gull diets. *Canadian Journal of Zoology*, 65(5), 1210-1213.

- Jackson, A. L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, 80(3), 595-602.
- Jamieson, A. J., Fujii, T., Bagley, P. M., & Priede, I. G. (2011). Scavenging interactions between the arrow tooth eel *Synaphobranchus kaupii* and the Portuguese dogfish *Centroscymnus coelolepis*. *Journal of Fish Biology*, 79(1), 205-216.
- Koslow, J.A., Boehlert, G.W., Gordon, J.D.M., Haedrich, R.L., Lorange, P., & Parin, N. (2000). Continental slope and deep-sea fisheries: implications for a fragile ecosystem. *ICES Journal of Marine Science: Journal du Conseil*, 57(3), 548-557.
- Layman, C. A., Arrington, D. A., Montana, C. G., & Post, D. M. (2007). Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology*, 88(1), 42-48.
- Logan, J. M., Jardine, T. D., Miller, T. J., Bunn, S. E., Cunjak, R. A., & Lutcavage, M. E. (2008). Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *Journal of Animal Ecology*, 77(4), 838-846.
- MacAvoy, S. E., Carney, R. S., Fisher, C. R., & Macko, S. A. (2002). Use of chemosynthetic biomass by large, mobile, benthic predators in the Gulf of Mexico. *Marine Ecology Progress Series*, 225, 65-78.
- MacAvoy, S. E., Morgan, E., Carney, R. S., & Macko, S. A. (2008). Chemoautotrophic production incorporated by heterotrophs in Gulf of Mexico hydrocarbon seeps: An examination of mobile benthic predators and seep residents. *Journal of Shellfish Research*, 27(1), 153-161.
- Marques, A. (1998). A note on the diet *Synaphobranchus kaupii* (Pisces : Synaphobranchidae) from the Porcupine Seabight, north-east Atlantic. *Journal of the Marine Biological Association of the United Kingdom*, 78(4), 1385-1388.
- McClelland, J. W., & Montoya, J. P. (2002). Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. *Ecology*, 83(8), 2173-2180.

- Moncreiff, C. A., & Sullivan, M. J. (2001). Trophic importance of epiphytic algae in subtropical seagrass beds: evidence from multiple stable isotope analyses. *Marine Ecology Progress Series*, 215, 93-106.
- Morato, T., Watson, R., Pitcher, T. J., & Pauly, D. (2006). Fishing down the deep. *Fish and Fisheries*, 7(1), 24-34.
- Nakamura, Ikuo, & Parin, Nikolai Vasil'evich. (1993). *FAO species catalogue. v. 15: snake mackerels and cutlassfishes of the world (families Gempylidae and Trichiuridae). An annotated and illustrated catalogue of the Snake Mackerels, Snoeks, Escolars, Gemfishes, Sackfishes, Domine, Oilfish, Cutlassfishes, Scabbardfishes, Hairtails and Frostfishes known to date*: FAO.
- Parnell, Andrew C, & Jackson, Andrew L. (2013). siar: Stable Isotope Analysis in R (Version R package version 4.2).
- Perez, JAA, Wahrlich, R, Pezzuto, PR, Schwingel, PR, Lopes, FRA, & Rodrigues-Ribeiro, M. (2003). Deep-sea Fishery off Southern Brazil: Recent Trends of the Brazilian Fishing Industry. *Journal of Northwest Atlantic Fishery Science*, 31, 1.
- Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, 18, 293-320.
- Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, 83(3), 703-718.
- Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., & Montana, C. G. (2007). Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, 152(1), 179-189.
- Powell, Shawna M, Haedrich, Richard L, & McEachran, John D. (2003). The deep-sea demersal fish fauna of the northern Gulf of Mexico. *Journal of Northwest Atlantic Fishery Science*, 31, 19-33.
- R Core Team. (2013). R: A language and environment for statistical computing (Version 2.15.2). Vienna, Austria: R Foundation for Statistical Computing. Retrieved from <http://www.R-project.org/>

- Royal Dutch Shell. (2010). Shell starts production at Perdido - world's deepest offshore drilling and production facility.
- Smith, C. R., & Baco, A. R. (2003). Ecology of whale falls at the deep-sea floor. *Oceanography and Marine Biology an Annual Review*, 41, 311-354.
- Steimle, Frank W. (1999). *Essential fish habitat source document. Tilefish, *Lopholatilus chamaeleonticeps*, life history and habitat characteristics*: DIANE Publishing.
- Tieszen, L. L., Boutton, T. W., Tesdahl, K. G., & Slade, N. A. (1983). Fractionation and turnover of stable carbon isotopes in animal tissues: implication for $\delta^{13}\text{C}$ analysis of diet. *Oecologia*, 57, 32-37.
- Vander Zanden, M. J., & Rasmussen, J. B. (2001). Variation in delta N-15 and delta C-13 trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, 46(8), 2061-2066.
- Vasilakopoulos, P., Pavlidis, M., & Tserpes, G. (2011). On the diet and reproduction of the oilfish *Ruvettus pretiosus* (Perciformes: Gempylidae) in the eastern Mediterranean. *Journal of the Marine Biological Association of the United Kingdom*, 91(4), 873-881.
- Viana, Danielle de Lima, Tolotti, Mariana Travassos, Porto, Mariana, Vale de Araujo, Rodolfo Jorge, Vaske Junior, Teodoro, & Vieira Hazin, Fabio Hissa. (2012). Diet of the oilfish *Ruvettus pretiosus* (Perciformes: Gempylidae) in the Saint Peter and Saint Paul Archipelago, Brazil. *Brazilian Journal of Oceanography*, 60(2), 181-188.
- Wada, E., Mizutani, H., & Minagawa, M. (1991). The use of stable isotopes for food web analysis. *Critical Reviews in Food Science and Nutrition*, 30(4), 361-371.
- Wang, X. C., Chen, R. F., & Gardner, G. B. (2004). Sources and transport of dissolved and particulate organic carbon in the Mississippi River estuary and adjacent coastal waters of the northern Gulf of Mexico. *Marine Chemistry*, 89(1-4), 241-256.
- Wells, R. J. D., & Rooker, J. R. (2009). Feeding ecology of pelagic fish larvae and juveniles in slope waters of the Gulf of Mexico. *Journal of Fish Biology*, 75(7), 1719-1732.

Yeh, John, & Drazen, Jeffrey C. (2009). Depth zonation and bathymetric trends of deep-sea megafaunal scavengers of the Hawaiian Islands. *Deep-Sea Research Part I-Oceanographic Research Papers*, 56(2), 251-266.

Table 1 Summary of all muscle tissue samples collected from teleosts in the Gulf of Mexico.

Species	Common Name	April 2011		August 2011		October 2011	April 2012		Total	Mean depth ± SD (m)
		NGS	WFS	NGS	WFS	WFS	NGS	WFS	n	
<i>Caulolatilus chrysops</i>	Goldface tilefish	1	2	0	0	0	0	0	3	291±54
<i>Caulolatilus microps</i>	Grey tilefish	0	0	0	0	0	0	4	4	249±5
<i>Coryphaenoides mediterraneus</i>	Mediterranean Grenadier	1	0	1	0	0	0	0	2	1550±655
<i>Coryphaenoides mexicanus</i>	Mexican Grenadier	1	0	0	1	0	1	0	3	1030±586
<i>Gymnothorax kolpos</i>	Blacktail morey	0	0	0	0	0	2	0	2	222±42
<i>Helicolenus dactylopterus</i>	Blackbelly rosefish	0	0	1	0	0	1	0	2	526±42
<i>Hyporthodus flavolimbatus</i>	Yellowedge grouper	0	1	0	0	0	2	2	5	248±10
<i>Hyporthodus nigrilus</i>	Warsaw grouper	0	0	0	0	0	0	2	2	267±21
<i>Lepidocybium flavobrunneum</i>	Escolar	0	0	0	0	5	0	0	5	N/A
<i>Lopholatilus chamaeleonticeps</i>	Great northern tilefish	12	0	25	0	0	31	0	69	276±54
<i>Merluccius albidus</i>	Offshore silver hake	0	0	0	1	0	0	0	1	315
<i>Ophichthus rex</i>	King snake eel	0	0	8	0	0	12	0	20	249±6
<i>Pontinus longispinis</i>	Longspine scorpionfish	0	0	1	0	0	0	0	1	325
<i>Ruvettus pretiosus</i>	Oilfish	0	0	0	0	7	0	0	7	N/A
<i>Synaphobranchus brevidorsalis</i>	Shortdorsal Cutthroat Eel	1	0	0	0	0	0	0	1	2014
<i>Synaphobranchus oregoni</i>	No common name	5	0	3	2	0	16	1	27	1242±202
<i>Urophycis cirrata</i>	Gulf Hake	41	3	29	0	0	67	3	143	411±92
<i>Urophycis floridana</i>	Southern hake	32	18	25	10	0	48	16	149	285±46
<i>Urophycis regia</i>	Spotted hake	0	1	0	3	0	0	3	7	318±42

Table 2 Variation in stable isotope values by sampling time in teleosts on the northern gulf slope (NGS) and along the west Florida slope (WFS). Bold values indicate statistically significant differences and dashes are where sample sizes were too small (n<5).

Species	NGS				WFS			
	$\delta^{13}\text{C}$		$\delta^{15}\text{N}$		$\delta^{13}\text{C}$		$\delta^{15}\text{N}$	
	Test statistic	p-value	Test statistic	p-value	Test statistic	p-value	Test statistic	p-value
<i>Lopholatilus chamaeleonticeps</i>	$F_2=0.64$	0.53	$F_2=9.44$	<0.001	-	-	-	-
<i>Ophichthus rex</i>	W=34	0.297	W=42.5	0.700	-	-	-	-
<i>Synaphobranchus oregoni</i>	W=48.5	0.510	W=29	0.386	-	-	-	-
<i>Urophycis cirrata</i>	$F_2=4.43$	0.014	$F_2=3.77$	0.025	-	-	-	-
<i>Urophycis floridana</i>	$F_2=1.09$	0.339	$F_2=15.1$	<0.001	$F_2=0.05$	0.951	$F_2=1.70$	0.196

Table 3 Results for linear regression analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by size for five teleost species. Significant values are bold.

Species	Region	Cruise	Measurement	n	R^2	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
						t	p	R^2	t	p	
<i>Lopholatilus chamaeleonticeps</i>	NGS	Apr 11/Aug 11	SL	37	0.35	4.35	<0.001	0.22	3.15	0.003	
<i>Ophichthus rex</i>	NGS	Aug 11/Apr 12	TL	19	0.45	3.74	0.002	0.20	2.08	0.053	
<i>Synaphobranchus oregoni</i>	NGS	All	TL	23	0.10	1.52	0.143	0.49	4.45	<0.001	
<i>Urophycis cirrata</i>	NGS	Apr 12	SL	64	0.18	3.65	<0.001	<0.01	-0.09	0.927	
<i>Urophycis floridana</i>	WFS	All	SL	44	0.28	4.02	<0.001	0.11	2.32	0.025	

Table 4 Results for linear regression analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by depth for five teleost species. Significant values are bold.

Species	n	R^2	$\delta^{13}\text{C}$		R^2	$\delta^{15}\text{N}$	
			t	p		t	p
<i>Lopholatilus chamaeleonticeps</i>	69	0.09	-2.60	0.011	<0.01	-0.033	0.742
<i>Ophichthus rex</i>	20	<0.01	0.14	0.890	0.11	-1.52	0.145
<i>Synaphobranchus oregoni</i>	27	0.01	0.60	0.555	0.03	0.10	0.329
<i>Urophycis cirrata</i>	143	0.10	4.04	<0.001	<0.01	1.08	0.280
<i>Urophycis floridana</i>	149	<0.01	-0.54	0.590	<0.01	0.18	0.854

Table 5 The percent of total area (TA) and individuals occupying unique isotopic niche space for each teleost species.

Species	n	% of TA unique	% of ellipse unique
NGS			
<i>Lopholatilus chamaeleonticeps</i>	69	1.5	9.5
<i>Ophichthus rex</i>	20	84.4	100
<i>Synaphobranchus oregoni</i>	24	80.3	100
<i>Urophycis cirrata</i>	137	8.9	11.6
<i>Urophycis floridana</i>	105	9.4	17.3
WFS			
<i>Lepidocybium flavobrunneum</i>	5	100	100
<i>Ruvettus pretiosus</i>	7	38.7	66.3
<i>Urophycis cirrata</i>	6	8.5	54.5
<i>Urophycis floridana</i>	44	93.4	44.6
<i>Urophycis regia</i>	7	0	34.4

Figure 1 Teleosts were sampled at multiple sample stations located along the northern slope (NGS, circles) and on the west Florida slope (WFS, triangles) of the Gulf of Mexico. Filled shapes indicate stations where teleosts were collected. The star indicates the location of the Deepwater Horizon oil spill (DwH).

Figure 2 The C:N ratios of bulk and lipid-extracted teleost muscle tissue.

Figure 3 Spatial and depth distributions of teleosts collected along the northern and west Florida slopes of the Gulf of Mexico.

Figure 4 Size distributions of teleosts collected along the northern and west Florida slopes of the Gulf of Mexico.

Figure 5 Temporal variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for *Urophycis floridana* on the northern gulf slope and along the west Florida slope. Groupings with different letters are significantly different determined by post-hoc Tukey's tests with capital letters for carbon and lower case for nitrogen.

Figure 6 Variation in stable isotope values (C and N) with body size. Only significant relationships are shown (see Table 3).

Scientific Name (Figure Code)	n	δ ¹³ C	δ ¹⁵ N
<i>Caulolatilus chrysops</i> (Cchr)	1	-	-
<i>Coryphaenoides mediterraneus</i> (Cmed)	2	-	-
<i>Coryphaenoides mexicanus</i> (Cmex)	2	-	-
<i>Gymnothorax kolpos</i> (Gkol)	2	-	-
<i>Helicolenus dactylopterus</i> (Hdac)	2	-	-
<i>Hyporthodus flavolimbatus</i> (Hfla)	2	-	-
<i>Lopholatilus chamaeleonticeps</i> (Lcha)	69	BC	C
<i>Ophichthus rex</i> (Orex)	20	A	A
<i>Pontinus longispinis</i> (Plon)	1	-	-
<i>Synaphobranchus brevadorsalis</i> (Sbire)	1	-	-
<i>Synaphobranchus oregoni</i> (Sore)	24	A	D
<i>Urophycis cirrata</i> (Ucir)	137	B	B
<i>Urophycis floridana</i> (Uflo)	105	C	C

Scientific Name (Figure Code)	n	δ ¹³ C	δ ¹⁵ N
<i>Caulolatilus chrysops</i> (Cchr)	2	-	-
<i>Coryphaenoides mexicanus</i> (Cmex)	1	-	-
<i>Caulolatilus microps</i> (Cmic)	4	-	-
<i>Hyporthodus flavolimbatus</i> (Hfla)	3	-	-
<i>Hyporthodus nigritus</i> (Hnig)	2	-	-
<i>Lepidocybium flavobrunneum</i> (Lfla)	5	A	B
<i>Merluccius albidus</i> (Malb)	1	-	-
<i>Ruvettus pretiosus</i> (Rpre)	7	B	A
<i>Synaphobranchus oregoni</i> (Sore)	3	-	-
<i>Urophycis cirrata</i> (Ucir)	6	BC	A
<i>Urophycis floridana</i> (Uflo)	44	C	B
<i>Urophycis regia</i> (Ureg)	7	C	B

Figure 7 Temporal and spatial variation in stable isotopic values of teleosts captured along the northeastern Gulf of Mexico (NGS) and along the West Florida Slope (WFS). Error bars are $\pm$ SD. Species with different letters in inset tables are significantly different based on post-hoc Tukey's tests; "-" denotes species that were not included in pairwise comparisons.

Figure 8 Relationship between the mean body size and $\delta^{15}\text{N}$ for teleosts. Only significant relationships are shown. See Fig. 7 for species codes.

Figure 9 Total area convex hulls and Bayesian ellipses of scavengers from the NGS and WFS sampling regions. See Fig. 7 for species codes.

CHAPTER V

TROPHIC INTERACTIONS OF COMMON ELASMOBRANCHS IN DEEP-SEA COMMUNITIES OF THE GULF OF MEXICO REVEALED THROUGH STABLE ISOTOPE AND STOMACH CONTENT ANALYSIS

Abstract

Deep-water sharks are abundant and widely distributed in the northern and eastern Gulf of Mexico. As mid and upper-level consumers that can range widely, sharks likely are important components of deep-sea communities and their trophic interactions may serve as system-wide baselines that could be used to monitor the overall health of these communities. We investigated the trophic interactions of deep-sea sharks using a combination of stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and stomach content analyses. Two hundred thirty-two muscle samples were collected from elasmobranchs captured off the bottom at depths between 200 - 1100 m along the northern slope (NGS) and the west Florida slope (WFS) of the Gulf of Mexico during 2011 and 2012. Although we detected some spatial, temporal, and interspecific variation in apparent trophic positions based on stable isotopes, there was considerable isotopic overlap among species, between locations, and through time. Overall $\delta^{15}\text{N}$ values in the NGS region were higher than in the WFS. The $\delta^{15}\text{N}$ values also increased between April 2011 and 2012 in the NGS, but not the WFS, within *Squalus cf. mitsukurii*. We found that stable isotope values of *S. cf. mitsukurii*, the most commonly captured elasmobranch, varied between sample regions, through time, and also with sex and size. Stomach content analysis (n=105) suggested relatively similar diets at the level of broad taxonomic categories of prey among the taxa with sufficient sample sizes. We did not detect a relationship between body size and relative trophic levels inferred from $\delta^{15}\text{N}$, but patterns within several species suggest increasing trophic levels with increasing size. Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values suggest a substantial degree of overlap among most deep-water shark species. This study provides

the first characterization of the trophic interactions of deep-sea sharks in the Gulf of Mexico and establishes system baselines for future investigations.

Key Words:

Stable isotopes, deep-sea, shark, feeding behavior, food web, trophic structure, Gulf of Mexico

Introduction

Human exploitation and use of the deep-sea, including expansion of deep-water fisheries (Morato et al., 2006), is increasing on a global scale. Therefore, it is important to understand the structure and dynamics of communities in these habitats including upper trophic level predators, like sharks. Deep-water sharks are widely distributed and can be locally abundant in deep-sea habitats around the world, including in the northern and eastern Gulf of Mexico (Castro, 2011). Though no deep-water fisheries currently exist in the Gulf of Mexico, because sharks possess a number of biological attributes that make them vulnerable to overfishing (White et al., 2012), they could be negatively affected should these activities be initiated (Kyne & Simpfendorfer, 2010). Indeed deep-water sharks tend to be particularly slow to mature and have relatively low reproductive rates and many populations experience intensive harvesting from both directed fisheries and as bycatch (reviewed in Nicholas & Robyn, 2010). Unfortunately, the status and ecological roles of most of the deep-water sharks in the Gulf and other deep-water ecosystems are largely unknown (Kyne & Simpfendorfer, 2010). Consequently, most of the common deep-water sharks in the Gulf of Mexico are listed as data deficient by the International Union for the Conservation of Nature (IUCN).

As mid and upper-level predators (Cortés, 1999) that occupy diverse horizontal and vertical habitats and have the capacity for long-distance movements (Kyne & Simpfendorfer, 2010), sharks may serve as sentinel species for understanding anthropogenic impacts on deep-sea communities. Specifically, sharks would be expected to integrate and respond to changes that occur throughout the food web (e.g., Rooney et al., 2006). This may be particularly true for sharks at the top of deep-sea food webs, like

bluntnose sixgill sharks (*Hexanchus griseus*) which are one of the dominant predators on continental slopes (Ebert, 1994). Therefore, quantifying trophic interactions of deep-water sharks may help establish system-wide baselines that could be used to monitor the overall health of these communities.

Diet and trophic ecology has traditionally been studied through stomach content analysis (Cortés, 1997; Hyslop, 1980). This technique allows for fine resolution of diets, but has limitations, particularly in the deep sea. For example, the large sample sizes that are often required to fully characterize diets are difficult and expensive to obtain in deep-water studies due to ship costs, logistical difficulties in sampling, low population densities, and the high proportion of individuals recovered with empty stomachs. In addition, deep-dwelling teleosts and elasmobranchs often regurgitate food as they are brought to the surface (Bowman, 1986) and prey items from deep-water communities often are fragile and difficult to identify (Cailliet et al., 1999; Drazen et al., 2001; Robinson et al., 2007). Finally, stomach contents provide only a snapshot of the diet of an individual and may not reflect the proportions of prey groups ingested or assimilated because of variability in the digestibility of prey items and the potential for temporal variation in diets.

Stable isotope analysis, particularly of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, is becoming increasingly important in studies of trophic interactions (Fry, 2006; Layman et al., 2012) because it provides time-integrated information on assimilated, rather than ingested, biomass (Peterson & Fry, 1987; Vander Zanden & Rasmussen, 2001). The utility of stable isotope analysis derives from the fact that isotopic ratios in consumer tissues reflect those of their prey (Bearhop et al., 2004; Vander Zanden & Rasmussen, 2001). $\delta^{15}\text{N}$ values can

be used to estimate relative trophic position because ^{15}N is generally enriched through each trophic transfer (reviewed in Caut et al., 2009; Post, 2002). Because carbon ratios are generally conserved through trophic transfers (Fry & Sherr, 1989) and vary among groups of primary producers or habitats (O'Leary, 1981; Smith & Epstein, 1971), $\delta^{13}\text{C}$ values can be used to elucidate foraging locations and/or sources of primary production across many trophic levels. Since isotopes provide integrated views of diets and changes in stable isotope values of consumers reflect changes not only in their direct prey, but also in lower level trophic pathways, stable isotope analysis is attractive for assessing overall changes in communities. Since shark tissues have slow incorporation rates (Kim et al., 2012b), the stable isotope values would be less affected by stochastic variation and display changes reflecting long-term shifts in the environment and trophic interactions. However, stable isotope analysis cannot identify specific prey items and multiple diet combinations can result in similar $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of a consumer, and discrimination factors can vary within and among taxa (Caut et al., 2009; del Rio et al., 2009; Hussey et al., 2014; Post, 2002). Therefore, isotopic data must be interpreted with caution and, where possible, benefit from the insights of stomach content analysis.

The goal of this study was to examine the trophic interactions of shark species in the deep-water habitats of the northern and eastern Gulf of Mexico. Because of their different strengths and weaknesses, we used a combination of stable isotope analysis and, when samples allowed, stomach content analysis to investigate regional and temporal variation in trophic interactions of sharks of a variety of body sizes.

Materials and Methods

Study Site

Sampling took place along the continental slope from off the south-central Gulf coast of Florida to Louisiana (Figure 1). The southern sampling sites were located on the West Florida Slope (WFS), approximately 270 km southeast of Tampa, Florida. The northern sites were spread across the northern slope of the Gulf (NGS) from an area approximately 25 km offshore of the Mississippi River delta through DeSoto Canyon and east onto the north Florida slope to an area 85 km southwest of Cape San Blas, Florida (Figure 1). These locations included sites on the continental slope to the east and west of DeSoto Canyon, along the canyon's eastern rim, and on the canyon floor. Stations in both areas were concentrated in depths of approximately 250 to 2000 m, however all sharks used in this study were captured shallower than 1200 m. The northern and southern sites were chosen to match depth and topography along a broad shelf and onto the slope.

Sampling Methods

Samples were collected during research cruises aboard the R/V *Weatherbird II* in April 2011, August 2011, and April 2012, which all occurred after the Deepwater Horizon Spill that began on April 20, 2010 (BP, 2010). Additional samples were obtained from an April 2005 cruise to the WFS. Each cruise in 2011-2012 included sampling in both the NGS (39 stations and 85 total sets) and the WFS (14 stations and 68 total sets; Figure 1).

Our fishery-independent surveys involved making two or three successive sets of bottom longlines with integrated traps. A standard longline/trap set consisted of 550 m of mainline on the bottom with 50 baited gangions including five hook sizes and three

different types of baited traps. The mainline consisted of 5.6-mm tarred twisted, hard-laid nylon (Everson Cordage TG-28) and components of each set were attached to the mainline in the following order: 1) a 4-kg grapnel anchor was attached to the end of the mainline, 2) a small box trap (60 cm x 60 cm x 38 cm with 2.5-cm mesh) was attached three meters from the anchor, 3) five 4-m gangions terminated with 18/0 circle hooks spaced 10 m apart, 4) five 3-m gangions terminated with 14/0 circle hooks spaced 10 m apart, 5) ten 50-cm gangions terminated with 11/0 circle hooks spaced 10 m apart, 6) ten 50-cm gangions terminated with 12/0 circle hooks spaced 10 m apart, 7) a cylindrical (60 cm x 35 cm with 1.0-cm mesh) trap, 8) twenty 50-cm gangions terminated with 10/0 circle hooks spaced 10 m apart, 9) small shrimp trap (40 cm x 20 cm x 15 cm with 1.0-cm mesh), 10) a sash weight (2.0-3.0 kg) if needed, 11) a Lotek LAT1400 temperature/depth recorder (TDR), 12) mainline scoped at least 1.5 times the depth, 13) a Polyform inflatable buoy (size A-2, 30-kg floatation), 14) 10-m slack line, and 15) a strobed highflier. Longlines were baited with little tunny (*Euthynnus alletteratus*) except for three 18/0 gangions on each set that were baited with elasmobranch. All traps were baited with menhaden (*Brevoortia* sp.) or Atlantic mackerel (*Scomber scombrus*). Each set was deployed in 20 to 30 minutes and was retrieved following a soak of 4-8 hr. Soak time varied due to depth-mediated differences in sinking time. Three hours was the targeted on-bottom soak time and was confirmed using the TDR data. Lines were slowly retrieved using a Lindgren-Pitman hydraulic winch (model LP-28X36-G3) and animals were brought on board by hand or with a 2.4-m aluminum-framed sling webbed with 3.0-cm trawl mesh for larger individuals.

Once the animals were on board, each individual was sexed, weighed and measured. We collected muscle from the dorsal region using sterile biopsy punches and removed the entire gastrointestinal track from all sharks that were not tagged and released or kept as taxonomic voucher specimens for other studies. All samples were frozen for later processing.

Stable Isotope Analysis

All muscle tissue was thawed and then rinsed in deionized water for several seconds, dried a minimum of 48 hours in a 60°C oven and ground to a fine powder. Elasmobranch stable isotope values may be affected by the presence of lipids and urea in the muscle tissue. Lipids tend to be depleted in ^{13}C (DeNiro & Epstein, 1977; Tieszen et al., 1983) and urea in ^{15}N (Fisk et al., 2002) relative to carbohydrates and protein. A chloroform-methanol extraction was used to remove both lipids and urea from muscle samples examined in this study (Hussey et al., 2012b). Dried, powdered samples were immersed in a mixture of chloroform and methanol (2:1) with a solvent volume three to five times greater than that of the sample. Samples were then vortexed for 30 seconds, left undisturbed for at least 30 minutes and centrifuged for 10 min at 1318 g. The extraction process was repeated at least three times or until the supernatant was clear. Samples were then re-dried (Bligh & Dyer, 1959; Logan et al., 2008).

All stable isotope analyses were performed on a ThermoFinnigan Delta C mass spectrometer coupled with a NA 1500 NE elemental analyzer at the Florida International University Stable Isotope Laboratory. The error of the isotope-ratio mass spectrometer based on repeat analyses of an internal glycine standard was less than 0.1‰ (n=72)

during this study. Approximately one sixth of the samples were run in duplicate. The mean absolute difference between duplicates was $0.1 \pm 0.1\text{‰}$ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. All reported C:N ratios are based on atomic mass.

We used analysis of variance and student's t-tests to examine spatial and temporal variation in trophic interactions within species. Because of low sample sizes for most species and spatial and temporal variation in the abundance of even common species (see Appendix A), we were not able to complete full factorial analyses. For *Centrophorus* cf. *granulosus*, we were able to investigate variation across seasons (August 2011 and April 2012) in NGS. For *Centrophorus* cf. *niaukang*, we tested whether isotopic values differed between April 2005 and April 2012. We then were able to contrast temporally pooled data from individuals in the WFS to those in the NGS. Because of large differences in sample sizes for this species, we used a non-parametric Wilcoxon rank sum test to compare stable isotope values between sampling times and regions. We were able to compare *Squalus cubensis* individuals from April 2011, August 2012 and April 2012 in the NGS region. For *Squalus* cf. *mitsukurii* we were able to compare across all sampling times (2005 & 2011-2012) in the WFS and across all sampling times and both regions for the 2011-2012 cruises.

For among-species comparisons, we used a one-way analysis of variance with a Tukey's poc-hoc test for paired contrasts. Based on the within-species analyses, groups that were significantly different from each other were treated separately. For species with insufficient sample sizes, we pooled individuals of the same species from different times (April 2005, April 2011, August 2011, April 2012). Species with less than five captured individuals were not included in analyses. This analysis was performed separately for the

NGS and WFS sampling regions. We used linear regression to investigate the relationship between mean size (pre-caudal length [PCL]) and mean $\delta^{15}\text{N}$ values across all deep-sea shark species collected as part of this study.

We used metrics from Layman et al. (2007) to compare community-level trophic interactions. Total area is an indication of the isotopic niche width of a population or community in isotopic bi-plot space. The $\delta^{13}\text{C}$ range is an estimate of the diversity of basal resources while the range of $\delta^{15}\text{N}$ provides an estimate of the trophic length of the community. We also calculated the percent of the total area that was unique to each species in the NGS and WFS sampling regions.

We used linear regression to investigate the effects of size (PCL) on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within species across both sampling regions where sample size was ≥ 5 . For *S. cf. mitsukurii*, we used ANCOVA to examine the effects of size, region (NGS or WFS), and sex on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. For individuals captured in the northern sampling region, we used ANCOVA to determine the effects of both size and sex. This analysis was not possible in the southern sampling region because no females were observed. We also used linear regression to assess the effects of capture depth on $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within each species where the sample size with accurate depth data was ≥ 5 .

Unless otherwise noted, all data were normally distributed and equal in variance. The critical level of significance was $\alpha=0.05$ for all analyses/statistical tests. All analyses were completed in JMP, version 10 (SAS Institute Inc. SAS Institute Inc., 2012).

Stomach Contents Analysis

Each gastrointestinal track was thawed and the stomach was separated from the intestine. Stomachs were carefully cut open and the contents, if any, were rinsed in deionized water and identified to the lowest possible taxonomic level. Prey items were counted, blotted dry and all items of a given taxon were weighed to the nearest 0.01g. In order to facilitate analyses, items were grouped into seven functional categories (see results). Diets were quantified using the following four metrics: frequency of occurrence (%FO, proportion of stomachs with prey that contain a given prey category), mean percent abundance (%MN, the mean calculated from the proportional abundance of diet taxa in individual stomachs), and mean percent weight (%MW, the mean calculated from individual stomach prey weight proportions). We use %MN and %MW, rather than pooled indices (e.g. %N, %W) because they are less biased and allow for the calculation of confidence intervals (Chipps & Garvey, 2007). However, to facilitate comparison with literature values, the index of relative importance using pooled indices (IRI; Pinkas et al., 1971) was calculated for each prey category using $IRI = \%FO \times (\%N + \%W)$, where %N is the proportion of the total number of prey items within a given prey category and %W is the proportion of the total weight of all prey items within a given category pooled across all stomachs. To make interspecific comparisons, the IRI was expressed as %IRI as suggested by Cortés (1997). Because of the difficulty in identifying deep-sea prey items, our prey categories are at broad taxonomic levels. We did not directly calculate overlap of diets based on stomach contents analysis because the low taxonomic resolution could amplify apparent dietary overlap.

Results

Stable Isotope Analysis

We captured 17 species of sharks at depths ranging from *ca.* 200 to 1100 meters. From these individuals, a total of 232 muscle tissue samples were collected and processed for analysis (Appendix A). All C:N ratios of chloroform/methanol extracted muscle tissue were close to those of protein (i.e., C:N ~ 3; Appendix B).

Centrophorus cf. granulosus caught in the NGS sampling region from August 2011 (n= 19) and April 2012 (n=20) did not differ significantly in $\delta^{13}\text{C}$ ($t=-0.83$, $p=0.41$) or $\delta^{15}\text{N}$ values ($t=2.00$, $p=0.06$). There was no significant difference in *C. cf. niaukang* $\delta^{13}\text{C}$ (S=110, $Z=-0.98$, $p=0.32$) or $\delta^{15}\text{N}$ values (S=144, $Z=0.63$, $p=0.52$) in the WFS region between April 2005 (n=9) and April 2012 (n=19) or between sampling regions (WFS n=30, NGS n=7; $\delta^{13}\text{C}$: S=158, $Z=0.95$, $p=0.34$, $\delta^{15}\text{N}$: S=171, $Z=1.45$, $p=0.14$), but sample sizes were low during some sampling periods. *Squalus cubensis*, captured only in the NGS, did not vary across years in $\delta^{13}\text{C}$ ($t=-2.05$, $p=0.52$) or $\delta^{15}\text{N}$ values ($t=0.87$, $p=0.39$) from April 2011 (n=9) to April 2012 (n=16). Based on data from 2005 and 2011-2012, *S. cf. mitsukurii* showed no significant differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ across time in the WFS sampling region ($F_{3,45}=0.94$, $p<0.43$, $F_{3,45}=2.13$, $p=0.11$, respectively; Figure 2). Restricting *S. cf. mitsukurii* data to those from cruises in 2011 and 2012, both $\delta^{13}\text{C}$ ($F_{5,64}=3.08$, $p=0.01$) and $\delta^{15}\text{N}$ ($F_{5,64}=13.13$, $p<0.001$) were influenced by an interaction of region and sampling time. $\delta^{15}\text{N}$ increased significantly in the NGS from April 2011 to April 2012, but did not in the WFS (Figure 2).

Overall, samples collected from NGS tended to have $\delta^{15}\text{N}$ values that were higher than those from WFS ($t=-8.55$, $p<0.001$), though this is likely driven by different species

compositions in the two regions. The $\delta^{13}\text{C}$ values between the sampling regions were not significantly different ($t=-0.49$, $p=0.62$). In the NGS region, there was significant variation among species/groups in both $\delta^{13}\text{C}$ ($F_{7, 114}=20.54$, $p<0.001$; Figure 3) and $\delta^{15}\text{N}$ values ($F_{7, 114}=36.89$, $p<0.001$; Figure 3). *Centrophorus* cf. *granulosus* had the highest $\delta^{15}\text{N}$ values. *Hexanchus griseus* and *Galeocerdo cuvier* had moderately higher $\delta^{13}\text{C}$ values compared to other sampled species. *Squalus cubensis* and *Squalus* cf. *mitsukurii* had the lowest $\delta^{13}\text{C}$ values in this region. The two *Squalus* species, along with *G. cuvier*, also displayed the lowest $\delta^{15}\text{N}$ values.

In the WFS region, there was also significant variation among species/groups in both $\delta^{13}\text{C}$ ($F_{5,86}=2.99$, $p<0.01$; Figure 3) and $\delta^{15}\text{N}$ values ($F_{5,86}=8.65$, $p<0.001$; Figure 3). In this region, some of the most extreme isotopic values were displayed by species with sample sizes that were insufficient for inclusion in analysis. *Centrophorus* cf. *niaukang* and *H. griseus* had the highest $\delta^{15}\text{N}$ values, and *Galeus arae* and *Mustelus canis* the lowest. *H. griseus* exhibited the highest $\delta^{13}\text{C}$ values and *G. arae* the lowest.

Despite some interspecific variation in mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, there was overlap among the isotopic niche spaces of species within both the NGS and WFS (Table 1; Figure 4). In the NGS region, four of the seven species (*C. cf. granulosus*, *G. cuvier*, *H. griseus*, and *S. cubensis*) had isotopic niche spaces where >50% of the area was unique to that species (Table 1). For two species (*G. cuvier* and *S. cubensis*), the total unique area also included >50% of the sampled individuals. In the WFS, only one species (*C. cf. niaukang*) had a total area with limited overlap. At the level of the elasmobranch community, the NGS and WFS regions had similar ranges for $\delta^{15}\text{N}$ (NGS: 4.4‰, WFS:

5.1‰) and $\delta^{13}\text{C}$ values (NGS: 3.2‰, WFS: 3.0‰). This led to similar total areas in isotopic space for both sampling regions (NGS: 8.9‰², WFS: 9.0‰²).

At the community level, there was no relationship between mean body size (PCL) and mean $\delta^{15}\text{N}$ (regression, $t=0.66$, $p=0.53$; Figure 5). There was, however, variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ based on body size (PCL, see Table 2) within several taxa. *Centrophorus* cf. *granulosus*, *Etmopterus bigelowi*, *G. cuvier*, *H. griseus*, and male *S. cf. mitsukurii* in the NGS sampling region showed no relationship between size (PCL) and either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$. *Centrophorus* cf. *niaukang*, *M. canis*, and male *S. cf. mitsukurii* in the WFS region displayed no significant relationship between PCL and $\delta^{13}\text{C}$, but did for $\delta^{15}\text{N}$ values. *Centrophorus* cf. *niaukang* showed a positive relationship while *M. canis* displayed a negative relationship (Figure 6). The relationship between PCL and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in *S. cf. mitsukurii* varied between sampling regions (ANCOVA, $F_{3,82}=8.35$, $p<0.001$, and $F_{3,82}=21.20$, $p<0.001$, respectively; Figure 7). In the NGS region, there was a significant positive relationship between size and both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, but only the relationship between size and $\delta^{15}\text{N}$ values was significant in the WFS. Both sex and size influenced $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *S. cf. mitsukurii* in the NGS region (ANCOVA, $F_{3,32}=4.36$, $p=0.01$ and $F_{3,32}=6.12$, $p=0.002$, respectively; Figure 8). For females, there was a positive relationship between size and both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. In males, there were no significant relationships. We were not able to perform this analysis in the WFS region because only larger males were collected in this sampling region.

We were able to examine the relationship between capture depth and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in six species (Table 3). *Centrophorus* cf. *granulosus* displayed a significant negative relationship between capture depth and $\delta^{15}\text{N}$ values, while *S. cubensis* showed a

negative relationship between depth and $\delta^{13}\text{C}$ values. When all individuals were included in the analysis, *S. cf. mitsukurii* exhibited significant relationships with depth and both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. With analyses completed separately for each sampling region, no significant relationships were observed. All other species had no significant relationships.

Stomach Contents Analysis

We recovered stomach contents from 105 individuals (12 *C. cf. granulosus*, 2 *C. cf. niaukang*, 2 *M. canis*, 11 *S. cubensis*, and 78 *S. cf. mitsukurii*; Appendix C). We found an octopus in one stomach sample collected for *C. cf. niaukang*. The two stomachs from *M. canis* contained portunid crabs. Due to small sample sizes, diet metrics were only calculated for *C. cf. granulosus*, *S. cubensis*, and *S. cf. mitsukurii*. For these species, teleosts were the dominant prey group, found in >50% of the samples from each (Appendix D). Of the identified teleosts, myctophids were most common. Squid also made a large contribution to all diet metrics for *S. cf. mitsukurii* and were also present in *C. cf. granulosus* and *S. cubensis* to a lesser extent. Sharks made a large contribution to %IRI in *C. cf. granulosus*, but this was the result of a single, large sample.

Discussion

Intraspecific Patterns

Stable isotopic data suggest that *Squalus cubensis* and female *Squalus cf. mitsukurii* undergo small ontogenetic shifts in stable isotope values. In the NGS, individuals from both species showed a positive relationship between body size and $\delta^{13}\text{C}$

values. However, this shift is small (-16.9 to -16.2‰) and the relationships are weak ($R^2 < 0.5$). *S. cubensis*, and *S. cf. mitsukurii* in the NGS also appear to feed at progressively higher trophic levels as they grow, as do *C. cf. niaukang* females in this region. All three species displayed a positive relationship between body size and $\delta^{15}\text{N}$. This pattern, however, could also be the result of shifting prey types that differ in their $\delta^{15}\text{N}$ values. Interestingly, *M. canis* $\delta^{15}\text{N}$ values decreased as body size increased. This may indicate ontogenetic shifts in prey or foraging locations.

Isotopic values of three shark species varied across depths in which they were captured. The patterns of this variation, however, were not consistent across taxa and were not particularly strong. *Centrophorus cf. granulosus* displayed a weak ($R^2=0.3$) negative relationship between $\delta^{15}\text{N}$ values and capture depth. *Squalus cubensis* had a weak ($R^2=0.2$) negative relationship between $\delta^{13}\text{C}$ values and depth. The ecological drivers of these patterns are unclear but could be related to shifts in diets or sources of productivity with depth. Patterns in *S. cf. mitsukurii* are even more difficult to interpret because individuals captured in the NGS region were collected at shallower depths than those in the WFS and depth effects may be more reflective of differences in isotopic baselines or food web structure between regions.

Of particular interest, we found that the most abundant and widespread species, *S. cf. mitsukurii* exhibited region-specific shifts in $\delta^{15}\text{N}$ values, which could be due to a temporal change in trophic position or shifts in the isotopic baseline within the sampling region. *Squalus cf. mitsukurii* displayed similar $\delta^{15}\text{N}$ values in the WFS region in April 2011 and 2012 April. In the NGS region, $\delta^{15}\text{N}$ values increased between April 2011 and April 2012. Although the NGS was subject to disturbance from the Deepwater Horizon

Oil Spill that occurred before sampling began, it is not possible to determine the mechanism underlying the observed shift. Further analyses of other, non-elasmobranch taxa from the NGS and WFS, employing other biochemical markers (e.g., compound specific isotope values; Chikaraishi et al., 2009), and continued long-term sampling may provide further insights into whether this shift might represent broad-based shifts in trophic structure and interactions in NGS.

Ecological Dynamics

We found a substantial degree of overlap in both stomach contents and stable isotope results across 13 species of deep-water sharks. Previously reported data for the diets of *Squalus cubensis*, *Squalus* cf. *mitsukurii*, *Centrophorus* cf. *niaukang*, *Centrophorus* cf. *granulosus*, *Deania calcea*, and *Etmopterus bigelowi* indicate these species consume squid and small fishes, including myctophids (Castro, 2011). Many species occupied little unique isotopic niche space, despite some differences in mean isotopic values. This overlap suggests that there may be a limited degree of resource partitioning within this predator guild in the deep-sea waters of the Gulf of Mexico. *Centrophorus* cf. *granulosus* and *H. griseus* stood out as two exceptions to this overall trend; both species displayed a high percentage of unique isotopic space and number of individuals within the unique space. This may be indicative of dietary or metabolic variation that differentiates these species from the rest of the deep-water shark community in the NGS.

The stable isotope analysis of the deep-sea elasmobranch community in the Gulf of Mexico revealed a relatively small range of $\delta^{13}\text{C}$ values that likely represents a limited

number of resource pathways. Possible carbon sources include plankton and detritus (-23.0‰ to -22.4‰; Wells & Rooker, 2009), *Sargassum* sp. (-16.6‰ to -16.2‰; Wells & Rooker, 2009), terrestrial sources through riverine input (-23.8‰ to -26.8‰; Wang et al., 2004), out-welled coastal seagrass (-13.4‰ to -11‰; Moncreiff & Sullivan, 2001), organic components in the sediment (-21.5‰ to -20‰; Gearing et al., 1977), whale falls (-32‰ to -20‰; Smith & Baco, 2003), and deep-sea chemosynthetic communities (-65‰ to -25‰; MacAvoy et al., 2008). Given the substantially lower $\delta^{13}\text{C}$ values of chemosynthetic systems, it seems likely that the deep-sea elasmobranchs from the areas of the Gulf of Mexico we sampled are reliant on photosynthetic inputs from surface waters. The higher $\delta^{13}\text{C}$ values observed in the sharks (-17‰ to -15.1‰), relative to many possible carbon sources, may be due to additional nutrient recycling between surface production and higher trophic level deep-water consumers (assuming small positive fractionation; McCutchan et al., 2003) or possibly large inputs of floating algae. Given the limited resource pool available in the deep-sea habitats we sampled, it is perhaps not surprising that the stable isotope analysis revealed interspecific overlap in $\delta^{13}\text{C}$ values and, therefore, obscures potential resource partitioning. It is interesting to note that even though there is likely some seasonality associated with the carbon inputs to the deep-sea in the Gulf of Mexico (Rowe, 2013), we did not detect evidence for this among the sharks sampled as part of this study. While the long stable isotope incorporation rates in shark tissues (Kim et al., 2012b; Logan & Lutcavage, 2010) would dampen the signal, we would still expect to observe seasonal shifts even if the muscle tissue does not reach a steady state (Matich & Heithaus, 2014).

There was a relatively limited range in $\delta^{15}\text{N}$ values across deep-sea sharks in both the NGS and WFS sampling regions (4.4‰ and 5.1‰, respectively). Observed differences between the sampling regions may be the result of different resource pathways or variation in the isotopic baselines. Stomach contents analysis suggests that the three species of sharks with sufficient sample sizes in these two regions are consuming similar prey items. Therefore, it is likely that differences in isotopic values among regions is a result of different $\delta^{15}\text{N}$ values in basal resources supporting these ecosystems or variation in the food webs below the prey of sharks.

A scaled discrimination factor framework, where higher dietary $\delta^{15}\text{N}$ values result in lower trophic discrimination factors (Caut et al., 2013; Hussey et al., 2014; Olin et al., 2013), suggests that the deep-sea sharks sampled in this study span approximately two trophic levels. Experimental studies done on a limited number of elasmobranch species revealed $\delta^{15}\text{N}$ discrimination factors ranging from 2.3‰ to 3.7‰ using lipid and urea extracted samples (Hussey et al. 2010; Kim et al. 2012) and 1.1‰ to 1.8‰ using bulk muscle tissue (Hussey et al., 2010; Kim et al., 2012a; Malpica-Cruz et al., 2012), but none of these experiments were conducted on deep-sea taxa. While the considerable differences in body size between adult *Galeus arae* (<20 cm PCL) and adult *Hexanchus griseus* (>400 cm PCL) might suggest foraging at vastly different trophic levels, isotopic data suggest they are separated by two levels. This may be driven by relatively short food chains along with high levels of scavenging contributing to compressed food webs. Further studies of discrimination factors across taxa and environments and compound specific isotope analysis (Chikaraishi et al., 2009) would provide important insights into the structure and dynamics of these communities.

In many fish species, there is a positive relationship between body size and trophic position (Romanuk et al., 2011) and $\delta^{15}\text{N}$ values, which is attributed to the consumption of larger, higher trophic level prey at larger body sizes (Hussey et al., 2011; Papastamatiou et al., 2010). This relationship has been observed in shark and dolphin species present in coastal waters in Western Australia (Heithaus et al., 2013). We found no overall relationship between average body size and mean $\delta^{15}\text{N}$ values in deep-sea sharks, even with the large range in body sizes sampled (22 cm to 350 cm PCL). In fact, in the NGS region, the species with the highest $\delta^{15}\text{N}$ value was *C. cf. granulatus*, which was higher than much larger-bodied sharks such as *H. griseus* and *G. cuvier*. This may be due to reduced resource pathways in the deep-sea habitat or related to the various isotopic discrimination issues that affect elasmobranchs (Hussey et al., 2012a). There is also the potential for scavenging in the species sampled in this study, which might cause $\delta^{15}\text{N}$ values to converge.

At a broad taxonomic resolution of prey taxa, the three species examined (*C. cf. granulatus*, *S. cubensis*, and *S. cf. mitsukurii*) showed somewhat similar values for the relative importance (%IRI) of squid, shrimp and teleosts in their diets based on stomach contents. It is quite possible that divergence in diets would be detected at finer taxonomic resolution for prey, but this was not achievable due to the digested state of stomach contents. Indeed, stable isotopes suggested average trophic interactions of *C. cf. granulatus* differed from those of the two species of *Squalus*. Further research using genetic barcoding of prey from stomach contents could assist in resolving such questions (Barnett et al., 2010).

Conclusions

Investigations of trophic interactions of sharks in deep-sea habitats have been limited by the difficulty of obtaining samples. Here, using a combination of stomach content analysis and stable isotope analysis we have been able to provide a preliminary characterization of the trophic structure of the shark community in deep-sea habitats of the Gulf of Mexico that may be applicable to other continental slope communities. Additional sampling of elasmobranch and other taxa, along with the application of additional biochemical markers, may reveal whether there is, indeed, greater inter- and intraspecific variation in trophic interactions as well as the generality of potential trophic shifts in the NGS. Such studies are of high priority given the growing potential for anthropogenic impacts to these communities (e.g., increasing exploitation of deep-sea oil reserves and expansion of deep-water fisheries). We are still in the early stages of our understanding of deep-sea shark communities and major human impacts like the Deepwater Horizon oil spill highlight the need for a better characterization of these unique habitats.

Acknowledgements

This research was made possible by two grants from BP/The Gulf of Mexico Research Initiative through the Florida Institute of Oceanography as well as a grant to the Deep-C Consortium. The 2005 research cruise was funded by a Florida Institute of Oceanography Shiptime Grant and FIU's Department of Biological Sciences. We thank Chip Cotton and Ron Schatman for logistical support, the staff of the FIU Stable Isotope Laboratory, and all of the research cruise volunteers.

References

- Barnett, A., Redd, K. S., Frusher, S. D., Stevens, J. D., & Semmens, J. M. (2010). Non-lethal method to obtain stomach samples from a large marine predator and the use of DNA analysis to improve dietary information. *Journal of Experimental Marine Biology and Ecology*, 393(1-2), 188-192.
- Bearhop, S., Adams, C. E., Waldron, S., Fuller, R. A., & Macleod, H. (2004). Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology*, 73(5), 1007-1012.
- Bligh, E.S., & Dyer, W.J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37, 911-917.
- Bowman, R.E. (1986). Effect on regurgitation on stomach content of marine fishes. *Environmental Biology of Fishes*, 16, 171-181.
- BP. (2010). Deepwater Horizon accident investigation report (pp. 192).
- Cailliet, G. M., Andrews, A. H., Wakefield, W. W., Moreno, G., & Rhodes, K. L. (1999). Fish faunal and habitat analyses using trawls, camera sleds and submersibles in benthic deep-sea habitats off central California. *Oceanologica Acta*, 22(6), 579-592.
- Castro, J. I. (2011). *Sharks of North America*. New York: Oxford University Press.
- Caut, S., Angulo, E., & Courchamp, F. (2009). Variation in discrimination factors (Delta N-15 and Delta C-13): the effect of diet isotopic values and applications for diet reconstruction. *Journal of Applied Ecology*, 46(2), 443-453.
- Caut, Stephane, Jowers, Michael J, Michel, Loïc, Lepoint, Gilles, & Fisk, Aaron T. (2013). Diet-and tissue-specific incorporation of isotopes in the shark *Scyliorhinus stellaris*, a North Sea mesopredator. *Marine ecology. Progress series*, 492, 185-198.
- Chikaraishi, Yoshito, Ogawa, Nanako O, Kashiyama, Yuichiro, Takano, Yoshinori, Suga, Hisami, Tomitani, Akiko, Miyashita, Hideaki, Kitazato, Hiroshi, & Ohkouchi, Naohiko. (2009). Determination of aquatic food-web structure based on

- compound-specific nitrogen isotopic composition of amino acids. *Limnology and Oceanography*, 7(11), 740-750.
- Chipps, S. R., & Garvey, J. E. (2007). Assessment of food habits and feeding patterns. In C. Guy & M. Brown (Eds.), *Analysis and interpretation of freshwater fisheries data* (pp. 473-514). Bethesda: American Fisheries Society.
- Cortés, E. (1997). A critical review of methods of studying fish feeding based on analysis of stomach contents: Application to elasmobranch fishes. *Canadian Journal of Fisheries and Aquatic Sciences*, 54(3), 726-738.
- Cortés, E. (1999). Standardized diet compositions and trophic levels of sharks. *ICES Journal of Marine Science*, 56(5), 707-717.
- del Rio, C. M., Wolf, N., Carleton, S. A., & Gannes, L. Z. (2009). Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews (Cambridge)*, 84(1), 91-111.
- DeNiro, M.J., & Epstein, S. (1977). Mechanisms of carbon isotope fractionation associated with lipid synthesis. *Science*, 197, 261-263.
- Drazen, J. C., Buckley, T. W., & Hoff, G. R. (2001). The feeding habits of slope dwelling macrourid fishes in the eastern North Pacific. *Deep-Sea Research Part I Oceanographic Research Papers*, 48(3), 909-935.
- Ebert, DA. (1994). Diet of the sixgill shark *Hexanchus griseus* off southern Africa. *South African Journal of Marine Science*, 14(1), 213-218.
- Fisk, A. T., Tittlemier, S. A., Pranschke, J. L., & Norstrom, R. J. (2002). Using anthropogenic contaminants and stable isotopes to assess the feeding ecology of greenland sharks. *Ecology*, 83(8), 2162-2172.
- Fry, B. (2006). *Stable Isotope Ecology*. New York: Springer.
- Fry, B., & Sherr, E. B. (1989). $\delta^{13}\text{C}$ Measurements as Indicators of Carbon Flow in Marine and Freshwater Ecosystems. In P. W. Rundel, J. R. Ehleringer, & K. A. Nagy (Eds.), *Stable Isotopes in Ecological Research* (Vol. 68, pp. 196-229): Springer New York.

- Gearing, P., Plucker, F. E., & Parker, P. L. (1977). Organic carbon stable isotope ratios of continental margin sediments. *Marine Chemistry*(5), 251-266.
- Heithaus, M. R., Vaudo, J. J., Kreicker, S., Layman, C. A., Krutzen, M., Burkholder, D. A., Gastrich, K., Bessey, C., Sarabia, R., Cameron, K., Wirsing, A., Thomson, J. A., & Dunphy-Daly, M. M. (2013). Apparent resource partitioning and trophic structure of large-bodied marine predators in a relatively pristine seagrass ecosystem. *Marine Ecology Progress Series*, 481, 225-237.
- Hussey, N. E., Brush, J., McCarthy, I. D., & Fisk, A. T. (2010). $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ diet-tissue discrimination factors for large sharks under semi-controlled conditions. *Comparative Biochemistry and Physiology A*, 155(4), 445-453.
- Hussey, N. E., Dudley, S. F. J., McCarthy, I. D., Cliff, G., & Fisk, A. T. (2011). Stable isotope profiles of large marine predators: viable indicators of trophic position, diet, and movement in sharks? *Canadian Journal of Fisheries and Aquatic Sciences*, 68(12), 2029-2045.
- Hussey, N. E., MacNeil, M. A., Olin, J. A., McMeans, B. C., Kinney, M. J., Chapman, D. D., & Fisk, A. T. (2012a). Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. *Journal of Fish Biology*, 80(5), 1449-1484.
- Hussey, N. E., Olin, J. A., Kinney, M. J., McMeans, B. C., & Fisk, A. T. (2012b). Lipid extraction effects on stable isotope values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of elasmobranch muscle tissue. *Journal of Experimental Marine Biology and Ecology*, 434, 7-15.
- Hussey, Nigel E., MacNeil, M. Aaron, McMeans, Bailey C., Olin, Jill A., Dudley, Sheldon F. J., Cliff, Jeremy, Wintner, Sabine P., Fennessy, Sean T., & Fisk, Aaron T. (2014). Rescaling the trophic structure of marine food webs. *Ecology Letters*, 17(2), 239-250.
- Hyslop, E. J. (1980). Stomach content analysis - a review of methods and their application. *Journal of Fish Biology*, 17(4), 411-429.
- Kim, SL, Casper, DR, Galva'n-Magaña, F, Ochoa-Díaz, R, Berenice Hernández-Aguilar, S, & Koch, PL. (2012a). Carbon and nitrogen discrimination factors for

elasmobranch soft tissues based on a long-term controlled feeding study. *Environmental Biology of Fishes*, 95(1), 37-52.

Kim, Sora Lee, del Rio, Carlos Martínez, Casper, Dave, & Koch, Paul L. (2012b). Isotopic incorporation rates for shark tissues from a long-term captive feeding study. *The Journal of Experimental Biology*, 215(14), 2495-2500.

Kyne, P. M., & Simpfendorfer, C. A. (2010). Deepwater Chondrichthyans. In J. C. Carrier, J. A. Musick, & M. R. Heithaus (Eds.), *Sharks and Their Relatives II: Biodiversity, Adaptive Physiology and Conservation* (pp. 37-114). Boca Raton, FL: CRC Press.

Layman, C. A., Araujo, M. S., Boucek, R., Hammerschlag-Peyer, C. M., Harrison, E., Jud, Z. R., Matich, P., Rosenblatt, A. E., Vaudo, J. J., Yeager, L. A., Post, D. M., & Bearhop, S. (2012). Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biological Reviews (Cambridge)*, 87(3), 545-562.

Layman, C. A., Arrington, D. A., Montana, C. G., & Post, D. M. (2007). Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology*, 88(1), 42-48.

Logan, J. M., Jardine, T. D., Miller, T. J., Bunn, S. E., Cunjak, R. A., & Lutcavage, M. E. (2008). Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *Journal of Animal Ecology*, 77(4), 838-846.

Logan, John M., & Lutcavage, Molly E. (2010). Stable isotope dynamics in elasmobranch fishes. *Hydrobiologia*, 644(1), 231-244.

MacAvoy, S. E., Morgan, E., Carney, R. S., & Macko, S. A. (2008). Chemoautotrophic production incorporated by heterotrophs in Gulf of Mexico hydrocarbon seeps: An examination of mobile benthic predators and seep residents. *Journal of Shellfish Research*, 27(1), 153-161.

Malpica-Cruz, L., Herzka, S. Z., Sosa-Nishizaki, O., & Lazo, J. P. (2012). Tissue-specific isotope trophic discrimination factors and turnover rates in a marine elasmobranch: empirical and modeling results. *Canadian Journal of Fisheries and Aquatic Sciences*, 69(3), 551-564.

- Matich, P., & Heithaus, M. R. (2014). Multi-tissue stable isotope analysis and acoustic telemetry reveal seasonal variability in the trophic interactions of juvenile bull sharks in a coastal estuary. *Journal of Animal Ecology*, 83(1), 199-213.
- McCutchan, J. H., Lewis, W. M., Kendall, C., & McGrath, C. C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, 102(2), 378-390.
- Moncreiff, C. A., & Sullivan, M. J. (2001). Trophic importance of epiphytic algae in subtropical seagrass beds: evidence from multiple stable isotope analyses. *Marine Ecology Progress Series*, 215, 93-106.
- Morato, T., Watson, R., Pitcher, T. J., & Pauly, D. (2006). Fishing down the deep. *Fish and Fisheries*, 7(1), 24-34.
- Nicholas, K. Dulvy, & Robyn, E. Forrest. (2010). Life Histories, Population Dynamics, and Extinction Risks in Chondrichthyans *Sharks and Their Relatives II* (pp. 639-679): CRC Press.
- O'Leary, M.H. (1981). Carbon Isotope Fractionation in Plants. *Phytochem.*(20), 553-567.
- Olin, Jill A, Hussey, Nigel E, Grgicak-Mannion, Alice, Fritts, Mark W, Wintner, Sabine P, & Fisk, Aaron T. (2013). Variable $\delta^{15}\text{N}$ Diet-Tissue Discrimination Factors among Sharks: Implications for Trophic Position, Diet and Food Web Models. *PLoS ONE*, 8(10), e77567.
- Papastamatiou, Y. P., Friedlander, A. M., Caselle, J. E., & Lowe, C. G. (2010). Long-term movement patterns and trophic ecology of blacktip reef sharks (*Carcharhinus melanopterus*) at Palmyra Atoll. *Journal of Experimental Marine Biology and Ecology*, 386(1-2), 94-102.
- Peterson, B. J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annual Review of Ecology and Systematics*, 18, 293-320.
- Pinkas, L., Oliphant, M.S., & Iverson, I. L. K. (1971). Food habits of albacore, bluefin tuna and bonito in Californian waters. *California Department of Fish and Game Fish Bulletin*, 152, 1-105.

- Post, D. M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology*, 83(3), 703-718.
- Robinson, H. J., Cailliet, G. M., & Ebert, D. A. (2007). Food habits of the longnose skate, *Raja rhina* (Jordan and Gilbert, 1880), in central California waters. *Environmental Biology of Fishes*, 80(2-3), 165-179.
- Romanuk, Tamara N, Hayward, April, & Hutchings, Jeffrey A. (2011). Trophic level scales positively with body size in fishes. *Global Ecology and Biogeography*, 20(2), 231-240.
- Rooney, N., McCann, K., Gellner, G., & Moore, J. C. (2006). Structural asymmetry and the stability of diverse food webs. *Nature*, 442(7100), 265-269.
- Rowe, Gilbert T. (2013). Seasonality In Deep-Sea Food Webs—A Tribute To The Early Works Of Paul Tyler. *Deep-Sea Research Part II Topical Studies in Oceanography*, 92, 9-17.
- SAS Institute Inc. (2012). JMP, Version 10. Cary, NC: SAS Institute Inc.
- Smith, B.N., & Epstein, S. (1971). Two Categories of $^{13}\text{C}/^{12}\text{C}$ ratios for higher plants. *Plant Physiology (Rockville)*(47), 380-384.
- Smith, C. R., & Baco, A. R. (2003). Ecology of whale falls at the deep-sea floor. *Oceanography and Marine Biology an Annual Review*, 41, 311-354.
- Tieszen, L. L., Boutton, T. W., Tesdahl, K. G., & Slade, N. A. (1983). Fractionation and turnover of stable carbon isotopes in animal tissues: implication for $\delta^{13}\text{C}$ analysis of diet. *Oecologia*, 57, 32-37.
- Vander Zanden, M. J., & Rasmussen, J. B. (2001). Variation in delta N-15 and delta C-13 trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, 46(8), 2061-2066.
- Wang, X. C., Chen, R. F., & Gardner, G. B. (2004). Sources and transport of dissolved and particulate organic carbon in the Mississippi River estuary and adjacent coastal waters of the northern Gulf of Mexico. *Marine Chemistry*, 89(1-4), 241-256.

- Wells, R. J. D., & Rooker, J. R. (2009). Feeding ecology of pelagic fish larvae and juveniles in slope waters of the Gulf of Mexico. *Journal of Fish Biology*, 75(7), 1719-1732.
- White, W. T., Blaber, S. J. M., & Craig, J. F. (2012). The current status of elasmobranchs: biology, fisheries and conservation. *Journal of Fish Biology*, 80(5), 897-900.

Table 1 The percent of total area (TA) and individuals occupying unique isotopic niche space for each shark species.

Species	n	Sampling Region	% of TA unique	% of individuals in unique space (no. of ind.)
<i>Centrophorus</i> cf. <i>granulosus</i>	39	NGS	52.3	92.3 (36)
<i>Centrophorus</i> cf. <i>niaukang</i>	7	NGS	2.4	0 (0)
<i>Galeocerdo cuvier</i>	3	NGS	51.8	33.3 (1)
<i>Hexanchus griseus</i>	7	NGS	75.9	71.4 (5)
<i>Mustelus canis</i>	5	NGS	0.0	0 (0)
<i>Squalus cubensis</i>	27	NGS	52.1	40.7 (11)
<i>Squalus</i> cf. <i>mitsukurii</i>	37	NGS	0.0	0 (0)
<i>Centrophorus</i> cf. <i>niaukang</i>	32	WFS	69.5	31.2 (10)
<i>Carcharhinus signatus</i>	4	WFS	26.0	25.0 (1)
<i>Etmopterus bigelowi</i>	11	WFS	49.5	45.4 (5)
<i>Squalus</i> cf. <i>mitsukurii</i>	49	WFS	12.9	18.4 (9)

Table 2: Results for linear regression analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by size (PCL) for eight shark species. Starred values indicate a significant relationship.

Species	Region, Sex	n	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
			R^2	t	p	R^2	t	p
<i>Centrophorus cf. granulosus</i>	All	38	<0.01	-0.36	0.72	0.07	-1.62	0.11
<i>Centrophorus cf. niaukang</i>	All	39	<0.01	-0.17	0.87	0.16	2.70	0.01*
<i>Etmopterus bigelowi</i>	All	9	0.09	-0.84	0.43	0.17	1.20	0.27
<i>Galeocerdo cuvier</i>	All	5	0.14	-0.72	0.52	0.07	0.49	0.66
<i>Hexanchus griseus</i>	All	8	0.11	0.86	0.42	0.01	0.31	0.77
<i>Mustelus canis</i>	All	6	0.48	1.94	0.12	0.96	-9.80	<0.001*
<i>Squalus cubensis</i>	All	27	0.23	2.76	0.01*	0.25	2.89	0.008*
<i>Squalus cf. mitsukurii</i>	WFS, male	48	0.01	0.78	0.44	0.10	-2.32	0.02*
<i>Squalus cf. mitsukurii</i>	NGS, all	37	0.28	3.70	<0.001*	0.40	4.84	<0.001*
<i>Squalus cf. mitsukurii</i>	NGS, male	10	<0.01	-0.10	0.92	0.06	0.69	0.51
<i>Squalus cf. mitsukurii</i>	NGS, female	26	0.41	4.09	<0.001*	0.43	4.30	<0.001*

Table 3 Results for linear regression analysis of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ by capture depth for six shark species. Starred values indicate a significant relationship.

Species	n	$\delta^{13}\text{C}$			$\delta^{15}\text{N}$		
		R^2	t	p	R^2	t	p
<i>Centrophorus</i> cf. <i>granulosus</i>	39	<0.01	-0.10	0.92	0.33	-4.27	<0.001*
<i>Centrophorus</i> cf. <i>niaukang</i>	30	0.04	-1.07	0.29	0.07	-1.51	0.14
<i>Etmopterus bigelowi</i>	7	0.31	-1.52	0.19	0.03	0.41	0.70
<i>Galeocerdo cuvier</i>	5	0.53	1.85	0.16	0.40	-1.43	0.25
<i>Hexanchus griseus</i>	9	0.04	0.55	0.60	0.37	2.06	0.08
<i>Mustelus canis</i>	6	0.46	-1.87	0.13	0.02	0.27	0.80
<i>Squalus cubensis</i>	27	0.2	-2.50	0.02*	0.06	-1.29	0.21
<i>Squalus</i> cf. <i>mitsukurii</i>	64	0.10	2.69	0.009*	0.20	-3.90	<0.001*
<i>Squalus</i> cf. <i>mitsukurii</i> – NGS	37	<0.01	0.52	0.60	0.04	1.23	0.23
<i>Squalus</i> cf. <i>mitsukurii</i> – WFS	27	0.04	0.96	0.34	0.04	-1.13	0.27

Figure 1 Deep-sea sharks were sampled at multiple sample stations (dots) located along the northern slope of the Gulf of Mexico (NGS) and waters of the west Florida slope (WFS).

Sampling Time and Region (Figure Code)	n	δ ¹³ C (WFS)	δ ¹⁵ N (WFS)	δ ¹³ C (NGS & WFS)	δ ¹⁵ N (NGS & WFS)
NGS April 2011 (SmNApr11)	44	-	-	B	B
NGS August 2011 (SmNAug11)	5	-	-	A B	A
NGS April 2012 (SmNApr12)	25	-	-	A B	A
WFS April 2005 (Sm05)	31	A	A	-	-
WFS April 2011 (SmWApr11)	7	A B	A B	A B	B C
WFS August 2011 (SmWAug11)	11	B	B	A B	C
WFS April 2012 (SmWApr12)	16	A B	A B	A	B C

Figure 2 Temporal variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for *Squalus cf. mitsukurii* on the northern gulf slope and along the west Florida slope. Groupings with different letters in the inset table are significantly different based on post-hoc Tukey's tests; "-" denotes species that were not included in pairwise comparisons.

Figure 3 Temporal and spatial variation in stable isotope values of sharks of the northeastern Gulf of Mexico (NGS) and along the West Florida Slope (WFS). Error bars are $\pm$ SE. Species with different letters in inset tables are significantly different based on post-hoc Tukey's tests; "-" denotes species that were not included in pairwise comparisons.

Figure 4 Total area convex hulls of sharks from the NGS and WFS sampling regions. See Figure 3 for species codes.

Figure 5 Relationship between the mean size (PCL) and $\delta^{15}\text{N}$ for sharks. Only significant relationships are shown. See Figure 3 for species codes.

Figure 6 Variation in stable isotope values with body size in *Centrophorus cf. niaukang* ($\delta^{15}\text{N}$), *Mustelus canis* ($\delta^{15}\text{N}$), and *Squalus cubensis* ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$).

Figure 7 Variation in $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) with length for *Squalus cf. mitsukurii* in the northeastern Gulf and West Florida Slope. Regression lines were fit to NGS ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and WFS ($\delta^{15}\text{N}$) data.

Figure 8 Sex differences in the relationship between size and $\delta^{13}\text{C}$ (a) and $\delta^{15}\text{N}$ (b) in *Squalus cf. mitsukurii* collected from NGS. Regression lines were fit to the data for females but not males.

CHAPTER VI
CONCLUSION

Our knowledge of the deep-sea is limited largely as a consequence of the logistical constraints of deep-water research. The lack of information is a potentially important knowledge gap because anthropogenic impacts in the deep water environments—fishing, disposal of litter and waste, mining for deep-sea mineral deposits, underwater cables, and climate change—are increasing (Ramirez-Llodra et al., 2011). Without basic information about deep-sea ecosystems, it will be difficult to manage human activities or predict future impacts. The Deepwater Horizon oil spill, that occurred on 20 April 2010, was an unprecedented disaster not only in the amount of oil released, but also the depth of the release, the volume of dispersants and depth at which they were used. Many of the impacts of the oil spill on epipelagic and coastal animal communities are more readily observable, including sea bird, sea turtle and cetacean mortalities (Antonio et al., 2011); changes in plankton abundances (Abbriano et al., 2011); and toxicity to early stages life stages of blue crabs (Lively & McKenzie, 2014). The potential impacts on deep-sea communities are difficult to determine. Our lack of understanding is, in part, the result of the disaster occurring in an ecosystem that was poorly understood before the spill occurred, highlighting the need for baseline data. To help fill the knowledge gap in deep-sea ecosystems, I investigated the trophic interactions of three important consumer groups (scavengers, teleosts, and sharks) and provide one of the first characterizations of the trophic ecology of the deep-water communities in the Gulf of Mexico (GoM).

Stable isotope analysis is often employed to investigate questions related to diet and trophic ecology (Fry, 2006). In order to make appropriate comparisons among multiple taxa, it is necessary to standardize values to account for interspecific differences

in factors that affect isotopic ratios. In Chapter II, I examine how varying concentrations of soluble nitrogen compounds, such as urea or trimethylamine oxide, can affect the analysis and interpretation of $\delta^{15}\text{N}$ values. I assessed the effects of a standard chloroform/methanol extraction on the stable isotope values of muscle tissue obtained from ten species of sharks and three species of hagfish collected from deep-water communities. There were significant differences in $\delta^{15}\text{N}$, %N, and C:N values as a result of extractions in eight of ten shark and all three hagfish species. In addition, I observed increased $\delta^{15}\text{N}$ values, but shifts in %N and C:N values were not unidirectional. Mathematical normalizations for $\delta^{15}\text{N}$ values were successfully created for four shark and two hagfish species. However, they were not successful for two shark species. Although the exact correction factors presented may not be broadly applicable, my results highlight the need to extract urea/TMAO from all individuals sampled, or to generate species-specific corrections for these deep-sea taxa.

Scavengers often are important links in food webs and can be important to ecosystem dynamics through recycling energy and nutrients back into food webs quickly. The foraging guild is likely an important component of deep-sea food webs because these communities derive much of their energy resources via allochthonous inputs from surface waters in the form of photodetritus (Billett et al., 1983) or discrete food-falls of marine carrion (Smith 1985, Tyler et al. 1993). In Chapter III, I examined the trophic interactions of mobile, benthic scavengers in the deep-sea habitats of the northern and eastern GoM. I was able to create taxa-specific mathematical corrections for $\delta^{13}\text{C}$ values to account for the high levels of lipids present in the sampled muscle tissue. My results suggest that taxa-specific normalizations or chemical extractions are more accurate than correcting

$\delta^{13}\text{C}$ values with previously published equations. I observed significant spatiotemporal variation within four of the scavenger species sampled (*Bathynomus giganteus*, *Chaceon quinquedens*, *Eptatretus springeri* and *Myxine mcmillanae*). There were no strong relationships between isotopic values and either depth or body size. Among invertebrates (isopods, crabs, shrimp) and paravertebrates (hagfish), I found no relationship between body size and $\delta^{15}\text{N}$ values at the taxa level. Among the more traditional scavenger community, although there was some variation among species in mean values of both carbon and nitrogen within each sampling region, there was a moderate degree of isotopic niche overlap among species, suggesting that while species may specialize in foraging to some degree there is likely overlap in diets.

The deep waters of the GoM include a diverse demersal fish community; however, the status and ecological roles of many of these demersal fish species remains largely unknown. In Chapter IV, I describe the trophic interactions of deep-water demersal teleosts in the northern and eastern GoM using stable isotope analysis (C and N). There was significant intraspecific spatial and temporal variation in stable isotope values within several species (*Lopholatilus chamaeleonticeps*, *Urophycis cirrata*, and *Urophycis floridana*), as well as evidence for ontogenetic shifts in trophic interactions of all five of the most commonly captured teleosts (*L. chamaeleonticeps*, *Ophichthus rex*, *Synphobranchus oregoni*, *U. cirrata*, and *U. floridana*). The stable isotope analysis of this deep-sea community revealed a relatively small range of $\delta^{13}\text{C}$ values (NGS: 2.62‰ and WFS: 2.21‰) that likely represent a limited pool of basal resources. There was a larger range of $\delta^{15}\text{N}$ values in the NGS (6.2‰) than in the WFS (3.2‰) sampling region, suggesting that NGS teleost community spans at least two trophic levels, whereas that in

the WFS spans approximately one level. I observed a high degree of isotopic niche overlap for a number of species, which is consistent with previously reported stomach contents analysis and is predicted for oligotrophic ecosystems.

Deep-water sharks are abundant and widely distributed in the northern and eastern GoM. As mid and upper-level consumers that can range widely, sharks likely are important components of deep-sea communities and their trophic interactions may serve as system-wide baselines that could be used to monitor the overall health of these communities. In Chapter V, I investigated the trophic interactions of deep-sea sharks using a combination of stable isotope (C and N) and stomach content analyses. Although I detected some spatial, temporal, and interspecific variation in apparent trophic positions using stable isotopes, there was considerable isotopic overlap among species, between locations, and through time. I found that stable isotope values of *S. cf. mitsukurii*, the most commonly captured elasmobranch, varied between sample regions, through time, and also with sex and size. Stomach content analyses suggested relatively similar diets at the level of broad taxonomic categories of prey among the taxa with sufficient sample sizes. A relationship between body size and relative trophic levels was not inferred from $\delta^{15}\text{N}$, but patterns within several species suggest increasing trophic levels with increasing size. Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values suggest a substantial degree of overlap among most deep-water shark species.

Taken together, the results from my studies provide interesting insights into the dynamics of the deep-sea ecosystems of the eastern Gulf of Mexico. For example, overall $\delta^{15}\text{N}$ values in the NGS region were higher than in the WFS across all consumer groups. This pattern likely reflects isotopic baseline differences between these two

regions. The $\delta^{13}\text{C}$ values among all three consumer groups also provide a consistent view of relatively few sources of basal resources in the systems and little reliance on chemosynthetic communities. Instead, it appears that surface-derived allochthonous inputs are critical in these systems. This is consistent with models of nutrient fluxes into the deep waters of the Gulf of Mexico (Rowe, 2013).

In general, it appears that the oligotrophic nature of the system drives a fairly high degree of dietary overlap and less differentiation in trophic levels than might be expected. For example, across all taxa sampled in this study (invertebrates/paravertebrates, teleosts, and elasmobranchs) there is no relationship between mean taxa body size (mass) and mean $\delta^{15}\text{N}$ values along the northern gulf slope ($t=0.96$, $p=0.34$; Figure 1a) and, although the relationship is significant along the west Florida shelf, it is weak ($t=2.31$, $p=0.03$, $R^2=0.20$; Figure 1b). No strong relationship between body size and apparent trophic position is surprising given the large range in body sizes sampled. In the deep-sea many large predators, including teleosts and sharks, are likely to scavenge at least opportunistically as a response to the reduced availability of resources in this system (Compagno, 1984; Gage, 2003). The feeding behavior may upset the relationship between size and trophic level (Romanuk et al., 2011) and subsequently higher $\delta^{15}\text{N}$ values (Hussey et al., 2011; Papastamatiou et al., 2010) observed in other systems (Heithaus et al., 2013).

At the level of individual taxa, sharks appear to have a somewhat separate isotopic niche than teleosts in both the northern gulf slope and west Florida shelf sampling regions (Figure 2), indicating that despite a potentially limited amount of nutrient resources and broadly similar trophic levels, there is some niche partitioning

between these two consumer groups. Indeed, both groups display >57% unique isotopic niche space in both sampling regions (Table 1). In both regions, the scavenger isotopic niche has considerable overlap with the shared niche space of sharks and teleosts, with <51% unique space in both regions (Table 1). Carbon ranges of the benthic scavengers suggest that these organisms may have shared resources with both sharks and teleosts via both predation and the consumption of large food falls, such as animal carcasses. Such trophic overlap is to be expected of large-bodied scavengers in an oligotrophic system.

Investigations of trophic interactions of organisms in deep-water environments have been limited by the difficulty of obtaining samples in a remote system. Here, using stable isotope analysis, I have been able to provide a preliminary characterization of the trophic structure of three deep-water consumer groups in the deep-sea habitats of the GoM. In addition, I have established a dataset upon which future studies and conservation efforts can be based. Sampling of additional taxa, along with the application of additional biochemical markers, may reveal additional details regarding the sources of energy and nutrients input into this system and how these resources transfer to the wider community. Such studies are of high priority given the increasing exploitation of deep-sea resources, the potential for anthropogenic impacts to these communities, and the still early stage of our understanding of these communities.

References

Abbriano, Raffaella, Carranza, Magdalena, Hogle, Shane, Levin, Rachel, Netburn, Amanda, Seto, Katherine, Snyder, Stephanie, & Franks, Peter. (2011). Deepwater Horizon Oil Spill: A Review of the Planktonic Response. *Oceanography*, 24(3), 294-301.

- Antonio, F. J., Mendes, R. S., & Thomaz, S. M. (2011). Identifying and modeling patterns of tetrapod vertebrate mortality rates in the Gulf of Mexico oil spill. *Aquatic Toxicology (Amsterdam)*, 105(1-2), 177-179.
- Billett, D. S. M., Lampitt, R. S., Rice, A. L., & Mantoura, R. F. C. (1983). Seasonal sedimentation of phytoplankton to the deep-sea benthos. *Nature*, 302(5908), 520-522.
- Compagno, L.J.V. (1984). *Sharks of the World: Hexanchiformes to Lamniformes*: United Nations Development Programme.
- Fry, B. (2006). *Stable Isotope Ecology*. New York: Springer.
- Gage, John D. (2003). Food Inputs, Utilization, Carbon Flow and Energetics. In P. A. Tyler (Ed.), *Ecosystems of the Deep Oceans* (pp. 315-382). New York: Elsevier.
- Heithaus, M. R., Vaudo, J. J., Kreicker, S., Layman, C. A., Krutzen, M., Burkholder, D. A., Gastrich, K., Bessey, C., Sarabia, R., Cameron, K., Wirsing, A., Thomson, J. A., & Dunphy-Daly, M. M. (2013). Apparent resource partitioning and trophic structure of large-bodied marine predators in a relatively pristine seagrass ecosystem. *Marine Ecology Progress Series*, 481, 225-237.
- Hussey, N. E., Dudley, S. F. J., McCarthy, I. D., Cliff, G., & Fisk, A. T. (2011). Stable isotope profiles of large marine predators: viable indicators of trophic position, diet, and movement in sharks? *Canadian Journal of Fisheries and Aquatic Sciences*, 68(12), 2029-2045.
- Lively, Julie A. Anderson, & McKenzie, Jon. (2014). Toxicity of the Dispersant Corexit 9500 to Early Life Stages of Blue Crab, *Callinectes sapidus*. *Bulletin of Environmental Contamination and Toxicology*, 93(6), 649-653.
- Papastamatiou, Y. P., Friedlander, A. M., Caselle, J. E., & Lowe, C. G. (2010). Long-term movement patterns and trophic ecology of blacktip reef sharks (*Carcharhinus melanopterus*) at Palmyra Atoll. *Journal of Experimental Marine Biology and Ecology*, 386(1-2), 94-102.
- Ramirez-Llodra, Eva, Tyler, Paul A., Baker, Maria C., Bergstad, Odd Aksel, Clark, Malcolm R., Escobar, Elva, Levin, Lisa A., Menot, Lenaick, Rowden, Ashley A.,

- Smith, Craig R., & Van Dover, Cindy L. (2011). Man and the Last Great Wilderness: Human Impact on the Deep Sea. *PLoS ONE*, 6(8), e22588.
- Romanuk, Tamara N, Hayward, April, & Hutchings, Jeffrey A. (2011). Trophic level scales positively with body size in fishes. *Global Ecology and Biogeography*, 20(2), 231-240.
- Rowe, Gilbert T. (2013). Seasonality In Deep-Sea Food Webs—A Tribute To The Early Works Of Paul Tyler. *Deep-Sea Research Part II Topical Studies in Oceanography*, 92, 9-17.

Table 1 The percent of total area (TA) and ellipse area occupying unique isotopic niche space for each consumer group.

Species	% of TA unique	% of ellipse unique
NGS		
Scavenger	25.6	41.6
Teleost	67.7	66.9
Shark	59.0	57.8
WFS		
Scavenger	31.1	50.3
Teleost	63.2	75.0
Shark	68.1	81.7

Figure 1 The relationship between body size (mass) and $\delta^{15}\text{N}$ values along the northern gulf slope (NGS) and the west Florida shelf (WFS) among scavenger, teleost and shark taxa.

Figure 2 Total area convex hulls and Bayesian ellipses of scavengers, sharks and teleosts from the northern gulf slope (NGS) and west Florida shelf (WFS) sampling regions.

VITA

DIANA CHURCHILL

2000-2004

B.S. Marine Biology
University of California, Santa Cruz

PUBLICATIONS AND PRESENTATIONS

Churchill, D.A., Heithaus, M.R., Vaudo, J.J., and Grubbs, R.D (August, 2012). *Trophic interactions of common elasmobranchs in deep-sea communities of the Gulf of Mexico*. Paper presented at the World Congress of Herpetology, Vancouver, British Columbia.

Churchill, D.A., Heithaus, M.R., and Grubbs, R.D. (July, 2013). *Trophic variation within and among hagfish species, a numerically dominant predator-scavenger in the Gulf of Mexico*. Paper presented at the Joint Meeting of Ichthyologists and Herpetologists, Albuquerque, New Mexico.

Churchill, D.A., Heithaus, M.R., Vaudo, J.J., Grubbs, R.D., Gastrich, K., Castro, J.I., (2015). *Trophic interactions of common elasmobranchs in deep-sea communities of the Gulf of Mexico revealed through stable isotope and stomach content analysis*. Deep Sea Research Part II: Topical Studies in Oceanography 115, 92-102.

Churchill, D.A., Heithaus, M.R., Dean Grubbs, R., (2015). *Effects of lipid and urea extraction on $\delta^{15}N$ values of deep-sea sharks and hagfish: Can mathematical correction factors be generated?* Deep Sea Research Part II: Topical Studies in Oceanography 115, 103-108.