THE EFFECTS OF OIL ON THE DEEP GULF OF MEXICO BENTHOS

A Dissertation

By

TRAVIS WILLIAM WASHBURN

BS, University of Alabama, 2005 MS, College of Charleston, 2008

Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR of PHILOSOPHY

in

MARINE BIOLOGY

Texas A&M University – Corpus Christi Corpus Christi, Texas

May 2017

© Travis William Washburn

All Rights Reserved

May 2017

EFFECTS OF OIL ON THE DEEP GULF OF MEXICO BENTHOS

A Dissertation

by

TRAVIS WILLIAM WASHBURN

This dissertation meets the standards for scope and quality of Texas A&M University-Corpus Christi and is hereby approved.

Paul A. Montagna, Ph.D. Chair Amanda W.J. Demopoulos, Ph.D. Committee Member

Jennifer B. Pollack, Ph.D. Committee Member David W. Yoskowitz, Ph.D Committee Member

Steven D. Seidel, Ph.D. Graduate Faculty Representative

May 2017

ABSTRACT

The deep sea (> 200 m) is the largest habitat on Earth. However, the deep sea ecosystem is poorly understood relative to most other habitats due to the difficultly in accessing it. As human activities increase in the deep sea, the need to understand processes occurring in the deep and impacts on these processes by human activities also increases. This study examines the importance of the deep sea to humans as well as the impacts of oil on deep-sea communities.

Approximately 5 million barrels of oil were released during the Deepwater Horizon spill, much of which remained in the deep sea. Shortly after the spill ended, benthic diversity and abundance were lower near the Deepwater Horizon wellhead compared to deep-sea areas not affected by the spill. Diversity increased with increasing distance from the wellhead while abundances peaked at intermediate distances suggesting a toxicity vs. enrichment effect. There were also several benthic taxa identified as potential indicators of oil-contaminated and uncontaminated areas.

Oil is released from the seafloor via natural seepage as well. Benthic abundance and diversity differed among different types of seep communities (microbial mats, tubeworms, and soft-bottom seeps), between seep and non-seep areas, and between seep and spill areas. Unlike communities impacted by the DWH spill, there did not appear to be taxa specifically associated with natural seepage. In fact, high variability in communities not only different from background and spill communities, but also different from other seep communities.

Oil that enters the oceans does not remain there indefinitely. Oil released by both natural and anthropogenic processes is removed from the marine environment naturally by burial in the seafloor, degradation by bacteria, and dilution in the water column. The removal of oil by the

v

environment is an example of an ecosystem service termed waste regulation. Waste regulation was examined in the context of the Deepwater Horizon spill by calculating the monetary value of the natural removal of oil spilled. Estimations of fates of the DWH oil as well as cleanup costs were examined. It was estimated that 10's of billions of dollars were saved from offshore waste regulation following the spill.

This dissertation concludes that the differences among communities at natural seeps, areas impacted by the spill, and areas not impacted by oil were numerous. Benthic communities associated with deep oil spills were defined, allowing for the future assessment of damages caused by deep-sea spills. Communities associated with natural seepage were different from other habitats as well as other seep communities, emphasizing the unique nature of each seep location in the Gulf of Mexico. Valuation of deep-sea services will provide monetary costs for destructive practices in the deep sea. Knowledge of deep-sea services is also important to communicate to the public to ensure these services will be protected. This dissertation provides information on the effects of the first deep-sea oil release on benthic communities, differences between impacts of natural and anthropogenic oil required to assess spill damages, a unique comparison of several different seep communities throughout the Gulf of Mexico, as well as an initial, partial value of waste regulation provided by the deep-sea environment. The work performed can help guide future policies concerning deep-sea drilling and assist in the identification and protection of unique habitats in the deep sea. Communication of deep-sea benefits can provide the public with motivation to care about the fate of the deep sea, which is far beyond the reach of most people.

vi

ACKNOWLEDGEMENTS

I would first like to thank my committee for all the help they gave me during the course of my Ph.D. research. My major professor, Dr. Paul Montagna, provided me the opportunity to perform my dissertation research on the deep sea. Paul, you were always available to discuss work and open with many useful tips and tricks to find future success in this career, which I really appreciate (even if this advice was often told in a very matter-of-fact way). Dr. Amanda Demopoulos provided me with invaluable deep-sea seep samples and kept my dream alive of working with unique, chemosynthetically driven, deep-sea environments. I hope that we will continue our research relationship with future deep-sea studies. Dr. David Yoskowitz helped to open my eyes to all the valuable work that can be done outside of ecology. Getting involved with ecosystem services may have been the smartest decision I (eventually) made during my time at Harte, and I thank you for the opportunity and guidance to do so. Finally, Dr. Jennifer Pollack provided me with a fresh perspective on many aspects of my research and some of the hardest qualifying exam questions one could get.

My dissertation research could not have been done without many, many people at the Harte Research Institute and Texas A&M University – Corpus Christi. Dr. Adelaide Rhodes helped guide me during my initial years in Texas and pushed me to always look for opportunities. Rick Kalke, Larry Hyde, Michael Reuscher, Bobby Guteirrezz, Kate Lavelle, Noe Barrera, and Elani Eckert not only helped me with taxonomy work but also made my time at the scope fun. Sara Smith and Meagan Hardagree put in some manpower to help sort. Talking with Melissa Rohal, Terry Palmer, Evan Turner, Lauren Hutchison, and Brittany Blomberg about dissertation nonsense really helped keep me sane and out of the weeds much of the time.

vii

The Harte Research Institute was a great place to work, and I am very grateful to have spent time here. Dr. Larry McKinney gave me several opportunities to benefit from my time at Harte from letting me go on scuba trips in the Flower Gardens to providing funds for our BLUE on Tour Film Festival. Gail Sutton, you helped me in so many little ways over the years as did Kat Santrock, Barbara Howard, Allison Knight, Patricia Rodriguez, Mike Grubbs, Luke Eckert, and so many others. And of course I would like to thank all of my friends that I made during my time in Corpus Christi: Matt, Judd, Larry, Dominic, Brett, Jason, Debbie, Brian (the Canadian), Anthony, Karen, Ryan, Zoe, Frank, Jeff, Eli... I am sure there are many more I am forgetting.

I would like to thank my funding sources for allowing me to eat, sleep, and have a little fun while obtaining my doctorate. Much of my funding came from NOAA via the NRDA on the Deepwater Horizon spill. Exxon Mobile also provided funding to help examine offshore ecosystem services. Both the marine biology program at Texas A&M University – Corpus Christi and Harte Research Institute provided fellowships to fund my last years of work. Deepsea seep samples were provided by Amanda from both the USGS and the ECOGIG consortium.

Finally, I would like to thank my family. First to my new, beautiful wife Alison Washburn (formerly Whelan), you had the crazy idea to move with me to Texas after dating for 10 months, and you never looked back. I honestly am not sure if I could have finished this thing without you, the constant support and flexibility meant more to me than you can know. To my mom, dad, step-mom, sister, and brother, (Gary, Dave, and Kristen, you guys are in there too) I know that you all are still rather confused as to what I am going to do with my life. I am somewhat confused as well. But you never stopped believing in me, no matter how confused you got. Your confidence in me gave me the confidence in myself to pursue my dreams. Now all I have to do is make a difference in the world, and I'll be set. The hard parts over right?

viii

CONTENTS	PAGE
ABSTRACT	v
ACKNOWLEDGEMENTS	vii
LIST OF FIGURES	xi
LIST OF TABLES	xiii
CHAPTER I: INTRODUCTION	1
1.1 Deep Sea	
1.2 The Deepwater Horizon Oil Spill.	
1.3 Hydrocarbon Seeps in the GoM	
1.4 Ecosystem Services Provided by the Deep GoM	
CHAPTER II: BENTHIC TAXA AS POTENTIAL INDICATORS OF A DE	EEP-SEA
OIL SPILL	
Abstract	
2.1. Introduction	
2.2. Methods	
2.2.1. Sample Collection	
2.2.2. Sample Design	
2.2.3. Bioindicator and Community Analyses	
2.2.4. Environmental Factors	
2.3. Results	
2.3.1. Abundance and Diversity	
2.3.2. Bioindicators and Community Structure	
2.3.3. Environmental Factors	
2.4. Discussion	
2.4.1. Offshore Oil Releases	
2.4.2. Blowout Impacts on Benthic Abundance and Diversity	
2.4.3. Blowout Benthic Bioindicators	39
References	44
CHAPTER III: BENTHIC COMMUNITIES ASSOCIATED WITH DEEP-S	SEA
HYDROCARBON SEEPS IN THE GULF OF MEXICO	65
Abstract	65
3.1. Introduction	
3.1.1. Hydrocarbon Seeps	
3.1.2. Importance of Deep-Sea Seeps	67
3.1.3. Seep Communities	
3.1.4. Seep Communities and Oil Spills	
3 2 Methods	74
3.2.1. Seep Collections	

TABLE OF CONTENTS

3.2.2. Background DGoMB and DWH Collections	75
3.2.3. DWH Oil Blowout Collection	77
3.2.4. Seep Community Descriptions	77
3.2.5. Seep vs. Background Comparisons	79
3.2.6. Seep vs. Spill Comparisons	80
3.3 Results	81
3.3.1. Community Analyses	82
3.3.2. Seep vs. Background Comparisons	85
3.3.3. Seep vs. Spill Comparisons	87
3.3.4. Sample Size	89
3.4 Discussion	89
3.4.4. Seep vs. Spill	
3.4.5. Sampling Methods	
References	100
CHAPTER IV: ECOSYSTEM SERVICES OF THE OFFSHORE GOM:	
IDENTIFICATION AND WASTE REGULATION OF OIL	
Abstract	126
4.1. Introduction	126
4.1.1. General Ecosystem Services	126
4.1.2. Deep-Sea Services in the GoM	129
4.1.3. Waste Removal and Burial	
4.2. Methods	134
4.3. Results	136
4.4. Discussion	
References	
UHAPIER V: CONCLUDING SUMMARY	159

LIST OF FIGURES

FIGURES PAGE
Figure 2.1. Station locations. Triangle is location of the MC252 site, and concentric rings are at 25 km intervals. Zones one and two were the most heavily impacted by the DWH spill while zones four and five were not impacted. Isobath intervals: light blue lines at 100 m intervals, and black lines at 500 m
Figure 2.2. Radial design station locations overlaid on the effects zones identified by Montagna et al. 2013. Triangle is location of the MC252 site, and concentric rings are at 1, 3, and 10 km intervals. The red area represents the most heavily impacted zone one, the orange area the moderately impacted zone 2, the yellow area zone 3, and the green area the background conditions of zones 4 and 5
 Figure 2.3. Vertical distribution of macrofauna within surface (0 - 5 cm) and deeper (5 - 10 cm) sediment. A) Macrofaunal abundance (average number of individuals per section). B) Macrofaunal diversity (N1, number of dominant species per section)
Figure 2.4. Nonmetric multi-dimensional scaling ordination of similarities of samples based on macrofaunal taxa occurrences. A) All stations examined with symbols representing zones, and circles representing 40% similarity level from cluster analysis. B) Radial design stations within 10 km from the wellhead with symbols representing distance categories, and circles representing 40% similarity level from cluster analysis
Figure 3.1. A map of all locations sampled during 2009 - 2013 (green circle = 2009, blue square = 2010, red triangle = 2012, and orange diamond = 2013) to examine communities associated with cold seeps in the Northern Gulf of Mexico as well as locations sampled during 2000-2002 on DGoMB cruises (black triangles) and during 2010 on the DWH response cruise (black circles). Depth contours are in 500 m increments
Figure 3.2. Box plots comparing seep types where diamonds represent means while lines through the rectangles represent medians. A) Diversity (N1) and B) Abundance (n m ⁻²) found at different seep types (microbial mat, tubeworm, or soft-bottom seep)
Figure 3.3. Rarefaction curves for A) each seep independently, where blue represents soft- bottom seeps, yellow represents tubeworm seeps, and red represents microbial mat seeps, and for B) All seep samples combined
Figure 3.4. Cluster plot of community structure at all seeps sampled between 2009 – 2013. Different symbols indicate different seeps separated into 3 depth bins (0 - 1000 m, 1000 – 2000 m, and > 2000 m). The dashed line represents the 50% similarity threshold. Blue represents soft-bottom seeps, yellow represents tubeworm seeps, and red represents microbial mat seeps
Figure 3.5. Average proportion of taxa comprising seep communities at shallow (0 – 1000 m), intermediate (1000 – 2000 m), and deep (> 2000 m) seeps

Figure 3.7. The nMDS plot of macrobenthic community structure collected during seep,	
DGoMB, and DWH cruises. A) Symbols by cruise type. B) Symbols by station where	
red represents microbial mats, blue represents soft-bottom seeps, yellow represents	
tubeworms, and green represents background (light green = DGoMB, dark green =	
DWH-B, and green = seep cruises)1	18
Figure 3.8. Rarefaction curves for each cruise type. Samples in the DWH category include only	y

-0	······································	-5
	background stations (DWH-B).	119

LIST OF TABLES

TABLES PAGE
Table 2.1 The 20 dominant taxa collected during the fall 2010 sampling. Number of stations where a taxon was found, mean abundance in all stations, percent of total abundance, and cumulative percent abundance. Abbreviations: P = Polychaete, N = Nemertea, M = Mollusca, C = Crustacea. 57
Table 2.2. Analysis of zones. Stations in zones 1 and 2 were heavily or moderately impacted by the DWH spill while zones 4 and 5 were unaffected. A) Results for the 2-way nested ANOVA. B) Tukey tests listed from highest to lowest where underlined categories are not significantly different at the 0.05 level. Abbreviations: df = degrees of freedom 58
Table 2.3. Analysis of the radial design within 10 km of the DWH wellhead. A) Results for the 4-way nested partially hierarchical ANOVA, df = degrees of freedom. B) Tukey tests with means listed from highest to lowest where underlined are not significantly different at the 0.05 level. Distance is in kilometers.59
Table 2.4. Bioindicator classification is based on P-values from 2-way ANOVAs performed on each taxon found at 10 or more stations during the fall of 2010. Impact:Background ratios are based on abundances in the two zones. *NA is due to no organisms being found in the impacted area
Table 2.5. Pairwise community structure differences among zones for the one-way ANOSIM. 62
Table 2.6. The ten most abundant taxa responsible for the similarity among stations for A) zones, and B) distances from the wellhead. A dash (-) indicates that the taxon was not listed in the 1-way SIMPER analysis because it was lower than the lowest value required to get 90% similarity. All taxa are polychaete families except for the phylum Nemertea and class Bivalvia.
Table 2.7. Station groups identified by the LINKTREE procedure using chemical and environmental variables obtained from the BEST procedure. Only groups with 50% or higher similarity were included. Similarity is the percent similarity within groups, R is the amount of variability associated with the physical-chemical variable and group and its value obtained from the BEST procedure, and distances of the stations within the group from the DWH wellhead. 64
Table 3.1. All stations sampled from 2009-2013 used to explore macrobenthic communities are natural hydrocarbon seeps in this study. Rep = replicate, PC = pushcore, and MC = multicore.120
Table 3.2. The average abundance (n m ⁻²) (Dens), richness (n) (ts), diversity (N1), and evenness (J') of macrobenthos at individual seep sites. Standard errors are given in parentheses. The number of cores for each calculation is given. These parameters were also averaged for all seep samples across depths (< 1000, 1000 – 2000, and > 2000 m), seep habitats (Microbial Mat, Soft-bottom Seep, and Tubeworm), and collection method (Multicore and pushcore). Similarity and multivariate dispersion (MVDISP) were also included. 121

- Table 3.4. Taxa making up 95% of the similarity of samples within A) background soft-bottomcommunities and B) natural hydrocarbon seep communities.123

- Table 4.1. The values for the fates of oil (% of total) released during the Deepwater Horizon oil spill in 2010. Percentages were obtained from various sources (BP = BP, 2010; Lubchenco = Lubchenco et al., 2010; Ramseur = Ramseur, 2010; Ryerson = Ryerson et al., 2012; and USCG = USCG, 2011).

CHAPTER I: INTRODUCTION

1.1 Deep Sea

The deep sea (generally defined as areas off the continental shelf and/or deeper than 200 m (Gage and Taylor, 1996)) constitutes the largest single habitat on Earth; however, it is still largely unexplored. We know more about the surface of the moon than we know about the deep sea (Thurman and Trujillo, 2003). The deep sea and seafloor form a vast, complex system linked by matter, energy, and biodiversity exchanges (Suttle, 2005; Cochonat et al., 2007; Armstrong et al., 2010). At the broadest level, the deep Gulf of Mexico can be divided into the continental slope, continental rise, and abyssal plain. Habitats can be divided further into hydrocarbon seeps, brine pools, submarine canyons, deep-water corals, soft-bottom mud, and hard-bottom habitats.

Benthic habitats in the ocean are estimated to contain as much as 98% of all marine species with the majority of these likely living in the deep seafloor (Gjerde, 2006). The deep sea may account for over 90% of the global bacterial biomass (Head et al., 2003). The number of deep-sea metazoan species is unknown, but estimates have ranged from 500,000 (May, 1992) to 100 million (Gianni, 2004). Benthic organisms are not uniformly present in the ocean, and changes in benthic abundance and biomass show similar trends along a depth gradient around the world. A review of 128 studies in deep-sea benthic habitats world-wide found both biomass and abundance of three major size classes of benthic organisms (megafauna, macrofauna and meiofauna) to each decrease with depth while bacterial biomass and abundance did not change (Rex et al., 2006). Macrofauna had the highest rates of decline having the highest biomass of any group at shallow depths but less biomass than smaller groups below around 3000 m.

Physical and chemical parameters shape benthic community structure (Etter and Grassle, 1992; Gage and Tyler, 1992), including changes with depth (Rex et al., 2006), and are affected by both environmental and anthropogenic processes. The deep sea is far removed from photosynthetic sources of energy, and organic matter is generally thought to be the limiting resource for animals living in this habitat (Gage and Taylor, 1996; Rex et al., 2006). Elevated concentrations of hydrogen sulfide and methane released around hydrocarbon seep environments provide alternative sources of energy via chemosynthetic activity which in turn provides additional organic matter for increased macrobenthic abundances and diversity around seeps compared to the surrounding environment (Levin et al., 2006; Cordes et al., 2010). Sediment grain size affects organic content, mobility and feeding of infauna (Etter and Grassle, 1992) and can be altered by deep-sea activities such as drilling (Montagna and Harper Jr., 1996). Several heavy metals and PAHs released by human activities are toxic to many macrobenthic taxa and can lead to decreases in richness and/or abundance (Montagna et al., 2013). All of these parameters must be measured with macrofauna to understand what is driving community differences and similarities.

1.2 The Deepwater Horizon Oil Spill

The DWH blowout resulted in the largest oil spill in American history. The accident began on April 20, 2010 and released an estimated 5 million barrels before it was finally capped on July 15, 2010 (Griffiths, 2012). The blowout occurred at a depth of 1525 meters in Mississippi Canyon Block 252 (Fig. 1) and resulted in the release of oil as two incidents, the common surface slick with short residence times and a subsurface deep-water plume with relatively unknown persistence (Peterson et al., 2012). Some of the released oil was consumed by microbial communities in the GOM or removed by human cleanup activities, but up to 35%

of the total hydrocarbons may have been trapped in underwater deep plumes and transported several kilometers horizontally from the spill site (Ryerson et al., 2012).

Effects of oil spills in the shallow marine environment have been well studied. A review of oil spills on coastal wetlands by Mendelssohn et al. (2012) found spill effects to vary and depended on many different factors such as habitat, oil type (light vs. heavy or crude vs. refined), time of release, and length of exposure. Following the DWH blowout, approximately 430 miles of coastal marsh were oiled (Zengel and Michel, 2011), resulting in high mortality of dominant vegetation with minimal recovery as of 2012 in heavily oiled areas (Lin and Mendelssohn, 2012).

Effects of deep-sea drilling on the surrounding environment have also been examined more recently as the technology and need to drill deeper has increased. Several such studies were performed under the Gulf of Mexico Offshore Operations Monitoring Experiment (GOOMEX) (Kennicutt et al., 1996). This work examined sediment toxicity, detoxification, genetic structure, megafaunal ecology, and meiobenthic and macrobenthic community responses to offshore oil and gas production. Effects were generally found on toxicology, sediment contaminants, and benthic ecology up to 100-200 m from the platform (Carr et al., 1996; Green and Montagna, 1996; Montagna and Harper, 1996).

The unprecedented nature of the underwater oil plume (Peterson et al., 2012) combined with a lack of information on deep-sea ecosystems resulted in relatively little being known about the impacts of the blowout on deep-sea biota. The water column was extensively sampled during and immediately after the spill, showing most oil and gas products had dispersed or metabolized beyond measurable quantities shortly after the wellhead was capped (Ryerson et al., 2012).

There was also a focus on deep-water coral communities, which demonstrated large effects on corals several kilometers from the wellhead (White et al., 2012).

Effects from the DWH spill on deep-sea benthic communities, such as reduced abundances and diversity, are still being examined. DWH effects are likely to persist for long periods of time, decades or longer, due to low deposition of sediment for burial and depressed metabolic activity. Benthic diversity, which is strongly positively correlated with ecosystem functioning (Danovaro et al., 2008), is also at a maximum in the GoM between 1200 and 1600 m (Haedrich et al., 2008). This coincides with the well site and underwater plume. Initial results from Montagna et al. (2013) showed strong and moderate effects on deep sea macrofauna and meiofauna, such as decreased benthic diversity and increased N:C ratios, within 3 km of the DWH wellhead in all directions and up to 15 km to the southwest of the wellhead.

Chapter II attempts to address the following null hypotheses: the DWH spill did not have effects on the deep-sea macrobenthic communities, any effects from the DWH spill on benthic communities would not be different between surface and deeper sediments, and no taxa could be used as indicators of the DWH spill. To answer these hypotheses macrobenthic communities in the deep-sea GoM were examined at various distances from the DWH wellhead. This information is not only useful for determining damages and costs associated with anthropogenically caused disasters such as the DWH spill, but also for enacting future policies to mitigate damages from offshore spills.

1.3 Hydrocarbon Seeps in the GoM

Hydrocarbon seeps and brine seeps are prevalent throughout the Gulf of Mexico. There are approximately 12,000 hydrocarbon seep features releasing approximately 400,000 bbl/year (63,600 m³/year) of oil into the Gulf of Mexico (<u>http://www.boem.gov/ -and-Gas-Energy-</u>

Program/Mapping-and-Data/Map-Gallery/Seismic-Water-Bottom-Anomalies-Map-

Gallery.aspx). However, seep flows are ephemeral, with hydrocarbon releases from the seafloor lasting for years to decades before stopping (Juniper and Sibuet, 1987), making an accurate count of such features difficult to impossible to maintain. Seeps support many chemosynthetic organisms and often contain high densities of organisms that may be endemic, colonists or vagrants (Carney, 1994; Barry et al., 1996; reviewed by Sibuet and Olu, 1998). Thus there is both spatial and temporal variability associated with seeps (Juniper and Sibuet, 1987; Olu et al., 1996,1997; Sibuet and Olu, 1998).

As reviewed by Sibuet and Olu (1998) and Levin (2005), there is a distinct lack of knowledge of macroinfauna compositions associated with deep-sea seeps. Because of methodological constraints, few sediment cores have been collected near deep-sea seeps hindering comparisons with the surrounding macrobenthic community. Little knowledge has been obtained for infaunal organisms associated with deep-sea seeps, as animals from these habitats are primarily identified from images obtained from ROVs. Recent studies on infaunal seep communities have explored differences among seep habitats and between seep and background areas. Macrobenthic infauna tend to reach their highest densities and lowest diversities at seeps dominated by microbial mats compared to other seep habitats and background areas (Bernardino et al., 2016; Bourque et al., 2016). Diversity often reaches a maximum at areas with less intense seepage such as seeps dominated by tubeworms or clams (Guillon et al., 2016). However, most studies on seep infaunal communities are isolated to one or a few seeps (e.g., Demopoulos et al., 2010; Borque et al., 2016; Guillon et al., 2016).

Seeps have been found throughout the world's oceans including the Pacific Ocean, Northern Atlantic, Gulf of Mexico, Mediterranean Sea, and Northern Indian Ocean (Sibuet and

Olu, 1998, Levin, 2005). Communities associated with seeps are often very different from region to region and even within kilometers of each other due to geological and biochemical heterogeneity (Cordes et al., 2010). Similarities and differences among seeps appear to be most closely related with depth. Abundances around seeps are generally higher than non-seep habitats (e.g., Carney, 1994, Levin et al., 2006); however, the difference in densities between seep and non-seep habitats generally increase with depth possibly due to increased food limitation (Levin and Michener, 2002).

Seeps are one of the most heterogeneous environments found on the continental margin due to local (meters) and regional (kilometers to 100's of kilometers) variability in fluid flow, geochemistry, substrate, microbes, and megafaunal communities (Cordes et al., 2010). Comparing hydrocarbon seep communities in various locations to one another and to non-seep communities may lead to new ecological insights. Food and reproductive limitations of deep-sea organisms as well as animal dispersal, energy sources, and distribution patterns can be examined by comparing physical/chemical/biological parameters between seep and non-seep sites.

Understanding the fate of hydrocarbons in the deep sea will greatly enhance existing knowledge of biogeochemistry within the deep GOM ecosystem. There is a timely need for this type of information, in particular due to the increase of drilling in deeper waters and likelihood of spills there. One recent event that underscores this lack of knowledge is the April, 2010, Deepwater Horizon (DWH) blowout, which released large amount of oil into the northwestern Gulf of Mexico (GOM). There have been several studies since 2010 that have shown impacts from the blowout on coastal, pelagic, and deep megafaunal organisms. Much less is known about how the blowout impacted deep-sea macrobenthic and meiobenthic communities where, due to low deposition of organic material and metabolic activity (Jannasch and Wirsen, 1973;

Smith and Teal, 1973; Rowe, 1983), impacts are more likely to persist for long periods of time, decades or longer.

Chapter III attempts to address the following null hypotheses: macrobenthic communities are not different at seeps compared to background areas, communities at seeps are not different at different depths, communities are not different among different seep habitats, no taxa are indicative of deep-sea seeps in the GoM, and communities are not affected differently by naturally and anthropogenically released hydrocarbons. To answer these hypotheses macrobenthic communities were examined near several seeps in the northern Gulf of Mexico as well as several background soft-bottom areas. Benthic communities were analyzed to look for characteristics associated with natural hydrocarbon seepage. Communities near seeps were also compared to communities near the Deepwater Horizon wellhead to examine similarities/ differences between communities exposed to natural and anthropogenic hydrocarbon releases.

1.4 Ecosystem Services Provided by the Deep GoM

Benefits that humans receive from the deep sea, or ecosystem services, may be affected by human-induced changes to benthic communities, e.g. from oil spills. Examining the deep sea from an ecosystem services perspective does not account for many of the physical/chemical/biological processes shaping this environment but is necessary for communicating to the general population why the deep sea must be protected. The Millennium Ecosystem Assessment (MEA, 2005) produced a global framework illustrating many of the ways healthy ecosystems, generally measured as biodiversity, benefit human well-being. Services include direct benefits such as basic materials, food and recreation as well as indirect benefits such as mitigation of greenhouse gases and exchange of carbon and nutrients with other environments. An understanding of ecosystem services provided by the deep sea has become

even more important in light of the Deepwater Horizon oil spill and possible damages that ensued. Quantifying the intrinsic values of some of these services will help in quantifying the amount of money to be paid for damages to the deep sea and possible restoration activities needed to compensate for events such as the DWH blowout.

In order to examine how changes to the environment, such as the DWH oil spill, affect ecosystem functions and in turn affect benefits to humans, these functions must first be measured. Biotic components of ecosystems are complex and, in any given ecosystem, are composed of various bacterial, plant, and animal constituents as well as the countless interactions among organisms and between organisms and the abiotic environment (MEA, 2005). There are many different ways to attempt to describe communities in simpler, univariate and multivariate measures. Community measures can then be linked to ecosystem functions and used as indicators of the quality and quantity of specific functions. The simplest measure of a community is abundance, which is simply the number of organisms found in a specific area. Several services, especially provisioning services, are dependent on the amount of organisms in a location. For example, the service of food production via fishing is greater where there are greater quantities of specific fish.

Another way to measure a community is diversity, which is a measure of the variety of organisms found at a location. Biodiversity can be defined many different ways, from genetic diversity between individuals of the same species to different biogeographical provinces, and at various scales, from microscopic to global (MEA, 2005). Measuring the effects of changes in biodiversity on ecosystem functioning is one way to link ecological communities to ecosystem services. One problem with valuing biodiversity is a lack of understanding by the public of what biodiversity means (Spash and Hanley, 1995; Turpie, 2003). In the United Kingdom the

department for Environment Food and Rural Affairs found that over a quarter of survey respondents had never heard of the term biodiversity before (DEFRA, 2002). Informing the public is therefore an important outcome of any study as the public has been found to assign a positive value to biodiversity (Christie et al., 2006).

While biodiversity is indispensable to keeping natural processes and thus all ecosystem services functioning, it is perhaps the most difficult service to value. Biodiversity is intertwined within practically every service provided to humans, and putting a value on biodiversity (Turner et al., 2003) would involve teasing apart the role biodiversity plays in services from carbon sequestration to fish production to whale watching. Biodiversity not only serves as an indirect service, crucial to the delivery of other services, but also as a direct service in many cases and even cultural or non-use values (Atkinson et al., 2012). The economics and ecosystems and biodiversity (TEEB) report even went so far as to include habitat as an entire category of ecosystem services (TEEB, 2007). As stated above, the biological components of a habitat can be represented by biodiversity.

Many studies have shown that a decrease in diversity in a community is often associated with a decrease in ecosystem functions provided by that habitat (Balvanera et al., 2006; Tilman et al., 2012). Decreased biodiversity has been linked to decreases in the resilience and resistance of habitats, which causes an increase in recovery times after a disturbance or a complete lack of recovery (e.g., Steneck et al., 2002; Reusch et al., 2005; Tilman et al., 2006). A decrease in biodiversity has also been linked to a decrease in primary production and likely reduced fisheries production (Runge, 1988). Ecosystems with high species richness have been found to have lower rates of species collapse and higher rates of post-collapse recovery (Worm et al., 2006). Changes in biodiversity will most likely have far reaching impacts on the biogeochemical cycles

of carbon and other nutrients and therefore the atmosphere and even climate (Legendre and Rivkin, 2005). A decrease in production associated with decreases in biodiversity may also result in a decrease in carbon sequestration (Tilman et al., 2006).

Biodiversity is an important factor influencing ecosystem functioning, but the underlying reasons behind this are still not well understood. Diversity may be positively correlated to ecosystem functioning because greater diversity increases the probability of including a few species which improve function or due to an increase in species interactions (Hector, 1998; Loreau and Hector, 2001).

The degree of ecosystem decline due to a loss in biodiversity is still being examined and most likely varies with starting community composition and habitat. Meta-analyses on studies exploring the effects of biodiversity changes on ecosystem functioning found diversity to have a positive but saturating effect on functioning across trophic levels and ecosystems. Unfortunately, the majority of such studies are from terrestrial plant and coastal environments where communities were assembled under controlled field experiments (Balvanera et al., 2006; Worm et al., 2006; Cardinale et al., 2012). The relationship between biodiversity and ecosystem functioning may be exponential on the deep-sea floor (Danovaro et al., 2008), suggesting that even a slight reduction in biodiversity could significantly alter global biogeochemical processes (Loreau, 2008).

Exploitable resources, primarily oil/natural gas and food, are the most obvious and direct ecosystem services provided by the deep sea. The Gulf of Mexico currently contains more than 4,000 active platforms and 20,000 miles of pipeline (USDOI, 2012). There are also many species of deep-water organisms harvested for food and other goods (Armstrong et al., 2010). As coastal resources continue to be exploited, industrial fishing moves deeper and deeper to

capture the remaining groups of economically harvestable biomass (Pauly et al., 2003; Morato et al., 2006). Bioprospecting of animals, or searching for animals with commercially important compounds, in the deep sea is becoming more common as technology continually improves. The deep sea contains the greatest quantity of genetic material with many organisms having unique adaptations to surviving under extreme pressures and temperatures (Synnes, 2007; Armstrong et al., 2010).

Possibly the most important service that the deep sea provides is the mitigation of global climate change. One mechanism of climate regulation important in the deep sea is the "biological pump," which results in the transfer and sequestration of carbon from the atmosphere and ocean surface to the deep sea and seafloor (Gage and Tyler, 1996). The deep sea also traps CO₂ for hundreds of years in the global ocean conveyer belt, absorbs greenhouse gases released from far under the sediment before they reach the surface, and stores large amounts of carbon in the form of methane hydrate nodules (Glover and Smith, 2003). These represent just some examples of ecosystem services provided by the deep sea.

Chapter IV examines the question: what ecosystem services are provided to humans by the deep GoM. To address this question, valuation efforts were performed to assign a value to one service provided by the deep Gulf of Mexico, waste regulation, in the context of a recent disaster, the DWH oil spill. Costs associated with DWH cleanup efforts were used to estimate the amount of money saved by the natural degradation or storage of oil released by the spill.

The overall goal of this dissertation is to provide new information on the deep Gulf of Mexico. A review of ecosystem services provided by the deep sea will illustrate why this immense habitat is important to humans. Exploration of data from the DWH blowout will be used to examine how humans can impact the deep sea and possibly hinder the benefits they

provide. Additional focus on seep communities will explore one unique and somewhat large habitat in the deep GOM and possibly help discern between natural and anthropogenic oiling of the macrobenthos.

References

- Armstrong, C.W., N. Folely, R. Tinch and S. van den Hove (2010). Ecosystem goods and services of the deep sea. Deliverable D6.2 of the HERMIONE project: 68 pp.
- Atkinson, G. I. Bateman, and S. Mourato (2012). Recent advances in the valuation of ecosystem services and biodiversity. Oxford Review of Economic Policy, 28, 22-47.
- Balvanera, P., A.B. Pfisterer, N. Buchmann, J. He, T. Nakashizuka, D. Raffaelli, and B. Schmid (2006). Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecology Letters, 9. 1146-1156.
- Barry, J.P., H.G. Greene, D.L. Orange, C.H. Baxter, B.H. Robison, R.E. Kochevar, J.W.
 Nybakken, D.L. Reed, and C.M. McHugh (1996). Biologic and geologic characteristics of cold seeps in Monterey Bay, California. Deep-Sea Research, 43, 1739-1762.
- Bernardino, A.F., L.A. Levin, A.R. Thurber, and C.R. Smith (2012). Comparative composition, diversity and trophic ecology of sediment macrofauna at vents, seeps and organic falls.PLoS One, 7.
- Bourque, J.R., C.M. Robertson, S. Brooke, and A.W.J. Demopoulos (2016). Macrofaunal communities associated with chemosynthetic habitats from the U.S. Atlantic margin: A comparison among depth and habitat types. Deep-Sea Research II,

http://dx.doi.org/10.1016/j.dsr2.2016.04.012.

- Cardinale, B.J., J.E. Duffy, A. Gonzalez, D.U. Hooper, C. Perrings, P. Venail, A. Narwani, G.M. Mace, D. Tilman, D.A. Wardle, A.P. Kinzig, G.C. Daily, M. Loreau, J.B. Grace, A. Larigauderie, D.S. Srivastava, and S. Naeem (2012). Biodiversity loss and its impact on humanity. Nature, 486, 59-67.
- Carney, R.S. (1994). Consideration of the oasis analogy for chemosynthetic communities at Gulf of Mexico hydrocarbon vents. Geo-Marine Letters, 14, 149-159.
- Carr, R.S., D.C. Chapman, B.J. Presley, J.M. Biedenback, L. Robertson, P. Boothe, R. Kilada, T. Wade, and P. Montagna (1996). Sediment porewater toxicity assessment studies in the vicinity of offshore oil and gas production platforms in the Gulf of Mexico. Canadian Journal of Fisheries and Aquatic Sciences, 53, 2618-2628.
- Christi, M., N. Hanley, J. Warren, K. Murphy, R. Wright, and T. Hyde (2006). Valuing the diversity of biodiversity. Ecological Economics, 58, 304-317.
- Cochonat, P., S. Durr, V. Gunn, P. Herzig, C. Mevel, J. Mienert, R. Schneider, P. Weaver and A. Winkler (2007). The deep-sea frontier: science challenges for a sustainable future.Luxembourg, Office for Official Publications of the European Communitie: 53 pp.
- Cordes, E.E., M.R. Cunha, J. Galeron, C. Mora, K.O. Roy, M. Sibuet, S. Van Gaever, A. Vanreusel, and L. Levin (2010). The influence of geological, geochemical, and biogenic habitat heterogeneity on seep biodiversity. Marine Ecology, 31, 51-65.
- Danovaro, R., C. Gambi, A. Dell'Anno, C. Corinaldesi, S. Fraschetti, A. Vanreusel, M. Vincx, and A.J. Gooday (2008). Exponential decline of deep-sea ecosystem functioning linked to benthic biodiversity loss. Current Biology, 18, 1-8.
- DEFRA (2002). Survey of public attitudes to quality of life and to the environment-2001. DEFRA, London, UK.

- Demopoulos, A.W.J., D. Gualtieri, and K. Kovacs (2010). Food-web structure of seep sediment macrobenthos from the Gulf of Mexico. Deep-Sea Research II, 57, 1972-1981.
- Etter, R.J. and J.F. Grassle (1992). Patterns of species diversity in the deep sea as a function of sediment particle size diversity. Letters to Nature, 360, 576-578.
- Gage, J.D. and P.A. Taylor (1996). Deep-sea biology: a natural history of organisms at the deepsea floor. 3rd edn. Cambridge University Press, Cambridge, UK.
- Gianni, M., (2004). High seas bottom trawl gisheries and their impacts on the biodiversity of vulnerable deep-sea ecosystems: Options for international actions. Gland, Switzerland, IUCN.
- Gjerde, K.M., (2006). Critical ocean issues: ecosystems and biodiversity in deep waters and high seas. UNEP/World Conservation Union (IUCN), Switzerland. Available at: http://www.iucn.org/dbtw-wpd/edocs/2006-007.pdf
- Glover, A.G. and C.R. Smith (2003). The deep-sea floor ecosystem: current status and prospects of anthropogenic change by the year 2025. Environmental Conservation, 30, 219-241.
- Green, R.H. and P. Montagna (1996). Implications for monitoring: study designs and interpretation of results. Canadian Journal of Fisheries and Aquatic Sciences, 53, 2629-2636.
- Griffiths, S.K., (2012). Oil release from Macondo well MC252 following the Deepwater Horizon accident. Environmental Science and Technology, 46, 5616.
- Guillon, E., L. Menot, C. Decker, E. Krylova, and K. Olu (2016). The vesicomyid bivalve habitat at cold seeps supports heterogeneous and dynamic macrofaunal assemblages. Deep-Sea Research I, <u>http://dx.doi.org/10.1016/j.dsr.2016.12.008</u>.

- Haedrich, R.L., J.A. Devine, and V.J. Kendall (2008). Predictors of species richness in the deepbenthic fauna of the northern Gulf of Mexico. Deep Sea Research II, 55, 2650-2656.
- Head, I.M., D.M. Jones, and S.R. Larter, (2003). Biological activity in the deep subsurface and the origin of heavy oil. Nature, 426, 344-352.
- Hector, A. (1998). The effects of diversity on productivity: detecting the role of species complementarity. Oikos, 82, 597-599.
- Jannasch, H.W. and C.O. Wirsen (1973). Deep-sea microorganisms: in situ response to nutrient enrichment. Science, 180, 641-643.
- Juniper, S.K. and M. Sibuet (1987). Cold seep benthic communities in Japan subduction zones: spatial organization, trophic strategies and evidence for temporal evolution. Marine Ecology Progress Series, 40, 115-126.
- Kennicutt II, M.C., P.N. Boothe, T.L. Wade, S.T. Sweet, R. Rezak, F.J. Kelly, J.M. Brooks, B.J. Presley, and D.A. Wiesenburg (1996). Geochemical patterns in sediments near offshore production platforms. Canadian Journal of Fisherieis and Aquatic Sciences, 53, 2554-2566.
- Legendre, L. and R.B. Rivkin (2005). Integrating functional diversity, food web processes, and biogeochemical carbon fluxes into a conceptual approach for modeling the upper ocean in a high-CO₂ world. Journal of Geophysical Research, 110, C09S17.
- Levin, L.A., and Michener, R. (2002). Isotopic evidence of chemosynthesis-based nutrition of macrobenthos: the lightness of being at Pacific methane seeps. Limnology and Oceanography, 47, 1336-1345.
- Levin, L.S. (2005). Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. Oceanography and Marine Biology: An Annual Review, 43, 1-46.

- Levin, L.A., W. Ziebis, G.F. Mendoza, V. Growney-Cannon, and S. Walther (2006).
 Recruitment response of methane-seep macrofauna to sulfide-rich sediments: An in situ experiment. Journal of Experimental Marine Biology and Ecology, 330, 132-150.
- Lin, Q. and I.A. Mendelssohn (2012). Impacts and recovery of the Deepwater Horizon oil spill on vegetation structure and function of coastal salt marshes in the Northern Gulf of Mexico. Environmental Science and Technology, 46, 3737-3743.
- Loreau, M (2008). Biodiversity and ecosystem functioning: The mystery of the deep sea. Current Biology, 18, 126-128.
- Loreau, M. and A. Hector (2001). Partitioning selection and complementarity in biodiversity experiments. Nature, 412, 72-76.
- May, R.M., (1992). Bottoms up in the ocean. Nature, 357, 278-279.
- Mendelssohn, I.A., G.L. Anderson, D.M. Baltz, R.H. Caffrey, K.R. Carmen, J.W. Fleeger, S.B. Joye, Q. Lin, E. Maltby, E.B. Overton and L.P. Rozas (2012). Oil impacts on coastal wetlands: implications for the mississippi river delta ecosystem after the Deepwater Horizon oil spill. BioScience, 62, 562-574.
- Morato, T., R. Watson, T.J. Pitcher, and D. Pauly (2006). Fishing down the deep. Fish and Fisheries, 7, 24-34
- MEA (Millennium Ecosystem Assessment) (2005). Ecosystems and human well-being: biodiversity synthesis. World Resources Institute, Washington, DC.
- Montagna, P.A. and D.E. Harper Jr. (1996). Benthic infaunal long-term response to offshore production platforms in the Gulf of Mexico. Canadian Journal of Fisheries and Aquatic Sciences, 53, 2567-2588.

Montagna, P.A., J.G. Baguley, C. Cooksey, I. Hartwell, L.J. Hyde, J.L. Hyland, R.D. Kalke,
L.M. Kracker, M. Reuscher, and A.C.E. Rhodes (2013). Deep-sea benthic footprint of the
Deepwater Horizon blowout. PLoS ONE, 8: e70540.

http://doi:10.1371/journal.pone.0070540.

- Olu, K., M. Sibuet, F. Harmegnies, J.P. Foucher, and A. Fiala-Medioni (1996). Spatial distribution of diverse cold seep communities living on various diapiric structures of the southern Barbados prism. Progress in Oceanography, 38, 347-376.
- Olu, K., S. Lance, M. Sibuet, P. Henry, A. Fiala-Medioni, and A. Dinet (1997). Cold seep communities as indicators of fluid expulsion patterns through mud volcanoes seaward of the Barbados Accretionary Prism. Deep Sea Research, 44, 811-841.
- Pauly, D., J. Alder, E. Bennett, V. Christensen, P. Tyedmers, and R. Watson (2003). The future of fisheries. Science, 302, 1359-1361.
- Peterson , C.H., S.S. Anderson, G.N. Cherr, R.F. Ambrose, S. Anghera, S. Bay, M. Blum, R. Condon, T.A. Dean, M. Graham, M. Guzy, S. Hampton, S. Joye, J. Lambrinos, B. Mate, D. Meffert, S.P. Powers, P. Somasundaran, R.B. Spies, C.M. Taylor, R. Tjeerdema, E.E. Adams, (2012). A tale of two spills: novel science and policy implications of an emerging new oil spill model. Biosience, 62, 461-469.
- Reusch, T.B.H., A. Ehlers, A. Hämmerli, and B. Worm (2005). Ecosystem recovery after climatic extremes enhanced by genotypic diversity. PNAS, 102, 2826-2831.
- Rex, M.A., R.J. Etter, J.S. Morris, J. Crouse, C.R. McClain, N.A. Johnson, C.T. Stuart, J.W. Deming, R. Thies, and R. Avery (2006). Global bathymetric patterns of standing stock and body size in the deep-sea benthos. Marine Ecology Progress Series, 317, 1-8.

- Rowe, G.T. (1983). Biomass and production of the deep-sea macrobenthos. In Deep-Sea Biology, vol. 8, The Sea. Ed. G.T. Rowe. John Wiley & Sons, New York. 560 pp.
- Runge, J.A. (1988). Should we expect a relationship between primary production and fisheries?The role of copepod dynamics as a filter of trophic variability. Hydrobiologia, 167/168, 61-71.
- Ryerson, T.B., R. Camilli, J.D. Kessler, C.M. Reddy, D.L. Valentine, E.L. Atlas, D.R. Blake,
 J.A. De Gouw, S. Meinardi, D.D. Parrish, J. Peischl, J.S. Seewald, and C. Warneke
 (2012). Chemical data quantify Deepwater Horizon hydrocarbon flow rate and
 environmental distribution. Proceedings of the National Academy of Sciences, 109,
 20246-20253.
- Sibuet M. and K. Olu (1998). Biogeography, biodiversity and fluid dependence of deep-sea coldseep communities at active and passive margins. Deep-Sea Research II, 45, 517-567.
- Smith Jr., K.L. and J.M. Teal (1973). Deep-sea benthic community respiration: An in situ study at 1850 meters. Science, 179, 282-283.
- Spash, C.L. and N. Hanley (1995). Preferences, information and biodiversity preservation. Ecological Economics, 12, 191-208.
- Steneck, R., M.H. Graham, B.J. Bourque, D. Corbett, and J.M. Erlandson (2002). Kelp forest ecosystems: Biodiversity, stability, and resilience and future. Environmental Conservation, 29, 436-459.

Suttle, C.A. (2005). Viruses in the sea. Nature, 437, 356-361.

Synnes, M. (2007). Bioprospecting of organisms from the deep sea: scientific and environmental aspects. Clean Technologies and Environmental Policy, 9, 53-59.

- TEEB (2007). The economics of ecosystems and biodiversity: Ecological and economic foundations. in: Earthscan, edited by: P. Kumar, London and Washington.
- Thurman, H.V. and A.P. Trujillo (2003). Introductory Oceanography 10th Edition, Pearson Publishers.
- Tilman, D, P.B. Reich, and F. Isbell (2012). Biodiversity impacts ecosystem productivity as much as resources, disturbance, or herbivory. PNAS, 109, 10394-10397.
- Tilman, D., P.B. Reich, and J.M.H. Knops (2006). Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature, 441, 629-632.
- Turner, R.K., J. Paavola, P. Cooper, S. Farber, V. Jessamy, and S. Georgiou (2003). Valuing nature: lessons learned and future research directions. Ecological Economics, 46, 493-510.
- Turpie, J.K. (2003). The existence value of biodiversity in South Africa: how interest, experience, knowledge, income and perceived level of threat influence local willingness to pay. Ecological Economics, 46, 199-216.
- USDOI (U.S. Department of Interior Bureau of Ocean Management) (2012). Deepwater Production Summary by Year.

http://www.gomr.boemre.gov/homepg/offshore/deepwatr/summary.asp

White, H.K., P.Y. Hsing, W. Cho, T.M. Shank, E.E. Cordes, A.M. Quattrini, R.K. Nelson, R.
Camilli, A.W.J. Demopoulos, C.R. German, J.M. Brooks, H.H. Roberts, W. Shedd, C.M.
Reddy, and C.R. Fisher (2012). Impact of the Deepwater Horizon oil spill on a deepwater coral community in the Gulf of Mexico. Proceedings of the National Academy of Sciences, 109, 20303-20308.

- Worm, B., E.B. Barbier, N. Beaumont, J.E. Duffy, C. Folke, B.S. Halpern, J.B.C. Jackson, H.K. Lotze, F. Micheli, S.R. Palumbi (2006). Impacts of biodiversity loss on ocean ecosystem services. Science, 314, 787-790.
- Zengel, S.A. and J. Michel (2012). Testing and implementation of treatment methods for marshes heavily oiled during the Deepwater Horizon spill. Gulf Oil Spill SETAC Focused Meeting, Pensacola, FL.

CHAPTER II: BENTHIC TAXA AS POTENTIAL INDICATORS OF A DEEP-SEA OIL SPILL

Abstract

The effect of the Deepwater Horizon oil spill on benthic macrofauna in the deep-sea Gulf of Mexico was measured in September-October 2010. Macrofauna community diversity and abundance were lowest closest to the wellhead and increased with distance from the wellhead up to 10 km. The macrofauna loss was primarily in surface sediments, which could be due to the deposition of oil and other toxic chemicals. Several taxa were classified as sensitive or resistant to the deep-sea blowout by comparing their distributions among impacted and non-impacted zones. Many crustacean taxa, which are often considered sensitive to pollution, were found to be sensitive to the spill while several taxa found to be resistant of the spill such as Thyasiridae, Capitellidae, and Dorvilleidae have often been found in areas associated with pollution, specifically hydrocarbons. Dorvilleidae, which is a polychaete often associated with organic enrichment, was responsible for the largest amount of dissimilarity between stations close and far from the wellhead. The macrobenthic communities in the deep Gulf of Mexico exhibit a toxic response to the blowout on the Deepwater Horizon well, and this is correlated with barium and petroleum hydrocarbons.

2.1. Introduction

The well blowout on the Deepwater Horizon platform located at Macondo 252 (MC252) from 20 April to 15 July 2010 released nearly 5 million barrels of crude oil before it was capped (Griffiths, 2012). Due to the extreme depths and volume of oil spilled, the blowout resulted in an unprecedented two-part spill: a surface slick with short residence times and a subsurface deep water plume with unknown persistence times (Peterson et al., 2012). As much as 35% of the

released hydrocarbons were likely trapped in deep plumes (>1000 m) and transported for several kilometers (Ryerson et al., 2012). Hydrocarbon plumes formed in the water column at a depth between 1100 and 1200 m depth (Griffiths, 2012). Additionally, 2.1 million gallons of dispersant were applied at the time of the spill and incorporated into the hydrocarbon plumes (Kujawinski et al., 2011). Because oil reached the seafloor, the potential for effects on the deep-sea benthos is a concern (Montagna et al., 2013).

Benthic abundance and diversity in the deep sea are affected by many different physical, chemical, and biological parameters such as depth (Rex et al., 2006), grain size (Etter and Grassle, 1992), natural hydrocarbon seepage (Levin, 2005), and organic matter input from the surface (Gage and Tyler, 1992). Benthic communities generally exhibit decreased abundances, diversity, and evenness when subjected to anthropogenic activities like waste disposal, trawling, or drilling for hydrocarbons; however, abundances of specific taxa with tolerances to disturbance or pollution may greatly increase (e.g., Pearson and Rosenberg, 1978; Gage and Tyler, 1992; Glover and Smith, 2003).

Communities around oil/gas platforms have been found to exhibit both a response to organic enrichment and a response to increased toxicity levels. Montagna and Harper (1996) found increased abundances of meiofaunal and macrofaunal deposit-feeders near gas platforms indicating enrichment, but they also found decreased meiofaunal crustaceans indicating toxic effects. Meiofaunal crustacean abundances decreased with increasing impacts of the DWH spill while nematode abundances increased suggesting a balance between enrichment and toxicity (Baguley et al., 2015). Immediately after the Hebei Spirit oil spill in coastal South Korea, there was a mass mortality of amphipods, generally considered pollution sensitive, followed by a rapid increase in opportunistic polychaetes roughly one year after the spill (Seo et al., 2014). The

changes in meiofaunal and macrofaunal communities after oil spills follow patterns of succession after an enrichment event described by Pearson and Rosenberg (1978). In the immediate vicinity of the discharge point benthic macrofauna are generally sparse. Outside of this defaunated zone abundances increase dramatically within a short distance to a maximum, due to a few opportunistic species, before gradually decreasing to background conditions. Species numbers generally increase from the discharge point reaching a maximum further from the discharge point than the abundance maximum, where toxicity is minimal and enrichment still occurs, before dropping to background levels. Organic enrichment may cause the abundances of some taxa to increase while causing other taxa to decrease; however, most taxa decline with an increase of toxic substances such as heavy metals (Bilyard, 1987).

Macrobenthic responses to oil spills have been examined in shallow-marine systems (e.g., Teal and Howarth, 1984) and around properly functioning drilling rigs on the continental shelf (Montagna and Harper, 1996; Hyland et al., 1990; Santos et al., 2009), but less is known about responses to deep-water blowouts such as the Deepwater Horizon spill. Compounding the difficulty of determining the effects of contaminants on macrobenthic communities, different geographic regions tend to have different assemblages of taxa that can be used to represent good or bad environmental health (Dean, 2008). Taxa whose presence may indicate a polluted environment in one location may be representative of a pristine environment in another location (e.g., Washburn and Sanger, 2011; Gillette et al. 2015). To date nearly all studies examining pollution effects on specific marine taxa have dealt with shallow-water organisms (e.g., Reish and Gerlinger, 1997; Bat, 2005; Dean, 2008; Diepens et al., 2014).

Changes in benthic diversity would likely result in changes to many important ecosystem functions such as bioturbation, organic matter decomposition, nutrient regeneration, secondary
production, and energy flow to higher trophic levels (Dayton and Hessler 1972, Danovaro et al. 2008, Tyler 2003). Effects of oil spills can last years or longer in the marine sediment compared to the water column (Teal and Howarth, 1984). Deep-sea infauna may also take many years to recolonize azoic sediments (Grassle, 1977) further increasing the time until the deep seafloor recovers from disturbance. The benthic communities between 1200 m and 1600 m in the Gulf of Mexico are highly diverse and highly endemic (Rowe and Kennicutt, 2009). An extreme perturbation to these communities could lead to species extinctions and decreased diversity.

An initial analysis on samples taken in the fall of 2010 from 58 stations surrounding the DWH wellhead found measurable adverse effects on benthic diversity, abundance, and relative proportions of meiofaunal indicator taxa in an area 148 km² and up to 15 km to the southwest from the wellhead (Montagna et al., 2013). This "DWH footprint" area was associated with high levels of barium (Ba), polycyclic aromatic hydrocarbons (PAH), and total petroleum hydrocarbons (TPH).

In the present study 10 additional stations which were sampled from 2010 but not included in Montagna et al (2013) were included in analyses bringing the total number of stations to 68. In addition to abundance and diversity, this manuscript examined how macrobenthic communities may have been altered due to the oil spill by analyzing community structure and spatial differences in individual taxa. Specific questions addressed here include: was the macrobenthic community structure different between areas close to and far from the DWH spill? What specific taxa were responsible for spatial differences? Did benthic responses differ in surface and subsurface sediments?

2.2. Methods

2.2.1. Sample Collection

Sixty-eight stations were sampled during cruises aboard the R/V Gyre (September 16 through October 19, 2010) and R/V Ocean Veritas (September 24 through October 30, 2010) (Fig. 1). A subset of 16 stations within 3 km of the wellhead were arrayed in a "bulls-eye" design close to the wellhead site (Fig. 2). The remaining 52 stations were located along a suspected contamination gradient primarily to the southwest of the wellhead and the likely trajectory of the deep-sea plume. The stations were located at depths ranging from 76 m to 2767 m and distances 0.33 km to 199 km from the DWH wellhead.

Sediment was collected with an ocean scientific international ltd. (OSIL) multicorer, which collected 8 or 12 sediment cores simultaneously per deployment with one deployment per station. Cores were 10 cm inner diameter and 60 cm in length. For every station, three cores were reserved for benthic macrofauna analysis (Montagna et al., 2013), and the data is available at https://dwhdiver.orr.noaa.gov/. Cores for benthic faunal analysis were extruded into two vertical sections (0 - 5 cm and 5 - 10 cm) immediately after collection and preserved in 4% buffered formalin with Rose Bengal.

In the laboratory, samples were sieved on a 0.3-mm mesh screen and transferred to 70% ethanol for taxonomic analysis. Total abundance and richness for each core at each station were analyzed by summing the two vertical sections (0 - 5 cm and 5 - 10 cm) for each replicate. All benthic macrofauna were sorted, counted, and identified. Taxa richness was calculated as the sum of: families for polychaetes, crustaceans, and mollusks; classes for echinoderms and oligochaetes; and phyla for all other taxa. Identifying species to the family level is justified for three reasons: (1) it increases the speed at which the analyses can be performed; (2) very few of

the deep-sea species are known to species level — for example, only 40% (207 of 517) of polychaete species and 25% (31 of 124) of the amphipod species found in the DGOMB (Rowe and Kennicutt 2009) study could be identified to the species level by taxonomic experts; and (3) benthic data at the family level have been shown to detect the same basic community structure patterns as those developed at the species level, and often with less noise due to eliminating the influence of individual rarer species (Heip et al. 1988, Warwick 1988, Warwick et al. 1988, Montagna and Harper 1996). A synthesis of the literature by Peterson et al. (1996) of benthic responses to marine pollution suggests that macroinfaunal and meiofaunal communities exhibit repeatable patterns of response to sedimentary contamination generally detectable at high taxonomic levels, even the phylum level. It has also been shown that higher taxonomic levels produce similar findings as analyses at the species level in many areas affected by oil spills, including in the Gulf of Mexico (Gesteira et al., 2003).

2.2.2. Sample Design

Macrobenthic communities from all stations examined were analyzed to address the following null hypotheses: 1) benthic communities did not altered by proximity to the DWH wellhead; 2) there were no differences in spatial community structure between surface (0-5 cm) and deeper (5-10 cm) sediments; and 3) no taxa in the deep GoM can be used as possible indicators of a deep-sea oil spill. A subset of stations within the heavily and moderately impacted areas < 10 km from the wellhead were also analyzed separately to address the following null hypotheses: 1) macrobenthic density, richness, and diversity do not differ at smaller spatial scales within the area impacted by the DWH blowout; and 2) macrobenthic density, richness, and diversity patterns are similar in surface sediments (0 – 5 cm in depth) vs deeper sediments near the wellhead (5 - 10 cm).

To analyze the extent that the DWH blowout affected the deep-sea macrobenthos, stations were divided into 5 zones (1 = severely impacted, 2 = moderately impacted, 3 =uncertain impacts, 4 and 5 = background conditions) based on conclusions from previous analyses of 58 stations (Montagna et al., 2013). The ten additional stations in our analysis were not included in Montagna et al. (2013) because of missing physical or chemical data and therefore were dropped from the multivariate analysis to define the footprint of the oil spill. These 10 stations were assigned a zone category by overlaying these stations on a map of the zones (Montagna et al., 2013, Figs. 4 and 5). If stations were near a zone boundary, then imputed PCA scores were also used to help classify stations into zones (Montagna et al., 2013, Table S1). Stations within 10 km of the DWH wellhead were selected for a radial analysis to explore smaller scale (km) benthic changes (Fig. 2). The stations were within the severely and moderately impact zones identified by Montagna et al. (2013). Stations were divided into 5 distance categories: within 1 km of the wellhead, 1 - 2 km away, 2 - 3.5 km away, 3.5 - 8 km away, and 8 - 10 km away from the wellhead. Direction from the wellhead was divided into 8 regions: NE (22.5° - 67.5°), E (67.5° - 112.5°), SE (112.5° -157.5°), S (157.5° - 202.5°), SW (202.5° - 247.5°), W (247.5° - 292.5°), NW (292.5° - 337.5°), and N (337.5° - 22.5°).

Analysis of variance (ANOVA) and Tukey's pairwise comparison tests were performed on the data set including all stations using PROC GLM in SAS 9.3 (SAS, 2011). For these analyses, each core within a drop was treated as a replicate. While cores within a drop are actually pseudoreplicates, Montagna et al. (2016) found that when comparing cores among different drops at the same station, within drop variability was much higher than among drop variability. A 2-way partially hierarchical ANOVA model was used with station nested within zone to test for differences in abundance and richness. A 3-way ANOVA model was used to test

for differences among benthic community responses with changes in sediment depth (i.e., the 0 - 5 cm and 5 - 10 cm vertical sections) with zone and station nested within zone as additional factors. To examine differences among communities within the severely and moderately impacted stations within 10 km of the DWH wellhead, a 4-way partially hierarchical ANOVA model was used with main effects being: vertical section depth, distance category within 10 km of the wellhead, station nested within zone, and direction from the DWH wellhead. Multiple comparison tests were performed using Tukey Honestly Significant Differences, which adjusts for differences in samples sizes, and maintains experiment-wise error at the 0.05 level.

2.2.3. Bioindicator and Community Analyses

Individual taxa were analyzed independently to assess their possible use as bioindicators of a deep-sea blowout. Zones were divided into two categories: impacted (zones 1 and 2) and background conditions (zones 4 and 5). A 2-way partially hierarchical ANOVA model was used with "station nested within zone" for each taxa present. Taxa that were found in fewer than 10 of the 68 stations were considered too rare to be useful bioindicators in this study. Of the 171 taxa identified, 104 (i.e., 60% of the total taxa) were found at 9 or fewer stations and not analyzed as a possible bioindicator. If the abundance of a taxon was significantly different when comparing impact (zones 1 and 2) vs. background conditions (zones 4 and 5), then it was classified as a deep-sea blowout indicator. Taxa were "pollution resistant" if higher abundances were found in the impact zone; or "pollution sensitive" if lower. If the ANOVAs were close to significant (p = 0.05-0.2), and abundances were at least 50% higher in one area compared to the other, then the taxa were considered as "possibly pollution resistant" or "possibly pollution sensitive". Taxa that were not significantly different between impact and background zones were considered as "possibly pollution neutral".

Community assemblages were examined using Primer-e version 6 software (Clarke and Gorley, 2006). Abundances were square-root transformed for Primer analyses. Non-metric multi-dimensional scaling (nMDS) was based on a Bray-Curtis similarity matrix among stations. Relationships within each nMDS were identified by cluster analysis using the group average method. A 1-way analysis of similarity (ANOSIM) for differences among zones was constructed using Bray-Curtis distances calculated on square-root transformed abundance data. A SIMPER analysis identified which taxa were most responsible for similarities within and differences among zones.

2.2.4. Environmental Factors

A stepwise analysis of environmental (e.g., sediment grain size) and chemical (e.g., PAHs and heavy metals) factors was conducted using the Bio-Env Stepwise (BEST) analysis. All environmental and chemical data were normalized before doing further analyses. Approximately 150 variables including various PAHs (as well as total PAHs here defined as PAH44 by NOAA), heavy metals, and grain size measures were included in the initial analysis. If two variables had a correlation coefficient of 95% or greater than one of the two variables was removed. After removing highly correlated variables 50 remained for the BEST analysis. Twenty restarts were performed, and the BEST analysis was stopped at rho > 0.95.

Environmental and chemical variables were also used to cluster stations without regard to biological factors using the LINKTREE analysis. The variables found to be best correlated with benthic community structure in the BEST analysis were included. Only groups which were 50% or more similar (B% > 50) were considered separate groupings and explored further. After groups were defined by LINKTREE (A, B, C, etc.), distances of stations within each group from the DWH wellhead were used to help define each group as impacted or not.

2.3. Results

A total of 13,981 macrofaunal organisms were collected from 171 different taxa. The top 20 taxa comprised approximately 80% of the total abundance (Table 1). Annelida, Mollusca, Nemertea, and Arthropoda were the dominant phyla. Polychaete families Spionidae and Paraonidae were in approximately 95% of all cores collected, and each accounted for nearly 10% of total abundance. 16 of the top 20 dominant taxa comprised polychaetes and accounted for almost 75% of macrofaunal abundance but only 25% of the taxa richness. Crustaceans accounted for 11% of macrofaunal abundance but over 40% of taxa richness. Tanaidacea (3.3% abundance and 13.5% richness), Ostracoda (3.4% abundance and 1.8% richness), Amphipoda (1.9% abundance and 11.1% richness), Isopoda (1.2% abundance and 7% richness), and Cumacea (1.1% abundance and 3.5% richness) represent over 99% of crustacean abundance. Mollusks accounted for 9.5% of total abundance and 24% of taxa richness. Bivalvia (5.6% abundance and 12.9% richness), Aplacophora (3.2% abundance and 2.9% richness), Gastropoda (0.6% abundance and 7% richness), and Scaphopoda (0.2% abundance and 1.2% richness) represent all mollusks found. Finally, eight additional phyla were collected, which accounted for 4.9% of total abundance and were not identified any further. Nemertea (3.8% abundance) and Sipuncula (0.75% abundance) represent approximately 95% of the miscellaneous taxa collected.

2.3.1. Abundance and Diversity

Total macrofaunal abundance was significantly different among zones when examining the 2-way nested ANOVA (Table 2). Zones 2 and 3 (moderate and unknown impacts) had significantly higher abundances than zones 4 and 1 (background and highly impacted sites). Abundances were lowest in zone 1 and were 74% and 85% higher in zones 2 and 3, respectively. Zones 4 and 5 had intermediate abundances 30% and 51% higher than zone 1, respectively.

Taxa richness, taxa diversity (N1), and taxa evenness (J') were all significantly different among zones in the 2-way ANOVA (Table 2). Zone 1, closest to the wellhead, was significantly lower than all other zones for richness, diversity, and evenness. Zone 2 was the second lowest zone for all parameters and was always significantly different from zone 5 and either zone 3 or 4. Zone 5 had the highest scores for richness, diversity, and evenness. There was a constant increase in J' with increasing distance from the wellhead with zone 1 having the lowest value and zone 5 the highest (Table 2).

Abundance was also significantly different among stations within 10 km of the DWH wellhead (Table 3). Stations 0 - 1 and 1 - 2 km from the wellhead had significantly lower abundances than stations 2 - 3.5 km away, and all were significantly lower than stations 8 - 10 km away. Richness, diversity, and evenness all increased with increased distance from the wellhead out to 10 km (Table 3). Direction from the wellhead was not significant for any parameter measured suggesting that the effects of the DWH spill were similar in all directions out to 10 km. Stations > 3.5 km from the wellhead were always significantly different than those < 2 km away (Table 3); however, the sampling design beyond approximately 3.5 km of the wellhead was focused on the likely trajectory of the deep-sea plume. There was a greater than 100% increase in abundance, total taxa, and N1 from station 0 - 1 km to 2 - 3 km from the wellhead.

Differences in macrofauna between different vertical layers of sediment (0-5 and 5-10 cm depth) were observed. The 3-way ANOVA which included vertical zonation as a factor found it to be significant for all parameters measured. However, the interaction of vertical section with zone and station was also significant for all analyses. Vertical distribution of the macrofauna is thus different among the different zones and stations. In zones 1 and 2, animals in the deeper

sediments (5-10 cm) accounted for over 10% of the total abundance per core on average: 11.4 and 12.8% respectively. In zones 3, 4, and 5 the deeper sediments contained lower proportions of the total core abundance (7.7, 8.2 and 7.1%, on average, respectively). Vertical section was also significant for abundance, N1, and richness when looking at stations within 10 km of the wellhead. The interaction of section and distance was also significant for all parameters but J'. Abundance, diversity, and richness all increased substantially with distance from the wellhead in the top 5 cm of the sediment but remained fairly constant in the 5-10 cm section (Fig. 3). Only evenness appeared different among distances in the deep sediments.

2.3.2. Bioindicators and Community Structure

Of the 67 taxa found at 10 or more stations, 23 taxa were found to be pollution neutral, with little difference in abundance between impacted and background stations. There were more than 3 times as many taxa identified as "pollution sensitive" compared to "pollution resistant", 21 and 6 respectively. The same was true for taxa "possibly pollution sensitive" compared to "possibly pollution resistant", 13 vs 4 respectively. Over half of the pollution sensitive taxa were crustaceans (12 taxa), followed by annelids (7). Two-thirds of the pollution resistant taxa were polychaetes (4 taxa). Pollution neutral taxa were primarily comprised of annelids (14 taxa), followed by crustaceans (5). Thirty eight percent of all polychaetes examined were possibly sensitive to the DWH blowout while 74% of crustaceans examined were sensitive. Seventeen percent of polychaetes were possibly enhanced by the blowout while only 4% of crustaceans were more abundant around the wellhead (Table 4).

Macrofauna community structure was significantly different among the five zones (ANOSIM, P < 0.001, Table 5). Stations in zone 4 were not significantly different from zones 5 (also background) and 3 (unknown impacts), but all other pairwise comparisons showed

significant differences (Table 5). When examining community structure via the nMDS plots, zone 2 was more closely grouped than the other zones suggesting more variability in stations very close to the wellhead and much further away (Fig. 4). When using a similarity threshold of 40%, the majority of stations collected from zone 1 was grouped together in the upper left portion of the plot and were found within 2 km of the wellhead. Most cores collected outside 2 km were grouped together (Fig. 4b). There were two stations greater than 100 km from the wellhead that did not group together. It is likely that these stations were so geographically distant from the remainder of the stations analyzed that they represent a different environment (Fig. 4a). These stations included 2 of 3 total stations sampled at depths greater than 2100 m. An nMDS plot examining stations within 10 km from the wellhead found all but one of the stations within 1 km grouped with most of the stations 1 - 2 km from the wellhead at 40% similarity. The rest of the stations formed a second group. However, there was a clear correlation between distance and the x axis of the graph with stations within 1 km of the wellhead on the far left, followed by stations 1 - 2 km away, stations 2 - 3 and 3.5 - 8 km away further to the right and stations 8 - 10 km away furthest right (Fig. 4b).

SIMPER analysis found the similarity of communities within a zone to range from 37% to 54% with zone 2 having the highest similarity and zones 4 and 5 having the lowest (Table 6A). Spionidae and Paraonidae, the two most abundant taxa, contributed the most to within-zone similarity for zones 3, 4, and 5. Nemertea was also important in zone 5. Paraonids were associated with much of the similarity for zones 1 and 2; however, Capitellidae and Maldanidae contributed approximately 12% each to similarity in zone 2, and Dorvilleidae contributed over 25% to similarity in zone 1. Zones 1 and 2 appear to be more similar due to a higher proportion

of dominant taxa. Only nine taxa are associated with 90% of the similarity in zone 1, 16 taxa for zone 2, and 24-27 taxa for zones 3, 4, and 5.

When examining stations less than 10 km from the wellhead, similarity within distance categories was somewhat higher than observed within zones. Stations within 1 km were 44% similar while all other categories had similarities of approximately 55%. Dorvilleidae contributed roughly 30% of the similarity within stations 0 - 1 and 1 - 2 km away from the wellhead. Paraonidae and Capitellidae combined contributed another 30% of the similarity within these 2 distance categories. Approximately 50% of the similarity within stations 2 - 3.5 and 3.5 - 8 km from the wellhead was attributed to Maldanidae, Capitellidae, Paraonidae, Spionidae and Nemertea (ranked highest to lowest). Roughly 40% of the similarity within stations 8 - 10 km from the wellhead were associated with Paraonidae, Maldanidae, Spionidae and Capitellidae (ranked highest to lowest) (Table 6B). Fewer taxa were associated with similarity within stations closer to the wellhead as was observed in closer zones. Only six taxa are associated with 90% of the similarity for stations 0 - 1 km away from the wellhead, nine taxa for stations 1 - 2 km away, 16 taxa for stations 2-3.5 km away, 20 taxa for stations 3.5 - 8 km away, and 23 taxa for stations 8 - 10 km away.

Pairwise comparisons in SIMPER show that the two most similar zones are 2 and 3 (46% similarity) while the least similar are zones 1 versus zones 4 and 5 (28-32%). Dorvilleidae explained the most dissimilarity of any taxa when looking at any pairwise comparisons examining either zone 1 or 2, except for the comparison between zones 2 and 5. Maldanidae explained the most dissimilarity when looking at only zones 3, 4 and 5. Pairwise comparisons for stations within 10 km show that stations 2 -3 .5 km, 3.5 - 8 km, and 8 - 10 km from the wellhead are all greater than 50% similar. Stations within 1 km of the wellhead are 65 - 70 %

dissimilar from stations greater than 2 km away while station 1 - 2 km from the wellhead are 54 -60 % dissimilar from stations greater than 2 km away. Dorvilleidae is associated with the highest amount of dissimilarity in all pairwise comparisons of stations within 10 km from the wellhead except between stations 2 - 3.5 and 8 - 10 km away from the wellhead.

2.3.3. Environmental Factors

The BEST analysis in Primer found nine environmental and chemical variables that were associated with 73% of the variability in macrobenthic community structure among stations. These variables included biphenyl, perylene, barium, copper, manganese, percent moisture, n_nonatriacontane_C39, percent fine sand, and percent very fine silt. However, two variables from the BEST were significantly correlated with other variables that were not included in the analyses. Biphenyl was strongly correlated (> 95 %) with acenaphthylene, C2_naphthalenes, dibenzothiophene, fluorine, naphthalene, phenanthrene, decane, dodecane, dotriacontane, N_nonane_C9, N_pentadecane, tetradecane, tridecane and undecane. Barium was strongly correlated with saturated hydrocarbons.

When examining how stations were separated based on these environmental and chemical variables using a LINKTREE, barium (or saturated hydrocarbons) was the variable most strongly affecting groupings followed to a lesser extent by perylene, % fine sand, and copper (Table 7). Two groups of stations were separated out at approximately 80% similarity based off of high and low barium concentrations found at these stations. Group A consisted of stations within 1 km of the wellhead and had the highest barium concentrations of all stations (2860 - 12700 ppm) while group B consisted of stations 60-197 km from the wellhead and had the lowest concentrations of barium (126 - 194 ppm). There were not clear patterns within other groups associated with distance. Groups C, D, and E were associated with high perylene, high

fine sand, and low copper, respectively. Groups C, D, and E consisted of stations at various distances from the wellhead (1 - 145 km, 10 - 194 km and 17 - 199 km respectively); however all but one station were at least 10 km away (Table 7).

2.4. Discussion

2.4.1. Offshore Oil Releases

Several oil spills in the marine environment have been associated with surface releases to shallow waters from tanker accidents (e.g., *Exxon Valdez, Arrow, Argo Merchant, Amoco Cadiz,* etc.). Recent drilling activities in deeper waters have raised the specter of a new type of oil spill, one in which oil and gas are released far below the water surface under extremely high pressures and low temperatures. Despite a need for deep-sea research, most funding for research associated with DWH impacts on the environment was awarded to studies examining coastal and water column habitats (Peterson et al., 2012).

Many studies have shown that macrobenthic communities in shallow marine systems generally respond to oil contamination with decreased diversity and/or increased abundances of pollution-tolerant species (e.g., Gray, 1979; Teal and Howarth, 1984). This trend is also true for changes around offshore oil and gas platforms, but in a more limited area (Peterson et al. 1996). Impacts to benthic community structure and genetic diversity due to sediment contamination were observed up to 100 - 200 m from drilling platforms in GOOMEX studies (Green and Montagna, 1996; Montagna and Harper, 1996). In contrast, Montagna et al. (2013) found that the DWH blowout had a significant effect on the levels of various concentrations of heavy metals and hydrocarbon compounds, such as barium and total PAHs, up to 15 km from the wellhead. Montagna et al. (2013) generated a benthic footprint of oil spill effects from the DWH blowout using a principal components analysis including various environmental, chemical, and

biological data. However, the only biological data included in the analysis were macrofauna and meiofauna abundance and diversity. The current study used the impact zones defined by Montagna et al. (2013) to further describe the details of the impacts on deep-sea macrobenthic community structure as a response to the DWH blowout.

2.4.2. Blowout Impacts on Benthic Abundance and Diversity

The DWH blowout generally decreased macrobenthic diversity, richness, evenness, and abundance near the wellhead (Tables 2-3). Biodiversity (e.g., diversity, richness, and evenness) has been associated with ecosystem functioning in several studies. For example, a larger number of species may contribute to ecosystem functions due to an increase in complementarity, or facilitative interactions among species (Loreau et al., 2002). Similarly, a decrease in diversity has been found to correspond to decreases in several ecosystem functions provided by a habitat, including productivity, nutrient cycling, and resistance to invasive species (Balvanera et al., 2006). Likewise, higher biodiversity is thought to improve the capacity of the deep-sea benthic communities to convert organic matter settling from the surface into biomass and channel this energy to other trophic levels (Danovaro et al., 2008). Studies have also suggested that decreases in biodiversity would have negative impacts on carbon sequestration and pollutant regulation (Snelgrove, 1999; Beaumont et al., 2008).

Macrobenthic diversity and abundance were examined in the deep-sea Gulf of Mexico after the DWH blowout to ascertain the possible extent of damages to the seafloor. The heavily impacted zone had the lowest abundance and number of taxa while the intermediate zone had the highest abundances and number of taxa (Table 2). This is in contrast to the meiobenthic communities around the DWH wellhead, in which diversity metrics were found to be lowest in the zone closest to the wellhead but abundances to be much higher in the most heavily impacted

zones (Baguley et al., 2015). Macrofaunal abundance has been shown to increase near offshore drilling platforms, most likely due to organic enrichment (Green and Montagna, 1996; Montagna and Harper, 1996). A combination of organic enrichment combined with toxicity may explain effects observed from the DWH blowout (Peterson et al., 1996). Close to the wellhead, where concentrations of nutrients, carbon, and toxic compounds are all high, fewer animals were able to survive. Pollution-resistant components of communities at intermediate distances from the wellhead likely benefited from increased levels of nutrients and carbon while pollution sensitive components experienced some amount of toxicity or were out-competed. Thus, beyond zone 2 it appears that toxicity effects of the DWH blowout on the macrobenthic communities are substantially diluted.

The most likely explanation for the observed changes in macrobenthic communities among zones is that the DWH spill had primarily negative effects on the deep-sea macrobenthic communities. There was a large range in depths among stations sampled (~2700 m), but average depth for stations within any zone was between 1050 – 1520 m. Decreases of benthic metrics with depth are well known in the Gulf of Mexico. Meiofauna abundance decreases linearly with depth from 300 to 3900 m (Baguley et al., 2006), as do benthic formanifera (Bernhard et al., 2008), nematodes (Sharma et al., 2012), and benthic macrofaunal isopods (Wilson, 2008). However, studies found little difference in community richness and diversity between 1000 – 2000 m in the northern GoM (Haedrich et al., 2008; Wilson, 2008). These same studies also found no difference in community richness and diversity among communities at similar depths across large areas of the northern GoM. In the present study, an increase in depth was also associated with an increase in distance from the wellhead in these heavily impacted areas. The fact that evenness decreased and abundance increased with depth in zone 1, and diversity and

richness increased with depth in zone 2 indicates that proximity to the DWH blowout had a greater effect on the macrobenthic community than a change in depth.

Within the impacted zones 1 and 2, smaller scale patterns (within 10 km of the wellhead) in macrobenthic community structure were observed. For nearly all metrics of benthic health examined, values were lowest within 1 km of the wellhead and continually increased with increased distance from the wellhead up to 10 km (Table 3). The polychaete families Paraonidae and Dorvilleidae, which are often associated with natural oil seeps (Levin, 2005), are responsible for much of the similarity in stations within 2 km of the wellhead.

Macrofaunal community response was largely restricted to surface sediments, consistent with responses to a recent disturbance where pollutants settle to the seafloor from the water column (e.g., the DWH spill). A large amount of oil reached the sea floor via the "dirty blizzard" mechanism, essentially sinking of oil-rich marine snow particles (Brooks et al., 2014). It has been estimated that approximately 10% of the spilled oil from the DWH blowout was deposited to the seafloor via sinking of aggregates as well as direct contact of the deep-sea hydrocarbon plume with sediments between 1000-1200 m (Romero et al., 2014). Due to this mechanism of transport, oil was deposited on the surface of the sediments. Our analyses show that while abundance, richness, and diversity were significantly different between stations close to the wellhead vs further away when examining the top 5 cm of sediment, many of these differences were not observed in the deep 5 - 10 cm of sediment.

2.4.3. Blowout Benthic Bioindicators

There were more than three times as many taxa classified as sensitive or possibly sensitive to the blowout compared to those classified as resistant or possibly resistant (Table 3). Animals of the phyla Echinodermata and Crustacea, such as urchins or amphipods, are often

more sensitive to contaminants than many families of the phyla Annelida (Newton and McKenzie, 1995; Gesteira and Dauvin, 2000; Lenihan et al., 2003), and this appeared to be true in the current study as well (Table 4). Ophiuroids may be useful as sensitive taxa to deep-water blowouts; however, most echinoderm taxa were not found at enough stations to be useful as bioindicators, and holothuroids were found throughout the study area. Crustaceans appeared to be much more useful than echinoderms as indicators of oil blowouts, at least within months of the spill event when these samples were collected. There were 17 crustacean families that appeared to be sensitive to the DWH blowout, while only five families were pollution neutral and only one family, the tanaid Sphyrapidae, was resistant. Many studies have shown that amphipods are useful bioindicators of many types of pollution including hydrocarbons (Gesteira and Dauvin, 2000; Bat, 2005). The sensitivities of other types of marine infaunal crustaceans have not been analyzed as often as amphipods, but studies that have examined benthic community structure in shallow marine areas have generally found that ostracod (Ruiz et al., 2005), isopod, cumacean and tanaid abundances decrease with increased pollution and other stressors (Lenihan et al., 1995; Thompson et al., 2007). Crustacean families as a whole appear to be useful indicators of a relatively healthy benthic habitat in the northern deep-sea Gulf of Mexico.

A large majority of mollusk taxa were not found in great enough abundances to analyze individually. The bivalve family Nuculanidae appeared to be sensitive to the DWH blowout, while Nuculidae was pollution neutral, and Thyasiridae was one of the few taxa found with much greater abundances near the wellhead. Several thyasirid species have been found around hydrocarbon seeps and host chemosynthetic bacteria (Oliver and Drewery, 2014; Amano et al., 2015). This adaptation to take advantage of natural oil seeps may have made them less

susceptible to the petroleum contamination stemming from the DWH blowout. Interestingly, when unidentifiable bivalves, gastropods, and scaphopods were summed, all three classes were either sensitive or possibly sensitive. Because there are several mollusk families within each class which can be considered either pollution tolerant or sensitive (Mouthon and Charvet, 1999) it is unclear how useful these higher taxa are as indicators of environmental health. Future studies should explore the sensitivity of mollusks as a whole to pollution in the deep sea.

Changes in macrobenthic abundance and diversity due to proximity to the DWH wellhead were caused primarily by polychaete taxa. Specifically, the family Dorvilleidae was responsible for the largest proportion of similarity in zone 1 and the largest proportion of dissimilarity in all pairwise comparisons between the impacted zones 1 or 2 and other zones. Many studies have found that dorvilleids are often associated with methane seep communities and high sulfide concentrations in the deep sea (Baco and Smith, 2003; Levin, 2005). Thornhill et al. (2012) explored the adaptive radiation of dorvilleid species associated with reducing environments and found at least four instances where dorvilleid taxa independently evolved the ability to inhabit seep habitats. Dorvillied taxa have also been associated with organic enrichment in many shallow marine studies (e.g., Pearson and Rosenberg, 1978; Dean, 2008; Salvo et al., 2015). Thus dorvilleids may be more tolerant to anthropogenically oiled areas.

Capitellidae, Maldanidae, and Paraonidae were the three other polychaete families which appeared to be resistant to the DWH blowout (Table 4). Capitellids have been shown to be tolerant of various forms of pollution, including hydrocarbons, in many different studies (Reish and Gerlinger, 1997; Dean, 2008). They are often used as indicators of polluted areas (Ganapati and Raman, 1976; Tsutsumi, 1990; Tomassetti and Porrello, 2005). Maldanids have been shown to be ineffective at metabolizing PAHs and therefore somewhat pollution sensitive (Rust et al.,

2004; Dean, 2008) and paraonids have not been examined much as bioindicators (Dean, 2008). One reason for the possible differences in classification in maldanids and paraonids may be the lack of deep-sea taxa used in bioindicator studies. All three families were also numerically abundant in both impacted and background stations making them less useful than dorvilleids in identifying impacted areas.

Spionidae and Paraonidae appear to be representative of background conditions as the two families are responsible for the first and second highest proportions of similarity in zones 3, 4, and 5. Paraonids are also responsible for a large proportion of similarity in zones 1 and 2 suggesting that they are not particularly susceptible to pollution from the DWH blowout or are so abundant that any decreases in abundance still leave them as a dominant taxon. Spionids account for half of the similarity within zones 1 and 2 compared to zones 4 and 5 (Table 6). Many studies examining spionids in shallow marine systems have found them to be generally tolerant of pollution including hydrocarbons (Dean, 2008), but again, they may be sensitive to the DWH blowout because the deep sea is a very different environment than shallow systems. Many of the other polychaete taxa found to be sensitive to the DWH spill such as Ampharetidae, Onuphidae, and Trichobranchidae have been found to be sensitive to pollution in shallow marine systems as well (Dean, 2008).

While it is a useful exercise to examine the responses of individual taxa to pollution events to determine possible bioindicators, the classifications determined in this paper must be considered in the context of the time and location of this study. Taxa found to be useful indicators of a deep blowout in the northern Gulf of Mexico may not be so in other deep areas of the ocean. There has historically been little examination of the tolerances of deep-sea taxa to pollutants. Thus differences in taxa abundance in impacted and background areas may be due to

the DWH blowout but may also be due to other underlying environmental factors such as depth, predation, or organic matter input. Finally, the list of bioindicators in this paper is the first step in identifying specific deep-sea taxa useful for this task, but studies on future events as well as laboratory analyses examining the tolerances of specific deep-sea taxa to pollutants should be used to add to and refine this list.

Macrobenthic organisms close to the wellhead were exposed to elevated concentrations of hydrocarbons, toxic metals, and dispersants that reached the bottom sediments after the initial blowout. How long effects will persist in deep-sea benthic communities is unknown, but recovery of soft-bottom benthos after oil spills in coastal waters has been shown to require years or decades (Teal and Howarth, 1984; Dauvin, 1998). In the deep sea, where deposition rates and metabolic activity are much slower (Rowe et al., 2008), recovery could take decades or centuries.

The responses of deep sea macrobenthic communities to the Deepwater Horizon oil blowout appear to follow a classic toxicity gradient. Low diversity, low abundance, and high dominance at stations very close to the wellhead are likely due to high levels of toxic chemicals. PAHs and barium are the most likely agents in this regard. The increase in observed abundance and decrease in observed diversity in zone 2 and low variability in community structure suggests that the toxicity here is still somewhat high, keeping many pollution sensitive species from surviving, but low enough to allow resistant families to take advantage of enrichment and reach elevated abundances relative to background conditions. Zone 3 had the highest abundances and diversity metrics suggesting that toxicity was minimal here. The increase in abundance and diversity from zones 1 to 3 was most likely due to a decrease in toxicity from the DWH wellhead with greater distance and changes associated with organic enrichment. However, the decrease in abundance and diversity from zones 3 to 5 was most likely due to a transition into a somewhat

different environment as evidenced by the shallow depths of zone 3 when compared to other zones. A steady increase in abundance and diversity was observed in the impacted zones out to 10 km. The majority of taxa examined were found in different abundances between impact and background areas. This study is a crucial first step in identifying macrobenthic indicators of deep-sea hydrocarbon blowouts. It also suggests that oil spill effects on deep-sea benthic communities mirror classic enrichment vs. toxicity effects observed in previous, shallow-water and terrestrial polluted sites.

References

- Amano, K, C.T.S. Little, K.A. Campbell, R.G. Jenkins, and K.P. Saether (2015). Paleocene and Miocene Thyasira sensu stricto (Bivalvia: Thyasiridae) from chemosynthetic communities from Japan and New Zealand. The Nautilus, 129, 43-53.
- Baco, A.R. and C.R. Smith (2003). High species richness in deep-sea chemoautotrohic whale skeleton communities. Marine Ecology Progress Series, 260, 109-114.
- Baguley J.G., P.A. Montagna, L.J. Hyde, R.D. Kalke, and G.T. Rowe (2006). Metazoan meiofauna abundance in relation to environmental variables in the northern Gulf of Mexico deep sea. Deep Sea Research Part I, 53, 1344-1362.
- Baguley, J.G., P.A. Montagna, C. Cooksey, J.L. Hyland, H.W. Bang, C. Morrison, A.
 Kamikawa, P. Bennetts, G. Saiyo, E. Parsons, M. Herdener, and M. Ricci (2015).
 Community response of deep-sea soft-sediment metazoan meiofauna to the Deepwater
 Horizon blow out and oil spill. Marine Ecology Progress Series, 528, 127-140.
- Balvanera P., A.B. Pfisterer, N. Buchmann, J. He, T. Nakashizuka, D. Raffaelli, and B. Schmid (2006). Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecology Letters, 9, 1146-1156.

- Bat, L. (2005). A review of sediment toxicity bioassays using the amphipods and polychaetes. Turkish Journal of Fisheries and Aquatic Sciences, 5, 119-139.
- Beaumont, N.J., M.C. Austen, S.C. Mangi, and M. Townsend (2008). Economic valuation for the conservation of marine biodiversity. Marine Pollution Bulletin, 56, 386-396.
- Bernhard, J.M., B.K. Sen Gupta, and J.G. Baguley (2008) Benthic foraminifera living in Gulf of Mexico bathyal and abyssal sediments: community analysis and comparison to metazoan meiofaunal biomass and density. Deep-Sea Research Part II, 55, 2617-2726.
- Bilyard, G.R. (1987). The value of benthic infauna in marine pollution monitoring studies. Marine Pollution Bulletin, 18, 581-585.
- Brooks, G.R., R.A. Larson, B. Flower, D. Hollander, P.T. Schwing, I. Romero, C. Moore, G.J. Reichart, T. Jilbert, J. Chanton, and D. Hastings (2014). Sedimentation pulse in the NE Gulf of Mexico following the 2010 DWH blowout. Deep-Sea Research II, 10: e0132341, <u>https://doi.org/10.1371/journal.pone.0132341</u>.
- Clarke, K.R. and R.N. Gorley, (2006). Primer v6: User Manual/Tutorial. Primer-E, Plymouth, UK.
- Danovaro, R., C. Gambi, A.D. Anno, C. Corinaldesi, S. Fraschetti, A. Vanreusel, M. Vincx, and A.J. Gooday (2008). Exponential decline of deep-sea ecosystem functioning linked to benthic biodiversity loss. Current Biology, 18, 1-8.
- Dauvin, J.C. (1998). The fine sand *Abra alba* community of the Bay of Morlaix twenty years after the Amoco Cadiz oil spill. Marine Pollution Bulletin, 36, 669-676.
- Dayton, P.K. and R.R. Hessler (1972). Role of biological disturbance in maintaining diversity in the deep sea. Deep Sea Research and Oceanographic Abstracts, 19, 199-204.

- Dean, H.K. (2008). The use of polychaetes (Annelida) as indicator species of marine pollution: a review. International Journal of Tropical Biology and Conservation, 56, 11-38.
- Diepens, N.J., G.H.P. Arts, T.C.M. Brock, H. Smidt, P.J. Van Den Brink, M.J. Van Den Heuvel-Greve, and A.A. Koelmans (2014). Sediment toxicity testing of organic chemicals in the context of prospective risk assessment: A review. Critical Reviews in Environmental Science and Technology, 44, 255-302.
- Etter, R.J. and J.F. Grassle (1992). Patterns of species diversity in the deep sea as a function of sediment particle size diversity. Nature, 360, 576-578.
- Gage, J.D. and P.A. Tyler (1992). Deep-sea biology: A natural history of organisms at the deepsea floor. Cambridge, UK, Cambridge University Press.
- Ganapati, P.N. and A.V. Raman (1976). *Capitella capitata* (fabricius, 1780) (Polychaeta:Capitellidae): An indicator of pollution in Visakhapatnam Harbour. International Journal of Molecular Sciences, 5, 251.
- Gesteira, J.L. and J.C. Dauvin (2000). Amphipods are good bioindicators of the impact of oil spills on soft-bottom macrobenthic communities. Marine Pollution Bulletin, 40, 1017-1027.
- Gesteira, J.L.G., J.C. Dauvin, and M.S. Fraga (2003). Taxonomic level for assessing oil spill effects on soft-bottom sublittoral benthic communities. Marine Pollution Bulletin, 46, 562-572.
- Gillette, D.J., S.B. Weisberg, T. Grayson, A. Hamilton, V. Hansen, E.W. Leppo, M.C. Pelletiere,
 A. Borja, D. Cadien, D. Dauer, R. Diaz, M. Dutch, J.L. Hyland, M. Kellogg, P.F. Larsen,
 J.S. Levinton, R. Llansó, L.L. Lovell, P.A. Montagna, D. Pasko, C.A. Phillips, C.
 Rakocinski, J.A. Ranasinghe, D.M. Sanger, H. Teixeira, R.F. Van Dolah, R.G. Velarde, and

K.I. Welch (2015). Effect of ecological group classification schemes on performance of the AMBI benthic index in US coastal waters. Ecological Indicators, 50, 99–107.

- Glover, A.G. and C.R. Smith (2003). The deep-sea floor ecosystem: current status and prospects of anthropogenic change by the year 2025. Environmental Conservation, 30, 210-241.
- Gray, J.S. (1979). Pollution-induced changes in populations. Philosophical Transactions of the Royal Society of London, B286, 545-561.

Grassle, J.F. (1977). Slow recolonisation of deep-sea sediment. Nature, 265, 618-619.

- Green, R.H. and P. Montagna (1996). Implications for Monitoring: Study Designs and Interpretation of Results. Canadian Journal of Fisheries and Aquatic Sciences, 53, 2629-2636.
- Griffiths, S.K. (2012). Oil release from Macondo well MC252 following the Deepwater Horizon accident. Environmental Science and Technology, 46, 5616.
- Haedrich, R.L., J.A. Devine, and V.J. Kendall (2008). Predictors of species richness in the deepbenthic fauna of the northern Gulf of Mexico. Deep-Sea Research II, 55, 2650-2656.
- Heip, C., R.M. Warwick, M.R. Carr, P.M.J. Herman, R. Huys, N. Smol, and K. Van Holsbeke (1988). Analysis of community attributes of the benthic meiofauna of Frierfjord/Langesundfjord. Marine Ecology Progress Series, 46, 171-180.
- Hyland, J., D. Hardin, E. Crecelius, D. Drake, P. Montagna, and M. Steinhauer (1990).
 Monitoring long-term effects of offshore oil and gas development along the southern
 California outer continental shelf and slope: Background environmental conditions in the
 Santa Maria basin. Oil & Chemical Pollution, 6, 195-240.

- Kujawinski, E.B., M.C.K. Soule, D.L. Valentine, A.K. Boysen, K. Longnecker, and M.C.Redmond (2011). Fate of dispersants associated with the Deepwater Horizon oil spill.Environmental Science & Technology, 45, 1298-1306.
- Lenihan, H.S., K.A. Kiest, K.E. Conlan, P.N. Slattery, B.H. Konar, and J.S. Oliver (1995).
 Patterns of survival and behavior in Antarctic benthic invertebrates exposed to contaminated sediment: field and laboratory bioassay experiments. Journal of Experimental Marine Biology and Ecology, 192, 233-255.
- Lenihan, H.S., C.H. Peterson, S.L. Kim, K.E. Conlan, R. Fairey, C. McDonald, J.H. Grabowski, and J.S. Oliver (2003). Variation in marine benthic community composition allows discrimination of multiple stressors. Marine Ecology Progress Series, 261, 63-73.
- Levin, L.A. (2005). Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. Oceanography and Marine Biology: An Annual Review, 43, 1-46.
- Loreau, M., S. Naeem, P. Inchausti (Eds). (2002). Biodiversity and ecosystem functioning, synthesis, and perspectives. Oxford, UK; Oxford University Press.
- Montagna, P. and D.E. Harper Jr. (1996). Benthic infaunal long-term response to offshore production platforms in the Gulf of Mexico. Canadian Journal of Fisheries and Aquatic Sciences, 53, 2567-2588.
- Montagna, P.A., J.G. Baguley, C. Cooksey, J.L. Hyland, R.D. Kalke, L.J. Hyde, M. Reuscher, A. Rhodes, and I. Hartwell (2013). Deep-sea benthic footprint of the Deepwater Horizon blowout. PloS ONE, 8, e70540, <u>http://doi:10.1371/journal.pone.0070540</u>.
- Montagna, P.A., J.G. Baguley, C.Y. Hsiang, and M.G. Reuscher (2016). Comparison of sampling methods for deep-sea infauna. Methods in Oceanography, 15, 166-183.

- Mouthon, J. and S. Charvet (1999). Compared sensitivity of species, genera and families of Molluscs to biodegradable pollution. International Journal of Limnology, 35, 31-39.
- Newton, L.C. and J.D. McKenzie (1995). Echinoderms and oil pollution: A potential stress assay using bacterial symbionts. Marine Pollution Bulletin, 31, 453-456.
- Oliver, P.G. and J. Drewery (2014). New species of chemosynthetic clams (Bivalvia: Vesicomyidae and Thyasiridae) from putative 'seep' in the Hatton-Rockall Basin, north-east Atlantic. Journal of the Marine Biological Association of the United Kingdom, 94, 389-403.
- Pearson, T.H. and R. Rosenberg (1978). Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology Annual Review, 16, 229-311.
- Peterson, C.H., M.C. Kennicutt II, R.H. Green, P. Montagna, D.E. Harper Jr., E.N. Powell, and P.F. Roscigno (1996). Ecological consequences of environmental perturbations associated with offshore hydrocarbon production: A perspective on long-term exposures in the Gulf of Mexico. Canadian Journal of Fisheries and Aquatic Sciences, 53, 2637-2654.
- Peterson, C.H., S.S. Anderson, G.N. Cherr, R.F. Ambrose, S. Anghera, S. Bay, M. Blum, R.
 Condon, T.A. Dean, M. Graham, M. Guzy, S. Hampton, S. Joye, J. Lambrinos, B. Mate, D.
 Meffert, S.P. Powers, P. Somasundaran, R.B. Spies, C.M. Taylor, R. Tjeerdema, and E.E.
 Adams (2012). A tale of two spills: Novel science and policy implications of an emerging new oil spill model. Biosience, 62, 461-469.
- Reish, D.J. and T.V. Gerlinger (1997). A review of the toxicological studies with polychaetous annelids. Bulletin of Marine Science, 60, 584-607.

- Rex, M.A., R.J. Etter, J.S. Morris, J. Crouse, C.R. McClain, N.A. Johnson, C.T. Stuart, J.W. Deming, R. Thies, and R. Avery (2006). Global bathymetric patterns of standing stock and body size in the deep-sea benthos. Marine Ecology Progress Series, 317, 1-8.
- Romero, I.C., G.A. Toro-Garmer, R.A. Larson, P. Schwing, and D.J. Hollander (2014).
 Hydrocarbon deposition in deep-sediments following the Deepwater Horizon blowout:
 Spatial analysis of organic geochemical signatures. Proceedings of the 2014 Ocean Sciences
 Meeting. Honululu, HI, February, 23-28.
- Rowe, G.T., C. Wei, C. Nunnally, R. Haedrich, P. Montagna, J.G. Baguley, J.M. Bernhard, M. Wicksten, A. Ammons, E.E. Briones, Y. Soliman, and J.W. Deming (2008). Comparative biomass structure and estimated carbon flow in food webs in the deep Gulf of Mexico. Deep-Sea Research II, 55, 2699-2711.
- Rowe, G.T. and M.C. Kennicutt II, eds. (2009). Northern Gulf of Mexico continental slope habitats and benthic ecology study. Final report. U.S. Dept. of the Interior, Minerals Management. Service, Gulf of Mexico OCS Region, New Orleans, LA. OCS Study MMS 2009-039. 456 pp.
- Ruiz, F., M. Abad, A.M. Bodergat, P. Carbonel, J. Rodriguez-Lazaro, and M. Yasuhara (2005).Marine and brackish-water ostracods as sentinels of anthropogenic impacts. Earth-Science Reviews, 72, 89-111.
- Rust, A.J., R.M. Burgess, B.J. Brownawell, and A.E. McElroy (2004). Relationship between metabolism and bioaccumulation of benzo[a]pyrene in benthic invertebrates. Environmental Toxicology and Chemistry, 23, 2587-2593.
- Ryerson, T.B., R. Camilli, J.D. Kessler, C.M. Reddy, D.L. Valentine, E.L. Atlas, D.R. Blake, J.A. De Gouw, S. Meinardi, D.D. Parrish, J. Peischl, J.S. Seewald, and C. Warneke (2012).

Chemical data quantify Deepwater Horizon hydrocarbon flow rate and environmental distribution. Proceedings of the National Academy of Sciences, 109, 20246-20253.

- Salvo, F., D. Hamoutene, and S.C. Dufour (2015). Trophic analyses of opportunistic polychaetes (Ophryotrocha cyclops) at salmonid aquaculture sites. Journal of the Marine Biological Associations of the United Kindgom, 95, 713-722.
- Santos, M.F.L., P.C. Lana, J. Silva, J.G. Fachel, and F.H. Pulgati (2009). Effects of non-aqueous fluids cuttings discharge from exploratory drilling activities on the deep-sea macrobenthic communities. Deep-Sea Research II, 56, 32-40.

SAS Institute Inc. (2011). SAS/STAT® 9.3 User's Guide. Cary, NC.

- Seo, J.-Y., K. Moonkoo, H.-S. Lim, and J.-W. Choi (2014). The macrofaunal communities in the shallow subtidal areas for the first 3 years after the Hebei Spirit oil spill. Marine Pollution Bulletin, 82, 208-220.
- Sharma, J., J.G. Baguley, P.A. Montagna, and G.T. Rowe (2012). Assessment of longitudinal gradients in nematode communities in the deep northern Gulf of Mexico and concordance with benthic taxa. International Journal of Oceanography Article ID 903018, 15 pages. http://doi:10.1155/2012/903018.
- Snelgrove, P.V.R. (1999). Getting to the bottom of marine biodiversity: Sedimentary habitats. Bioscience, 49, 129-138.
- Teal, J.M. and R.W. Howarth (1984). Oil spill studies: A review of ecological effects. Environmental Management, 8, 27-44.
- Thompson, B.A.W., P.M. Goldworthy, M.J. Riddle, I. Snape, and J.S. Stark (2007). Contamination effects by a 'conventional' and a 'biodegradable' lubricant oil on infaunal

recruitment to Antarctic sediments: a field experiment. Journal of Experimental Marine Biology and Ecology, 340, 213-226.

- Thornhill, D.J., T.H. Struck, B. Ebbe, R.W. Lee, G.F. Mendoza, L.A. Levin, and K.M Halanych (2012). Adaptive radiation in extremophilic Dorvilleidae (Annelida): Diversification of a single colonizer or multiple independent lineages? Ecology and Evolution, 2, 1958-1970.
- Tomassetti, P. and S. Porrello (2005). Polychaetes as indicators of marine fish farm organic enrichment. Aquaculture International, 13, 109-128.
- Tsutsumi, H (1990). Population persistence of *Capitella* sp. (Polychaeta; Capitellidae) on a mud flat subject to environmental disturbance by organic enrichment. Marine Ecology Progress Series, 63, 147-156.
- Tyler, P.A. (2003). Ecosystems of the deep. Elsevier Science, Amsterdam, Netherlands.
- Warwick, R.M. (1988). Analysis of community attributes of the macrobenthos of Frierfjord/Langesundfjord at taxonomic levels higher than species. Marine Ecology Progress Series, 46, 167-170.
- Warwick, R.M., M.R. Carr, K.R. Clarke, J.M. Gee, and R.H. Green (1988). A mesocosm experiment on the effects of hydrocarbon and copper pollution on a sublittoral soft-sediment meiobenthic community. Marine Ecology Progress Series, 46,181-191.
- Washburn, T. and D. Sanger (2011). Land use effects on macrobenthic communities in southeastern United States tidal creeks. Environmental Monitoring and Assessment, 180, 177-188.
- Wilson, G.D.F. (2008). Local and regional species diversity of benthic Isopoda (Crustacea) in the deep Gulf of Mexico. Deep-Sea Research Part II, 55, 2634-2649.



Figure 2.1. Station locations. Triangle is location of the MC252 site, and concentric rings are at 25 km intervals. Zones one and two were the most heavily impacted by the DWH spill while zones four and five were not impacted. Isobath intervals: light blue lines at 100 m intervals, and black lines at 500 m.



Figure 2.2. Radial design station locations overlaid on the effects zones identified by Montagna et al. 2013. Triangle is location of the MC252 site, and concentric rings are at 1, 3, and 10 km intervals. The red area represents the most heavily impacted zone one, the orange area the moderately impacted zone 2, the yellow area zone 3, and the green area the background conditions of zones 4 and 5.



Figure 2.3. Vertical distribution of macrofauna within surface (0 - 5 cm) and deeper (5 - 10 cm) sediment. A) Macrofaunal abundance (average number of individuals per section). B) Macrofaunal diversity (N1, number of dominant species per section).



A)



B)

Figure 2.4. Nonmetric multi-dimensional scaling ordination of similarities of samples based on macrofaunal taxa occurrences. A) All stations examined with symbols representing zones, and circles representing 40% similarity level from cluster analysis. B) Radial design stations within 10 km from the wellhead with symbols representing distance categories, and circles representing 40% similarity level from cluster analysis.

Table 2.1 The 20 dominant taxa collected during the fall 2010 sampling. Number of stations where a taxon was found, mean abundance in all stations, percent of total abundance, and cumulative percent abundance. Abbreviations: P = Polychaete, N = Nemertea, M = Mollusca, C = Crustacea.

Rank	Taxa (Phylum)	Stations (n)	Abundance (n/m ²)	Percent	Cumulative Percent
1	Spionidae (P)	67	885	9.8%	9.8%
2	Paraonidae (P)	67	882	9.8%	19.6%
3	Maldanidae (P)	59	801	8.9%	28.5%
4	Dorvilleidae (P)	57	709	7.9%	36.4%
5	Capitellidae (P)	65	662	7.4%	43.8%
6	Cirratulidae (P)	62	383	4.3%	48.0%
7	Nemertea (N)	67	341	3.8%	51.8%
8	Syllidae (P)	62	292	3.2%	55.1%
9	Cossuridae (P)	49	276	3.1%	58.1%
10	Bivalvia (M)	54	269	3.0%	61.1%
11	Prochaetodermatidae (M)	49	229	2.5%	63.7%
12	Podocopida (C)	50	219	2.4%	66.1%
13	Terebellidae (P)	49	209	2.3%	68.4%
14	Sigalionidae (P)	52	195	2.2%	70.6%
15	Lumbrineridae (P)	52	153	1.7%	72.3%
16	Thyasiridae (P)	37	142	1.6%	73.9%
17	Ampharetidae (P)	52	137	1.5%	75.4%
18	Acrocirridae (P)	53	135	1.5%	76.9%
19	Opheliidae (P)	41	114	1.3%	78.2%
20	Nereididae (P)	50	109	1.2%	79.4%
151	Other Families		1,856	20.6%	100.0%
171	Total	68	8,998	100.0%	

Table 2.2. Analysis of zones. Stations in zones 1 and 2 were heavily or moderately impacted by the DWH spill while zones 4 and 5 were unaffected. A) Results for the 2-way nested ANOVA. B) Tukey tests listed from highest to lowest where underlined categories are not significantly different at the 0.05 level. Abbreviations: df = degrees of freedom.

A) ANOVA		P-Value for Macrofauna Trait						
Source			Abundance		Richness	N1	J'	
Zone	4	0.0174		0.0005	< 0.0001	< 0.0001		
Station(Zone)		63	< 0.0001		< 0.0001	< 0.0001	< 0.0001	
Error		128						
D) Tukov To	ata							
Abundance	Zone		3	2	5	4	1	
	Mean		88.2	82.8	71.6	62.1	47.6	
Richness	Zone		3	5	4	2	1	
	Mean		26	25.8	22.1	21.4	12	
N1	Zone		5	3	4	2	1	
	Mean		18.9	16.5	15.4	13.6	7.2	
T'	Zone		5	4	3	2	1	
J	Mean		.91	- 0.89	0.86	0.85	0.74	

Table 2.3. Analysis of the radial design within 10 km of the DWH wellhead. A) Results for the 4-way nested partially hierarchical ANOVA, df = degrees of freedom. B) Tukey tests with means listed from highest to lowest where underlined are not significantly different at the 0.05 level. Distance is in kilometers.

A) ANOVA			P-Value for Macrofauna Trait					
Source			df	Abundance	Richn	ess	N1	J'
Distance			4	0.0002	< 0.00)01 <	< 0.0001	0.0118
Direction(Distance)			63	0.6678	0.83	3337 0.802		0.9071
Station(Distance*Direction)			6	0.3900	0.33	0.3313 0.334		0.0103
Section			1	< 0.0001	< 0.00)01 <	< 0.0001	0.0683
Distance*Section			4	0.0018	<0.0001 <0.00		< 0.0001	0.5928
Direction(Section(Distance))			18	0.4846	0.7899 0.68		0.6876	0.0460
Sec*Station(Distance*Direction)			6	0.0452	$<\!0.00$	01	0.0072	0.8017
Error			114					
B) Tukey Tests								
Abundance	Distance	8-10	3.5-8	2-3.5	1-2	0-1		
	Mean	52.9	43.8	39.3	27.4	18.′	7	
	-	-						
Richness	Distance	8-10	5	2-3.5	1-2	0-1		
Ittellitess	Moon	166	14.2	12.0	74	50		
	Mean	10.0	14.3	13.0	7.4	5.2	,	
		_						
N1	Distance	8-10	3.5-8	2-3.5	1-2	0-1		
	Mean	11.5	9.8	9.4	4.6	3.6		
		-					<u> </u>	
T'	Distance	8-10	3 5-8	2-3.5	1_2	0_1		
J	Maan	0.00	0.95	2-3.5	0.65	0-1	ר	
	Mean	0.88	0.85	0.85	0.65	0.62	2	
Table 2.4. Bioindicator classification is based on P-values from 2-way ANOVAs performed on each taxon found at 10 or more stations during the fall of 2010. Impact:Background ratios are based on abundances in the two zones. *NA is due to no organisms being found in the impacted area.

				Impact:	%
			Bioindicator	Background	Stations
Taxa	Class	P-Value	Classification	Ratio	Found
Oedicerotidae	Amphipoda	0.0059	Sensitive	1:17.1	32.4
Phoxocephalidae	Amphipoda	0.0115	Sensitive	1:4.4	50.0
Leuconidae	Cumacea	0.0444	Sensitive	1:4.9	23.5
Nannastacidae	Cumacea	0.0116	Sensitive	1:13.9	33.8
Desmosomatidae	Isopoda	0.0003	Sensitive	1:7.3	44.1
Ischnomesidae	Isopoda	0.0459	Sensitive	1:7.5	26.5
Munnopsidae	Isopoda	0.0045	Sensitive	1:4.3	29.4
Podocopida	Ostracoda	0.001	Sensitive	1:3.8	72.1
Agathotanaidae	Tanaidacea	0.001	Sensitive	1:5.1	55.9
Akanthophoreidae	Tanaidacea	0.0054	Sensitive	*NA	20.6
Colletteidae	Tanaidacea	0.0023	Sensitive	1:3.5	57.4
Leptognathiidae	Tanaidacea	0.0129	Sensitive	1:5.8	20.6
Typhlotanaidae	Tanaidacea	0.0316	Sensitive	1:14.4	25.0
Nuculanidae	Bivalvia	0.0229	Sensitive	1:6.3	29.4
Scaphopoda	Scaphopoda	0.0181	Sensitive	1:11.7	19.1
Oligochaeta	Oligochaeta	0.0455	Sensitive	1:5.4	16.2
Ampharetidae	Polychaeta	0.0029	Sensitive	1:2.9	76.5
Flabelligeridae	Polychaeta	0.034	Sensitive	1:3	51.5
Onuphidae	Polychaeta	0.0352	Sensitive	1:3.9	27.9
Opheliidae	Polychaeta	0.0016	Sensitive	1:8.9	60.3
Spionidae	Polychaeta	0.0046	Sensitive	1:1.9	91.2
Trichobranchidae	Polychaeta	0.0209	Sensitive	1:3.7	47.1
Ampeliscidae	Amphipoda	0.167	Possibly Sensitive	1:5.4	16.2
Diastylidae	Cumacea	0.0655	Possibly Sensitive	1:3.5	32.4
Myodocopida	Ostracoda	0.0869	Possibly Sensitive	1:4.4	38.2
Pseudotanaidae	Tanaidacea	0.0529	Possibly Sensitive	1:2.4	41.2
Bivalvia	Bivalvia	0.1529	Possibly Sensitive	1:1.6	77.9
Gastropoda	Gastropoda	0.0594	Possibly Sensitive	1:5.4	23.5
Aberrantidae	Polychaeta	0.094	Possibly Sensitive	1:3.6	14.7
Fauveliopsidae	Polychaeta	0.0725	Possibly Sensitive	1:2.8	51.5
Longosomatidae	Polychaeta	0.0721	Possibly Sensitive	1:5.7	26.5
Orbiniidae	Polychaeta	0.0851	Possibly Sensitive	1:3.4	23.5
Oweniidae	Polychaeta	0.1573	Possibly Sensitive	1:2	55.9

Sabellidae	Polychaeta	0.1394	Possibly Sensitive	1:10.6	42.6
Ophiuroidea	Ophiuroida	0.1157	Possibly Sensitive	1:2	42.6
Eusiridae	Amphipoda	0.3769	Neutral	1:2.1	23.5
Anarthuridae	Isopoda	0.3727	Neutral	1:2.7	14.7
Munnidae	Isopoda	0.8675	Neutral	1:1.1	17.6
Neotanaidae	Tanaidacea	0.6444	Neutral	1:1.6	16.2
Tanaellidae	Tanaidacea	0.3177	Neutral	1:1.8	26.5
Prochaetodermatidae	Aplacophora	0.2757	Neutral	1:1.6	69.1
Nuculidae	Bivalvia	0.9755	Neutral	1:1.1	25.0
Holothuroidea	Holothuroidea	0.4267	Neutral	1.4 : 1	47.1
Acrocirridae	Polychaeta	0.4846	Neutral	1:1.2	77.9
Chaetodermatidae	Polychaeta	0.574	Neutral	1:1.7	25.0
Cirratulidae	Polychaeta	0.2529	Neutral	1:1.5	89.7
Cossuridae	Polychaeta	0.8107	Neutral	1.2 : 1	72.1
Glyceridae	Polychaeta	0.2292	Neutral	1.6 : 1	45.6
Hesionidae	Polychaeta	0.7659	Neutral	1.1:1	61.8
Lumbrineridae	Polychaeta	0.7179	Neutral	1.2 : 1	76.5
Nephtyidae	Polychaeta	0.8841	Neutral	1:1.1	41.2
Phyllodocidae	Polychaeta	0.4161	Neutral	1:1.3	55.9
Pilargidae	Polychaeta	0.6585	Neutral	1.6 : 1	30.9
Polynoidae	Polychaeta	0.929	Neutral	1:1.1	19.1
Sphaerodoridae	Polychaeta	0.6321	Neutral	1:1.6	14.7
Syllidae	Polychaeta	0.3942	Neutral	1.2 : 1	91.2
Terebellidae	Polychaeta	0.9797	Neutral	1:1	72.1
Nemertea	Nemertea	0.5116	Neutral	1.1:1	98.5
Nereididae	Polychaeta	0.1955	Possibly Resistant	1.5 : 1	73.5
Sigalionidae	Polychaeta	0.1006	Possibly Resistant	1.6 : 1	76.5
Sipuncula	Sipuncula	0.1163	Possibly Resistant	1.7 : 1	69.1
Thyasiridae	Bivalvia	0.0055	Resistant	3:1	54.4
Sphyrapidae	Tanaidacea	0.0387	Resistant	3:1	29.4
Capitellidae	Polychaeta	0.0233	Resistant	1.6 : 1	89.7
Dorvilleidae	Polychaeta	<.0001	Resistant	7.6:1	80.9
Maldanidae	Polychaeta	0.035	Resistant	1.7:1	76.5
Paraonidae	Polychaeta	0.0088	Resistant	1.7:1	91.2

Zone		Significance
Comparison	R Statistic	Level
1,2	0.350	0.001
1,3	0.413	0.001
1,4	0.340	0.001
1,5	0.331	0.001
2,3	0.220	0.001
2,4	0.123	0.001
2,5	0.267	0.001
3,4	0.034	0.095
3,5	0.040	0.030
4,5	0.028	0.201

Table 2.5. Pairwise community structure differences among zones for the one-way ANOSIM.

Table 2.6. The ten most abundant taxa responsible for the similarity among stations for A) zones, and B) distances from the wellhead. A dash (-) indicates that the taxon was not listed in the 1-way SIMPER analysis because it was lower than the lowest value required to get 90% similarity. All taxa are polychaete families except for the phylum Nemertea and class Bivalvia.

A)		Zone (% Similarity)			
Taxa	1	2	3	4	5
Spionidae	8.1	9.33	12.75	17.51	15.97
Paraonidae	21.02	13.07	10.89	12.04	7.42
Maldanidae	6.49	12.07	8.27	6.64	3.73
Dorvilleidae	25.67	6.97	3.43	1.95	1.06
Capitellidae	13.45	12.25	7.55	7.31	5.77
Cirratulidae	1.87	4.95	4.17	3.31	4.06
Nemertea	8.51	6.27	6.17	5.7	7.58
Syllidae	4.73	4.92	4.14	5.04	5.11
Cossuridae	-	3.14	3.22	-	1.61
Bivalvia	-	2.05	4.29	3.4	3.44
B)		Dista	nce (% Simila	arity)	
Taxa	0-1 km	1-2 km	2-3.5 km	3.5-8 km	8-10 km
Spionidae	6.46	8.57	9.08	7.91	9.12
Paraonidae					
1 uruomaac	22.78	17.35	10.81	11.86	10.54
Maldanidae	22.78 7.33	17.35 4.30	10.81 12.97	11.86 13.88	10.54 9.43
Maldanidae Dorvilleidae	22.78 7.33 31.54	17.35 4.30 29.21	10.81 12.97 4.1	11.86 13.88 2.93	10.54 9.43 4.36
Maldanidae Dorvilleidae Capitellidae	22.78 7.33 31.54 12.09	17.35 4.30 29.21 13.24	10.81 12.97 4.1 10.92	11.86 13.88 2.93 12.49	10.54 9.43 4.36 8.51
Maldanidae Dorvilleidae Capitellidae Cirratulidae	22.78 7.33 31.54 12.09 2.46	17.35 4.30 29.21 13.24 1.49	10.81 12.97 4.1 10.92 4.29	11.86 13.88 2.93 12.49 2.36	10.54 9.43 4.36 8.51 5.12
Maldanidae Dorvilleidae Capitellidae Cirratulidae Nemertea	22.78 7.33 31.54 12.09 2.46 9.98	17.35 4.30 29.21 13.24 1.49 6.29	10.81 12.97 4.1 10.92 4.29 7.35	11.86 13.88 2.93 12.49 2.36 6.45	10.54 9.43 4.36 8.51 5.12 5.07
Maldanidae Dorvilleidae Capitellidae Cirratulidae Nemertea Syllidae	22.78 7.33 31.54 12.09 2.46 9.98 2.07	17.35 4.30 29.21 13.24 1.49 6.29 8.44	10.81 12.97 4.1 10.92 4.29 7.35 6.00	11.86 13.88 2.93 12.49 2.36 6.45 4.61	10.54 9.43 4.36 8.51 5.12 5.07 3.90
Maldanidae Dorvilleidae Capitellidae Cirratulidae Nemertea Syllidae Cossuridae	22.78 7.33 31.54 12.09 2.46 9.98 2.07	17.35 4.30 29.21 13.24 1.49 6.29 8.44	10.81 12.97 4.1 10.92 4.29 7.35 6.00 2.77	11.86 13.88 2.93 12.49 2.36 6.45 4.61 1.47	10.54 9.43 4.36 8.51 5.12 5.07 3.90 2.15
Maldanidae Dorvilleidae Capitellidae Cirratulidae Nemertea Syllidae Cossuridae Bivalvia	22.78 7.33 31.54 12.09 2.46 9.98 2.07	17.35 4.30 29.21 13.24 1.49 6.29 8.44	$10.81 \\ 12.97 \\ 4.1 \\ 10.92 \\ 4.29 \\ 7.35 \\ 6.00 \\ 2.77 \\ 2.24$	11.86 13.88 2.93 12.49 2.36 6.45 4.61 1.47 4.08	10.54 9.43 4.36 8.51 5.12 5.07 3.90 2.15 3.12

Table 2.7. Station groups identified by the LINKTREE procedure using chemical and environmental variables obtained from the BEST procedure. Only groups with 50% or higher similarity were included. Similarity is the percent similarity within groups, R is the amount of variability associated with the physical-chemical variable and group and its value obtained from the BEST procedure, and distances of the stations within the group from the DWH wellhead.

Grouping	Similarity	R	Variable Group	Well Distance (km)
А	84	0.66	Barium > 2860 ppm	0, 1, 1, 1, 1
В	79	0.68	Barium < 194 ppm	60, 74, 145, 158, 197
С	66	0.61	Perylene > 21.8 ppm	1, 45, 51, 132, 145
D	59	0.65	Fine Sand > 1.2%	10, 98, 123, 128, 194
Е	50	0.67	Copper < 20.8 ppm	17, 21, 22, 125, 199

CHAPTER III: BENTHIC COMMUNITIES ASSOCIATED WITH DEEP-SEA HYDROCARBON SEEPS IN THE GULF OF MEXICO

Abstract

Macrobenthic communities were sampled near natural hydrocarbon seeps and oil-spill impacted deep-sea sediments in the northern Gulf of Mexico at various depths and locations. Infaunal community composition, diversity, and abundance were examined at individual seeps, among different seep habitats (microbial mats, tubeworms, and soft-bottom seeps), between seep and background habitats, and between seep habitats and areas affected by the Deepwater Horizon Spill. Abundance and diversity differed among microbial mat, tubeworm, and soft-bottom seep habitats. Abundances were also higher at seep sites compared to either background or spill areas. While seep communities differed from other habitats, they were also generally unique to a specific site. High variability in community structure defined seep communities rather than specific taxa. Analyses found variability was 75% greater within communities near seeps compared to either communities in background areas or areas near the Deepwater Horizon wellhead. The similar grouping of background communities sampled with either a boxcorer or multicorer indicates that seepage had a much larger effect on benthic communities than sampling method. The benthic community structure is highly variable and unique to individual seeps in the northern Gulf of Mexico, and different from natural background areas and areas affected by the anthropogenic oil releases in the deep sea.

3.1. Introduction

3.1.1. Hydrocarbon Seeps

Hydrocarbon and brine seeps are dynamic, organic-rich areas in an otherwise organicpoor deep sea. They occur where methane or reduced sulfur is released into pore waters, which

are forced towards the sediment surface via pressure gradients (Gage and Taylor, 1996; Levin 2005.). Hydrocarbon and brine seeps are generally referred to as cold seeps due to a lack of increased temperatures in these areas. Unlike hydrothermal vents, cold seeps are often found on passive plate boundaries or within a plate. Macdonald et al., (2015) identified over 900 active seep areas in the Gulf of Mexico. In general, an area of seepage was roughly 2000 m in diameter and the majority of seeps were in the northwest area of the basin. However, seep flows are ephemeral, with hydrocarbon releases from the seafloor lasting for years to decades before stopping, making an accurate count of such features difficult to maintain.

Deep-sea seeps are found throughout the world's oceans including the Pacific Ocean, Northern Atlantic, Gulf of Mexico, Mediterranean Sea, Arctic, and Northern Indian Ocean (Sibuet and Olu, 1998, Levin, 2005). Communities associated with seeps are often very different from region to region and even within kilometers of each other due to geological and biochemical heterogeneity (Cordes et al., 2010b). Similarities and differences among seeps appear to be related to depth. Sahling et al. (2003) found that seeps above 370 m lacked seependemic, symbiont-bearing organisms. Davis and Spies (1980) found that in shallow waters, about 18 meters, macrobenthic abundances were higher within communities associated with seeps compared to the surrounding environment, but there was no difference in taxa present. Abundances around seeps are generally higher than non-seep habitats (e.g., Carney, 1994, Levin et al., 2005); however, it is still not clear how far natural seepage affects communities away from the source of the flow. The difference in abundances between seep and non-seep habitats generally increase with depth possibly due to increased food limitation (Levin and Michener, 2002; Sahling et al., 2003).

There is both spatial and temporal variability associated with seeps (Juniper and Sibuet, 1987; Olu et al., 1996, 1997; Sibuet and Olu, 1998), which causes seeps to be one of the most heterogeneous environments found on the continental margin. Spatial variability in fluid flow, geochemistry, substrate, and microbial and megafaunal communities occurs at both local (meters) and regional (kilometers to 100's of kilometers) scales (Cordes et al., 2010b). Pore-water fluids structure microbial communities and epibenthic colonizers while colonizers influence the underlying microbes even further (Cruaud et al., 2015). Comparing hydrocarbon seep communities in various locations to one another and to non-seep communities may lead to new ecological insights into the role of heterogeneity on speciation, food and reproductive limitations of deep-sea organisms. Animal dispersal, energy sources, and distribution patterns can also be explored by comparing physical/chemical/biological parameters between seep and non-seep sites.

3.1.2. Importance of Deep-Sea Seeps

Deep-sea chemosynthetic systems are important habitats to study for several reasons. They are unique habitats for epifaunal and infaunal organisms to grow and evolve in, important sources of deep-sea primary productivity, areas where large amounts of greenhouse gases are consumed, and biodiversity hotspots (Carney, 1994; Cordes et al., 2010b; Armstrong et al., 2012; Kiel, 2015). The dependence of seep communities on relatively small areas in the deep sea where hydrogen sulfide, methane, or other hydrocarbons are released provides opportunities to explore dispersal and connectivity in the marine environment. Because the deep-sea may have served as refugia during past mass-extinction events, and fauna at seeps do not rely on energy originating from the sun, they are evolutionarily independent of organic matter inputs from the shallow ocean and terrestrial environment allowing the examination of evolutionary processes in

novel environments (Van Dover et al., 2002; Ross et al., 2012; Kiel, 2015). Deep-sea seep and vent fauna experienced widespread extinctions in the mid-Cretaceous due to deposition of sulfur in the newly opening South Atlantic. There was a subsequent widespread radiation of modern clades in the early Eocene roughly 50 million years ago due to erosion of this deposited sulfur. The early age of most modern clades at seep communities makes them very useful for studying evolution over shorter geological timescales as well as understanding conditions in the deep oceans over this period (Kiel, 2015).

Deep-sea hydrocarbon seeps not only provide many opportunities for scientific discovery but also provide many benefits to humans or "ecosystem services." Habitat itself is often considered a supporting ecosystem service (Farber et al., 2006; Armstrong et al., 2010), and hydrocarbon seeps provide unique habitats for organisms to live. Sediments around these seeps experience many unique conditions such as high pressures, low temperatures, and high concentrations of chemicals including methane or hydrogen sulfide. Seeps are home to many endemic species, especially at greater depths, and these animals have developed novel adaptations to deal with various environmental obstacles (Sibuet and Olu, 1998; Levin, 2005).

In addition to provision of habitat and diversity, seeps provide many benefits to human health and well-being. Organisms around seeps had to develop novel genes, organic products, and processes to deal with high pressures, low temperatures, and toxic chemicals. Harvesting some of these organisms will most likely yield new pharmaceutical, agricultural, biotechnological, or cosmetic products (Glover and Smith, 2003; Armstrong et al, 2010). Increased deep-sea diversity would likely increase the amounts and types of organic and chemical pollutants biodegraded and regulated (Beaumont et al., 2008). Several deep-sea fishes, such as longspine thornyhead (*Sebastolobus altivelis*) and Pacific dover sole (*Microstomus*

pacificus) which may be targets for deep-sea fisheries, appear to congregate around seeps, which suggests they rely on chemosynthetically-derived organic matter (Grupe et al., 2015). The proportion of energy that background organisms derive from seeps seems to increase with depth (Sahling et al., 2003). Thus at greater depths, fisheries exploited by humans likely receive a larger proportion of their food from organic matter derived from seep habitats.

3.1.3. Seep Communities

There is a lack of knowledge on infaunal communities associated with deep-sea seeps. A majority of information known on seep ecology is isolated to large megafauna assemblages captured by submersible images (Sibuet and Olu, 1998; Levin, 2005; Levin and Mendoza, 2007). Seeps support many chemosynthetic organisms and often contain high abundances of organisms that may be endemic, colonists, or vagrants (Carney, 1994; Barry et al., 1996; reviewed by Sibuet and Olu, 1998; Levin, 2005). Because of methodological constraints, few sediment cores have been taken near deep-sea seeps hindering our understanding of infauna associated with seeps as well as preventing comparisons with the surrounding macrobenthic community.

Previous studies on infaunal seep communities have mostly focused on one or a few seep sites (Demopoulos et al., 2010; Decker et al., 2012; Plum et al., 2015; Borque et al., 2016; Guillon et al., 2016). The seep studies that do examine several different seeps often compare communities among seeps in different ocean basins (Levin and Mendoza, 2007; Bernardino et al., 2012). The present study examined infauna at 11 seeps represented by different epibenthic megafaunal assemblages throughout the Louisiana slope at depths from 100 - 2600 m. This is the first study comparing macrofaunal communities at several seeps in the northern Gulf of Mexico to one another and background areas over a wide depth range and large geographical area.

Large, symbiont-containing bivalve or tubeworm epifauna dominate communities at many deep-sea hydrocarbon seeps (Sibuet and Olu, 1998). Bacterial mats comprising the genus *Beggiatoa* can also be important structures at seeps (Montagna and Spies, 1985; Levin, 2005). It has been hypothesized that the types of epibenthic megafaunal colonizers found at a seep location may be used as an indicator of the chemistry at the habitat (Cordes et al., 2010a). Habitats associated with microbial mats are often found in areas with high methane releases and large concentrations of hydrogen sulfide in the sediments (Levin et al., 2003; Sahling et al., 2002). As the seep ages, mussel and tubeworm communities settle on precipitated carbonate from microbial processes with tubeworms becoming more dominant as methane fluxes decrease (Bergquist et al., 2003; Cordes et al., 2005).

Infaunal communities differ among different seep habitats and between seeps and background areas (Levin, 2005; Bernardino et al., 2012). Macrobenthic infaunal communities in microbial mat habitats have generally been found to contain higher densities (Robinson et al., 2004; Bourque et al., 2016) and lower diversity (Levin et al. 2003; Bernardino et al., 2012; Bourque et al., 2016) compared to communities in other seep habitats or background areas. In contrast, areas where methane seepage is less intense, such as tubeworm and clam-dominated habitats, are associated with lower macrofaunal densities but higher diversity of macrofaunal communities (Guillon et al., 2016). The low oxygen penetration in the sediments often leads to a larger proportion of the infaunal community being found in surface (0 - 2 cm) sediments at microbial mat seeps compared to background areas (Levin, 2005; Bourque et al., 2016). Clams and tubeworms at seeps pump oxygen and sulfates into the sediments possibly allowing infaunal communities to live deeper in the sediments where these megafauna are present (Levin, 2005; Guillon et al., 2016).

Areas of methane seepage are associated with high sulfide concentrations and low oxygen which are physiologically stressful for most taxa (Diaz and Rosenberg, 1995). Polychaetes in the families Dorvilleidae, Ampharetidae, and Hesionidae often dominate macroinfaunal communities associated with deep-sea seeps. These polychaete taxa are some of the only taxa able to tolerate high sulfide, low oxygen conditions (Sahling et al., 2002; Levin, 2003; Decker et al., 2012).

3.1.4. Seep Communities and Oil Spills

Although the deep sea appears isolated from direct contact by human beings, recent anthropogenic disasters such as the Deepwater Horizon oil blowout have illustrated the need to understand the effects humans may have on the deep-sea environment. This is especially true for drilling activities that have seen large increases in hydrocarbon production in deeper and deeper waters in the last decade. Hydrocarbon production in the deep sea (> 1000 ft or 305 m) has increased over 70% from 2000 to 2010 accounting for more than 80% of oil (~460 million bbl or 73 million m³ per day) and 45% of natural gas (~28.32 million m³ per day) production in the GoM (USDOI, 2012). Understanding the fate of hydrocarbons in the deep sea and the effects on communities is confounded in the Gulf of Mexico by the natural release of large amounts of hydrocarbons from the seafloor. It is estimated that roughly 100,000 tons of oil enters the GoM every year from various sources including: natural seeps (73%), oil drilling activities (3%), transportation (4%), and oil byproduct combustion (16%) (OSBMB, 2003). The estimated release of hydrocarbons by the DWH spill was over seven times that of annual natural releases (McNutt et al., 2012).

The first step to assessing damages caused by anthropogenic releases of oil is to identify whether there are differences between impacts of natural and anthropogenically released

hydrocarbons. If the effects of deep-sea oil blowouts to the surrounding environment were the same as effects of natural seeps then it would be nearly impossible to distinguish spill damages from natural seepage. If communities near spills are different from those near seeps and non-seep environments, then the communities around the spill were impacted, and the extent and effects of any changes can be quantified to assign damages to the offending parties. These observed damages could also be used to help shape policies to limit impacts from future drilling activities.

There is a timely need for information concerning communities around natural hydrocarbon seeps, in particular due to the increase of drilling in deeper waters and likelihood of spills there. Because hydrocarbon seeps are often associated with large hydrocarbon reservoirs beneath the seafloor (Levin, 2005), areas with seepage are likely to experience oil drilling. Key differences between spills and natural seeps include the presence of heavy metals, drilling muds, and large concentrations of hydrocarbons, which may remain toxic for many years; all of which are associated with spills (Peterson et al., 1996; Peterson et al., 2003; Hussain and Gondal, 2008; Montagna et al., 2013). In contrast, hydrocarbon seeps generally only release organic materials such as methane or inorganic reduced compounds such as hydrogen sulfides (Sibuet and Olu, 1998; Levin, 2005) at much lower concentrations than spills.

Seep communities may experience less toxic effects than communities near oil spills allowing taxa at seeps to better take advantage of organic enrichment due to increased organic matter. It has been shown that seep petroleum has similar effects on macrobenthic communities as other forms of organic enrichment such as kelp debris (Spies et al., 1988). In the case of the DWH blowout, 2.1 million gallons of dispersant, a possibly toxic chemical, were also released into the deep sea and at the sea surface (Kujawinski, 2011). Organic enrichment generally has

the effect of increasing abundances of tolerant taxa while decreasing diversity due to the death of sensitive taxa; in contrast, toxicity leads to a declined abundances and diversity (Pearson and Rosenberg, 1978). Enrichment and toxicity can both lead to the removal of large bioturbators, which reduces subsurface oxygen and concentrates infauna in surface sediments (Pearson and Rosenberg, 1978).

Damages to the environment by deep-sea oil releases are important to understand not only for the purpose of assigning costs to the parties responsible, but also to improve policies concerning drilling activities and to decrease damages from possible future spills. If spills cause a change in benthic abundance, diversity, or both then they may damage the environment and affect the benefits humans derive from the deep sea (Balvanera et al., 2006; Tilman et al., 2012). If a deep-sea spill causes benthic communities to artificially resemble seeps then alterations could still be construed as damages caused by the spill. More information regarding the influence of decreased abundance or diversity on human benefits from the environment is found in Chapter I of this dissertation.

One recent event that underscores the lack of knowledge on human impacts on deep-sea communities is the April 2010, Deepwater Horizon (DWH) blowout, which released a large amount of oil into the northwestern Gulf of Mexico (GOM). Several studies were performed to examine the effects of the spill on macro- and meiobenthic infauna (Montanga et al., 2013; Baguley et al., 2015; Demopoulos et al., 2016; Washburn et al., 2016). Shortly after the DWH spill, effects on the seafloor were observed in a 148 km² area up to 17 km from the wellhead (Montagna et al., 2013). Meiofauna abundances were highest in the most severely impacted areas due to increased nematode counts while meiofaunal diversity increased with increased distance from the wellhead (Baguley et al., 2015). In contrast macrofaunal abundances were

lowest in the most severely impacted areas while diversity was greatest at intermediate distances from the wellhead (Washburn et al., 2016). Long-term effects of the blowout are still unknown, but due to low deposition of organic material and metabolic activity in the deep sea (Jannasch and Wirsen, 1973; Smith and Teal, 1973; Rowe, 1983), impacts are more likely to persist for long periods, decades or longer.

The present study examined the effects of hydrocarbons in the deep sea and the role of hydrocarbon seeps in the larger deep-sea habitat by addressing the following null hypotheses: 1) Macrobenthic communities associated with hydrocarbon seepage were not different than communities in background, soft-bottom habitats, 2) Communities were not different among seeps at different depths, 3) Communities were not different among different types of seeps, and 4) Communities associated with natural hydrocarbon seepage were not different than communities associated with anthropogenic hydrocarbon releases.

3.2 Methods

3.2.1. Seep Collections

Sediment cores were collected near seep features in the northern Gulf of Mexico between 2009 and 2013 by the United States Geological Survey (USGS)-Wetland and Aquatic Research Center and the Ecosystem Impacts of Oil and Gas Inputs to the Gulf (ECOGIG) consortium. Stations included soft-bottom hydrocarbon seeps, seep microbial mats, tubeworm communities, near-seep control, and far-seep control conditions. Stations ranged from 137 m to 2601 m in depth; however, only one station was shallower than 500 m. Five stations were represented by only one core per station (Table 1).

Eleven seeps were sampled over the course of this study: one in 2009, three in 2010, four in 2012, and three in 2013. Two seeps that were sampled in 2009 and 2010 were represented by

five or six cores, respectively, and were composed of several cores taken within the seep field as well as cores collected close to but outside the seep field (Table 1). Samples collected via ROV were considered within the seep field if ROV footage showed seep structures within a few meters of the core. Samples collected via multicorer were considered within the seep field habitat if hydrocarbons were visible in the sediment sample or if there was a hydrocarbon odor to the samples. If at least one core from a drop had visible oil or an odor then all cores collected in that drop were considered representative of seep habitat (Table 1). All multicore samples from seep communities were assigned the seep type of "soft-bottom seep." Five stations represented general soft bottom habitat in the deep GoM and were not located immediately adjacent to any seep activity. These five stations were considered representative of background conditions.

Benthic cores were collected via a pushcorer attached to a remotely operated vehicle (ROV) as well as a ship-deployed multicoring device. Multicores had a diameter of 9.5 cm while ROV cores had a diameter of 6.35 cm. Cores were divided at various sediment depths aboard the boat and preserved in 10% formalin or 95% ethanol. Samples collected in 2009 were divided into 6 sections (0 - 1, 1 - 2, 2 - 3, 3 - 5, 5 - 7, and 7 - 10 cm) while samples collected in 2019 were divided into only 3 sections (0 - 2, 2 - 5, and 5 - 10 cm). Samples collected in 2012 and 2013 were divided into 4 vertical sections (0 - 1, 1 - 3, 3 - 5, and 5 - 10 cm). Samples were kept in storage for 2 - 5 years then sorted and identified between 2013 and 2015.

3.2.2. Background DGoMB and DWH Collections

Because of the opportunistic nature of the seep collections and relative lack of nearby control stations, samples collected from 2000 - 2002 during the Deep Gulf of Mexico Benthos cruises (DGoMB; Rowe and Kennicutt, 2009) and in 2010 during the Deepwater Horizon (DWH-B) Response cruises (Montagna et al., 2013) were included in analyses as additional

deep-water soft-bottom background control stations. Background stations were within 100 km and 100 m depth of a station where seep samples were collected (Fig. 1). One to two stations closest to each seep at similar depths were chosen for comparisons between seep and background communities.

The DGoMB samples were collected using a 2209 cm² (47 cm x 47 cm) GOMEX box corer (Boland and Rowe, 1991). Six subcores were mounted within the boxcore for various sample collections, which had a combined area of 307 cm² resulting in a final area for each macrofauna sample of 1901 cm². The top 15 cm of sediment were sieved onboard immediately after collection on a 300 μ m mesh, and all material retained on the sieve was preserved with the addition of 10% buffered formalin. While boxcores collected macrobenthos down to 15 cm, it has been shown that the vast majority (>95%) of macrofaunal communities are isolated to the top 10 cm of sediment in the deep Gulf of Mexico (Montagna et al., 2017). Thus the comparisons between boxcores and multicores in this study are appropriate. All specimens were sorted and identified to lowest taxonomic level by various taxonomic laboratories.

The DWH background samples (DWH-B) were collected using an OSIL multicorer with 12 separate cores. Only stations collected in the non-impacted zones 4 and 5 were included in DWH-B samples. Cores collected 80 cm^2 (10 cm diameter) of sediment, and samples were collected to 10 cm in depth. Three cores were collected for macrofaunal analysis from each drop. Cores were divided into two sediment depths for 2010 samples (0 - 5 and 5 - 10 cm), and each core section was preserved by adding 10% buffered formalin to the sample in the field. Samples were later sorted in the laboratory and identified to family level (Montagna et al., 2013).

3.2.3. DWH Oil Blowout Collection

Samples collected during the 2010 DWH response sampling cruises were also used to compare macrobenthic communities associated with natural oil seeps to communities impacted by a deep-sea oil spill. Cores from seep habitats were the same samples as those collected in section 2.1. DWH samples from the impacted area (DWH-I) were collected on the same cruises and with the same methods as DWH-B samples in section 2.2; however, only samples collected close to the wellhead were used to represent spill conditions. Samples near the DWH wellhead were not considered representative of soft-bottom deep-sea GoM background conditions, and different DWH samples were used for background (DWH-B) vs. seep comparisons and spill (DWH-I) vs. seep comparisons. Montagna et al. (2013) classified the benthic area around the DWH wellhead into 5 separate impact zones. Only samples within 5 km of the wellhead and within the most heavily impacted zone, zone 1, were used to represent deep-sea oil spill habitats, which equated to 10 stations with 3 replicate cores at each station.

3.2.4. Seep Community Descriptions

All polychaetes were identified to family level, while mollusks, crustaceans, and oligochaetes were identified to class or order level, and other taxa (e.g., nemerteans, sipunculans, echinoderms, etc.) were identified to phylum level. Polychaetes were the focus of this study because they dominated the samples collected, representing approximately two-thirds of all organisms found. Taxonomic level within this study is justified because previous studies have found that data at the family level could be used to show deep-sea benthic community differences using multivariate techniques (Warwick, 1988; Narayanaswamy et al., 2003). The lack of knowledge on deep-sea species is additional justification for using higher taxonomic levels. For example, only 40% (205 of 517) of polychaetes and 25% (31 of 124) of amphipods

found in the DGoMB study (Rowe and Kennicutt, 2009) could be identified to the species level by taxonomic experts. Furthermore, Peterson et al. (1996) reviewed benthic responses to marine pollution and found macroinfaunal communities exhibit repeatable patterns of response to contamination at high taxonomic levels, even at the phylum level. Grouping animals at higher taxonomic levels can even reduce the noise within the data by removing the influence of rare species and grouping organisms into similar niches more relevant to pollution tolerances (Warwick, 1988; Montagna and Harper, 1996).

Because of the difference in sample sizes between multicores and ROV cores, abundances per m² were used in analyses rather than abundances per sample. All abundances were converted to abundances per m² as follows: for multicores, abundance data was multiplied by 141.08, for ROV cores, abundance data were multiplied by 315.77. Univariate community measures, which included abundance per m² as well as taxa diversity, richness, and evenness, were analyzed using PROC GLM in SAS 9.4 (SAS, 2013). A 1-way nested ANOVA with seep type (microbial mat, tubeworm, soft-bottom) nested within depth category (<1000, 1000 – 2000, >2000 m) was used to test the seep dataset for differences between seep and background communities. Analyses were performed to describe community compositions for the various seeps sampled using Primer v7. Rarefaction analysis was used to calculate diversity for the various seeps sampled. Multivariate dispersion (MVDISP) analysis was used to examine multivariate variability among different seep habitats. Individual vertical sediment sections (0 – 1, 0 – 3, 0 – 5, and 5 – 10 cm) were also examined to determine if similarities or differences among habitats were observed throughout the sediment.

Analysis of similarity (SIMPER) was used to determine the similarity of cores within a seep as well as to identify taxa shared among cores in all seep habitats. If a seep station was

represented by only one core, then that seep community was omitted from SIMPER analysis due to insufficient replication. Seep samples were grouped into different depth and habitat categories in order to compare taxa among microbial mat, tubeworm, or soft-bottom seeps. Seep samples were grouped into the following depth categories: shallow (< 1000 m), intermediate (1000 – 2000 m), and deep (> 2000 m), to examine similarities among seeps at various depths.

3.2.5. Seep vs. Background Comparisons

To compare macrobenthic communities across several sampling methods, the data had to first be standardized for all three studies. Abundances were converted to individuals per m² for each study. Macrofaunal abundances for seep cores were converted to abundances per m² as described in section 2.4. Abundance data from DGoMB samples were multiplied by 5.2604, and abundances collected from DWH samples were multiplied by 125.5 to convert to individuals m⁻². Several taxa, including Anthozoa, Bivalvia, Decapoda, Echinodermata, Gastropoda, Nemertea, Ostracoda, Scaphopoda, Sipuncula, Tanaidacea, and Turbellaria were not identified beyond phylum, class, or order for DGoMB macrofauna samples for various reasons. These taxa were removed from DWH samples and samples collected during seep cruises when comparisons with DGoMB samples were performed. All taxa were included in descriptions of seep communities and comparisons between seep and spill communities.

Total macrobenthic abundance was analyzed for all samples using a 2-way ANOVA with habitat (seep, background) and depth zone (< 1000 m, 1000 – 2000 m, > 2000 m) as variables. Abundances were first standardized by sample using Primer software (Clarke and Gorley, 2015) to help remove effects of different sampling areas collected via boxcorers, multicorers, and ROV cores then square-root-transformed. A nonmetric multidimensional scaling (nMDS) plot was created using the Bray-Curtis resemblance matrix. Stations were compared among sampling

gears, depth zones, and between seep and background locations. SIMPER analysis examined which taxa were responsible for differences among groups. An analysis of similarity (ANOSIM) test was performed with habitat (seep or background) nested within depth category. Diversity was calculated using the rarefaction method because it is less sensitive to differences in sample size (Simberloff, 1972), and it is well known that diversity is positively correlated to sample area (Bunge and Fitzpatrick, 1993). With larger sample areas comes an increased probability of collecting rare organisms, which are more easily missed by smaller samplers.

An examination of differences between seep and background stations was performed at various sediment depths. Because DGoMB samples were collected to a depth of 15 cm with no differentiation among sediment depths, they were excluded from this analysis. Likewise, DWH samples in 2010 were only divided into 2 sections. Most macrobenthic organisms in deep-sea sediments are found only in the top 5 cm of sediment (Montagna et al. 2016). In order to examine differences among specific layers of sediment within the top 5 cm, only samples collected between 2009 - 2013 during seep cruises were used. To examine communities present in the 0 - 1, 1 - 3, 3 - 5 cm of sediment, samples from 2009, 2012, and 2013 were used, representing 7 background and 17 seep samples. To examine the top 2 cm of sediment, samples from 2009 and 2010 were used for a total of 5 background and 11 seep samples. Finally, all samples collected for seep study could be used to compare sediments at 5 – 10 cm depth, for a total of 10 background and 25 seep samples. Abundances per m² and community similarity (via SIMPER analysis) were examined at each sediment depth.

3.2.6. Seep vs. Spill Comparisons

All stations heavily impacted by the DWH spill were located within several km of the wellhead, and their depths ranged from 1400-1600 m. Only seep samples collected between

1000 – 1800 m in depth were used to compare with these samples. Abundances of organisms were analyzed in Primer. Samples were first standardized due to the differences in sample sizes and then square-root transformed. An nMDS plot was created, and an ANOSIM analysis was used to examine differences among communities. Diversity was calculated using rarefaction analysis. Finally, a 1-way ANOVA was performed in SAS version 9.4 with hydrocarbon type (natural seepage vs. oil spill) as the factor to examine differences in community abundances.

3.3 Results

A total of 1421 organisms were collected from seven phyla during seep sampling cruises between 2009 and 2013. Annelids, crustaceans, and mollusks were the dominant taxa found representing 62%, 22%, and 14% of all the organisms found, respectively. Nemerteans and sipunculids each represented 1% of the total number of organisms while only three echinoderms and two cnidarians were found.

A total of 35 polychaete families were identified. The five most abundant polychaete families were Chrysopetalidae, Cirratulidae, Dorvilleidae, Paraonidae, and Ampharetidae, which represented 33% of total organisms found. The two most abundant non-polychaete taxa were tanaid and cumacean crustaceans, representing 9% and 8% of all organisms collected based on abundance. However, previous work in the same area of the deep-sea Gulf of Mexico found a similar proportion of crustaceans in their samples which represented nearly half of all taxa diversity when crustaceans were identified to family (Washburn et al., 2016). Thus, it is very likely that the tanaid and cumacean taxa in this study represent many different families. The same can be said for the molluscan bivalves and gastropods, which each represented 5% of all organisms collected based on abundance.

3.3.1. Community Analyses

When examining only samples collected near seeps, the hierarchical model found significant differences in abundance, richness, and diversity between macrobenthos found at microbial mat, tubeworm, or soft-bottom seep communities. Microbial mat communities had two to three times the abundances as tubeworm and soft bottom seep communities (Fig. 2A). However, microbial mat communities also had approximately half of the diversity (N1) compared to tubeworm and soft-bottom seep diversity (Fig. 2B).

After combining all seep types, the MVDISP analysis indicated seep communities as a whole were nearly twice as variable (1.062) as background communities (0.584). Further dispersion analyses were performed only on seep communities. Variability was similar at seeps < 1000 m and seeps between 1000 - 2000 m, but was 20 - 30% lower at seeps > 2000 m. There appeared to be higher variability in tubeworm communities compared to soft-bottom seep and microbial mat communities (Table 2).

When examining diversity at individual seep sites, many of the rarefaction curves appeared to reach an asymptote (Fig. 3a). Species accumulations began to level off at roughly 100 - 150 individuals at seeps where over 100 organisms were collected. There also appeared to be two different rarefaction patterns; two seeps at GC600 and one at GC246 had gentler slopes than the other seeps. The less diverse seeps appeared to approach roughly 15 taxa as a maximum while the remaining seeps approached roughly 25 taxa. When all seep cores were combined into one rarefaction curve, seeps in the northern GoM approached > 40 taxa suggesting little overlap in taxa between the less diverse and more diverse seep groupings or a lack of sensitive taxa in less diverse seeps (Fig. 3b). All but one Chrysopetalidae polychaete collected were found at microbial mat seeps, but all other taxa found at mat seeps were also found in soft-bottom or

tubeworm seeps. Three of the four communities with the highest abundances/ m^2 of Hesionidae and Gastropoda were found at microbial mat seeps as were two of the four communities with the highest abundances/ m^2 of Ampharetidae.

Because seeps were located at different depths and sampled in different years, individual seeps were first examined. In 2009 five cores were collected at the same seep (DC 583), three within the seep field (< 1m) and two outside (approximately 10 and 75 m from the seep mound). The background and seep communities were 22% similar. The background community was only 16% similar, and this similarity was due to only 1 taxon, Paraonidae. The seep communities at DC583 were 32% similar due to 2 taxa, Spionidae and Opheliidae.

In 2010 six cores were collected at the same seep site (GC354), three within the seep field and three outside (approximately 25 m from the seep mound). In contrast to DC583, there was higher similarity within background (49%) and seep (48%) communities. However, the background and seep communities were only 34% similar, much less than the similarity of either group. The only taxon responsible for similarity within the background community was Isopoda. Three taxa were responsible for similarity within the DC583 seep community: Ampharetidae, Amphipoda, and Cossuridae.

At several seeps, only one sample was collected, which makes it impossible to determine whether animals collected at that seep location were representative of the habitat or collected by chance. There were seven seep locations represented by two or three samples. Similarities within these seven seeps ranged from 26% to 64%. The five seep communities that occurred at intermediate depths (1000 - 2000m) had \geq 50% similarity (Table 2). While replicate samples from individual seeps tended to cluster together, four seep samples from DC583, GC354, GC246, and OC26 were very different from the other replicates (Fig. 4). Two seeps were

shallow (GC354 and GC246) and one was deep (DC583) while the intermediate seep, OC26, was also very close to the DWH wellhead and was sampled following the blowout.

Seep communities differed as a function of depth. Taxa composition was very similar between seep communities at shallow (< 1000 m) and intermediate (1000 – 2000 m) depths. Deep seep (> 2000 m) communities were comprised of far fewer taxa than other seeps (Fig. 5). Tanaids were a dominant taxon at three of the seeps at intermediate depths (GC415, GC600-1, and GC600-3), while dorvilleids were prominent at two intermediate seeps (GC600-1 and GC600-3). Both deep seeps were dominated by spionids (DC583 and DC673). The two shallow seeps were very different with one dominated by gastropods (GC246) and the other by dorvilleids (GC354) (Table 3). Seeps between 1000 - 2000 m were more similar (57%) than shallower or deeper seeps (43%); however, there were twice as many samples collected at intermediate depths. Only seven taxa were responsible for 95% of the similarity in deep seep stations compared to 12 or 14 taxa in shallow or intermediate depths, respectively.

Benthic abundance patterns throughout the sediment column were not significantly different among different seep habitats at any particular sediment section (0 - 1, 0 - 3, 3 - 5, 0 - 5,or 5 - 10 cm). Only one station represented microbial mat or tubeworm community cores divided into 0 - 1, 0 - 3, and 3 - 5 cm in depth, so for these seep habitats, only the 0 - 5 and 5 - 10 cm sections were examined separately. Abundances in the surface sediments (0 - 5 cm) of microbial mats (26,414 individuals/m⁻²) were 3 times that of abundances in surface sediments of the tubeworm (9,022) or soft-bottom seep habitats (7,863). Abundances in deeper sediments (5 - 10 cm) of tubeworm habitats (1,685) were 5 times that of abundances in deeper sediments of microbial mat (316) or soft-bottom seep habitats (387). Macrofaunal densities (5,510) in surface sediments (0 - 5) at background stations collected during the seep cruises were lower than those

quantified from surface sediments at any seep habitat. Tubeworm habitats had a higher proportion of fauna in deeper sediments (5 - 10 cm) than microbial mats, soft-bottom seeps, or background deeper sediments (Fig. 6).

3.3.2. Seep vs. Background Comparisons

When background samples from different years (2000-2002, 2009-2013) and from different collection methods (boxcorer, multicorer, ROV core) were compared to seep samples, communities within all seep habitats were still 74% more variable (1.592) than background communities (0.917) using MVDISP. Abundances were higher at seeps compared to background sites and were highest at depths between 1000 and 2000 m (2-way ANOVA, df = 90, F = 7.32, p < 0.0001). Macrofaunal communities were not significantly different at seep sites compared to background sites (nested ANOSIM, R = 0.389, P = 0.133) although presence or absence of seepage explained nearly 40% of the variability in the communities. Macrofaunal communities present in background habitats were more similar (49%) than those found in seep habitats (29%). Seep communities were nearly as similar to background communities (31%) as they were to other seep communities.

All taxa found at seeps were also collected in background sediments, except for the polychaete Trochochaetidae which had three specimens collected at seep DC673. However, some taxa were more important in explaining similarities among seeps while other taxa explained more similarity among background areas. Paraonidae and Maldanidae explained much more similarity within background communities while Dorvilleidae, Ampharetidae, Hesionidae, Nereididae, and Aplacophora explained more similarity within seep communities (Table 4). Likewise, Cumacea, Oligochaeta, and Sphaerodoridae were only responsible for similarity within seep communities while Glyceridae, Lumbrineridae, Nephtyidae, Onuphidae, Opheliidae,

Pilargidae, Sabellidae, Sigalionidae, and Terebellidae were only responsible for similarity within background communities.

When looking at different depths, background and seep communities were more similar to one another at shallow and intermediate depths (40% and 39%, respectively) compared to deeper depths (32%). Within either background or seep habitats, communities were most similar to each other at intermediate depths (52% and 56%, respectively) and least similar at deeper locations (40% and 43%, respectively). The polychaete families Spionidae (16% - 23%) and Paraonidae (14% - 31%) explained the most similarity within each depth range explored (15% - 29%) in background habitats. Seep communities had much more variable compositions at different depths than background communities. Spionidae (48%) explained the most similarity for seep communities > 2000 m in depth, followed by Paraonidae (14%) and Oligochaeta (11%). However, shallow seep communities were dominated by Dorvilleidae (18%) and Cossuridae (15%), and seep communities at intermediate depths were dominated by Cumacea (16%) and Hesionidae (10%).

Polychaeta dominated at most seeps; however, different families were associated with different seeps. SIMPER analysis identified the following polychaete families that were dominant at individual seeps: Spionidae, Cirratulidae, Maldanidae, and Dorvilleidae (Table 3). Other polychaete families, which appeared to be associated with seeps from SIMPER analyses, included Ampharetidae, Cossuridae, Capitellidae, Hesionidae, and Paraonidae (Table 3). While several other phyla were collected in seep sediments (e.g., Cnidaria, Echinodermata, Nemertea, and Sipuncula), only Nemertea was identified as important in defining seep communities, contributing 5% of the similarity within seep DC673.

Seep and background samples collected between 2009 - 2013 during seep cruises were used to compare macrobenthos in different depth layers of sediment. Surface sediments (0 - 1 cm) had similar numbers of taxa in seeps and background sediments. However, seep sediments had 2.5 times the number of individuals (4635 individuals/m²) as background sediments (1795 individuals/m²). Similar patterns were observed in the top 0 - 2 cm of sediment, with average macrofaunal abundance at seeps > 3 times that of background abundances. In deeper fractions (1 - 3 cm) seep macrofaunal abundances dropped to 1.7 times that of background stations, while at 3 - 5 cm depth, abundances at background stations were 1.2 times greater than seep sites. In the deepest sediment fractions (5 - 10 cm), seep macrofaunal abundances were 1.2 times that of background abundances. The largest differences in macrobenthic communities between seep and background stations were confined to the top 3 cm of sediment, except for microbial mat communities (Table 5).

Examining community structure at each of the different seep types and among sediment depths revealed that there was very little similarity within seep types below 5 cm in sediment depth. Tanaidacea, Amphipoda, Bivalvia, and Aplacophora explained much more of the similarity in surface communities at soft-bottom seep sites compared to other seep habitats. Similarity at microbial mats was caused almost exclusively by Cumacea, Ampharetidae, Dorvilleidae, and Hesionidae. Similarity within tubeworm communities was largely due to Spionidae. Similarity at background habitats was in a large part due to Cirratulidae, Paraonidae, Spionidae, and Maldanidae (Table 5).

3.3.3. Seep vs. Spill Comparisons

When examining only seeps between 1000 – 1800 m in depth, seep communities (1.582 MVDISP) were 73% more variable than communities heavily impacted by the DWH blowout

(0.912). This is consistent with the SIMPER results, which found DWH-I macrofaunal communities were nearly twice as similar (50.36%) as seep communities (28.17%) at similar depths. Seep communities between 1000 – 1800 m were only slightly more similar (28.17%) than seep communities across the entire depth range (137 – 2601 m, 24.98%). There were significantly higher abundances at seep sites (12,732 ind. m⁻²) compared to spill sites (5,953 ind. m⁻²) (ANOVA, df = 54, F = 7.33, p = 0.009), and seep community composition was significantly different from spill sediment communities (ANOSIM, R = 0.663, P = 0.0001).

The higher similarity among DWH-I communities appeared to be due to lower taxa richness in these areas. Twelve taxa explained 95% of the similarity among DWH-I communities while 17 taxa explained 95% of the similarity among seep communities. The polychaete families Dorvilleidae (25.57%), Paraonidae (19.95%), and Capitellidae (13.33%) explained ~59% of the similarity among spill sites. In contrast, the crustaceans, Tanaidacea and Cumacea and polychaete Dorvilleidae explained 43% of the similarity among seep sites. There were no crustaceans among the 12 taxa explaining DWH-I community similarities (Table 6).

Dorvilleidae was responsible for 26% of the similarity at spill sites compared to 15% of the similarity at seep sites. Bivalves and aplacophorans were found in both communities but accounted for more than 2 times the similarity at seep sites compared to spill sites. Common polychaete families associated with disturbance including Paraonidae, Capitellidae, Spionidae, and Maldanidae (Reish and Gerlinger, 1997; Dean, 2008) were found in both seep and spill communities but accounted for 2 - 5 times the similarity among spill communities. Polychaete families generally associated with deep-sea hydrocarbon seeps including Hesionidae and Ampharetidae (Levin, 2005) were scarce at DWH-I sites and were not among the 12 taxa explaining 95% similarity there.

3.3.4. Sample Size

While the communities appeared to be different between seep and background conditions and among different depth zones, there was a large confounding factor when comparing samples across studies; the difference in sample areas. All DGoMB samples were collected with boxcorers, which had roughly 25 times the area of DWH samples collected with multicorers. Multicorers had roughly twice the area of seep samples collected via ROV cores.

When gear type was examined in the MDS plot, all DGoMB samples were clustered close together near the center of the graph (Fig. 6a). Samples collected during the DWH response cruise were also clustered together around the DGoMB samples but were less tightly grouped. The difference in the tightness of clustering between DGoMB and DWH-B samples was likely due to the difference in collection methods. Samples collected via ROV and multicorer during the seep cruises were spread across the entire MDS plot (Fig. 7). Rarefaction analysis for the different studies found boxcorers to underrepresent diversity for a given number of animals collected compared to multicorers and ROV pushcores. There were not enough samples taken during the seep cruises for rarefaction analysis to approach an asymptote (Fig. 8).

3.4 Discussion

3.4.1. Seep vs. Background

Macrofaunal abundances and community composition differed between seep and background samples, consistent with the alternative hypothesis that macrobenthic communities at seeps in the Gulf of Mexico are different then communities in background, soft-bottom sediments. Infaunal abundances/m² were higher near seeps compared to background conditions (Table 2), which is similar to results in other studies (Levin, 2005; Bernardino et al., 2012). Abundances are often higher near seeps in deeper waters due to a lack of surface-derived organic

matter in background areas and organic enrichment via chemosynthetic processes at seeps (Levin and Michener, 2002). However, there is not a linear relationship between fluid flow at seeps and macrobenthic abundance. Guillon et al. (2016) found macrofaunal abundances had a parabolic correlation to methane and sulfide flows with the highest numbers at intermediate fluxes.

While community composition was different between seeps and background areas, all taxa identified from seeps were also present in background sediments except for one polychaete. However, there were several taxa that were more important in describing either background or seep habitats (Table 4). Seep infauna are generally comprised of background taxa which are tolerant of high hydrogen sulfide concentrations (Bernardino et al., 2012). Organic enrichment has been found to favor small deposit feeders over suspension feeders or larger animals (Pearson and Rosenberg, 1978). In contrast, many of the taxa responsible for similarity in background communities but not seep communities included carnivores and deposit feeders.

Taxa that dominated in seep habitats but not background areas included the polychaete familes Dorvilleidae, Hesionidae, and Ampharetidae among others (Table 4). These polychaete taxa are often considered characteristic of seep communities (Levin et al., 2003; Levin, 2005; Bernardino et al., 2012). High abundances of dorvilleids are often found at seep with high methane and sulfide fluxes where few other taxa are present (Levin, 2005; Bernardino et al., 2012; Decker et al., 2012). The two microbial mat seeps were the only locations with a large abundance of the polychaete family Chrysopetalidae while the crustacean Cumacea were also abundant at microbial mats.

There did not appear to be specific taxa that were representative of all seeps in the northern Gulf of Mexico. Even taxa that explained similarity among seeps, such as dorvilleids and ampharetids, were absent at several seeps (Table 3). Almost every seep examined appeared

to have different dominant taxa regardless of whether the samples were collected from the same seep habitat (microbial mat, tubeworm assemblage, or soft bottom). Studies have shown high species turnover (beta diversity) among different seeps, even within similar geographic regions (Cordes et al., 2010b; Bourque et al., 2016). This study found that macrobenthic communities associated with seepage were more variable than communities associated with the background, soft-bottom habitat. Background stations collected with multiple sampling devices and over the course of thirteen years were much more tightly clustered in the nMDS plot compared to stations collected near seeps with similar sampling devices over the course of four years (Fig. 4). High variability in seep communities has also been observed in shallow seeps within the Santa Barbara Channel, which had much larger fluctuations in abundance then nearby areas outside of the seepage field (Davis and Spies 1980).

3.4.2. Variability

Most studies and statistical analyses attempting to measure ecological changes rely on means of variables such as abundance, diversity, and contaminant concentrations. While means are easy and straightforward to use, variability within a specific habitat or impacted region can mask any differences observed among areas or treatment levels if large enough, and increased variability itself may be indicative of impacts of pollution (Warwick and Clarke, 1993; Demopoulos et al., 2016). Schmalhausen's law states that when stressed, organisms show greater variance in life history traits (Lewontin and Levins, 2000). Seep habitats are associated with low sediment oxygen content and high levels of methane or hydrogen sulfide (Levin, 2005; Bernardino et al., 2012) making them stressful environments for many animals.

A likely cause of much of the variability observed among seep locations was the wide range in water depths of individual seeps. Macrobenthic communities at seeps in the Gulf of

Mexico were different at different depths (Table 2, Fig. 5). Depth generally has a negative relationship with food availability derived from surface waters and thus a negative relationship with macrofaunal densities (Pequegnat et al., 1990; Rex and Etter, 2010). There was a clear shift in community structure from shallow to deep stations in both background and seep samples. At shallow seep sites, those still within or near the photic zone, seep communities often resemble background communities. In shallow seep macrobenthic communities off the coast of Santa Barbara, CA, 90% of the individuals examined were taxa shared between seep and background stations, although abundances were generally higher at seep locations (Davis and Spies, 1980). Some studies have suggested few species are shared among upper and lower slope seep communities (Carney, 1994; Cordes et al., 2007). In the present study background and seep communities were more similar at depths above 2000 m (Fig. 5), indicating that even in the lower slope organic matter is not as limiting as the deepest areas of the GoM. Organic matter not only comes from the sinking of surface production, but also via transport along shelf areas (Rex and Etter, 2010). Organic matter may cascade down submarine canyons in the GoM and provide food for deeper communities. Given these different mechanisms, food availability in the northern GoM may be heavily influenced by distance to shore even when compared with depth.

Food availability decreases with depth in background areas but not necessarily at seeps, where chemosynthesis provides an additional food source. The differences in response between seep and background communities with depth would suggest that seeps would grow more dissimilar to background communities with depth. Chemosynthetic nutritional pathways have been shown to contribute more to invertebrate diets at deeper seeps compared to shallower ones, possibly due to lower external organic matter inputs at deeper sites (Sahling et al., 2003; Levin, 2005; Levin et al., 2016). Seep communities <1000 m and 1000 – 2000 m in depth were more

similar to one another than either was to communities below 2000 m. Stable isotope analysis showed that methane-derived carbon contributed up to 20% of invertebrate diets in shallow California slope seeps (500 m) while it contributed 20-50% of invertebrate diets in the deepest Alaska or Florida margin seeps (> 3000 m) (Levin and Michener, 2002; Sahling et al., 2003). At deeper (2000 m) oligotrophic areas in the Mediterranean, invertebrates at methane seeps derive practically all of their carbon from a chemosynthetic origin (Carlier et al., 2010). Demopoulos et al. (2010) found macrofauna at seeps on the lower slope in the GoM derived 60 - 100% of their food from *Beggiatoa* mats. A lack of differences in seep community abundances with differences in depth would suggest that the quality and quantity of organic matter associated with seeps does not change with depth.

Another likely source of variability among seep communities is the difference in porewater fluids and thus initial food sources for organisms at different seeps. There are large scale differences associated with methane seepage in the northern Gulf of Mexico. Most methane along the upper Louisiana slope is thermogenic in origin (Sassen et al., 1999) while methane has a larger biogenic component in some other areas along the upper slope and the Florida escarpment (Martens et al., 1991). Decker and Olu (2012) found that the majority of organic carbon consumed by macrofauna at both an active mud volcano and less active seep on the Norwegian margin was derived from sulfide-oxidizing bacteria rather than marine snow or methane-derived carbon. In this study sulfide and methane concentrations were not measured, but epibenthic megafaunal colonizers (e.g., microbial mats, tubeworms, and mussels) were noted. Many studies have found these megafauna to be representative of sulfide and methane concentrations in the seep habitat they are found (MacDonald et al., 1989; Levin et al., 2003; Cordes et al., 2010b; Guillon et al., 2016). The seeps in this study represented several types of

epibenthic communities, partly explaining the large amount of variability in macrobenthic communities among seeps.

3.4.3. Seep Habitats

Macrobenthic communities were different among the different types of seeps examined in this study (i.e., microbial mat, tubeworm, and soft-bottom seeps) with higher abundances and lower diversity occurring at microbial mat seeps compared to tubeworm or soft-bottom seeps. The differences in macrobenthic communities among microbial mat, tubeworm, and soft-bottom seep communities may be a function of many factors, including fluid flow, seep successional stage, megafaunal communities, and habitat suitability (or geochemical differences). When seepage begins in an area, microbial mats bloom and methanogenesis occurs, creating carbonates (Levin, 2005). As fluid flow from the sediment decreases and hard substrate begins to appear in the form of carbonates, larger chemosynthetic organisms such as mussels and tubeworms move into the area (Cordes et al., 2006). There were higher abundances at microbial mats, but higher taxa richness and diversity at tubeworm and soft-bottom seeps (Fig. 2). Previous studies have found microbial mat habitats to support the highest abundances of macrofauna compared to other seep habitats as well as background areas (Robinson et al., 2004; Levin et al., 2006; Bourque et al., 2016). High macrofaunal abundances at microbial mats compared to other seeps are most likely caused by the ability of taxa tolerant to high sulfide conditions to take advantage of the large amount of chemosynthetically derived organic matter at these habitats (Sahling et al., 2002; Bernardino et al., 2012). The higher abundances at microbial mats coupled with lower diversity and richness correspond to community patterns associated with organic enrichment (Pearson and Rosenberg; 1978).

Microbial mat seeps had lower taxa richness and diversity compared to other seep habitats; however, all taxa found at microbial mats were also found within some tubeworm or soft-bottom seep communities. Microbial mat seeps often have low macrofaunal diversity compared to other seep or background habitats (Sahling et al., 2002; Levin et al., 2003; Bernardino et al., 2012; Bourque et al., 2016). Microbial mats are found at seeps where hydrogen sulfide concentrations are high, and when a seep contains several habitats, microbial mats are often located at the center of the seep formation where fluid concentrations are highest (Tryon and Brown, 2001; Levin, 2005). Pore-water methane and sulfide concentrations are important in shaping microbial community structure and megafaunal epibenthos (Cruaud et al., 2015). Sahling et al. (2002) found distinct macrofaunal assemblages within meters of each other associated with different epifauna/megafauna. Macrobenthic communities living under Beggiatoa mats exhibited low abundance and diversity, characteristic of disturbance. This was most likely caused by the high sulfide concentrations (> 25 mM) in the sediment. Microbial mats themselves may be partly responsible for the lower taxa richness at these sites. A microbial film over the sediments may be more difficult to burrow through than sediment or make it more difficult for organisms living underneath to acquire oxygen.

The differences in taxa richness and diversity among seep habitats may be due to other factors than sulfide and oxygen concentrations. Seep epibenthic megafauna often exhibit successional patterns at seeps over time with new, high flux seeps being first colonized by microbial mats before decreases in seepage allow larger tubeworm and mussel communities to thrive (Levin et al., 2005). Thus, more macrobenthic taxa may be found in tubeworm habitats compared to microbial mat habitats because organisms have had more time to colonize them. Vestimentiferan tubeworms also uptake hydrogen sulfide from the sediment in their roots
(Freytag et al., 2001), which may make the sediments more hospitable to infauna. Tubeworm communities provide much more structure than microbial mat or soft-bottom habitats. Tubeworms slow water movement around them allowing for greater settlement of materials, including organic matter, from the water column to the sediment around them. The 3-D structure also provides for more niches for different types of organisms (Cordes et al., 2007), possibly explaining the increased number of taxa within the tube-worm associated sediments.

3.4.4. Seep vs. Spill

Macrobenthic communities at seeps were not only different than communities in background areas but also different than communities impacted by the Deepwater Horizon oil spill. Comparisons of community abundances between seep and spill communities mirrored comparisons among seep and background communities, with abundances much higher at seeps. While the depth of the DWH blowout was unprecedented, there have been many spills and wellhead leaks in shallower waters, some near natural seepage. Off the coast of Trinidad, macrobenthic communities near an oil refinery were moderately to grossly stressed when examining abundance/biomass comparison plots while those close to a very large natural oil seep nearby were not (Agard et al., 1993). Non-metric multidimensional scaling plots showed communities near the refinery differed from natural seep and background communities.

The differences in abundances and taxa are most likely due to the fact that seeps are primarily a source of organic enrichment to the surrounding environment (Sibuet and Olu, 1998; Levin, 2005), while the DWH blowout released both hydrocarbons causing organic enrichment as well as heavy metal depositions which have toxic effects on organisms (Montagna et al., 2013). Agard et al. (1993) suggested that the reason seep taxa do not thrive near a refinery site was because of the episodic input of refined products and dispersants near the refinery. They

found heavy metals to be more strongly correlated with macrobenthic abundances than PAH concentrations. The addition of dispersants to the oil being released at the DWH wellhead most likely increased the toxicity of the materials being released there (Goodbody-Gringley et al., 2013, Almeda et al., 2014), further depressing abundances and excluding sensitive taxa.

Likewise, variability at seeps was much higher than at spill areas (Table 5). As mentioned above, variability is often associated with increased stress (Lewontin and Levins, 2000). Demopoulos et al. (2016) found higher variance in macrofaunal abundance and diversity as well as higher multivariate dispersion in sediment communities adjacent to deep-sea corals impacted by the DWH spill compared to healthy deep-sea coral infaunal communities. Since previous work has shown that background and spill communities in the deep GoM are different (Washburn et al., 2016), natural seepage appears to alter background deep-sea communities but is not associated with the losses of abundance and diversity common to anthropogenic oil releases. The large amount of heterogeneity at seeps associated with differences in depth, chemistry, fluid flux, and epibenthic megafauna appear to cause even greater variability in macrobenthic communities than even the stress from an oil spill.

The biggest difference between communities at seeps vs. near the DWH wellhead was that a large portion of the similarity among seeps was due to crustacean taxa while no crustaceans were responsible for even 1% of similarity within spill communities (Table 5). Crustaceans are often considered more sensitive to stressors, such as hydrocarbon concentrations, than polychaetes (Gesteira and Dauvin, 2000; Lenihan et al., 2003). Washburn et al. (2016) found that infaunal communities near the Deepwater Horizon wellhead following the blowout contained far fewer crustaceans and mollusks than communities unaffected by the blowout. Several polychaete taxa were responsible for similarity within both seep and spill

communities. Dorvilleids, ampharetids, and hesionids were found at many seeps in this study and have often been found representative of seeps (Levin et al., 2003; Levin, 2005; Bernardino et al., 2012). Ampharetids and hesionids were not responsible for similarity in spill communities, but dorvilleid abundances were nearly twice as high near the spill as seeps. Finally, many more taxa were found at seeps vs. spills suggesting that deep-sea spills are more toxic to the macrobenthos than seeps, removing taxa that are more sensitive.

3.4.5. Sampling Methods

While background habitats were sampled over a much larger range of years and very different sampling methods (i.e., boxcorer, multicorer, and ROV core) than seep habitats, seep communities were still more variable than background communities (Fig. 7A). With DGoMB and DWH-B samples grouped together, there appeared to be a strong relationship between sampling area and variability among samples (Fig. 7A). DGoMB samples were collected with a boxcorer, which was over an order of magnitude larger than samples collected with a multicorer. Multicore samples had twice the area of samples collected via ROV.

In this study background habitats sampled via multicorer were more similar to background samples collected via boxcorer then seep samples collected via multicorer or ROV core. When examining rarefaction curves for all samples collected away from seeps, samples collected via a multicorer or ROV core appeared to have very similar curves (Fig. 7). Both multicores and ROV cores sampled roughly 30 taxa over the first 200 individuals found while boxcorers collected only 22 taxa over the first 200 individuals (Fig. 6). The same number of individuals consistently represented fewer taxa in boxcores compared to the smaller cores until approximately 2500 individuals were collected. Only three multicores were used for macrofauna

collection at a specific station in 2010, which means that several different stations were required to equal the abundances of one boxcore station.

Montagna et al. (2016) compared benthic communities collected in the deep Gulf of Mexico at the same place and time by both a multicorer and boxcorer. They found that that the boxcorer underestimated macrofaunal abundance by 3x while the multicorer collected 60% fewer taxa than the boxcorer. This study also found that abundances per m² were much lower in boxcorer samples vs. multicorer samples. Bow waves from the boxcorer may wash away small, surface dwelling animals during collections (Hulings and Gray, 1971). The washing and sieving of samples on the boat deck during boxcorer operations may also be responsible for the loss of organisms (Montagna et al., 2017). However, Montagna et al. (2017) found that communities collected via boxcorer and multicorer at the same locations were very different, making comparisons between methods extremely difficult. In spite of these large differences among sampling methods, much more variability was associated with seep communities compared to sampling methods (Fig. 7).

3.4.6 Conclusion

In the deep Gulf of Mexico, macrofaunal seep communities were different than communities in background or spill habitats. Within the seep group, communities were different among different seep habitats (i.e., microbial mat, tubeworm, and soft-bottom seeps). In fact, every seep seemed to represent different macrobenthic communities. All variability in background habitats associated with depth, time, location, and sampling method were masked by the large variability among seeps. The variability associated with stress from the DWH spill was also masked by the variability among seeps. Thus it appears that most seeps in the deep Gulf represent unique macrobenthic communities. Seeps have often been thought of as extremely

heterogeneous environments, representing wide ranges in depth, chemical composition, fluid flux, geomorphology, age, and epibenthic megafaunal communities. This study confirms their heterogeneity in the Northern Gulf of Mexico

There is a timely need for information on seep communities in the deep GoM. Determining baseline community structure at these seeps is important for understanding patterns of global biodiversity. One goal of many policies and non-governmental organizations is to conserve as much biodiversity as possible, and seeps may be a rather large repository of this diversity, especially compared to their small area. Based off of this study, more taxa may be preserved by protecting many smaller seeps compared to a small amount of large seeps. Differentiating the effects of natural seepage and oil spills on benthic communities is also important. Many people have argued that oil spills in areas of natural seepage will have minimal environmental effects due to the natural presence of oil. However, this study showed macrobenthic communities responded differently to spills than seeps. The lower abundances and diversity associated with the DWH spill suggests that the spill caused damage to the deep-sea GoM benthos despite their acclimation to seepage.

References

- Agard, J.B.R., J. Gobin, and R.M. Warwick (1993). Analysis of marine macrobenthic community structure in relation to pollution, natural oil seepage and seasonal disturbance in a tropical environment (Trinidad, West Indies). Marine Ecology Progress Series, 92, 233-243.
- Almeda, R., C. Hyatt, and E.J. Buskey (2014). Toxicity of dispersant Corexit 9500A and crude oil to marine microzooplankton. Ecotoxicology and Environmental Safety, 106, 76-85.

- Armstrong, C.W., N. Folely, R. Tinch and S. van den Hove (2010). Ecosystem goods and services of the deep sea. Deliverable D6.2 of the HERMIONE project: 68 pp.
- Armstrong, C.W., N.S. Foley, R. Tinch, and S. van den Hove (2012). Services from the deep: Steps towards valuation of deep sea goods and services. Ecosystem Services, 2, 2-13.
- Baguley, J.G., P.A. Montagna, C. Cooksey, J.L. Hyland, H.W. Bang, C. Morrison, A.
 Kamikawa, P. Bennetts, G. Saiyo, E. Parsons, M. Herdener, and M. Ricci (2015).
 Community response of deep-sea soft-sediment metazoan meiofauna to the Deepwater
 Horizon blow out and oil spill. Marine Ecology Progress Series, 528, 127-140.
- Barry, J.P., H.G. Greene, D.L. Orange, C.H. Baxter, B.H. Robison, R.E. Kochevar, J.W.
 Nybakken, D.L. Reed, and C.M. McHugh (1996). Biologic and geologic characteristics of cold seeps in Monterey Bay, California. Deep-Sea Research, 43, 1739-1762.
- Balvanera, P., A.B. Pfisterer, N. Buchmann, J. He, T. Nakashizuka, D. Raffaelli, and B., Schmid (2006). Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecology Letters, 9, 1146-1156.
- Beaumont, N.J., M.C. Austen, S.C. Mangi, and M. Townsend (2008). Economic valuation for the conservation of marine biodiversity. Marine Pollution Bulletin, 56, 386-396.
- Bergquist, D.C., T. Ward, E.E. Cordes, T. McNelis, S. Howlett, R. Kosoff, S. Hourdez, R. Carney, and C.R. Fisher (2003). Community structure of vestimentiferan-generated habitat islands from Gulf of Mexico cold seeps. Journal of Experimental Marine Biology and Ecology, 289, 197-222.
- Bernardino, A.F., L.A. Levin, A.R. Thurber, and C.R. Smith (2012). Comparative composition, diversity and trophic ecology of sediment macrofauna at vents, seeps and organic falls.
 PLoS One, 7, e33515. <u>https://doi.org/10.1371/journal.pone.0033515</u>.

- Boland, G.S. and G.T. Rowe (1991). Deep-sea benthic sampling with the GOMEX box corer. Limnology and Oceanography, 36, 1015-1020.
- Bourque, J.R., C.M. Robertson, S. Brooke, and A.W.J. Demopoulos (2016). Macrofaunal communities associated with chemosynthetic habitats from the U.S. Atlantic margin: A comparison among depth and habitat types. Deep-Sea Research II, http://dx.doi.org/10.1016/j.dsr2.2016.04.012.

- Bunge, J. and M. Fitzpatrick (1993). Estimating the number of species; a review. Journal of the American Statistical Association, 88, 364-373.
- Carlier A., B. Ritt, C. Rodrigues, J. Sarrazin, K. Olu, J. Grall, and J. Clavier (2010).
 Heterogeneous energetic pathways and carbon sources on deep eastern Mediterranean cold seep communities. Marine Biology, 157, 2545–2565.
- Carney, R.S. (1994). Consideration of the oasis analogy for chemosynthetic communities at Gulf of Mexico hydrocarbon vents. Geo-Marine Letters, 14, 149-159.
- Clarke, K.R. and R.N. Gorley (2015). PRIMER v7: User Manual/Tutorial. PRIMER-E, Plymouth, UK, 296 pp.
- Cordes, E.E. M.A. Arthur, K. Shea, R.S. Arvidson, and C.R. Fisher (2005). Modeling the mutualist interactions between tubeworms and microbial consortia. PLoS Biology, 3, 497-505.
- Cordes, E.E., E.L. Becker, S. Hourdez, and C.R. Fisher (2010a). Influence of foundation species, depth, and location on diversity and community composition at Gulf of Mexico lower-slope cold seeps. Deep-Sea Research II, 57, 1870-1881.
- Cordes, E.E., D.C. Bergquist, B.L. Predmore, C. Jones, P. Deines, G. Telesnicki, and C.R. Fisher (2006). Alternate unstable states: convergent paths of succession in hydrocarbon-seep

tubeworm-associated communities. Journal of Experimental Marine Biology and Ecology, 339, 159-176.

- Cordes, E.E., S.L. Carney, S. Hourdez, R.S. Carney, J.M. Brooks, and C.R. Fisher (2007). Cold seeps of the deep Gulf of Mexico: community structure and biogeographic comparisons to Atlantic equatorial belt seep communities. Deep-Sea Research I, 54, 637-653.
- Cordes, E.E., M.R. Cunha, J. Galeron, C. Mora, K.O. Roy, M. Sibuet, S. Van Gaever, A.
 Vanreusel, and L. Levin (2010b). The influence of geological, geochemical, and biogenic habitat heterogeneity on seep biodiversity. Marine Ecology, 31, 51-65.
- Cruaud, P. A. Vigneron, P. Pignet, J.C. Caprais, F. Lesongeur, L. Toffin, A. Godfroy, and M.A. Cambon-Bonavita (2015). Microbial communities associated with benthic fauna assemblages at cold seep sediments of the Sonora Margin, Guaymas Basin. Frontiers in Marine Science, 2, <u>http://doi.org/10.3389/fmars.2015.00053</u>.
- Davis, P.H. and R.B. Spies (1980). Infaunal benthos of a natural petroleum seep: study of community structure. Marine Biology, 59, 31-41.
- Dean, H.K. (2008). The use of polychaetes (Annelida) as indicator species of marine pollution: a review. International Journal of Tropical Biology and Conservation, 56, 11-38.
- Decker, C. and K. Olu (2012). Habitat heterogeneity influences cold-seep macrofaunal communities within and among seeps along the Norwegian margin. Part 2: contribution of chemosynthesis and nutritional patterns. Marine Ecology, 33, 231-245.
- Decker, C., N. Zorn, N. Potier, E. Leize-Wagner, F.H. Lallier, K. Olu, and A.C. Anderson (2012). Habitat heterogeneity influences cold-seep macrofaunal communities within and among seeps along the Norwegian margin. Part1: Macrofauna community structure. Marine Ecology, 33, 205-230.

- Demopoulos, A.W.J., D. Gualtieri, and K. Kovacs (2010). Food-web structure of seep sediment macrobenthos from the Gulf of Mexico. Deep-Sea Research II, 57, 1972-1981.
- Demopoulos, A.W.J., J.R. Bourque, E. Cordes, and K.M. Stamler (2016). Impacts of the *Deepwater Horizon* oil spill on deep-sea coral-associated sediment communities. Marine Ecology Progress Series, 561, 51-68.
- Diaz, R.J. and R. Rosenberg (1995). Marine benthic hypoxia: A review of its ecological effects and the behavioral responses of benthic macrofauna. Oceangraphy and Marine Biology Annual Review, 33, 245-303.
- Farber, S., R. Costanza, D.L. Childers, J. Erickson, K. Gross, M. Grove, C.S. Hopkinson, J. Kahn, S. Pincetl, A. Troy, P. Warren, and M. Wilson (2006). Linking ecology and economics for ecosystem management. Bioscience, 56, 121-133.
- Freytag, J.K., P.R. Girguis, D.C. Bergquist, J.P. Andras, J.J. Childress, and C.R. Fisher (2001). A paradox resolved: Sulfide acquisition by roots of seep tubeworms sustains net chemoautotrophy. Proceedings of the National Academy of Sciences, 98, 13408-13413.
- Gage, J.D. and P.A. Taylor (1996). Deep-sea biology: a natural history of organisms at the deepsea floor. 3rd edn. Cambridge University Press, Cambridge, UK.
- Gesteira, J.L.G. and J.-C. Dauvin (2000). Amphipods are good bioindicators of the impact of oil spills on soft-bottom macrobenthic communities. Marine Pollution Bulletin, 40, 1017-1027.
- Glover, A.G. and C.R. Smith (2003). The deep-sea floor ecosystem: current status and prospects of anthropogenic change by the year 2025. Environmental Conservation, 30, 219-241.

- Goodbody-Gringley, G. D.L. Wetzel, D. Gillon, E. Pulster, A. Miller, and K.B. Ritchie (2013).
 Toxicity of Deepwater Horizon source oil and the chemical dispersant, Corexit® 9500, to coral larvae, PLoS ONE, 8, e45574. <u>http://doi:10.1371/journal.pone.0045574</u>.
- Grupe, B.M., M.L. Krach, A.L. Pasulka, J.M. Maloney, L.A. Levin, and C.A. Frieder (2015).Methane seep ecosystem functions and services from a recently discovered southernCalifornia seep. Marine Ecology, 36, 91-108.
- Guillon, E., L. Menot, C. Decker, E. Krylova, and K. Olu (2016). The vesicomyid bivalve habitat at cold seeps supports heterogeneous and dynamic macrofaunal assemblages. Deep-Sea Research I, <u>http://dx.doi.org/10.1016/j.dsr.2016.12.008</u>.
- Hulings, N.C. and J.S. Gray (1971). A manual for the study of meiofauna. Smithsonian Contributions to Zoology, 78, 1-84.
- Hussain, T. and M.A. Gondal (2008). Monitoring and assessment of toxic metals in Gulf War oil spill contaminated soil using laser-induced breakdown spectroscopy. Environmental Monitoring and Assessment, 136, 391-399.
- Jannasch, H.W. and C.O. Wirsen (1973). Deep-sea microorganisms: in situ response to nutrient enrichment. Science, 180, 641-643.
- Juniper, S.K. and M. Sibuet (1987). Cold seep benthic communities in Japan subduction zones: spatial organization, trophic strategies and evidence for temporal evolution. Marine Ecology Progress Series, 40, 115-126.
- Kiel, S. (2015). Did shifting seawater sulfide concentrations drive the evolution of deep-sea methane-seep ecosystems? Proceedings of the Royal Society B: Biological Sciences, 282, <u>http://dx.doi.org/10.1098/rspb.2014.2908</u>.

- Kujawinski, E.B., M.C.K. Soule, D.L. Valentine, A.K. Boysen, K. Longnecker, and M.C.Redmond (2011). Fate of dispersants associated with the Deepwater Horizon oil spill.Environmental Science & Technology, 45, 1298-1306.
- Lenihan, H.S., C.H. Peterson, S.L. Kim, K.E. Conlan, R. Fairey, C. McDonald, J.H. Gabowski, and J.S. Oliver (2003). Variation in marine benthic community composition allows discrimination of multiple stressors. Marine Ecology Progress Series, 261, 63-73.
- Levin, L.A. (2005). Ecology of cold seep sediments: interactions of fauna with flow, chemistry and microbes. Oceanography and Marine Biology: An Annual Review, 43, 1-46.
- Levin, L.A., A.R. Baco, D.A. Bowden, A. Colaco, E.E. cordes, M.R. Cunha, A.W.J.
 Demopoulos, J. Gobin, B.M. Grupe, J. Le, A. MeTaxas, A.N. Netburn, G.W. Rouse, A.R.
 Thurber, V. Tunnicliffe, C.L. Van Dover, A. Vanreusel, and L. Watling (2016).
 Hydrothermal vents and methane seeps: Rethinking the sphere of influence. Frontiers in
 Marine Science, 3, 1-23.
- Levin, L.A. and G.F. Mendoza (2007). Community structure and nutrition of deep methane-seep macrobenthos from the North Pacific (Aleutian) Margin and the Gulf of Mexico (Florida Escarpment). Marine Ecology, 28, 131-151.
- Levin L.A. and H.M. Michener (2002). Isotopic evidence for chemosynthesis-based nutrition of macrobenthos: the ligntness of being at Pacific methane seeps. Limnology and Oceanography, 47, 1336–1345.
- Levin, L.A., W. Ziebis, G.F. Mendoza, V.A. Growny; M.D. Tryon, K.M. Brown, C. Mahn, J. Gieskes, and A.E. Rathburn (2003). Spatial heterogeneity of macrofauna at northern California methane seeps: influence of sulfide concentration and fluid flow. Marine Ecology Progress Series, 265, 123-139.

- Levin, L.A., W. Ziebis, G.F. Mendoza, V. Growney-Cannon, and S. Walther (2006). Recruitment response of methane-seep macrofauna to sulfide-rich sediments: An in situ experiment. Journal of Experimental Marine Biology and Ecology, 330, 132-150.
- Lewontin, R., and R. Levins (2000). Schmalhausen's law. Capitalism Nature Socialism, 11, 103-108.
- Martens, C.S., J.P. Chanton, and C.K. Paull (1991). Biogenic methane from abyssal brine seeps at the base of the Florida escarpment. Geology, 19, 851–854.
- MacDonald, I.R., G.S. Boland, J.S. Baker, J.M. Brooks, M.C. Kennicutt, and R.R. Bidigare (1989). Gulf of Mexico hydrocarbon seep communities. II. Spatial distribution of seep organisms and hydrocarbons at Bush Hill. Marine Biology, 101, 235-247.
- MacDonald, I.R., O. Garcia-Pineda, A. Beet, S. Daneshgar Asl, L. Feng, G. Graettinger, D.
 French-McCay, J. Holmes, C. Hu, F. Huffer, I. Leifer, F. Muller-Karger, A. Solow, M.
 Silva, and G. Swayze (2015). Natural and unnatural oil slicks in the Gulf of Mexico.
 Journal of Geophysical Research, 120, 8364-8380.
- McNutt, M.K., S. Chu, J. Lubchenco, T. Hunter, G. Dreyfus, S.A. Murawski, and D.M. Kennedy (2012). Applications of science and engineering to quantify and control the Deepwater Horizon oil spill. PNAS, 109, 20222-20228.
- Montagna, P.A., J.G. Baguley, C. Cooksey, I. Hartwell, L.J. Hyde, J.L. Hyland, R.D. Kalke, L.M. Kracker, M. Reuscher, and A.C.E. Rhodes (2013). Deep-sea benthic footprint of the Deepwater Horizon blowout. PLoS ONE, 8, e70540.

http://doi:10.1371/journal.pone.0070540.

- Montagna, P.A., J.G. Baguley, C.-Y. Hsiang, and M.G. Reuscher (2017). Comparison of sampling methods for deep-sea infauna. Limnology and Oceanography Methods, 15, 166-183
- Montagna, P. and D.E. Harper Jr. (1996). Benthic Infaunal Long-term Response to Offshore Production Platforms in the Gulf of Mexico. Canadian Journal of Fisheries and Aquatic Sciences, 53, 2567-2588.
- Montagna, P.A. and R.B. Spies (1985). Meiofauna and chlorophyll associated with Beggiatoa mats of a natural submarine petroleum seep. Marine Environmental Research, 16, 231-242.
- Narayanaswamy, B.E., T.D. Nickell, and J.D. Gage (2003). Appropriate levels of taxonomic discrimination in deep-sea studies: species vs family. Marine Ecological Progress Series, 257, 59-68.
- Olu, K., S. Lance, M. Sibuet, P. Henry, A. Fiala-Medioni, and A. Dinet (1997). Cold seep communities as indicators of fluid expulsion patterns through mud volcanoes seaward of the Barbados Accretionary Prism. Deep Sea Research, 44, 811-841.
- Olu, K., M. Sibuet, F. Harmegnies, J.P. Foucher, and A. Fiala-Medioni (1996). Spatial distribution of diverse cold seep communities living on various diapiric structures of the southern Barbados prism. Progress in Oceanography, 38, 347-376.
- OSBMB (Ocean Studies Board and Marine Board) (2003). Oil in the Sea III. Inputs, fates, and effects. National Academies Press, Washington, D.C.
- Pearson, T.H. and R. Rosenberg (1978). Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology Annual Review, 16, 229-311.

- Pequegnat, W.E., B.J. Gallaway, and L.H. Pequegnat (1990). Aspects of the ecology of the deepwater fauna of the Gulf of Mexico. American Zoologist, 30, 45-64.
- Peterson, C.H., M.C. Kennicutt II, R.H. Green, P. Montagna, D.E. Harper, Jr., E.N. Powell, and P.F. Roscigno (1996). Ecological consequences of environmental perturbations associated with offshore hydrocarbon production: a perspective on long-term exposures in the Gulf of Mexico. Canadian Journal of Fisheries and Aquatic Sciences, 53, 2637-2654.
- Peterson, C.H., S.D. Rice, J.W. Short, D. Esler, J.L. Bodkin, B.E. Ballachey, and D.B. Irons (2003). Long-term ecosystem response to the Exxon Valdez oil spill. Science, 302, 2082-2086.
- Plum, C., S. Gollner, P. Martinez-Arbizu, and M. Bright (2015). Diversity and compositions of the copepod communities associated with megafauna around a cold seep in the Gulf of Mexico with remarks on species biogeography. Marine Biodiversity, 45, 419-432.
- Reish, D.J. and T.V. Gerlinger (1997). A review of the toxicological studies with polychaetous annelids. Bulletin of Marine Science, 60, 584-607.
- Rex, M.A. and R.J. Etter (2010). Deep-sea biodiversity: Pattern and scale. Harvard University Press, Cambridge, MA.
- Robinson, C.A., J.M. Bernhard, L.A. Levin, G.F. Mendoza, and J.K. Blanks (2004). Surficial hydrocarbon seep infauna from the Blake Ridge (Atlantic Ocean, 2150 m) and Gulf of Mexico (690-2240 m). Marine Ecology, 25, 313-336.
- Ross, S.W., A.W.J. Demopoulos, C.A. Kellogg, C.L. Morrison, M.S. Nizinski, C.L. Ames, T.L.Casazza, D. Gualtieri, K. Kovacs, J.P. McClain, A.M. Quattrini, A.Y. Roa-Varon, andA.D. Thaler (2012). Deepwater program: studies of Gulf of Mexico lower continental

slope communities related to chemosynthetic and hard substrate habitats. U.S. Geological Survey Open-File Report 2012-1031, 301 p.

- Rowe, G.T. (1983). Biomass and production of the deep-sea macrobenthos. In Deep-Sea Biology, vol. 8, The Sea. Ed. G.T. Rowe. John Wiley & Sons, New York. 560 pp.
- Rowe, G.T. and M.C. Kennicutt II, eds. (2009). Northern Gulf of Mexico continental slope habitats and benthic ecology study: final report. U.S. Dept. of the Interior, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, LA. OCS Study MMS 2009-039. 456 pp.
- Sahling H., S.V. Galkin, A. Salyuk, J. Greinert, H. Foerstel, D. Piepenburg, and E. Suess (2003)
 Depth-related structure and ecological significance of cold-seep communities a case
 study from the Sea of Okhotsk. Deep-Sea Research I, 50, 1391–1409.
- Sahling, H., D. Rickert, R.W. Lee, P. Linke, and E. Seuss (2002). Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin.
 Marine Ecology Progress Series, 231, 121-138.

SAS Institute Inc. (2013). SAS/STAT® 9.4 User's Guide. Cary, NC.

- Sassen, R., S. Joye, S.T. Sweet, D.A. DeFreitas, A.V. Milkov, and I.R. MacDonald (1999). Thermogenic gas hydrates and hydrocarbon gases in complex chemosynthetic communities, Gulf of Mexico continental slope. Organic Geochemistry, 30, 485–497.
- Sibuet M. and K. Olu (1998). Biogeography, biodiversity and fluid dependence of deep-sea coldseep communities at active and passive margins. Deep-Sea Research II, 45, 517-567.
- Simberloff, D. (1972). Properties of the rarefaction diversity measurement. The American Naturalist, 106, 414-418.

- Smith Jr., K.L. and J.M. Teal (1973). Deep-sea benthic community respiration: An in situ study at 1850 meters. Science, 179, 282-283.
- Spies, R.B., D.D. Hardin, and J.P. Toal (1988). Organic enrichment or toxicity? A comparison of the effects of kelp and crude oil in sediments on the colonization and growth of benthic infauna. Journal of Experimental Marine Biology and Ecology, 124, 261-282.
- Tilman, D., P.B. Reich, and F. Isbell (2012). Biodiversity impacts ecosystem productivity as much as resources, disturbance, or herbivory. PNAS, 109, 10394-10397.
- Tryon, M.D. and B.M. Brown (2001). Complex flow patterns through Hydrate Ridge and their impact on seep biota. Geophysical Research Letters, 28, 2863-2866.
- USDOI (U.S. Department of Interior Bureau of Ocean Management) (2012). Deepwater Production Summary by Year.

http://www.gomr.boemre.gov/homepg/offshore/deepwatr/summary.asp

- Van Dover, C.L., C.R. German, K.G. Spear, L.M. Parson, and R.C. Vrijenhoek (2002). Evolution and biogeography of deep-sea vent and seep invertebrates. Science, 295, 1253-1257.
- Warwick, R.M. (1988). Analysis of community attributes of the macrobenthos of Frierfjord/Langesundfjord at taxonomic levels higher than species. Marine Ecological Progress Series, 46, 167-170.
- Warwick, R.M. and K.R. Clarke (1993). Increased variability as a symptom of stress in marine communities. Journal of Experimental Marine Biology and Ecology, 172, 215-226.
- Washburn, T., A.C.E. Rhodes, and P.A. Montagna (2016). Benthic taxa as potential indicators of a deep-sea oil spill. Ecological Indicators,



Figure 3.1. A map of all locations sampled during 2009-2013 (green circle = 2009, blue square = 2010, red triangle = 2012, and orange diamond = 2013) to examine communities associated with cold seeps in the Northern Gulf of Mexico as well as locations sampled during 2000-2002 on DGoMB cruises (black triangles) and during 2010 on the DWH response cruise (black circles). Depth contours are in 500 m increments.



Figure 3.2. Box plots comparing seep types where diamonds represent means while lines through the rectangles represent medians. A) Diversity (N1) and B) Abundance (n m⁻²) found at different seep types (microbial mat, tubeworm, or soft-bottom seep).



Figure 3.3. Rarefaction curves for A) each seep independently, where blue represents softbottom seeps, yellow represents tubeworm seeps, and red represents microbial mat seeps, and for B) All seep samples combined.



Figure 3.4. Cluster plot of community structure at all seeps sampled between 2009 - 2013. Different symbols indicate different seeps separated into 3 depth bins (0 - 1000 m, 1000 - 2000 m, and > 2000 m). The dashed line represents the 50% similarity threshold. Blue represents soft-bottom seeps, yellow represents tubeworm seeps, and red represents microbial mat seeps.



Figure 3.5. Average proportion of taxa comprising seep communities at shallow (0 - 1000 m), intermediate (1000 - 2000 m), and deep (> 2000 m) seeps.



Figure 3.6. The average percent of the community found in surface (0 - 5 cm) and deeper (5 - 10 cm) fractions of sediments collected during the 2009-2013 seep cruises. Abundances were averaged across seep type before converting to percentages.



B)

Figure 3.7. The nMDS plot of macrobenthic community structure collected during seep, DGoMB, and DWH cruises. A) Symbols by cruise type. B) Symbols by station where red represents microbial mats, blue represents soft-bottom seeps, yellow represents tubeworms, and green represents background (light green = DGoMB, dark green = DWH-B, and green = seep cruises).



Figure 3.8. Rarefaction curves for each cruise type. Samples in the DWH category include only background stations (DWH-B).

Site	Year	Rep	Latitude	Longitude	Depth	Seep	SampleType	Method
DC583	2009	1	28.3856	87.3886	2461	Background	Background	PC
DC583	2009	2	28.3855	87.3885	2449	Near	Tubeworms	PC
DC583	2009	3	28.3849	87.3883	2447	Near	Tubeworms	PC
DC583	2009	4	28.3855	87.3884	2452	Background	Background	PC
DC583	2009	5	28.3855	87.3885	2449	Near	Tubeworms	PC
DC673	2010	1	28.31	87.311	2601	Near	Tubeworms	PC
DC673	2010	2	28.31	87.311	2601	Near	Tubeworms	PC
DC673	2010	3	28.31	87.311	2601	Near	Tubeworms	PC
GC246	2010	1	27.7016	90.6486	834	Near	Microbial mat	PC
GC246	2010	2	27.702	90.649	834	Near	Microbial mat	PC
GC354	2010	1	27.598	91.823	567	Near	Tubeworms	PC
GC354	2010	2	27.5977	91.827	527	Background	Background	PC
GC354	2010	3	27.598	91.823	567	Near	Tubeworms	PC
GC354	2010	4	27.5981	91.8231	567	Near	Tubeworms	PC
GC354	2010	5	27.5977	91.827	527	Background	Background	PC
GC354	2010	6	27.598	91.827	527	Background	Background	PC
GC600-1	2012	1	27.3644	90.5643	1263	Near	Seep	PC
GC600-1	2012	2	27.3644	90.5643	1263	Near	Seep	PC
GC600-2	2012	1	27.3698	90.5712	1221	Near	Microbial mat	PC
GC600-2	2012	2	27.3698	90.5712	1221	Near	Microbial mat	PC
GC600-2	2012	3	27.37	90.571	1220	Near	Microbial mat	PC
GC600-3	2012	1	27.3728	90.5749	1181	Near	Seep	MC
GC600-3	2012	2	27.3728	90.5746	1178	Near	Seep	MC
MC252	2012	1	28.7235	88.3773	1639	Background	Background	PC
MC252	2012	2	28.7235	88.3773	1639	Background	Background	PC
MC252	2012	1	28.7417	88.352	1477	Background	Background	MC
OC26	2012	1	28.7035	88.3598	1669	Near	Seep	PC
OC26	2012	2	28.7035	88.3598	1669	Near	Seep	PC
OC26	2012	3	28.7035	88.3598	1669	Near	Seep	PC
GC185	2013	1	27.7848	91.503	562	Background	Background	MC
GC232	2013	1	27.7418	91.3188	575	Near	Seep	MC
GC415	2013	1	27.53	90.9918	1046	Near	Seep	MC
GC415	2013	2	27.541	90.9952	1050	Near	Seep	MC
NN001	2013	1	28.3255	88.3862	1786	Background	Background	MC
TE001	2013	1	28.9331	88.9591	137	Near	Seep	MC

Table 3.1. All stations sampled from 2009 - 2013 used to explore macrobenthic communities are natural hydrocarbon seeps in this study. Rep = replicate, PC = pushcore, and MC = multicore.

Table 3.2. The average abundance (n m⁻²) (Dens), richness (n) (ts), diversity (N1), and evenness (J') of macrobenthos at individual seep sites. Standard errors are given in parentheses. The number of cores for each calculation is given. These parameters were also averaged for all seep samples across depths (< 1000, 1000 – 2000, and > 2000 m), seep habitats (Microbial Mat, Soft-bottom Seep, and Tubeworm), and collection method (Multicore and pushcore). Similarity and multivariate dispersion (MVDISP) were also included.

Seep	Ν	Dens	ts	N1	J,	% similarity	MVDISP
DC583-09	3	2421 (278)	4.7 (0.33)	4.18 (0.38)	0.93 (0.02)	31.7	1.667
GC246-10	2	40892 (16577)	7.0 (4.00)	3.41 (1.00)	0.71 (0.09)	26.0	1.8
GC354-10	3	25156 (3738)	13.0 (1.73)	9.29 (1.14)	0.87 (0.01)	48.4	1
DC673-10	3	4526 (862)	8.7 (0.67)	7.65 (0.64)	0.94 (0.02)	55.1	0.833
GC600-12-1	2	17525 (1105)	9.0 (0)	4.03 (0.53)	0.63 (0.06)	64.3	0.3
GC600-12-2	3	17262 (12055)	7.3 (1.76)	4.51 (1.21)	0.77 (0.12)	60.4	0.567
GC600-12-3	2	9475 (63)	16.5 (0.5)	9.93 (1.12)	0.82 (0.05)	50.0	1.4
OC26-12	3	5052 (365)	9.7 (0.67)	8.33 (0.95)	0.93 (0.03)	54.3	0.8
GC232-13	1	5146	15.0	10.46	0.87	NA	NA
GC415-13	2	4142 (126)	10.5 (1.5)	7.27 (0.63))	0.85 (0.01)	52.2	0.9
TE001-13	1	3640	10.0	6.37	0.80	NA	NA
> 2000 m	6	3473 (623)	6.7 (0.95)	5.92 (0.84)	0.93 (0.01)	43.4	0.733
1000 - 2000 m	12	10769 (3117)	10.3 (1.00)	6.75 (0.76)	0.81 (0.04)	56.7	0.956
< 1000 m	7	23720 (6862)	11.1 (1.62)	7.36 (1.22)	0.81 (0.03)	42.8	1.075
Tubeworm	9	10701 (3793)	8.8 (1.32)	7.04 (0.85)	0.91 (0.02)	45.0	1.145
Soft-bottom	11	7839 (1572)	11.5 (0.94)	7.66 (0.72)	0.82 (0.03)	54.9	0.937
Microbial Mat	5	26714 (10227)	7.2 (1.59)	4.07 (0.78)	0.74 (0.7)	51.8	0.824
Multicorer	6	6003 (1117)	13.2 (1.35)	8.54 (0.79)	0.83 (0.02)	51.1	0.525
Pushcore	19	14741 (3542)	8.5 (0.75)	6.15 (0.60)	0.84 (0.03)	49.4	1.042
All Seeps	25	12644 (2792)	9.6 (.076)	6.72 (0.53)	0.84 (0.02)	25.0	

Station	TE 001	GC 354	GC 232	GC 246	GC 415	GC 600-3	GC 600-2	GC 600-1	OC 26	DC 583	DC 673
Year	2013	2010	2013	2010	2013	2012	2012	2012	2012	2009	2010
Depth	137	567	575	834	1048	1180	1221	1263	1669	2449	2601
Replicates	1	3	1	2	2	2	3	2	3	3	3
Arthropoda											
Tanaidacea	0	6	3	1.5	12.5	19.5	0	10	1	1	1
Cumacea	0	1.3	0	3	2.5	1	32	0.5	0.3	0	0
Amphipoda	0	5.7	0	0	1.5	5	0	2	0	0	0
Isopoda	0	0	1	1	4	0	0	0	0.3	0.3	0.7
Annelida											
Chrysopetalidae	0	0.3	0	47.5	0	0	2.3	0	0	0	0
Ampharetidae	12	5.7	0	16.5	0	0.5	7.3	0	0	0	0
Dorvilleidae	0	14.3	10	1	1.5	3.5	2	5.5	0	0.3	0
Cirratulidae	1	2.3	1	1.5	0	1.5	0	31.5	0	0.3	0.3
Paraonidae	3	9.3	5	0	1	0	0	0.5	1.7	0	2
Capitellidae	1	8	4	0	0.5	4.5	0.3	0.5	3	0	0
Spionidae	2	4	5	0	1	0	0	0	2	2.7	3.3
Cossuridae	0	9.7	0	0	0	6	0.3	0	0.3	0	0
Hesionidae	0	2	1	4.5	0	2.5	6.7	0.5	0	0	0
Oligochaeta	0	1.7	5	0	0.5	0	0	0	0	0	2.3
Syllidae	0	1.3	0	0.5	0	3.5	0	0.5	0	0.3	1
Acrocirridae	0	0	0	0	0	4.5	0	0	0.3	0.3	0.3
Maldanidae	0	0.3	0	0	0	1	0	0	2.3	0	0
Pilargidae	0	1	1	0	0	0	0.3	0	0	0.7	0.3
Nephtyidae	1	0	0	0	0	3	0	0	0	0	0
Nereididae	0	0	0	0.5	0	0.5	0	0	1.3	0	0
Sphaerodoridae	0	0.3	0	0	1	1.5	0	0	0	0	0
Sigalionidae	0	0	1	0	1	0	0	0	0.3	0.3	0
Trichobranchidae	0	0	1	0	0	1	0	0.5	0.3	0	0
Lumbrineridae	5	0	0	0	0	0	0	0	0	0	0
Molluska											
Gastropoda	0	0	0	33.5	0	2.5	1.3	0.5	0	0.3	0
Bivalvia	1	2.7	1	18.5	1	0.5	0.3	2	1	0	0
Aplacophora	2	2.3	1	0	2.5	10.5	0.3	1	0.3	0	0
Other											
Nemertea	0	0.3	0	0	0	1	1	0	0.7	0	0.7
Total	29	79.7	41	129.5	33	75.5	54.7	55.5	16	7.7	14.3

Table 3.3. Taxonomic composition of each seep site. Values represent average abundance per core. If less than 5 organisms of a specific taxa were found throughout all seep sites, then those taxa were not included.

Table 3.4. Taxa making up 95% of the similarity of samples within A) background soft-bottom communities and B) natural hydrocarbon seep communities.

A) Group Backgroud Average similarity 40.6%

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Paraonidae	0.8	7.02	2.08	17.29	17.29
Spionidae	0.78	6.57	2.35	16.19	33.48
Syllidae	0.48	2.45	0.65	6.03	39.51
Capitellidae	0.43	2.36	1.08	5.8	45.31
Isopoda	0.41	2.23	0.58	5.48	50.79
Maldanidae	0.33	1.76	0.74	4.33	55.12
Nephtyidae	0.25	1.37	0.47	3.37	58.49
Pilargidae	0.28	1.36	0.56	3.35	61.84
Cirratulidae	0.23	1.35	1.16	3.34	65.18
Opheliidae	0.37	1.33	0.52	3.28	68.46

B)

Group Seep Average similarity 25.18%

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Dorvilleidae	0.59	3.54	0.69	14.04	14.04
Spionidae	0.49	3.4	0.56	13.51	27.55
Hesionidae	0.48	2.1	0.4	8.33	35.88
Capitellidae	0.39	2.07	0.7	8.21	44.09
Aplacophora	0.4	1.86	0.47	7.38	51.47
Ampharetidae	0.4	1.74	0.36	6.89	58.36
Paraonidae	0.47	1.7	0.47	6.75	65.11
Syllidae	0.44	1.52	0.32	6.03	71.14
Cumacea	0.23	1.32	0.62	5.23	76.37
Nereididae	0.27	0.85	0.23	3.37	79.74

	BG	MM	SBS	TW	BG	MM	SBS	ΤW	BG	MM	SBS	ΤW	BG	MM	SBS	ΤW
Depth (cm)	0-1	0-1	0-1	0-1	0-3	0-3	0-3	0-3	0-5	0-5	0-5	0-5	5-10	5-10	5-10	5-10
Similarity	40.3	49.6	28.7	0.0	47.5	61.3	31.9	35.6	43.7	52.8	34.3	38.2	14.7	0.0	7.2	0.0
Tanaidacea			38.5				27.5		7.8		24.7	7.9				
Cumacea	5.7	20.7	5.3		2.5	39.8	5.0			34.8	5.9					
Ampharetidae		25.7				20.4				22.2						
Dorvilleidae	2.9	19.8	4.9		1.4	13.1	7.0			11.3	6.4	5.2				
Cirratulidae	23.8		3.4		17.1		3.6		12.7		3.3	1.5				
Paraonidae	8.6		1.6		20.1		7.7		17.3		6.9	6.4	73.5			
Capitellidae			4.9				8.2				10.6	2.7			82.6	
Spionidae	2.6		12.4		15.5		7.6	80.4	15.5		6.4	47.6			7.8	
Hesionidae		30.4				14.6				22.3						
Bivalvia	4.1		7.2				8.4		5.5		11.4					

Table 3.5. The ten taxa responsible for the most amount of similarity of macrobenthic communities among all seeps. The % similarity of each seep habitat (BG = background, MM = microbial mat, SBS = soft-bottom seep, and TW = tubeworm) broken down by dominant taxa and different sediment depths (0-1, 0-3, 0-5, and 5-10 cm).

Table 3.6. Taxa making up 95% of the similarity of samples within A) the area impacted by the DWH wellhead and B) natural hydrocarbon seep communities.

A)

Wellhead Average similarity: 50.36

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Dorvilleidae	5.08	12.88	0.96	25.57	25.57
Paraonidae	3.74	10.05	2.39	19.95	45.52
Capitellidae	2.57	6.71	1.76	13.33	58.84
Nemertea	1.85	3.96	1.1	7.86	66.7
Spionidae	1.89	3.76	0.95	7.47	74.17
Maldanidae	2.02	3.27	0.8	6.5	80.67
Syllidae	1.32	2.36	0.77	4.68	85.35
Bivalvia	1.29	1.64	0.58	3.25	88.6
Aplacophora	0.85	0.96	0.49	1.91	90.51
Sigalionidae	0.89	0.95	0.44	1.89	92.39
Cirratulidae	0.8	0.83	0.45	1.64	94.04
Cossuridae	0.68	0.56	0.37	1.12	95.15

B)

Seep

Average similarity: 28.17

Species	Av.Abund	Av.Sim	Sim/SD	Contrib%	Cum.%
Tanaidacea	2.92	4.56	0.66	16.2	16.2
Cumacea	2.6	4.14	0.72	14.71	30.91
Dorvilleidae	1.85	3.37	0.72	11.98	42.89
Bivalvia	1.31	2.36	0.75	8.37	51.26
Capitellidae	1.7	2.23	0.72	7.92	59.18
Hesionidae	1.51	1.66	0.44	5.88	65.07
Aplacophora	1.34	1.4	0.49	4.97	70.04
Amphipoda	1.04	1.21	0.5	4.29	74.32
Paraonidae	1.12	1.12	0.4	3.96	78.29
Spionidae	1.17	1.04	0.4	3.68	81.97
Ampharetidae	0.91	0.73	0.28	2.6	84.57
Maldanidae	1.05	0.68	0.29	2.4	86.98
Cirratulidae	1.41	0.63	0.17	2.23	89.2
Nereididae	0.82	0.52	0.29	1.86	91.07
Nemertea	0.78	0.47	0.27	1.66	92.72
Isopoda	0.79	0.42	0.21	1.49	94.21
Cossuridae	0.72	0.36	0.22	1.27	95.48

CHAPTER IV: ECOSYSTEM SERVICES OF THE DEEP-SEA GOM: IDENTIFICATION AND WASTE REGULATION OF OIL

Abstract

The deep sea provides many benefits to humans, or ecosystem services, from exploitable resources to processes which enable life on Earth. Unfortunately, the remote nature of this environment makes it easy for people to overlook. One service in particular, waste regulation, was further examined in the context of the Deepwater Horizon oil spill. Using values for spilled oil cleanup efforts, which included capping the wellhead and collecting oil, surface combustion, and surface skimming, it was calculated that the natural removal of spilled oil saved BP between \$20 and \$40 billion. This research illustrates the importance of the offshore environment to humans as well as the large monetary values associated with services provided by the deep sea.

4.1. Introduction

4.1.1. General Ecosystem Services

This study examines the ecosystem services provided by the deep-sea (> 200 m) Gulf of Mexico. The term ecosystem service (ES) has been defined many different ways in the past (Fisher et al., 2009); however, a commonly used description comes from the Millennium Ecosystem Assessment (MEA, 2005), which defines ES as "the benefits that people obtain from ecosystems." Ecosystem services are produced by ecosystem functions, which consist of abiotic and biotic components of ecosystems as well as their interactions. Ecosystem functions exist outside of human involvement and may or may not provide one or more ecosystem services (Thurber et al., 2014).

Ecosystem services have often been divided into services that benefit humans indirectly and services humans directly benefit from (Fisher et al., 2009; Luisetti et al., 2011). Direct

services such as obtaining food by fishing or the joy of SCUBA diving are much easier for people to understand and value than indirect services such as the sequestration of carbon in the deep sea or the recycling of nutrients by sea grasses. Many indirect services occur throughout large portions of the Earth's habitats (Thurber et al., 2014) and require an understanding at the global scale to fully value. However, recent studies have begun to focus on only direct services (Landers and Nahlik, 2013). Ecosystem functions must relate to humans in order to be considered services (Costanza et al., 2014). Indirect services are also required for various direct services to take place, meaning a valuation including both direct and indirect services in an area would most likely run into the problem of double counting (Fu et al., 2011; Landers and Nahlik, 2013). For these reasons, current ecosystem functions than benefits to humans. The first step in understanding ecosystem services provided by an environment is to assess the full suite of services provided by that habitat (TEEB, 2010).

There are many different ways to attempt to value ecosystem services. Economists often refer to total economic value (TEV), which is comprised of various environmental values. Two categories of values tend to come from this TEV approach, use values, which are benefits humans currently receive, and non-use values, which are intrinsic values in addition to usage (Turner et al., 2003). Valuations are generally limited to use values. A convenient mechanism to value a given service is via market prices, which reveal at least a lower bound on the monetary value that individuals place on a service. However, this only works for goods and services directly used by humans (e.g., food and raw materials) and can also be problematic if market conditions are not optimal (Costanza et al., 1998; Hussen, 1999).

When markets for services are not available, monetary values must be estimated through other methods. Estimates of willingness to pay (WTP) include avoided costs, replacement costs, factor income, travel costs, and hedonic costs (de Groot et al., 2002; Farber et al., 2002). Avoided costs can be used when valuing services that prevent costs that would have incurred without those services while replacement costs can be used when human activities or equipment could perform the same service as natural systems (de Groot et al., 2002). However, replacement and avoidance values assume that the replacement or avoidance systems are equivalent to the original system, the systems are the least costly option, and the willingness to pay for the system exceeds the costs (Spangenberg and Settele, 2010). Factor incomes measure the values added to incomes by ecosystem services, travels costs measure the value spent traveling to take advantage of services, and hedonic pricing measures the prices of goods associated with service demand (de Groot et al., 2002).

Valuation efforts often focus on monetary measures; however, there are several ways to attempt to value services in non-monetary terms. Non-monetary valuations can measure changes to ecosystem services in ecological terms. Habitat, species population, and energy flow models can be used as foundations for non-monetary methodologies (Feather et al., 1997). One such model is the Habitat Evaluation Procedure (HEP), which combines measurements of habitat quality and quantity to calculate habitat units (USFWS, 1980). Non-monetary values require an understanding of which ecological responses contribute to human well-being, predictions of change to these responses, and a consideration of a large range of valuation methods. Services can also be measured in social or civil norms, moral or spiritual beliefs, attitudes or judgements, societal good, and constructed or uninformed preferences (EPA, 2009).

When attempting valuation of ecosystem services, several considerations must be taken into account. Ecosystem services are associated with different ecosystem functions and processes, which are not uniform throughout an ecosystem (Chen et al., 2009). The quality of a habitat may impact the quality of the services provided by that habitat (NRC, 2013). Likewise, different locations, ages, etc. of the same habitat may be better for different services (Maes et al., 2011), causing tradeoffs in the quality/quantity of different services provided by a habitat. Putting an all-encompassing valuation number on a service is often impossible, for instance biodiversity is priceless as human life cannot exist without it (Toman, 1998). It is often better to examine the effects of marginal changes in a service. Many functions and processes are not exclusive to one service and double-counting is a possibility. There is not necessarily a one to one relationship between costs, services, and benefits. And there are often thresholds or instances where the unit loss or gain in service will cause an ecosystem to change into an alternative stable state and drastically change the value of the service (Farber et al., 2002; Luisetti et al., 2011). However, without placing a value on natural services the actual value is often considered to be lower than other, more easily assessed services or completely ignored.

4.1.2. Deep-Sea Services in the GoM

In order to measure the services provided to humans by ecosystems, there must first be adequate data on the ecosystems themselves. While many ecosystem services are provided by several different habitats some services are often associated with particular habitats. A vast majority of the literature to date examining ecosystem services have focused on terrestrial and shallow-water ecosystems compared to deep-water ecosystems for obvious logistical reasons. There are different services associated with terrestrial vs. marine environments and shallow vs. deep environments. Galparsoro et al. (2014) suggested that there is a gradient of services in

relation to depth with humans deriving more benefits from coastal benthic habitats, and benefits decreasing with depth. An early estimation of the global value of ecosystem services also found that coastal areas provided more services than deeper areas with the coastal zone covering 8% of the planet's surface but providing 43% of total global value (Costanza et al., 1997). However, these studies relied on literature reviews that were dominated by studies on terrestrial and coastal ecosystem services.

The deep-sea ecosystem is far removed from most forms of direct contact from humans. Thus much of the value that humans derive from the deep sea is from indirect services. Regulating and supporting services are primarily indirect services and often undervalued and less understood than direct services (Christie et al., 2006; Farber et al., 2006). However, they are necessary for final services to take place. Life could not persist on Earth without many supporting and regulating services. Without economic valuation it will remain difficult for deepsea environments to factor in to decision-making processes, or for the public to understand why the deep-sea is important. The lack of knowledge about the costs of damages to the deep sea also results in an inability to determine appropriate penalties when ecological disasters take place. While the remoteness of the deep sea hinders human access to the benefits it provides, it also makes it easier to conserve biodiversity and services provided by this ecosystem. Conservation costs for a specific benefit are dependent on human pressure in an area (Balmford et al., 2008).

Much of the importance of deep-sea functions and services is derived from the vast size of this habitat and the large amount of time it is separated from the atmosphere, aspects that also result in the lack of information on this ecosystem. Water masses in the deep sea are primarily formed in the North Atlantic and will not have contact with the atmosphere for several hundred

years after sinking (Broecker, 1979). There are more than one billion cubic kilometers of water in the deep sea, which allows this habitat to serve as a global buffer for nutrients, carbon, human wastes, and even temperature (Thurber et al., 2014).

While the deep sea provides many services, these services are not provided uniformly throughout the water column. The 3-dimensional nature of the ocean adds another element to understanding ocean services that is not present in terrestrial environments. The water column is often delineated between surface waters where light penetrates (< 200 m) and deeper waters which represent approximately 98.5% of the permanently inhabitable volume of the planet (Gage and Tyler, 1991). Another component of the deep sea is the deep seafloor which represents 63% of the total surface area of the Earth. The sea floor community is comprised of organisms which either live on or within the sediments. This study will focus on the deep sea and seafloor. Most studies exploring services provided by the deep sea are abstract with a lack of ties to biophysical information. Reviews by Armstrong et al. (2010) and Thurber et al. (2014) attempted to identify and describe the many ecosystem services provided by the deep sea but were forced to extrapolate functions measured in a particular location across an ocean basin or the entire globe.

The anthropogenic activities most associated with detrimental effects on the deep-sea environment have changed in recent years. For most of the 20th Century the direct disposal of various substances such as garbage, sewage, radioactive wastes, and munitions was the human activity which most affected the deep sea (Glover and Smith, 2003; Ramirez-Llodra et al., 2011). However, with increased technology and need for resources, exploitation from fishing is currently having the greatest impact on the deep sea. Fish in the deep sea have life histories associated with low fecundity and long times till maturation making their exploitation more like mining than sustainable fishing, as populations are not replenished after harvests. Deep-sea
fishing also results in large amounts of bi-catch, which cannot survive after being brought to the surface, as well as the destruction of structure, such as deep-sea corals, which are important habitats for many organisms (Glover and Smith, 2001; Ramirez-Llodra et al., 2011). Exploitation of deep-sea minerals has the potential to directly disturb 10's to 100's of millions of square kilometers. There are approximately 46 million km² of manganese nodule-rich seafloor (Ghosh and Mukhopadhyay, 2000). Mineral deposits around hydrothermal vents are often rich and have also been suggested as resources for future exploitation (Hoagland et al., 2009).

4.1.3. Waste Removal and Burial

The deep sea provides many services to humans, several examples of which were listed above in Chapter I; however, assessments in this study will focus on the ability of the environment to process and remove contaminants introduced by humans, or "waste regulation." The removal of pollutants by the marine environment is generally considered an indirect service. Microbial communities can often consume and degrade many pollutants such as hydrocarbons (Valentine et al., 2010). Microbial communities with resistant strains have been shown to bioremediate heavy metals associated with sewage sludge (Watanabe, 2001). Marine benthic organisms store, bury, and transform various types of wastes. Macrobenthic organisms on the deep-sea floor assimilate and chemically alter many wastes through bioturbation as well as simple burial and removal of wastes from the system. Macrofaunal communities can also modify the distribution, bioavailability, and toxicity of metals via complexation (Abdullah and Fredriksen, 2004). There are various types of wastes and contaminants which need to be regulated. A decline in biodiversity would most likely reduce the amounts and types of wastes which could be regulated and result in a decline in marine health (Beaumont et al., 2008).

Several contaminants such as radiation from nuclear testing, chlorofluorocarbons, and polychlorinated biphenyls have been found over 1000 m in depth in all the world's oceans. This is especially true in the Arctic and around Antarctica as much of the deep-ocean water masses are formed here (Theil, 2003). Many of the chemicals and materials deposited on the seafloor can persist for decades or longer, as seen by the abundance of unburnt coal and clinker left from steamship travel (Theil, 2003). The production of wastes by humans has been estimated to be as high as 40 kg per person per day in some industrialized countries (Oslo Commission, 1989). Dumping in the deep sea may be a practical option in the future as terrestrial and coastal areas become more and more developed.

The deep sea plays a major role in the global regulation of pollutants in several ways. Pollutants reach the deep sea via sinking of large water masses, adsorption onto larger sinking particles or marine snow (Dachs et al., 2002), dense shelf water cascades (Canals et al., 2006), or direct dumping by humans (Thiel, 2003). Once in the deep sea, pollutants may be deposited and buried, where they remain for very long periods of time. The level of bioturbation also regulates the amount of wastes exposed to oxidizing and reducing environments that determines rates of decomposition and sequestration (Armstrong et al., 2010). On the other hand, the pollutants may be detoxified through biotic or abiotic processes in the deep sea, which is considered to be a regulating service (Thurber et al., 2014).

Because of the almost limitless number of pollutants entering the environment, many studies which examine waste regulation focus on bioremediation of specific wastes in a given habitat. One of the most obvious and heavily studied human waste is sewage waste (Mangi, 2011; Murillas-Maza et al., 2011). Other pollutants include pesticides, heavy metals, and hydrocarbons.

The recent Deepwater Horizon oil blowout was a disaster that illustrated the ability of the deep sea to manage and remove pollutants released by human activities. In April 2010, the Deepwater Horizon (DWH) blowout occurred, and in the subsequent months approximately five million barrels of oil were released (Peterson et al., 2012). Du and Kessler (2012) examined a deep-water bloom in bacterial biomass following the DWH blowout by measuring reductions in dissolved oxygen (DO) at various locations and depths through the capping of the wellhead. They found that the majority of hydrocarbon mass in the underwater plume was respired by bacteria.

4.2. Methods

In order to assess the ecosystem services in an area, the first step is to determine which services to examine and what variables to use to measure said services. Pendleton et al. (2015) developed an approach for ecosystem service assessments which involved three steps: 1) determine reason and scope of assessment, 2) identify specific services, and 3) identify measures to quantify services. This study is meant to partly explain the importance of the deep-sea environment to humans, especially given the lack of direct contact. The specific service examined is waste regulation. The deep sea represents most of the Earth's volume, and this provides space for chemical and biological activities to metabolize and transform waste products. Deposition on the seafloor and burial also allows for the removal of wastes from the environment. Finally, measures of waste regulation can include avoided costs, or how much money it costs for human clean-up efforts.

The deep sea provides one benefit to humans with the removal of wastes from the marine environment. Biological and chemical processes can change or degrade pollutants and physical processes can bury and remove pollutants (Armstrong et al., 2010; Thurber et al., 2014). During

the Deepwater Horizon oil blowout there was a large microbial plume in the deep GoM of hydrocarbon degrading bacteria (Valentine et al., 2010; Crespo-Medina et al., 2014). However, the microbial plume was mostly feeding on natural gases, primarily ethane, methane, and propane (Valentine et al., 2010). Figures included in cleanup efforts reflected only barrels of oil released and removed. The ratio of gas-to-oil released from the DWH wellhead of 1,600 standard cubic feet per barrel of petroleum oil was used to calculate the total amount of natural gas released into the deep sea (Reddy et al., 2012).

In order to quantify the amount of money saved by natural processes that removed hydrocarbons from the DWH spill, the amount of oil which was actually removed from the environment by anthropogenic means was first calculated. British Petroleum (BP) undertook three major activities in attempts to remove oil leaked from the Maconda wellhead: skimming oil from the sea surface, capturing oil from the wellhead with a capping device, and burning oil at the surface (BP, 2010). It is estimated that approximately 4.9 million barrels of oil were released into the deep sea (Griffiths, 2012). Information regarding the amount of oil recovered from cleanup efforts following the DWH blowout was obtained from BP annual business reports (BP, 2010; BP, 2011) as well as several independent studies (Lubchenco et al., 2010; Ramseur, 2010; USCG, 2011; Ryerson et al., 2012).

After the quantity of hydrocarbons removed by human activities was determined, the cost of said cleanup was then examined. British Petroleum annual business reports were examined to identify the amount of money spent during cleanup operations (BP, 2010; BP, 2011). The BP annual business reports for 2010 and 2011 (BP, 2010; BP, 2011) provided information on money spent on legal claims, money put into a trust for future expenditures regarding the DWH spill, and money spent on efforts to respond to the spill. Only money spent on containing and cleaning

up spilled oil was considered to serve as an avoided cost for natural microbial degradation of oil and burial in the deep sea. Money spent on stopping the flow of oil from the wellhead was not included.

4.3. Results

British Petroleum posted a \$41 billion dollar loss in 2010 solely related to the DWH oil spill. Of this \$41 billion, it has been estimated that BP spent approximately \$14 billion dollars in spill response operations (BP, 2010; Ramseur, 2010). These response operations included many activities meant to capture or remove released oil including: placing a containment device over the riser to collect oil directly from the wellhead, aerial surveillance looking for oiled locations, mechanical surface skimming, surface burning, dispersant application, booms, and many techniques for oil cleanup from coastal areas. Approximately 48,000 people and 6,500 vessels were deployed during the peak of the spill response (BP, 2010; Ramseur, 2010).

Much of the oil and nearly all the natural gas released from the DWH oil spill never reached the water surface or even left the deep sea. A large amount of hydrocarbons released during the blowout (40%) comprised natural gases which were almost completely respired (Joye et al., 2011). While methane concentrations in the deep-sea plume reached values as high as 183 µmol/kg, less than 0.01% of the methane released reached the surface and entered the atmosphere (Reddy et al., 2012). Microorganisms that degrade methane were readily available in the deep sea Gulf of Mexico as methane and other hydrocarbons naturally escape the ocean subsurface at thousands of locations in the northern GOM.

Natural gases including methane, ethane, and propane were found at concentrations several orders of magnitude higher in waters below 800 m compared to shallower depths (Valentine et al., 2010). Using Reddy et al. (2012) ratio of gas-to-oil of 1,600 cubic feet per

barrel, the release of 4.9 million barrels of oil would correspond to 2.0×10^{11} g (200,000 tonnes) of C₁-C₅ natural gases being released. Joye et al. (2011) estimated that as much as 500,000 tonnes of natural gas were discharged from the DWH blowout. Replacement costs for natural gases removed naturally by the deep-sea environment are not easily calculated as cleanup efforts were solely focused on observable oil. However, it is likely that humans benefited from the degradation of these gases before they entered the atmosphere.

While a total of \$14 billion dollars was spent by BP on spill response, spill response operations included not only efforts to capture and remove released oil, but also efforts to stifle the spill from the wellhead such as the drilling of two relief wells and top-kill efforts (BP, 2010). Details of the costs of halting the spill were not in financial reports, but it was estimated that a relief well drilled near the Macondo wellhead would cost approximately \$100 million (Rigzone, 2010). Thus the two relief wells drilled would likely have cost twice as much or approximately \$200 million. The relief wells took 5 months to finish and marked the end of the DWH spill (McNutt et al., 2012). Costs for top-kill efforts were not found; however, deep-sea drilling activities are costly, and this study assumes drilling the relief wells was more expensive than top-kill efforts. For this study top-kill efforts were estimated at \$50 million, but even at costs an order of magnitude higher they would only account for 3.5% of the total spill response costs. For a much more conservative value, top-kill efforts were estimated at \$750 million, much more than the costs of drilling 2 relief wells.

The United States Geological Survey estimated that approximately 1/3rd of the oil released during the DWH spill was captured, dispersed, or burned. It was estimated that 17% (830,000 barrels) of the oil was captured from capping mechanisms on the riser or blowout preventer, 8% (400,000 barrels) was dispersed either at the wellhead or sea surface, 5% (250,000

barrels) was burned, and 3% (150,000) was skimmed from the surface (USCG, 2011) (Table 3). The USCG figures for oil removal were very similar to figures provided by BP for oil captured at depth from capping mechanisms (827,000 barrels) and oil burned (265,450 barrels). However, BP estimated 828,000 barrels of oily liquid were skimmed from the surface, much more than the 150,000 barrels found by the USCG (Table 3). This discrepancy is most likely due to the fact that BP looked at the total amount of liquid captured, which would include sea water, but the USGS looked at only oil captured. There were no figures for the amount of oil dispersed in BP's annual reports (BP, 2010).

The fate of hydrocarbons released during the DWH spill is still not fully understood. Ryerson et al. (2012) estimated that 5% of the total spill mass evaporated into the atmosphere, 10% formed surface slicks, and 25% was removed mechanically or burned. A deep-water plume at approximately 1200 m depth also formed containing approximately 35% of the total spill mass. Lubchenco et al. (2010) also estimated removal efforts to have captured 25% of the total oil released in addition to 25% which was evaporated or dissolved, 24% which was dispersed, and 26% unaccounted for or residual. The federal government had the same estimate for captured oil but found 29% of the oil to be dispersed, 16% chemically and 13% naturally. They found slightly lower numbers for evaporated or dissolved hydrocarbons (24%) and residual (22%) (Ramseur, 2010) (Table 3).

The cost associated with removing or cleaning one barrel of oil released by the DWH blowout (/barrel) was calculated as the total amount spent on BP oil spill response (SP_t) minus the amount spent attempting to kill the DWH wellhead (SP_k) divided by the total amount of oil cleaned (O_c):

$$(\text{s/barrel}) = (SP_t - SP_k)/O_c$$

Because oil that was chemically dispersed was still left in the system, this fraction may be included in the amount of oil naturally removed or buried. However, dispersants were intentionally added to minimize damages in coastal areas so it may also be considered removed from the system via human activities. Likewise, residual oil refers to oil left in the water column, mixed in the sediments, degraded by microbes, collected on shore, and various other unknown fates (Ramseur, 2010). Thus several different calculations for the amount of money spent per barrel of cleaned oil and amount naturally degraded/buried were performed to get an estimated range of costs.

If we estimate costs for killing the well at \$250 million and 25% of the oil was cleaned then $\frac{1}{250,000,000} - \frac{250,000,000}{1,225,000}$ barrels) or $\frac{11,200}{1,220}$ barrel

If chemically dispersed oil was considered cleaned then 33% of the oil was cleaned for $\frac{14,000,000,000 - 250,000,000}{1,617,000}$ barrels) or ~\$8,500/barrel

For a larger cost for killing the well of \$1 billion and 25% of the oil cleaned \$/barrel = (\$14,000,000,000 - \$1,000,000,000)/1,225,000 barrels) or ~\$10,600/barrel

Finally, for kill costs of \$1 billion dollars and 33% of the oil cleaned

\$/barrel = (\$14,000,000,000 - \$1,000,000,000)/1,617,000 barrels) or ~\$8,000/barrel

Once the costs per barrel of oil cleaned up are calculated, this number can be used to determine how much money was saved by natural cleanup methods. The total value of natural

removal of oil (NV_r) would be equal to the cost of cleaning one barrel of oil (\$/barrel) multiplied by the number of barrels of oil naturally degraded or stored (O_r):

$$NV_r = (\$/barrel) \times (O_r)$$

The lowest values for natural removal of oil would be obtained for the lowest value of cleanup costs (\$1 billion for kill costs) and highest value of oil removed by humans (33%). While the highest values for natural removal would be for opposite conditions. These two scenarios give us total values for natural waste regulation following the DWH spill of:

If costs for killing the well were \$1 billion and 33% oil cleaned

 $NV_r =$ \$8,000/barrel x 3,283,000 barrels or ~\$26.25 billion

If costs for killing the well were \$250 million and 25% oil cleaned

NV_r = \$11,200/barrel x 3,675,000 barrels or ~\$41.16 billion

While this analysis focuses on the removal of hydrocarbons released into the environment by human activities, there was also over 2 million gallons of dispersant intentionally added to the marine environment. Approximately 1.4 million gallons of the dispersant COREXIT® EC9500A were applied to the sea surface while an additional 0.77 million gallons were injected directly at the wellhead (Kujawinski et al., 2011). COREXIT has been shown to be toxic to many different organisms, especially larval forms (Goodbody-Gringley et al., 2013; Almeda et al., 2014). Hamdan and Fulmer (2011) even found that the presence of COREXIT inhibited hydrocarbon-degrading bacteria, possibly hindering the environment from regulating wastes. Regardless of the pros and cons of dispersant usage during the DWH spill, the GoM was forced

to regulate 2.1 million gallons of an artificial chemical. The removal of dispersants from the system was undoubtedly a benefit to humans.

4.4. Discussion

This study estimated that natural processes in the offshore Gulf of Mexico provided roughly \$25 - \$40 billion dollars in value by removing oil released during the Deepwater Horizon spill. The removal of wastes, such as oil, from the environment is considered an ecosystem service and often termed waste regulation (Farber et al., 2006). There are many benefits, or ecosystem services, that humans receive from the deep sea (Armstrong et al., 2010; Thurber et al., 2014) with waste regulation being only one of them. The values for this study are only a fraction of the value for waste regulation provided by the GoM as they do not include values for other wastes released into the Gulf such as trash, sewage, or pesticides. These values do not even capture the entirety of hydrocarbon removal by the Gulf as they do not include oil released by various drilling activities, boats, or other spills/leaks.

The DWH spill was unique in that it released hydrocarbons directly into the deep sea. There are several mechanisms by which human wastes may reach the deep sea. While mostly banned now, munitions and radioactive wastes were actively dumped into the deep sea up till the mid-to-late twentieth century (Glover and Smith, 2003). Pollutants from land enter the marine environment via runoff into the continental shelf where dense shelf waters cascade along with sediments into the deep sea (Armstrong et al., 2010). Waste particles can also become attached to elements of marine snow and sink through the water column as oil particles did following the Deepwater Horizon spill (UAC, 2010). Due to a lack of knowledge on the deep-sea environment and processes that occur here, little is known about monetary values associated with deep-sea

ecosystem services. Values obtained for oil degradation in the deep GoM are an important first step in understanding the benefits of deep-sea processes following human-made disasters.

Unlike many coastal and terrestrial services, a large number of benefits provided by the deep sea to humans do not pass through the economy but are provided directly to humans. These services, such as the removal of wastes by the environment, cannot be measured by market values and in many cases are not evident to the people benefiting from them (Costanza et al., 1997). The DWH spill gave us a unique opportunity to examine the value of deep-sea oil degradation. During and after the spill a known quantity of money was spent cleaning up a known amount of hydrocarbons. Using the concept of avoided costs (de Groot et al., 2002), the amount spent on oil cleanup can be used to calculate the amount of money saved on natural storage and degradation of oil. Avoidance costs are used when services provided by nature can be replaced with man-made systems (Farber et al., 2002).

Relatively few studies have attempted to assign monetary values for the ecosystem service waste regulation, and the waste examined is usually waste water or sewage. Most studies that do focus on waste regulation in the marine environment are performed in wetlands and base values per hectare of wetland instead of the amount of wastes removed (Kazmierczak Jr., 2001; Patton et al., 2013; Zhang et al., 2013). Studies which examined waste regulation in the offshore marine environment were also often focused on waste water. Using replacement costs, Murillas-Maza et al. (2011) estimated a cost of 1,216 euros (\$1,300) to eliminate one ton of biochemical oxygen demand (BOD) contained in wastewater. Mangi et al. (2011) estimated a cost of 2,100 pounds (2,500 euros or \$2,700) to remove one ton of BOD in wastewater. It was estimated that it cost between \$60,000 to \$90,000 to remove one ton of DWH oil.

Values from this study on waste regulation do not include the savings associated with the removal of natural gases released from the spill nor the removal of introduced dispersants. As much as 500,000 tonnes of natural gases were released during the DWH spill, and most of these gases were bacterial respired before reaching the surface (Joye et al., 2011). Methane is also created throughout the ocean floor via methanogenesis which has been estimated to produce between 85 and 300 Tg CH₄ annually. Microorganisms associated with the seafloor consume more than 90% of this methane (Knittel and Boetius, 2009). Anaerobic oxidation of methane in the seafloor results in minimal efflux of methane from the ocean to the atmosphere (< 2% of global flux) (Armstrong et al., 2012). Methane is also produced via organic waste decomposition. Methane released from municipal and industrial wastewater in the United States increased over 33% from 1990 to 2010 (USEPA, 2006). Methane has been shown to contribute approximately 20% to global warming caused by anthropogenic activities. The primary sources of anthropogenic methane emissions are agriculture (53%), energy (28%), and waste (19%) (Armstrong et al., 2012). The natural removal of methane released during the DWH spill was not valued, but if included it is likely that the values for natural hydrocarbon degradation in this study would increase.

Cleanup efforts were also focused on areas with high concentrations of easily collected oil. As removal efforts move to areas with lower hydrocarbon concentrations, the cost per unit of oil removed from the Gulf of Mexico will increase. These removal activities don't take into account possible cleanup efforts after the spill was stopped. Oil removal activities are often performed for extended periods of time in coastal areas following shallow or surface water spills (Teal and Howarth, 1984). Compared to work in shallow areas, similar efforts often cost orders of magnitude more in deep-sea habitats. Thus this estimate is most likely a conservative one.

Past oil spills and cleanup efforts can be used to help put the DWH spill and subsequent cleanup activities into context. On March 24, 1989 the tank vessel *Exxon Valdez* grounded in Prince William Sound, Alaska and released roughly 10.8 million gallons (~260,000 barrels) of oil (Wolfe et al., 1994; Paine et al., 1996). Exxon Mobil was estimated to have spent \$2 billion on cleanup activities (Exxon Corporation, 1993; Trustee Council, 1995) that removed approximately 10% of the oil released or 26,000 barrels (Wolfe et al., 1994; Paine et al., 1996). Using avoided costs it can thus be estimated that roughly \$80,000 were spent on the removal of one barrel of oil following the *Exxon Valdez* spill, or 6-10 times the cost to remove a barrel of DWH oil. Much of this money was spent on shoreline cleanup suggesting that additional cleanup efforts following the DWH spill would greatly increase the costs associated with removing a barrel of oil from the shore or deep seafloor.

On the other hand the calculations from this study do not take into account damages associated with the spill. Previous studies have illustrated that the DWH spill caused extensive damages in the deep sea, affecting benthic infauna (Montagna et al., 2013; Baguley et al., 2015; Washburn et al., 2016) as well as deep-sea corals (Fisher et al., 2014). Any damages that would result in a decrease of value of other ecosystem services would reduce the value of waste regulation calculated in this study. Another source of possible error is the fact that the total costs associated with oil response not directly related to the removal of oil (ex., relief wells and top kill efforts) are not completely understood. If more than \$1 billion dollars out of the \$14 billion associated with oil spill response was spent on non-removal efforts, then the amount of money spent per barrel of removed oil and thus total value associated with natural removal would decrease.

This study was an attempt at assigning monetary values to a service provided by the offshore marine environment with no market value. Services are not provided uniformly throughout the water column. While the 35% of oil released that was trapped in the underwater plume at 1,200 m (Ryerson et al., 2012) was most likely dealt with entirely in the deep sea, some of the oil which reached the surface was removed from the system via evaporation, biodegraded at the surface, or transported to shallow or coastal areas. Thus it cannot be said that the entire value of natural oil removal following the DWH spill was provided by the deep sea. Ryerson et al. (2012) estimated 5% of the leaked mass evaporated while 10% formed sheens and slicks. However, due to the lack of details associated with costs for cleanup efforts at depth vs. cleanup efforts at the surface, it was not possible to partition the value of oil degradation by depth.

It is unclear the exact amount of oil removed by deep-sea processes; however, the natural release of nearly 70,000 tons of oil annually into the Gulf of Mexico through natural seepage (OSBMB, 2003) has created a deep-sea environment adapted to the input of oil. A large portion of the \$25 - \$40 billion worth of oil removal likely occurred in the deep GoM. While the services provided by humans from deep-sea processes generally do not directly benefit humans, the DWH spill provided an example of the deep-sea environment directly mitigating a man-made disaster. Offshore stakeholder workshops have found that services associated with market values (e.g., fisheries and oil/gas extraction) are generally the services most greatly valued. However, other services such as waste regulation were also considered important to humans (Yoskowitz et al., 2016).

Human civilization and even life as we know it is dependent on functions in the deep-sea that drive global biogeochemical cycles (Cochonat et al. 2007; Danovaro et al., 2008). Thus, knowledge of the services provided by the deep as well as values for these services is imperative

when making policies concerning activities in this environment. One example of humans impacting the deep sea was the Deepwater Horizon oil spill where 4.9 million barrels of oil were released at 1500 m depth (Griffiths, 2012). Impacts of this spill are still being examined, but the natural burial and degradation of hydrocarbons from the spill will help in not only reducing these impacts but also in the recovery of the offshore environment.

References

- Abdullah, M.I. and S. Fredriksen (2004). Production, respiration and exudation of dissolved organic matter by the kelp *Laminaria hyperborea* along the west coast of Norway.Journal of the Marine Biological Association of the United Kingdom, 84, 887-894.
- Almeda, R., C. Hyatt, and E.J. Buskey (2014). Toxicity of dispersant COREXIT 9500A and crude oil to marine microzooplankton. Ecotoxicology and Environmental Safety, 106, 76-85.
- Armstrong, C.W., N. Foley, R. Tinch, and S. van den Hove (2010). Ecosystem good and services of the deep sea. Deliverable D6.2 HERMIONE Project, pp. 68.
- Armstrong. C.W., N.S. Foley, R. Tinch, and S. van den Hove (2012). Services from the deep: Steps towards valuation of deep sea goods and services. Ecosystem Services, 2, 2-13.
- Baguley, J.G., P.A. Montagna, C. Cooksey, J.L. Hyland, H.W. Bang, C. Morrison, A.
 Kamikawa, P. Bennetts, G. Saiyo, E. Parsons, M. Herdener, and M. Ricci (2015).
 Community response of deep-sea soft-sediment metazoan meiofauna to the Deepwater
 Horizon blow out and oil spill. Marine Ecology Progress Series, 528, 127-140.
- Balmford, A., A. Rodrigues, M. Walpole, P. ten Brink, M. Kettunen, L. Braat, and R. de Groot (2008). Review on the economics of biodiversity loss: scoping the science. European Commission.
- Beaumont, N.J., M.C. Austen, S.C. Mangi, and M. Townsend (2008). Economic valuation for the conservation of marine biodiversity. Marine Pollution Bulletin, 56, 386-396.
- BP (British Petroleum) (2010). Annual report and form 20-F 2010. London, UK: <u>http://www.bp.com/content/dam/bp/pdf/investors/bp-annual-report-and-form-20f-</u> <u>2010.pdf</u>.

- BP (British Petroleum) (2011). Annual report and form 20-F 2010. London, UK: <u>http://www.bp.com/content/dam/bp/pdf/investors/bp-annual-report-and-form-20f-2011.pdf</u>.
- Broecker, W.S. (1979). A revised estimate for radiocarbon age of North Atlantic deep water. Journal of Geophysical Research, 84, 3218-3266.
- Canals, M., P. Puig, X.D. de Madron, S. Heussner, A. Palanques, and J. Fabres (2006). Flushing submarine canyons. Nature, 444, 354-357.
- Chen, N., H. Li, and L. Wang (2009). A GIS-based approach for mapping direct use value of ecosystem services at a county scale: Management implications. Ecological Economics, 68, 2768-2776.
- Christi, M., N. Hanley, J. Warren, K. Murphy, R. Wright, and T. Hyde (2006). Valuing the diversity of biodiversity. Ecological Economics, 58, 304-317.
- Cochonat, P., S. Durr, V. Gunn, P. Herzig, C. Mevel, J. Mienert, R. Schneider, P. Weaver, andA. Winkler (2007). The deep-sea frontier: Science challenges for a sustainable future.Luxembourg, Office for Official Publications of the European Communities.
- Costanza, R., J. Cumberland, H. Daly, R. Goodland, and R. Norgaard (1998). An introduction to ecological economics. CRC Press LLC, Boca Rotan, FL, USA.
- Costanza, R., R. D'Arge, R. De Groot, S. Farber, M. Grasso, B. Hannon, K. Limburg, S. Naeem,R.V. O'Neill, J. Paruelo, R.G. Raskin, P. Sutton, and M. van den Belt (1997). The value of the world's ecosystem services and natural capital. Nature, 387, 253-260.
- Costanza, R., R. de Groot, P. Sutton, S. van der Ploeg, S.J. Anderson, I. Kubiszewski, S. Farber, and R.K. Turner (2014). Changes in the global value of ecosystem services. Global Environemtnal Change, 26, 152-158.

- Crespo-Medina, M. C.D. Meile, K.S. Hunter, A-R. Diercks, V.L. Asper, V.J. Orphan, P.L
 Tavormina, L.M Nigro, J.J. Battles, J.P. Chanton, A.M Shiller, D-J. Joung, R.M.W.
 Amon, A. Bracco, J.P. Montoya, T.A. Villareal, A.M. Wood, and S.B. Joye (2014). The rise and fall of methanotrophy following the deepwater oil-well blowout. Nature Geoscience, 7, 423-427.
- Dachs, J., R. Lohmann, W.A. Ockenden, L. Mejanelle, S.J. Eisenreich, and K.C. Jones (2002).
 Oceanic biogeochemical controls on global dynamics of persistent organic pollutants.
 Environmental Science & Technology, 36, 4229-4237.
- Danovaro, R., A., Dell/'Anno, C. Corinaldesi, M. Magagnini, R. Noble, C. Tamburini, and M.Weinbauer (2008). Major vial impact on the functioning of benthic deep-sea ecosystems.Nature, 454, 1084-1087.
- De Groot, R.S., M.A. Wilson, and R.M.J. Boumans (2002). A typology for the classification, description and valuation of ecosystem functions, goods and services. Ecological Economics, 41, 393-408.
- Du, M. and J.D. Kessler (2012). Assessment of the spatial and temporal variability of bulk hydrocarbon respiration following the Deepwater Horizon oil spill. Environmental Science and Technology, 46, 10499-10507.
- EPA (Environmental Protection Agency) (2009). Valuing the protection of ecological systems and services: A report of the EPA Science Advisory Board. Science Advisory Board, Washington D.C., U.S.
- Exxon Corporation (1993). Form 10-K. EDGAR. U.S. Securities and Exchange Commission. <u>https://web.archive.org/web/20080304025742/http://yahoo.brand.edgar-</u> online.com/fetchFilingFrameset.aspx?FilingID=512563&Type=HTML.

- Farber, S.C., R. Costanze, and M.A. Wilson (2002). Economic and ecological concepts for valuing ecosystem services. Ecological Economics, 41, 375-392.
- Farber, S., R. Costanza, D.L. Childers, J. Erickson, K. Gross, M. Grove, C.S. Hopkinson, J. Kahn, S. Pincetl, A. Troy, P. Warren, and M. Wilson (2006). Linking ecology and economics for ecosystem management. Bioscience, 56, 121-133.
- Feather, T.D., C.S. Russell, K.W. Harrington, and D.T. Capan (1997). Review of monetary and nonmonetary valuation of environmental investments. Planning and Management Consultants, Carbondale, IL, U.S. Army Corps of Engineers.
- Fisher, B., R.K. Turner, and P. Morling (2009). Defining and classifying ecosystem services for decision making. Ecological Economics, 68, 643-653.
- Fisher, C.R., P.Y. Hsing, C.L. Kaiser, D.R. Yoerger, H.H. Roberts, W.W. Shedd, E.E. Cordes, T.M. Shank, S.P. Berlet, M.G. Saunders, E.A. Larcom, and J.M Brooks (2014). Footprint of *Deepwater Horizon* blowout impact to deep-water coral communities. Proceedings of the National Academy of Sciences, 111, 11744-11749.
- Fu, B.J., C.H. Su, Y.P. Wei, I.R. Willett, Y.H. Lu, and G.H. Liu (2011). Double counting in ecosystem services valuation: causes and countermeasures. Ecological Research, 26, 1-14.
- Gage, J.D. and P.A. Tyler (1991). Deep-sea biology: A natural history of organisms at the deepsea floor. Cambridge University Press, Cambridge, 504.
- Galparsoro, I., A. Borja, and M.C. Uyarra (2014). Mapping ecosystem services provided by benthic habitats in the European North Atlantic Ocean. Frontiers in Marine Science, <u>http://doi.org/10.3389/fmars.2014.00023</u>.

Ghosh, A.K. and R. Mukhopadhyay (2000). Mineral Wealth of the Ocean. Rotterdam, Netherlands: A.A. Balkema.

- Glover, A.G. and C.R. Smith (2003). The deep-sea floor ecosystem: Current status and prospects of anthropogenic change by the year 2025. Environmental Conservation, 30, 219-241.
 - Goodbody-Gringley, G., D.L. Wetzel, D. Gillon, E. Pulster, A. Miller, and K.B. Ritchie (2013). Toxicity of Deepwater Horizon source oil and the chemical dispersant, Corexit 9500, to coral larvae. PLOSone, 8, e45574, <u>http://doi.org/10.1371/journal.pone.0045574</u>.
- Griffiths, S.K. (2012). Oil release from Macondo Well MC252 following the Deepwater Horizon accident. Environmental Science & Technology, 46, 5616-5622.
- Hamdan, L.J., and P.A. Fulmer (2011). Effects of COREXIT® EC9500A on bacteria from a beach oiled by the Deepwater Horizon spill. Aquatic Microbial Ecology, 63, 101-109.
- Hoagland, P., S. Beaulieu, M.A. Tivey, R.G. Eggert, C. German, L. Glowka, and J. Lin (2009). Deep-sea mining of seafloor massive sulfides. Marine Policy, 34, 728-732.
- Hussen, A.M. (1999). Principles of environmental economics. Routledge, London/New York.
- Joye, S.B, I.R. MacDonald, I. Leifer, and V. Asper (2011). Magnitude and oxidation potential of hydrocarbon gases released from the BP oil well blowout. Nature Geoscience, 4, 160-164.
- Kazmierczak Jr., R.F. (2001). Economic linkages between coastal wetlands and water quality: A review of value estimates reported in the published literature. Department of Agricultural Economics. Louisiana State University, Baton Rouge, LA, USA, 1-22.
- Knittel, K. and A. Boetius (2007). The anaerobic oxidation of methane progress with an unknown process. Annual Reviews of Microbiology, 63, 311-334.

- Kujawinski, E.B., M.C. Kido Soule, D.L. Valentine, A.K. Boysen, K. Longnecker, and M.C.Redoment (2011). Fate of dispersants associated with the Deepwater Horizon oil spill.Environmental Science and Technology, 45, 1298-1306.
- Landers, D. and A. Nahlik (2013). Final ecosystem goods and services classification (FEGS-CS). U.S. Environmental Protection Agency, Washington, DC.
- Lubchenco, J., M. McNutt, B. Lehr, M. Sogge, M. Miller, S. Hammond, and W. Conner (2010). BP Deepwater Horizon oil budget: What happened to the oil? <u>http://www.noaanews.noaa.gov/stories2010/PDFs/OilBudget_description_%2083final.pd</u> <u>f</u>.
- Luisetti, T., R.K. Turner, I.J. Bateman, S. Morse-Jones, C. Adams, and L. Fonseca (2011). Coastal and marine ecosystem services valuation for policy and management: Managed realignment case studies in England. Ocean and Coastal Management, 54, 212-224.
- MEA (Millennium Ecosystem Assessment) (2005). Island Press, Washington, DC. World Resources Institute.
- Maes, J., M.L. Paracchini, and G. Zulian (2011). A European assessment of the provision of ecosystem services – towards an atlas of ecosystem services. Publications Office of the European Union, Luxembourg. European Commission.
- Mangi, S.C., C.E. Davis, L.A. Payne, M.C. Austen, D. Simmonds, N.J. Beaumont, and T. Smyth (2011). Valuing the regulatory services provided by marine ecosystems. Environmetrics, 22, 686-698.
- McNutt, M.K., S. Chu, J. Lubchenco, T. Hunter, G. Dreyfus, S.A. Murawski, and D.M. Kennedy (2012). Applications of science and engineering to quantify and control the Deepwater Horizon oil spill. PNAS, 109, 20222-20228.

- Montagna, P.A., J.G. Baguley, C. Cooksey, J.L. Hyland, R.D. Kalke, L.J. Hyde, M. Reuscher, A. Rhodes, and I. Hartwell (2013). Deep-sea Benthic Footprint of the Deepwater Horizon
 Blowout. PloS ONE, 8, e70540, <u>http://doi:10.1371/journal.pone.0070540</u>.
- Murillas-Maza, A., J. Virto, M.C. Gallastegui, P. González, and J. Fernandez-Mácho (2011). The value of open ocean ecosystems: A case study for the Spanish exclusive economic zone. Natural Resources Forum, 35, 122-133.
- NRC (National Research Council) (2013). An ecosystem services approach to assessing the impacts of the Deepwater Horizon oil spill in the Gulf of Mexico. National Academy Press, Washington D.C., USA.
- OSBMB (Ocean Studies Board and Marine Board) (2003). Oil in the sea III. Inputs, fates, and effects. National Academies Press, Washington, D.C.
- Oslo Commission, 1989. Review of sewage sludge disposal at sea. Oslo Commission, London, 84 pp.
- Paine, R.T., J.L. Ruesink, A. Sun, E.L. Soulanille, M.J. Wonham, C.D.G. Harley, D.R.
 Brumbaugh, and D.L. Secord (1996). Trouble on oiled waters: Lessons from the *Exxon Valdez* oil spill. Annual Review of Ecology and Systematics, 27, 197-235.
- Patton, D., J. Bergstrom, R. Moore, and A. Covich (2013). A meta-analysis of ecosystem services associated with wetlands in USFWS National Wildlife Regues. Proceedings of the 2013 Georgia Water Resources Conference, April 10-11, 2013, University of Georgia, Athens, GA.
- Pendleton, L., R. Mongruel, N. Beaumont, T. Hooper, and M. Charles (2015). A triage approach to improve the relevance of marine ecosystem services assessments. Marine Ecology Progress Series, 530, 183-193.

- Peterson , C.H., S.S. Anderson, G.N. Cherr, R.F. Ambrose, S. Anghera, S. Bay, M. Blum, R. Condon, T.A. Dean, M. Graham, M. Guzy, S. Hampton, S. Joye, J. Lambrinos, B. Mate, D. Meffert, S.P. Powers, P. Somasundaran, R.B. Spies, C.M. Taylor, R. Tjeerdema, and E.E. Adams (2012). A Tale of Two Spills: Novel Science and Policy Implications of an Emerging New Oil Spill Model. Biosience, 62, 461-469.
- Ramirez-Llodra, E., P.A. Tyler, M.C. Baker, O.A. Bergstad, M.R. Clark, E. Escobar, L.A. Levin,
 L. Menot, A.A. Rowden, C.R. Smith, and C.L. Van Dover (2011). Man and the last great
 wilderness: Human impact on the deep sea. PLoS one, 6, e22588,

http://doi.org/10.1371/journal.pone.0022588.

- Ramseur, J.L. (2010). Deepwater Horizon oil spill: The fate of the oil. Congressional Research Service, R41531.
- Reddy, C.M., J.S. Arey, J.S. Seewald, S.P. Sylva, K.L. Lemkau, R.K. Nelson, C.A. Carmichael,
 C.P. McIntyre, J. Fenwick, G.T. Ventura, B.A.S. Van Mooy, and R. Camilli (2012).
 Composition and fate of gas and oil released to the water column during the Deepwater
 Horizon oil spill. Proceedings of the National Academy of Sciences, 109, 20292-20297.

Rigzone (2010). BP's relief well to halt oil spill will cost \$100MM:

http://www.rigzone.com/news/oil_gas/a/91728/BPs_Relief_Well_to_Halt_Oil_Spill_Will _Cost_100MM.

Ryerson, T.B., R. Camilli, J.D. Kessler, E.B. Kujawinski, C.M. Reddy, D.L. Valentine, E. Atlas,
D.R. Blake, J. de Gouw, S. Meinardi, D.D. Parrish, J. Peischl, J.S. Seewald, and C.
Warneke (2012). Chemical data quantify *Deepwater Horizon* hydrocarbon flow rate and environmental distribution. Proceedings of the National Academy of Sciences, 109, 20246-20253.

- Spangenberg, J.H. and J. Settele (2010). Precisely incorrect? Monetising the value of ecosystem services. Ecological Complexity, 7, 327-337.
- Teal, J.M. and R.W. Howarth (1984). Oil spill studies: A review of ecological effects. Environmental Management, 8, 27-44.
- TEEB (The Economics of Ecosystems and Biodiversity) (2007). The economics of ecosystems and biodiversity: Ecological and economic foundations. in: Earthscan, edited by: P. Kumar, London and Washington.
- Thiel, H. (2003). Anthropogenic impacts on the deep sea. In: P.A. Tyler, Ecosystems of the world: the deep sea. Amsterdam, Elsevier: 431-474.
- Thurber, A.R., A.K. Sweetman, B.E. Narayanaswamy, D.O.B. Jones, J. INgels, and R.L. Hansam (2014). Ecosystem function and services provided by the deep sea. Biogeosciences, 11, 3941-3963.
- Toman, M. (1998). Why not to calculate the value of the world's ecosystem services and natural capital. Ecological Economics, 25, 57-60.
- Trustee Council, Exxon Valdez Oil Spill (1995). Fiscal Year 1996 Work Plan. Anchorage, AL.
- Turner, R.K., J. Paavola, P. Cooper, S. Farber, V. Jessamy, and S. Georgiou (2003). Valuing nature: lessons learned and future research directions. Ecological Economics, 46, 493-510.
- UAC (Unified Area Command) (2010). Deepwater Horizon MC252 response unified area command – strategic plan for sub-sea and sub-surface oil and dispersant detection, sampling, and monitoring. November 13, 2010 Final, U.S. Coast Guard and BP Exploration and Production, Inc. New Orleans, LA. USA.

- USCG (United States Coast Guard) (2011). Final action memorandum incident specific preparedness review (ispr) Deepwater Horizon oil spill. Washington, DC: https://www.uscg.mil/foia/docs/DWH/BPDWH.pdf.
- USEPA (United States Environmental Protection Agency) (2011). Inventory of U.S. greenhouse gas emissions and sinks: 1990-2009. Washington, DC, USA: U.S. Environmental Protection Agency, EPA, 430-R-11-005.
- USFWS (United States Fish and Wildlife Service) (1980). Habitat evaluation procedures (HEP) manual.
- Valentine, D.L., J.D. Kessler, M.C. Redmond, S.D. Mendes, M.B. Heintz, C. Farwell, L. Hu,
 F.S. Kinnaman, S. Yvon-Lewis, M. Du, E.W. Chan, F.G. Tigreros, and C.J. Villanueva
 (2010). Propane respiration jump-starts microbial response to a deep oil spill. Science,
 330, 208-211.
- Washburn, T., A.C.E. Rhodes, and P. Montagna (2016). Benthic taxa as potential indicators of a deep-sea oil spill. Marine Pollution Bulletin, 71, 587-597.
- Watanabe, K. (2001). Microorganisms relevant to bioremediation. Current Opinion in Biotechnology, 12, 237-241.
- Wolfe, D.A., M.J. Hameedi, J.A. Galt, G. Watabayashi, J. Short, C. O'Claire, S. Rice, J. Michel,J.R. Payne, J. Braddock, S. Hanna, and D. Sale (1994). The fate of the oil spilled from the *Exxon Valdez*. American Chemical Society, 28, 561-568.
- Yoskowitz, D.W., S.R. Werner, C. Carollo, C. Santos, T. Washburn, and G.H. Isaksen (2016).Gulf of Mexico offshore ecosystem services: Relative valuation by stakeholders. Marine Policy, 66, 132-136.

Zhang, Y., D. Zhou, Z. Niu, and F. Xu (2013). Valuation of lake and marsh wetlands ecosystem services in China. Chinese Geographical Science, 23, 1-10.

Table 4.1. The values for the fates of oil (% of total) released during the Deepwater Horizon oil spill in 2010. Percentages were obtained from various sources (BP = BP, 2010; Lubchenco = Lubchenco et al., 2010; Ramseur = Ramseur, 2010; Ryerson = Ryerson et al., 2012; and USCG = USCG, 2011).

	BP	Lubchenco	Ramseur	Ryerson	USCG
Cleaned	39	25	25	26	25
Captured at depth	17		17		17
Skimmed at surface	^c 17		3		3
Mechanically Recovered				20	
Burned	5		5	6	5
^a Naturally					
degraded/removed	61	75	75	74	75
^b Chemically dispersed		8	16		8
Naturally dispersed		16	13		
Dispersed		24	29		
Evaporated/dissolved		25	24	5	
Sheens/slicks				10	
Residual/unaccounted		26	22	^d 24	
Underwater plume				35	

a) Naturally degraded/removed oil was not listed in BP, 2010 or USCG, 2011. For these numbers the percentages given were subtracted from 100, and the remainder listed.

b) Chemically dispersed oil was considered cleaned in conservative estimates of waste regulation values but naturally degraded for remaining calculations.

c) This number represents "oily liquid" collected which would include seawater.

d) Residual amounts were not given in Ryerson et al., 2012. For these numbers the percentages given were subtracted from 100, and the remainder listed.

CHAPTER V: CONCLUDING SUMMARY

In the past, human activities in the marine environment have been limited to shallow, coastal areas due to technological constraints associated with accessing the deep sea. With increases in demand and over-exploitation of more easily accessible resources, human activities have pushed further offshore and into deeper waters. The ability of humans to impact the deep sea was brought to light in the 2010 Deepwater Horizon oil spill. The release of millions of barrels of oil in the deep Gulf of Mexico and need to assess damages from the spill helped to illustrate not only the lack of knowledge on human effects in the deep sea, but also the lack of baseline data on deep-sea functions necessary to examine human effects. Using samples collected during the DWH response and knowledge of deep-sea floor areas impacted by the spill, Chapter II examined how macrobenthic communities in the deep GoM were impacted by the DWH spill. It was hypothesized that the spill would have impacts on benthic community measures including abundance, taxa richness, and taxa diversity. Community composition was also analyzed in an attempt to identify specific taxa which may serve as indicators of both areas in the deep Gulf impacted by anthropogenic deep-sea oil releases and areas representative of deep Gulf soft-bottom background conditions.

Differences were found in macrobenthic communities between deep-sea areas within the impact zone of the DWH spill and areas outside this zone representative of background softbottom habitat. Macrobenthic abundance, richness, evenness, and diversity were all significantly lower in the area closest to the wellhead compared to all other zones. Diversity and evenness were highest in the zones furthest from the wellhead while abundance and richness were highest at intermediate distances. This suggests that while the spill may have been highly toxic in close proximity to the wellhead where oil concentrations were highest, there may have also been an

enrichment effect at intermediate distances with an increase in abundances of taxa able to survive the impacts of the spill and utilize the increased organic matter. Within the most heavily impacted zone all benthic metrics measured increased with increased distance from the wellhead. The impacts of the spill on benthic communities out to at least 10 km from the wellhead illustrates a major difference between the impacts of deep-sea spills and the impacts from normal drilling activity, which have generally been found to occur within 200 – 500 m of the drilling site.

Various benthic taxa were identified as either indicative of a deep-sea oil spill or nonimpacted soft-bottom conditions in the deep GoM. Over three times as many taxa were associated with background communities compared to spill communities, highlighting the negative effects of the spill on the benthos. Taxa sensitive to the spill were comprised largely of crustacean taxa compared to polychaete taxa that made up a large majority of benthic abundances. Taxa found to be indicative of the spill, including the families Dorvilleidae and Thyasiridae, have been found indicative of natural seepage as well. Studies examining future anthropogenic oil releases in the deep GoM may be able to use the indicator taxa from Chapter II to identify an area impacted by a spill by simply looking at macrobenthic community composition. Future work on the value provided by deep-sea benthos will assist in converting these changes in benthic communities to monetary damages done to the deep sea environment by future subsurface spills.

While Chapter II explored the effects of anthropogenic oil in the deep GoM, due to the unique nature of the spill at depth and the large amount of oil naturally being released in the deep Gulf, it was not clear if the DWH oil spill would have impacts on the deep-sea benthos distinguishable from natural oil seepage. It was argued by some that if the effects of oil spills in

the deep GoM were similar to effects of natural seepage then the spill would not cause damage to the deep-sea environment. While the spill artificially changing soft sediment communities to resemble seeps could still be construed as damage, it would also make it difficult or even impossible to distinguish between impacts of the spill and impacts from the thousands of naturally occurring seeps. Thus Chapter III examined macrobenthos around several seeps in the Northern GoM to get a baseline for benthic communities associated with deep GoM seeps as well as compare communities near natural seeps to communities near the DWH wellhead. It was hypothesized that there would be differences in community measures and compositions between seeps and both background and spill-impacted areas.

Seep communities were explored at various locations throughout the Northern Gulf, but there are various types of seepage with different types of fluid being released (e.g., hydrogen sulfide and methane), different concentrations of fluid being released, different underlying geology, and different ages of seeps. Chapter III examined three different types of hydrocarbon seep communities identified by the associated epibenthic megafauna: microbial mat, tubeworm, and soft-bottom seeps. In general the presence of different epibenthic communities at seeps corresponds to differences in released fluid concentrations. Macrobenthic infaunal diversity was lower and abundance higher at seeps associated with mats compared to other seep types. When examining community composition, nearly every seep had different taxa associated with similarity within a given seep. This was not only true at the family level but also at the phylum level, with some seeps dominated by polychaetes, others by crustaceans, and still others by mollusks. Community structure was similar for background habitats sampled with three different methods (boxcorer, multicorer, and ROV core). Seep communities, which were sampled by similar methods, were as dissimilar from other seep communities as they were to

background communities. Natural seep and oil spill community comparisons had similar results with communities in the spill impact zone more similar to one another and different from seep communities while seep communities were as different from one another as they were to communities near the DWH spill.

The attempt to define taxa associated with hydrocarbon seepage in the Northern Gulf yielded few similarities among seeps. The lack of similarity, or high variability, among seeps appeared to be the best indicator of seep communities. This high variability among seep communities is likely due to the spatial, temporal, fluid, and faunal heterogeneity associated with seepage. While there were many differences between spill and seep communities, there was not a general description of seep macrobenthic community structure. The variability among seeps highlights the unique nature of each seep assemblage examined. Future efforts to preserve unique deep-sea habitats may increase the preservation of diversity by protecting many small areas of seepage throughout the GoM rather than focus efforts on a few large seep areas. The differences found between seep and spill communities can also be used as evidence to refute the claim of natural seepage masking spill effects.

After Chapters II and III found impacts from the spill on deep GoM macrobenthic communities and differences between communities associated with natural and anthropogenic oil, another question arose. Why should people care about impacts to the deep sea? If changes in the deep-sea environment do not translate to effects to humans, then are these changes important? In order to answer these questions, Chapter IV examined benefits humans derived from the offshore marine environment. Because of the focus of this dissertation, waste regulation was further examined to put a rough monetary value on this service in the context of the removal of oil by the environment after the DWH spill.

Monetary valuation of the natural degradation of anthropogenically released oil in the deep sea was performed by comparing the cleanup costs and amount of oil removed by human activities associated with the DWH oil spill. The fate of some oil is still unclear, as was whether chemically dispersed oil was considered removed from the system or still in need of degradation so ranges of oil naturally degraded and removed by human activities were used. The amount of money used for cleanup efforts not associated with the removal of oil (i.e., relief wells and top kill efforts) was also not specifically listed so a range of values for cleanup costs were used. It was calculated that the natural degradation of oil by the GoM saved between ~\$25 - \$40 billion in cleanup costs, not including the value of dispersant and natural gas removal. While a rough estimate these values help to illustrate just one of the many ways that humans can and do benefit from deep sea ecosystem services.

Communities in the deep sea appear to exhibit a classic enrichment vs. toxicity effect when subjected to both anthropogenically and naturally released oil. However, oil spills appear to represent highly stressed areas while spills represent more moderately stressed areas. When examining the DWH spill, abundance and diversity were lowest closest to the wellhead where toxicity was highest. This was also true when comparing seep communities to spill communities. Likewise, when examining seep habitats, the most stressful seep type with highest sulfide and methane concentrations (microbial mat seeps) had the lowest diversity but highest abundances. Along a pollution gradient, areas closest to the source are often devoid of animals due to high toxicity, which was seen near the wellhead. As toxicity decrease, abundances peak but diversity is still low, which was seen at seeps. As toxicity and enrichment reach background levels, abundances are somewhat lower and diversity increases, which was seen at background areas.

This dissertation increased scientific knowledge on deep sea infaunal communities, which are relatively under-studied compared to most other habitats. Understanding the size and degree of impacts from deep-sea oil spills will assist in the assignment of damages not only for the DWH spill but also future spills, which are becoming more likely with increased drilling activity. While dispersants were released to mitigate damages done to surface and coastal habitats, the large amount of oil trapped in the deep sea likely increased damages in this habitat. Thus future efforts to stop deep-sea spills will hopefully take damages to deep-sea habitats into consideration before implementation. The differences found between impacts of natural and anthropogenic oil releases also undermines the argument that deep-sea oil spills will have little to know observable impacts in areas associated with natural hydrocarbon seepage. Baseline information on seep communities in the Gulf will also assist with the identification of damages caused to these communities by nearby spills. The high variability among seep communities in the Gulf will be helpful in the designation of marine protected areas meant to conserve diversity as well as the identification of currently unknown seep habitats. The variability among seeps also suggests that seep communities, and the deep sea in general, may be repositories for a substantial amount of global biodiversity. Finally, while public knowledge on deep-sea ecosystems may be minimal, increased knowledge on the offshore may help the public place more value on and increase conservation efforts in the deep sea. This knowledge may also assist the public in holding people accountable for damages they cause to this environment. The billions of dollars saved by offshore processes following the DWH spill was just one example to illustrate how valuable this environment can be to humans.