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Methods for the Characterization of Deep-Sea Benthic Megafauna in the Vicinity of the Deepwater Horizon Macondo Well

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METHODS FOR THE CHARACTERIZATION OF DEEP-SEA BENTHIC MEGAFAUNA IN THE VICINITY OF THE DEEPWATER HORIZON MACONDO WELL

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This dissertation is dedicated to my parents, who have provided continual and unwavering love and support in the pursuit of my dreams over the years.

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ABSTRACT

Soft-sediment benthic environments are amongst the largest marine ecosystems in the world and play important roles in many ecosystem functions. In recent years, exploitation of resources and unintentional impacts on deep-sea benthic environments has increased. The Deepwater Horizon oil spill of 2010 in the northern Gulf of Mexico (GoM) represented a prime example of this. The oil spill not only highlighted deficiencies of data and information on baseline conditions, but also represented an opportunity to learn more and develop better methods for the future. Deep-sea imaging platforms such as autonomous underwater vehicles (AUVs) and remotely operated vehicles (ROVs) have been growing in popularity as a minimally invasive means of exploring the deep-sea; and the use of industrial resources has increased due to availability of these technologies in the GoM region. This dissertation explores the use of industrial-based AUVs and ROVs as a means of studying benthic megafauna in the vicinity of the Deepwater Horizon Macondo well, and outlines a methodology that can be applied to current and future environmental monitoring efforts. Industrial ROVs were found to be generally superior to AUVs for specifically studying benthic megafauna. Simulations comparing different radial survey designs found that designs featuring longer transects with smaller transect spacing were more effective at estimating animal populations. In particular, the 15°, 250 m long transect radial survey design employed by the ROVs in this study was found to perform well for surveying benthic megafauna. To improve collection and analysis of the wealth of data extracted from the imagery, a customized database system was developed for use in this study and for similar future studies. Data collected via ROV one year after the oil spill was used to characterize benthic megafaunal communities and evaluate potential influences on them. It was found that community composition was primarily related to depth and, to a lesser degree, location in the northern GoM and anthropogenic disturbance to the seafloor. Overall, the methodology and results explored here represent an opportunity to standardize and improve future environmental monitoring efforts of this kind in the GoM and beyond.

CHAPTER 1. GENERAL INTRODUCTION

1.1. Deep-Sea Soft-Sediment Environments and Benthic Megafauna

Ocean habitats are divided into two zones: pelagic (the water column) and benthic (the seafloor). Benthic habitats associated with the mesopelagic (200-1000 m) and bathypelagic zones (1000-4000 m) are generally considered to include those at continental slope depths from about 200 to 4000 m depth. These deep-sea environments are harsh. They feature cold temperatures (< 3-4°C), high pressures (300 to 600 atmospheres at abyssal depths), and are impenetrable by sunlight (Gage and Tyler, 1991; Rex and Etter, 2010; Miller and Wheeler, 2012).

Soft-sediment environments comprise some of the largest benthic ecosystems in the world, with much of the deep ocean bottom being covered in sediments (Miller and Wheeler, 2012). These environments are dominant in the deep-sea, encompassing vast ranges across the continental shelf and into the deepest parts of the ocean across the abyssal plain and oceanic trenches. Composition of the sediments can vary greatly based on location, distance from land, and depth. Large grain size and highly porous sediments, such as sand, are more common in shallower coastal benthic environments. Deeper areas of the ocean that are further away from land are generally comprised more of fine muds, silts, and siliceous and calcareous oozes.

Living in soft-sediment ecosystems are a wide variety of animals, including demersal (on or near the bottom), epifaunal (living on the surface of the seafloor), and infaunal (living in the seafloor sediments) species. Demersal and epifaunal benthic megafauna (also referred to as megabenthos) are generally considered to include organisms that live at the sediment-water interface and are greater than 1 cm in size, or are visible with a camera (Grassle et al., 1975; Gage and Tyler, 1991). These animals are often classified and discussed in terms of their motility. Mobile benthic megafauna are those that can readily move around and generally include a variety of fishes, crabs, and shrimps. Low mobility animals (e.g. holothurians, sea stars) typically move slowly through the benthic environment. Sessile animals like anemones spend most of their life history immobile on the seafloor or attached to objects (Gage and Tyler, 1991; Rex and Etter, 2010; Miller and Wheeler, 2012).

Many of the animals living in benthic environments are deposit feeders that ingest sediments and extract organic matter as a source of nutrition. Other animals include suspension or filter feeders that are attached to objects on the seafloor or the seafloor directly and obtain food by passively or actively filtering water. In some deep-sea environments, predators and scavengers that feed on other animals or remains are more common (Gage and Tyler, 1991; Rex and Etter, 2010; Miller and Wheeler, 2012). Despite often low abundances, megabenthos play an important role in the deep-sea environment by contributing substantially to benthic biomass and playing an important role in a number of ecosystem processes (Meyer et al., 2013).

Expansion of human activities into the deep-sea has made it necessary to continually increase our knowledge of deep-sea environments and the benthic megafaunal communities that live there. As a result, investigations into megafaunal abundances and biodiversity need to be coupled with evaluations of natural environmental factors and the impacts of anthropogenic influences on marine communities as a whole. Understanding variability in environmental conditions and corresponding influences on species composition is important because it contributes to insights into ecosystem functioning; therefore, how ecosystem processes may be impacted by changes to the benthic environment (Bremner et al., 2006).

1.2. Natural Influences on the Benthic Environment

A variety of natural environmental factors (e.g. oceanographic parameters such as temperature, oxygen concentration and currents, along with seafloor characteristics such as substrate type, sediment composition, grain size, and disturbance) can influence abundances, diversity and distributions of benthic organisms (Grassle, 1991; Etter and Grassle, 1992; Engle and Summers, 1999; Levin et al., 2001; Williams et al., 2010; Pitcher et al., 2012). Depth is a well-known factor influencing benthic faunal composition in the deep-sea (Rex, 1981; Pires-Vanin, 2001; Barry et al., 2003; Miller and Wheeler, 2012). Substrate type often plays an important role in determining the types and quantities of benthic fauna present. More specifically in the case of soft-sediment deep-sea environments, it is often sediment composition and texture that influence the megabenthos (Pires-Vanin, 2001; Barry et al., 2003; Jones et al., 2007a; Miller and Wheeler, 2012).

Furthermore, food supply, which is generally tied to ocean surface productivity and changes with depth, is often considered a highly limiting factor that can greatly influence benthic communities (Grassle, 1991; Miller and Wheeler, 2012). Food production via photosynthesis is not possible because sunlight does not reach the deep-sea. Most benthic communities, with the possible exception of chemosynthetic communities, rely largely on surface production as a primary food source. Organic carbon flux from surface waters reaches the deep-sea primarily in the form of marine snow drifting down through the water column. The further these materials move down through the water column, the more they are scavenged. This results in a decrease in abundance by the time these materials reach the deep-sea floor. It has been suggested that on average only about 1-2% of surface production actually reaches the seafloor and this can greatly influence benthic communities that rely on this source of nutrition (Miller and Wheeler, 2012). Since changes to oceanographic parameters, nutrient input, and surface productivity often exhibit seasonal variations, the resulting impacts on trophic web dynamics also have the potential to contribute to seasonal variations in megabenthos communities (Kojima and Ohta, 1990; Cartes, 1998; Pires-Vanin, 2001; Gooday, 2002; Sumida et al., 2008; Corliss et al., 2009; Glover et al., 2010; Rowe, 2013).

Benthic diversity trends vary globally but often exhibit a mid-depth maximum along continental margins at depths between 1500 to 2500 m (Rex, 1983; Grassle and Maciolek, 1992; Levin et al., 2001; Rex and Etter, 2010). Animal abundances and biomass typically decrease with depth (Rex et al., 2006). There are different theories as to what mechanisms govern diversity, abundances, and biomass of benthic fauna. As food supplies dwindle and the ocean environment becomes harsher at deeper depths along the continental margin, organisms are required to adapt in order to accommodate the additional limitations being placed on them. In many cases, organisms become smaller in size with depth, which contributes to an overall decline in biomass. Adaptations for survival will ultimately facilitate greater diversity largely through specialized biological interactions such as those that lead competitive niche diversification (Gage and Tyler, 1991; Rex and Etter, 2010; Miller and Wheeler, 2012). This is intimately linked to the popular spatial mosaic theory (Thompson, 2005), which proposes that small-scale disturbances at the seafloor (in an otherwise generally stable deep-sea

environment) allow for high local diversities through creation of successional sequences that are temporally out of phase.

These concepts are encompassed by the two main groups of theories on the maintenance of high diversity in the deep-sea: equilibrium vs. disequilibrium processes. Equilibrium-based explanations suggest that resource partitioning combined with general stability of the deep-sea environment promote diversity. When conditions become less stable, it has been suggested that stress will instead favor tolerance to a wide range of conditions and thus limit diversity. It is hard to fully support this hypothesis since the deep-sea environment, particularly along the continental slopes, where conditions are not necessarily stable and prone to disturbances from natural physical events that can cause erosion, transport and re-deposition of sediments (Rex, 1981; Grassle and Morse-Porteous, 1987; Gage and Tyler, 1991; Rex and Etter, 2010).

On the other hand, disequilibrium may help better to account for disturbance as a cause of observed peaks in diversity on the continental slope. As the interval between disturbances increases, diversity will initially increase due to more time available for immigration and colonization from background communities; yet as the interval increases further, a decline in diversity will eventually begin due to competitive interactions and exclusions. Therefore, areas along the slope that experience more disturbance will have a consequent increase in diversity that will then once again drop back off as the more-stable and food-limiting environment of the abyss is approached (Gage and Tyler, 1991; Rex and Etter, 2010).

1.3. Anthropogenic Impacts

The growth of human activities into the deep-sea has made it necessary to expand beyond traditional investigations of natural environmental factors and into also evaluating impacts of anthropogenic influences on marine communities. One of the primary types of potential anthropogenic influences is in the form of physical disturbances to the seafloor including those from fishing and industrial offshore exploration and extraction activities. Widespread commercial fishing has led to many studies that have investigated the impacts of physical disturbance by fishing gear on the seafloor and associated communities (Kaiser et al., 1998; Thrush and Dayton, 2002; Hermsen et al., 2003; Asch and Collie, 2008). These studies have

shown that benthic communities are often impacted by disturbance, resulting in different abundances, diversity and overall community compositions associated with disturbed areas. Recent studies have also evaluated impacts and recovery of benthic megafaunal communities in response to anthropogenic disturbances associated with offshore hydrocarbon drilling wells, with similar results (Jones et al., 2006; Jones et al., 2007a,b; Jones et al., 2011; Gates and Jones, 2012; Jones et al., 2012).

1.4. Challenges for Studying the Deep-Sea Environment

Increasing demands on global resources has resulted in a push to explore and develop resources deeper in the ocean. Pressure from fisheries and offshore industries is increasing. The impact of humans on deep-sea resources has grown considerably (Gage, 2001; Pinder, 2001; Glover and Smith, 2003; Redden, 2010; Ramirez-Llodra et al., 2011). There is a need to develop standardized methods for surveying and monitoring deep-sea ecosystems in order to better assess and interpret possible impacts of anthropogenic activities. An important component of environmental monitoring activities is the ability to compare newly acquired and existing data. This is challenging when data collected during different studies is spread out across many sources and is not always readily accessible by everyone. Often, this is the case when data is considered sensitive or confidential in nature and there is a consequent hesitancy to make the data widely available.

Despite this need for information, monitoring in the deep-sea still proves challenging in many ways. One of the biggest reasons is that depth presents a major logistical obstacle. Also, visibility can limit the types and quality of visual data that can be obtained, particularly in soft-sediment environments like those of the northern GoM. These benthic environments are even more challenging for exploration and monitoring. While depth and visibility due to lack of light are known to be complicating factors in all deep-sea studies, these environments often have additional complications resulting from the nature of the substrate itself. Unlike hard seafloor substrates, softer sediments are easily disturbed and re-suspended by currents, animal activities, sampling equipment, or other disturbances. This can impose major limitations on seafloor visibility, especially when combined with the presence of large quantities of marine snow.

1.5. Sampling the Benthic Environment

What some may consider as the most significant challenges for deep-sea research are the relatively limited availability of deep-sea technologies and the overall invasiveness of the most common sampling procedures. Benthic sampling of megafauna traditionally employs the use of disruptive sampling methods capable of easily disturbing the sampling area (Miller and Wheeler, 2012). These methods include nets (Husebø et al., 2002), trawls (Rowe and Menzel, 1971; Haedrich et al., 1980; Gordon and Duncan, 1985; Escobar-Briones and Soto, 1997; Powell et al., 2003), and traps (Gage and Bett, 2005). This creates a challenge when conducting environmental monitoring studies where it is ideal to study a desired environment without causing any impacts to that environment. Advancements in technology have prompted a shift away from these traditional methods of studying benthic megafaunal communities. As a result, deep-sea technologies including acoustic-based technologies, towed cameras, autonomous underwater vehicles (AUVs), and remotely operated vehicles (ROVs) are being increasingly considered for and used in benthic monitoring programs (Miller and Wheeler, 2012).

Continual development of deep-sea technologies has contributed greatly to the evolution of deep-sea ecosystems studies. Some deep-sea technologies have the ability to provide a wealth of information on seafloor features, but result in data that is more general in nature. High resolution side-scan sonar (McRea et al., 1999; Kendall et al., 2005; Brown and Collier, 2008), multi-beam sonar (Kostylev et al., 2001; Kendall et al., 2005; Whitmire et al., 2007), and laser line scan (Amend et al., 2007) have been shown as useful means of mapping out larger areas of seafloor, but are limited in their ability to collect the detailed imagery that is necessary when studying benthic communities.

Deep-sea imagery-based platforms have been gaining popularity in recent years. These technologies have an advantage over more traditional sampling methods because they can be used more easily in areas with seafloor habitats in which trawls and nets are considered too difficult to use (Tolimieri et al., 2008; Clarke et al., 2010). AUVs and ROVs represent the two primary platforms used for deep-sea investigations that aim to visualize benthic ecosystems. AUVs are programmable, free-moving robotic vehicles that do not require any real-time control

by human operators. ROVs are robotic submersibles tethered to vessels and require human operation by ROV pilots who remotely control their movement and actions.

The rise of these more advanced deep-sea technologies has resulted in a need to determine how these technologies can be used for evaluation of deep-sea benthic habitats and faunal community characteristics. Some studies aim to classify and map benthic habitats and bottom features in order to predict where important habitats or species are for the purposes of science and management activities. Studies involving multiple technologies traditionally focus on how one particular technology can be used to validate another. For example, Kendall et al. (2005) conducted a study using scuba and towed camera video transects as a means of validating side-scan sonar data. Brown and Collier (2008) similarly used underwater video surveys to ground-truth side-scan sonar data for the purposes of benthic habitat mapping in regions of gradational substrata. This approach of using imagery-based technologies as means of validation or ground-truthing of sonar-derived data appears to be a common trend.

Other studies gaining popularity aim to evaluate the use of deep-sea technologies for determining specific habitat or faunal characteristics. This is becoming increasingly important for improving temporal and spatial monitoring of important habitats or species. As an example, Tolimieri et al. (2008) have evaluated *SeaBED* AUV for the use of monitoring groundfish in rocky, untrawlable areas of the U.S. West Coast. Similarly, video and photo transects derived from a towed camera were completed in a submarine canyon environment by Schlacher et al. (2009) in order to determine species diversity and bottom cover. Trenkel et al. (2004a,b) have specifically evaluated ROV video transects in context of their ability to provide population density estimates for deep-water fishes.

Many recent benthic studies use a combination of different deep-sea technologies to determine multiple parameters that are then combined to create benthic habitat maps that include both seafloor characteristics as well as benthic faunal information. Kostylev et al. (2001) used a combination of multi-beam bathymetry and geophysical profiling technologies in conjunction with seafloor photographs to create benthic habitat maps of the Scotian Shelf. Other studies have aimed to compare different sampling technologies in order to determine

the accuracy and effectiveness of each for particular types of sampling. One particular example of this is a study in which Cailliet et al. (1999) compared fish faunal and habitat data collected via trawls, camera sleds and submersibles. Similarly, Trenkel et al. (2004a,b) have compared the ability of trawls and ROVs to determine deep-water fish population data.

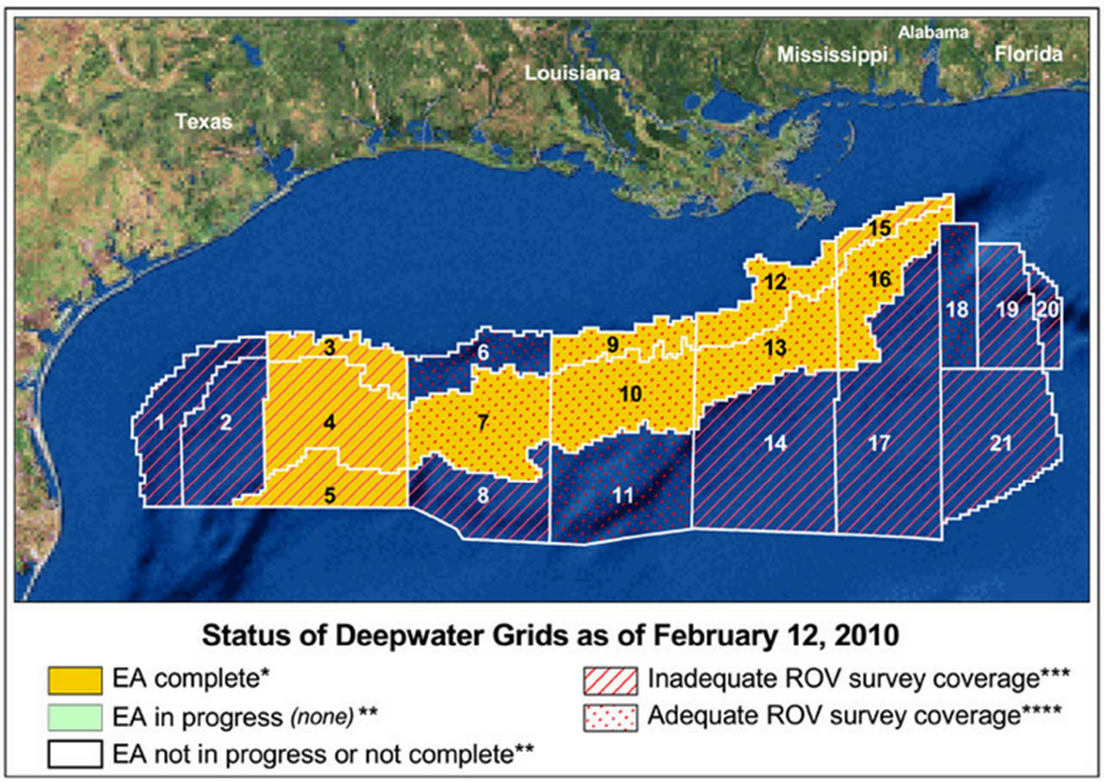
Photographic surveys of benthic megafauna are typically conducted according to one of several survey designs. Strip transects are often used, particularly when using towed cameras (Jones et al., 2007; Schlacher et al., 2009; McIntyre et al., 2013) or AUVs for collection of photographic data. This survey design is commonly practiced for habitat mapping and assessment studies because strip transects consisting of overlapping photographs can be readily patched together into larger-scale photomosaics. These photomosaics provide a continuous view of the seafloor, and minimize gaps in information on the benthic environment being explored.

Industrial-based technologies such as work class ROVs are becoming more common due to their availability and proximity to areas of interest for studying impacts of anthropogenic activities on benthic communities. Use of industrial ROVs has led to an increase in the number of radial survey designs employed in benthic studies because of the overall practicality of these configurations. Studies employing radial survey designs using industrial ROVs have been conducted on several occasions in order to determine impacts of anthropogenic disturbances associated with drilling activities on deep-sea benthic megafauna (Jones at al., 2007), as well as the recovery of these communities from disturbances (Gates and Jones, 2012; Jones et al., 2012; Valentine and Benfield, 2013).

The U.S. Department of the Interior's Bureau of Ocean Energy Management (BOEM) has established guidelines associated with meeting statutory responsibilities under the National Environmental Policy Act (NEPA) related to development of outer continental shelf oil and natural gas resources. A biologically based grid system (Fig. 1.1) was created in the deepwater northern GoM for use with environmental impact statements (EISs) and environment assessments (EAs) related to offshore activities. A major part of the EIS and EA requirements includes ROV surveys within these grids. These surveys allow for expansion of general knowledge on deepwater benthic habitats in the area and protection of sensitive or densely

populated hard-bottom communities, as well as monitoring of the effects of particular activities on these habitats (MMS, date unknown).

Amongst other regulations, BOEM requires two limited ROV visual habitat surveys to be completed before and after drilling operations. These surveys consist of recording biological and physical information, generally including the types of animals present and appearance of the seafloor (BOEM, 2011). It is recommended that the ROV be flown close enough to the seafloor to observe relatively small animals and features. Six survey track lines radiating from a central point of origin at intervals of 60° and extending at least 100 meters from the launch point are the minimum survey requirements.



* This grid is no longer considered a "relatively untested deepwater or remote area. "You may omit the following information per NTL 2008-G04:
1.Waste and Discharge Information (�� 250.217 and 250.248) (a) Projected generated wastes. (5) Deepwater development operations.
2.Waste and Discharge Information (�� 250.217 and 250.248) (b) Projected ocean discharges. (4) Deepwater development operations.
3.Support Vessels and Aircraft Information (�� 250.224 and 250.257) (d) Solid and liquid wastes transportation. (4) Deepwater development operations. Preparation of a Grid EA is not applicable to Grids 18, 19, 20, or 21.

** This grid is still considered a "relatively untested deepwater or remote area. "You must submit the following information per NTL 2008-G04: 1.Waste and Discharge Information (�� 250.217 and 250.248) (a) Projected generated wastes. (5) Deepwater development operations. 2.Waste and Discharge Information (�� 250.217 and 250.248) (b) Projected ocean discharges. (4) Deepwater development operations. 3.Support Vessels and Aircraft Information (�� 250.224 and 250.257) (d) Solid and liquid wastes transportation. (4) Deepwater development operations. Preparation of a Grid EA is not applicable to Grids 18, 19, 20, or 21.

*** You must submit a plan for an ROV monitoring survey per NTL 2008-G04.

**** You do not have to submit a plan for an ROV monitoring survey per NTL 2008-G04.

Fig. 1.1. BOEM grid EA and ROV survey status report (BOEM, 2010).

1.6. Studies in the Northern Gulf of Mexico

Over the last half-century, human activities have continually expanded throughout the GoM as the demand for fisheries and energy resources has created a push to go deeper into the ocean. This has underscored the necessity for reducing deficiencies of deep-sea benthic ecosystem data, particularly as the need for data on baseline conditions increases. Baseline data such as species abundances and diversity, as well as community composition and dynamics are important for use, not only for general long-term environmental monitoring, but also for investigating anthropogenically-induced environmental impacts. Despite being one of the most economically important deep-sea regions in the world, the GoM often lacks adequate data on baseline conditions associated with benthic megafauna.

A variety of studies of benthic fauna have been conducted in the GoM over the past several decades. Yet, the focus of these studies has not necessarily been on megabenthos associated with soft-sediment areas of the seafloor. Some studies have focused on unique benthic communities associated with hydrocarbon seep sites (MacDonald et al., 1989; MacDonald et al., 2010) or corals, while many others have aimed to study infaunal communities (Rowe and Menzel, 1971; Flint and Holland, 1980; Montagna and Harper, 1996; Escobar-Briones and Soto, 1997; Hernández-Arana et al., 2003; Hyland et al., 2003). Despite an overall lack of published GoM studies, recent studies of deep-sea macrobenthos in the GoM were conducted as part of the Deep Gulf of Mexico Benthos (DGoMB) project (Powell et al., 2003; Rowe and Kennicutt, 2009). DGoMB described benthic and benthopelagic communities in the GoM, along with evaluating various biological, physical, and environmental processes influencing the structure and function of these communities.

Overall, the GoM has been recognized to have a paucity of benthic megafauna compared to other deep-sea benthic ecosystems. Dredge and photographic surveys conducted by Rowe and Menzel (1971) in the deep GoM indicated that deep-sea benthic fauna numbers and biomass are relatively low. In this study, noted variations in abundances were attributed to differences resulting from local patchiness. Another trawl study as part of the DGoMB studied deep-sea demersal fish fauna and observed similar low abundances (Powell et al., 2003). This study also noted that fish fauna assemblages exhibited depth zonation. It was found that the most

abundant shelf species were small caproids, Macrouridae, and Steindachneriidae. Mid-slope fauna were dominated by Macrouridae, and Ophidiidae were found to be predominant in the deep zone. Once again, species richness and abundance were found to be greater in shallower zones, with lower abundances at depth. Escobar-Briones and Soto (1997) found that the predominant shelf epibenthic macrofauna in the GoM included a diverse group of decapod crustaceans and stomatopods, as well as brachyuran crabs and penaeid shrimp. In general, GoM studies have noted benthic biomass exhibits a decrease with depth and distance from the coast (Rowe and Menzel 1971, Escobar-Briones and Soto 1997, Powell et al. 2003).

In the northern Gulf of Mexico (GoM), collection of imagery data has been growing. ROV videos are being collected as part of the SERPENT (Scientific and Environmental ROV Partnership Using Existing iNdustrial Technology) Project. Some recent studies using industrial ROVs have also been conducted to specifically investigate deep-sea benthic megafauna in the vicinity of the Deepwater Horizon Macondo well immediately after the oil spill of 2010. Valentine and Benfield (2013) reported on benthic megafaunal abundances and diversity obtained from surveys conducted in August 2010 at five sites located within 2000 m of the Macondo well. Red shrimps, mobile holothurians from the family Elpididae, and the red crab *Chaceon quinquedens* generally comprised the most abundant mobile invertebrates. Sea stars represented the most abundant of the sessile or low mobility invertebrates. The most abundant fishes were observed to be halosaurs, *Synaphobranchus* and *Facciolella* eels, the tripod fish *Bathypterois quadrafilis*, and two cusk eels, *Bassogigas gillii* and *Dicrolene* sp. (Valentine and Benfield, 2013).

1.7. Deepwater Horizon Oil Spill of 2010

The 2010 Deepwater Horizon oil spill incident has underscored the need for more information on northern GoM ecosystems and, in particular, for additional data on benthic ecosystems in soft-sediment environments in this area. Deepwater Horizon was a semi-submersible rig located in Mississippi Canyon block 252 (MC252), approximately 50 miles southeast of the Mississippi River delta. Well control was lost on April 20, 2010, resulting in an explosion and sinking of the rig on April 22, 2010. Oil and gas from the Macondo Prospect oil field flowed from the well until it was finally capped on July 15, 2010. While the exact amount of oil and gas spilled into the GoM as a result of this accident has been widely debated, the Deepwater

Horizon oil spill has been acknowledged as the largest oil spill in the history of the United States (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2011).

In the wake of the spill, there was a marked increase in demand for information on GoM ecosystems, including the lesser known deep-sea megabenthos. This became even more important as a necessary part of environmental monitoring of areas potentially affected by the spill in order to determine how GoM marine communities have been affected and will recover in the future. A prominent issue that has arisen is the difficulty in evaluating impacts of environmental incidents and subsequent recovery of benthic ecosystems without having adequate data. This is particularly an issue in situations where baseline data are limited. The question of which technologies are most suitable for collecting sufficient benthic ecosystem and megafaunal data for environmental monitoring activities needs to be explored more thoroughly.

1.8. Objectives

In order to facilitate more effective investigations of potential impacts and recovery of megabenthos in the wake of an environmental incident such as the Deepwater Horizon oil spill, methods for the characterization of deep-sea benthic megafaunal communities need to be explored. Expanded use of imagery-based technologies in deep-sea studies has further added precedence for evaluating these technologies specifically for studying the megabenthos. This dissertation includes the following four components related to the use of imagery-based technologies for investigating deep-sea benthic megafaunal communities in the vicinity of the Deepwater Horizon Macondo well:

1. A 15° radial benthic survey methodology that can be used by industrial ROVs to survey deep-sea benthic sites at a range of locations around the MC252 well site is described. This methodology is intended to be used as part of environmental monitoring to observe the deep-sea benthic ecosystem and evaluate potential responses to the oil spill. The effectiveness of the 15° radial survey design was evaluated and compared to other radial designs including the 60° BOEM design, along with showing how it can be used to investigate benthic communities. Simulations were completed to evaluate

detection of theoretical random and clustered organism populations by ten radial survey designs of different transect lengths and degrees of separation between transects. Preliminary results exemplifying how this methodology can be used for observing and monitoring deep-sea benthic environments and associated megafauna are presented using a case study of benthic surveys completed at one study site (MC118) in the northern GoM. An initial set of surveys was conducted at this site prior to crab-trapping that took place as part of a Natural Resource Damage Assessment (NRDA) research cruise; and the results of these surveys were compared to follow-up surveys conducted after crab-trapping was completed. This provided an opportunity to explore the effectiveness of the 15° radial survey design in a situation of known impact to the megabenthos.

2. Industrial AUV and ROV surveys were evaluated and compared with respect to their abilities to collect benthic megafaunal community and habitat information in reduced-visibility soft-sediment environments that are prominent throughout much of the northern GoM. The effectiveness of the industrial AUV and ROV technologies was evaluated using imagery collected at two study sites (MC252 2000 m N and MC208) near the Macondo well. Several aspects of the imagery collected were investigated, including: 1) quality and detail of imagery; 2) ability to detect and identify different kinds of organisms, and consequently determine megafaunal abundances and diversity; and 3) ability to identify and distinguish aspects of the seafloor habitat, including visual characterization of sediment composition, seafloor features, and areas of physical disturbance to the seafloor.

3. A simplified database system approach was developed for use with imagery obtained by a variety of deep-sea technologies in the northern GoM. The purpose of this database system is to provide an efficient, un-complicated option for analyzing and storing a variety of data extracted from imagery. This data ranges from habitat and other environmental variables to anthropogenic influences and information on megafaunal communities. The database system is also specifically applicable for use with imagery

acquired during industrial environmental surveys since was originally designed for analysis of industrial ROV video imagery collected during NRDA cruises in response to the Deepwater Horizon oil spill. Examples of types of data extracted from ROV imagery are presented along with a description of the database system approach used for imagery and corresponding data analysis. Further applications and future developments of the database system are also discussed.

4. Benthic megafaunal community compositions were quantified and compared across seven study sites using the results obtained from a set of industrial ROV surveys conducted in 2011, approximately one year after the Deepwater Horizon oil spill. The ROV surveys were collected using the 15° survey design and analysis of the resulting imagery including use of the previously-mentioned database system. The seven sites evaluated are located in the northern GoM, at varying distances and directions from the Deepwater Horizon MC252 wellhead. Several natural and anthropogenic environmental variables observable in the ROV imagery were also evaluated in order to provide insights into possible factors contributing to observed variations in benthic megafaunal communities in the northern GoM.

This dissertation is structured such that it includes a general introduction (Chapter 1); four body chapters (Chapters 2-5) that represent stand-alone reports on individual projects comprising the dissertation research; and a summary conclusion (Chapter 6).

CHAPTER 2. A COMPARATIVE ANALYSIS OF IMAGERY FROM AUVS AND ROVS FOR EVALUATING MEGABENTHOS IN SOFT-SEDIMENT ENVIRONMENTS

2.1. Introduction

Expansion of human activities into the deeper ocean has facilitated a need for enhanced abilities to explore and monitor deep-sea ecosystems. Pressure from fisheries and offshore oil, gas and mining industries is increasing; and the impacts of humans on deep-sea resources has grown considerably (Gage, 2001; Pinder, 2001; Glover and Smith, 2003; Redden, 2010; Ramirez-Llodra et al., 2011). Benthic sampling of deep-sea megafauna traditionally employs the use of disruptive sampling methods such as nets (Husebø et al., 2002), trawls (Rowe and Menzel, 1971; Haedrich et al., 1980; Gordon and Duncan, 1985; Escobar-Briones and Soto, 1997; Powell et al., 2003), and traps, which disturb the sampling area. This creates a challenge for environmental monitoring where it is ideal to study the environment without causing any impacts. Because of this, deep-sea platforms like acoustic-based technologies, towed cameras, autonomous underwater vehicles (AUVs), and remotely operated vehicles (ROVs) are being increasingly considered for use in benthic monitoring programs.

Continuous technological developments have contributed greatly to the evolution of deep-sea ecosystems studies. High-resolution side-scan sonar (McRea et al., 1999; Kendall et al., 2005; Brown and Collier, 2008), multi-beam sonar (Kostylev et al., 2001; Kendall et al., 2005; Whitmire et al., 2007), and laser line-scan (Amend et al., 2007) technologies are useful means of mapping out larger areas of seafloor. The data retrieved by these methods frequently shows enough detail that general habitat features can be evaluated, particularly in situations where rocky or other distinguishable features are present; however, these technologies often do not provide enough detail of the seafloor and associated fauna as would be ideal for studying specific benthic habitat or faunal characteristics.

As a result, deep-sea imaging platforms are being used with increasing frequency because of their ability to collect detailed imagery that is both qualitatively and quantitatively meaningful in nature. AUVs and towed camera systems have been used in a wide variety of surveys of

benthic habitats and associated megabenthos, including off the U.S. coast (Rowe and Menzel, 1971; Cailliet et al., 1999; Kendall et al., 2005; Moline et al., 2007; Tolimieri et al., 2008; Clarke et al., 2010), Canada (Schneider et al., 1987; Kostylev et al., 2001), and Australia (Schlacher et al., 2010; Williams et al., 2010a). ROV and submersible surveys likewise have the ability to encompass a wide range of benthic studies such as habitat mapping (Romsos et al., 2007; Brown and Collier, 2008), investigating unique deep-sea ecosystems (MacDonald et al., 1989; MacDonald et al., 2010), studying faunal community characteristics (Grassle et al., 1975; Cailliet et al., 1999; Beaulieu, 2001; Parry et al., 2003; Tissot et al., 2007; Gonzalez-Mirelis et al., 2009; Baker et al., 2012;), observations of animal behavior in response to deep-sea technologies (Lorance and Trenkel, 2006; Raymond and Widder, 2007; Stoner et al., 2008; Uiblein, 2011), and evaluating the effects of offshore drilling activities and consequent recovery of benthic fauna from disturbance events (Gates and Jones, 2012; Jones et al., 2012).

The rise of advanced deep-sea technologies developed for industry has resulted in a need to determine different ways in which these technologies can be used for evaluation of the deep-sea environment. Imagery-based platforms have been used to classify and map benthic habitats and bottom features, in order to predict where important habitats or species may be located for science and management activities. Other studies aim to evaluate the use of deep-sea technologies for determining more specific habitat or faunal characteristics. Imagery-based technologies have also been used as means of validation or ground-truthing of sonar-derived data. These types of applications are becoming increasingly important for improving temporal and spatial monitoring of important habitats or species. Despite many studies evaluating imagery-based platforms, deep-sea soft-sediment environments and associated benthic megafauna are often overlooked in favor of infauna or fauna associated with more fragile deep-sea ecosystems such as seeps and coral reefs.

Soft-sediment environments represent one of the largest marine ecosystems, especially in the deep-sea. These benthic environments are challenging for exploration and monitoring. While depth and low visibility due to lack of light are known to be complicating factors in all deep-sea studies, soft-sediment environments often have additional complications resulting from the nature of the substrate itself. Unlike hard seafloor substrates, soft sediments are easily stirred

up and re-suspended, whether by currents, animal activities, sampling equipment, or other disturbances. This can impose major limitations on seafloor visibility, especially when combined with the presence of large quantities of marine snow. It is thus important to evaluate different imagery-based platforms to determine which are more suitable for soft-sediment benthic studies under these lower-visibility conditions.

In the northern Gulf of Mexico (GoM) specifically, the 2010 Deepwater Horizon oil spill underscored the need for benthic ecosystem data for soft-sediment environments in the area. Deepwater Horizon, a semisubmersible rig, which was located approximately 50 miles southeast of the Mississippi River delta in the GoM, lost well control on April 20, 2010. The resulting explosion and sinking of the rig on April 22, 2010 led to oil and gas from the Macondo Prospect oil field flowing from the well at a depth of approximately 1500 m until it was capped on July 15, 2010. A prominent issue that has arisen in the wake of the spill is the difficulty in evaluating impacts of environmental incidents and subsequent recovery of benthic ecosystems without having adequate data, particularly when baseline data are limited. Furthermore, the question of which technologies are most suitable for collecting sufficient benthic ecosystem data for environmental monitoring activities needs to be explored more thoroughly.

Approximately one year after the spill, AUV and ROV surveys were conducted at sites in close proximity to the Macondo well as part of post-spill surveys. These surveys were important because, although they may not have provided data on conditions prior to the spill, they did provide information on the state of the benthic environment afterwards. As such, they contributed to a post-spill baseline to which future changes can be evaluated. Having imagery collected using AUVs and ROVs also presented an opportunity to compare the two platforms for collecting benthic megafaunal community and habitat information in the reduced-visibility soft-sediment environments prominent throughout much of the Northern GoM.

The goals of this study were to explore the effectiveness of industrial AUV and ROV technologies for studying deep-sea benthic megafaunal communities in soft-sediment environments in the northern GoM using imagery collected at two study sites (MC252 2000 m N and MC208) near the Macondo well. Several aspects of the imagery collected were

investigated: 1) quality and detail of imagery; 2) ability to detect and identify different kinds of organisms, and consequently evaluate organism abundances and diversity; and 3) ability to identify and distinguish seafloor habitat characteristics, including sediment composition, seafloor features, and areas of physical disturbance to the seafloor.

2.2. Methods

2.2.1. Image Acquisition

2.2.1.1. ROV

Two different sets of surveys using industrial ROVs (Table 2.1) were completed at two study sites – MC252 2000 m North of the MC252 wellhead (28°45’ 22.295” N 88°21’ 58.520” W) and MC208 (28° 45’ 55.102” N, 88° 22’ 00.779” W). The first set of surveys was conducted from the Transocean Development Driller 3 (DD3) on March 10, 2011 and March 11, 2011 for MC252 2000 m N and MC208, respectively. The second set of surveys occurred during Natural Resource Damage Assessment (NRDA) cooperative cruises conducted by the HOS Sweetwater in the northern GoM during June and August of 2011, respectively. Sweetwater ROV surveys were conducted at MC252 2000 m N on June 15-16, 2011 and MC208 was surveyed August 19-20, 2011.

Both the work-class Millennium 82 and Triton XLS 150 ROVs used in the DD3 and Sweetwater surveys, respectively, were equipped with multiple cameras, including a standard definition (SD) video camera, high definition (HD) video camera, and a digital still camera (DSC) used for taking still images during survey transects. The ROVs were also outfitted with laser scalers (green for DD3 and red for Sweetwater) to aid in measuring sizes of animals and other features of the seafloor. An ultra-short baseline (USBL) system was used to guide the ROV to the precise central point of the survey and log the position of the ROV during surveys. Twelve 250-m long transects radiating from a common point of origin with a 30° offset were conducted at each study site during the DD3 surveys; and twenty-four radiating 250-m long transects with a 15° offset were conducted during the Sweetwater surveys (Fig. 2.1). The total areas surveyed during MC208 and MC252 2000 m N Sweetwater surveys were 48,113 m^2 and 11,007 m^2, respectively. Outbound transects were flown at a constant velocity with an approximate

altitude of 2-4 m above the seafloor in order to maintain consistency amongst transects. Video imagery was collected using both SD and HD cameras oriented downwards for an oblique view of the seafloor. At the end of each transect, the ROV turned on a reciprocal heading and proceeded to collect close-up video and DSC images of organisms encountered along the return transect.

2.2.1.2. AUV

MC252 2000 m N and MC208 were also surveyed in 2011 using an autonomous underwater vehicle (AUV), *C-Surveyor III*™ (Hugin 4500), which is designed to collect high-resolution deep-sea geophysical data in water depths up to 4,500 m. The *C-Surveyor III*™ AUV is equipped with geophysical survey sensors including a Simrad EM 2000 Swath Bathymetric System (200 kHz), EdgeTech DF4500 Side Scan Sonar (410 kHz standard or 230 kHz dynamic focusing) and an EdgeTech DW106 Subbottom Profiler (Chirp 1 to 6 kHz). The AUV was also outfitted with a digital underwater photography system developed by C&C Technologies that provides grayscale still frame images of the seafloor at a resolution of 4 mm at an AUV altitude of approximately 7.25 m (C&C Technologies, 2011).

The original set of AUV surveys for these two sites were both completed on April 22, 2011; however, processing of camera photographs upon return of the AUV determined that several of the transects from each site were not of adequate quality. As a result, MC252 2000 m N transects were re-surveyed on April 29, 2011; and transects from MC208 were resurveyed on April 30, 2011 (Table 2.1). The camera investigation survey grids for each site consisted of 29 North-South parallel track-lines spaced 3.5 meters apart with a total length of 350 meters each. Photographic surveys were conducted by the AUV at an altitude of about 7 m. The total areas surveyed were approximately 58,989 m^2 and 53,515 m^2 for MC208 and MC252 2000 m N, respectively.

Table 2.1. AUV, DD3 ROV, and Sweetwater ROV surveys completed at MC208 and MC252 2000 m N.

Platform	Survey Site	Survey Month	Survey Design	Transects
AUV	MC208	April 2011	Parallel, North-South	29
AUV	MC252 2000 m N	April 2011	Parallel, North-South	29
DD3 ROV	MC208	March 2011	Radial	12
DD3 ROV	MC252 2000 m N	March 2011	Radial	12
Sweetwater ROV	MC208	August 2011	Radial	24
Sweetwater ROV	MC252 2000 m N	June 2011	Radial	24

2.2.2. Data Analysis

2.2.2.1. ROV

Outbound ROV video transects were analyzed using VideoReDo Plus (DRD Systems, Inc., 2003) video playback software in order to collect information including organism counts, identifications and locations. Frame grabs were taken each time an organism was encountered on, or just above, the seafloor along the transect, and the date and time from the video was recorded. Frame grabs were sorted into broad taxonomic groups and the organism in each photo was identified down to the lowest possible taxonomic level. Broad taxonomic groups considered for this study consisted of anemones, crabs, eel-like fishes, other fishes, holothurians, sea pens, sea stars, shrimps, sponges, and "other". This last category included organisms that did not fall within any of the other groups and possible organisms for which snapshots were taken but group placement was uncertain.

Width of the field of view (FOV) was calculated for each transect using laser scalers visible on the seafloor in the HD video, using ten frame grabs evenly spaced in time. For transects in which laser scalers were not visible or where fewer than three frame grabs were obtained, a FOV corresponding to the mean FOV for all other transects was used. Areas surveyed by each transect were calculated by multiplying FOV values by total distance traveled. Densities were then obtained for each of the broad taxonomic groups of organisms for each transect using calculated areas. A mean density for each group was then calculated for each survey site as a whole and the total density of all organisms at the site was calculated.

2.2.2.2. AUV

Geospatially-referenced AUV photographs were imported into ArcMap (ESRI, 2014) for data analysis using a NAD 1927 UTM Zone 16N coordinate system projection with a measurement unit in “feet”. Once imported into ArcMap, the overlapping photos (with approximately 20-30% overlap between photos) created a mosaic map of the seafloor. Because of the grayscale nature of the photos and lack of specific seafloor identifying features, the photomosaic was not able to be processed further to blend areas of overlap. Analysis of the photomosaic involved systematically viewing each individual photo in order to locate organisms. Photos were viewed in greater detail and to allow for increased accuracy of detection by zooming in on areas throughout the photo, which allowed for better detection of smaller or less conspicuous organisms.

A line shapefile was created for marking locations of organisms observed in the photos. In each photo, a line was drawn across each observed organism according to the following scheme: a) fishes (including eels) – head to tail; b) circular organisms (ex. sponges and anemones) – across radius; c) crabs – across carapace width; d) shrimp – end to end of main body. For the matching data entries in the attributes table corresponding to each organism, organism type was listed according to the same groups used for ROV data analysis. This process was repeated for each organism observed in the AUV photos. After all photos had been analyzed, sizes of organisms were calculated by using the geometry calculation tool in ArcMap to measure lengths of the shapefile lines demarking each organism. Attribute tables were then exported as text files containing organism identification, length, and spatial coordinates.

The total area surveyed at each site was determined by creating a polygon shapefile and drawing around the borders of the entire photo survey area, then calculating area using the geometry tool in ArcMap. Once area was calculated, the total number of organisms was counted for the site as a whole and divided by total area in order to obtain the mean density of organisms at each site. As with the ROV-collected data, densities were calculated for each of the broad taxonomic groups of organisms, as well as the total density of all organisms at each survey site.

2.2.3. Comparison of ROV and AUV Imagery

In order to compare the use of ROV and AUV imagery in evaluating deep-sea benthic characteristics in this study, several features of the imagery were explored. AUV images and ROV still images were compared to evaluate aspects including: image quality and overall visibility of the seafloor; ability to distinguish sediment characteristics and seafloor features (i.e. presence and size of depressions, mounds and other surficial features); ability to observe and identify different types of organisms; and effectiveness for observing anthropogenic disturbances on the seafloor.

In order to quantitatively compare the ROV and AUV imagery for evaluating benthic megafaunal communities, densities of the broad organism taxonomic groups were calculated and initially compared using a “difference in detection” (DD) factor. This factor was determined by dividing ROV-derived organism mean densities by AUV-derived mean densities for each organism group, and represents how many times an organism was observed in the ROV imagery relative to the AUV. The two ROV imagery sets (DD3 and Sweetwater) were similarly compared by dividing the Sweetwater ROV results by the DD3. A DD value of greater than one means that ROV-derived organism densities were greater than AUV-derived densities, and a value less than one means that AUV-derived densities were greater.

Densities derived from the two ROV data sets were further statistically compared using a t-test to test the null hypothesis that the mean densities were equal. Since the AUV-derived densities of each taxonomic group represent a single value (i.e. a population census) rather than a mean density like the ROV, a modified t-test was used (Sokal and Rohlf, 1981). This modified t-test compared the single observations (the AUV density for each taxonomic group) with the sample means derived from the ROV; and was used to test the null hypothesis that the AUV density could belong to a population with the same mean as the ROV. Both the DD and density comparisons were made for the most abundant taxonomic groups comprising approximately 5% or more of the total animal population.

Finally, a broad comparison of diversity at each site was also made among the AUV, DD3 ROV, and Sweetwater ROV data sets. Several diversity indices were calculated to measure

biodiversity at a broad taxonomic level, including Margalef's index of community diversity (d), Shannon-Wiener index (H'), Pielou's evenness index (J'), and Simpson's index (λ). It should be noted that, although these indices are intended to be used with species-level data, this was not possible in this study since taxonomic placement was often limited to broad groups instead of specific species. Therefore, these indices act as approximations for the purpose of general comparisons among the three data sets.

Since the AUV data represents a complete census of all animals observed in the area surveyed, select dispersion indices were also calculated to evaluate general spatial distribution of the animals observed in the AUV imagery. The Index of Dispersion (ID), Index of Cluster Size (ICS), Green's Index (GI), and Morisita's Index (I_M; Morisita, 1959) were calculated in PASSaGE (Rosenberg and Anderson, 2011) software for the animals observed at both MC208 and MC252 2000 m N. These indices, obtained by overlaying a grid on the data and using quadrat counts to determine mean and variance, provide an approximate estimation of the deviation of the spatial distribution of the animals from a random (Poisson) distribution.

2.3. Results

2.3.1. Data Summary

Both ROV surveys consisted of a set of transects radiating out from a central point of origin at each of the study sites, MC208 and MC252 2000 m N. Twelve transects were completed with 30° offsets during the DD3 ROV surveys, and a total of 24 transects with 15° offset comprised the Sweetwater ROV surveys. AUV surveys conducted at each study site consisted of parallel north-south transects, which were slightly offset from parallel as a result of currents in the area. The top areas of the AUV surveys at each study site were located near the point of origin for the ROV surveys; and the AUV transects then extended south past the southernmost point of the ROV survey area. The overall shape of the ROV surveys consisted of a 500 m by 500 m square area, while the AUV survey areas were long and rectangular in shape extending longer from north to south. Figure 2.1 shows an example of the radial ROV survey design overlaid on top of the AUV survey area (shown as a plot of all animals observed in the AUV photo images).

Relative abundances of animals obtained from the ROV for the different broad taxonomic groups (refer to Table 2.2 and 2.4) were similar for both sites, with shrimps, fishes, eel-like fishes, and sea stars representing the four largest animal groups. Shrimps observed at both sites consisted mostly of multiple undeterminable species of red shrimps (~80-85%), along with the benthic armored shrimp, *Glyphocrangon* (~15-20%). Fishes were dominated by multiple indeterminable species of Macrourids, with other genera including *Acanthochaenus*, *Acanthonus*, *Bathypterois*, and chimaeras representing other taxa observed in smaller numbers. *Aldrovandia* and *Synaphobranchus* accounted for the majority of eel-like fishes present at both sites. Sea pens, crabs, and anemones were seen in intermediate numbers, while sponges and holothurians had the lowest observed densities. The majority of crabs seen were red deep-sea crabs, *Chaceon quinquedens*, along with the occasional large neolithodid crab, *Neolithodes agassizii*. Two types of anemones were observed as well – small purple cerianthid anemones and the Venus flytrap anemone, *Actinoscyphia aurelia*. Holothurians and sponges represented the least abundant groups by far at both sites.

Figures 2.2 and 2.3 show the locations of all animals (mobile and sessile or low mobility) observed in the overlapping grayscale AUV photos overlaid on the AUV photomosaics for MC208 and MC252 2000 m N, respectively. Benthic megafaunal composition was very similar to that observed in the ROV video (refer to Table 2.2 and 2.4), with shrimps, fishes, eel-like fishes, and sea stars again exhibiting the largest abundances. Holothurians and sponges once again had the lowest abundances observed at both study sites.

At MC208, opposite results were obtained for the two ROV surveys compared to the AUV with respect to animal densities and differences in detection of organisms (Tables 2.2 and 2.3). Animal densities derived from the DD3 ROV data were more often higher than those from the AUV. On the other hand, Sweetwater ROV-derived densities were almost all lower than those from the AUV. DD values were generally larger and often greater than 1 for DD3 ROV densities compared to the AUV densities (average DD = 1.14); and Sweetwater ROV values were smaller and rarely greater than 1 (average DD = 0.61). Statistical comparison of densities did not indicate significant differences between the DD3 ROV with the AUV data, while statistically significant differences were observed between the Sweetwater ROV and AUV data for almost

all broad taxonomic groups. These results were further supported by the statistically significant differences between the DD3 and Sweetwater ROV data, in which densities were observed to be almost half (average DD = 0.53) in the Sweetwater compared to the DD3. Despite these differences, densities of certain taxonomic groups (i.e. crabs, eel-like fishes, shrimp, and sponges) were consistently lower for both ROV data sets compared to the AUV.

Table 2.2. AUV and ROV-derived organism densities at MC208.

Taxonomic Group	AUV Densities (ha^{-1})	DD3 ROV Densities (ha^{-1} ± 95% CI)	Sweetwater ROV Densities (ha^{-1} ± 95% CI)
Anemones	3.7	11.5 ± 5.4	2.4 ± 1.8
Crabs	20.7	13.8 ± 10.0	8.4 ± 3.0
Eel-Like Fishes	100.7	51.3 ± 18.8	30.4 ± 6.2
Other Fishes	56.8	96.7 ± 31.8	72.9 ± 19.4
Holothurians	0.8	6.4 ± 4.4	0.4 ± 0.6
Sea Pens	15.1	42.8 ± 22.1	12.6 ± 4.5
Sea Stars	60.3	74.8 ± 21.9	25.2 ± 4.8
Shrimp	849.5	786.7 ± 165.9	435.4 ± 71.5
Sponges	1.4	0.0 ± 0.0	0.0 ± 0.0
All Organisms	1135.5	1480.6 ± 343.9	624.2 ± 97.4

Table 2.3. Modified t-test comparison of ROV and AUV-derived organism densities at MC208.

	DD3 ROV vs. AUV		Sweetwater ROV vs. AUV		Sweetwater vs. DD3 ROV	
Taxonomic Group	**DD**	**p-value**	**DD**	**p-value**	**DD**	**p-value**
Eel-Like Fishes	0.51	0.1327	0.30	**<0.0001**	0.59	**0.0360**
Other Fishes	1.70	0.4547	1.28	0.7277	0.75	0.1783
Sea Stars	1.24	0.6905	0.42	**0.0054**	0.34	**0.0003**
Shrimp	0.93	0.8194	0.51	**0.0227**	0.55	**0.0006**
All Organisms	1.30	0.5478	0.55	**0.0368**	0.42	**0.0001**

Results obtained at MC252 2000 m N were more consistent for the two ROV surveys compared to the AUV (Tables 2.4 and 2.5) than at MC208. Both DD3 and Sweetwater ROV-derived animal densities were mostly higher than densities obtained from the AUV data. While DD values were generally larger than 1 for DD3 and Sweetwater (average DD = 1.03 and 2.02, respectively), these values were overall much higher for Sweetwater than DD3. Only a few statistical differences were observed in comparing the DD3 and Sweetwater organisms densities to the AUV-derived densities. Statistically significant differences were observed between the DD3 and Sweetwater ROV data, where the latter had densities approximately twice (average DD = 2.11) that of DD3. As observed at MC208, densities of crabs and eel-like fishes were once again both lower in the ROV data sets compared to the AUV.

With respect to biodiversity, a few trends were evident amongst the three data sets (Table 2.6). Overall, the highest diversity and evenness were observed in the DD3 ROV data for both MC208 and MC252 2000 m N. Sweetwater ROV data had the next highest diversity and evenness, with the AUV having the lowest. A comparison between study sites showed higher diversity and evenness at MC252 2000 m N than MC208.

Table 2.4. AUV and ROV-derived organism densities at MC252 2000 m N.

Taxonomic Group	AUV Densities (ha^{-1})	DD3 ROV Densities (ha^{-1} ± 95% CI)	Sweetwater ROV Densities (ha^{-1} ± 95% CI)
Anemones	5.0	26.7 ± 15.2	19.7 ± 12.2
Crabs	17.0	13.9 ± 7.3	12.5 ± 9.5
Eel-Like Fishes	146.1	44.1 ± 20.9	128.3 ± 20.7
Other Fishes	92.3	108.9 ± 31.5	254.9 ± 42.1
Holothurians	0.9	14.6 ± 8.4	2.6 ± 3.0
Sea Pens	16.4	49.1 ± 14.4	32.3 ± 15.4
Sea Stars	53.6	81.1 ± 20.0	162.8 ± 20.5
Shrimp	1059.7	913.8 ± 75.1	1741.8 ± 195.0
Sponges	2.2	2.9 ± 3.4	7.0 ± 5.0
All Organisms	1454.2	1899.5 ± 235.9	2582.2 ± 218.5

Table 2.5. Modified t-test comparison of ROV and AUV-derived organism densities at MC252 2000 m N.

	DD3 ROV vs. AUV		Sweetwater ROV vs. AUV		Sweetwater vs. DD3 ROV	
Taxonomic Group	**DD**	**p-value**	**DD**	**p-value**	**DD**	**p-value**
Eel-Like Fishes	0.30	**0.0117**	0.88	0.7255	2.91	**<0.0001**
Other Fishes	1.18	0.7502	2.76	0.1239	2.34	**<0.0001**
Sea Stars	1.51	0.4158	3.03	**0.0377**	2.01	**<0.0001**
Shrimp	0.86	0.2550	1.64	0.1614	1.91	**<0.0001**
All Organisms	1.31	0.2680	1.78	**0.0435**	1.36	**<0.0001**

Table 2.6. Diversity indices, including Shannon-Wiener index (H'), Margalef's index of community diversity (d), Pielou's evenness index (J'), and Simpson's index (λ).

Index	AUV		DD3 ROV		Sweetwater ROV	
	MC208	**MC252**	**MC208**	**MC252**	**MC208**	**MC252**
H'	0.7701	0.7852	1.2255	1.2550	0.9588	1.0193
d	0.9081	0.8929	0.9662	1.0571	0.8743	1.0060
J'	0.3505	0.3574	0.5893	0.5712	0.4611	0.4639
λ	0.5660	0.5381	0.3619	0.3526	0.5065	0.4762

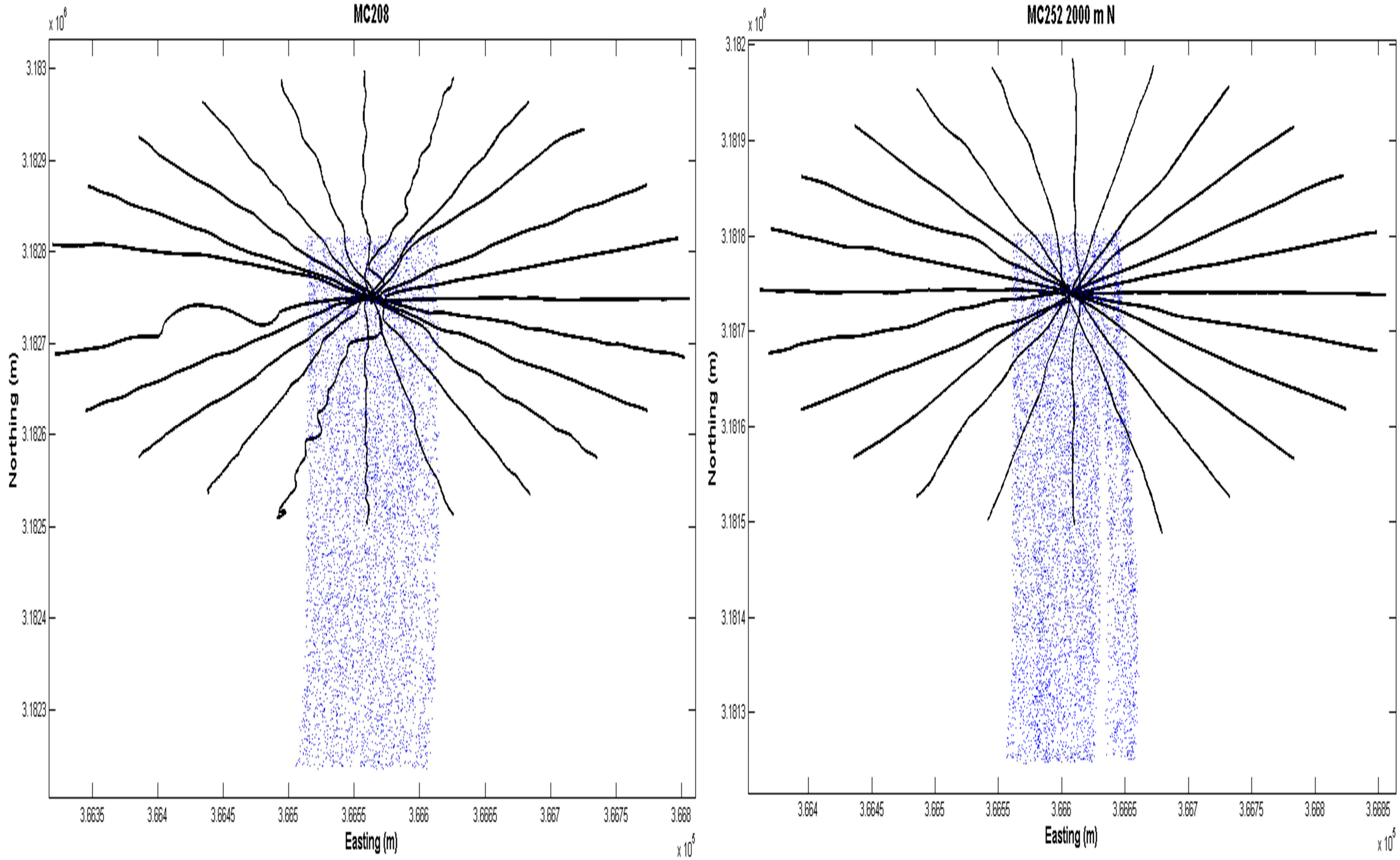
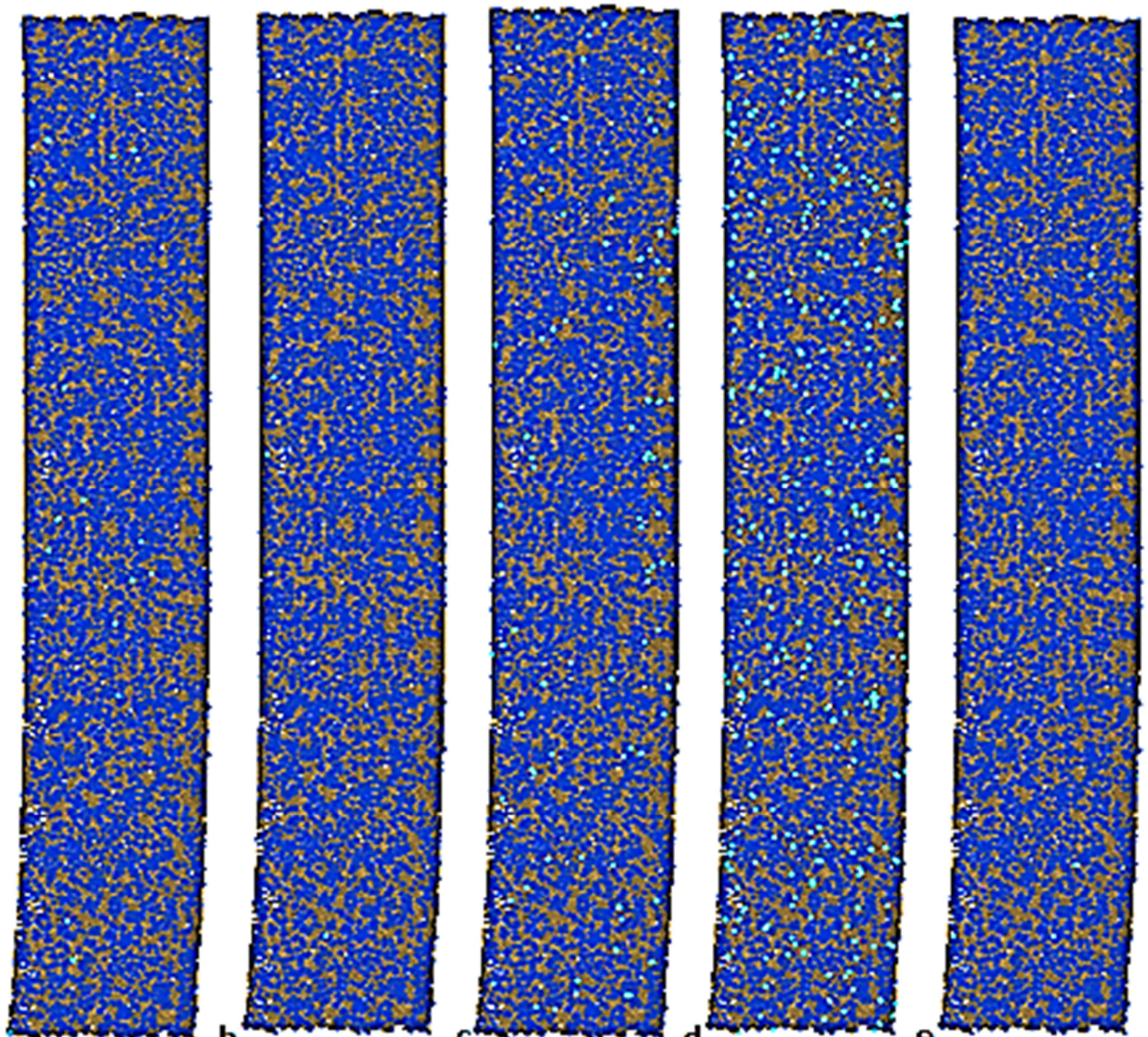


Fig. 2.1. Sweetwater ROV survey overlaid over the AUV surveys at MC208 (left) and MC252 2000 m N (right). Black lines represent ROV track lines and blue points represent the AUV survey areas and show all animals observed in the AUV surveys.



a b c d e

Fig. 2.2. Plots of organisms overlaid on AUV photomosaic imagery at MC208. Dark blue points are mobile animals including crabs, eel-like fishes, other fishes, and shrimps. Sessile and low mobility organisms are highlighted in light blue on each of the plots: a) anemones, b) holothurians, c) sea pens, d) sea stars, and e) sponges.

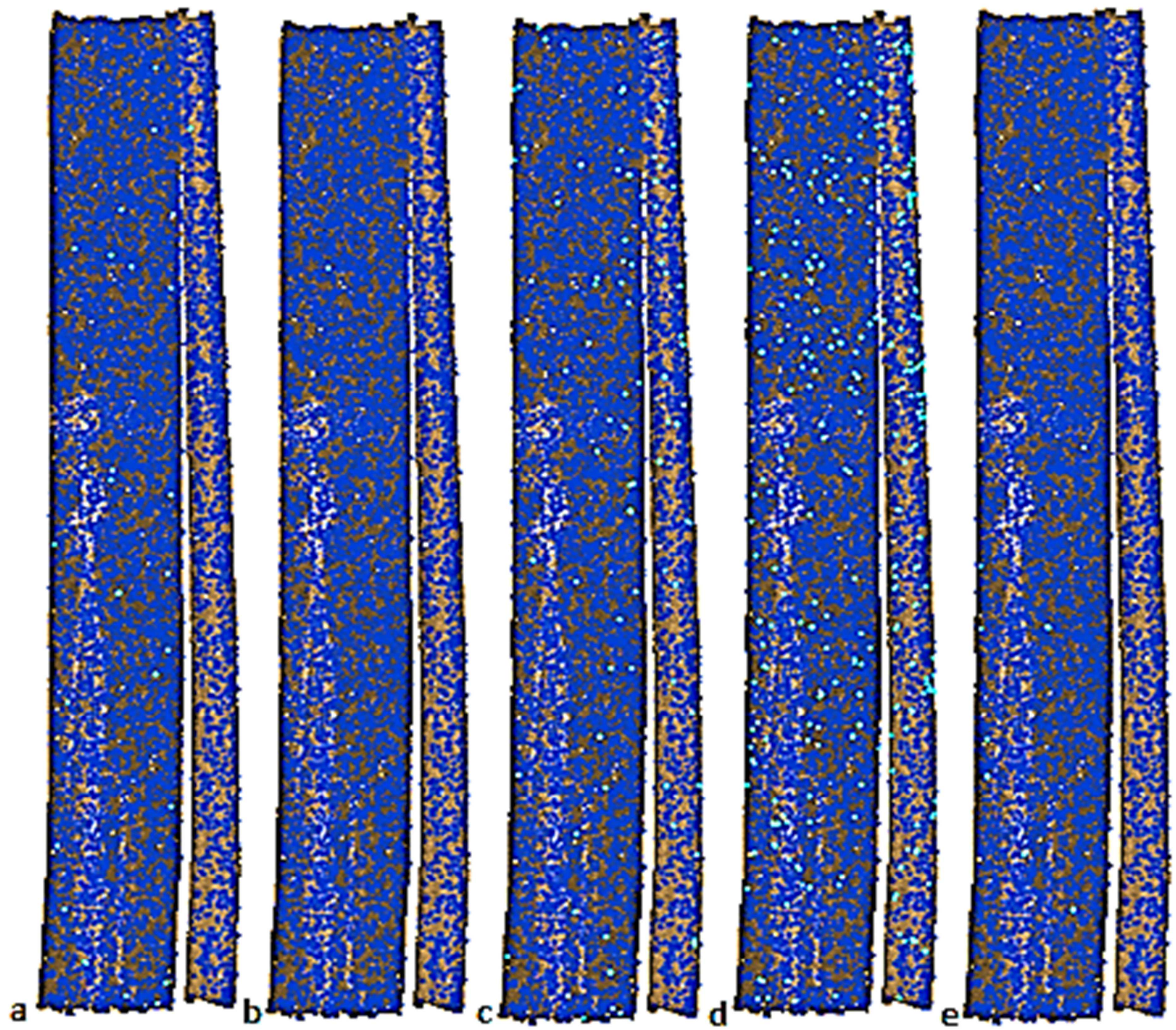


Fig. 2.3. Plots of organisms overlaid on AUV photomosaic imagery at MC252 2000 m N. Dark blue points are mobile animals including crabs, eel-like fishes, other fishes, and shrimps. Sessile and low mobility organisms are highlighted in light blue on each of the plots: a) anemones, b) holothurians, c) sea pens, d) sea stars, and e) sponges.

Dispersion indices calculated for MC208 and MC252 2000 m N AUV data revealed similar spatial distributions of the animals at both sites (Table 2.7). Values of ID and ICS that are greater than 1 and 0, respectively, indicate a deviation from a Poisson distribution and suggest clumping of the data. Green's Index ranges from 0 for a random to 1 for a maximally clumped distribution; and, similarly, higher values of Morisita's Index reflect a greater degree of clumping in the distribution. ID at both sites (3.49780 and 3.07691 for MC208 and MC252 2000 m N, respectively) indicated an over-dispersed distribution of animals that differed significantly (χ^2 test, $p < 0.000001$ in both cases) from a random (Poisson) distribution. ICS values also indicated clumping for both sites. Even though the spatial patterns were found to significantly deviate from a random distribution, the values of GI and I_M suggest that the degree of clumping, although present, was relatively low at both MC208 and MC252 2000 m N.

Table 2.7. Dispersion indices, including Index of Dispersion (ID), Index of Cluster Size (ICS), Green's Index (GI), and Morisita's Index (I_M), for MC208 and MC252 2000 m N obtained from AUV organism plots.

Index	MC208	MC252 2000 m N
ID	3.49780	3.07691
ICS	2.49780	2.07691
GI	0.00511	0.00275
I_M	1.18238	1.20152

2.3.2. Identification of Animals

Figure 2.4 and 2.5 show cropped images taken from original AUV photographs at MC208 and MC252 2000 m N and enlarged to show detail. Most larger animals were visible in the AUV photos at low magnification. Smaller animals or those that blended in well with the seafloor were more difficult to detect without increasing magnification. AUV images provided enough detail that animals were easily placed into broad taxonomic groups. Finer taxonomic resolution was not consistently achievable, however, for much of the AUV imagery due to inconsistencies in the clarity of photographs within, and between each study site. This affected the ability to observe specific identifiable features of the animals.

Principle identifying features for animals in the AUV photos consisted of shape since photos were grayscale, and color was not available. In addition, georeferencing and GIS analysis allows

for quick animal size measurements from the AUV photos. A major disadvantage of the AUV imagery, however, was a common lack of clarity of the animals. This lack of clarity resulted in a loss of specific distinguishing features, which contributed to inconsistencies in achieving finer taxonomic resolution. This was a particular issue for taxonomic groups where species look similar to one another and can only be identified by specific characteristics such as particular body features or color.

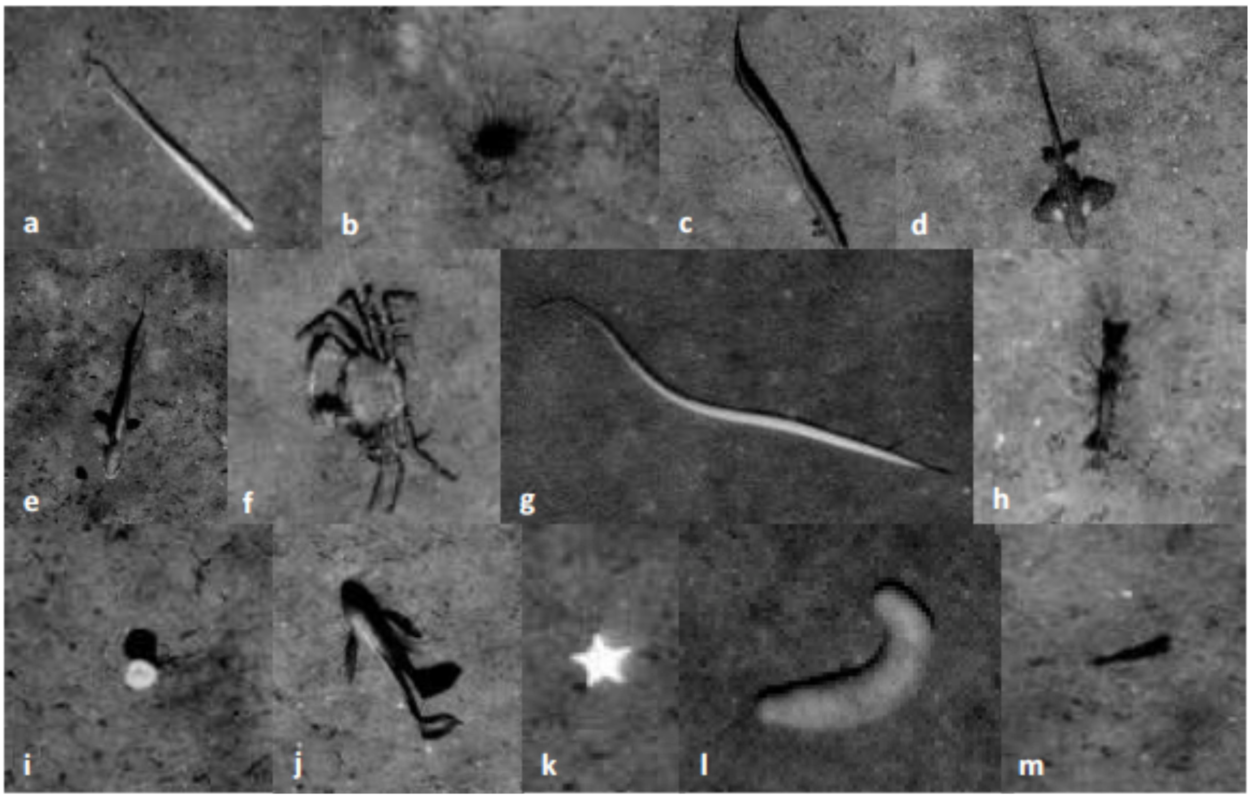


Fig. 2.4. Example pictures of animals from MC208 AUV photo surveys: a) *Aldrovandia* sp; b) unidentified cerianthid anemone; c) *Synaphobranchus* sp; d) unidentified chimaera; e) Macrourid fish; f) *Chaceon quinquedens*; g) unidentified eel; h) unidentified shrimp; i) unidentified sponge; j) unidentified fish; k) unidentified sea star; l) *Mesothuria* holothurian; and m) unidentified red shrimp.

A number of valuable characteristics useful in identifying benthic animals were observable in still photos taken from the ROV video imagery. Figures 2.6 and 2.7 show how color was one of the most notable observable features and can consequently be an important feature for identifying animals, especially down to more specific taxonomic groups. Overall coloration,

along with patterns and variations in color, aids in distinguishing between different species. It also makes animals with coloration similar to the seafloor more noticeable than they would be in a grayscale image. In conjunction with color, the multi-dimensional aspect that video imagery provides from an oblique viewing angle contributes further to identifying power.

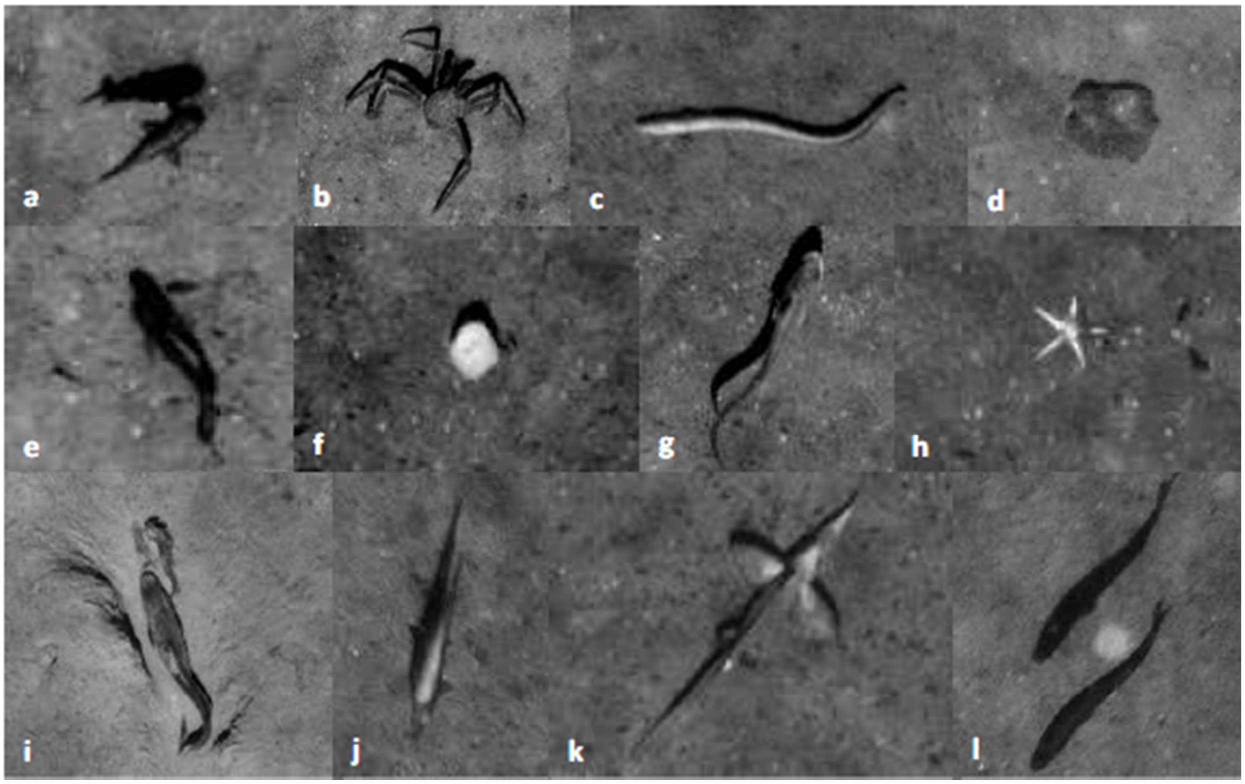


Fig. 2.5. Example pictures of animals from MC252 2000 m N AUV photo surveys: a) unidentified fish; b) *Neolithodes agassizii*; c) *Synaphobranchus* sp; d) unidentified octopus; e) unidentified fish; f) unidentified sponge; g) Macrourid fish; h) unidentified sea star; i) *Bassogigas gillii*; j) unidentified fish; k) unidentified *Rhinochimaera*; and l) unidentified fish.

Although not specifically considered in this study, sizes of organisms can be approximated from ROV imagery by using laser scalers of known length visible on the seafloor. Notable issues of poor visibility throughout the ROV surveys at these two sites, however, often greatly hindered visibility of the laser scalers and animals. This was a greater issue for the Sweetwater ROV video with red laser scalers than for the green laser scalers in the DD3 surveys. As with the AUV imagery, clarity of the ROV imagery was highly variable both within, and between the two study sites.

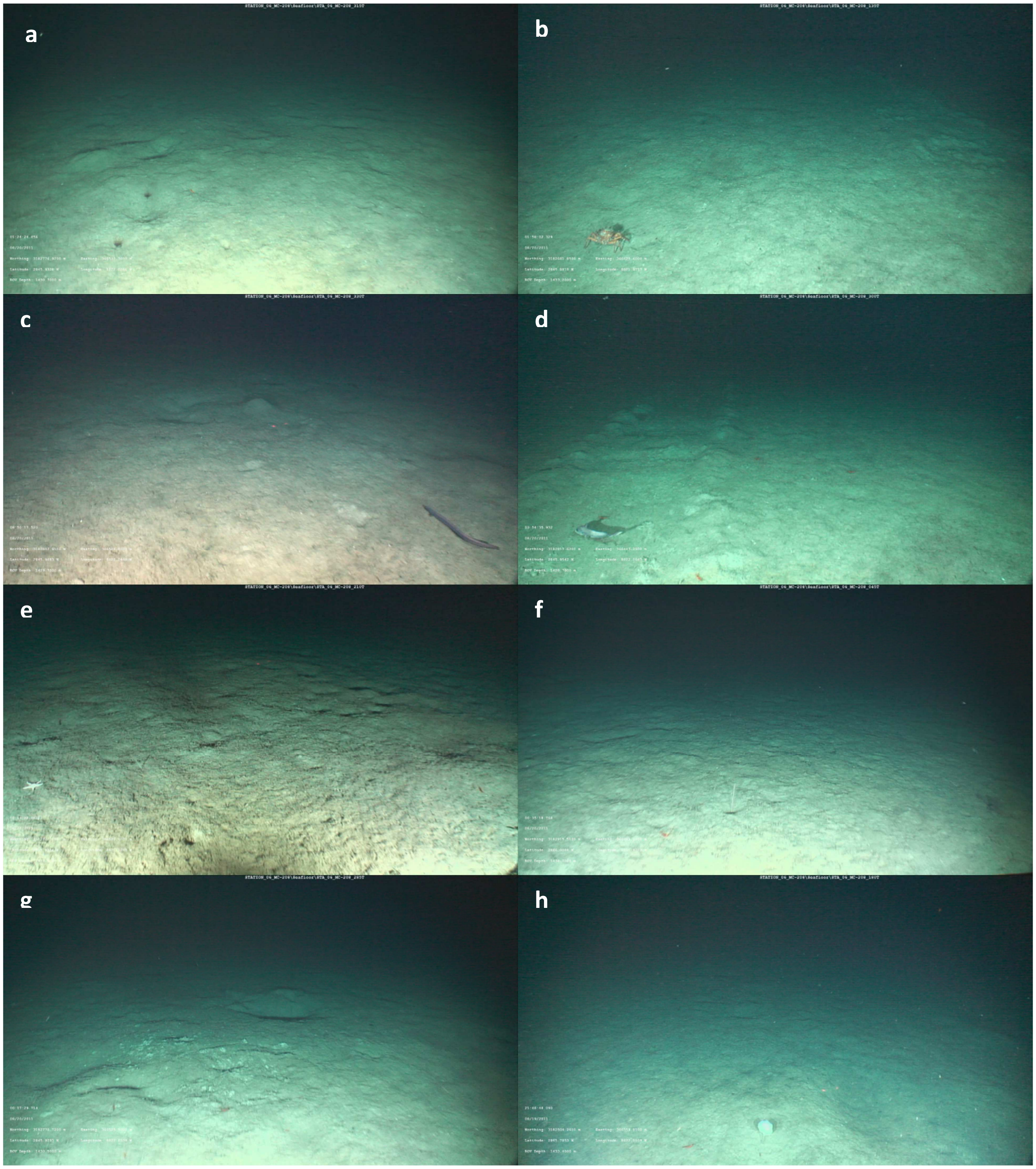


Fig. 2.6. Example video snapshot pictures of animals from MC208 Sweetwater ROV video surveys: a) cerianthid anemones; b) *Chaceon quinquedens*; c) *Synaphobranchus* sp; d) Macrourid fish; e) unidentified sea star; f) unidentified sea pen; g) unidentified red shrimp; h) unidentified sponge.

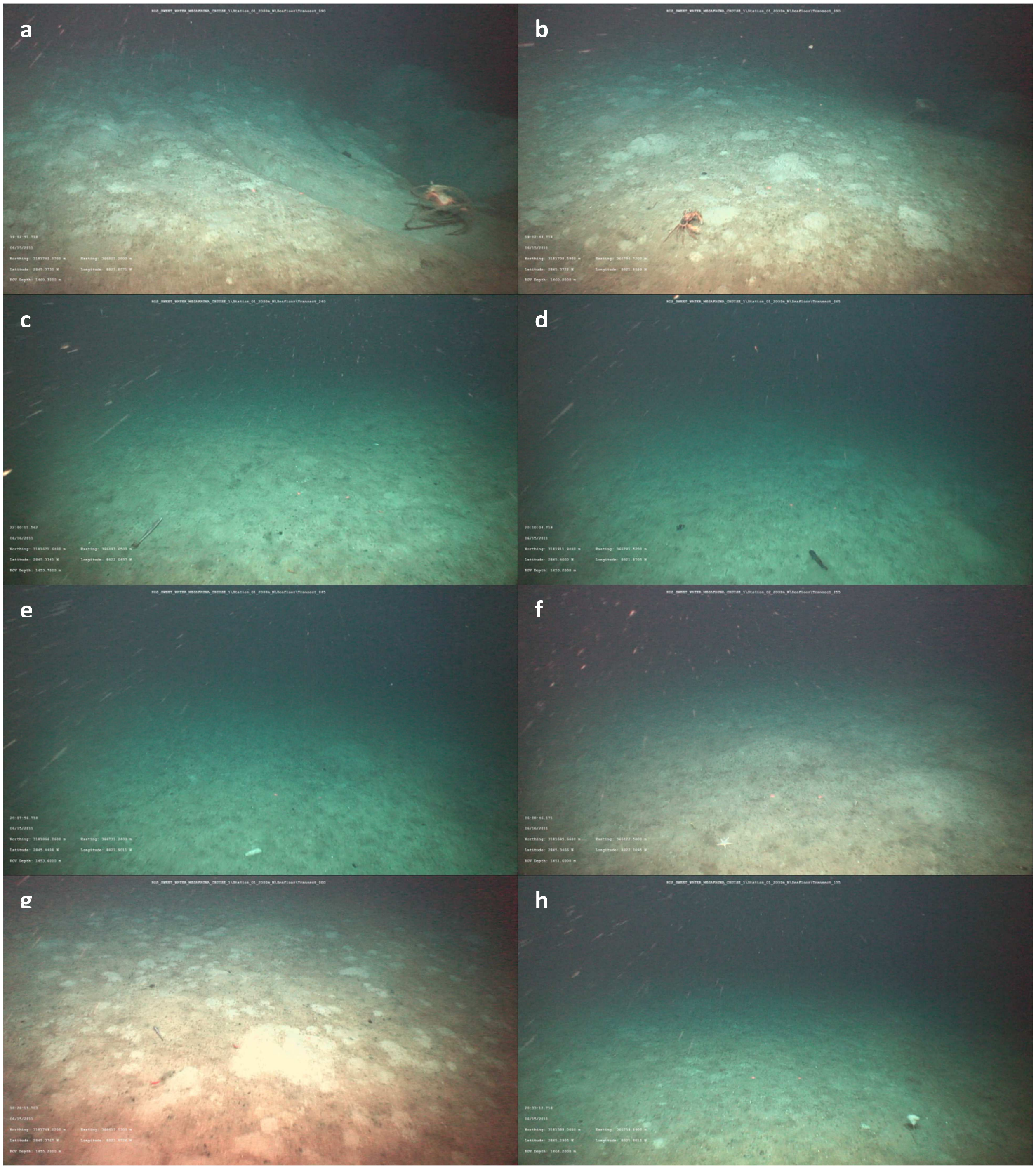


Fig. 2.7. Example video snapshot pictures of animals from MC252 2000 m N Sweetwater ROV video surveys: a) *Actinoscyphia aurelia*; b) *Chaceon quinquedens*; c) *Aldrovandia* sp; d) unidentified fish; e) *Mesothuria* holothurian; f) unidentified sea pen; g) unidentified red shrimp; h) unidentified sponge.

2.3.3. Identification of Seafloor Features and Disturbances

A variety of abiotic seafloor features were observable in the ROV imagery. Sediment type and mix were distinguishable except when water column visibility greatly inhibited a clear view of the seafloor. Seafloor features of a variety of sizes ranging from small pits to larger depressions and mounds (Fig. 2.8) were observable in the ROV imagery where visibility permitted. The oblique angle at which the seafloor was viewed allowed these features to readily stand out, and there is the possibility to measure the size of these features using the laser scalars. In addition to naturally occurring seafloor features, areas of man-made disturbance to the seafloor were also visible. Small-scale disturbances to the seafloor were readily detectable in the ROV imagery; however, it was more difficult to observe larger-scale disturbance patterns across the whole study sites.

Sediment type and mix along with finer-scale seafloor features were difficult to distinguish in most of the AUV imagery due largely to the lack of color and full downward-looking view with which the photos were taken. Exceptions to this were areas of different sediment coloration or where seafloor features were of a large enough scale to create visible elevation of the feature in the photos (Fig. 2.9). Small seafloor features such as pits and burrow holes or low-height mounds were difficult to differentiate in the AUV imagery.

Unlike smaller features, because of the downward-looking and overlapping, mosaicable nature of AUV photographs, large-scale seafloor features such as areas of disturbance were easily observable for both sites. This allows for detection of central points of disturbance as well as the direction (from point of origin) and extent of secondary disturbance. On a smaller scale, however, it was more difficult to identify areas of man-made disturbance versus naturally-occurring seafloor features (i.e. mounds, pits, etc.) in individual photos.

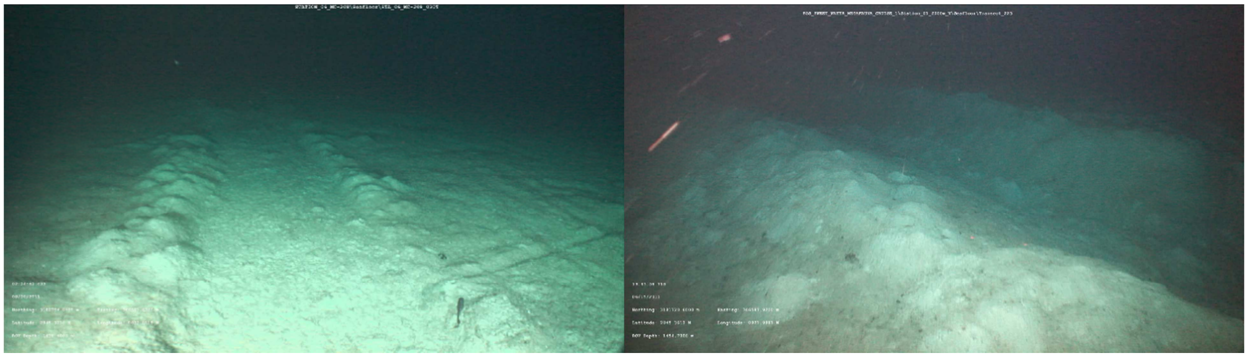


Fig. 2.8. Example Sweetwater ROV still photos from MC208 (left) and MC252 2000 m N (right). Note the variety of seafloor features visible including small pits and larger depressions, mounds, and areas of physical disturbance to the seafloor.

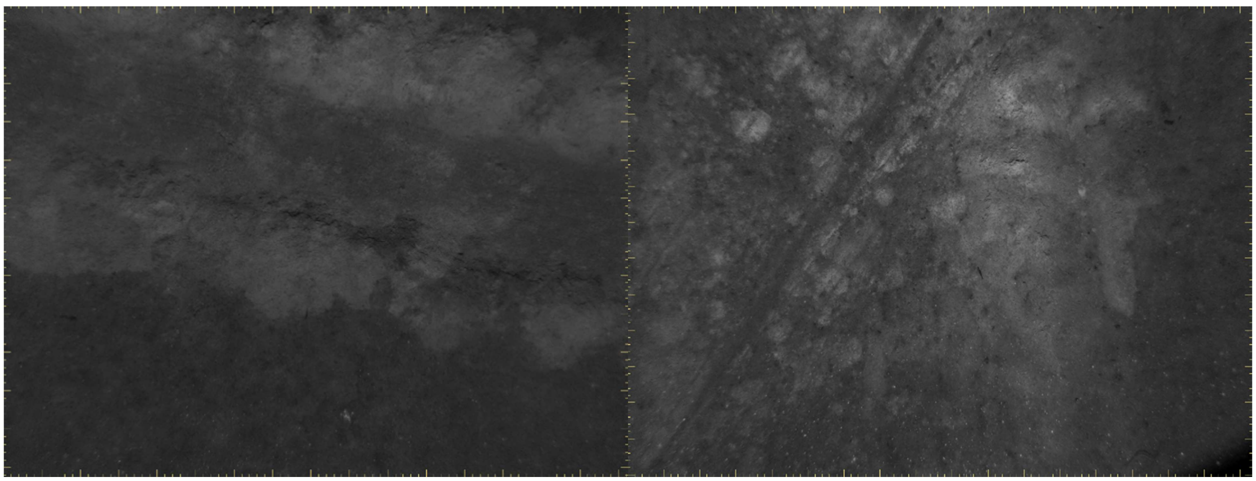


Fig. 2.9. Example AUV still photos from MC208 (left) and MC252 2000 m N (right). Note the variety of seafloor features visible including small pits and larger depressions, mounds, and areas of physical disturbance to the seafloor.

2.4. Discussion

2.4.1. Resolving Seafloor Features

Both AUV and ROV imagery were capable of resolving seafloor features and disturbances to the seafloor. In most respects, ROV imagery was superior at detecting smaller-scale features and disturbances largely because of the oblique viewing angle of the seafloor and three-dimensional quality video imagery provides. The downward-looking view of AUV imagery made it difficult to detect smaller seafloor features because it was hard to distinguish the dimensional aspects of these features. ROV imagery also provided a better means of determining sediment

characteristics such as type and mix because color aids in distinguishing variations in these characteristics.

In comparison, the larger-scale view of the seafloor in the AUV imagery makes it more suitable for benthic studies in which “the bigger picture” is highly desirable. This is of particular importance for investigating the extent of natural or man-made disturbances to the seafloor, which are often more easily distinguished at a larger-scale. The overlapping, mosaicable nature of the AUV imagery allows for analyses of the spatial distribution of the animals on the seafloor. This can provide valuable information for studies where spatial distributions and patchiness in the benthic environment are desirable to evaluate. It is more challenging to determine this from ROV imagery, where transects are narrower and typically do not overlap one another.

2.4.2. Identifying Animals

The use of color and movement as identifying features in the ROV video in many cases gave ROV imagery an advantage over the AUV images. Many animals exhibited distinct coloration or patterns that were not seen in the grayscale photos. These were often features that made the animals stand out in imagery, even in situations of lowered visibility. Other recognizable features useful for identifying animals included size, shape or unique appendages. These represented the principle factor that allowed for benthic animals to be placed into broad taxonomic groups. Within those broad groups, taxonomy was further narrowed down by looking at unique identifying features distinguishing between different animals in the same group. While this worked for many animal groups, fishes were more problematic since many species look very similar and the angle from which they were viewed greatly impacted appearance of notable features. This was where the viewing perspective of the imagery became important. The difference in viewing angle between ROV (oblique) and AUV (down-looking) imagery played a major role in identifying animals and, in particular, for fishes because the body shapes and unique appendages were often more easily viewable in the ROV imagery because of the oblique viewing angle.

Shrimps represent an ideal example for exploring differences in identification between the two imagery types because of the importance of color and movement for their identification. Red

shrimps were more easily identified in color ROV videos because of their notable red coloration, which stood out against the surrounding environment even in cases where visibility was low. In grayscale AUV photos, it was often challenging to differentiate between a shrimp on the seafloor and non-living objects such as detritus. *Glyphocrangon* shrimp were also generally easier to see in ROV video imagery because they were easily disturbed by the ROV and swam up off the seafloor as the ROV approached. The lighter coloration and reflection of light by their eyes further aided in detection of these shrimp in both imagery types, yet was much more noticeable in ROV imagery and may have been missed in AUV photos where these shrimp typically blended in well with seafloor. For these reasons, ROV video imagery represented the more reliable and effective means of detecting shrimp in this soft-sediment benthic environment.

Observations and identification of animals in both kinds of imagery was highly dependent on overall visibility within the water column. Visibility varied greatly amongst the ROV videos as a result of differences in altitude above the seafloor, and varying presence of suspended sediments and marine snow in the water column. The altitude of the ROV during data acquisition was ideally kept low (2-3 meters off the seafloor), but consistency in this parameter sometimes varied greatly based ROV pilot ability. Occasionally sediments were disturbed if the ROV got too close to the sediments or if the ROV tether hit the seafloor. The lights used by the ROV sometimes caused overexposure within the videos and consequently further complicated visibility, especially when higher concentrations of suspended particles were present.

In many instances where visibility was low in the ROV videos, small animals or those that blended in well with the seafloor could not be easily seen and it was hard to specifically identify other animals beyond observing that the animal was present. In the latter case, finer taxonomic resolution within the broad taxonomic groupings was greatly hindered for the ROV data. In addition to this, lowered visibility may have impacted animal counts if objects that were not animals were mistaken for being living organisms. This was a particular concern for the fishes taxonomic group because low visibility conditions sometimes obscured the ability to distinguish between fishes and non-living objects with absolute certainty.

While decreased water column visibility also impacts AUV images, the observed impacts in this study were smaller than for the ROV. AUVs are programmed to fly at a specific height (in this case, approximately 7 meters) off the seafloor, partly in order to prevent the AUV from touching the seafloor. The higher altitude and consistency throughout surveying makes it unlikely that the AUV would disturb the seafloor or otherwise contribute to additional suspended particles in the water column. In addition to this, lighting used by the AUV was different than the ROV and was not as affected by the presence of suspended particles in the water column. These factors contributed to the much more consistent visibility observed in the AUV photographs compared to the ROV videos. This, in turn, likely increases the consistency of animal densities derived from the AUV imagery. Despite this benefit, through a combination of grayscale imagery and higher altitude, the AUV photos in this study often lacked definition of the animals and there was a resultant blurring of the animals with the surrounding sediments. As a result, it was challenging to achieve identifications of finer taxonomic resolution due to difficulty in observing distinguishing features.

2.4.3. Measurements of Abundances and Diversity

Overall, the comparisons of the animal densities determined from the three data sets – DD3 ROV, AUV, and Sweetwater ROV – did not demonstrate consistent density differences among the sites or survey types. DD3 densities at MC208 were on average higher than AUV densities, while Sweetwater ROV densities were lower compared to the AUV. These differences were significant for the Sweetwater ROV-AUV comparison, but not for DD3-AUV. This lack of differences between AUV and ROV densities in the spring suggests that both surveys quantified densities equally well. Significant differences were observed for both the AUV-Sweetwater and DD3-Sweetwater comparisons at MC208. These observed significant differences suggest possible temporal influences resulting from gaps in time between the spring DD3 ROV and AUV surveys, and late-summer Sweetwater ROV surveys. If these temporal differences were related to seasonality within this deep-sea ecosystem, one would expect potentially significant differences in mobile animal densities, but not necessarily for sessile fauna. The observed lower mobile and sessile animal densities during the summer Sweetwater surveys is thus more likely attributed to differences in visibility or mortality between the two seasons. In this

respect, temporal variations may also be an influencing factor in the observed lower diversity, particularly for the Sweetwater ROV surveys, at MC208.

Compared to MC208, surveys at MC252 2000 m N were completed closer in time to one another and therefore minimized temporal gaps between surveys. ROV surveys at this site were completed one month before (DD3) and less than two months after (Sweetwater) the AUV surveys in spring of 2011. This presumably limits the likely temporal variation in organism abundances observed at MC208. At MC252 2000 m N, there were fewer significant differences observed between each of the ROV surveys and the AUV densities. DD3 densities were similar to those obtained from the AUV and were generally not significantly different. Despite Sweetwater densities being higher than AUV densities, there were also fewer significant differences between the two than were observed for the MC208 surveys.

Where large statistically significant differences did exist at MC252 2000 m N, however, were between the two ROV surveys. Animal densities derived from Sweetwater ROV imagery were approximately twice as high as those observed from DD3. It is highly unlikely in this case that temporal influences such as seasonality are responsible for the differences since the two ROV surveys were conducted only a couple months apart and both in the spring season. Instead, it is more likely that the observed differences are the result of differences between the two ROV video imagery sets. Transects and video imagery collection were generally more consistent and thorough during Sweetwater surveying. ROV altitude and thus FOV were likewise more consistent. These factors contribute to a likely increase in ability to effectively detect benthic megafauna in the video imagery and therefore minimized variations in visibility.

2.4.4. Additional Considerations

Differences in survey design and location were likely responsible for some of the observed differences between the AUV and two ROV surveys. The ROV and AUV survey areas have different dimensions – square versus rectangle – and therefore different areal coverages. There was also an offset in location between the ROV and AUV surveys, with the latter located starting from the approximate point of origin location of the ROV surveys and moving further to the southern region of each study site. Using a radial survey design (ROV) instead of

overlapping strip transects (AUV) not only results in different areas surveyed, but in the amount of possible error associated with re-counting or missing organisms observed during the surveys. Studies have indicated that benthos often exhibit patchiness or general deviation from a perfectly random distribution (Jumars, 1975; Jumars, 1976; Schneider et al., 1987; Parry et al., 2003; Gonzalez-Mirelis et al., 2009). This is the result of a variety of factors ranging from resource availability, disturbances, and variations in seafloor characteristics or hydrographic conditions to predator-prey relationships and interspecific competition (Gage, 2001; Levin et al., 2001; Powell et al., 2003; Smith et al., 2008). As a result, even small differences in location or areal coverage of surveys could affect the likelihood of observing megafauna and in particular those animals that are less abundant or rare.

The much higher densities of sessile and low-mobility organisms observed by the ROV at both MC208 and MC252 2000 m N may possibly be attributed to these differences in ROV and AUV survey designs and area coverage. Overlap of ROV transects in some areas where transects were not straight or close to one another may have contributed to higher densities of these animals since animals may have been unintentionally re-counted. When the AUV imagery was analyzed, careful attention was paid to avoid double-counting of organisms where overlap of photos occurred. Minimizing this error was easier for the AUV imagery since the area of overlap was known and easily observable on all sides of each photo. Another issue for both the ROV and AUV imagery was potential counting of mobile organisms multiple times in cases where that animal moved from one survey transect to another. The likelihood of this occurring should be similar for both imagery types and was therefore not a main consideration when comparing the two technologies. Causes of variation in mobile animal abundances, therefore, were more likely attributed to differences in behavior of these animals to the two technologies used.

An increasing number of studies have aimed to determine effects of stimuli from these technologies on behaviors of deep-sea benthos in order to evaluate effectiveness of each respective technology for studying benthic megafauna (Trenkel et al., 2004a,b; Lorance and Trenkel, 2006; Raymond and Widder, 2007; Stoner et al., 2008; Trenkel and Lorance, 2011; Uiblein, 2011). The nature and occurrence of these behaviors still remain widely varied and

controversial between and within different studies. Studies have indicated that stimuli from deep-sea technologies induces a variety of behaviors depending on the type of deep-sea vehicle used, lighting, types of animals being studied, and other environmental conditions. While most of studies have focused on evaluating animal behaviors in response to the presence of ROVs, fewer studies have explored the effects of AUVs on the animals they are surveying. A logical assumption would be that AUVs have less potential influence on behaviors since they are generally smaller, employ less lighting, and fly higher above the seafloor – thus, minimizing the effects of close proximity to the benthic communities and habitats being surveyed.

Compared to sessile animals, mobile animals are likely more affected by deep-sea vehicles, and in particular ROVs, since they have the ability to readily move towards or avoid the vehicle. Mobile animals and especially fishes have the ability to swiftly react to stimuli and will generally exhibit either attraction or avoidance behaviors. Densities of some mobile animals, including eel-like fishes and crabs, were smaller in both ROV surveys than those determined from the AUV imagery. Other mobile animals (including all other fishes) had higher ROV-derived densities. The lower detection of crabs and eel-like fishes could be the result of avoidance behaviors of these animal groups with respect to the ROVs. Avoidance behaviors were in fact observed on many occasions with crabs in the ROV videos, where they often would start moving quickly away upon approach of the ROV. Cerianthid anemones similarly exhibited obvious avoidance behaviors by pulling into the sediment as the ROV approached. Contrarily, the much higher detection of other fishes may be the result of attraction to the survey area by the presence of the ROV. Consequently, considerations of animal behaviors need to be studied further and taken into account when comparing different benthic surveying platforms.

2.5. Conclusion

Unique characteristics of animals are often used when detecting and identifying benthic megafauna in imagery. Identifying features such as notable coloration or patterns, size, presence of unique appendages, overall body shape, and movement provide means for recognizing particular animal groups and, more specifically, species within a broad taxonomic group. Detection of different animal types varied between the ROV and AUV imagery. In this

study, there were two major differences between the ROV and AUV imagery that created challenges in detecting and identifying animals: 1) the ROV imagery analyzed was taken from video whereas AUV imagery consisted of still photos; and 2) the ROV imagery was in color, and the AUV photos were grayscale.

Both ROV and AUV technologies have advantages for studying benthic ecosystems and are readily capable of detecting benthic organisms, as well as small-scale seafloor characteristics. One of the primary advantages of ROVs is that they allow for "live" video observations with color and movement visible. ROVs generally allow more flexibility to make changes during surveys and obtain close-ups of organisms since human pilots are actively in control during surveying. AUV surveys have excellent areal coverage since they are not limited by physical attachment to a ship. This, in turn, allows for better observation of larger-scale seafloor characteristics along with the opportunity to geospatially map or "mosaic" the resulting AUV images with ease. AUV surveys are more consistent with regards to altitude, field of view and other survey parameters since they are programmed to follow an exact survey pattern. For this same reason, AUV surveys are typically low maintenance to complete.

There are inevitably going to be limitations to these technologies as well. Survey coverage by ROVs is limited to the design of the survey and the physical limitations of the ROV itself. Differences in pilot ability can create differences in survey parameters such as altitude, which can affect visibility. Similarly, the presence of suspended sediments and marine snow can greatly impact ability to detect and identify organisms on the seafloor. Avoidance or attraction behaviors of benthic animals can likewise affect detection by ROVs. Industrial AUVs such as the one used in this study often provide grayscale still life imagery. This can make it hard to distinguish organisms that are small or blend into the seafloor since color can be very useful for distinguishing between organisms and seafloor features. Another downside of AUV data is that it can be extremely time-consuming to analyze, since it requires viewing each photo individually in order to minimize errors resulting from photo overlap or animal movement between subsequent photos.

Based on the comparison of AUV and ROV imagery in this study, ROV imagery is more highly recommended for studies of benthic megafauna in soft-sediment environments of the northern Gulf of Mexico. Because continuous soft-sediment environments are fairly homogeneous in nature, it is important to be able to detect small differences in these environments such as variations in sediment type or mix. The ability to view benthic characteristics (including megafauna) in color and with sufficient clarity and dimensionality is also an important characteristic for observing and identifying animals associated with the benthic environment. These are all features that were superior in the color video imagery collected by the industrial ROVs compared to the more monotone and less dimensional industrial AUV grayscale photographic imagery of the particular AUV used in this study.

That being said, further strides need to be made to continually improve upon the ROV imagery to produce better-quality video with greater clarity and lessened effects of reduced visibility from suspended materials and light overexposure. This may mean working with new camera system technologies and data storage capabilities to reduce loss of clarity during data acquisition, transfer, and storage. While there may be incentive to use a fixed set of lighting during ROV surveying to maintain consistency, different lighting schemes need to be evaluated and/or selectively employed depending on water column conditions in order to increase visibility and minimize issues such as overexposure.

Regardless of which technology is chosen, consistency is key for environmental monitoring activities since it allows for more accurate comparability of results, and therefore more accurate decision-making considerations. When addressing a particular monitoring and management issue, consistently using the same technology, timing for surveys, and analysis methods will help minimize variability and errors associated with different technologies and temporal influences in the deep-sea benthic environment.

CHAPTER 3. A RADIAL ROV SURVEY METHODOLOGY FOR USE IN EVALUATING DEEP-SEA BENTHIC MEGAFAUNA IN THE NORTHERN GULF OF MEXICO

3.1. Introduction

Growing demands on global resources have resulted in a push to explore and develop resources deeper in the ocean. Increased exploration for hydrocarbons and deep-sea mining has led to greater concerns of the impacts of these activities on deep-sea ecosystems (Gage, 2001; Pinder, 2001; Glover and Smith, 2003; Jones et al., 2006; Redden, 2010; Ramirez-Llodra et al., 2011). Over the last half-century, human activities have continually progressed throughout the Northern Gulf of Mexico (GoM) as the demand for fisheries and energy resources has created a push to go deeper into the ocean. This has underscored the necessity for expanding on deficiencies of deep-sea benthic ecosystem data, particularly as the need for data on baseline conditions increases.

Baseline data such as species abundances and diversity, and community composition and dynamics are important not only for general long-term environmental monitoring, but also for investigating anthropogenically-induced environmental impacts. Despite being one of the most economically important deep-sea regions in the world, the GoM often lacks adequate data on baseline conditions associated with benthic megafauna. There is a need to develop standardized methods for surveying and monitoring deep-sea ecosystems in order to assess and interpret impacts of anthropogenic activities.

Deep-sea sampling of megabenthos – those animals greater than 1 cm in size or visible with a camera – traditionally employs the use of nets (Husebø et al., 2002), trawls (Rowe and Menzel, 1971; Haedrich et al., 1980; Gordon and Duncan, 1985; Escobar-Briones and Soto, 1997; Powell et al., 2003), and traps. These methods are generally considered to be destructive in nature because they disturb the sampling area and less invasive technologies such as remotely operated vehicles (ROVs) are often more available to industry than to researchers. Despite this limited availability, these technologies are becoming increasingly used because of their ability to collect imagery from which a variety of both quantitative and qualitative data can be

extracted. These technologies also have an advantage over more traditional sampling methods since they can be used more easily in areas with seafloor habitats in which trawls and nets are considered too difficult to use (Tolimieri et al., 2008; Clarke et al., 2010).

In recent years, industrial-based platforms such as work-class ROVs and AUVs are becoming more common because of their availability and proximity to areas of interest for studying benthic communities. Photographic surveys of benthic megafauna by these platforms can be conducted using several kinds of survey designs. Strip transects are often used, particularly when using towed cameras (Jones et al., 2007; Schlacher et al., 2009; McIntyre et al., 2013) or AUVs for collection of photographic imagery. This type of survey design is commonly practiced for habitat mapping and assessment studies because strip transects consisting of overlapping photographs can be readily patched together into larger-scale photomosaics.

Use of industrial ROVs has contributed to an increase in use of radial survey designs for benthic studies because of the overall practicality of these configurations. Often, scientists and industry use these ROVs to evaluate the benthic environment in proximity to a current or proposed offshore well location. ROVs attached to offshore rigs provide an excellent opportunity to readily study benthic communities surrounding the rigs. In recent years, studies employing radial survey designs using industrial ROVs have been conducted in order to determine impacts of anthropogenic disturbances associated with drilling activities on deep-sea benthic megafauna (Jones at al., 2007), as well as recovery of these communities from disturbances (Gates and Jones, 2012; Jones et al., 2012; Valentine and Benfield, 2013).

Currently, the only quasi-consistent monitoring studies of deep-sea GoM ecosystems are biological surveys required by the U.S. Department of the Interior’s Bureau of Ocean Energy Management (BOEM), as part of regulation of the development of outer continental shelf oil and natural gas resources. BOEM requires a limited ROV visual habitat survey to be completed both before and after drilling and anchor placement. These surveys consist of recording biological and physical information, including the types of animals present and appearance of the seafloor (BOEM, 2011). Recommendations for these surveys include that the ROV is flown close enough to the seafloor to observe relatively small animals and features. Minimum survey

requirements include conducting six survey track lines radiating from a central point of origin at intervals of 60° and extending at least 100 meters from the launch point.

The BOEM survey design provides a simple methodology for obtaining basic benthic data directly around a developed well site. These surveys place primary emphasis on observing sensitive communities and enforcing stand-off requirements rather than evaluating all benthic communities in the surrounding areas. Therefore the design has limitations in its ability to provide adequate information for more in-depth environmental monitoring purposes. Smaller surveys generally have more room for error in estimating diversity and abundances of animals. This limits the ability to observe less abundant or rare species. Similarly, it may not provide enough area coverage to adequately study animal populations that exhibit patchy distributions. The BOEM surveys are also generally limited only to areas directly at the site of offshore activities rather than including areas further away that may still be impacted by drilling or in the event of an environmental incident.

The recent Deepwater Horizon oil spill incident has further emphasized the need to expand knowledge on GoM ecosystems. Deepwater Horizon was a semisubmersible rig located in Mississippi Canyon block 252 (MC252), approximately 50 miles southeast of the Mississippi River delta in the GoM. An explosion and consequent loss of well control resulted in oil and gas flowing from the Macondo Prospect oil field from April 22, 2010 to July 15, 2010. While the exact amount of oil and gas spilled into the GoM as a result of this accident has been widely debated, the Deepwater Horizon oil spill has been acknowledged as the largest oil spill in the history of the United States (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2011). In the wake of the spill, there was a marked increase in demand for knowledge on GoM ecosystems, including the lesser-known deep-sea megabenthos. This became even more important as a component of environmental monitoring of areas potentially affected by the spill.

In response to a need to understand potential impacts of the Deepwater Horizon oil spill, a methodology was developed for industrial ROVs to survey deep-sea benthic sites at a range of locations around the MC252 well site. Industrial ROVs represent a readily available resource

located in proximity to areas potentially affected from offshore drilling activities and oil spills. The methodology described here is further intended to be used as part of an environmental monitoring program to observe the deep-sea benthic ecosystem responses to the 2010 Deepwater Horizon oil spill. This study presents a 15°, radial benthic survey methodology that can be employed for use in environmental monitoring programs for deep-sea benthic ecosystems. The goals were to evaluate effectiveness of the 15° radial survey design compared to other radial designs including the 60° BOEM design, along with showing how it can be used to investigate benthic megafaunal communities.

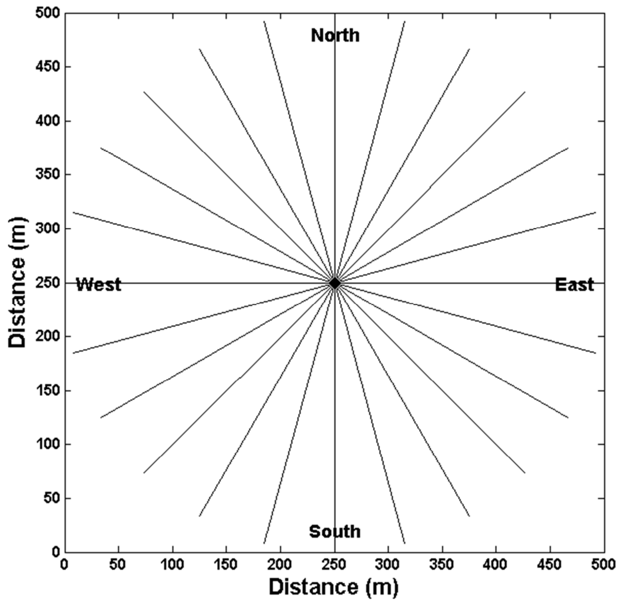
Simulations were created to evaluate detection of theoretical organism populations by nine radial survey designs of different transect lengths and degrees of separation between transects, as well as the BOEM design. These simulations were run on both random and clustered animal populations in order to evaluate effectiveness of determining animal densities under different population distribution conditions. A preliminary field test exemplifying how this methodology can be used for observing the deep-sea benthic environment and its associated megafauna is presented using a case study of benthic surveys completed at site Mississippi Canyon 118 (MC118) in the northern GoM. An initial set of surveys was conducted at MC118 prior to crab-trapping taking place as part of a Natural Resource Damage Assessment (NRDA) research cruise, followed by a set of surveys after crab-trapping. This provided an opportunity to explore the effectiveness of the 15° radial survey design in a situation of known impact to the deep-sea benthic communities.

3.2. Methods

3.2.1. 15° Survey Design

In order to quantify the biodiversity and abundance of benthic megafauna in the vicinity of MC252 and at sites further away, an expanded version of the 60°, 100 m long BOEM survey design was developed. This new design incorporated the use of industrial ROVs to perform a radial transect survey pattern; however, instead of 6 transects in the BOEM design, there were a total of 24 transects. These 24 transects were more finely spaced at 15° intervals with longer

lengths of 250 m. Figure 3.1 shows a diagram of the theoretical 15° survey design along with an example of navigation data from a real survey employing the 15° design in the field.



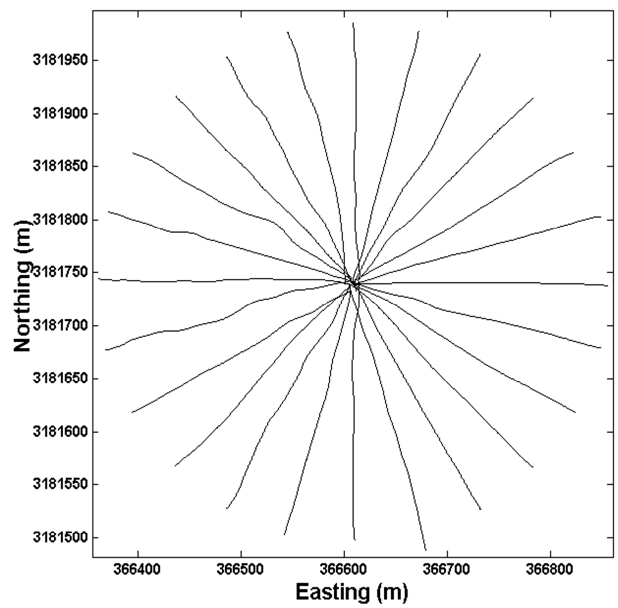


Fig. 3.1. Diagram showing a theoretical ROV transect survey design with 250 m long transects separated by 15° (on left) and an example of an actual ROV survey track employing the 15° radial survey pattern (on right).

3.2.2. Modeling Survey Design Effectiveness for Observing Benthic Animal Densities

Simulations were conducted using MATLAB (The Mathworks INC., 2014) to evaluate the effectiveness of different radial ROV survey designs for measuring benthic megafaunal densities. Two types of animal populations were used in the simulations – random and clustered (or clumped). Random distributions consisted of organisms plotted randomly throughout the model survey grid, using MATLAB to create pseudorandomly chosen x and y coordinates. Clustered populations were created by placing ten “center points” at random locations in the survey grid, with organisms then randomly located in a clustered fashion around these centers to produce observable “clustering” of the population. Both random and clustered organism distributions were compared for a 500 m by 500 m area using organism densities ranging from 10 to 2,560 ha^{-1} or 250 to 64,000 animals for the entire area (at intervals of 80 ha^{-1} up until a density of 800 ha^{-1} and intervals of 160 ha^{-1} for the remainder).

A transect was overlaid on top of the theoretical populations. Instead of rotating the transect, the transect was fixed in a north-south orientation and the survey domain containing the animals was rotated by increments reflecting the angles corresponding to each of the transects. This was done for computational efficiency. The density of animals detected within each transect was estimated by calculating how many animals were found in the transect and dividing by the total area of the transect. The mean density (averaged over all transects) was then calculated to estimate mean density of animals at the site. Figure 3.2 illustrates simulations run on example random and clustered animal populations.

A total of ten radial survey designs were evaluated (Table 3.1), including both the 100 meter-long BOEM 60° (60degBOEM) survey design, as well as the 250 meter-long, 15° (15deg250) survey. Simulation nomenclature reflects the degree angle and length of the transects in the survey design – e.g. a 250 m long transect with 15° spacing between transects is named 15deg250. In all cases the swath width of each transect was fixed at 5 m. This allowed for investigating the effects of transect length and degree of separation between transects on the ability to accurately estimate animal densities. Each of the ten simulations were run a total of twelve times for both random and clustered population distributions for each of the ten survey designs in order to account for some of the variation that occurs as a result of differences in the distributions of the random or clustered populations. Estimated animal densities obtained from the simulations were determined for each true density inputted into the simulation. Estimated densities were then plotted against true densities, and slopes and y-intercept values were determined for the results of each simulation. A multivariate ANCOVA was then used to test the null hypothesis that the slopes and y-intercept values were equal for all simulations. Slopes and y-intercept values for each simulation were also compared to the ideal values of 1 and (0,0), respectively.

Monetary costs associated with conducting each of the 10 survey designs were estimated to allow for additional consideration of each design as a reasonable means of evaluating benthic megafaunal densities. An overall estimated cost of $75,000 per day, covering the costs of operating an ROV to conduct benthic surveys, was assumed. Transect times were based on actual times observed when conducting the 250 meter long transects during field applications

of the 15deg250 survey design. Each transect (outbound and return, equaling 500 m total) was estimated at 30 minutes, which translates to a time of approximately 0.06 minutes per meter and a resulting cost of about $52 per minute. These values were then used to calculate the costs of conducting each of the survey designs evaluated using the simulations.

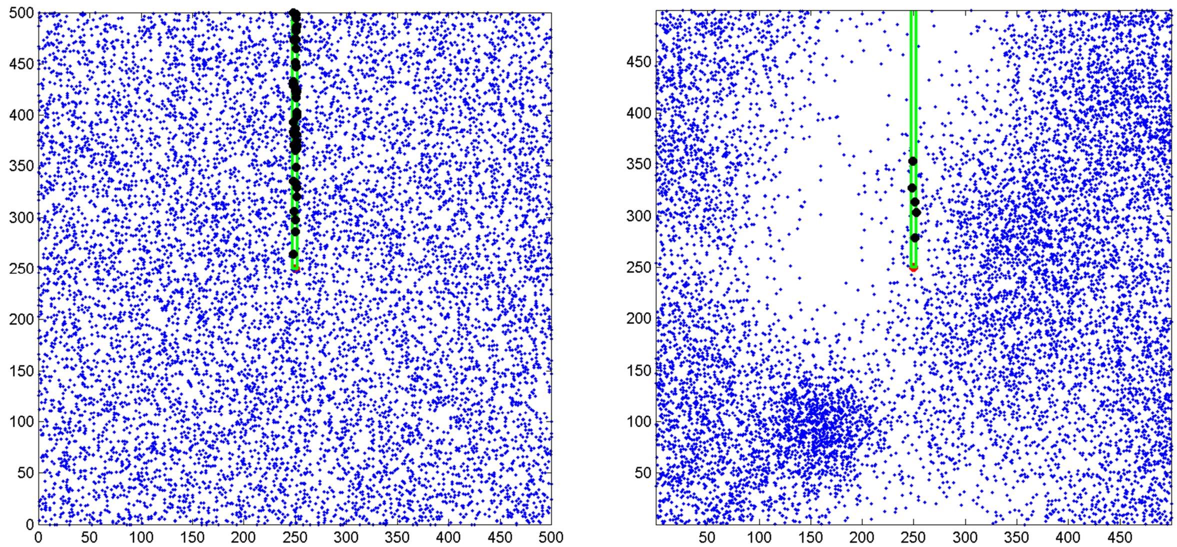


Fig. 3.2. Example of a simulation transect overlaid on top of random (on left) and clustered (on right) animal populations distributed across a 500 m by 500 m area. Blue dots represent animals; green shows the transect; and dots in black are animals within the transect detected by the simulation.

Table 3.1. Survey designs used for simulations.

Survey Design	Degree Angle (°)	Number of Transects	Transect Length (m)	% of Total Area
15deg100	15	24	100	4.8
15deg150	15	24	150	7.2
15deg200	15	24	200	9.6
15deg250	15	24	250	12
30deg250	30	12	250	6
45deg250	45	8	250	4
60deg250	60	6	250	3
75deg250	75	4	250	2
90deg250	90	3	250	1.5
60degBOEM	60	6	100	1.2

3.2.3. Mississippi Canyon 118 Case Study

To more specifically explore the applications and effectiveness of the modified 15°, 250 m radial survey design for evaluating benthic megafaunal populations, a case study exemplifying use of this design was examined. Data was collected at a study sites surveyed during one of two NRDA cooperative cruises conducted aboard the HOS Sweetwater in the northern GoM during the summer of 2011. An industrial-class remotely operated vehicle (ROV) was used to collect video and still imagery at Mississippi Canyon 118 (MC118) located at 28° 51' 07.992" N and 88° 29' 30.000" W. At this site, ROV surveys were conducted both before and after crabs were trapped and collected as part of a NRDA research cruise (trapping occurred at 28° 49' 04.44" N and 88° 32' 53.592" W). Pre-crab trapping surveys were conducted from August 14, 2011 to August 15, 2011, and the post-crab trapping surveys were completed one day later from August 16, 2011 to August 17, 2011.

3.2.4. Data Collection and Survey Design

A Triton XLS 150 ROV capable of operating to a maximum depth of approximately 3000 m was used for all surveys in this study. The ROV was equipped with multiple still image and video cameras including: standard definition video (SD) camera with 480i (640 x 480 pixel) resolution; ROS MantaHD (high definition) video camera with 1080i (1920 x 1080 pixel) interlaced resolution; 5 megapixel Kongsberg Digital Still Camera (DSC) OE14-208 with strobe OE11-242; or a 12 megapixel Imenco Shark Digital Still Camera with strobe. An ultra-short baseline (USBL) navigation system was used to guide the ROV to the precise central point of the survey and log the position of the ROV during surveys. After the camera systems were aimed obliquely downward (to an approximate angle of 30° from horizontal) and a predetermined set of lights were switched on, the ROV proceeded to move at a constant velocity along the transect. A pair of red laser scalers was used to measure width of the field of view (FOV) of the camera, as well as to measure organisms or other features encountered.

The ROV conducted a series of twenty-four 250-m long transects radiating from a point of origin with a 15° offset for the pre-crab trapping survey. Due to time constraints, only a half-survey consisting of 12 transects spaced 30° apart was completed for the post-crab trapping

data collection. In preparation for the survey, the ROV descended to an altitude of approximately 30 meters above the point of origin and detached from the tether management system (TMS); and then continued to slowly descend to approximately 2-3 meters above the seafloor. At the end of each transect, the ROV ascended to approximately 10 meters above the seafloor in order to avoid disturbing the sediment, at which point it turned to a reciprocal heading. The ROV descended back to 2-3 meters above the seafloor and moved back along the transect, collecting close-up video and DSC images of organisms encountered. Return transect data were used to improve identification of organisms and provide high quality, close-up images.

3.2.5. Video Analysis

Each outbound ROV HD video transect was analyzed using VideoReDo Plus (DRD Systems, Inc., 2003) video playback software. Counts, identifications and locations were recorded to be used in estimating overall abundances of each taxon and diversity. Analysis of each video began at the official transect start time taken from the cruise data logbook, and concluded at the specified transect end. Video playback speeds during the analysis varied depending on the speed at which the ROV flew the transect, with faster transects being played at less than 100% of the normal playback speed to ensure accurate analysis of the video. In order to minimize subjective detection and identification during video analysis, the same person analyzed all videos in this study.

Each time an organism on or just above the seafloor was encountered along the transect, a frame grab was taken using the VideoReDo Plus snapshot tool, and the date and time from the video was recorded in the snapshot file name. Only organisms that fell within a specific area of the FOV where optimal illumination occurred, approximately the bottom 30% of the video FOV, were counted in order to maintain consistency between transects. Efforts were made to avoid possible double-counting of organisms by generally excluding organisms that approached the field of view in the video from behind the ROV, except where it was known that a particular organism had not already been counted.

Organisms in snapshots were classified into broad taxonomic groups and then each organism was identified down to the lowest taxonomic level possible. The broad taxonomic groups used were: anemones, crabs, eel-like fishes, fishes, holothurians, sea pens, sea stars, shrimps, sponges, and other. The "other" category included: a) organisms that did not fall within any of the previously defined broader taxonomic groups; and/or b) all possible organisms for which snapshots were taken but broad taxonomic group placement was uncertain.

3.2.6. Data Analysis

Width of the FOV was calculated using the red laser scalers (separated by 17 cm) visible on the seafloor in the HD video. Where visibility permitted, ten frame grabs separated by equal time intervals were taken from each transect; and width of the video FOV was estimated using the reference distance from the laser scalers. FOV values from each photo from a particular transect were averaged in order to determine a mean FOV for that transect. Any transects for which the laser scalers were not visible or where fewer than three usable frame grabs were obtained were assigned a FOV corresponding to the mean for all other transects at the site.

The area surveyed by each transect was calculated using mean FOV of the transect multiplied by the total distance traveled along the transect as obtained from the ROV navigation data. Using these areas, densities were calculated for each of the broad taxonomic groups of organisms occurring along each transect. A mean density for each group was then determined for the survey site as a whole. Furthermore, a total density of all organisms at the site was also calculated by summing the data from all broad taxonomic groups with that of the "other" category. Since two sets of surveys were completed (before and after crab trapping took place at MC118), this procedure was carried out twice so that organism densities were obtained for both sets of surveys.

Inconsistencies in altitude of the ROV combined with varying amounts of suspended sediments and marine snow affected visibility. Differences in visibility consequently affected observations and identifications of organisms along the video transects, requiring that a correction factor be employed (Refer to Appendix A for rationale). In general, visibility was better during the post-trapping survey at MC118 than during the pre-trapping survey. Densities of limited-mobility

fauna (sea stars, sea pens and non-swimming holothurian taxa) were compared for the pre- and post-trapping surveys, and were used as a correction factor for all other organisms. It was assumed that densities of these three groups would not have changed since they were either stationary or possessed limited mobility. Consequently, their densities would have changed very little during the single-day gap between surveys. Any change in calculated densities was instead attributed to be largely an artifact of visibility differences. Densities of sea stars, sea pens and holothurians were averaged for both pre- and post-crab trapping data sets and the pre-trapping value (poorer visibility) was divided into the post-trapping to determine a correction factor. This reflects the average difference in densities observed for the three groups and is an approximation representing the influence of visibility differences on animal detection (and, consequently, density estimates) between the pre- and post-trapping surveys. The mean correction factor of 1.8 (holothurians = 1.7, sea pens = 0.9, sea stars = 2.9) was then applied to densities of all taxa for the pre-trapping survey in order to correct for effects of the decreased visibility during that survey.

Taxonomic group densities were statistically compared for the pre- and post-crab trapping data. Levene's test (Trujillo-Ortiz and Hernandez-Walls, 2003) was used to test for homogeneity of variances in order to evaluate the null hypothesis that the variances for the pre- and post-trapping data were equal. An Anderson-Darling test was performed to test whether the two sets of data followed a normal distribution. The results of the Levene's and Anderson-Darling tests were such that the two sets of data did not have equal variances nor both exhibit a normal distribution. Before and after densities of each taxonomic group were then compared using a non-parametric Wilcoxon rank sum test in order to test the null hypothesis that the densities were equal.

3.3. Results

3.3.1. Effectiveness and Accuracy of Survey Design

Animal densities obtained by the simulations were plotted in order to evaluate how simulation results compared to a 1:1 detection ratio. On the x axis of each density plot is the "true density", which is the density that was input into the simulation (0.001 to 0.256 m^{-2}); and on

the y axis is the “estimated density”, which is the mean density estimated from the simulated surveys. True and estimated densities were plotted for each population density tested (Figs. 3.3 and 3.6). The closer the estimated density is to the 1:1 line, the more accurate the detection by the simulated survey was. For example, if a true density of 40 ha^{-1} put into the simulation were to result in an estimated density of 40 ha^{-1}, there was 100% accurate estimation of organism densities by the survey design.

3.3.1.1. Random Population Simulations

Results from the simulations run on random populations were very similar amongst the ten survey designs. Overall, estimated densities were very close in value to the true densities across the range of animal densities. Changing transect length did not greatly impact accuracy of density estimations, with estimated densities falling very closely along the 1:1 detection line (Fig. 3.3). Similarly, animal densities obtained for the simulations using different degrees of transect separation were relatively consistent along the 1:1 ratio line (Fig. 3.3). However, the overall fit of the estimated density points along the 1:1 line was not as uniform as observed for different transect lengths. While not significant, variation was observed amongst the survey designs within specific input densities, particularly for higher animal densities.

Table 3.2 shows a compilation of mean estimated animal densities and 95% confidence intervals (CIs) measured as animals per hectare obtained for each input animal density across the different survey design simulations. The values in the table reflect the trends observed in Figure 3.3. There was very little variation in the estimated densities with respect to the input densities, particularly for the simulations using different transect lengths.

A different trend emerges if one compares the mean CIs associated with the mean densities. As a whole, the CIs were smaller with increasing transect length, especially at higher population densities. The 250 m transect length generally exhibited the smallest CIs, while the 100 m transect length typically had the largest. This trend was less observable across the simulations of different angles of transect separation; however, CIs were noticeably lower and less variable for the 15° simulation than for the 90° transect separation.

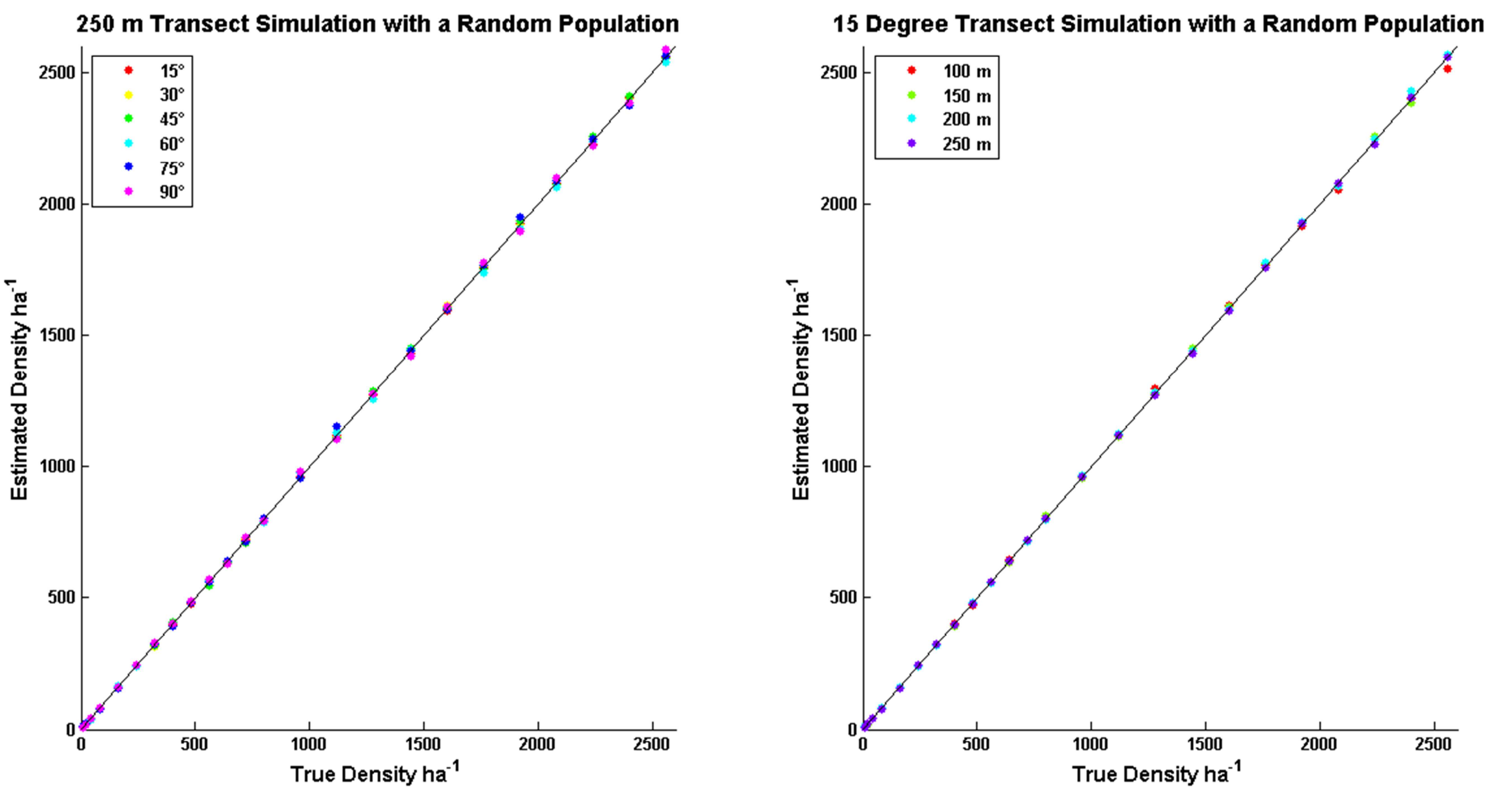


Fig. 3.3. Mean estimated animal densities obtained by simulations run for a random population distribution using: (left) 15° degree transect separation with different transect lengths (simulations: 15deg100, 15deg150, 15deg200, and 15deg250); and (right) 250 m long transects with different degrees of separation between transects (simulations: 15deg250, 30deg250, 45deg250, 60deg250, 75deg250, and 90deg250).

Table 3.2. Mean estimated animal densities obtained across different input animal densities for simulations run on random animal populations.

Input Density (ha^{-1})	Estimated Densities (ha^{-1} ± 95% CI)									
	15deg100	**15deg150**	**15deg200**	**15deg250**	**30deg250**	**45deg250**	**60deg250**	**75deg250**	**90deg250**	**60deg100**
10	9 ± 9	9 ± 5	11 ± 8	10 ± 5	11 ± 4	8 ± 6	11 ± 6	10 ± 6	9 ± 8	10 ± 13
20	22 ± 9	20 ± 6	20 ± 8	20 ± 7	21 ± 8	19 ± 6	19 ± 9	20 ± 9	19 ± 10	21 ± 19
40	43 ± 19	40 ± 13	40 ± 9	40 ± 6	40 ± 11	42 ± 13	38 ± 15	42 ± 22	42 ± 12	41 ± 30
80	75 ± 20	81 ± 24	80 ± 16	79 ± 13	80 ± 17	77 ± 14	79 ± 29	76 ± 9	83 ± 21	81 ± 56
160	163 ± 34	160 ± 20	159 ± 19	157 ± 13	161 ± 21	161 ± 37	164 ± 23	155 ± 37	161 ± 28	148 ± 29
240	246 ± 34	240 ± 36	240 ± 20	244 ± 36	241 ± 33	245 ± 43	238 ± 39	243 ± 49	246 ± 46	230 ± 56
320	324 ± 71	317 ± 38	318 ± 27	322 ± 17	316 ± 49	321 ± 24	322 ± 45	324 ± 35	327 ± 42	323 ± 129
400	403 ± 37	395 ± 31	398 ± 20	396 ± 29	406 ± 33	405 ± 59	393 ± 63	393 ± 56	404 ± 40	404 ± 51
480	472 ± 60	478 ± 22	483 ± 41	475 ± 32	486 ±40	482 ± 45	484 ± 31	483 ± 46	485 ± 81	497 ± 130
560	561 ± 53	561 ± 48	558 ± 42	561 ± 39	566 ± 53	549 ± 33	568 ± 77	561 ± 58	570 ± 43	570 ± 77
640	643 ± 64	638 ± 51	641 ± 32	639 ± 23	642 ± 51	637 ± 56	638 ± 47	638 ± 93	632 ± 67	620 ± 92
720	718 ± 72	721 ± 55	713 ± 69	719 ± 31	724 ± 40	708 ± 50	728 ± 75	716 ± 50	727 ± 89	713 ± 140
800	797 ± 73	815 ± 55	797 ± 33	803 ± 54	794 ± 36	803 ± 60	791 ± 52	806 ± 92	791 ± 58	791 ± 93
960	958 ± 64	955 ± 35	966 ± 81	962 ± 40	956 ± 75	982 ± 87	975 ± 74	956 ± 91	980 ± 90	956 ± 200
1120	1121 ± 86	1114 ± 71	1125 ± 36	1118 ± 45	1114 ± 61	1112 ± 77	1130 ± 86	1153 ± 74	1106 ± 125	1114 ± 168
1280	1296 ± 92	1278 ± 76	1281 ± 35	1275 ± 33	1279 ± 72	1287 ± 69	1260 ± 87	1280 ± 75	1276 ± 83	1296 ± 101
1440	1433 ± 92	1449 ± 43	1441 ± 75	1432 ± 44	1446 ± 71	1451 ± 57	1446 ± 95	1441 ± 108	1421 ± 121	1416 ± 206
1600	1615 ± 122	1610 ± 95	1600 ± 57	1596 ± 55	1614 ± 78	1605 ± 107	1611 ± 64	1598 ± 69	1610 ± 148	1603 ± 158
1760	1765 ± 48	1773 ± 106	1775 ± 67	1759 ± 61	1762 ± 62	1752 ± 89	1739 ± 103	1768 ± 110	1776 ± 208	1725 ± 270
1920	1916 ± 134	1930 ± 98	1929 ± 82	1923 ± 70	1921 ± 68	1937 ± 166	1908 ± 100	1951 ± 77	1896 ± 127	1962 ± 162
2080	2055 ± 206	2081 ± 96	2067 ± 98	2079 ± 72	2075 ± 83	2072 ± 106	2064 ± 143	2088 ± 135	2098 ± 120	2051 ± 248
2240	2255 ± 120	2254 ± 82	2246 ± 96	2229 ± 61	2236 ± 127	2255 ± 124	2239 ± 131	2247 ± 164	2220 ± 128	2270 ± 161
2400	2399 ± 178	2384 ± 126	2428 ± 73	2406 ± 59	2398 ± 78	2411 ± 82	2374 ± 135	2375 ± 128	2386 ± 154	2452 ± 222
2560	2512 ± 78	2557 ± 88	2567 ± 82	2556 ± 100	2553 ± 92	2555 ± 110	2537 ± 165	2562 ± 125	2586 ± 141	2534 ± 240

Results from the simulations were each fit with a linear best fit line in order to see how they compare to the 1:1 detection line. Slopes and y-intercepts were obtained for the best fits lines (refer to Table 3.3, Fig. 3.4) to evaluate overall accuracy of each simulation compared to the ideal 1:1 line with a slope of 1 and y-intercept at (0,0). For simulations run on a random population, the 15deg250 and 30deg250 simulations had slopes closest to 1, with 60deg250 and 15deg100 having slopes furthest from 1. The 15deg150, 15deg250, 45deg250, and 75deg250 simulations had y-intercepts closest to (0,0), while 15deg100, 90deg250, and 60deg100 had y-intercepts furthest from the ideal. Despite these mild trends, there were no significant differences observed in the values of the slopes and y-intercepts of the best fit lines between the ten simulations. R^2 values were found to generally increase with increasing transect length and finer spacing between transects, with the 15deg250 simulation having the highest correlation between true and estimated densities (R^2 = 0.9994) and the 60degBOEM simulation having the lowest (R^2 = 0.9938).

Costs of conducting each survey design are also shown in Table 3.3. A linear cost trend is observed associated with survey transect lengths. For every 50 meters of transect length added, there is an added cost of approximately $7488. Similarly, there is an increase in cost as the degrees of transect separation becomes finer. In this case, however, there is a much greater increase in cost (along the lines of a power function vs. linear fit) as separation between transects becomes smaller.

Table 3.3. Values of the slope, intercept, and R^2 of the fit lines associated with each simulation run on a random population, along with the estimated monetary cost associated with conducting each survey design.

Simulation	Slope	Intercept	R^2	Cost ($)
15deg100	0.9948	0.0004	0.9977	14976
15deg150	1.0014	-0.00002	0.9989	22464
15deg200	1.0042	-0.0002	0.9991	29952
15deg250	0.9990	-0.00003	0.9994	37440
30deg250	0.9987	0.0002	0.9991	18720
45deg250	1.0025	-0.00005	0.9985	12480
60deg250	0.9927	0.0004	0.9981	9360
75deg250	1.0018	0.00005	0.9977	7800
90deg250	0.9985	0.0002	0.9975	6240
60deg100BOEM	1.0033	-0.0003	0.9938	3744

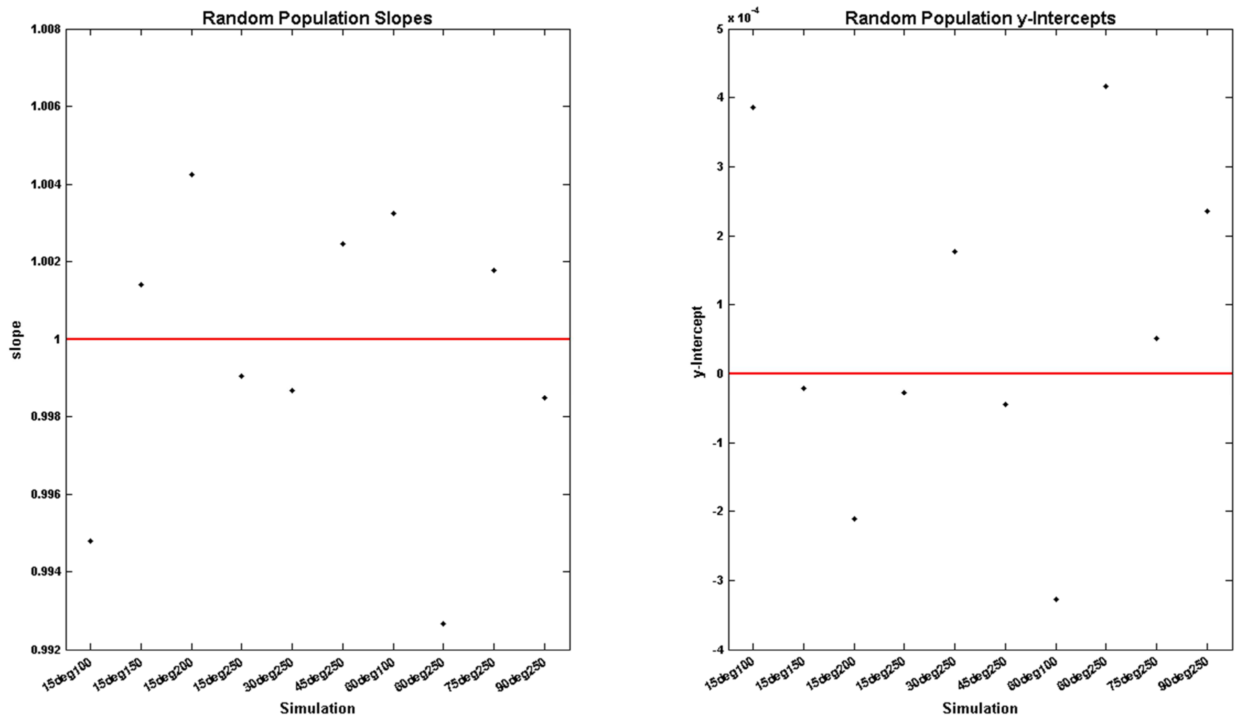


Fig. 3.4. Slopes (left) and y-intercept values (right) for the ten different simulations run on a random population distribution. Note that slopes with values closer to 1 and y-intercepts closer to (0,0), both marked by red lines, are ideal.

3.3.1.2. Clustered Population Simulations

Unlike the simulation results for a random population where results were generally consistent with the 1:1 detection line, more variation was observed for a clustered population. Figure 3.5 shows the simulation results for simulations of varying transect lengths and degrees of separation, respectively, run on a clustered population. There was not only greater variation in proximity to the 1:1 line between different true animal densities, but also between different simulations at a given true density. This was particularly the case at larger true densities. These trends were even more pronounced among the simulations using different transect lengths when compared to those using different degrees of separation between transects, with less variation and closer overall proximity to the 1:1 line observed for the latter.

When comparing mean animal densities (refer to Table 3.4) obtained for the various simulations run on a clustered population, several trends emerged. Simulations using smaller

transect lengths were much more variable over the range of input densities, with over- or under-estimation of densities commonplace. While still also exhibiting variability, longer transect lengths were as a whole more consistently closer to the 1:1 line. This was likewise the trend observed amongst the simulations of different degrees of transect separation. Variability of estimated densities was lower as separation between transects decreases.

95% CIs for simulations on the clustered populations were more similar across simulations than for the random populations. No obvious trends were observed for the 95% CIs between simulations with different degrees of transect separation; however, there was still a trend with respect to transect length. In general, CIs were smaller for longer transect lengths, with 250 m exhibiting smaller CIs than the 100 m simulation. Variability in the CIs was also less pronounced for the longer transect lengths.

As with the simulations run on a random population, slope and y-intercept values were also determined for the best fit lines for simulations on a clustered population (Table 3.5, Fig. 3.6). The 15deg200, 15deg150, and 30deg250 had slopes closest to 1, while the 15deg100, 45deg250, and 75deg250 slopes were furthest from the 1:1 line. The 90deg250, 15deg250, and 30deg250 simulations had y-intercepts closest to 0,0; and 15deg100, 75deg250, 60deg100, and 45deg250 were furthest away from the ideal y-intercept of 0,0. No discernable trend between simulations was observed for y-intercept values; however, a decrease in slope was observed as transect lengths increased from 100 to 250 meters. Compared to the slope and y-intercept values for a random population, those for a clustered population exhibited greater variation across the simulations. As with the simulations on random populations, there were no significant differences observed in the slopes or y-intercepts of the best fit lines between the ten simulations. The R^2 values for simulations run on clustered populations exhibited a large increase with increasing transect length, with the 15deg100 and 60deg100BOEM having the smallest values by far (R^2 = 0.5692 and 0.5605, respectively) compared to the highest values observed for the 15°-60°, 250 m simulations (R^2 ranging from 0.8647 to 0.8727). The costs associated with the different survey designs are also shown in Table 3.5 and are the same as described for the random population simulations.

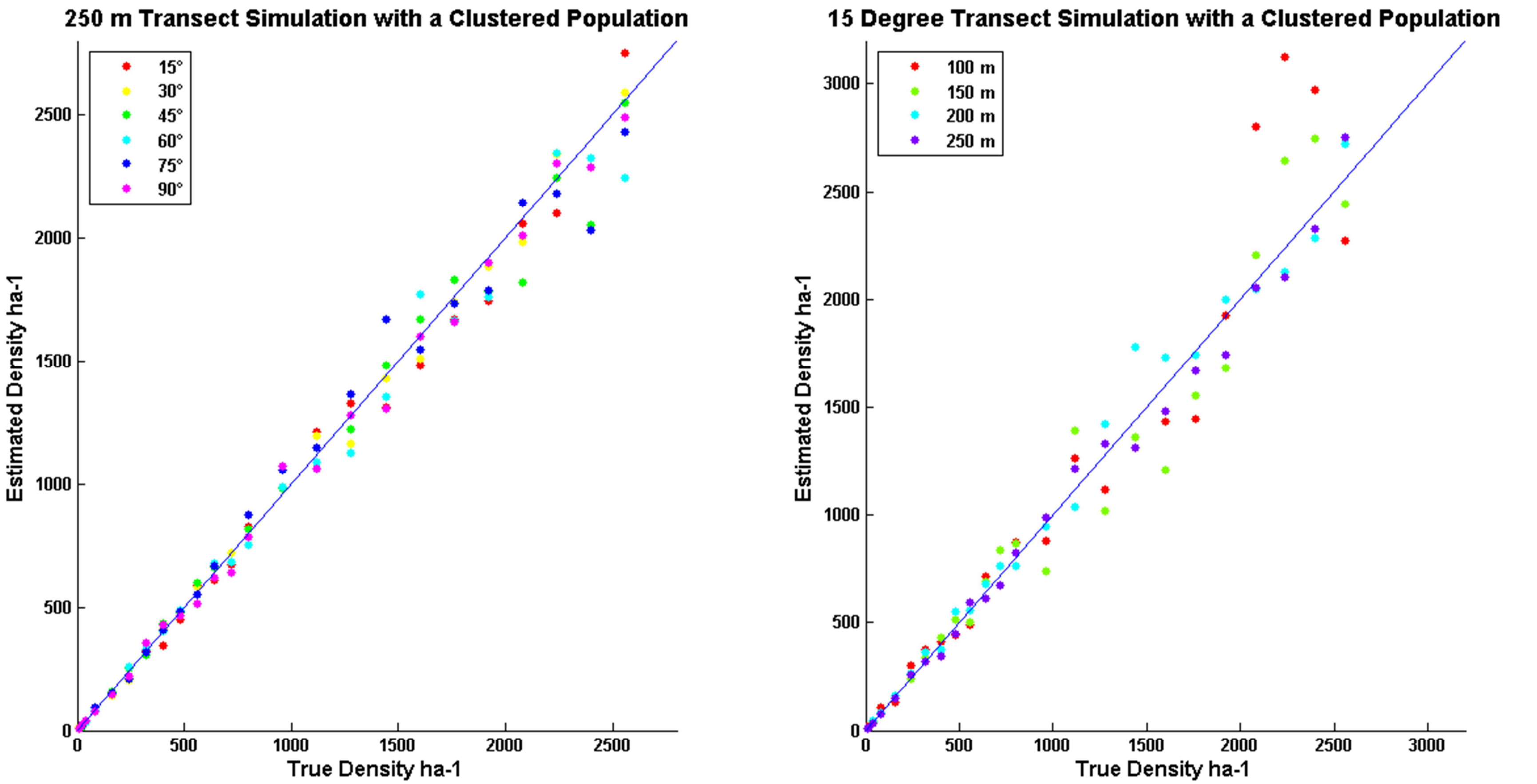


Fig. 3.5. Mean estimated animal densities obtained by simulations run for a clustered population distribution using: (left) 15° degree transect separation with four different transect lengths (simulations: 15deg100, 15deg150, 15deg200, and 15deg250); and (right) 250 m long transects with different degrees of separation between transects (simulations: 15deg250, 30deg250, 45deg250, 60deg250, 75deg250, and 90deg250).

Table 3.4. Mean estimated animal densities obtained across different input animal densities for simulations run on clustered animal populations.

Input Density (ha^{-1})	**Estimated Densities (ha^{-1} ± 95% CI)**									
	15deg100	**15deg150**	**15deg200**	**15deg250**	**30deg250**	**45deg250**	**60deg250**	**75deg250**	**90deg250**	**60deg100**
10	11 ± 20	12 ± 13	10 ± 8	9 ± 3	11 ± 7	9 ± 9	10 ± 7	10 ± 11	10 ± 8	10 ± 14
20	21 ± 26	16 ± 25	17 ± 12	18 ± 12	21 ± 15	15 ± 14	17 ± 7	17 ± 15	23 ± 18	22 ± 45
40	31 ± 47	41 ± 43	45 ± 38	36 ± 26	41 ± 29	38 ± 26	35 ± 27	40 ± 31	41 ± 17	40 ± 69
80	105 ± 99	75 ± 79	83 ± 64	78 ± 44	75 ± 50	77 ± 56	81 ± 32	93 ± 74	75 ± 38	109 ± 181
160	133 ± 88	150 ± 181	163 ± 147	152 ± 76	139 ± 44	158 ± 61	148 ± 73	153 ± 109	145 ± 71	151 ± 264
240	300 ± 333	240 ± 163	264 ± 130	258 ± 141	208 ± 104	253 ± 82	260 ± 104	211 ± 83	222 ± 220	239 ± 399
320	372 ± 481	335 ± 231	363 ± 190	320 ± 185	317 ± 168	309 ± 218	328 ± 224	319 ± 171	352 ± 171	270 ± 392
400	413 ± 570	429 ± 446	375 ± 258	342 ± 102	412 ± 175	434 ± 288	403 ± 245	405 ± 285	427 ± 399	462 ± 575
480	443 ± 630	512 ± 317	549 ± 444	448 ± 142	465 ± 246	479 ± 259	485 ± 388	484 ± 220	465 ± 395	648 ± 676
560	492 ± 465	504 ± 580	556 ± 438	591 ± 274	580 ± 378	598 ± 317	515 ± 198	552 ± 359	515 ± 287	614 ± 978
640	717 ± 1011	691 ± 674	679 ± 384	612 ± 457	669 ± 373	661 ± 405	680 ± 235	666 ± 483	619 ± 303	643 ± 643
720	670 ± 652	838 ± 795	766 ± 239	674 ± 433	720 ± 352	685 ± 458	682 ± 507	644 ± 470	639 ± 245	810 ± 1066
800	874 ± 2059	865 ± 1001	766 ± 498	825 ± 393	817 ± 600	818 ± 435	754 ± 492	876 ± 625	783 ± 542	685 ± 484
960	879 ± 921	739 ± 1056	943 ± 426	986 ± 442	1057 ± 307	981 ± 467	989 ± 741	1055 ± 965	1072 ± 761	844 ± 639
1120	1264 ± 1420	1391 ± 943	1038 ± 634	1211 ± 944	1197 ± 438	1062 ± 547	1087 ± 697	1146 ± 613	1061 ± 591	1251 ± 1250
1280	1118 ± 1579	1018 ± 638	1423 ± 857	1329 ± 507	1163 ± 697	1222 ± 560	1127 ± 503	1365 ± 1122	1280 ± 739	1018 ± 1323
1440	1311 ± 1183	1360 ± 926	1778 ± 1106	1313 ± 655	1428 ± 538	1482 ± 832	1356 ± 513	1670 ± 950	1309 ± 1086	1736 ± 1550
1600	1431 ± 1841	1207 ± 1680	1733 ± 966	1483 ± 900	1510 ± 876	1668 ± 806	1769 ± 1138	1547 ± 323	1600 ± 746	2135 ± 1774
1760	1446 ± 1040	1552 ± 1140	1741 ± 930	1668 ± 882	1741 ± 1475	1830 ± 1128	1663 ± 1056	1732 ± 1015	1658 ± 1240	2160 ± 2884
1920	1924 ± 2468	1684 ± 1495	1996 ± 1573	1745 ± 918	1883 ± 1166	1780 ± 1113	1758 ± 941	1788 ± 1398	1895 ± 986	2081 ± 2681
2080	2803 ± 3768	2208 ± 1674	2045 ± 1421	2055 ± 1511	1984 ± 872	1816 ± 830	2141 ± 822	2142 ± 1313	2009 ± 1676	1945 ± 3196
2240	3124 ± 1851	2646 ± 2568	2127 ± 1586	2099 ± 1177	2340 ± 1392	2242 ± 1006	2344 ± 1367	2178 ± 1262	2304 ± 1323	2648 ± 3496
2400	2969 ± 3836	2747 ± 2720	2284 ± 1718	2325 ± 1369	2286 ± 1106	2053 ± 876	2323 ± 660	2032 ± 1298	2284 ± 1089	2258 ± 1553
2560	2274 ± 2753	2440 ± 1760	2723 ± 2256	2752 ± 1897	2590 ± 1280	2548 ± 2301	2245 ± 1573	2432 ± 1612	2491 ± 1402	2177 ± 2727

Table 3.5. Values of the slope and intercept of the fit lines associated with each simulation run on a clustered population, along with the estimated monetary cost associated with conducting each survey design.

Simulation	Slope	Intercept	R^2	Cost ($)
15deg100	1.1120	-0.006	0.5692	14976
15deg150	1.0122	-0.002	0.7031	22464
15deg200	1.0089	0.002	0.7971	29952
15deg250	0.9817	-0.0003	0.8672	37440
30deg250	0.9869	0.0006	0.8647	18720
45deg250	0.9453	0.003	0.8665	12480
60deg250	0.9618	0.001	0.8727	9360
75deg250	0.9515	0.004	0.8426	7800
90deg250	0.9751	0.0002	0.8498	6240
60deg100BOEM	1.0181	0.003	0.5605	3744

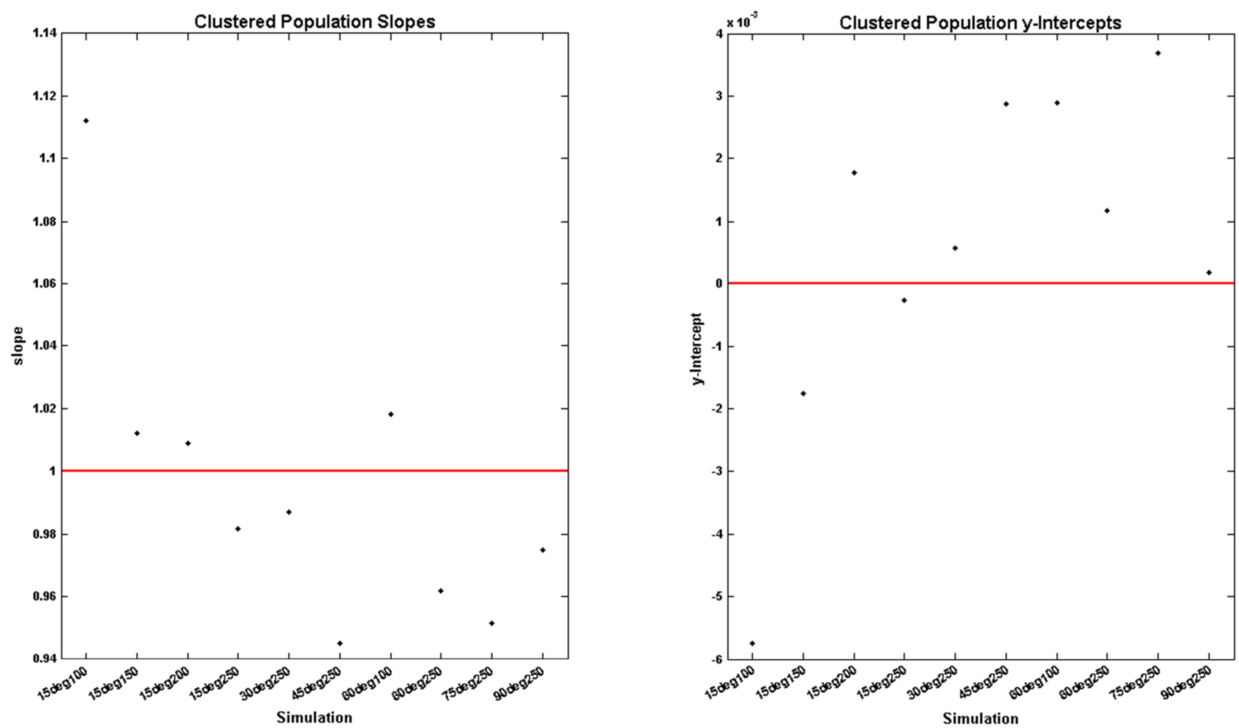


Fig. 3.6. Slopes (left) and y-intercept values (right) for the ten different simulations run on a clustered population distribution. Note that slopes with values closer to 1 and y-intercepts closer to (0,0), both marked by red lines, are ideal.

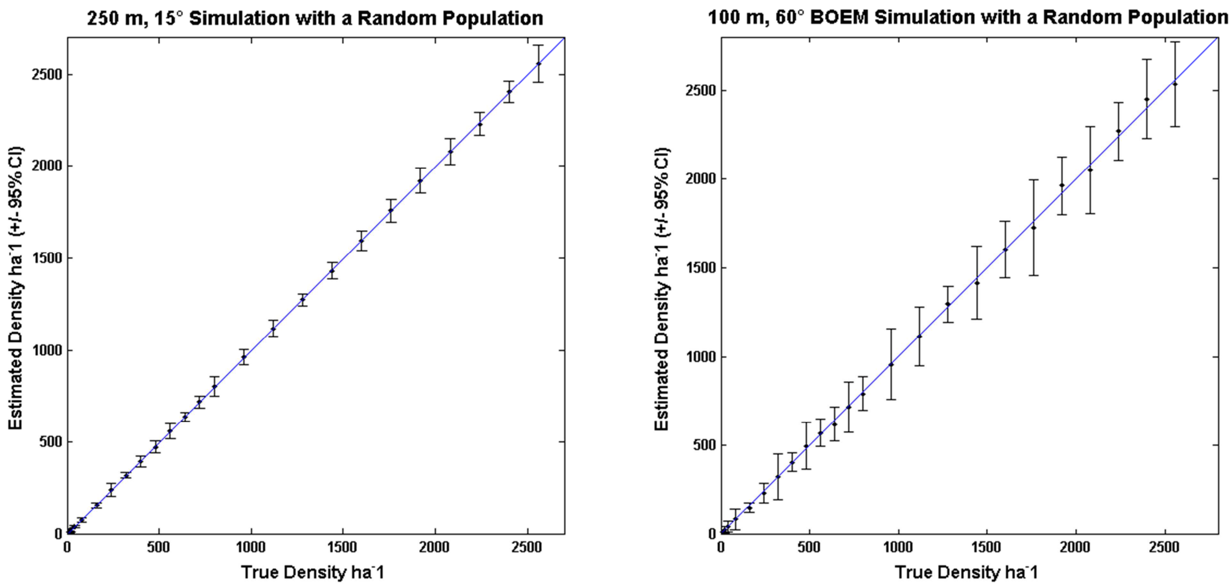


Fig. 3.7. Mean estimated animal densities, including 95% CIs, obtained for a random population distribution for the 15°, 250 m long (left) and 60°, 100 m BOEM (right) radial survey designs.

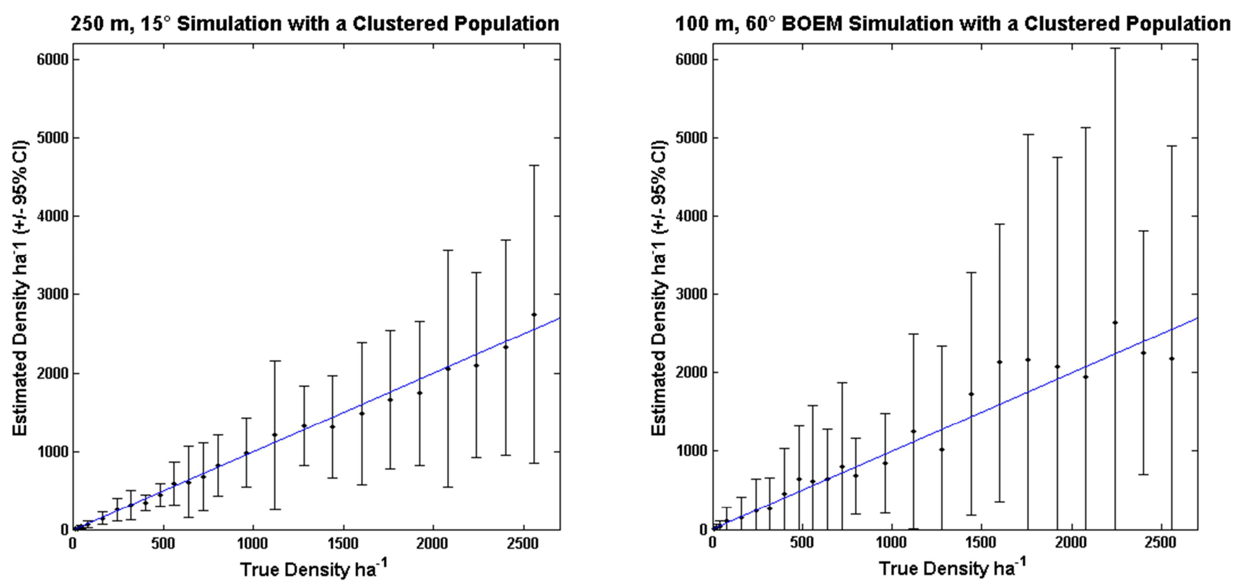


Fig. 3.8. Mean estimated animal densities, including 95% CIs, obtained for a clustered population distribution for the 15°, 250 m long (left) and 60°, 100 m BOEM (right) radial survey designs.

Figures 3.7 and 3.8 show a more specific comparison of the 60°, 100 meter long transect BOEM survey design (60degBOEM) with the 250 meter 15° design (15deg250). For a random population distribution, both survey designs were similarly effective at estimating true animal densities. The majority of values, within 95% confidence, were in close proximity to the 1:1 detection ratio line. Estimated densities obtained by the 15° survey fall almost exactly along the 1:1 line and had small 95% CIs; while estimate densities from the BOEM design exhibited a less consistent fit with the 1:1 line and had larger 95% CIs. These differences between the surveys were even more pronounced in the results for clustered populations. There was more variability and much larger 95% CIs and, in general, estimated mean densities were less consistent with the 1:1 line for the BOEM design. Similar to the random population simulations, differences between the two survey designs were more pronounced at larger population densities. For these reasons, the 15° modified survey design was found to be more consistent and effective at estimating animal populations that exhibit both a random or clustered population distribution than the 60° BOEM design.

3.3.2. MC118 Case Study

In order to evaluate the use of a radial ROV survey design for evaluating the benthic environment and its associated megafauna, ROV video imagery from a site in the Northern Gulf of Mexico was analyzed. A variety of biotic and abiotic features can be observed in still photos taken from the ROV videos (refer to Fig. 3.9), including the presence of animals and seafloor features such as mounds, depressions, pits and burrow holes, and areas of physical disturbance to the seafloor. While abiotic features are not the focus of the MC118 case study, it is important to note the kinds of benthic data obtainable from ROV benthic surveys such as this one.

A total of 1,830 frame grabs were taken of organisms from the 24 pre-crab trapping MC118 survey transect videos, and 1,072 were taken from the 12-transect post-crab trapping survey. Typical organisms seen in greater abundances at this site consisted of many varieties of red shrimps, including *Plesiopenaeus* sp. and others; the red crab, *Chaceon quinquedens*; eel-like fishes, predominantly *Aldrovandia* sp. and *Synaphobranchus* sp.; and a variety of fish including

several types of Macrourids, several species of tripod fish *Bathypterois*, and Chimaeras. Other prominent organisms that were found in lower abundances included white *Mesothuria* holothurians and several unidentified species of sea stars. Less commonly seen organisms at this study site were the white armored shrimp, *Glyphocrangon* sp.; flytrap anemone, *Actinoscyphia aurelia*; and sea pens. It must be noted that observations were limited to what could readily be seen in the ROV video flown at 2-3 m altitude, which likely excluded some smaller organisms like small anemones. Variability in visibility throughout both sets of surveys further impacted the ability to adequately observe smaller animals that may have been present.

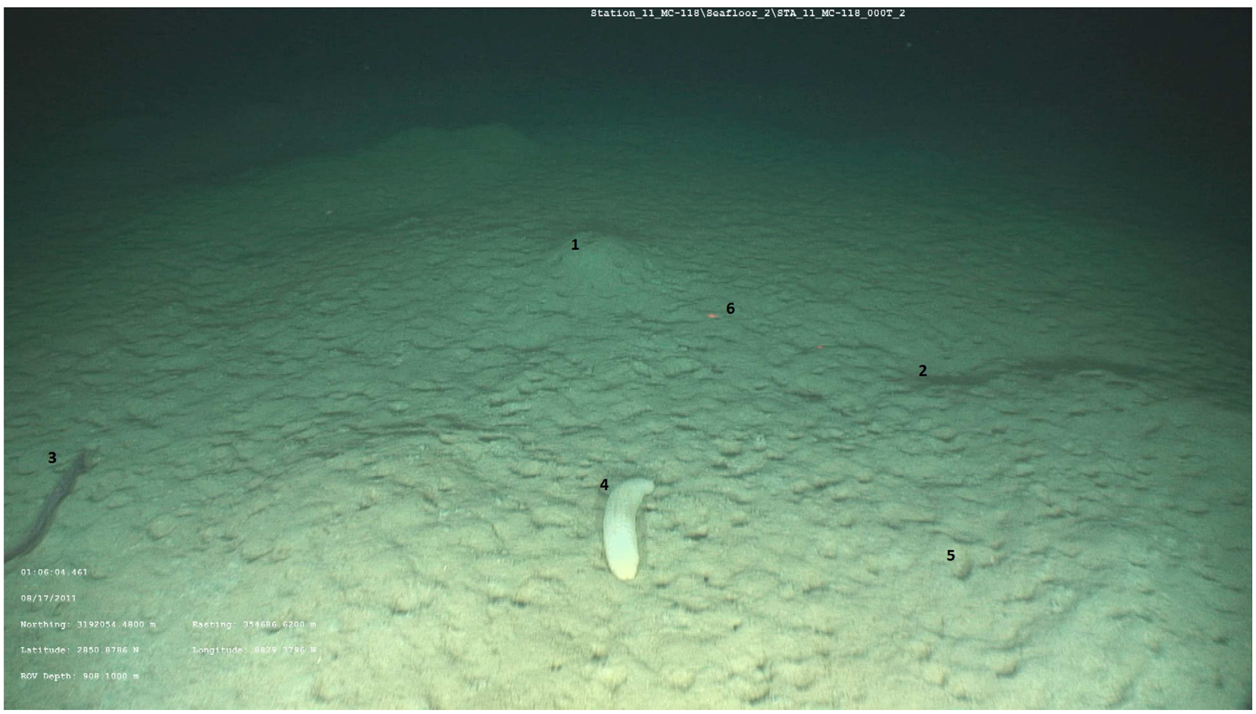


Fig. 3.9. Example still photo taken from ROV video at MC118 during post-crab-trapping surveys. The following biotic and abiotic seafloor features are observable in the still photo: 1) mound; 2) depression; 3) *Synaphobranchus* eel; 4) *Mesothuria* holothurian; 5) small pits or burrow holes; and 6) ROV laser scalers that can be used for measuring features.

Densities calculated for the broad taxonomic groups at MC118 exhibited a range of values for both the pre- and post-crab trapping surveys. Table 3.6 shows the densities obtained for each of the taxonomic groups after the pre-crab trapping survey densities were corrected for

visibility differences using the correction factor. Anemones by far had the lowest densities with only one (density of 0.7 ha^{-1}) observed in the pre-trapping survey and zero observed during the post-trapping survey. Sea pens similarly had low densities (<10 ha^{-1}), with sea stars, holothurians and crabs exhibiting the next lowest densities in the range of 10 to 50 ha^{-1}. Overall, fishes, eels and shrimps, respectively, had the highest densities by at least an order of magnitude greater than all the taxonomic groups for this study site.

Within each set of surveys the densities for each taxonomic group varied greatly, with transects often having no observable occurrences of particular organisms. Eel-like fishes (p = 0.0008) showed significant differences in densities between the pre- and post-trapping surveys. Densities for anemones, fishes, holothurians, sea pens, and shrimps did not show significant differences between the pre- and post-crab trapping surveys (p-values >> 0.05, Table 3.6). While there was also no significance difference obtained for crab, sea star, or total organism densities, the p-values for crabs (p = 0.298), sea stars (p=0.102), and total density of all organisms (p = 0.079) were closer to the significance threshold of p = 0.05 than for the other groups. The latter result is likely influenced by the extremely significant difference observed for the eel-like fishes group, which were significantly higher post-trapping. Crab densities, though not significant, were lower in the post-crab trapping survey than before crab trapping took place and equated to a net loss of approximately 168 crabs.

Table 3.6. Mean densities of organisms at MC118 pre- and post-crab-trapping, as obtained from photos taken from ROV video surveys.

Taxonomic Group	MC118 Pre Density Uncorrected (ha^{-1} ± 95% CI)	MC118 Pre Density Corrected (ha^{-1} ± 95% CI)	MC118 Post Density (ha^{-1} ± 95% CI)	p-value
Anemones	0.3 ± 0.7	0.7 ± 1.4	0.0 ± 0.0	0.5079
Crabs	10.0 ± 3.4	19.8 ± 6.7	13.1 ± 8.4	0.2976
Eel-like Fishes	132.6 ± 28.8	261.2 ± 56.7	555.2 ± 182.0	**0.0008**
Fishes	312.6 ± 64.3	615.9 ± 126.7	768.0 ± 258.4	0.4980
Holothurians	20.4 ± 7.6	40.3 ± 15.0	34.4 ± 22.2	0.6631
Sea Pens	3.2 ± 2.1	19.5 ± 4.1	28.7 ± 4.4	0.4833
Sea Stars	9.9 ± 6.0	6.2 ± 11.9	2.9 ± 13.3	0.1022
Shrimps	69.5 ± 16.8	137.0 ± 33.1	150.7 ± 60.2	0.8213
All Organisms	592.8 ± 83.6	1168.0 ± 164.6	1628.7 ± 467.5	0.0793

NOTE: P-values indicate the difference in density of organisms after crab trapping compared to before trapping took place, using the corrected pre-trapping mean densities. Values that are significantly different are highlighted in bold.

3.4. Discussion

3.4.1. Simulations

Simulations exploring the abilities of different survey designs for evaluating animal populations represent valuable tools for determining the most effective methods for conducting environmental surveys. The ability to detect species of varying abundances is important for deep-sea benthic studies, as populations of many species are often small with the exception of a few dominant groups. General stability of the deep-sea environment can mean that deep-sea animals have a greater likelihood of being impacted by environmental disturbances. An ideal design for environmental surveys will need to maximize the accuracy and consistency with which animals across a variety of abundances can be detected.

Simulation results suggested that, in general, longer transect lengths and finer spacing between transects in the radial design led to more effective estimations of animal densities. Increased consistency was observed in estimated densities as transect length increased and transect spacing decreased. This was observed both within and between true densities used in the simulations, and was more pronounced at higher animal densities where results from different simulations varied a lot more. This implies that the ability to accurately estimate true densities is generally higher with longer transects and closer transect spacing. While these trends were relatively weak for random populations where simulation results were similar, they were more pronounced for simulations run on clustered populations.

In addition to this, there were notable trends in the R^2 and 95% CI values between simulations on both random and clustered populations, particularly with respect to transect length. Larger R^2 values associated with longer transect lengths, and in particular the 15°, 250 m survey design, suggest stronger associations between true density and the corresponding densities estimated by the simulations for survey designs featuring longer transects. The smaller CIs associated with increasing length indicated an overall increase in confidence in the mean densities associated with longer transects. These observations suggest that animal densities determined from radial designs with longer transects are more likely to reflect true animal densities in the environment. Trends in R^2 and CI values between simulations of different

transect spacing were less discernable. For random populations, a mild trend of increasing CIs with increased spacing between transects was observed. This trend was not evident for clustered populations where CIs were typically within the same general range of one another.

Despite trends in consistency and 95% CIs, statistically significant differences between simulations were not observed for the slopes or y-intercepts. There were no strong trends observed in relation to transect length or degree spacing. Decreases in slope moving closer to 1 were observed with increasing transect length for cluster populations, indicating possible increased accuracy in this case. For both random and clustered populations, the 15°, 250 m design was consistently in the top performers with respect to having a slope closest to 1 and y-intercept closest to (0,0). Lower performing designs typically included (with some exceptions) those with smaller transect lengths and greater spacing between transects.

More specifically, there are many benefits to the modified 250 meter, 15° survey design that make it superior for deep-sea benthic megafaunal studies, particularly in comparison to the current BOEM 60°, 100 m long surveys. Greater survey area resulting from longer transects and finer transect spacing can lend to more accurate results than a smaller survey design. It was evident from the simulations that the modified 15° design provides much more accurate and consistent results than the 60° BOEM survey design, especially for clustered populations. This is likely a combined result of shorter transect length and larger separation between transects in the latter, both of which decreased the effectiveness of the survey design. Longer transects with greater area coverage and finer spacing between transects also allow for better detection of less abundant species and localized patterns of patchiness in organism communities that may be missed by surveying smaller areas.

These are all important considerations since some studies indicate that deep-sea communities may be more clustered or patchy than random in distribution. Evidence of patchiness has been observed in a variety of benthic studies ranging from subtidal benthic environments (Parry et al., 2003) to studies of deep-sea epibenthic megafauna (Schneider et al., 1987; Gonzalez-Mirelis et al., 2009). A recent study of benthic megafauna using AUV imagery at two sites in the Northern Gulf of Mexico has indicated a deviation from a random spatial distribution (refer to

Chapter 2). It was found that observed animal populations did not follow a random (Poisson) distribution and instead exhibited evidence of mild to moderate clumped distributional patterns across the survey area. This tendency for possible clustering and patchiness amongst deep-sea benthic communities is shaped by a variety of physical and biological environmental factors, including: resource availability; predator-prey relationships; interspecific competition; variations in seafloor characteristics such as substrate and sediment type; local and larger-scale hydrographic conditions; and influences from anthropogenic and natural disturbances (Levin et al., 2001; Gage, 2003; Powell et al., 2003; Jones et al., 2007; Smith et al., 2008).

While the focus of this study is not on the actual monetary costs associated with carrying out the various radial benthic survey designs, a few brief comments are warranted. The costs of conducting surveys can greatly increase as complexity of the surveys increases. By increasing the overall area being surveyed either through increased transect lengths or a greater number of transects, there is an associated increase in time and, thus, cost. This increase in cost is linear in relation to increasing transect lengths; while increasing the number of transects through finer separation between them results in a much more significant change in cost more along the lines of a power function. In this respect, the BOEM 60°, 100 meter-long surveys are considered to be more cost-effective than the longer, finer transect spacing of the 15°, 250 m survey design. The question of whether these cost increases are worth the changes in accuracy and/or consistency reflected by the simulations will depend on factors such as availability of research time and funding or accessibility of technologies. This decision is therefore situation-dependent. What is important to note once again is that increasing the complexity of the survey design also contributes to greater ability in detecting rare or less abundant species. This is ultimately a very important consideration depending on the specific goals of a research project, as well as for environmental monitoring activities.

3.4.2. MC118 Case Study

While highly mobile animal densities are likely to change over short periods of time, it is not expected that densities of less mobile organisms would change much, if it all. These organisms and other smaller, less mobile animals on the seafloor often blend into the seafloor and are less

visible in situations of decreased visibility. As a result, sea stars, sea pens, and holothurians represented opportune organisms on which to base the correction factor used in the MC118 case study. This ultimately allowed for better comparison between the results of the pre- and post-crab trapping surveys, and presented a means for attempting to minimize errors in the data associated with variations in visibility.

The case study of MC118 presented an interesting opportunity to investigate use of industrial ROV video imagery for evaluating benthic megafaunal communities. Even though a significant difference was not observed in values obtained before (using corrected densities) and after crab-trapping at MC118, the densities of crabs were of particular interest due to the crab trapping activities that took place between surveys. A total of 86 crabs were collected at MC118 as part of the NRDA baited trapping efforts. This value is less than the post-trapping deficit of approximately 168 crabs that was obtained from the ROV visual surveys. Because crabs are large in size and easily distinguishable in shape and color, it was assumed that very little of this difference in crabs was attributed to differences in visibility between the surveys.

A decrease in number of crabs was expected because of trapping efforts, yet the observed decrease was larger than the anticipated 86 crabs known to be removed. This suggests that there was some movement of crabs between the ROV survey location and the nearby area where trapping occurred. Similar to eels, crabs are known to be attracted to bait (which was used during trapping efforts). It is therefore likely that there was movement of additional crabs into the area where the traps were set as a result of attraction to the bait. The offset in location between the crab trapping and MC118 ROV surveys would therefore have possibly drawn crabs away from the immediate ROV survey area.

Bait is a highly effective means of attracting animals from adjoining areas to where the bait is located. Studies have indicated that eels such as the northern cutthroat eel, *Synaphobranchus kaupii*, are highly attracted to bait, particularly when foraging (Trenkel and Lorance, 2011). In addition, these eels, which are common in the deep-sea GoM, have been shown to be attracted to ROVs and in particular the lights associated with them (Stoner et al., 2008). It is likely these reasons that account for the observed significant increase in densities of eel-like fishes

observed in the post-crab-trapping surveys since the traps used recently in the area were baited. Heavy activity near the seafloor during both sets of ROV surveys and during trapping may have contributed to attracting these animals to the area.

For both the crabs and eels, some of the observed differences may be attributed to errors associated with determining animal densities from visual surveys, as well as resulting from having to use a correction factor to make other taxonomic group densities more comparable. If the correction factor was ignored and the uncorrected pre-trapping crab densities were compared to the post-trapping densities, there would be a calculated net increase of 78 crabs post-trapping. This alternative still does not yield a net loss of crabs consistent with the known number of crabs removed. It consequently suggests that other factors, including general movement of the animals from one area to another, are more likely responsible for determining actual numbers of crabs observed.

Unlike traditional sampling methods, the use of ROVs to collect video imagery represents a much more minimally invasive data collection methodology. It allows for *in situ* observation of the deep-sea benthic realm with little or no interaction with the seafloor environment and organisms. A large amount of information can be obtained from ROV videos – including sediment characteristics such as size and type; geophysical seafloor features; biological features on the seafloor; organism abundances, diversity, distributions, behaviors, and more – depending on the needs of a particular study.

There are, inevitably, limitations to ROV video data. As evidenced in the case study of MC118, visibility can significantly impact the ability to see and identity animals. This is particularly the case for organisms that live directly on the seafloor, are similar colors to or blend easily into sediments, may become partially or fully covered by sediments, or are very small in size. Video clarity and light over-exposure resulting from video compression or data transfer issues and changes in ROV elevation above the seafloor during surveys further contribute to visibility issues. Large quantities of marine snow and suspended sediments likewise play a role in lowering visibility by increasing reflection of ROV lighting and obscuring the view of the seafloor. Differences in ROV pilot ability further impacts transect precision and consistency. All

of these factors contribute to differences in the quality of data collected within and between study sites. As a result, organisms may be missed and difficulties in animal identifications along with inconsistencies in the level of taxonomic resolution are more likely to occur.

In this respect, using a correction factor such as the one used for the MC118 data, can prove a valuable tool; however, this correction factor also has limitations. The correction factor of approximately 1.8 was specifically intended for use only with the two data sets collected from the same study site presented here, MC118. While a correction factor may also be of value to use with other similar data sets, it cannot be used in the same form as was used here. Differences in visibility can occur from a number of factors including differences in ROV height above the seafloor and presence of suspended materials in the water. Therefore, if this correction factor were to be considered for other survey sites, it would need to re-evaluated in order to take into consideration these differences before applying it to the other data sets. Ideally, surveys at each site would need to be at the very least duplicated (as was done at MC118) to obtain an estimate of how observed animal densities vary at that site within a short time frame, particularly with respect to short-term variability in visibility.

In conjunction with measuring consistency in ROV survey parameters (e.g. altitude), development of a method to quantitatively evaluate visibility in the video imagery would be of great value to explore as well. This could lead to a more uniform method for correcting for differences in data resulting from both in- and between-site variations in visibility. The relationship between visibility in the video imagery and ROV altitude is something that needs to be explored further in order to facilitate increased accuracy in future ROV surveys completed in soft-sediment Northern GoM environments such as the one explored here.

3.5. Conclusion

Overall, the results obtained from the MC118 surveys exemplify how the modified 15° radial survey design can be used for collecting information on the benthic environment. Depending on video quality and image clarity, a wide variety of organisms ranging in size from small shrimp, squat lobsters, and cerianthid anemones to larger fishes can be observed and counted. These organisms can be classified into broad taxonomic groups for comparison, as shown by

the MC118 example. In addition, more specific taxonomic identification may be possible through high-quality photo snapshots taken from HD video or from DSC images. Other applications of the video data include observing organism distributions and sizes, investigating animal behaviors, and evaluating habitat and corresponding environmental factors and conditions.

Studies have indicated that ROVs represent a highly effective, relatively non-invasive means of studying deep-sea megabenthos. This is supported by Stoner et al. (2008), who recommend continuation of ROV surveys since there is no sufficient substitute for the direct observations made using ROVs, albeit with due care to minimize survey biases where possible. Factors such as distance from the bottom, depth, current speed, relative surveying direction, behaviors of deep-sea fishes, and organism abundances and distributions have all been shown to impact organism counts and, consequently, population density estimates (Trenkel and Lorance, 2003; Trenkel et al., 2004a,b). Due to the general effectiveness and capacity of ROV video imagery to allow for many different kinds of studies, it may be concluded that the use of ROVs in deep-sea benthic megafaunal studies can provide a wealth of valuable scientific data. Continued development of ROV-related technologies and camera systems will further allow for expansion of ROVs as a means of studying and monitoring the deep-sea.

Compared to other survey designs, the radial transect design represents a relatively time-effective alternative while still providing excellent area coverage. The 15°, 250 m modified design used in our study represents an excellent option for benthic megafaunal monitoring studies. This is due to its overall accuracy and consistency in estimating animal densities from both random and clustered populations. In addition to this, the longer transect lengths and finer transect spacing increase the potential to detect rare animals with lower abundances in the benthic environment.

The general results of the simulations suggest that a highly effective radial survey design should be designed favoring longer transects and finer spacing between transects. This, of course needs to be considered in conjunction with availability of time, funding and technology resources. This study has indicated that a more finely-scaled sampling design such as the 15°

radial survey design can greatly increase the effectiveness of benthic megafauna sampling via ROV compared to survey designs like the current BOEM recommendation. Therefore, where possible, considerations should be made to modify or replace the 60°, 100 m survey design employed by BOEM in order to improve environmental monitoring of deep-sea benthic communities, particularly with respect to offshore oil and gas activities.

Particularly in the context of offshore activities, however, one of the weaknesses of purely radial survey designs (including both the 15° and BOEM 60°) will need to be investigated further. These designs lack the ability to statistically assess directional differences associated with environmental variables and benthic organisms. A radial design consisting of a series of parallel transects at different headings would therefore be of value to evaluate and compare further to the simplified radial design. This type of design may provide a better understanding of benthic communities in relation to, for example, patchiness within the benthic environment or the impacts of drilling activities.

CHAPTER 4. A DATABASE APPROACH FOR MANAGING AND EVALUATING DEEP-SEA BENTHIC MEGAFAUNAL COMMUNITIES USING DATA EXTRACTED FROM MARINE IMAGERY

4.1. Introduction

Expansion of human activities into the deep-sea has made it necessary to improve our understanding of deep-sea environments. Investigations into megafaunal abundances and biodiversity need to be coupled with evaluations of natural environmental factors and the impacts of anthropogenic influences on marine communities. Understanding variability in environmental conditions and their corresponding influences on species composition is important because it contributes to insights into ecosystem functioning and therefore how ecosystem processes may be impacted by changes to the benthic environment (Bremner et al., 2006).

Advancements in technology have prompted a shift from traditional methods of studying benthic megafaunal communities through trawling, nets and traps towards minimally invasive methods using optical deep-sea technologies. Towed cameras, remotely operated vehicles (ROVs), and autonomous underwater vehicles (AUVs) have been gaining popularity due to their ability to obtain imagery from which both seafloor habitat and megafaunal community information can be extracted. These technologies provide a continuous view of the seafloor and allow for in-situ observations of benthic megafaunal communities. Quantitative data on both benthic habitats and associated fauna can be obtained; however, with this comes a very large set of observations that pose a challenge for analysis. Extraction of useful data from imagery is a very time consuming process that produces large data sets that are challenging to manage.

One approach to exploring the marine benthic environment and associated megafaunal communities that has been growing in popularity over the last decade is the use of photographic and video imagery analysis in conjunction with development of database systems to store the extracted data. This combination can be a very useful tool, particularly as image acquisition improves and imagery data sets expand. Several institutions have been developing

and employing this type of approach for managing their vast depositories of imagery data. Many of these database systems incorporate image annotation into them so that imagery can be viewed and the resulting data stored in a single place. The Video Annotation and Reference System (VARS; Schlining and Stout, 2006) is a software tool used by Monterey Bay Aquarium Research Institute for making detailed annotations during or after ROV video is recorded. FISH_ROCK (Ferrini et al., 2006; Ferrini and Singh, 2006) is a similar system using a Graphical User Interface (GUI) created in MATLAB to acquire and store benthic organism identifications, measurements, and locations. Woods Hole Oceanographic Institute also has similar systems for processing and storing imagery information from AUV and ROV data sets (WHOI, 2014).

In the northern Gulf of Mexico (GoM), collection of imagery data has been growing. ROV videos are being collected as part of the Gulf SERPENT (Scientific and Environmental ROV Partnership Using Existing iNdustrial Technology) Project. A variety of other studies using both AUVs and ROVs for image acquisition have also been conducted. Some of these studies, including the one presented here, use imagery collected as part of the Natural Resource Damage Assessment (NRDA) in response to the Deepwater Horizon oil spill of 2010. An important component of environmental monitoring activities and investigations into the impacts of anthropogenic influences and consequent recovery of the benthic marine environment is the ability to evaluate newly acquired data against data collected previously. This is difficult when data collected from different studies is spread out and not readily accessible.

This paper discusses a simplified database system approach that can be used with imagery obtained by a variety of deep-sea technologies in the northern GoM. The principles of this database system are to provide an easy-to-use, efficient option for analyzing and storing a variety of data extracted from imagery. This data ranges from habitat and other environmental variables to anthropogenic influences and information on megafaunal communities. The database system is specifically applicable for use with imagery acquired during industrial environmental surveys since it was originally designed for analysis of industrial ROV video imagery collected during the NRDA cruises. Example data extracted from this ROV imagery will be presented in conjunction with a description of the database system approach used for

imagery analysis in this study. Further applications and future developments of the database system will also be discussed.

4.2. Methods

4.2.1. Study Sites

In the summer of 2011, two NRDA cooperative cruises were conducted aboard the HOS Sweetwater in the northern GoM. Industrial ROVs were used to conduct benthic megafaunal surveys at 11 survey sites (Fig. 4.1, Table 4.1) located at a range of distances and directions from the MC252 wellhead blowout preventer.

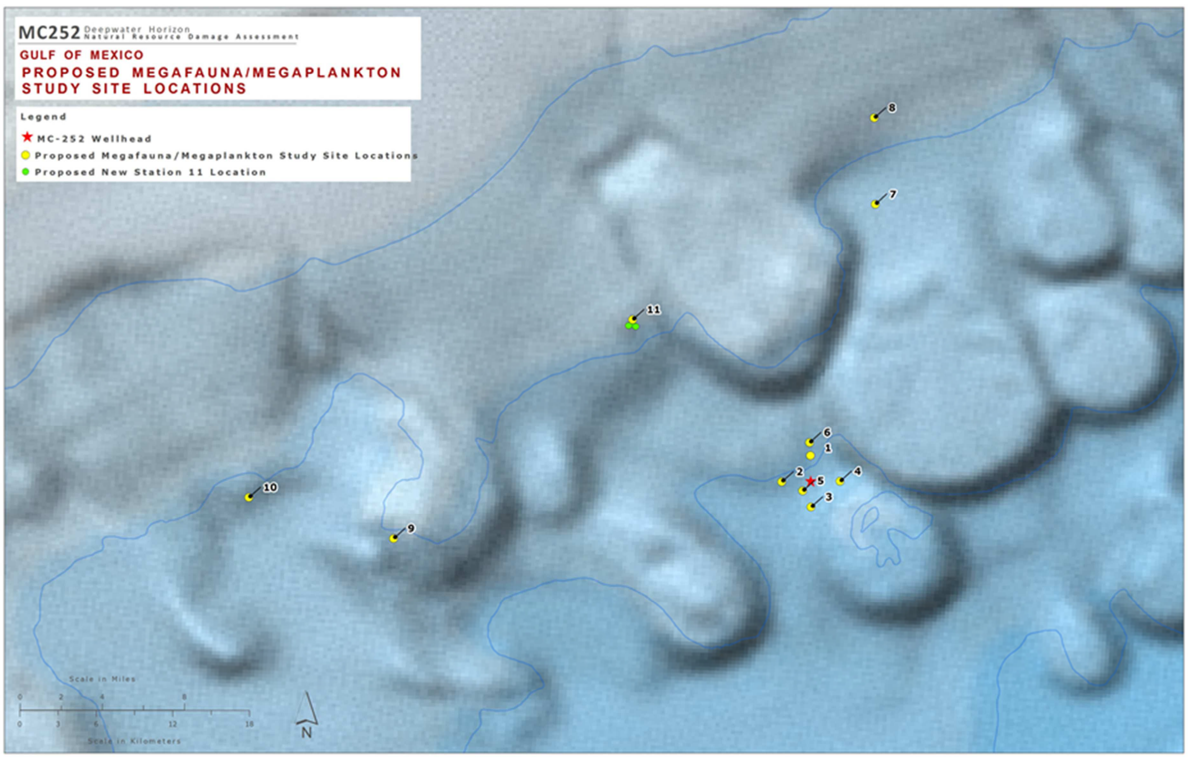


Fig. 4.1. Location of ROV survey sites in the vicinity of the Deepwater Horizon Macondo well. The red star represents the location of the wellhead. Yellow points with numbers are the locations of the survey sites (refer to Table 4.1 for site information).

Table 4.1. Locations of survey sites in the vicinity of the Macondo well evaluated in this study.

Site	Description	Depth (m)	Latitude	Longitude
1	MC252 2000 m N	942	28° 45' 22.295" N	88° 21' 58.520" W
6	MC208 Photomosaic	1450	28° 45' 55.102" N	88° 22' 00.779" W
7	Red Crab 1D	1473	28° 56' 01.200" N	88° 19' 13.800" W
8	Red Crab 1C	860	28° 59' 40.200" N	88° 19' 16.200" W
9	WSW Megafauna1	1043	28° 41' 51.900" N	88° 39' 37.000" W
10	WSW Megafauna2	1043	28° 43' 36.600" N	88° 45' 44.600" W
11	MC118	1044	28° 51' 07.992" N	88° 29' 30.000" W

4.2.2. Data Acquisition

A work-class Triton XLS 150 ROV equipped with a standard definition video (SD) camera, high definition (HD) video camera, and digital still camera (DSC) was used to collect video and still imagery at each of the 11 study sites. A series of twenty-four 250-m long transects with 15° offset (radiating from a common point of origin) were completed at each site. An ultra-short baseline (USBL) system was used to guide the ROV to the point of origin for the survey and log the path of the ROV over the seabed. The ROV then proceeded to move at a constant velocity and altitude (approximately 2-3 meters) along the transect. Upon completion of the outbound transect, the ROV turned on a reciprocal heading and flew back to the point of origin while collecting close up still images of representative organisms. A pair of red laser scalers was fixed to the pan and tilt unit on the ROV to allow for measurement of the field of view of the camera and to measure organisms.

Outbound ROV video transects were analyzed from the beginning to end of each transect as denoted in the cruise data log book using VideoReDo Plus (DRD Systems, Inc., 2003) video playback software. Each time an organism on or just above the seafloor was encountered along the transect, a frame grab was taken and the date and time the organism was recorded. In order to maintain consistency between transects and study sites, only organisms that fell within a specific area of the field of view where the best light illumination (approximately the bottom 30% of the video FOV) occurred were included. USBL navigation data for each transect was smoothed (using a point running mean) and used to calculate transect lengths.

Locations of each photo observation were determined by matching the time of the photo with the navigation data corresponding to that time. Frame grabs were then sorted into broad

taxonomic groups with the organism in each photo identified down to the lowest possible taxonomic level. The broad taxonomic groups used for this study included: anemones, crabs, eel-like fishes, other fishes, holothurians, sea pens, sea stars, shrimps, sponges, and “other” (Fig. 4.2). The latter category includes all organisms that did not fall into any of the other groups and all possible organisms for which snapshots were taken but group placement was uncertain.

4.2.3. Database Approach for Imagery Analysis

In order to facilitate easier collection and analysis of data extracted from ROV imagery obtained via industrial ROVs, a customized Microsoft Access database system was created. The core design and components of this database system were adapted from a Microsoft Access database system employed by University of Victoria in British Columbia, Canada for use with ROV imagery (Gauthier, 2012). The database was specifically designed to incorporate data collected from the northern GoM, and consequently includes organism and other features relevant to benthic environments in this area. Data extracted from each piece of imagery was entered into a new database form, which is shown in Fig. 4.3.

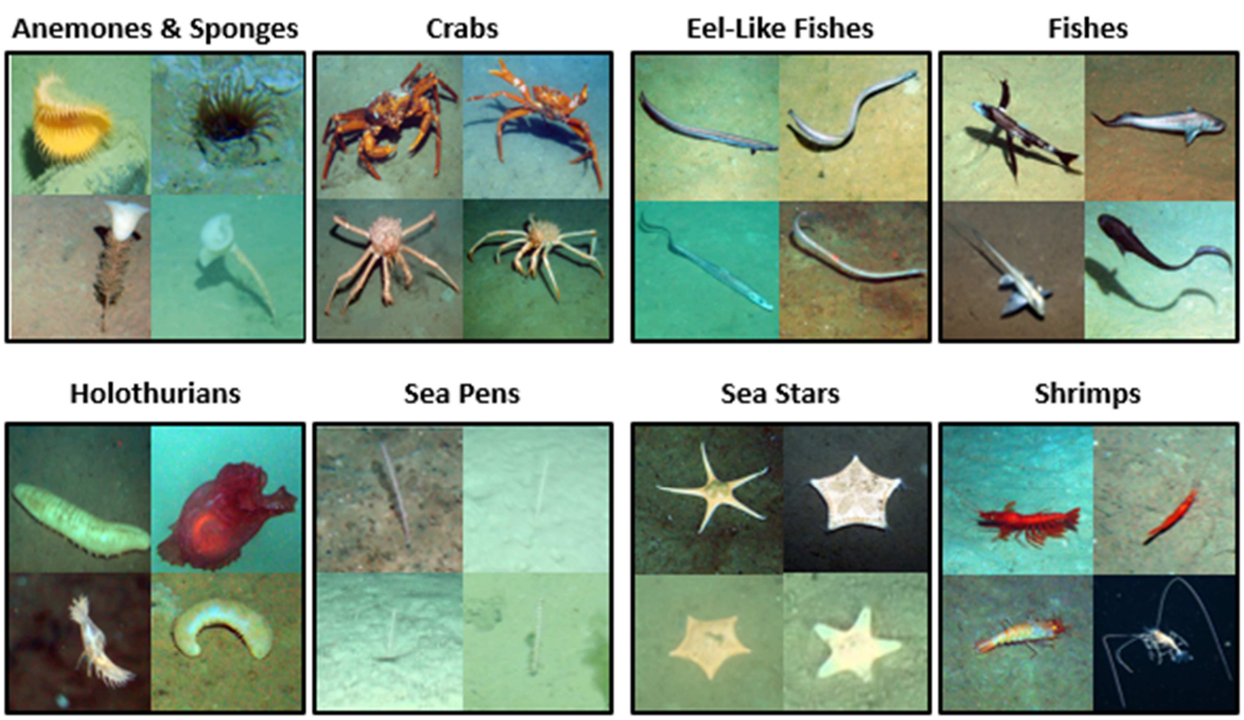


Fig. 4.2. Broad taxonomic groups used for sorting ROV frame grab photographs.

The primary key for this database is set to automatically create a unique number identifier for each new form entry, such that no two entries have the exact same identifier. Individual images from this particular study are further linked to form entries based on their file names, which consist of date and time identifiers corresponding to the date and time of the image obtained from the ROV video. Individual observations are similarly linked to the database and can be identified through the date and time fields. Certain fields, including date and time, have been created as "mandatory" fields in the database such that a form and associated database entry cannot be saved without these fields being filled in according to the set pre-determined formatting. The user will be unable to move on to a new form or entry, even without saving, if these mandatory fields are not completed correctly. The use of mandatory fields such as filename, date, and time maintain consistent inclusion of important information and allow for data collected independently of imagery to be more easily linked into the database system if required.

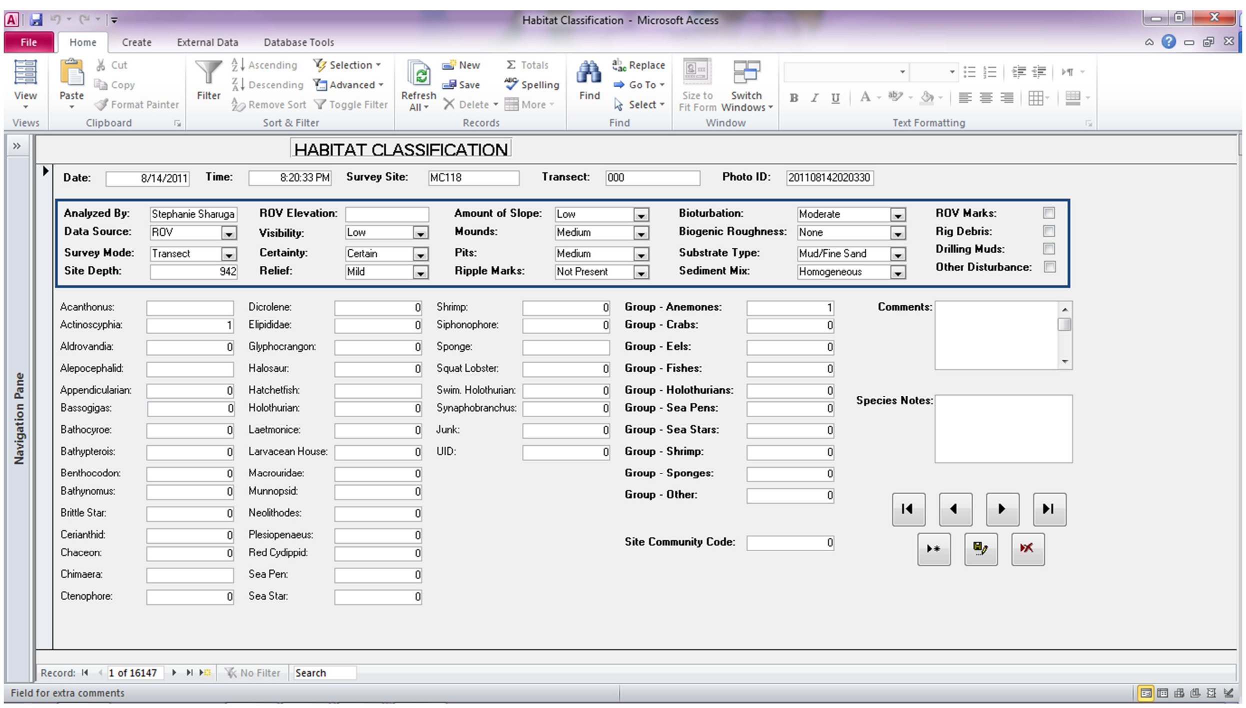
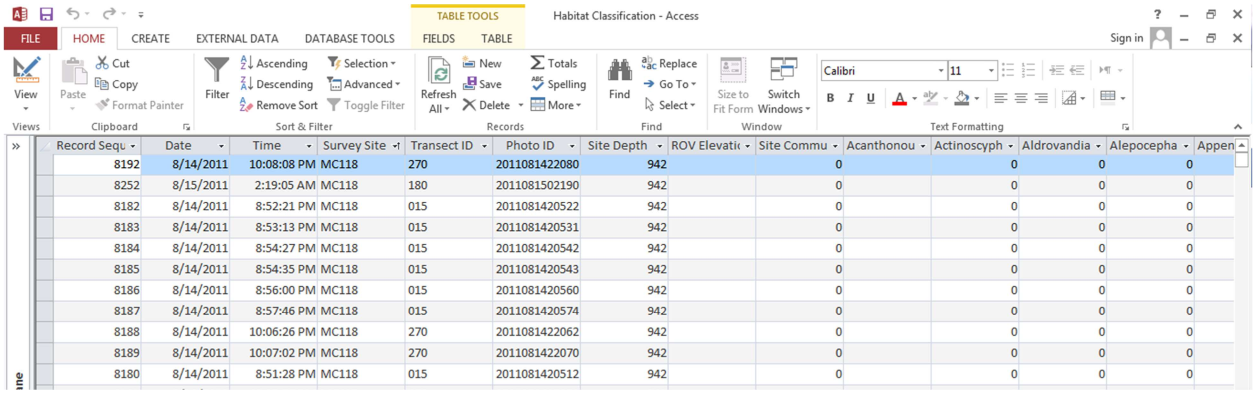


Fig. 4.3. Example of Microsoft Access database form used for inputting survey, biological and abiotic data extracted from photographic or video imagery.

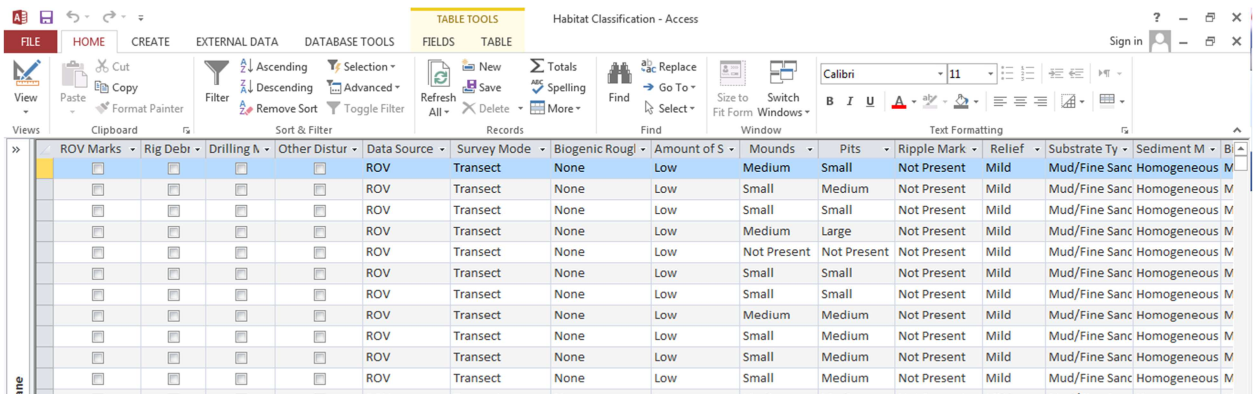
As data is entered in the form, it populates a cumulative table of observations. The large number of categories and entries create an extensive all-inclusive table (refer to Fig. 4.4 for an example of parts of the database table). This table can be easily exported in a number of different file formats into other programs for analysis, including MATLAB, Microsoft Excel, and ArcGIS. Data collected independently of the imagery (e.g. CTD or navigation data) can be linked in with the imagery data table by joining different tables together using date and time or other similar identifiers. Additional information and specific instructions on how to add and modify fields and data in the database system can be found in Appendix B.

Modifications can be made to the database system as required over the course of data analysis by adding new fields to the database form. These new fields will automatically be added into the corresponding database table; and the addition of new fields will not affect any fields or data already present in the database system. This is an important component of the database because it allows for flexibility and inclusion of new or possibly unanticipated types of data being extracted from imagery even after imagery analysis has begun. For example, a situation may arise where a new type of animal or seafloor feature is observed that has not previously been encountered in imagery being analyzed.

This database system has been designed to store a variety of survey, biological, and abiotic or environmental data extracted from photographic or video imagery of the seafloor. Survey and abiotic seafloor data are entered in to the top part of the form, with biological data in the bottom portion. Survey data includes information such as date, time and other identifying details associated with the imagery, along with information on the survey site (e.g. site name, transect ID). Abiotic and environmental data entered into the form may consist of observations on a range of larger-scale site characteristics (e.g. depth, relief, slope, and sediment type and mix), as well as smaller-scale characteristics such as seafloor features (e.g. presence of pits or depressions, mounds, ripple marks, bioturbation, and presence of physical disturbance to the seafloor). The final component of the form, the organism data, allows for counts of organisms observed to be entered both at a broad group taxonomic level, as well as at a finer taxonomic resolution where possible.



Record Sequ	Date	Time	Survey Site	Transect ID	Photo ID	Site Depth	ROV Elevatio	Site Commu	Acanthonou	Actinoscyph	Aldrovandia	Alepocepha	Appen
8192	8/14/2011	10:08:08 PM	MC118	270	2011081422080	942		0		0	0	0	
8252	8/15/2011	2:19:05 AM	MC118	180	2011081502190	942		0		0	0	0	
8182	8/14/2011	8:52:21 PM	MC118	015	2011081420522	942		0		0	0	0	
8183	8/14/2011	8:53:13 PM	MC118	015	2011081420531	942		0		0	0	0	
8184	8/14/2011	8:54:27 PM	MC118	015	2011081420542	942		0		0	0	0	
8185	8/14/2011	8:54:35 PM	MC118	015	2011081420543	942		0		0	0	0	
8186	8/14/2011	8:56:00 PM	MC118	015	2011081420560	942		0		0	0	0	
8187	8/14/2011	8:57:46 PM	MC118	015	2011081420574	942		0		0	0	0	
8188	8/14/2011	10:06:26 PM	MC118	270	2011081422062	942		0		0	0	0	
8189	8/14/2011	10:07:02 PM	MC118	270	2011081422070	942		0		0	0	0	
8180	8/14/2011	8:51:28 PM	MC118	015	2011081420512	942		0		0	0	0	



ROV Marks	Rig Debi	Drilling N	Other Distur	Data Source	Survey Mode	Biogenic Rougl	Amount of S	Mounds	Pits	Ripple Mark	Relief	Substrate Ty	Sediment M	Bi
				ROV	Transect	None	Low	Medium	Small	Not Present	Mild	Mud/Fine Sanc	Homogeneous	M
				ROV	Transect	None	Low	Small	Medium	Not Present	Mild	Mud/Fine Sanc	Homogeneous	M
				ROV	Transect	None	Low	Small	Small	Not Present	Mild	Mud/Fine Sanc	Homogeneous	M
				ROV	Transect	None	Low	Medium	Large	Not Present	Mild	Mud/Fine Sanc	Homogeneous	M
				ROV	Transect	None	Low	Not Present	Not Present	Not Present	Mild	Mud/Fine Sanc	Homogeneous	M
				ROV	Transect	None	Low	Small	Small	Not Present	Mild	Mud/Fine Sanc	Homogeneous	M
				ROV	Transect	None	Low	Small	Small	Not Present	Mild	Mud/Fine Sanc	Homogeneous	M
				ROV	Transect	None	Low	Medium	Medium	Not Present	Mild	Mud/Fine Sanc	Homogeneous	M
				ROV	Transect	None	Low	Small	Medium	Not Present	Mild	Mud/Fine Sanc	Homogeneous	M
				ROV	Transect	None	Low	Small	Medium	Not Present	Mild	Mud/Fine Sanc	Homogeneous	M
				ROV	Transect	None	Low	Small	Medium	Not Present	Mild	Mud/Fine Sanc	Homogeneous	M

Fig. 4.4. Example of the beginning (top) and end (bottom) sections of the database table.

Although the database was designed to incorporate a large variety of data, only specific types were extracted from the photo frame grabs taken from the ROV videos in this example study. Survey data including site and photo information were included in as much detail as available. Features such as relief, slope and biogenic roughness were not considered since these features were not readily observable. Important factors considered were presence and size of seafloor features such as pits or depressions, mounds, and seafloor disturbance (see Figs. 4.5 and 4.6). The latter consisted of any and all visible physical disturbance to the seafloor resulting from deep-sea equipment (including ROVs) or other sources.

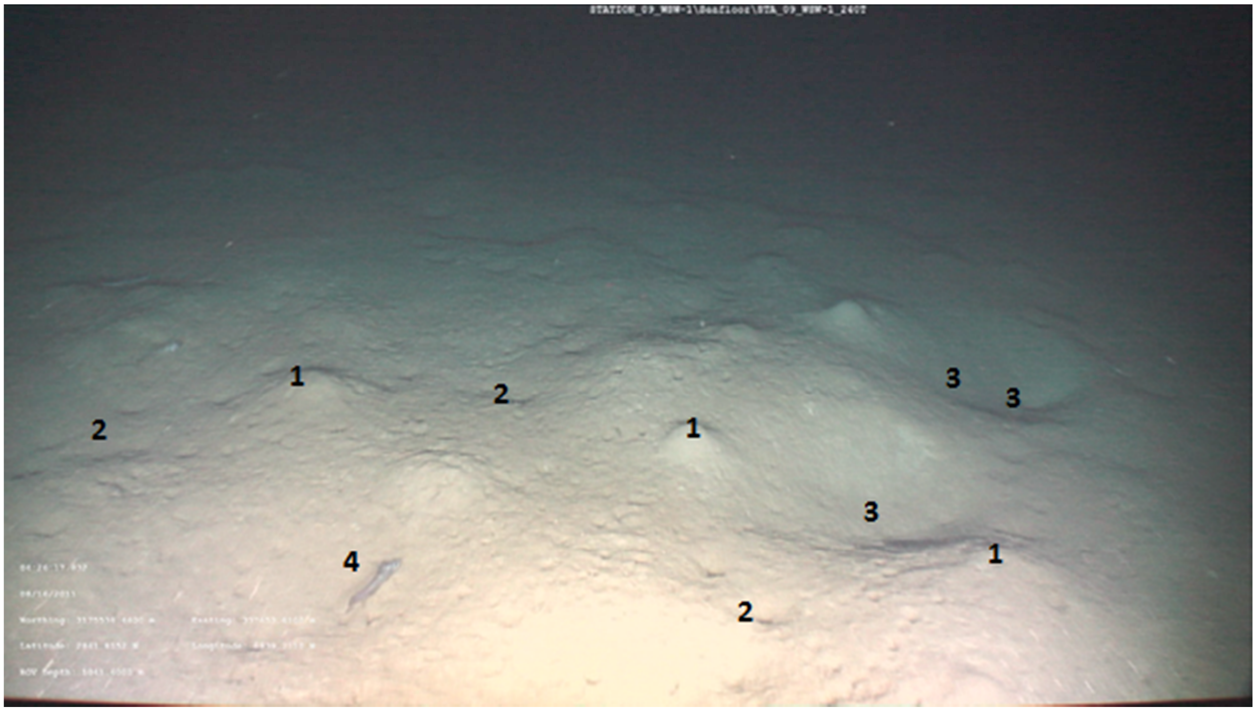


Fig. 4.5. Example of a seafloor environment with finer-grained, homogeneously mixed sediments. Naturally occurring seafloor features are present including mounds (1) of different sizes, small pits (2), and larger depressions (3). A Macrourid fish (4) is also visible in the image.

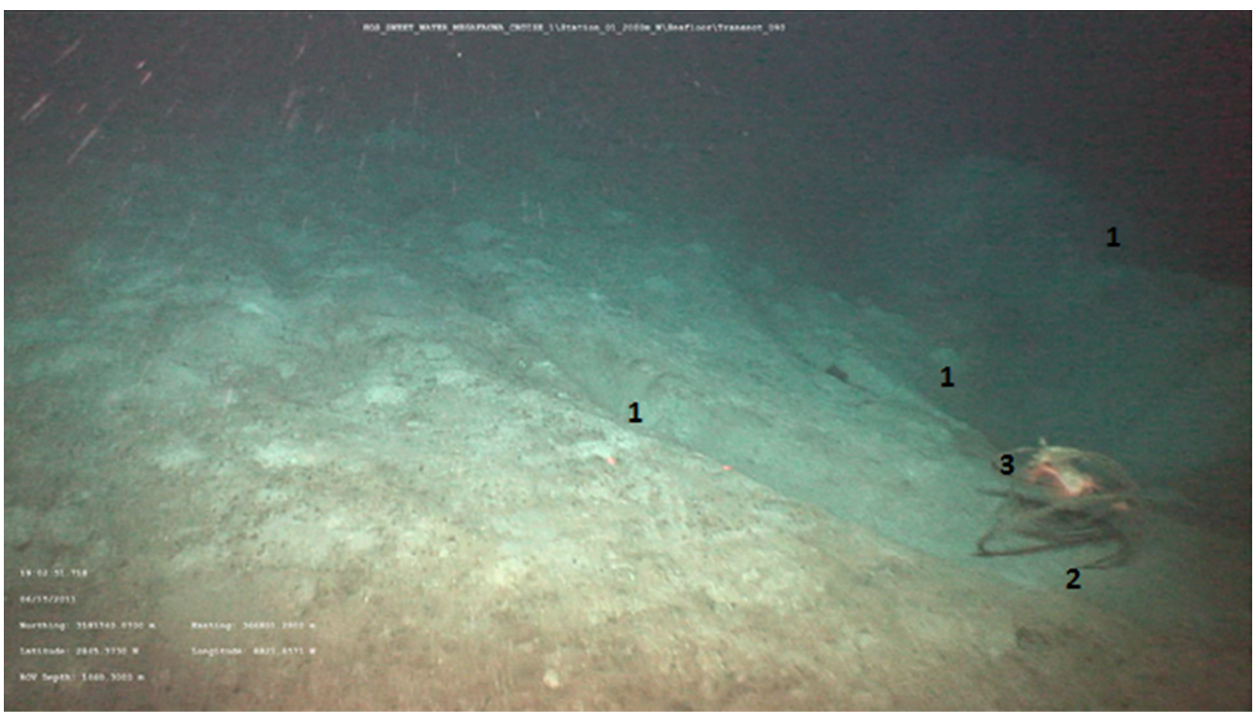


Fig. 4.6. Example of a seafloor characterized by coarser-grained, less homogeneously mixed sediments. Areas of anthropogenic physical disturbance to the seafloor (1) are visible along with trash (2). *Actinoscyphia aurelia* anemones (3) are often found growing affixed to trash on the seafloor.

In this study, physical seafloor disturbance was most often attributed to anthropogenic causes (instead of natural), which were generally recognizable by linear features or identifiable debris observable on the seafloor. All the seafloor feature components were determined for each photo by noting their presence (or absence) and estimating the approximate sizes of the features (small, medium or large), if applicable, within the specific field of view of the photo being analyzed. Since each photo was taken because of the presence of an animal observed on the seafloor, the animal in each photo was also identified. Each identified animal was recorded at both a broad (indicated as "group" in the database form) taxonomic level, as well as lower taxonomic resolution when possible.

4.2.4. Example Applications of the Database

Two sets of features were plotted using data collected from the ROV imagery including: 1) seafloor features such as pits/depressions and mounds; and 2) locations of physical disturbance to the seafloor. Since each still photograph taken from the ROV video was associated with a specific date and time of collection, approximate locations were determined by matching the time with locations obtained from ROV navigation data. This then allowed for spatial plotting of data obtained from the imagery in order to look at how these features varied within and between each survey site. For each type of seafloor feature, a table containing locations and numbers representing the corresponding size classes was exported from the database system. The tables were then imported into MATLAB in order to plot the seafloor features.

The database system was also used to explore the benthic megafaunal communities present in the northern GoM. Total densities of organisms at each survey site were determined for broad taxonomic groups by calculating densities for each transect then determining the mean density. General associations of different organisms to particular seafloor features were likewise explored by pooling data across the seven study sites in order to evaluate the percentage of each animal group found associated with particular seafloor features.

4.3. Results

4.3.1. Seafloor Features and Disturbance

Data on seafloor features and areas of physical disturbance were extracted from the database system in order to create plots mapping the locations of varying size classes or presence/absence of the features. Figures 4.7 and 4.8 show distributions of pits/depressions and mounds, respectively, on the seafloor at each of the study sites. Figure 9 shows the locations of observed physical disturbances to the seafloor at each of the sites.

4.3.2. Benthic Organisms

Total benthic megafaunal densities varied between the seven study sites (Fig. 4.10). Densities observed at MC252 2000 m N were by far the highest; and the lowest densities were observed at MC118 and MC208. The other four sites had densities comparable to one another. The largest percentage of animal observations were found associated with small and medium sized (pits/depressions and mounds) seafloor features (Fig. 4.11). There was greater variation between animal groups in associations with extreme (i.e. not present or large) features. The majority of animals were not found highly associated with seafloor disturbance (Fig. 4.12); however, some groups such as anemones, sea stars, and particularly shrimps were found much more often associated with disturbance than other groups.

4.4. Discussion

Mapping of seafloor features and areas of physical disturbance to the seafloor is beneficial in that it creates a visual picture of patchiness of seafloor environmental conditions. In terms of environmental monitoring, these seafloor maps not only can be compared to look at differences between study sites but also for observing patchiness of the physical environment within a single site. By mapping seafloor features each time a site is surveyed and comparing subsequent maps, temporal changes in the seafloor can be evaluated. This is of particular interest for observing changes in anthropogenic influences to the seafloor, and can consequently be valuable when evaluating impacts of physical seafloor disturbances on benthic communities. After the seafloor is disturbed, natural processes will begin to reshape the disturbed areas; and just as the seafloor changes after a disturbance, so is it likely that the benthic communities potentially affected by the disturbance may change in kind.

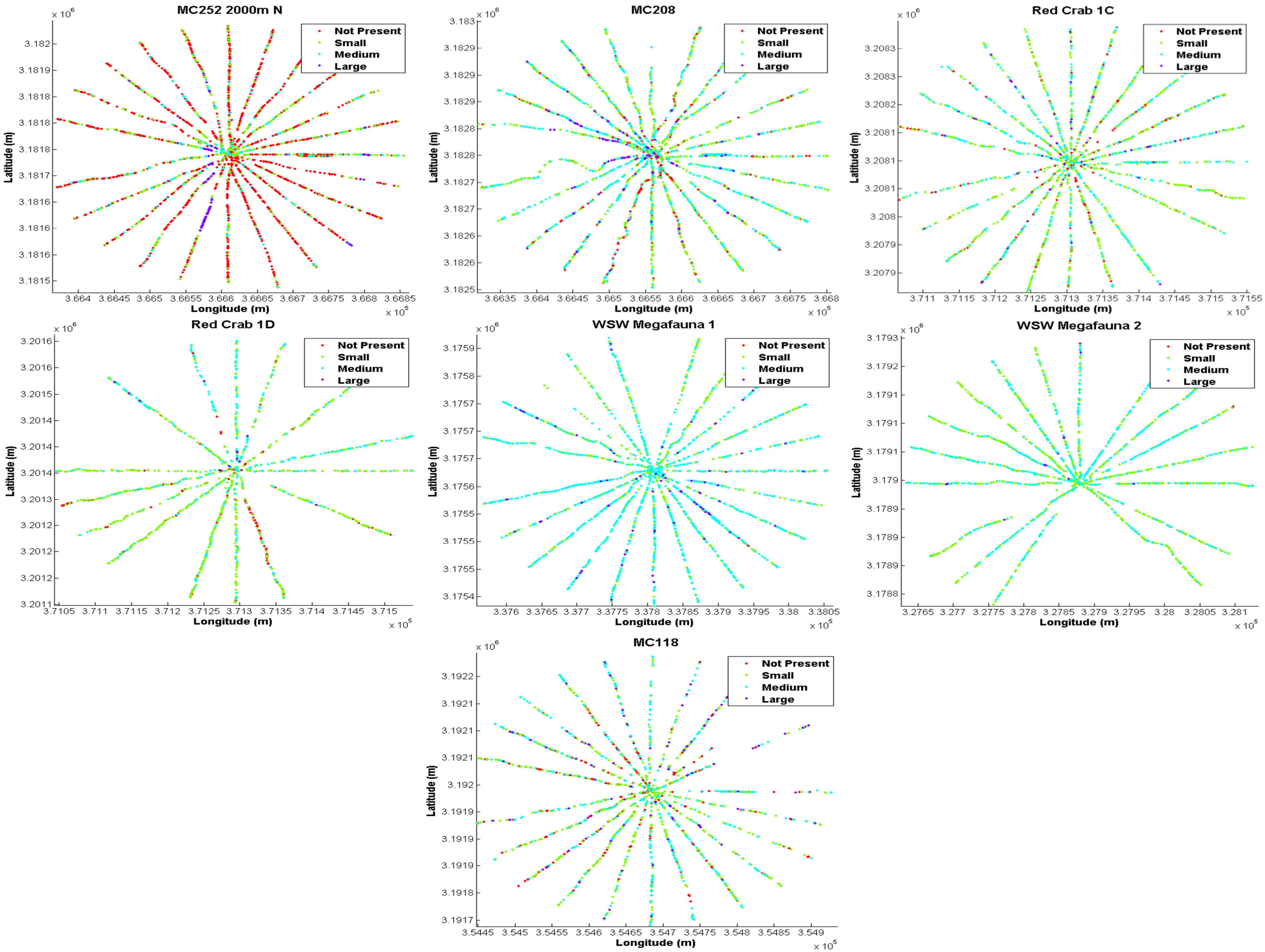


Fig. 4.7. Seafloor feature plots showing location and size of pits/depressions on the seafloor at each of the seven study sites.

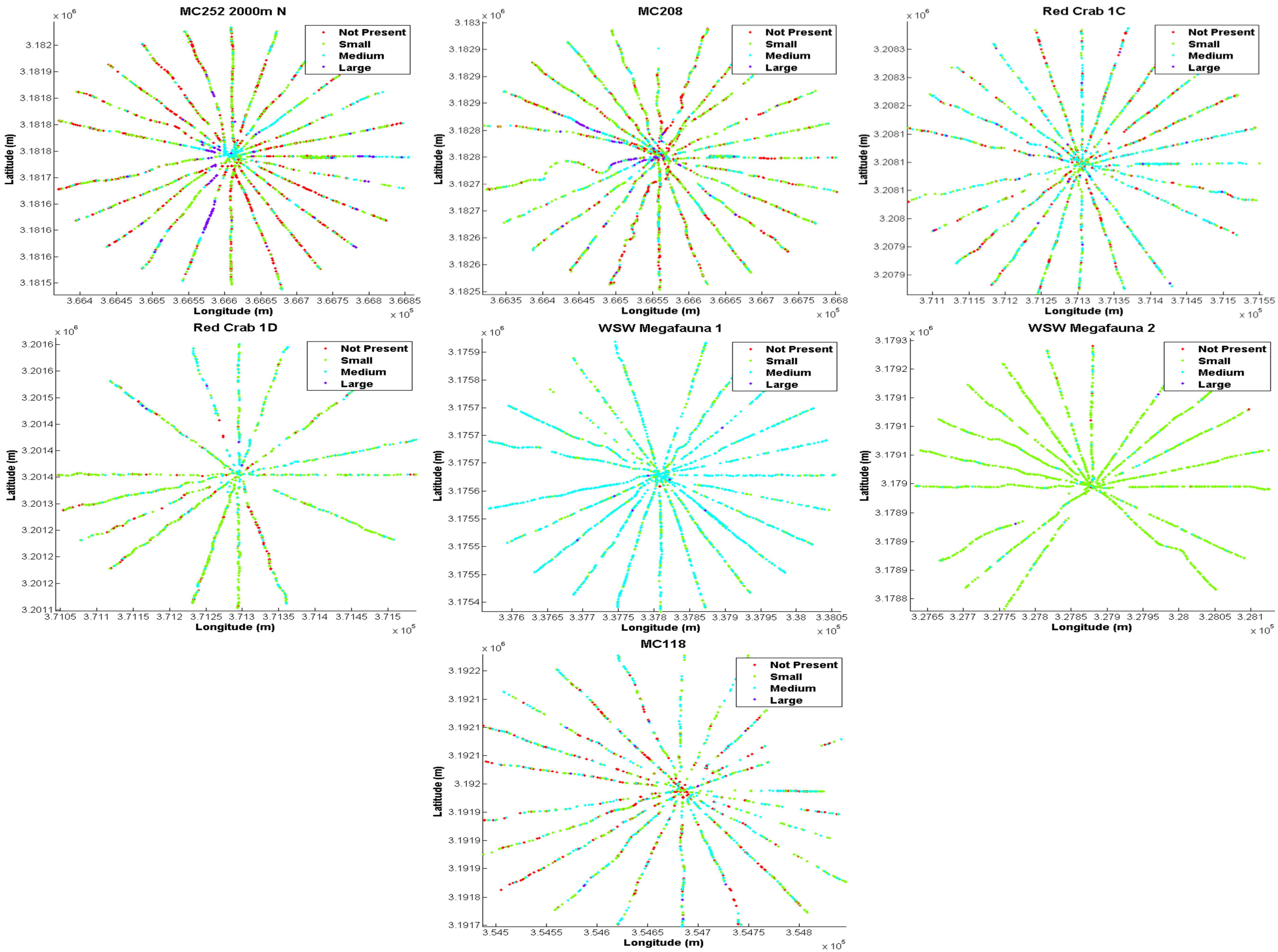


Fig. 4.8. Seafloor feature plots showing location and size of mounds on the seafloor at each of the seven study sites.

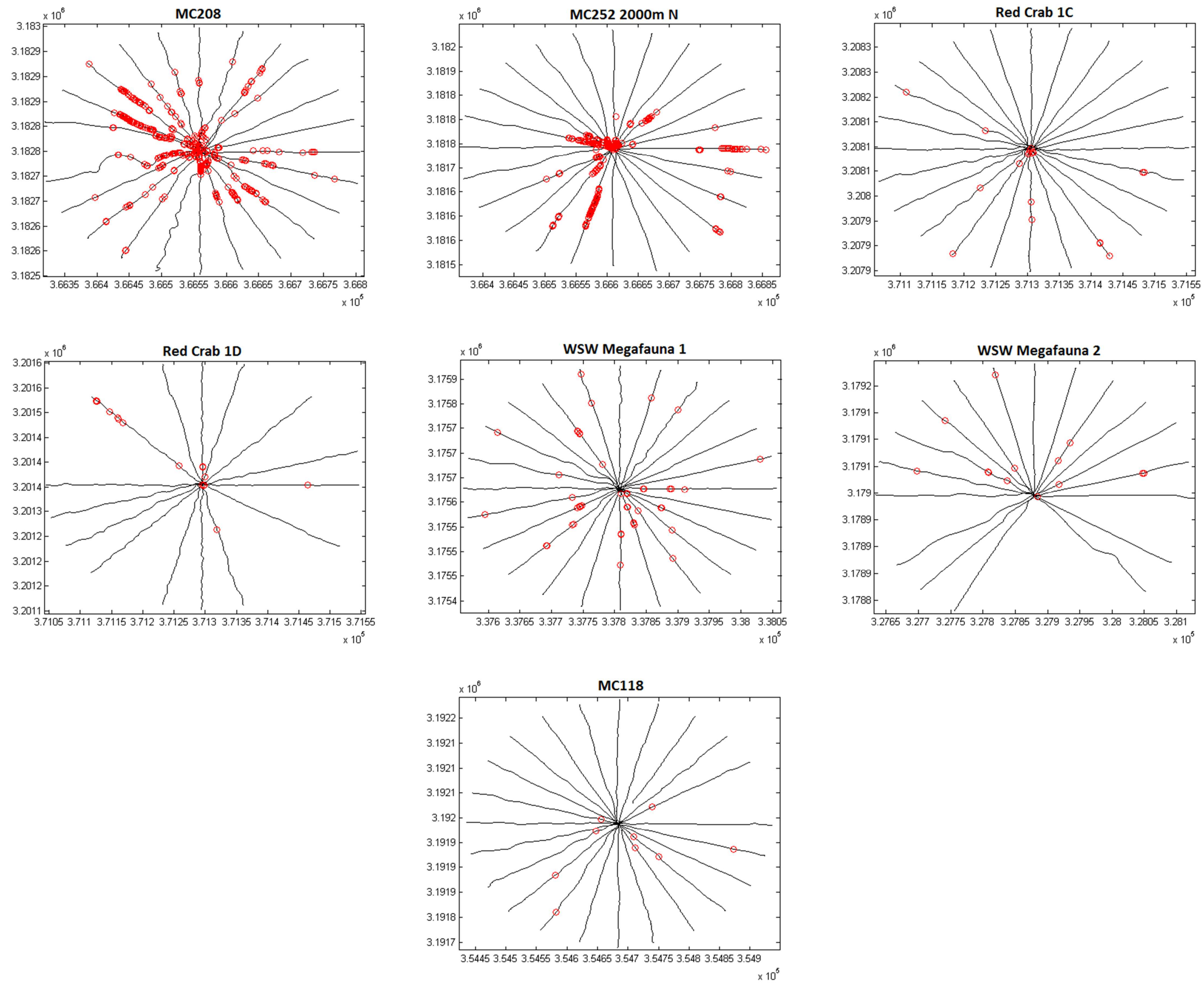


Fig. 4.9. Seafloor feature plots showing location of physical disturbances to the seafloor (red circles) at each of the seven study sites.

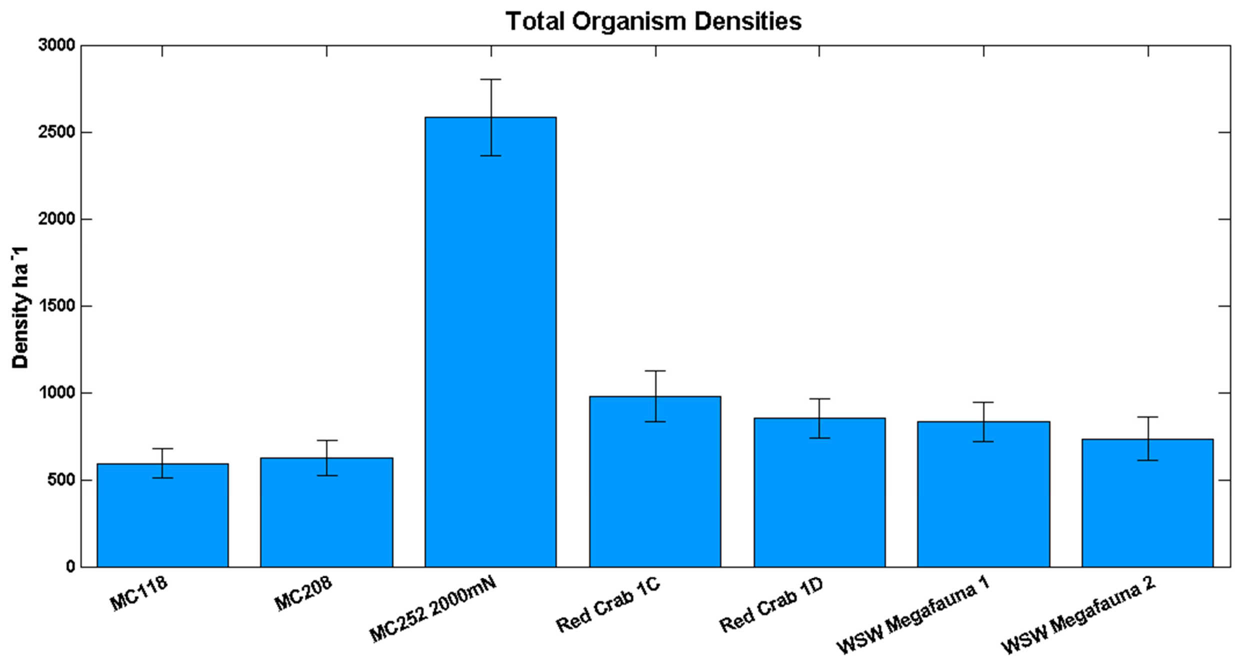


Fig. 4.10. Total densities of all benthic organisms (including 95% CI) at each of the survey sites.

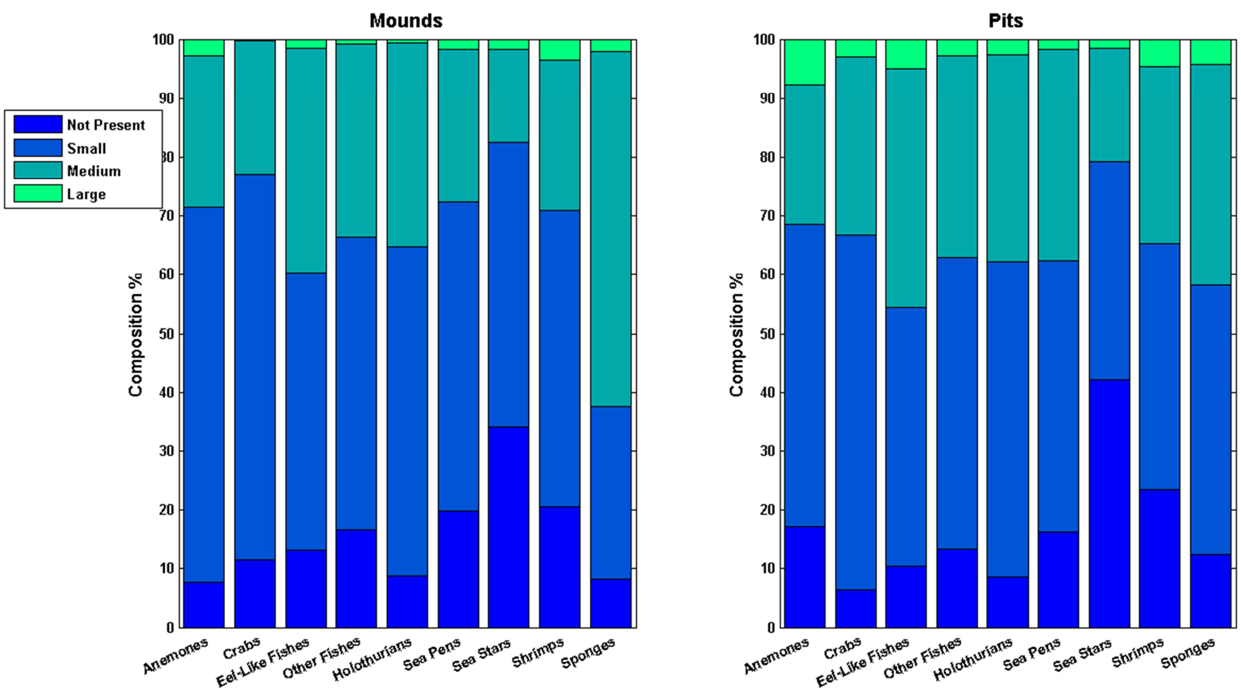


Fig. 4.11. Percentage of animal observations, by broad taxonomic group, associated with different size classes of mounds and pits or depressions.

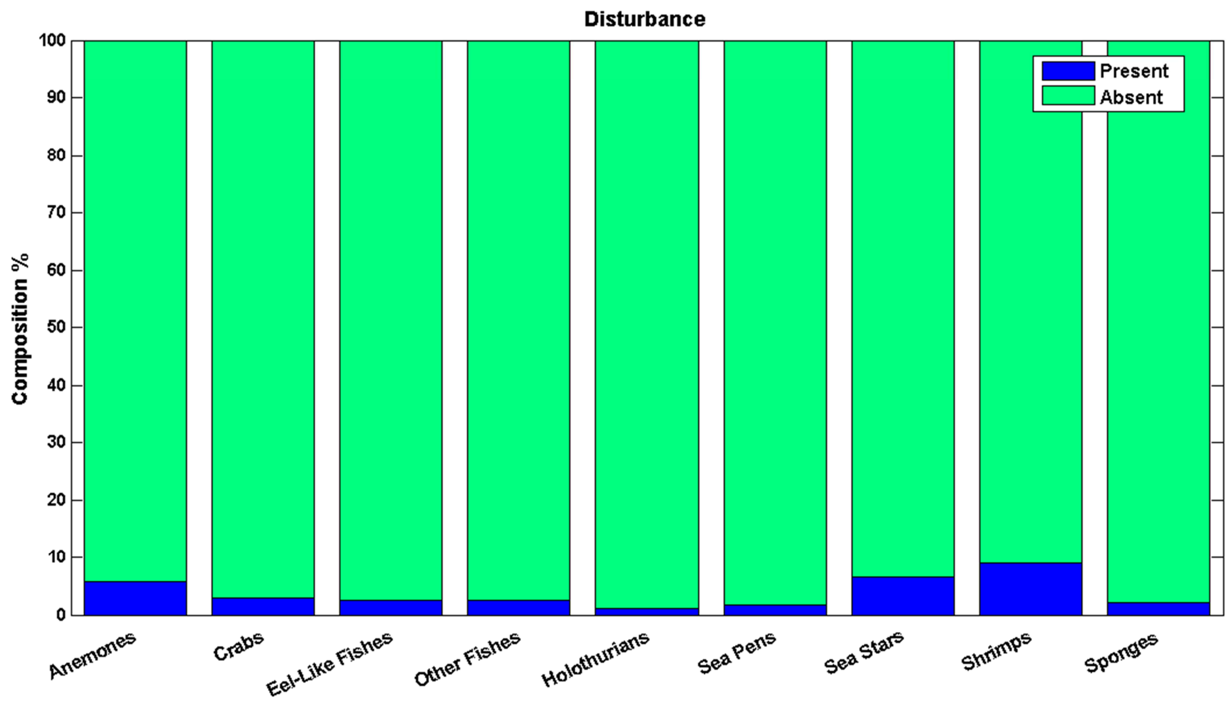


Fig. 4.12. Percentage of animal observations, by broad taxonomic group, associated with presence or absence of physical disturbance to the seafloor.

An important component of environmental monitoring programs is evaluation of benthic megafaunal communities not only in terms of abundances and diversity, but also with respect to relationships between organisms and their environment. A better understanding of associations of different taxonomic groups with specific environmental variables or conditions is critical for evaluating the effects of anthropogenic influences on the benthic communities because it helps distinguish between natural variation and possible anthropogenic influences. The database system, because it compiles all relevant environmental and organism data in one location, simplifies acquisition of data and the subsequent analysis of these associations. Combined with the ability to add new data into the database, this facilitates more effective spatial and temporal investigations into how benthic communities vary and change.

Modifications to the database system can be made easily as required over the course of data analysis, and adding new database fields won't affect the original fields or data that have already been entered. This is an important characteristic because it allows for broader

applications and increased flexibility in longer-term uses of the database system. Another major benefit is that data collected independently of the images can be linked in with the data tables created in the database system. Also, a database system allows for easier management of large and multiple datasets since they are all in one location and can be combined or separated as deemed necessary.

In the northern GoM, a large quantity of imagery data collected comes from ongoing SERPENT Project activities and industrial environmental surveys in the vicinity of offshore drilling locations. More recently, efforts to collect additional information on deep-sea benthic communities in the wake of the Deepwater Horizon oil spill have further contributed to the depository of video imagery collected in this area. Those involved with imagery collection and analysis of this data often consist of persons who may not always have advanced technical training working with complex software, including industrial ROV pilots or data collectors as well as both undergraduate and postgraduate students acting in the capacity of research assistants or completing academic research. Compared to other database systems such as VARS or FISH_ROCK, the database system presented here features a more simplified design that limits complex features or annotation functions. It is set up in a way that enables quick entry of data and is simple to use, even with minimal training. Not only does this save time during analysis of large quantities of imagery, but it creates additional opportunities to more readily include less experienced users such as students or crowdsourcing in the research being completed. Overall, a simplified design with fool-proof mandatory fields built in to ensure minimum information standards are met more appropriately suits the audience to which this database system targets – students, industrial surveyors, etc. working with environmental and imagery-based data in the northern GoM.

Using a database system overall provides a valuable resource for collection and storage of environmental data from different kinds of imagery. Both short- and long-term environmental studies typically result in very large quantities of diverse data, which can be difficult to manage when spread out across different systems and computer programs. Having the ability to combine and manipulate the structure of different data sets facilitates more comprehensive

analyses since all potentially relevant data will be accessible in one compiled location. This ideally facilitates better spatial and temporal comparisons of environmental data, ultimately contributing to a better understanding of ecosystem and community changes, as well as impacts and subsequent recoveries of benthic communities from environmental stressors.

4.5. Conclusion

Incorporating a formal, uniform database system into data analysis and management procedures for studies in the northern Gulf of Mexico can prove a valuable future tool. Partnerships between industry and the science community are becoming increasingly important as part of environmental monitoring programs related to offshore exploration and drilling activities. SERPENT is an example of one of these partnerships that has contributed to the acquisition of large quantities of ROV imagery in the northern GoM. The database system presented here provides an opportunity to compile data from across a variety of studies including those that are part of NRDA studies related to the Deepwater Horizon oil spill as well as from SERPENT. This database has the potential to become a long-term depository of data that would not only increase our understanding of benthic megafaunal communities but also improve our ability to evaluate changes to these communities resulting from human impacts on the deep-sea environment.

While maintaining records and ensuring availability of collected imagery itself is important, the data extracted from imagery is often of greater importance in long-term studies, particularly with respect to environmental monitoring efforts. Rather than concentrating efforts on developing a database system where video or other imagery can be annotated within the database itself, the focus here was to create a simplified place where observations could be added, stored, and extracted to answer whatever scientific questions may be of value for a given study.

Future development of the database system will include the ability to add and view an image directly in the database form, so that each form entry has the corresponding image readily available. Additional environmental variables and observational fields will also be added to the database system to expand the types of data that can be stored and investigated using the

database. This will increase the applicability of the database system to a larger variety of investigations beyond what was used in this particular study. In addition, the database form will be further modified in a way that will make data entry even simpler for inexperienced users such as students and industrial ROV pilots, who may not be as familiar features and animals observed in the imagery. This new design will combine elements from the current database with elements of the Bureau of Ocean Energy Management (BOEM) biological survey requirements for offshore activities. The premise is to create an uncomplicated, standardized system of image data entry that can be used in a variety of applications for research in the northern GoM.

CHAPTER 5. AN EVALUATION OF DEEP-SEA BENTHIC MEGAFAUNAL COMMUNITIES IN THE NORTHERN GULF OF MEXICO ONE YEAR AFTER DEEPWATER HORIZON

5.1. Introduction

On April 20, 2010, the Deepwater Horizon oil rig located in Mississippi Canyon block 252 (MC252), approximately 50 miles southeast of the Mississippi River delta in the northern Gulf of Mexico (GoM), lost well control. The resulting explosion and sinking of the rig on April 22, 2010 resulted in oil and gas flowing from a bathymetric source until it was finally capped on July 15, 2010. Even though the exact amount of oil and gas spilled into the GoM has been widely debated, it has been acknowledged that the Deepwater Horizon oil spill was the largest oil spill in the history of the United States (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2011).

The Deepwater Horizon oil spill highlighted deficiencies in knowledge of northern GoM ecosystems, and especially the lesser-known deep-sea megabenthos. Filling in these gaps has now become even more important in order to interpret the results from monitoring of areas potentially affected by the spill. While a variety of studies of benthic fauna have been conducted in the GoM over the past several decades, the focus of most has not necessarily been on the megabenthos. Instead, many studies have evaluated infaunal communities (Rowe and Menzel, 1971; Flint and Holland, 1980; Montagna and Harper, 1996; Escobar-Briones and Soto, 1997; Hernández-Arana et al., 2003; Hyland et al., 2003). Of those studies that have explored megafauna, more emphasis is generally placed on unique benthic communities associated with hydrocarbon seep sites (MacDonald et al., 1989; MacDonald et al., 2010) or deep-sea corals. Despite an overall lack of published GoM studies, recent studies of deep-sea benthos have included those conducted as part of the Deep Gulf of Mexico Benthos (DGoMB) project (Powell et al., 2003; Rowe and Kennicutt, 2009). DGoMB aimed to describe benthic and benthopelagic communities in the GoM, along with evaluating the biological, physical, and environmental processes influencing structure and function of these communities.

Based on studies completed to-date, the GoM has a paucity of benthic megafauna compared to other deep-sea benthic ecosystems. Dredge and photographic surveys conducted by Rowe and Menzel (1971) in the deep GoM indicated that deep-sea benthic fauna numbers and biomass are relatively poor; and the authors attribute noted variations in abundances to differences resulting from local patchiness. Another trawl study as part of the DGoMB studied deep-sea demersal fish fauna and observed similar low abundances (Powell et al., 2003). This study also noted that fish fauna assemblages exhibited depth zonation. It was found that the most abundant shelf species were Caproidae, Macrouridae and Steindachneriidae, while mid-slope fauna were dominated by Macrouridae, and Ophidiidae predominated in the deep zone. Once again, species richness and abundance were found to be greater in shallower zones, with lower abundances at depth. Escobar-Briones and Soto (1997) found that the predominant shelf epibenthic macrofauna in the GoM included a diverse group of decapod crustaceans and stomatopods, as well as brachyuran crabs and penaeid shrimp. In general, GoM studies have noted benthic biomass exhibits a decrease with depth and distance from the coast (Rowe and Menzel 1971, Escobar-Briones and Soto 1997, Powell et al. 2003).

Recent studies using industrial remotely operated vehicles (ROVs) have investigated deep-sea benthic megafauna in the vicinity of the Deepwater Horizon Macondo well immediately after the oil spill. Valentine and Benfield (2013) reported on benthic megafaunal abundances and diversity obtained from surveys conducted in August 2010 at five sites located within 2000 m of the Macondo well. Red shrimps, mobile holothurians from the family Elpidiidae, and the red crab *Chaceon quinquedens* generally comprised the most abundant mobile invertebrates, with sea stars representing the most abundant of the sessile or low mobility invertebrates. The most abundant fishes were observed to be halosaurs, *Synaphobranchus* and *Facciolella* eels, the tripod fish *Bathypterois quadrafilis*, and two cusk eels, *Bassogigas gillii* and *Dicrolene* sp. (Valentine and Benfield, 2013).

Expanding knowledge on deep-sea megabenthos will better facilitate understanding of potential effects and recovery of GoM marine communities in response to anthropogenic influences such as the Deepwater Horizon oil spill. Collection of baseline data on species abundances and diversity along with community composition and dynamics are critical

components for use in general monitoring programs, as well as those investigating anthropogenically-induced environmental impacts and subsequent recovery. The GoM often has limited data on baseline conditions associated with benthic megafauna, as has become evident in the wake of the 2010 oil spill. There is also a need to go beyond collection of basic data on megafaunal abundances and diversity to explore possible environmental influences contributing to observed benthic megafaunal community compositions. Without this information, it is impossible to conclude whether observed differences in these communities are the result of impacts from the oil spill or simply attributable to other variations in the marine benthic environment.

A variety of natural environmental factors (i.e. physical oceanographic parameters such as oxygen concentration and currents, seafloor characteristics such as substrate type, sediment composition, and disturbance) can influence abundances, diversity and distributions of benthic organisms (Levin et al., 2001). Depth is a well-known factor influencing benthic faunal composition in the deep-sea (Rex, 1981; Pires-Vanin, 2001; Barry et al., 2003). Substrate type often plays an important role in determining the types and quantities of benthic fauna present. More specifically in the case of soft-sediment deep-sea environments, it is often sediment composition and texture that influence the megabenthos (Pires-Vanin, 2001; Barry et al., 2003; Jones et al., 2007a).

Food supply, which is generally tied to ocean surface productivity and changes with depth, is often considered a highly limiting factor that can greatly influence benthic communities. Changes to oceanographic parameters, nutrient input, and surface productivity, and the consequent impacts on trophic web dynamics also have the potential to contribute to seasonal variations in benthic communities (Cartes, 1998; Kojima and Ohta, 1990; Pires-Vanin, 2001; Gooday, 2002; Sumida et al., 2008; Corliss et al., 2009; Glover et al., 2010; Rowe, 2013;).

The growth of human activities into the deep-sea has made it necessary to expand beyond traditional investigations into natural environmental factors and into evaluating impacts of anthropogenic influences on marine communities. One of the primary types of potential anthropogenic influences is in the form of physical disturbances to the seafloor including from

fishing and industrial offshore exploration and extraction activities. Widespread commercial fishing has led to many studies that have investigated the impacts of physical disturbance to the seafloor and associated communities resulting from fishing gear (Kaiser et al., 1998; Thrush and Dayton, 2002; Hermsen et al., 2003; Asch and Collie, 2008). These studies have shown that benthic communities are often impacted by disturbance, resulting in different abundances, diversity and overall community compositions associated with disturbed areas. Recent studies have also evaluated impacts and recovery of benthic megafaunal communities in response to anthropogenic disturbances associated with offshore hydrocarbon drilling wells (Jones et al., 2006; Jones et al., 2007a,b; Jones et al., 2011; Gates and Jones, 2012; Jones et al., 2012).

The study described herein presents the results obtained from a set of industrial ROV surveys conducted in 2011, approximately one year after the Deepwater Horizon oil spill. Seven sites in the northern GoM, located at varying distances and directions from the Deepwater Horizon MC252 wellhead, were studied. The goal was to quantify and compare benthic megafaunal abundances and diversity across the seven study sites and evaluate trends in community composition. Several natural and anthropogenic environmental variables observable in the ROV imagery were evaluated in order to provide insights into possible factors influencing the benthic megafaunal communities in the northern GoM.

5.2. Methods

5.2.1. Study Location and Survey Sites

This study consists of data collected from two Natural Resource Damage Assessment (NRDA) cooperative cruises conducted aboard the HOS Sweetwater in the northern GOM during the summer of 2011. Sweetwater Megafauna Cruise 1 took place between June 8, 2011 to June 22, 2011, and Cruise 2 from August 10, 2011 to August 22, 2011. An industrial ROV was used to collect video and still image data at survey sites (Fig. 5.1) located around the MC252#1 well.

Benthic surveys were conducted at a total of 11 survey sites, with six located in close proximity (< 3.5 km) to the MC252 wellhead blowout preventer and five more distant stations that were previously survey during pre-spill studies on the deep-sea red crabs, *Chaceon quinquedens*. Figure 5.1 shows the locations of the 11 survey sites in relation the Macondo wellhead. The

analysis presented here aims to characterize benthic megafaunal communities at 7 of the 11 survey sites – MC252 2000 m N, MC208, MC118, Red Crab 1C, Red Crab 1D, WSW Megafauna 1, and WSW Megafauna 2 – that were chosen because they represent a range of locations and distances with respect to the MC252 well (Table 5.1).

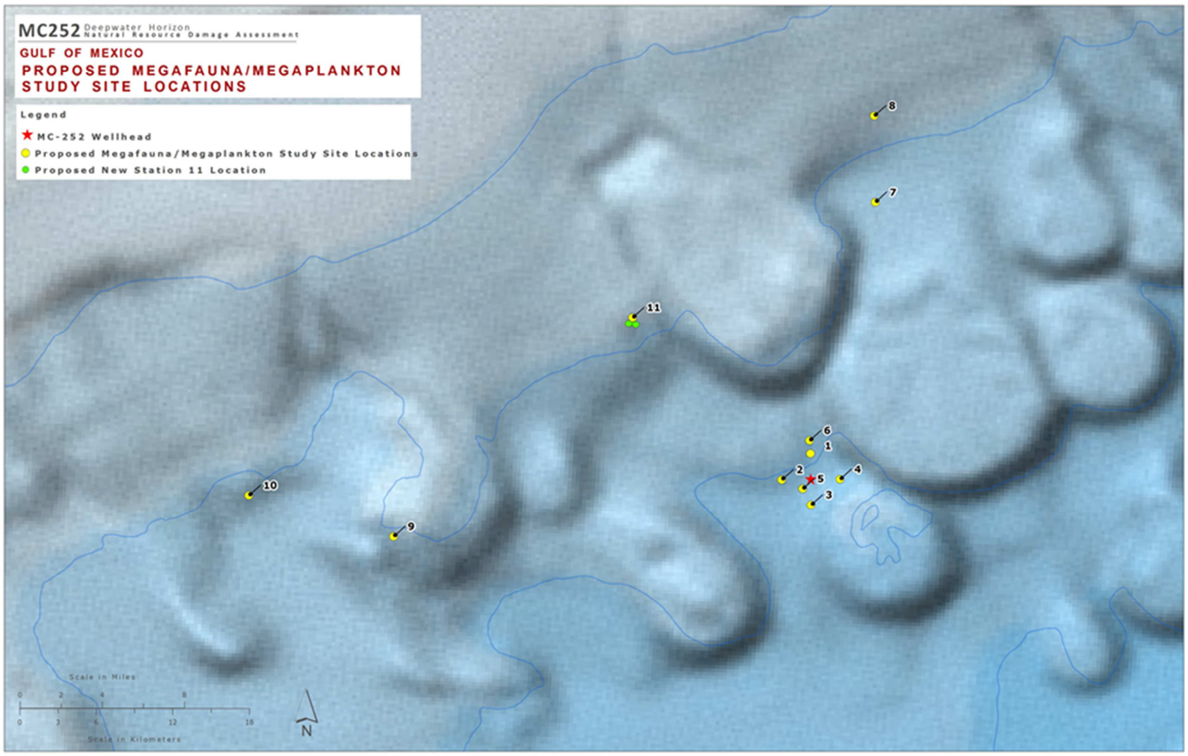


Fig. 5.1. Location of ROV survey sites in the vicinity of the Deepwater Horizon Macondo well. The red star represents the location of the wellhead. Yellow points with numbers are the locations of the survey sites (refer to Table 5.1 for site information).

Table 5.1. Locations of sites surveyed in the vicinity of the Deepwater Horizon Macondo well.

Site	Full Site Name	Abbreviated Site Name	Latitude	Longitude	Distance from MC252 Well (km)
1	MC252 2000 m N	MC252	28° 45’ 22.295” N	88° 21’ 58.520” W	2.0
6	MC208 Photomosaic	MC208	28° 45’ 55.102” N	88° 22’ 00.779” W	3.0
7	Red Crab 1D	RC1D	28° 56’ 01.200” N	88° 19’ 13.800” W	22.2
8	Red Crab 1C	RC1C	28° 59’ 40.200” N	88° 19’ 16.200” W	28.8
9	WSW Megafauna1	WSWMF1	28° 41’ 51.900” N	88° 39’ 37.000” W	29.1
10	WSW Megafauna2	WSWMF2	28° 43’ 36.600” N	88° 45’ 44.600” W	38.7
11	MC118	MC118	28° 51’ 07.992” N	88° 29’ 30.000” W	17.6

5.2.2. Data Collection and Analysis

A work-class Triton XLS 150 ROV equipped with multiple still image and video cameras including a standard definition video (SD) camera, high definition (HD) video camera, and digital still camera (DSC) was used to collect the video and still imagery evaluated in this study. A series of twenty-four 250-m long transects radiating from a common point of origin with a 15° offset were completed at each survey site. An ultra-short baseline (USBL) system was used to guide the ROV to the central point of the survey, and the ROV was oriented to the appropriate transect heading. The ROV then proceeded to move at a constant velocity along the transect while attempting to maintain a constant altitude of approximately 2 meters off the seafloor. Figure 5.2 shows transect tracklines, where navigation data was available, obtained by using a running mean, completed at each site. After the outbound transect was completed, the ROV turned on a reciprocal heading and conducted an inbound transect, which allowed for close-up video and DSC images of organisms encountered. This data collected during the inbound transect was used to improve identification of organisms. During both outbound and inbound transects, a pair of red laser scalers fixed to the ROV were used to allow for measurement of the field of view of the camera.

Outbound ROV video transects were analyzed from the beginning to end of each transect using VideoReDo Plus (DRD Systems, Inc., 2003) video playback software. Frame grabs were taken each time an organism on or just above the seafloor was encountered along the transect, and the date and time the organism was viewed in the video was recorded as the file name. In order to maintain consistency between transects and study sites, only organisms that fell within a specific area of the field of view (FOV) where the best light illumination occurs (approximately the bottom 30% of the video FOV) were included. The USBL navigation data for each transect was smoothed in MATLAB using a running point mean and the length of each transect was calculated. For transects where navigation data was missing, a transect length of 250 meters was assumed. A location for each photo observation was determined by matching the time of the photo with the navigation data corresponding to that time. From the frame grabs, organism counts and identifications were obtained in order to estimate species abundances and diversity at each study site.

The frame grabs were then sorted into broad taxonomic groups with the organism in each photo identified down to the lowest taxonomic level possible. The broad groups used for this study included: anemones, crabs, eel-like fishes, other fishes, holothurians, sea pens, sea stars, shrimps, sponges, and “other”. The latter category includes all organisms that did not fall into any of the other groups and all possible organisms for which frame grabs were taken but taxonomic placement was uncertain.

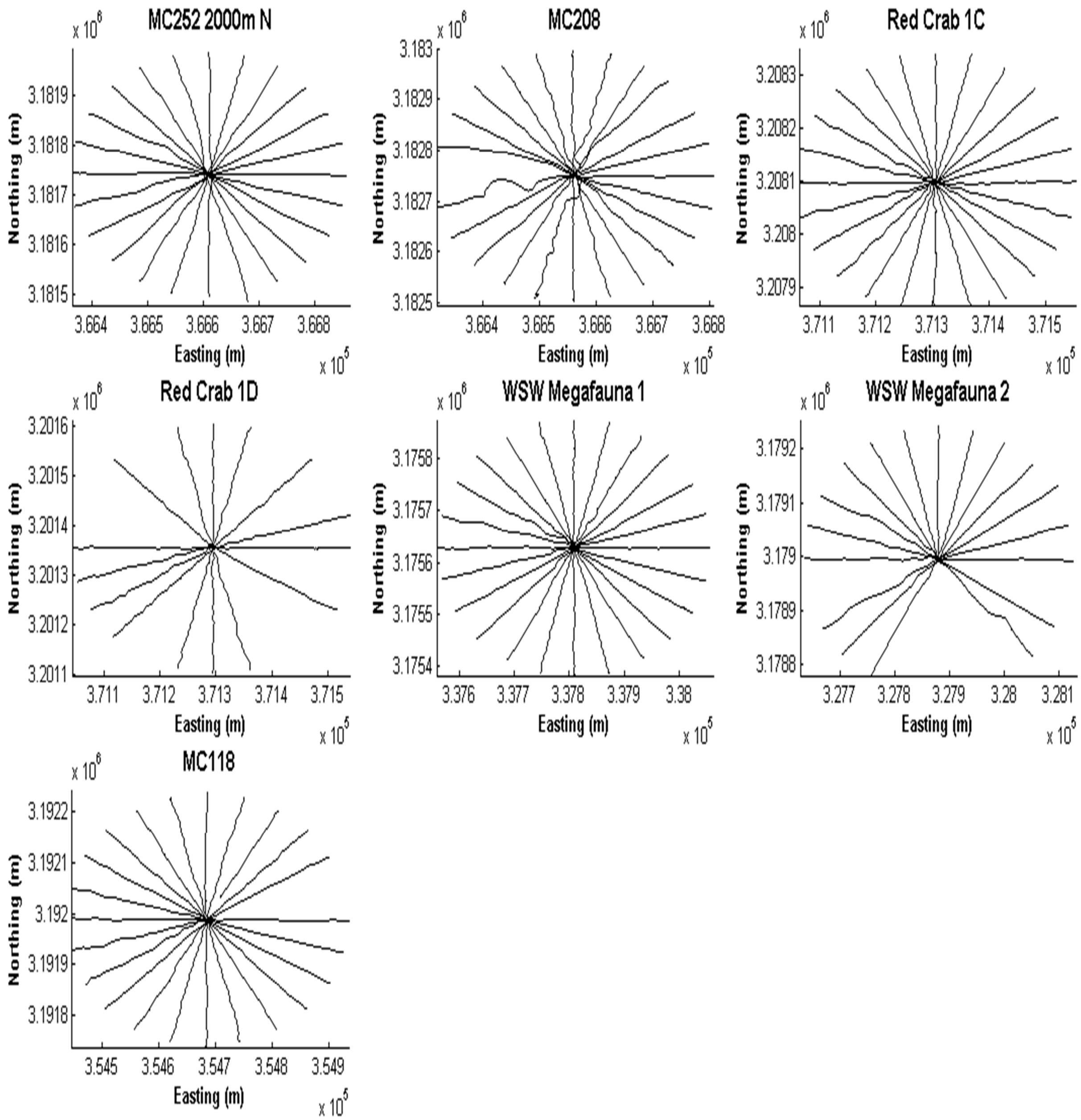


Fig. 5.2. Transect tracklines at the seven study sites. The black lines represent the smoothed and interpolated tracks obtained from the raw navigation data.

In order to calculate densities of each of the organism groups, first the field of view (FOV) was calculated using the red laser scalers visible on the seafloor in the HD video. Ten evenly spaced frame grabs were taken from throughout the video for each transect, where visibility permitted. The pixels of the laser scalers, the known distance between the lasers, and the known pixel width across the width of the video were used to determine the FOV. The FOV values from each photo were then averaged to give a mean FOV for each transect. For any transects in which the laser scalers were not visible or where less than three frame grabs were obtained, a FOV corresponding to the mean FOV for all other transects at that survey site was used. Total area surveyed was then calculated for each transect using the mean FOV multiplied by the total distance traveled for the transect. Refer to Chapter 2 for more specific details on the ROV survey methodology employed in this study.

5.2.3. Community Composition and Diversity

Organism densities were obtained for each individual transect, then mean densities for each taxonomic group were calculated for each of the survey sites as a whole. A total density for all organisms at each site was also calculated by combining the data from all groups with that of the "other" category. The resulting broad taxonomic group densities were statistically compared to evaluate differences in densities of each group among the seven study sites. Homogeneity of variances were tested using Levene's test (Trujillo-Ortiz and Hernandez-Walls, 2003) in order to evaluate the null hypothesis that the variances for the different sites were equal. An Anderson-Darling test was also performed to test whether the sets of data followed normal distributions. The results of the Levene's and Anderson-Darling tests were such that the data did not have equal variances nor exhibit normal distributions. Densities of each broad taxonomic group were then compared using a non-parametric Kruskal-Wallis test in order to test the null hypothesis that the densities were equal. A follow-up Dunn's test (Cardillo, 2006) further differentiated which densities were significantly different from one another. All density and significance test calculations were completed in MATLAB (The Mathworks INC., 2014).

In order to compare overall community composition amongst the study sites, several approaches were used. Percentage compositions of all organisms at the sites were calculated for each broad taxonomic group. Non-parametric multivariate analyses were performed using

PRIMER-E (Clarke and Gorley, 2006) software to explore trends in community composition amongst the study sites. Data was fourth root transformed to minimize bias resulting from highly abundant species, then a Bray-Curtis resemblance matrix was calculated. Using the Bray-Curtis matrix, a hierarchical cluster analysis with group-averaged linkage and multidimensional scaling (MDS) were performed (Clarke, 1993; Clarke and Warwick, 2001) to look at grouping of the study sites based on overall community composition. This process was performed for both broad and more specific taxonomic groups. In addition to this, several diversity indices were calculated (using MATLAB) to measure biodiversity at each of the seven study sites using the lowest reasonable taxonomic resolution possible. These indices included species richness (S), Margalef's index of community diversity (d), Shannon-Wiener index (H'), Pielou's evenness index (J'), and Simpson's index (λ).

5.2.4. Environmental Factors

General sediment characteristics were noted for each site by examining the appearance of the sediment in the video to determine approximate texture and mix. Other environmental factors explored in this study consisted of those that could also be readily seen in the ROV imagery. These included the presence and size of seafloor features such as pits or depressions, mounds, and seafloor disturbance. The latter consisted of any and all visible physical disturbance to the seafloor resulting from deep-sea equipment (including ROVs) or other sources. These factors were determined for each photo by noting the presence (or absence) and estimating the average size of the features within the applicable field of view of the photo. The number of observations from each category (and corresponding size class within it) of environmental data were totaled at each survey site to allow for comparison of the sites. A Bray-Curtis resemblance matrix was created using fourth root transformed data to perform a hierarchical cluster analysis in order to compare differences in overall composition of the environmental variables between sites. MDS analyses were performed in order to explore similarities in environmental variables between the sites (Clarke, 1993; Clarke and Warwick, 2001). Percentage of observations from each site contributing to the total observations associated with each seafloor feature (pits/depressions, mounds, and disturbance) were also calculated.

General relationships between organisms and environmental factors were investigated in order to explore possible causes of variation in organism communities between the study sites. Data from all sites was pooled by animal group such that each animal group was represented by a specific value for every environmental factor being considered. By pooling the data, it allowed for better comparison of relationships between organisms and environment.

Similar to the analysis of community and environmental variable compositions, a non-parametric multivariate analysis was performed in PRIMER-E. Fourth root transformed data was used to calculate a Bray-Curtis resemblance matrix, which was then used in MDS analyses to look at grouping of broad taxonomic groups based on environmental variables. Environmental variables considered included: seafloor features (four size classes of pits or depressions and four size classes of mounds); disturbance to the seafloor (present or not); and site. The site variable incorporates a number of components that were within each study site: depth, location, sediment type and mix, and month when the site was surveyed. In addition to this, the percentage of organisms associated with each of the different seafloor features was calculated.

5.3. Results

5.3.1. Study Sites

The seven sites surveyed in this study were divided into three depth ranges: shallow (MC118 and Red Crab 1C), medium (Red Crab 1D, WSW Megafauna 1, and WSW Megafauna 2), and deep (MC208 and MC252 2000 m N). Six of the seven sites were surveyed during August of 2011, while MC252 2000 m N was surveyed in June 2011. A total of 1717 photo snapshots were taken from video at MC118; 2918 and 2670 photos were taken at MC208 and MC252 2000 m N, respectively; 2175 and 2066 at Red Crab 1C and Red Crab 1D, respectively; and 2523 and 2148 at WSW Megafauna 1 and WSW Megafauna 2, respectively.

5.3.2. The Seafloor Environment

Four of the study sites (MC118, Red Crab 1C, Red Crab 1D, and WSW Megafauna 1) had similar sediments consisting of homogeneously mixed unconsolidated fine-grained sediments (e.g. mud and/or fine sand). Two of the remaining sites (MC208 and WSW Megafauna 2) consisted

of mostly homogeneous unconsolidated coarse-grained sediments (e.g. sand); and the final site, MC252 2000 m N, was characterized by patchy and mixed soft sediments. Sediments at all sites were generally light grey in color with the exception of MC252 2000 m N, which had slightly darker sediments with frequent patches of lighter colored sediments. Table 5.2 summarizes environmental characteristics at all the survey sites.

Table 5.2. Environmental characteristics of the survey sites in this study.

Site	Characteristic			
	Survey Month	**Depth (m)**	**Sediment Type**	**Sediment Mix**
MC118	August 2011	942	Fine-grained	Homogeneous
MC208	August 2011	1450	Coarse-grained	Mostly Homogeneous
MC252 2000 m N	June 2011	1473	Mixed	Patchy
Red Crab 1C	August 2011	860	Fine-grained	Homogeneous
Red Crab 1D	August 2011	1043	Fine-grained	Homogeneous
WSW MF 1	August 2011	1043	Fine-grained	Homogeneous
WSW MF 2	August 2011	1044	Coarse-grained	Mostly Homogeneous

Seafloor features and areas of physical disturbance to the seafloor were evaluated as possible factors influencing megafaunal community composition. Seafloor data was pooled across the seven study sites in order to compare overall seafloor compositions (Table 5.3). MC208 and MC252 2000 m N overall had the least consistent seafloors. MC118, Red Crab 1C, and Red Crab 1D have moderately consistent seafloors; while WSW Megafauna 1 and WSW Megafauna 2 have the most consistent seafloors out of all the sites. MC252 2000 m N and, in particular, MC208 both have much more seafloor disturbance than the other sites, accounting for 31.7% and 52.3% of seafloor disturbance, respectively, observed amongst the sites. The other four sites have relatively little seafloor disturbance in amounts comparable to one another.

There was a clear grouping of sites based on overall composition of their seafloor features (Fig. 5.3 and 5.4). While there was greater than 80% similarity in environmental variables between all of the sites, there was further grouping by additional similarities. An initial grouping divided the two western sites from the others. Further grouping was related to site location and depth: the deepest sites closest to the well (MC208 and MC252 2000 m N), mid-depth sites to the west (WSW Megafauna 1 and WSW Megafauna 2), and sites to the north and northwest (Red Crab

1C, Red Crab 1D, and MC118). In this latter group, there was a division by depth with the two shallower sites having more similarities than the deeper site.

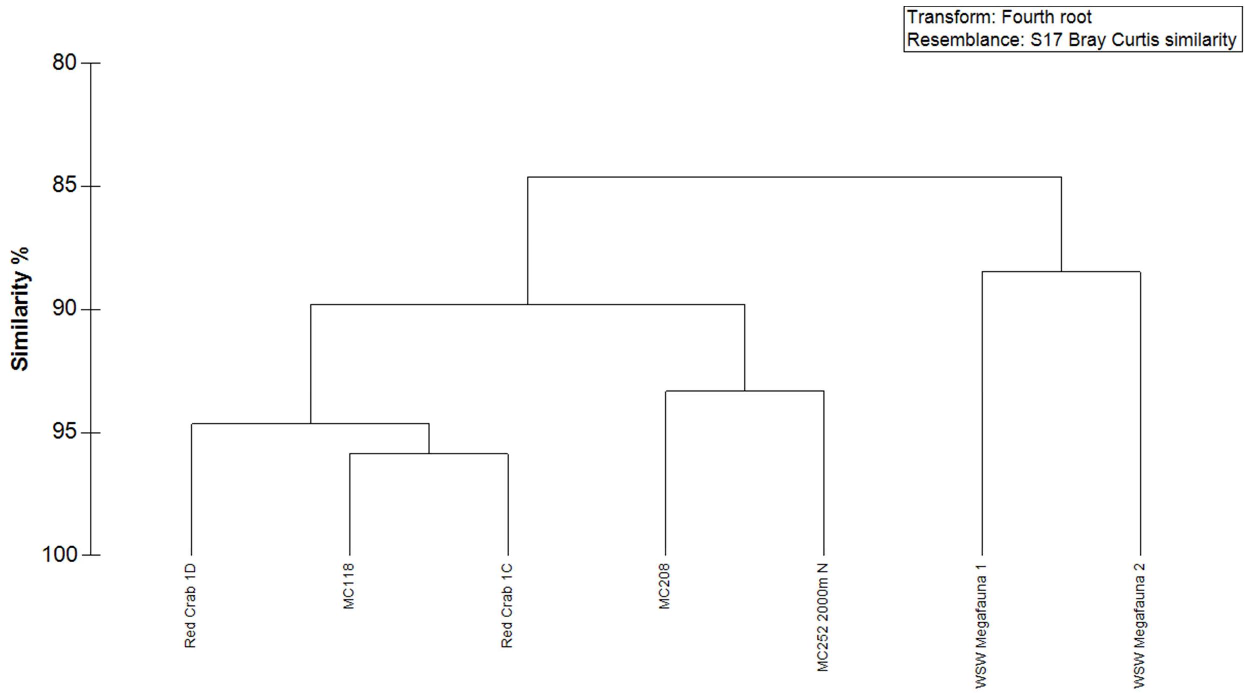


Fig. 5.3. Hierarchical cluster analysis of similarities in environmental variables (pits or depressions, mounds, and seafloor disturbance) between study sites.

5.3.3. Community Composition

Abundances of animals from the broad and more specific taxonomic groups (Table 5.4) varied greatly amongst study sites; however, there were some general trends. The most dominant groups were shrimp, fishes, and eel-like fishes. Two main groups of shrimps were observed at all sites – an abundant group consisting of multiple undeterminable species of red shrimps, and a less abundant group of benthic armored shrimp, *Glyphocrangon* sp. *Aldrovandia* sp. and *Synaphobranchus* sp. accounted for the majority of eel-like fishes present at all sites. Other fishes were typically dominated by multiple indeterminable species of Macrourids, with *Acanthochaenus* sp., *Acanthonus* sp., *Alepocephalidae*, *Bathypterois* sp., and chimaeras representing other species observed in smaller numbers. The majority of crabs observed were the red deep-sea crabs, *Chaceon quinquedens*, with the occasional large king crab, *Neolithodes agassizii*, seen at about half of the sites.

Table 5.3. Percentage of observations each study site contributes to total observations from all sites, associated with different size classes of pits or depressions and mounds, and presence or absence of physical disturbance to the seafloor.

Study Site	Pits or Depressions (%)				Mounds (%)				Seafloor Disturbance (%)	
	Not Present	Small	Medium	Large	Not Present	Small	Medium	Large	Present	Not Present
MC118	10.3	11.1	9.1	17.7	15.3	8.5	11.6	4.1	1.3	11.1
MC208	14.1	16.7	20.9	29.6	26.4	17.5	12.9	35.5	52.3	16.2
MC252 2000 m N	58.5	8.7	4.1	22.3	31.3	14.0	9.9	46.7	31.7	15.7
Red Crab 1C	9.1	16.5	11.9	11.3	17.7	10.1	17.0	9.5	3.5	14.0
Red Crab 1D	7.7	17.6	10.0	3.2	8.7	15.7	11.1	1.5	3.2	13.3
WSW Megafauna 1	0.1	11.7	28.6	13.2	0.2	9.3	35.0	2.1	6.2	15.7
WSW Megafauna 2	0.2	17.8	15.4	2.7	0.5	24.9	2.5	0.6	1.9	13.9

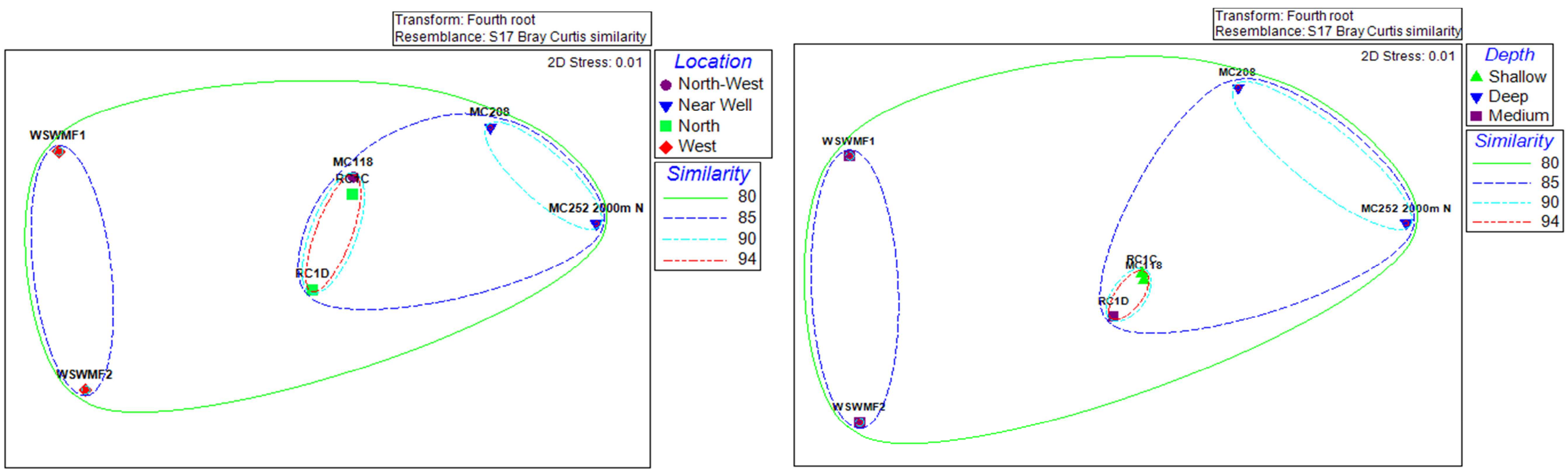


Fig. 5.4. MDS analysis of similarities in environmental variables between study sites using location (left) and depth (right) as a factor.

Densities of mobile taxonomic groups (Fig. 5.5) were quite variable, with few readily observable trends. MC208 and MC252 2000 m N differed from the other sites in that they had much higher abundances of shrimps. MC208 also had much lower abundances of eel-like and other fishes. Significant differences were observed when comparing mobile taxon from many of the sites (Fig. 5.5). The most significant differences were observed for eel-like and other fishes amongst the majority of study sites. Red Crab 1C and WSW Megafauna 2 were most significantly different (lower and higher, respectively) from other sites for crab densities. MC208 and MC252 2000 m N had significantly higher densities of shrimps.

Least abundant groups at all the sites generally consisted of sessile or low-mobility animals including anemones, holothurians, sea pens, sea stars, and sponges. Two types of anemones were observed, the small purple cerianthid anemones and the Venus flytrap anemone, *A. aurelia*, at almost all study sites. Densities of holothurians (mostly *Mesothuria*), sea pens, and sea stars were generally low and varied greatly amongst sites. Sponges represented the smallest group by far with no observations at three out of the seven sites (Table 5.4).

Densities of sessile and low mobility animals were highly variable amongst sites (refer to Fig. 5.6). Aside from low abundances of holothurians and slightly higher abundances of sea stars at MC208 and MC252 2000 m N compared to other sites, there were no obvious trends in sessile or low mobility animals. Significance testing revealed many significant differences between sessile animal densities amongst the sites (Fig. 5.6). Abundances at MC252 2000 m N were significantly different (and mostly much higher) than the majority of other sites for almost all taxonomic groups. For anemones, MC252 2000 m N was most significantly different (with higher densities) than the other sites. MC252 2000 m N and WSW Megafauna 1 had significantly higher abundances of sponges; and MC208 and MC252 2000 m N exhibited significantly higher abundances of sea stars. Overall, significantly different abundances of sea pens and holothurians were observed amongst all sites.

Table 5.4. Mean organism densities for each of the survey sites in the vicinity of the Deepwater Horizon well in the Northern GoM.

Taxonomic Group	Density (ha^{-1} ± 95% CI)						
	MC118	**MC208**	**MC252 2000mN**	**Red Crab 1C**	**Red Crab 1D**	**WSW MF 1**	**WSW MF 2**
Anemones	**0.3 ± 0.7**	**2.4 ± 1.8**	**19.7 ± 12.2**	**0.7 ± 1.1**	**1.9 ± 2.0**	**10.1 ± 4.9**	**10.8 ± 6.6**
Actinoscyphia aurelia	0.3 ± 0.7	-	1.8 ± 3.6	0.4 ± 0.9	-	6.9 ± 5.0	3.2 ± 2.5
Cerianthidae	-	2.4 ± 1.8	16.4 ± 10.3	0.3 ± 0.7	1.9 ± 2.0	3.2 ± 2.6	7.5 ± 4.7
Other	-	-	1.5 ± 3.2	-	-	-	-
Crabs	**10.0 ± 3.4**	**8.4 ± 3.0**	**12.5 ± 9.5**	**0.4 ± 0.9**	**14.2 ± 4.9**	**18.7 ± 6.5**	**35.4 ± 6.4**
Chaceon quinquedens	10.0 ± 3.4	7.9 ± 2.9	12.5 ± 9.5	0.4 ± 0.9	13.0 ± 4.3	18.0 ± 6.3	35.1 ± 6.0
Neolithodes agassizii	-	0.4 ± 0.6	-	-	1.2 ± 1.4	0.6 ± 0.9	0.3 ± 0.7
Eel-Like Fishes	**132.6 ± 28.8**	**30.4 ± 6.2**	**128.3 ± 20.7**	**187.4 ± 33.4**	**82.1 ± 18.0**	**159.9 ± 26.1**	**78.0 ± 14.7**
***Aldrovandia* sp.**	47.6 ± 15.9	5.2 ± 2.7	3.5 ± 3.4	26.4 ± 9.4	30.5 ± 10.6	94.9 ± 21.4	38.9 ± 11.5
***Synaphobranchus* sp.**	34.9 ± 10.3	8.6 ± 2.9	27.9 ± 10.0	18.6 ± 5.9	10.3 ± 5.2	8.7 ± 3.7	15.0 ± 4.7
Other	50.0 ± 12.2	16.7 ± 4.9	96.9 ± 19.5	142.4 ± 27.8	41.3 ± 10.2	56.2 ± 11.6	24.1 ± 6.0
Other Fishes	**312.6 ± 64.3**	**72.9 ± 19.4**	**254.9 ± 42.1**	**467.8 ± 89.4**	**400.8 ± 69.0**	**327.5 ± 52.1**	**234.4 ± 46.9**
***Acanthochaenus* sp.**	-	1.2 ± 1.1	-	-	-	-	-
***Acanthonus* sp.**	0.4 ± 0.7	0.2 ± 0.4	-	-	-	-	7.6 ± 4.9
Alepocephalidae	0.7 ± 1.0	-	-	3.5 ± 3.0	-	0.3 ± 0.7	-
***Bathypterois* spp.**	1.3 ± 1.6	4.1 ± 1.7	2.7 ± 3.1	0.4 ± 0.9	1.5 ± 1.5	2.0 ± 1.7	3.1 ± 2.2
Chimaeriformes	3.8 ± 2.5	-	-	4.4 ± 2.2	5.9 ± 3.3	0.6 ± 0.8	-
Macrouridae	103.3 ± 28.7	5.2 ± 5.2	33.9 ± 9.5	134.3 ± 37.7	108.8 ± 24.3	70.8 ± 17.6	21.7 ± 8.0
Other	203.1 ± 51.8	62.2 ± 16.8	254.9 ± 42.1	325.2 ± 58.3	284.6 ± 73.8	253.7 ± 44.0	201.9 ± 44.6
Holothurians	**20.4 ± 7.6**	**0.4 ± 0.6**	**2.6 ± 3.0**	**17.1 ± 6.7**	**57.7 ± 13.3**	**34.0 ± 9.1**	**22.5 ± 7.6**
Mesothuria	20.1 ± 7.4	0.4 ± 0.6	1.8 ± 2.6	16.0 ± 6.0	57.3 ± 13.2	33.7 ± 8.7	22.5 ± 7.6
Elpididae	0.3 ± 0.7	-	-	0.6 ± 0.9	0.4 ± 0.8	0.3 ± 0.7	-
Enypniastes eximia	-	-	0.9 ± 1.8	0.4 ± 0.9	-	-	-
Sea Pens	**3.2 ± 2.1**	**12.6 ± 4.5**	**32.3 ± 15.4**	**2.9 ± 2.1**	**21.4 ± 10.0**	**11.3 ± 3.7**	**3.1 ± 2.6**
Sea Stars	**9.9 ± 6.0**	**25.2 ± 4.8**	**162.8 ± 20.5**	**4.5 ± 2.2**	**5.8 ± 3.6**	**5.4 ± 2.7**	**7.6 ± 5.0**

(Table 5.4. continued)

Taxonomic Group	Density (ha^{-1} ± 95% CI)						
	MC118	**MC208**	**MC252 2000mN**	**Red Crab 1C**	**Red Crab 1D**	**WSW MF 1**	**WSW MF 2**
Shrimps	**69.5 ± 16.8**	**435.4 ± 71.5**	**1741.8 ± 195.0**	**165.6 ± 32.0**	**212.8 ± 54.0**	**206.0 ± 50.9**	**264.2 ± 58.1**
***Glyphocrangon* sp.**	0.4 ± 0.7	76.7 ± 18.5	240.2 ± 58.7	10.6 ± 5.0	31.9 ± 10.7	46.9 ± 12.4	132.1 ± 28.2
Red Shrimps	69.2 ± 16.8	358.7 ± 60.6	1501.6 ± 168.3	155.0 ± 30.5	180.8 ± 46.2	159.1 ± 45.6	132.1 ± 35.4
Sponges	**-**	**-**	**7.0 ± 5.0**	**-**	**0.3 ± 0.7**	**11.3 ± 4.2**	**0.7 ± 1.0**
Other	**34.2 ± 12.2**	**36.0 ± 11.4**	**220.2 ± 64.9**	**127.7 ± 31.5**	**53.9 ± 15.3**	**44.6 ± 10.6**	**74.4 ± 16.8**
Bathynomus giganteus	-	0.8 ± 0.8	0.8 ± 1.7	-	-	0.3 ± 0.6	0.5 ± 1.1
Cnidaria	3.2 ± 2.2	3.7 ± 2.6	7.9 ± 5.1	35.6 ± 17.6	12.5 ± 17.3	3.5 ± 2.8	7.4 ± 4.4
Cydippid Ctenophores	-	0.3 ± 0.6	-	0.4 ± 0.8	-	-	0.3 ± 0.6
Hermit Crabs	-	1.2 ± 1.1	-	0.7 ± 1.1	-	-	-
Larvaceans	0.6 ± 0.9	1.5 ± 1.4	-	1.4 ± 1.3	1.4 ± 1.4	-	0.3 ± 0.6
Munnopsid Isopods	-	0.4 ± 0.9	-	1.7 ± 1.7	0.4 ± 0.9	-	0.3 ± 0.7
Cirroteuthidae	-	-	-	3.9 ± 4.3	-	0.7 ± 1.0	0.3 ± 0.6
Solitary Coral	0.3 ± 0.6	0.2 ± 0.4	-	-	-	-	-
Squat Lobster	-	-	-	0.9 ± 1.3	0.4 ± 0.7	0.9 ± 1.4	0.6 ± 0.8
Squid	0.4 ± 0.8	-	1.8 ± 2.6	1.3 ± 1.5	-	0.3 ± 0.7	0.5 ± 1.1
Other	29.7 ± 11.4	27.9 ± 8.7	209.6 ± 64.0	81.8 ± 19.4	39.1 ± 12.5	38.9 ± 9.1 45.6	64.2 ± 14.2
All Organisms	**592.8 ± 83.6**	**623.7 ± 97.4**	**2582.2 ± 218.5**	**974.2 ± 146.4**	**850.8 ± 111.8**	**828.7 ± 112.7**	**730.9 ± 123.7**

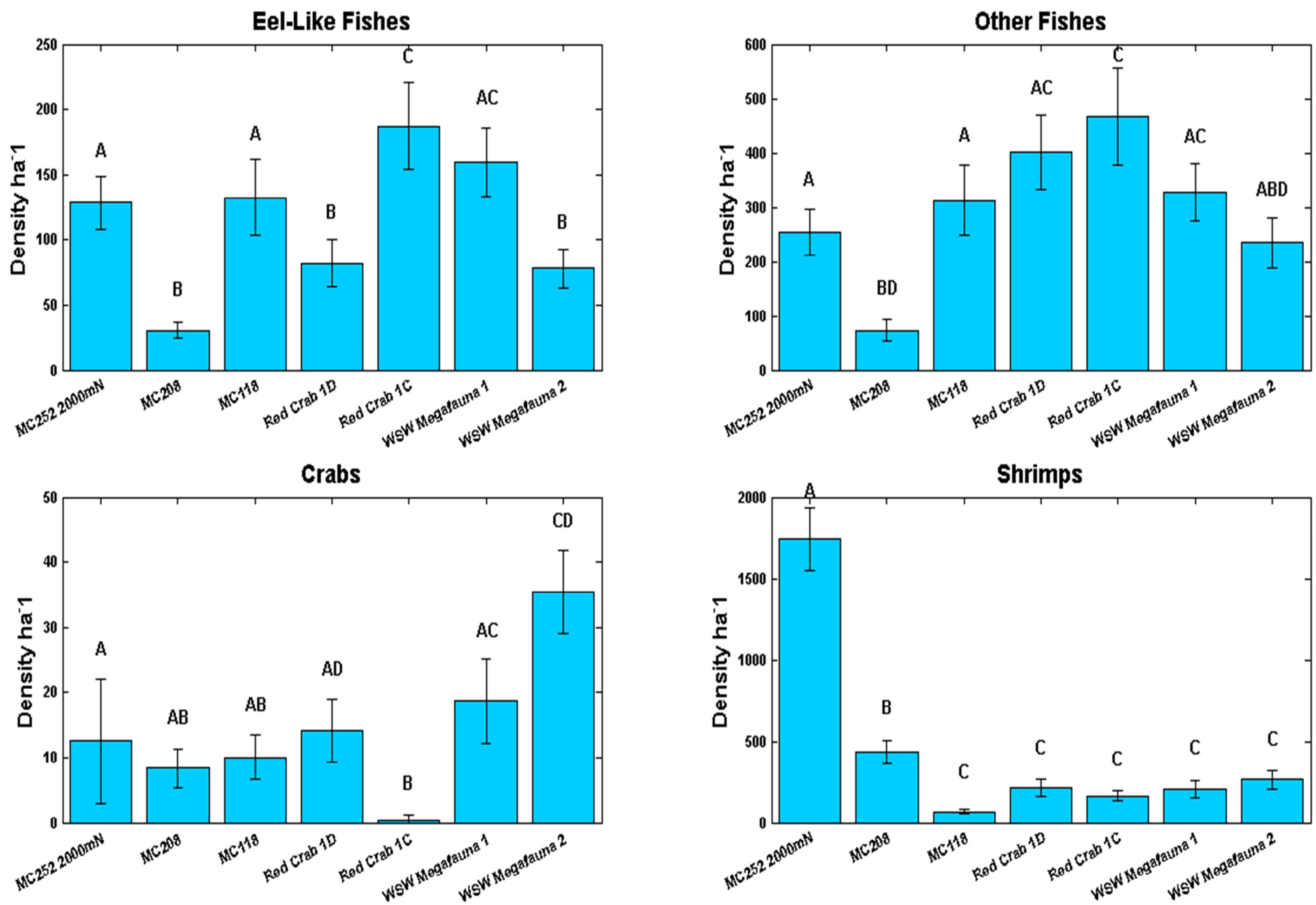


Fig. 5.5. Densities of mobile organisms, by broad taxonomic group, at each study site. Bars with the same letters above them are not statistically significantly different from one another; and bars that do not share a letter are significantly different with 95% CI. Sites are shown in order of increasing approximate distances from the MC252 well (MC252 2000 m N = 2.0 km, MC208 = 3.0 km, MC118 = 17.6 km, Red Crab 1D = 22.2 km, Red Crab 1C = 28.8 km, WSW Megafauna 1 = 29.1 km, and WSW Megafauna 2 = 38.7 km).

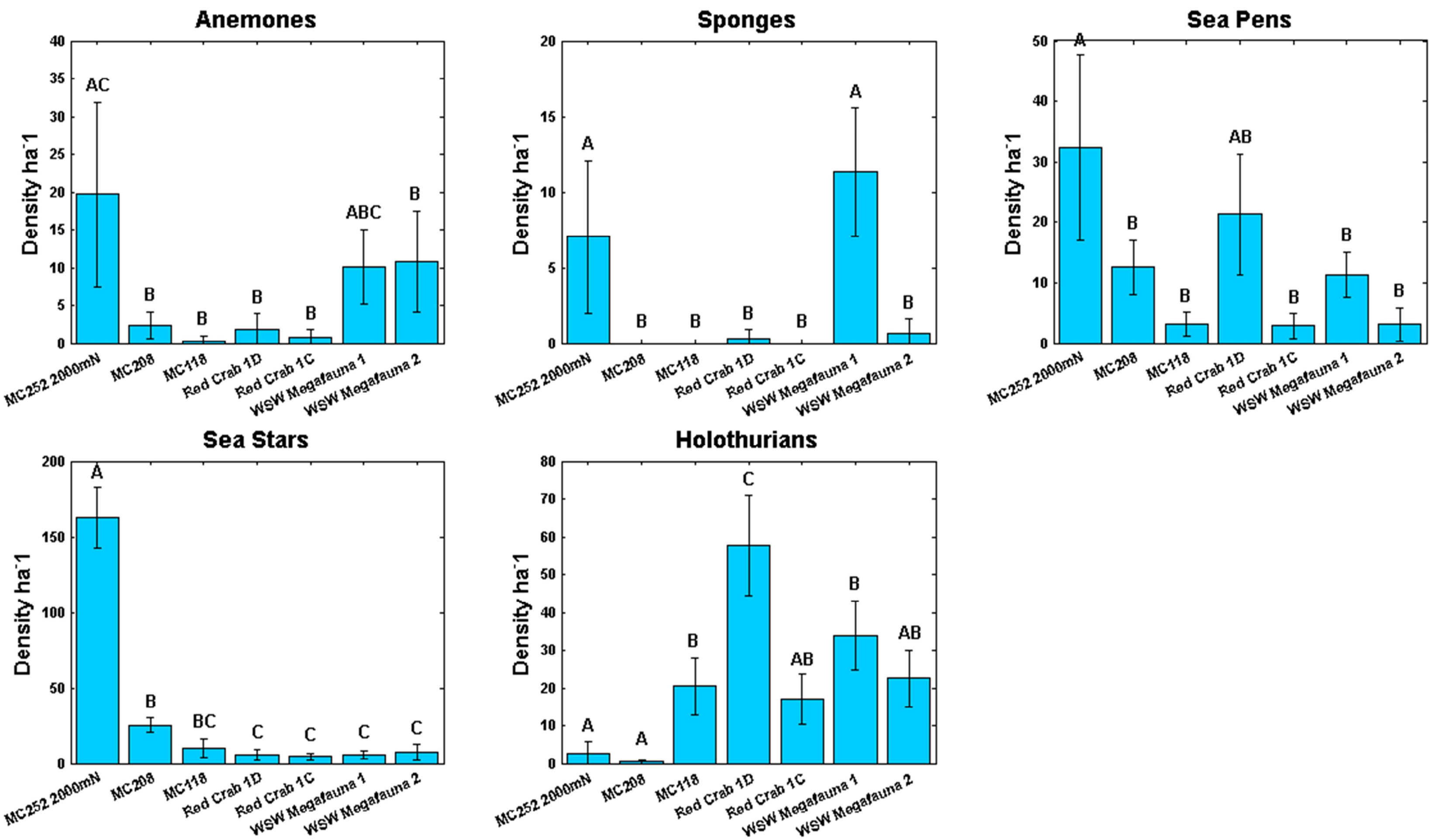


Fig. 5.6. Densities of sessile or low mobility organisms, by broad taxonomic group, at each study site. Bars with the same letters above them are not statistically significantly different from one another; and bars that do not share a letter are significantly different with 95% CI. Sites are shown in order of increasing approximate distances from the MC252 well (MC252 2000 m N = 2.0 km, MC208 = 3.0 km, MC118 = 17.6 km, Red Crab 1D = 22.2 km, Red Crab 1C = 28.8 km, WSW Megafauna 1 = 29.1 km, and WSW Megafauna 2 = 38.7 km).

Table 5.5. Percentage community composition of broad taxonomic groups.

Taxonomic Group	Community Compositions (%)						
	MC118	MC208	MC252 2000m N	Red Crab 1C	Red Crab 1D	WSW MF 1	WSW MF 2
Anemones	0.1	0.4	0.8	0.1	0.2	1.2	1.5
Crabs	1.7	1.3	0.5	0.0	1.7	2.3	4.8
Eel-Like Fishes	22.4	4.9	5.0	19.2	9.6	19.3	10.7
Other Fishes	52.7	11.7	9.9	48.0	47.1	39.5	32.1
Holothurians	3.4	0.1	0.1	1.8	6.8	4.1	3.1
Sea Pens	1.7	4.0	6.3	0.5	0.7	0.7	1.0
Sea Stars	0.5	2.0	1.3	0.3	2.5	1.4	0.4
Shrimps	11.7	69.8	67.5	17.0	25.0	24.9	36.1
Sponges	0.0	0.0	0.3	0.0	0.0	1.4	0.1
Other	5.8	5.8	8.5	13.1	6.3	5.4	10.2

In terms of relative abundances (Table 5.5) of each broad taxonomic group in relation to total abundances at each site, a few trends emerge. MC208 and MC252 2000 m N have very high abundances of shrimps (almost twice as many as the site with the next highest abundances), but the lowest abundances of fishes (~10% vs. ~30-50% at other sites) and eel-like fishes (~5% vs. 10-20% at other sites). Sea pens made up a larger part of the communities at these two sites by at least two times the amount of other sites. Almost no holothurians (almost 20 times fewer than at the next lowest site) were found at MC208 and MC252 2000 m N. The organism communities at the other five sites (MC118, Red Crab 1C, Red Crab 1D, WSW Megafauna 1, and WSW Megafauna 2) were dominated mostly by other fishes and shrimps and, to a slightly lesser degree, eel-like fishes. Anemones, sea pens, sea stars, and sponges were generally minor components of the communities.

Larger-scale trends are more apparent for community compositions as a whole. A trend emerged from the cluster (Fig. 5.7) and MDS (Fig. 5.8) analyses in that the main groupings were associated with site depth and, to a lesser degree, location – the shallower sites (MC118 and Red Crab 1C), mid-depth sites (Red Crab 1D, WSW Megafauna 1, and WSW Megafauna 2), and deep sites (MC208 and MC252 2000 m N). The most similarity existed between sites located at medium depths, likely because the depths of these three sites are within only a couple meters of one another versus larger depth differences between the other sites. Mid-depth sites were

further divided into two smaller groups that correspond to similarity in other environmental characteristics of the sites.

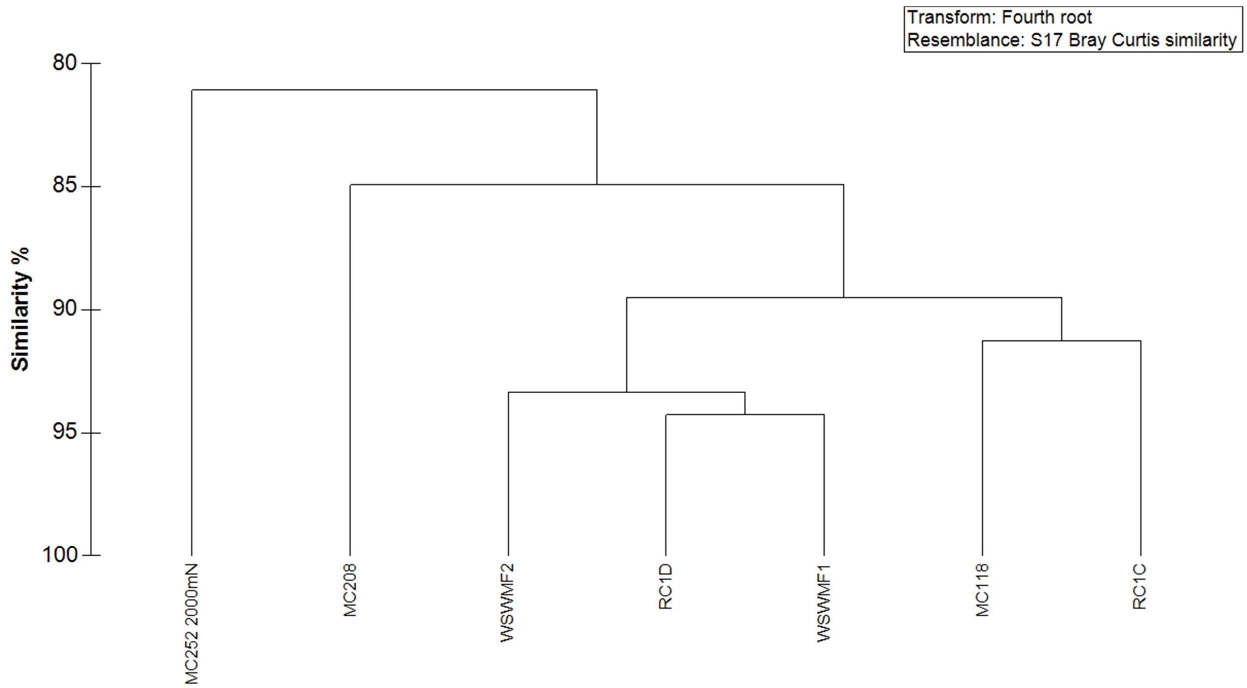


Fig. 5.7. Hierarchical cluster analysis of similarities in overall community compositions between study sites using broad taxonomic groups.

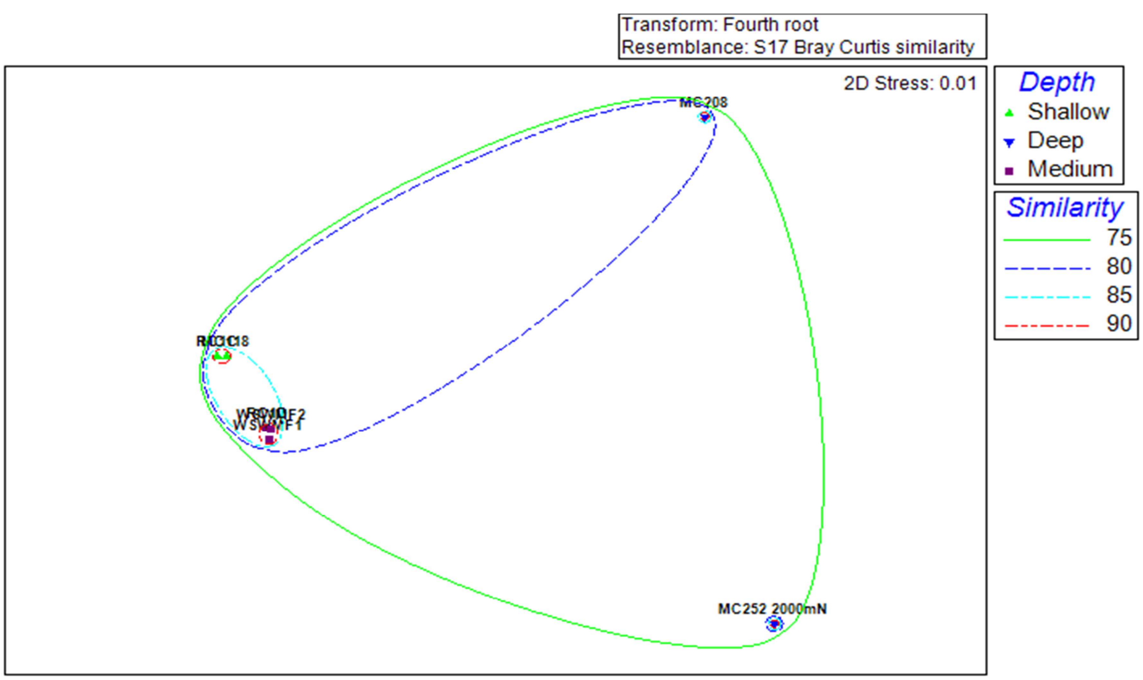


Fig. 5.8. Non-metric MDS plot showing similarities in the community composition of the seven study sites, using site depth as a factor.

5.3.4. Species Diversity

Diversity indices were calculated for each of the sites using the lowest reasonable taxonomic resolution possible (Table 5.6, Fig. 5.9). Due to issues with visibility and the resulting inability to achieve ideal taxonomic resolution, the values of the calculated diversity indices represent approximated measures that allow for comparison of general trends in diversity and evenness. MC208 and MC252 2000 m N generally exhibited lower diversity and evenness than other sites, which is largely due to the dominance of certain animal groups. As with community composition, there appears to be a mild depth-related trend in evenness, but not necessarily overall richness and diversity. The deepest sites (MC208 and MC252 2000 m N) exhibited lowest evenness; the mid-depth sites (Red Crab 1D, WSW Megafauna 1, WSW Megafauna 2) have greatest evenness; and the shallowest sites (MC118 and Red Crab 1C) fall near but lower than the mid-depth sites.

Table 5.6. Diversity indices including species richness (S), Margalef's index of community diversity (d), Shannon-Wiener index (H'), Pielou's evenness index (J'), and Simpson's index (λ).

Index	Study Site						
	MC118	**MC208**	**MC252 2000m N**	**Red Crab 1C**	**Red Crab 1D**	**WSW MF 1**	**WSW MF 2**
S	22	24	21	27	22	25	26
d	2.811	2.873	2.515	3.310	2.729	3.054	3.231
H'	2.046	1.600	1.560	2.006	2.070	2.172	2.191
J'	0.662	0.503	0.512	0.609	0.670	0.675	0.672
λ	0.183	0.361	0.369	0.187	0.186	0.164	0.158

5.3.5. Organism Associations with Environmental Variables

A few trends emerge from the pooled data with regards to organism associations with seafloor features (refer to Table 5.7). Most taxa were associated with average (small = ~37-60% and medium = ~19-41%) size classes of depressions and mounds features. A higher percentage of anemones were found associated with large depressions (7.6%) and larger percentages of sea stars and shrimps were associated with a smoother seafloor with no pits or depressions (42.1% and 23.5%, respectively) than for other animal groups. Anemones and, even more so, shrimps also were found associated with larger mound features (2.9% and 3.5%, respectively) in higher

percentages than other groups. These three groups were found in higher abundances at MC208 and MC252 2000 m N, which had more of those seafloor features.

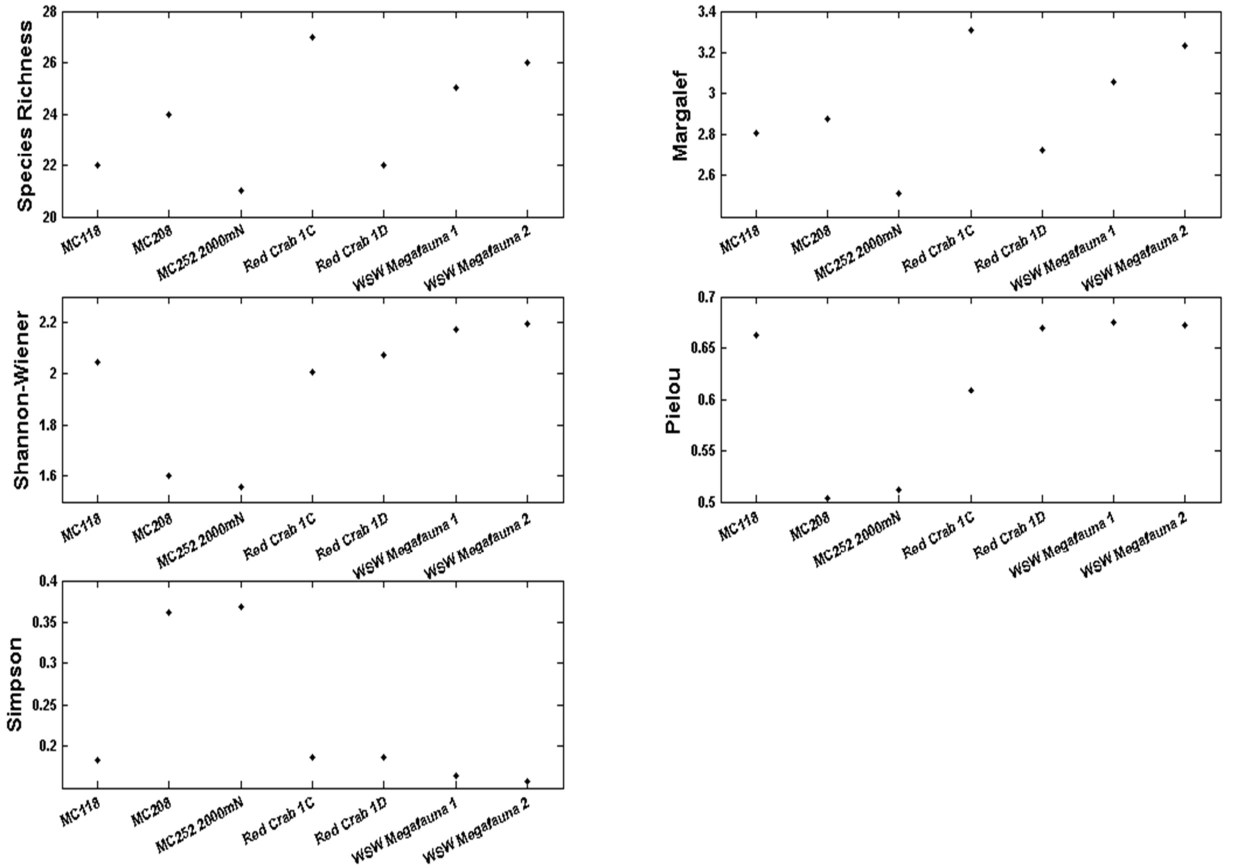


Fig. 5.9. Diversity indices of dominant taxa observed at seven sites located at varying distances and directions from the MC252 Macondo well.

The most notable observations are, perhaps, the relative occurrences of different animal groups in association with the presence of seafloor disturbance (Table 5.7). Shrimps, sea stars and anemones had higher associations (9.0%, 6.7% and 5.7%, respectively) with areas of noted seafloor disturbance than any other taxonomic group. Shrimps and sea stars were found in significantly higher abundances at the two most disturbed sites, MC208 and MC252 2000 m N. With the exception of sea stars, most sessile or low mobility groups exhibited fewer associations with areas of disturbance; and correspondingly, were found in lower abundances at the more highly disturbed study sites.

Analysis of taxonomic group occurrences in relation to environmental factors (Fig. 5.10) revealed a division into two primary groups – 1) highly mobile animals, including shrimp, eel-like fishes, and other fishes, and 2) sessile or low-mobility animals, including sponges, anemones, holothurians, sea stars, crabs (representing the one mobile animal exception), and sea pens. If site variables are excluded, this latter group can be further subdivided into sessile animals (anemones and sponges) and low-mobility (sea stars, sea pens, crabs, and holothurians). Looking strictly at location of the taxonomic groups on the plots (Fig. 5.10) with respect to the axes (and excluding crabs), there are the sessile groups located in the bottom left, low mobility in the middle, and highly mobile towards the top right.

5.4. Discussion

5.4.1. Patchiness

General trends in benthic megafaunal community compositions observed in this study were similar to those of other studies in the northern GoM. As was observed previously by Rowe and Menzel (1971), Escobar-Briones and Soto (1997), Powell et al. (2003), and Valentine and Benfield (2013), there were marked low overall abundances and diversity of most taxon across all seven sites in this study. Macrourid fish represented one of the most abundant taxa, particularly at the shallower and mid-depth sites. Further, shrimps and, to a lesser extent, crabs were observed as the dominant mobile invertebrate taxa, which was similar to observations made by Escobar-Briones and Soto (1997) and Valentine and Benfield (2013).

The seven sites in this study varied greatly with respect to megafaunal abundances and diversity. Abundances were often very different amongst sites, even with sites that were in close proximity to each other and characterized by similar environmental factors. In many cases, these differences were statistically significant. This suggests that there is a general lack of homogeneity in benthic megafaunal communities across different areas of the northern GoM. Much of this lack of consistency for the more specific taxonomic groups is attributed to difficulty in species identifications resulting from highly variable visibility amongst and within the study sites. This does not account for the often large differences in abundances for the broad taxonomic groups and, in conjunction with more specific taxa, suggests that there is an overall patchiness of megabenthos.

Table 5.7. Percentage of organisms in each broad taxonomic group associated with different size classes of pits or depressions and mounds, and presence or absence of physical disturbance to the seafloor.

Taxonomic Group	Pits or Depressions (%)				Mounds (%)				Seafloor Disturbance (%)	
	Not Present	Small	Medium	Large	Not Present	Small	Medium	Large	Present	Not Present
Anemones	17.1	51.4	23.8	7.6	7.6	63.8	25.7	2.9	5.7	94.3
Crabs	6.4	60.3	30.3	3.0	11.4	65.7	22.6	0.3	3.0	97.0
Eel-Like Fishes	10.4	44.1	40.5	5.0	13.1	47.1	38.2	1.6	2.6	97.4
Other Fishes	13.3	49.6	34.3	2.9	16.6	49.7	32.8	0.9	2.4	97.6
Holothurians	8.6	53.6	35.1	2.7	8.8	55.9	34.6	0.7	1.1	98.9
Sea Pens	16.3	46.2	35.7	1.8	19.9	52.5	25.8	1.8	1.8	98.2
Sea Stars	42.1	37.2	19.2	1.5	34.2	48.3	15.8	1.7	6.7	93.3
Shrimp	23.5	41.8	30.1	4.7	20.5	50.4	25.7	3.5	9.0	91.0
Sponges	12.5	45.8	37.5	4.2	8.3	29.2	60.4	2.1	2.1	97.9

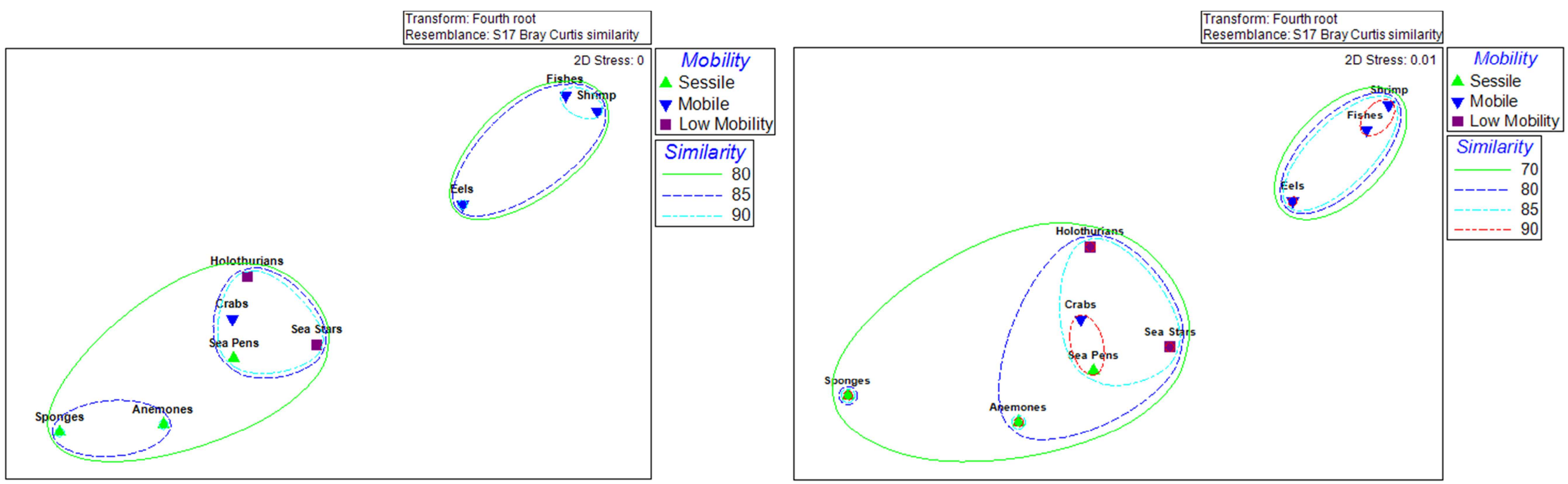


Fig. 5.10. Environmental factors without site variable (left) and with site variable (right).

Patchiness of the deep-sea benthic communities and seafloor environments in the northern GoM, as observed in this study, can prove challenging for evaluating impacts and subsequent recovery of these communities after anthropogenic disturbance events such as Deepwater Horizon oil spill of 2010. There is an additional challenge in evaluating the megafaunal communities as part of an environmental monitoring program because of the notable dominance of mobile animals. Most sessile groups like anemones and sponges were relatively rare at all sites. In order to more fully understand these benthic communities, potential underlying factors influencing community structure need to therefore be investigated. The results from this study suggest there were several underlying environmental factors contributing to differences in observations at the seven study sites.

5.4.2. Environmental Factors Affecting Community Characteristics

The limited number of previous studies or well-established baseline conditions for deep-sea benthic megafaunal communities in the vicinity of the Deepwater Horizon Macondo well or other sites investigated in this study makes it difficult to accurately assess how these communities may have been impacted by the 2010 oil spill. Despite concerns over potential impacts of the predicted subsea plume associated with the oil spill (Camilli et al., 2010), no clear evidence or trends were observed in this study that suggested impacts to these deep-sea benthic megafaunal communities. There was no evidence of oil observed in the imagery collected at any of the seven study sites, though it should be noted that our imaging systems were not designed to detect any hydrocarbons other than visible oil. In addition to this, while some evidence of mortality (in the form of carcasses of megafauna and plankton) was observed at the sites previously surveyed close to the Macondo wellhead in the study by Valentine and Benfield (2013), there was no evidence of mortality observed in this study. The present study consists of surveys completed one year after the spill, and mortality would possibly be expected in the event of sustained presence of oil, continued exposure or lasting impacts of the spill. The lack of observed mortality may reflect the absence of acutely toxic conditions affecting the benthic megafaunal communities. Given the high degree of scavenging from megafaunal organisms such as crabs, isopods, and fishes, low levels of mortality might not be detectable. There were also no directional or proximal trends observed in organism abundances or diversity

at the study sites in relation to direction or distance from the MC252 wellhead location. Overall, the results of this study instead suggested that it is a combination of natural environmental variables in conjunction with physical disturbance to the seafloor that are influencing the benthic megafaunal communities observed.

Analysis of seafloor features revealed two primary factors influencing seafloor composition: site location and depth. MC208 and MC252 2000 m N represented the group of the two deepest sites, closest to the MC252 well; and WSW Megafauna 1 and WSW Megafauna 2 comprised the mid-depth group to the west. The three remaining sites to the north and north-west were grouped initially by location and then by depth. Distance from land, proximity to the Mississippi River delta, and other near-seafloor oceanographic processes throughout the northern GoM are the likely contributors to these observed differences in naturally-occurring seafloor features since these factors are directly linked to both location and depth. While there was some evidence of anthropogenically-caused physical disturbance to the seafloor at all of the sites surveyed, MC208 and MC252 2000 m N by far had the most highly disturbed seafloors. This further distinguished these two sites from the others. Both natural environmental factors and presence of anthropogenic physical disturbances were found to be responsible for influencing seafloor characteristics at the sites considered in this study.

Community composition showed similar trends in that there was an observed grouping of sites primarily by depth, along with a secondary influence of location. As with seafloor compositions, depth and location appeared to be the primary influences on the benthic megafaunal communities. This then implies that there is consequently a link between seafloor characteristics and the animal communities observed. Additional analysis of relationships between abundances of the broad taxonomic groups and environmental variables further emphasized this link.

Taxonomic group associations with environmental factors were found to be based largely on mobility of the animals. The greater observed association of certain taxonomic groups with particular seafloor features explains why those animal groups were found in higher abundances at sites with those seafloor features. For example, anemones, sea stars and shrimps were

found in higher abundances associated with seafloor features more prominent at MC208 and MC252 2000 m N, and were consequently found in higher abundances at those two sites. This was particularly the case with respect to seafloor disturbances. Anemones, sea stars and especially shrimps were found associated with disturbance in much higher numbers than any of the other taxonomic groups. These three groups are largely opportunistic feeders that are common in the deep-sea; and may therefore be present more often associated with disturbance than other groups because of the additional food supply that physical disturbance to the seafloor potentially creates.

In comparison, the majority of sessile or low mobility groups were found to have fewer associations with areas of seafloor disturbance, which in turn relates to the lower abundances of these groups observed at the more highly disturbed study sites. Sessile or low-mobility animals are limited in their ability to move out of an area when faced with an environmental stressor such as seafloor disturbance, which can possibly kill or displace the animal. The ability for mobile animals to move away from areas of disturbance to those of more favorable conditions may explain why fishes (both the eel-like and other fishes groups) were found to compose much smaller proportions of the overall megafaunal communities at MC252 2000 m N and MC208.

5.4.3. MC208 and MC252 2000 m N

Beyond the differences in dominant taxonomic groups mentioned previously, MC252 2000 m N and MC208 (located only about 1000 m further north) had several other characteristics that set them apart from the other sites. It was difficult to determine, however, if any of the differences at these sites can be attributed to the 2010 oil spill since there are other factors that differentiate these two sites. These sites were deeper than the other study sites, had much more physical disturbance to the seafloor, and were in closer proximity to the Macondo well. Based on the trends observed in this study, it is these factors that were likely the contributing factors distinguishing benthic communities at these sites from the others.

Both MC208 and MC252 2000 m N had lower diversity and evenness of the communities compared to other sites; yet, they were represented by very different overall animal

abundances. Although total animal abundances at MC208 were comparable to other sites with lower abundances, MC252 2000 m N had much higher abundances than any of the other sites. This could possibly be attributed to general patchiness within the benthic environment or differences in the physical seafloor disturbance regimes between the sites, since MC208 had slightly higher incidences of disturbance than MC252 2000 m N and had lower overall animal abundances.

More likely, this difference is due to effects of seasonal differences, which is possibly a consequence of MC252 2000 m N being surveyed during a different month than the other sites. Rather than being surveyed in August, MC252 2000 m N was surveyed earlier in the year at the start of June. Based on this study, there were no observable factors that were different enough between the two sites to readily explain the significant differences in abundances. While it is still widely debated to what extent seasonality is observed in deep-sea ecosystems, studies are beginning to support the idea that some semblance of seasonality does indeed exist. Factors such as food supply can be greatly limiting for deep-sea communities. Consequently, seasonal variations in surface productivity, trophic web dynamics, and many other environmental variables can impact benthic ecosystems (Kojima and Ohta, 1990; Cartes, 1998; Gooday, 2002; Sumida et al., 2008; Corliss et al., 2009; Glover et al., 2010; Rowe, 2013). In fact, seasonal variability in benthic community assemblages has recently been observed through the use of long-term time-series studies (Glover et al., 2010; Juniper et al., 2013). In the particular case of MC252 2000 m N, the larger animal abundances observed in June may reflect an increase in food supply to the deep-sea, resulting from spring run-off from the Mississippi River into the northern GoM and the corresponding increase in primary productivity associated with it.

5.5. Conclusion

Patchiness, dominance of mobile animals, and a general lack of information on deep-sea benthic megafaunal communities in the vicinity of the Deepwater Horizon Macondo well make it difficult to adequately assess how these communities may have been impacted by the 2010 oil spill. Without exploring further both the natural and anthropogenic environmental factors potentially influencing these communities, it is difficult to make accurate inferences on whether observed differences in the megabenthos are the result of impacts from the oil spill or simply

attributable to other variations in the marine benthic environment. It also limits our ability to adequately evaluate recovery of benthic communities in the wake of the oil spill, if they were in fact affected in the first place.

This study, however, lends some valuable insights into some of the environmental factors influencing these communities – depth, site location and seafloor characteristics, and presence of physical disturbances to the seafloor. These trends were established solely through evaluating environmental components that were visible in ROV imagery, and emphasize the potential that imaging technologies have for deep-sea benthic environmental monitoring. While there were no observations or obvious trends in the data that indicate the megafaunal communities at the seven sites in this study were impacted by the 2010 oil spill, further data is required. In order to more fully explore trends and search for possible links to the oil spill, data that cannot be readily seen in imagery must also be evaluated. This includes additional oceanographic information ranging from physical and chemical oceanographic components (i.e. currents, oxygen concentration, trace chemicals) to additional information on the seafloor environment (i.e. sediment composition, hydrocarbon content). It is only through a comprehensive evaluation of both imagery and imagery-independent data that possible impacts of the spill acting as an influence determining benthic megafaunal communities can be completely ruled out.

In order to truly evaluate changes in the benthic megafaunal communities over time, studies such as the one presented here need to be repeated as part of a regular, consistent monitoring program. Ideally, this type of environmental monitoring program would include surveys conducted at least once a year (or preferably several times a year to properly investigate seasonality) for at least the first 5-7 years after the environmental incident occurred, with follow-up surveys every few years after that. The limited availability of certain deep-sea technologies such as ROVs has made partnerships between industry and the science community increasingly important, particularly with respect to monitoring in relation to offshore exploration and drilling activities. Partnerships, such as the SERPENT (Scientific and Environmental ROV Partnership using Existing Industrial Technology) program, help increase the availability of deep-sea technologies for researchers to use, while allowing companies to

fulfill environmental monitoring and social responsibility requirements more effectively. The success of this program allows for continued expansion of knowledge on the benthic megafaunal communities of the northern GoM that will be critical for future environmental monitoring of these communities.

CHAPTER 6. CONCLUSIONS

Expanding knowledge on deep-sea megabenthos will better facilitate understanding of potential effects and recovery of GoM marine communities in response to anthropogenic influences such as the Deepwater Horizon oil spill. Collection of baseline data on species abundances and diversity along with community composition and dynamics are critical components for use in general monitoring programs, as well as those investigating anthropogenically-induced environmental impacts and subsequent recovery. The GoM, however, often has limited data on baseline conditions associated with benthic megafauna, as has become evident in the wake of the 2010 oil spill. There is also a need to go beyond collection of basic data on megafaunal abundances and diversity to explore possible environmental influences contributing to observed benthic megafaunal community compositions. Without this information, it is impossible to conclude whether observed differences in these communities are the result of impacts from the oil spill or simply attributable to other variations in the marine benthic environment.

Overall, based on the comparison of industrial AUV and ROV imagery in this dissertation, ROV imagery is more highly recommended for studies of benthic megafauna in soft-sediment environments of the northern Gulf of Mexico. Soft-sediment environments are generally fairly homogeneous in nature and it is therefore important to be able to detect small differences such as variations in sediment type or mix in these environments. The ability to visually observe characteristics of the benthic environment in color and with sufficient clarity and dimensionality is important because it improves observations of and the ability to accurately identify megafauna. These are all features that were superior in color video imagery collected by the industrial ROVs compared to the more monotone and less dimensional AUV grayscale photographic imagery in this study.

In general, studies have indicated that ROVs represent an effective, relatively non-invasive means of studying deep-sea benthic megafaunal communities. This is supported by Stoner et al. (2008), in which a recommendation was made to continue ROV surveys since there is no sufficient substitute for the direct observations made using ROVs, albeit with due care to

minimize survey biases where possible. Factors such as distance from the bottom, depth, current speed, relative surveying direction, behaviors of deep-sea fishes, and organism abundances and distributions have all been shown to impact organism counts and, consequently, population density estimates (Trenkel and Lorance, 2003; Trenkel et al., 2004a; Trenkel et al., 2004b). As a result, further strides need to be made to continually improve upon ROV imagery to produce better-quality imagery with greater clarity and lessen the effects of decreased visibility resulting from suspended materials. In the case of the studies in the northern GoM described in this dissertation, future improvements in image quality may possibly be obtained through working with new camera system technologies and data storage capabilities to reduce loss of clarity during data acquisition, transfer, and storage. Further, limited and variable visibility, as was often observed in the soft-sediment environments here, can prove problematic. While there may be incentive to maintain a fixed set of lighting during ROV surveying to maintain consistency, different lighting schemes need to be evaluated and/or selectively employed depending on water column visibility in order to increase visibility and minimize issues such as overexposure.

Due to the general effectiveness and capacity of ROV video imagery to allow for many different kinds of studies, it may be concluded that the use of ROVs in deep-sea benthic megafaunal studies can provide a wealth of valuable scientific data. Continued development of ROV-related technologies and camera systems will further allow for expansion of ROVs as a means of studying and improving monitoring efforts in the deep-sea. This will be particularly important as the use of industrial-based technologies expands in response to increased offshore exploration and extraction activities.

Recommendations with respect to the best radial survey design to use for deep-sea benthic megafaunal environmental monitoring studies using industrial ROVs in the northern GoM come two-fold. Overall, the 250 meter-long, 15° design used in this study represents an excellent option for monitoring studies, and especially for those in the vicinity of oil or gas platforms. This is due to its practicality and the accuracy and consistency of the design with respect to detecting animal populations from both random and clustered spatial distributions. In addition to this, longer transect lengths and finer transect separation help increase the potential for

surveys to detect rare animals that may exhibit lower abundances in the benthic environment. Generally, the most effective radial survey design should be designed to favor longer transects and finer spacing between transects. This, of course needs to be considered in conjunction with availability of time, funding, and technology resources. Where possible, considerations should be made to modify or replace the original 60° survey design employed by BOEM in order to improve environmental monitoring of deep-sea benthic communities, particularly with respect to offshore oil and gas activities.

Incorporation of a formal, uniform database system into data analysis and management procedures for imagery-based studies in the northern Gulf of Mexico can prove a valuable future tool as well. SERPENT is an example of a program that has contributed to the acquisition of ROV imagery in the northern GoM. A database system such as the one created for this study provides an opportunity to compile data from across a variety of sources and types, including those that are part of NRDA studies and from SERPENT. Furthermore, this database has the potential to become a long-term depository of data that would not only increase our understanding of benthic megafaunal communities but also improve our ability to evaluate changes to these communities resulting from human impacts on the deep-sea environment.

Future development of the database system will increase the applicability of the database to a larger variety of studies beyond what was used in this particular study. In addition, the database form will be reorganized in a way that will make data entry even simpler for inexperienced users such as students and industrial ROV pilots, who may not be as familiar features and animals observed in the imagery. This new design will combine elements from the current database with elements of the Bureau of Ocean Energy Management (BOEM) biological survey requirements for offshore activities. The premise is to create an uncomplicated, standardized system for image data entry that can be used in a variety of applications, including in SERPENT and BOEM-mandated environmental surveys. This system then has the potential to be further modified and applied on an even wider scale to similar studies in other regions of the U.S. and world.

Patchiness, dominance of mobile animals, and a general lack of information on deep-sea benthic megafaunal communities in the vicinity of the Deepwater Horizon Macondo well make it difficult to adequately assess how these communities may have been impacted by the 2010 oil spill. Without exploring further both the natural and anthropogenic environmental factors potentially influencing these communities, it is difficult to make accurate inferences on whether observed differences in the megabenthos are the result of impacts from the oil spill or simply attributable to other variations in the marine benthic environment. Consequently, it also limits our ability to adequately evaluate recovery of benthic communities in the wake of the oil spill, if they were in fact affected in the first place.

The study presented here lends valuable insights into some of the environmental factors influencing these communities – depth, site location and seafloor characteristics, and the presence of physical disturbances to the seafloor. These trends were established solely through evaluating environmental components that were visible in ROV imagery, and emphasize the potential that imaging technologies have for deep-sea benthic environmental monitoring. While there were no observations or obvious trends in the data that indicate the megafaunal communities at the seven sites in this study were impacted by the 2010 oil spill, further data is required. In order to more fully explore trends and search for possible links to the oil spill, data that cannot be readily seen in imagery must also be evaluated. This includes additional oceanographic information ranging from physical and chemical oceanographic parameters (e.g. currents, oxygen concentration, trace chemicals) to additional information on the seafloor environment (e.g. sediment composition, hydrocarbon content). It is only through a comprehensive evaluation of both imagery and imagery-independent data that possible impacts of the spill acting as an influence determining benthic megafaunal communities can be completely ruled out.

In order to truly evaluate changes in the benthic megafaunal communities over time, studies such as the one presented here need to be repeated as part of a regular, consistent monitoring program. Temporal comparisons are key in evaluating not only possible impacts but also the subsequent recovery of affected benthic communities. An ideal environmental monitoring program would include surveys conducted at least once a year (or preferably several times a

year to properly investigate seasonality) for at least the first 5-7 years after the environmental incident occurred, with follow-up surveys every few years after that. The limited availability of certain deep-sea technologies such as ROVs has made partnerships between industry and the science community increasingly important, particularly with respect to monitoring in relation to offshore exploration and drilling activities. Partnerships such SERPENT help increase the availability of deep-sea technologies for researchers to use, while allowing companies to fulfill environmental monitoring and social responsibility requirements more effectively. Continued success of this program allows for continued expansion of knowledge on the benthic megafauna of the northern GoM that will be critical for future environmental monitoring of these communities.

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APPENDIX A. MC118 DENSITY CORRECTION FACTOR FEASIBILITY ANALYSIS

A.1. Purpose

To determine an appropriate method for correcting organism densities at site MC118, along with all Sweetwater cruise survey sites, due to differences in detection in the ROV video data resulting from variations in visibility of the seafloor and corresponding organisms. Two sets of ROV surveys were conducted within a day of each other at MC118, and this correction will be used to correct the densities obtained from each survey in order to facilitate ease of comparison between the two surveys. This will contribute to a more accurate evaluation of the effectiveness of the ROV benthic survey methodology in estimating benthic organism densities.

A.2. Problem

Because ROV video data relies on being able to see what is in the video, visibility can potentially impact organism density values calculated from the ROV data. In the deep-sea, visibility of organisms in ROV video imagery is effected by a number of factors including lack of light, type of camera used for data collection, altitude of the ROV above the seafloor, marine snow, suspended sediments, and type of organisms. The first two factors can be maintained relatively constant through the use of a specific set of ROV lighting and same type of camera employed throughout all surveys. ROV altitude should be maintained as constant as possible, however this may vary due to differences in pilot skill level or changes in elevation above the seafloor resulting from avoidance of seafloor features or suspended sediment clouds.

Factors that are harder to control and can significantly impact visibility include the presence of marine snow and/or suspended sediments and the type of organisms being observed. Marine snow and suspended sediments have the potential to change with time of day, area on the seafloor (along the transect), currents, or from (potentially anthropogenic) disturbance of the seafloor. Also, ability to see different types of organisms changes with respect to other visibility factors because some organisms are smaller, are colored to blend into the seafloor, may be partially or fully buried beneath sediments, or easily obscured by sediments and snow in the water column. It is most often through a combination of factors, especially altitude, marine

snow and suspended sediments, and type of organism, that visibility in the ROV video can vary greatly over the course of a survey transect, within a survey area, and between survey sites.

A.3. Correction Factor Options:

A.3.1. Laser Scalers

This approach involves evaluating how laser scaler color intensity varies with depth and determining the depth at which the laser scalers are no longer visible, then comparing average intensities between the two sets of surveys. One advantage of this approach is that it is using a fixed component of known intensity that is always on the ROV. This approach is also largely site independent in that the intensities could be measured at all the sites. Once the relationship between laser scaler intensity and the differences in organism densities between the two MC118 surveys is determined, this could then potentially be extrapolated to correct across the other sites.

However, the laser scaler approach is very complicated. Intensity of the laser scalers is dependent on a number of factors including both the properties of the reflecting surface (i.e. seafloor) as well as turbidity of the medium through which the light is being transmitted (i.e. deep-sea water). Factors potentially affecting visibility and corresponding intensity of the laser include depth and ROV altitude, ROV lighting, placement of the laser scalers on the ROV, angle of incidence onto the seafloor, seafloor and sediment characteristics and disturbances (e.g. depressions, mounds, bioturbation), and amount of suspended sediments and marine snow in the water column and at the sediment-water interface. Overall, water clarity, altitude and bottom reflectivity are amongst the factors that can significantly impact laser visibility and intensity.

Based on observations during ROV video analysis, many of these factors are highly variable and inconsistent even within a single transect. In many cases, altitude changes (sometimes up to a couple meters) within a transect and there is thus a corresponding loss of visibility resulting from this. This is compounded by highly variable marine snow and suspended sediments in the water column, with areas along a single transect and/or within a survey site where the seafloor is sometimes partially or completely obscured. There are instances where reflection of the ROV

lighting off the seafloor or suspended sediments/snow has resulted in severe overexposure in the video, even if the ROV is close enough to the seafloor where the lasers would normally be easily seen with good intensity. Similarly, seafloor characteristics such as the presence of features like mounds, depressions or intense bioturbation can impact the angle of incidence of the lasers on the seafloor as well as potentially create variations in altitude (particularly for larger mounds and depressions) for the specific area of the seafloor where the lasers fall.

The location of the lasers within the video field of view varies with ROV altitude due to the fact that the laser scalers were placed on a different part of the ROV than the camera tilt mechanism. Many of the factors potentially impacting laser scaler visibility and intensity cannot adequately be calculated based on the particular deep-sea benthic characteristics and ROV configuration in this particular set of Gulf of Mexico surveys. All of these issues lend to the non-feasibility of using the laser scalers as a means for correcting densities since it is too difficult to evaluate all of these factors in order to determine the relationship between laser scalers intensity and depth.

A.3.2. Non-Highly Mobile (Sessile) Organisms

This approach is a simpler, all-encompassing "averaging" approach to the visibility problem. The idea behind this approach is to determine the difference in organism densities between the two MC118 surveys for non-highly mobile organism groups, as these densities can be assumed to be the same between the surveys since the surveys were completed at the same site and within a short period of time (one day) of one another. Instead of having to calculate many variables to determine the variable that is being used as a correction, this method simplifies the correction by comparing values that are logically assumed to be the same between the two MC118 surveys.

The least conservative approach would be to correct all organism densities based on the difference obtained from one sessile group (e.g. sea stars). A more conservative approach would be to incorporate several such groups (e.g. sea stars, sea pens, holothurians) into the correction, since visibility differences will affect the ability to detect each group a little differently. As with the laser scaler approach, there is potential to possibly apply this correction

across other study sites as well. This could be done by comparing the calculated correction factor against the difference in altitude (or FOV in the case of these surveys) to determine the relationship between the two components.

Even though a moderate level of site independence could be achieved in this manner, correcting across all sites in this manner still poses a problem. As mentioned in the discussion of using laser scalers as a correction, visibility is affected by many other factors than just altitude and these factors vary greatly and cannot easily be quantified. This leads to one significant advantage of using sessile organism densities as a correction factor, at least for the MC118 surveys, through averaging the effects of visibility. Variations in visibility resulting from altitude or presence of marine snow and suspended sediments will affect each organism group a bit differently.

The ability to detect sea stars is greatly impacted loss of visibility by altitude in conjunction with presence of suspended sediments and snow, since these organisms are often smaller in size, similar in color to the sediments, partially or completely buried in sediments, etc. Sea pens, due to their thin profile, light colors and elevation off the seafloor are similarly impacted by altitude, suspended materials, and lighting. The ability to detect holothurians tends not to be as dependent on altitude since they are larger organisms but can be greatly affected by suspended materials and lighting. By averaging the differences in densities of these three organism groups then applying this correction factor across all organism groups, it essentially allows for these visibility effects to be averaged out in a manner that likely gives an accurate estimation of the true impacts of visibility on detecting organisms of different kinds.

A.4. Recommendation

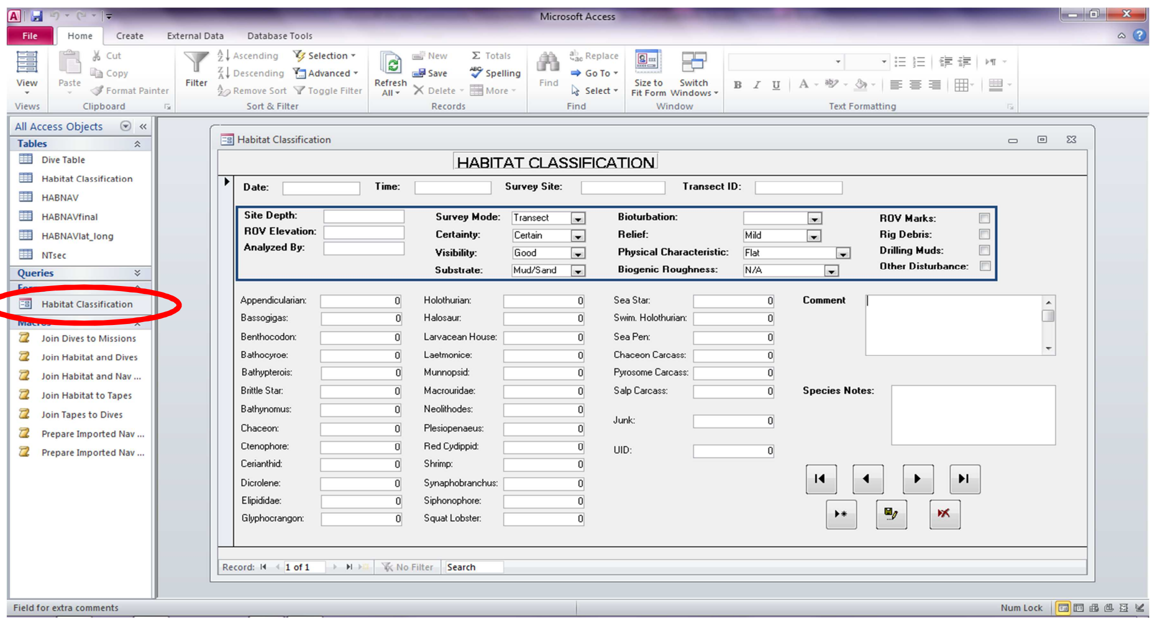
Using laser scaler intensity to create a correction factor allows for a relatively site-independent approach to evaluating changes in visibility. This approach requires measurement of many different factors that cannot adequately be evaluated given the nature of the data from the Sweetwater surveys. A much simpler and less specific approach, using density differences in sessile organisms between two surveys conducted at the same site and within a short time of each other represents a more “averaging” approach to the correction issue. This latter

approach not only requires fewer pieces of hard-to-evaluate information, but also provides a method for correcting that potentially better represents the true impacts of visibility on detection ability since it effectively averages the various factors affecting visibility. A correction approach using differences in densities of sessile organisms is therefore recommended for the data from MC118 and can further be potentially applied to the remainder of the Sweetwater survey sites.

APPENDIX B. CUSTOMIZED MICROSOFT ACCESS DATABASE SYSTEM – INSTRUCTIONS ON HOW TO PERFORM BASIC FUNCTONS

B.1. Entering Data into the Database

1. Open "Habitat Classification" database form from the Access Objects list on the left-hand side of the window. It will open a window that contains the form showing all data entry fields.



Form Components

a) The first row on the form includes Date (dd/mm/yyyy), Time (hh:mm:ss), Survey Site, and Transect ID. As a fail-safe, these fields are set so that they MUST be filled in completely or else the form will not allow you to save the entry or move to the next entry.

b) In the blue box below the first row are a variety of other site characteristics corresponding to that data entry. These include a number of physical and habitat characteristics, as well as basic site information.

c) The bottom portion of the form includes a list of possible organisms observed. This is where you fill in what organism is present at a given time, which will correspond with a still photo taken from the ROV video. Specific taxonomic groups as well as general broader groups are given, and should be filled in with as much taxonomic detail as possible.

d) Two sections are also given for additional Comments and any applicable "Species Notes" (like important or unique species observed, etc.).

e) At the end of the form is a set of 7 buttons that are used as follows:

Button 1 – First Record.

Button 2 – Previous Record.

Button 3 – Next Record.

Button 4 – Last Record.

Button 5 – Add Record.

Button 6 – Save Record.

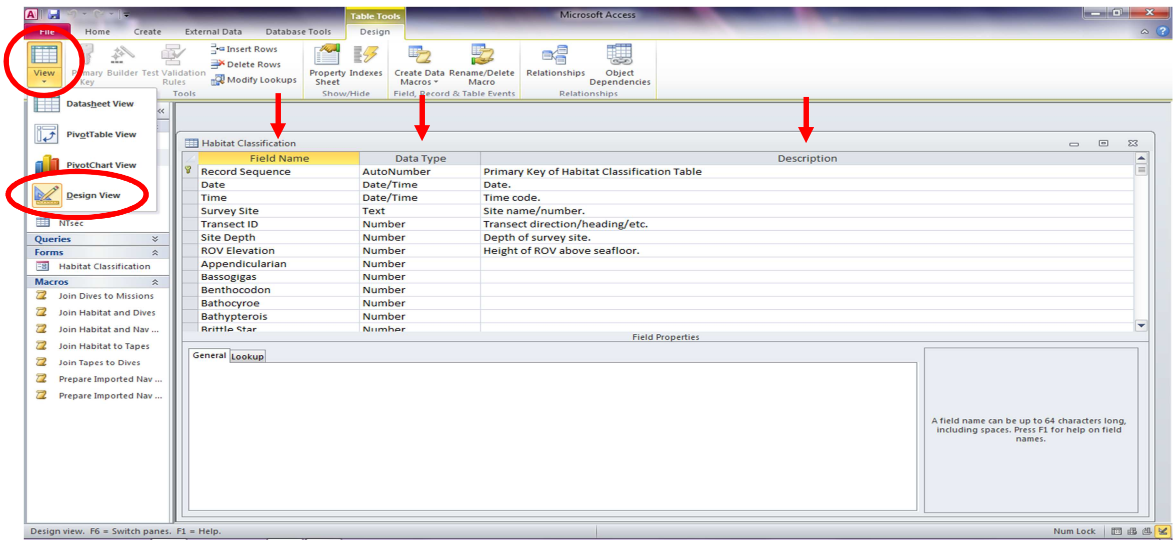
Button 7 – Delete Record.

2. To enter information into the form, simply fill in each of the fields by either typing in the information or picking an option from the drop-down menu. Make sure as many fields are filled in as possible.
3. Once filled in, click on the Add Record and/or Save Record buttons. Add Record will save and add the record to the corresponding Access table, then move you to the next new record. Save Record will only save the record, and is particularly useful when adding or making changes to fields within an existing record.

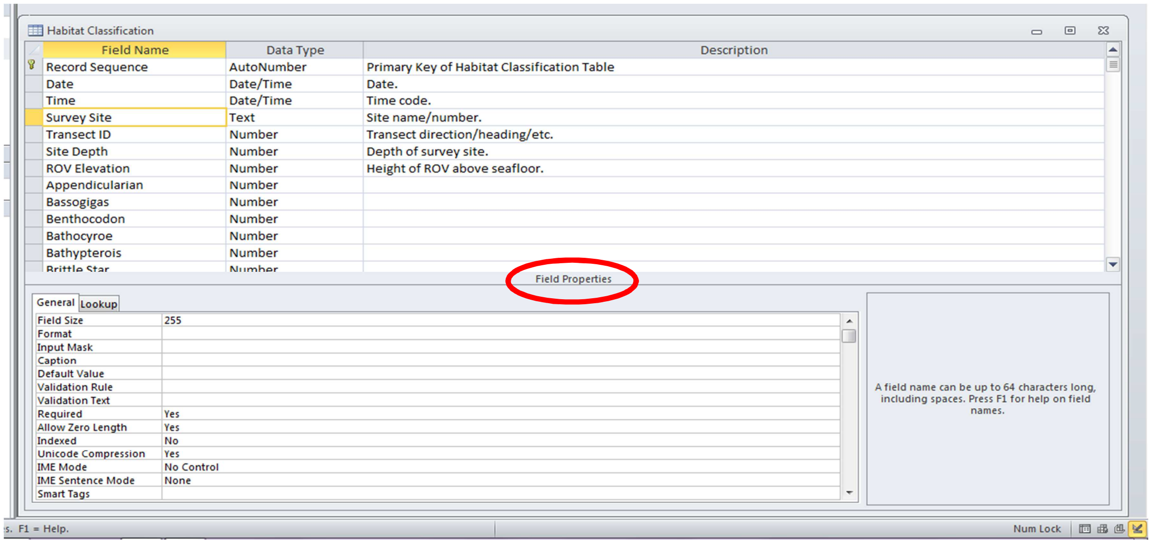
4. Repeat previous steps for each new observation.

B.2. Modifying Table Parameters

1. In the "Home" tab, click on "View" and then select "Design View". This will take you to a view of the table that allows for you to make changes to the table, including adding or removing fields in the table.



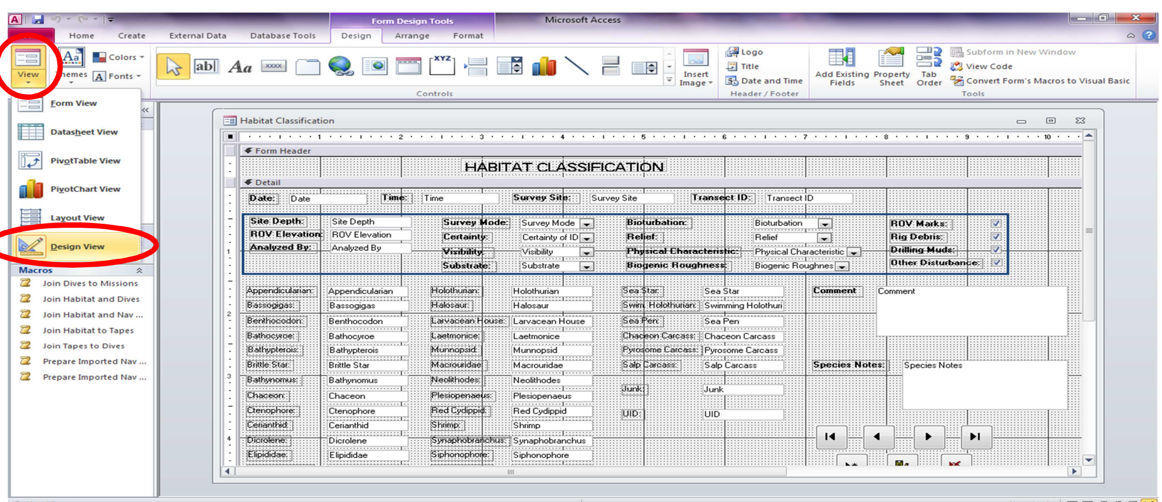
2. To add a field, right click and insert a row where desired or your new field in an empty row at the end of the list. Type your desired field name in the "Field Name" column.
3. In the "Data Type" column, click and select from the drop down menu what kind of data you will be entering in that field.
4. Add notes describing details about the field in the "Description" column.
5. Once a new field has been added, the properties of that field can be further specified in the bottom part of the window in the "Field Properties" area. Properties that can be specified include field size, validation rules, default values, whether the field is mandatory, etc. These properties will vary slightly depending on the data type of that field.



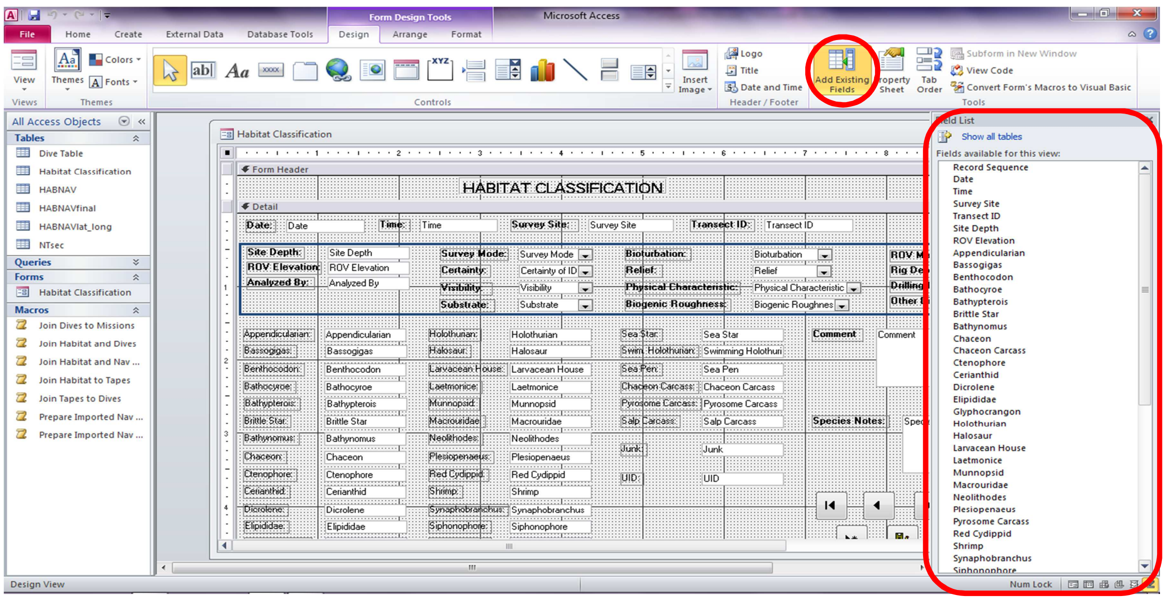
6. To delete a field, simply select/highlight the row you want to delete, right click and then select “Delete Rows”.

B.3. Modifying the Form

1. In the “Home” tab, click on “View” and then select “Design View”. This will take you to a view of the form that allows for you to make changes to the form, including adding or removing fields and buttons as well as moving things around.

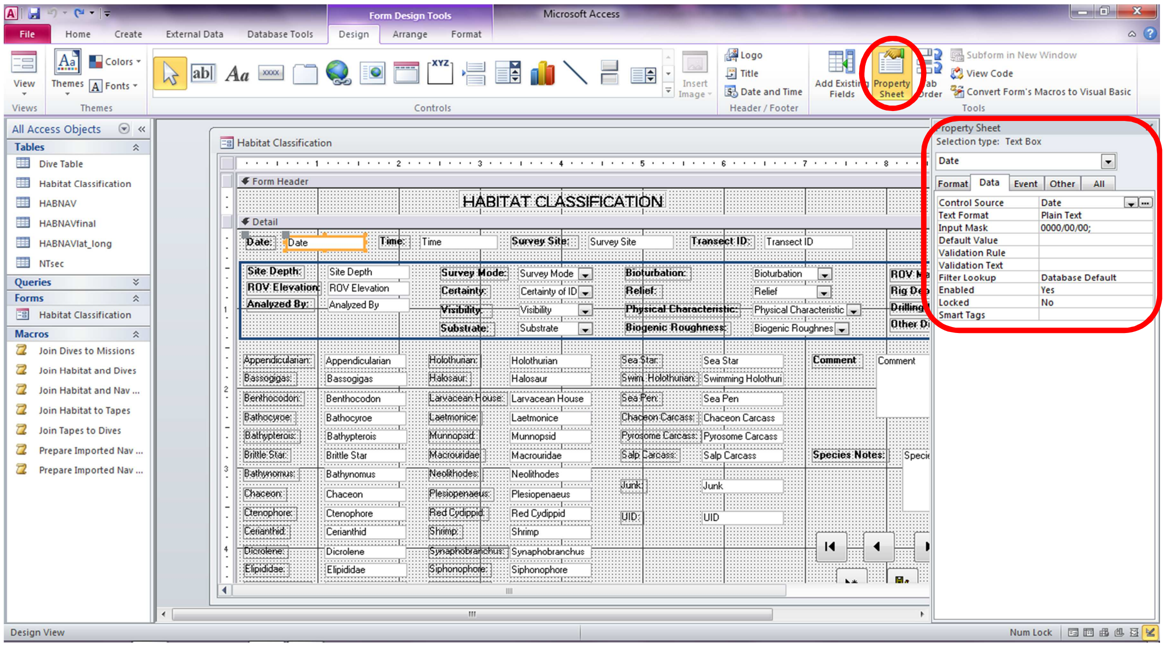


2. Move objects by clicking and dragging, or using arrow keys to move back/forth and up/down.
3. To add a new field that is already present in the Access table, go to the "Design" tab and click on "Add Existing Fields". This will open a list on the right-hand side of the window that shows all fields present in the table. Double-click on the field name that you would like to add to the form and it will show up in the form. Drag to where you would like it.



4. To change properties of a field in the form, select the field in the form. Then, under the "Design" tab, click on "Property Sheet". This will bring up a section on the right-hand side that has multiple tabs corresponding to various properties of the field that can be changed including changes to format, data source, name, etc.

Most of these properties should not or do not need changing. However, one important one is to ensure the data source corresponds to the correct field in the matching table. This can be done in the "Data" tab and selecting the correct source under the "Control Source" property.



5. A variety of other things can be added to the form by making selections within the design tab. Buttons similar to the ones already in the form can be added. Text boxes and drop boxes can likewise be added and can be linked to fields in the Access table in the same manner as in Step 4 above.

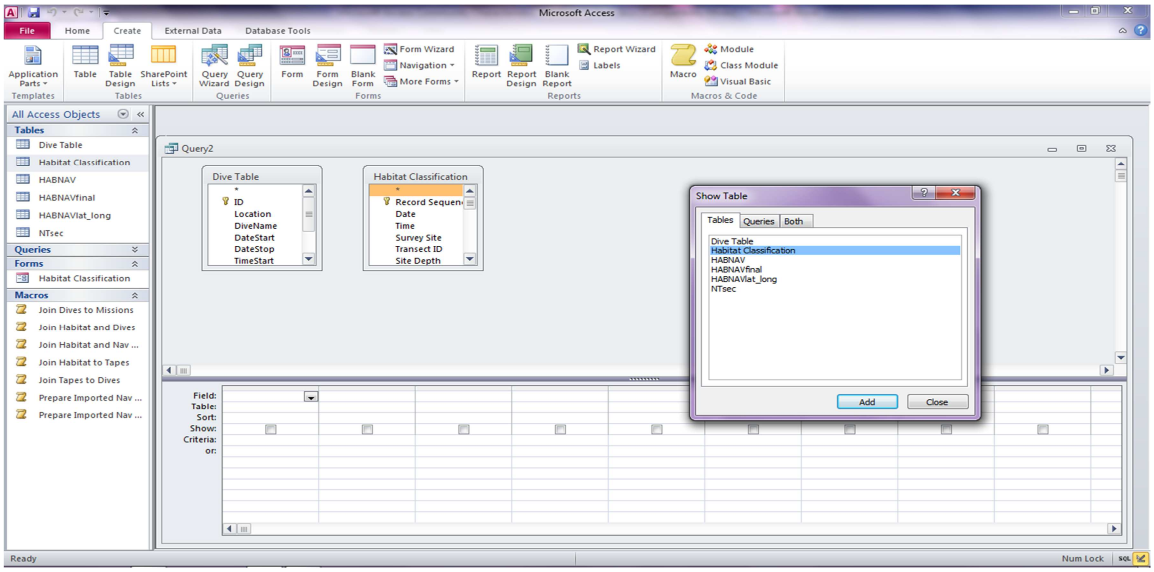
B.4. Important Notes

1. Do not make modifications to any of the coding unless you are very experienced with working on this type of database. Changing parts of the underlying code for the forms and tables can render aspects non-functioning and can cause errors. If you end up in an area where coding comes up by accident, close it without making or saving any changes.
2. At all times, fields in the form must have a corresponding field in the matching Access table. Make sure these are linked properly, or else you may not have all your data transferred between form and table properly.

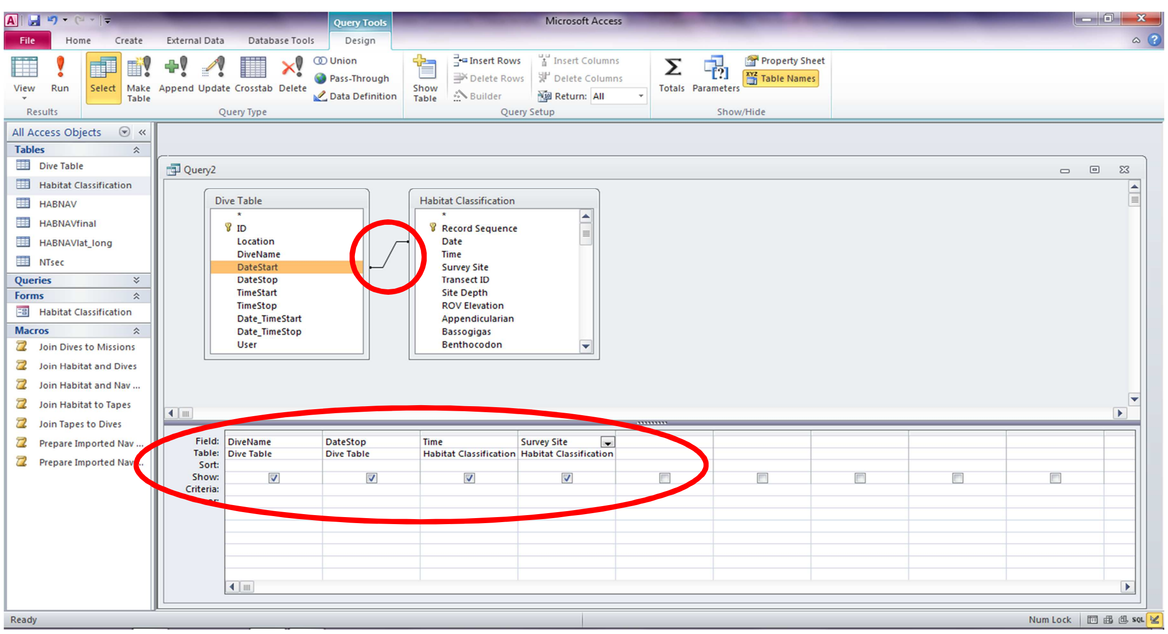
B.5. Joining Database Tables

1. Go to the “Create” tab and click on “Query Design”.

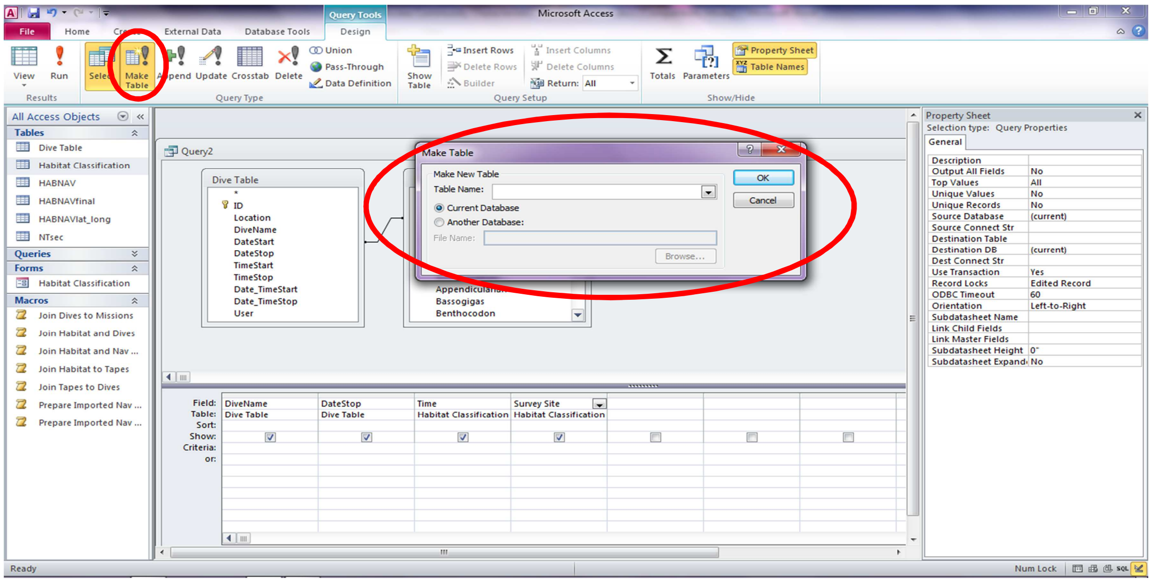
2. “Show Table” opens up, and you can choose which tables you would like to join by selecting them and clicking “Add”.



3. Drag field names from each of the tables down to the bottom half of the window to add desired columns to the new table that will be built.
4. To join, click on first table column and drag it to the matching column in second table. A line will show joining the selected field of both tables.



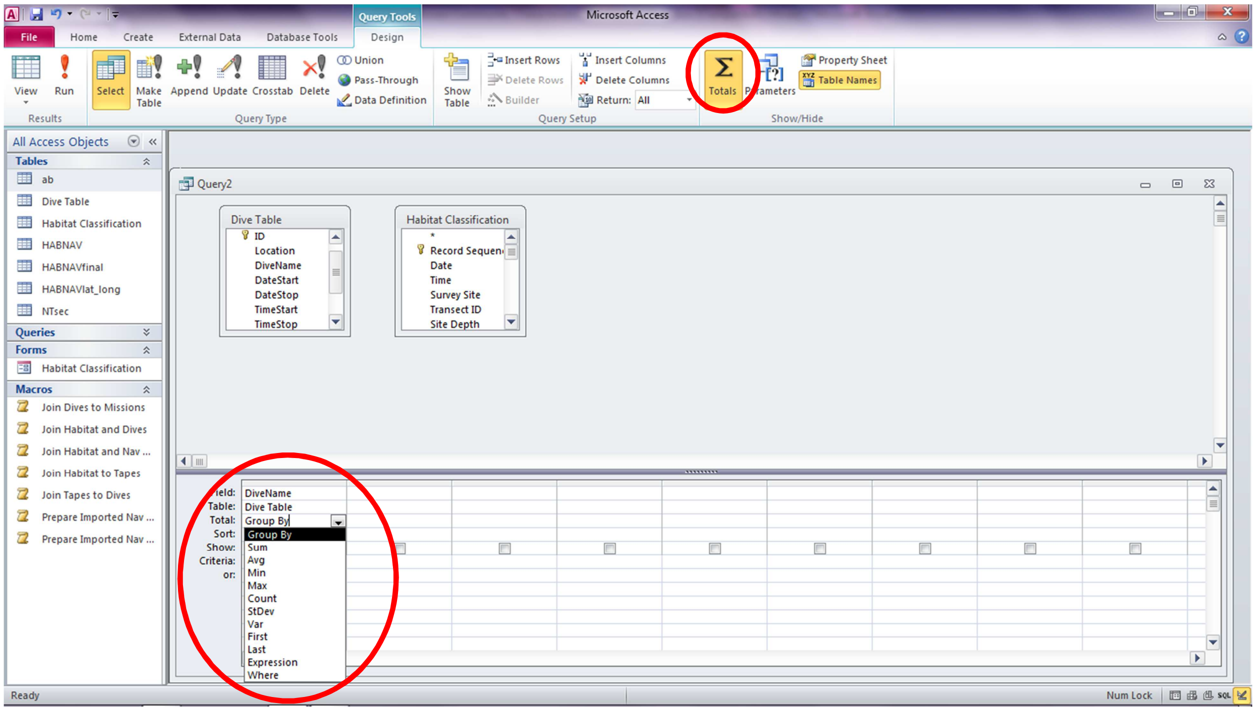
5. Right click on line joining tables and go to “Properties”.
6. Select desired properties.
7. Click “Make Table” at top of toolbar and type in your new table name, then click OK.



8. Click “Run” (near the left-hand side of the “Create” tab).

B.6. Grouping/Combining Table Data by Specified Variables

1. Procedure is similar to joining tables above.
2. Go to “Create” tab and click on “Query Design”.
3. Pick tables you want to group/combine data from. Drag to add desired columns.
4. Click on Σ_{totals} or other parameter you want for combining the data (near right-hand side of “Create” tab).
5. Choose “Group By” for columns you want to group the data by.
6. Then choose how you want other variables to be treated (i.e. average, sum, etc.).



7. Click “Make Table”, then “Run”.

VITA

Stephanie Marie Sharuga is originally from Canada and now is a citizen of the United States. In 2008, she received her Bachelor of Science degree majoring in Biology and Earth & Ocean Sciences (environmental emphasis) from the University of Victoria in British Columbia, Canada. She then went on to complete a Master of Science in Environmental Management and Sustainability from the Stuart School of Business at Illinois Institute of Technology, with a focus on marine and coastal policy and management. Prior to beginning her PhD in Oceanography and Coastal Sciences at Louisiana State University, Stephanie was involved with a variety of research, volunteer, and consulting projects, including working for the biogeochemistry lab at the Smithsonian Environmental Research Center. Over the years, she has been actively involved as both a ship and shore-based scientist in multiple oceanographic research cruises, including those utilizing telepresence technologies. Stephanie has continued sharing her passion for the ocean, the environment and science through science education and outreach activities. She is also an active member of three national and international Honors societies. Stephanie is currently pursuing opportunities using innovative and novel technologies and approaches for marine research, education, policy, and management.