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## Characterization of Epibenthic and Demersal Megafauna at Mississippi Canyon 252 Following the Deepwater Horizon Oil Spill

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CHARACTERIZATION OF EPIBENTHIC AND DEMERSAL MEGAFUNA AT  
MISSISSIPPI CANYON 252 FOLLOWING THE DEEPWATER HORIZON OIL SPILL

A Thesis

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Master of Science

in

The Department of Oceanography and Coastal Sciences

by  
Marla Maxine Valentine  
B.S. Salem College, 2009  
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## **ABSTRACT**

Increased demand for new sources of oil and gas has resulted in an expansion of drilling into deeper waters. With this exploratory drilling come increased risks, which were realized on April 20, 2010 when the blow out preventer on the Macondo Well failed, resulting in the release of a large quantity of oil and gas into the Northern Gulf of Mexico from a bathypelagic source. This unprecedented environmental disaster was coined the Deepwater Horizon Oil Spill by the popular news media. In the months that followed the spill, the lack of knowledge about the pre-spill condition of deep-sea communities in this area of the Gulf of Mexico became apparent. This made it difficult to determine the effects of the spill on deep-water megafauna.

The objective of this study was to characterize the epibenthic and demersal megafaunal community immediately following and one year after the spill. Remotely operated vehicles conducted a series of video surveys over an extended time series (11 surveys Aug 4 – Nov 1, 2010) of a site located 750 m to the Southwest of the Macondo Well and at five additional study sites during August and September 2010: 2000m north, west, south, and east, and 500m north of the Macondo well. The 750 m Southwest site was revisited in July of 2011 to determine what, if any, changes had occurred in the deep-water megafaunal community.

These study sites were dominated by demersal fishes and mobile benthic invertebrates both in 2010 and 2011. The results indicate both diversity and densities of organisms declined over time in 2010, while densities appeared to increase in 2011 to levels similar to those observed immediately following the spill. The presence of carcasses of pyrosomes, salps, and crabs in 2010 indicated some short-term or acute mortality following the spill.

It is hoped that these data will be used as a post-spill baseline against which future surveys of diversity and abundance of deep-water megafauna can be compared.

## **CHAPTER 1: GENERAL INTRODUCTION**

### **1.1 Deep-sea Megafauna**

The deep-sea is a unique environment characterized by widely unexplored regions and untold species not known to science. The development of new technologies such as remotely operated vehicles (ROVs) have improved our ability to reach these deeper waters thereby enabling a better understanding of the ecology of deep-sea communities. ROVs capable of reaching great depths are expensive to purchase, and require a large retinue for deployment and repair. This often limits the scientific community's access to this research tool. However, larger ROVs are widely used in the oil and gas trade and by partnering with this industry, scientists now have the ability to study the deep-sea environment in a cost effective manner.

As the demand for new sources of oil and gas increases, deep-water drilling has also expanded. Deep-water drilling projects in the Gulf of Mexico increased from 16 in 1997 to 51 by 2002 (Baud et al., 2002). As of 2012 a total of 67 new deep-water drilling permits were issued. The potential impacts of oil drilling in shallow waters have been well studied; however, limited work has been performed on the consequences of drilling in deep waters.

The deep-sea is a dynamic habitat in terms of the physical and chemical processes affecting the distributions and abundances of biota that dwell in this environment. Most notably, limited primary production may have the largest influence of the composition and abundance of these fauna. The majority of energy found in the deep-sea benthos is supplied through the descent of surface production (Levin et al 2001, Rex and Etter 2010). On average the deep-sea benthos only receives 1% of surface production, which can be affected by depth, distance from productive waters, and seasonality (Rex and Etter 2010). Seasonal variation in surface primary productivity results in a temporally varying supply of food and nutrients to the benthos resulting

in large seasonal changes to abundance, diversity, and reproduction. In regards to the Gulf of Mexico there appears to be a higher fish and invertebrate density in the fall versus the spring, but little effect in diversity (Pequenat et al., 1990). Globally, there also appears to be pronounced latitudinal variation in nutrient supply to the deep sea with regions of higher latitude receiving far greater quantities of allochthonous inputs of nutritional materials than seen in tropical and temperate regions (Rex and Etter 2010, Corliss et al., 2009).

Deep-sea fauna are also distinctive in their ability to inhabit an environment with no light, high pressure, and very low temperatures. Once below 1000m in depth, light effectively disappears and organisms adapt to this in a variety of ways, for example, utilizing other sensory organs or via bioluminescence to find food and mates. It is well known that pressure increases at a rate of 1 atm for every 10-m increase in depth, so at 1000 m the pressure will be 101 atm. Temperatures in the deep-sea are also cold relative to shallow water, ranging from 10°C at 200m depth on down to about 2°C at depths below 3000m (Carney 2001). This high pressure in combination with low temperatures affects the rate and type of enzymatic reactions that occur, effectively changing metabolic pathways for deep-sea fauna (Gage and Tyler 1991, Somero, 1998).

Deep-sea fauna are often categorized based on their body size into one of three groupings: megafauna, macrofauna, and meiofauna. Macro- and meiofauna consist of smaller (0.3mm and 0.063 mm respectively) organisms and are frequently studied due to ease of sampling and their higher abundances (Kropp, 2004). In terms of size, the largest group of organisms inhabiting the seafloor interface is megafauna. These are also some of the most poorly studied of all of the deep-sea organisms. Megafauna are defined as any animal able to be visibly photographed or retained in trawls, and typically greater than 1-2 cm (Rex and Etter

2010; Carney, 2001; Rowe and Kennicutt 2000; Gage and Tyler 1991). The megafauna can be further divided into four categories: demersal fishes, demersal invertebrates (e.g. cnidarians, ctenophores), mobile invertebrates (e.g. crabs and shrimps), and sessile or limited mobility invertebrates (e.g. sea stars, sea cucumbers).

The majority of deep-sea exploration has been conducted in the Pacific and Atlantic Oceans (Rex and Etter, 2010). There is limited available quantitative information about deep-sea organisms in the Gulf of Mexico making it difficult to describe patterns of abundance and distributions. The focus of many of these studies is on descriptive characterizations of the megafaunal community composition. The two most comprehensive data sets in this region were made available from extensive trawl studies conducted by Pequenat et al. (1990) and the Deep Gulf of Mexico Benthos (DGOMB) (Rowe and Kennicutt 2000, Powell et al. 2003). These studies do show that, for fish and invertebrate assemblages, species richness and abundances generally decline with depth in the gulf (Pequenat et al., Rowe and Kennicutt 2000 Powell et al 2003). It also appears that deep-sea biota exhibit an east to west gradient in terms of density and diversity (Pequenat et al., 1990 Rowe and Kennicutt 2000, Powell et al 2003).

## **1.2 Deep-sea Sampling Methods**

Historically trawling has been the predominate method for sampling deep sea organisms. Commercial fleets and scientific studies rely heavily of trawling because of its effectiveness at sampling large volumes of sparsely distributed water and ability to procure physical samples enabling identification and physical analysis (Heino et al, 2011). While trawling is an effective and simple method to procure information on overall abundances, there are drawbacks to studying megafauna with this methodology. Trawling is highly invasive, often disturbing the

seafloor and damaging fauna. This method also destroys fragile organisms such as siphonophores and medusa. These gelatinous organisms are often extruded through the mesh or physically destroyed, effectively preventing identification or enumeration. Larger organisms also have the potential to escape trawl nets, leading to under-estimations of biomass.

Development of new technologies such as manned submersibles, remotely operated vehicles (ROVs) and autonomous underwater vehicles (AUVs) have enabled more in depth studies of the deep-sea fauna. ROVs enable minimally invasive, in situ observations of deep-sea biota's distribution, orientation, and behavior. The use of these electro-hydraulic platforms is not without flaw. The deep-sea is a dark quiet environment with minimal disturbance. These ROVs produce a large quantity of noise and are equipped with bright lighting both of which are foreign in this environment. This can lead to either avoidance or attraction by mobile organisms, but should have little impact of sessile or limited mobility fauna. This can potentially lead to bias causing either over- or under- estimation of organismal abundances.

In the wake of the Deepwater Horizon oil spill there was a great need to fill in the gaps in the scientific communities' knowledge about Gulf of Mexico deep-sea megafaunal communities. This thesis reflects that need through analysis of a series of deep-water surveys.

### **1.3 Deepwater Horizon Oil Spill**

The scarcity of data concerning deep-water megafauna in the northern Gulf of Mexico became apparent in the wake of an environmental tragedy that occurred in Mississippi Canyon Lease Block 252 (MC 252). The failure of the Macondo blowout preventer (BOP) resulted in the release of 200 million gallons of oil from a point source in the bathyl zone (1511 m) (National Oil Commission, 2011). Subsequently the Deepwater Horizon drilling rig sank to the

seafloor causing a large physical disruption to the benthos. An additional 1.8 million gallons of chemical dispersants were also applied to accelerate the breakdown and dilution of this oil (National Oil Commission, 2011). There also appeared to be evidence of a subsurface plume of dispersed oil and gas extending 35km southwest of the Macondo BOP at approximately 1100m in depth (Camilli et al., 2010). The spill occurred on April 20, 2010 and continued until July 15 when the well was finally capped. It was not until Sept that the well was officially sealed. In addition to this effort, two relief wells were drilled to ease the flow from the Macondo BOP. While oil was flowing from the Macondo BOP the majority of resources (ships, ROVs, personnel) were focused on controlling the discharge of oil and gas and sealing the well. It was not until after the well had been capped that these resources were made available for use in biological assessments of the surrounding area.

The two prior studies by (Pequenat et al.1990; Rowe and Kennicutt 2000; Powell et al 2003) were unfortunately conducted too far to the east and west of the spill site to provide a description of baseline conditions at the impact site. Without detailed information pertaining to the organisms inhabiting this area, it is difficult, verging on nearly impossible, to determine the impacts of this oil spill and the potential resiliency of these inhabitants. As such, it was necessary, for assessment purposes, to implement extensive and comprehensive surveys both in the impacted area and at sites with similar depths and benthic morphology outside of the impacted area. ROVs were used exclusively for these surveys.

#### **1.4 Thesis Goals and Objectives**

This thesis documents the findings of a series of surveys conducted on a spatial scale and temporally over a two year period. This study served a dual purpose: providing the opportunity

to greatly enhance the scientific community's understanding of deep sea megafauna and an attempt to classify the condition of deep-sea megafauna following the Deepwater Horizon oil spill. ROVs were utilized to determine organism condition, diversity, abundance, and distribution during and after the oil spill.

As part of a larger study, the chapters in this thesis are devoted to assessing the impacts of the Deepwater Horizon oil spill on epibenthic and demersal megafauna in the immediate vicinity of the drilling rig.

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## **CHAPTER 2: CHARACTERIZATIONS OF EPIBENTHIC AND DEMERSAL MEGAFUNA AT MISSISSIPPI CANYON 252 SHORTLY AFTER THE DEEPWATER HORIZON OIL SPILL**

### **2.1 Introduction**

Following the Deepwater Horizon (DWH) Oil Spill, approximately 200 million gallons of oil (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2011) were accidentally released into the Gulf of Mexico from the Macondo well located in Mississippi Canyon lease block 252 (MC252). This spill was unique, not only for its large volume, but also because its discharge depth of 1511 m, introduced oil into the bathypelagic zone near the seafloor. This resulted in a plume of oil and gas that rose through the bathypelagic, mesopelagic, and epipelagic zones and ultimately reached the surface. Approximately 2,900,000 L of dispersant were injected directly into the oil and gas discharge at the seafloor (Kujawinski et al., 2011). In addition to surface oil there was evidence of a deep, subsurface plume of dispersed oil and gas (Camilli et al., 2010). This plume consisted of a mixture of complex hydrocarbons, including methane, ethane, and propane (Kessler et al., 2011; Reddy et al., 2011), that could have produced chronically- or acutely-toxic conditions in its vicinity. The subsurface plume was estimated to stretch 35 km centered at a depth of 1100 m extending to the southwest from MC252 (Camilli et al., 2010).

The potential impacts of the hydrocarbons and dispersants on deep-sea fauna living on or near the seafloor at depths of approximately 1500 m are unknown. The presence of toxins originating from the DWH spill had the potential to alter the species composition and abundances of demersal and benthic taxa. Moreover, sublethal concentrations of pollutants have been shown to produce avoidance behavior by some marine crustaceans in laboratory studies (Møhlenberg and Kiørboe, 1983; Benfield and Aldrich, 1994), suggesting the potential for alteration of the distribution and abundance mobile deep-sea organisms.

During the spill, the majority of resources were focused on containing and ending the discharge of oil and gas. There do not appear to have been many attempts to quantify the impact of the spill on the organisms inhabiting the seabed around the Macondo well. Remotely operated vehicles (ROVs) were an essential component of the response to contain the MC252 well. Equipped with color cameras and lights, these systems enabled visual assessment of the distribution and abundance of marine life at the depths prevalent around MC252. Ultrasonic navigation beacons permitted the positions of the ROVs to be tracked and documented. Limitations of the resolution of the cameras typically used on industrial ROVs restricted their utility to observations of larger organisms (megafauna), that are defined as those typically large enough to be viewed in photographs or caught with trawls (Gage and Tyler, 1991). In an ideal assessment of the impact of any oil spill, communities residing in the area would have been surveyed before the accident and the survey would have been replicated at impact and control sites following the before-after-control-impact (BACI) experimental design (Smith, 2002). In the case of the DWH spill, there were no quantitative data on the species composition and abundance near MC252 prior to the accident, which complicated the assessment of the spill's impact on megafauna using community metrics such as taxonomic composition and abundances. Limited qualitative data existed from ROV surveys conducted as part of the Gulf SERPENT Project during February and March 2010.

Once the Macondo well had been controlled and the flow of oil ended on July 15, 2010, some of the ROV resources that had been dedicated to responding to the spill became available for biological surveys. During August and September of 2010, two such ROVs were tasked to quantify the distribution and abundance of benthic megafauna, demersal, and planktonic organisms around the Macondo well. As part of those surveys, this paper describes attempts to

characterize and quantify benthic megafauna and demersal fishes and invertebrates within a 2 km radius of the Macondo well. Such data provide a baseline against which, subsequent changes in the composition and abundance of marine life may be compared.

## **2.2 Methods**

### **2.2.1 Post-Spill Surveys**

Sea floor surveys were conducted by industrial work-class ROVs. A pair of Schilling Robotics Ultra-Heavy-Duty ROVs (UHD30 and UHD31) were deployed from the MV Olympic Challenger to survey the fauna at four sites (Table 2.1) located 2000 m from the Macondo blowout preventer (BOP) at bearings of 0° (2000-N), 90° (2000-E), 180° (2000-S), and 270° (2000-W). To evaluate conditions closer to the BOP, a fifth site located 500 m due north (500-N) of the BOP was also surveyed (Table 2.1). Owing to the very large number of vessels operating at the incident site, the selection of these survey sites reflected a compromise between our desire to survey sites at different ranges and bearings from the BOP and vessel access restrictions around the incident site. Details of the dates, times, and coordinates of each site are summarized in Table 2.1.

At each of the 2000 m sites, a series of radial 250 m-long transects were surveyed by one or both ROVs from the center point of each site (Table 2.1). The survey design called for a total of 24 transects, beginning at 0° and separated by 15° increments; however at some sites fewer transects were conducted due to time or other operational constraints. At the 500 m site, a series of nine 250 m-long transects were surveyed from 90 – 270° at 22.5° increments. Coarser angular resolution was necessary because we had been allocated limited time at that location. All transects at 500-N were conducted at bearings north of a line running east to west in order to avoid operating in a region where there was substantial debris from the DWH. All surveys were

conducted by ROVs at altitudes of approximately 1 – 2 m above the seabed. A subsea navigation system (ultra-short base-line system: USBL), which had been installed for the MC252 response was used to determine the location of each ROV in geographical coordinate space at intervals of approximately 7 s. Based on the USBL data, the mean horizontal velocity of the ROV at each site was: 0.09 m s<sup>-1</sup> (2000-N); 0.10 m s<sup>-1</sup> (2000-W); 0.12 m s<sup>-1</sup> (2000-S); 0.16 m s<sup>-1</sup> (2000-E); and 0.15 m s<sup>-1</sup> (500-N).

Table 2.1 Locations, survey dates, and depths of each of the five stations that were surveyed by the ROVs in this study.

Station	Coordinates	Start Date	Start Time	End Date	End Time	Depth (m)
2000-N	28° 45' 22.295" N 88° 21' 58.520" W	08/09/2010	14:00	08/11/2010	00:43	1443-1456
2000-W	28° 44' 16.360" N 88° 23' 11.190" W	08/11/2010	10:31	08/12/2010	00:32	1493-1506
2000-S	28° 43' 12.310" N 88° 21' 56.530" W	08/19/2010	21:04	08/20/2010	14:14	1585-1591
2000-E	28° 44' 17.280" N 88° 20' 43.490" W	08/26/2010 <sup>a</sup> 09/09/2010 <sup>a</sup>	16:25 19:02	08/27/2010 09/10/2010	02:16 16:40	1526-1528
500-N	28° 44' 34.780" N 88° 21' 57.930" W	08/21/2010	11:42	08/21/2010	18:35	1493-1509

<sup>a</sup>Surveys were begun on 8/26-27/10. The vessel was re-tasked prior to completion, and it completed the surveys on 9/09-10/2010.

In order to determine the area surveyed during each transect, the width of the field of view was estimated during surveys of the 500-N site. At this site it was necessary to traverse a section of the drilling riser, which was lying on the seabed. The riser provided an opportunity to quantify the field of view referenced to an object of known dimensions. The diameter of the riser including the outer flotation collar was 132 cm and the upper half of this structure (clearly demarked by a seam) was used as a reference point. When the ROV was at a distance from the riser that was typically the range at which organisms could be detected, a video frame was

digitized and used to estimate the width of the field of view. Measurements from four of these frames collected on independent transects were averaged to estimate the width of the field of view. This mean width (7.1 m, standard deviation of 0.7) was multiplied by the length of each transect, as measured by the USBL navigation system, to derive a measure of the area surveyed during each transect. Since the altitude of the ROV was usually very similar during surveys of all sites, the field of view measured from the 500 m site was also used to estimate the area surveyed at each of the 2000 m sites.

As each ROV flew over the bottom, the seabed and water column immediately above the seafloor were observed for marine life. When organisms were detected, the ROV attempted to obtain close-up video using one of the two color video cameras. The ROV attempted to continue moving along the transect while these close-ups were collected. Video was stamped with an overlay containing navigational and date/time information and then stored on a surface hard drive. After the surveys, software (Avermedia USB Playback Console) was used to review the video and save representative still image (JPEG) and video files (AVI) of organisms for use in identification. Each still image was assigned a filename that included the ROV that collected the image and the date and time of the observation. Identifications were made to the finest possible taxonomic resolution; however, the high compression used to store the standard definition video generally limited the degree to which organisms could be identified to genus or species.

Organisms were grouped according to five categories: mobile benthic invertebrates; sessile or limited mobility benthic invertebrates; demersal invertebrates; fishes; and dead organisms.

Organisms that could not be recognized and placed into any category with at least taxonomic resolution to class level, were assigned a label of unidentified and were not used in subsequent analyses.

The navigation data were imported into Matlab as text files containing date, time, UTM (easting, northing), latitude, longitude and depth. Eastings, northings, and pressure data were examined for outliers, which were removed. Then the resultant tracklines were individually smoothed using a Lowess Quadratic filter in Matlab (Curve Fitting Toolbox) and then merged with the corresponding time data. Because the original ROV position data were updated at intervals of approximately 7 s, the survey data were linearly interpolated on to a 1 s time base using the `interp1` function in Matlab (Fig. 2.1).

The time of each observation was used to determine where the organisms were observed within the navigation dataset. The areal density of each taxon was estimated along individual transects by dividing the number of organisms encountered in each transect by the product of the transect length and swath field of view. By treating each radial transect as an independent estimate of the density of each taxon, the mean density of organisms in each site was calculated by averaging densities over all transects at each site. Mean densities and 95% confidence intervals on the mean were estimated for each taxon. We also computed an index of abundance (observations  $\text{min}^{-1}$ ) for each taxon that was observed during pre-spill surveys by dividing the number of selected taxa observed during each transect by the corresponding transect. Then a mean and 95% confidence interval were computed for these abundance indices at each site in the same manner as for the density calculations.

For statistical comparisons of mean densities of selected taxa among sites, a Levene's Test was performed (Trujillo-Ortiz and Hernandez-Walls, 2003) to evaluate the null hypothesis that the means were drawn from a population with the same variance and a Kolmogorov-Smirnov test was performed to evaluate the null hypothesis that the densities from all transects at each site were normally distributed. In cases where either the assumptions of homogeneity or

normality of variances were not met, we performed a non-parametric Kruskal-Wallis analysis of variance of ranks to evaluate the null hypothesis that the densities of a particular taxon were the same at all sites. In cases where the null hypothesis was rejected, we performed a non-parametric multiple comparison test using the Dunn's test (Cardillo, 2006). All statistical tests were run in Matlab.

### **2.2.2 Pre-Spill Surveys**

Quantitative pre-spill data on the abundances of benthic and demersal taxa were not available for comparison. Instead we utilized video data that were collected by the ROV on the DWH as part of the Gulf SERPENT Project. On February 19, 2010 and March 27, 2010 the EMAG-1 ROV operated by Oceaneering recorded video of the seafloor in the immediate vicinity of the MC252 Macondo BOP as they searched for marine life. The ROV flew slowly above the seafloor at an altitude of 1 – 2 m while recording video on to DVD in NTSC (480i) resolution. There was no subsea navigation system and therefore we could not determine where the surveys were conducted. Given the limited length of their ROV's tether, these data are likely from within a radius of 250 m around the Macondo BOP. The video was examined for the presence of organisms and still images were extracted and stored with a filename that included the date and time of each observation. The start and end time of each survey was used to compute the total

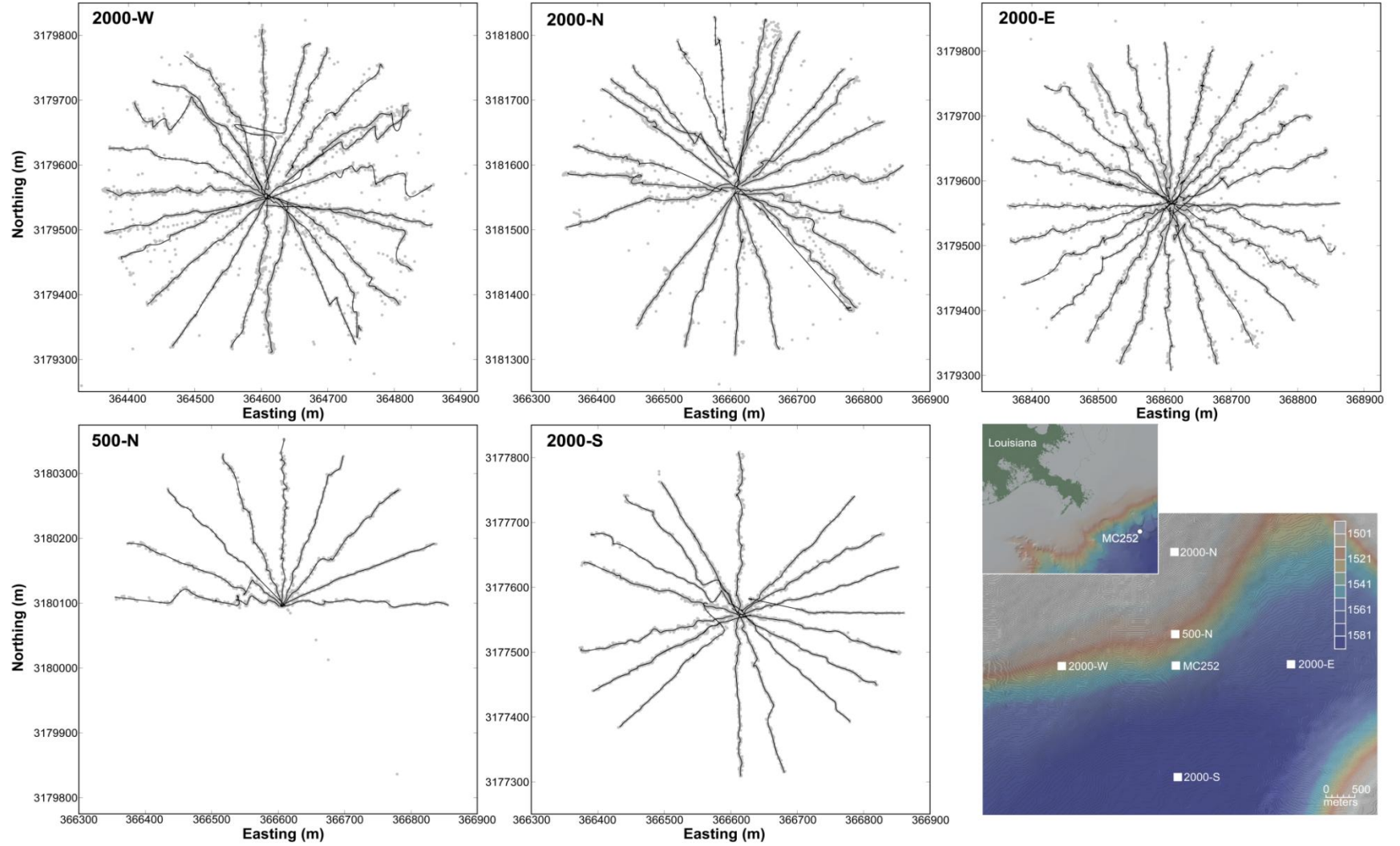


Figure 2.1. Survey tracks at each of the sites and the location of the study sites in relation to the bathymetry (m). Grey dots indicate raw navigation data and black lines follow filtered, interpolated trajectories of the ROV.



survey time. This was corrected in a few cases by subtracting any time that the ROV spent loitering at an animal. Then an index of abundance was computed for each taxon (observations  $\text{min}^{-1}$ ).

In order to evaluate whether the indices of abundance were different during pre- and post-spill surveys, we treated the two Gulf SERPENT pre-spill surveys as replicate surveys. We employed the same approach used to evaluate homogeneity and normality of variances for the pre- and post-spill abundance index data as was used for the density data. None of the data met the assumptions of homogeneity and normality of variance. For each taxon, a Kruskal-Wallis analysis of variance of ranks was used to evaluate the null hypothesis that the abundance indices of a particular taxon were the same during pre-spill and post-spill surveys (at each site). In cases where the Kruskal-Wallis test was significant ( $p < 0.05$ ) we conducted a Dunn's non-parametric multiple comparison test at a significance level of  $p = 0.05$ .

### **2.3 Results**

A total of nine different megafauna categorized as mobile invertebrates were observed at our sites around the Macondo BOP (Fig. 2.2). Four of these were identifiable to species: red crabs *Chaceon quinquidens*, the large lithodid crab *Neolithodes agassizii*, the giant isopod *Bathynomus giganteus* and individuals of the holothurian *Enypniastes eximia*. One group of red shrimps contained a variety of unidentified species. A second group of shrimp contained the genus *Glyphocrangon*. Three additional groups were identifiable to family: deep sea hermit crabs (Parapaguridae), squat lobsters (Galatheidae), and mobile holothurians (Elpidiidae). One group, the brittle stars were identifiable to class level (Ophiuroidea).

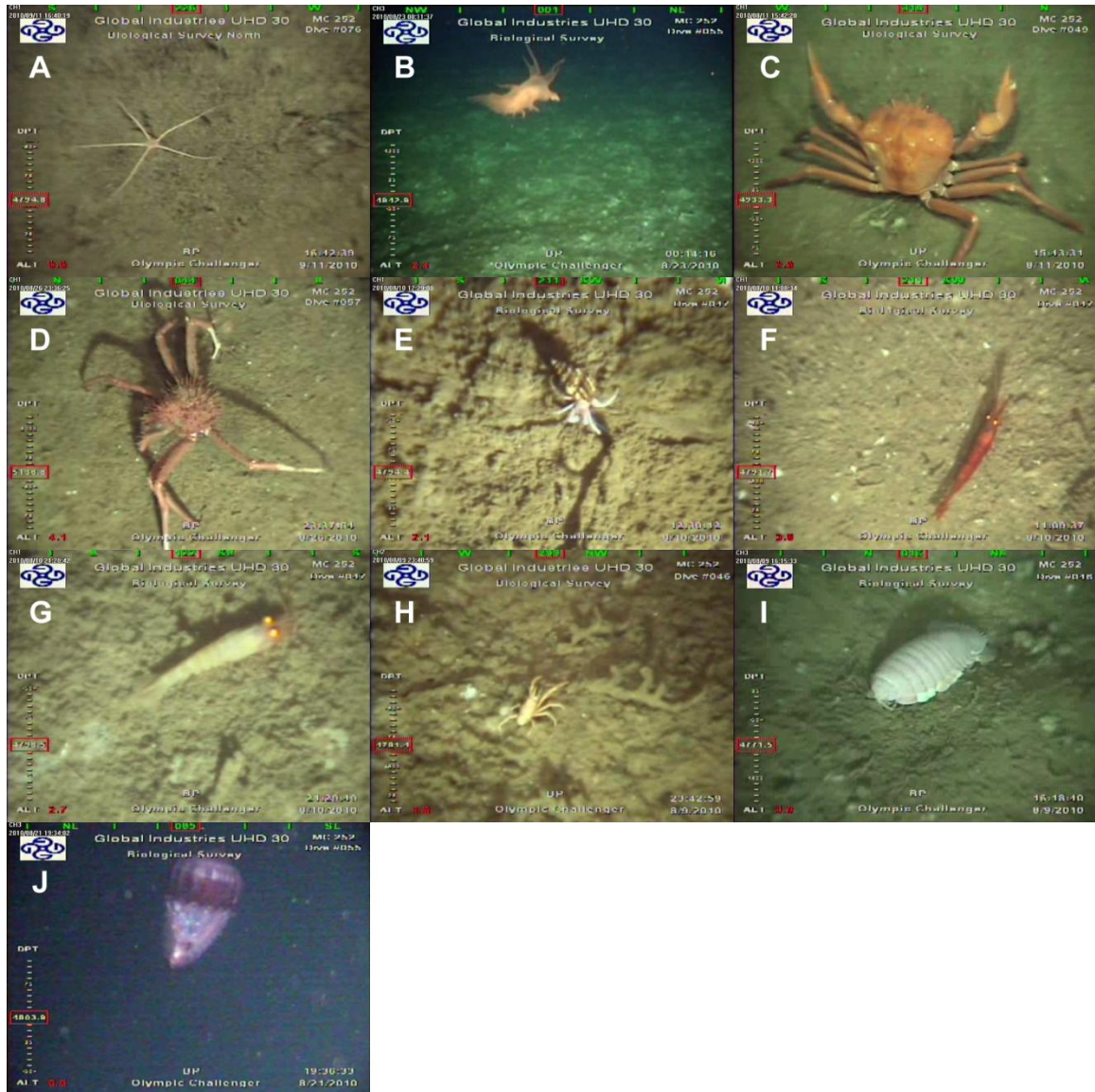


Figure 2.2. Example images of mobile benthic invertebrates. A: brittlestar; B: Holothuroidean Family Elpididae; C: *Chaceon quinquedens*; D: *Neolithodes agassizii*; E: deep sea hermit crab; F: Red shrimps; G: *Glyphocrangon* sp.; H: squat lobster; I: *Bathynomus giganteus*; J: *Enypniastes eximia*.

Six invertebrate megafaunal taxa classed as being sessile or possessing limited mobility were observed (Fig. 2.3). Two of these were identifiable to genus: the stalked glass sponge *Hyalonema* sp. and the Aphroditid polychaete *Laetmonice* sp. Two more taxa were identified to

family level: cerianthid anemones (Cerianthidae) and holothuroideans. The remaining two taxa were identifiable to class level: sea pens (Anthozoa) and sea stars (Asteroidea).

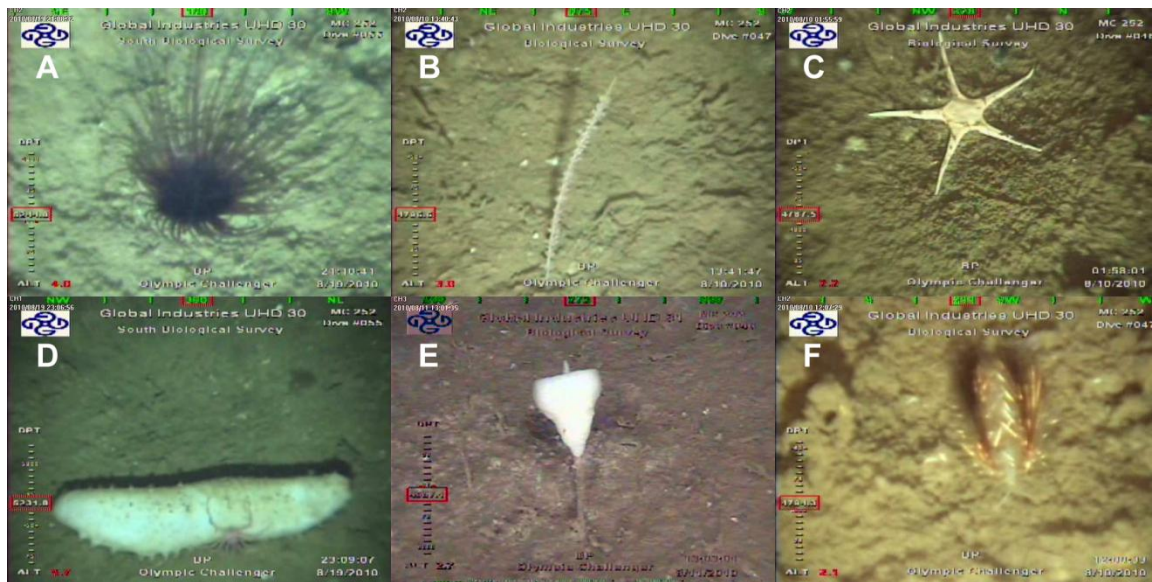




Figure 2.4. Example images of demersal organisms: A: *Bathocyroe fosteri*; B: an undescribed cydippid ctenophore; C: *Benthocodon peduncula*; D: Unidentified hydromedusa; E: *Poralia rufescens*; F: a munnopsid isopod.

There were nineteen different fish taxa observed at our study sites (Fig. 2.5). Of these 8 were identifiable to species and included one elasmobranch: the six-gilled shark *Hexanchus griseus*; and 7 teleosts. The latter included one species of tripod fishes: *Bathypterois quadrifilis*; two species of cusk eels: the bony-eared assfish *Acanthonus armatus* and *Bassogigas gillii*; one species of pricklefis *Acanthochaenus leutkenii*; one species of duckbilled eel *Venefica procera*; one spiny eel *Polyacanthonotus merretti*; and one slickhead eel *Talismania antillarum*. Five fish taxa were identified to genus level halosaurs belonging to the genus *Aldrovandia*; the cutthroat eel genus *Synaphobranchus*; the cusk eel *Dicrolene* sp.; a genus of tripod fish *Bathypterois* sp. and the duckbilled eel *Facciolella* sp.. Four taxa were identified to family level: an unidentified genus of cusk eel (Ophiididae); and three different types of grenadiers (Macrouridae). Two taxa could not be identified below the class Actinopterygii.



Five groups of dead organisms were noted during our surveys: pyrosome carcasses, salp carcasses, and dead benthic holothurians, sea pens and glass sponges (Fig. 2.6).

### **2.3.1 Taxonomic Composition Among Sites**

Taxonomic richness, defined as the number of different live taxa present varied among sites (Fig. 2.7, Tables 2.2 and 2.3) with the highest values at the 2000-N site (n=40) followed by 2000-W (n=31), 2000-E (n=23), 2000-S (n=18) and 500-N (n=14). Similar trends were evident within most of the different megafauna categories. For mobile invertebrates the rankings (highest to lowest taxonomic richness) were 2000-N (n=9) > 2000-W (n=8) > 2000-E (n=7) > 2000-S (n=6) > 500-N (n=4). Sessile or limited mobility invertebrates showed the pattern: 2000-N (n=6) > 2000-W (n=5) > 2000-S (n=3) > 2000-E (n=2) = 500-N (n=2). Of the six different demersal taxa, the site rankings were: 2000-N (n=6) > 2000-W (n=5) > 2000-S (n=4) = 2000-E (n=4) > 500-N (n=3). For fishes the site ranking was: 2000-N (n=18) > 2000-W (n=13) > 2000-E (n=10) > 2000-S (n=5) = 500-N (n=5).

### **2.3.2 Density Among Sites**

In general the densities within each category of organisms showed a pattern of highest densities ( $n\ m^{-2}$ ) at the 2000-N site with lower densities at 2000-W followed by 2000-E or 2000-S. Densities at the site closest to the Macondo well (500-N) were as low, or lower than those observed at the 2000-S site.

Within the mobile invertebrate category, the most abundant organisms were red shrimps, mobile holothurians belonging to the family Elpidiidae, and the red crab *Chaceon quinquidens* (Fig. 2.8). Red shrimps dominated at all sites except 2000-E (Fig. 2.7).



Figure 2.5. Example images of fishes observed at sites around MC252. A: *Hexanchus griseus*; B: *Aldrovandia* sp.; C: *Synaphobranchus* sp.; D: *Venefica procera*; E: *Polyacanthonotus Merretti*; F: *Facciolalla* sp.; G: *Alepocephalidae*; H: *Bathypterois* sp.; I: *B. quadrifilis*; J: *Dicrolene* sp.; K: *Acanthonus armatus*; L: *Bassogigas gillii*; M: *Acanthochaenus leutkeni*; N: Unidentified Macrouridae Type 1; O: Unidentified Macrouridae Type 2; P: Unidentified Macrouridae Type 3; Q: Unidentified Ophiididae; R: Unidentified fish Type 1; S: Unidentified Fish Type 2.



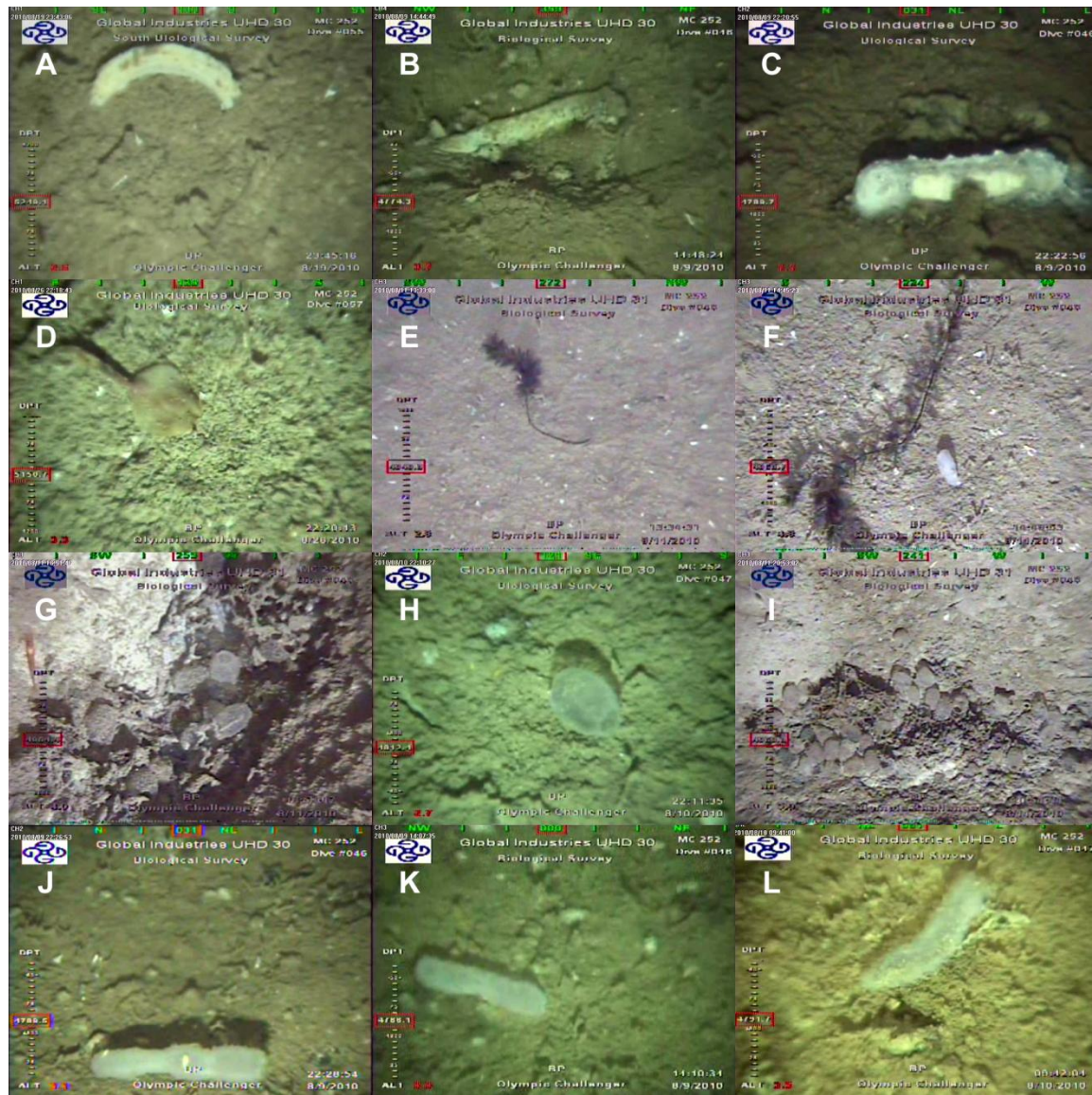


Figure 2.6. Examples of dead invertebrates observed in the vicinity of MC252. A – C: holothurians; D: hexactinellid; E – F: sea pens; G – I: salps; J – L: *Pyrosoma atlanticum*.

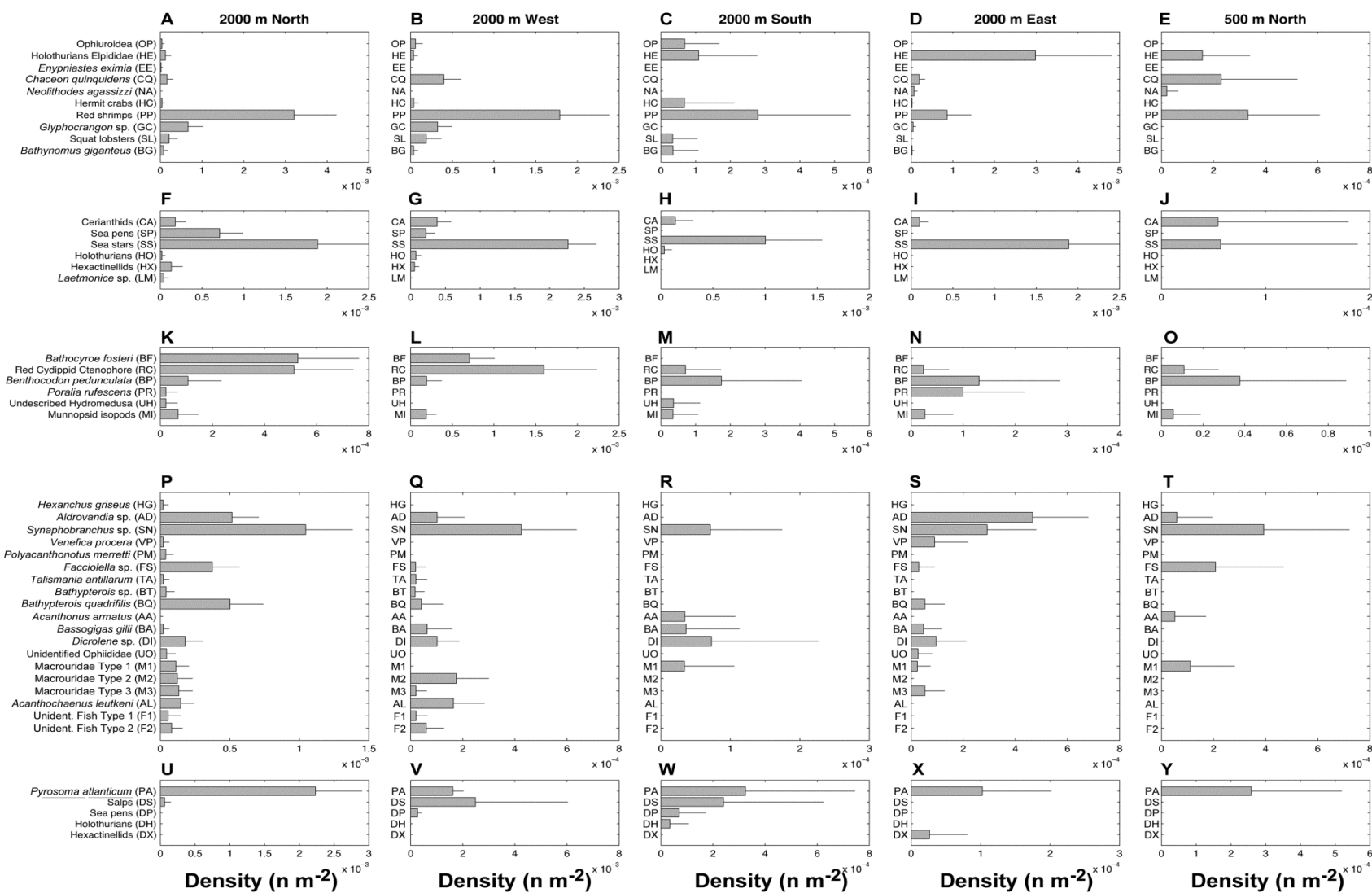


Figure 2.7. Mean densities of mobile invertebrates (A-E), sessile or limited mobility invertebrates (F-J), demersal invertebrates (K-O), fishes (P-T), and dead organisms (U-Y) at each of the survey sites. Error bars are 95% confidences on the means.



Table 2.2. Taxonomic classification and distribution of invertebrates observed in vicinity of MC252.

Mobile Benthic Megafauna						2000m				500m
Phylum	Class	Family	Genus	Species	Common Name	N	W	S	E	N
Echinodermata	Ophiuroidea	Unidentified	Unidentified	Unidentified	Brittle stars	•	•	•		
Echinodermata	Holothuroidea	Elpidiidae	Unidentified	Unidentified		•	•	•	•	•
Echinodermata	Holothuroidea	Pelagothuridae	<i>Enypniastes</i>	<i>E. eximia</i>		•				
Arthropoda	Malacostraca	Geryonidae	<i>Chaceon</i>	<i>C. quinquedens</i>	Red crab	•	•		•	•
Arthropoda	Malacostraca	Lithodidae	<i>Neolithodes</i>	<i>N. agassizii</i>		•			•	
Arthropoda	Malacostraca	Parapaguridae	Unidentified		Deep sea hermit crabs	•	•	•	•	
Arthropoda	Malacostraca	Superfamily Penaeoidea	Unidentified	Unidentified	Red shrimp	•	•	•	•	•
Arthropoda	Malacostraca	Glyphocrangonidae	<i>Glyphocrangon</i>	Unidentified		•	•		•	
Arthropoda	Malacostraca	Galatheididae	Unidentified	Unidentified	Squat lobsters	•	•	•		
Arthropoda	Malacostraca	Cirolanidae	<i>Bathynomus</i>	<i>B. giganteus</i>	Giant isopod	•	•	•	•	
Sessile or Limited Mobility Megafauna						N	W	S	E	N
Cnidaria	Anthozoa	Cerianthidae	Unidentified	Unidentified	Cerianthid anemones	•	•	•	•	•
Cnidaria	Anthozoa	Unidentified	Unidentified	Unidentified	Sea pens	•	•			
Echinodermata	Asteroidea	Unidentified	Unidentified	Unidentified	Sea stars	•	•	•	•	•
Echinodermata	Holothuroidea	Cucumariidae	Unidentified	Unidentified	Sea cucumbers	•	•	•		
Porifera	Hexactinellida	Hyalonematidae	<i>Hyalonema</i>	Unidentified	Stalked glass sponge	•	•			
Annelida	Polychaeta	Aphroditidae	<i>Laetmonice</i>	Unidentified		•				
Demersal Megafauna and Plankton						N	W	S	E	N
Ctenophora	Tentaculata	Bathycyroidae	<i>Bathocyroe</i>	<i>B. fosteri</i>		•	•			
Ctenophora	Tentaculata	Unidentified	Unidentified	Unidentified		•	•	•	•	•
Cnidaria	Hydrozoa	Rhopalonematidae	<i>Benthocodon</i>	<i>B. pedunculata</i>		•	•	•	•	•
Cnidaria	Scyphozoa	Ulmaridae	<i>Poralia</i>	<i>P. rufescens</i>		•	•		•	
Cnidaria	Hydrozoa	Unidentified	Unidentified	Unidentified		•		•		
Arthropoda	Malacostraca	Munnopsidae	Unidentified	Unidentified	Munnopsid Isopods	•	•	•	•	•
Dead Organisms						N	W	S	E	N
Chordata	Thaliacea	Pyrosomatidae	<i>Pyrosoma</i>	<i>P. atlanticum</i>	Pyrosomes	•	•	•	•	•
Chordata	Thaliacea	Salpidae	Unidentified	Unidentified	Salps	•	•	•		
Cnidaria	Anthozoa	Unidentified	Unidentified	Unidentified	Sea pens		•	•		
Echinodermata	Holothuroidea	Unidentified	Unidentified	Unidentified	Sea cucumbers	•		•		
Porifera	Hexactinellida	Hyalonematidae	<i>Hyalonema</i>	Unidentified	Stalked glass sponge				•	

Table 2.3. Taxonomic classification and distribution of fishes observed in vicinity of MC252.

Mobile Benthic Megafauna						2000m				500m
Class	Order	Family	Genus	Species	Common Name	N	W	S	E	N
Elasmobranchii	Hexanchiformes	Hexanchidae	<i>Hexanchus</i>	<i>H. griseus</i>	Six gill shark	•				
Actinopterygii	Notacanthiformes	Halosauridae	<i>Aldrovandia</i>	Unidentified	Halosaurs	•	•		•	•
Actinopterygii	Anguilliformes	Synphobranchidae	<i>Synphobranchus</i>	Unidentified	Cutthroat eels	•	•	•	•	•
Actinopterygii	Anguilliformes	Nettostomatidae	<i>Venefica</i>	<i>V. procera</i>	Duckbilled eels	•			•	
Actinopterygii	Notacanthiformes	Notacanthidae	<i>Polyacanthonotus</i>	<i>P. merretti</i>	Spiny eel	•				
Actinopterygii	Anguilliformes	Nettostomatidae	<i>Facciolella</i>	Unidentified	Duckbilled eel	•	•		•	•
Actinopterygii	Osmeriformes	Alepocephalidae	<i>Talismania</i>	<i>T. antillarum</i>	Slickheads	•	•			
Actinopterygii	Aulopiformes	Ipnopidae	<i>Bathypterois</i>	Unidentified	Tripod fish	•	•			
Actinopterygii	Aulopiformes	Ipnopidae	<i>Bathypterois</i>	<i>B. quadrifilis</i>	Tripod fish	•	•		•	
Actinopterygii	Ophidiiformes	Ophiidae	<i>Acanthonus</i>	<i>A. armatus</i>	Bony-eared assfish			•		•
Actinopterygii	Ophidiiformes	Ophiidae	<i>Bassogigas</i>	<i>B. gillii</i>		•	•	•	•	
Actinopterygii	Ophidiiformes	Ophiidae	<i>Dicrolene</i>	Unidentified	Digitate cusk-eel	•	•	•	•	
Actinopterygii	Ophidiiformes	Ophiidae	Unidentified	Unidentified	Unidentified	•			•	
Actinopterygii	Gadiformes	Macrouridae	Unidentified	Unidentified	Type 1	•		•	•	•
Actinopterygii	Gadiformes	Macrouridae	Unidentified	Unidentified	Type 2	•	•			
Actinopterygii	Gadiformes	Macrouridae	Unidentified	Unidentified	Type 3	•	•		•	
Actinopterygii	Stephanoberyciformes	Stephanoberycidae	<i>Acanthochaenus</i>	<i>A. leutkeni</i>	Pricklefish	•	•			
Actinopterygii	Unidentified	Unidentified	Unidentified	Unidentified	Type 1	•	•			
Actinopterygii	Unidentified	Unidentified	Unidentified	Unidentified	Type 2	•	•			

A statistical comparison of the mean densities of red shrimps among sites indicated that their densities were not significantly different among the 2000-N and 2000-W sites and that the densities at the 2000-S, 2000-E, and 500-N were not different (Fig. 2.8). The red crab *Chaceon quinquidens* was another relatively abundant mobile crustacean (Fig. 2.6). Its mean density was not significantly different among any of the sites with the exception of the 2000-S where it was absent (Fig. 2.8). Mobile holothurians (family Elpidiidae) were present at statistically comparable densities at 2000-N, 2000-W, 2000-S, and 500-N; however, the only location where they were relatively abundant was 2000-E (Fig. 2.8), where their densities were significantly higher than at any station except 500-N.

Densities of sea stars were highest and statistically similar at 2000-N, 2000-W, and 2000-E. Densities at 2000-S were significantly lower only in comparison to 2000-W and the density at 500-N was lowest overall and statistically not different from 2000-S, but significantly lower than at 2000-N, 2000-W, and 2000-E. Cerianthid anemones were present at all sites at statistically similar densities (Fig. 2.7). Sea pens and glass sponges were only present at 2000-N and 2000-W. An undescribed, red species of cydippid ctenophore was present at all sites (Fig. 2.7). Its mean densities were highest and statistically similar at 2000-N and 2000-W (Fig. 2.8) while its densities were lower and statistically similar at 2000-S, 2000-E, and 500-N. Densities at 2000-N and 500-N were also not statistically different (Fig. 2.8). The hydromedusa *Benthocodon pedunculata* was also present at all sites (Fig. 2.7) at statistically similar mean densities (Fig. 2.8). Munnopsid isopods were also present at all sites in statistically similar densities (Fig. 2.8). The lobate ctenophore *Bathocyroe fosteri* was only observed at 2000-N and 2000-W (Fig. 2.7).

Among the fishes, the halosaur *Aldrovandia* sp., the cutthroat eel *Synaphobranchus* sp., the duck bill eel *Facciolella* sp., the tripodfish *Bathypterois quadrifilis*, and two cusk eels:

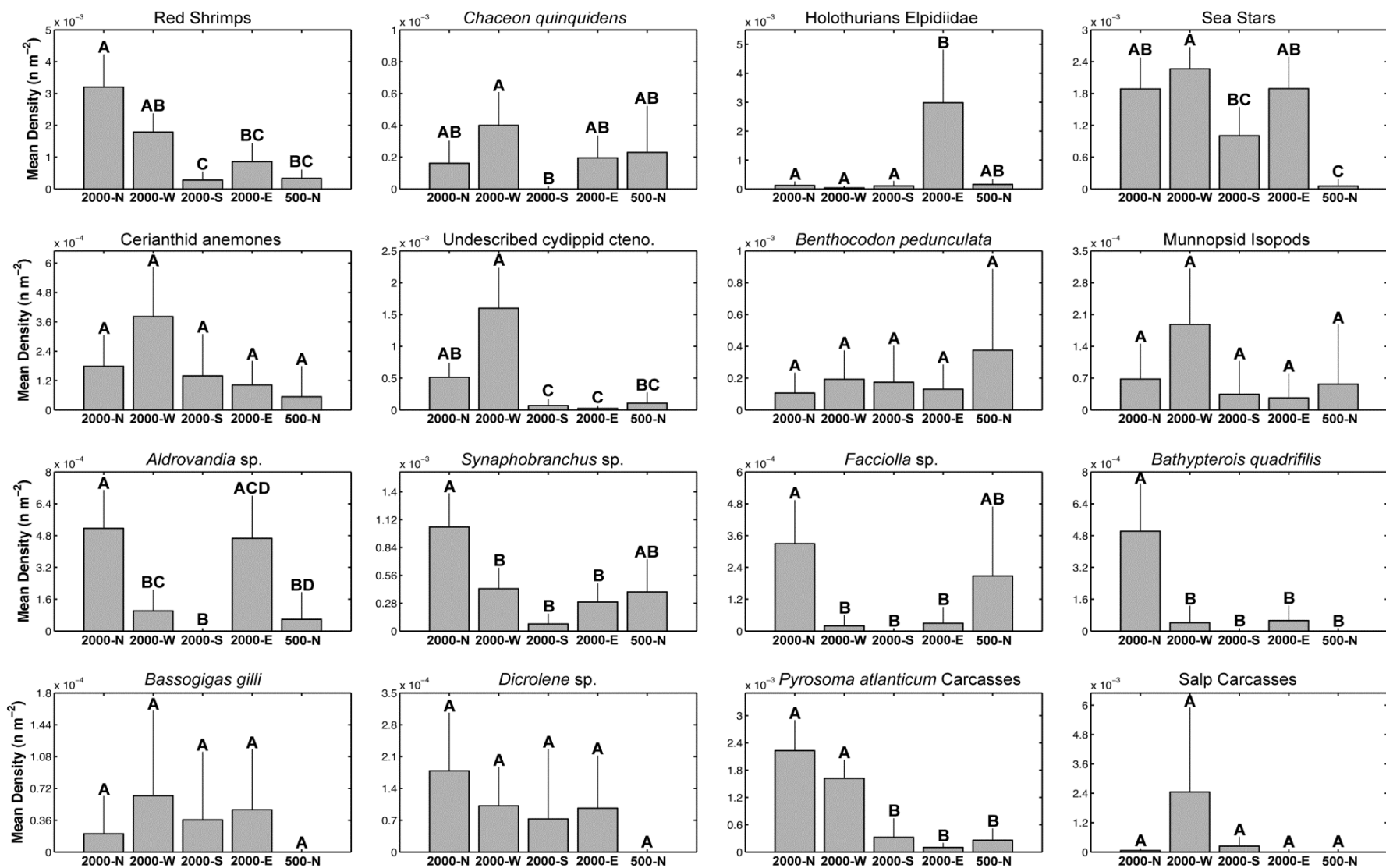
*Bassogigas gillii* and *Dicrolene* sp. were most abundant and widely distributed (Fig. 2.8).

*Aldrovandia* sp. was most abundant at 2000-N and 2000-E and present at significantly lower but similar densities at 2000-W and 500-N (Fig. 2.8). It was absent from 2000-S. *Synaphobranchus* sp. was present at all sites (Fig. 2.7). Its densities at 2000-N and 500-N were statistically similar, while its densities at 2000-W, 2000-S, and 2000-E were significantly lower than 2000-N and were not significantly different from each other (Fig. 2.8). *Facciolella* sp., displayed a similar distribution pattern to *Synaphobranchus* sp. although it was absent from 2000-S (Fig. 2.8). The mean density of *Bathypterois quadrifilis* was greatest at 2000-N and significantly lower at 2000-W and 2000-E, while it was absent from 2000-S and 500-N (Fig. 2.8). Both cusk eel species displayed a similar pattern with densities at all sites except 500-N (where they were absent) that were statistically similar (Fig. 2.8).

### **2.3.3 Mortality**

Carcasses of the pyrosome *Pyrosoma atlanticum* were present at all sites (Fig. 2.7). Mean densities were highest and statistically similar at 2000-N and 2000-W, while at 2000-S, 2000-E, and 500-N their densities were lower and not significantly different from each other (Fig. 2.8). Dead salps of an unrecognizable species were found at 2000-N, 2000-W, and 2000-S (Fig. 2.7). Their densities were not significantly different among sites or from zero (Fig. 2.8). Dead sea pens were restricted to 2000-W and 2000-S (Fig. 2.7) while dead holothurians (n=3) were found at 2000-S and a single dead glass sponge was observed at 2000-E (Fig. 2.7).

Figure 2.8. Mean densities of selected taxa at the five survey sites. Error bars are 95% confidences on the mean. Sites that share the same letter are not significantly different ( $p>0.05$ ) based on a Dunn's multiple comparison test following a significant Kruskal-Wallis test.



### **2.3.4 Pre- and Post-Spill Abundance Indices**

During the two Gulf SERPENT surveys conducted prior to the spill, a total of nine different taxa were observed: *Chaceon quinquidens*, *Plesiopenaeus* sp., cerianthid anemones, *Adrovandia* sp., *Synaphobranchus* sp., *Venefica procera*, *Bathypterois quadrifilis*, *Dicrolene* sp., and an unidentified species of macrourid (type 3). The encounter rates for these taxa during the two pre-spill surveys provided an estimate of their abundance (Table 2.4). Comparisons of the mean encounter rates of these taxa pre- and post-spill indicated that for all taxa with the exception of *B. quadrifilis* at 2000-N, the mean encounter rates at our sites were below the pre-spill means (Table 2.4). For the red crab, post-spill mean encounter rates were statistically lower at 2000-N and 2000-S (Table 2.4). Red shrimps had a significantly lower mean encounter rate at 2000-S. *Venefica procera* had significantly lower encounter rates post-spill at 2000-N and 2000-E and was not encountered at 2000-W, 2000-S, or 500-N. *Dicrolene* sp. was encountered at statistically lower rates at 2000-W, 2000-S, and 2000-E than during the pre-spill surveys and was not encountered at 500-N.

### **2.4 Discussion**

Our surveys revealed substantial differences in the abundances and biodiversity of marine life around the Macondo BOP. The sites located 2000 m to the north and west of the BOP generally contained the greatest taxonomic richness while the sites to the south and east at 2000 m range had lower densities and numbers of taxa. It is not possible to determine whether all four of the 2000 m sites had similar numbers and densities of taxa prior to the spill given the absence of quantitative pre-spill sampling. We do note that these sites share relatively close proximity, similar depths, and bottom types. Given that a subsea hydrocarbon plume was reported to extend SW from the BOP, the low numerical densities and numbers of taxa at 2000-S might be

indicative of an impact from the plume due to either emigration or mortality of animals. The location of the plume, which was centered at a depth of approximately 1100 m (Camilli et al. 2010), would place it some 300 – 400 m above the seafloor. The spatial and temporal extent of its interaction with the seafloor is not well understood and it is difficult to know whether hydrocarbon concentrations at the seabed were sufficiently high to impact animal densities. The site located 500 m north of the BOP usually had the lowest numbers and taxonomic richness, which may indicate that the concentrations of hydrocarbons near the BOP were sufficiently high to prevent most organisms from occupying that site. It might also reflect the high levels of sustained subsea activity (e.g. lights, noise, and hydrodynamic disturbances) in the area close to the BOP associated with the attempts to control the well. Although we were unable to survey other sites located on different bearings at the same range from the BOP, the most extreme contrasts in densities and taxonomic richness were between 500-N and 2000-N. This may indicate that, at least to the north of the BOP, the greatest acute impacts of the spill occurred at distances of less than 2000 m. It is also possible that the lower densities and diversity at the 500-N site were a consequence of the reduced survey effort at that location (only the northern half of the site could be surveyed).

The presence of relatively low taxonomic richness and densities at 2000-E are more difficult to relate to the spill given the reported trajectory of the subsea plume. Unlike the surveys at the remaining four sites, 2000-E was the last site to be surveyed and it was investigated without the presence of either of the authors. The site was surveyed on two different dates. Some transects, apparently selected arbitrarily were surveyed initially and other transects, were surveyed on the second date.



**Table 2.4.** Pre-spill abundance indices ( $n \text{ min}^{-1}$ ) for all taxa observed during Gulf SERPENT surveys around the Macondo BOP on 02/19/10 and 03/27/10 and post-spill abundance mean indices estimated for each of our survey sites for the same taxa. Hyphens indicate the taxon was not detected during post-spill surveys. The p values are the outcomes of Kruskal-Wallis analysis of variance of ranks for the mean pre-spill index and the five post-spill surveys. Asterisks indicate significant differences between pre-spill and post-spill indices (Dunn's multiple comparison test,  $p < 0.05$ ).

	<b>Pre-Spill Survey Abundance Index (<math>n \text{ min}^{-1}</math>)</b>			<b>Post-Spill Survey Abundance Index (<math>n \text{ min}^{-1}</math>)</b>					
<b>Taxon</b>	<b>02/2010</b>	<b>03/2010</b>	<b>Pre- Spill Mean</b>	<b>2000-N</b>	<b>2000-W</b>	<b>2000-S</b>	<b>2000-E</b>	<b>500-N</b>	<b>p value</b>
<i>Chaceon quinquidens</i>	0.118	0.427	0.272±0.665	0.005*	0.017	-	0.011	0.019	0.0004
Red shrimps	0.221	0.133	0.177±0.188	0.036	0.011	0.017*	0.009	0.020	<0.0001
Cerianthid anemone	0.029	0.000	0.014±0.063	0.005	0.012	0.004	0.005	0.005	0.0873
<i>Aldrovandia</i> sp.	0.044	0.013	0.029±0.066	0.020	0.009	0.004	0.004	0.006	<0.0001
<i>Synaphobranchus</i> sp.	0.074	0.013	0.043±0.130	0.036	0.017	0.003	0.017	0.024	<0.0001
<i>Venefica procera</i>	0.000	0.120	0.060±0.258	0.001*	-	-	0.004*	-	0.0108
<i>Bathypterois quadrifillis</i>	0.015	0.013	0.014±0.003	0.015	0.001	-	0.011	-	<0.0001
<i>Dicrolene</i> sp.	0.074	0.040	0.057±0.072	0.007	0.003*	0.002*	0.006*	-	0.0032
Macrouridae type 3	0.000	0.027	0.013±0.057	0.005	0.001	-	0.003	-	0.0182

The implications of temporally separating the surveys of different transects at 2000-E on estimates of densities and biodiversity may be different for mobile or sessile/limited mobility taxa. For the sessile taxa, we assume that a relatively short interval of two weeks between surveys would not substantially change the density or diversity estimates. Mobile organisms were potentially problematic because of the possibility that the same animal could be detected on multiple transects leading to an overestimation of their true density. While we did not have any means to control for this, nor assess the magnitude of double counting, we assumed that animals moving through the study area would be as likely to be counted twice as they would to be missed entirely. As a consequence we assumed that we were on average, approximating their true densities. For mobile taxa, we have to assume that the taxonomic composition and numerical densities were similar during the two surveys. If they were not, then it is possible that we either under- or over-estimated biodiversity and density.

We noted that the average survey velocity at 2000-E was  $0.16 \text{ m s}^{-1}$ , which was significantly higher (Dunn's multiple comparison test following a significant Kruskal-Wallis test,  $p < 0.0001$ ) than the mean velocities at either 2000-N ( $0.09 \text{ m s}^{-1}$ ), 2000-W ( $0.10 \text{ m s}^{-1}$ ), though not statistically different from the mean velocities at 2000-S ( $0.12 \text{ m s}^{-1}$ ) or 500-N ( $0.15 \text{ m s}^{-1}$ ). The velocity of the survey platform can have a significant impact on the probability of detecting targets. This has been demonstrated for strip-transect aerial counts of seabirds (Certain and Bretagnolle, 2008), diver transect counts of temperate rocky reef fishes (Lincoln Smith, 1988), and ROV counts of flatfishes in coastal waters (Norcross and Mueter, 1998). In general speed impacts detection because speed is inversely related to the amount of time available to scan the scene for targets. This issue is compounded with interlaced video because motion blur becomes more of a problem at higher survey speeds. Thus, the more rapidly the ROV is moving,

the more likely it is that organisms will not be detected. This may present more of a problem for detecting small organisms or animals with low contrast relative to the background. If the lower densities at 2000-E were due to bias induced by the higher survey speed of the ROV at that site and the true animal densities were in fact similar to those at 2000-N or 2000-W, then we would expect similar relative abundances with proportionally lower densities. This premise assumes that the slope of the relationship between ROV speed and abundance is similar for all taxa. The relative abundance patterns for the most abundant taxa do not appear to support the hypothesis that lower densities at 2000-E are due to the higher ROV velocity. Densities of Elpidiid holothurians were highest at 2000-E (Fig. 2.8). These animals are quite difficult to observe when they are on the seabed due to their translucence and pale coloration. Densities of sea stars were also similar at 2000-E to their abundance at the other 2000 m sites. A similar pattern was noted for the halosaur *Aldrovandia* sp. Thus, while we cannot completely rule out some underestimation of abundances at this site due to ROV speed, there does not appear to be evidence of a clear relationship. Moreover, even our highest mean velocities were lower than those employed by other studies using ROVs to quantify megafauna (e.g. Jones et al., 2006, 2007; Trenkel et al., 2004a).

Our survey design was an enhancement of the Bureau of Ocean Energy Management (BEOM: formerly Minerals Management Service) pre-bottom survey. That design was prescribed for characterization of biological conditions on the seafloor prior to drilling operations. It consisted of six radial transects, 100 m in length on bearings offset by 60 degrees (Minerals Management Service, 2008). That design has been used to assess the impact of subsea spills on megafauna (Boland et al., 2004). Our design extended the length of each transect to 250 m and increased the number of individual radial transects to 24, although we had to reduce the

number of transects at some sites due to time constraints or the necessity of moving the vessel to allow other ships access to the site for higher-priority operations. Radial surveys of a similar design are common in benthic ecology. Examples include studies of the impacts of drilling on megafauna in the Faroe-Shetland Channel (Jones et al., 2006, 2007) who used 18 x 250 m radial transects flown at  $0.3 \text{ m s}^{-1}$ , an assessment of the responses of megabenthos to deepwater drilling in the Orinoco Basin, Venezuela (Jones et al., 2011) who employed 8 x 80 m radial transects flown at  $0.3 \text{ m s}^{-1}$ . While some of these studies have attempted to use the radial design to evaluate hypotheses about distributional patterns of megafauna around a drilling site, the lack of replicated transects in each direction complicates the interpretation of such data. In our case, we were not interested in whether there were differences in the densities of organisms along different bearings from the center point. Rather we were using each transect as a replicate to determine whether the mean density within each study site is different from the means at other sites. In many cases, the variance of our data was high possibly owing to patchy distributions and the generally low densities of organisms. This high variance, combined with the need to employ non-parametric tests with lower statistical power, to compare differences among sites, meant that we did not find significant differences in densities among sites even though there were trends in the means. The solution to this problem may require longer transects so that greater areas are sampled, or finer angular separation between transects. Such an approach could reduce the number of transects containing zero organisms and therefore reduce the overall variance. We also recognize that in cases where the time required to completely survey a site was long, overestimation of the densities of mobile taxa may have occurred due to aliasing of the same organisms on different transects. While we did not have any means to control for this, nor assess the magnitude of double counting, we assumed that animals moving through the study area

would be as likely to be counted twice as they would to be missed entirely. As a consequence we assumed that we were on average, approximating their true densities.

Electro-hydraulic ROVs are loud systems with bright lights. In a dark, quiet environment such as the deep sea, they are likely to affect the behavior of some mobile taxa that are sensitive to light or sound. As Trenkel et al. (2004a) point out, both avoidance of, and attraction to ROVs by fishes have been documented. We recognize the potential for biases when ROVs are utilized to estimate abundance. This is especially important when bias leads to under- or over estimation of organismal abundances. Such errors may arise from accidental multiple counting of organisms, organism avoidance or attraction to ROV (via lighting, noise, pressure changes, or olfactory cues), ROV speed, and possibly detection of electric or magnetic fields (Stoner et al, 2008). Despite knowledge of these factors, there have been few quantitative studies on how stimuli associated with ROVs may bias estimates of animal densities. The impact of lighting on presence appears to be dependent on taxonomic grouping with reactions ranging from strong avoidance to attraction (Trenkel et al., 2004a; Ryer et al., 2009). Trenkel et al (2004a) performed surveys at 2 different velocities ( $0.25 \text{ m s}^{-1}$  and  $0.5 \text{ m s}^{-1}$ ) to determine whether survey speed affected density estimates. They found that while the slower survey speed resulted in greater detections of individuals, the mean densities of most taxa were not statistically significantly different at the two survey speeds with the exception of two taxa, one of which was only documented at the faster survey speed. Our survey speeds ranged from  $0.09 - 0.16 \text{ m s}^{-1}$ , which were substantially slower than the speeds used in the Trenkel et al. (2004a) study.

While double counting is generally a non-issue for organisms with limited or no mobility, it is potentially problematic for mobile fishes and invertebrates. During a study of rockfishes off the coast of Alaska, Rooper et al. (2012), presumed that multiple counting of the same organisms

was a limited occurrence as the ROV traveled in a uniform direction and typically passed fish after they were documented. We have assumed that animals moving through the study area would be as likely to be counted twice as they would to be missed entirely. Our study was not designed to evaluate this potential source of bias and we have assumed that on average we were approximating their true densities.

Identification of organisms from video is a challenge, particularly in the absence of physical samples. This has been recognized in many studies (e.g. Busby et al., 2005; Howell et al., 2010; Trenkel et al., 2004b). Although we have referred to our organisms as taxa with various levels of taxonomic resolution, we recognize that they are in fact morphotaxa in the absence of physical samples and higher-resolution images. Some of our taxonomic groups are quite coarse and certainly contain more than one unique species. Examples of such categories are red shrimps, and sea stars. During the Deep Gulf of Mexico Benthos (DGoMB) Project, Rowe and Kennicutt (2009) collected six species of benthic red shrimps (*Aristaeopsis edwardsianus*, *Benthesicymus bartletti*, *Hemipenaeus carpenter*, *Plesiopenaeus armatus*, *Nematocarcinus ensifer*, and *N. rotundus*) from two stations (S36 and S37) located 70 km ENE, and 62 km ESE of the Macondo BOP in 1784 m and 2369 m of water, respectively. It is likely that some or all of these species were encompassed within our coarse category of red shrimps. Similarly, the DGoMB samples from S36 and S37 contained three different species of sea stars: *Nymphaster arenatus*, *Plutonaster agassizii*, and *Benthopecten simplex* (Rowe and Kennicutt, 2009), which were likely encompassed within our sea star category. While we accept that our video data imposed limits on our ability to resolve unique species within many of our groups, this also means that we cannot distinguish subtle or gross differences in the spatial distributions of the constituent species due to differential responses to hydrocarbons.

Our data suggest that some taxa were potentially more sensitive to exposure to hydrocarbons than others. Comparisons of the densities of taxa present at 2000-N and 2000-W in relation to 2000-S and 500-N may provide some insights into their potential responses to the spill. Examples of taxa that were present at 2000-N and 2000-W but absent at 2000-S and 500-N include the invertebrates: *Glyphocrangon* sp., sea pens, hexactinellids, the ctenophore *Bathocyroe fosteri*; and fishes: Alepocephalidae, *Bathypterois grallator*, an unidentified Macrouridae (type II), *Acathochaenus leutkeni*, and both types of unidentified fishes (Fig. 2.7). A second approach to identify taxa potentially-vulnerable to impacts of the spill is to use the data from the Gulf SERPENT pre-spill surveys to identify organisms that were absent post-spill from 2000-S or 500-N. This approach suggests that Nettastomatid eels, *Bathypterois quadrifilis*, and an unidentified macrourid (type 3) were also potentially sensitive taxa.

Direct observations of dead organisms provide stronger potential evidence of a spill-related mortality, as no carcasses were documented during the pre-spill assessment. Aside from carcasses of *Pyrosoma atlanticum* and salps, relatively few dead organisms were observed. In part this may be because the high likelihood of scavenging of any dead organism made it unlikely that any soft-bodied organism would remain unconsumed for very long. The presence of dead sea pens at both 2000-W and 2000-S may suggest that these cnidarians are more sensitive to hydrocarbons. Apparently dead holothurians were observed at 2000-S. They were probably not very abundant prior to the spill around the Macondo BOP because of their absence from the Gulf SERPENT pre-spill surveys, so it is difficult to know whether the generally low numbers of live and dead individuals was a response to the spill.

The pyrosome and salp carcasses were the two most abundant groups of dead organisms. Both of these taxa are planktonic. In the case of the pyrosomes, the densities were higher and not

statistically different at 2000-N and 2000-W but lower and statistically-similar at the remaining sites (Fig. 2.8). Mass deposition of pyrosome carcasses have been reported elsewhere. Lebroto and Jones (2009) described a large accumulation of dead *P. atlanticum* on the seafloor off the Ivory Coast during 2006. The cause of this mortality was not known. They also cited earlier records of dead pyrosomes off the Cape Verde Islands and the Madeira Abyssal Plain. They noted that the carcasses were slow to degrade and consumed by very few scavengers. The densities of carcasses at their deepest survey depths (>1100 m) ranged from 0.11 to 28.0 pyrosomes  $\text{m}^{-2}$ , which were orders of magnitude greater than were observed at 2000-N where our highest densities occurred. Why pyrosomes were not distributed at statistically-similar densities among all our sites is not clear. We do know that *P. atlanticum* is a strong vertical migrator. Unpublished data collected on planktonic organisms during the same Olympic Challenger cruises reported in the present study indicate that pyrosomes reside in the upper 100 m during the night and descend to 300 – 450 m by 06:00 in the morning. If the animals observed close to 06:00 were still migrating downward then these data are consistent with Gulf SERPENT observations from elsewhere in the northern Gulf including Mississippi Canyon that indicate a daytime distribution of 475 – 640 m. If pyrosome mortality occurred within the upper ~600 m of the water, then their heterogeneous distribution on the seafloor may reflect prevailing water mass movements immediately following mortality, patchiness in their distributions in the waters over the study area, the spatial distribution of toxic fractions in the water column that contributed to mortality, or some combination of these and perhaps other factors. Lebroto and Jones (2009) also noted patchiness in the distributions of carcasses off West Africa. Moreover, dead pyrosomes were reported by a variety of journalists on the surface of the Gulf within the spill area. What factors could carry pyrosomes to the surface remain conjectural, however, once on the surface



they would have been subject to the same physical processes that produce localized accumulations of sargassum and other flotsam. If they ultimately lost buoyancy and sank to the bottom then one would expect patchy distributions on the seafloor.

In the absence of quantitative pre-spill data from the vicinity of the Macondo BOP, it is a challenge to evaluate the degree to which the densities of organisms have changed in response to the spill. The DGoMB dataset includes samples collected during June 2000 from their stations S36 and S37 (described above). By consolidating their megafaunal data into categories comparable to our taxonomic groups, one can see that for many of our taxa, the densities of organisms in these groups were similar (Fig. 2.9). With the exception of the brittle stars and Elpidiidae, all non-zero densities at our sites were of similar or greater magnitude than were observed in the DGoMB study 2000 prior to the spill. It is important to emphasize that none of the DGoMB stations were close to MC252, and both the sites closest to our study area were 62-70 km distant. Moreover, the depths of their sites were both deeper than the MC252 stations.

Many studies have shown that both species richness and abundance decline with depth (e.g. Pequegnat, 1983; Powell et al., 2003; Rowe, 1983; Yeh and Drazen, 2009). This may partially explain why the densities of invertebrate megafauna were generally lower at the deeper DGoMB stations than at our sites; however, the magnitude of the differences was often so large that depth alone cannot explain all of the differences observed. It is important to note that direct comparisons of trawl and ROV-derived estimates of abundance are problematic (Trenkel et al., 2004a, 2004b), due to differential catchability among taxa. Adams et al. (1995) found that ROV-derived abundances tend to be higher than trawl-based metrics in a study of demersal fishes. In a shallower study (73 – 366 m), Uzmann et al. (1977) found that an otter trawl produced

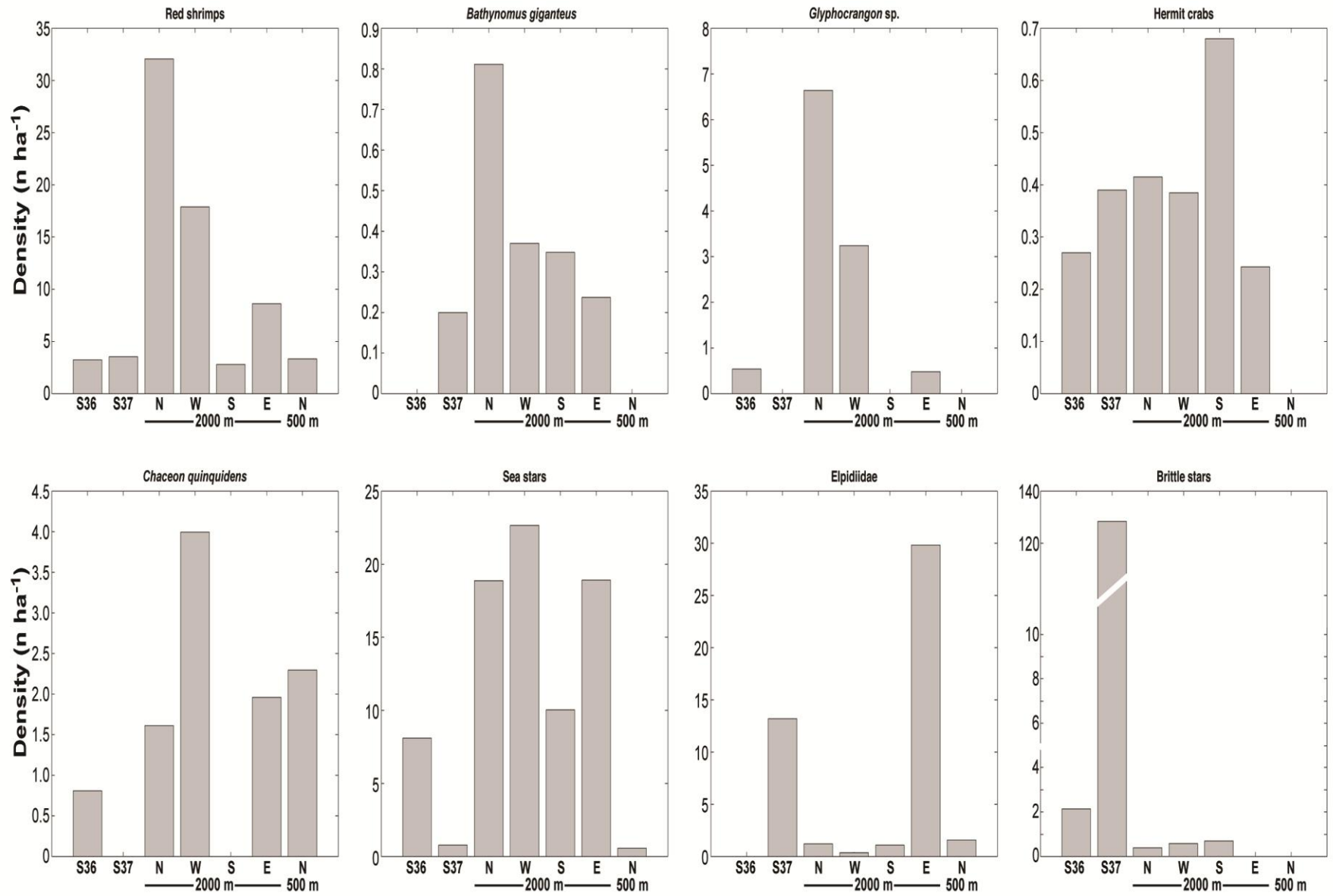
significantly lower estimates of the abundances of crabs, lobsters, some fish species compared to visual observations from a manned submersible.

With the exception of the lobate ctenophore *Bathocyroe fosteri* and an undescribed cydippid ctenophore, demersal invertebrate taxa generally showed densities that were statistically similar among all stations including 500-N. This pattern was consistent with the continual influx of water containing similar albeit patchy concentrations of animals through the study area. The lower densities of *B. fosteri* may reflect difficulty in observing these largely transparent organisms. The undescribed cydippids are similar in size and color to the medusa *Benthocodon pedunculata*, the latter of which did not show a statistical difference in densities among sites. It is possible that the cydippids were more sensitive to hydrocarbons than the medusa, which might have led to a widespread reduction in their abundances in some areas. If the water flowing through the sites where their abundance was low came from areas where their populations had been substantially reduced, then it could explain observed differences in their densities. In the absence of synoptic near-bottom current data and plankton abundance estimates, we cannot explain the observed differences in distributions.

## **2.5 Conclusions**

The DWH Spill had the potential to impact benthic and demersal megafauna communities surrounding the spill site. Much of the focus during the period that the Macondo well was discharging oil and gas into the Gulf of Mexico (response phase) was directed towards collecting physical and chemical measurements within the water column. Relatively little of the sampling conducted during the response phase was directed towards collection of biological data. This study is to our knowledge, the earliest comprehensive attempt to characterize biological conditions around the Macondo well.

Figure 2.9. Densities of selected taxa enumerated during June 2000 using deepwater trawls at two sites (S36: 28.93323°N, 87.64504°W, 1784 m and S37: 28.58750°N, 87.74785°W, 2369 m) as part of the Deep Gulf of Mexico Benthos Program in comparison with data from the present study. To facilitate this comparison we combined their abundances of following taxa to enable comparison with our groups: red shrimps (*Aristaeopsis edwardsianus*, *Benthescymus bartletti*, *Hemipenaeus carpenteri*, *Plesiopenaeus armatus*, *Nematocarcinus ensifer*, *N. rotundus*), sea stars.



There were clear differences in the taxonomic composition and abundances of benthic megafauna, demersal plankton, and fishes among our five study sites. The generally lower taxonomic richness and densities at the site located to the south, were consistent with the reported trajectory of a subsea plume of dispersed hydrocarbons. These data suggest that mortality, emigration, or some other unmeasured factor was responsible for the lower densities and taxonomic richness of mobile taxa at the southern site. The generally lower densities and diversity at the 500-N site suggested that the concentrations of hydrocarbons could have been sufficiently high within 500 m of the well to induce mortality, emigration, or both responses. The widespread presence of carcasses of pyrosomes and salps suggests that the spill impacted planktonic assemblages within at least a 2000 m radius of the BOP.

The data collected during these surveys provides a post-spill baseline that could be used to determine whether the biodiversity and abundances at these sites are changing. We conducted follow-on ROV surveys employing the same survey protocols at most of these sites during March, June, and August 2011. When analyses of those surveys are completed, we will be better able to determine if and how the communities around the Macondo well are responding to the DWH Spill.

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## **CHAPTER 3: COMPOSITION OF EPIBENTHIC AND DEMERSAL MEGAFUNA COMMUNITIES IN THE VICINITY OF THE MC252 RELIEF WELL 2, DURING THE LATE SUMMER AND FALL OF 2010**

### **3.1 Introduction**

Discharge of oil and gas into marine systems has the potential to impact deep-sea fauna in a variety of ways. These impacts include acute lethal toxicity, hydrocarbon stress-related diseases, physical smothering from oil, disruption of behavioral activities, reduced food availability, physiological uptake of hydrocarbons, and habitat alteration (Moore and Dwyer, 1974; Sinderman et al., 1982; Allison et al., 2003). Opportunistic species can also benefit from organic enrichment. There is also the possibility that potentially-toxic subsurface oil persists in the environment leading to chronic exposures, which even at sub-lethal levels, may continue to impact the health of these organisms, thus delaying recovery (Peterson et al., 2003). Long-term exposure could ultimately result in changes in local fauna demography, genetic structure, reproductive success, and subsequent recruitment (Suchanek, 1993)

The Deepwater Horizon (DWH) oil spill resulted in approximately 200 million gallons of oil being released from the Macondo well (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2011) into the Northern Gulf of Mexico (GoM). In addition, nearly 1.8 million gallons of chemical dispersants were applied near the source and at the surface to expedite the natural breakdown of this oil (National Commission on the BP Deepwater Horizon Oil Spill and Offshore Drilling, 2011). This combination of oil, gas, and dispersants, which originated in the bathypelagic zone resulted in an ascending plume of oil and gas, a surface oil slick, and a subsurface oil plume (centered at a depth of 1100 m) of highly dispersed oil (Camilli et al. 2010). The sinking of the DWH rig, the release of oil near the sea floor, the application of dispersants into the oil and gas at the discharge point, and other subsea activities

associated with the well control effort had the potential to induce both physical and chemical stresses on benthic and demersal organisms in the vicinity of the spill site. If acute toxicity and smothering occurred in areas closest to the wellhead, there would likely be widespread absence of megafauna and faunal diversity. In this instance abundance would be greatest away from the well due to dilution.

Determining the impact of the spill on marine life in the vicinity of the well is a central goal of the Natural Resource Damage Assessment. This is particularly challenging in the deep-sea because of our limited understanding of the taxonomic composition, distribution, seasonality, and abundance of deep sea invertebrates and fishes in the vicinity of the Macondo well prior to the spill. The absence of quantitative pre-spill data on the condition of the near-bottom ecosystem is particularly problematic. Moreover, during the response phase of the spill, activities were primarily directed towards well control and contaminant monitoring. As a consequence, it was not until after the well had been contained that the focus shifted to include more biological characterization. Qualitative surveys were conducted with the aid of remotely operated vehicles (ROV) deployed from the DWH as part of the Gulf SERPENT Project during February and March 2010 in the vicinity of the Macondo well; however it should be noted that these surveys provided an incomplete picture of which taxa were numerically abundant on the seafloor near the Macondo well and the only metrics that can be derived from these surveys are based on encounter rates (numbers per unit survey time). Thus, determining the magnitude of the impact of the DWH spill on benthic and demersal megafauna and fishes is challenging in the absence of quantitative pre-spill information about the composition and abundances of the community.

A large number of vessels equipped with ROVs were deployed to support the response effort to the DWH spill. A mobile offshore drilling unit called the Development Driller 2 (DD2)

was drilling one of the relief wells (MC252#2) at a site located approximately 750 m SW of the Macondo well. The DD2 was equipped with a pair of industrial ROVs that were tasked by BP to conduct periodic video transect surveys of the sea floor around MC252#2 in order to quantify the taxonomic composition and abundances of marine life during the fall of 2010.

The primary objectives of this study were to: (1) characterize the composition and abundances of megafaunal benthic and demersal organisms surrounding MC252#2 immediately following the DWH spill; and (2) determine how variable these parameters were during a period of 16 weeks. Here we present the results of a study designed to assess the acute effects of the oil on the megafauna in the area surrounding the accident site.

### **3.2 Methods**

Eleven seafloor surveys were conducted at approximately 2 week intervals between August 4, 2010 and November 1, 2010) by a pair of industrial ROVs deployed from the DD2 at the MC252 #2 site. This site is located approximately 750 m SW ( $28^{\circ} 43' 53.338''$  N,  $88^{\circ} 22' 17.927''$  W) of the MC252 Macondo wellhead at an average depth of 1578 m (Fig. 3.1). Surveys spanned a three-month period (Aug 4 – Nov 1, 2010) (Table 3.1). In addition, one survey was also performed on May 16, 2010, prior to the onset of drilling MC252 relief well #2, as part the Bureau of Ocean Energy Management (BOEM) requirement for a pre-bottom assessment of biological conditions. The ROV's aboard the DD2 were Innovator 8 (I8) and Innovator 14 (I14) operated by Saipem America under contract to BP. These industrial vehicles are 150 HP systems equipped with standard definition (SD: 480p) Insight Pacific Pegasus color video cameras on pan and tilt mounts. Each ROV was equipped with incandescent lighting.

The August – November surveys consisted of 24 250 m long radial transects offset by 15° angles, originating from a point of origin located within 3.6 – 4.6 m of the blow out preventer of relief well #2 (BOP) (Fig. 3.1). The BOEM survey conducted in May also employed a radial design with six, 100 m-long transects offset by 60° intervals (Fig. 3.1). There was no subsea navigation system to record the location of the ROV and surveys were conducted by flying the ROVs along fixed headings using their gyroscope. Transect distances were estimated by monitoring the amount of tether paid out.

The ROV attempted to maintain a constant heading and speed at an altitude of approximately 2 m above the benthos throughout each transect. The pilot attempted to keep the ROV tether off of the seafloor during the outbound transects in order to minimize sediment disturbance. The presence of hazardous debris required some transects to be terminated before r

Video was recorded throughout each survey; however, only video recorded during the outbound section of each transect was used for faunal identification and estimation of invertebrate and fish abundances. Cameras were aimed forward and downward at an oblique angle so the field of view was of the sea floor directly in front the ROV and unobscured by other equipment on the ROV. Video cameras maintained zero zoom (wide angle) throughout most of each transect and only briefly zoomed in on possible biological targets as they were encountered. The level of incandescent lighting was held constant throughout the entire length of the survey.

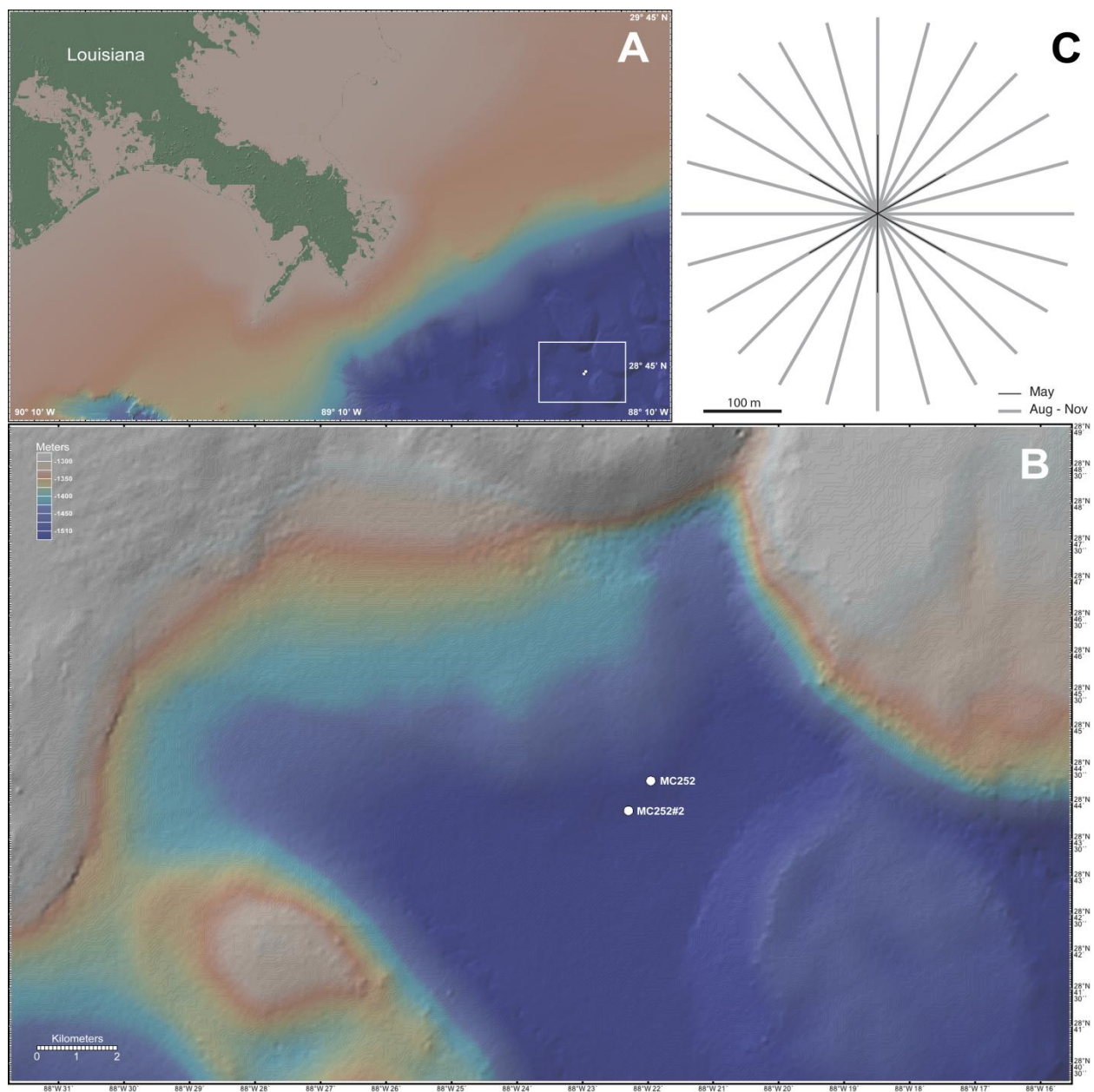


Figure 3.1.A: Study area in relation to the Louisiana Coast. The white rectangle indicates the area defined by the higher resolution map (B), which shows the locations of the Macondo Well (MC252) and the study site (MC252#2). C: The survey design used in Aug – Nov 2010 surveys and May 2010.

were processed with VideoRedo Plus imaging software after correcting for time code errors and conversion to Motion Pictures Expert Group (MPEG) format. Video footage was reviewed at 1x normal playback speed. ROV heading and altitude were manually recorded at one minute intervals during the length of each transect to assist in determining ROV position along transect over time while altitude was recorded for use in calculating of field of view. When possible biological targets were present, still images were extracted for documentation and identification. The level of taxonomic resolution varied due to differences in water clarity and image quality.

To determine the area surveyed, it was necessary to calculate field of view (FOV) and swath width at various altitudes. The ROVs were not equipped with optical reference points from laser scalers. Thus the measurements of five manmade objects (beer cans: 13 mm tall x 66 mm wide) of known dimensions combined with information about the angular field of view of the camera (48° horizontal, 37° vertical) were used to estimate the average downward viewing angle (slant angle) of the camera (Equation 1).

$$slant\ angle\ (^{\circ}) = \sin^{-1} \left( \frac{can\ length\ (pixels) \times 2 \times \tan\left(\frac{48}{2}\right) \times altitude\ (m)}{image\ width\ (pixels) \times can\ length\ (m)} \right) \quad (1)$$

Once the slant angle had been determined, a function describing the linear relationship between field of view and altitude (equation 2) based on the mean downward viewing angle was calculated as:

$$fov\ (m) = 0.4822 \times altitude\ (m) \quad (2)$$

Altitudes were recorded from the video overlay at 1 min intervals and smoothed using a spline function (Matlab Curve Fitting Toolbox). These data were linearly interpolated on to a 1 s time interval (Matlab interp1 function) and Equation 2 was then used to estimate the FOV at 1 s

intervals along each transect. The mean FOV for each transect was multiplied by the transect distance (250 m) to estimate the area surveyed by the ROV. The May video overlay lacked altitude data. Depth and altitude data from the August transects run on the same headings as in May were used to estimate altitudes for the latter survey. The FOV during May was then calculated the same manner as the Aug – Nov surveys assuming the same slant angle.

Observed megafauna were sorted into 5 categories, mobile benthic invertebrates, invertebrates with limited or no mobility, demersal invertebrates, and carcasses. Density was calculated as the number of each taxon observed divided by the area surveyed on each transect. A mean site density and 95% confidence interval on the mean was estimated by averaging all transect densities. To evaluate the null hypothesis that mean densities of selected taxa were drawn from populations with the same variance, a Levene's Test was performed (Trujillo-Ortiz, and Hernandex-Walls, 2003). Then a Kolmogorov-Shmirnov test was performed to evaluate the null hypothesis that densities from all transects during each survey period were normally distributed. If the assumptions of homogeneity or normality of variances were not met, a non-parametric Kruskal-Wallis analysis of variance of ranks was performed to evaluate the null hypothesis that the densities of the selected taxon were similar for all survey periods. When the null hypothesis was rejected, a non-parametric multiple comparison test, known as the Dunn's test (Cardillo, 2006) was performed. These statistical tests were performed using Matlab.

### **3.3 Results**

A total of 215 transects were conducted during 18 days of surveys. On average each transect had a swath width of 4.07 m. The average survey area of this 500 m x 500 m site was 24,420 m<sup>2</sup> was surveyed.

A total of nine different mobile benthic invertebrates were observed over the survey period (Table 3.2). These included four organisms classifiable to species: the swimming holothurians, *Enypniastes eximia*, the red crab *Chaceon quinquedens*, the large lithodid crab *Neolithodes agassizii*, and the giant isopod *Bathynomus giganteus*. One shrimp *Glyphocrangon* sp. was identified to genus. Three other types of megafauna were identified to family: the mobile holothurian (Elpidiidae), red shrimps (consisting of a variety of species), and the squat lobsters (Galatheididae). The remaining taxon was only identifiable to class level, the brittlestars (Ophiuroidea).

Six other megafaunal taxa, categorized as sessile or limited mobility invertebrates were also recorded. Two were identifiable to genus: the stalked glass sponge *Hyalonema* sp. and the Aphroditid polychaete *Laetmonice*. Two taxa were only identifiable to the level of family: cerianthid anemones (Cerianthidae) and holothuroideans. The remaining two taxa were identifiable to class level: sea pens (Anthozoa) and sea stars (Asteroidea).

Four groups of megafauna were grouped as demersal and planktonic invertebrates. Four taxa were identifiable to the species level: a lobate ctenophore *Bathocyroe fosteri*, a hydrozoan medusa *Benthocodon hyalinus*, and scyphozoan medusa *Poralia rufescens* and the lobate ctenophore *Lampocteis cruentiventer*. One taxon was identifiable to family level: the isopod (Munnopsidae). Two taxa were only identifiable to class level: an undescribed red cydippid ctenophore and an undescribed hydrozoan medusa.

Nine demersal fish taxa were also observed. Three of these were identifiable to species level: two species of cusk eel were present, the bony-eared assfish *Acanthonus armatus* and *Bassogigas gilli*; and the spiny eel *Polyacanthonotus merretti*. Five taxa were identifiable to



genus level: the halosaur *Aldrovandia* sp., the cutthroat eel *Synaphobranchus* sp., the cusk eel *Dicrolene* sp., the duckbilled eel *Facciolella* sp., and the tripod fish *Bathypterois* sp. The remaining taxon was only identifiable to the level of family: grenadiers (Macrouridae).

The carcasses of three groups of dead organisms were also observed during the surveys. These included pyrosomes *Pyrosoma atlanticum*, salps, and the red crab *Chaceon quinquedens*.

### **3.3.1 Density Over Time**

Taxonomic richness, defined as the number of different live taxa present varied over time (Tables 3.1 and 3.2) with the highest values occurring on 5-Aug, 217 (n=24) followed by 24-Aug, 236, (n=20), 9-Sep, 252 (n=12), 13-Sep, 256 (n=8), 21-Sep, 264 (n=8), 29-Sep, 272 (n=12), 4-Oct, 277 (n=9), 11-Oct, 284 (n=10), 19-Oct, 292 (n=6), 25-Oct, 298 (n=3), and 1-Nov, 305 (n=9). Similar trends were present for the megafauna categories.

### **3.3.2 Densities**

For the majority of organisms, densities were not statistically different over time. In general, the densities of megafauna, within each taxon, peaked during late-August through September (tables 3.1 and 3.2 and fig. 3.2). From late September through November densities remained low for most of the organisms. There was however a small peak in densities during November for a limited number of taxa (*Synaphobranchus* sp., and red shrimps). Organisms documented in May had highest densities of the survey; often orders of magnitude greater than the summer/fall survey (e.g. seastars were 4 orders of magnitude greater in May).

Table 3.1. Taxonomic classification and distribution of demersal fishes observed in vicinity of MC252 #2 over time.

Demersal Fishes			Sampling Date (mm/dd) Julian Day											
Phylum: Class: Family	Genus	Species	(05/16) 136	(8/05) 217	(8/24) 236	(9/09) 252	(9/13) 256	(9/21) 264	(9/29) 272	(10/04) 277	(10/11) 284	(10/19) 292	(10/25) 298	(11/01) 305
Actinopterygii: Notacanthiformes: Halosauridae	<i>Aldrovandia</i>	Unidentified	-	$1.0 \times 10^{-4}$	$4.0 \times 10^{-4}$	$4.0 \times 10^{-4}$	-	-	$3.79 \times 10^{-5}$	-	$9.87 \times 10^{-5}$	-	-	$1.0 \times 10^{-4}$
Actinopterygii: Anguilliformes: Synphobranchidae	<i>Synphobranchus</i>	Unidentified	0.3611	$1.2 \times 10^{-3}$	$2.0 \times 10^{-3}$	$1.2 \times 10^{-3}$	$5.0 \times 10^{-3}$	$9.0 \times 10^{-4}$	$2.0 \times 10^{-3}$	$8.0 \times 10^{-4}$	$1.4 \times 10^{-3}$	$1.3 \times 10^{-3}$	$9.0 \times 10^{-4}$	$2.0 \times 10^{-3}$
Actinopterygii: Notacanthiformes: Notacanthidae	<i>Polyacanthopus</i>	<i>P. merretti</i>	-	$4.1 \times 10^{-5}$	-	-	-	-	-	-	$6.73 \times 10^{-5}$	$6.54 \times 10^{-5}$	-	$7.17 \times 10^{-5}$
Actinopterygii: Anguilliformes: Nettostomatidae	<i>Facciolella</i>	Unidentified	-	-	$8.3 \times 10^{-5}$	-	-	-	-	-	-	-	-	-
Actinopterygii: Aulopiformes: Ipnopidae	<i>Bathypetrolis</i>	Unidentified	0.0556	$4.7 \times 10^{-5}$	-	$4.56 \times 10^{-5}$	$7.09 \times 10^{-5}$	$3.68 \times 10^{-5}$	-	-	-	-	-	$4.37 \times 10^{-5}$
Actinopterygii: Ophidiiformes: Ophiidae	<i>Acanthonus</i>	<i>A. armatus</i>	-	-	$9.36 \times 10^{-5}$	-	-	-	-	-	-	-	-	-
Actinopterygii: Ophidiiformes: Ophiidae	<i>Bassogigas</i>	<i>B. gillii</i>	-	$1.5 \times 10^{-4}$	$1.4 \times 10^{-4}$	$5.18 \times 10^{-5}$	-	-	-	-	-	-	-	-
Actinopterygii: Ophidiiformes: Ophiidae	<i>Dicrolene</i>	Unidentified	0.4167	$3.0 \times 10^{-4}$	-	-	-	-	$4.71 \times 10^{-5}$	$1.0 \times 10^{-4}$	-	-	-	-

Table 3.1. continued. Taxonomic classification and distribution of demersal fishes observed in vicinity of MC252 #2 over time.

Actinopterygii: Ophidiiformes: Ophiidae	Unidentified	Unidentified	-	$1.4 \times 10^{-4}$	-	-	-	$3.92 \times 10^{-5}$	-	-	-	-	-	-
Actinopterygii: Notacanthiformes: Halosauridae	<i>Aldrovandia</i>	Unidentified	0.4167	$1.0 \times 10^{-4}$	$4.0 \times 10^{-4}$	$4.0 \times 10^{-4}$	-	-	$3.79 \times 10^{-5}$	-	$9.87 \times 10^{-5}$	-	-	$1.0 \times 10^{-4}$
Actinopterygii: Gadiformes: Macrouridae	Unidentified	Unidentified	-	$3.0 \times 10^{-4}$	$1.0 \times 10^{-4}$	-	-	-	$2.0 \times 10^{-4}$	-	-	-	-	-

Table 3.2. Taxonomic classification and distribution of invertebrates observed in vicinity of MC252 #2 over time.

Mobile Benthic Invertebrates			Sampling Date (mm/dd) Julian Day											
Phylum: Class: Family	Genus	Species	(05/16) 136	(8/05) 217	(8/24) 236	(9/09) 252	(9/13) 256	(9/21) 264	(9/29) 272	(10/04) 277	(10/11) 284	(10/19) 292	(10/25) 298	(11/01) 305
Echinodermata: Ophiuroidea: Unidentified	Unidentified	Unidentified	-	5.49 $\times 10^{-5}$	-	-	-	-	-	-	-	-	-	-
Echinodermata: Holothuroidea: Elpidiidae	Unidentified	Unidentified	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Arthropoda: Malacostraca: Geryonidae	<i>Chaceon</i>	<i>C. quinquedens</i>	-	2.0 $\times 10^{-3}$	5.0 $\times 10^{-4}$	1.0 $\times 10^{-4}$	3.0 $\times 10^{-4}$	6.58 $\times 10^{-5}$	4.29 $\times 10^{-5}$	3.0 $\times 10^{-4}$	3.0 $\times 10^{-4}$	4.0 $\times 10^{-4}$	2.0 $\times 10^{-4}$	4.0 $\times 10^{-4}$
Arthropoda: Malacostraca: Lithodidae	<i>Neolithodes</i>	<i>N. agassizii</i>	-	-	6.97 $\times 10^{-5}$	7.20 $\times 10^{-5}$	-	2.86 $\times 10^{-5}$	8.14 $\times 10^{-5}$	6.43 $\times 10^{-5}$	-	1.0 $\times 10^{-4}$	-	-
Arthropoda: Malacostraca: Penaeoidea	Unidentified	Unidentified	0.4444	1.8 $\times 10^{-2}$	2.3 $\times 10^{-3}$	8.9 $\times 10^{-3}$	8.1 $\times 10^{-3}$	1.9 $\times 10^{-3}$	1.7 $\times 10^{-3}$	2.3 $\times 10^{-3}$	4.1 $\times 10^{-3}$	2.4 $\times 10^{-3}$	1.3 $\times 10^{-3}$	7.1 $\times 10^{-3}$
Arthropoda: Malacostraca: Glyphocrangonidae	<i>Glyphocrangon</i>	Unidentified	-	4.0 $\times 10^{-4}$	-	1.0 $\times 10^{-4}$	2.0 $\times 10^{-4}$	9.06 $\times 10^{-5}$	9.66 $\times 10^{-5}$	1.0 $\times 10^{-4}$	6.36 $\times 10^{-5}$	-	-	-
Arthropoda: Malacostraca: Galatheidae	Unidentified	Unidentified	-	-	-	-	-	-	-	1.0 $\times 10^{-4}$	-	-	-	-
Arthropoda: Malacostraca: Cirolanidae	Bathynomus	B. giganteus	0.2222	5.47 $\times 10^{-5}$	-	-	-	-	-	-	-	-	-	-
<b>Sessile or Limited Mobility Megafauna</b>			<b>136</b>	<b>217</b>	<b>236</b>	<b>252</b>	<b>256</b>	<b>264</b>	<b>272</b>	<b>277</b>	<b>284</b>	<b>292</b>	<b>298</b>	<b>305</b>
Cnidaria: Anthozoa: Cerianthidae	Unidentified	Unidentified	-	2.0 $\times 10^{-4}$	-	-	-	-	-	-	-	-	-	-
Cnidaria: Anthozoa: Unidentified	Unidentified	Unidentified	-	6.85 $\times 10^{-5}$	6.40 $\times 10^{-5}$	-	5.53 $\times 10^{-5}$	-	-	-	-	-	-	-

Echinodermata: Asteroidea: Unidentified	Unidentified	Unidentified	3.9444	$1.5 \times 10^{-3}$	$1.5 \times 10^{-3}$	-	-	-	-	-	-	-	-	-
Echinodermata: Holothuroidea: Unidentified	Unidentified	Unidentified	0.0556	$1.5 \times 10^{-4}$	$4.0 \times 10^{-3}$	$11.0 \times 10^{-3}$	-	-	-	$1.0 \times 10^{-4}$	-	-	-	-
Porifera: Hexactinellida: Hyalonematidae	Hyalonema	Unidentified	-	-	$7.60 \times 10^{-5}$	-	-	-	-	-	-	-	-	-
Annelida: Polychaeta: Aphroditidae	Laetmonice	Unidentified	-	-	$7.60 \times 10^{-5}$	-	-	-	-	-	-	-	-	-
<b>Demersal Megafauna and Plankton</b>			<b>136</b>	<b>217</b>	<b>236</b>	<b>252</b>	<b>256</b>	<b>264</b>	<b>272</b>	<b>277</b>	<b>284</b>	<b>292</b>	<b>298</b>	<b>305</b>
Ctenophora: Tentaculata: Bathocyroidae	<i>Bathocyroe</i>	<i>B. fosteri</i>	-	$4.70 \times 10^{-5}$	-	-	-	-	$4.71 \times 10^{-5}$	-	$7.31 \times 10^{-5}$	-	-	$7.02 \times 10^{-5}$
Cnidaria: Hydrozoa: Rhopalonematidae	<i>Benthocodon</i>	<i>B. hyalinus</i>	-	$1.2 \times 10^{-4}$	$3.0 \times 10^{-4}$	$1.3 \times 10^{-4}$	-	-	$3.16 \times 10^{-5}$	$3.77 \times 10^{-5}$	$9.87 \times 10^{-5}$	$1.0 \times 10^{-4}$	-	$1.0 \times 10^{-4}$
Cnidaria: Scyphozoa: Ulmaridae	<i>Poralia</i>	<i>P. rufescens</i>	-	-	-	-	-	-	-	-	-	-	-	-
Arthropoda: Malacostraca: Munnopsidae	Unidentified	Unidentified	-	$5.66 \times 10^{-5}$	$9.36 \times 10^{-5}$	-	-	-	-	-	-	-	-	-
<b>Dead Organisms</b>			<b>136</b>	<b>217</b>	<b>236</b>	<b>252</b>	<b>256</b>	<b>264</b>	<b>272</b>	<b>277</b>	<b>284</b>	<b>292</b>	<b>298</b>	<b>305</b>
Chordata: Thaliacea: Pyrosomatidae	<i>Pyrosoma</i>	<i>P. atlanticum</i>	-	$2.6 \times 10^{-3}$	$2.7 \times 10^{-3}$	$6.88 \times 10^{-5}$	-	$7.52 \times 10^{-5}$	$8.46 \times 10^{-5}$	-	-	-	-	$2.0 \times 10^{-4}$
Chordata: Thaliacea: Salpidae	Unidentified	Unidentified	-	$2.0 \times 10^{-3}$	$7.02 \times 10^{-5}$	-	$7.32 \times 10^{-5}$	-	-	-	-	-	-	-
Arthropoda: Malacostraca: Geryonidae	<i>Chaceon</i>	<i>C. quinquedens</i>	-	$3.07 \times 10^{-5}$	$8.29 \times 10^{-5}$	$5.13 \times 10^{-5}$	-	-	-	-	$5.27 \times 10^{-5}$	-	-	-

The most abundant organism observed to occur at the study site was a mobile holothurian belonging to the family Elpidiidae. There was a massive influx of these animals into the survey area during October. These organisms were so numerous in the water column above the seabed and on the seafloor that it was not possible to accurately estimate their abundances because the field of view in the water column ahead of the ROV was not known and because their semi-transparent coloration made them very difficult to enumerate on the seabed. The other commonly observed organisms during the survey period included red shrimps, cutthroat eels, and red crabs.

Among the mobile invertebrate taxa that were present throughout the survey period, there was a general decline in abundance over time (Table 3.2). *Chaceon quinquidens* was present in significant higher densities during the survey performed on day 217 than in any other survey with the exception of the surveys that occurred on 236 and 256. During these latter two days the densities were lower than on day 217 though not significantly different. Red crab densities were statistically similar at low densities for the remainder of the surveys and this species was absent during the pre-drilling survey on day 136. Red shrimps were present for throughout the survey period. Red shrimp were recorded at significantly higher densities on day 136 than during any of the post-spill surveys. Densities on days 217 and 252 were statistically similar; however, only 217 was statistically greater than the remaining surveys. *Glyphocrangon* sp. was only present on days 217, 252, 256, 264, 272, 277, and 284 and none of its densities were significant different.

Within the limited to no mobility invertebrate taxa, seastars were the numerically dominant organism, followed by Holothuroidea (Table 3.2). Seastars were present at a significantly higher density during the survey conducted on 136 than in the remainder of the surveys except the survey on 217 and were only present during surveys 126-236. Seastars were present at similar densities during the surveys on 217 and 236. The surveys performed on 236



and 252 were statistically similar and recorded Holothuroidea at statistically significantly higher values than the remaining surveys. Holothuroidea recorded on 136 and 217 were only significantly different than survey 236 and 252. The remainder of the surveys recorded statistically similar densities of Holothuroidea. Of the remaining invertebrates with limited or no mobility, there were no significant differences in seapen densities, which were only present on days 217, 236, and 256. A limited number of cerianthid anemones and sea pens were present, while only one glass sponge was observed during the surveys.

Among the demersal invertebrate megafauna, the medusa *Benthocodon hyalinus* and an undescribed red cydippid ctenophore were present in the highest densities (Table 3.2). *Benthocodon hyalinus* was present in the highest densities from late-August through early-September, although none of the densities during this survey period were significantly different. The red cydippid ctenophore showed no clear pattern of abundance over time, with densities oscillating but remaining statistically similar over the survey period for the dates when it was present. The ctenophore *Bathocyroe fosteri* was present only sporadically during the study and none of the densities of this taxon were significantly different.

Among the fishes, the cut throat eel *Synaphobranchus* sp. was the most abundant taxon followed by the cusk eel, *Dicrolene* sp., and the halosaur *Aldrovandia* sp (Table 3.1). The abundance of *Synaphobranchus* sp., fluctuated throughout the survey period. This taxon was present in significantly higher densities during the pre-drilling survey performed on day 136. The densities on day 305 were significantly higher than during all surveys except on days 217 and 298. The mean density on day 217 was significantly greater than on days 252, 264, and 272. *Aldrovandia* sp. and *Dicrolene* sp. were present at significant higher densities during the pre-drilling survey on day 136 than during the remaining surveys, which were all statistically similar.

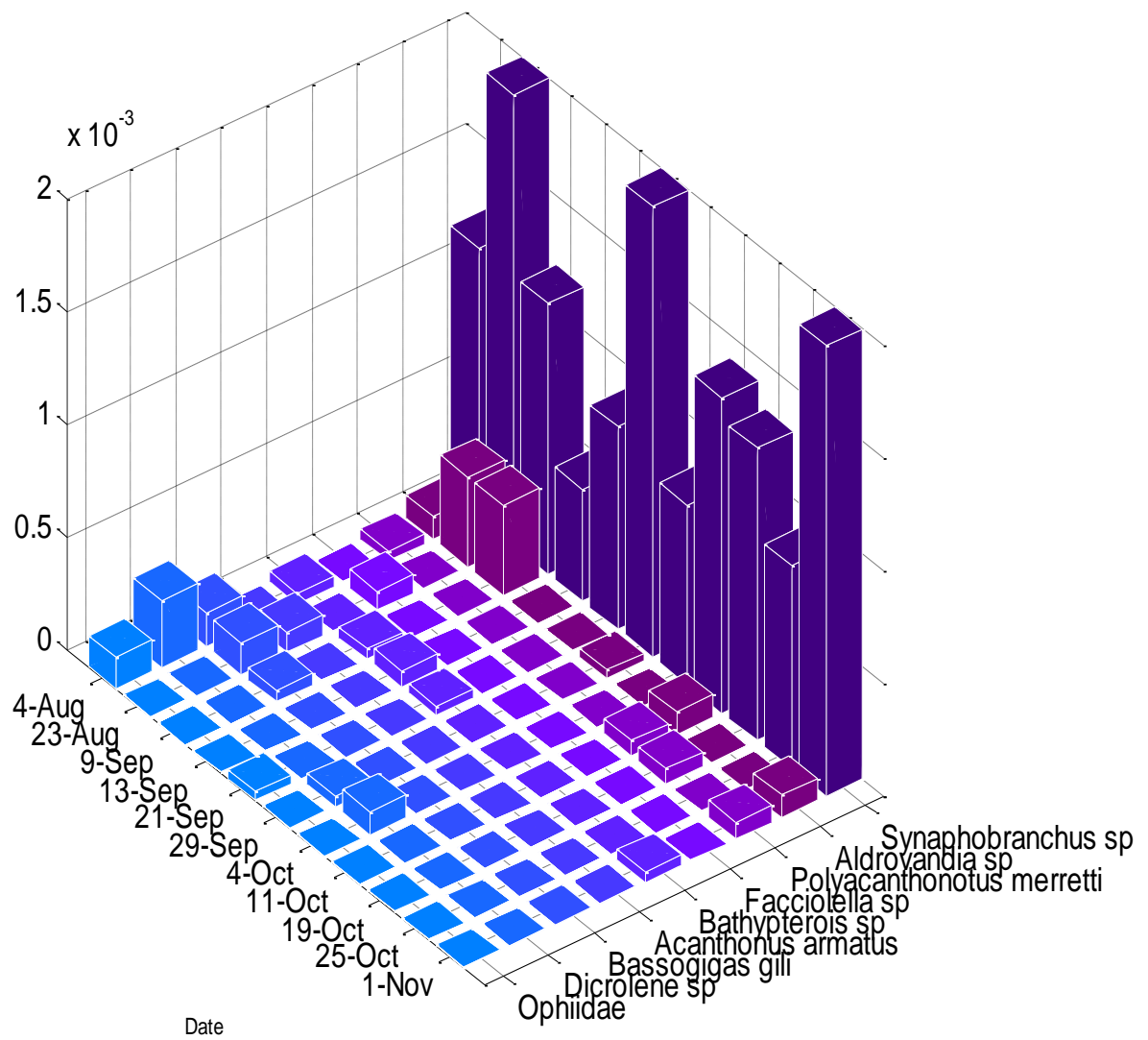


Figure 3.2. Mean densities of demersal fishes

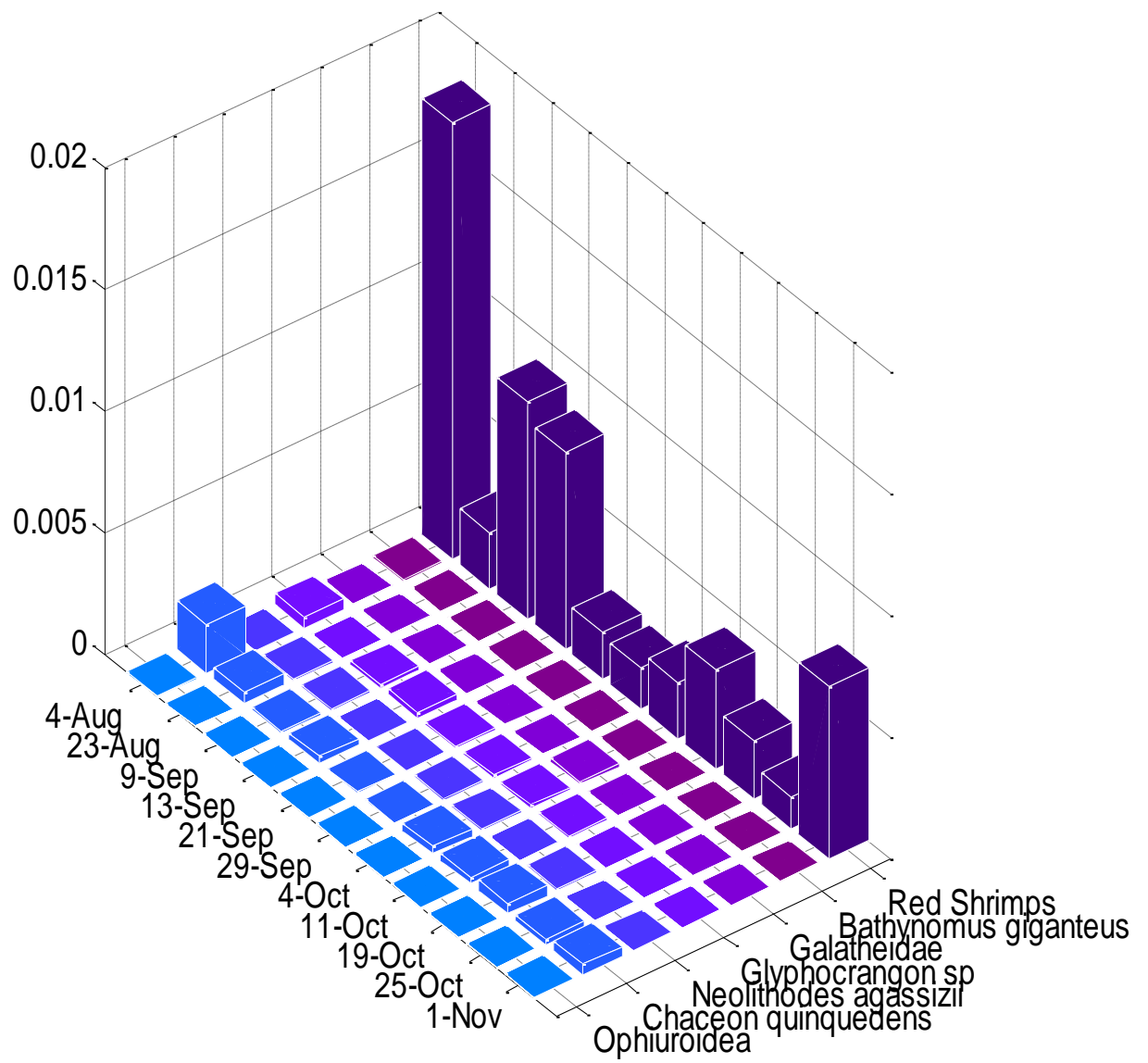


Figure 3.3. Mean densities of mobile invertebrates over time.

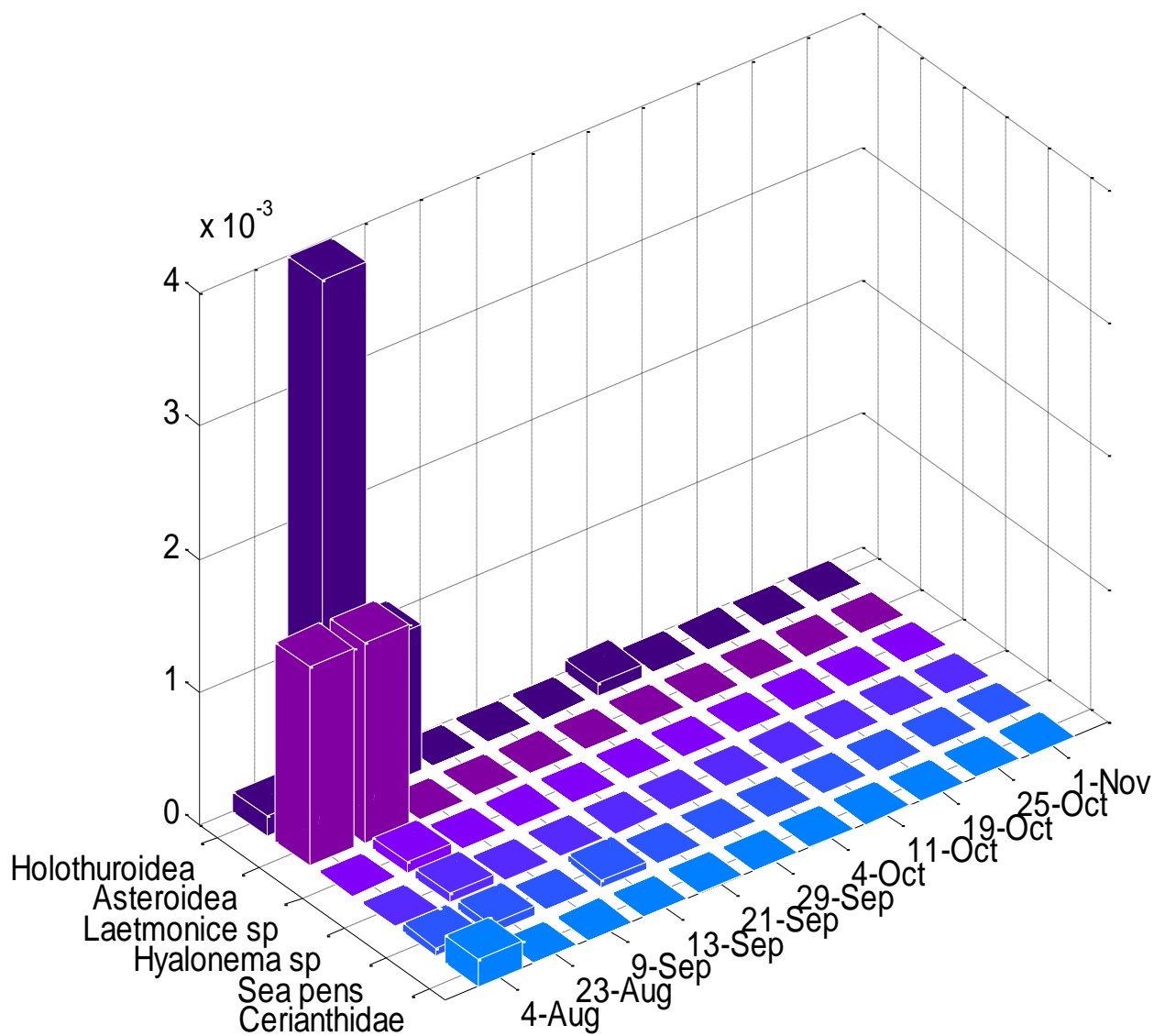


Figure 3.4. Mean densities of limited mobility invertebrates over time

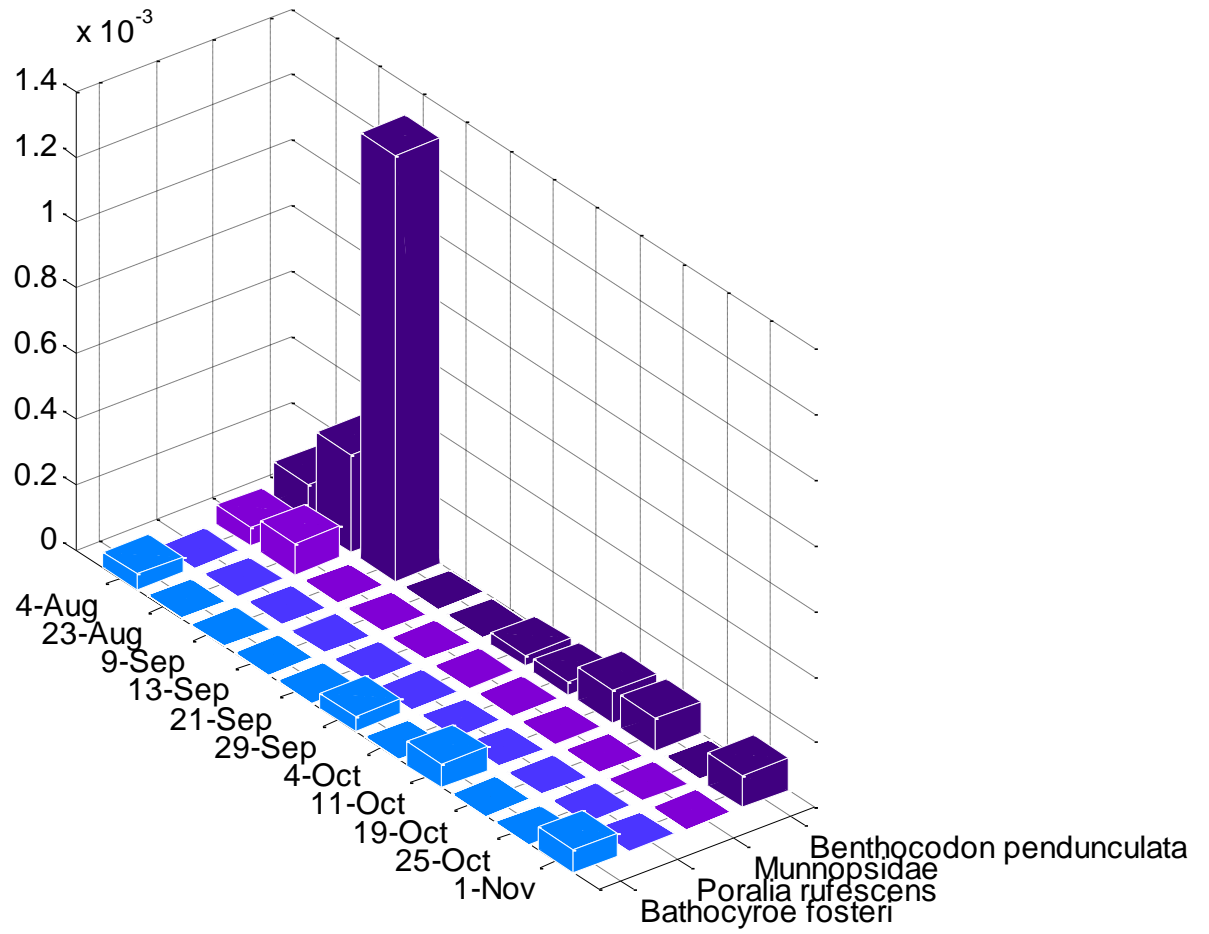


Figure 3.5. Mean densities of demersal invertebrates over time

### **3.3.3 Mortality**

Dead organisms from three taxa were observed during the surveys. Pyrosome carcasses were documented during five surveys: 217, 236, 252, 264, and 305. During surveys on days 217 and 236, significantly greater densities of pyrosome carcasses were observed than during any other time during this study. Salp carcasses were only present on three surveys: 217, 236, and 256. They were present at the highest densities during the survey on day 217; however, none of their densities were significantly different throughout the survey period. *Chaceon quinquidens* carcasses were present at low densities during four surveys: 217, 236, 252, and 284. None of these densities were statistically significant. No carcasses were documented on 136.

### **3.4 Discussion**

The results of this study indicate that both abundance and biodiversity of benthic megafauna in close proximity to the Macondo BOP varied over a three-month period that began shortly after the well had been capped. In general, both abundances and taxonomic richness were highest during the initial portion of this time-series study (August-September) and then declined through the fall (October-November). While this trend is common for most taxa (especially sessile and limited mobility taxa), some groups of mobile organisms were present in fluctuating densities throughout the survey. For example red shrimps, which were present in the highest densities throughout the survey, showed both declines and gains over time; however, overall abundances did decline between the first and final surveys. The trend of fluctuating densities was most common in the demersal fishes. Perhaps the clearest example of this was for *Synphobranchus* sp., which reached peak densities on three different survey dates near the beginning, middle, and end of the study. Comparisons with the pre-drilling survey that was

undertaken in May while the Macondo well was still flowing indicated that when present, densities of most taxa were much higher at that time compared to the mean post-spill densities. However, many organisms prevalent during the later surveys were not documented during the May survey, including *Chaceon quinquidens*, *Benthocodon hyalinus*, and *Glyphocrangon* sp. Their absence during May could have been a consequence of the substantially smaller area surveyed (10% of the fall survey area) or avoidance of the area while the well was flowing.

While we lack quantitative pre-spill density estimates, comparisons based on encounter rates ( $n \text{ min}^{-1}$ ) permitted comparisons of the relative abundances of selected taxa from pre-spill (Feb, Mar 2010), spill (May 2010) and post-spill (Aug – Nov, 2010) time periods. One consistent pattern was the near complete absence during the spill, of taxa that were present prior to the spill. Comparisons of pre-spill and post-spill encounter rates revealed two general patterns: some taxa (red shrimps and *Synaphobranchus* sp.) were as abundant following the spill or appeared to return to pre-spill levels; while others (*Chaceon quinquidens*, Cerianthid anemones, *Venefica procera*, and *Aldrovandia* sp.)) were either absent following the spill or present at levels that were substantially lower than prior to the spill.

A subsea hydrocarbon plume of dispersed oil and gas was reported to extend 35 km SW of the Macondo BOP at a depth of approximately 1100 m (Camilli et al. 2010). While we do not know the exact mechanism of how this plume may have interacted with the seafloor, two hypotheses were presented. The first is the dirty blizzard hypothesis, in which the fall of marine snow and other particulate matter carries oil to the benthos. The second is the toxic bathtub ring hypothesis, in which higher topographic portions of the seafloor make direct contact with the plume.

Table 3.3. Pre-spill abundance indices ( $\text{n min}^{-1}$ ) for all taxa observed during Gulf SERPENT surveys around the Macondo BOP on 02/19/10 and 03/27/10 and post-spill abundance mean indices estimated for each of our survey efforts over time. Hyphens indicate the taxon was not detected during post-spill surveys.

Taxon	Pre-Spill Survey Abundance Index ( $\text{n min}^{-1}$ )			Development Driller 2 Post-Spill Survey Abundance Index ( $\text{n min}^{-1}$ )											
	Feb-10	Mar-10	Pre-Spill Mean	136	217	236	252	256	264	272	277	284	292	298	305
<i>Chaceon quinquidens</i>	0.118	0.427	0.272±0.665	0	0.05 3	0.036	0.002	0.011	0.005	0.002	0.009	0.017	0.017	0.012	0.021
Red shrimps	0.221	0.133	0.177±0.188	2.22 $\times 10^{-06}$	0.41 6	0.19	0.214	0.367	0.102	0.121	0.094	0.211	0.097	0.098	0.409
Cerianthid anemone	0.029	0	0.014±0.063	0	0.00 4	0	0	0	0	0	0	0	0	0	0
<i>Aldrovandia</i> sp.	0.044	0.013	0.029±0.066	1.90 $\times 10^{-06}$	0.00 4	0.029	0.013	0	0	0.002	0	0.0053	0	0	0.013
<i>Synaphobranchus</i> sp.	0.074	0.013	0.043±0.130	1.59 $\times 10^{-06}$	0.04 3	0.15	0.032	0.019	0.053	0.137	0.046	0.0899	0.062	0.059	0.136
<i>Venefica procera</i>	0	0.12	0.060±0.258	0	0	0	0	0	0	0	0	0	0	0	0
<i>Bathypterois</i> sp.	0.015	0.013	0.014±0.003	3.17 $\times 10^{-07}$	0.00 2	0	0.001	0.002	0.001	0	0	0	0	0	0.002
<i>Dicrolene</i> sp.	0.074	0.04	0.057±0.072	1.90 $\times 10^{-06}$	0.00 9	0	0	0.002	0	0.002	0.005	0	0	0	0



Since the depth of this site was deeper than the reported depth of the subsea plume, the latter hypothesis seems unlikely. In the absence of sediment chemistry data on hydrocarbon concentrations, it is difficult to evaluate whether the reduced abundances of some taxa were the result of acute or chronic toxicity or due to other factors.

Variable visibility and image quality may have had an effect on successful documentation of more cryptic organisms, those that blend with their surrounding environment or are particularly small. Based on analysis of ROV footage, both salp and pyrosome carcasses appeared to have accumulated in small depressions in the sea floor. This has likely led to an underestimation of the presence of salp and pyrosome carcasses as only the carcasses at the top of a mound could be seen and enumerated. Cerianthid anemones frequently retreated into the sediment in the presence of the ROV and were also likely underestimated. The mobile holothurian Elpidiidae was not present in May and increased in abundance during the August through September transects. Surveys in October recorded a massive influx of Elpidiidae to the area. The Elpidiid's semi-transparent white coloration combined with its small body size; cause it to be difficult to see when they were on the sea floor. Due to the limitations of the ROV video footage it is impossible to enumerate the Elpidiids settled on the seafloor. During many transects the water column was filled with Elpidiidae; however, we could not determine the volume of water that was in the images and this made it impossible to enumerate swimming Elpidiidae. Thus no quantitative results are presented on the abundance of Elpidiidae during this study; however we can qualitatively state that Elpidiidae were the most abundant organism during the month of October and likely during the entire survey.

During this survey period the Development Driller II was tasked with drilling the second relief well (May 16- August 21). Drilling in the deep sea can affect organisms in the vicinity

through noise production, physical smothering from drill cuttings, and exposure to chemical constituents of drilling mud (Kropp, 2004; Gates and Jones, 2012). This can often lead to reductions in abundance and diversity due to toxicity or smothering (Gates and Jones, 2012) or increases in abundances of opportunistic species due to organic enrichment (Kingston, 1992). The drilling of the relief well during this survey could have led to emigration of mobile organisms from the area, but should have had little impact on sessile or limited mobility organisms. It is also unlikely that the presence of salp and pyrosome carcasses can be attributed to the drilling of the well. In a survey conducted in August of 2010 both salp and pyrosome carcasses were reported at 5 sites in varying distances from the Macondo well (Valentine and Benfield, Under Review).

### **3.5 Conclusion**

A lack of baseline data on megafauna communities in the Northern Gulf of Mexico, makes determining the exact impacts of the DWH spill very difficult if not impossible. This study represents a preliminary attempt to characterize the epibenthic and demersal communities near the DWH spill epicenter.

A clear trend in diversity and abundance was evident. These parameters were highest in August, declined throughout September, and the lowest diversity and abundance were recorded in October and November. While pre-drilling surveys conducted in May recorded low diversity, abundance of encountered organism was much higher than the average post-spill survey. Mortality was also highest in August.

A secondary goal of this study was to establish a post-spill baseline of epibenthic and demersal megafauna communities in Mississippi Canyon 252. This baseline can be used to

determine how abundance and diversity changes in the years following the DWH oil spill. Follow-on cruises were conducted in March, June, and August of 2011, once analysis is complete, a better understanding on how the DWH oil spill impacted surrounding megafauna will be possible.

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## **CHAPTER 4: CHARACTERIZATION OF CHANGES TO EPIBENTHIC AND DEMERSAL MEGAFUNA COMMUNITIES AT MISSISSIPPI CANYON 252 RELIEF WELL 2 ONE YEAR AFTER THE DEEPWATER HORIZON OIL SPILL**

### **4.1 Introduction**

A disturbance can generally be defined as any event in time that disrupts ecosystem, community, or population structure and changes resources, substrate availability, or the physical environment (Pickett and White 1985). Disturbance is a major source of temporal and spatial heterogeneity in the structure and dynamics of natural communities and is also an agent of natural selection (Sousa 1984). As such, acute disturbances are capable of precipitating regime shifts in species composition and abundance resulting in alternative stable state assemblages.

The Deepwater Horizon oil spill was a source of major disturbance both physically (the sinking of the Deepwater Horizon rig and sustained subsurface control and cleanup efforts) and chemically (release of gas and oil and the application of chemical dispersants). In addition to this the Development Driller II (DD2) was tasked with drilling a relief well to relieve pressure on the failed Macondo blow out preventer. Drilling of the secondary relief well, Mississippi Canyon Relief Well 2 (MC252#2), began on May 16, 2010 and was completed on Aug 21, 2010. Extensive seafloor biological surveys were conducted at MC 252#2 from Aug-Nov 2010 (Valentine and Benfield in review). This survey enabled the creation of a post-spill ecological baseline which may be used for comparison to future estimates of biodiversity and megafauna abundance at MC252#2; thus enabling a determination of community resilience and recovery.

Disturbance can result in a general reduction in biomass, reduced body size, and shifts in the trophic structure of the community (Weston 1990). It has been suggested that deep sea benthic communities affected by major physical disturbances often exhibit an initial decrease in species richness, but then show an increase in the total number of individuals present as a result

of the rapid immigration of highly motile and opportunistic species (Weston 1990). Chemical disturbances can result in decreases both in the total number of species and in the number of individuals (Olsgard and Gray, 1995). Hydrocarbon drilling in deeper waters (>380m) has been documented as a cause of initial declines in megafauna diversity and abundance in the immediate vicinity the drill site (100-200 m) (Gates and Jones 2012; Jones et al 2006).

Recovery may be defined as a return to pre-disturbance conditions; however, the post-disturbance system may deviate in terms of traditional function, diversity, and abundances (O'Neill 1998; Jones et al 2012). Recovery of megafauna abundance and diversity in deeper systems from drilling and other physical disturbances appears to be slower in comparison to shallow water systems. Recovery in deeper systems also appears to be highly dependent upon the frequency and magnitude of disturbance as well as the physical conditions of the ambient environment (Gates and Jones 2012). Communities can respond to these events via different mechanisms including migration of new species into the area, or recruitment (Reice 1994).

The primary objective of this study was to return to MC252#2 approximately 13 months after the Deepwater Horizon oil spill and determine what, if any, changes have occurred with the community composition and abundances since it was surveyed in 2010.

## **4.2 Methods**

A deep-water survey cruise was conducted to characterize and quantify benthic and demersal megafaunal communities around MC252 Relief Well # 2 (MC252#2). The site is located approximately 750 m SW (28° 43' 53.338" N, 88° 22' 17.927" W) of the MC252 Macondo well head. MC252#2 has an average depth of 1578m and is characterized by a primarily soft mud substrate type. This survey was conducted from the M/V HOS Sweetwater in

June 2011 as part of a cooperative Natural Resource Damage Assessment (NRDA) cruise (Fig. 4.1).

A Perry Triton XLS work-class ROV (150 hp) was utilized for this survey. This ROV was equipped with a high definition camera (ROS Mantis HD, 1980i) and a standard definition (Kongsberg OE1366MKII, 480i) video camera. An additional digital camera (Imenco Shark 12 megapixel) was also deployed to collect high-resolution still images of target organisms for taxonomic identification. To maximize visibility, the ROV was equipped with an array of forward-facing survey lighting gear including light emitting diode (LED), high-intensity discharge (HID), and standard incandescent lighting. Additionally, a pair of red diode lasers was mounted 17cm apart, in parallel, on the top of the standard definition camera. A 1200 kHz acoustic Doppler current profiler (ADCP: Teledyne RDI Workhorse) was mounted, facing down, at the rear of the ROV. This was used to quantify the ROV's velocity. The geographic position of the ROV was determined using an ultra-short baseline (USBL) acoustic transponder that was tracked via a vessel-mounted hydrophone.

The same radial survey design was used for this sampling event as was used for the DD2 survey. The seafloor survey consisted of 24 transects conducted from a central point of origin. Transects were separated by 15° intervals and had a horizontal length of 250 m (Fig. 4.1C). ROV pilots attempted to maintain a constant velocity and altitude (1-2 m off seafloor) throughout each transect while the USBL system recorded the ROV locations along each transect.

Determination of the area surveyed at this site required an estimate of the width of the field of view (FOV) on each transect. This was accomplished by measuring the pixel width of the image and the pixel distance between the two red diode lasers at one minute intervals. A

ratio of the known distance between the lasers (17 cm) and the pixel distance in each image was used to determine actual image width. A mean FOV was then calculated for each transect. This mean FOV was then multiplied by the transect distance to estimate the area surveyed by the ROV.

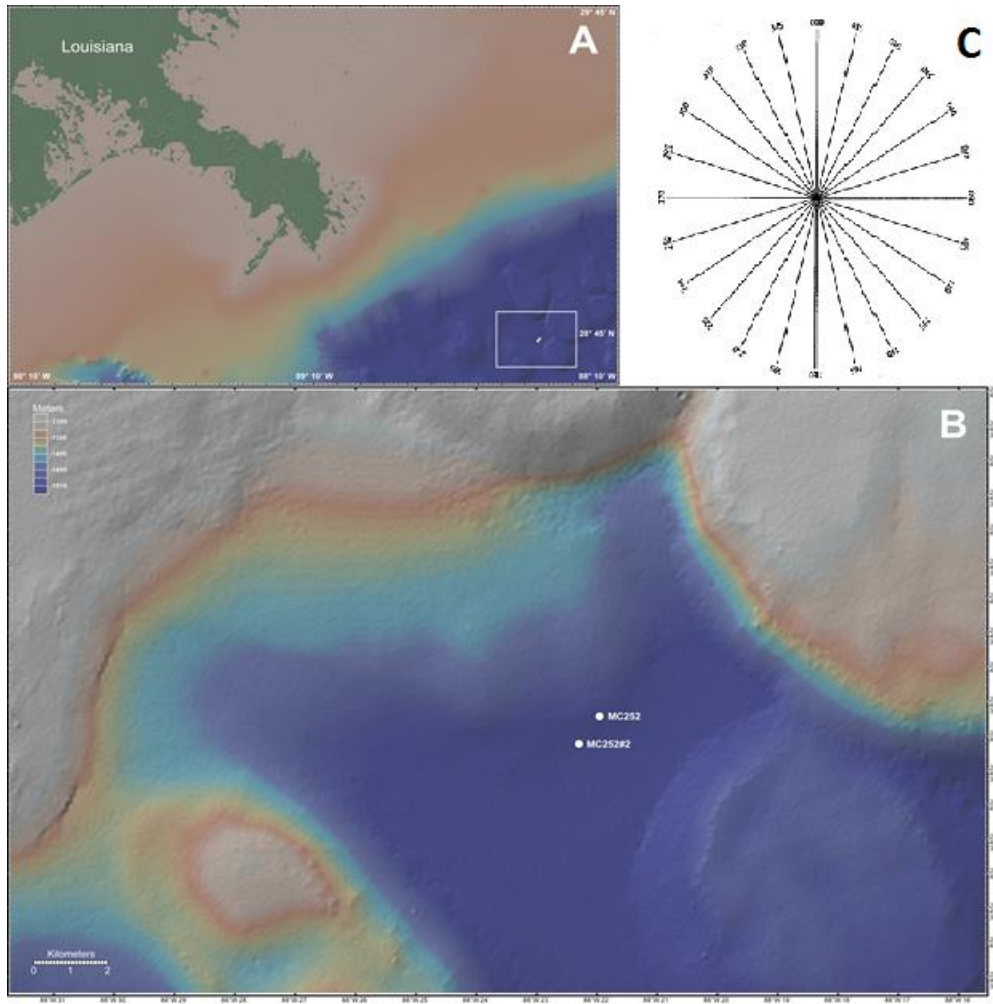


Figure 4.1. A: Study area in relation to the Louisiana Coast. The white rectangle indicates the area defined by the higher resolution map (B), which shows the locations of the Macondo Well (MC252) and the study site (MC252#2). C: The survey design used in June of 2011 study.



Based on examinations of the quality of the video collected during surveys, it was determined that the high definition footage provided the best quality images for quantification. Videos were reviewed using Vreveal software at 1x playback speed. A still image of targets of interest was then extracted from the footage for identification and enumeration. The level of taxonomic resolution was dependent on the quality of each image, which varied due to water clarity, animal movement and other factors that affected image quality.

### **4.3 Results**

The average swath width for transects conducted during this survey was 2.94 m. The total area surveyed at MC252#2 was 17207.29 m<sup>2</sup>.

A total of five different mobile benthic invertebrate taxa were observed over the survey period (Table 4.1). These included two taxa that were identified to species: the red crab *Chaceon quinquedens* and the large lithodid crab *Neolithodes agassizii*. One taxon was identified to genus: the shrimp *Glyphocrangon* sp. Two taxa were only classifiable to family: the mobile holothurian (Elpidiidae) and red shrimps (consisting of a variety of species).

Five megafaunal taxa were characterized as sessile, or limited mobility invertebrates (Table 4.1). Two taxa were identified to genus: the stalked glass sponge, *Hyalonema* sp., and the aphroditid polychaete, *Laetmonice* sp.. Two organisms were classified to the level of family: cerianthid anemones (Cerianthidae) and holothuroideans. The remaining taxon was only identifiable to class level: the sea stars (Asteroidea).

Four groups of megafauna were categorized as demersal and planktonic invertebrates (Table 4.1). Three of these taxa were classified to the species level: a lobate ctenophore, *Bathocyroe fosteri*, and a hydrozoan medusa, *Benthocodon hyalinus*. One taxon was identifiable

to family level: isopods belonging to the family Munnopsidae. One taxon was only discernible to class level: an undescribed red cydippid ctenophore.

Eight taxa were categorized as demersal fishes (Table 4.1). Two of these were classifiable to species level: the cusk eel, *Bassogigas gilli*, and the spiny eel, *Polyacanthonotus merretti*. Four taxa were identifiable to genus level: the halosaur, *Aldrovandia* sp., the cutthroat eel, *Synaphobranchus* sp., the cusk eel, *Dicrolene* sp., the duckbilled eel, *Facciolella* sp.. The remaining taxon was only identifiable to the level of family: grenadiers (Macrouridae).

#### **4.3.1 Densities**

Densities of demersal fishes varied widely between taxa. *Synaphobranchus* sp. was the dominant fish at this site as densities of this taxon were an order of magnitude greater than other documented fishes. *Aldrovandia* sp. and *Dicrolene* sp. were also recorded at relatively high densities. *Bassogigas gillii*, *Polyacanthonotus merretti*, *Facciolella* sp., and several species of Macrourids were present in lesser densities.

Among the mobile invertebrates, red shrimps were the dominant taxa. These were also the most abundant group of organisms at this site. The red crab *Chaceon quinquedens* and the shrimps, *Glyphocrangon* spp. were also present in high densities. *Neolithodes agassizii* was present in lower densities.

Within the sessile or limited mobility category, cerianthid anemones and sea stars were found in the highest densities. The aphroditid polychaete, *Laetmonice* sp., was also relatively common. The stalked glass sponge, *Hyalonema* sp., and holothuroideans were present in the lowest densities.

Demersal invertebrates were not abundant. Of these, the undescribed red cydippid ctenophore and the hydrozoan medusa, *Benthocodon hyalinus*, were present in the highest densities. The lobate ctenophore, *Bathocyroe fosteri*, and munnopsid isopods were also documented in lower densities.

Table 4.1. Taxonomic classification and distribution of demersal fishes in vicinity of MC252 #2 in June-2011.

<b>Phylum: Class: Family</b>	<b>Genus</b>	<b>Species</b>	<b>Density (n m<sup>-2</sup>)</b>
Actinopterygii: Notacanthiformes: Halosauridae	<i>Aldrovandia</i>	Unidentified	8.01X10 <sup>-4</sup>
Actinopterygii: Anguilliformes: Synphobranchidae	<i>Synphobranchus</i>	Unidentified	1.91X10 <sup>-3</sup>
Actinopterygii: Notacanthiformes: Notacanthidae	<i>Polyacanthonotus</i>	<i>P. merretti</i>	5.63X10 <sup>-4</sup>
Actinopterygii: Anguilliformes: Nettostomatidae	<i>Facciolella</i>	Unidentified	1.31X10 <sup>-4</sup>
Actinopterygii: Ophidiiformes: Ophiidae	<i>Bassogigas</i>	<i>B. gillii</i>	1.20X10 <sup>-4</sup>
Actinopterygii: Ophidiiformes: Ophiidae	<i>Dicrolene</i>	Unidentified	4.94X10 <sup>-4</sup>
Actinopterygii: Gadiformes: Macrouridae	Unidentified	Unidentified	2.46X10 <sup>-4</sup>

Table 4.2. Taxonomic classification and distribution of mobile invertebrates in vicinity of MC252 #2 in June-2011.

<b>Phylum: Class: Family</b>	<b>Genus</b>	<b>Species</b>	<b>Density (n m<sup>-2</sup>)</b>
Echinodermata: Holothuroidea: Elpidiidae	Unidentified	Unidentified	-
Arthropoda: Malacostraca: Geryonidae	<i>Chaceon</i>	<i>C. quinquedens</i>	2.19X10 <sup>-3</sup>
Arthropoda: Malacostraca: Lithodidae	<i>Neolithodes</i>	<i>N. agassizii</i>	3.07X10 <sup>-4</sup>
Arthropoda: Malacostraca: Penaeoidea	Unidentified	Unidentified	9.03X10 <sup>-2</sup>
Arthropoda: Malacostraca: Glyphocrangonidae	<i>Glyphocrangon</i>	Unidentified	1.93X10 <sup>-3</sup>

Table 4.3. Taxonomic classification and distribution of limited mobility invertebrates in vicinity of MC252 #2 in June-2011.

<b>Phylum: Class: Family</b>	<b>Genus</b>	<b>Species</b>	<b>Density (n m<sup>-2</sup>)</b>
Cnidaria: Anthozoa: Cerianthidae	Unidentified	Unidentified	8.54X10 <sup>-3</sup>
Echinodermata: Asteroidea: Unidentified	Unidentified	Unidentified	1.65X10 <sup>-3</sup>
Echinodermata: Holothuroidea: Unidentified	Unidentified	Unidentified	4.31X10 <sup>-4</sup>
Porifera: Hexactinellida: Hyalonematidae	Hyalonema	Unidentified	5.82X10 <sup>-5</sup>
Annelida: Polychaeta: Aphroditidae	Laetmonice	Unidentified	1.03X10 <sup>-3</sup>

Table 4.4. Taxonomic classification and distribution of demersal invertebrates in vicinity of MC252 #2 in June-2011.

<b>Phylum: Class: Family</b>	<b>Genus</b>	<b>Species</b>	<b>Density (n m<sup>-2</sup>)</b>
Ctenophora: Tentaculata: Bathocyroidae	Bathocyroe	B. fosteri	1.19X10 <sup>-4</sup>
Cnidaria: Hydrozoa: Rhopalonematidae	Benthocodon	B. hyalinus	2.62X10 <sup>-4</sup>
Arthropoda: Malacostraca: Munnopsidae	Unidentified	Unidentified	5.66X10 <sup>-5</sup>
Ctenophora: Tentaculata: Unidentified	Unidentified	Unidentified	1.21X10 <sup>-3</sup>

#### **4.4 Discussion**

The results of this post-spill sampling event conducted in the summer of the year following the spill indicate the presence of some taxa at abundances similar to those measured during May 2010, while the well was flowing. MC 252 #2 was sampled 11 times prior to the 2011 survey. These surveys consisted of the Aug-Nov of 2010 surveys by the Development Driller II and the BOEM pre-bottom survey in May of 2010 (Valentine and Benfield, In Review). In addition, five nearby sites located 2000m from MC252 on headings of 0°, 90°, 180°, and 270° and at 500 m due north of MC252 were also surveyed from August through September of 2010 (Valentine and Benfield, In Review). These studies provide the only post-spill baseline estimates of biodiversity and abundance of megafauna. The comparisons of megafaunal diversity and abundance in 2011 did not reveal dramatic changes for most taxa and underscore the need for a continued and consistent monitoring program to assess recovery from the spill.

In terms of diversity, fewer taxonomic categories were recorded compared to surveys conducted in early August 2010 (Fig. 4.2). On average, however, 15 different taxonomic categories of megafauna were documented at the more distal sites and only 11 during the Aug-Nov survey. In comparison, 21 taxa were recorded during the 2011 sampling event. More common organisms, such as *Synaphobranchus* sp., *Chaceon quinquidens*, and red shrimps continued to be documented. More rare taxa, however, such as *Bathypterois* sp. were not observed during this sampling event.

Mobile invertebrates were generally documented in higher densities in 2011 than in Aug-Nov, 2010 at all study locations. In 2011, the red crab, *Chaceon quinquidens*, was recorded at densities statistically similar to those measured on Aug 4 2010 (Fig. 4.3). The 2011 density was significantly higher than all other surveys conducted in 2010. In addition, during 2011 there were several encounters with mating pairs of red crabs, something that was not observed in 2010. Red shrimps were present in significantly higher densities during 2011 than in all 2010 surveys with the exception of May (Fig. 4.4). In comparison to the May 2010 survey, red shrimp densities are still quite low. An unpublished survey conducted by the Development Driller III in March 2011 documented several size classes of red shrimp, including many that qualitatively appeared smaller than the red shrimps found at this site in June of 2011. It is possible that red shrimp reproduction in the late winter/spring produced a large number of recruits.

Comparisons of demersal fish densities between 2010 and 2011 appear to vary dependent of taxa. Densities of *Synaphobranchus* sp. fluctuated during 2010. The densities of this mobile fish in 2011 were statistically similar to those observed in Aug-Nov 2010 but with slightly lower than peak densities. These 2011 densities were greater than those recorded at sites further afield in 2010 (Fig. 4.5). *Synaphobranchus* sp. was recorded in densities an order of magnitude higher

in 2011 than in May of 2010 (Fig. 4.6). This appears to be a fairly resilient mobile fish that may have moved into the site as conditions improved after the spill. Less abundant fishes such as *Aldrovandia* sp. (Fig. 4.7), *Bassogigas gili*, and *Dicrolene* sp were only documented sporadically and in low densities during 2010. In 2011, these fishes were documented in higher mean densities than in 2010, although these means were not statistically different among years.

Limited mobility organisms, such as sea stars and holothuroideans (excluding *Peniagone* sp.), were documented intermittently and in limited concentrations in 2010. Sea stars were documented in marginally higher but statistically similar densities in 2011 compared to the Aug-2010 survey and to the further afield sites in later 2010 surveys (Fig. 4.8). These densities are three orders of magnitude smaller than those documented in May-2010 (Fig. 4.9). Holothuroideans were documented in significantly lower densities in 2011 than on Aug-23 and Sept-9 of 2011 (Figure 4.10). Densities are, however, similar between 2011 and Aug-4, 2010. Compared with May 2010, densities of holothuroideans documented in 2011 are still extremely low. This may indicate that the DWH oil spill had a greater impact on organisms with more limited mobility.

There was no clear trend within demersal invertebrates. *Benthocodon hyalinus* and *Bathocyroe fosteri* were both found in similar densities during 2010 and 2011 (Figs. 4.11 and 4.12). This likely indicates that these organisms, which often travel with currents, were either more resilient to the effects of the oil spill or that their advection from outside the affected area resulted in a rapid return to pre-spill densities.

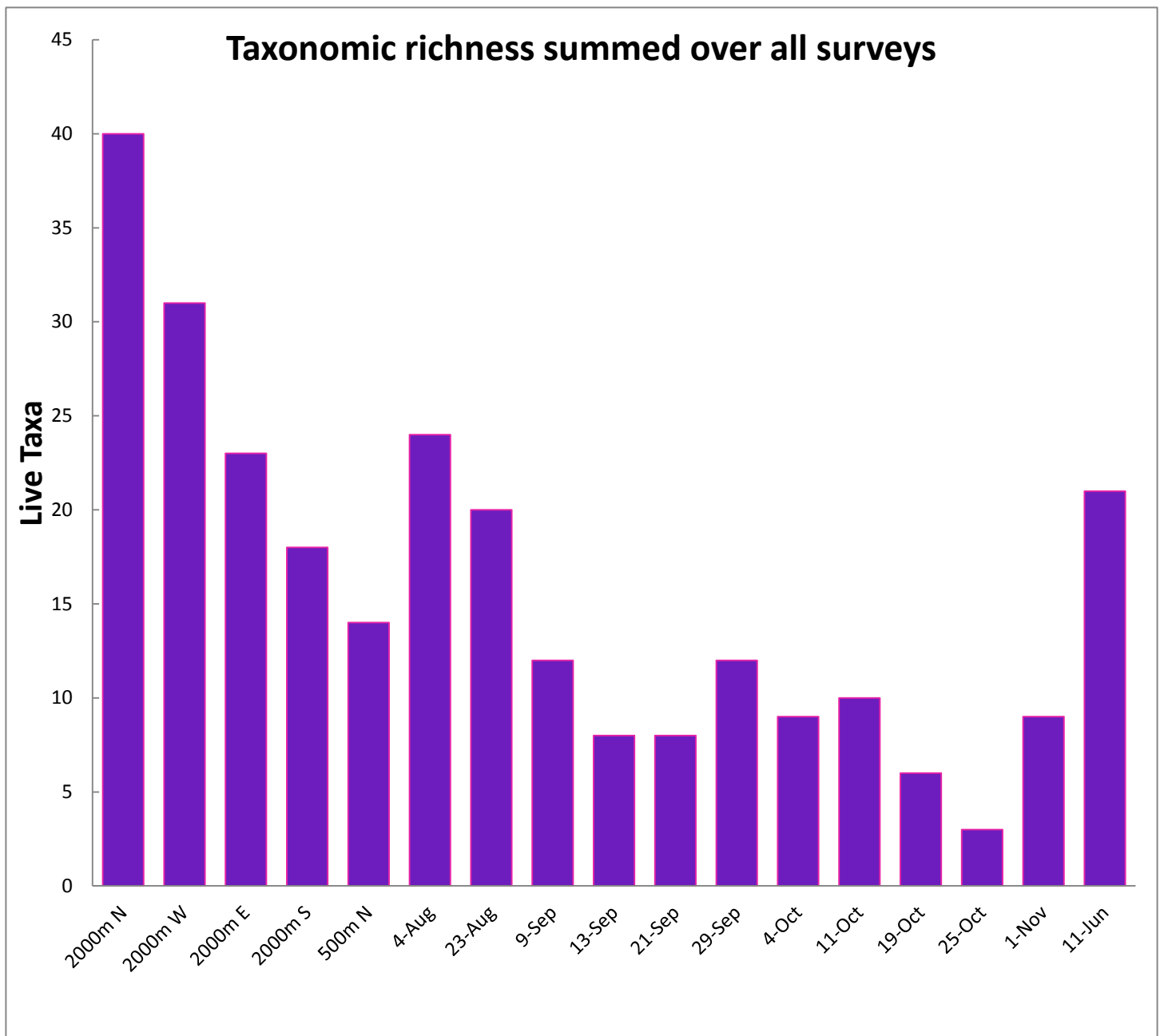


Figure 4.2. Taxonomic richness summed across taxa on each sampling event.



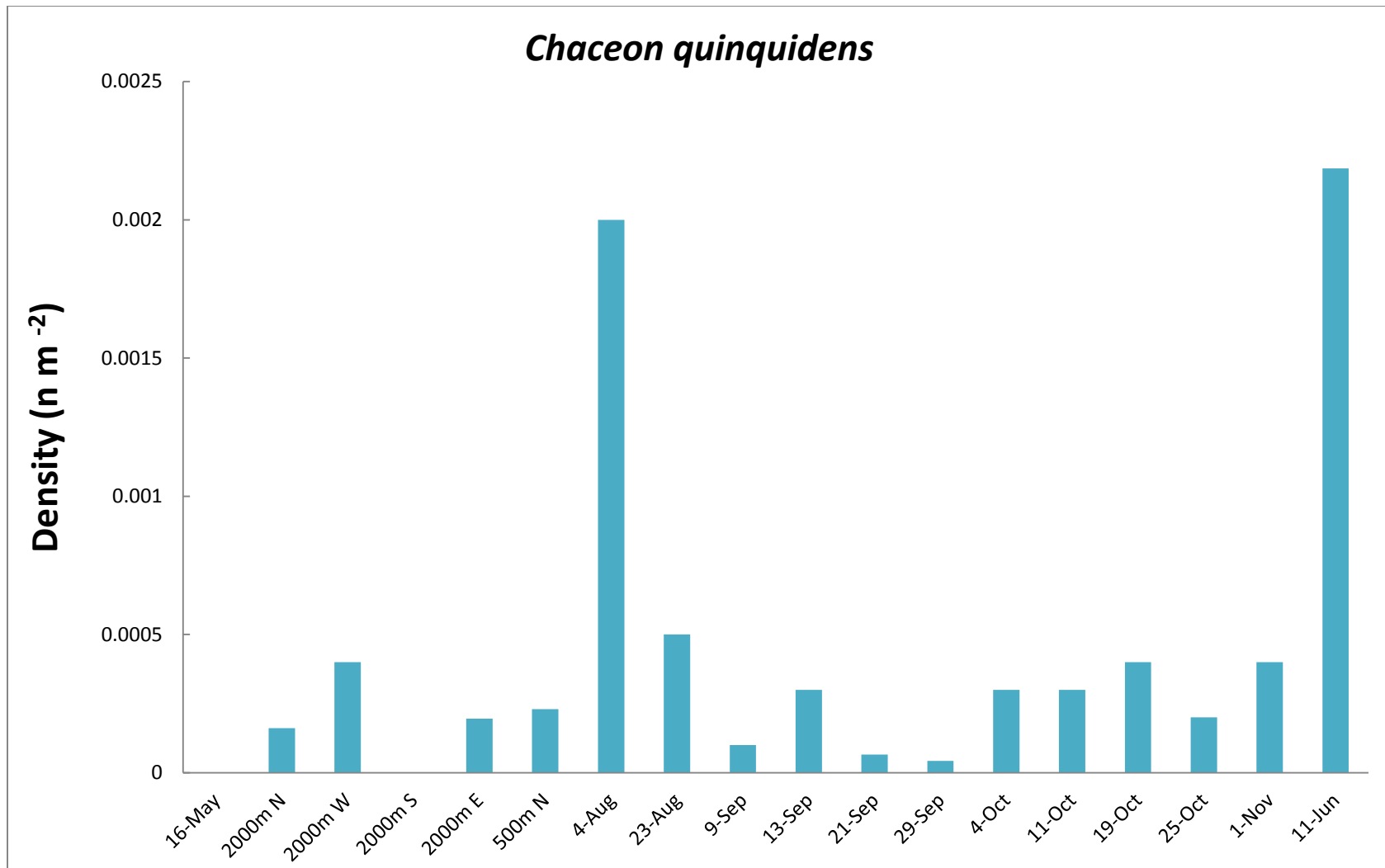


Figure 4.3. Mean density of *Chaceon quinquidens* from August-November 2010 and May 2010.

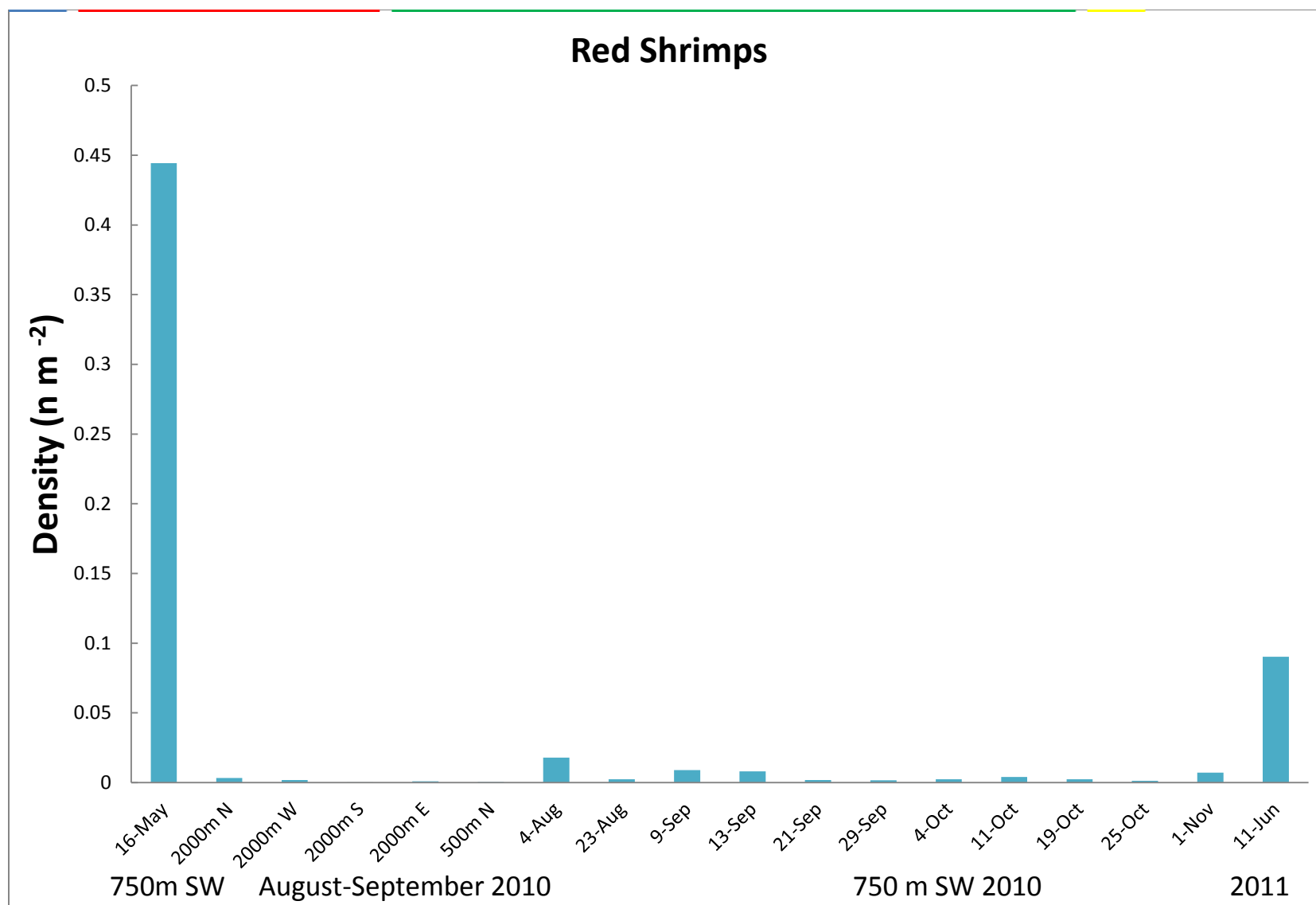


Figure 4.4. Mean density of Red Shrimps from August-November 2010 and May 2010.

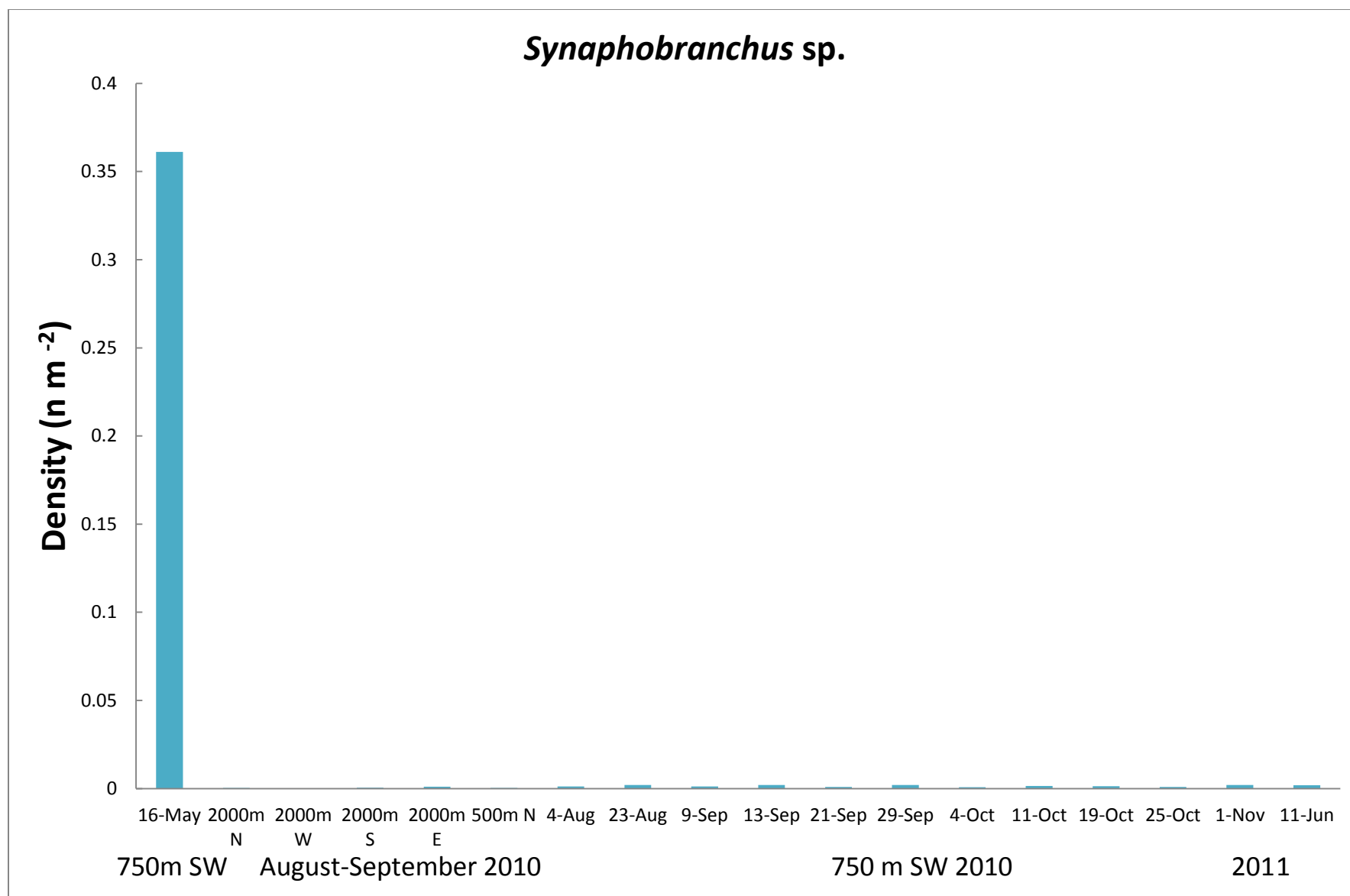


Figure 4.5. Mean density of *Synaphobranchus* sp. from August-November 2010 and May 2010.

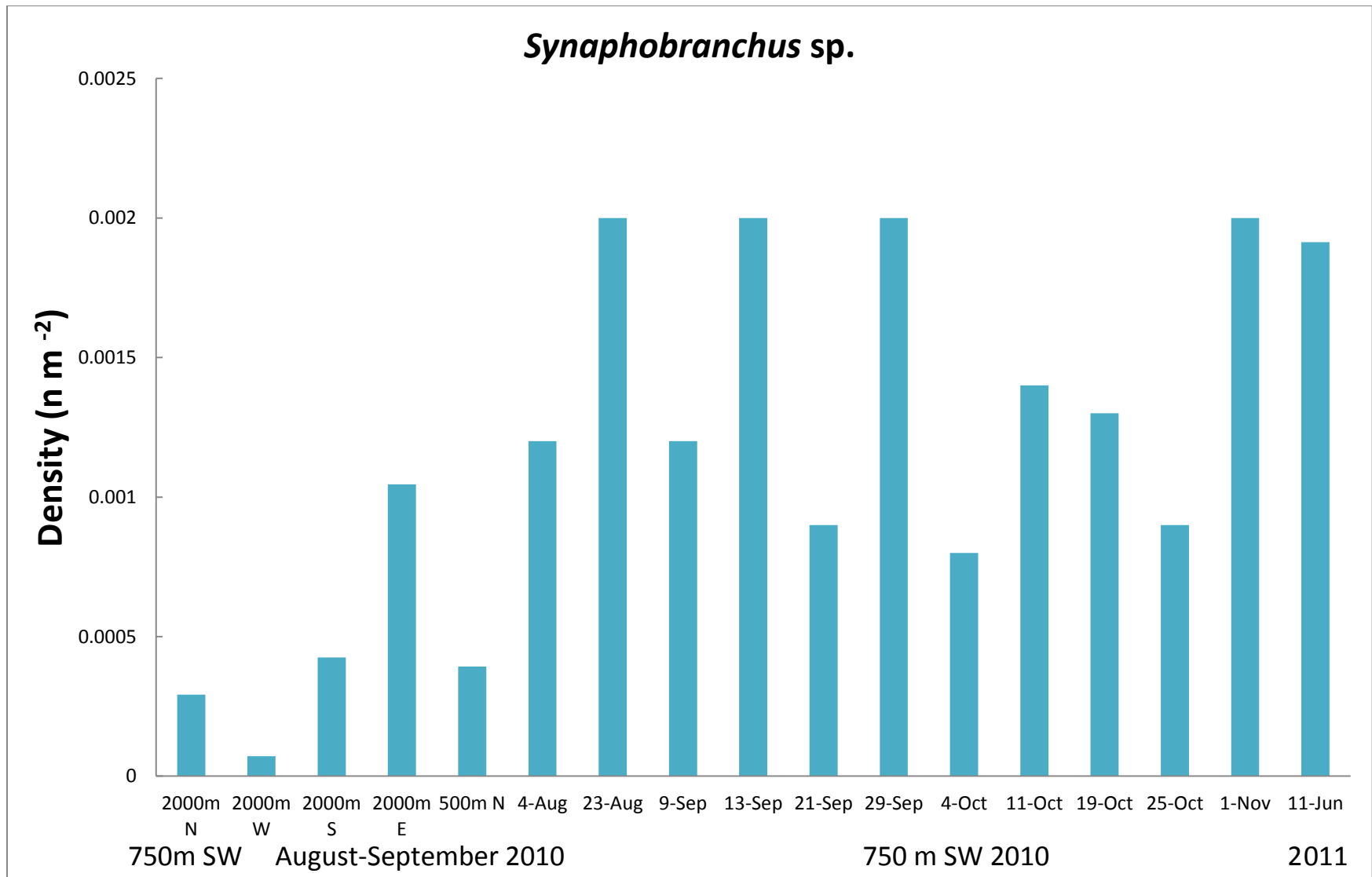


Figure 4.6. Mean density of *Synaphobranchus* sp. from August-November 2010.

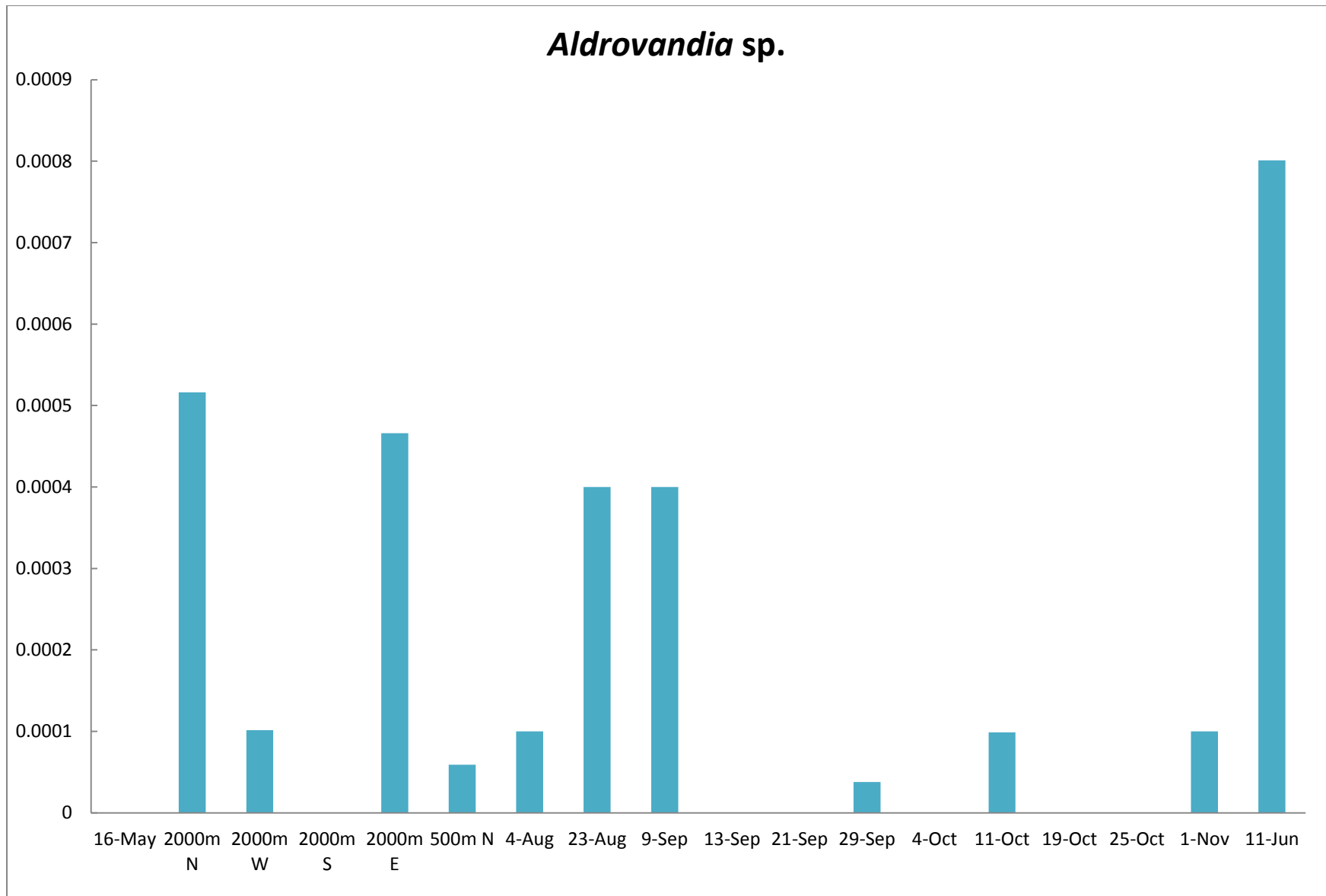


Figure 4.7. Mean density of *Aldrovandia* sp. from August-November 2010 and May 2010.

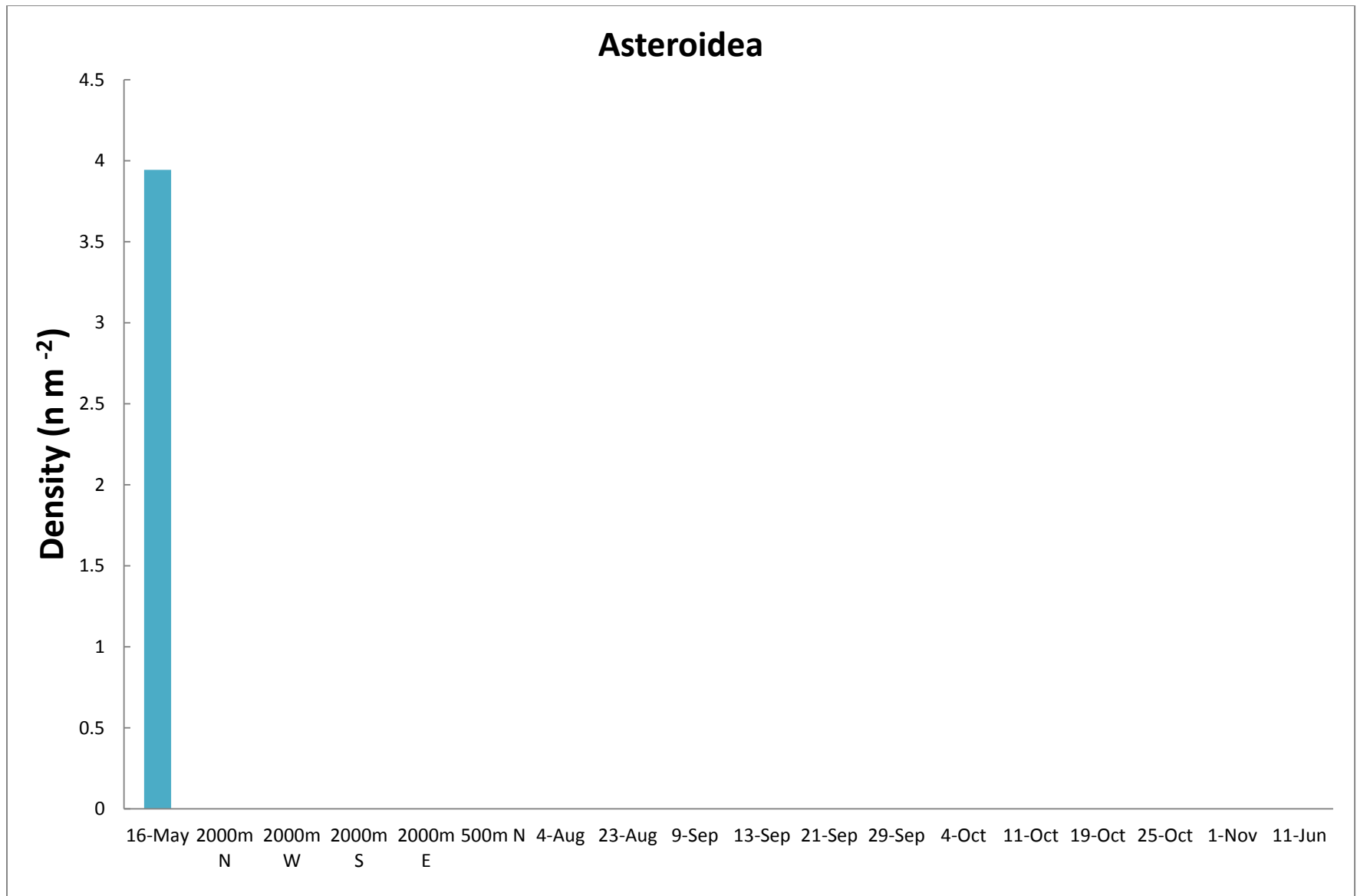


Figure 4.8. Mean density of Asteroidea from August-November 2010 and May 2010.

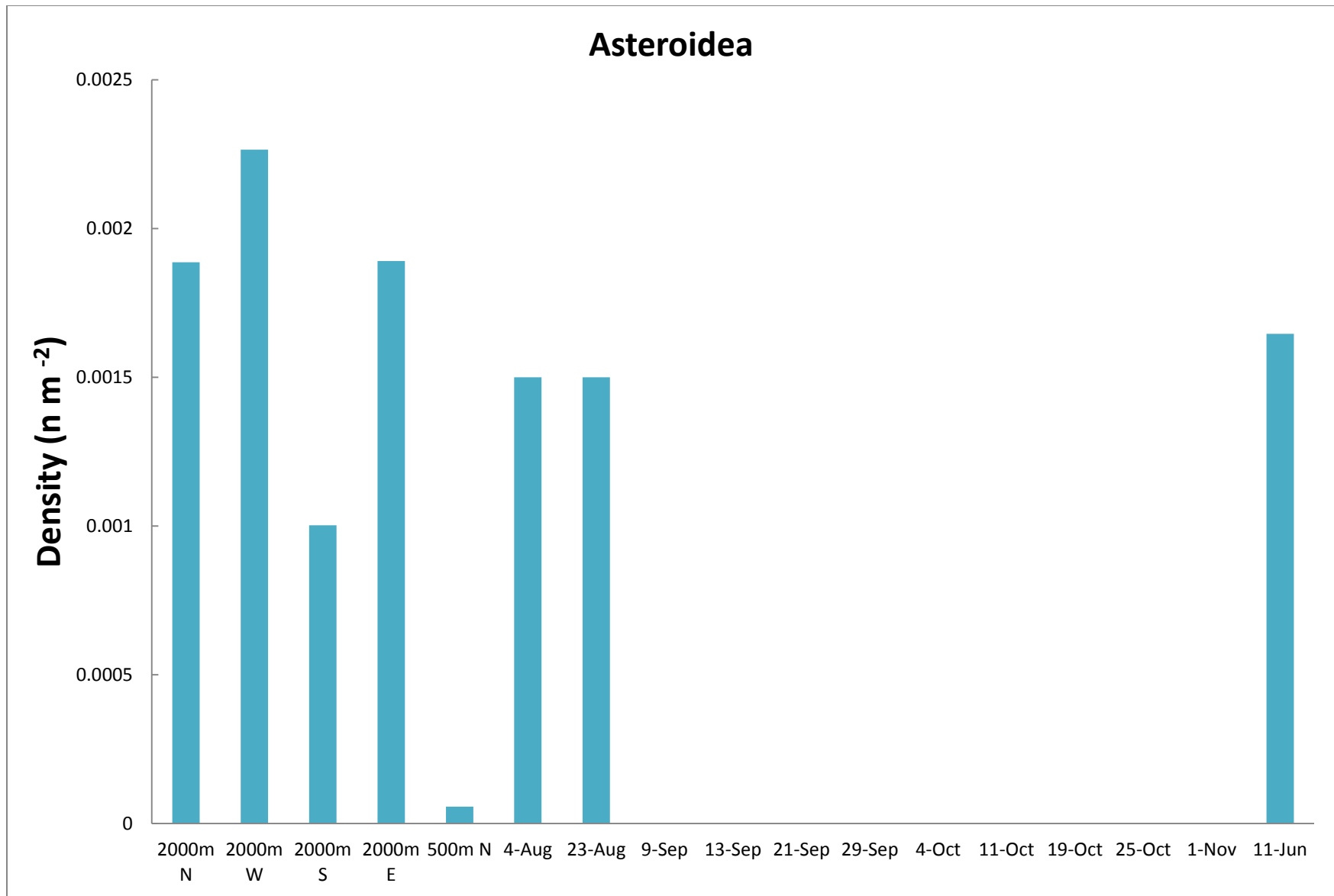


Figure 4.9. Mean density of Asteroidea from August-November 2010.

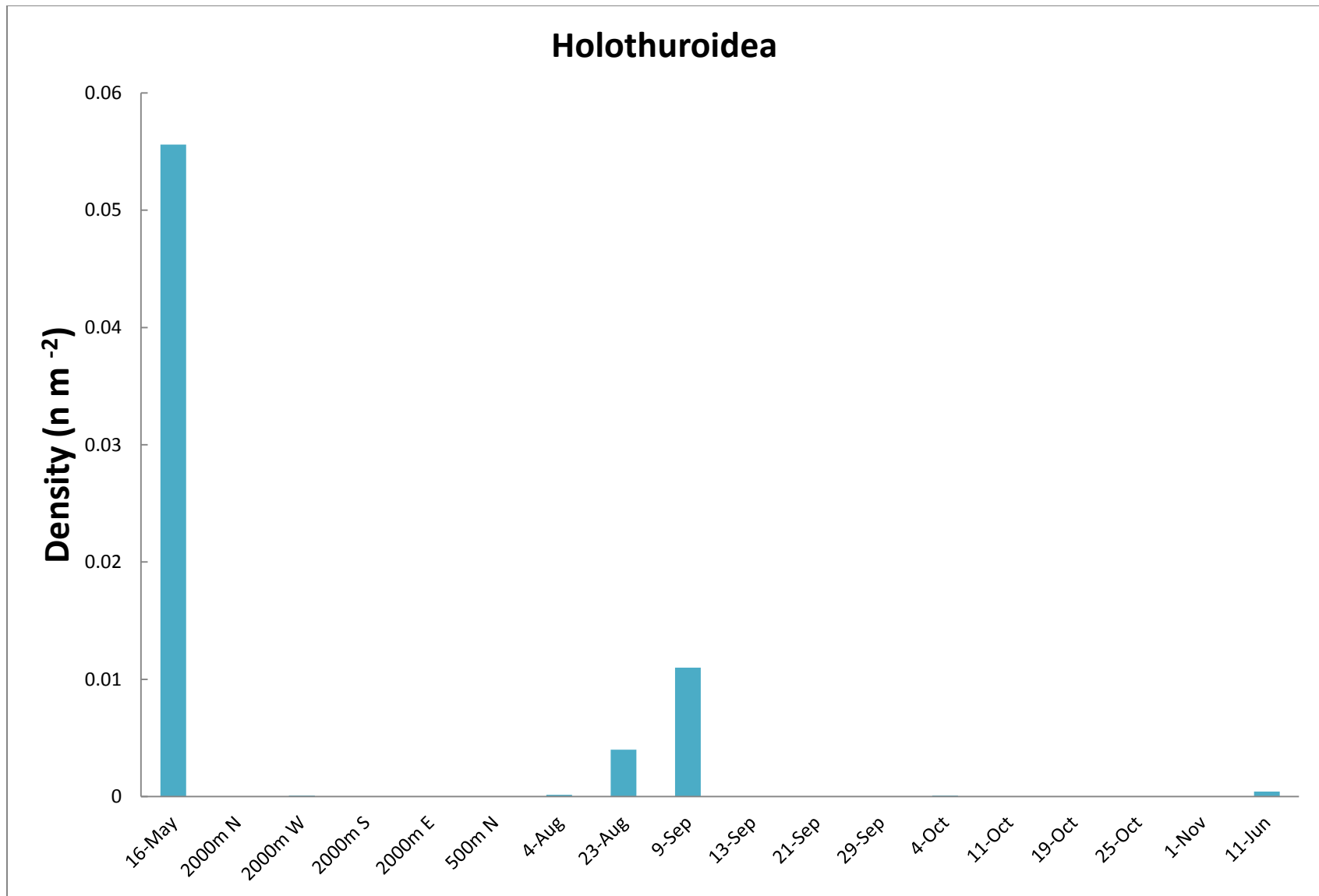


Figure 4.10. Mean density of Holothuroidea from August-November 2010 and May 2010.



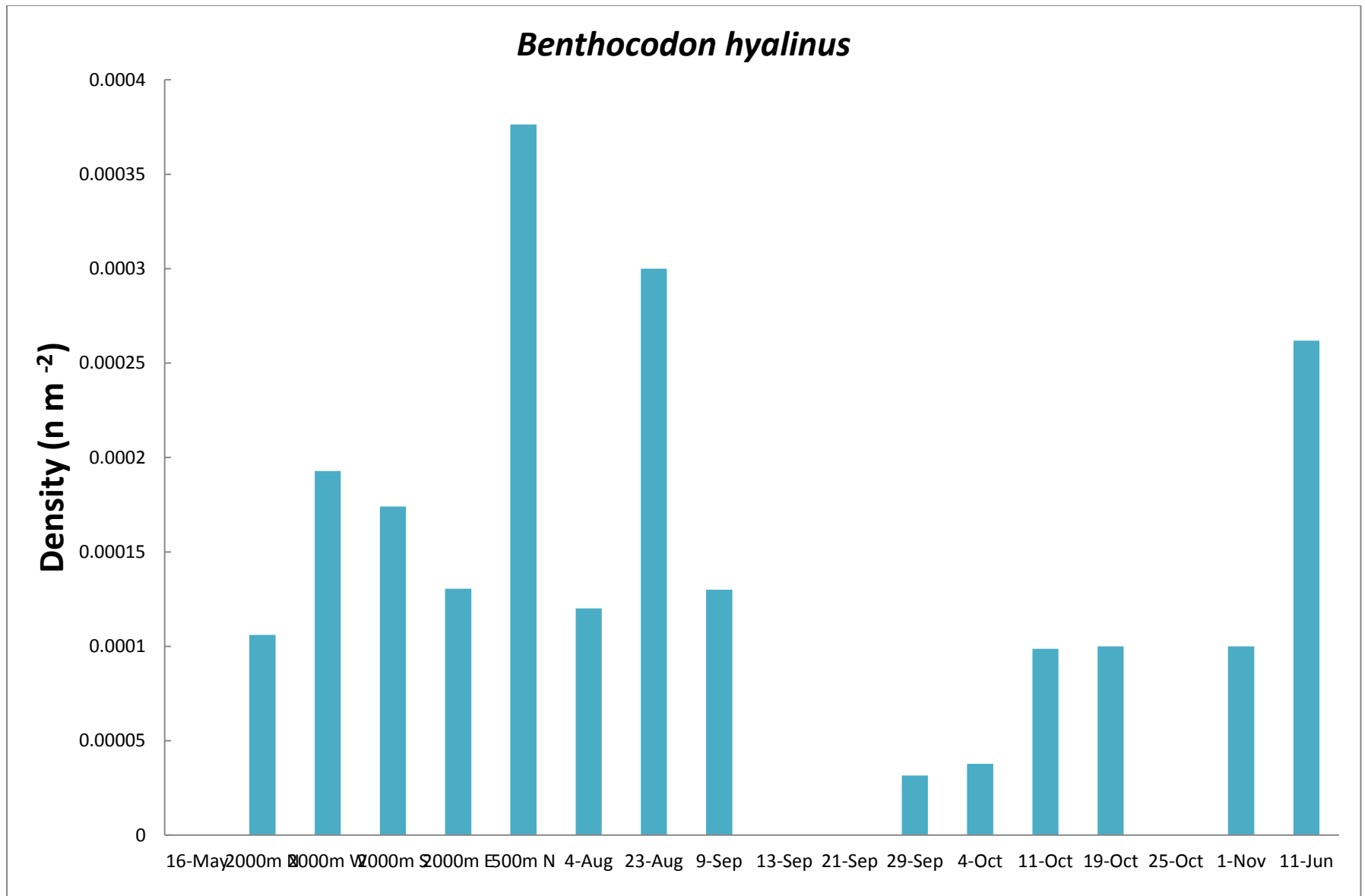


Figure 4.11. Mean density of *Benthocodon hyalinus* from August-November 2010 and May 2010.

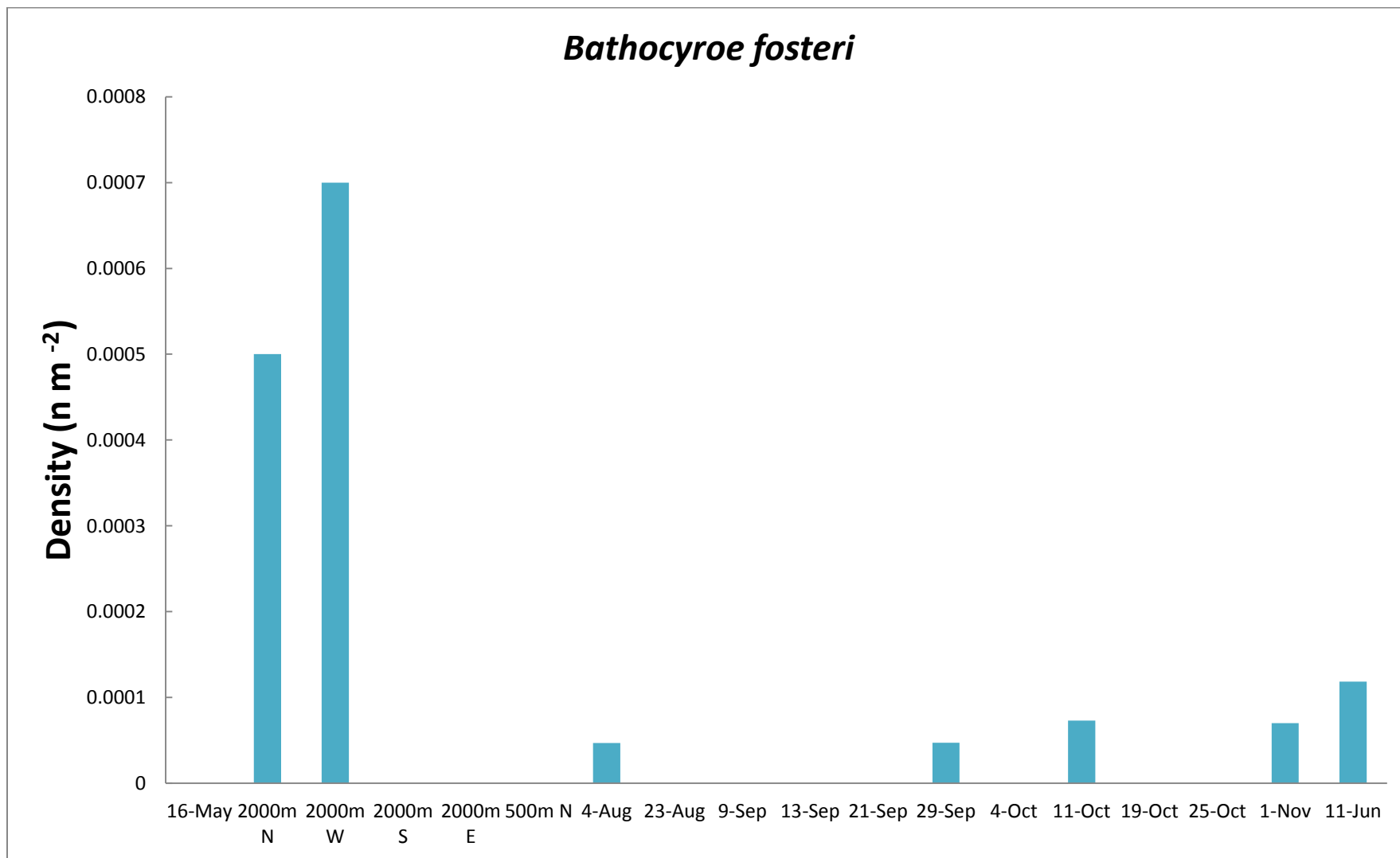


Figure 4.12. Mean density of *Bathocyroe fosteri* from August-November 2010 and May 2010.

The high prevalence of carcasses (pyrosomes and salps) documented during 2010 were not seen in 2011. This likely indicates that the mass deposition of the pelagic organisms during 2010 was a one-time or limited duration acute mass mortality. Continued mortality of organisms due to the spill is either not occurring or occurring at such low levels that we were unable to document it.

Surveys conducted in 2010 utilized standard-definition cameras, while in 2011, ROVs were equipped with high-definition cameras. More advanced camera systems enabled better detection of more cryptic organisms, such as red shrimps and sea stars. Detection of larger megafauna, such as fish and crabs should not be affected as they are more distinct, both on the seafloor and in the water column.

The lack of pre-spill abundance data limits the ability to determine whether or not recovery has occurred over the short-term. While there does appear to be an increase in abundances in 2011 as compared to late 2010, this could be attributed to seasonal variation. There is a need for long term monitoring to determine the true extent of community changes in the wake of the Deepwater Horizon oil spill.

#### **4.5 Literature Cited**

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## **CHAPTER 5: SUMMARY AND CONCLUSIONS**

### **5.1 Summary**

The objective of this study was to characterize and evaluate the deep-water epibenthic and demersal megafauna communities surrounding the Deepwater Horizon oil spill (DWHOS) site. Previous studies have broadly documented megafauna communities in the Northern Gulf of Mexico; however, fine-scale distribution and structure of these communities relative to new oil and gas exploration has been understudied. Studies of the effects of deep-water drilling (>1500m) on benthic megafauna community structure and how these effects vary spatially and temporally are severely limited and there were no quantitative pre-spill data available. I sought to establish initial estimates of community composition and abundance immediately following, and approximately one year after the DWHOS. The final goal of this thesis was to create a post-spill ecological baseline against which future studies of community structure and dynamics in this area can be compared.

In chapter 2, I evaluated the diversity and abundance of megafauna relative to distance and direction from the Macondo well. This was accomplished utilizing remotely operated vehicles (ROVs) at six study sites of varying distances and direction from the Macondo well. The results of this study indicated that the effects of the DWHOS appeared greatest at the sites located 2000 m to the south and east of the Macondo well and at the site located 500 m north of the BOP. Spill-related mortality was evident in the form of carcasses, primarily of planktonic salps and pyrosomes not previously documented at these sites, although some dead benthic organisms were also observed.

In chapter 3, I evaluated the diversity and abundance of megafauna at a single study site located approximately 750 m southwest of the Macondo well: Mississippi Canyon Block 252

relief well 2 (MC252#2) over an extended time period (Aug-Nov 2010) to evaluate any temporal differences in megafauna abundance and diversity relative to the DWHOS. The results of this study indicate the greatest abundance and diversity of megafauna occurred in August 2010 and then proceeded to decline through the fall of 2010. Carcasses of five taxonomic groupings were documented at MC 252#2, mostly in Aug 2010, likely indicating that mortality was acute and does not appear to have been persistent.

In chapter 4, I evaluated the diversity and abundance of megafauna at MC 252 # 2, one year after the DWHOS to determine what, if any, changes had occurred in the surrounding communities. Abundance varied greatly between taxonomic categories. The densities of fishes and other mobile organisms were similar to those documented in Aug 2010, while sessile organisms were found to be less abundant.

Variations in abundance and diversity, based on either time or distance, suggest that some taxonomic groups are either more sensitive or more resilient to disturbances of their habitat. In an ideal assessment of the impact of any oil spill, communities residing in the area would have been surveyed before the accident and the surveys would have been replicated at both affected and control sites. Hopefully, the results of this study will be used to establish post-spill baseline which may be of use in assessing long-term changes at the site over time.

## **5.2 Conclusions**

While extensive surveys of deep-sea megabenthic communities were conducted in 2010-2011, it is impossible to know the true extent to which recovery has occurred after such a brief period. In the case of the DWHOS, there were no quantitative data on the species composition and abundance near the Macondo well prior to the incident. This paucity of baseline ecological

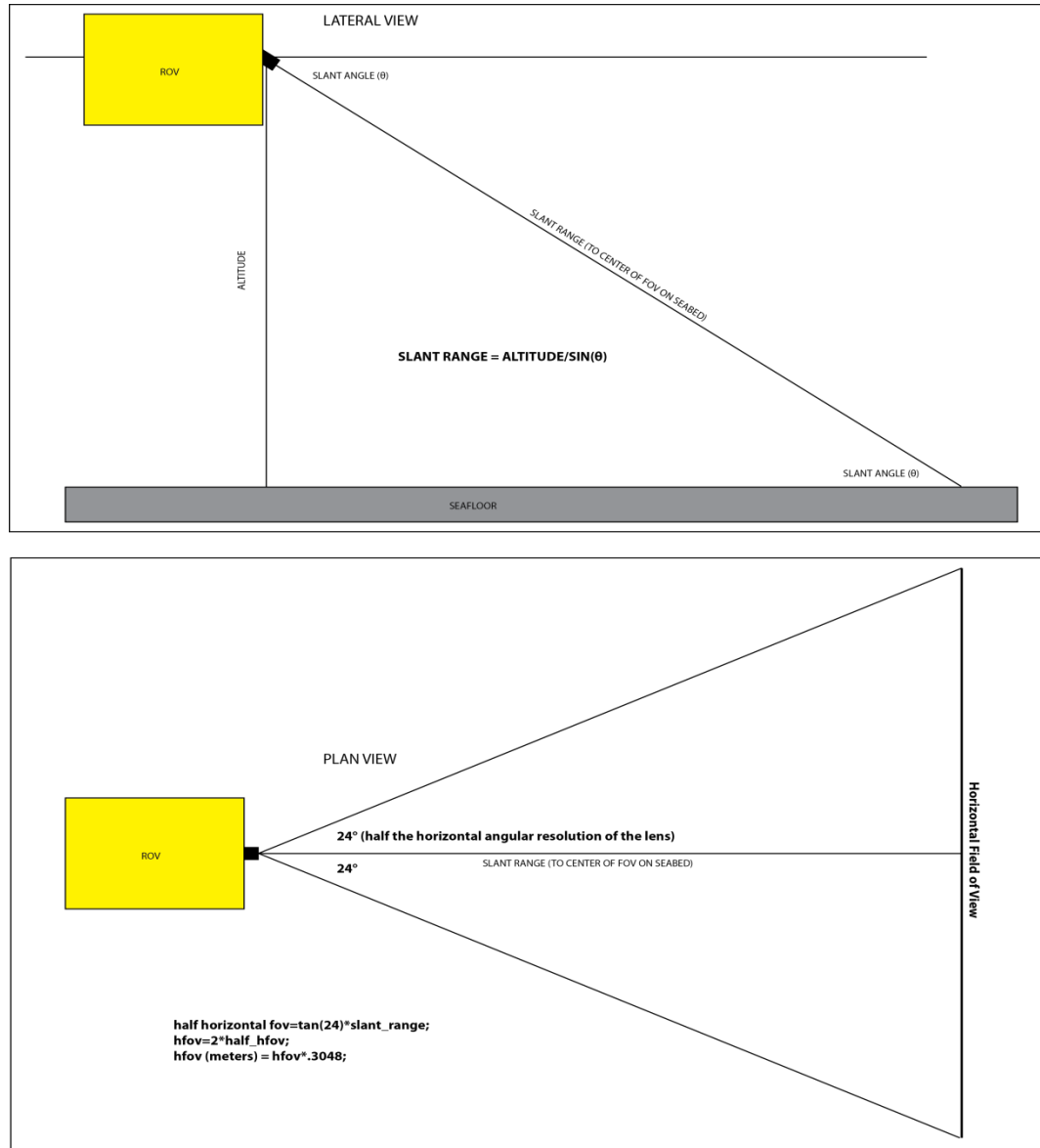
data complicated the assessment of the spill's impact on the surrounding megafauna communities.

ROVs are expensive to deploy and operate and conducting surveys with these vehicles present a unique set of logistical difficulties. In terms of the DWHOS, I was limited to survey sites and dates based the availability of the ships and equipment. One major limitation of this work was the lack of greater spatial coverage. This study was also limited to one year of sampling. A true assessment of the ultimate changes in community function, composition, and structure will undoubtedly require several additional years of repetitive sampling to tease apart the effects of seasonality, nutrient variation, and drilling on these deep sea megafaunal communities.

There is a great need for a comprehensive ecological survey of the entire Gulf of Mexico, especially in the face of ever increasing exploratory oil drilling activity in the region. A lack of synoptic sampling makes it difficult to tease apart the effects of the oil spill from those of natural perturbations and phenomena such as seasonality and spatial distribution. Cooperative collaboration between Industry and the scientific community through projects like serpent will facilitate the collection of this data, so that if there should there be a "next time," hopefully we will be better prepared to respond quickly and effectively to minimize the deleterious effects of such a catastrophe.

## APPENDIX

### Calculation of the downward viewing angle of the camera



In order to solve for theta we next needed to calculate the angle that would accurately estimate the size of a beer can on the seafloor. The dimension of the beer can is 124 mm long. The equation to calculate this for an arbitrarily selected downward angle of  $\theta = 30^\circ$  at an altitude of 7 feet would be:



A. Slant Range (ft) = altitude/sin( $\theta$ ) = 7/sin(30) = 14 feet

B. Half Horizontal FOV (ft) = tan(horizontal angular resolution/2) x slant range (ft) =  
 $\tan(48/2) \times 14 = 6.233$  feet.

C. Horizontal FOV (m) = half Horizontal FOV x 2 x 0.3048 = 3.7998 m

#### Matlab Code

```
sl=30; % Assume 30 degree slant angle
```

```
alt=7; % Set altitude to 7 feet
```

```
slant_range=alt/sin(sl*pi/180);
```

```
half_hfov=tan(24*pi/180)*slant_range;
```

```
hfov=2*half_hfov;
```

```
hfov_m=hfov*.3048;
```

This code yields an answer of 3.7998 m.

Next calculate the size of a beer can measured from an altitude of 7 feet with an assumed horizontal fov of 3.7998 m. The can has an apparent size of 23 pixels in an image that is 626 pixels wide. The true size of the can is 0.124 m.

A. Can size (m) = can size (pixels) x horizontal field of view (m)/image width (pixels)

B. Can size (m) = 23 pixels x 3.7998 m/626 pixels = 0.1396 m

Conclusion is that the horizontal FOV is too wide because the slant angle is too shallow. The above equations can be rearranged to solve for the slant angle:

$\theta$

$$= \sin^{-1} \left( \frac{\text{can size (px)} \times 2 \times \tan \left( \frac{\text{horizontal angular resolution}}{2} \right) \times \text{altitude (ft)} \times 0.3048}{\text{image width (px)} \times \text{can size (m)}} \right)$$

So for our example of a 0.124 m long can with an apparent length of 23 pixels viewed from an altitude of 7 feet we get:

$$\theta = \sin^{-1} \left( \frac{23 \times 2 \times \tan \left( \frac{48}{2} \right) \times 7 \times 0.3048}{626 \times 0.124} \right) = \sin^{-1}(0.5629) = 34.2566$$

Matlab Code:

```
can_dim_px=23;
```

```
can_dim_m=0.124
```

```
alt=7*.3048;
```

```
theta=asin(can_dim_px*2*tan(24*pi/180)/626*alt/can_dim_m)/(pi/180)
```

Check the can dimension calculation using a slant angle of 34.2566 degrees by running the matlab code shown in the previous section:

```
sl=34.2566; % Assume 30 degree slant angle
```

```
alt=7; % Set altitude to 7 feet
```

```
slant_range=alt/sin(sl*pi/180);
```

```
half_hfov=tan(24*pi/180)*slant_range;
```

```
hfov=2*half_hfov;
```

$hfov\_m = hfov * .3048;$

This yields a slant range estimate of 3.3752 m.

Check the can size estimate:

Can size (m) = can size (pixels) x horizontal field of view (m)/image width (pixels)

Can size =  $23 \times 3.3752 / 626 = 0.1240$ .

Next we calculated the slant angle for each of the five cans and averaged them:

Can Number	Can Length (pixels)	Image Width (pixels)	ROV Altitude (feet)	Slant Angle
------------	---------------------	----------------------	---------------------	-------------

1	23	626	7	34.3
2	20	626	8	34.0
3	9	318	7	25.7
4	27	626	7	41.4
5	23	626	7	34.3

We will use the consensus value of 34 degrees as the estimate of the mean slant angle.

## **VITA**

Marla Maxine Valentine graduated from the International Baccalaureate Organization at S.S. Murphy High School in 2005. She moved to Winston-Salem, North Carolina, and received a Bachelor of Science degree in biology with a chemistry concentration from Salem College in 2009. Marla worked as a fisheries technician under the direction of Dr. Sean Powers at the Dauphin Island Sea lab until May of 2011. Marla then moved to Baton Rouge, Louisiana, where she became she became a graduate assistant under Dr. Mark C. Benfield in the Department of Oceanography and Coastal Sciences at L.S.U. Marla will receive a Master of Science degree in oceanography and coastal sciences in May of 2013.