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Deepwater Horizon Impact on Deep-sea Meiofauna Biomass

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by

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Abstract

The Deepwater Horizon oil spill occurred in 2010, oil and dispersants were spread across the surface and the deep-sea. Meiofauna community biomass increased regardless of whether the station had signatures of the oil spill or not. Increases in meiofauna biomass possibly indicate a bentho-pelagic coupling of surface waters and benthic communities. The increase in biomass was mainly due to nematode abundance increases and not individual biomass size. In the years following the oil spill, meiofauna community biomass remained elevated compared to pre-spill conditions. Biomass decreased in the years following returning closer to pre-spill values, possibly indicating signs of recovery in the meiofauna community.

Dedication

This would not have been possible without the love and support of my family and friends. I would never have been able to make it through the hardships, trials, and ultimately success that this project has been.

This journey would never have started without the support of my high school biology teacher Mr. Peevyhouse. He introduced me to the “magic” of zoology and botany, and gave me my first steps into a scientific world.

“I want to stay as close to the edge as I can without going over. Out on the edge you see all kinds of things you can't see from the center.”

Kurt Vonnegut, *Player Piano*

“To invent your own life’s meaning is not easy…

…but it’s still allowed…

…and I think you’ll be happier for the trouble”

Bill Watterson

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Introduction

The Gulf of Mexico (GOM) is the Earth's ninth largest body of water and serves as both an important ecosystem and economic resource (Tunnel, 2011). It spans from the Yucatan peninsula to Florida with warm productive waters (Love et al., 2013). Shallow areas (<20m) and the continental shelf (20 to 180 m) make up roughly half of the GOM while the continental slope and abyssal (180 to 3,000 m, and >3,000 m) make up the remaining area (Love et al., 2013). Its deepest areas are in west-central GOM, known as the Sigsbee Deep reaching approximately 4,000 m (Love et al., 2013). The GOM is home to 11 critically endangered species, 8 endangered species and 13 other threatened species as listed under the Endangered Species Act ("Endangered and Threatened Marine Species under NMFS' Jurisdiction"). This study focuses on the benthic community of the GOM and the impact of the Horizon oil spill on meiofaunal benthic community biomass. Benthic communities are composed of different types of animals defined by their size, with ecologically distinct functions. In the last century, the term *meiobenthos* has been used to describe metazoan meiofauna and protist species of intermediate size (Mare, 1942). The taxa that comprise deep-sea meiobenthos are sized between 300 to 45 µm. There are 20 phyla present in the meiobenthos, with five meiofauna phyla, Gnathostomulida, Kinoryncha, Loricifera, Gastrotricha, Tardigrada, and Micrognathozoa (Giere, 2009). Meiofauna are ubiquitous through the marine sediment and become a relatively larger portion of faunal biomass of the deep-sea when compared to larger fauna.

Meiofauna has a major role in the carbon cycle, aiding in the re-mineralization of organic carbon and release of nutrients within the sediment as well as an important food source for higher trophic levels (Coull and Chandler, 2001; Solan et al., 2004; Montagna 1984; 1995). Shallow water meiofauna communities typically have much higher abundances compared to those of the deep sea (Coull and Chandler, 2001). Nematoda and Copepoda were the dominant taxa found with less than 5% of the remaining abundance being represented by Polychaeta, Kinoryncha, Ostracoda, Cyclopoida, Tardigrada, Tanaidacea, Acari, Isopoda, Bivalvia, Gastrotricha, Loricifera and other minor taxa for a total of 21 taxa found (Baguley et al., 2006). In the GOM, nematodes and copepods remain the dominant taxa regardless of depth, but the number of taxa found decreases as a function of depth (Baguley et al., 2006). Meiofauna are present across the marine environment and compete for resources with other benthic size classes. Many species use different habitats as larval and adult stages switching between pelagic and benthos zones depending on the life stage (Bertness et al., 348).

Benthic communities depend on surface waters for enrichment. Ungrazed phytoplankton decompose and pass from the pelagic zone to the benthic community (Falkowski, 2012). Decomposing fecal matter from zooplankton also provides an energy source to the lower water column and benthos (Bertness et al., 2014). Meiofauna have low chances of survival compared to fauna with larger clutch sizes and that may be able avoid predation and ecosystem disturbances due to being more motile (Giere, 2009). With limited resources and low survival, meiofauna populations increase their persistence probabilities with relatively continuous reproduction together juveniles hatching as self-sufficient individuals, i.e., lack of larval forms in permanent meiofaunal taxa (Giere, 2009). Food resources are limited in the deep sea and competition for nutritional resources results in spatial niche segregation within the meiofaunal community (Joint et al., 2012). Predation also impacts meiofauna communities as many macrobenthic species prey on these organisms, along with “bulk uptake” by macrofauna and larger animals that indiscriminately consume the sediment (Giere, 2009).

The benthic community has been disturbed by a variety of human activities and natural phenomena (Halls, 1994; Posey et al., 1994). Disturbances can greatly affect meiofauna community structure (Huston, 1979). The rate and frequency of these disturbances may determine whether they enhance or devastate meiofauna communities. Following a disturbance, meiofauna assemblages may shift from K-selected to a more r-selected one (Giere, 2009). Opportunistic species may be able to re-colonize quicker due to relatively quick reproduction cycles within meiofauana. Animal size would also be expected to be smaller overall following a disturbance event (Pearson and Rosenberg, 1976; Schwinghammer, 1981). However, depending on the taxon and the disturbance type, the re-colonization rates and sizes might be different due to the various life strategies within meiofauna (Giere, 2009). The time to recovery is highly variable as nearby donor communities may have a role in re-colonization (Azovsky, 1988; Sherman and Coull, 1980). If nearby communities are low in diversity and abundance then recruitment would be limited following a disturbance event.

In 2010, the Deepwater Horizon (DWH) oil spill occurred in the GOM (Unified Area Command (UAC), 2010). The spill persisted for 87 days after the initial blowout, and released approximately 4.9 million barrels of oil prior to permanent capping of the wellhead (Lubchenco et al., 2010). Impacts across the GOM were ecologically and economically significant (Peterson et al., 2012). It was predicted that an $8.7 billion loss to the fishing industry alone was seen in the years following the spill (Sumaila et al., 2012). British Petroleum (BP) settled in April of 2017 for $20.8 billion with potentially an additional $14.8 billion in private claims not covered by this amount. BP claims to have recovered approximately 33% of the released oil via surface recapture methods (UAC, 2010). After calculating rates of chemical and natural dispersion, evaporation/dissolved, burning, and skimming, there is estimated to still be around 1.1 million barrels of oil considered "other" left in the GOM (Lehr et al., 2010). Thirty-five percent of the "other" oil was left as a deep-sea plume traveling across the GOM (UAC, 2010).

In addition to surface application, dispersants were used near the seafloor and directly injected into the flow of oil from the wellhead (Kujawinski, et al., 2011). Released dispersant and oil mixture spread laterally across the deep-sea GOM (Kujawinski et al., 2011; Valentine et al., 2014). This deep-sea plume was estimated to have spread to an area of 3,200 km^2 around the wellhead at depths of ~900 - 1,700 m but a heterogeneous distribution suggests the oil footprint may be larger than what was detected in deep-sea water and sediment samples (Valentine et al., 2014). Oil and dispersants attached to marine snow, organic material from surface waters, to create a marine oil snow (MOS) event following the DWH (Passow and Ziergovel, 2016). This MOS event increased deposition of oil-laden particles to deep-sea sediments following the spill.

There is evidence that oil related bacterial blooms occurred in the upper zones of the GOM resulting in increased transport rates of MOS to benthic habitats (Valentine et al., 2014). Evidence of oil in the deep sea benthos found that there were distinct communities of impact and non-impact areas along the GOM (Montagna et al., 2013; Valentine et al., 2014). In particular, meiofauna communities had decreased diversity and increased nematode to copepod (N: C) ratios in areas of high impact (Baguley et al., 2015). Changes to the benthic community structure have been described immediately following the spill (Montagna et al., 2013; Baguley et al., 2015; Hsing et al., 2013). Impacts to the benthic fauna are of concern due to their role in deep-sea ecosystem services (Gage, 2003; Gray, 1981). Loss of diversity in the benthic communities has been linked to exponential losses of ecosystem function and efficiency (Danovaro et al., 2008).

Previous data from the GOM indicate that relative meiofauna biomass increases with depth, and the majority of meiofauna biomass is comprised by nematodes and harpacticoid copepods (Rowe et al., 2008; Baguley et al., 2008). Because meiofaunal biomass contributes significantly to deep-sea food webs, and is proportionally greater than other benthic size classes, it is important to gain a more complete understanding of their role in ecological dynamics, ecosystem services, and how pollution events such as the DWH spill may affect the trophic structure of the deep sea.

Links between high biodiversity and ecosystem functions makes understanding changes in diversity important to assessing impacts (Danovaro et al., 2008). The biodiversity within the GOM includes bacterial communities that may have aided in the initial recovery of the DWH spill (Edwards et al., 2011). Previous to the spill the GOM had high benthic diversity with the maximum values observed being around the same depth as the DWH wellhead (Stuart et al., 2003; Rowe and Kennicutt, 2009; Baguley et al., 2006). Initial impacts to the benthic communities showed decreased diversity in areas affected by the oil spill (Montagna et al., 2013; Baguley et al., 2015). During investigations into the impacts a new genus and species of copepod was found within the meiofauna community (Bang et al., 2014). Benthic communities do have important roles in ecosystem function, however, the ecosystem services and underlying mechanisms of the deep-sea ecosystem are still not well understood (Armstrong, 2012). Investigations into changes of the deep-sea trophic structure may help in elucidating these mechanisms and services.

Changes to community function have not been well documented following the oil spill. Changes in animal size or abundance affect the rate of respiration at an individual, and community level (Baguley et al., 2004; 2008). Meiofauna biomass becomes a relatively larger portion of the community as depth increases (Pequegnat et al., 1990; Rowe et al., 2008). Their proportionally larger role in the deep-sea indicates they could have a proportionally larger impact on community function.

Deep-sea communities depend on outside carbon sources for sustenance and would experience a food deficit without it (Smith et al., 2001). Most of that carbon is released via the benthic community's respiration with relatively smaller portions being cycled via predation and export, and burial (Rowe et al., 2008). Following the DWH oil spill extensive sampling was conducted in the northern GOM deep sea to investigate potential ecological effects (UAC, 2010). In total, 170 stations were sampled for to assess benthic community impacts, with 68 of those designated priority for understanding post-spill alterations to benthic diversity and abundance (Lubchenco et al., 2010). Analysis of the priority stations found that sites with the highest chemical loads also had the lowest diversity (N1) along with high nematode to copepod ratios, which indicate impacts from the oil spill (Montagna et al., 2013). At stations impacted by the oil spill, meiofauna abundance increased by ~190% while diversity had gone down by ~40% compared to background conditions (Baguley et al., 2015). Previous studies have not included biomass in their assessment of the impacts of the DWH spill. Diversity has been theorized in the importance of ecosystem services but the cycling of nutrients, such as carbon, is a metric of community function (Smith et al., 2001; Wei et al., 2010), and is in need of further study with respect to the DWH spill.

In this study the aim was to determine whether the DWH oil spill impacted meiofauna biomass in the northern Gulf of Mexico deep sea. Several key questions were addressed concerning how the oil spill may have impacted meiofauna community function, and hypotheses centered around these questions were tested.

1. Did average individual animal size decrease at impacted vs. non-impacted sites?
2. Was total community biomass different at impacted vs. impacted sites, and was there a spatial relationship with respect to distance from the wellhead?
3. Was water depth a significant factor influencing meiofauna biomass and did depth affect impacted vs. non-impacted stations differently?
4. Were meiofauna biomass patterns different post-spill compared to pre-spill observations? By testing hypotheses centered on these questions, I hope to more fully understand meiofauna biomass in the deep Gulf of Mexico, and how the largest marine oil spill in US history impacted the function of this important animal community.

Oil and its related hydrocarbon compounds have known toxic effects on a variety of species. Oil degrades, volatilizes, forms secondary compounds, and can be trapped beneath sedimentation. The deep-sea is subject to sedimentation from surface waters but has much colder temperatures and higher pressure. There is evidence from the literature that Nematodes have a higher tolerance to pollution than other meiofauna while copepods are more susceptible (Warwick, 1981; Amjad and Gray, 1983; Baguley et al., 2015). Nematode to Copepod ratio (N: C) has been used as an indicator for pollution level but may only be useful in certain conditions. It has also been suggested that there is a balance between enrichment and toxicity after oil spill events, where some tolerant and opportunistic taxa may become dominant. Biomass could therefore be useful as an indicator of functional community response since it represents a carbon stock that has the potential to flow through higher trophic levels.

Because the DWH oil spill has toxic impacts from the oil and dispersant related compounds, I predict that copepod community biomass will be lower in impacted areas due to their susceptibility to pollutants. However, I predict nematode community biomass to increase at impact sites due to their pollution tolerance. Pearson and Rosenberg (1978) previously found that smaller body sizes are expected in a post disturbance community. I predict that in impact zones I will find smaller body sizes than compared to non-impact zones, regardless of taxa. Reuscher et al. (2017) found that meiofauna abundance has begun to return to background conditions. I predict that meiofauna community biomass will be closer to background conditions in the years following the oil spill due to the loss of enrichment from hydrocarbon degrading bacteria.

Methods and Materials

Field Methods

Detailed descriptions of field sampling have been previously reported (Montagna et al. 2013). Briefly, an Ocean Scientific International (OSIL) multicorer was used to take deep-sea benthic samples. The OSIL multicorer takes 12 simultaneous sediment cores; each being 10 cm in diameter and 60 cm in height. Meiofauna was subsampled by using smaller 5.5 cm diameter subcores, consisted with prior sampling in the region (Baguley et al. 2006). Subcores were extruded to isolate the 0-1 cm from the 1-3 cm subsection overlying water within subcores was siphoned and added to the 0-1 cm sections. Samples were relaxed using 7% $MgCl_2$, and preserved in a 4% buffered formalin solution stained with Rose Bengal. Samples were packaged and delivered to the University of Nevada, Reno, where all laboratory analyses of the meiofauna community were conducted.

Laboratory Methods

Enumeration

In the laboratory, meiofauna samples were rinsed over a 42 µm mesh sieve to remove formalin and fine sediment particles (Giere, 2009). Isopycnic centrifugation with Ludox^{TM} HS-40 was used to separate organisms from the sediment. The rinse and isopycnic centrifugation were done twice to achieve a retention rate of ~ 95-99% of organisms (Burgess, 2001). A final rinse removed the Ludox^{TM} HS-40 and samples were then rinsed with tap water into a beaker for enumeration and taxonomic identification. Approximately 95% of meiofauna biomass is dominated by two taxa, Nematoda and Harpacticoida (Baguley et al., 2008). This study focuses on those two taxa as a proxy for total meiofauna community biomass. 60 nematode and 60 copepod individuals were taken from each sample for digital micro-photography. If samples that contained less than 60 individuals from each taxon, then all individuals were collected for biomass estimates.

Microphotography

Nematode and copepod biomass was estimated using a well-established digital microphotographic approach (Baguley et al. 2004). Organisms were placed into drops of glycerol on a slide with ~10 organisms per drop. Individuals were arranged to ensure no overlap and that the largest cross section of the organism was in view. The prepared slides were viewed with a Leica DM2500 compound microscope and photographed with a Leica DFC295 camera. Leica Application Suite v.6 was used to capture photographs of organisms. In a few cases a nematode was too large to be measured via one photograph, in those instances multiple photos were taken with reference to one and other. Photographs were then analyzed using Sigma Scan Pro 5, measuring body area (A) and mid-body width (W). Area and mid-body width are used to estimate biovolume, which can then be converted to units of biomass (Baguley et al. 2004, and references therein).

Biomass Estimation

Nematode

Nematode biovolume was estimated using an equation suggested by Warwick and Price (1979) and supported by Baguley et al. (2004). It assumes that the body of a nematode approximates a cylinder and determines biovolume using equation 1.

Copepod

Copepod biovolume was approximated using an equation by Warwick and Price, (1979) and modified by Baguley et al. (2004) based on direct measurement techniques (Equation 2). Feller and Warwick (1988) suggested the use conversion factors for body form and orientation to account for the diversity found within the Copepoda as well as differences in how organisms settle on the slide within the glycerol medium. Baguley et al. (2004) established that a conversion body factor (C_{bf}) and an orientation factor (C_O) of 440 and 1.5, respectively, would be appropriate for an analysis of Copepods in the GOM. The C_{bf} was determined using the five most common body types found in the GOM and averaging them based on recommendations of Feller and Warwick (1988).

Community Biomass Estimates

After estimating biovolumes for individual organisms, estimates were converted into wet weight (µg), then to dry weight, to carbon content, and then to mg of Carbon m^{-2} (TC) (Equation 3). Baguley et al. (2004) used direct estimate to find a more accurate carbon ratio (CC) for nematodes (CC= 51.4%) and copepods (CC= 45.8%) as previous studies had used a CC based off direct measurement of chaetognaths. The final factor of equation 3 is to scale TC to a per meter squared estimate based on the diameter of the subcores used for sampling.

Equations

$$\boldsymbol{V(nL) = \pi r^2 L/10^6}$$

Equation 1: Nematode biovolume (V, nL) is determined using the radius (r, µm), and length (L, µm).

$$V(nL) = [A\ (mm) x W(mm)](C_{bf} x C_O)/10^9$$

Equation 2: Copepod biovolume (nL) is estimated using area (A) by width (W), modified by a body factor (C_{bf}) and body orientation (C_O), to correct for differences between families and organism's orientation on the slide, over 10 to the ninth.

$$C\ mg\ m^{-2} = VxWWxDWxCCx420.9$$

Equation 3: Milligrams of Carbon per meter squared were determined by multiplying the biovolume (V) of the organism by its wet weight conversion factor (WW=1.13) by the dry weight conversion factor (DW=0.25) by their measured carbon ratio (CC=0.514, Nematode, and 0.458, Copepod) by 420.9 (Determined using area of the core used for sampling) (Baguley et al., 2004).

Experimental Design

Statistical Analysis

To analyze meiofauna community biomass, I used a zone classification scheme similar to Montagna et al. (2013) and Baguley et al., (2015), where Zone 1: severe impacts, Zone 2: moderate impacts, Zone 3: uncertain impacts, Zone 4: background conditions, Zone 5: background conditions). Montagna et al. (2013) used principal components analysis to designate zones of impact and non-impact based on conditions of stations measured post-spill. Baguley et al. (2015) reduced Zones 4 and 5 to single background condition zone for meiofauna community analysis. Both previous studies described benthic community structure but did not consider community biomass in their approach.

In this study zones were simplified into 22 Impact and 31 Non-impact zones (Zones 1-3 and Zones 4 and 5, respectively). Biomass photographs were obtained for 55 stations from 2010 which included stations that had already been used for copepod taxonomic identification. In 2010, 2011, and 2014 a subset of 21 stations of the priority stations were used for biomass analysis (13 Impact and 7 Non-impact).

Univariate hypothesis testing of total biomass between Impact and Non-impact zones was done with one-way Analysis of Variance (ANOVA). Two-way ANOVA was used to test for differences among depths, sections (0-1 and 1-3 cm), and years. Copepod biomass in 2010 was log transformed before analysis to meet the assumptions of the general linear model (GLM). Between years comparison had nematode, copepod and total biomass transformed to meet the assumption of GLM. Bartlett's test of homogeneity of variance was used to assess assumptions of the GLM. Tukey's test was used for post hoc comparison of the 1-way ANOVA. R 3.2.3 was used for analysis and Appendix 1 has a complete list of library's and functions used.

The reduced number of stations in 2011 and 2014 was suitable for ANOVA, but not spatial analysis. I used ArcMap Geostatistical Analyst and the the inverse distance weighted method of spatial interpolation (Power: 2, Max neighbors: 6, Minimum neighbors: 4, weighted by depth, n: 65). The data were not separated into zones for spatial analysis, instead depths were weighted allowing for non-priority stations to be incorporated creating a larger sample size. Interpolated surfaces were mapped on to a map of the DWH oil spill station map projected on the GOM.

Results

2010

General Results

Samples were collected from water depths of 76-2767 m (n=55), in the northern Gulf of Mexico deep sea. A total of 10,618 individuals were sorted and used to obtain body volume estimates, 4,168 of those were copepods and 6,450 were nematodes. Copepods had an average of 75 individuals per sample, with a minimum of 31 and a maximum of 120. Nematodes had an average of 112 individuals per sample, with a minimum of 60 and a maximum of 122.

Overall, individual biomass for both taxa differed between zones and years (Table 1) (Figure 2, 3). Average copepod biomass exhibited a weak decreasing trend with depth at non-impact stations (Copepod, R^2=0.0131) (Figure 2). Nematodes had a slight increase in biomass size with depth at impact stations while non-impact had the inverse (Impact nematode, R^2=0.0034; Non-impact nematode, R^2=0.0045) (Figure 2). Average community biomass (mg C m^{-2}) was higher at impact stations (131.61±77.46) than non-impact stations (104.81±80.69) (Table 1). The lowest biomass was found at a non-impact station (6.45 mg C m^{-2}), while the largest biomass was found at an impact station (343.34 mg C m^{-2}). Regression of total community biomass by depth, regardless of zone, was found to be highly significant (R^2=0.134, F=9.098, p=0.004, df= 51,). Whereas, regression between depth and biomass at impact sites was non-significant likely due to the relatively small depth range (~1500m±200) and small sample size of impact stations.. Overall total biomass for both taxa decreased with depth.

ANOVA

Individual animal biomass was significantly different between zones for both nematode and copepods ($F=25.245$, $p<0.001$; $F=32.167$, $p<0.001$, respectively) (Table 2). Depth also had significant effects on individual biomass (Nematode $F=7.443$, $p=0.006$; Copepod $F=4.826$, $p=0.028$). Nematode individual biomass was significantly different by upper and lower section ($F=141.645$, $p<0.001$) and had a significant depth by section interaction ($F=5.459$, $p=0.0195$). Copepod individual biomass had significant interaction effect of zone and section ($F=4.809$, $p=0.0284$) (Table 2).

There were no significant differences in meiofauna community biomass between zones ($F=3.067$, $p=0.08$) (Table 8), but significant interaction effects were observed between section and zone ($F= 6.454$, $p=0.01$) and between depth, zone, and section ($F=5.785$, $p=0.02$) for total biomass. Main effects of section ($F=18.08$, $p<0.001$) and depth were also highly significant ($F=17.89$, $p<0.001$). The difference observed in total biomass is mostly due to nematode biomass as it was typically 2 or 3 factors higher than copepod biomass (Table 2).

Significant differences were observed in total nematode community biomass by zone ($F=10.53$, $p<0.01$), section ($F=4.35$, $p=0.04$), and depth ($F=25.34$, $p<0.001$) (Table 8). There was also an interaction effect between section and zone ($F=14.81$, $p<0.001$) and a weaker interaction effect of section and depth ($F=4.77$, $p=0.03$).

Total copepod community biomass showed no significant difference between zones. Section had the highest significant observed effect on total copepod biomass ($F=205.88$, $p<0.001$), while depth had a weaker but significant effect ($F=7.72$, $p=0.006$).

Although an interaction effect from section and zone was observed (F=7.72, p=0.01), it was mainly due to the strong effect of section (Table 2).

Spatial Analysis

Inverse distance squared weighted interpolation had an estimation of meiofauna community biomass had a of range of 6.16-385.69 mg C m^{-2} across 10 groups (Figure. 4) The estimated biomass was higher around the wellhead and higher estimations consistent with the trajectory of the surface plume (Figure 4). The model generally overestimated meiofauna community biomass at lower depths, but underestimated at greater depths (Predicted Regression= 0.329x+77.598; Error Regression= -0.671x+77.598, RMS= 74.671, n=65).

Across Years Comparison

General Results

The stations with among year comparisons had water depths that ranged from 1002-2389 m (n=63, per year n=21). A total of 12,723 individuals were collected from the three sampling years (2010, n=3744 individuals; 2011, n= 4,251; 2014, n= 4728), 5,336 of those are copepods and 7387 were nematodes.

Average individual biomass did not have a consistent pattern across years (Figure 2 and Figure 3) (Table 1). Average biomass size of individuals was relatively equal when considering the high standard deviation between zones within years (Table 1). Nematodes were generally larger in the deeper 1-3 cm section, with impact zones having slightly larger biomass size (Figure 1). Copepods had larger biomass size in the upper 0-1 cm section and tended to be larger in impact zones across years (Table 1) (Figure 2).

Total community biomass was highest in 2010, and decreased with time regardless of zone (Table 2) (Figure 5). Nematode community biomass was greater than copepod community biomass in all three years (Table 2). Nematode community biomass was higher at impact stations in 2010 and 2011, but was higher at non-impact stations in 2014. Copepod community biomass was higher at non-impact stations in 2010, but higher at impact stations in 2011 and 2014.

ANOVA

In 2011, average biomass size was significantly different among sections for both nematode and copepods ($F=8.356$, $p=0.004$; $F=5.632$, $p=0.02$, respectively) (Table 3). There was significant interaction effects of section and zone for copepods ($F=5.357$, $p=0.02$), while interactions between section and depth was significant for nematodes ($F=5.572$, $p=0.02$). There was also a significant depth by zone interaction for nematodes ($F=40.182$, $p < 0.001$).

Both nematode and copepod individual biomass was significantly affected by depth and zone ($F=8.592$, $p=0.003$; $F=4.601$, $p=0.03$, respectively), whereas nematodes were affected by interactions among depth, zone, and section ($F=11.317$, $p<0.001$) (Table 4).

There was an among year effect on individual biomass for both nematodes and copepods ($F=22.947$, $p<0.001$; $F=15.434$, $p<0.001$) (Table 7). Nematodes individual biomass was also significantly different among years and zones ($F=3.533$, $p=0.03$) and there was an interaction effects of year and depth, and of year, zone, and depth ($F=10.01$, $p<0.001$; $F=13.128$, $p < 0.001$, respectively).

Total community biomass was significantly different by section across years and by section and year interaction ($F=20.46$, $p<0.001$; $F=3.72$, $p=0.03$, respectively). There was a highly significant year effect in nematode community biomass ($F=8.596$, $p<0.001$) and a significant interaction of year by section ($F=7.15$, $p=0.001$). Significant main effects were observed on copepod biomass by year ($F=6.79$, $p=0.002$), and section ($F=363.22$, $p<0.001$) and year by section interaction ($F=13.6$, $p<0.001$).

Discussion

Immediately following the oil spill there was a significant difference between zones in total nematode biomass, but not a significant difference for total meiofauna biomass. Estimation of areas between sampling points had an increased biomass around the wellhead with increases towards the north. Individual biomass was significantly different between zones for both taxa. Copepods were larger in impact areas than non-impact, while the inverse was observed for nematodes. In the years following the DWH oil spill total meiofauna biomass decreased. Individual biomass generally decreased in copepods with a slight increase in 2011 at non-impact stations. Nematodes had an inconsistent pattern in individual biomass regardless of zone.

The data presented in this study provide new insights into how the DWH oil spill impacted deep-sea meiofauna community biomass. Prior to the DWH event, a comprehensive investigation of meiofauna biomass was performed in the northern Gulf of Mexico (Baguley et al. 2008), providing a strong basis for comparison to results generated here. A central theory in deep-sea ecology is that benthic abundance and biomass decrease with increasing water depth, which represents a response to decreasing food resources (Priede et al., 2013; Wei et al., 2010; Rowe et al., 2008). Baguley et al. (2008) observed this general trend in meiofauna community biomass. In another study, the average size of nematode and harpacticoid copepod individuals varied with depth where average copepod body size increased, but nematode average body size decreased over a depth range of 200-3000 m (Baguley et al. 2004). In this study, samples were taken over a depth range of 300-2700 m, but most stations were at depths of 800-2000 m, and impact stations were all between 1350-1600 m. I observed weak, yet significant, relationships of average body size with depth for both nematodes and copepods, and the trends were also weak regardless of zone. I also observed significant effects of depth on overall community biomass, and the depth effect generally remained significant across the tree years of this study.

While depth effects are known to structure deep-sea benthic biomass (Wei et al. 2010), meiofauna biomass in this study was also affected by DWH impacts. The results of this study show that meiofauna biomass has begun to return to background conditions over a few years. This is contrary to previous studies of meiofauna following disturbance showing a much quicker return to background conditions, however these studies focused on mechanical disturbance not a toxicity and enrichment balance (Azovsky, 1988; Sherman and Coull, 1980). The highest values of biomass occurred at impact stations but non-impact stations biomass values were higher than pre-spill values (Baguley et al., 2008). This overall increase in biomass suggests an enrichment occurring across the deep-sea. Community biomass had a significant interaction of section and zone, which suggest that animals were vertically distributed within sediments differently at impact vs. non-impact stations. Average individual body size was also significantly different by zone for both nematodes and copepods. The differences observed between zones are mainly due to shifts in abundance (mainly nematodes) driving the amount of community biomass rather than individual size. Impacted stations had higher nematode to copepod ratios, and greater nematode abundance than non-impact stations (Montagna et al. 2013; Baguley et al. 2015).

Significant temporal dynamics in both individual body size and community biomass were also observed in this study. Average meiofauna community biomass is estimated to be 43.3 mg C m^{-2} across the northern GOM prior to the oil spill (Baguley et al., 2008). However, the current study estimated meiofauna community biomass at more than double that historical average, regardless of zone. Temporal analysis indicated significant differences for both nematode and copepod total biomass. Nematode average body size did not have a consistent pattern across depth between years. Average copepod body size generally decreased with depth regardless of year. The single sampling event within years may not have been able to fully address a theory of smaller individuals dominating a community post-disturbance (Pearson and Rosenberg, 1976). While individual body sizes showed inconsistent temporal patterns, average community biomass did move closer to background levels in the years following the oil spill. Previous studies noted that stations with the strongest DWH impacts had decreased meiofauna abundance and diversity, but sites that had moderate impacts showed increased abundance (Baguley et al., 2015). The apparent movement toward background conditions observed here, is corroborated by similar trends in meiofauna community diversity following the oil spill (Reuscher et al., 2017), collectively suggesting that recovery of the community is beginning.

As mentioned above, no consistent pattern or trend was observed in body size in the temporal analysis. Each of the sampling years only had one sampling event at each station and samples were taken in September/October 2010, but taken in May/June in 2011 and 2014. Inconsistent patterns in body size could indicate shifts in community diversity, but could also be due to relatively quick reproductive events of meiofauna and age-class variation within the community (Giere, 2009). Meiofauna reproduction events are relatively quick depending on the taxon (Giere, 2009). If the samples were taken during a period of high reproduction, smaller individuals may have been over represented in the sampling efforts. Variation in sediment properties, depth, and life stage may have also contributed to inconsistent body size across years (van der Grient and Rogers, 2015; Giere, 2009). Copepod size differences may have been marked by changes in community diversity since nauplii were not analyzed for biomass in this study. An analysis of copepod community diversity could highlight these differences within the GOM. Studies across many aquatic habitats have supported that copepods display a range of pollution tolerance, while still being considered sensitive taxon (van Damme et al., 1983; Hicks, 1980; Giere, 2009). Before the spill, copepod diversity declined with proximity to oil platforms (Montagna and Harper, Jr., 1996). Post-spill copepod diversity was also shown to decrease at impacted stations, and both tolerant and sensitive copepod families have been identified (Baguley and Bang, in prep). Taken together, these results stress the need for both seasonal and long-term multi-year studies in deep-sea benthic community structure and function to fully understand temporal dynamics, and to be able to separate impact and recovery from natural background variation.

Spatial interpolation of meiofauna community biomass in 2010 estimates show highest biomass values occurred near the DWH wellhead and with high biomass values north of the wellhead. This pattern is consistent with the surface oil plume that traveled generally northward toward the Louisiana, Mississippi, and Alabama shorelines (UAC, 2010). The increases in observed biomass within this northward trajectory could be due to enrichment from the breakdown of lighter hydrocarbons by bacteria at the surface (Passow and Ziervogel, 2016). The spatial trend corroborates the depth by zone interaction observed in the ANOVA. Areas farther to the west and east of the wellhead exhibit a decrease in biomass with depth as would be expected (Wei et al., 2010; Baguley et al., 2008; Giere, 2009). However, near the wellhead and north of the wellhead biomass is: 1) greatly enhanced, and 2) highest at depths similar to that of the wellhead. Prior studies did find mid-depth abundance and biomass maxima (Baguley et al. 2006, 2008). However, in those prior studies highest meiofauna community biomass was observed in the Mississippi and De Soto Canyons. The DWH wellhead is located generally between those two bathymetric features, but the nearest prior-sampled station HIPRO (see Baguley et al. 2008) did not experience elevated meiofauna biomass. Furthermore, biomass estimates from 2010 are 2-3 times higher than those observed by Baguley et al. (2008).

As mentioned above, the spatial trend is consistent with the surface plume that extended across the GOM (UAC, 2010). The DWH initial footprint was estimated to have various sizes depending on the focus varying between surface and subsurface oil spread and observed impacts on community structure (Montagna et al. 2013; Valentine et al. 2014; Baguley et al. 2015; Passow and Ziergovel 2016). When specifically focusing on prior estimates of deep-sea benthic impacts of the DWH event, the deep-sea oil plume was presumed to be more significant that the surface plume (Montagna et al. 2013; Baguley et al. 2015). However, the data presented here suggest a potential benthic-pelagic coupling event.

The formation of Marine Oil Snow (MOS) could have led to enrichment of the sediments below through sedimentation processes. Post-spill investigation showed increased rates of MOS sedimentation, but the long-term impact of these oil related compounds is unclear (Joye et al., 2016). Additionally, post-spill analysis of bacterial communities showed that surface communities were dominated by small hydrocarbon metabolizing bacteria, while sediments contained a wide variety of hydrocarbon metabolizing bacteria (Liu and Liu, 2016). The increased oil footprint from MOS formation and sedimentation derived from the surface plume, along with increased bacterial community biomass, may have allowed pollution tolerant nematode and copepod families a chance to dominate in response to the spill. A balance between enrichment effects and pollution effects was suggested in prior studies as a dramatic increase in nematode to copepod ratios were observed near the wellhead (Spies et al., 1988; Peterson et al., 1996; Baguley et al., 2015). Increased food and decreased competition from other taxa could have allowed nematodes initial increase in biomass. Both taxa had significant differences between zones by section in 2010 but the years following the spill only significant interaction effects of section by year were observed. While not part of this study, preliminary data suggests that copepod diversity was significantly differences between zones (Baguley and Bang, in prep). Copepod body size did have a consistent trend of smaller body sizes coinciding with greater depths but 2014 had the smallest average individual size regardless of zone. The differences in body size between years could be due to a shift in diversity but this study does not have enough information to make that conclusion.

It is well established that benthic communities are dependent on organic input from surface waters, ranging from whale falls to marine snow (Tecchio et al, 2013). However, these inputs vary regionally and seasonally in their intensity. The few studies that have addressed this topic with respect to meiofauna suggest that metazoan meiofauna do not respond as quickly as their protist counterparts (Gooday, 2002). Further studies are needed to understand the bentho-pelagic coupling meiofauna have with surface waters (Giere, 2009).

In 2014 the meiofauna community structure metrics were similar between impact and non-impact stations compared to 2010, suggesting the zones have begun to approach each other in similarity. This supports the possibly that meiofauna communities are showing signs of post oil spill recovery (Reuscher et al., 2017). However, there are large deposits of heaver oil related compounds remaining in the GOM deep-sea, and their role in nutrient cycling in the deep-sea that has yet to be completely understood (Romero et al., 2017). Further research should investigate potential impacts humans are having on the deep-sea. As individual biomass is tied to remineralization of organic carbon and community biomass is potentially food for higher trophic sources, this study estimates meiofauna community biomass as a proxy for community function. Future studies could use both lab-based and *in situ* experiments to more fully understand deep-sea meiofauna community function. Interruption of deep-sea carbon sequestration could have large scale impacts to terrestrial life. Meiofauna can be used as complex indicators of environmental change or impact based on their variety of life history and diverse taxonomy (Zeppilli et al., 2017). They have a relatively high abundance, and small sediment cores can help monitor fluxes within environments. However, studies need multi-year sampling and representation of the environment and region under scrutiny to provide accurate metrics. Finally, continued monitoring studies at both impacted and non-impacted stations with the GOM would help improve resolution of recovery rates and return to background conditions.

Appendix

R version 3.2.3 (2015-12-10) -- "Wooden Christmas-Tree"

Install.packages(“psych”)

Install.packages (“dplyr”)

References

Amjad, S., & Gray, J. S. (1983). Use of the nematode-copepod ratio as an index of organic pollution. *Marine Pollution Bulletin*, *14*(5), 178–181. http://doi.org/10.1016/0025-326X(83)90229-1

Armstrong, C. W., Foley, N. S., Tinch, R., & van den Hove, S. (2012). Services from the deep: Steps towards valuation of deep sea goods and services. *Ecosystem Services*, *2*, 2–13. http://doi.org/10.1016/j.ecoser.2012.07.001

Azovsky, A. I. (1988). Colonization of Sand " Islands " by Psammophilous Ciliates : The Effect of Microhabitat Size and Stage of Succession. *Oikos*, *51*(1), 48–56.

Baguley, J. G., Hyde, L. J., & Montagna, P. A. (2004). A semi-automated digital microphotographic approach to measure meiofaunal biomass. *Limnology and Oceanography: Methods*, *2*(6), 181–190. http://doi.org/10.4319/lom.2004.2.181

Baguley, J. G., Montagna, P. A., Hyde, L. J., Kalke, R. D., & Rowe, G. T. (2006). Metazoan meiofauna abundance in relation to environmental variables in the northern Gulf of Mexico deep sea. *Deep-Sea Research Part I: Oceanographic Research Papers*, *53*(8), 1344–1362. http://doi.org/10.1016/j.dsr.2006.05.012

Baguley, J. G., Montagna, P. A., Hyde, L. J., & Rowe, G. T. (2008). Metazoan meiofauna biomass, grazing, and weight-dependent respiration in the Northern Gulf of Mexico deep sea. *Deep-Sea Research Part II: Topical Studies in Oceanography*, *55*(24–26), 2607–2616. http://doi.org/10.1016/j.dsr2.2008.07.010

Baguley, J., Montagna, P., Cooksey, C., Hyland, J., Bang, H., Morrison, C., … Ricci, M. (2015). Community response of deep-sea soft-sediment metazoan meiofauna to the Deepwater Horizon blowout and oil spill. *Marine Ecology Progress Series*, *528*, 127–140. http://doi.org/10.3354/meps11290

Bang, H. W., Baguley, J. G., & Moon, H. (2014). A new genus of cletopsyllidae (Copepoda, Harpacticoida) from gulf of Mexico. *ZooKeys*, *391*(March), 37–53. http://doi.org/10.3897/zookeys.391.6903

Bertness, M.D., Bruno, J.F., Silliman, B.R. and J.J. Stachowicz, eds. *Marine Community Ecology and Conservation*, 348. Sunderland: Sinauer Associates, 2014. Print

Burgess, R. (2001). An improved protocol for separating meiofauna from sediments using colloidal silica soils. *Marine Ecology Progress Series*, *214*, 161–165. http://doi.org/10.3354/meps214161

Coull, B.C. and G.T. Chandler (2001). Meiobenthos. In J.H. Steele (Ed.), *Encyclopedia of Ocean Sciences* (Second Edition). Retrieved from https://doi.org/10.1016/B978-012374473-9.00212-5.

Danovaro, R., Gambi, C., Dell'Anno, A., Corinaldesi, C., Fraschetti, S., Vanreusel, A., … Gooday, A. J. (2008). Exponential Decline of Deep-Sea Ecosystem Functioning Linked to Benthic Biodiversity Loss. *Current Biology*, *18*(1), 1–8. http://doi.org/10.1016/j.cub.2007.11.056

Edwards, B. R., Reddy, C. M., Camilli, R., Carmichael, C. a, Longnecker, K., & Van Mooy, B. a S. (2011). Rapid microbial respiration of oil from the Deepwater Horizon spill in offshore surface waters of the Gulf of Mexico. *Environmental Research Letters*, *6*(3), 35301. http://doi.org/10.1088/1748-9326/6/3/035301

"Endangered and Threatened Marine Species under NMFS' Jurisdiction." *NOAA Fisheries*, 13 May 2014, www.nmfs.noaa.gov/pr/species/esa/listed.htm.

Falkowski, P. (2012). Ocean science: The power of plankton. *Nature*, *483*(7387), S17–S20. http://doi.org/10.1038/483S17a

Feller RJ, and Warwick RM (1988) Energetics. In: RP Higgings & H Thiel, editors. *Introduction to the study of meiofauna*. pp: 181–196. Smithsonian Institution Press, Washington, DC

Gage JD (2003) Food inputs, utilization, carbon flow, and energetics. In: PA Tyler (ed) *Ecosystems of the deep* p 313−380. Elsevier Science BV, Amsterdam

Giere, Olav (2009). *Meiobenthology: The Microscopic Motile Fauna of Aquatic Sediments.* Springer, Berlin

Gooday, A.J. (2002). Biological Responses to Seasonally Varying Fluxes of Organic Matter to the Ocean Floor: A Review. *Journal of Oceanography,* 58(2):305-332

Gray JS (1981) *The ecology of marine sediments*.Cambridge, Cambridge University Press

Hall, S.J. (1994). Physical disturbance and marine benthic communities: life in unconsolidated sediments. *Oceanography and Marine Biology: An Annual Review*, 32:179-239

Hicks, G. R. F. (1980). Structure of phytal harpacticoid copepod assemblages and the influence of habitat complexity and turbidity. *Journal of Experimental Marine Biology and Ecology*, *44*(2), 157–192. http://doi.org/10.1016/0022-0981(80)90151-3

Hsing, P.-Y., Bo Fu, B., Elizabeth Larcom, B. A., Samantha Berlet, B. P., Timothy Shank, B. M., Annette Govindarajan, B. F., … Charles Fisher, B. R. (2013). Evidence of lasting impact of the Deepwater Horizon oil spill on a deep Gulf of Mexico coral community. *Elementa: Sicence of the Anthropocene*, *1*(12). http://doi.org/10.12952/journal.elementa.000012

Huston, M. (1979). A General Hypothesis of Species Diversity. *The American Naturalist*, *113*(1), 81–101. http://doi.org/10.1086/283366

Joint, I. R., Gee, J. M., & Warwick, R. M. (1982). Determination of fine-scale vertical distribution of microbes and meiofauna in an intertidal sediment. *Marine Biology*, *72*(2), 157–164. http://doi.org/10.1007/BF00396916

Joye, S. B., Bracco, A., Özgökmen, T. M., Chanton, J. P., Grosell, M., MacDonald, I. R., … Passow, U. (2016). The Gulf of Mexico ecosystem, six years after the Macondo oil well blowout. *Deep-Sea Research Part II: Topical Studies in Oceanography*, *129*, 4–19. http://doi.org/10.1016/j.dsr2.2016.04.018

Kujawinski, E. B., Kido Soule, M. C., Valentine, D. L., Boysen, A. K., Longnecker, K., & Redmond, M. C. (2011). Fate of dispersants associated with the Deepwater Horizon oil spill. *Environmental Science and Technology*, *45*, 1298–1306. http://doi.org/10.1021/es103838p

Lehr, B., Bristol, S., & Possolo, A. (2010). *Oil Budget Calculator*. *Review Literature And Arts Of The Americas*.

Liu, Z., & Liu, J. (2013). Evaluating bacterial community structures in oil collected from the sea surface and sediment in the northern Gulf of Mexico after the Deepwater Horizon oil spill. *MicrobiologyOpen*, *2*(3), 492–504. http://doi.org/10.1002/mbo3.89

Love, M., Baldera, A., Yeung, C., & Robbins, C. (2013). The Gulf of Mexico Ecosystem: A Coastal and Marine Atlas. New Orleans, LA: Ocean Conservancy, Gulf Restoration Center.

Lubchenco, J., Mcnutt, M. K., Lehr, B., Sogge, M., Miller, M., Hammond, S., & Conner, W. (2010). BP Deepwater Horizon Oil Budget: What Happened To the Oil? Retrieved from NOAA/USGS Federal Science Report. Available at: www.noaanews.noaa.gov/

Mare, M. (1942). A study of a marine benthic community with special reference to the micro-organisms. *Journal of the Marine Biological Association of the United Kingdom*, 25(3), 517-554. doi:10.1017/S0025315400055132

Montagna, P. A. (1984). In situ measurement of meiobenthic grazing on sediment bacteria and edaphic diatoms. *Marine Ecology Progress Series*, *18*(1), 119–130.

Montagna, P.A., 1995. Rates of metazoan meiofauna microbivory: a review. *Vie et Milieu* 45, 1–9

Montagna, P.A. and D.E. Harper, Jr. (1996). Benthic infaunal long-term response to offshore production platforms in the Gulf of Mexico. *Canadian Journal of Fisheries and Aquatic Sciences*, 53(11). P2567-2588

Montagna, P. A., Baguley, J. G., Cooksey, C., Hartwell, I., Hyde, L. J., Hyland, J. L., … Rhodes, A. C. E. (2013). Deep-Sea Benthic Footprint of the Deepwater Horizon Blowout. *PLoS ONE*, *8*(8), e70540. http://doi.org/10.1371/journal.pone.0070540

Passow, U., & Ziervogel, K. (2016). Marine Snow Sedimented Oil Released During the Deepwater Horizon Spill. *Oceanography*, *29*(3), 118–125. http://doi.org/10.5670/oceanog.2016.76

Pearson, T. H., & Rosenberg, R. (1978). Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. *Oceanography and Marine Biology Annual Review*, *16*(January), 229–311. http://doi.org/10.1111/j.1540-5834.2012.00707.x

Pequegnat, W.E., Gallaway, B.J., Pequegnat, L.H., 1990. Aspects of the ecology of the deepwater fauna of the Gulf of Mexico. *American Zoologist* 30, 45–64

Peterson, C. H., Anderson, S. S., Cherr, G. N., Ambrose, R. F., Anghera, S., Bay, S., … Adams, E. E. (2012). A Tale of Two Spills: Novel Science and Policy Implications of an Emerging New Oil Spill Model. *BioScience*, *62*(5), 461–469. http://doi.org/10.1525/bio.2012.62.5.7

Peterson, C. H., Kennicutt Ii, M. C., Green, R. H., Montagna, P., Harper, D. E., Powell, E. N., & Roscigno, P. F. (1996). Ecological consequences of environmental perturbations associated with offshore hydrocarbon production: a perspective on long-term exposures in the Gulf of Mexico. *Canadian Journal of Fisheries and Aquatic Sciences*, *53*, 2637–2654.

Posey, M., Lindberg, W., Alphin, T., & Vose, F. (1996). Influence of Storm Disturbance on an offshore benthic community. *Bulletin of Marine Science*, *59*(3), 523–529.

Priede, I. G., Bergstad, O. A., Miller, P. I., Vecchione, M., Gebruk, A., Falkenhaug, T., … Lorance, P. (2013). Does Presence of a Mid-Ocean Ridge Enhance Biomass and Biodiversity? *PLoS ONE*, *8*(5), 1–10. http://doi.org/10.1371/journal.pone.0061550

R Core Team (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Reuscher, M. G., Baguley, J. G., Conrad-Forrest, N., Cooksey, C., Hyland, J. L., Lewis, C., … Washburn, T. (2017). Temporal patterns of Deepwater Horizon impacts on the benthic infauna of the northern Gulf of Mexico continental slope. *PLoS ONE*, *12*(6), e0179923. http://doi.org/10.1371/journal.pone.0179923

Romero, I. C., Toro-Farmer, G., Diercks, A. R., Schwing, P., Muller-Karger, F., Murawski, S., & Hollander, D. J. (2017). Large-scale deposition of weathered oil in the Gulf of Mexico following a deep-water oil spill. *Environmental Pollution*, *228*, 179–189. http://doi.org/10.1016/j.envpol.2017.05.019

Rowe, G. T., Wei, C., Nunnally, C., Haedrich, R., Montagna, P., Baguley, J. G., … Deming, J. W. (2008). Comparative biomass structure and estimated carbon flow in food webs in the deep Gulf of Mexico. *Deep-Sea Research Part II: Topical Studies in Oceanography*, *55*(24–26), 2699–2711. http://doi.org/10.1016/j.dsr2.2008.07.020

Rowe, G. T., & Kennicutt, M. C. (2009). *Northern Gulf of Mexico Continental Slope Habitats and Benthic Ecology Study Final Report*. *U.S. Dept. of the Interior Minerals Managment*. New Orleans, LA.

Schwinghamer, P (1981). Characteristic Size Distributions of Integral Benthic Communities. *Canadian Journal of Fisheries and Aquatic Sciences*, 38(10) :1255-1263

Sherman, K.M. and B.C. Coull (1980). The response of meiofauna to sediment disturbance. *Journal of Experimental Marine Biology and Ecology*, 46: 59-71

Smith, K. L., Kaufmann, R. S., Baldwin, R. J., & Carlucci, A. F. (2001). Pelagic–benthic coupling in the abyssal eastern North Pacific: An 8-year time-series study of food supply and demand. *Limnology and Oceanography*, *46*(3), 543–556. http://doi.org/10.4319/lo.2001.46.3.0543

Solan, M., Cardinale, B. J., Downing, A. L., Engelhardt, K. A. M., Ruesink, J. L., & Srivastava, D. S. (2004). Extinction and Ecosystem Function in the Marine Benthos. *Science*, *306*, 1177–1180. http://doi.org/10.1126/science.1103960

Spies, R. B., Hardin, D. D., & Toal, J. P. (1988). Organic enrichment or toxicity? A comparison of the effects of kelp and crude oil in sediments on the colonization and growth of benthic infauna. *Journal of Experimental Marine Biology and Ecology*, *124*(3), 261–282. http://doi.org/10.1016/0022-0981(88)90175-X

Stuart, C.T., Rex, M.A., and R.J. Etter, “Large-scale spatial and temporal patterns of deep-sea benthic species diversity.” *Ecosystems of the Deep Oceans* Ed. Tyler P.A. Amsterdam: Elsevier, 2003. Print

Sumaila, U. R., Cisneros-Montemayor, A. M., Dyck, A., Huang, L., Cheung, W., Jacquet, J., … Pauly, D. (2012). Impact of the Deepwater Horizon well blowout on the economics of US Gulf fisheries. *Canadian Journal of Fisheries and Aquatic Sciences*, *69*, 499–510. http://doi.org/10.1139/F2011-171

Tecchio, S., van Oevelen, D., Soetaert, K., Navarro, J., & Ramírez-Llodra, E. (2013). Trophic Dynamics of Deep-Sea Megabenthos Are Mediated by Surface Productivity. *PLoS ONE*, *8*(5). http://doi.org/10.1371/journal.pone.0063796

Tunnell, J. W. (2011). *An expert opinion of when the Gulf of Mexico will return to pre-spill harvest status following the BP Deepwater Horizon MC 252 oil spill*. Gulf Coast Claims Facility, Feinberg Rozen, LLP.

Unified Area Command (UAC). (2010). *Deepwater Horizon MC 252 Response*.

Valentine, D. L., Fisher, G. B., Bagby, S. C., Nelson, R. K., Reddy, C. M., Sylva, S. P., & Woo, M. A. (2014). Fallout plume of submerged oil from Deepwater Horizon. *PNAS*, *111*(45), 15906–15911. http://doi.org/10.1073/pnas.1414873111

van Damme, D., Heip, C, and K.A. Williams. (1983). Influence of pollution on the harpacticoid copepods of two North Sea estuaries. *Hydrobiologia*, 112(2). https://doi.org/10.1007/BF00006919

van der Grient, J.M.A. and A.D. Rogers (2015). Body Size Versus Depth: Regional and Taxonomical Variation in Deep-Sea Meio- and Macrofaunal Organisms.” *Advances in Marine Biology* (71-97). In B.E. Curry (Ed). London: Academic Press,

Warwick, R. M. (1981). The nematode/copepod ratio and its use in pollution ecology. *Marine Pollution Bulletin*, *12*(10), 329–333. http://doi.org/10.1016/0025-326X(81)90105-3

Warwick, R.M. and R. Price (1979). Ecological and metabolic studies on free-living nematodes from an estuarine mud-flat. Estuarine and Coastal Marine Science, 9 (3), 257-271

Wei, C.-L., Rowe, G. T., Escobar-Briones, E., Boetius, A., Soltwedel, T., Caley, M. J., … Romanuk, T. N. (2010). Global Patterns and Predictions of Seafloor Biomass Using Random Forests. *PLoS ONE*, *5*(12), e15323, doi:10.1371/journal.pone.0015323. http://doi.org/10.1371/journal.pone.0015323

Zeppilli, D., Sarrazin, J., Leduc, D., Arbizu, P. M., Fontaneto, D., Fontanier, C., … Fernandes, D. (2015). Is the meiofauna a good indicator for climate change and anthropogenic impacts? *Marine Biodiversity*, *45*(3), 505–535. http://doi.org/10.1007/s12526-015-0359-z

Table 1- Average individual meiofauna biomass (µg of C) with standard deviation (between zone, impact and. non-impact, following the Deepwater Horizon oil spill. 2010's complete station average and standard deviation (n=55) is followed by the reduced number of stations for between year comparison (n=63). Broken down by taxon and summed, Nematode (N), Copepod (C).

		Impact			Non-impact		
2010*	N	0.0403	±	0.0713	0.0556	±	0.14
	C	0.351	±	0.523	0.255	±	0.492
2010¹	N	0.045	±	0.082	0.058	±	0.217
	C	0.339	±	0.544	0.262	±	0.448
2011¹	N	0.0436	±	0.06	0.0412	±	0.065
	C	0.29	±	0.663	0.293	±	0.698
2014¹	N	0.0306	±	0.124	0.029	±	0.067
	C	0.22	±	0.516	0.199	±	0.44

* 2010 samples of full set (n=55)
¹ Used for between year comparisons (n=21)

Table 2- Significant ANOVA results of 2010 individual biomass sizes (µg of C) of Nematodes (n= 6,450) and Copepods (n= 4,168).

	IV			Df	SS	F	p
Copepod	Depth			1	0.08	7.021	**0.008**
	Zone			1	0.75	68.161	<**0.001**
	Zone	x	Section	1	0.05	4.382	**0.036**
	Depth	x	Section	1	0.05	4.124	**0.042**
Nematode	Depth			1	0.022	21.156	<**0.001**
	Zone			1	0.037	35.364	<**0.001**
	Section			1	0.247	237.7	<**0.001**
	Section	x	Depth	1	0.016	15.292	<**0.001**

Table 3- Significant ANOVA results of 2011 individual biomass sizes (µg of C) of Nematodes (n=2344) and Copepods (n=1907).

	IV			Df	SS	F	p
Copepod	Section			1	2.6	5.632	**0.02**
	Section	x	Zone	1	2.4	5.357	**0.02**
Nematode	Section			1	0.031	8.356	**0.004**
	Section	x	Depth	1	0.021	5.572	**0.02**
	Depth	x	Zone	1	0.151	40.182	<**0.001**

Table 4- Significant ANOVA results of 2014 individual biomass sizes (µg of C) of Nematodes (n=2713) and Copepods (n=2015).

	IV	Df	SS	F	p
Copepod	Depth x Zone	1	1.1	4.601	**0.03**
Nematode	Section	1	0.031	2.801	0.09
	Depth x Zone	1	0.096	8.592	**0.003**
	Depth x Zone x Section	1	0.127	11.317	**<0.001**

Table 5-Summary of meiofauna community biomass (mg of C m^{-2}) between zones, impact and non-impact, following the Deepwater Horizon oil spill. 2010's complete station average and standard deviation (n=55) is followed by the reduced number of stations for between year comparison (n=63). Broken down by taxon and summed, Nematode (N), Copepod (C), and total (T).

		Impact			Non-impact		
2010*	N	99.81	±	72.94	68.93	±	65.03
	C	31.80	±	14.03	35.88	±	37.97
	T	**131.61**	±	**77.46**	**104.81**	±	**80.69**
2010 [1]	N	108.49	±	74.21	98.96	±	73.30
	C	31.69	±	14.44	38.56	±	31.30
	T	**140.17**	±	**74.46**	**137.52**	±	**81.00**
2011[1]	N	83.19	±	47.39	73.42	±	41.62
	C	67.26	±	57.31	42.74	±	45.59
	T	**150.45**	±	**74.35**	**116.16**	±	**72.18**
2014[1]	N	43.53	±	36.70	52.11	±	25.22
	C	22.35	±	21.50	15.04	±	5.12
	T	**65.88**	±	**35.40**	**67.15**	±	**28.32**

* 2010 samples of full set (n=55)

[1] Used for between year comparisons (n=21 per year)

Table 6- ANOVA of 2010 meiofauna community biomass (mg C m $^{-2}$) following the Deepwater Horizon oil spill (n=55).

	IV	Df	SS	F	p
Copepod [1]	Section	1	35.4	205.88	**<0.001**
	Section x Zone	1	1.18	6.86	**0.01**
	Depth	1	1.33	7.72	**0.006**
Nematode [1]	Section	1	0.66	4.35	**0.04**
	Section x Zone	1	2.29	14.81	**<0.001**
	Section x Depth	1	0.72	4.77	**0.03**
	Zone	1	1.59	10.53	**0.002**
	Depth	1	3.81	25.34	**<0.001**
Total	Section	1	35644	18.08	**<0.001**
	Section x Zone	1	12724	6.454	**0.01**
	Zone	1	6047	3.067	0.08
	Zone x Depth	1	5931	3.01	0.08
	Depth	1	35280	17.89	**<0.001**
	Depth x Zone x Section	1	11405	5.785	**0.02**

[1] log transformed to satisfy assumptions of ANOVA

Table 7- Between year ANOVA comparison individual biomass size (µg of C) of copepods (n=5336) and nematodes (n=7387).

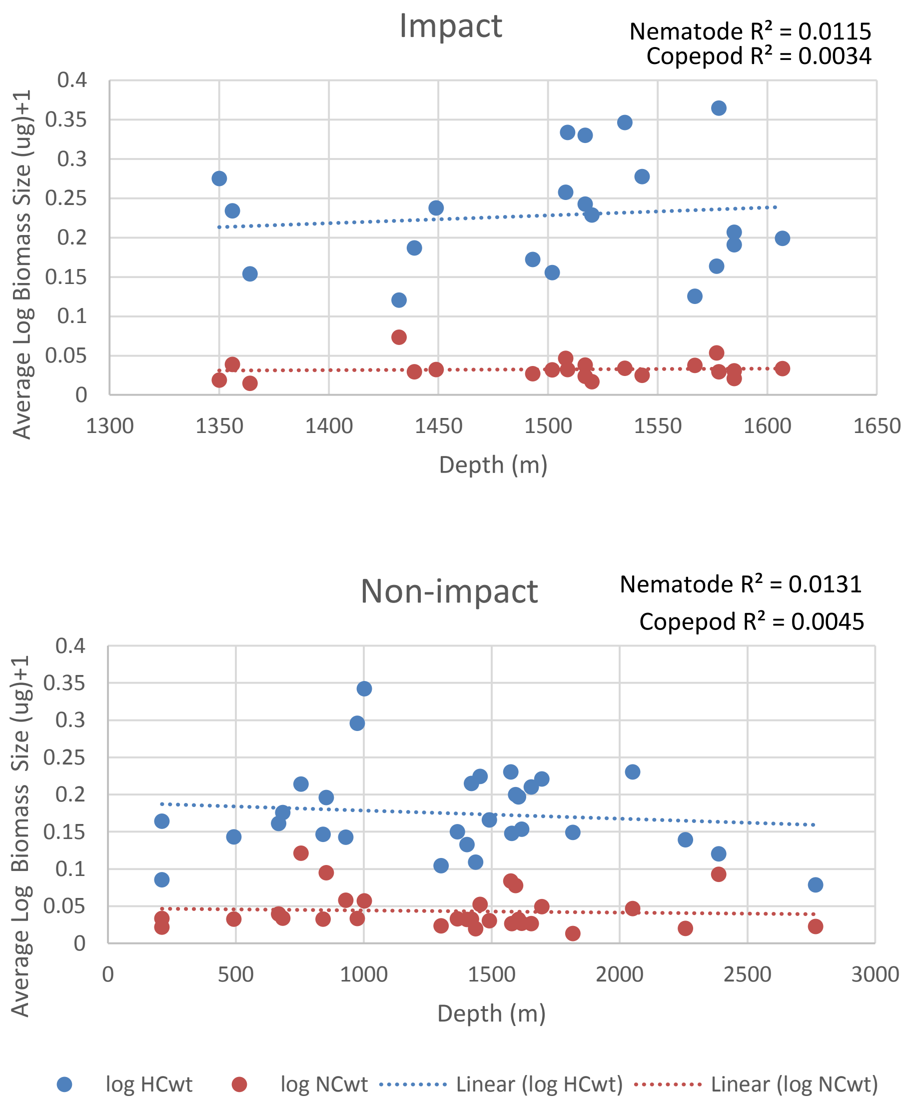
	IV					Df	SS	MS	F	p
Copepod	Zone					1	0.9	0.941	2.918	0.09
	Year					2	10	4.978	15.434	<**0.001**
Nematode	Year					2	0.54	0.271	22.497	<**0.001**
	Year	x	Depth			2	0.24	0.121	10.01	<**0.001**
	Year	x	Zone			2	0.09	0.0426	3.533	**0.03**
	Year	x	Zone	x	Depth	2	0.32	0.15	13.128	<**0.001**

Table 8- Between year ANOVA comparison of meiofauna community biomass (mg C m $^{-2}$) following the Deepwater Horizon oil spill (n=63).

	IV	Df	SS	MS	F	p
Copepod $_1$	Year	2	1.44	0.72	6.79	**0.002**
	Year x Section	2	2.88	1.44	13.6	<**0.001**
	Year x Section x Zone	2	0.55	0.27	2.579	**0.08**
	Section	1	38.48	38.48	363.22	<**0.001**
Nematode $_1$	Year	2	1.92	0.96	8.596	<**0.001**
	Year x Section	2	1.56	0.8	7.15	**0.001**
Total $_2$	Section	1	0.02	0.02	20.464	<**0.001**
	Year x Section	2	0.007	0.004	3.72	**0.03**

$_1$ log transformed to satisfy assumptions of ANOVA

$_2$ reciprocal transformation to satisfy assumption of ANOVA



A

B

Figure 1- Shows log (x+1) transformed average 2010 animal biomass (µg of C) in relation to depth (m) (n=53). **A-** Shows impact stations average individual biomass of Copepods (HCwt, blue) and nematode (NCwt, red) and their respective trend lines. **B-** Shows non-impact stations average individual biomass of Copepods and nematodes and their respective trend lines.

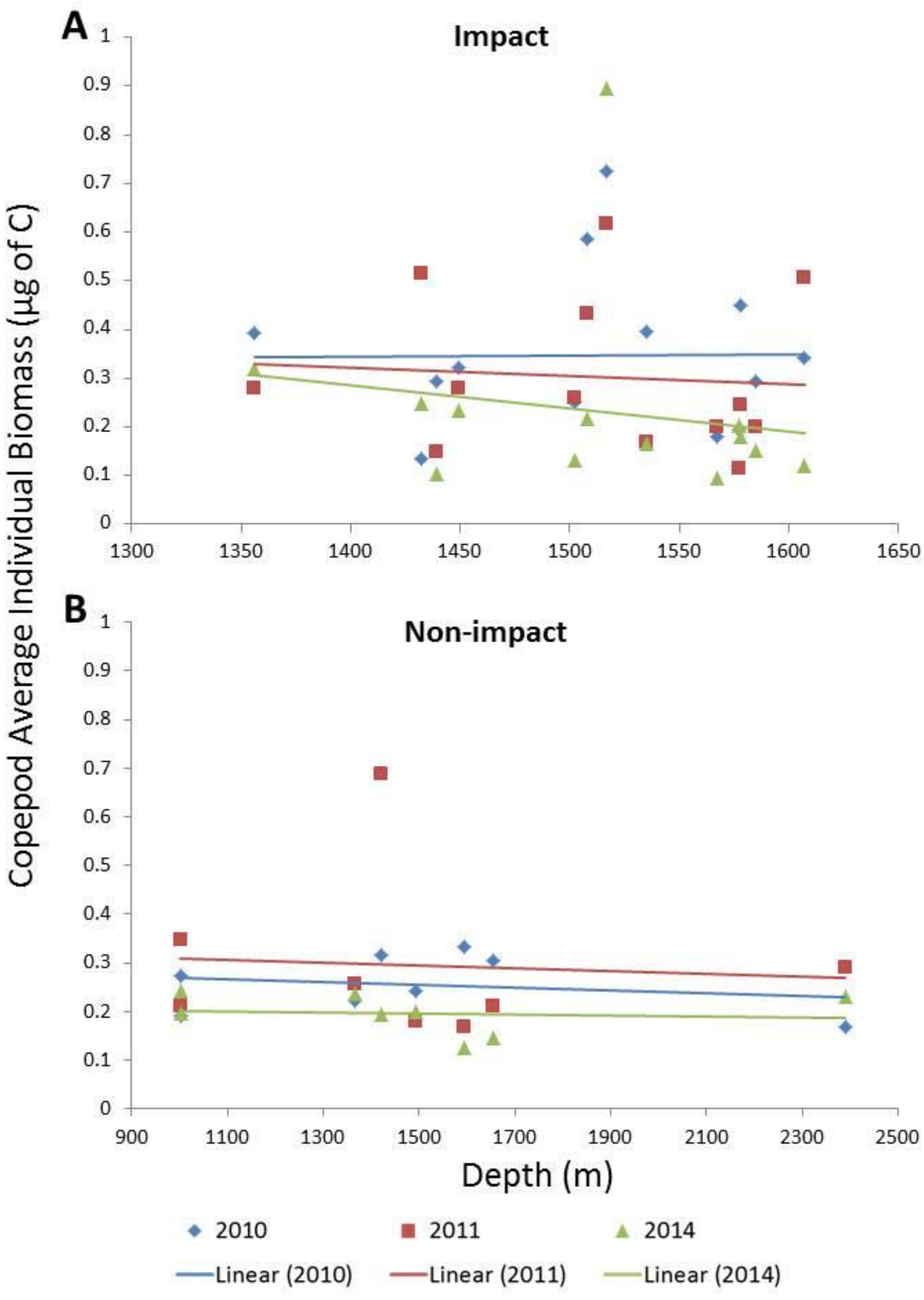


Figure 2- Shows the average individual biomass size (µg of C) of copepods across depth (m) (n= 63). **A**- Impact stations from the three sampling periods (2010, blue, 2011, red, and 2014, green). **B**- Non-impact stations from the three sampling periods (2010, blue, 2011, red, and 2014, green).

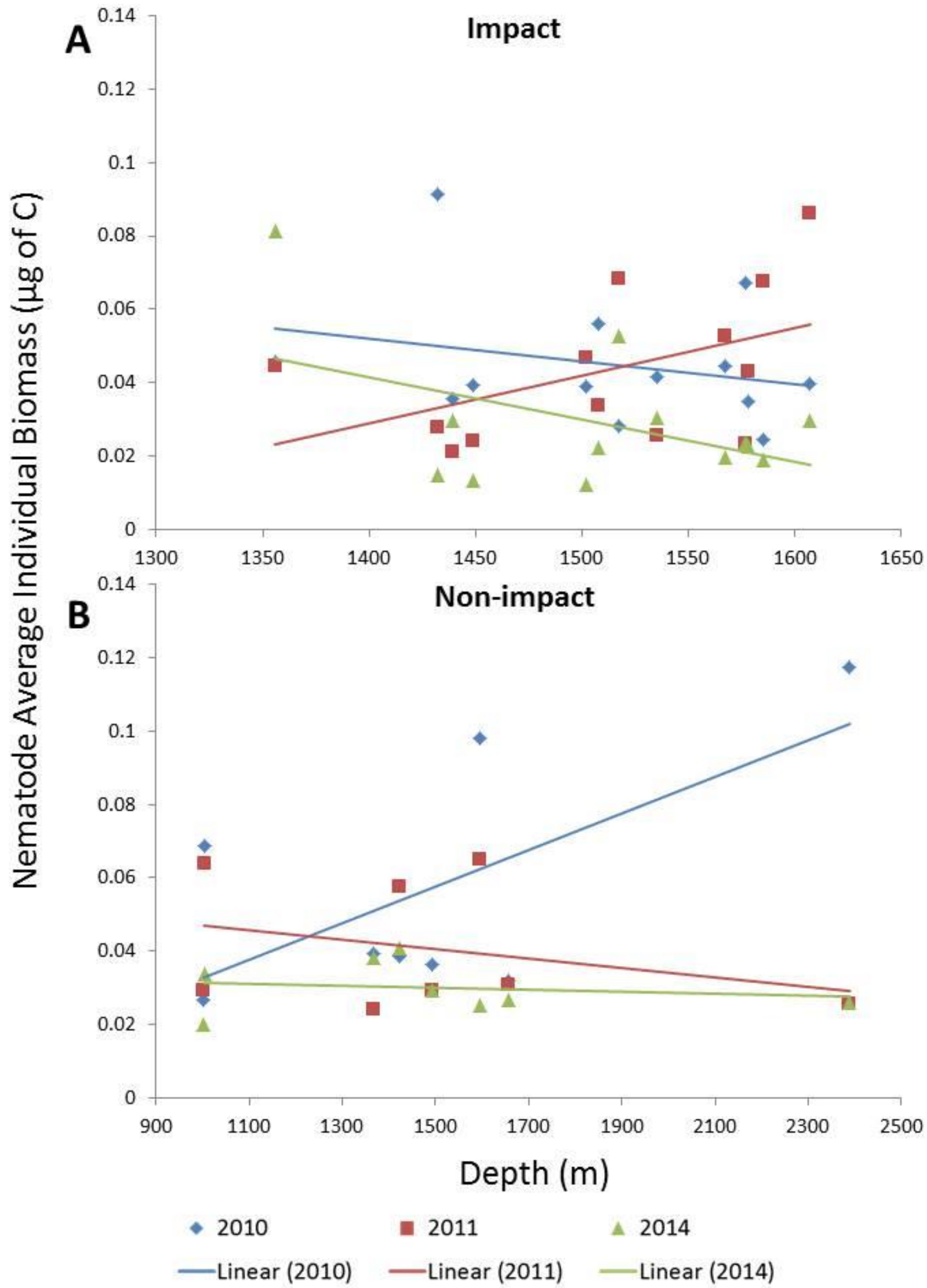


Figure 3- Shows the average individual biomass size (µg of C) of nematodes across depth (m) (n= 63). **A**- Impact stations from the three sampling periods (2010, blue, 2011, red, and 2014, green). **B**- Non-impact stations from the three sampling periods (2010, blue, 2011, red, and 2014, green).

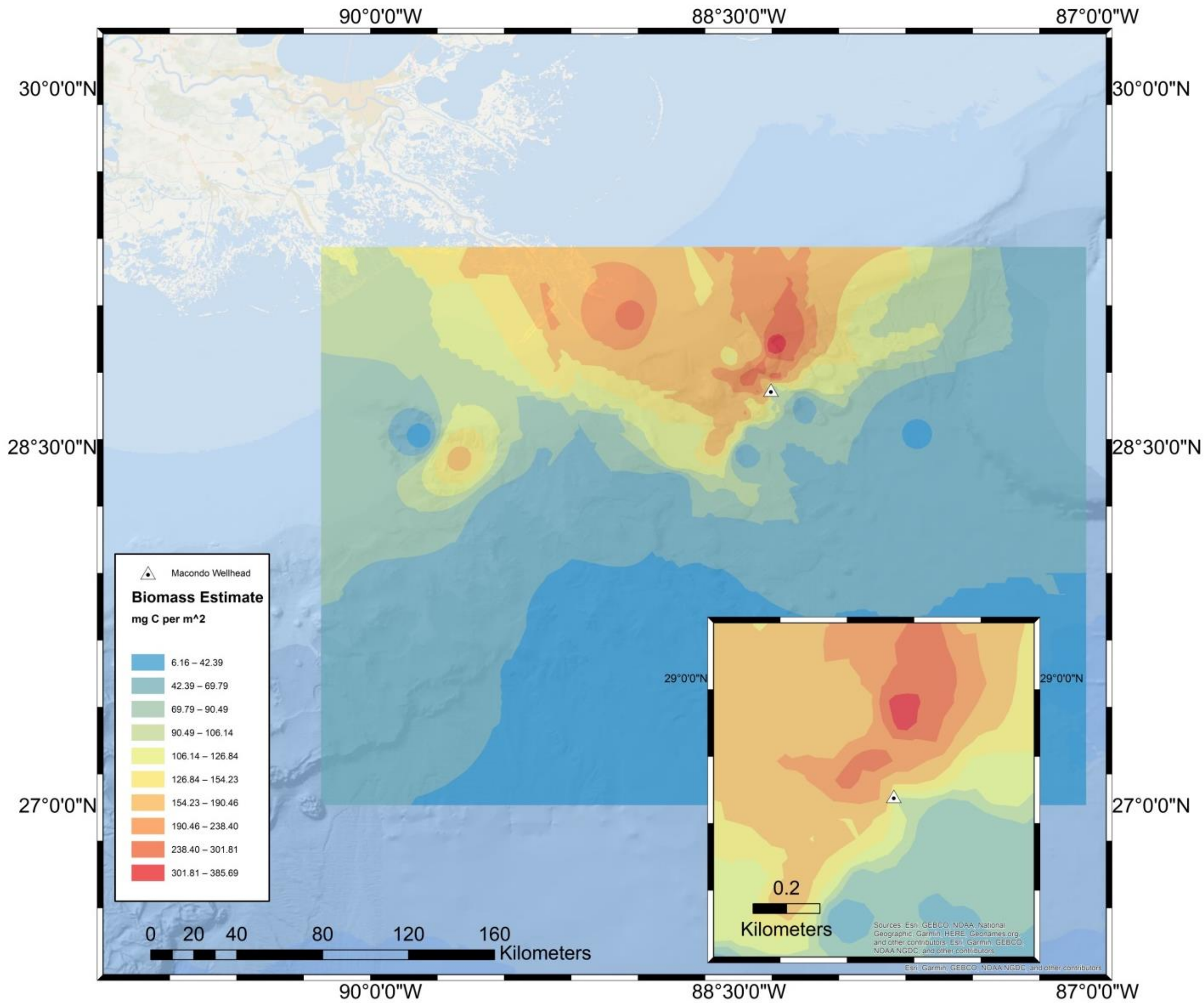


Figure 4- Inverse distance weighted interpolation of 66 stations of meiofauna community biomass (mg C m^{-2}) (Min. neighbor: 4, Max neighbor: 6) surrounding the wellhead (white triangle). Bottom right shows a close up view of the area directly around the oil spill. Estimations were weighted by their depth. Calculations were made in and map was acquired from ArcGIS 10.1.

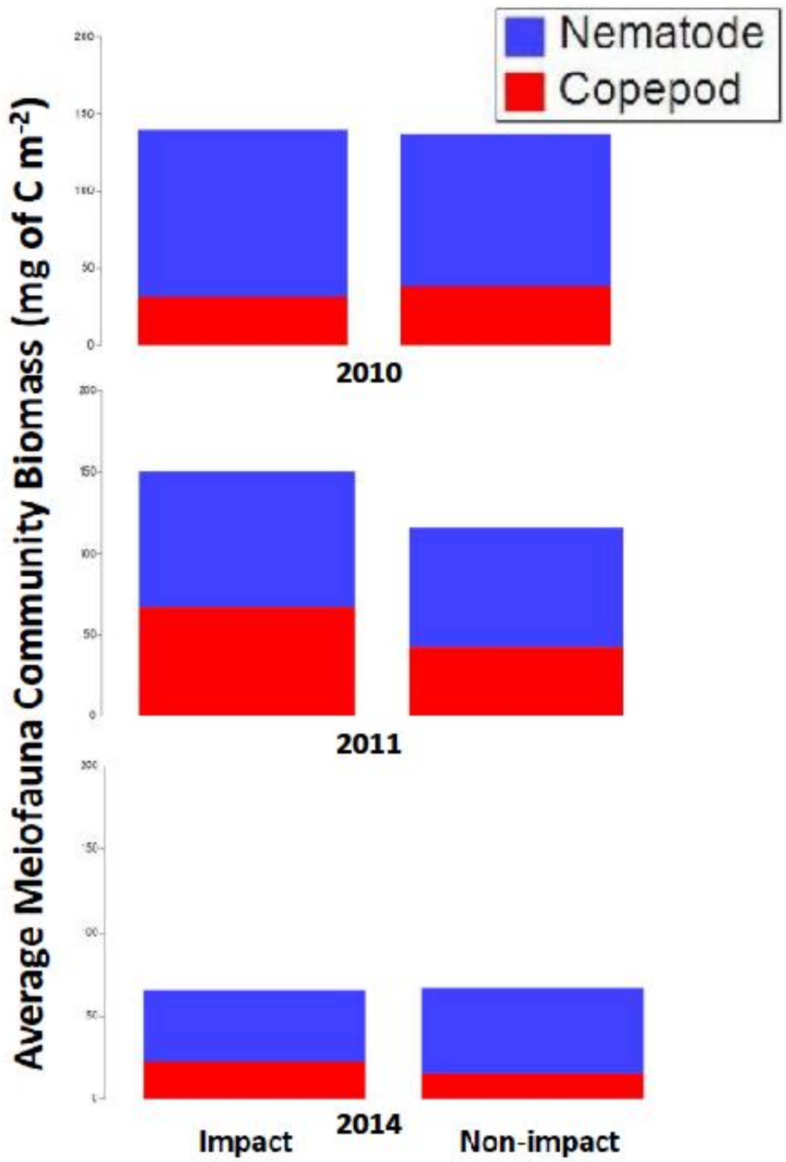


Figure 5- Average nematode (red) and Copepod (blue) biomass (mg C m^{-2}) from each sampling year with impact (left) and non-impact (right) stations (n=63). Copepod biomass was significantly different between years (F: 15.434, p: <0.001). Nematode biomass was significantly different between years (F: 22.497, p<0.001), and had significant interaction with zone (F: 3.533, p: 0.03).