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Macrobenthic infaunal communities associated with deep-sea hydrocarbon seeps in the northern Gulf of Mexico

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Abstract

There are thousands of seeps in the deep ocean worldwide; however, many questions remain about their contributions to global biodiversity and the surrounding deep-sea

environment. In addition to being globally distributed, seeps provide several benefits to humans such as unique habitats, organisms with novel genes, carbon regulation, etc. The purpose of this study is to determine if there are unique seep macrobenthic assemblages, by comparing seep and non-seep environments, among different seep habitats and at different depths and locations. Infaunal community composition, diversity, and abundance were examined between seep and non-seep background habitats and among three seep habitats (i.e., microbial mats, tubeworms, and soft-bottom seeps). Abundances were higher at seep sites compared to background areas. Abundance and diversity also differed among microbial mat, tubeworm, and soft-bottom seep habitats. While seeps contained different macrobenthic assemblages than non-seep areas, infaunal communities were also generally unique for each seep. Variability was 75% greater within communities near seeps compared to communities in background areas. Thus, high variability in community structure characterized seep communities rather than specific taxa. The lack of similarity among seep sites supports the idea that there are no specific infauna that can be used as indicators of seepage throughout the northern Gulf of Mexico, at least at higher taxonomic levels.

Introduction

Hydrocarbon and brine seeps are dynamic, organic-rich areas in an otherwise organic-poor deep sea. They occur where methane or reduced sulfur is released into pore waters, which are forced towards the sediment surface via pressure gradients (Gage and Taylor, 1996; Levin 2005). Deep-sea seeps are found throughout the world's oceans including the Pacific Ocean, Northern Atlantic, Gulf of Mexico, Mediterranean Sea, Arctic, and Northern Indian Ocean (Sibuet and Olu, 1998, Levin, 2005). MacDonald et al. (2015) identified over 900 active seep areas in the Gulf of Mexico. In general, an area of seepage was roughly 2000 m in diameter, and the majority of seeps were in the northwest area of the basin.

Deep-sea chemosynthetic systems are unique habitats for epifaunal and infaunal organisms to grow and evolve in, important sources of deep-sea primary productivity, areas where large amounts of greenhouse gases are consumed, and biodiversity hotspots (Carney, 1994; Cordes et al., 2010b; Armstrong et al., 2012; Kiel, 2015). Habitat itself is often considered a supporting ecosystem service (Farber et al., 2006; Armstrong et al., 2010), and hydrocarbon seeps provide unique habitats for organisms to live. Several deep-sea fishes, such as longspine

thornyhead (*Sebastolobus altivelis*) and Pacific dover sole (*Microstomus pacificus*), which may be targets for deep-sea fisheries, appear to congregate around seeps, which suggests they rely on chemosynthetically-derived organic matter (Grupe et al., 2015). Also, due to the extreme conditions at seeps, harvesting of organisms here will likely yield new pharmaceutical, agricultural, biotechnological, or cosmetic products (Glover and Smith, 2003; Armstrong et al, 2010).

Seeps support many chemosynthetic organisms and often contain high abundances of organisms that may be endemic, colonists, or vagrants (Carney, 1994; Barry et al., 1996; reviewed by Sibuet and Olu, 1998; Levin, 2005). Large, symbiont-containing bivalve or tubeworm epifauna dominate communities at many deep-sea hydrocarbon seeps (Sibuet and Olu, 1998), while bacterial mats comprising the genus *Beggiatoa* can also be important structures (Montagna and Spies, 1985; Levin, 2005). There is a lack of knowledge on infaunal communities associated with deep-sea seeps (Sibuet and Olu, 1998; Levin, 2005; Levin and Mendoza, 2007); however, studies have found higher densities (Robinson et al., 2004; Bourque et al., 2017), lower diversity (Levin et al. 2003; Bernardino et al., 2012), or lower densities and higher diversity (Guillon et al., 2017) at seeps compared to background areas depending on the type/magnitude of seepage. The low oxygen penetration in the sediments often leads to a larger proportion of the infaunal community being found in surface (0 – 2 cm) sediments at microbial mat seeps compared to background areas (Levin, 2005; Bourque et al., 2017). Clams and tubeworms at seeps pump oxygen and sulfates into the sediments possibly allowing infaunal communities to live deeper in the sediments where these megafauna are present (Levin, 2005; Guillon et al., 2017).

There is both spatial and temporal variability associated with seeps (Juniper and Sibuet, 1987; Olu et al., 1996, 1997; Sibuet and Olu, 1998), causing seep habitats to be some of the most heterogeneous environments found in the deep sea. Local temporal variations in hydrocarbon releases can occur over months or years making an accurate count of seep features difficult to maintain (Levin, 2005). Spatial variability in fluid flow, geochemistry, substrate, and microbial and megafaunal communities occur at both local (meters) and regional (kilometers to 100's of kilometers) scales (Cordes et al., 2010b). Temporal variability in fluid flow can coincide with tidal or lunar cycles while regional changes in methane and hydrocarbon releases may occur over centuries or longer (Levin, 2005). Pore-water fluids structure microbial communities and

epibenthic colonizers while colonizers influence the underlying microbes even further by providing additional habitat and altering pore-water chemistry (Cruaud et al., 2015).

Seep communities follow a general pattern of succession, with chemosynthetic microbes first colonizing new seeps. Bacterial mats comprising the genus *Beggiatoa* can also be important structures at seeps (Montagna and Spies, 1985; Levin, 2005). As the seep ages, carbonate slowly precipitates in the sediments via microbial processes providing hard substrate for mussel and tubeworm communities to settle, with tubeworms becoming more dominant as methane fluxes decrease (Bergquist et al., 2003; Cordes et al., 2005a,b). While this succession can occur over time, it can also take place over spatial scales with the center of the seep dominated by microbial mats and mussels, clams, or tubeworms located more in the seep periphery where pore-water hydrogen sulfide concentrations are moderate (Fischer et al., 2012). It has been hypothesized that the types of epibenthic colonizers found at a seep location may be used as an indicator of the chemistry at the habitat (Cordes et al., 2010a). Habitats associated with microbial mats are often found in areas with high methane releases and large concentrations of hydrogen sulfide in the sediments (Levin et al., 2003; Sahling et al., 2002). As seeps age, carbonates form, fluid fluxes decrease, and bivalve or tubeworm epifauna begin to colonize and dominate seep communities (Sibuet and Olu, 1998; Levin et al., 2003).

The deep sea is a remote environment and due to the expense, time, and labor required to obtain and process deep-sea infaunal samples, most data gathered on seep ecology have been isolated to large megafaunal assemblages captured by submersible images (Sibuet and Olu, 1998; Levin, 2005; Levin and Mendoza, 2007). Previous studies that have focused on infaunal seep communities have mostly been isolated to one or a few seep sites (Demopoulos et al., 2010; Decker et al., 2012; Plum et al., 2015; Bourque et al., 2017; Guillon et al., 2017). The seep studies that do examine several seeps often compare communities among seeps in different ocean basins (Levin and Mendoza, 2007; Bernardino et al., 2012).

The objective of the present study is to examine the effects of natural hydrocarbon seepage on macrobenthic infaunal communities in the deep-sea Gulf of Mexico by answering the following questions: 1) Are communities different between seeps and background, soft-bottom habitats, 2) Are macrobenthic communities associated with hydrocarbon seepage in the deep GoM different among different types of seeps?**Material and Methods**

Study Area

Sediment cores were collected near seep features in the northern Gulf of Mexico (GoM) in 2009 and 2010 by the U.S. Geological Survey (USGS) aboard the R/V Ron Brown and in 2012 and 2013 aboard the R/V Falkor and R/V Endeavor, respectively, by the Ecosystem Impacts of Oil and Gas Inputs to the Gulf (ECOGIG) consortium (data DOI: 10.7266/N70R9MV2). The ROV Jason was used to collect ROV cores for this study. Sampling was opportunistic, with the focus of some cruises on seeps; however, cores collected for infaunal analyses were not collected with any detailed hypotheses in mind, only that infauna near megafaunal communities would differ from background communities. Stations included five habitats which were not chosen a priori: soft-bottom hydrocarbon seeps, microbial mats, tubeworm communities, near-seep controls (within 20 – 400 m but outside the area containing seep-characteristic epifauna), and control conditions far from seeps (several km away). Stations ranged from 137 m to 2601 m in depth; however, only one station was shallower than 500 m. Five stations were represented by only one core per station (Table S1).

Because of the opportunistic nature of the seep collections and relative lack of nearby reference stations, samples collected from 2000 - 2002 during the Deep Gulf of Mexico Benthos cruises (DGoMB; Rowe and Kennicutt, 2009) and in 2010 during the Deepwater Horizon (DWH) Response cruises (Montagna et al., 2013) were included in analyses as additional deep-water soft-bottom background control stations. Background stations were within 100 km and 100 m depth of a station where seep samples were collected (Fig. 1 & Table S1). One to two stations closest to each seep at similar depths were chosen for comparisons between seep and background communities.

Sample Collections

Eleven seeps were sampled over the course of this study (Table S1). Samples collected via remotely operated vehicle (ROV) were considered within the seep field if ROV footage showed seep structures (i.e., tubeworms, mussel beds, or microbial mats) within a few meters of the core. Samples collected via multicorer were considered within the seep field habitat if hydrocarbons were visible in the sediment sample (as either a surface sheen when sieving the sediment or black tarry substance) or if there was a hydrocarbon odor to the samples. If at least one core from a drop had visible oil or an odor then all cores collected in that drop were considered representative of seep habitat. All multicore samples from seep communities were

assigned the seep type of "soft-bottom seep." Five stations represented general soft bottom habitat in the deep GoM and were not located immediately adjacent to any seep activity; therefore, they were considered representative of background conditions (Table S1).

Seep sediment cores were collected via a pushcorer attached to the ROV Jason as well as a ship-deployed multicoring device. Multicores had a diameter of 9.5 cm while ROV cores had a diameter of 6.35 cm. Cores were divided at various sediment depths aboard the boat and preserved in 10% formalin or 95% ethanol. Samples collected in 2009 were divided into 6 sections (0 - 1, 1 - 2, 2 - 3, 3 - 5, 5 - 7, and 7 - 10 cm) while samples collected in 2010 were divided into only 3 sections (0 - 2, 2 - 5, and 5 - 10 cm). Samples collected in 2012 and 2013 were divided into 4 vertical sections (0 - 1, 1 - 3, 3 - 5, and 5 - 10 cm). All sediment was sieved on a 300 µm mesh before sorting and taxa identification.

The DWH samples were collected using an Ocean Scientific International Ltd. (OSIL) multicorer with 12 separate cores. Only stations collected in the non-impacted zones 4 and 5 (Montagna et al., 2013) were included in our analyses. Cores collected were 78.5 cm^2 in area (10 cm diameter) and 10 cm in depth. Three cores were collected for macrofaunal analysis from each drop. Cores were divided into two sediment depths (0 - 5 and 5 - 10 cm), and each section was preserved with the addition of 10% buffered formalin. Samples were later sieved on a 300 µm mesh, sorted in the laboratory, and identified to family level (Montagna et al., 2013). The DGoMB samples were collected using a 2209 cm^2 (47 cm x 47 cm) GOMEX box corer. After subcores were removed, 1901 cm^2 of sediment remained for macrofaunal analysis. The top 15 cm of sediment were sieved onboard immediately after collection on 300 µm mesh, and all material retained on the sieve was preserved with the addition of 10% buffered formalin. While DGoMB samples collected sediment 5 cm below DWH samples, it has been shown that little information on benthic community structure is obtained below 10 cm in sediment depth (Montagna et al., 2017). All specimens were sorted and identified to lowest taxonomic level by various taxonomic laboratories (Boland and Rowe, 1991).

Benthic Community Descriptions

All polychaetes were identified to family level, while mollusks, crustaceans, and oligochaetes were identified to class or order level. Other taxa (e.g., nemerteans, sipunculans, echinoderms, etc.) were identified to phylum level. Polychaetes were the focus of taxonomic effort for this study because they dominated the samples collected, representing approximately

two-thirds of all organisms found. Taxonomic level within this study is justified because previous studies have found that data at the family level could be used to show deep-sea benthic community differences using multivariate techniques (Warwick, 1988; Narayanaswamy et al., 2003). The lack of knowledge on deep-sea species is additional justification for using higher taxonomic levels. For example, only 40% (205 of 517) of polychaetes and 25% (31 of 124) of amphipods found in the DGoMB study (Rowe and Kennicutt, 2009) could be identified to the species level by taxonomic experts. Furthermore, Peterson et al. (1996) reviewed benthic responses to marine pollution and found macroinfaunal communities exhibit repeatable patterns of response to contamination at high taxonomic levels, even at the phylum level.

Because of the difference in sample sizes among the multicores, ROV push cores, and box cores, abundances per m^2 were used in analyses rather than abundances per sample. All abundances were converted to abundances per m^2 as follows: for multicores collected during seep cruises, abundance data were multiplied by 141.08 cores/m^2; for multicores collected during DWH response cruises, abundance data were multiplied by 125.5 cores/m^2; for ROV cores, abundance data were multiplied by 315.77 cores/m^2, and for boxcores, abundance data were multiplied by 5.2604 cores/m^2. Seep samples were grouped into different depth and habitat categories in order to compare taxa among microbial mat, tubeworm, or soft-bottom seeps. A transition between upper and lower slope communities has been found between 1300 and 1700 m in the northern Gulf of Mexico (Cordes et al., 2007). Seep samples were grouped into the following depth categories to capture upper slope, transition, and lower slope communities: shallow (< 1000 m, 4 seeps), intermediate (1000 – 2000 m, 5 seeps), and deep (> 2000 m, 2 seeps), to examine similarities among seeps at various depths.

After seep communities were examined, comparisons were made between seep and background habitats. Again, to compare macrobenthic communities across several sampling methods, the data had to first be converted to abundances per m^2. Analyses were limited to taxonomic groups encountered and identified in DGoMB samples and thus excluded: Anthozoa, Bivalvia, Decapoda, Echinodermata, Gastropoda, Nemertea, Ostracoda, Scaphopoda, Sipuncula, Tanaidacea, and Turbellaria. These taxa were removed from DWH samples and samples collected during seep cruises when comparisons with DGoMB samples were performed. Diversity was calculated using the rarefaction method because it is less sensitive to differences in sample size (Simberloff, 1972), and it is well known that diversity is positively correlated to

sample area (Bunge and Fitzpatrick, 1993). Rarefaction curves were assumed to represent communities only if they approached an asymptote, and rarefaction scores were used for descriptive purposes. With larger sample area comes an increased probability of collecting rare organisms, which are more easily missed by smaller samplers.

An examination of differences between seep and background stations was performed at various sediment depths. Because DGoMB samples were collected to a depth of 15 cm with no differentiation among sediment depths, they were excluded from this analysis. The DWH samples collected in 2010 were only divided into 2 sections. To examine communities present in the 0 - 1, 1 - 3, 3 - 5 cm of sediment, samples from 2009, 2012, and 2013 were used, representing 7 background and 17 seep samples. To examine the top 2 cm of sediment, samples from 2009 and 2010 were used for a total of 5 background and 11 seep samples. Finally, all samples collected for the seep study could be used to compare sediments at 0 – 5 and 5 – 10 cm depth, for a total of 10 background and 25 seep samples. Abundances per m^2 and community similarity (via SIMPER analysis) were examined at each sediment depth.

Statistical Analyses

Because of the differences in methodology between seep and background DWH/DGoMB samples, taxa richness (number of taxa), diversity (N1 [Hill, 1973]), and evenness (J' [Pielou, 1969]) were only statistically analyzed among seep communities. Univariate community measures, which included abundance per m^2 as well as taxa diversity, richness, and evenness, were analyzed using PROC GLM in SAS 9.4 (SAS, 2013). A 1-way nested ANOVA with seep type (microbial mat, tubeworm, soft-bottom) nested within depth category (<1000, 1000 – 2000, >2000 m) was used to test the seep dataset for differences among seep habitats, residuals and homoscedasticity were not examined. Analyses were performed to describe community compositions for the various seeps sampled using Primer v7. Rarefaction analysis was used to calculate and compare diversity between boxcore and pushcore samples. Multivariate dispersion (MVDISP) analysis was performed in Primer to examine the amount of dispersion (i.e., variability in community composition) among different seep habitats, where higher values equate to higher levels of variability. Individual vertical sediment sections (0 – 1, 0 – 3, 0 – 5, and 5 – 10 cm) were also examined to determine if similarities or differences among habitats were observed throughout the sediment. Analysis of similarity (SIMPER) was used to determine the similarity of cores within a seep as well as to identify taxa shared among cores in all seep

habitats. The majority of results are descriptive in nature and thus have no p-values associated with them.

Total macrobenthic abundance was the only univariate parameter statistically analyzed for all samples using a 1-way nested ANOVA with habitat (seep, background) nested within depth zone (< 1000 m, 1000 – 2000 m, > 2000 m) as variables. While cores collected within one multicore drop are considered pseudoreplicates, Montagna et al. (2017) found that more variance in macrobenthic abundance and richness was associated with cores within a deployment compared to cores among replicate deployments. Thus, cores within a multicore deployment were treated as replicates for statistical analyses. For multivariate community analyses, abundances were first standardized by sample using Primer software (Clarke and Gorley, 2015) to help remove effects of different sampling areas collected via boxcorers, multicorers, and ROV cores, then they were square-root-transformed to help prevent changes in dominant taxa masking changes in the rest of the community. A non-metric multidimensional scaling (nMDS) plot was created in Primer using the Bray-Curtis resemblance matrix. Stations were compared among sampling gears, depth zones, and between seep and background locations. A similarity percentages (SIMPER) analysis in Primer examined which taxa were responsible for differences among groups. An analysis of similarity (ANOSIM) test was first performed with habitat (seep or background) nested within depth category in Primer. However, due to the low amount of replication of seeps in each depth category, there were not enough possible permutations (10) to obtain test statistics. Thus a 1-way ANOSIM was performed testing differences between seep and background communities.

Results

A total of 1421 organisms were collected from seven phyla during seep sampling cruises between 2009 and 2013. Annelids, crustaceans, and mollusks were the dominant taxa found representing 62%, 22%, and 14% of all the organisms found, respectively. Nemerteans and sipunculids each represented 1% of the total number of organisms, while only three echinoderms and two cnidarians were found. A total of 35 polychaete families were identified. The five most abundant polychaete families were Chrysopetalidae, Cirratulidae, Dorvilleidae, Paraonidae, and Ampharetidae (in descending order of abundance), which represented 33% of the total organisms found. The two most abundant non-polychaete taxa were tanaidacean and cumacean

crustaceans, representing 9% and 8% of all organisms collected. Molluscan bivalves and gastropods each represented 5% of all organisms collected.

Macrofaunal community patterns between seeps and background sediments

When samples from seep, DWH, and DGoMB cruises were examined, abundances were higher at seeps compared to background sites and were highest at depths between 1000 and 2000 m ($df = 90$, $F = 7.32$, $p < 0.0001$). Due to the different sampling methods, diversity, evenness, and richness were not statistically analyzed while differences among specific sediment depths were not statistically analyzed because of the differences in sample processing, including sectioning. Surface sediments (0 - 1 cm) had similar taxa richness in seeps and background sediments. However, seep sediments had 2.5 times the abundances (4635 N/m^2) as background sediments (1795 N/m^2). Similar patterns were observed in the top 0 - 2 cm of sediment (Table 1). In the 1 – 3 cm fractions, seep macrofaunal abundances dropped to 1.7 times that of background stations, while at 3 - 5 cm depth, abundances at background stations were 1.2 times greater than seep sites. In the deepest sediment fractions (5 - 10 cm), seep macrofaunal abundances were 1.2 times that of background. The largest differences in macrobenthic communities between seep and background stations were confined to the top 3 cm of sediment, except for microbial mat communities (Table 1). Comparisons between abundance, diversity, evenness and richness were purely descriptive because differences in core sectioning among sampling efforts prevented statistical tests.

Macrofaunal communities were significantly different at seep sites compared to background sites (1-way ANOSIM, $R = 0.48$, $P = 0.001$), and presence or absence of seepage explained nearly 50% of the dissimilarity among communities. Seep communities were nearly as similar to background communities (31%) as they were to other seep communities. When background sites from different years (2000-2002, 2009-2013) and from different collection methods (boxcorer, multicorer, ROV core) were compared to seep sites, communities within all seep habitats were still 74% more variable (1.592) than background communities (0.917) using MVDISP. Variability was similar at seeps < 1000 m and seeps between 1000 – 2000 m, but MVDISP was 20 – 30% lower at seeps > 2000 m (Table S2).

All taxa found at seeps were also collected in background sediments, except for the polychaete Trochochaetidae, which had three specimens collected at seep DC673. However, Paraonidae and Maldanidae explained more similarity within background communities while

Dorvilleidae, Ampharetidae, Hesionidae, Nereididae, and Aplacophora explained more similarity within seep communities (Table 2). Likewise, Cumacea, Oligochaeta, and Sphaerodoridae were only responsible for similarity within seep communities, while Glyceridae, Lumbrineridae, Nephtyidae, Onuphidae, Opheliidae, Pilargidae, Sabellidae, Sigalionidae, and Terebellidae were only responsible for similarity within background communities.

Background and seep communities were more similar to one another at shallow and intermediate depths (40% and 39%, respectively) compared to deeper depths (32%). Within either background or seep habitats, communities were most similar to each other at intermediate depths (52% and 56%, respectively) and least similar at deeper locations (40% and 43%, respectively). The polychaete families Spionidae (16% - 23%) and Paraonidae (14% - 31%) explained the most similarity within each depth range of background habitats. Spionidae (48%) explained the most similarity for seep communities > 2000 m in depth, followed by Paraonidae (14%) and Oligochaeta (11%). However, shallow seep communities were dominated by Dorvilleidae (18%) and Cossuridae (15%), and seep communities at intermediate depths were dominated by Cumacea (16%) and Hesionidae (10%). Different taxa were responsible for similarity for each seep (Table 3).

When gear type was examined in the MDS plot, all DGoMB samples (boxcores) were clustered close together near the center of the graph (Fig. 2). Samples collected during the DWH response cruise (multicores) were also clustered together around the DGoMB samples but were less tightly grouped. Samples collected via ROV and multicorer during the seep cruises were spread across the entire MDS plot (Fig. 2). Background stations collected with multiple sampling devices and over the course of thirteen years were much more tightly clustered in the nMDS plot compared to stations collected near seeps with similar sampling devices over the course of four years (Fig. 2B).

Macrofaunal community descriptions for northern GoM seeps

When examining only samples collected near seeps, the hierarchical model found significant differences in abundance/m^2 (p-value = 0.0014), richness (p-value = 0.0322), and diversity (p-value = 0.0448) between macrobenthos found at microbial mat, tubeworm, or soft-bottom seep communities while evenness was not statistically different (p-value = 0.1878). Microbial mat communities had two to three times the abundances as tubeworm and soft-bottom seep communities, respectively, but approximately half of the diversity (Figs. 3A & 3B).

Richness, diversity, and evenness were all lowest in microbial mat communities (Fig. 3A &Table S2).

When examining diversity at individual seep sites, rarefaction curves began to level off at roughly 100 - 150 individuals (Fig. 4A). There also appeared to be two different rarefaction patterns. Microbial mats were the least diverse, approaching roughly 15 taxa as a maximum, while the remaining seeps approached roughly 20 – 25 taxa, although some sites did not reach an asymptote (Fig. 4A). When all seeps were considered together, rarefaction settled around 40 taxa (Fig. 4B). All taxa found at mat seeps were also found in soft-bottom or tubeworm seeps. However, only one Chrysopetalidae polychaete was found outside microbial mats. Three of the four communities with the highest N/m^2 of Hesionidae and Gastropoda were found at microbial mats, as were two of the four communities with the highest N/m^2 of Ampharetidae.

Benthic abundance patterns throughout the sediment column were not significantly different among different seep habitats for any sediment section (0 - 1, 0 - 3, 3 - 5, 0 - 5, or 5 - 10 cm) due to high variability among and within seeps. Despite a lack of significant differences, abundances in the surface sediments (0 - 5 cm) of microbial mats (26,414 N/m^2) were 3 times that of abundances in surface sediments of tubeworm (9,022 N/m^2) or soft-bottom seep habitats (7,863 N/m^2). Abundances in deeper sediments (5 - 10 cm) of tubeworm habitats (1,685 N/m^2) were 5 times that of abundances in deeper sediments of microbial mat (316 N/m^2) or soft-bottom seep habitats (387 N/m^2). There was very little similarity within seep types below 5 cm in sediment depth. Tanaidacea, Amphipoda, Bivalvia, and Aplacophora explained more similarity in surface communities at soft-bottom seeps compared to other seep habitats. Similarity at microbial mats was caused almost exclusively by Cumacea, Ampharetidae, Dorvilleidae, and Hesionidae. Similarity within tubeworm communities was largely due to Spionidae. Similarity at background habitats was largely due to Cirratulidae, Paraonidae, Spionidae, and Maldanidae (Table 1).

There were nine seep locations represented by more than one sample, enabling statistical analysis of community similarity among replicates. Similarities within these nine seeps ranged from 26% to 64%. Each seep habitat type had a similarity of 45 – 55% while all seeps combined only had a similarity of 25%, illustrating that each seep and seep habitat had more similarity within their communities than when seep communities were combined into one group (Table 2S). Seep communities differed as a function of depth. Taxa composition was very similar

between seep communities at shallow (< 1000 m) and intermediate (1000 – 2000 m) depths (Fig. 5). Deep-seep (> 2000 m) communities were comprised of far fewer taxa than other seeps (Fig. 6). Seeps between 1000 - 2000 m were more similar (57%) than shallower or deeper seeps (43%); however, there were twice as many samples collected at intermediate depths. Deep seeps were less variable than intermediate or shallow seeps (Table S2). Only seven taxa were responsible for 95% of the similarity in deep-seep stations compared to 12 or 14 taxa in shallow or intermediate depths, respectively.

Discussion

Differences and similarities between seep and background communities

Macrofaunal abundances and community composition differed between seep and background samples, consistent with the alternative hypothesis that macrobenthic communities at seeps in the Gulf of Mexico are different from communities in background, soft-bottom sediments. Infaunal abundances/m^2 were higher near seeps compared to background conditions , which is a similar result to other studies (Levin, 2005; Bernardino et al., 2012; Bourque et al., 2017). Abundances are often higher near seeps compared to background areas in deeper waters due to organic enrichment via chemosynthetic processes at seeps and the low amount of surface-derived organic matter reaching the deep seafloor in background areas and (Levin and Michener, 2002).

While community composition was different between seeps and background areas, all taxa identified from seeps were also present in background sediments except for one polychaete. Seep infauna are generally comprised of a subset of background taxa, which are tolerant of high hydrogen sulfide concentrations (Bernardino et al., 2012). Taxa that dominated in seep habitats but not background areas included the polychaete families Dorvilleidae, Hesionidae, and Ampharetidae among others (Table 2). These polychaete taxa are often considered characteristic of seep communities (Levin et al., 2003; Levin, 2005; Bernardino et al., 2012). High abundances of dorvilleids are often found at seeps with high methane and sulfide fluxes where few other taxa are present (Levin, 2005; Bernardino et al., 2012; Decker et al., 2012). The two microbial mat seeps were the only locations with a large abundance of the polychaete family Chrysopetalidae, while the crustacean Cumacea was also abundant at microbial mats. While little is known about Chrysopetalidae ecology, some species of cumaceans have shown a preference for sulfide seeps (Levin, 2005). Cumaceans were dominant taxa at several organicly enriched environments

including methane seeps, kelp- and whale-falls where sulphide concentrations are high, likely due to their opportunistic nature (Bernardino et al., 2012).

There did not appear to be specific taxa that were representative of seep communities in the northern Gulf of Mexico. However, this is most likely due in part to the coarse taxonomic resolutions of the analyses. Even taxa that explained similarity among seeps, such as dorvilleids and ampharetids, were absent at several seeps. Almost every seep examined appeared to have different dominant taxa regardless of whether the samples were collected from the same seep habitat (microbial mat, tubeworm assemblage, or soft bottom) (Table 3). Studies have shown high species turnover (beta diversity) among different seeps, even within similar geographic regions (Cordes et al., 2010b; Bourque et al., 2017). This study found that macrobenthic communities associated with seepage were more variable than communities associated with the background, soft-bottom habitat.

Variability in macrofaunal communities associated with seeps

Most studies and statistical analyses attempting to measure ecological changes rely on means of variables such as abundance, diversity, and chemical concentrations. While means are easy and straightforward to use, variability within a specific habitat or impacted region can mask any differences observed among areas or treatment levels if large enough, and increased variability itself may be indicative of impacts of pollution (Warwick and Clarke, 1993; Green and Montagna, 1996; Demopoulos et al., 2016). Schmalhausen's law states that organisms living under stressful conditions are more susceptible to any environmental changes (Lewontin and Levins, 2000). Therefore, variance in the data may be indicative of stressful conditions. Seep habitats are associated with low sediment oxygen content and high levels of methane or hydrogen sulfide (Levin, 2005; Bernardino et al., 2012). Low oxygen and high methane or hydrogen sulfide cause seeps to be more stressful environments for many animals than background areas and may lead to increased variability (Demopoulos et al., 2016). Warwick and Clarke (1993) found that univariate measures of macrobenthic communities had increased variance at polluted sites compared to control sites.

A possible cause of the variability of macrofaunal assemblages among seep locations was the wide range in water depths of individual seeps. Macrobenthic community composition at seeps in the Gulf of Mexico were different at different depths (Table S2, Fig. 6). Depth generally has a negative relationship with food availability derived from surface waters and thus

a negative relationship with macrofaunal densities (Pequegnat et al., 1990; Rex and Etter, 2010). At shallow seep sites, those still within or near the photic zone, seep communities often resemble background communities. In shallow seep macrobenthic communities off the coast of Santa Barbara, CA, 90% of the individuals examined were taxa shared between seep and background stations (Davis and Spies, 1980). Some studies have suggested few species are shared among upper and lower slope seep communities (Carney, 1994; Cordes et al., 2007). In the present study, background and seep communities were more similar at depths shallower than 2000 m, indicating that even in the lower slope, organic matter is not as limiting as the deepest areas of the GoM. Food availability decreases with depth in background areas, but not necessarily at seeps, where chemosynthesis provides an additional food source. Chemosynthetic nutritional pathways have been shown to contribute more to invertebrate diets at deeper seeps compared to shallower ones (Sahling et al., 2003; Levin, 2005; Levin et al., 2016). Seep communities <1000 m and 1000 – 2000 m in depth were more similar to one another than either were to communities below 2000 m. Demopoulos et al. (2010) found macrofauna at seeps on the lower slope in the GoM derived 60 – 100% of their food from *Beggiatoa* mats. Therefore, as depth increases, the difference in food availability to communities associated with seeps and background areas likely increases as well.

Another likely source of variability among seep communities is the difference in pore-water fluids and thus initial food sources for organisms at different seeps. In this study, sulfide and methane concentrations were not measured, but epibenthic megafaunal colonizers (e.g., microbial mats, tubeworms, and mussels) were noted. Many studies have found these megafauna to be representative of sulfide and methane concentrations in the seep habitat they are found (MacDonald et al., 1989; Levin et al., 2003; Cordes et al., 2010b; Guillon et al., 2017). The seeps in this study represented several types of epibenthic communities, partly explaining the large amount of variability in macrobenthic communities among seeps.

Seep Habitats

Macrobenthic communities were different among the different types of seeps examined in this study (i.e., microbial mat, tubeworm, and soft-bottom seeps) (Fig. 3A & 3B), which may be a function of many factors, including fluid flow, seep successional stage, megafaunal communities, habitat suitability, or geochemical differences. When seepage begins in an area, microbial mats form and methanogenesis occurs, creating carbonates (Levin, 2005). As fluid

flow from the sediment decreases and hard substrate begins to appear in the form of carbonates, larger chemosynthetic organisms such as mussels and tubeworms begin to colonize the area (Cordes et al., 2006). Previous studies have found microbial mat habitats supporting the highest abundances of macrofauna compared to other seep habitats as well as background areas (Robinson et al., 2004; Levin et al., 2006; Bourque et al., 2017). These high abundances are most likely due to taxa tolerant to high sulfide conditions taking advantage of the large amount of chemosynthetically derived organic matter released (Sahling et al., 2002; Bernardino et al., 2012).

Microbial mat seeps often have low macrofaunal diversity compared to other seep or background habitats (Sahling et al., 2002; Levin et al., 2003; Bernardino et al., 2012; Bourque et al., 2017). Microbial mats are found at seeps where hydrogen sulfide concentrations are high, or when there is a gradient of seep habitats (e.g., microbial mats, mussel beds, tubeworm clusters) radiating from a central seepage area, mats are often found near the source where fluid concentrations are highest (Tryon and Brown, 2001; Levin, 2005). Macrobenthic communities living under *Beggiatoa* mats exhibited low abundance and diversity, characteristic of disturbance (Pearson and Rosenberg, 1978). Microbial mats themselves may be partly responsible for the lower richness at these sites. A microbial film over the sediments may be difficult to burrow through or make it more difficult for organisms living underneath to acquire oxygen. While these stressful conditions generally reduce richness and diversity, they may not necessarily reduce abundances as illustrated in this study.

Alternative factors that may be influencing taxa richness and diversity include successional processes and epibenthic structures. Seep epibenthic megafauna often exhibit successional patterns at seeps over time with new, high flux seeps being first colonized by microbial mats before decreases in seepage allow larger tubeworm and mussel communities to thrive (Levin et al., 2005). Thus, more macrobenthic taxa may be found in tubeworm habitats compared to microbial mat habitats because organisms have had more time to colonize them. Vestimentiferan tubeworms also uptake hydrogen sulfide from the sediment in their roots (Freytag et al., 2001), which may make the sediments more hospitable to infauna. Tubeworms slow water movement around them allowing for greater settlement of materials, including organic matter, from the water column to the sediment around them. The 3-dimensional

structure also provides additional niches for different types of organisms (Cordes et al., 2007), possibly explaining the increased number of taxa within the tubeworm associated sediments.

Variability due to Sampling Methods

Communities exhibited different patterns between seep and background environments and among different depth zones in the nMDS plots, but there was a large confounding factor when comparing samples across studies; the difference in sample areas. All DGoMB samples were collected with boxcorers, which had roughly 25 times the area of DWH samples collected with multicorers. Multicorers had roughly twice the area of seep samples collected via ROV cores. While background habitats were sampled over a much larger range of years and very different sampling methods (i.e., boxcorer, multicorer, and ROV core) than seep habitats, seep communities were still more variable than background communities (Fig. 2). With DGoMB grouped most tightly, followed by DWH samples, there appeared to be a strong relationship between sampling area and variability among samples (Fig. 2).

Montagna et al. (2017) compared benthic communities collected in the deep Gulf of Mexico at the same place and time by both a multicorer and boxcorer. They found that the boxcorer underestimated macrofaunal abundance by 3x while the multicorer collected 60% fewer taxa than the boxcorer. Bow waves from the boxcorer may wash away small, surface dwelling animals during collections (Hulings and Gray, 1971). The washing and sieving of samples on the boat deck during boxcorer operations may also be responsible for the loss of organisms (Montagna et al., 2017). However, Montagna et al. (2017) found that communities collected via boxcorer and multicorer at the same locations were very different, making comparisons between methods extremely difficult. In spite of these large differences among sampling methods, much more variability was associated with seep communities compared to sampling methods, highlighting the large amount of variability associated with seeps (Fig. 2).

Conclusion

In the deep Gulf of Mexico, macrofaunal community structure and abundance were different between seep and background habitats (Fig. 2, Table 2). Community structure, abundance, richness, and diversity were also different among different seep habitats (i.e., microbial mat, tubeworm, and soft-bottom seeps) (Fig. 3A & 3B). In fact, every seep seemed to have a different macrobenthic community although this may partly be an artifact of the lack of replication. All variability in background habitats associated with depth, time, location, and

sampling method were masked by the large variability among seeps. Thus, it appears that many seeps in the deep Gulf represent unique macrobenthic communities. Seeps have often been thought of as extremely heterogeneous environments, representing wide ranges in depth, chemical composition, fluid flux, geomorphology, age, and epibenthic megafaunal communities. This study confirms their heterogeneity in the Northern GoM.

There is a timely need for information on seep communities in the deep GoM. Determining baseline community structure at these seeps may be important for understanding patterns of global biodiversity. Some policies and non-governmental organizations may work to conserve as much biodiversity as possible, and seeps may be a rather large repository of this diversity, especially considering their small area. Further effort in collecting deep-sea organisms as well as taxonomic work is needed to determine the amount of endemism present at seeps. Given the high among-site variability in seep infaunal communities observed in this study, more taxa may be preserved by protecting many smaller seeps compared to a small number of large seeps.

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Horizon NRDA. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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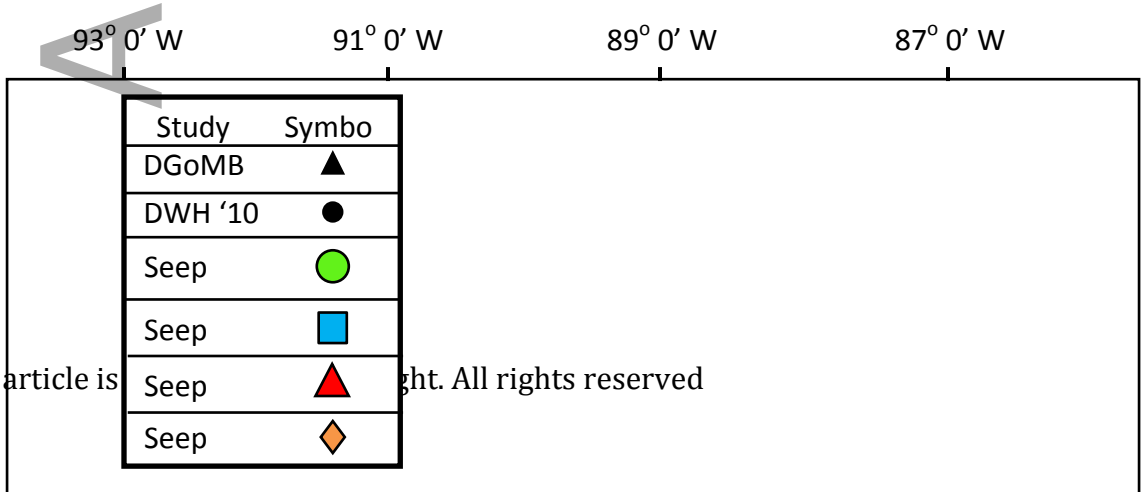


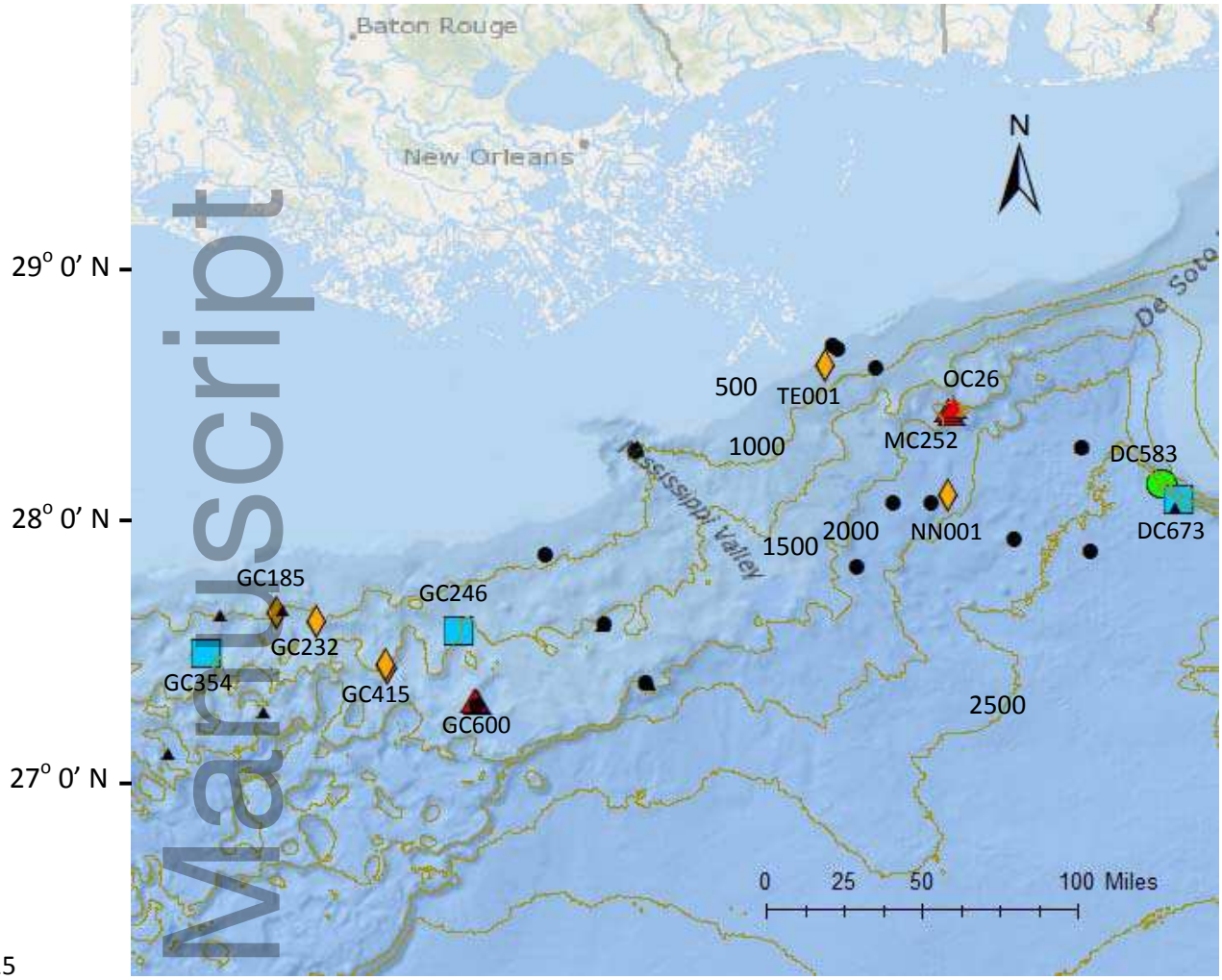
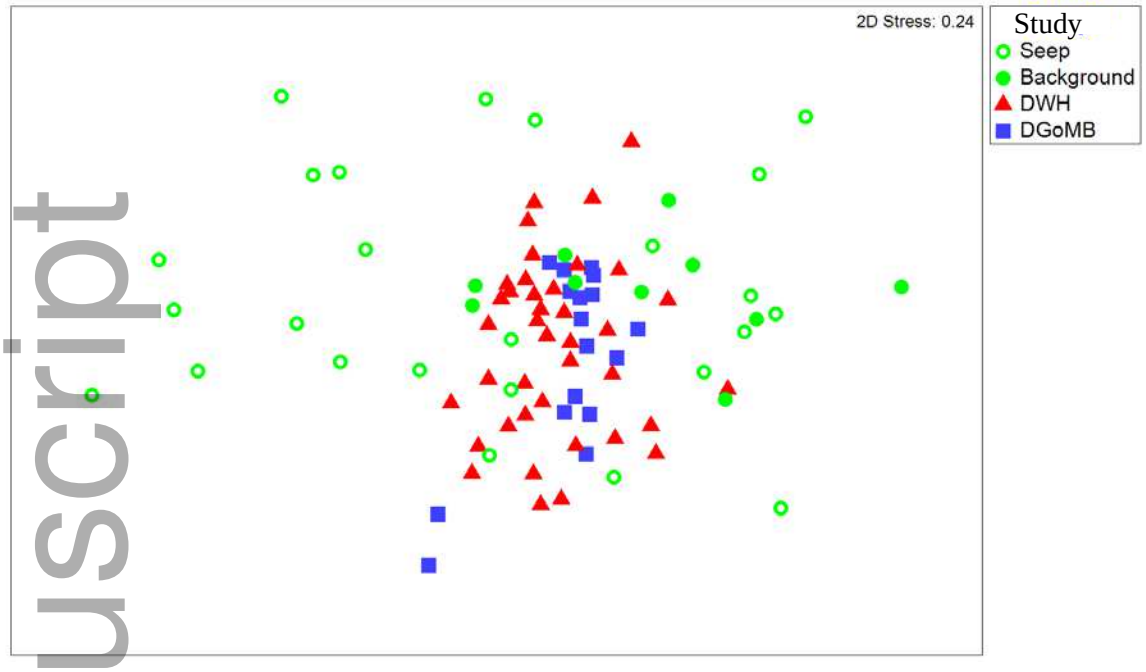
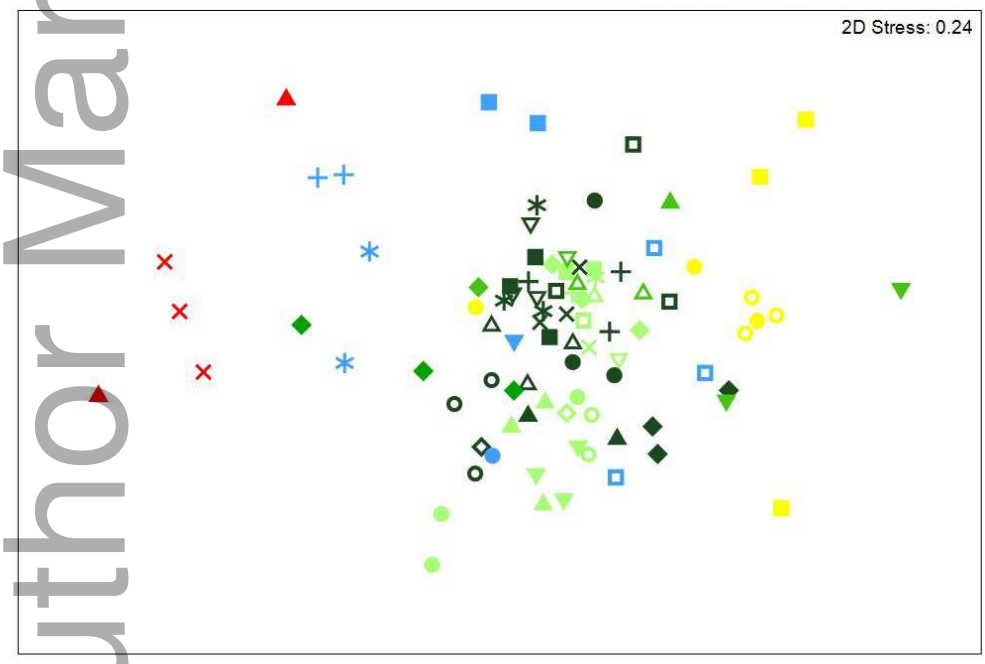


Figure 1. A map of all locations sampled during 2009-2013 (green circle = 2009, blue square = 2010, red triangle = 2012, and orange diamond = 2013) to examine communities associated with cold seeps in the Northern Gulf of Mexico as well as locations sampled during 2000-2002 on DGoMB cruises (black triangles) and during 2010 on the DWH response cruise (black circles). Station names are shown only for samples collected during seep cruises. Depth contours are in 500 m increments.



A)



B)

Figure 2. The nMDS plot of macrobenthic community structure collected during seep, DGoMB, and DWH cruises. A) Symbols by cruise type. B) Symbols by station where red represents microbial mats, blue represents soft-bottom seeps, yellow represents tubeworms, and green represents background (light green = DGoMB, dark green = DWH, and green = seep cruises).

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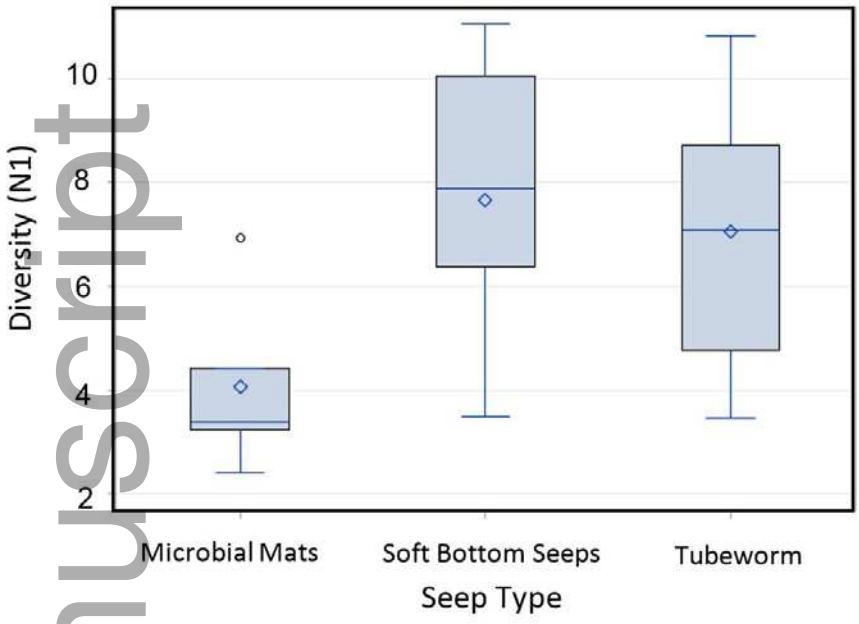

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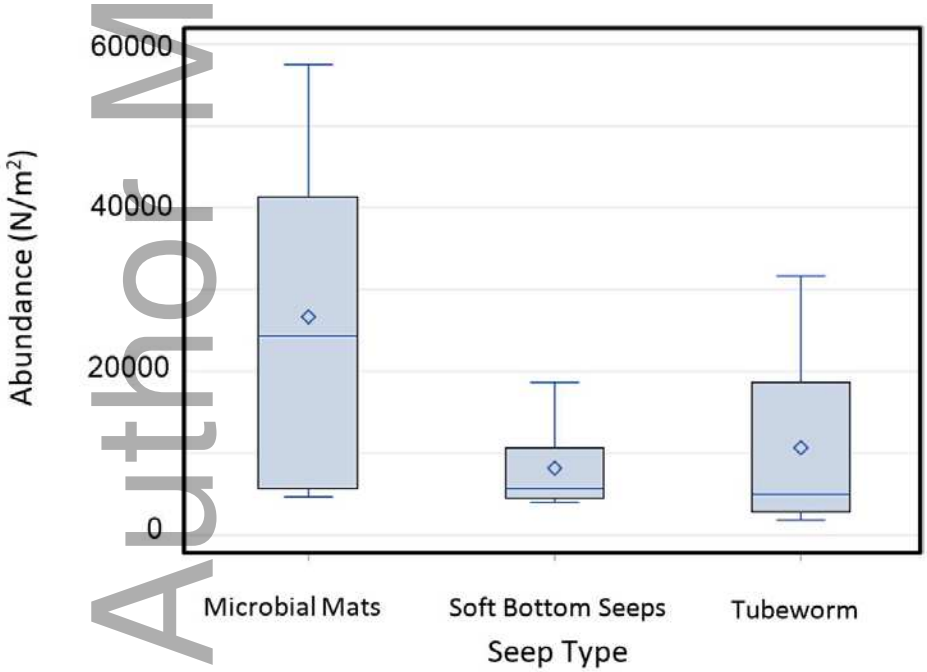

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747 B)

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749 Figure 3. Box plots comparing seep types where diamonds represent means while lines through the
750 rectangles represent medians. A) Diversity (N1) and B) Abundance (N/m^2) found at different seep types
751 (microbial mat, tubeworm, or soft-bottom seep).
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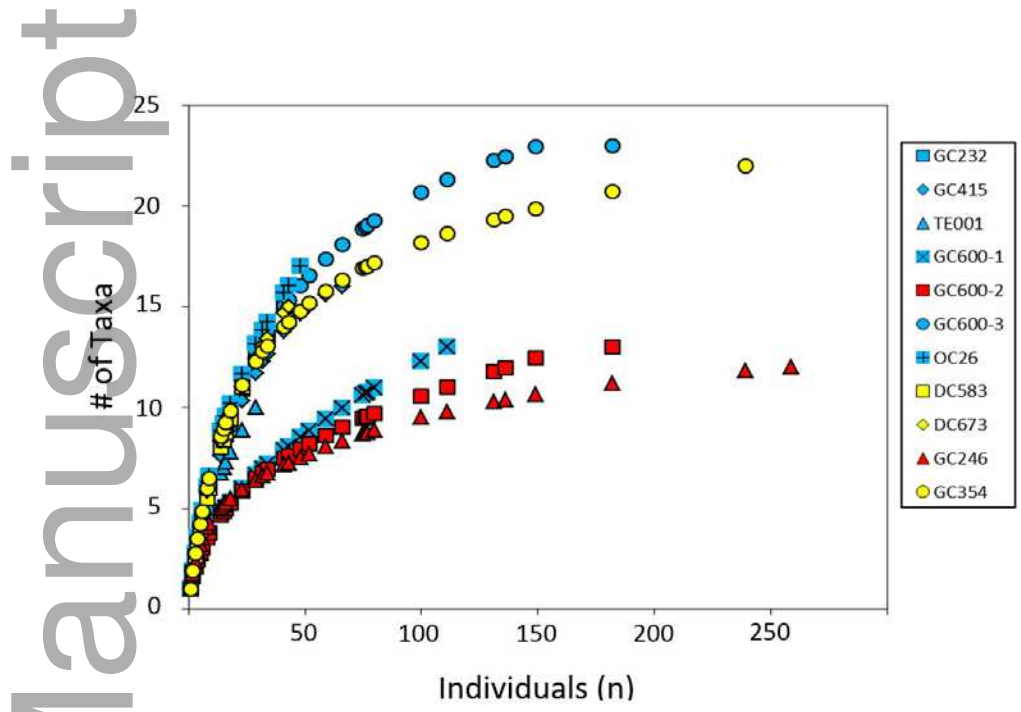

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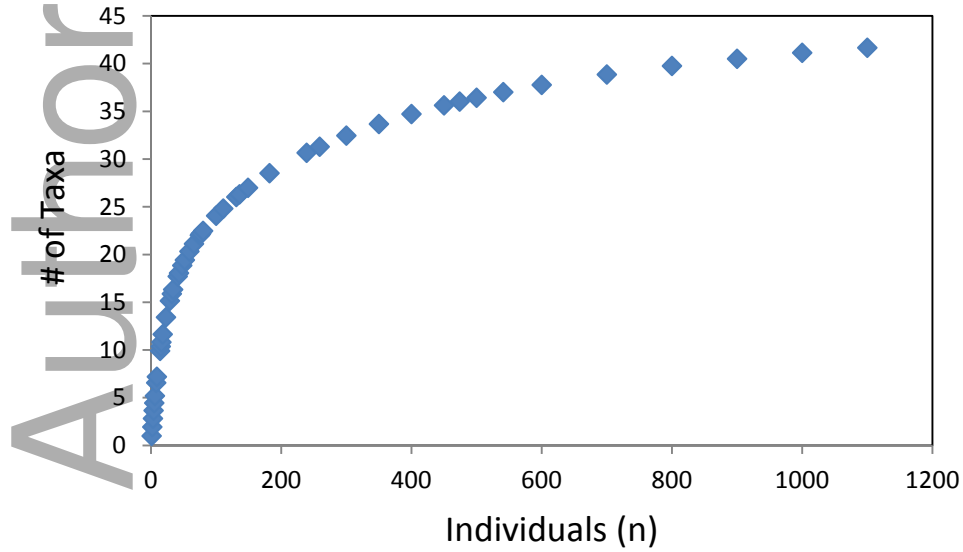
754 A)



755 B)

756 Figure 4. Rarefaction curves for A) each seep independently, where blue represents soft-bottom
757 seeps, yellow represents tubeworm seeps, and red represents microbial mat seeps, and for B) All

seep samples combined. GC = Green Canyon, TE = Taylor Energy, and DC = Desoto Canyon, while OC was in Mississippi Canyon.

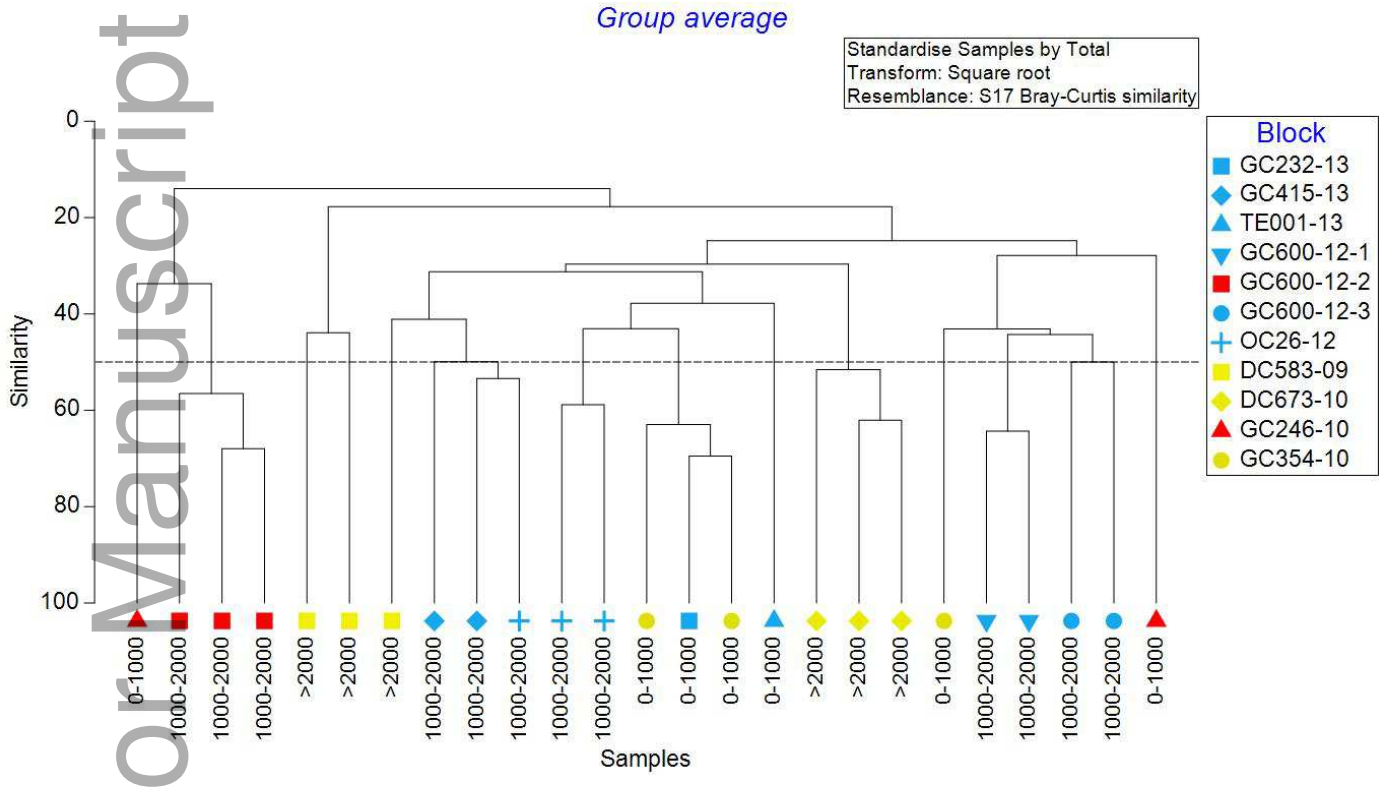


Figure 5. Cluster plot of community structure at all seeps sampled between 2009 – 2013. Different symbols indicate different seeps separated into 3 depth bins (0 - 1000 m, 1000 – 2000 m, and > 2000 m). The dashed line represents the 50% similarity threshold. Blue represents soft-bottom seeps, yellow represents tubeworm seeps, and red represents microbial mat seeps. GC = Green Canyon, TE = Taylor Energy, and DC = Desoto Canyon, while OC was in Mississippi Canyon.

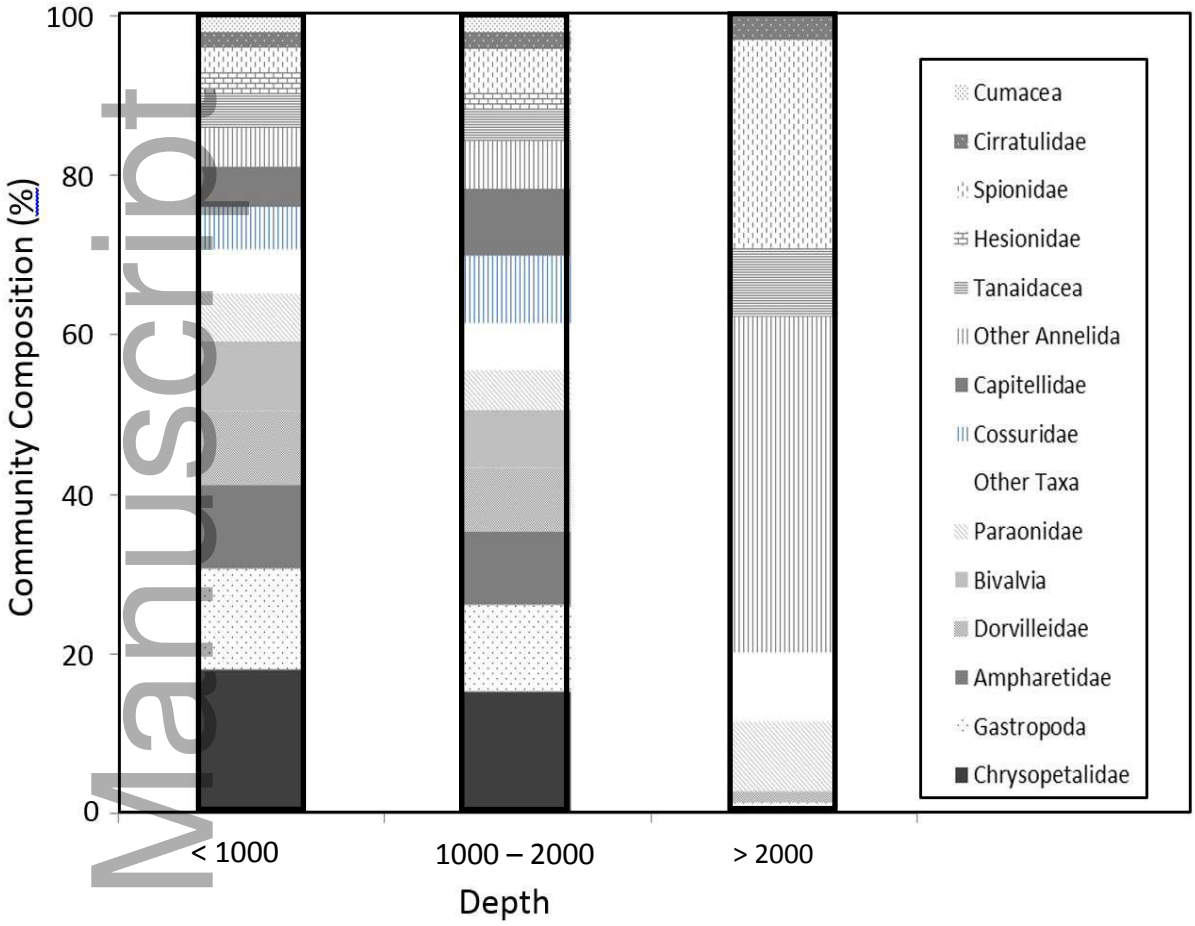


Figure 6. Average proportion of taxa comprising seep communities at shallow (0 – 1000 m), intermediate (1000 – 2000 m), and deep (> 2000 m) seeps.

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

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

Table 2. Taxa making up 95% of the similarity of samples within A) all background soft-bottom communities and B) all natural hydrocarbon seep communities. Abbreviations: Abundance = average abundance/m^2 at each habitat, Sim = average similarity attributed to each taxa, % Contribution = percent of overall similarity each taxa accounts for, and Cumulative = cumulative percent similarity.

A) Group Background, Average similarity 34.37%

Taxa	Abundance	Similarity	% Contribution	Cumulative
Spionidae	875.06	8.58	24.95	24.95
Paraonidae	679.31	8.19	23.84	48.79
Capitellidae	447.48	2.33	6.77	55.56
Maldanidae	340.83	2.23	6.49	62.06
Cirratulidae	377.19	1.81	5.27	67.33
Syllidae	137.86	1.36	3.94	71.27
Isopoda	108.88	1.32	3.83	75.1
Cossuridae	434.79	1.04	3.03	78.13
Lumbrineridae	327.84	0.83	2.42	80.56
Amphipoda	783.02	0.8	2.33	82.89

B) Group Seep, Average similarity 20.34%

Taxa	Abundance	Similarity	% Contribution	Cumulative
Spionidae	505.5	4.99	24.53	24.53
Dorvilleidae	908.6	2.45	12.03	36.56
Cumacea	1403.63	1.88	9.25	45.82
Capitellidae	526.73	1.49	7.31	53.12
Ampharetidae	982.78	1.35	6.62	59.74
Paraonidae	561.66	1.33	6.56	66.3
Aplacophora	302.59	0.9	4.43	70.73
Hesionidae	488.57	0.86	4.25	74.98
Cirratulidae	975.53	0.83	4.07	79.05

Syllidae	165.81	0.71	3.48	82.53

Table 3. Taxonomic composition of each seep site. Taxa in each phylum are ordered by abundance, from greatest to least. Values represent average abundance/m^2. If less than 5 organisms of a specific taxa were found throughout all seep sites, then those taxa were not included.

	Station, Year, Depth, and Replicates										
	TE001	GC354	GC232	GC246	GC415	GC600-3	GC600-2	GC600-1	OC26	DC583	DC673
	2013	2010	2013	2010	2013	2012	2012	2012	2012	2009	2010
	137	567	575	834	1048	1180	1221	1263	1669	2449	2601
Taxa	1	3	1	2	2	2	3	2	3	3	3
Arthropoda											
Tanaidacea	0	1895	423	474	1764	2751	0	3158	316	316	316
Cumacea	0	411	0	947	353	141	10105	158	95	0	0
Amphipoda	0	1800	0	0	212	705	0	632	0	0	0
Isopoda	0	0	141	316	564	0	0	0	95	95	221
Annelida											
Chrysopetalidae	0	95	0	14999	0	0	726	0	0	0	0
Ampharetidae	1693	1800	0	5210	0	71	2305	0	0	0	0
Dorvilleidae	0	4516	1411	316	212	494	632	1737	0	95	0
Cirratulidae	141	726	141	474	0	212	0	9947	0	95	95
Paraonidae	423	2937	705	0	141	0	0	158	537	0	632
Capitellidae	141	2526	564	0	71	635	95	158	947	0	0
Spionidae	282	1263	705	0	141	0	0	0	632	853	1042
Cossuridae	0	3063	0	0	0	846	95	0	95	0	0
Hesionidae	0	632	141	1421	0	353	2116	158	0	0	0
Oligochaeta	0	537	705	0	71	0	0	0	0	0	726
Syllidae	0	411	0	158	0	494	0	158	0	95	316

Acrocirridae	0	0	0	0	0	635	0	0	95	95	95
Maldanidae	0	95	0	0	0	141	0	0	726	0	0
Pilargidae	0	316	141	0	0	0	95	0	0	221	95
Nephtyidae	141	0	0	0	0	423	0	0	0	0	0
Nereididae	0	0	0	158	0	71	0	0	411	0	0
Sphaerodoridae	0	95	0	0	141	212	0	0	0	0	0
Sigalionidae	0	0	141	0	141	0	0	0	95	95	0
Trichobranchidae	0	0	141	0	0	141	0	158	95	0	0
Lumbrineridae	705	0	0	0	0	0	0	0	0	0	0
Mollusca											
Gastropoda	0	0	0	10578	0	353	411	158	0	95	0
Bivalvia	141	853	141	5842	141	71	95	632	316	0	0
Aplacophora	282	726	141	0	353	1481	95	316	95	0	0
Nemertea	0	95	0	0	0	141	316	0	221	0	221
Total	4091	25167	5784	40892	4656	10652	17273	17525	5052	2431	4516