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High-Throughput Sequencing Characterizes Intertidal Meiofaunal Communities in Northern Gulf of Mexico (Dauphin Island and Mobile Bay, Alabama)

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Abstract. Meiofauna are important components of food webs and for nutrient exchange between the benthos and water column. Recent studies have focused on these communities in the Gulf of Mexico due to potential impacts of the *Deepwater Horizon* Oil Spill (DWHOS). In particular, intertidal meiofaunal communities from Mobile Bay and Dauphin Island, Alabama, were previously shown to shift from predominately metazoan taxa prior to DWHOS to a fungal-dominated community after the spill. However, knowledge of variability within these communities remains unknown. Herein, we used Illumina high-throughput amplicon sequencing to examine variation throughout a year for the same locations for which the organismal shift was noted. Sediment samples were collected bi-monthly for a year (July 2011–July 2012) from which the meiofaunal community was examined by sequencing the eukaryotic hypervariable V9 region of the 18S rRNA gene. Results showed that the presence of fungal taxa was limited within these communities, suggesting that previously reported acute impacts of the DWHOS on meiofauna were apparently short term. However, these meiofaunal communities show shifts in proportions of metazoan taxa compared to pre-spill samples. Whether this change is due to prolonged impacts of the spill or variation in community composition is unclear. Taxonomic variation within and between sampled locations throughout the study was observed, suggesting potential yearly variation in communities. Continued sampling over a longer timeframe will provide a more complete understanding of seasonality and variation within these communities. Such a baseline is required to assess future anthropogenic impacts.

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Introduction

Meiofauna are generally defined as animals 45 μm to 1 mm that live between sediment grains (Mare, 1942; Giere, 2009). These organisms are a key component of food webs, nutrient exchange, and sediment stability (Snelgrove, 1997). More specifically, meiofauna serve as a trophic linkage between bacteria and macrofauna (Coull, 1999) as well as play an important role in transport between the sediment and water column (Aller and Aller, 1992; Coull, 1999). Despite their ecosystem importance, meiofauna are often overlooked and not easily studied because sieving and using microscopy to taxonomically identify individuals is extremely time consuming and usually requires experts to properly identify organisms. Furthermore, because expertise is needed, researchers typically focus on a few taxonomic groups within meiofauna, limiting the ability of studies to be extrapolated across the community. High-throughput sequencing approaches along with bioinformatic tools provide the ability to examine the composition of communities as a whole more quickly than traditional methods, allowing for a better understanding of community composition and variation. Even with the usefulness of this technology, only a handful of studies have implemented high-throughput genomic approaches to assess meiofaunal diversity (Fonseca *et al.*, 2010; Creer *et al.*, 2010; Bik *et al.*, 2012a, b, c).

In recent years, interest in the Gulf of Mexico (GOM) ecosystem has grown greatly in light of the *Deepwater Horizon* Oil Spill (DWHOS). Unfortunately, current knowledge of meiofauna community composition and variation

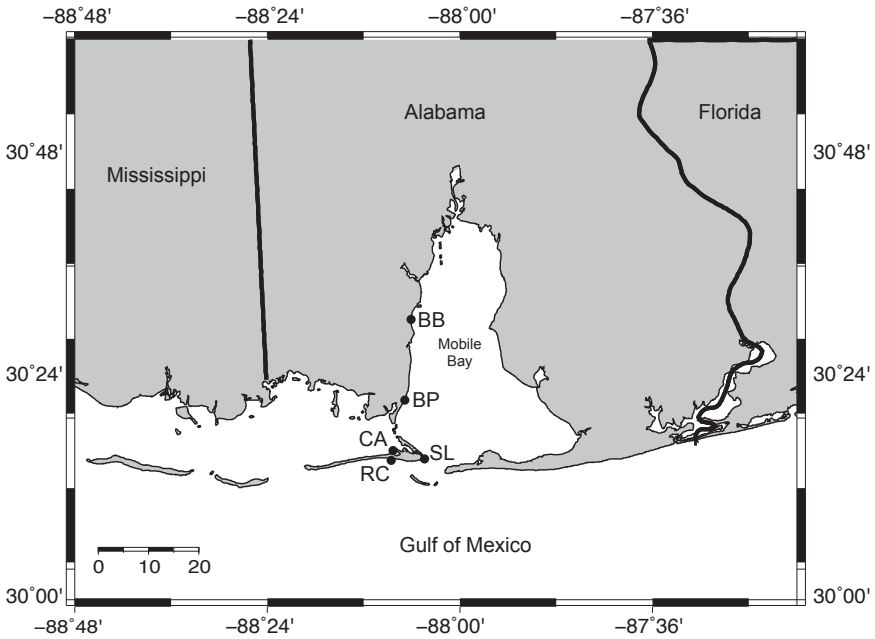

Figure 1. Sample locations within Mobile Bay and along Dauphin Island, Alabama. RC = Ryan Court, CA = Cadillac Ave, SL = Shellfish Lab, BP = Bayfront Park, and BB = BelleAir Blvd.

within the GOM is limited, especially in intertidal systems. The few studies that have examined subtidal meiofauna community composition in GOM focused either on deep sea (Pequegnat *et al.*, 1990; Baguley *et al*., 2006, 2008; Montagna *et al*., 2013), on continental shelf locations (Montagna and Harper, 1996; Escobar-Briones and Soto, 1997; Escobar *et al.*, 1997; Landers *et al.,* 2012*)*, or within Texas estuaries (Montagna and Kalke, 1992; Montagna *et al*., 2002). Nematodes and copepods were reported as the two dominant taxa within communities (Montagna *et al.,* 2002; Baguley *et al.*, 2006, 2008; Landers *et al.*, 2012). Additionally, variation in meiofauna abundance has been related to water depth and longitudinal locations (Pequegnat *et al.*, 1990; Baguley *et al*., 2006; Landers *et al*., 2012). These studies provided baseline knowledge of community composition and allowed for exploration of the impact of the DWHOS, which greatly reduced abundance and diversity of meiofauna in subtidal areas close to the source of the spill (Montagna *et al.*, 2013).

In contrast to information on subtidal meiofaunal populations, data on community composition and variation within intertidal meiofauna communities within the GOM are lacking. To the best of our knowledge, there have been no studies that examine variation and yearly composition within GOM intertidal meiofauna populations. In the wake of the DWHOS, Bik *et al.* (2012a) explored intertidal meiofauna communities along Dauphin Island and in Mobile Bay, Alabama, using high-throughput sequencing approaches. They showed a dramatic community shift from predominantly metazoan composition prior to the DWHOS to communities dominated by fungal taxa after the spill (Bik *et al.*, 2012a). What was not known in this study was the degree of variability or changes in community composition over time (*e.g.,* months, seasons, years).

Herein, we explore intertidal meiofaunal community variation along sites along Dauphin Island and in Mobile Bay, Alabama, over the course of a year. To facilitate comparison, we chose the same localities as Bik *et al.* (2012a). This study is the first within the GOM region to examine community composition of intertidal meiofauna populations over the course of a year and the first to incorporate Illumina platform technologies to assess meiofaunal diversity. Goals of this work included (1) assessing spatial and temporal variation within intertidal meiofaunal communities, (2) determining whether the large fungal composition seen in Bik *et al.* (2012a) was persistent, and (3) ascertaining whether the DWHOS has a lingering impact on intertidal communities. This study provides a fundamental baseline to examine similar intertidal meiofauna community impacts of future natural and anthropogenic disturbances in the GOM region.

Materials and Methods

Sampling

Between July 2011 and July 2012, bi-monthly sediment samples were taken from the same five intertidal locations (Fig. 1 and Appendix Table A1) along Dauphin Island and in Mobile Bay, Alabama, as Bik *et al.* (2012a). Eight sediment cores 10 cm in depth were collected from each location at each sampling date using a PVC pipe with an

interior diameter of 4 cm. All samples were covered by water at the time of collection. In the field, each core was divided into 0–3-cm and 3–10-cm sections. Six cores were immediately frozen on dry ice for genetic analysis and two cores were preserved in DMSO EDTA Salt Solution (DESS) (Yoder *et al.*, 2006) for a nematode morphological study to be published later. Upon return to Auburn University, frozen samples were stored at −80 °C until processed.

Meiofauna isolation and DNA extraction

For each sampling locality and time, three cores were haphazardly chosen for processing, and the two depth sections were processed separately. Depth fractions (either 0–3 cm or 3–10 cm) from the three cores were weighed and combined into one sample in an attempt to reduce the sample-specific heterogeneity typically found in meiofaunal communities. Meiofauna were isolated from sediment by decanting. Sediment was agitated by vigorously inverting the sample 10 times in a flask with a 32-ppt Instant Ocean solution (Blacksburg, VA) up to a total of 950 ml, the larger particles were allowed to settle (30-s maximum), and then the aqueous layer was carefully poured over a 45-μm sieve. This decantation protocol was repeated 10 times per sample, except for Bayfront Park's (BP) 3–10-cm fractions, which were decanted only five times due to a large amount of small silt particles that hindered filtration. Material retained on the sieve was transferred to a 50-ml conical tube and stored at −80 °C until DNA was extracted. Most of the material obtained with this protocol falls in the defined size range of meiofauna. However, some slightly larger organisms (<2 mm) might have been retained as well, and unpublished data (Brannock and Halanych) show that decanting preferentially retains metazoans compared to some other eukaryotic taxa. Herein, we use the term "meiofauna" to reflect the entire biotic sample retained on the sieve.

Total genomic DNA was extracted using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Cat #12888). The bead solution was transferred from each PowerBead tube to a sterile 1.5-ml tube. Decanted material was mixed, and 1 ml was placed into the PowerBead tube and centrifuged at 15,700 × *g* for 1 min; most of the supernatant was discarded. The bead solution was returned to the PowerBead tubes, and the manufacturer's protocol was followed except the 10-min vortexing step was replaced with a 2-min Mini Beadbeater (BioSpec) step and DNA was eluted in a final volume of 50 μl. DNA integrity was checked by gel electrophoresis, and DNA was stored at −20 °C until sent for amplification and sequencing.

Sequencing and bioinformatics

Genomic DNA was sent to the Genomic Services Laboratory at Hudson Alpha Institute for Biotechnology (Huntsville, Alabama) for amplicon sequencing using the eukaryotic-specific hypervariable V9 region of the 18S small subunit ribosomal RNA (SSU rRNA) gene utilizing primers 1380F and 1510R (Amaral-Zettler *et al.*, 2009). Samples were amplified using 20 ng per sample per technical replicate for the input for PCR, except for where DNA concentration was extremely low. In such cases, the DNA volume sent for amplification was divided evenly between two PCR replicates. Maximum DNA volume per PCR was 18 μl due to reaction limits. Each sample was amplified in duplicate. These technical replicates remained separated through operational taxonomic unit (OTU) clustering in the analysis pipeline. Paired-end 100-bp reads were obtained by sequencing dual barcoded amplicons on an Illumina HiSeq 2500 platform or paired-end 150-bp reads on an Illumina MiSeq. All samples had at least one technical replicate run on the HiSeq 2500, while only a subset of samples had one replicate run on the MiSeq. All samples from March 2012, May 2012, and July 2012 as well as Cadillac Ave 3–10-cm sample from July 2011 and BelleAir Blvd 3–10-cm sample from September 2011 had one technical replicate run on the MiSeq and one technical replicate run on the HiSeq 2500. All other samples had both technical replicates run on the HiSeq 2500. Previous studies have showed consistent results in amplicon analysis between the HiSeq and MiSeq platforms (Caporaso *et al.*, 2012). Reads were demultiplexed by the Genomic Services Laboratory. Raw high-throughput sequencing reads were deposited in the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database (Accession Number SRP042047).

Raw forward and reverse reads were overlapped using PandaSeq ver. 2.5 (Masella *et al.*, 2012) . During the overlapping process, any reads not containing the 1380F and 1510R primers were discarded, and forward and reverse primers were trimmed after overlapping. In addition, sequences that contained uncalled bases (N) or were longer than 200 bp were also discarded. Remaining sequences were quality filtered utilizing the FASTX-Toolkit ver. 13.2 fastq_quality_trimmer script (Hannon Lab, 2009) with a quality score cutoff of 30. Chimeras were identified and filtered in QIIME ver. 1.8 (Caporaso *et al.*, 2010b) using USEARCH ver. 6.1 (Edgar, 2010), and the remaining sequences were combined into one fasta file with properly formatted labels using the QIIME script add_qiime_labels.py. Sequences less than 75 bp were discarded.

Sequences were clustered into OTUs at 97% similarity using QIIME's pick_open_reference_otus.py workflow with the UCLUST (Edgar, 2010) clustering method disallowing singletons. Alternative clustering methods (*e.g.,* USEARCH and UPARSE—Edgar, 2010 and 2013, respectively) were tested on another smaller dataset (PMB and KMH, unpubl. data) and did not differ greatly in the resulting taxonomic assignments; therefore, since the current dataset is too large to utilize the 32-bit ver. of USEARCH,

clustering was conducted using UCLUST. The SILVA 111 (Quast *et al.*, 2013) 99% clustered database was utilized as the reference set. The most abundant sequence was chosen as the reference sequence for the resulting OTU cluster. Taxonomy was assigned to representative sequences by using MegaBLAST (sequence identity ≥90%, e-value 0.000001) to retrieve the top-scoring hit against the SILVA 111 99% clustered database. Alternative taxonomic assignment methods (*e.g.*, RDP Classifier and UCLUST—Wang *et al.*, 2007, and Edgar, 2010, respectively) did not result in OTU designations more specific than Eukaryota or Metazoa and were therefore not useful for these analyses. OTUs that did not result in a hit were classified as a "no hit." We restricted the sequence identity match to ≥90% to be consistent with Bik *et al.* (2012a) methods as well as to be more conservative in our taxonomic assignments. Representative OTUs were aligned against the same reference database (SILVA 111, 99% clustered) using PYNAST (Caporaso *et al.,* 2010a) (75-bp minimum length, 70% minimum ID). A minimum evolution tree was constructed with the resulting alignment using FASTTREE (Price *et al.*, 2009). The resulting tree was utilized for alpha and beta-diversity measures (see the following section). Sequences classified as Bacteria and Archaea as well as those that failed to align with PYNAST were filtered from the resulting OTU table. Hereafter, the OTU table that excluded those sequences that failed to align with PYNAST and were classified as Bacteria or Archaea will be referred to as the final OTU table.

Analysis

To determine whether technical replicates resulted in similar OTU composition, a hierarchical clustering analysis on the proportional OTU table for the 140 samples (70 samples with two PCR replicates each) was performed using the BiodiversityR package (Kindt and Coe, 2005) in R ver. 3.0.0 (R Development Core Team, 2008) utilizing an average linkage agglomerative clustering algorithm. The distance matrix was calculated using the Bray-Curtis dissimilarity metric. In the resulting dendrogram, samples with more similar OTU composition are clustered together and samples with more distinct OTU compositions cluster separately. As the dendrogram showed that technical replicates were more similar in OTU composition to each other than to any other sample (Appendix Fig. A1), sequences for technical replicates were combined and treated as one sample for the remainder of the analyses. Likewise, differences in replicate runs on the two platforms (Illumina HiSeq 2500 and MiSeq) were not observed. Hereafter, the data that include the combined replicates will be referred to as the complete dataset.

To allow direct comparison of diversity measures between samples, we normalized the number of sequences compared from each sample using a rarefaction analysis that subsampled the complete data set at 10 levels, from 10 to 300,010 sequences, in steps of 30,000 sequences. Ten replicates for each subsampling level were conducted, and average alpha-diversity measurements for each level were plotted against depth to determine a sufficient subsampling level. We subsampled the OTU table to 69,000 sequences in subsequent alpha and beta-diversity analyses. In order to be conservative in our community measurements, this number was chosen because it was close to the number of sequences (69,224) in our smallest sample that contained greater than 50,000 reads. For each subsample, three separate alpha-diversity measurements were calculated: Chao1, phylogenetic distance (PD), and Shannon diversity. Both Chao1 and PD examine species richness, while Shannon diversity examines species evenness. Beta diversity was measured by calculating the weighted Unifrac distance (Lozupone and Knight, 2005), Bray–Curtis dissimilarity, and the binary Jaccard dissimilarity metrics in QIIME. The weighted Unifrac metric is a quantitative metric based on phylogenetic relationship of the OTUs that looks at the evenness of samples and takes into account the relative abundance of the OTUs. The Bray-Curtis metric measures species turnover on the basis of the raw counts of the OTUs, and the Jaccard metric is based on presence-absence of an OTU. Neither the Bray–Curtis nor the Jaccard metrics take into account species relatedness and therefore are not based on any phylogenetic information. Moreover, use of a presence-absence based approach allows assessment of OTU data irrespective of abundance or counts. In essence, the Jaccard metric allows one to more fully understand whether differences between samples represent changes in community composition by negating variation in abundances between OTUs due to differences in copy number of ribosomal genes. Jackknifed ($n = 100$) beta-diversity matrices were utilized in both principle coordinate analysis (PCoA) and unweighted pair group method with arithmetic mean (UPGMA) hierarchical clustering analysis.

Community composition was examined by summarizing taxonomic assignments with the summarize_taxa_through_plots.py script in QIIME. This was performed on both the complete dataset and the subsampled OTU table normalized to 69,000 sequences. This study focused on the metazoan fraction of the eukaryotes, and for purposes of reporting, any metazoan phylum that had an average abundance less than 0.5% throughout the whole OTU table was classified as "Other Metazoa." The reasoning behind creating the "Other Metazoa" category was to reduce the number of very low abundance taxa that would otherwise render the bar graph unreadable. In addition, OTUs classified as fungi or stramenopiles, as well the "no hit" category, were reported separately when discussing the proportion of taxa. All other OTUs were grouped into an "Other Eukaryote" category that included unicellular eukaryotes. Phyla comprising the

"Other Metazoa" and "Other Eukaryote" categories are listed in Appendix Table A2.

Results

Amplicon data

In all, 70 environmental samples were obtained representing five localities, seven time points, and two depth fractions. Five samples had extremely low initial DNA concentrations and therefore had less than 20 ng of input into the amplicon PCR. These included Shellfish Lab (SL) 0–3 cm from September 2011 and from November 2011, Ryan Court (RC) 0–3 cm from November 2011, BelleAir Blvd (BB) 0–3 cm from November 2011 and 3–10 cm from November 2011. All of these samples except BB 3–10 cm from November 2011 had greater than 100,000 sequences once the technical replicates were combined. BB 3–10 cm from November 2011 had only 1757 sequences after combining technical replicates.

The 70 environmental samples, each with two technical replicates, yielded a total of 24,381,423 demultiplexed Illumina reads in each paired-end direction. After preliminary overlapping (2,404,230 reads removed, $<10\%$ of original reads), quality (86 sequences removed, $<0.0004\%$ of overlapped sequences), chimera (5442 sequences removed, $<0.03\%$ of quality checked sequences), and length filtering (770 sequences removed, $<0.004\%$ of sequences after chimera checking), 90% (21,970,895 sequences) of original reads went into the OTU clustering process. Filtered sequences from the MiSeq ranged from 75 to 200 bp with both an average and median length of 138 bp. Sequences from the HiSeq 2500 ranged from 75 to 159 bp with an average of 138.5 bp and a median of 138 bp in length. After filtering, the final OTU table consisted of 98,765 OTUs across all samples. Removal of OTUs that failed to align with PYNAST (1953 OTUs, 1.9% of OTUs, containing 277,816 sequences, 1.3% of sequences) or that were classified as Bacteria or Archaea (656 OTUs, 0.6% of PYNAST filtered OTU table, containing 3164 sequences, 0.015% sequences after failed PYNAST alignment filtered) was limited. Of the 98,765 OTUs, only 7% (6931 OTUs containing 74,860 sequences, $<0.4\%$ of sequences in the final OTU table) were classified as "no hit." The "no hit" OTUs were blasted (blastn, e-value 0.000001) against the NCBI Genbank database, resulting in a majority (5971 OTUs, 86.1% of "no hit" OTUs and 6% of all OTUs in the final table) of reference sequences having no found match. The remaining 960 OTUs matched an rRNA entry in the database, with a majority (644 OTUs, 9.3% of "no hit" OTUs and $<0.7\%$ of all OTUs in the final table) matching an entry less than the initial 90% sequence identity cutoff. The remaining 316 "no hit" OTUs (4.6% of "no hit" OTUs and $<0.4\%$ of all OTUs in the final table) did match an NCBI entry with $\geq 90\%$ sequence identity. Discrepancies could be caused by the version of the SILVA database used (current QIIME compatible version is 111) or the fact that the SILVA database is curated and some NCBI entries were discarded during the curation process. The 70 samples ranged from 1,757 to 814,604 sequences, with a mean of 301,799 sequences.

Rarefaction analyses suggest that species richness (Choa1 and PD) were not saturated at a sampling depth of 300,010 sequences, although evenness (Shannon diversity) leveled off at greater than 30,000 sequences per sample (Appendix Fig. A2). Four samples (BP 3–10 cm on July 2011, November 2011, and July 2012 as well as BB 3–10 cm on November 2011) were below the subsampling threshold of 69,000 sequences and therefore were removed from further analyses.

Community composition

Taxonomic assignments did not differ greatly between the OTUs based on the complete and subsampled datasets. These two datasets differed only by, at most, 0.3% for a given taxonomic grouping. Therefore, presentation of community taxonomic composition is based on the complete dataset. However, since the number of sequences in each OTU could vary depending on the sequence depth, the subsampled OTU table was used for all alpha- and beta-diversity measurements.

Communities examined were predominantly composed of metazoan taxa, and fungal OTUs were nonexistent or present in very low abundance (Fig. 2). A majority (94%) of communities had 72%–99% metazoan composition, while 6% (4 samples) contained 40%–63% metazoans. Annelids, arthropods, nematodes, and platyhelminths were the most abundant taxa throughout the samples (Fig. 2). Sample locations, however, differed in dominant taxa present. Locations were heterogeneous in amount of community variation observed over the year sampled. For example, RC and BB varied little in community composition in comparison to SL and BP (Fig. 2). Depth fractions (0–3 cm and 3–10 cm) differed in composition, and this difference varied temporally and spatially (Fig. 2). When comparing community composition between July 2011 and July 2012, some of the locations showed differences in taxa composition between years (Fig. 2), suggesting potential for yearly variation within the composition of these communities as well; however, additional data are required to test the yearly variation in these communities.

Alpha- and beta-diversity

For the three alpha-diversity measures, sample locations showed limited variation in alpha-diversity over the year sampled, except for SL (Appendix Fig. A3). Thus, even though community composition was variable in reference to the taxa present at some locations, the overall number of species present stayed relatively consistent.

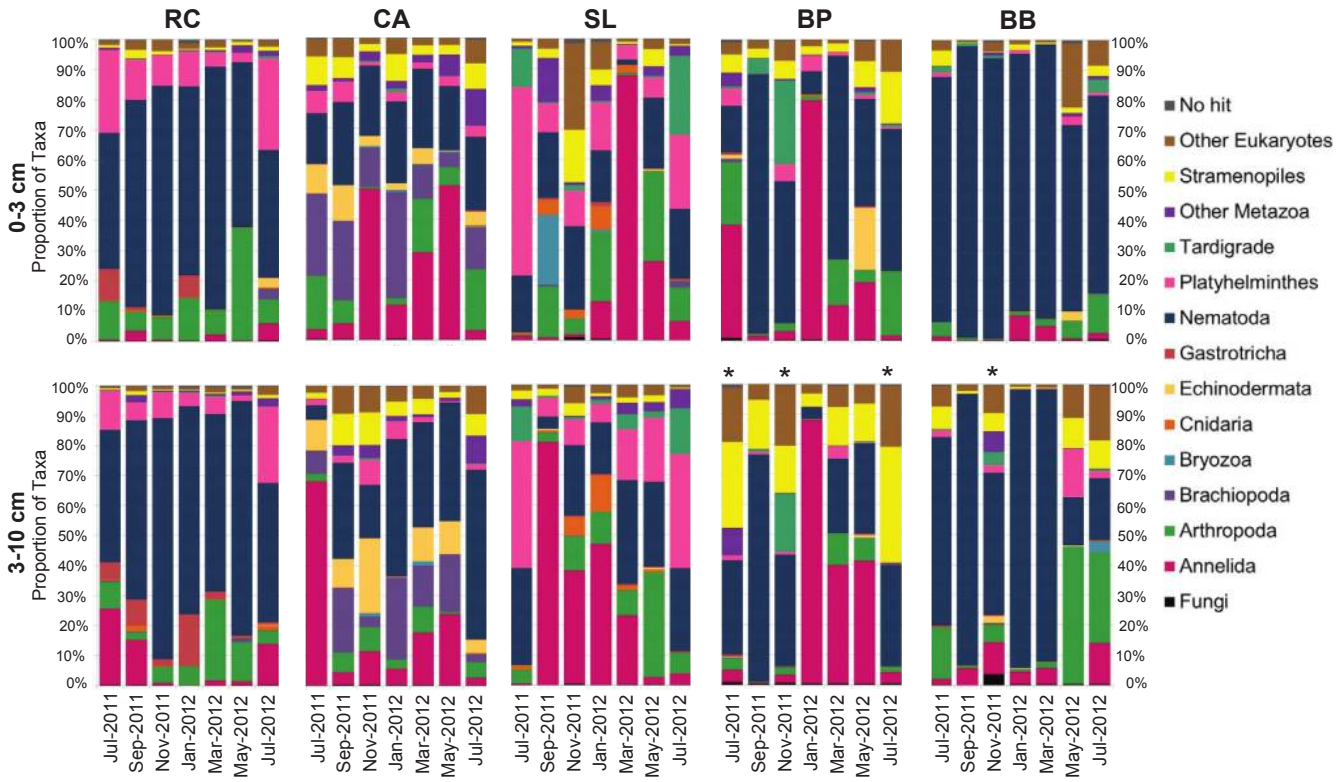

Figure 2. Community composition of dominant microscopic eukaryotic taxa at two sediment depth fractions (0–3 cm top panel and 3–10 cm lower panel) collected bimonthly from July 2011 to July 2012 at five Mobile Bay and Dauphin Island, Alabama, locations. Location abbreviations are defined in Fig. 1 legend and Appendix Table A1. Asterisks (*) indicate four samples that had below 69,000 sequences and were dropped from the diversity analyses.

Jackknifed UPGMA hierarchical clustering analysis (Fig. 3) and PCoA analysis (Fig. 4) revealed that samples showed separation by collection site. This clustering by site was apparent in all three beta-diversity matrices (Fig. 4), and Analysis of Similarity (ANOSIM) showed significant groupings of the beta-diversity of communities in reference to collection site (Table 1). However, the weighted unifrac showed a lower R-statistic in comparison to Jaccard or Bray-Curtis metrics (Table 1). When samples were grouped by location type, bay side (CA, BP, and BB) or Gulf side (RC and SL), there was a significant difference in the diversity of communities, and the finding was consistent for all three beta-diversity measures (Table 1). Samples did not appear to cluster by depth fraction or season (Figs. 3 and 4). ANOSIM results indicated that sample variation in relation to depth or season was significantly different from random (Table 1). Results for the Jaccard beta-diversity for season showed a significant P value ($P = 0.011$); however, the R-statistic was close to zero ($R = 0.0712$), indicating the group was not quite random. In addition, similar to the proportion of taxa breakdown, the two July sampling time points for a given location did not cluster together (Figs. 3 and 4), indicating potential yearly variation in community composition.

Discussion

Illumina amplicon sequencing of the eukaryotic-specific hypervariable V9 SSU rRNA gene region proved useful in examining the community composition in intertidal meiofauna locations from Mobile Bay and Dauphin Island, Alabama. The high-throughput sequencing approach incorporated in this study showed variability in community composition over both spatial and temporal scales. Even though the number of species did not vary greatly throughout the data (alpha-diversity, Appendix Fig. A3), there was variability in which taxa were present. For example, during the September 2011 sampling of SL we noted an abundance of bryozoan biomass floating in the surf as well as washed up on the shore (Brannock, pers. obs.). In the community composition breakdown for SL 0–3 cm (Fig. 2) there was a bryozoan signature not found in other samples.

Overall results showed that annelids, arthropods, nematodes, and platyhelminthes were the most abundant taxa throughout the dataset; however, community composition

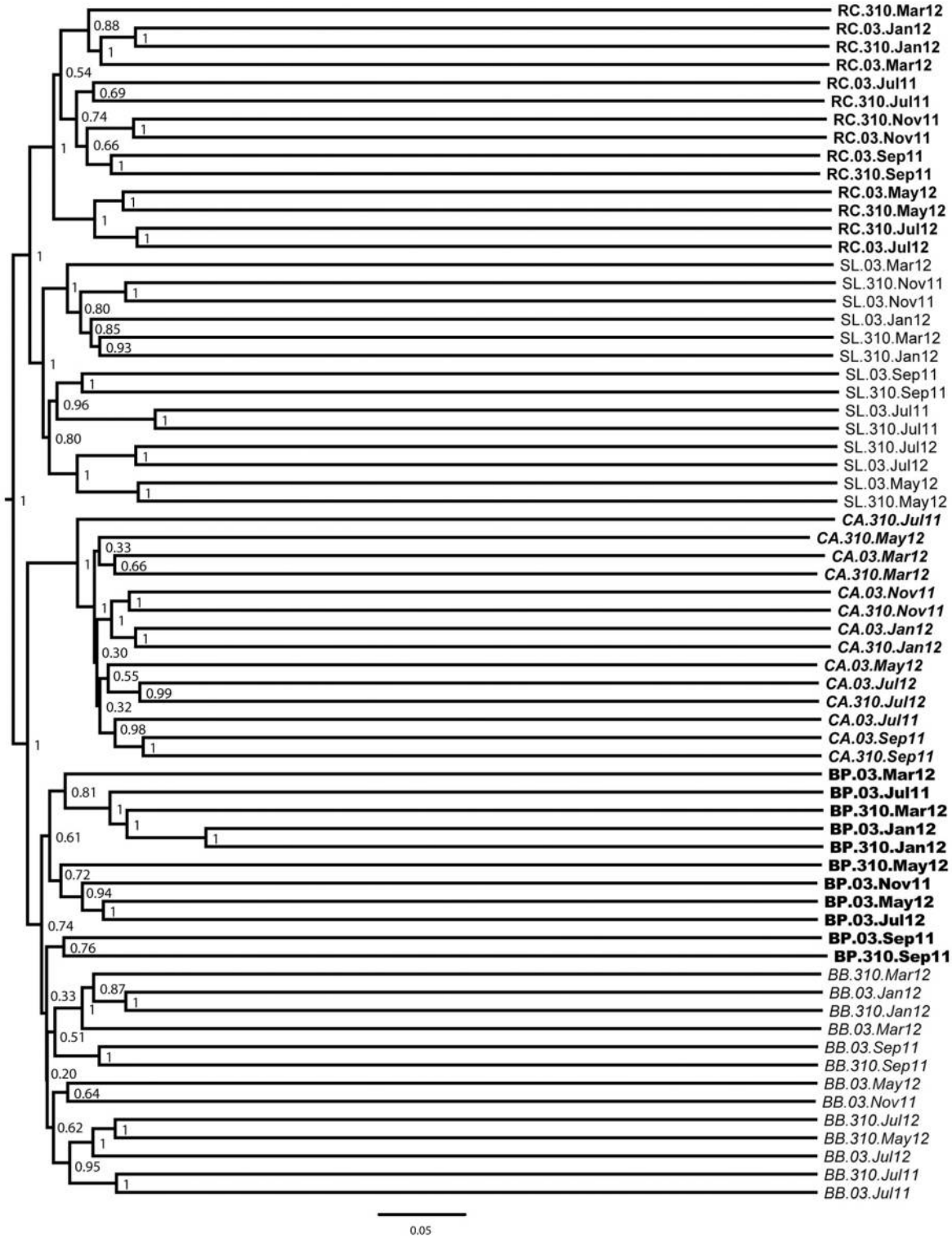

Figure 3. Jackknifed unweighted pair group method with arithmetic mean (UPGMA) hierarchical clustering of the binary Jaccard distance metric calculated for each sample from the subsampled dataset. The operational taxonomic unit (OTU) table was subsampled for 100 replications to get representative support. Samples are named by sampled location (defined in Fig. 1 legend and Appendix Table A1), followed by depth fraction (0–3 cm or 3–10 cm), month, and year sampled. Different sample locations have different fonts for the branch tips. Percentage near the node represents nodal support. Jackknifed UPGMA for Bray-Curtis and weighted Unifrac are not presented but show similar results of samples clustering by location.

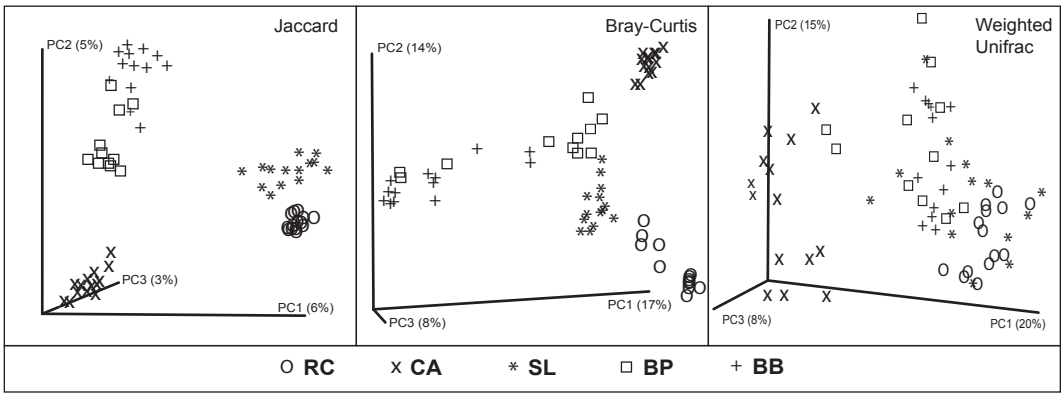

Figure 4. Jackknifed principal coordinate analysis (PCoA) of the binary Jaccard (left), Bray-Curtis (middle), and weighted UniFrac (right) distance matrices calculated for each sample from the subsampled dataset. The operational taxonomic unit (OTU) table was subsampled for 100 replications to get representative support. O = RC, X = CA, * = SL, □ = BP, and + = BB. Location abbreviations are defined in Fig. 1 legend and Appendix Table A1.

showed spatial and temporal variation throughout the year (Fig. 2). RC and BB were heavily dominated by nematodes year round as well as in both depth fractions, and variation in community composition between sampling dates was low in comparison to SL (Fig. 2). Community composition was highly variable throughout the year at SL, especially for the 0–3-cm fraction, where from September 2011 through May 2012 community composition fluctuated greatly between several metazoan taxa (Fig. 2). On the other hand, the SL 3–10-cm fraction gradually progressed from a predominately annelid composition to a community composition of arthropod, nematode, and playhelminthes taxa during the same time frame. The CA 0–3-cm fraction appeared to have a fairly consistent nematode taxa proportion throughout the year, but varied in the amount of annelid, arthropod, brachiopod, and echinoderm taxa present. The 3–10-cm fraction showed variation in the proportions of major taxa present (annelids, arthropods, brachiopods, and echinoderms) and had a high nematode proportion except for July 2011, which was dominated by annelids. The BP 3–10-cm fraction consisted mainly of nematode and stramenopile taxa up to January 2012, at which time it drastically shifted to a predominately annelid community. After January 2012, annelid taxa decreased in this depth fraction, and the community returned to mainly nematode and stramenopile composition by July 2012. That same fluxuation in annelid taxa in January 2012 was present in the 0–3-cm fraction as well. Previous studies have shown meiofauna vary seasonally (Harris, 1972; Herman and Heip, 1983; Coull, 1985) and yearly (Harris, 1972; Herman and Heip, 1983; Coull, 1985; Pequegnat *et al.*, 1990) in both species densities (Harris, 1972; Herman and Heip, 1983; Coull, 1985; Pequegnat *et al.*, 1990) and vertical distribution (Harris, 1972); however, the degree of variation observed was species-dependent (Harris, 1972; Herman and Heip, 1983; Coull, 1985).

Community composition varied by location, as shown by samples clustered mainly by collection site (Figs. 3 and 4, Table 1). Likewise, samples collected from bay and gulf locations clustered separately from each other (Fig. 3 and Table 1). This suggests that location rather than season or

Table 1

Analysis of Similarity (ANOSIM): resulting ANOSIM R-statistic and* P *value for all three beta-diversity distance matrices†*

	Bray-Curtis		Jaccard		Weighted Unifrac	
	R-statistic	*P*	R-statistic	*P*	R-statistic	*P*
Location Type	0.6422	0.001	0.7219	0.001	0.2777	0.001
Depth	0.0133	0.204	0.0241	0.102	−0.0142	0.846
Season	0.0212	0.185	0.0712	0.011	0.0050	0.406
Site	0.8129	0.001	0.8547	0.001	0.4817	0.001

* An R-statistic of 0 represents complete random grouping, a value of +1 indicates samples are most similar within the same group, and a value of −1 indicates samples most similar outside of the group.

† For each distance matrix, four separate comparisons were made to determine whether significant differences were seen between groups.

depth potentially plays a larger role in community composition. These groupings were more apparent in Jaccard analyses, but were also seen in both the Bray-Curtis and weighted Unifrac analyses (Fig. 4 and Table 1), suggesting that locations may have different operational taxonomic units (OTUs) present in their communities; however, when comparing raw and relative abundances of taxa, the differences become less apparent. A corresponding morphological nematode study shows similar clustering of samples by location rather than season (Sharma, unpubl. data). Sediment composition, location characteristics, environmental variables, or surrounding vegetation could all be contributing to the differences in community composition observed. Differences in sediment size, type, and structure have been noted to influence meiofaunal community composition (Coull, 1988; Giere, 2009). Sediment analysis at these five locations using Bik *et al.*'s (2012a) samples as well as a March 2011 (H. Bik, University of Birmingham, UK; unpubl. data) time point shows differences in grain size, sorting ability, and amount of organic carbon (Williams, 2013). Locations and sample dates both show these differences (Williams, 2013). Likewise, Coull (1985) noted meiofauna in a muddy location had higher abundance variation in comparison to a more sandy location. More recently, Baguley *et al*. (2006) reported an increase in meiofauna abundance with increase in silt concentration. RC and SL are both more sandy coastal locations, while CA, BP, and BB are more protected bay locations that have higher silt content than RC and SL (Brannock, pers. obs.). Sediment composition and properties should be incorporated into future studies to examine the influence on community composition further. In addition, environmental factors such as temperature and salinity have also been reported as playing a role in the composition of meiofauna communities (Coull, 1985, 1999; Moens *et al.*, 2013). Coull (1985) reported that salinity was correlated with meiofaunal abundance in the muddy location, while temperature was correlated with the sandy locations. During the current study, RC consistently had the highest salinity, whereas both BP and BB had relatively lower salinities in comparison to the other locations (Appendix Table A1). Temperature, on the other hand, showed less variation between the sample locations at a given time point (Appendix Table A1). Although environmental measurements were obtained only during sample collection, our limited number of time points for specific sampling localities precluded the ability to meaningfully correlate salinity and temperature data to variations observed in OTU data. To have a better understanding of the influences of these variables on the community composition, more regular measurements need to be obtained to grasp the daily and monthly fluctuations.

For Gulf of Mexico (GOM) meiofaunal localities sampled here, by July 2011 communities were similar to pre-spill communities (fig. 1 in Bik *et al*., 2012a) in that metazoan taxa dominated the composition (Fig. 2). In contrast, samples in September 2010 were dominated by fungal species (Bik *et al*., 2012a). By July 2011, fungal OTUs were not detectable or were present in extremely low abundances (Fig. 2), suggesting that the drastic shift in community composition that Bik *et al.* (2012a) reported as a potential impact of the *Deepwater Horizon* oil spill (DWHOS) was an acute phenomenon. Even though fungal impact on these sites might have been short term, communities could be experiencing prolonged impacts from the DWHOS disturbance. When combining the two depth fractions (0–3 cm and 3–10 cm) in this study for the May 2012 time point (Fig. 5) and comparing the taxa composition to those reported in Bik *et al.* (2012a) for May 2010, community compositions differ between the two years. One of the most notable differences is that the RC May 2012 community composition of predominantly nematode and arthropod taxa (Fig. 5) resembled the post-spill community in Bik *et al.* (2012a, Fig. 1) more closely than it resembled the May 2010 composition of predominately annelid taxa.

There were methodological differences between Bik *et al*. (2012a) and the current study that could also potentially explain the observed variation in community compositions between the two studies. First, Bik *et al.* (2012a) used a smaller amount (200 μl) of decanted material in their DNA extractions and used a different extraction kit. We attempted to use 200 μl of decanted material in our extractions at first with little success in DNA yield. Therefore, we increased the amount of initial extraction material. However, when using a larger amount of starting material one would not expect to find taxa absent among the fungi. In addition, the 18S gene region targeted differed between the two studies. Bik *et al.* (2012a) used primer pairs that targeted a larger fragment (~450 bp) toward the beginning of the 18S gene, while we targeted a smaller fragment (87–187 bp, Amaral-Zettler *et al.*, 2009) toward the end of the 18S gene. Primers used in the current study were chosen because at the time of the experiment the Illumina platform was unable to sequence through the full fragment used in Bik *et al.* (2012a).

Determining whether the *Deepwater Horizon* oil spill (DWHOS) may have a long-term impact on meiofaunal communities is difficult because knowledge of seasonality and variability in these communities is still limited. The current study did illustrate seasonal and yearly variation in community composition at the sample locations. However, whether this variation is normal or indicative of a community re-stabilizing after the DWHOS disturbance is unknown. Continued sampling over a longer timescale as well as the inclusion of environmental parameters is necessary to comprehend the drivers in variation observed in these communities.

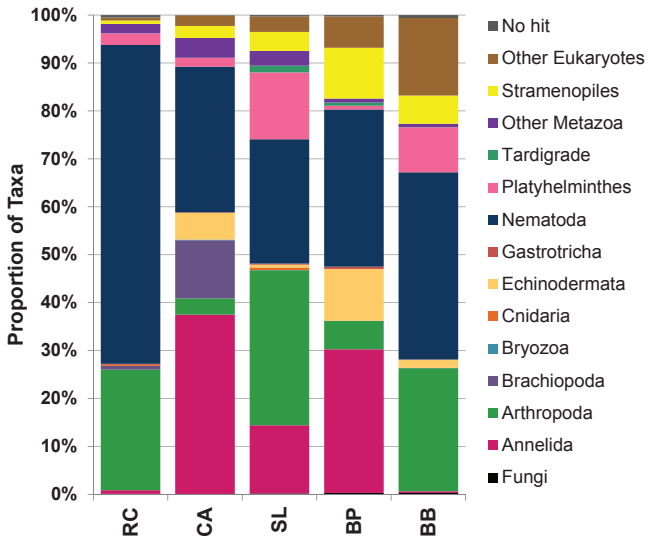

Figure 5. Community composition for combined 0–3 cm and 3–10 cm for each sample location for May 2012. Location abbreviations are defined in Fig. 1 legend and Appendix Table A1.

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Appendix

Table A1

The Mobile Bay and Dauphin Island, Alabama, sample locations with the corresponding abbreviations and GPS coordinates as well as salinity and water temperature measurements taken at the time of sampling (image of the sample locations can be found in Fig. 1)

		Ryan Court	Cadillac Ave	Shellfish Lab	Bayfront Park	BelleAir Blvd
Abbreviation		RC	CA	SL	BP	BB
Latitude		30.2500N	30.2532N	30.2465N	30.3538N	30.5080N
Longitude		88.1462W	88.1363W	88.0784W	88.1179W	88.1016W
Salinity (ppt)	Jul 2011	29.0	20.0	22.0	17.0	10.0
	Sep 2011	25.0	20.0	20.0	10.0	7.0
	Nov 2011	30.0	28.0	28.0	20.0	15.0
	Jan 2012	24.0	19.0	10.0	8.0	5.0
	Mar 2012	32.0	24.5	27.0	5.0	4.0
	May 2012	27.0	17.0	23.0	14.0	11.0
	Jul 2012	29.0	24.0	23.0	18.0	11.0
Water Temperature (°C)	Jul 2011	N/A	N/A	N/A	N/A	N/A
	Sep 2011	30.8	30.8	28.3	29.4	26.0
	Nov 2011	N/A	N/A	N/A	N/A	N/A
	Jan 2012	18.6	20.4	19.8	22.6	21.4
	Mar 2012	22.2	23.2	22.7	23.4	23.9
	May 2012	29.3	27.4	29.2	29.5	28.2
	Jul 2012	31.8	32.5	33.1	35.0	33.7

N/A, not available.

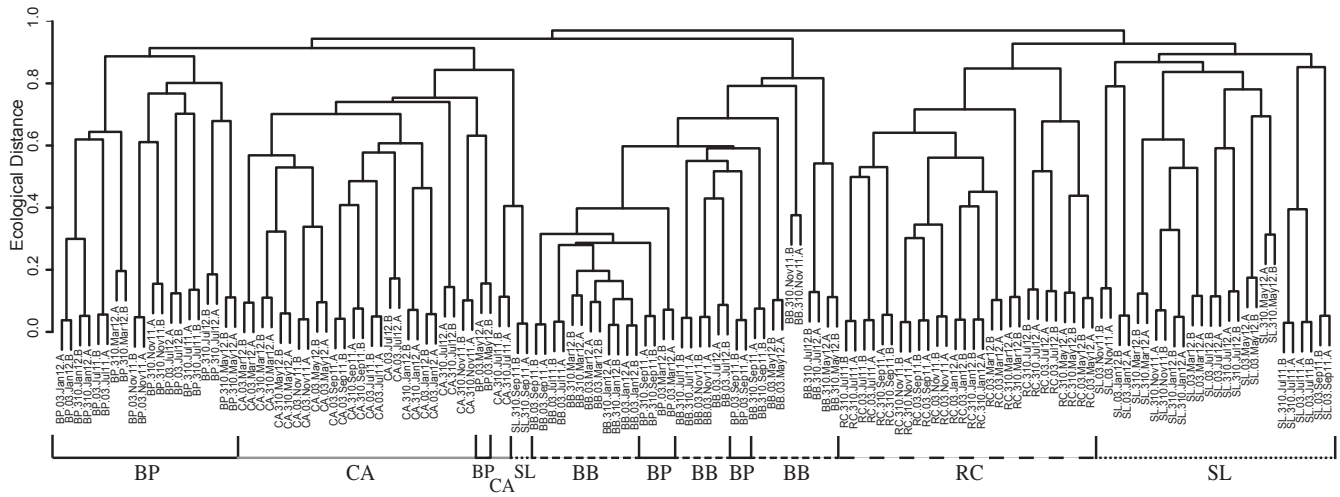

Figure A1. Hierarchical clustering analysis on all 140 samples employing an average linkage agglomerative clustering algorithm. The distance matrix was calculated using Bray-Curtis dissimilarity method. Samples are named by sampled location (defined in Fig. 1 legend and Appendix Table A1), followed by depth fraction (0–3 cm or 3–10 cm), month, and year sampled.

Table A2

List of phyla comprising either the "Other Metazoa" or the "Other Eukaryotes" groupings in Figs. 2 and 5

Other Metazoa Phyla	Other Eukaryote Phyla
Chaetognatha	Ciliophora
Ctenophora	Other Alvelata
Entoprocta	Amoebozoa
Hemichordata	Cryptophyta
Loricifera	Loukozoa
Gnathifera	Glaucophyta
Onochophora	Choanozoa
Porifera	Picozoa
Rotifera	Foraminifera
Xenoturbeiidea	Rhodophyta
Acanthocephala	Myzozoa
Chordata	Apusozoa
Cycliophora	Heliozoa
Gnathostomulida	Percolozoa
Kinohycha	Euglenozoa
Mesozoa	Haptophyta
Nematomorpha	Metamunada
Placozoa	Cercozoa
Priapulida	Radiozoa
Sipuncula	Uncultured unicellular Eukaryote
Uncultured Metazoa	

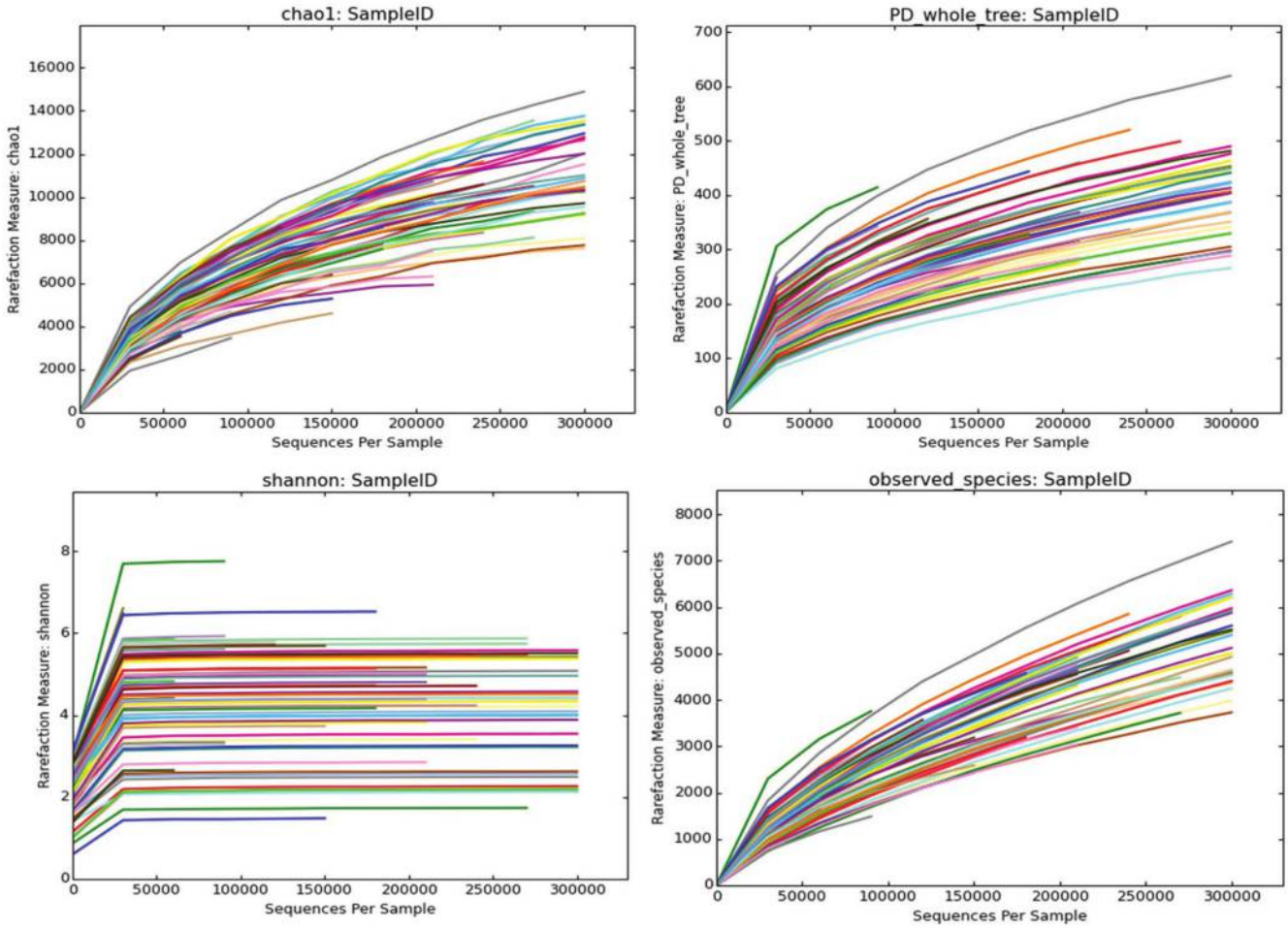

Figure A2. Rarefaction curves for the 140 samples from $n = 10$ sequences to $n = 300{,}010$ sequences with a step size of 30,000 sequences. Included are the three alpha-diversity measurements (Chao1, phylogenetic distance, and Shannon's diversity) as well as the observed number of species plotted against the number of sequences. Each line in the plots represents one of the 140 samples.

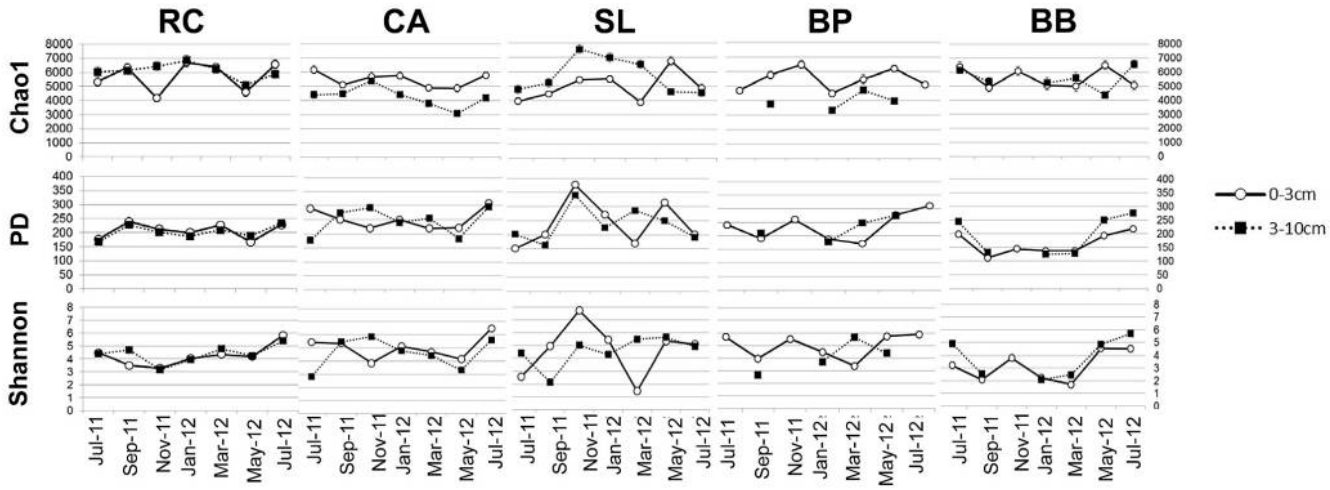

Figure A3. Alpha-diversity measurements (Chao1, phylogenetic distance: PD, and Shannon's diversity) for each sampled location, at each depth, for each time point. ○ = 0–3-cm and ■ = 3–10-cm depth fraction. Location abbreviations are defined in Fig. 1 legend and Appendix Table A1.