A DIVERSITY BASELINE OF BENTHIC MACROFAUNA ALONG THE NORTHWESTERN INSULAR SLOPE OF CUBA (GULF OF MEXICO)

A Thesis

by

SAMANTHA SCHIERECK

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This thesis meets the standards for scope and quality of
Texas A&M University-Corpus Christi and is hereby approved.

Paul Montagna, PhD
Chair

Jennifer Pollack, PhD
Committee Member

Patrick Schwing, PhD
Committee Member

December 2023

ABSTRACT

The Gulf of Mexico (GoM) is a unique ecosystem due to the physical characteristics influenced primarily by the Mississippi River in the North and the Loop Current, which originates in the south, resulting in a gradient of organic to carbonate sediment composition. The continental slope of the northern (US) and southwestern (Mexico) portions of the GoM are generally well studied; however, very little is known about the southeastern GoM along the slope of Cuba. To fill this knowledge gap, sediment cores were collected in 2017 at nine stations (974–1580 m depth) to gather baseline data and determine controls on the deep-sea benthic macrofauna community. Oceanographic data indicated a stratified water column typical of an oligotrophic ocean and no evidence of hypoxia. Sediment texture and composition indicated a west-east gradient likely determined by downslope transport of terrigenous material in the eastern part with a high proportion of carbonate in the west. Heavy metals (Cu, Hg, Pb, and Zn) at concentrations known to cause benthic effects were present in the east near the major city of Havana, with the macrofauna community showing characteristics indicative of environmental stress. Stations had a low overall average diversity (15 families/79 cm2) and abundance (7,980/ m2), with high variability among replicates within the stations. The diversity was 48% less, and the abundance was 14% less than in the northern GoM. The major factors influencing macrofauna communities in the continental slope off northwestern Cuba are most likely the lack of organically rich sediment, low sediment deposition rates, and the strong current.

ACKNOWLEDGEMENTS

Sediment samples were collected aboard R/V Weatherbird II cruise WB-0517 in the Gulf of Mexico from 2017-05-11 to 2017-05-23. The cruise was a collaborative effort between the University of South Florida, College of Marine Science, the Florida Institute of Oceanography, Eckerd College, the University of Havana, Centro de Investigaciones Marinas, and the Harte Research Institute, Texas A&M University-Corpus Christi. Steven Murawski and David Hollander were co-Chief Scientists. Much of the cruise effort was funded by a grant to the Center for Integrated Modeling and Analysis of Gulf Ecosystems (C-IMAGE: http://www.marine.usf.edu/c-image/) from the Gulf of Mexico Research Initiative (GOMRI). Taxonomic expertise was provided by Rick Kalke and Larry Hyde.

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1. INTRODUCTION

The Gulf of Mexico (GoM) is an oligotrophic, semi-enclosed sea with diverse, interconnected ecosystems varying across temporal and spatial gradients (Sutton et al., 2020). It is unique compared to other oceans due to the physical characteristics primarily being influenced by the Mississippi river in the north and the Loop Current (LC) originating in the south through the Yucatan Straits carrying nutrient poor water from the Caribbean Sea (Rowe & Kennicutt, 2008). Reflective of this oceanographic setting, the GoM deep benthos is a heterogenous environment with variation in sediment composition, nutrient levels, and biological diversity. In the southern GoM the Yucatan Shelf, Florida shelf, and Cuban shelf are carbonate platforms (Davis, 2017; Balsam & Beeson, 2003). These carbonate platforms contribute to a gradient in sediment composition with the north GoM represented primarily by terrigenous sediment from the Mississippi River (Balsam & Beeson, 2003; Davis, 2017). The deep-sea is a cold and food limited environment with low rates of sediment deposition and low metabolic rates of the organisms living there (Gage & Tyler, 1991; Rowe et al., 2008). As food availability is determined by surface fluxes (Baguley et al., 2006; Fisher et al., 2016; Rowe, 2007) and impacted by sediment pulses along with other geological, physical, and geochemical processes, the deep-sea is especially vulnerable to pollution (Danovaro et al., 2008). The Loop Current influences many processes within the GoM along with the associated eddies causing upwelling and downwelling events, which leads to temporal and spatial variability even at a relatively small scale. Towards the southeast the Loop Current turns into the Florida Current and eventually becomes the Gulf Stream connecting the GoM to the Atlantic Ocean through the Florida Straits between Florida and Cuba. This area of high interconnectivity has been identified as a high impact spot if an oil spill were to occur here having the potential to cause significant loss in biodiversity and far-reaching consequences (Androulidakis et al., 2020).

The deep-sea benthos perform numerous ecosystem functions including nutrient cycling, carbon storage and sequestering, oil bioremediation, as well as being important food sources for many commercially and environmentally important species (Armstrong et al., 2012; Danovaro et al., 2008). Benthic macroinvertebrates are important bioindicators because they tend to have limited mobility and live long enough to be reflective of longer-term changes as opposed to seasonal or daily fluctuations (Gage & Tyler, 1991). This means that the community composition is a good indicator for overall ecosystem health. Additionally changes in community composition are a good indicator of environmental change since they cannot leave the area and are affected by deposition meaning they are reflective of the local conditions. They were identified as key taxa for estimating impact and recovery as the more sensitive species tend to disappear and the opportunistic, tolerant species increase in abundance (Montagna et al., 2013; Washburn et al., 2016). Therefore, understanding the communities and the processes influencing community composition is important for conservation efforts and risk assessments with the increasing amount of deep-sea exploration for oil and natural gas (Murawski et al., 2020; Schwing et al., 2020a). As the diversity and abundance can be affected by a variety of stressors, including long-term exposure to pollutants and heavy metals, changing environmental conditions, and other anthropogenically driven disturbances baseline data is useful for untangling the multitude of stressors to identify impacts associated with specific events such as an oil spill (Schwing et al., 2020b).

The 2010 Deepwater Horizon oil spill that occurred in the northern GoM had catastrophic effects on benthic communities (Fisher et al., 2016; Montagna et al., 2013; Reuscher et al., 2020), which highlighted the need for baseline data on deep-sea communities to be better prepared in case of future oil spills and other large-scale disturbances. The GoM is an important source of oil and natural gas and all three countries bordering it are exploring deep-sea sources for oil and natural gas wells within their Exclusive Economic Zone's (EEZ) which means there is an increasing likelihood of future oil spills to occur (Murawski et al., 2020). There have been data collected for the northern GoM and southwestern GoM on deep-sea benthic communities as well as sediment characteristics and other abiotic factors associated with them (Rowe & Kennicutt, 2008; reviewed by Wei et al., 2012; Carvalho et al., 2013; Montagna & Girard, 2020; Schwing et al., 2020b; Quintanar-Retama et al., 2023). The southern GoM has lower diversity compared to the northern GoM, which is subject to higher terrigenous input due to the Mississippi River (Schwing et al., 2020a). The lower diversity in the southern GoM may also be reflective of the nutrient poor Caribbean Sea inflow at the start of the Loop Current.

The northwestern portion of Cuba is the land boundary for the southeastern Gulf of Mexico with the Florida Straits on the eastern portion of Cuba connecting the Gulf to the Atlantic Ocean and the Yucatan Straits to the west as the connection to the Caribbean Sea. This region of the GoM is characterized by a narrow shelf and steep slope at an active margin setting influenced by tectonic processes and subject to the strong Florida Current flowing across the shelf from the west leaving it essentially devoid of sediments and instead having coarse, carbonate rubble (Brooks et al., 2019; Armenteros et al., 2020). The western tip of Cuba holds the Gulf of Guanahacabibes, which contains multiple Marine Protected Areas hosting abundant coral reef crests and patches, multispecies spawning aggregates and high biodiversity. It is also difficult to access due to the strong currents and has therefore stayed relatively pristine (Brooks et al., 2019; Le Hénaff et al., 2020). The eastern portion has had stronger anthropogenic influence due to the coastal development in Havana and the presence of a large port. Sediment samples showed a west-east gradient with carbonate and calcareous mud being the predominant components (Armenteros et al., 2020; Brooks et al., 2019). The LC is the dominant circulating feature affecting GoM waters off of Cuba as well as the mesoscale eddies, both cyclone and anticyclone, that make the hydrological and physical processes vary depending on the meandering of the LC and other factors associated with it (Le Hénaff et al., 2020).

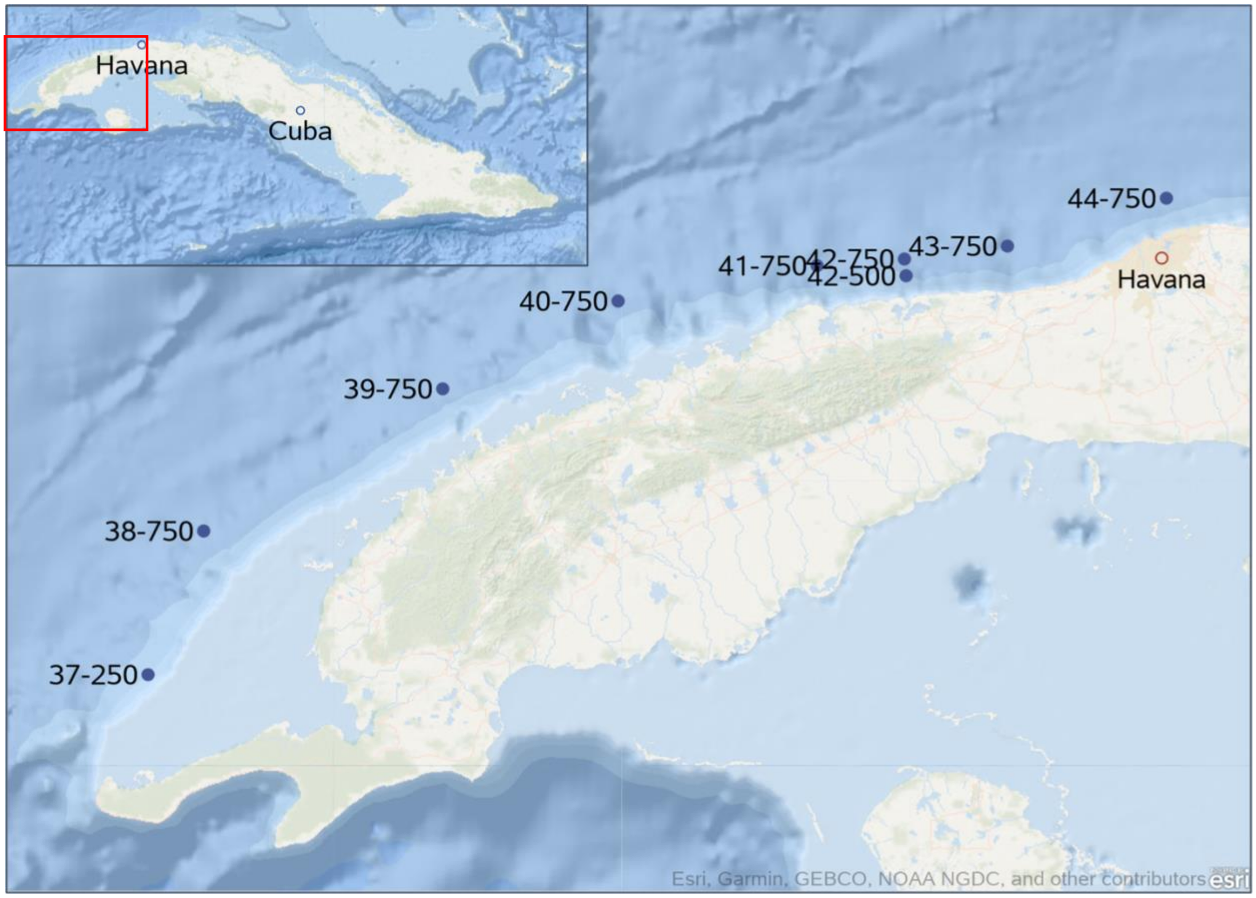
Due to geopolitical limitations, there is very little publicly available information on the southeastern GoM off the coast of Cuba. Much of what is available came from a limited number of research cruises that occurred after a joint statement between the USA and Cuba was signed in 2014 for cooperation on environmental protection to better inform policies related to the marine environment shared by both countries (see reference for access to statement: U.S. Department of State, 2015). The goals of this statement were to set plans and policies for the prevention of future oil spills as well as preparedness for future occurrences to aid in the response and recovery. To be better prepared in the case of future natural environmental disasters (i.e., hurricanes) or anthropogenic events (oil spills), baseline data are necessary for the southeastern GoM. Therefore, in this thesis I will be analyzing sediment cores retrieved from the NW slope of Cuba in the southeastern GoM for the benthic macrofauna and calculating diversity and abundance metrics. These values will then be compared to the environmental data, taken on the same cruise, as well as previous data reported from the northern GoM in order to identify processes impacting the community composition of deep-sea benthic macrofauna. Due to the reversal of the joint agreement, the data retrieved from these cores is an important look at the macrobenthic communities in this unique region where very little is known for, and sampling is not currently possible.

2. METHODS

2.1 Sample Collection

Samples were collected during a research cruise in May of 2017 aboard the RV Weatherbird II as part of the Center for Integrated Modeling and Analysis of Gulf Ecosystems (C-IMAGE: http://www.marine.usf.edu/c-image/) program, which was funded by the Gulf of Mexico Research Initiative (GoMRI). Data taken during this cruise has already been published for the abiotic factors collected during the cruise and is accessible from the GRIIDC data archive (Schwing, 2019), along with photos of sediment cores (Brooks & Larsen, 2019a), sediment texture and composition (Brooks & Larsen, 2019b), and pore water content (Brooks & Larsen, 2019c). Mollusk and meiofauna data (Armenteros, 2019), and trace metal data have been reported (Hastings et al., 2019). The macrofauna data used here is also publicly available and can be accessed from the GRIIDC data archive (Montagna & Schiereck, 2023).

Originally the plan was for 13 stations along 8 transects (37 - 44) representing nominal depths of 300 m, 500 m, 1000 m, and 1500 m; however due to the steep slope of the margin and lack of sediment deposits, cores were only able to be retrieved from the deeper sites (1000 m and 1500 m) and only station 42 has data for both depths. Actual depths range from 1000 m at 42-500 to 1670 at station 38-750. Due to the lack of depth profile among stations the effect of depth was not able to be investigated; however, the environmental data and macrofauna data were not always able to be calculated at the same depths for every transect therefore all station names correspond to the transect and nominal depth in fathoms (Fig. 1).



Esri, Garmin, GEBCO, NOAA NGDC, and other contributors

Figure 1. Map of station locations along the northwestern shelf of Cuba.

Sediment cores were collected using a multicorer device, which takes 12 cores simultaneously with a diameter of 11cm and an inner area of 79 cm^2. The 12 cores are then assigned to different analyses (such as sediment composition and texture, trace metals, meiofauna, foraminifera, etc.) in order to gather information on the environmental conditions and the biotic communities for each station. When possible 3 of the cores are assigned for macrofauna as replicates. If not all 12 cores are able to be retrieved cores were assigned to different analyses in such a way as to gain the most information possible for this region. For stations 37-500 and 42-500 only 2 replicates were able to be retrieved. These core samples were separated into 3 different vertical layers (0-3 cm, 3-5 cm, and 5-10 cm) allowing for the assessment of vertical profiles. Samples were preserved with 10% buffered formalin and Rose Bengal was added to aid in sorting individuals.

2.2 Sample Processing

During the sorting process, Rose Bengal, which stains protein, allows us to differentiate between the organisms that were alive in the benthic zone and the calcium carbonate shells of pelagic macrofauna deposited on the bottom. No living material was found in the calcium carbonate shells. The macrofauna are defined as organisms retained on a 0.3 mm sieve during extraction, which is comparable to previous studies on deep-sea macrofauna (Montagna et al., 2013; Reuscher et al., 2020). The benthic macrofauna isolated from these layers were sorted to the level of family for Polychaetes, Mollusks, Crustaceans, and Cnidaria, class for Oligochaetes and phylum for all others or to the lowest taxonomic group possible. Using families has been shown to be effective during the DWH assessment (Montagna et al., 2013; Reuscher et al., 2020) especially considering many deep sea macrofauna have not been identified at the species level. Organisms were then stored in 70% ethanol after extraction and separated by taxa and sample for storage.

2.3 Statistical Analysis

2.3.1 Univariate Analysis of Benthic Communities

Diversity was calculated using Hill's number one diversity index (N1) (Hill, 1973) and Pielou's (1975) evenness index (J'). Hill's N1 indicates the number of dominant species in a sample while Pielou's evenness index indicates the degree to which all species in a sample are equally abundant. Species richness (S), which is the number of species found in a sample, and Abundance (N), number of individuals per core, is also reported along with the density, which is calculated using the abundance per sediment volume collected and extrapolating to give individuals per meter squared (n/m^2). A plot with k-Dominance curves was created to show the degree of dominance seen in each sample (Lambshead et al., 1983). Diversity metrics are shown for each sample as well as the pooled data from all samples. The abundance and species richness for the 3 sections was also calculated and reported. Table A1 in the appendix shows the species richness, total abundance, average species richness, and average abundance and degree of overlap for the pooled replicates from each station.

2.3.2 Multivariate Analyses of Benthic Communities

Primer-7 software was used to preform nonparametric multidimensional scaling (nMDS) (Clarke & Gorley, 2001) to examine community structure (Clarke & Warwick, 2001). Vertical sections were pooled for each core then compared using a Bray-Curtis similarity matrix and then plotted onto an ordination plot. The SIMPROF procedure in Primer was used to determine significant clusters at a 5 % significance level. The Primer-7 ANOSIM procedure was used to determine if the differences in community composition between stations was significant compared to the differences between the replicates (Clarke, 1993).

2.3.3 Linking Environmental Data and Macrofauna Communities

Sediment data were previously analyzed by laboratories at Eckerd College and the University of South Florida and is available on the GRIIDC data server along with a description of the methods used for each analysis. Photographs were taken of each sediment core (Brooks & Larson, 2019a). Sediment composition and texture was reported as % Sand, % Silt, % Clay, % Gravel, and % Mud (% Silt + % Clay) for texture and % carbonate, % TOM (total organic matter), and % other (non-carbonate, non-organic) (Brooks & Larson, 2019b). Sediment density was represented by % Pore Water and Bulk Density (g/cm^3) (Brooks & Larson, 2019c). The metals analyzed were Vanadium (V), Chromium (Cr), Manganese (Mn), Iron (Fe), Zinc (Zn), Strontium (Sr), Molybdenum (Mo), Barium (Ba), Rhenium (Re), Mercury (Hg), Lead (Pb), Uranium (U), Cadmium (Cd), and Nickel (Ni) (Hastings et al., 2019). Vertical slices were taken with values measured for each slice. For our purposes the average value for the first 10 cm was used as that is comparable to the macrofauna sediment volume. Not all of the stations had both environmental and macrofauna samples. All of the available data were used, and correlations are reported for stations where both macrofauna and environmental data was collected. There was no metal data for Station 38-750 at any depth and Zn, Pb, and Hg were not reported for Station 39-750. Station 37-250, from a depth of 530 m, contains all available environmental data and Station 37-500, from a depth of 1209 m, contains the macrofauna data. Station 43-500 has metal data from a depth of 1000 m while all other environmental and macrofauna samples came from Station 43-750 with a depth of 1535 m. Station 44 has environmental data for 3 depths: 44-150 (316 m), 44-500 (970 m), and 44-750 (1475 m), and macrofauna data for 44-750.

The Principal Components Analysis (PCA) was used to reduce the intercorrelated abiotic variables into a smaller subset of orthogonal variables that describe environmental variability and visualized using biplots. The SAS FACTOR procedure with the varimax rotation method was utilized for the PCA (SAS, 2017). For strongly correlated variables, such as % Pore Water and Average Density, only one variable was used. The metal data was subset to those that encompassed the pattern seen in an initial PCA of only the metals (Appendix B), with those of greatest environmental concern preferentially used. Then, Spearman's correlation was performed with the SAS CORR procedure (H_o: $r = 0$) to link the environmental data to the biotic data.

3. RESULTS

3.1 Univariate Analysis of Macrofauna Communities

Out of 75 samples analyzed (25 cores with 3 vertical sections per core), covering an area of 1,975 cm^2, a total of 276 animals (34,647 n/m^2) were collected from 65 families in 8 different phyla, although not all were able to be identified to the level of family. The largest abundance found in a sample was 10 individuals from the family Cirratulidae with most samples containing 1-2 individuals per family found. The majority of the animals were found in the top section (0-3 cm) for all stations. Station 39-750 had zero abundance for the bottom sections while stations 42-500 and 37-500 had the largest amount of distribution between the three layers (Table 1).

Table 1. Average abundance, species richness, and proportion of the total for each station-section. A) Average abundance (n/m^2) and species richness by station-section depth (cm) per 79 cm^2. Standard deviation in parentheses. B) Proportion of the total for each station-section.

A)	Abundance			Species Richness		
Station	0-3 cm	3-5 cm	5-10 cm	0-3 cm	3-5 cm	5-10 cm
37-500	1318 (89)	439 (89)	188 (89)	6.0 (0.0)	2.0 (0.0)	1.0 (0.0)
38-750	1213 (619)	335 (316)	126 (126)	6.3 (2.1)	2.7 (2.5)	1.0 (1.0)
39-750	1130 (377)	0	0	7.7 (2.5)	0	0
40-750	418 (362)	42 (72)	42 (72)	3.0 (2.6)	0.3 (0.6)	0.3 (0.6)
41-750	1339 (384)	84 (145)	42 (72)	8.3 (1.2)	0.7 (1.2)	0.3 (0.6)
42-500	941 (89)	439 (444)	188 (89)	6.5 (0.7)	3.0 (2.8)	1.5 (0.7)
42-750	1046 (192)	42 (72)	0	5.0 (0.0)	0.3 (0.6)	0
43-750	879 (217)	126 (126)	42 (72)	6.3 (1.5)	1.0 (1.0)	0.3 (0.6)
44-750	1799 (566)	377 (251)	167 (72)	7.3 (2.5)	2.0 (1.7)	1.3 (0.6)
Average	1120 (322)	209 (168)	88 (66)	6.3 (1.5)	1.3 (1.2)	0.6 (0.5)
B)						
37-500	68%	23%	10%	67%	22%	11%
38-750	72%	20%	8%	63%	27%	10%
39-750	100%	0%	0%	100%	0%	0%
40-750	83%	8%	8%	83%	8%	8%
41-750	73%	18%	9%	89%	8%	3%
42-500	60%	28%	12%	59%	27%	14%
42-750	96%	4%	0%	94%	6%	0%
43-750	84%	12%	4%	83%	13%	4%
44-750	59%	28%	12%	69%	19%	12%
Average	77%	16%	7%	79%	14%	7%

Polychaetes had the highest relative abundance (67.2%) and were distributed into 31 families. The next largest contributors were Mollusca, Crustacea, and Nemertea with relative abundances of 10.5%, 7.6%, and 7.6% respectively and 13 different families were identified for both Mollusca and Crustacea. Polychaetes were the only group found in every station with a high relative abundance for most stations. The two stations that differ the most are station 40-750, where the relative abundance was slightly lower than half, and Stations 44-750 and 37-500, which are composed primarily of Polychaetes. Crustaceans were absent from stations 42-750 and 44-750 and most abundant at stations 38-750, 39-750, and 41-750. Mollusks were present at all but station 37-500, as was Nemertea, with relatively higher abundances seen at stations 38-750, 39-750, 41-750, and 43-750 and low abundances for stations 42-500, 42-750, and 44-750. All other groups were found in only a few stations at low abundances (Fig. 2).

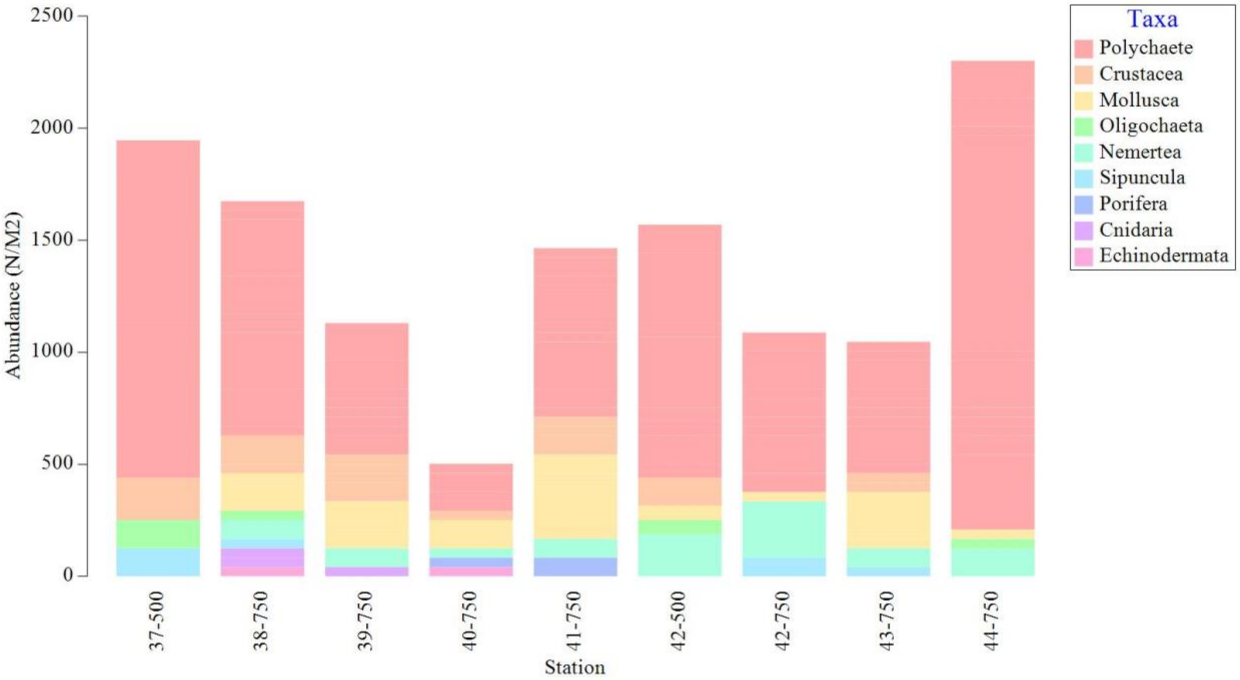


Figure 2. Bar plot of average abundance (n/m^2) for each station. Major taxa are differentiated by color.

All Mollusks, Nemertea, and Porifera came from the top 3 cm. Polychaetes made up 77.4% of the total abundance for the lower two sections of stations and Crustaceans made up 14.6%. Polychaetas and Crustaceans were the only taxa found in the lower two sections of all stations except for 38-750. There was one Crustacean found in the 5-10 cm layers with the rest being Polychaetes. The family Cirratulidae made up 25% of the total abundance found in the 5-10 cm section across all stations.

Station 40-750 has the lowest species diversity with only an average of 3 species and 4 individuals found (Table 2). Stations 38-750, 41-750 and 42-500 all have similar N1 values representing the highest amount of diversity among the stations while Station 44-750 has low diversity due to the lower J''. Station 44-750 had the highest degree of dominance as can be seen in Figure 3. Station 40-750 had very low abundance which made the dominance curve steeper, and one replicate only had a single species, so it is represented by a single point. 42-500_1 had the lowest dominance, as seen by the lower position on the plot, although the 42-500_2 had a much higher degree of dominance. Station 41-750 had low dominance with all three replicates showing a similar trend as opposed to most of the other station.

Table 2. Diversity indices for each sample (79 cm^2 to a depth of 10 cm) along with the total for all samples. Abbreviations: S = species richness, N = number of individuals per sample, N1 = Hill's N1, NM2 = abundance (n/m^2), J' = Pielou's evenness index.

Station	Replicate	S	N	NM2	J'	N1
37-500	1	8	14	1757	0.97	7.45
37-500	2	7	17	2134	0.92	5.98
38-750	1	11	17	2134	0.95	9.80
38-750	2	5	6	753	0.97	4.76
38-750	3	11	17	2134	0.94	9.50
39-750	1	5	6	753	0.97	4.76
39-750	2	8	9	1130	0.98	7.72
39-750	3	10	12	1506	0.98	9.52
40-750	1	4	6	753	0.96	3.78
40-750	2	1	1	126		1.00
40-750	3	5	5	628	1.00	5.00
41-750	1	11	17	2134	0.94	9.61
41-750	2	9	10	1255	0.98	8.71
41-750	3	7	8	1004	0.98	6.73
42-500	1	12	16	2009	0.98	11.31
42-500	2	7	9	1130	0.94	6.24
42-750	1	5	10	1255	0.93	4.50
42-750	2	5	8	1004	0.93	4.46
42-750	3	5	8	1004	0.93	4.46
43-750	1	6	8	1004	0.93	5.30
43-750	2	7	8	1004	0.98	6.73
43-750	3	8	9	1130	0.98	7.72
44-750	1	12	25	3138	0.83	7.81
44-750	2	7	19	2385	0.84	5.11
44-750	3	5	11	1506	0.80	3.65
Total		65	276	34773	0.83	32.24

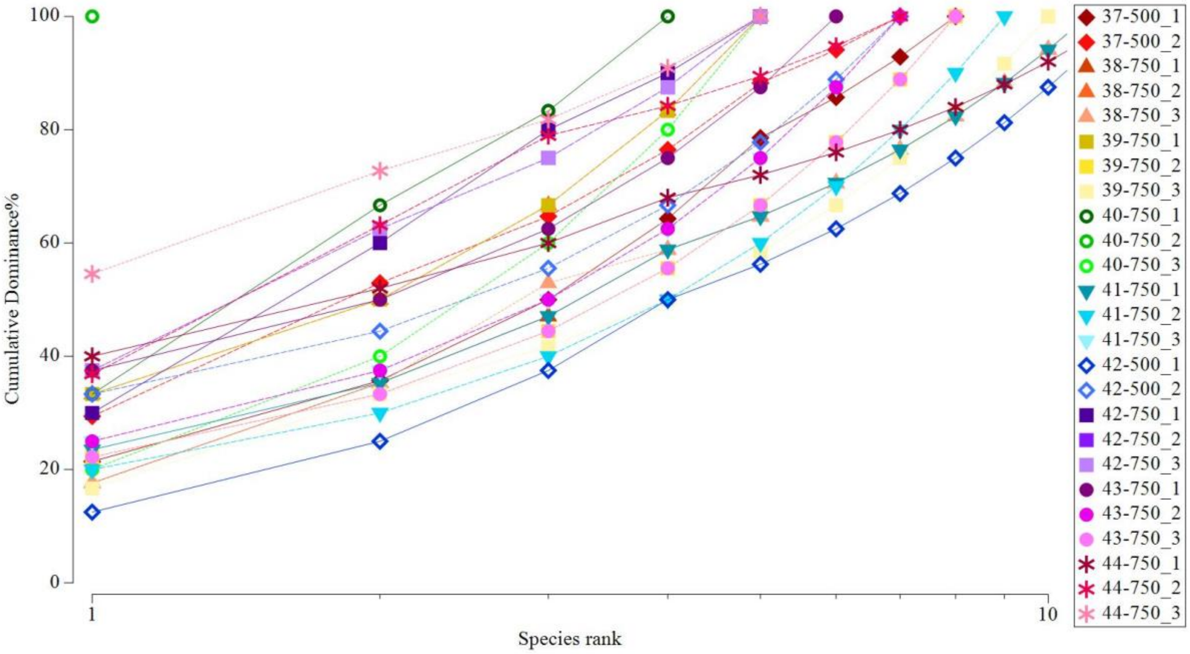


Figure 3. k-Dominance plot with the dominance curve for each sample. The x-axis has species rank in log scaled based on the abundance (n/m^2). Each station is represented by a symbol with a color gradient used for the corresponding replicates.

3.2 Multivariate Analysis of Macrofauna Community Composition

The ANOSIM procedure did not find significant differences between communities due to the high degree of variation among replicates therefore station replicates were considered independently. The nMDS plot for Bray-Curtis similarities between stations overlayed with the SIMPROF groups at 5% significance level is shown in Figure 4. The 2D ordination had a stress of 0.14 and showed most stations did not have close groupings between replicates, although stations 37-500 and 44-750 were exceptions. The group containing 42-750_3, 41-750_2, 41-750_3, and 40-750_3 showed a significant difference in community composition compared to the rest of the samples (Figure 4).

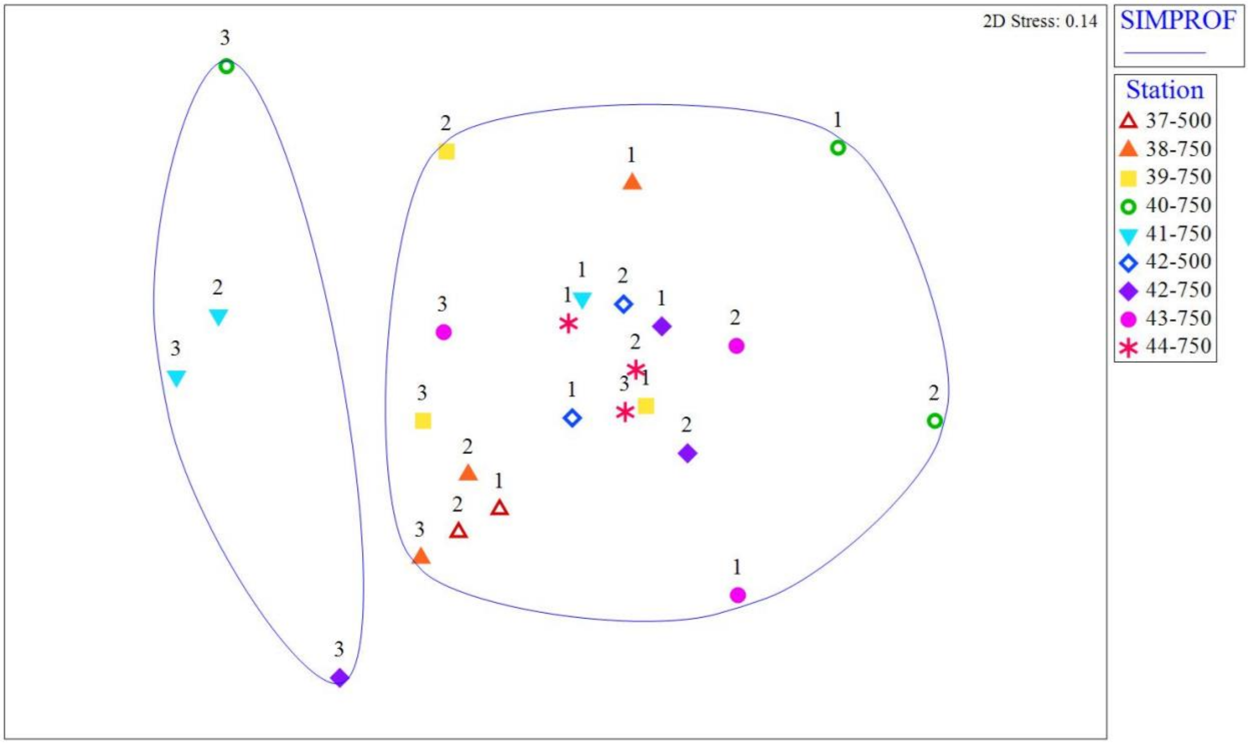


Figure 4. nMDS plot comparing the community compositions of the square root transformed abundances (n/m2) for each core, with overlaid SIMPROF groupings (Sig. level = 5%) circled in blue. Stations are differentiated by colors and symbols and labeled by replicates.

3.3 Environmental Data

A PCA of the combined environmental variables with the subset metals identified 3 significant PC’s which combined explained 89.2% of the variation. PCA 1 represented 53% of the variation and was primarily defined by a difference in sediment composition (% TOM vs % Carbonate). Most of the metals were closely related to TOM except for Sr which was related to Carbonate. PC2 explained 24% of the variation and was primarily a measure of the sediment texture with negative scores indicating a higher proportion of mud and positive scores a higher proportion of sand. Most of the metals were only loosely correlated with PC2 with a small skew towards positive. Mercury had the highest positive correlation to PC2 while Cadmium and Lead were also positive for PC2 though not as highly correlated (Figure 5, Eigenvalues and PC loads for each variable in Appendix C). PC3 accounted for 11.9% of the variation and is primarily related to negative correlation to Hg and Pb and positive correlations with Ba and pore water to a lesser extent (Appendix C). The stations were plotted on the PC axes (Figure 6) to show the major environmental factors at each station. Station 44 was strongly associated with PC1 at all depths showing high correlation with TOM as well as the presence of heavy metals. Station 44-750, which corresponds to the macrofauna data, did not show a strong relation to PC2. Station 37-250 had a high negative correlation with both PC1 and PC2 showing it is primarily carbonate mud. Station 38-750 was the next strongest correlated with a negative score for PC2 and a slightly negative PC1, while the rest of the stations were negative for PC1 and generally did not show strong associations with either axis.

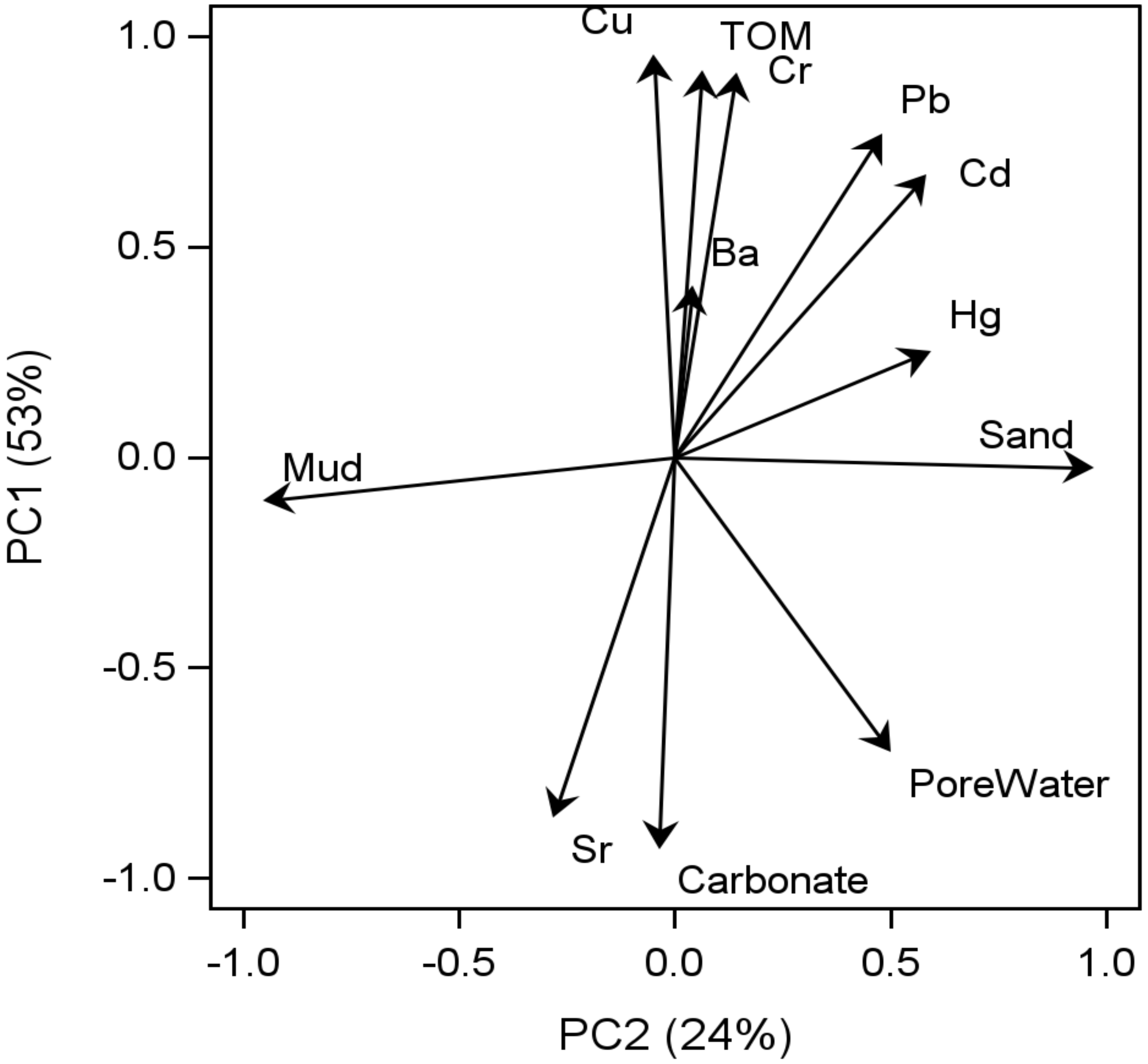


Figure 5. PCA for environmental sediment variable loads. Sediment texture is represented by Sand and Mud, Carbonate and TOM are indicative of the sediment composition and pore water shows the density. Metals included copper (Cu), Chromium (Cr), Cadmium (Cd), Barium (Ba), Mercury (Hg), Lead (Pb), and Strontium (Sr).

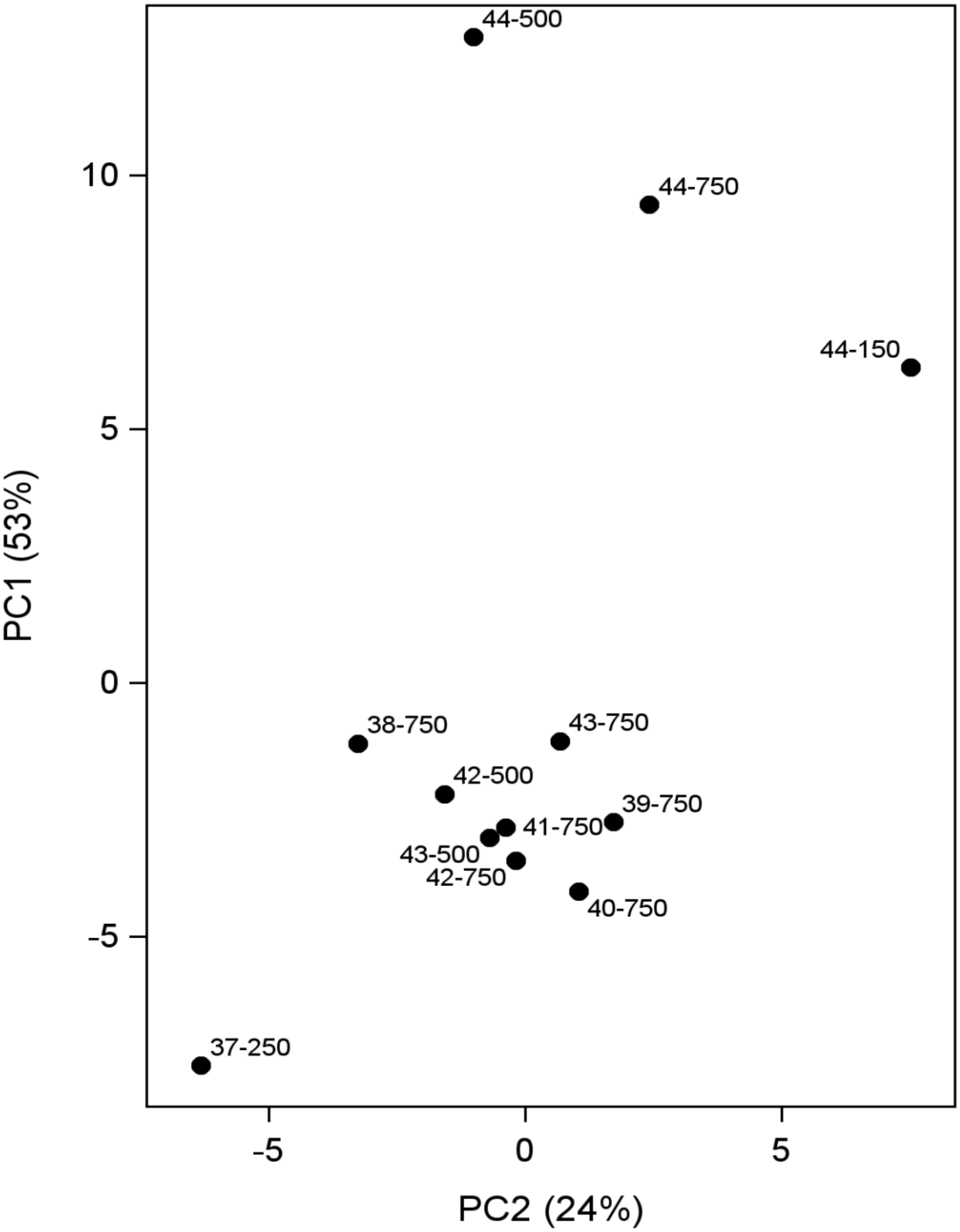


Figure 6. Environmental PCA with station scores plotted corresponding to the PC loads. The x axis shows PC2, and the y axis relates to PC1.

Table 3. Correlations between the biotic variables of density (n/m^2) and species richness (S), and the factors from the PCA of environmental variables. Under the column statistic, r = Spearman correlation coefficient, n = sample size, and P is the significance value.

	Statistic	**nm^2**	**S**
PC1	r	0.91	0.23
	P	0.002	0.581
	n	8	8
PC2	r	0.22	-0.41
	P	0.605	0.312
	n	8	8
PC3	r	-0.22	-0.43
	P	0.600	0.282
	n	8	8

Pearson's correlation found that the abundance (n/m^2) was significantly correlated to PC1 ($p = 0.0018$) but not significantly related to the species richness (Table 3). Station 44 -750 had a high, positive score for PC1 while Station 40-750 had a negative score, though not as strong as Station 44-750. Station 37-250 also had a strong negative score, although due to the lack of macrofauna data for this station it was not represented in the correlation. It is plausible that the conditions would be similar for Stations 37-250 and 37-500 so we can infer that the predominant features at this station are likely also calcareous mud with a low presence of heavy metals.

4. DISCUSSION

4.1 Comparison with the Northern Gulf of Mexico

There is a high number of rare species found and low overall abundances indicating the relatively pristine nature of the macrofauna assemblages in this region; however, they have a significantly lower abundance and lower diversity than what was found in the northern Gulf of Mexico (nGoM) including the samples taken at heavily impacted sites following the Deepwater Horizon (DWH) oil spill (Table 4; Reuscher et al., 2017; Reuscher et al., 2020). The sediment was primarily composed of carbonate mud whereas the nGoM have organic enrichment with an average clay content of 71% (Holmes, 1976). In the region near Cuba the average clay was 16% with station 37-250 being comprised of only 1.7% clay. Table 4 shows data comparing the depth, sediment composition (% mud which is % silt + % clay), porosity (%), trace metal concentrations (ppm), average abundance (individuals per core), average species richness, average N1, and average J' for nGoM, DWH impacted stations, and NW coast of Cuba. The stations from Cuba show a high degree of variability in many of the variables compared to the nGoM and DWH stations. On average the heavy metal concentrations were lower for the Cuba stations, with the exception of Hg and Cu, and the variation is higher. Barium is associated with oil drilling processes which explains the much lower concentrations from the Cuban samples. The diversity and abundance are lower as previously stated; however, the evenness index is higher for the Cuba samples. Fe and Mn are both naturally occurring and are indicative of different geochemical conditions. They are also important elements in redox reactions and, coupled with other elements, can indicate oxic and anoxic sediment boundaries (Smrzka et al., 2019) which have important implications for macrofauna communities and warrants further investigation.

In the nGoM it was found that the abundance for Mollusks and Crustaceans was significantly affected by clay content while Polychaeta were not, and Mollusk diversity increased with lower clay content and higher sand. While there was a general similarity with macrofauna communities, such as polychaetas being the dominant taxa followed by mollusks and crustaceans, this region showed a large amount of heterogeneity. All replicates came from the same deployment of the multicorer which means that for some of the stations there was more difference in the communities found within less than 1 m^2 then there were for this entire region. This is especially evident for stations 39-750, 40-750 and 43-750 which show a large amount of distance between replicates in the nMDS plot.

The community composition between the two regions was most different for the crustacean families which also showed low species turnover between stations within the northern GoM (Montagna et al., 2020) (Table 4). For the mollusks the dominant family identified for the nGoM (Reuscher et al., 2020), Thyasiridae, was not found in the Cuban samples. One of the most widely dispersed families for the Cuban region from the mollusk death assemblages (Peraza-Escarrá et al., 2022) was Rissoidae; however, only one station identified any living individuals for this family. Only two families of mollusks were found at more than two stations (Nuculidae and Yoldidae) with many being found in only one sample while the north GoM found the mollusks to have far less species turnover. The Aplacophorans were identified as the mollusk analogs of Polychaeta with opportunistic adaptability and wide species distributions in the nGoM; however, only a single individual of this group was found in the Cuban samples which was from 40-750. For the Polychaetes two of the more abundant families from the nGoM, Cossuridae and Nereididae, were absent from the Cuban samples. From the samples taken in 2010 following the DWH oil spill the dominant polychaete families identified were Dorvilleidae (highest abundances), Paraoinidae, Maldanidae, and Spionidae. By 2014 this had changed a bit with Cirratulidae and Syllidae becoming more dominant and Dorvilleidae less dominant. In the Cuban samples there were only 3 individuals from the family Dorvilledidae, all found in the same replicate from station 38-750 and only 2 Maldanidae, located at stations 42-500 and 42-750. At station 44-750, which showed more anthropogenic disturbance, the dominant family was Cirratulidae, which was also the most dominant family overall for this region. Capitellidae, Spionidae, Paraonidae, and Syllidae were also dominant families for this region which is similar to the findings at nGoM. Interestingly, Paraonidae was not found at station 44-750 which may indicate this family is more sensitive to heavy metal pollution despite being more tolerant to oil pollution. While more widely dispersed, Capitellidae was found in low abundance at all of the stations with only one or two individuals being found in a sample. Cirratulidae and Syllidae are the only two of the dominant families to show increased abundances at station 44-750. There were no crustaceans identified from station 44-750 and only one mollusk family (one individual from family Nuculidae).

Table 4. Comparison of environmental and macrofauna data between the NGoM, DWH, and Cuban stations. Mud is % silt + % clay, N is the avg. abundance (ind. per core), S is the avg. number of species, N1 is Hill's N1, and J' is Pielou's evenness index. The number of samples (n), mean (standard deviation), minimum, and maximum are shown for each region.

	NGOM (Reuscher et al. 2017)				**DWH (Reuscher et al. 2017)**				**CUBA**			
Variables	n	Mean	Min	Max	n	Mean	Min	Max	n	Mean	Min	Max
Depth (m)	14	1488 (333)	1002	2389	20	1517 (63)	1356	1607	13	1204 (404)	316	1682
Mud (%)	14	91 (0.4)	90.2	91.5	20	90.7 (0.6)	89.2	91.3	11	79.9 (9.6)	64	95.6
Ba (ppm)	14	862 (649)	313	2528	20	1425 (1140)	303	4853	10	74.7 (32.3)	11	137.9
Cd (ppm)	14	0.29 (0.09)	0.16	0.46	20	0.3 (0.04)	0.24	0.4	10	0.17 (0.05)	0.1	0.24
Cr (ppm)	14	29.6 (5.3)	25.4	42.4	20	28.5 (2.4)	23.9	32.2	10	18.5 (22.1)	0.8	65.5
Co (ppm)	14	11.9 (1.3)	9.8	13.9	20	12.5 (0.7)	11.3	13.9	10	5.8 (4.8)	0.7	15
Cu (ppm)	14	23.9 (3.3)	19.2	29.8	20	26.9 (7.2)	22.1	56.4	10	29.9 (32.1)	4.1	107.3
Fe (ppm)	14	24788 (1365)	22378	26476	20	24903 (2063)	20922	28337	10	10.4 (9.8)	1.2	29.2
Pb (ppm)	14	24.4 (9.6)	17.6	51.3	20	24.5 (6.4)	16.5	45.8	9	19.5 (19.7)	4.2	56.1
Mn (ppm)	14	13433 (5839)	6280	21778	20	16932 (5421)	7908	29071	10	571 (253)	205	1146
Hg (ppm)	14	0.06 (0.02)	0.04	0.12	20	0.06 (0.02)	0.05	0.13	9	0.16 (0.21)	0.03	0.68
Ni (ppm)	14	40.3 (8.3)	29.2	58	20	43.3 (3.6)	38.8	51.5	10	23.8 (20.3)	7.1	72.4
V (ppm)	14	58.7 (5.4)	48.7	65.3	20	59.7 (5.7)	48.6	68.7	10	19.7 (17.2)	0.4	46.9
Porosity (%)	42	70.6 (2.9)	64.3	75.1	60	71.2 (2.0)	65.1	74.2	12	54 (5.2)	44	64.9
N	42	77 (29.5)	23	137	60	78 (27.9)	16	176	25	11 (5.4)	1	25
S	42	25 (5.1)	13	34	60	20 (5.8)	4.3	29.7	25	7.2 (2.8)	1	12
N1	42	17 (3.1)	9.3	22.9	60	12.7 (4.5)	2.9	19.5	25	6.5 (2.4)	1	11.3
J'	42	0.88 (0.03)	0.79	0.94	60	0.82 (0.1)	0.42	0.91	25	0.94 (0.05)	0.80	1

4.2 Effects of Environmental Drivers

Station 44-750 had a lower evenness compared to the other stations with a few more dominant species (Cirratulidae, with higher abundances for Syllidae and Hesionidae at separate replicates). This station also had the highest relation to PC1 which corresponds to TOM along with the presence of a high number of heavy metals (Table 5), which indicates a stressed ecosystem with the more opportunistic species becoming more dominant. As this station is closest to Havana this would be a reasonable conclusion. The average metal concentrations (Hastings et al., 2019; Table 5) were highest at Station 44-750 for many of the heavy metals compared to the other stations including Zn, Ni, Pb, Hg, and Cu. Stations 42-750 and 43-500 had high levels of mercury as did station 44-150. Otherwise, all other stations were below the thresholds for metal concentrations. Another interesting characteristic is that Station 37-250 has generally lower concentrations of metals compared to the rest, except for Hg. As this station is also near the MPA at the Gulf of Guanahacabibes this isn't surprising, although the fact that the mercury concentrations are similar to the other stations is unexpected.

Table 5. Metal concentrations in parts per million (ppm) for each station (Hastings et al., 2019). Red text corresponds to values that exceed multiple of the sediment quality guidelines for metals (Burton, 2002).

Station	**Depth (m)**	**Cr**	**Cu**	**Zn**	**Hg**	**Pb**	**Cd**	**Ni**
37-250	530	0.79	4.14	15.73	0.09	4.22	0.08	7.11
*39-750	1250	7.16	12.64				0.16	12.32
*40-750	1590	9.80	15.69	73.48	0.03	8.89	0.17	18.43
*41-750	1511	10.92	14.78	21.38	0.04	8.79	0.19	13.38
*42-500	1156	9.65	14.77	29.74	0.03	8.96	0.17	12.63
*42-750	1420	1.34	12.79	35.17	0.16	6.33	0.14	14.93
43-500	1120	1.31	13.54	20.98	0.18	6.25	0.13	14.87
44-150	316	33.72	43.61	104.85	0.68	56.07	0.24	25.79
44-500	970	45.18	107.26	87.80	0.092	29.46	0.21	46.02
*44-750	1392	65.50	60.15	98.68	0.11	46.63	0.21	72.39

*Stations with macrofauna data

Station 38-750 is located in front of the Gulf of Guanahacabibes, which contains multiple Marine Protected Areas (MPA) with very little impact from anthropogenic sources due to the difficulties of accessing and legal protection for it. This station had a high species richness and abundance for 2 of the replicates; however, replicate 2 contained only 5 species and 6 individuals compared to the 11 species and average of 15 individuals found in the other two. Additionally, between the 3 replicates there were 23 different families found with only 4 families in common between 2 samples and none found in all 3. Given these findings it seems likely that the total extent of the diversity at this station was not well represented by the 3 replicates. Station 37-500 is also located nearby and did not possess the same level of diversity seen from Station 38-750. This may be an artifact of only having 2 replicates, but the total number of species found within the two is still much lower than I would have expected. Additionally, the 2 replicates shared 6 out of the 9 species, though none were in high abundance. As the sediment data does not correspond to the same depth as the macrofauna samples, and stations with data from multiple depths have shown to have inconsistent differences, it is difficult to draw any strong conclusions regarding the connection between sediment composition or texture. This station had the lowest proportions of Sand (4.3%) and the highest of Mud (95.6%) as well as Carbonate (96.2%), although that may not be true at the deeper depth (Brooks & Larson, 2019b). Although this is not significantly different than the values for Station 38-750 where there was much more diversity.

The sediment primarily consisted of carbonate (86% - 96% for stations 43-750 through 37-500, 49% for Station 44-750) with low proportions of organic matter (2.4% - 0.98%, for stations 43-750 – 37-500 and 7% for Station 44-750) (Brooks & Larson, 2019b). These low values are characteristic of the oligotrophic nature and lack of major rivers carrying large amounts of organic nutrients as is characteristic of the nGoM. The low level of diversity at station 37-500 may be reflective of this as this station is located at the tip of Cuba and would be primarily affected by the LC as it enters into the Gulf of Mexico through the Yucatan straits. That combined with the low sediment deposition, strong current, and steep slope is the most likely explanation for the very low diversity found. The cause for the incredibly low abundances at Station 40-750 and high degree of species found only at this station or one other station with no overlap between replicates is unclear. The sediment chemistry, texture and metal data does not show any distinguishing characteristics. The concentration of Zn is higher at Station 40-750 compared to all others except Station 44-750, but it is below the threshold limits. The % Pore Water is 65%, which is higher than any of the other stations. Of the 10 species found only Syllidae, Serpulidae, and Nemertea are at more than one other station. Interestingly, the group of polychaetas found at most stations (Cirratulids, Ampharetids, Capitellids, Hesionids, and Spionids) are all absent from Station 40-750. It is possible that conditions at this station are not suited for certain life strategies or reproductive strategies that limit the species that survive here. Another possibility is that there is a large degree of predatory species that limit the proximity of other organisms making organisms at this station more widely dispersed and limiting what can be collected with cores. Further sampling would help to shed light on the cause of the lack of diversity and abundance found at this station.

4.3 Sampling Size Effects

However, it is well known that a larger sampling area results in more species being found (Hessler & Sanders, 1967; Lomolino, 2000; Montagna et al., 2017). There were only 2-3 replicates collected for each station off Cuba while samples taken in the nGoM assessing the impact of DWH collected 1-3 replicate drops each containing 3 cores for every station for a total of 3-9 replicate cores per station. While it is clear that there is higher diversity of deep sea macrofauna in the nGoM compared to off the coast of Cuba, it is possible that differences in the number of animals collected and species richness between the two regions is inflated by this difference in sampling area.

4.4 Connection to Oceanographic Processes

The southeast GoM is highly impacted by a number of oceanographic processes, most influentially the variable nature of the LC. The degree of environmental variability and harsher conditions make this region unique compared to many other deep-sea systems including other areas in the GoM. Other studies have identified the presence of both cyclonic and anti-cyclonic eddies near the coast of Cuba that causes upwelling and downwelling (Le Hénaff et al., 2020). It has also been found that these eddies can reach depths of about 1500 m (Androulidakis et al., 2021), which would have direct impacts on the temperature, salinity, and nutrient availability for deep sea communities. The degree of seasonal variation due to the changing hydrological effects in community composition is unable to be assessed with the limited data we currently have available. This region is also impacted by periodical natural disturbances such as hurricanes and tropical storms which can cause changes in the deep-sea currents. Due to the narrow shelf the slope is in very close proximity to the coast which means debris from shore may be washed into the water and effecting this region of the deep sea more heavily than what you would see in the nGoM where the slope is much farther offshore. Additional sampling at this location, especially in relation to the state of the LC, could reveal interesting information on the effects of this variability on community composition.

5. CONCLUSION

Overall, there was a very low diversity of macrofauna found for the southeastern portion of the GoM along the NW Cuban slope. Despite the low diversity the community characteristics present were indicative of pristine assemblages with a high degree of evenness and a large proportion of rare species with the exception of Station 44-750 which was consistent with environmental stress due to heavy metal pollution. There was a large amount of variability within the replicates at each station that resulted in a lack of strong pattern between stations. The cause of the heterogeneity within stations is unclear because the only strong correlations with the environmental variables indicating disturbance was mainly found at Station 44-750. The major factors influencing macrofauna communities for the continental slope off NW Cuba are most likely the lack of organically rich sediment, low sediment deposition rates, and the strong current. In the future, samples for different depths along with information related to the trajectory of the Loop Current may reveal more information related to factors influencing community composition for deep-sea macrofauna in the GoM.

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APPENDIX A: SPECIES ABUNDANCE DISTRIBUTIONS ACROSS STATIONS

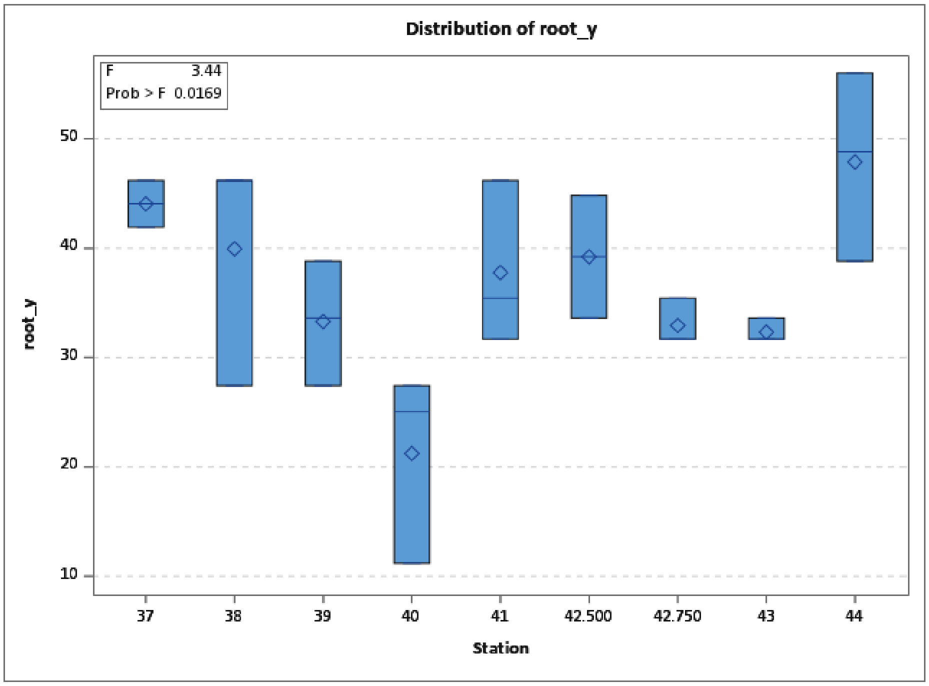

Figure A1. Boxplot with mean and variation for macrofauna density (n/m^2) at each station. F statistic is shown along with the probability of there being no difference in mean density between species.

Table A1. Table showing the species richness and abundance for the pooled replicates of each station, the averages of the replicates, and the overlap in species between replicates. S = Species Richness (pooled reps), N = Total number of individuals (pooled reps), Avg S = Average Species Richness, Avg N = Average number of individuals per core, and Sp. in Common is the number of families found in at least two replicates. Number of families found in all three replicates in parenthesis.

Station	S	N	Avg S	Avg. N	Sp. in Common
*37-500	9	31	7.5	15.5	6
38-750	23	36	9	13.3	4
39-750	18	27	7.7	9	4
40-750	10	12	3.3	4	0
41-750	21	35	9	11.7	6
*42-500	16	25	9.5	12.5	3
42-750	12	26	5	8.7	3
43-750	18	24	7	8.3	2
44-750	14	55	8	18.3	7 (4)

*Only 2 Replicates

APPENDIX B: PRINCIPAL COMPONENTS ANALYSIS FOR METALS

Table B1. Eigenvalues for PCs of metals. PC1 and PC2 explained 83% of the variation.

Eigenvalues of the Correlation Matrix: Total = 16 Average = 1				
	Eigenvalue	**Difference**	**Proportion**	**Cumulative**
1	10.1283555	6.9227563	0.6330	0.6330
2	3.2055993	1.6966242	0.2003	0.8334
3	1.5089751	0.9484436	0.0943	0.9277
4	0.5605315	0.2294691	0.0350	0.9627
5	0.3310624	0.1452531	0.0207	0.9834
6	0.1858093	0.1310072	0.0116	0.9950
7	0.0548020	0.0339432	0.0034	0.9984
8	0.0208589	0.0168528	0.0013	0.9997
9	0.0040061	0.0040061	0.0003	1.0000

Table B2: PC Scores for each metal variable.

Factor Pattern				
		Factor1	**Factor2**	**Factor3**
V	V (ppm)	0.95702	0.06055	0.08281
Cr	Cr (ppm)	0.93511	0.18801	0.29014
Mn	Mn (mg/g)	0.59890	-0.43308	0.53445
Fe	Fe (ppm)	0.96581	0.00703	0.10251
Co	Co (ppm)	0.94550	-0.14868	0.18894
Cu	Cu (ppm)	0.91738	-0.08216	-0.07126
Zn	Zn (ppm)	0.84955	0.33542	-0.04104
Sr	Sr (mg/g)	-0.94636	0.13068	0.25231
Mo	Mo (ppm)	-0.69331	0.31152	0.61327
Ba	Ba (ppm)	0.54017	-0.75934	-0.33753
Re	Re (ppb)	0.41294	0.88818	0.10488
Hg	Hg (ppb)	0.21970	0.79790	-0.48662
Pb	Pb (ppm)	0.78917	0.58961	-0.03251
U	U (ppm)	-0.77426	0.59262	0.11010
Cd	Cd (ppm)	0.83203	0.14653	-0.26366
Ni	Ni (ppm)	0.88762	0.03291	0.44175

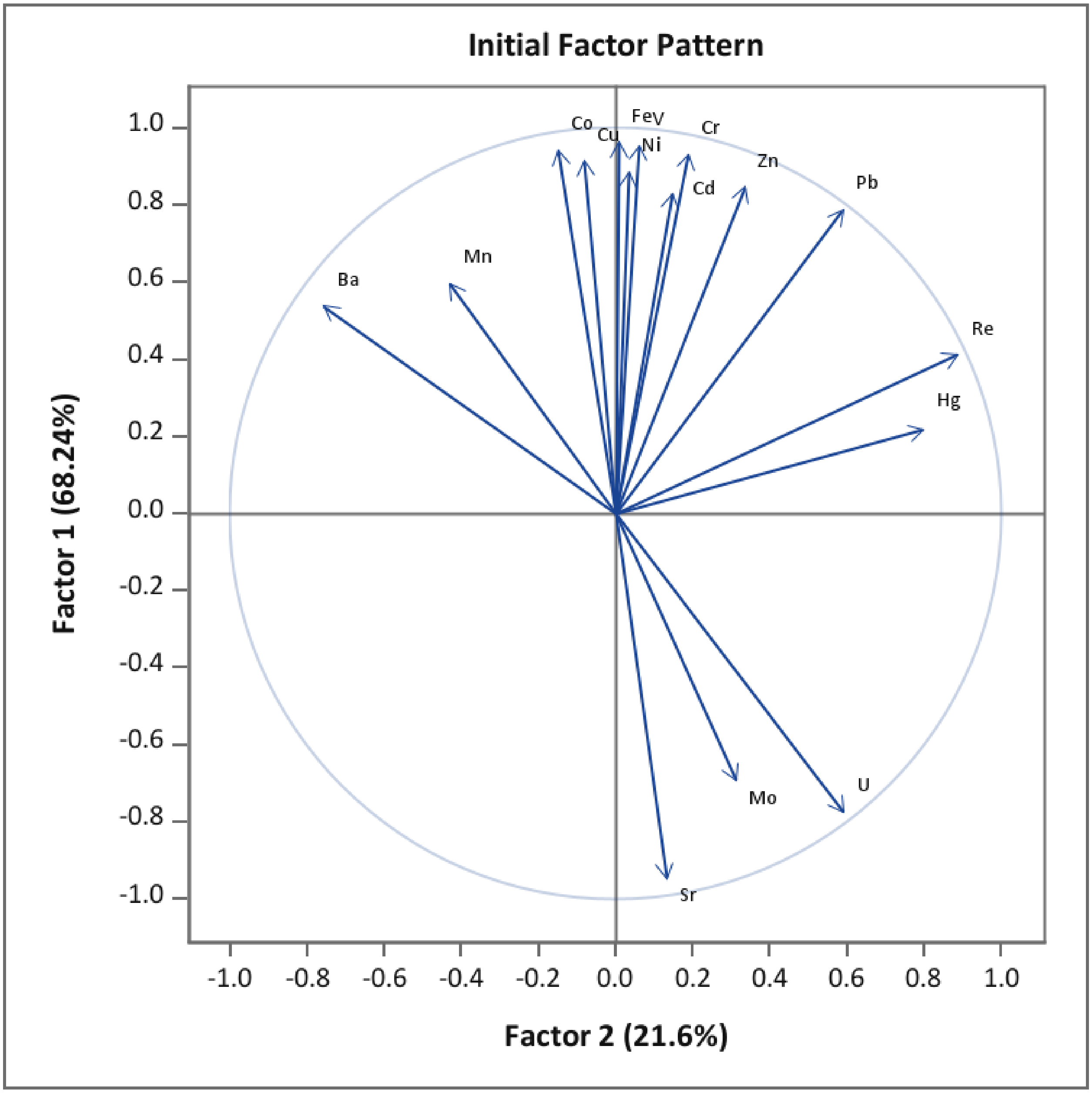

Figure B1. PC plot with metals corresponding to each factor.

APPENDIX C: PRINCIPAL COMPONENTS ANALYSIS STATISTICS

Table C1: Eigenvalues for PCs.

Eigenvalues of the Correlation Matrix: Total = 12 Average = 1				
	Eigenvalue	**Difference**	**Proportion**	**Cumulative**
1	6.36562463	3.46260954	0.5305	0.5305
2	2.90301509	1.46743761	0.2419	0.7724
3	1.43557748	0.94243713	0.1196	0.8920
4	0.49314036	0.02880664	0.0411	0.9331
5	0.46433372	0.24753132	0.0387	0.9718
6	0.21680240	0.15095870	0.0181	0.9899
7	0.06584370	0.02681281	0.0055	0.9954
8	0.03903088	0.02449368	0.0033	0.9986
9	0.01453720	0.01246614	0.0012	0.9998
10	0.00207106	0.00204756	0.0002	1.0000

Table C2: PC Loads for each variables using Varimax rotation method.

Rotated Factor Pattern				
		Factor1	**Factor2**	**Factor3**
PoreWater	Pore Water (%)	-0.70213	0.49966	0.27764
Sand	Sand (%)	-0.02546	0.97163	-0.03555
Mud	Mud (% Silt + % Clay)	-0.10433	-0.95452	0.04620
Carbonate	Carbonate (%)	-0.93133	-0.03509	-0.22061
TOM	TOM ((% LOI)	0.92011	0.06463	0.01260
Cu	Cu (ppm)	0.95725	-0.04948	0.20010
Cr	Cr (ppm)	0.91462	0.14347	-0.04264
Sr	Sr (mg/g)	-0.85822	-0.28050	-0.35845
Pb	Pb (ppm)	0.76945	0.48020	-0.37911
Cd	Cd (ppm)	0.67110	0.58392	0.15695
Ba	Ba (ppm)	0.40660	0.04120	0.86222
Hg	Hg (ppb)	0.25102	0.59355	-0.61368

Table C3: Variance explained by each factor.

Factor1	**Factor2**	**Factor3**
5.9786537	3.1375138	1.5880497

APPENDIX D: SAMPLE LOCATIONS

Table D1: Station locations (latitude and longitude) and depth along with the number of replicates collected.

Station	Latitude	Longitude	Depth (m)	Reps
37-500	22.18490	-84.87075	1209	2
38-750	22.47892	-84.69280	1670	3
39-750	22.80570	-84.10897	1296	3
40-750	23.00252	-83.67952	1580	3
41-750	23.08470	-84.19570	1511	3
42-500	23.06062	-82.94448	1000	2
42-750	23.09917	-82.97558	1455	3
43-750	23.12892	-82.73193	1535	3
44-750	23.23955	-82.35405	1430	3