The Role of Sponge Filtration in Transforming Coastal Water Quality in the Florida Keys

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CORAL REEF

The Role of Sponge Filtration in Transforming Coastal Water Quality in the Florida Keys

Final Report

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Management Summary

Declining water quality in the Florida Keys is a widespread concern and signifies a direct threat to coral reefs in the region. Along with various engineering solutions to reduce pollutants and bacteria in coastal waters, a promising avenue for water quality improvement is through the protection or rejuvenation of compromised coastal habitats, such as those for filter-feeding sponges that consume bacterioplankton and dissolved organic matter. To assess whether sponges are important contributors to water quality and the concentration of potential microbial pathogens, we compared water quality (water chemistry, bacterioplankton) at seagrass, sponge-dominated hardbottom, and open sand habitats (12 sites each; 36 total) in the Lower Florida Keys during winter (Dec 2022) and spring (May 2023). We also measured in mesocosms the efficiency of three common sponge species (Loggerhead, Vase, Sheepswool) to filter a potential waterborne pathogen: *E. coli*.

To summarize our findings:

1. **Field Results:** Characterization of the benthos in terms of sponge community volume or seagrass area and comparisons of those with bacterioplankton concentrations and water chemistry during one winter (Dec 2022) and spring (May 2023) period revealed no obvious relationships other than distinct differences in bacterial community structure between water column and sedimentary communities.

2. **Mesocosm Results:**
    a. On average, sponges removed approximately 10-40% of the *E. coli* in the water column in essentially one pass of seawater over the sponges at a rate of flow of 0.5cm/s which is empirically relevant for the shallow waters surrounding the Florida Keys.
    b. Removal of *E. coli* from the water column by all sponge species was approximately twice as high during our April experiments as compared to June experiments. This could be due to differences in water temperature, DO, or background picoplankton/bacteria concentrations in the water between the two time periods.
    c. Differences in *E. coli* removal from the water column among sponge species were minimal and inconsistent among time frames.
    d. Water flow has an appreciable effect on sponge filtration efficiency typified by lower filtration of *E. coli* at higher water flow speeds.

Executive Summary

Declining water quality in the Florida Keys and Florida Keys National Marine Sanctuary (FKNMS) is a widespread concern and signifies a direct threat to coral reefs in the region, including the recent devastation of corals by the Stony Coral Tissue Loss Disease (SCTLD). Harmful algal blooms and high bacterial concentrations are also concerning given their implications for the health of humans, wildlife, and ecosystems. A multitude of factors have contributed to this decades-long decline in water quality and billions of dollars have been spent on remedying the problem. Along with various engineering solutions to reduce pollutants and bacteria in coastal waters, another promising avenue for water quality improvement is through the protection or rejuvenation of compromised coastal habitats. For example, reconstruction of natural filtration systems (e.g., coastal wetlands, bivalve reefs) have been used to remove nutrients and pathogenic microorganisms from some coastal waters, but this approach has not been widely applied in the Florida Keys. Benthic filter feeders, in particular sponges and bivalves, can exert strong top-down control of plankton communities, control rates of biogeochemical cycling, reduce turbidity, and alter water chemistry. Over the past few decades our research team has developed the methods and investigated the feasibility of restoring sponge communities in coastal hardbottom areas destroyed by harmful algal blooms and hurricanes. However, demonstration of the effect of sponge filtration on microorganisms that are potentially pathogenic to wildlife (e.g., corals) or humans is untested, as is the relative effect of sponge communities and seagrass communities on water quality.

We had two objectives in this study:

Objective 1: Examine the relationship between water quality and coastal benthic community composition in hard-bottom, seagrass, and sand/open habitats in the Florida Keys.

To assess whether sponges are important contributors to water quality and the concentration of potential microbial pathogens, we compared water quality (water chemistry, bacterioplankton) at seagrass, sponge-dominated hardbottom, and open sand habitats (12 sites each; 36 total) in the Lower Florida Keys during winter (Dec 2022) and spring (May 2023). Our findings indicate that water quality among field sites differing in benthic substrate vary little, although differences exist between seasons.

Objective 2: Quantify species-specific and community-level filtration of waterborne bacterioplankton and alteration of water chemistry by sponges.

We also measured in mesocosms the efficiency of three common sponge species (Loggerhead, Vase, Sheepswool) to filter a potential waterborne pathogen: *E. coli*. Those experiments revealed that the sponge species tested are all capable of reducing *E. coli* in coastal waters by 10-40% depending on season and water velocity.

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Table of Contents

1. DESCRIPTION
    1.1 Background ………………………………………………………… 6
    1.2 Goals and Objectives ……………………………………………. 7
2 METHODS
    2.1 Objective 1
        2.1.1 Benthic Surveys ……………………………………………… 8
        2.1.2 Water Chemistry ……………………………………………... 9
        2.1.3 Phytoplankton/Bacteria Communities ……………………….. 10
        2.1.4 Microbial Community Composition …………………………. 10
    2.2 Objective 2 …………………………………………………………. 11
3 RESULTS
    3.1 Objective 1
        3.1.1 Benthic Surveys ……………………………………………… 12
        3.1.2 Water Chemistry ……………………………………………... 13
        3.1.3 Phytoplankton/Bacteria Communities ……………………….. 15
        3.1.4 Microbial Community Composition …………………………. 15
    3.2 Objective 2
        3.2.1 Species-specific Effects of Sponges on Bacterioplankton …… 25
        3.2.2 Species-specific Effects of Sponges on Water Chemistry …… 26
4 DISCUSSION ……………………………………………………………. 26
5 LITERATURE CITED …………………………………………………... 27
6 APPENDICES
    6.1 List of taxa identified in benthic surveys ………………………….. 30
    6.2 Total sequence reads for MiSeq runs ………………………………… 32

List of Figures

Figure 1 Map showing the 12 survey locations in the middle Florida Keys, FL .. 8
Figure 2 Diagram of the experimental sponge filtration mesocosm set-up ….. 12
Figure 3 Rarefaction analyses of all 16S rRNA amplicon data ………………… 16
Figure 4 NMDS analyses of 16S rRNA amplicon libraries by location ………. 17
Figure 5 NMDS analyses of 16S rRNA amplicon libraries by site type ………. 18
Figure 6 NMDS analyses of 16S rRNA amplicon libraries by sample type …….19
Figure 7 CCA analyses combining microbiome data and nutrient data ……….. 20
Figure 8 Top 20 Phyla from surface waters ……………………………… 21
Figure 10 Top 20 Phyla from sediments …………………………………… 22
Figure 11 Top 20 Orders from surface waters ………………………………… 22
Figure 12 Top 20 Orders from bottom waters …………………………………… 23
Figure 13 Top 20 Orders from sediments ……………………………………..… 23
Figure 14 Top 20 Families from surface waters …………………………………. 23
Figure 15 Top 20 Families from bottom waters ……………………………….…… 24
Figure 16 Top 20 Families from sediments …………………………………….. 24
Figure 17 Box plots of species richness and Shannon diversity indices by sites …25

List of Tables

Table 1 CAChE Nutrient Analysis Core Facility Detection Limits ………… 9
Table 2 Summary of sponge data for hardbottom and sand/open sites ……. 13
Table 3 Summary of seagrass data for seagrass sites ……………………….. 13
Table 4 Summary of the water chemistry data for field sites ……………….. 14
Table 5 Summary of microscopic autotroph and heterotroph concentrations...15
Table 6 Example of conversion of cells to DNA for quantitiation in qPCR … 25
Table 7 Summary of mesocosm sponge filtration experiment results ………..26

1. DESCRIPTION

1.1. Background

Declining water quality in the Florida Keys and Florida Keys National Marine Sanctuary (FKNMS) is a widespread concern and signifies a direct threat to coral reefs in the region, as signified by the establishment of the FKNMS Water Quality Protection Program by Congress in 1990. Eutrophication has had a long and deleterious effect on south Florida's marine ecosystem and has significantly altered species diversity, biogeochemical cycles, and ecosystem integrity[1-12]. A multitude of factors have contributed to this decades-long decline in water quality and billions of dollars have been spent on remedying the problem through the Comprehensive Everglades Restoration Plan and construction of a modern sewer system in the Florida Keys, among other actions. Harmful algal blooms (HABS) and high bacterial concentrations are especially ominous given their implications for the health of humans, wildlife, and ecosystems.
Inputs of sewage-related bacteria have been implicated in human infections and in coral diseases such as white pox disease[6, 13]. The culprit for the recent devastation to Caribbean corals by the Stony Coral Tissue Loss Disease (SCTLD) is thought to be a contagious consortium of bacteria. Though their precise identity or source is unknown, a hypothesized precursor to initiation of the disease was nearby sediment disturbance due to port dredging. In general, high bacterial concentrations in South Florida waters are associated with nonpoint source contamination by sewage and storm water [5, 14,15], sediment resuspension[16], and animal and human shedding of commensal bacteria[17,18] whose concentrations often spike sporadically and seasonally[21-23] due to changes in temperature and rainfall. A recent study by the co-PI contractor on this proposal has linked sediment microbe transfer from coastal dredging to coral reef sediments, including potential human and coral pathogens[19]. Along with various engineering solutions to reduce pollutants and bacteria in coastal waters, another promising avenue for water quality improvement is through the rejuvenation of compromised coastal habitats.

Reconstruction of natural filtration systems (e.g., coastal wetlands, bivalve reefs) have been used to remove nutrients and pathogenic microorganisms from some coastal waters[20-25], but this approach has not been applied in the Florida Keys where restoration has focused on repairing damaged habitats (i.e., coral, mangrove, seagrass, hard-bottom). Over the past few decades our research team has developed the methods and investigated the feasibility of restoring sponge communities in coastal hardbottom areas destroyed by harmful algal blooms and hurricanes[26]. One of our goals in doing so has been the improvement of water quality via filtration by sponges, but we have not explicitly tackled this aspect of coastal ecosystem restoration – until now.

In the tropical Pacific, the presence of healthy coastal habitats (e.g., seagrass) has been associated with reduced nutrients and bacterial loads with demonstrable benefits to nearby coral reefs[27], but such effects have not been examined in south Florida. Benthic filter feeders, in particular sponges and bivalves, can exert strong top-down control of plankton communities, control rates of biogeochemical cycling, reduce turbidity, and alter water chemistry[28,29]. Sponges are especially important in subtropical and tropical systems like those in south Florida[30-37], where they consume a diverse array of dissolved

and suspended materials. Together with their microbial symbionts, sponges improve water quality via their cycling of nutrients and uptake of dissolved organic material (DOM) and particulate organic material (POM) - the latter dominated by their consumption of bacteria. In fact, the filtration efficiency of bacteria-size particles by sponges typically exceeds 75% [38-41] and those rates tend to increase with increasing concentrations of available resources[24,25,43]. We have demonstrated in mesocosm experiments the pronounced species- and biomass-dependent effects that south Florida's shallow water sponges can have on bacterial concentrations and water chemistry[43,44]. However, demonstration of the effect of sponge filtration on microorganisms that are potentially pathogenic to wildlife (e.g., corals) or humans is untested, as is the relative effect of sponge communities and seagrass communities on water quality.

In south Florida, > 60 species of sponges dominate the animal biomass in shallow hard-bottom habitat, which covers >30% of the seafloor and where sponge abundance often exceeds 75,000 sponges/ha[45,46]. Prior to recent HABs, sponges once filtered Florida Bay's water column once every few days[29] - but no longer. Like Florida's coral reefs, the hard-bottom sponge community in large areas of south Florida has been severely degraded. HABs in 1991, 1992, 2007, 2013, and 2017 decimated or reduced the presence and diversity of sponges over much of the central Florida Keys and south-central Florida Bay[45-46]. Landfall of hurricanes (e.g., Hurricane Andrew in Biscayne Bay in 1992 and Hurricane Irma in the Florida Keys in 2017) also damaged sponge communities in shallow coastal areas, as can over-fishing of commercial sponges[47,48] and environmental stressors associated with urbanized coastal waters[49-51].

Given the important role of sponges in shallow marine ecosystems, their decline in south Florida – both in terms of biomass and biodiversity – is likely to have had deleterious ecological consequences, particularly for water quality. Our research team has been studying sponge ecology and the restoration of sponge communities in south Florida for over two decades and, in partnership with colleagues at the Florida Fish and Wildlife Conservation Commission (FWC), pioneered the methodology necessary for large-scale sponge community restoration[26]. This project constitutes the next logical step in the understanding of coastal habitat composition on water quality.

1.2. Goals and Objectives

The goal of this one-year project was to spearhead the investigation of the association between coastal habitat structure and water quality in the Florida Keys, with a particular focus on the hypothesized important role of sponges in modulating waterborne bacterial concentrations. We had two objectives:

Objective 1: Examine the relationship between water quality and coastal benthic community composition in hard-bottom, seagrass, and sand/open habitats in the Florida Keys.

Objective 2: Quantify species-specific and community-level filtration of waterborne bacterioplankton and alteration of water chemistry by sponges.

2. METHODS

2.1. Objective 1: Examine the relationship between water quality and coastal benthic community composition in hard-bottom, seagrass, and sand/open habitats in the Florida Keys.

2.1.1.1. Benthic Surveys

We compared the structure of hard-bottom, seagrass, and open-sand bottom sites communities at representative locations along the oceanside and bayside of the lower Florida Keys from Bahia Honda to Sugarloaf (Fig. 1) in May-June 2023. We surveyed 12 locations in this region with three sites per location: one hard-bottom site, one seagrass site, and one open/sand bottom site (n = 36 sites total). On hard-bottom sites, we used a combination of belt transect sampling (two 2m x 25m belt transects) and quadrat sampling (eight 1m x 1m quadrats) to estimate the density and size structure of the 28 most common sponge species, along with the density of other sessile flora (macroalgae; 10 species) and fauna (corals, octocorals; 6 species), the percent cover of the dominant red algae (*Laurencia* spp)(Appendix I), and sediment depth. In the same general location (i.e., within 1 KM) as each hard-bottom site, we also surveyed a seagrass site where we quantified seagrass species composition, density, and height in ten 25cm x 25cm quadrats. Finally, we characterized the benthos on open/sand bottom sites at each location using the same methods as described above for hardbottom sites. At each hard-bottom, seagrass, and open/sand field site, we also collected during winter (December 2022) and summer (May 2023) water samples for analysis of water quality characteristics (see below) associated with each habitat along with sediment samples to characterize bacterial abundance and composition.

Figure 1: Map showing the positions of the 12 survey locations in the middle Florida Keys, FL. At each of these 12 locations three types of sites (hard-bottom, seagrass, and sand/open bottom) <1 km from one another were surveyed and sampled.

2.1.2 Water Chemistry:

Water samples were obtained from each of the hard-bottom, seagrass, and sand/open bottom sites during the same tidal cycle and weather conditions: (1) ebb tide so as to assess inshore water characteristics that may also affect offshore locations via tidal transport and (2) winds below 15 kts to reduce mixing of surface and bottom waters from which we derived separate samples. Samples were collected just below the surface of the water column and 10 cm from the bottom and to account for potential differences in water characteristics with depth in the water column. The triplicate hardbottom, seagrass, and sand-open bottom sites sampled at each location were in the same general area (i.e., within 1 KM of one another) but far enough away so that water characteristics are likely to be independent. Water samples were collected during the winter (December 2022) and summer (late May 2023) in accord with FLDEP standard procedures for surface water sampling (DEP-SOP-001/01; FS 2100 Surface Water Sampling).

At each location, 2 liters of water was collected in acid-washed polyethylene bottles, immediately placed in a dark cooler filled with ice, and returned to the laboratory where half of each water sample was filtered (0.2 µm) for N and DOC analysis before freezing and temporary storage at -40°C. Water samples was analyzed to FL DEP required standards by the Water Quality Laboratory at Florida International University, a certified laboratory operating under EPA, FLDEP, and Florida Environmental Laboratory Accreditation Programs. The laboratory is currently responsible for water quality analyses conducted in the Everglades, the Florida Keys National Marine Sanctuary, and Biscayne Bay among other programs. The water quality constituents that were analyzed are listed in Table 1 along with the minimum and maximum detection limits for the procedures. Using these data, we assessed the independent effects of sponge and seagrass community structure on water quality parameters.

Table 1: CAChE Nutrient Analysis Core Facility Method Detection Limit (MDL) and Practical Quantitation Limit (PQL) values approved and effective as of 08/01/2022 for water quality constituents to measured in this project. Values are established equal to the lowest (non-blank) calibration standard of each analysis.Any reported value below PQL and above MDL was flagged and considered uncertain, as per LOQ definition.

Parameter	**MDL µmol/L**	**PQL µmol/L**
Nitrogen, Nitrite (NO2) + Nitrate (NO3) as N+N	0.130	0.960
Nitrogen, Nitrite as NO2-N	0.012	0.160
Nitrogen, Ammonia/Ammonium as NH3/NH4-N	0.128	1.000
Soluble Reactive Phosphorus as SRP	0.00069	0.0124
Total Organic Carbon	0.076	0.500

Turbidity (NTUs)	0.5 NTUs	----
Chlorophyll-a	0.1 µg/L	----

2.1.3. Phytoplankton/Bacteria Concentrations:

An additional 10 mL of seawater was collected as described above, preserved with glutaraldehyde, and kept frozen at -80ºC for < 1 month prior to processing. Concentrations of various planktonic components (Prochlorococcus, Synechococcus, pico/nanoeukaryotes, high nucleic acid (HNA) bacteria, low nucleic acid (LNA) bacteria, and viruses) was determined using a BD FACSCelesta Flow Cytometer and standard methods[24].

2.1.4. Microbial Community Composition:

All genomic DNA for both tasks 1 and 2 were extracted and purified using standard Qiagen Powerlyzer Powersoil kits. To generate 16S rRNA gene amplicon libraries, total genomic DNAs were extracted from each soil and sediment sample from Table 1 above. Samples were sequenced using standard Earth Microbiome Project (EMP) protocols for the Illumina MiSeq platform[62] .The 515F and 806R primers were used to amplify the ~300bp sequence of the V3 and V4 region of the 16S gene [62,63]. The PCR products were then cleaned using AMPure XP beads. This process is used to purify the 16S V3 and V4 amplicon away from free primers and primer dimer species. The final DNA (and RNA) concentrations were checked to high precision using a Qubit® 2.0 Fluorometer.

Once concentrations were obtained, each sample was diluted to a normalization of 4 pM. All the samples was library pooled and rechecked on the Qubit to make sure the concentration is between 4-6 ng/µL. When the pool passed, a final quality check was run using an Agilent 4150TS Tapestation, which checks the quality of DNA and for any possible contamination. The pooled DNA product was then loaded into the Illumina MiSeq in the Lopez Laboratory for 16S metagenomic DNA using the *MiSeq* Reagent Kit *v3 at 6*00 cycles of sequencing following a modified Illumina workflow protocol.

After 16S rRNA sequencing was completed, detailed alpha and beta diversity was assessed using the statistical software QIIME2 and R Studio following routine methods performed in the Lopez laboratory of HCNSO for analysis of other sample types such as South Florida surface waters samples and pelagic microbial plankton in coordination with the DEEPEND consortium [62, 64-66.] The Quantitative Insights into Microbial Ecology v.2 (QIIME2) pipeline was used to demultiplex, quality filter, assign taxonomy, reconstruct phylogeny, and produce diversity analysis and visualizations from the FASTQ DNA sequence files [62]. Quality filtering and data trimming was conducted in DADA2 using the "dada2 denoise" command, which will then be used to create a feature-table. The QIIME2-generated sequences was assigned taxonomy through a learned SILVA classifier (silva-132-99- 515-806-nb-classifier.qza). The R Studio statistical software packages "vegan" and "phyloseq" was utilized to assess diversity between samples. Alpha diversity which describes the species richness and evenness within a sampling location was looked at for each sample type and location. This was determined using multiple measures such as Observed and Chao1 for species richness estimators and Shannon and Inverse Simpson

indices for relative abundance diversity. Statistical differences between samples included SIMPER and ANOSIM for Beta diversity analyses.

2.2. Objective 2: Quantify species-specific and community-level filtration of waterborne bacterioplankton and alteration of water chemistry by sponges.

We experimentally measured using mesocosm experiments the effectiveness of individual sponge taxa in removing bacterioplankton and altering water chemistry in the water column. We have used this technique successfully in previous studies of shallow hard-bottom sponge community effects on water column properties [42, 43]. Given the 1-year duration of this funding opportunity, we did not plan to test the full suite of environmental conditions that are known to influence sponge filtration of bacterioplankton (e.g., sponge biomass, bacterioplankton taxon and concentration, water flow, temperature, etc.). Therefore, in this first set of experiments, we tested the effect of filtration by individual sponge taxa (monoculture) on ambient bacterioplankton, a cultured *Escherichia coli* (*E. coli*), and water chemistry. Initially we had planned to test sponge filtration of SCTLD-associated pathogens but instead tested *E. coli* in mesocosm experiments for several reasons. First, at the time of this writing the SCTLD pathogen has still not been unequivocally determined making it impossible to design a genetic probe to measure its concentration in seawater. Second, *E. coli* is a Gram-negative, facultative anaerobic coliform bacterium that is commonly found in the lower intestine of warm-blooded organisms and is also a common pathogenic contaminant of coastal waters. As pathogenic test organisms go, *E. coli* is a relatively safe and proven experimental pathogen and laboratory strains are not particularly harmful to humans. Importantly, *E. coli* specific primers for qPCR are readily available from the literature or can be easily designed [67,68].

The sponges used in the experiment included several species that are abundant in hardbottom habitat in the Florida Keys. Small individuals (~ 1000 cm^3 volume) of each sponge species chosen for the study was removed at their base from the substrate by divers, attached to ceramic tiles with cable ties, and placed back on the seafloor for 2 mos to heal and attach to the tile. We have used this approach to create replicate sponge transplants successfully for many sponge species over the past two decades.

The mesocosm experiments were conducted at the Newfound Harbor Marine Institute on Big Pine Key, FL within a specially designed experimental mesocosm system (Fig 2). comprised of a head tank (2000 liter) connected to a flow-through ambient seawater system that then fed ambient or pathogen-dosed seawater to 12 experimental mesocosms (25 liters each) that drained into a seawater treatment tank. Seawater (ambient or treated with a known pathogen concentration) entered each mesocosm on one end and flowed smoothly through a plastic honeycomb baffle to exit the mesocosm at the other end in a single pass. By measuring the characteristics of the water entering and leaving the mesocosm, we accurately determined changes in water column constituents due to sponge filtration similar to that *in situ* under normal tidal flow. Water leaving the mesocosms was drained into a 2000 liter holding tank where it was subject to UV

sterilization for 24 hours prior to release of the water onto the ground and not back into the sea as not to introduce potentially pathogenic bacterioplankton into the wild.

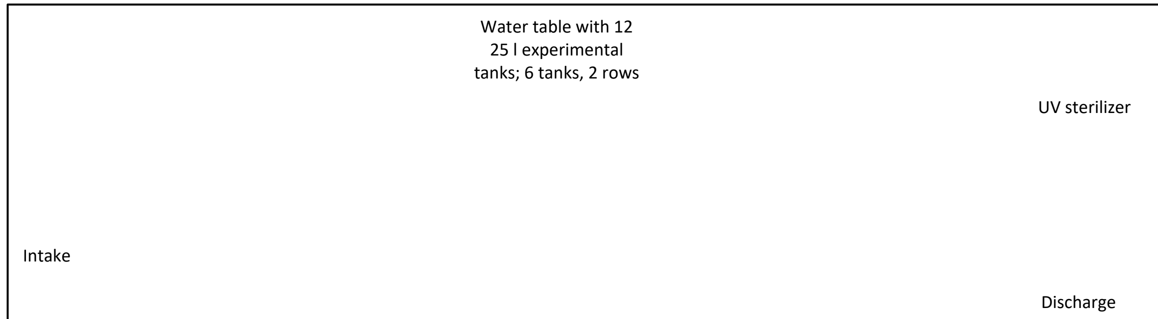

Figure 2: Diagram of the experimental sponge filtration mesocosm set-up.

Prior to the start of a mesocosm trial, a sponge transplant of known volume (~400ml) was introduced into to a mesocosm and then permitted to acclimate with flow-through seawater for 24 hrs. To begin a trial, water from the seawater-filled headtank was dosed with one of two target concentrations of *E. coli*: low concentration (~ 10^3 cells/ml) or a high concentration (~ 10^9 cells/ml) in keeping with the natural range of bacterial concentrations in south Florida[42,54]. Each sponge was tested under each target concentration and under two water flow regimes: static water (mimicking slack tide conditions) and water flowing through the mesocosm at 3 liters/min (peak tidal flow). Once the bacteria-dosed water entered the mesocosm the sponge was permitted to filter the water for 15 mins and at that time a 1 liter sample of water was taken from the headtank and from the water draining from each of the 12 mesocosms. These inflow (headtank sample) and mesocosm outflow water samples were then processed as described under Objective 1 for water chemistry, bacterioplankton, and *E. coli* concentration. Differences between the concentrations in the mesocosm inflow and outflow samples indicates changes caused by sponges.

3. RESULTS

3.1. Objective 1: Examine the relationship between water quality and coastal benthic community composition in hard-bottom, seagrass, and sand/open habitats in the Florida Keys

3.1.1 Benthic Surveys:

A total of 36 benthic sites were surveyed in the Lower Florida Keys at 12 separate locations where at each location one hardbottom site, one sand/open site, and one seagrass site were surveyed. The complete data set (Excel file) accompanies this report. Summary data for sponge communities and seagrass communities at these sites is presented in Tables 2 and 3, respectively.

Table 2: A summary of the volume, species richness, species diversity, and species evenness for each of the hardbottom and sand/open sites surveyed.

Site	Volume of All sponges per site/m2	Total # Sponge species/site	Sponge Shannon-Wiener Diversity	Sponge Simpson Eveness
Bahia Honda HB	3363	11	0.977	0.47
Bahia Honda Open	0	0	0	0
Cudjoe 1 HB	6720	7	0.16	0.099
Cudjoe 1 Open	0	0	0	0
Cudjoe 2 HB	6549	9	0.828	0.462
Cudjoe 2 Open	121	1	0	0
Friend HB	3075	14	1.42	0.5917
Friend Open	773	7	1.069	0.6639
Little Torch HB	2298	13	1.67	0.86
Little Torch Open	1266	11	1.51	0.8404
Middle Torch HB	567	8	0.8	0.4948
Middle Torch Open	307	7	1.24	0.8911
Newfound Harbor HB	4764	15	1.73	0.7532
Newfound Harbor Open	680	8	1.23	0.763
No Name HB	2641	13	1.86	0.8466
No Name Open	352	7	1.47	0.9118
Porpoise HB	1814	20	1.6	0.7297
Porpoise Open	777	13	1.54	0.7906
Ramrod HB	2835	7	1.24	0.6365
Ramrod Open	1594	6	0.58	0.4199
Sandfly HB	2796	12	1.78	0.7415
Sandfly Open	451	11	1.3	0.9385
Top Tree HB	814	9	1.25	0.6413
Top Tree Open	0	0	0	0

Table 3: A summary of the number of seagrass blades, blade height, total blade area, and sediment depth at each seagrass site surveyed.

Site	mean # blades / 10cm2	mean blade ht (cm)	Mean blade area (cm3)	Mean Sediment Depth (cm)
Bahia Honda Bay	22.8	14	319.2	27
Big Munson	15.4	11	169.4	75
Cudjo 1	20.6	16	329.6	25
Cudjo 2	12	20	240	22
Friend Key	15.6	26	405.6	16
Little Torch	13.8	20	276	49
Middle Torch	12.8	12	153.6	31
No Name Key	7.2	23	165.6	15
Porpoise Key	11.4	16	182.4	27
Ramrod	10.4	11	114.4	76
Sandfly Key	12	19	228	27
Top Tree	23.4	17	397.8	28

3.1.2 Water Chemistry:

Complete data sets for water chemistry results for samples collected in the surface and bottom (10cm from bottom) on ebb tides when winds were < 15kts that were collected in winter (Dec 2022) and spring (May 2023) at all 36 field sites accompany this report. Table 4 summarizes those data.

Table 4: (Top panel; blue) A summary of the water chemistry data collected at three types of survey sites (hardbottom, sand/open, seagrass) during spring (May 2023) and winter (Dec 2022) at the surface and 10cm from the bottom at each site. (Bottom panel; green) A summary of the water chemistry data shown above but condensed by surface vs. bottom samples, and winter vs. spring sampling. Cells in gold indicate sets of data where one or more samples differed appreciably from the others.

Habitat	Water column	Season	Mean concentrations						
			N+N ppm	NO2-N ppm	NH3/NH4-N	TN ppm	TP ppm	SRP ppm	TOC ppm
HB	SURFACE	WINTER	0.00529167	0.00041667	0.02014167	0.177825	0.0123	0.000325	5.19483333
HB	SURFACE	SPRING	0.01495667	0.00147083	0.02804333	0.22863	0.01573333	0.00126833	2.88575
HB	BOTTOM	WINTER	0.00875833	0.000725	0.02916667	0.17383333	0.01114167	0.0002	5.06633333
HB	BOTTOM	SPRING	0.01354167	0.0013825	0.02840333	0.236195	0.01555	0.00119083	52.2361667
OPEN	SURFACE	WINTER	0.00624167	0.000325	0.02675	0.16550833	0.01225833	0.00053333	6.20575
OPEN	SURFACE	SPRING	0.01129833	0.00105917	0.029495	0.231105	0.01571	0.00101833	3.60975
OPEN	BOTTOM	WINTER	0.00660833	0.0006	0.01861667	0.167625	0.01083333	0.00014167	7.04916667
OPEN	BOTTOM	SPRING	0.01580917	0.00205083	0.03835583	0.24548833	0.01537417	0.0012225	210.65675
SG	SURFACE	WINTER	0.006125	0.0005	0.03448333	0.17793333	0.01215	0.000625	4.18283333
SG	SURFACE	SPRING	0.01115909	0.00093727	0.02090545	0.24305364	0.01562636	0.00063727	3.07954545
SG	BOTTOM	WINTER	0.00748333	0.00056667	0.099625	0.23348333	0.01125833	0.00015	6.63583333
SG	BOTTOM	SPRING	0.01398818	0.00206818	0.03193	0.22483364	0.01485909	0.00125818	87.2398182

Habitat	Water column	Season	Mean concentrations						
			N+N ppm	NO2-N ppm	NH3/NH4-N	TN ppm	TP ppm	SRP ppm	TOC ppm
HB	SURFACE		0.01012417	0.00094375	0.0240925	0.2032275	0.01401667	0.00079667	4.04029167
OPEN	SURFACE		0.00877	0.00069208	0.0281225	0.19830667	0.01398417	0.00077583	4.90775
SG	SURFACE		0.00864205	0.00071864	0.02769439	0.21049348	0.01388818	0.00063114	3.63118939
HB	BOTTOM		0.01115	0.00105375	0.028785	0.20501417	0.01334583	0.00069542	28.65125
OPEN	BOTTOM		0.01120875	0.00132542	0.02848625	0.20655667	0.01310375	0.00068208	108.852958
SG	BOTTOM		0.01073576	0.00131742	0.0657775	0.22915848	0.01305871	0.00070409	46.9378258
HB		WINTER	0.007025	0.00057083	0.02465417	0.17582917	0.01172083	0.0002625	5.13058333
OPEN		WINTER	0.006425	0.0004625	0.02268333	0.16656667	0.01154583	0.0003375	6.62745833
SG		WINTER	0.00680417	0.00053333	0.06705417	0.20570833	0.01170417	0.0003875	5.40933333
HB		SPRING	0.01424917	0.00142667	0.02822333	0.2324125	0.01564167	0.00122958	27.5609583
OPEN		SPRING	0.01355375	0.001555	0.03392542	0.23829667	0.01554208	0.00112042	107.13325
SG		SPRING	0.01257364	0.00150273	0.02641773	0.23394364	0.01524273	0.00094773	45.1596818

3.1.3 Phytoplankton/Bacteria Concentrations:

Complete data sets for phytoplankton/bacteria concentrations as determined from flow cytometry on samples collected in the surface and bottom (10cm from bottom) on ebb

tides when winds were < 15kts that were collected in winter (Dec 2022) and spring (May 2023) at all 36 field sites accompany this report. Table 5 summarizes those data.

Table 5: A summary of the concentrations of microscopic autotrophs and heterotrophs present in water samples collected at three types of survey sites (hardbottom, sand/open, seagrass) during spring (May 2023) and winter (Dec 2022).

Habitat	Season	mean autotrophs	sd autotrophs	mean heterotrophs	sd heterotrophs	mean all cells	sd all cells
HB	Winter	130111	73287	1953143	1135747	2083253	1150304.38
HB	Spring	200848	324104	1720522	550835	1921370	431880.185
OPEN	Winter	122433	30665	1675741	728832	1798173	720690.906
OPEN	Spring	116474	24314	1703337	357812	1819810	355159.006
SG	Winter	131907	54807	1880098	731610	2012004	765779.048
SG	Spring	137261	95377	1919987	532023	2057249	535261.427
HB	BOTH	165479	198695	1836833	843291	2002312	791092
OPEN	BOTH	119453	177384	1689539	639834	1808992	576286
SG	BOTH	134584	27490	1900042	543322	2034627	537925

3.1.4 Microbial Community Composition:

Winter 2022 Data

For the winter (Dec 2022) water sampling, matching samples for surface, bottom and sediment samples (n = 99 total samples) were examined and those with greater than 10,000 reads after rarefaction analyses used for final analyses (Fig 3). Appendix 2 shows a total of 111 MiSeq sequence samples generated because a repeat run was made to remedy missing data. Samples that yielded fewer than this cut-off were not included. Overall, the total number of unique amplified sequence variants (ASVs) generated were 35,956 (Unfiltered dataset) and 13,941 (Filtered dataset (0.1% abundance). We completed microbiome analyses of Fall 2022 and Summer 2023 field samples. All samples have been analyzed with the same methods discussed in the preliminary report.

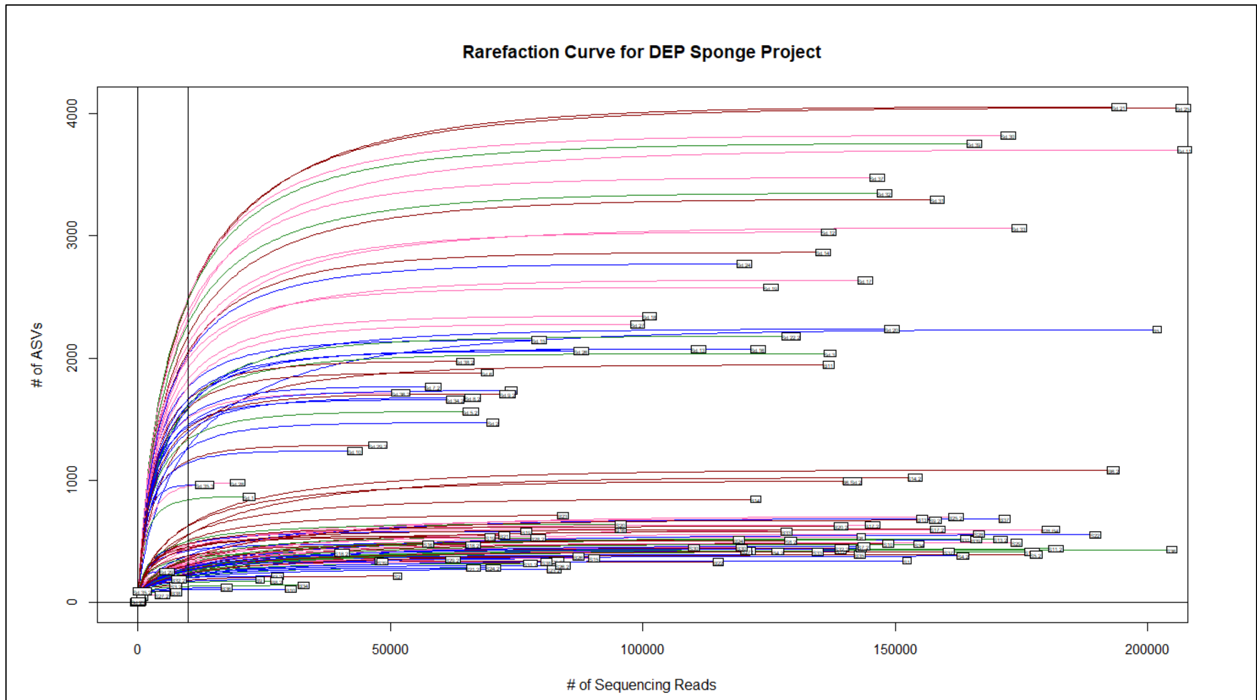

Figure 3: Rarefaction analyses of all 16S rRNA amplicon data indicate that inflection occurs at 10,000 reads, whereby the over 90% of sequence diversity is captured.

To obtain a broad understanding of microbial communities across FL Keys habitats, a cluster analyses through non-metric dimensional scaling (NDMS) was applied as a form of beta diversity analyses. Each point on the plot represents the taxonomic composition of the bacteria in the sample relative to all other samples in the dataset. Figures 4 and 5 show that most of the samples were well mixed in community composition based on geographic location and site type, respectively. This is expected since water column samples are more or less continuous and was mixed depending on the conditions of the water at the time (wind, underwater currents, convection and turbidity). On the other hand, NMDS plots show that in situ sediments and water samples are clearly separated (Figure 6).

Geographically, Bahia Honda microbiome samples (red and orange in Fig. 6) appeared the most distinct relative to other FL Keys sites. Although this distinction does not appear statistically significant, the differences stem from higher relative abundances of cyanobacteria, namely from the family Synechococcales (shown in stacked bar charts of relative abundances further below in Figs. 11-13).

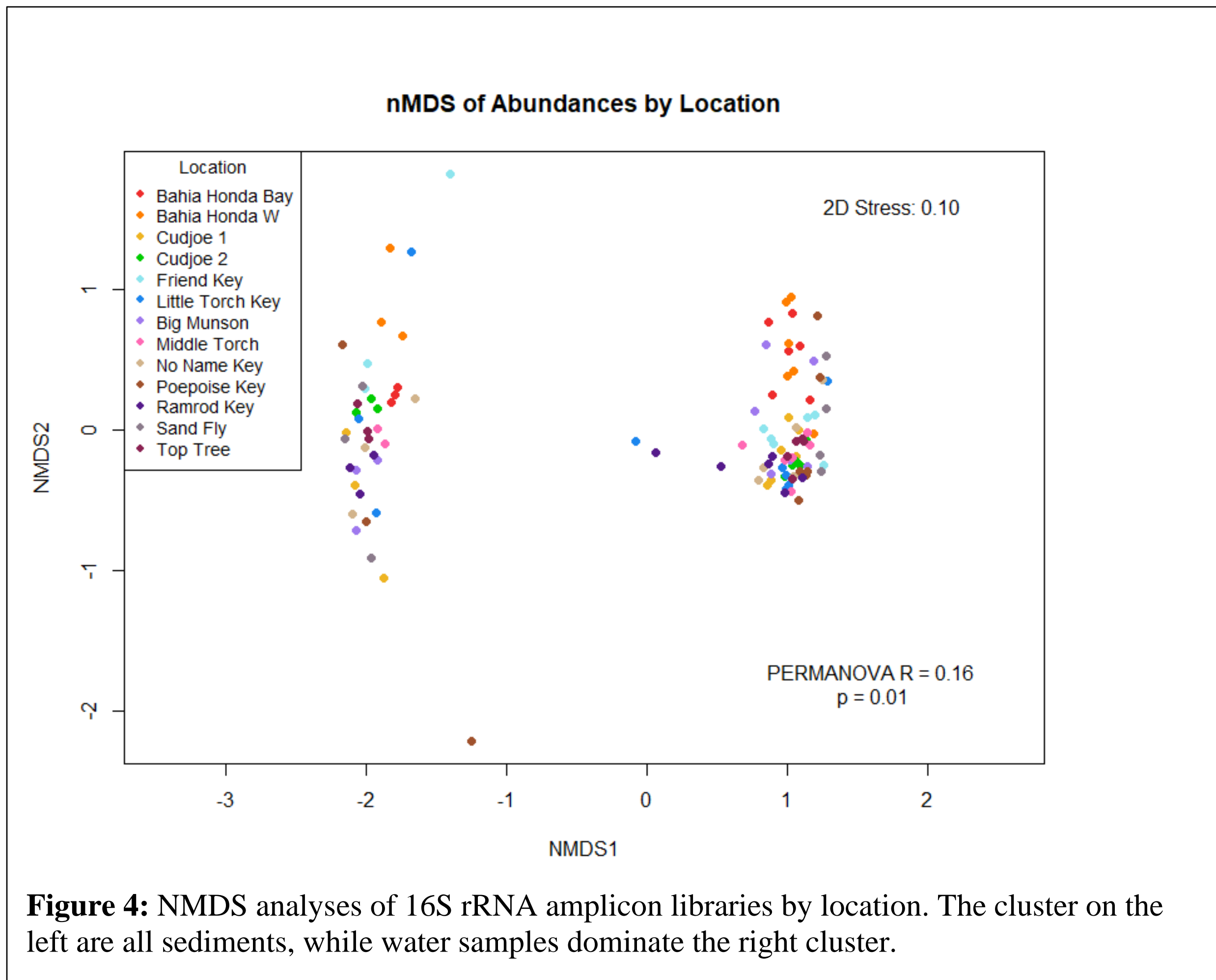

Figure 4: NMDS analyses of 16S rRNA amplicon libraries by location. The cluster on the left are all sediments, while water samples dominate the right cluster.

The clearest microbiome distinction in beta diversity occurs between sediment and water samples as shown in Fig. 6. This is also not unexpected, since it is well known that sediment samples often have a higher species richness than water column samples. Microbiome data was also combined with available nutrient data for a canonical-correlation analyses (CCA) (Fig. 7).

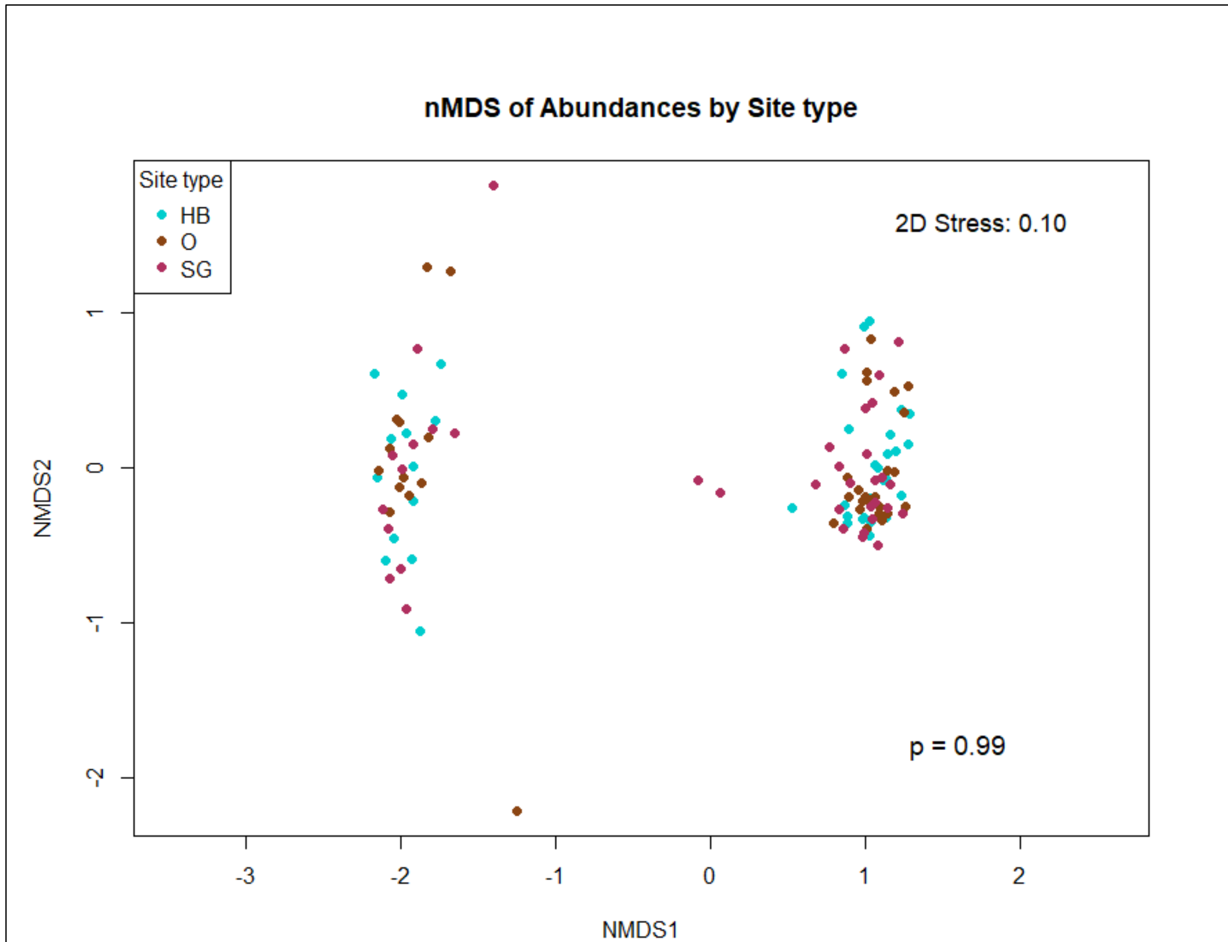

Figure 5. NMDS analyses of 16S rRNA amplicon libraries based on site type (hard-bottom (HB), seagrass (SG), and open-sand bottom (O) sites.

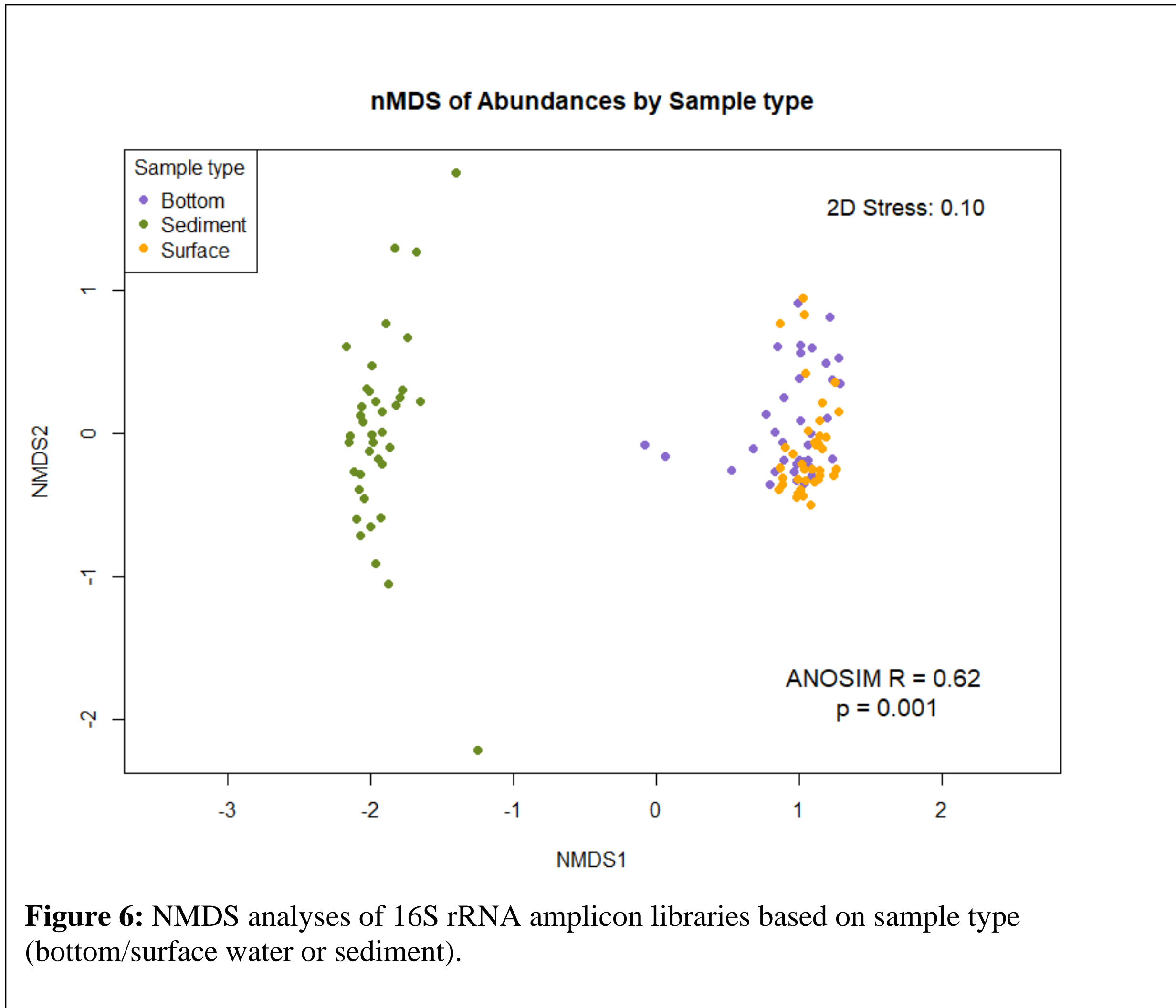

Figure 6: NMDS analyses of 16S rRNA amplicon libraries based on sample type (bottom/surface water or sediment).

To assess the relative abundances of bacterial taxa according to site types, stacked bar charts are shown in Figures 9 - 16. We have generated these comparisons at the Phyla, Order and Family levels. (Color coding of taxa was maintained consistently for each grouping for ease of comparisons). The taxonomic resolution of the 16S rRNA gene typically stops at the family level, though some taxa may be identified to genus. We only include the top 20 taxa for each group, since the majority of microbiome sequences have much lower abundances (< 1%) and cannot be fully represented graphically.

The comparisons show distinctions between certain sites, but also a high degree of homogeneity. Again, water conditions at the time can contribute to the differences. Also not yet included here are other parameters such as nutrients that may be present in the water samples.

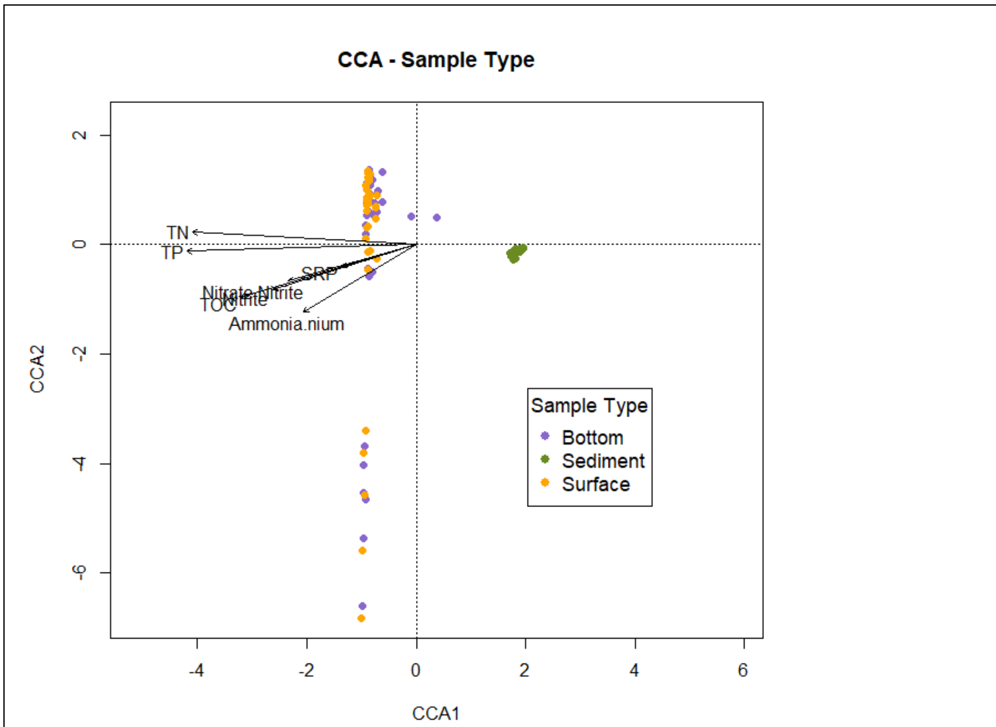

Figure 7: CCA analyses combining microbiome data and nutrient data of seawater.

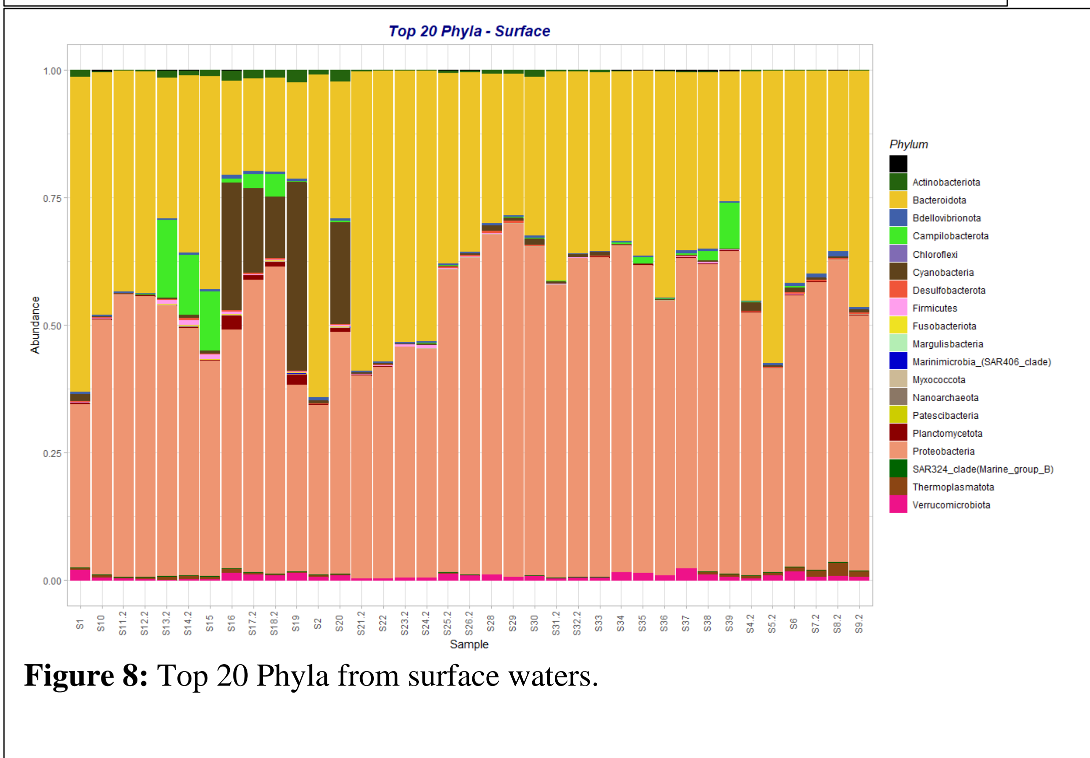

Figure 8: Top 20 Phyla from surface waters.

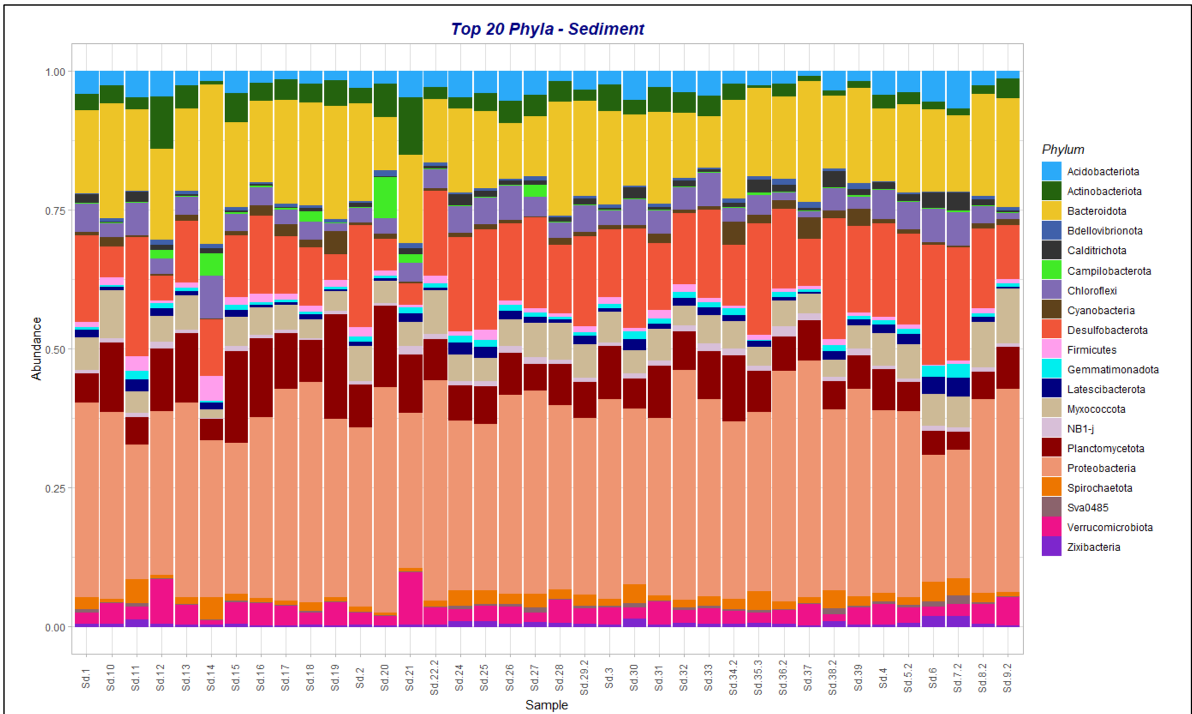

Figure 10: Top 20 Phyla from sediments.

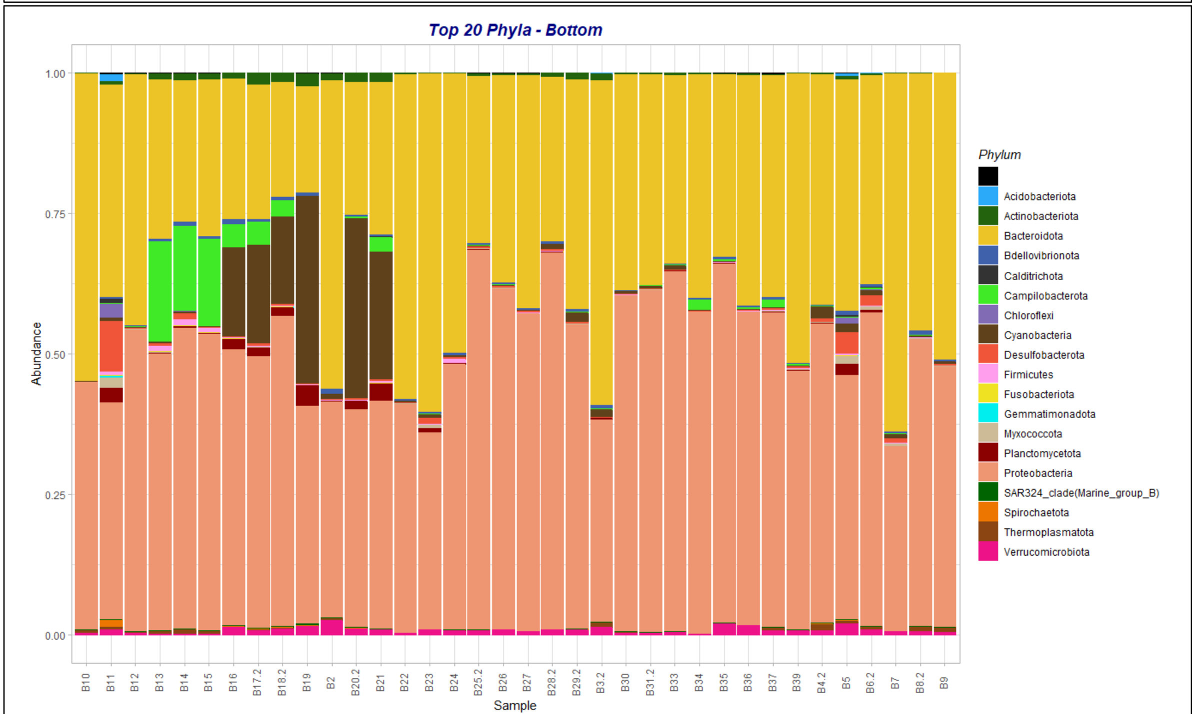

Figure 9: Top 20 Phyla from bottom water.

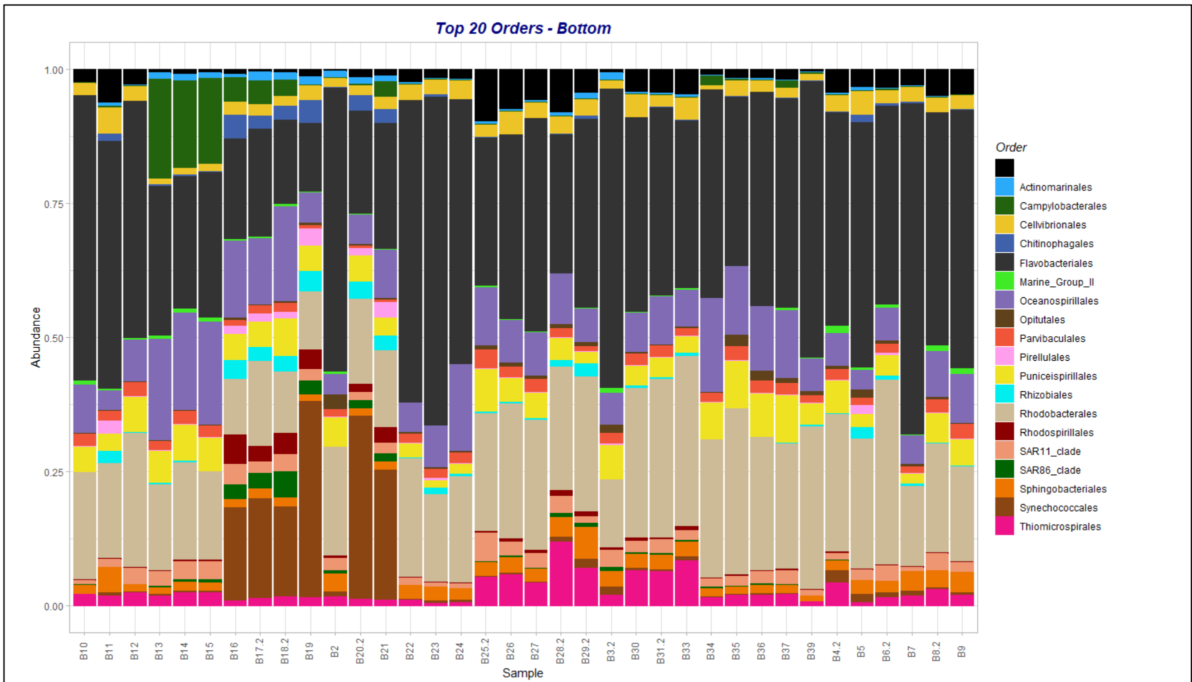

Figure 12: Top 20 Orders from bottom waters.

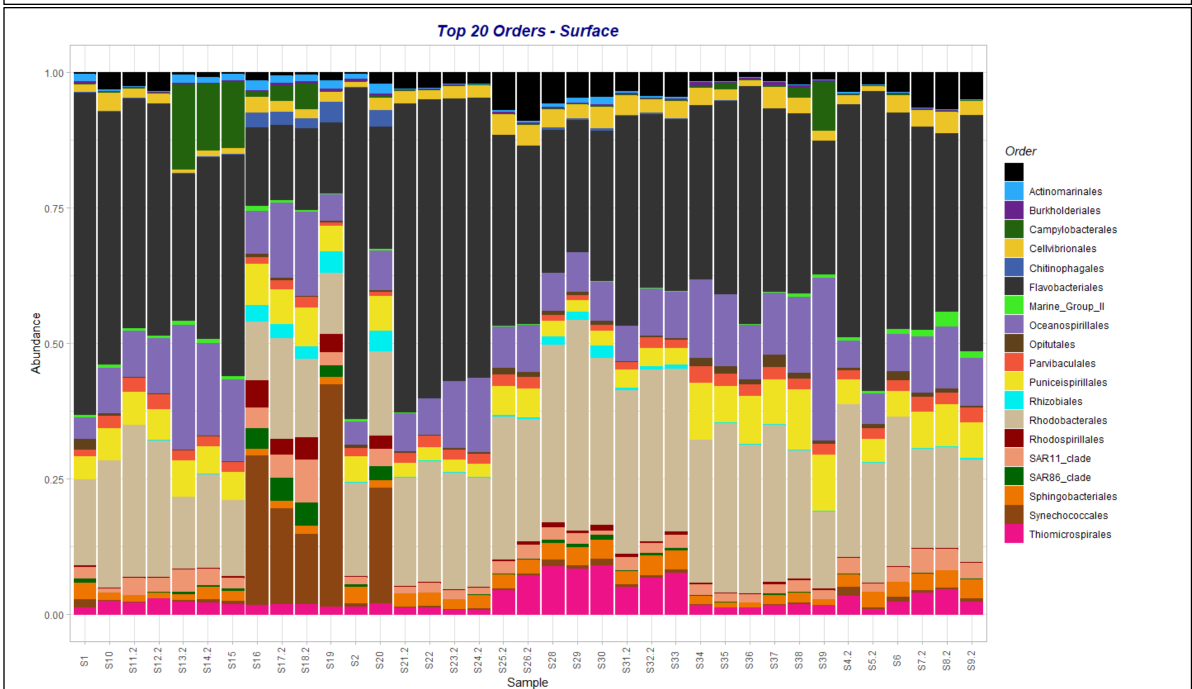

Figure 11: Top 20 Orders from surface waters.

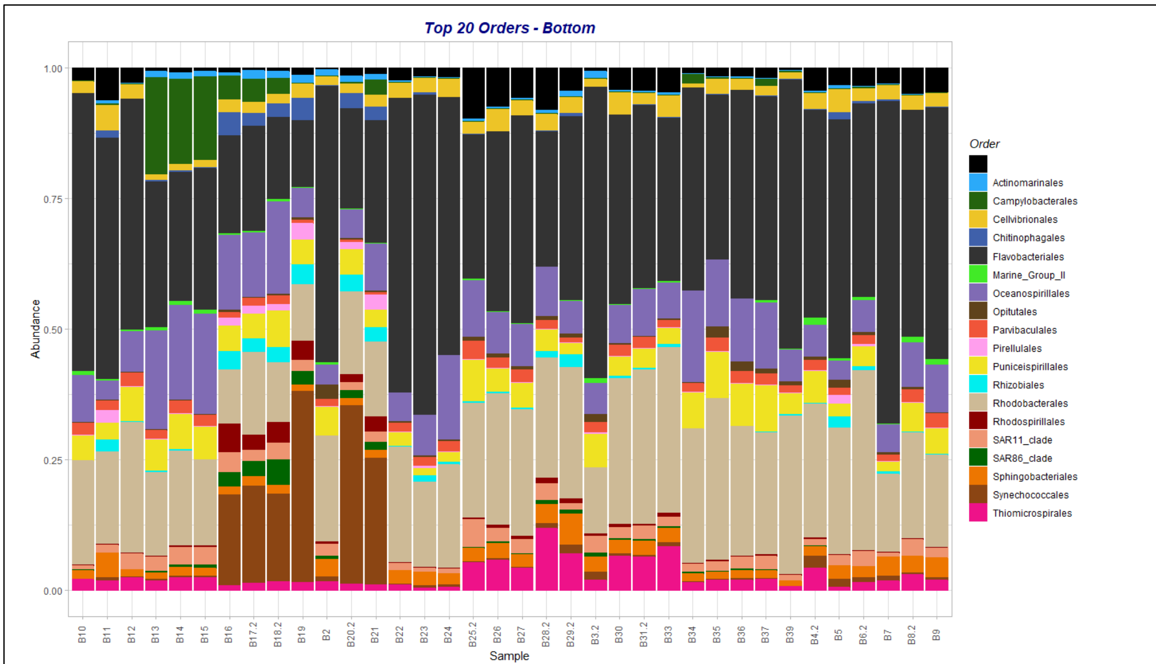

Figure 13: Top 20 Orders from sediments.

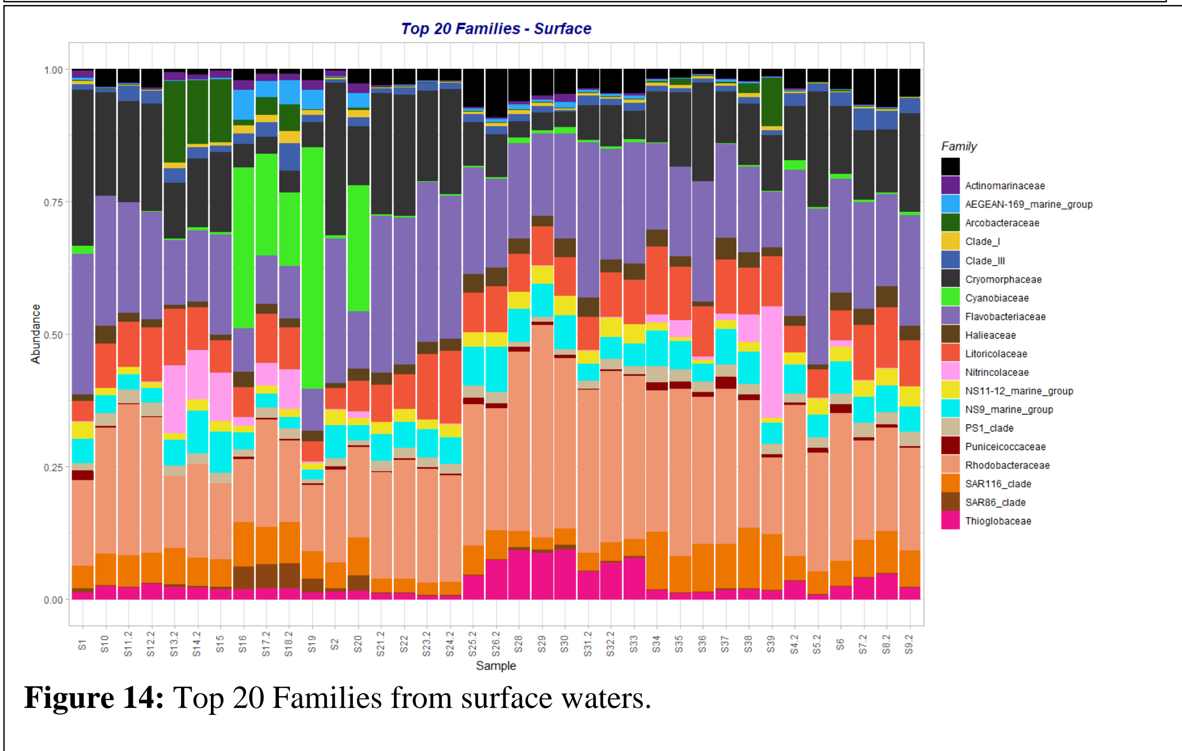

Figure 14: Top 20 Families from surface waters.

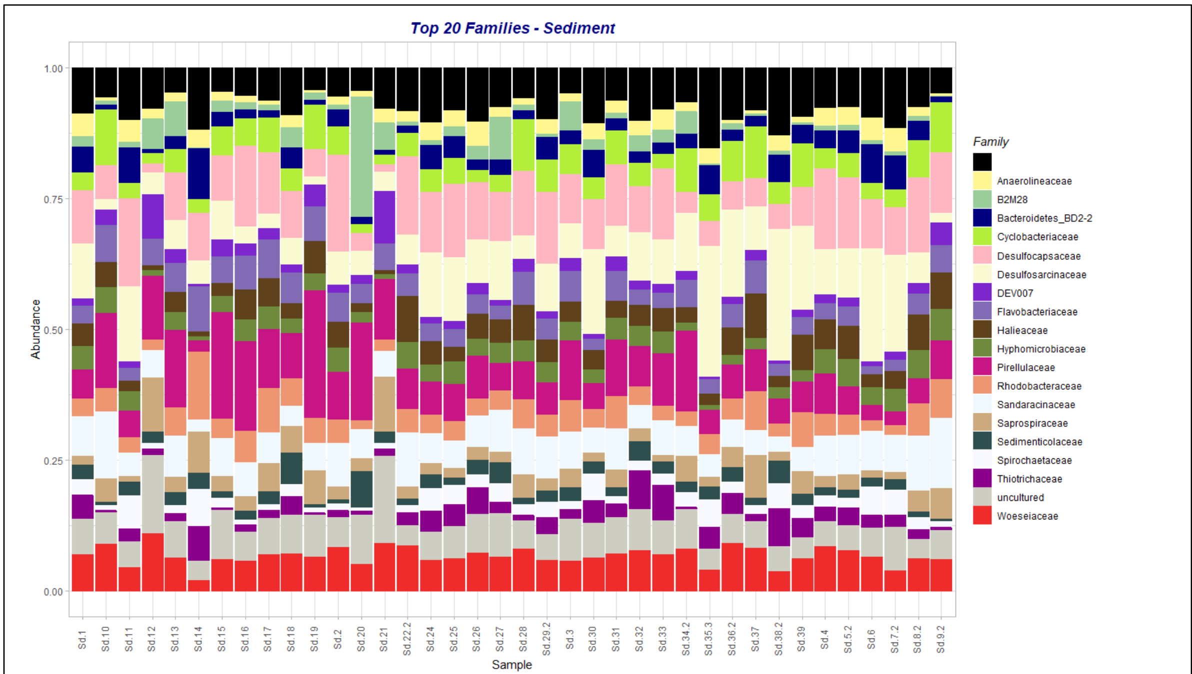

Figure 16: Top 20 Families from sediments.

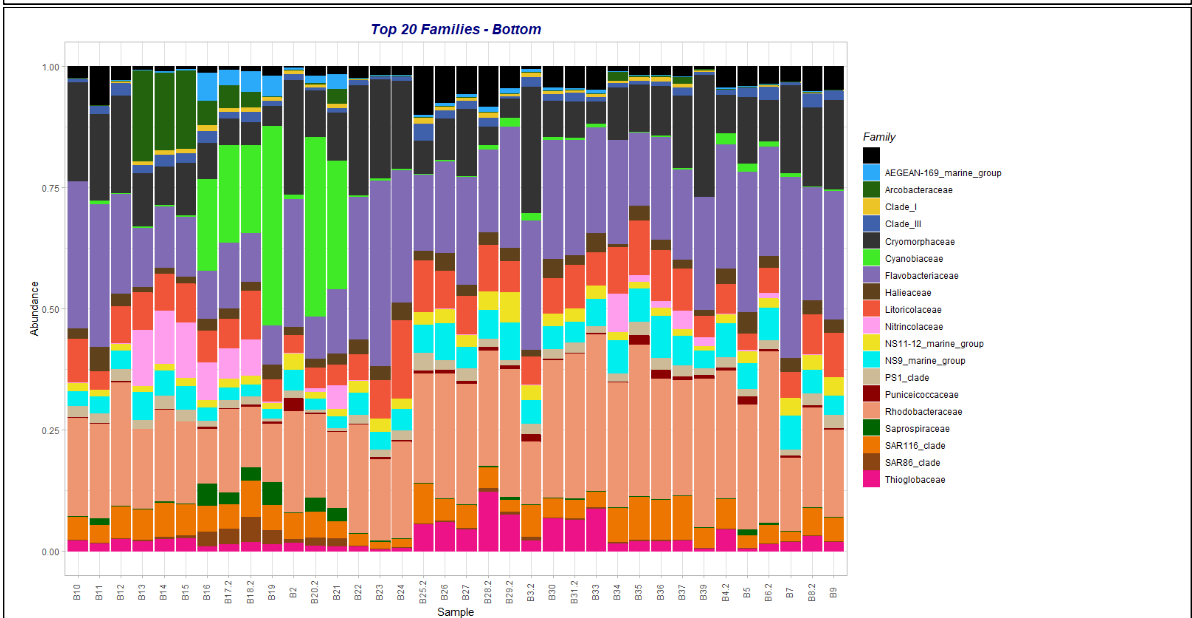

Figure 15: Top 20 Families from bottom waters.

Alpha diversity was also compared via species richness and diversity plots across all sites and different site types (Fig. 17). These provide natural habitat context for the experimental mesocosm results.

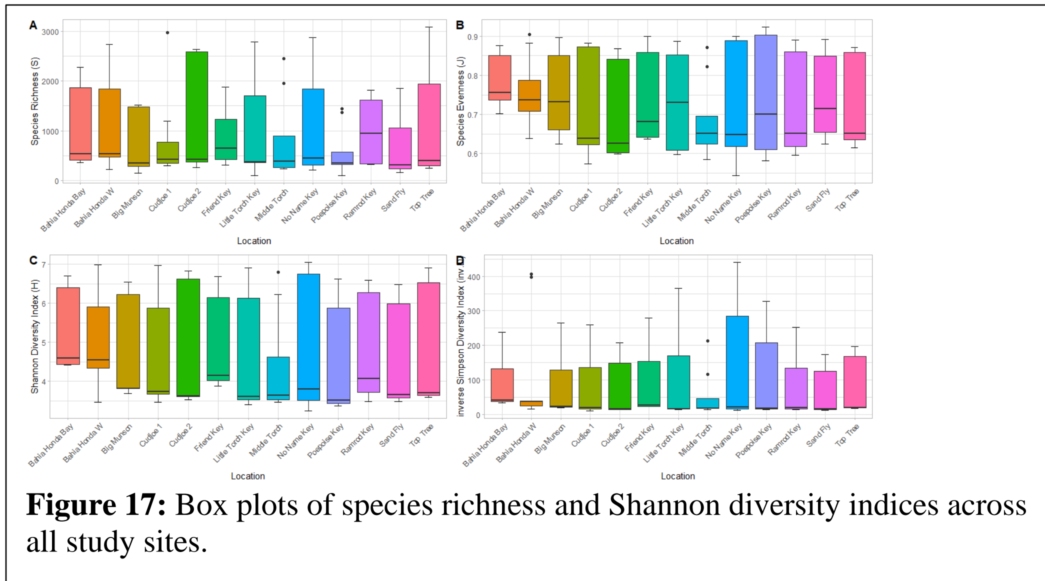

Figure 17: Box plots of species richness and Shannon diversity indices across all study sites.

Summer 2023 samples

Samples from FL Keys reefs were collected at the same sites as Fall/Winter 2022. Several similar results were retained in the summer datasets (Figures 20 – 34). Firstly, we also see the same distinct bacterial composition in the waters at the specific site of Bahia Honda. This difference appears due to higher relative abundances of cyanobacteria, order Synechococcales. These abundances were not as large as the winter samples. The alpha diversity indices corroborate the qualitative analyses, by showing Bahia Honda with the highest species richness and Shannon diversity (Figure 30). Within this scope, Bahia Honda ocean communities had higher species richness than bayside Bahia Honda. Sediment richness > both surface and bottom water communities (Figure 31). The expected dominance of Proteobacteria phyla was observed. Order Rhodobacterales appeared prevalent across most Keys sites, an order suggested as a possible member of SCTLD bacterial consortia (Rosales et al, 2020). More broadly, there were some differences between winter and summer bacterial communities. For example, smaller cyanobiaceae proportions appear in summer samples compared to winter.

Secondly, we see the same clear partitioning of both (shallow, deep) water collections apart from sediment microbial communities in all of the NMDS plots (Figures 33 – 35). There was no distinction between bottom and surface water communities as shown in Fig. 34.

All raw data are now available for downloading at this link:

https://www.dropbox.com/scl/fo/nhjbsfomzodovnzoebo8b/h?rlkey=3b6h8ed4nksuid18mwas5wntq&dl=0

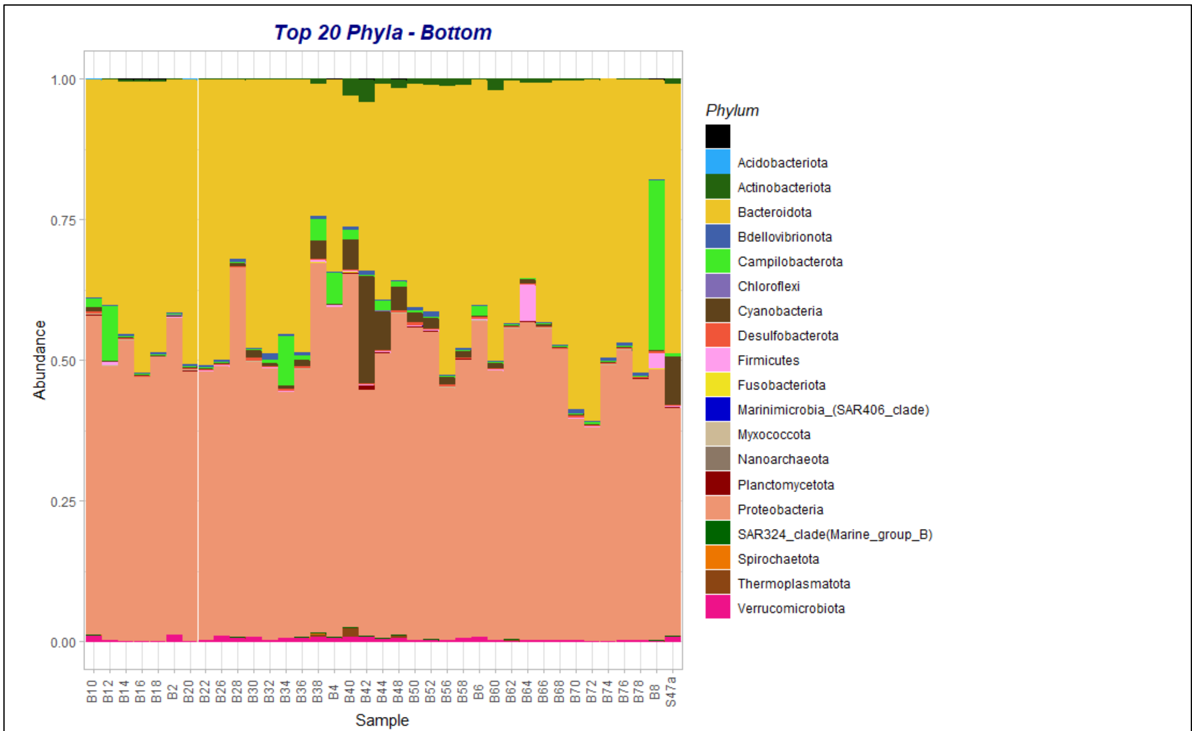

Figure 18: Top 20 Phyla from surface waters, Summer 2023

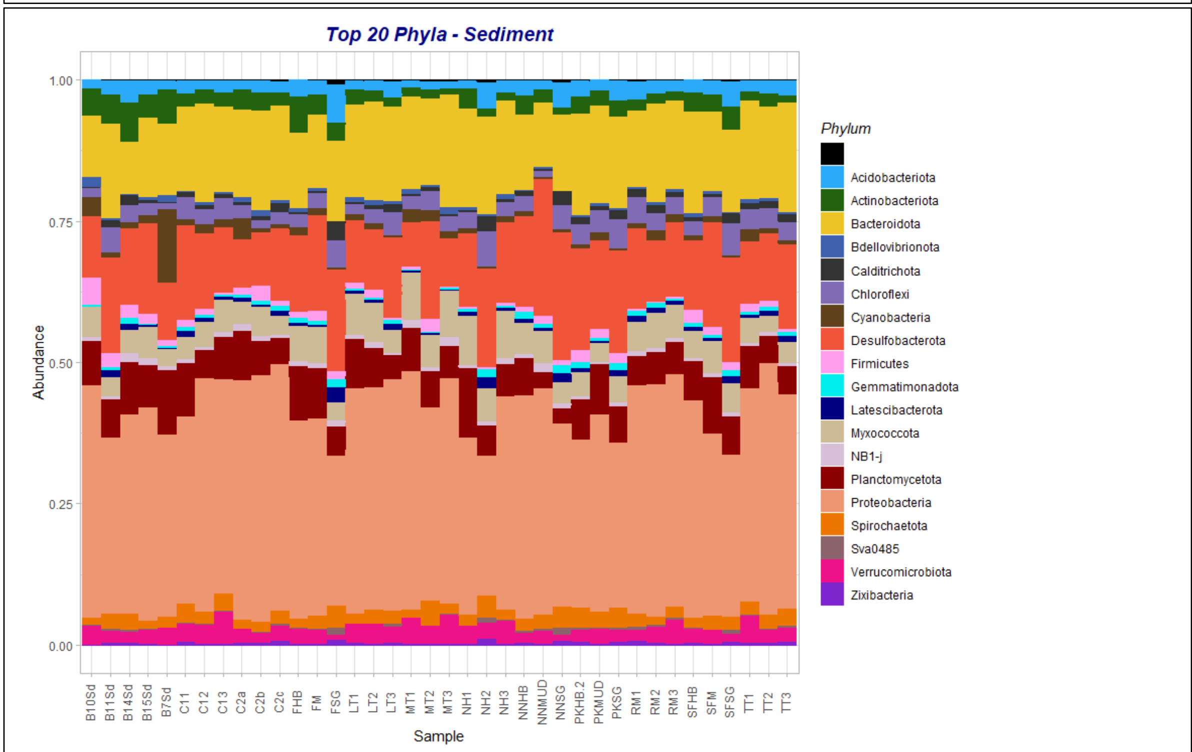

Figure 19: Top 20 Phyla from sediments, Summer 2023 samples.

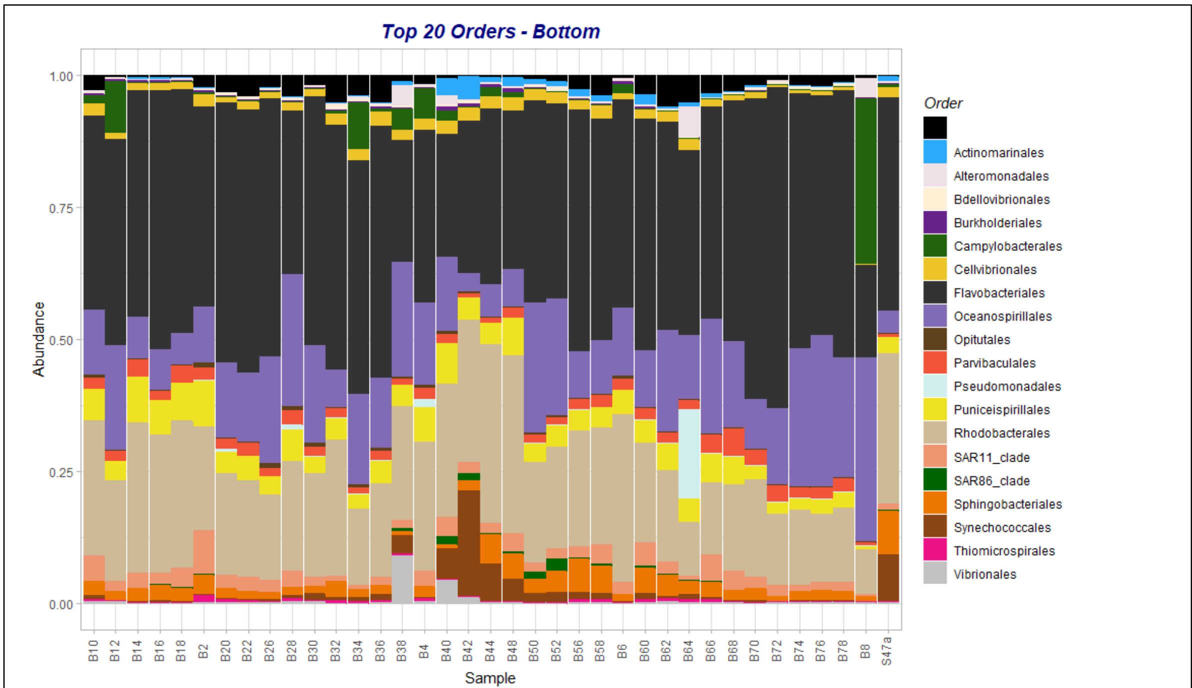

Figure 21: Top 20 Orders from bottom waters, Summer 2023 samples.

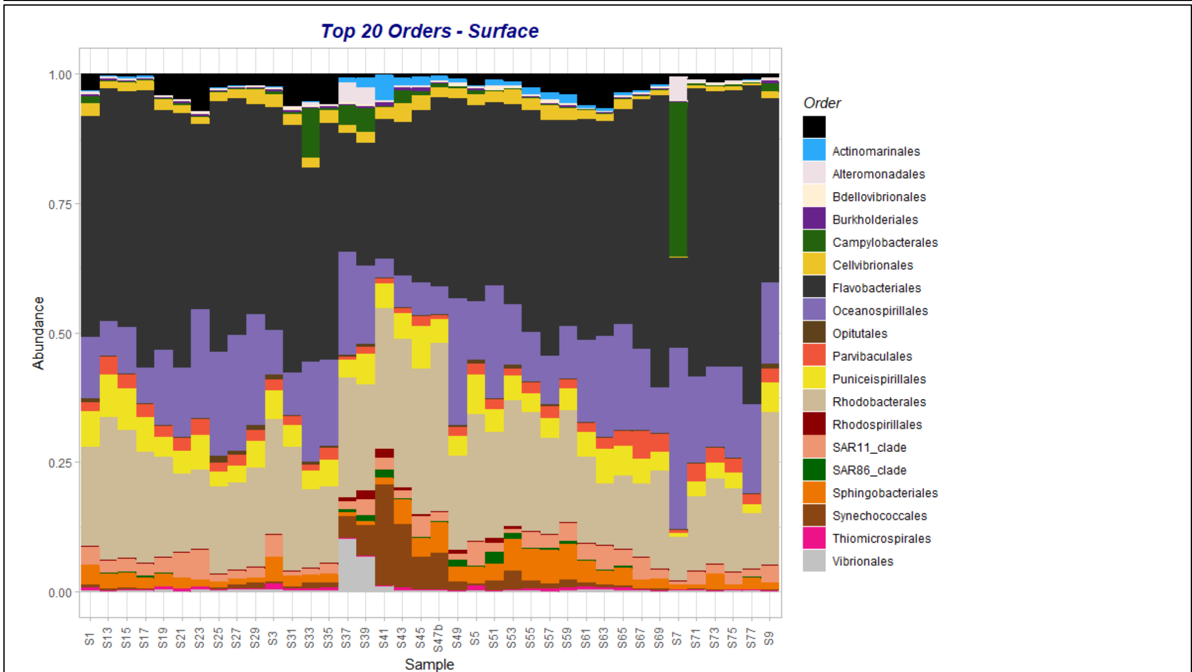

Figure 20: Top 20 Orders from surface waters, Summer 2023 samples.

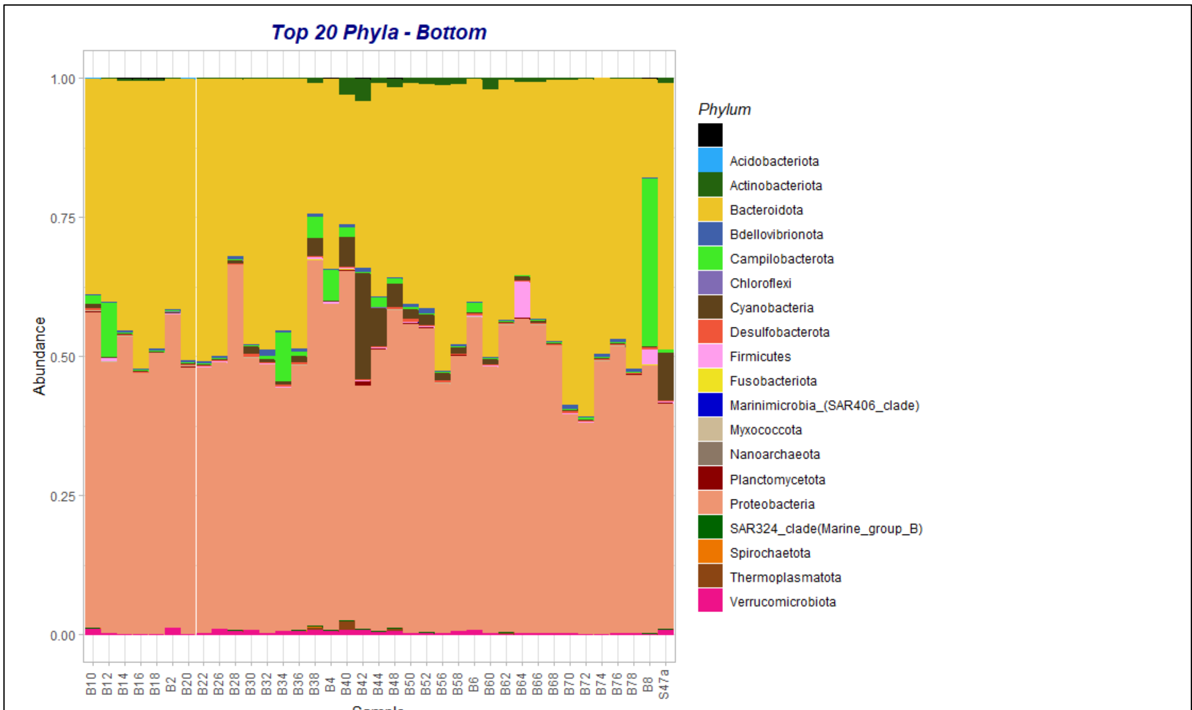

Figure 23: Top 20 Phyla from bottom water. Summer 2023 samples.

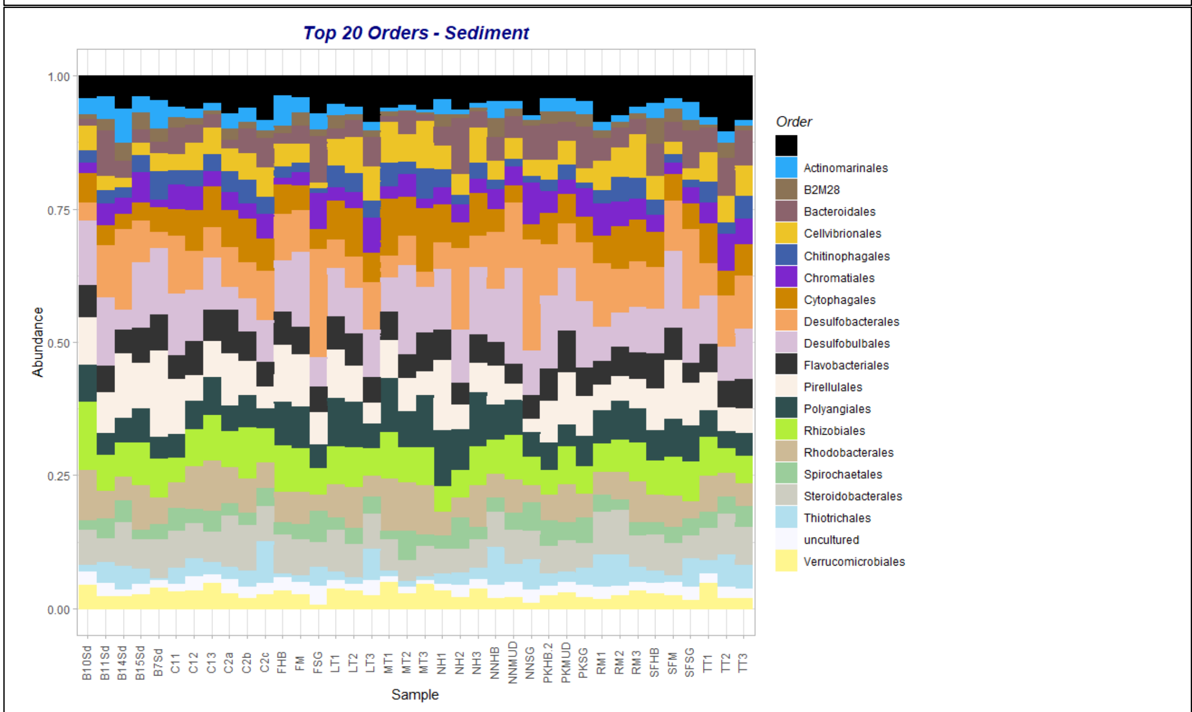

Figure 22: Top 20 Orders from sediments, Summer 2023 samples.

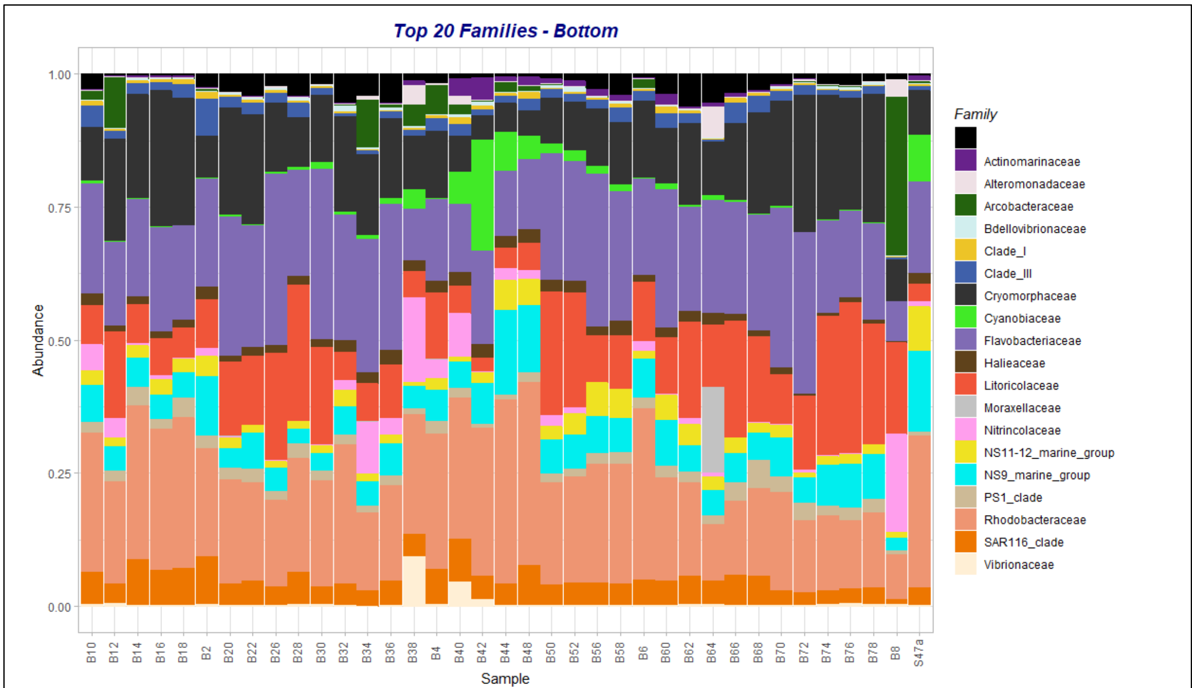

Figure 25: Top 20 Families from bottom waters, Summer 2023 samples.

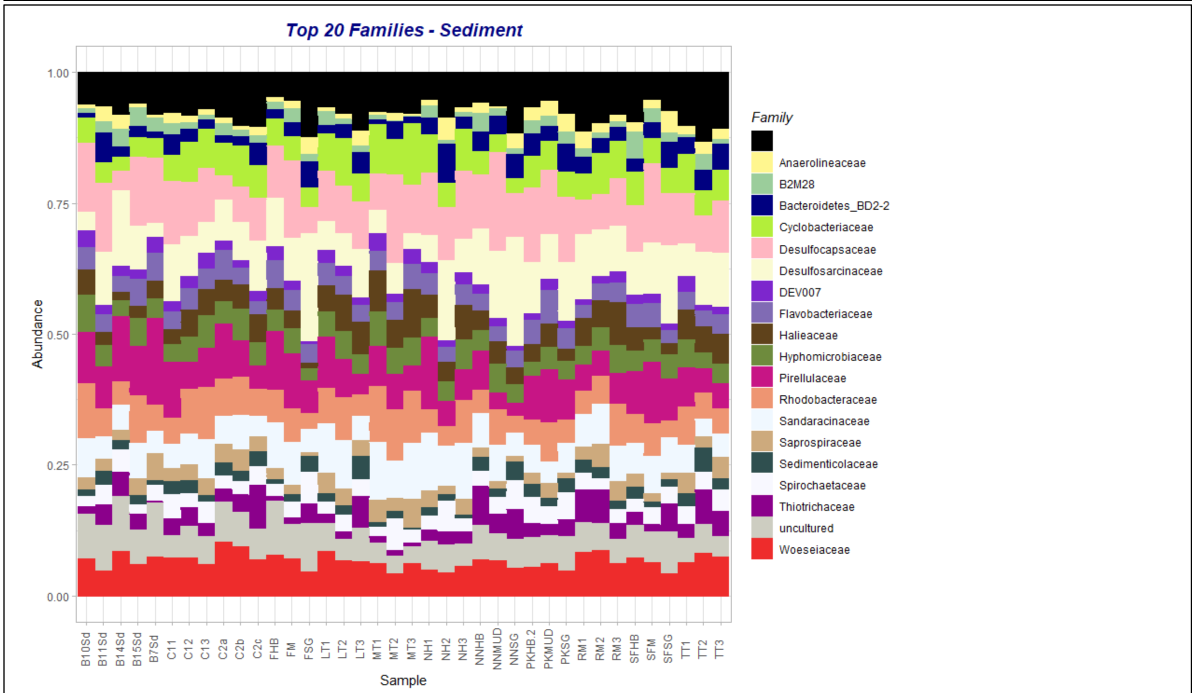

Figure 24: Top 20 Families from sediments, Summer 2023 samples.

Figure 27: Box plots of species richness and Shannon diversity indices across all study sites, Summer 2023 samples.

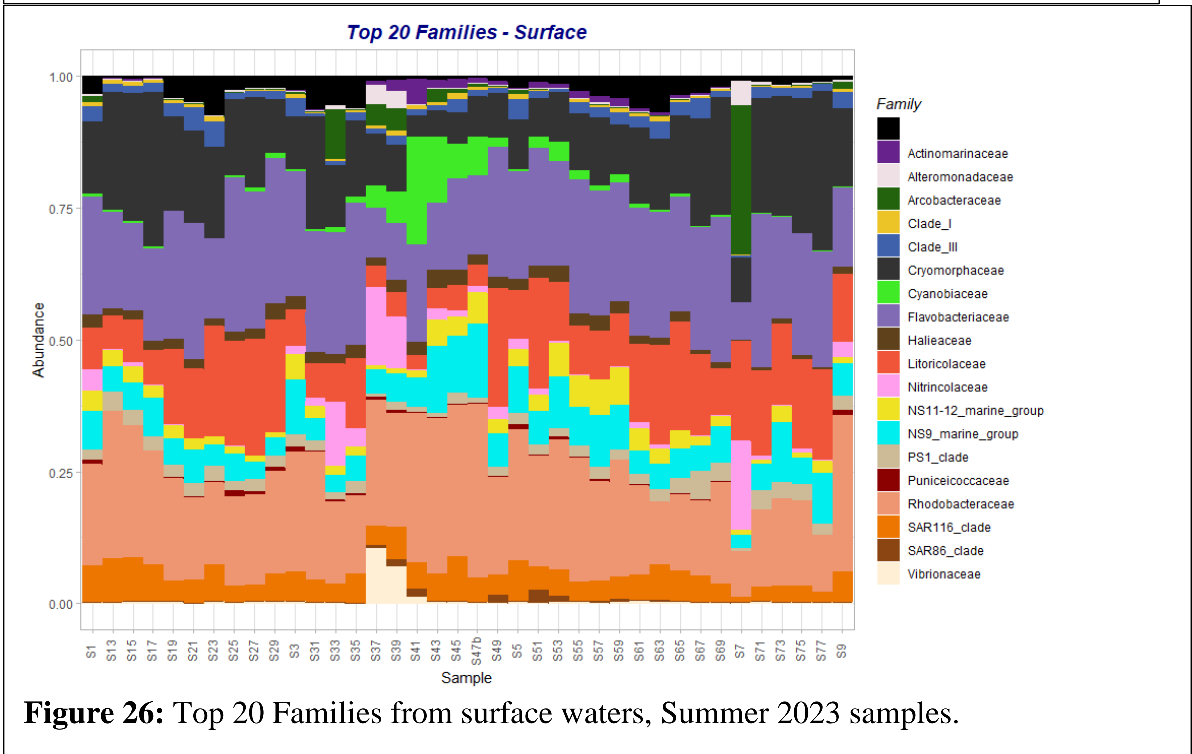

Figure 26: Top 20 Families from surface waters, Summer 2023 samples.

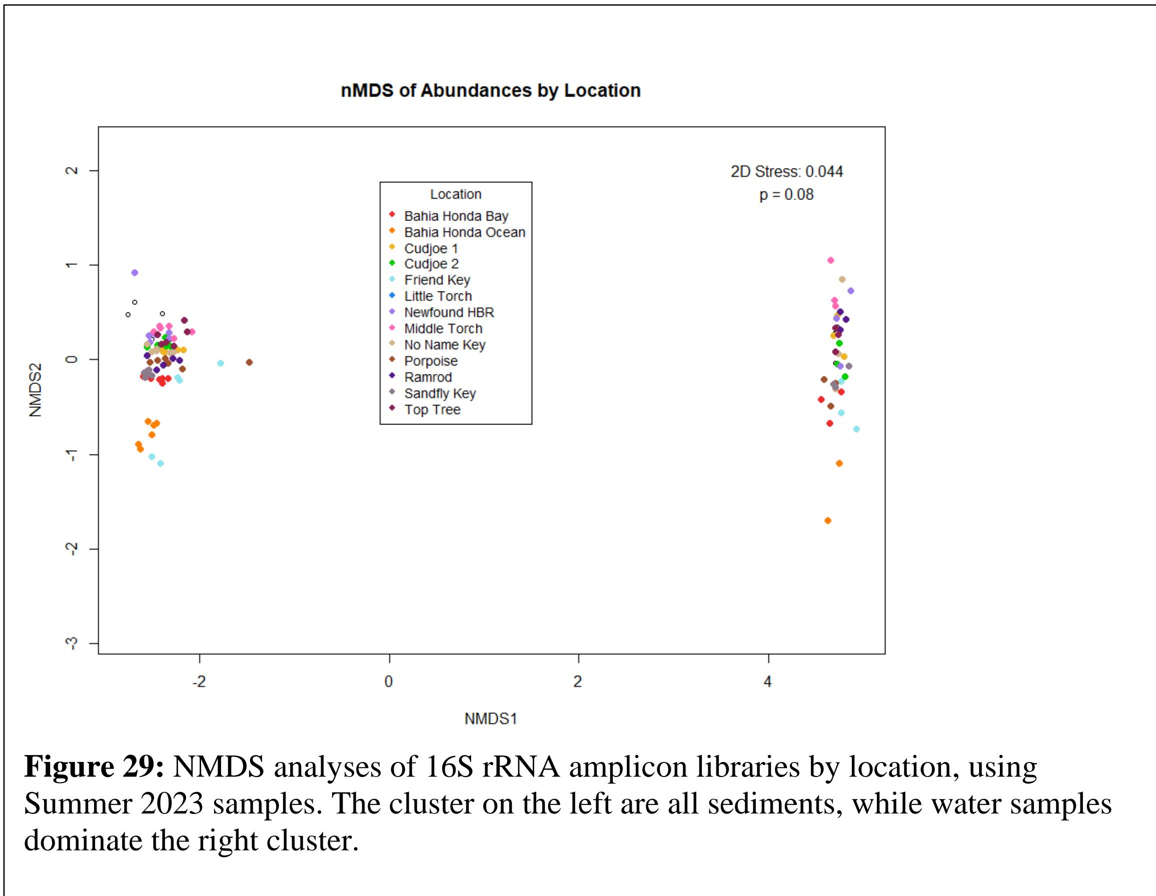

Figure 29: NMDS analyses of 16S rRNA amplicon libraries by location, using Summer 2023 samples. The cluster on the left are all sediments, while water samples dominate the right cluster.

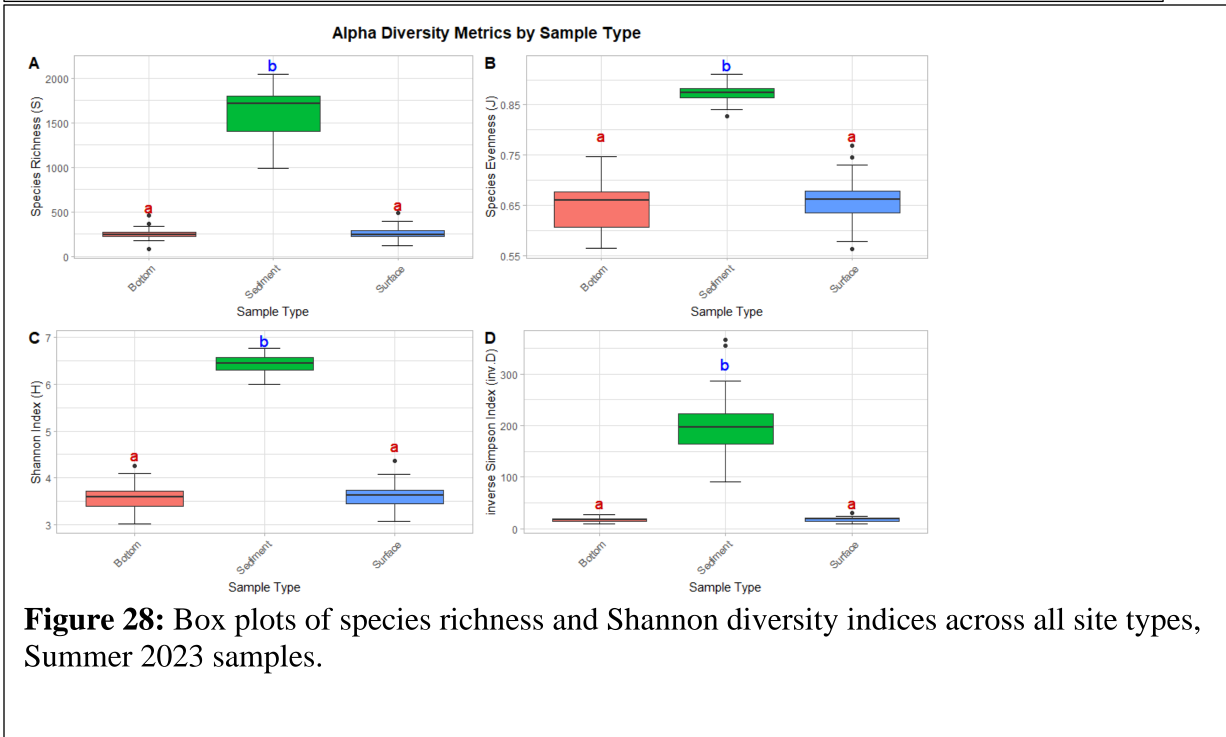

Figure 28: Box plots of species richness and Shannon diversity indices across all site types, Summer 2023 samples.

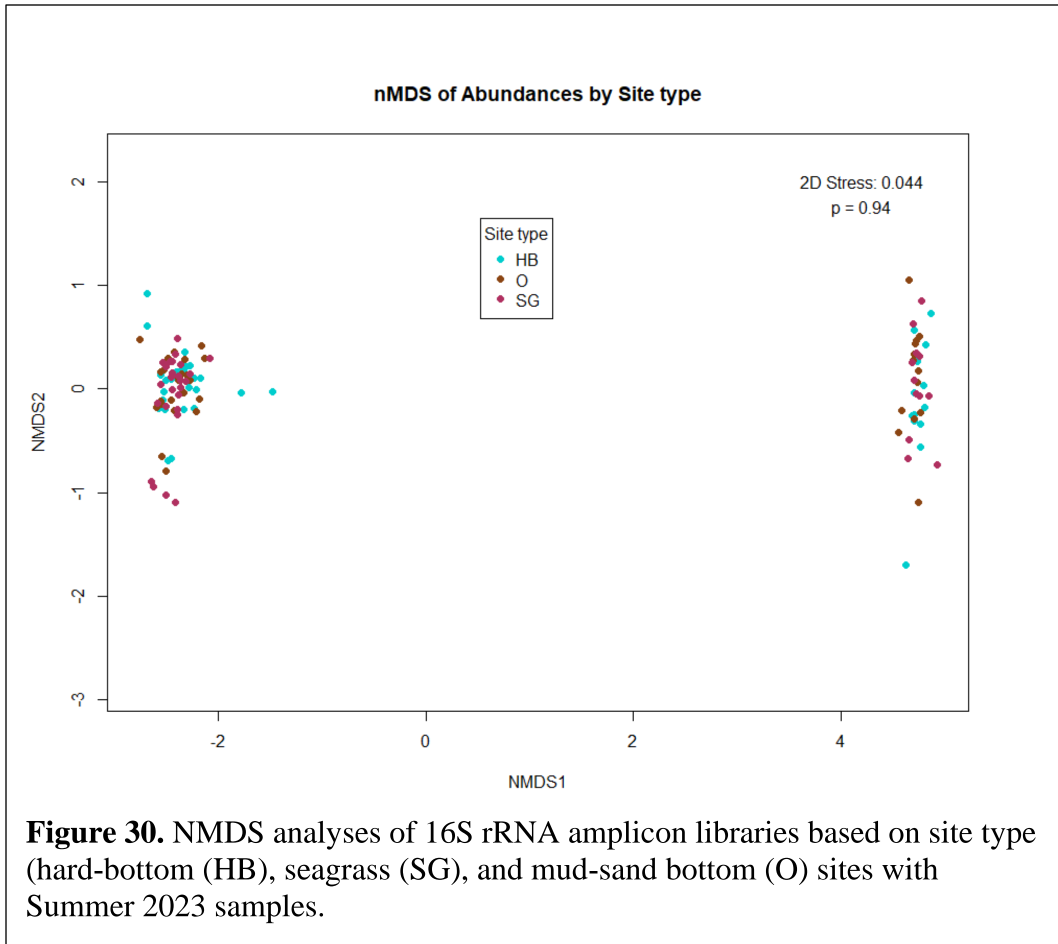

Figure 30. NMDS analyses of 16S rRNA amplicon libraries based on site type (hard-bottom (HB), seagrass (SG), and mud-sand bottom (O) sites with Summer 2023 samples.

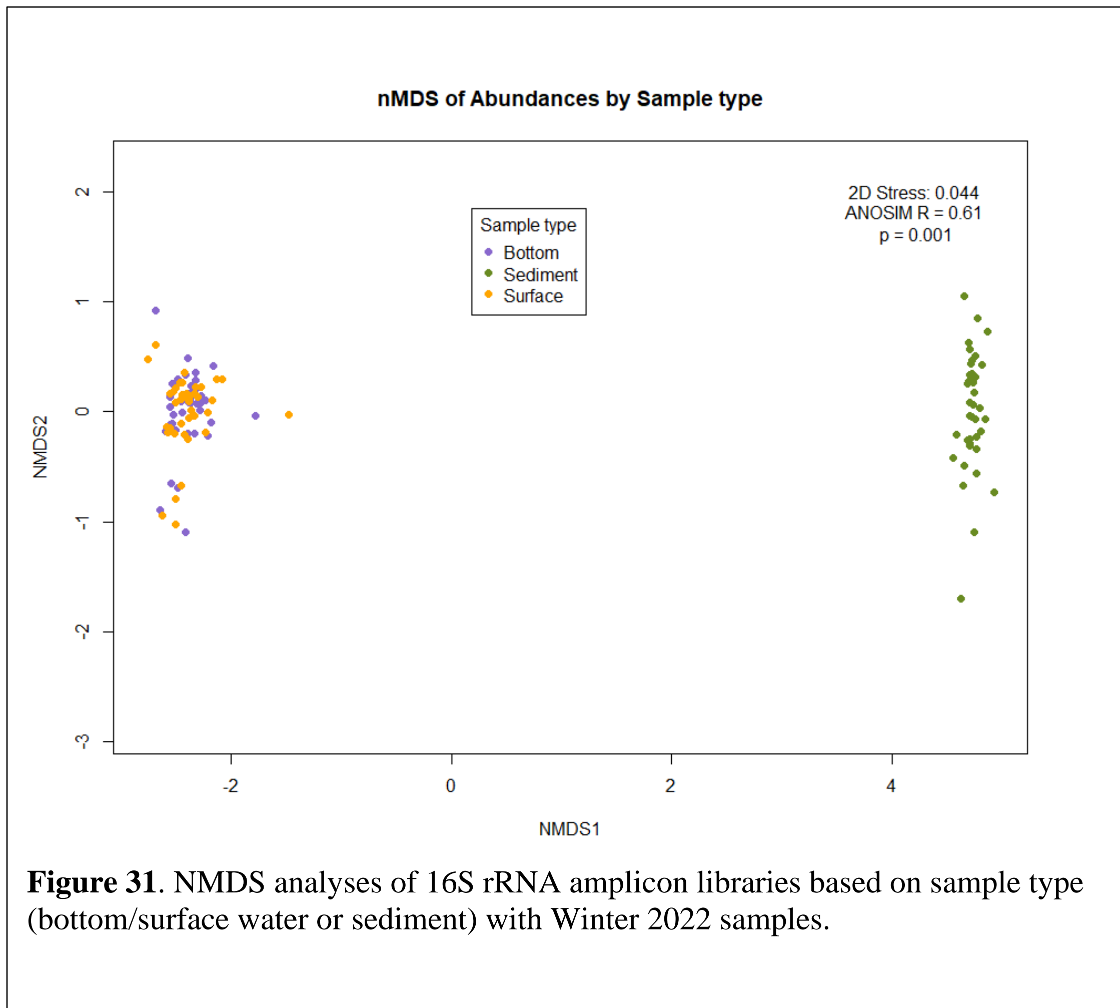

Figure 31. NMDS analyses of 16S rRNA amplicon libraries based on sample type (bottom/surface water or sediment) with Winter 2022 samples.

3.2 Objective 2: Quantify species-specific and community-level filtration of waterborne bacterioplankton and alteration of water chemistry by sponges in mesocosm experiments.

3.2.1 Species-specific Effects of Sponges on Bacterioplankton

Quantitative PCR (qPCR) has been used widely because of its sensitivity to amplify specific target templates and ability to quantitate these templates. All genomic DNA for both tasks were extracted and purified using standard Qiagen Powerlyzer Powersoil kits. DNA yields were quantified with a Qubit 4.0 fluorometer (Invitrogen). We used a Bio-Rad CFX96 with C1000 software to run qPCR reactions. Each sample was always run in triplicate.

An *E. coli* strain JM109 was grown from commercial competent cells in standard nutrient or Luria broth for over 18 hours in order to reach log and stationary phase. For the mesocosm experiments, quantitating the number of *E. coli* cells in the water column applied the following variables: If 1Mbp ~ 1fg (10^-15). DNA for a single molecule or cell, and the *E. coli* genome is 4.6 MB, then the DNA in a single cell weighs 4.6 x 10^{-15} g

Table 6: Example of conversion of cells to DNA for quantitiation in qPCR

Molecular Weight	Cells	Total DNA
a. 4.6 x 10^{-15} g	1000 (10^3)	4.6 x 10^12 (4.6 pg)
b. 4.6 x 10^{-14} g	10000 (10^4)	40.6 pg
c. 4.6 x 10^{-13} g	100000 (10^5)	406. pg (.406 ug)

Standard curves were generated using known quantities of purified *E coli* DNA and by carrying out serial (10 fold) dilutions. DNA quantities are inversely proportional to the Cq value, defined as the cycle number threshold crossed when sufficient fluorescently labeled PCR products are produced. Only true PCR products which are double stranded will bind fluorescent SYBR-green. Quantitative PCR (qPCR) has been used widely because of its sensitivity to amplify specific target templates and ability to quantitate these templates.

The choice of using *E. coli* in mesocosm experiments was based on several factors: 1) E. coli is relatively safe and proven experimental organism and laboratory strains are not harmful to humans; 2) *E. coli* specific primers (in Table 4) for qPCR were readily available from the literature or can be easily designed (Heijnen et al 2006; Miotto et al 2019); 3) in the literature E coli cells are known to be killed or stop replicating in seawater yet, do not readily lyse which allows the cells to be captured on Sterivex filters; 4) at the time writing, the SCTLD pathogen has still not been unequivocally determined. The last factor makes experimental work extremely difficult, since primer design cannot be applied to specific target pathogens.

Table 7: *E. coli* specific primers used for qPCR

Ecoli 45000 entero1 F	CGCGTCGATAACCTCGTACA
Ecoli 45000 entero1 RC	GCAATCAATACGCCGTCACC
Ecoli primer 7 entero1 F	CGCAAGCTTCCGTAACATCG
Ecoli primer 7entero1 RC	GGGTTTCCAGTTCAAACGGC

Our initial mesocosm filtration experiment took place in April 2023 at the new mesocosm facility that we constructed at the Newfound Harbor Marine Institute on Big Pine Key. In this first set of experiments we tested the three target sponge species (*Spheciospongia vesparium, Ircinia campana* and *Spongia cheris*) under three different experimental conditions:

(a) low *E. coli* concentration + static water (no flow in mesocosm)
(b) low *E. coli* concentration + low water flow (0.5 cm/s)
(c) high *E. coli* concentration + high water flow (1 cm/s)

Water samples were stored and filtered onto Sterivex immediately after the designed exposure to three different sponge species, which had technical replicates of varying size. Sterivex filters were then kept cold at 4oC until delivery to the NSU MMG laboratory for DNA extractions and qPCR described above. A summary of the results for the first mesocosm sponge filtration trial are presented in Table 7.

Table 8: A summary of the preliminary mesocosm sponge filtration experiment results listed according to the treatment conditions and sponge species tested. Statistics are presented for the Cq values, which represent the inverse of *E. coli* cell concentrations. Hence, larger Cq values represent greater removal of *E. coli* by filtration.

Treatment	Sponge Type	N	Mean Cq	SD Cq	% Change from Control	% SE
Static - Low Conc	Loggerhead	9	26.4	6.6	13.0	24.9
Static - Low Conc	Vase	6	25.8	6.7	11.0	25.8
Static - Low Conc	Glove	5	31.6	1.5	27.2	4.8
Static - Low Conc	Control	7	23.3	4.6		
Flow - Low Conc	Loggerhead	4	26.0	3.2	11.5	12.2
Flow - Low Conc	Vase	8	22.4	2.2	-2.8	9.8
Flow - Low Conc	Glove	7	25.1	4.6	8.5	18.4
Flow - Low Conc	Control	7	23.4			
Flow - High Conc	Loggerhead	3	23.0	10.4	4.3	45.2
Flow - High Conc	Vase	3	22.0	1.7	0.0	7.9
Flow - High Conc	Glove	5	24.2	7.4	9.1	30.4
Flow - High Conc	Control	3	21.7			

3.2.2 Species-specific Effects of Sponges on Water Chemistry

Our second set of mesocosm experiments were conducted in early June 2023 once the genomic results for the April experiments were available. For this set of experiments, we focused on increasing the replication for each sponge species under the low flow water conditions and with variable *E. coli* concentrations (Table 8). Raw data of the qPCR results are also available in a Dropbox repository: https://www.dropbox.com/scl/fo/nhjbsfomzodovnzoebo8b/h?rlkey=3b6h8ed4nksuid18mwas5wntq&dl=0

There was an initial problem with obtaining accurate dilutions for the standard curves but this was eventually resolved. Furthermore, the melting temperature of the qPCR with these specific *E. coli* primers was further optimized by testing various temperatures with gradient PCRs. As mentioned above, standard curves were generated in each qPCR run. Examples for this are shown in Figure 35.

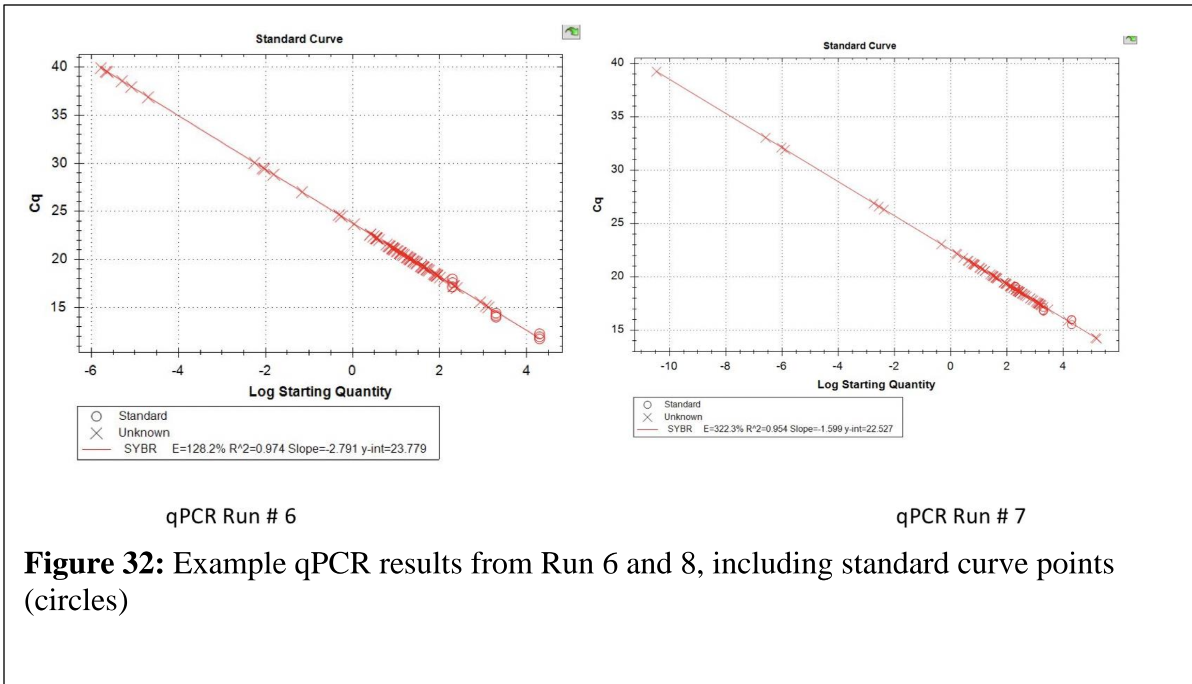

Figure 32: Example qPCR results from Run 6 and 8, including standard curve points (circles)

Table 9: Post hoc analyses of starting DNA concentrations

Sample	(ng/ µL)
Exp. 7	6.84
Exp. 8	5.82
Exp. 39	8.57
Exp. 40	6.09
Exp. 41	7.86
Exp. 53	9.23
Exp. 54	5.64
Exp. 55	5.84
Exp. 66	10.1
Exp. 67	6.98

We found much lower Cq values compared to the preliminary experiments. This is likely due to a higher inoculum of bacteria in these experiments whereas lower Cq values in many of the sponge tanks could also indicate a generally lower level of sponge filtering under warmer summer temperatures. Low Cq values indicate a higher presence of target *E. coli* or perhaps higher total DNA. This was confirmed in a Qubit quantitation of a subset of samples (Table 7) showing that starting DNA amounts will affect qPCR results.

There was more variance in the results from the control mesocosms than we anticipated, which may be a result of one or more variables that include: a) inconsistent quantities of *E. coli* cells seeded into the head tank due to lag times between culturing and inoculations, b) multiple staff assisted in DNA extractions and included a few staff with

less genetics laboratory experience, or c) variable numbers of cells reaching the sponge holding tanks due to differences in tubing, flow, or other unknown factors. All of these factors need to be better standardized in future studies.

After examination of the data, we believe the most realistic and consistent results are obtained when we use the lowest Cq value from replicate controls as the most logical representation of the starting concentration of E. coli in each of the seven independent trial runs. Those results are summarized below in two tables. The first table includes just the June 2023 data whereas the second table combines the results of the April and June experiments conducted under the same experimental condition; that is: low water flow regime.

Table 10: Results of mesocosm of *E. coli* filtration by trials conducted in April 2023 for three species of sponge under a low water flow regime (0.5 cm/s).

Sponge Type	N	Mean Cq	SD Cq	Mean % Change in Cq from Control
Loggerhead	4	26.0	3.2	44.4
Vase	8	22.4	2.2	24.3
Glove	7	23.1	4.6	39.7

Table 11: Results of mesocosm of *E. coli* filtration by trials conducted in June 2023 for three species of sponge under a low water flow regime (0.5 cm/s).

Sponge Type	N	Mean Cq	SD Cq	Mean % Change in Cq from Control
Loggerhead	17	21.2	1.8	12.5
Vase	16	20.8	5.0	14.3
Glove	14	20.8	5.3	17.3

Table 12: Results of mesocosm of *E. coli* filtration by trials conducted in April 2023 and June 2023 for three species of sponge under a low water flow regime (0.5 cm/s)..

Sponge Type	N	Mean Cq	SD Cq	Mean % Change in Cq from Control
Loggerhead	21	21.4	3.1	18.6
Vase	24	21.1	4.4	17.6
Glove	20	22.2	5.8	21.0

Three important findings are demonstrated by these results:

(1) On average, sponges removed approximately 10-40% of the *E. coli* in the water column in essentially one pass of seawater over the sponges at a rate of flow of

0.5cm/s which is empirically relevant for the shallow waters surrounding the Florida Keys.

(2) Removal of *E. coli* from the water column by all sponge species was approximately twice as high during our April experiments as compared to June experiments. This could be due to differences in water temperature, DO, or background picoplankton/bacteria concentrations in the water between the two time periods.

(3) Differences in *E. coli* removal from the water column among sponge species were minimal and inconsistent among time frames.

(4) Water flow has an appreciable effect on sponge filtration efficiency typified by lower filtration of *E. coli* at higher water flow speeds.

4. DISCUSSION

We successfully completed all of the field sampling of 36 sites during winter and spring, constructed a mesocosm facility in which to conduct species-specific sponge filtration of bacteria (including *E. coli* experimentally dosed into the system), conducted two separate filtration trials in those mesocosms, analyzed water chemistry samples from the two field surveys and two mescocosm trials, estimated bacterioplankton concentrations in samples from the two field surveys and two mescocosm trials using flow cytometry, analyzed *E. coli* concentrations in mesocosm trials using quantitative PCR, and characterized the taxonomic structure of waterborne and sediment bacteria communities from 36 field locations during winter and spring. That was an enormous amount of work to accomplish in the 8 months that we effectively had to conduct the study given administrative problems that delayed the project's start.

Objective 1: Examine the relationship between water quality and coastal benthic community composition in hard-bottom, seagrass, and sand/open habitats in the Florida Keys.

Characterization of the benthos in terms of sponge community volume or seagrass area and comparisons of those with bacterioplankton concentrations and water chemistry during one winter (Dec 2022) and spring (May 2023) period revealed no obvious relationships other than distinct differences in bacterial community structure between water column and sedimentary communities. This is counter to our initial hypotheses that waterborne bacterioplankton concentrations would be inversely correlated with sponge volume and perhaps with seagrass density. A more complete statistical analysis of those data that also incorporates other environmental aspects that we measured (e.g., sediment depth, macroalgae composition, other sessile animal abundances) along with a more detailed sponge species-by-species assessment of volume will soon be conducted and may perhaps alter these initial interpretations.

Objective 2: Quantify species-specific and community-level filtration of waterborne bacterioplankton and alteration of water chemistry by sponges.

We were unable to test for putative pathogens associated with the SCTLD coral disease because there remains considerable debate in the scientific literature as to just what those pathogens may be. One cannot design genomic probes to test pathogen concentrations if the pathogen is unknown, so we therefore pivoted and instead tested a known coastal bacteria (*E. coli*) associated with terrestrial run-off and sewage that can be pathogenic. Our mesocosm experiments testing the filtration of *E. coli* at different concentrations from the water column by three common species of shallow-water sponges yielded results consistent with our hypothesis that coastal sponges can effectively remove potentially pathogenic bacteria from seawater.

Previous experiments testing the efficiency of sponges at removing bacteria from water that they directly filtered (i.e., water entering and leaving an individual sponge) have yielded removal efficiencies exceeding 75% [38-41]. However, that over-estimates the actual removal of bacteria by sponges as a mass of water moves past, not necessarily through, a sponge. This difference is equivalent to measuring the removal of oxygen from the atmosphere by human respiration if measured: (a) comparing the difference in oxygen concentration in the air a person inhales vs. exhales, compared to (b) measuring the change in oxygen concentration in the air as it blows past a respiring human. The latter is similar to our experimental procedure and is a more relevant approach if one wishes to test the effect of sponge filtration on the local seawater environment.

Three important findings emerge from our mesocosm results:

(1) On average, sponges removed approximately 10-40% of the *E. coli* in the water column in essentially one pass of seawater over the sponges at a rate of flow of 0.5cm/s which is empirically relevant for the shallow waters surrounding the Florida Keys.

(2) Removal of *E. coli* from the water column by all sponge species was approximately twice as high during our April experiments as compared to June experiments. This could be due to differences in water temperature, DO, or background picoplankton/bacteria concentrations in the water between the two time periods.

(3) Differences in *E. coli* removal from the water column among sponge species were minimal and inconsistent among time frames.

(4) Water flow has an appreciable effect on sponge filtration efficiency typified by lower filtration of *E. coli* at higher water flow speeds.

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6. APPENDICES

Appendix A – List of taxa (common name; scientific name) identified and enumerated in benthic surveys.

Sponges	
Loggerhead	*Spheciospongia vesparium*
Vase	*Ircinia campana*
Brown Branching	Ircinia *sp.*
Stinker	*Ircinia strobilina*
Glove	*Spongia cheris*
Yellow	*Spongia barbara*
Sheepswool	*Hippospongia lachne*
Green Encrusting	*Haliclona viridis*
Fire	*Tedania ignis*
Yellow Fire	*Lissodendoryx sp.*
Grass	*Spongia graminea*
Cake	*Aaptos sp.*
Blue rope	*Niphates erecta*
White Encrusting	*Geodia gibberosa*
Yellow Rope	*Aplysinia fulva*
Brown Rope	*Anthosigmella varians*
Chicken Liver	*Chondrilla sp.*
Black Cake	*Aaptos sp.*
White encrusting	*Geodia gibberosa*
Volcano	*Tethya crypta*
Green Volcano	*Haliclona elanadocia*
Golfball	*Cinachyra sp.*

Delicate	*Adocia sp.*
Variable tube	*Haliclona sp.*
Lavender ridge	*Dysidea sp.*
Yellow cushion	*Biemna sp.*
Bumpy black pore	*Hyrtios sp.*
Corals & Octocorals	
Golfball coral	*Favia fragum*
Lesser Starlet	*Siderastrea radians*
Rose Coral	*Manicina areolata*
Finger coral	*Porites porites*
Angular Seawhip	*Pterogoriga anceps*
Slimy Plume	*Pseudopterogorgia americana*

Appendix B – Total sequence read data from samples (n=111) which provided sufficient sequence quality for analyses in two MiSeq runs. A number of samples (n=48) failed to provide sequence due to technical failures (no DNA, poor sequence quality).

Sample	Sequencing Reads
B10	30307
B11	136812
B12	139222
B13	155324
B14	122372
B15	90284
B16	95645
B17.2	145501
B18.2	66406
B19	76888
B2	121378
B20.2	139265
B21	72522
B22	114940
B23	84114
B24	118986
B25.2	162003
B26	87283
B27	120423
B28.2	79328
B29.2	62436
B3.2	27550
B30	119621
B31.2	164368
B33	128412
B34	32740
B35	143048
B36	17591
B37	171647
B39	166005
B4.2	126739
B5	201865
B6.2	193221
B7	47776
B8.2	27515
B9	24126

S1	152383
S10	148570
S11.2	181782
S12.2	177164
S13.2	170849
S14.2	153952
S15	90331
S16	57473
S17.2	158426
S18-2	40402
S19	69756
S2	51484
S20	95607
S21-2	66469
S22	189655
S23-2	82440
S24-2	70359
S25-2	82938
S26-2	84114
S28	166604
S29	173971
S30	110058
S31-2	77740
S32.2	139449
S33	134592
S34	154692
S35	143237
S36	204776
S37	160544
S38	80838
S39	48473
S4.2	163409
S5.2	177907
S6	143217
S7.2	143745
S8.2	129305
S9.2	157896
Sd.1	21946
Sd.10	43027
Sd.11	207338
Sd.12	136757

Sd.13	111013
Sd.14	135746
Sd.15	79411
Sd.16	122821
Sd.17	144105
Sd.18	101334
Sd.19	125394
Sd.2	70298
Sd.20	149309
Sd.21	194405
Sd.22.2	129380
Sd.24	120135
Sd.25	207128
Sd.26	87810
Sd.27	98982
Sd.28	19786
Sd.29-2	47485
Sd.3	137060
Sd.30	172441
Sd.31	158340
Sd.32	147838
Sd.33	174530
Sd.34-2	62830
Sd.35.3	13146
Sd.36-2	52092
Sd.37	146414
Sd.38-2	64822
Sd.39	165735
Sd.4	73979
Sd.5-2	65925
Sd.6	69181
Sd.7-2	58457
Sd.8-2	66325
Sd.9.2	73201