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Fine grained compositional analysis of Port Everglades Inlet microbiome using high throughput DNA sequencing

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Similar to natural rivers, manmade inlets connect inland runoff to the ocean. Port Everglades Inlet (PEI) is a busy cargo and cruise ship port in South Florida, which can act as a source of pollution and nutrients to surrounding beaches and offshore coral reefs. Understanding the composition and fluctuations of bacterioplankton communities ("microbiomes") in major port inlets is important due to their impacts on surrounding marine environments. We hypothesize annual microbial fluctuations based on seasons (wet vs dry), assessed by high throughput 16S rRNA amplicon library sequencing. Surface water samples were collected weekly for one year, creating a high sampling frequency and fine sampling scale. Over 1.4 million 16S rRNA V4 reads generated a total of 16,384 Operational Taxonomic Units (OTUs) from the PEI habitat. We observed Proteobacteria, Cyanobacteria, Bacteroidetes, and Actinobacteria as the most dominant phyla. Analysis of potentially pathogenic genera show the presence of *Staphylococcus* and Bacillus, albeit at lower relative abundances during peak shipping and tourist months (November –April), thus underscoring their relatively low presence**.** Statistical analyses indicated significant alpha diversity differences when comparing microbial communities with respect to time**.** This observation probably stems from the low community richness and abundance in August, which had lower than average rainfall levels for Florida's wet season. The lower rainfall levels may have contributed to less runoff, and subsequently fewer bacterial groups being introduced into the port surface waters. Bacterioplankton beta diversity differed significantly by month and season. The 2013-2014 dry season (October-April), was warmer and wetter than historical averages, which may have driven the significant differences in beta diversity. Increased nitrogen and phosphorous concentrations were also observed in these months, possibly creating favorable bacterial growth conditions. To our knowledge, this study represents the first to sample a large port at this fine sampling

scale. These data can help establish underlying inlet microbial community baselines, and supplement the vital monitoring of local marine and recreational environments, which appears more poignant in the context of local reef disease outbreaks and worldwide coral reef collapses in the wake of a harsh 2015-16 El Nino event.

Fine-Grained Compositional Analysis of Port Everglades Inlet Microbiome Using High Throughput DNA Sequencing

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Abstract

Background. Similar to natural rivers, manmade inlets connect inland runoff to the ocean. Port Everglades Inlet (PEI) is a busy cargo and cruise ship port in South Florida, which can act as a source of pollution and nutrients to surrounding beaches and offshore coral reefs. Understanding the composition and fluctuations of bacterioplankton communities ("microbiomes") in major port inlets is important due to their impacts on surrounding marine environments. We hypothesize annual microbial fluctuations based on season (wet vs dry), which will be profiled by high throughput 16S rRNA amplicon library sequencing and analysis.

Methods & Results. Surface water samples were collected weekly for one year, creating a high sampling frequency and fine sampling scale. Over 1.4 million 16S rRNA V4 reads generated a total of 16,384 Operational Taxonomic Units (OTUs) from the PEI habitat. We observed Proteobacteria, Cyanobacteria, Bacteroidetes, and Actinobacteria as the most dominant phyla. Analysis of potentially pathogenic genera show the presence of *Staphylococcus* and Bacillus, albeit at lower relative abundances during peak shipping and tourist months (November –April), thus underscoring their relatively low risk for public health concerns.

Discussion. Statistical analyses indicated significant alpha diversity differences when comparing microbial communities with respect to time**.** This observation probably stems from the low community richness and abundance in August, which had lower than average rainfall levels for Florida's wet season. The lower rainfall levels may have contributed to less runoff, and subsequently fewer bacterial groups being introduced into the port surface waters. Bacterioplankton beta diversity differed significantly by month and season. The 2013-2014 dry season (October-April), was warmer and wetter than historical averages, which may have driven

the significant differences in beta diversity. Increased nitrogen and phosphorous concentrations were also observed in these months, possibly creating favorable bacterial growth conditions. To our knowledge, this study represents the first to sample a large port at this fine sampling scale and sequencing depth. These data can help establish underlying inlet microbial community baselines, and supplement the vital monitoring of local marine and recreational environments, all the more poignant in the context of local reef disease outbreaks and worldwide coral reef collapses in the wake of a harsh 2015-16 El Nino event.

70

71 **Introduction**

72 A continental coastal zone can represent a gradient across distinct biogeographical
73 boundaries (freshwater, brackish and saltwater). Mangroves, streams, or manmade inlets in these
74 transition zones provide potential links. Fort Lauderdale's Port Everglades Inlet (PEI) (also
75 known as the Port Everglades Shipping Channel [PESC]), in Broward County, FL is a man-made,
76 dredged, deep-water port located along the southeastern coast of subtropical Florida (Stauble
77 1993; http://www.porteverglades.net; NOS, 2011). Located directly offshore from the PEI is a
78 major US coral reef tract (Banks et al., 2008; Rowher 2010; Staley et al., 2017), as well as
79 multiple recreational beaches, fishing piers, and watersport areas (Stamates et al., 2013). Coral
80 reefs, beaches, and recreational water sport areas are impacted both positively and negatively by
81 resident microbial communities of these areas. The ecosystem services of bacteria in marine
82 communities include nutrient cycling and symbiosis, while disadvantages include the possible
83 presence of marine pathogens, which may cause illness in the marine environment or to humans
84 utilizing it.

85 Marine microbes are major components of global biogeochemical cycles, especially
86 carbon, nitrogen and phosphorous cycles (Azam et al., 1983; Arrigo, 2004).
87 For example, marine nitrogen-fixing bacteria are responsible for the transformation of N_2 into
88 NO_3^-, maintaining the balance of biologically available nitrogen, and are therefore of paramount
89 importance to the nutrient cycling between the atmosphere and the world's oceans (Canfield et
90 al., 2010) as well as climate forcing feedbacks via the complicated production and flux of
91 greenhouse gases CO_2 and N_2O (Duce et al. 2008; Gao 2015). While marine microbes are crucial

components in biogeochemical cycling, certain microbes introduced into marine environments via land-based pollution sources can impact both coral reef and human health.

This study reports an extensive environmental genetics characterization of the bacterioplankton community (or "microbiome") from the surface seawater in PEI. Water samples were collected from June 2013 to May 2014 to determine monthly alpha (α) and beta (β) diversity fluctuations. This study examines changes in composition of PEI's surface water microbiome over a year, and differs from others because samples were taken on a weekly basis allowing for a finer sampling scale (or higher time resolution).

A primary hypothesis of this study predicted that during the typical wet and warm season (May-September), an increased diversity of bacterial species would occur in PEI water. Secondly, changes in water chemistry would correlate with changes in abundance of certain microbial genera. The third hypothesis predicted that harmful pathogens to both humans and marine life will be present in a higher abundance during the wet season.

This study applied Illumina MiSeq high-throughput sequencing technology to complete DNA sequencing of water samples, differing from previous studies which were largely restricted to culture-based or RT-qPCR methods (Symonds et al., 2016; Aranda et al., 2015; Carsey et al., 2012).

Materials & Methods

Water Sample Collection, DNA Extraction, and Chemical Analysis. A total of 82 surface seawater samples were collected weekly, at ebb tide, from PEI in Broward County, FL by kayak over a year-long timespan (2013-2014). Three 1.0 liter (L) water samples were collected at a depth of 0.5 meters every week from two different sites within in the inlet. Water

temperature was measured in-situ at time of sampling with a glass thermometer. Salinity measurements were taking immediately upon returning to lab (within 30 minutes of sample collection) with a refractometer. Precipitation values were obtained using NOAAs data from the National Center for Environmental Information (http://www.ncdc.noaa.gov/cag/time-series). For each site, 1.0 L of water was filtered using Pall GN Metricel® grid 47 mm, 0.45µm filters, through vacuum filtration.

Total microbial genomic DNA was extracted using MO BIO's PowerLyzer™ PowerSoil® kit (Carlsbad,CA). After extraction a 1% agarose gel was run to ensure that genomic DNA extraction was successful. After gel verification, DNA concentration was measured using the Qubit 2.0 (Life Technologies).

Surface water samples collected at each site were subjected to ion chromatography (IC) analysis using a Thermo Scientific Dionex ICS-1600 system (Bannockburn, IL). After filtration of particulates using syringe filters, samples were diluted 1000 times before injection into the IC. Ion chromatography analysis was used to detect the presence and measure the concentrations of chemical ions in the PEI surface water. A total of five anions - fluoride, chloride, nitrate, phosphate, and sulfate - were analyzed with calibration curves from standard solutions and detection limits at approximately 10 ppb.

Sequencing Sample Preparation. Samples were prepared for MiSeq® sequencing following Illumina's 16S Metagenomic Sequencing Library Preparation guide (Illumina, 2013). The final pooled DNA library was diluted to a concentration of 4 pM with a 50% spike in of 12.5 pM PhiX.

Sequence Analyses. Raw sequence analysis was performed using Quantitative Insights into Microbial Ecology (QIIME) 1.8.0 (Caporaso et al., 2010a). Raw sequence outputs were

analyzed using QIIME, where paired ends were joined using PANDAseq 2.8.1 at a 90% confidence level (Masalla *et al,* 2012). Chimera checking was completing using USEARCH 6.1 (Edgar, 2010). Operational Taxonomic Units (OTUs) were picked using the cd-hit method (Li and Godzik, 2006). Sequences were aligned using PyNAST (Caporaso et al*.,* 2010b) and assigned taxonomy using the greengenes 13_8 referene database (DeSantis et al*.,* 2006; McDonald et al*.,* 2012). Sequences with less than a 75% sequence identity with a minimum length of 150 basepairs (bp) were discarded from downstream analysis. Rarefaction curves were generated in QIIME to determine if existing diversity was fully captured with existing sampling efforts.

Statistical Analyses. All statistical analyses were completed using R Statistical Software Version 3.1.1. The R package phyloseq (McMurdie and Holmes, 2013) was used for downstream statistical analysis of MiSeq-generated sequences. Richness and evenness estimates were determined using the plyr package (Wickham, 2011). Shannon, Simpson, Observed, and Chao1 alpha diversity estimates were generated and plotted. To determine statistical significance in alpha diversity a non-parametric kruskal-wallis test was used to complete pairwise comparisons between month, location, and the interaction between month and location. Results were considered significant if *p*-values were less than 0.05.

Statistical analyses for beta diversity was completed by calculating both Bray-Curtis distance and weighted Unifrac distance using phyloseq. A non-parametric Adonis test was used to complete pairwise comparisons of samples for month, location, and season. A *p*-value less than 0.05 with high R^2 values are considered significant.

Regression Analysis. A series of Multiple Least Squares Regressions were used to assess possible relationships between each bacterial taxa and the environmental variables taken as part

of the study. A backward selection method was used with both entry and model retention set at alpha=0.10. All regression analyses were carried out using SAS Statistical Software (SAS Institute).

Pathogen Detection. Pathogenic bacteria were detected through filtering out orders known to contain pathogens of interest using the subset_taxa command in phyloseq. The filtered orders were pruned to contain only the top 50 OTUs from the subset of data in the previous step. The abundance, genus, and month were plotted using the plot_bar command in phyloseq.

Results

16S rRNA Sequence Output Overview. A total of 151 samples were collected weekly from PEI from July 2013-June 2014, with 82 samples used for DNA sequencing. Illumina MiSeq sequencing yielded a total of 1,435,072 raw sequences with Q scores greater than 30 and an average read length of 255 basepairs. The average number of reads per sample was 17,287 with the minimum number of reads per sample being 5,666 and the maximum number of reads per sample being 80,122. A total of 25,020 chimeric sequences were removed from the dataset with a total of 1,410,052 sequences left for OTU table generation and database alignment. After filtering of sequences to remove identical sequences and subsequences a total of 395,009 unique sequences were left for taxonomic assignment using the Greengenes 13_8 database. A total of 16,384 OTUs were generated.

Alpha Diversity. Alpha diversity is the diversity within an ecosystem and is often expressed in terms of species richness (Whittaker, 1972). The most abundant taxa in the bacterioplankton community were present in >1% in all samples. To compare alpha diversity metrics non-parametric (Monte Carlo) two samples t-tests were carried out for Chao1, Shannon, Simpson,

and Observed Species diversity indices. No significant differences were observed (P> 0.05) between alpha diversity indices. Community richness and relative abundance of PEI bacterioplankton species were assessed using a Kruskal-Wallis to test for significant differences. Bacterioplankton richness exhibited significant differences when comparing the month of August with the months of October, November, and December, as well as when comparing seasons (wet vs. dry) (p value <0.05). A rank abundance curve for the top 50 OTUs in the dataset was generated (Fig. 1). The slope of the curve is gradual indicating that species diversity in the inlet waters is high. The most abundant OTU (2717) is classified as being the family Rhodobacteraceae in the phylum Proteobacteria. The second most abundant OTU (1389) is classified as being the family Cryomorphaceae in the phylum Bacteroidetes (Fig. 1).

Abundant Taxa. Cyanobacteria, Bacteroidetes, Proteobacteria, and Actinobacteria are the most abundant bacterial phyla in this PEI study and are present in all samples. The frequencies of these bacterial phyla are above 1.0% in all samples, indicating that they are major components of the bacterial assemblage present in PEI surface water samples (Fig. 2). These results are consistent with previous studies completed not only on coastal marine environments, but also on the bacterioplankton community associated with PEI (Campbell et al., 2015; Gifford et al., 2014; Elifantz et al., 2013; Rappe et al., 2000). The most abundant taxonomic groups at the class level included Flavobacteriia, Alphaproteobacteria, Gammaproteobacteria, and Synechococcophycideae**.** *Synechococcus* and *Candidatus portiera* were the most abundant genera. Seasonal trends were observed with the Synechococcophycideae class, where relative abundance levels doubled in the wet season months.

Beta Diversity. Beta diversity for PEI surface water samples was determined by calculating both Bray-Curtis dissimilarity and weighted Unifrac distance. Adonis tests analyzed the strength of

significance that a specific group had in determining variations in distances between samples. Beta diversity was tested for differences in location, season, and month that the samples were taken.

Location. The results of the Adonis (PERMANOVA) tests for both Bray-Curtis dissimilarity and weighted Unifrac distance showed no significant differences in the beta diversity of samples taken at two different locations in PEI ($P > 0.05$, $R^2=0.0137$ (Bray-Curtis); $P > 0.05$, $R^2=0.0146$(Unifrac)).

Season. Southern Florida has two main seasons. The wet season, which ranges from May-September and the dry season, which ranges from October-April. Significant differences in microbial community composition were observed between seasons (Fig. 3). The Adonis test results for both the Bray-Curtis dissimilarity and weighted Unifrac distance are significant ($P<0.05$, $R^2 = 0.157$ (Bray-Curtis); $P<0.05$, $R^2 = 0.203$ (Unifrac)).

Month. A major objective in this study was to analyze the PEI surface water on a more frequent time scale then had previously been done. Water samples were taken weekly for a year to allow for samples to be analyzed by month. To determine if there were differences in the microbial community composition by week or month, an Adonis test applied Bray-Curtis dissimilarity values and weighted Unifrac values (Fig. 4). The results for these tests came back significant ($P<0.05$, $R^2 = 0.605$ (Bray-Curtis); $P<0.05$, $R^2 = 0.706$ (Unifrac)). After determining that community composition differed significantly by month, multiple pairwise comparisons between all months were done using Bray-Curtis dissimilarity values. Results of the Adonis test reveal that all month comparisons were significant ($p<0.05$), but not at the same level. Some months show lower p-values than others, indicating these months had more differences in microbial community composition (Table S1).

Linear Regression Analysis with Chemical and Environmental Metadata. Multiple least Squares regression analysis was completed using the number of reads for the top nine most abundant bacterial classes and the available environmental metadata: chloride ion, sulfate ion, rainfall, water temperature and salinity (Table 1). The level of alpha was set to 0.10 for statistical significance. The R^2 value is the measure used to determine how well the data fits the regression line. The higher R^2 values indicated better data fits with the model. Results for the class Gammaproteobacteria (R^2=0.21) show a significant relationship with salinity (p=0.0173) and water temperature (p=0.0601). The class Flavobacteriia(R^2=0.21) show a significant relationship with salinity (p=0.101) and water temperature (p=0.0204). The class Acidomicrobiia (R^2=0.21) show a significant relationship with salinity (p=0.0013). The class Alphaproteobacteria (R^2=0.13) show a recognizable relationship with salinity (p=0.0008). The class Chloroplast (R^2=0.13) show a recognizable relationship with chloride ion concentration (p=0.0016) and sulfate ion concentration (p=0.0016). The class Betaproteobacteria (R^2=0.14) show a recognizable relationship with salinity (p=0.0005). The class Synechococcophycideae (R^2=0.20) show a significant relationship with water temperature (p=0.001) and chloride ion concentration *(*p=0.0155). The class Actinobacteria (R^2=0.21) show a significant relationship with salinity (p=0.0514) and water temperature (p=0.0001). The unclassified bacterial groups (R^2=0.07) had a somewhat weak relationship with rainfall (p=0.0184). The R-squared values are lower than expected, but some of the variations can be explained by the model. An interesting trend seen in the data was that *Proteobacteria* and *Cyanobacteria* showed an inverse trend in abundances, which correlated with temperature and rainfall data to some degree (Fig. S1**).**

Potentially Pathogenic Bacteria. Orders known to contain pathogenic bacteria were partitioned out of the overall dataset and filtered to the top 100 OTUs. The top 50 OTUs were plotted using

stacked bar charts to determine relative abundance. This diagram highlighted possible pathogens present in PEI. In the *Bacillales, Clostridiales,* and *Lactobacillales* orders, the most abundant pathogenic genera were *Staphylococcus* and *Streptococcus. Staphylococcus* appeared in all months except for February, and was in highest abundance during the summer months of July and August (Fig. 5).

Streptococcus appeared in all months except November and May and was present in highest abundance during April, August, and September. It is important to note that a common fecal pathogen *Enterococcus*, was only seen in high enough abundance to be detected in the month of September (Fig 5).

The orders *Enterobacteriales, Campylobacterales,* and *Vibrionales* contained few pathogens in high enough abundance to be detected in the top 50 OTUs. In the *Enterobacteriales* order the genus *Citrobacter* was present in highest abundance over the entire year (Fig. 6). This genus is normally only pathogenic if an individual is immunocompromised. The other orders in this group did not contain any pathogenic genera in high abundance.

Discussion

Inlets are not rivers, but both share several similarities: large volumes of water flowing into and out of them; connection of inland city and agriculture runoff with the ocean; and generation of visible plumes distinct from offshore marine waters. However, as a mostly manmade construction, inlets may be subject to more detailed or controlled characterizations. For example, our study considered that different water masses (shallow and deeper) may be generated during ebb tides, which can lead to hydrodynamic complexity (Stamates et al., 2013). For example the mean volume of ebb tide surface water flow (4.41 million cubic meters per tidal

phase) is about half the water flow of the deep channel. Although our sampling site was slightly outside the canal on the ICW, we expected that most of our water samples reflect mostly surface inland and not oceanic water. In a study by Miller and colleagues (2016), it was found that dredging of the Port of Miami from late 2013 to early 2015 increased coral tissue loss on adjacent reefs due to sedimentation. The dredging in the Port of Miami appears to have exacerbated local environmental stress due to the overlap with increased water temperatures leading to a mass bleaching event and increases in reef disease (Miller et al., 2016). This points to the utility of the present report as a baseline prior to the PEI dredging slated to occur in the near future.

Previous to this study, the majority of the research and monitoring completed in the inlet used culture-based and qPCR approaches, and focused on presence of fecal indicator bacteria (Futch et al., 2011; Craig unpublished, 2012). An earlier study from our laboratory (Campbell et al 2015) utilized high-throughput 454-pyrosequencing technology to generate baseline knowledge of PEI microbiomes, but the sampling scale was quarterly. Beta diversity statistics run by Campbell et al (2015) suggest a more unique bacterioplankton profile from inlets (vs outfall and reefs), which is confirmed by the present analyses. The present study also shows that PEI bacterioplankton are comparable to other marine microbial communities found in different marine and coastal environments. The utilization of Illumina sequencing allows for a much higher number of 16S rRNA reads per sample than in the previous inlet study, but do not have as long of a read length compared with 454-pyrosequencing technology. Both studies on the PEI microbiome yielded complementary data creating a strong baseline of the microbiome present in the inlet. These studies can be used by local county and public health officials, who complete routine monitoring on port waters, and by environmental scientists looking to see what the

impacts of the microbial community in PEI might have on the surrounding coastal beaches and the adjacent Florida coral reef (Aranda et al, 2015).

Bacterioplankton Community Composition Taxa Fluctuations: Location, Month, and Season. The most abundant phyla in all samples (>1%) were Proteobacteria, Cyanobacteria, Bacteroidetes, Actinobacteria, Verrucomicrobia, and Euryarchaeota. These organisms are consistent with previous studies completed on marine coastal waters (Campbell et al., 2015; Gifford et al., 2014; Elifantz et al., 2013; Rappe et al., 2000). Significant differences in community composition were seen in alpha diversity when comparing the month of August with the months of December, October, and November. This is most likely due to the low species richness, diversity, and abundance seen in the samples for the month of August. The low species richness, diversity, and abundance values in August, could be due to the abnormally low rainfall for this month. Beta diversity compares differences among groups. In this study the groups examined were location, month, and season. Significant differences were observed only when comparing bacterial communities at month and season. Location was excluded as a variable for any further analysis. At the phylum taxonomic level only slight fluctuations in microbial community composition can be seen throughout the year. The most drastic shifts occur with Cyanobacteria, which decrease in relative abundance during the winter months, or dry season, and increase in abundance during the summer and early fall, or wet season months. Our data correlate with previous observations of increased cyanobacterial blooms in Florida's coastal and freshwater ecosystems in the late summer and early fall months. The blooms are caused by warm water conditions paired with increased sunlight levels, and nutrient loading from urban runoff (Flombaum et al., 2013). At the class taxonomic level the most common taxa were Alphaproteobacteria, Flavobacteriia, Synechoccophycideae, Chloroplast, Gammaproteobacteria,

Actinobacteria, and Acidimicrobiia. These results are similar to taxonomic composition seen in other coastal microbial community studies (Campbell et al., 2015; Gifford et al., 2014; Elifantz et al., 2013; Rappe et al., 2000). More pronounced fluctuations in microbial community composition are observed at the class taxonomic level, specifically with Synechoccophycideae, in which relative abundance doubles during the wet season months, while Flavobacteriia decreases during that same time. For this same biogeographic area of PEI, Campbell et al (2015) also showed that Flavobacteriaceae appeared to significantly fluctuate with season and salinity parameters, which are probably linked with the wet season. Although not measured in this study, total suspended solids (TSS) and nitrate levels also affected Synechococcus and Rhodobacteraceae abundance. Synechoccophycideae is the most abundant organism in the Cyanobacteria phylum, and therefore would be expected to have a similar shift in abundance to what was seen at the phylum level. The most pronounced shifts in microbial composition can be seen at genus level classification. The most abundant organisms at the genus level were *Candidatus portiera* and *Synechococcus*. These two organisms were also seen in the highest abundance in a previous study completed on Southeast Florida's inlets, outfalls, and reef environments (Campbell *et al.,* 2015).

Flavobacteriaceae is considered a major component in all ocean microbial communities. They are important organisms in the microbial loop, breaking down large organic molecules such as chitin and proteins (Tully et al., 2014). They are also associated with areas of high primary productivity and can break down algal polymers (Gomez-Pereira et al., 2010). The family Flavobacteriaceae also includes the genus *Psychroserpens*, which has been identified in Thailand and Bermuda reef communities and associated with amoebic gill disease in fishes (Somboonna et al. 2014; Bowman and Nowak, 2004; Giovannoni and Cho, 2003). In previous studies this genus

was found present year-round, with increases in abundance in the summer. In the current dataset, the genus *Psychroserpens* was present at its highest abundance in the months of February, March, and May and was almost absent in the month of September, and in very low abundance in October and August. The low abundance of this organism in August, September, and October could be due to the fluctuations in precipitation. The month of August had significantly low precipitation and increased salinity values. In comparison, the month of September had very high precipitation values and low salinity. These drastic fluctuations may have impacted the survivability of *Psychroserpens sp.*

Potential Pathogens

PEI represents a point source of pollution introducing harmful pollutants into the surrounding marine environments including the Florida coral reef tract and recreational beaches (Banks et al., 2008 and Stamates et al., 2013). Due to the influence of the inlet waters on the surrounding marine environments, it is important to examine the presence of pathogenic organisms in the inlet waters.

The order Bacillales contain three known pathogenic genera *Staphylococcus, Bacillus,* and *Paenibacillus*. The only one of these genera known to cause ocean-related illness is *Staphylococcus spp.*, which appeared in almost all months and had the highest abundance levels of all three genera. *Staphylococcus* abundance levels were highest in the months of July and August. *Staphylococcus* is a genus of gram-positive bacteria commonly found on the nails, skin, and hair of humans (Lian *et al,* 2012). This taxon can thus be shed directly into coastal waters from bathers. The well-known species in this genus, *S. aureus*, can also cause illness in humans. *S. aureus* has a high resistance to salinity, making it a potential threat to other humans using the contaminated water source for recreational purposes. While this species commonly links to both

368 human symbiosis and illnesses, marine mammals have also been infected (van Elk et al., 2012;
369 Bik et al., 2016). The origin of the strain of *S. aureus* that is contracted by marine mammals was
370 most likely from terrestrial sources introduced into the marine environment via runoff (van Elk *et*
371 *al,* 2012). Studies examining the abundance of *Staphylococcus* over a wet and dry season at a
372 heavily visited coastal area observed increased abundance of *S. aureus* during the wet season
373 (Curiel-Ayala et al., 2012). The trends of this data also showed increased abundance of
374 *Staphylococcus* during Florida's wet season.

375 Two pathogenic genera in the Lactobacillus class occurred in the PEI water samples.
376 *Streptococcus* was present year-round in this study. *Streptococcus* appeared in highest abundance
377 in the month of January. Increased freshwater input and warm water conditions could have been
378 the cause of the increased abundance of these organisms. This presence also coincided during the
379 prime shipping season in Port Everglades, which may have had an impact on abundance levels of
380 *Streptococcus spp.*

381 *Enterococcus spp.* are important fecal indicator bacteria, most often utilized to assess
382 fecal contamination on recreational beaches and coastal areas (Aranda et al*.,* 2015; Heaney et al*.,*
383 2014; Wade et al*.,* 2003; US Environmental Protection Agency, 1986 and 2004). A recent study
384 examining the number of exceedances of enterococci on recreational beaches in Miami-Dade
385 County, FL from 2000-2010 (Aranda et al., 2015) showed that beaches were only in exceedance
386 of the allowable levels of enterococci 3% of the time. This study examined data generated by the
387 Florida Healthy Beaches Program, which samples weekly. No patterns in regards to rainfall or
388 storms were seen in correlation with enterococci exceedances, although this may be due to the
389 sampling frequency and high decay rate of enterococci in marine waters (Aranda et al., 2015). In
390 contrast to this, a study completed by Curiel-Ayala and colleagues in Mexico (2012), showed

increased *Enterococcus* levels during the rainy season, and the highest concentrations of the genus corresponding to highest tourist presence. The highest levels of eneterococci seen in the Miami-Dade study were in March and October, which could be due to high tidal levels in October, and possible tourism influences in the month of March overlapping with spring break (Aranda et al, 2015). Interestingly, in a previous study examining presence of pathogens in PEI, no *Enterococcus spp.* were observed (Campbell *et al,* 2015). This may be due to the different sequencing platforms that were used in the studies, or that *Enterococcus spp.* are not prevalent inhabitants in PEI. More intensive studies would need to be completed to determine presence of *Enterococcus spp.* in PEI.

It was interesting that the causative agent of the white pox disease for coral *Acropora palmate,* human fecal bacteria *S. marcescens* was not observed in the top 100 OTUs in the Enterobacteriales class, although a recent high throughput molecular study of coral white band disease identified only 5 orders with large numbers of disease-associated OTUs: *Flavobacteriales, Alteromonadales, Oceanospirillales, Campylobacterale* and *Rhodobacterales* (Gignoux-Wolfsohn and Vollmer, 2015). Also of note, is the absence of *Vibrionales* pathogens in our dataset. A previous study which analyzed waters from PEI showed the presence of *Vibrionales,* although it was not present in high abundance (Campbell et al. 2015)

Significance and Conclusions

This study has provided one of the first in depth profiles of bacterioplankton in metropolitan S. Florida waters. Specific marine habitats, such as coral reefs or mangroves, have well defined optimal conditions for thriving and can be sensitive to small perturbations (Precht and Miller 2007; Hoegh-Guldberg et al., 2007). The data from this study should be helpful to local environmental managers, such as the Southeast Florida Coral Reef Initiative (SEFCRI,

414 http://www.dep.state.fl.us/coastal/programs/coral/sefcri. htm), which aims to protect and monitor
415 S. Florida reef habitats. Characterizing ecosystem inputs, such as nutrients and pollutants, and
416 more recently microbial loads will likely contribute to better management of their overall health.
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References

Aranda D, Lopez JV, Solo-Gabriele HM, Fleisher JM. 2015. Using probabilities of enterococci exceedance and logistic regression to evaluate long term weekly beach monitoring data. *Journal of Water and Health*. 14(1):81-89. DOI:10.2166/wh.2015.030.

Arrigo KR. 2004. Marine microorganisms and global nutrient cycles. *Nature*. 437: 349-355. DOI:10.1038/nature04159

Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil L, Thingstad F. 1983. The ecological role of water-column microbes in the sea. *Marine Ecology Progress Series*. 10:257-263. DOI:0171-8630/83/0010/0257/$ 02.00

Banks KE, Riegl BM, Richards VP, Walker BE, Helmle KP, Jordan LKB, Phipps J, Shivji M, Spieler RE, and Dodge RE. 2008. Chapter 5: The reef tract of continental Southeast Florida (Miami-Dade, Broward, and Palm Beach Counties, USA). In: *Coral Reefs of the USA*. Dordrecht: Springer Verlag,175-220.

Bik EM, Costello EK, Switzer AD, Callahan BJ, Holmes SP, Wells RS, Carlin KP, Jensen ED, Venn-Watson S, Relman DA. 2016. Marine mammals harbor unique microbiotas shaped by and yet distinct from the sea. *Nature Communications*. 7. DOI:10.1038/ncomms10516.

Bowman JP, Nowak B. 2004. Salmonid gill bacteria and their relationship to amoebic gill disease. *Journal of Fish Diseases.* 27(8): 483-492.

Campbell AM, Fleisher J, Sinigalliano C, White JR, Lopez JV. 2015. Dynamics of marine bacterial community diversity of the coastal waters of the reefs, inlets, and wastewater outfalls of southeast Florida. *MicrobiologyOpen*. 1-19. DOI: 10.1002/mbo3.245

Canfield DE, Glazer AN, Falkowski PG. 2010. The evolution and future of Earth's nitrogen cycle. *Science***.** 330(6001): 192-196. DOI:10.1126/science.1186120.

[a]Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley, GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R, 2010. QIIME allows analysis of high-throughput community sequence data. *Nature Methods*. *7*(5): 335-336. DOI:10.1038/nmeth.f.303

[b]Caporaso JG, Bittlinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R. 2010. PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics*. 26(2): 266-267. DOI: 10.1093/bioinformatics/btp636

Carsey TP, Stamates JS, Bishop JR, Brown CJ, Casanova HL, Featherstone CM, Gidley ML, Kosenko M, Kotkowski RM, Sinigalliano CD, Visser LA, Zhang, JZ 2013: Broward County coastal ocean: Water quality cruises, 2010-2012. NOAA Technical Report (in preparation).

Cho J-C, Giovannoni SJ. 2003. *Croceibacter atlanticus* gen. nov., sp. nov., a novel marine bacterium in the family *Flavobacteriaceae*. *Systematic and Applied Microbiology*. 26(1): 76-83. https://doi.org/10.1078/072320203322337344

Curiel-Ayala F, Quinones-Ramirez EI, Pless RC, Gonzalez-Jasso, E. 2012. Comparative studies on *Enterococcus, Clostridium perfringens,* and *Staphylococcus aureus* as quality indicators in tropical seawater at a Pacific Mexican beach resort. *Marine Pollution Bulletin,* 64(10): 2193-2198. DOI:10.1016/j.marpolbul.2012.07.052

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. 2006. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and Environmental Microbiology*. 72(7):5069-5072. DOI:10.1128/AEM.03006-05

Duce RA, LaRoche J, Altieri K, Arrigo KR, Baker AR, Capone DG, Cornell S, Dentener F, Galloway J, Ganeshram RS, Geider RJ, Jickells T, Kuypers MM, Langlois R, Liss PS, Liu SM, Middelburg JJ, Moore CM, Nickovic S, Oschlies A, Pedersen T, Prospero J, Schlitzer R, Seitzinger S, Sorensen LL, Uematsu M, Ulloa O, Voss M, Ward B, Zamora L. 2008. Impacts of Atmospheric Anthropogenic Nitrogen on the Open Ocean. *Science.* 320(5878): 893-897. DOI:10.1126/science.1150369

Edgar RC. 2010. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics.* 26(19): 2460-2461. DOI:10.1093/bioinformatics/btq461.

Elifantz H, Horn G, Ayon M, Cohen Y, Minz D. 2013. *Rhodobacteraceae* are the key members of the microbial community of the initial biofilm formed in Eastern Mediterranean coastal seawater. *FEMS Microbiology Ecology.* 85: 348-357. DOI:10.1111/1574-6941.12122

Flombaum P, Gallegos JL, Gordillo RA, Rincon J, Zabala LL, Jioa N, Karl DM, Li WKW, Lomas MW, Veneziano D, Vera CA, Vrugt JA, Martiny AC. 2013. Present and future global distributions on marine Cyanobacteria *Prochlorococcus* and *Synechococcus*. *PNAS.* 110(24): 9824-9829. www.pnas.org/cgi/doi/10.1073/pnas.1307701110.

Futch JC, Griffin DW, Banks K, Lipp EK. 2011. Evaluation of sewage source and fate on Southeast Florida coastal reefs. *Marine Pollution Bulletin*. 62(11):2308-2316. DOI:10.1016/j.marpolbul.2011.08.046

Gao S. 2015. Managing short-lived climate forcers in curbing climate change: An atmospheric chemistry synopsis. *Journal of Environmental Studies and Sciences.* 5: 130 - 137. DOI:10.1007/s13412-014-0207-7

Gifford SM, Sharma S, Moran MA. 2014. Linking activity and function to ecosystem dynamics in a coastal bacterioplankton community. *Frontiers in Microbiology*. 5:1–12. DOI:10.3389/fmicb.2014.00185

Gignoux-Wolfsohn SA, Vollmer SV. 2015. Identification of candidate coral pathogens on white band disease-infected staghorn coral. *PLOS ONE*. 10(11): e0142760. https://doi.org/10.1371/journal.pone.0142760

Gomez-Pereira PR, Fuchs BM, Alonso C, Oliver MJ, van Beusekom JE, Amann R 2010. Distinct flavobacterial communities in contrasting water masses of the North Atlantic. *ISME*. 4(4):472-487. DOI:10.1111/j.1462-2920.2011.02601

Heaney CD, Exum NG, Dufour AP, Brenner KP, Haugland RA, Chem E, Schwab KJ, Love DC, Serre ML, Noble R, Wade T. 2014. Water quality, weather and environmental factors associated with fecal indicator organiam density in beach sand at two recreational marine beaches. *Science of The Total Environment.* 497-498:440-447. DOI:10.1016/j.scitotenv.2014.07.113

Hoegh-Guldberg O, Mumby PJ, Hooten AJ, Steneck RS, Greenfield P, Gomez E, Harvell CD, Sale PF, Edwards AJ, Caldeira K, Knowlton N, Eakin CM, Iglesias-Prieto R, Muthiga N, Bradbury RH, Dubi A, Hatziolos ME. 2007. Coral reefs under rapid climate change and ocean acidification. *Science*. 318(5857):1737-1742. DOI:10.1126/science.1152509

Lian PY, Maseko T, Rhee M, Ng K. 2012. The antimicrobial effects of cranberry against *Staphylococcus aureus*. *Food Science and Technology International*, 18(2): 179-186. DOI:10.1177/1082013211415159.

Li W, Godzik A. 2006. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequencies. *Bioinformatics*. 22(13):1658-1659. DOI:10.1093/bioinformatics/btl158.

Masella AP, Bartram AK, Truszkowski JM, Brown DG, Neufeld JD. 2012. PANDAseq: paired-end assembler for illumina sequences. *Biomed Central Bioinformatics.* 13:31. DOI:10.1186/1471-2105-13-31

McDonald D, Price MN, Goodrich J, Nawrocki EP, DeSantis TZ, Probst A, Andersen GL, Knight R, Hugenholtz P. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME Journal*. 6(3): 610-618. DOI:10.1038/ismej.2011.139

McMurdie PJ, Holmes S. 2013. Phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE,* 8(4):e61217.

Miller MW, Karazsia J, Groves CE, Griffin S, Moore T, Wilber P, Gregg K. 2016. Detecting sedimentation impacts to coral reefs resulting from dredging the Port of Miami, Florida USA. *PeerJ.* 4(e2711):DOI:10.7717/peerj.2711.

NOS (National Ocean Service, National Oceanic and Atmospheric Administration), 2011. Coast Pilot 4. *Atlantic Coast Cape Henry to Key West, 43rd ed.* Chapter 10, St. John's River to Miami, 328-332.

Port Everglades Fiscal Year 2016 Commerce Report. Available at https://res.cloudinary.com/simpleview/image/upload/v1/clients/porteverglades/PEG779_Commerce_Report_FY2016_ddd1ccd4-f343-4aae-9382-11b5e1af0ebd.pdf (accessed 02 July 2017).

Precht WF, Miller, SL. 2007. Ecological shifts along the Florida Reef Tract: The Past is a key to the future. In Geological Approaches to Coral Reef Ecology. R.B. Aronson. Chapter 9:237-312. United States:Springer NY.

Rappe MS, Connan SA, Vergin KL, Giovannoni SJ. 2002. Cultivation of the ubiquitous SAR11 marine bacterioplankton clade. *Nature*. 418: 630-633. DOI:10.1038/nature00917

Rohwer F, Youle M, Vosten D, 2010. *Coral Reefs in the Microbial Seas* (Vol. 1). United States: Plaid Press.

Somboonna N, Wilantho A, Assawamakin A, Monanunsap S, Sangsrakru D, Tangphatsornruang S, Tongsima S. 2014. Structural and functional diversity of free-living microorganisms in reef surfacem Kra island, Thailand. *BMC Genomics* 15(607). https://doi.org/10.1186/1471-2164-15-607

Stauble DK. 1993. An overview of southeast Florida inlet morphodynamics. *Journal of Coastal Research*: 1-27. http://www.jstor.org/stable/25735671.

Staley C, Kaiser T, Gidley ML, Enochs IC, Jones PR, Goodwin KD, Sinigalliano CD, Sadowsky MJ, Chun CL. 2017. A next-generation sequencing approach to characterize the impacts of land-based sources of pollution on the microbiota of southeast Florida coral reefs. *Applied and Environmental Microbiology* 83; 1-27. DOI:**10.1128/AEM.03378-16**

Stamates SJ, Bishop JR, Carsey TP, Featherstone CM, Gidley ML, Kelble CR, Kotkowski, RM, Roddy RJ. 2013. Port Everglades Flow Measurement System. *NOAA Technical Report, OAR-AOML-42*.1-22.

Symonds EM, Sinigalliano C, Gidley M, Ahmed W, McQuaig-Ulrich SM, Breitbart M. 2016. Faecal pollution along the southeastern coast of Florida and insight into the use of pepper mild mottle virus as an indicator. *Journal of Applied Microbiology.* 121(5): 1469-1481. DOI:10.1111/jam.13252

The Official Site of the Broward County Port Everglades Department. Available at http://www.porteverglades.net (accessed 10 July 2017).

Tully B, Sachdeva R, Heidlberg KB, Heidlberg JF. 2014. Comparative genomics of planktonic *Flavobacteriaceae* from the Gulf of Maine using metagenomics data. *Microbiome*. 2(34). DOI:10.1186/2049-2618-2-34

US Environmental Protection Agency. (1986). Ambient water quality criteria for bacteria 1986. EPA 440/5-84-002. Office of Water Regulations and Standards, US Envrionmental Protection Agency, Washington, DC.

US Environmental Protection Agency. 2004. Implementation guidance for ambient water quality criteria for bacteria. EPA-823-B-04-002. US Environmental Protection Agency, Washington, DC.

United States Census Bureau. Available at https://www.census.gov/quickfacts/fact/table/browardcountyflorida,FL/PST045216. (accessed 2 July 2017).

US Army Corps of Engineers. Port Everglades Fact Sheet. Available at: http://www.saj.usace.army.mil/Portals/44/Port%20Everglades%20FS-FAQ%2024Mar17%20final.pdf (accessed 02 July 2017).

van Elk CE, Boelens HA, van Belkum A, Foster G, Kuiken T. 2012. Indications for both host-specific and introduced genotypes of Staphylococcus aureus in marine mammals. *Vetrinary Microbiology*. 156(3-4): 343-346. DOI:10.1016/j.vetmic.2011.10.034.

Wade TJ, Pai N, Eisenberg JN, Colford JM. 2003. Do US Environmental Protection Agency water quality guidelines for recreational water prevent gastrointestinal illness? A systematic review and meta-analysis. *Environmental Health Perspective*, 111(8): 1102-1109.

Whittaker RH. 1972. Evolution and measurement of species diversity. *Taxon.* 21(2/3): 213-251. DOI:10.2307/1218190

Wickham H. 2011. The split-apply-combine strategy for data analysis. *Journal of Statistical Software*. 40(1):1-29. DOI:10.18637/jss.v040.i01

Figure 1

Rank abundance plot of top 50 OTUs in dataset

Rank abundance plot of the top 50 OTUs. The top 10 OTUs are Rhodobacteraceae (2717), Cryomorphaceae (1389), Synechococcaceae (2398), Unclassified bacteria (1018), Stramenophiles (2842), Rhodobacteraceae (3433), Synechococcaceae (2865), Halomonadeceae (3416), Halomonadeceae (1932), Alphaproteobacteria (3046).

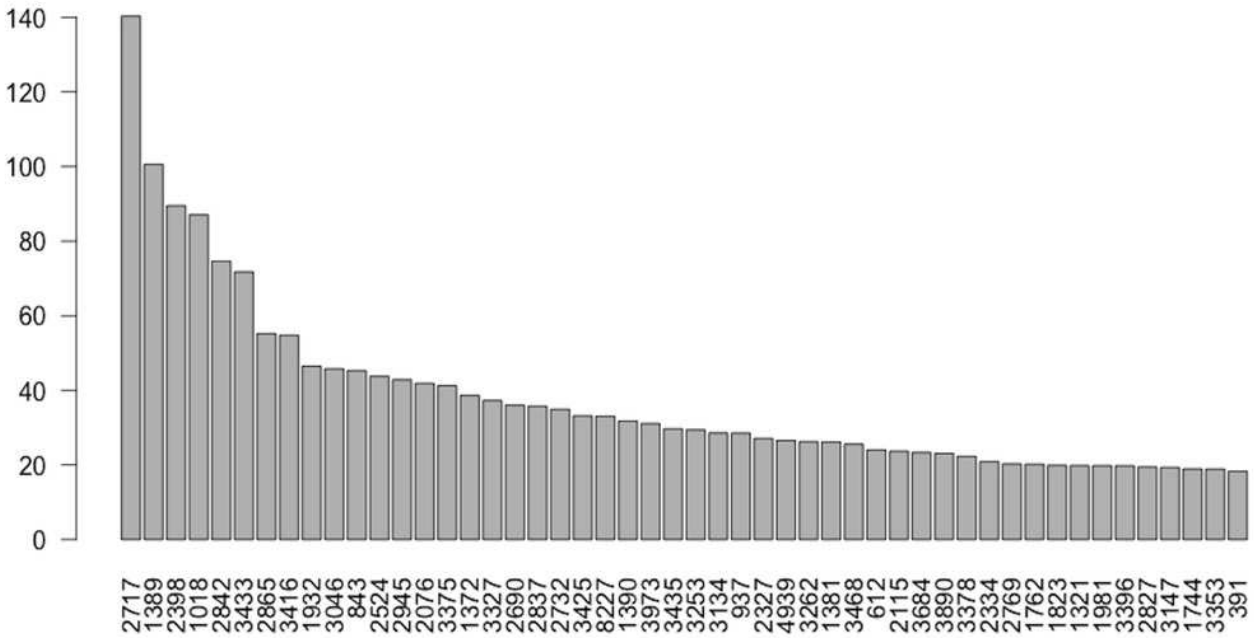

Figure 2

Average relative abundance vs. frequency plot for top 6 most abundance bacterial phyla in dataset.

Shows the average relative abundance of each vs. frequency observed for of the top 6 most abundant bacterial phyla present in Port Everglades Inlet surface water samples. All phyla were observed in >1% in all samples.

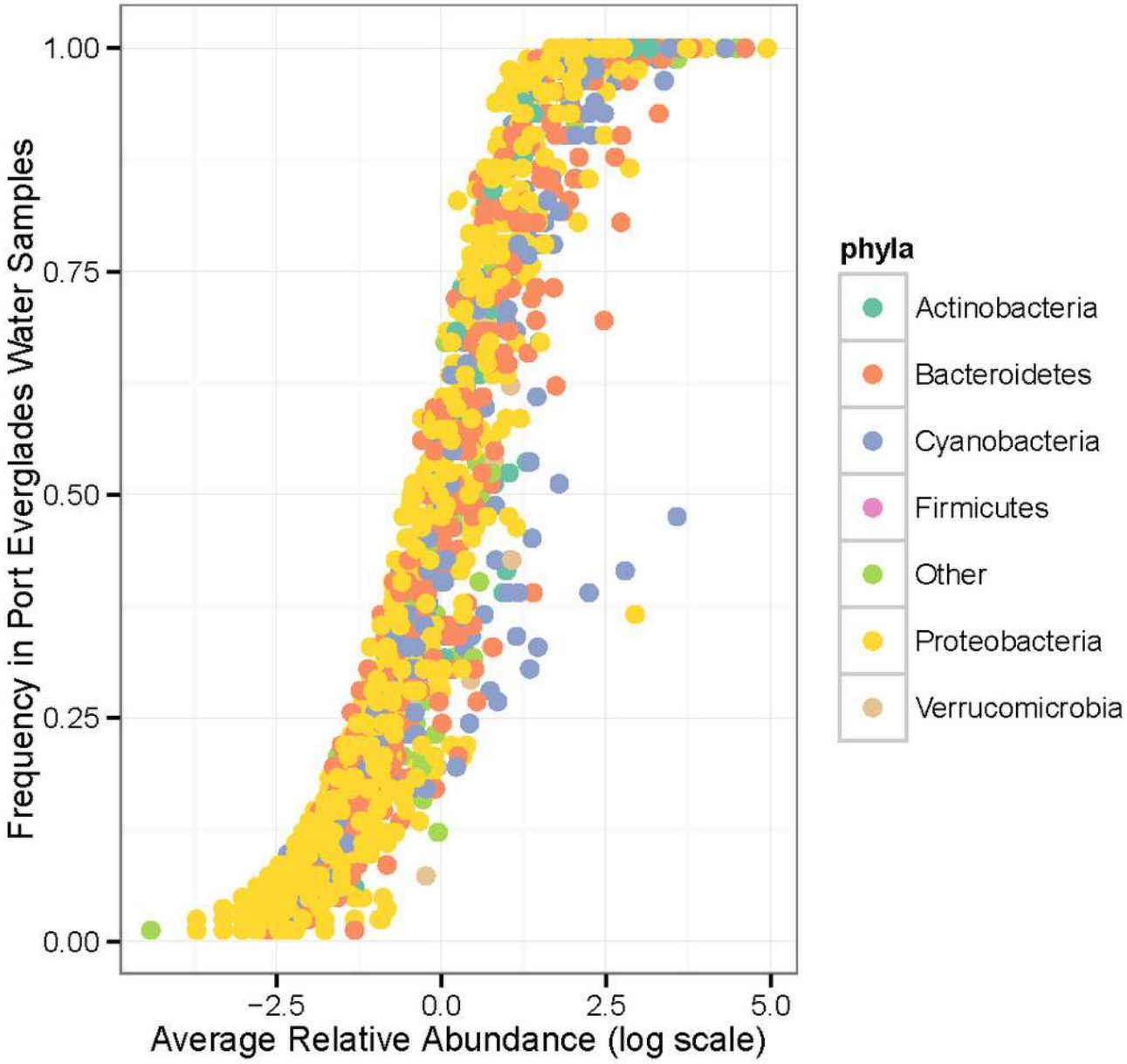

Figure 3

NMDS plot of weighted Unifrac distance by season.

Shows an NMDS analysis of weighted unifrac distance for the wet and dry seasons. The weighted unifrac distance of microbial communities in PEI varied significantly between seasons ($p<0.05$, $R2 = 0.203$).

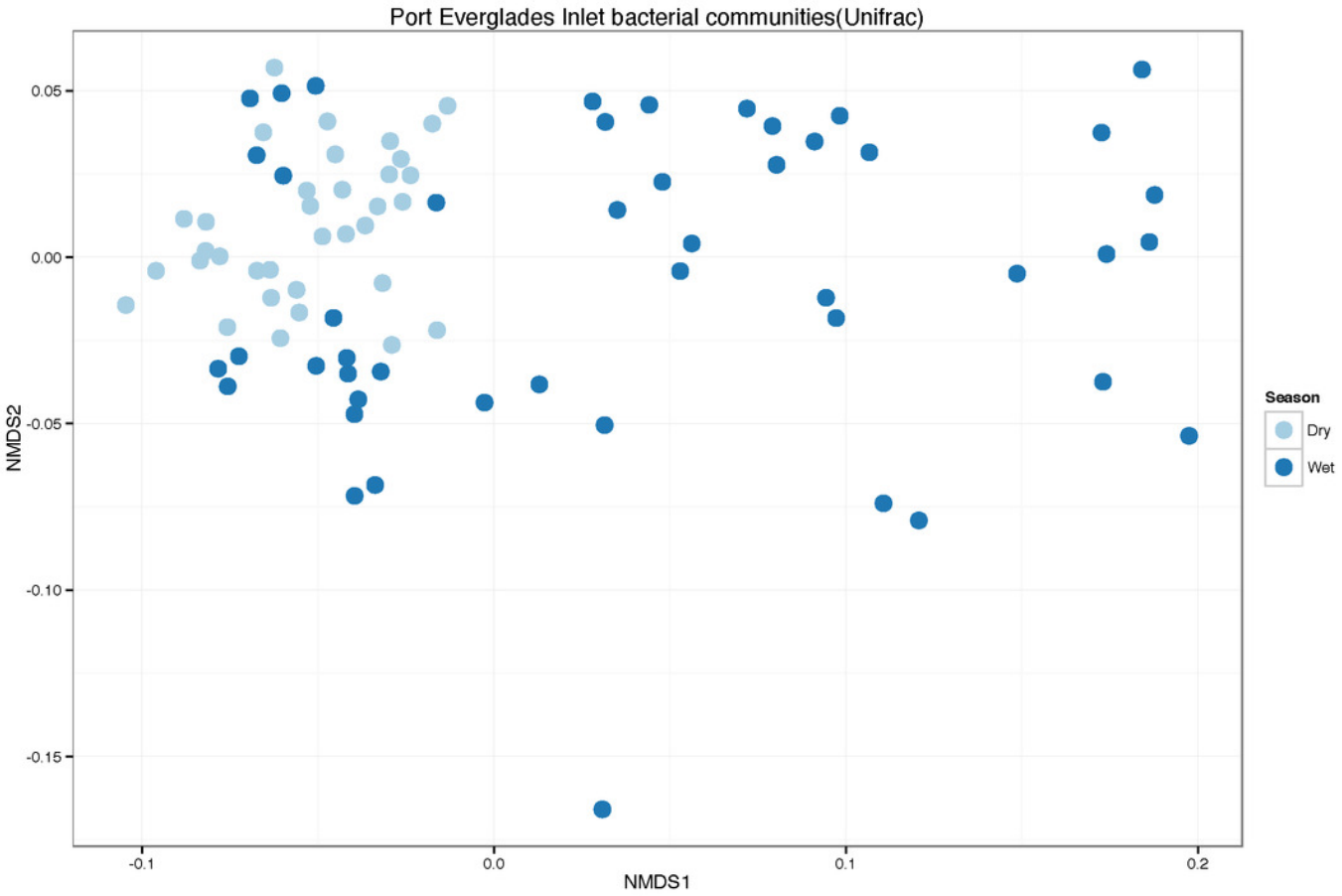

Figure 4

NMDS plot of weighted Unifrac distance by month.

Shows an NMDS analysis of weighted unifrac distance for all months sampled over a 1-year timespan. The weighted unifrac distance of microbial communities in PEI varied significantly between all months ($p<0.05$, $R2 = 0.706$).

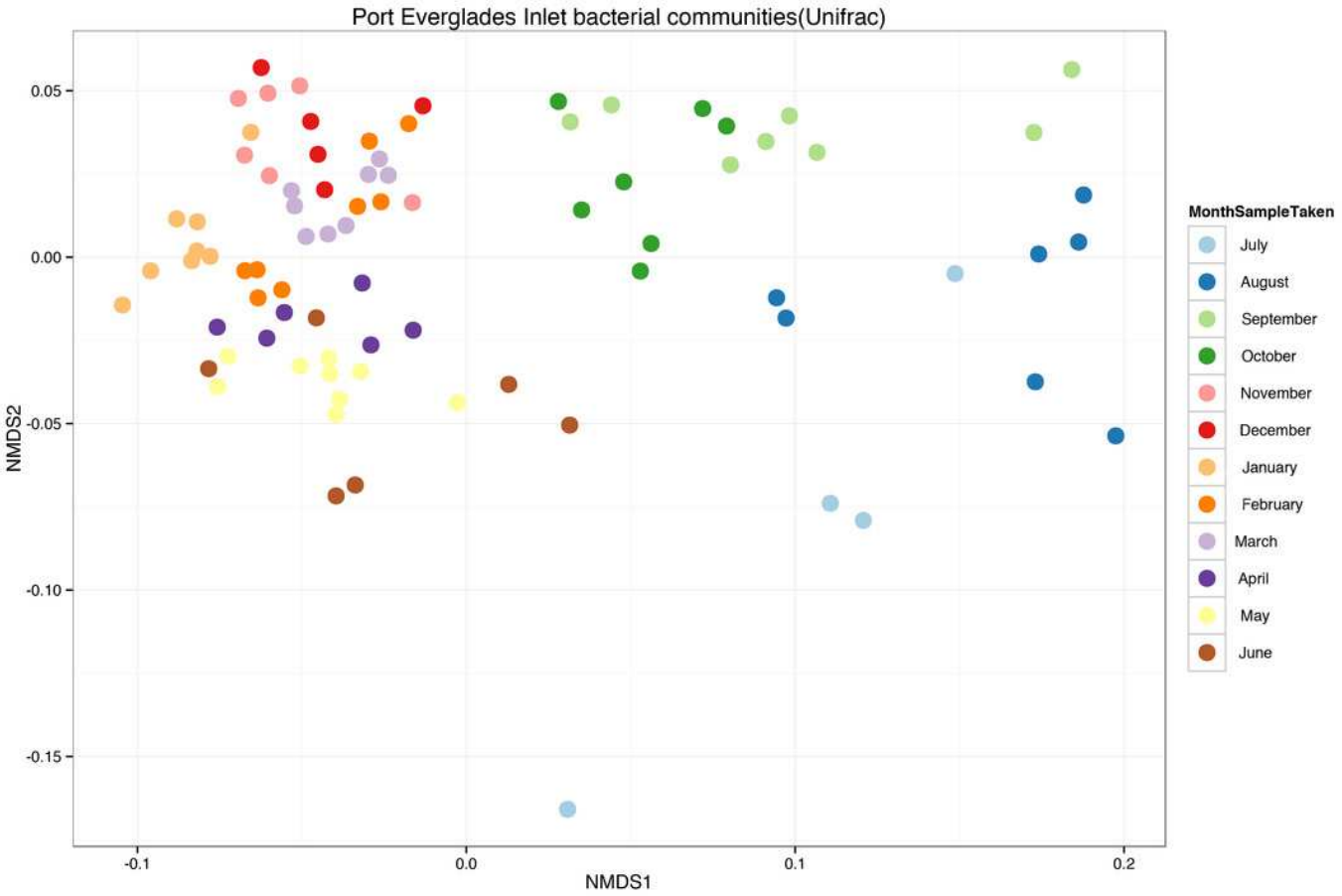

Table 1(on next page)

Table containing results of the multiple least squares regression analysis

Results of multiple least squares linear regression analysis using SAS. Alpha =0.10. All values greater than alpha were not included. Values less than 0.10 were considered to be statistically significant. Gamma, Alpha, and Beta table headings refer to the classes Gammaproteobacteria, Alphaproteobacteria, and Betaproteobacteria and were shortened for spatial reasons.

	Gamma	Flavobacteriia	Acidomicrobiia	Alpha	Chloroplast	Beta	Synechcoccophycideae	Actinobacteria	Unclassified
R-Squared	0.21	0.21	0.21	0.13	0.13	0.14	0.20	0.21	0.07
Salinity	0.0173	0.101	0.0013	0.0008	NA	0.0005	NA	0.0514	NA
Water Temperature	0.0601	0.0204	NA	NA	NA	NA	0.001	0.0001	NA
Chloride Ion	NA	NA	NA	NA	0.0016	NA	0.0155	NA	NA
Sulfate Ion	NA	NA	NA	NA	0.0016	NA	NA	NA	NA
Rainfall	NA	NA	NA	NA	NA	NA	NA	NA	0.0184

Figure 5

Relative abundance of the top 50 OTUs in the *Bacillales* order

Shows a stacked bar chart which represents the taxa summary and abundance levels of top 50 OTUs in the *Bacillales* order in PEI samples. The top 50 OTUs were filtered out to determine the presence of possible pathogens in PEI water samples. Color designations are shown on the right, while white indicates unclassified.

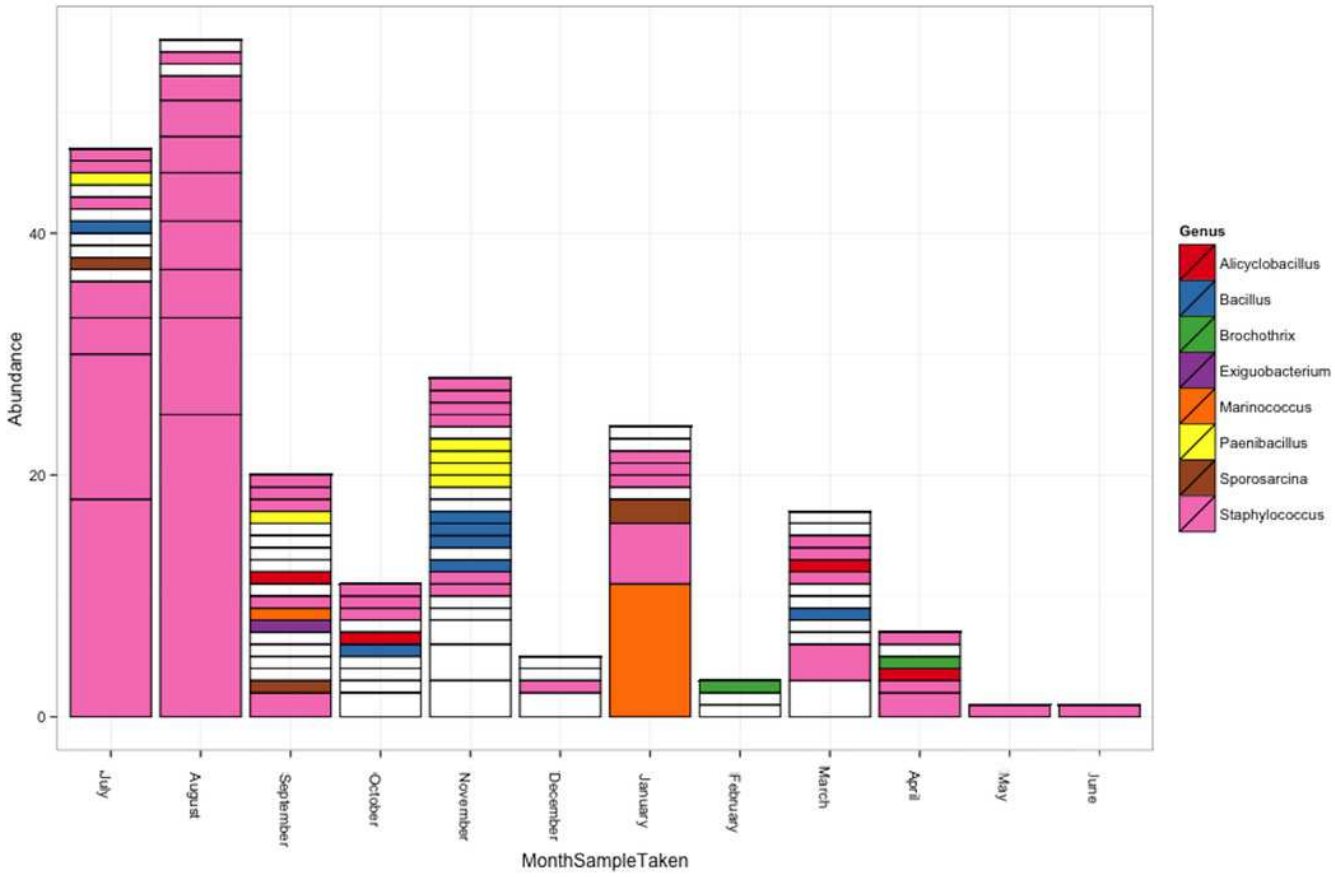

Figure 6

Relative abundance of top 50 OTUs in *Lactobacillales* order

Shows a stacked bar chart which represents the taxa summary and abundance levels of top 50 OTUs in the *Lactobacillales* order in PEI samples. The top 50 OTUs were filtered out to determine the presence of possible pathogens in PEI water samples. Color designations are shown on the right, while white indicates unclassified.