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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.

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

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25 **Abstract**

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

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

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

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

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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

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

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

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

105 This study applied Illumina MiSeq high-throughput sequencing technology to complete
106 DNA sequencing of water samples, differing from previous studies which were largely restricted
107 to culture-based or RT-qPCR methods (Symonds et al., 2016; Aranda et al., 2015; Carsey et al.,
108 2012).

109

110 **Materials & Methods**

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

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

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

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

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

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

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

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

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

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

161 of the study. A backward selection method was used with both entry and model retention set at
162 $\alpha=0.10$. All regression analyses were carried out using SAS Statistical Software (SAS
163 Institute).

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

168

169 **Results**

170 **16S rRNA Sequence Output Overview.** A total of 151 samples were collected weekly from
171 PEI from July 2013-June 2014, with 82 samples used for DNA sequencing. Illumina MiSeq
172 sequencing yielded a total of 1,435,072 raw sequences with Q scores greater than 30 and an
173 average read length of 255 basepairs. The average number of reads per sample was 17,287 with
174 the minimum number of reads per sample being 5,666 and the maximum number of reads per
175 sample being 80,122. A total of 25,020 chimeric sequences were removed from the dataset with
176 a total of 1,410,052 sequences left for OTU table generation and database alignment. After
177 filtering of sequences to remove identical sequences and subsequences a total of 395,009 unique
178 sequences were left for taxonomic assignment using the Greengenes 13_8 database. A total of
179 16,384 OTUs were generated.

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

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

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

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

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

210 **Location.** The results of the Adonis (PERMANOVA) tests for both Bray-Curtis
211 dissimilarity and weighted Unifrac distance showed no significant differences in the beta
212 diversity of samples taken at two different locations in PEI ($P > 0.05$, $R^2=0.0137$ (Bray-Curtis);
213 $P > 0.05$, $R^2=0.0146$ (Unifrac)).

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

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

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

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

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

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

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

267

268 Discussion

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

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

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

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

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

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

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

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

353 **Potential Pathogens**

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

359 The order Bacillales contain three known pathogenic genera *Staphylococcus*, *Bacillus*,
360 and *Paenibacillus*. The only one of these genera known to cause ocean-related illness is
361 *Staphylococcus spp.*, which appeared in almost all months and had the highest abundance levels
362 of all three genera. *Staphylococcus* abundance levels were highest in the months of July and
363 August. *Staphylococcus* is a genus of gram-positive bacteria commonly found on the nails, skin,
364 and hair of humans (Lian et al, 2012). This taxon can thus be shed directly into coastal waters
365 from bathers. The well-known species in this genus, *S. aureus*, can also cause illness in humans.
366 *S. aureus* has a high resistance to salinity, making it a potential threat to other humans using the
367 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

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

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

408 **Significance and Conclusions**

409 This study has provided one of the first in depth profiles of bacterioplankton in
410 metropolitan S. Florida waters. Specific marine habitats, such as coral reefs or mangroves, have
411 well defined optimal conditions for thriving and can be sensitive to small perturbations (Precht
412 and Miller 2007; Hoegh-Guldberg et al., 2007). The data from this study should be helpful to
413 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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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).

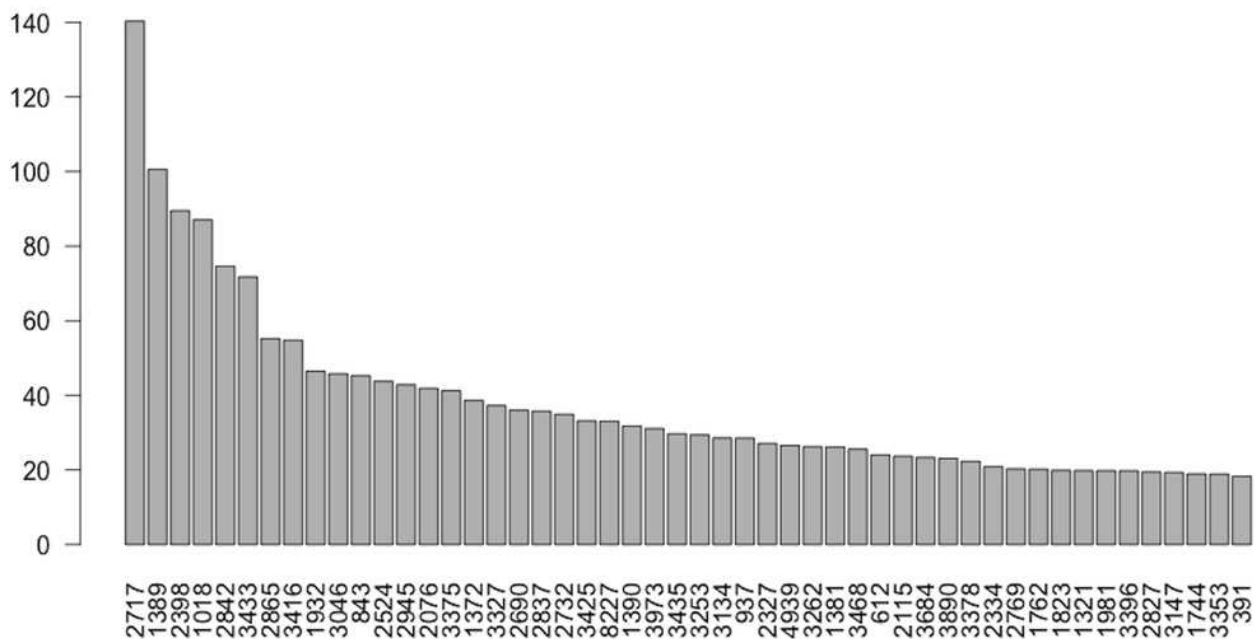


Figure 2

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.

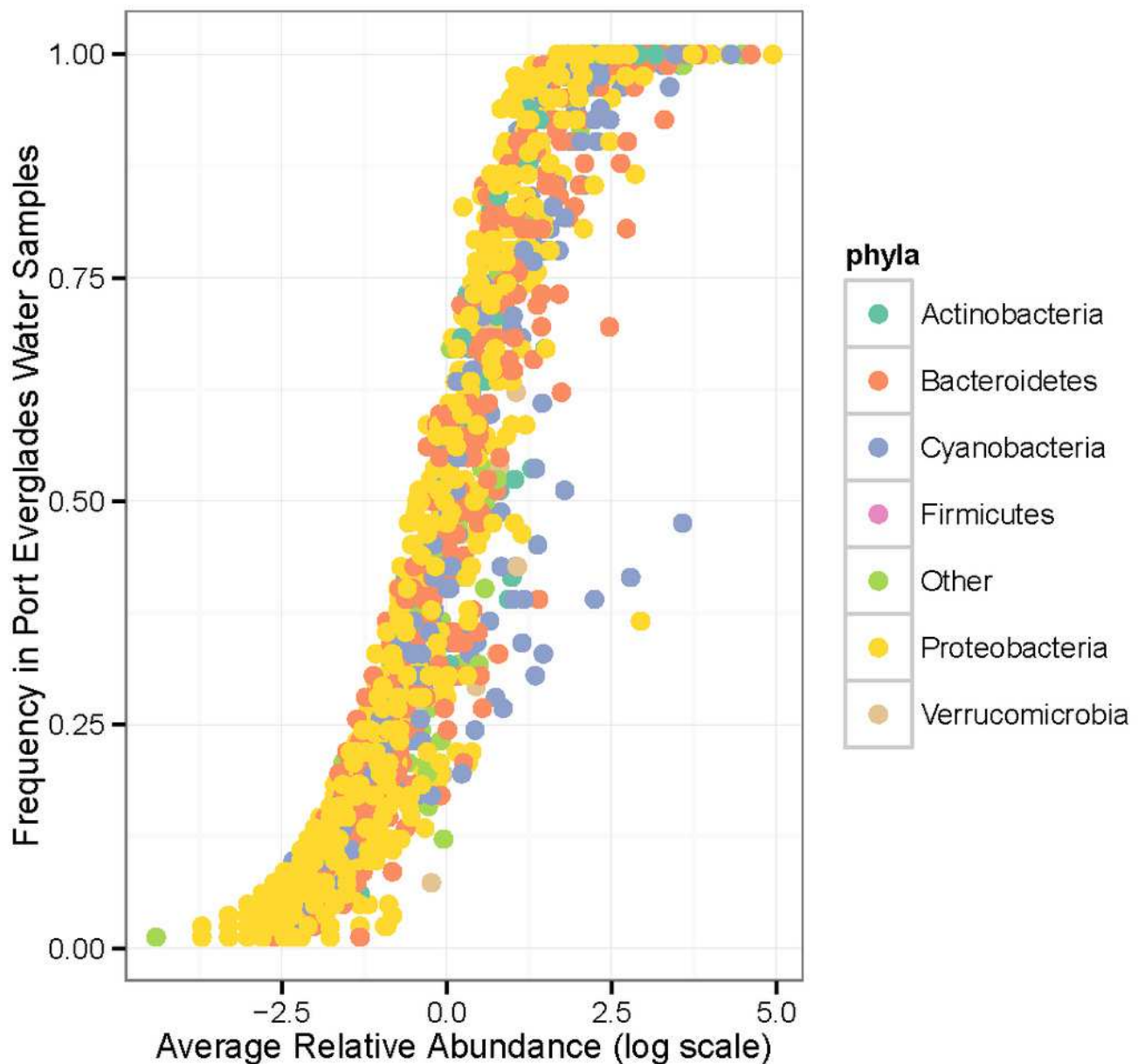


Figure 3

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 ($p < 0.05$, $R^2 = 0.203$).

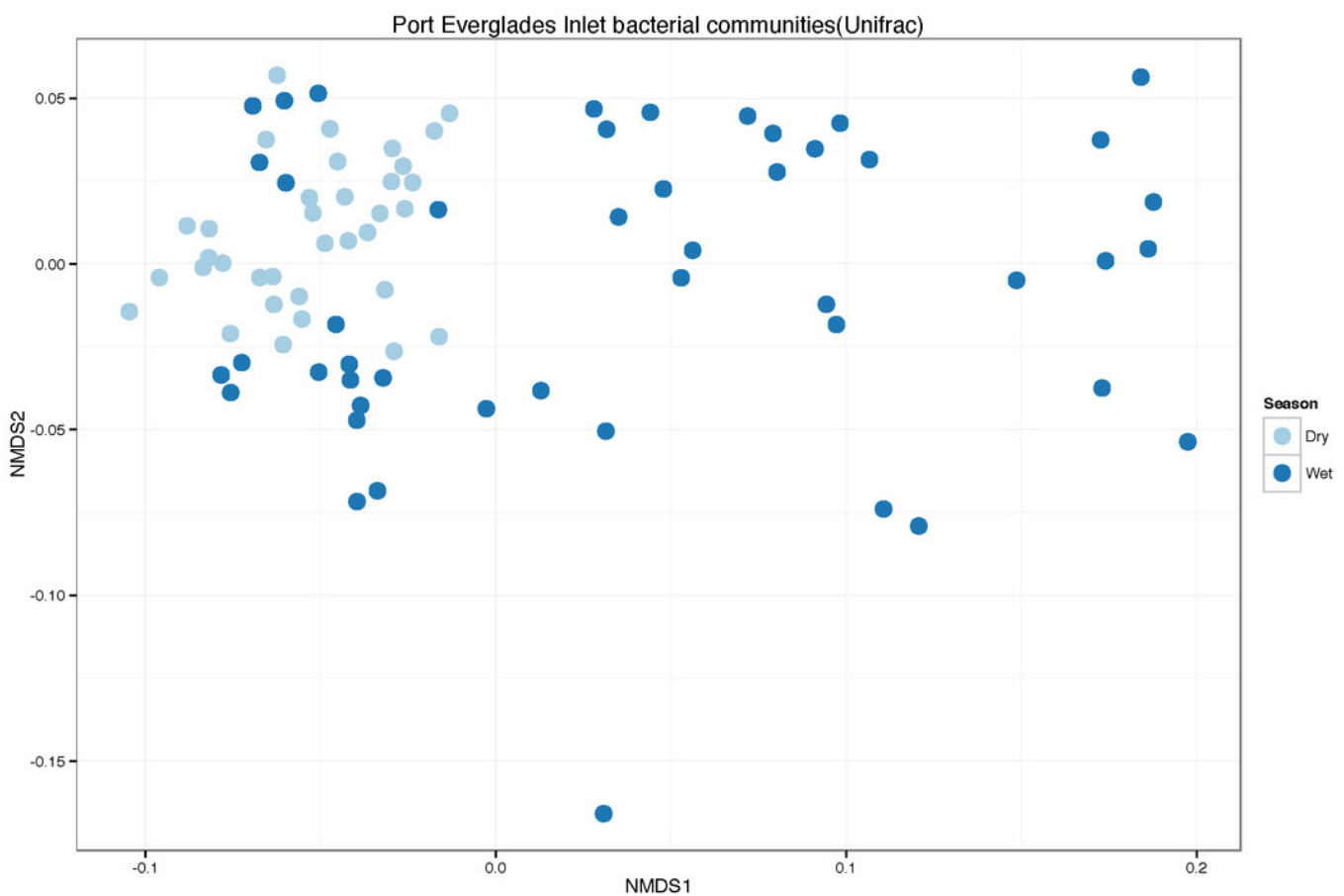


Figure 4

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 ($p < 0.05$, $R^2 = 0.706$).

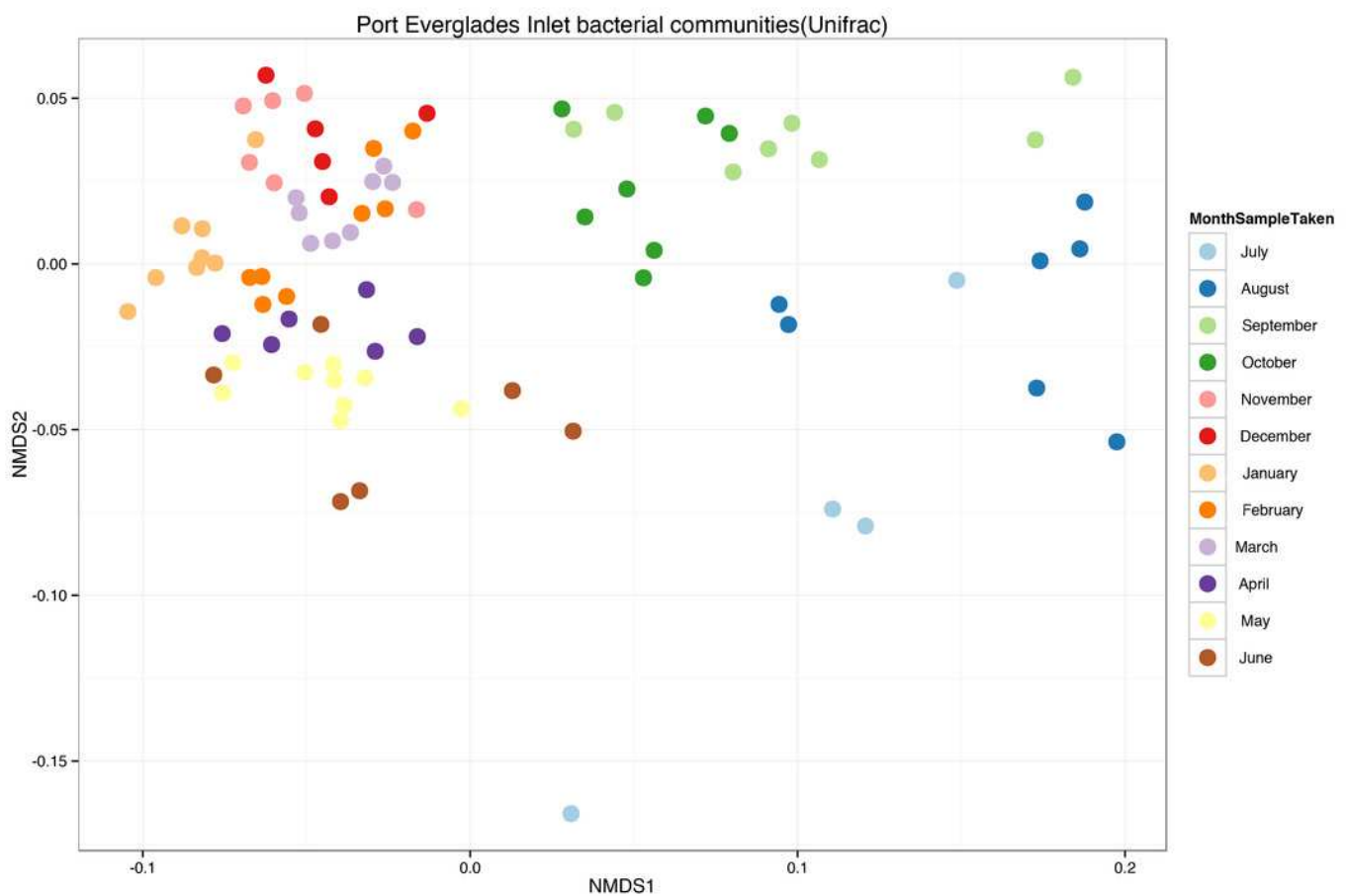


Table 1 (on next page)

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	Synechococcophycideae	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.

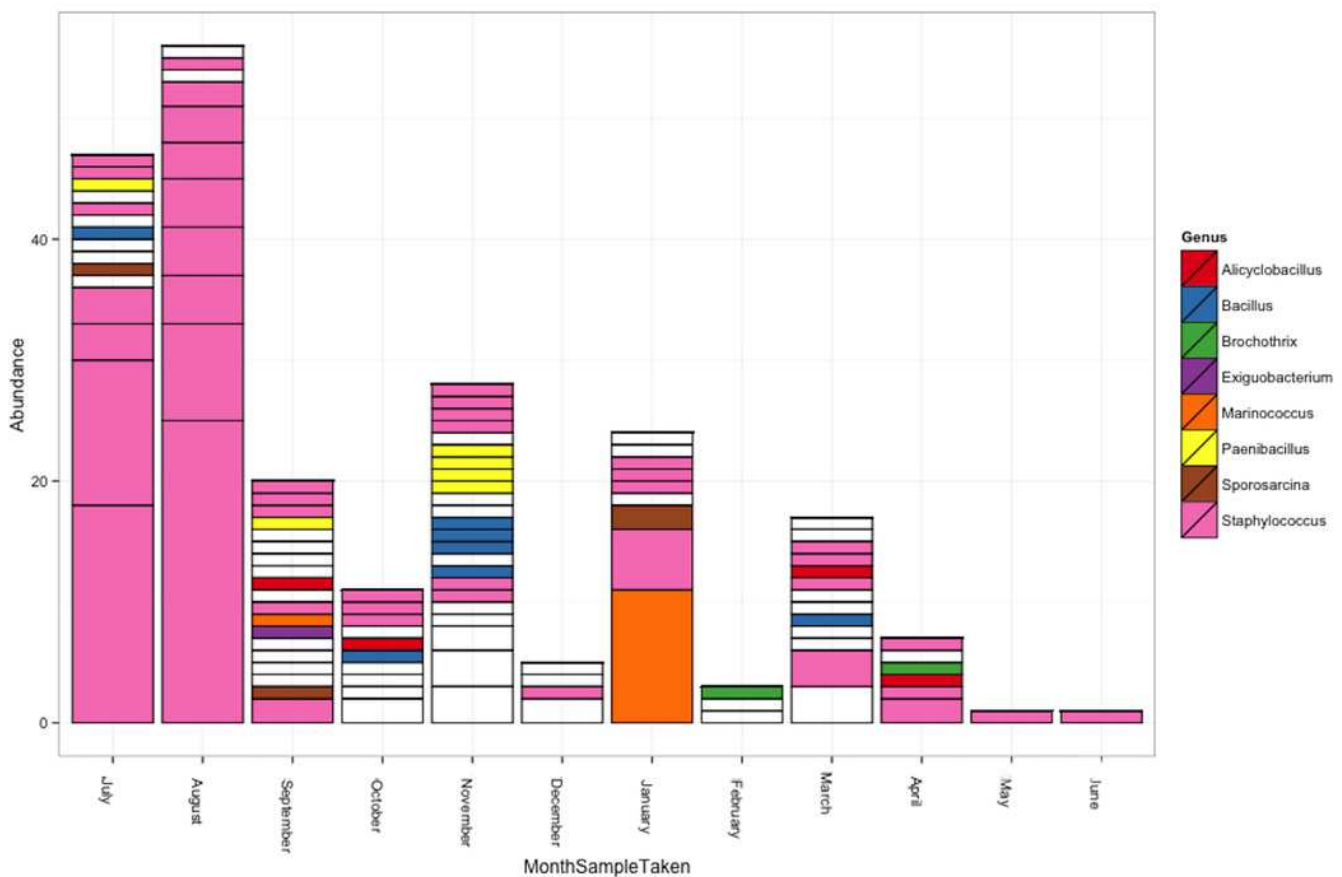


Figure 6

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.

