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

Lauren O'Connell [Corresp., 1] , **Song Gao** [2] , **Donald McCorquodale** [3] , **Jay Fleisher** [4] , **Jose V Lopez** [1]

[1] Department of Biological Sciences, Halmos College of Natural Sciences and Oceanography, Nova Southeastern University, Dania Beach, Florida, United States

[2] Division of Natural Sciences, Duke Kunshan University, Kunshan, Jiangsu, Florida, China

[3] Department of Marine and Environmental Sciences, Halmos College of Natural Sciences and Oceanography, Nova Southeastern University, Dania Beach, Florida, United States

[4] College of Osteopathic Medicine, Nova Southeastern University, Davie, Florida, United States

Corresponding Author: Lauren O'Connell
Email address: lo248@nova.edu

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 to surrounding beaches and offshore coral reefs. Understanding the composition and fluctuations of bacterioplankton communities ("microbiomes") in major port inlets is important due to potential impacts on surrounding environments. We hypothesize seasonal microbial fluctuations, which were profiled by high throughput 16S rRNA amplicon sequencing and analysis. **Methods & Results.** Surface water samples were collected every week for one year. A total of 4 samples per month, 2 from each sampling location, were used for statistical analysis creating a high sampling frequency and finer sampling scale than previous inlet microbiome studies. We observed significant differences in community alpha diversity between months and seasons. Analysis of composition of microbiomes (ANCOM) tests were run in QIIME 2 at genus level taxonomic classification to determine which genera were differentially abundant between seasons and months. Beta diversity results yielded significant differences in PEI community composition in regard to month, season, water temperature, and salinity. Analysis of potentially pathogenic genera showed presence of *Staphylococcus* and *Streptococcus*. However, statistical analysis indicated that these organisms were not present in significantly high abundances throughout the year or between seasons. **Discussion.** Significant differences in alpha diversity were observed when comparing microbial communities with respect to time. This observation stems from the high community evenness and low community richness in August. This indicates that only a few organisms dominated the community during this month. August had lower than average rainfall levels for a wet season, which may have contributed to less runoff, and fewer bacterial groups introduced into the port surface waters. Bacterioplankton beta diversity

differed significantly by month, season, water temperature, and salinity. The 2013-2014 dry season (October-April), was warmer and wetter than historical averages. This may have driven significant differences in beta diversity. Increased nitrogen and phosphorous concentrations were observed in these dry season months, possibly creating favorable bacterial growth conditions. Potentially pathogenic genera were present in the PEI. However their relatively low, non-significant abundance levels highlight their relatively low risk for public health concerns. This study represents the first to sample a large port at this sampling scale and sequencing depth. These data can help establish the inlet microbial community baseline and supplement the vital monitoring of local marine and recreational environments, all the more poignant in context of local reef disease outbreaks and worldwide coral reef collapse in wake of a harsh 2014-16 El Niño event.

1 **Fine-Grained Compositional Analysis of Port Everglades Inlet Microbiome Using High**

2 **Throughput DNA Sequencing**

4 Lauren M. O'Connell[1,3], Song Gao[2], Donald McCorquodale[3], Jay Fleisher[4], Jose V. Lopez[1]

6 [1]Department of Biological Sciences, Halmos College of Natural Sciences and Oceanography,

7 Nova Southeastern University, Dania Beach, FL, USA

8 [2]Division of Natural Sciences, Duke Kunshan University, Kunshan, Jiangsu, China

9 [3]Department of Marine and Environmental Sciences, Halmos College of Natural Sciences and

10 Oceanography, Nova Southeastern University, Dania Beach, Florida, USA

11 [4]College of Osteopathic Medicine, Nova Southeastern University, Davie, Florida, USA

13 Corresponding Author:

14 Lauren O'Connell[1]

15 Locon833@gmail.com

24 **Abstract**

25 **Background**. Similar to natural rivers, manmade inlets connect inland runoff to the ocean. Port

26 Everglades Inlet (PEI) is a busy cargo and cruise ship port in South Florida, which can act as a

27 source of pollution to surrounding beaches and offshore coral reefs. Understanding the

28 composition and fluctuations of bacterioplankton communities ("microbiomes") in major port

29 inlets is important due to potential impacts on surrounding environments. We hypothesize

30 seasonal microbial fluctuations, which were profiled by high throughput 16S rRNA amplicon

31 sequencing and analysis. **Methods & Results.** Surface water samples were collected every week

32 for one year. A total of 4 samples per month, 2 from each sampling location, were used for

33 statistical analysis creating a high sampling frequency and finer sampling scale than previous

34 inlet microbiome studies. We observed significant differences in community alpha diversity

35 between months and seasons. Analysis of composition of microbiomes (ANCOM) tests were run

36 in QIIME 2 at genus level taxonomic classification to determine which genera were differentially

37 abundant between seasons and months. Beta diversity results yielded significant differences in

38 PEI community composition in regard to month, season, water temperature, and salinity.

39 Analysis of potentially pathogenic genera showed presence of *Staphylococcus* and

40 *Streptococcus*. However, statistical analysis indicated that these organisms were not present in

41 significantly high abundances throughout the year or between seasons. **Discussion.** Significant

42 differences in alpha diversity were observed when comparing microbial communities with

43 respect to time. This observation stems from the high community evenness and low community

44 richness in August. This indicates that only a few organisms dominated the community during

45 this month. August had lower than average rainfall levels for a wet season, which may have

46 contributed to less runoff, and fewer bacterial groups introduced into the port surface waters.

47 Bacterioplankton beta diversity differed significantly by month, season, water temperature, and

48 salinity. The 2013-2014 dry season (October-April), was warmer and wetter than historical

49 averages. This may have driven significant differences in beta diversity. Increased nitrogen and

50 phosphorous concentrations were observed in these dry season months, possibly creating

51 favorable bacterial growth conditions. Potentially pathogenic genera were present in the PEI.

52 However their relatively low, non-significant abundance levels highlight their relatively low risk

53 for public health concerns. This study represents the first to sample a large port at this sampling

54 scale and sequencing depth. These data can help establish the inlet microbial community baseline

55 and supplement the vital monitoring of local marine and recreational environments, all the more

56 poignant in context of local reef disease outbreaks and worldwide coral reef collapse in wake of

57 a harsh 2014-16 El Niño event.

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72   **Introduction**

73   A continental coastal zone can represent a gradient across distinct biogeographical

74   boundaries (freshwater, brackish and saltwater). Mangroves, streams, or manmade inlets in these

75   transition zones provide potential links. Fort Lauderdale's Port Everglades Inlet (PEI) (also

76   known as the Port Everglades Shipping Channel [PESC]), in Broward County, FL is a man-

77   made, dredged, deep-water port located along the southeastern coast of subtropical Florida

78   (Stauble 1993; http://www.porteverglades.net; NOS, 2011). Located directly offshore from the

79   PEI is a major US coral reef tract (Staley et al., 2017), as well as multiple recreational beaches,

80   fishing piers, and watersport areas (Stamates et al., 2013). Coral reefs, beaches, and recreational

81   water sport areas are impacted both positively and negatively by resident microbial communities

82   of these areas. The ecosystem services of bacteria in marine communities include nutrient

83   cycling and symbiosis, while disadvantages include the possible presence of marine pathogens,

84   which may cause illness in the marine environment or to humans utilizing it.

85   Although large-scale bacterioplankton community (or "microbiome") studies such as the

86   Tara expeditions or the Earth Microbiome project, have their place providing important global

87   information on a diverse array of marine microbial habitats (Sunagawa et al, 2015; Thompson et

88   al, 2017), much value can also be obtained by focusing on finer scale microbial dynamics of

89   distinct local habitats especially when unique. PEI is one of largest cargo and cruise ship ports in

90   the country and is susceptible to pollution and pathogens from cargo and cruise ships via ballast

91   water flushing, oil leakage, and cargo spills, and through land-based pollution. The past decade

92   has marked rapid increases in human population and coastal development, resulting in the input

93   of human pathogens and enteric bacteria into coastal waters (Mallin et al., 2000; Lapointe et al.,

94   2015). The introduction of these pathogens can cause human illnesses such as skin, ear, and

95   gastric infections and can cause disease in marine organisms. This was seen with the introduction

96   of the human pathogen *Serratia marcescens,* into a coral reef ecosystem, which was found to be

97   the causative agent in the bleaching event seen in the Carribbean elkhorn coral (Sutherland et al.,

98   2011). Pollution into PEI also occurs via land-based runoff. Land-based runoff contributes to

99   nutrient, pathogen, and pollutant deposition into the marine environment, which can lead to

100 eutrophication (Carrie Futch et al., 2011; Lapointe et al., 2015; Staley et al, 2017).

101 Eutrophication leads to conditions of hypoxia and anoxia and decreased biodiversity causing

102 major shifts in community structure of an ecosystem. These shifts can result in an increase of

103 phytoplankton, which can cause harmful algal blooms, and can eventually lead to reduction of

104 zooplankton and bacterioplankton diversity (Marcus 2004; Paerl et al., 2003). Southern Florida

105 has two main seasons. The wet season mostly ranges from May-September, while the dry season

106 occurs from October-April. The wet season is mostly during the hot summer months, and is

107 characterized by increased rainfall levels and increased temperatures. With increased rainfall

108 levels, also comes increased runoff into the marine environment. Previous research has indicated

109 that influxes of nutrients from agriculture practices in near-shore environments are contributing

110 to degradation of Florida reef ecosystems via eutrophication and introduction of pathogens (Finkl

111 and Charlier, 2003; Banks et al., 2008; Walker et al., 2008; Lewis et al., 2017**).**

112   This study reports an extensive environmental next-generation sequencing

113 characterization of the bacterioplankton microbiome from the surface seawater in PEI. Water

114 samples were collected from June 2013 to May 2014 to determine monthly and seasonal alpha

115 (α) and beta (β) diversity fluctuations. Fluctuations of the bacterioplankton community in PEI's

116   surface water was monitored over a year. While samples were collected weekly, a total of 4

117   samples per month, 2 from each location in the port, were used for consistency in statistical

118   analyses. This study provides a higher sampling resolution than has been currently reported. To

119   date, inlet study data has been collected bi-monthly, quarterly, and for a shorter sampling periods

120   (Wallace et al. 2018; Staley et al. 2017; Campbell et al. 2015).

121   The primary hypothesis of this study predicted strong seasonal effects on the inlet

122   microbiome in PEI due to temperature and rainfall. It was also hypothesized that potentially

123   harmful pathogens to both humans would be present in a higher abundance during the wet

124   season.

125   This study applied high-throughput (HTP) sequencing technology to complete DNA

126   sequencing of surface seawater samples in PEI, extending from previous inlet studies, which

127   were largely restricted to a smaller sample sizes, lower sampling frequency, and culture-based or

128   RT-qPCR methods (Symonds et al., 2016; Aranda et al., 2015; Campbell et al., 2015).

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130   **Materials & Methods**

131   **Water Sample Collection, DNA Extraction, and Chemical Analysis.** A total of 151

132   surface seawater samples were collected weekly, at ebb tide, from PEI in Broward County, FL

133   by kayak over a year-long timespan (June 2013- July 2014). Three 1.0 liter water samples were

134   collected at a depth of 0.5 meters every week from two different sites within in the inlet. Water

135   temperature was measured *in situ* at time of sampling with a glass thermometer. Salinity

136   measurements were taking immediately upon returning to lab (within 30 minutes of sample

137   collection) with a refractometer. Precipitation values were obtained using NOAAs data from the

138   National Center for Environmental Information (http://www.ncdc.noaa.gov/cag/time-series). For

139   each site, 1.0 L of water was filtered using Pall GN Metricel® grid 47 mm, 0.45µm filters, by

140   vacuum filtration.

141   Total microbial genomic DNA was extracted using MO BIO's PowerLyzer™

142   PowerSoil® kit (Carlsbad,CA). Genomic DNA size and quality was verified with a 1% agarose

143   gel and Nanodrop measurements. A total of 81 samples were queued for sequencing based on

144   quality of extracted DNA. Samples that showed clear, non-degraded, high molecular weight

145   genomic DNA bands and had 260/280 ratios ~1.8 were considered quality DNA. After gel

146   verification, DNA concentration was measured using the Qubit 2.0 (Life Technologies).

147   Surface water samples collected at each site were subjected to ion chromatography (IC)

148   analysis using a Thermo Scientific Dionex ICS-1600 system (Bannockburn, IL). After filtration

149   of particulates using syringe filters, samples were diluted 1000 times before injection into the IC.

150   Ion chromatography analysis was used to detect the presence and measure the concentrations of

151   chemical ions in the PEI surface water. A total of five anions - fluoride, chloride, nitrate,

152   phosphate, and sulfate - were analyzed with calibration curves from standard solutions and

153   detection limits at approximately 10 ppb.

154   **Sequencing Sample Preparation.** Samples were prepared for MiSeq® sequencing

155   following Illumina's 16S Metagenomic Sequencing Library Preparation guide (Illumina, 2013).

156   The amplicon primers used for the first PCR were universal primers, MIDf-515F and 806rc

157   which amplified the V4 region of the 16S rRNA gene (Caporaso et al., 2010). Illumina adaptor

158   sequences were added to these primers as outlined in the aforementioned Metagenomic

159   Sequencing Library Preparation Guide (Illumina, 2013). A second PCR was completed to attach

160   Illumina Nextera indices using the Illumina Nextera indexing kit so that samples could be

161   demultiplexed (Illumina, 2013). The final pooled DNA library was diluted to a concentration of

162   4 pM with a 50% spike in of 12.5 pM PhiX.

163   **Sequence Analyses.** Sequence analysis was performed using Quantitative Insights into

164   Microbial Ecology 2 (QIIME2) (Caporaso et al., 2010a). Paired ends were joined using

165   PANDAseq 2.8.1 at a 90% confidence level (Masella *et al,* 2012). A total of 4 samples per

166   month, 2 from each location, resulting in a total of 48 samples were used for downstream

167   analysis so as not to skew statistical analysis. Paired end sequences were imported into QIIME2

168   and sequence quality control was completed using the DADA2 pipeline incorporated into

169   QIIME. The DADA2 program filtered out phiX reads, removed chimeric sequences, and

170   assigned reads into Operational Taxonomic Units (OTUs). The sequences were truncated to 220

171   basepairs because the quality of reverse reads after base 220 declined. OTUs were grouped into

172   clusters at 97% sequence similarity (Callahan et al., 2016). Taxonomy was assigned using the

173   DADA2 pipeline which implemented the Ribosomal Database Project (RDP) naïve Bayesian

174   classifier (Callahan et al., 2016).

175   **Statistical Analyses**. All statistical analyses were completed using QIIME2 and R Statistical

176   Software Version 1.1.383. The R package phyloseq was used to generate stacked bar charts

177   depicting relative abundance of taxa (McMurdie and Holmes, 2013). Richness and evenness

178   estimates were calculated using QIIME2 (https://qiime2.org). To determine statistical

179   significance in alpha diversity non-parametric Kruskal-Wallis tests using Pielou's evenness

180   values were used to complete pairwise comparisons between month, location, and weather at

181   time of sampling (rain vs. no rain). Non-parametric Kruskal-Wallis comparisons were performed

182   using Faith's Phylogenetic Diversity to determine if there were statistical differences in species

183   richness in the PEI samples. Box and whisker plots for species richness and evenness were

184   generated using QIIME2. To test non-discrete metadata (salinity and water temperature) for

185   correlations with alpha diversity, Spearman's Rank correlation analysis was completed using

186   Pielou's evenness values. Taxonomic stacked bar charts were generated using data from QIIME2

187   imported into Phyloseq. Analysis of composition of microbiomes (ANCOM) test were run to

188   determine if there were significant differences in the relative abundance of any taxa between

189   month or season (Mandal et al. 2015).

190   Statistical analyses for beta diversity were completed by calculating Bray-Curtis distance

191   using QIIME2. A PERMANOVA was run to complete pairwise comparisons of samples for

192   month, location, and season. Principle Coordinate Analysis (PCoA) plots were generated for

193   month sample taken and for season from Bray-Curtis distances using QIIME2. A Mantel test was

194   run to determine if there were statistically significant correlations in beta diversity in relation to

195   non-discrete metadata.

196   **Regression Analysis.** A series of Multiple Least Squares Regressions were used to assess

197   possible relationships between each bacterial class and the environmental variables taken as part

198   of the study. The R^2 value is the measure used to determine how well the data fits the regression

199   line. The higher R^2 values indicated better data fits with the model. The class level taxonomic

200   classification was used to evaluate relationships between environmental variables because

201   majority of the sequences were classified to the class level. A backward selection method was

202   used with both entry and model retention. All regression analyses were carried out using SAS

203   Statistical Software (SAS Institute).

204   **Potential Pathogen Detection.** Potentially pathogenic bacteria were detected by filtering

205   out genera that may be pathogenic. Statistical analysis was completed on *Staphylococcus* and

206   *Streptococcus spp.* using the ANCOM test. This test compares abundances of genera to

207   determine if they change significantly between populations or environments (Mandal et al.,

208   2015). To display presence of potential pathogens graphically, the relative abundance, genus, and

209   month were plotted and faceted by season using the plot_bar command in phyloseq.

210   **Results**

211   **16S rRNA Sequence Output Overview.** A total of 151 samples were collected weekly from

212   PEI from July 2013-June 2014; among them 81 samples were queued for DNA sequencing. A

213   total of 48 samples, 4 per month, 2 from each site, were used for downstream bioinformatics

214   analysis (Table S1). The MiSeq sequencing yielded a total of 1,435,072 raw sequences with Q

215   scores greater than 30 and an average read length of 255 basepairs. The average number of reads

216   per sample was 20,586 with the minimum number of reads per sample being 9,199 and the

217   maximum number of reads per sample being 35,108. A total of 25,020 chimeric sequences were

218   removed from the dataset with a total of 1,410,052 sequences left for OTU table generation and

219   database alignment. After filtering of sequences to remove identical sequences and subsequences

220   a total of 395,009 unique sequences were left for taxonomic assignment using the Greengenes

221   13_8 database. A total of 16,384 OTUs were generated.

222   **Alpha Diversity.** Alpha diversity was assessed through species evenness and species richness

223   measures. Results for species evenness were statistically significant for month and season

224   (p=0.015; p=0.020 respectively). There were no significant differences in species evenness for

225   PEI samples by location, or weather at time of sampling (raining vs. not raining) (p>0.05). The

226   month of August had the lowest species evenness values indicating that these samples were

227   dominated only by a few organisms. The month of December had the highest level of species

228   evenness (Fig. S1). The wet season has lower species evenness values than the dry season (Fig.

229   1). In contrast, no significant differences in alpha diversity appeared in relation to water

230   temperature or salinity (p>0.05).

231   Statistically significant differences were seen in species richness in relation to month and

232   season (p= 0.010; p= 0.025 respectively). For example, the month of December had the lowest

233   measure of species richness, while September had the highest measure of species richness (Fig.

234   S2). Concordantly, the wet season samples had higher species richness values than the dry

235   season (Fig. 1). In contrast, species richness analyses across samples indicated that there were no

236   statistically significant differences in species richness in relation to rain at time of sampling

237   versus no rain at time of sampling, water temperature values, salinity measures, or sampling

238   location (p>0.05).

239   **Beta Diversity.** The PEI samples were analyzed to determine if there were significant

240   differences in the microbial communities due to differences in sampling location, month that the

241   sample was taken, season, weather at time of sampling, salinity, and water temperature.

242   **Location.** The results of the PERMANOVA test using Bray-Curtis dissimilarity

243   indicated no significant differences in beta diversity of samples taken at two different locations

244   in PEI (p=0.99).

245   **Weather at Time of Sampling (Raining vs. Not Raining).** There were significant

246   differences in beta diversity of PEI samples in regards to weather (rain vs. no rain) at the time of

247   sampling (p=0.03).

248   **Season.** Results of the PERMANOVA indicated that there were significant differences in

249   the microbial community composition between seasons (p= 0.001) There is separation of points

250   in the PCoA plot between the wet and dry seasons, displaying that there are differences in the

251   microbial community composition (Fig. 2).

252   **Month.** The results of the PERMANOVA test using Bray-Curtis Dissimilarity indicated

253   that there were overall significant differences observed in the beta diversity of all months tested

254   (p=0.001). Multiple pairwise comparisons were completed to determine which months differed

255   significantly from one another. All months differed significantly in beta diversity except for

256   August and July, February and March, February and January, July and September, July and

257   October, and July and June (Table S2).

258   **Abundant Genera.** To better understand how the microbial community composition fluctuated

259   with season we examined which organisms were present at different taxonomic levels and their

260   relative abundance levels. Proteobacteria, Planctomycetes, Cyanobacteria, Bacteroidetes,

261   Actinobacteria, Verrucomicrobia, Tenericutes, and Euryarchaeota were the most abundant phyla

262   across all samples, with Proteobacteria making up at least 25% of the bacterial community in all

263   samples (Fig. S3). At the lower taxonomic levels, an increased number of taxa were observed in

264   wet season in comparison to the dry season (13 vs 11 taxa) (Fig. 3). While observable

265   fluctuations in relative abundance levels are seen in the *Opitutus* and *Anaerospora* genera, results

266   from the ANCOM tests indicated that at the genus level, only *Synechococcus* differed

267   significantly in its abundance levels from the dry to the wet season. *Synechococcus spp.* went

268   through large increases in abundance levels from the dry season to the wet season (Table 1).

269   **Linear Regression Analysis with Chemical and Environmental Metadata.** Multiple least

270   Squares regression analysis results showed that there were statistically significant relationships

271   between certain taxa and salinity measures, chloride ion concentration, water temperature, sulfate

272   ion concentration, and rainfall (Table 2). An interesting trend to point out seen in the data was

273   that *Proteobacteria* and *Cyanobacteria* showed an inverse trend in abundance, which correlated

274   with temperature and rainfall data to some degree (Fig. S4**).** Environmental metadata for average

275   precipitation levels and temperature was obtained through NOAA National Centers for

276   Environmental information (http://www.ncdc.noaa.gov/cag/). Rainfall averages for the month of

277   August were 3.4 inches lower than average historical data. The remaining wet season months

278   (May-October) generally had higher than average rainfall levels (Fig. 4). The average

279   temperature during the wet season months was generally higher than the historical average, while

280   the winter months had lower than average temperatures in comparison to historical data (Fig. 4).

281   **Potentially Pathogenic Bacteria.** Results of the ANCOM tests demonstrated that there were no

282   significant differences in the relative abundance levels of potential pathogens during the year

283   (Table S3; Table S4). In the *Bacillales, Clostridiales,* and *Lactobacillales* orders, the most

284   abundant potentially pathogenic genera were *Staphylococcus* and *Streptococcus. Staphylococcus*

285   was in highest relative abundance during the summer months of July and August, though this

286   increase in abundance was not statistically significant (Fig. 5). *Staphylococcus* was also present

287   during April and March, but this increase in abundance was not statistically significant.

288   *Streptococcus* was present in much lower abundance than *Staphylococcus sp.* It was present in its

289   highest abundance in April and was too low to be detected in other months. This increase in

290   abundance was not statistically significant (Fig. 5).

291   **Discussion**

292   Inlets are not rivers, but both share several similarities: large volumes of water flowing

293   out of them; connection of inland city and agriculture runoff with the ocean; and generation of

294   visible plumes distinct from offshore marine waters. However, as a mostly artificial construction,

295   inlets may be subject to more detailed or controlled characterizations. Our study considered that

296   different water masses (shallow and deeper) may be generated during ebb tides, which can lead

297   to hydrodynamic complexity (Stamates et al., 2013). For example, the mean volume of ebb tide

298   surface water flow (4.41 million cubic meters per tidal phase) through the PEI is about half the

299   water flow of the deep channel. Although our sampling site was slightly outside the Intracoastal

300   waterway (ICW), we expected our water samples to more closely resemble a brackish surface

301   water microbial community rather than an open ocean water microbial community. This was

302   expected as PEI is influenced by human development and is subject to land-based pollution,

303   which does not influence open-ocean waters. Beach samples and oceanic samples would need to

304   be collected and compared to see which of these environments the inlet most resembles. In a

305   study by Miller and colleagues (2016), it was found that dredging of the Port of Miami from late

306   2013 to early 2015 increased coral tissue loss on adjacent reefs due to sedimentation. The

307   dredging in the Port of Miami appears to have exacerbated local environmental stress due to the

308   coincidence with increased water temperatures leading to a mass bleaching event and increases

309   in reef disease (Miller et al., 2016). This points to the utility of the present report as a baseline

310   prior to the PEI dredging slated to occur in the near future.

311   An earlier study from our laboratory (Campbell et al., 2015) utilized high-throughput

312   454-pyrosequencing technology to generate baseline knowledge of PEI microbiomes, but this

313   was limited to sampling quarterly. In the current study multiple samples per month were

314   analyzed to assess variation and generate a more complete baseline of the microbial community

315   present in PEI. Utilization of Illumina MiSeq sequencing technology also provided more reads

316   per sample, and therefore more coverage, adding confidence to taxonomic assignments. Both

317   studies on the PEI microbiome yielded complementary data creating a strong baseline of the

318   microbiome present in the inlet. These studies can be used by local county and public health

319   officials, who conduct routine monitoring on port waters, and by environmental scientists

320   looking to see what the impacts of the microbial community in PEI might have on the

321   surrounding coastal beaches and the adjacent Florida coral reef (Aranda et al, 2015).

322   **Bacterioplankton Community Composition Taxa Fluctuations: Location, Month,**

323   **and Season**. Seasonal and monthly diversity fluctuations were observed for the PEI samples.

324   The dry season had much higher species evenness, but lower species richness when compared to

325   the wet season. This means that the communities in the dry season were dominated by a few

326   organisms in relatively equal abundances, while during the wet season more taxa were present,

327   but not in similar abundances. This was not unexpected. The wet season in Florida is

328   characterized by increased rainfall that can result in more runoff into the inlet. August had the

329   lowest levels of species evenness and average levels of species richness. August also

330   significantly differed in its microbial community composition when compared to July and

331   September, which did not differ significantly in their bacterioplankton composition. The lower

332   than average rainfall levels in August may have led to decreased sample diversity. Beta diversity

333   of PEI samples was examined and showed that community composition fluctuations were seen

334   between months, seasons, weather at time of sampling, and that there were strong correlations

335   between fluctuations in community composition relating to salinity levels and water temperature.

336   The difference in beta diversity due to weather at time of sampling (rain vs. no rain) was most

337   likely due to surface water dilution during sampling resulting in fewer bacterioplankton

338   organisms sampled during times with rain. Taxonomic classification of sequences at the phylum

339   level yielded similar bacterioplankton community composition results to previously completed

340   coastal seawater studies (Campbell et al., 2015; Gifford et al., 2014; Elifantz et al., 2013; Rappe

341   et al*., 2000). The most evident microbial abundance shifts are seen at lower taxonomic levels.

342   Interestingly, an inverse trend in abundance levels was observed between Proteobacteria and

343   Cyanobacteria throughout the year. It is unknown what caused this trend and more research

344   needs to be completed to determine if these taxa are impacting each other's growth and

345   abundance levels, or if it is other environmental factors causing this trend.

346   **Synechococcus.** The genus *Synechococcus*, decreased in relative abundance during the

347   winter months, or dry season, and increased in abundance during the summer and early fall, or

348   wet season months. *Synechococcus spp.* are in the Cyanobacteria phylum. Our data correlate

349   with previous observations of increased cyanobacterial blooms in Florida coastal and freshwater

350   ecosystems in the late summer and early fall months. The blooms are caused by warm water

351   conditions paired with increased sunlight levels, and nutrient loading from urban runoff

352   (Flombaum et al*., 2013).

353   **Thalassiosira.** Another taxon seen to fluctuate in abundance with the seasons was the

354   diatom *Thalassiosira spp.* Previous studies have shown that this genus is a common resident in

355   marine coastal surface waters, and tends shift in abundance on a seasonal cycle, increasing in

356   abundance with spring algal blooms (Heinzelmann et al., 2016). Our results indicate that this

357   organism increased in abundance during dry season months, however it was seen at its highest

358   relative abundance in early spring. While it would be expected that *Thalassiosira spp.* should

359   increase in abundance during the wet season, it was present in highest abundance in the month of

360   April, the month which marks the start of the wet season.

361   **Rhodovibrio.** Interestingly, members of the genus *Rhodovibrio* were seen only in samples

362   in Florida's wet season. *Rhodovibrio* is a genus commonly seen in oceanic biofilms or microbial

363   mats. There have not been many studies completed on ecosystem roles of *Rhodovibrio*, although

364   it is known that this organism is classified as a purple non-sulfur bacterium which can secure

365   energy through light by being an photoorganoheterotroph or can secure energy through organic

366   matter by being a chemoorganoheterotroph (Stal et al., 2017). The Florida wet season spans

367   summer months when typically, more light availability and warmer water temperatures occur.

368   These conditions could have stimulated biofilm formation and proliferation which could explain

369   why members of the *Rhodovibrio* genus were present in higher abundance during the wet season.

370   ***Acholeplasma.*** Fluctuations in abundance were seen in the genus *Acholeplasma*.

371   Microbes in this genus are in the class Mollicutes and are characterized by lacking a cell wall.

372   Microbes in this genus are known to be commensal organisms in plants, insects, and vertebrates

373   (Kube et al., 2014). It is unlikely that microbes from this taxon are residents of the surface water

374   microbial community, and most likely the presence of this genus in PEI samples is due to size

375   selection when filtering, or because this is the closest classified organism at 97% sequence

376   similarity in the RDP database.

377   **Potential Pathogens**

378   PEI represents a point source of pollution introducing harmful pollutants into the

379   surrounding marine environments including the Florida coral reef tract and recreational beaches

380   (Banks et al., 2008 and Stamates et al., 2013). Due to the influence of the inlet waters on the

381   surrounding marine environments, it is important to examine the presence of potentially

382   pathogenic organisms in the inlet waters.

383   The Bacilli class contained two known potentially pathogenic genera *Staphylococcus,*

384   *and Streptococcus*. *Staphylococcus* abundance levels were highest in the months of July and

385   August; however, members of this genus were also present in March and April. *Staphylococcus*

386   is a genus of gram-positive bacteria commonly found on the nails, skin, and hair of humans (Lian

387   *et al,* 2012). This taxon can thus be shed directly into coastal waters from bathers. The well-

388   known species in this genus, *S. aureus*, can also cause illness in humans. *S. aureus* has a high

389 resistance to salinity, making it a potential threat to other humans using the contaminated water

390 source for recreational purposes. While this species commonly links to both human symbiosis

391 and illnesses, marine mammals have also been infected (van Elk et al., 2012; Bik et al., 2016).

392 The origin of the strain of *S. aureus* that is contracted by marine mammals was most likely from

393 terrestrial sources introduced into the marine environment via runoff (van Elk *et al,* 2012).

394 Studies examining the abundance of *Staphylococcus* over a wet and dry season at a heavily

395 visited coastal area observed increased abundance of *S. aureus* during the wet season (Curiel-

396 Ayala et al., 2012). These data also showed the highest increased abundance of *Staphylococcus*

397 during Florida's wet season.

398   *Streptococcus sp.* were present in highest abundance in April. Increased freshwater input

399 and warm water conditions could have been the cause of the increased abundance of these

400 organisms. This presence also coincided during the prime shipping season in Port Everglades,

401 which may have had an impact on abundance levels of *Streptococcus spp.*

402   *Enterococcus spp.* are important fecal indicator bacteria, most often utilized to assess

403 fecal contamination on recreational beaches and coastal areas (Aranda et al.*,* 2015; Heaney et al.*,*

404 2014; Wade et al.*,* 2003; US Environmental Protection Agency, 1986 and 2004). A recent study

405 examining the number of exceedances of enterococci on recreational beaches in Miami-Dade

406 County, FL from 2000-2010 (Aranda et al., 2015) showed that beaches were only in exceedance

407 of the allowable levels of enterococci 3% of the time. This study examined data generated by the

408 Florida Healthy Beaches Program, which samples weekly. No patterns in regard to rainfall or

409 storms were seen in correlation with enterococci exceedances, although this may be due to the

410 sampling frequency and high decay rate of enterococci in marine waters (Aranda et al., 2015). In

411 contrast to this, a study completed by Curiel-Ayala and colleagues in Mexico (2012), showed

412   increased *Enterococcus* levels during the rainy season, and the highest concentrations of the

413   genus corresponding to highest tourist presence. The highest levels of enterococci seen in the

414   Miami-Dade study were in March and October, which could be due to high tidal levels in

415   October, and possible tourism influences in the month of March overlapping with spring break

416   (Aranda et al., 2015). It was expected that presence of *Enterococcus spp.* would be in highest

417   abundance in PEI during March-April when shipping season and tourist season start.

418   Interestingly, *Enterococcus spp.* were not observed in any of the samples taken in PEI. This was

419   also seen in the previous study examining presence of pathogens in PEI (Campbell et al., 2015).

420   It is possible that *Enterococcus spp.* are not prevalent inhabitants in PEI. More intensive studies

421   would need to be completed to determine if there is the presence of *Enterococcus spp.* in PEI.

422   It was interesting that the causative agent of the white pox disease for coral *Acropora*

423   *palmata,* human fecal bacteria *S. marcescens* was not observed in the top 100 OTUs in the

424   Enterobacteriales class. A recent high throughput molecular study of coral white band disease

425   identified only 5 orders with large numbers of disease-associated OTUs: *Flavobacteriales,*

426   *Alteromonadales, Oceanospirillales, Campylobacterale* and *Rhodobacterales* (Gignoux-

427   Wolfsohn and Vollmer, 2015). Also of note, is the absence of *Vibrionales* pathogens in our

428   dataset. The previous analysis of PEI microbiomes by Campbell et al (2015) showed a low

429   abundance of *Vibrionales.*

430   While it is intriguing that potentially pathogenic genera were detected in PEI, it is not

431   possible to conclude that these organisms are virulent without culturing, in depth metagenomic

432   sequencing, or oligotyping analyses (Eren et al, 2013). A general pitfall of 16S amplicon

433   sequencing is the lack of taxonomic resolution down to the species and strain level. Without this

434   level of classification pathogenicity cannot be confirmed. A previous 16S amplicon and shotgun

435   sequencing study completed on the microbiomes present in the subway system in Boston,

436   showed that there were very few antibiotic resistant genes and virulence factors found in the

437   samples. They also found that there were very few sequences that were identified as

438   opportunistic pathogens or potentially pathogenic organisms (Hsu et al. 2016). This suggests that

439   most likely the microbes identified in this study did not contain virulence factors and are non-

440   pathogenic commensals living in the inlet. However, future research utilizing deeper sequencing

441   and culture-based methods should be completed to further examine presence of pathogens in

442   PEI.

443   **Significance and Conclusions**

444   This study has provided one of the first in-depth profiles of the bacterioplankton

445   community in metropolitan S. Florida inlet waters. The current dataset complements and expands

446   upon a previous a pilot HTP study characterizing the bacterioplankton community of Port

447   Everglades inlet and surrounding coastal waters. Specific marine habitats, such as coral reefs or

448   mangroves, have well defined optimal conditions for thriving and can be sensitive to small

449   perturbations (Precht and Miller 2007; Hoegh-Guldberg et al., 2007). The data from this study

450   will be helpful to local environmental managers, such as the Southeast Florida Coral Reef

451   Initiative (SEFCRI, http://www.dep.state.fl.us/coastal/programs/coral/sefcri. htm), which aims to

452   protect and monitor S. Florida reef habitats. The timeframe of this study appeared poignant,

453   showing that anomalous weather patterns (dry August) can affect community composition. This

454   study also preceded a protracted 2014-2016 El Niño event outbreak of coral disease on the

455   Florida reef tract in 2014 and the planned expansion and deepening of PEI in the upcoming

456   years. Understanding the microbial composition in the inlet prior to these perturbations will

457   contribute to better management and mitigation of the environment and may help to protect the

458   adjacent coral reef where the inlet outflows.

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Figure 1

Alpha diversity measurements of PEI microbiome

(A) Comparison of species evenness in PEI between seasons. Pielou's Evenness values were used to calculate species evenness. Significant differences were observed between seasons of PEI for species evenness, with the dry season having lower species evenness (p=0.020). (B) The comparison of species richness in PEI between seasons. Faith's Phylogenetic Diversity values were used to calculate species richness. Significant differences were observed between seasons of PEI for species richness, with the wet season having higher species richness values (p=0.025).

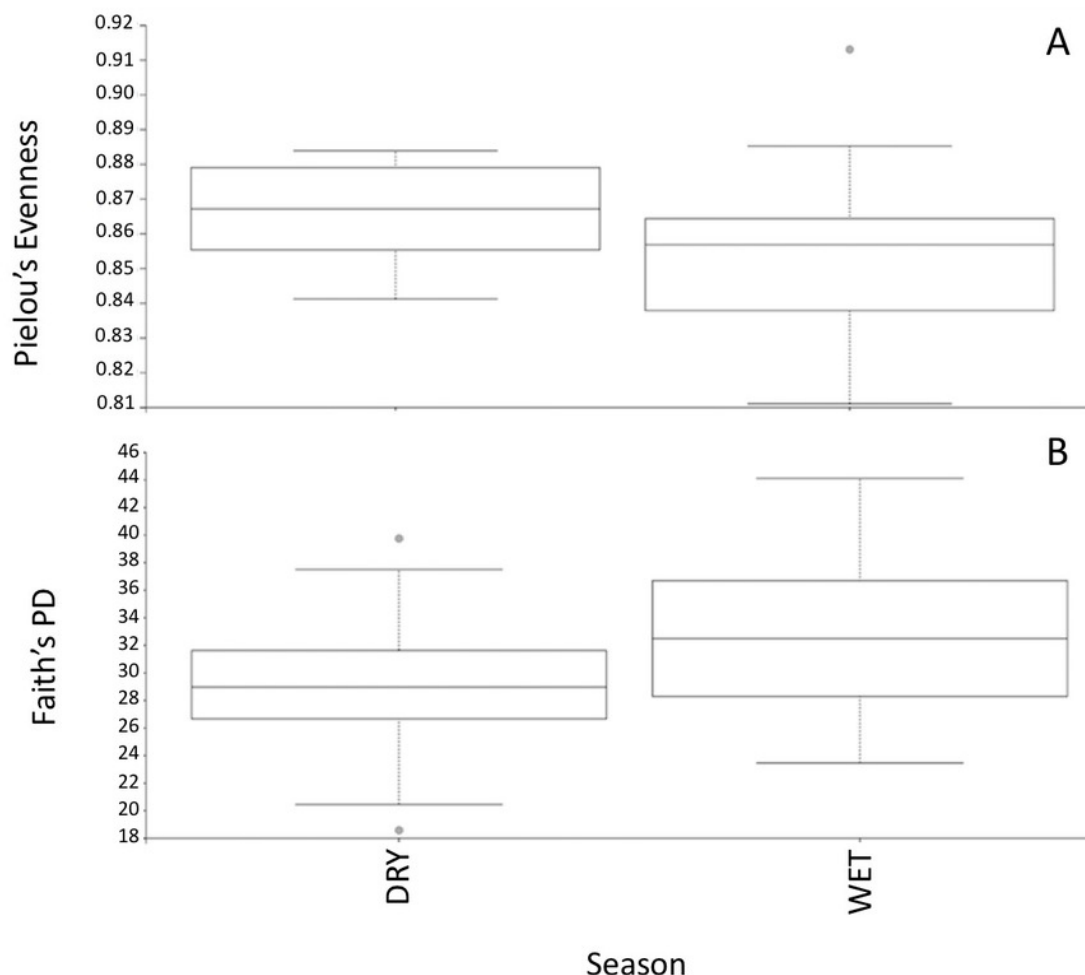



Figure 2

Beta diversity measurements of PEI microbiome

Principle Coordinate Analysis of PEI water samples using Bray-Curtis distance. Clustering of points indicates sample similarity. Significant differences in beta diversity were observed between the wet and the dry season (p=0.001).

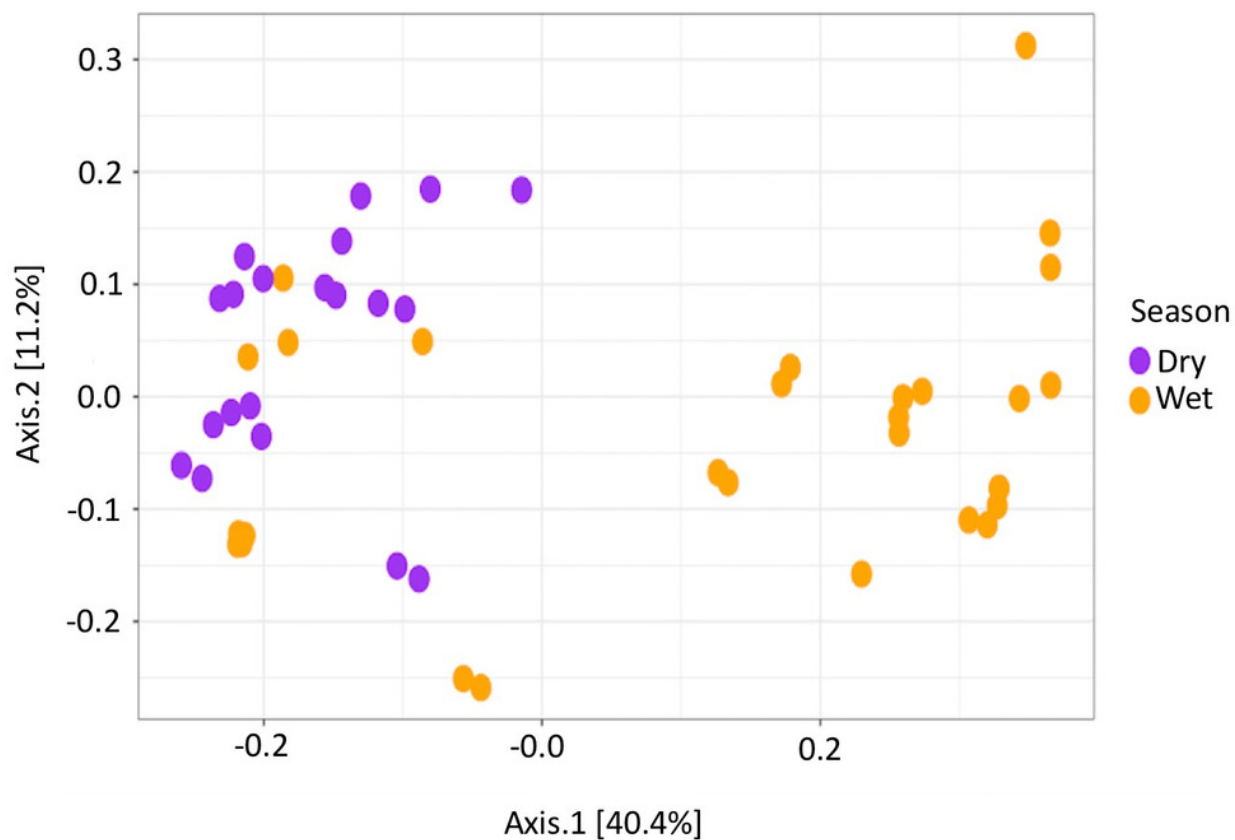



Figure 3

Comparison of relative abundance values of genus-level taxa between seasons.

Stacked bar chart displaying seasonal fluctuations of relative abundance levels at genus-level classification. *Synechococcus* was the only taxon to have significantly different abundance levels between the wet and the dry season.

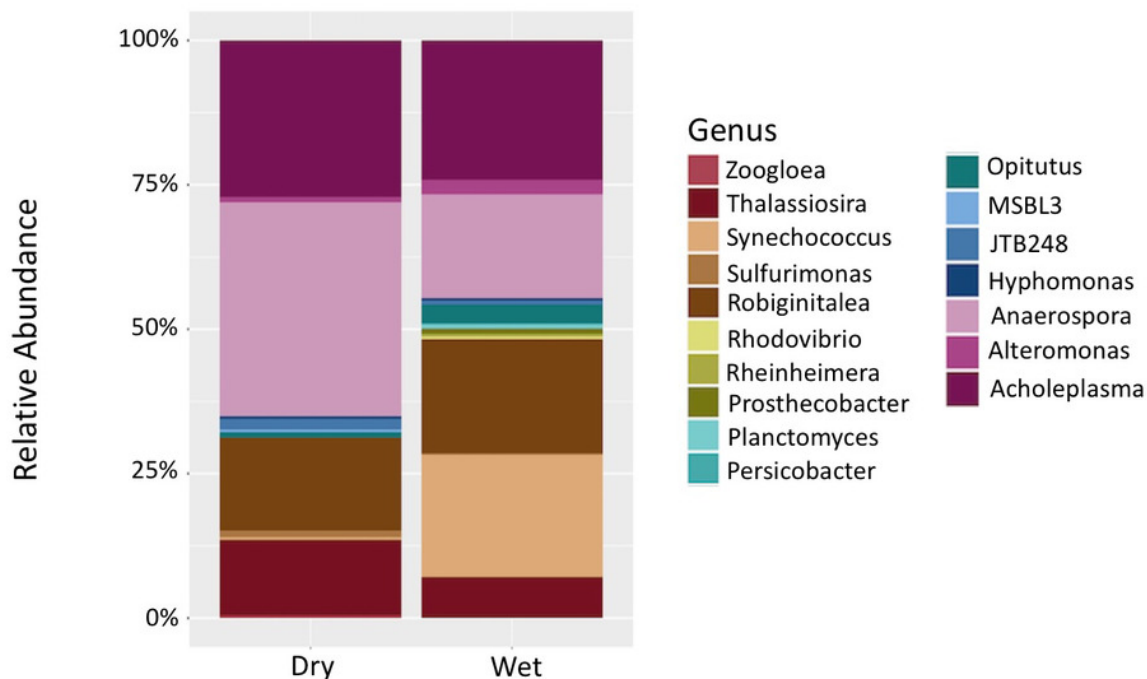



Figure 4

Average rainfall and temperature values for PEI from July 2013 to June 2014.

(A) The average rainfall values for PEI and surrounding areas. The red line indicates the average rainfall levels for PEI during the study. The black line indicates the historical average of rainfall levels for PEI and surrounding areas. The black line is comprised data collected from 1981-2010. (B) The average temperature values for PEI. The red line indicates the average temperature values during the current study. The black line indicates the historical average of temperature values for PEI. The black line is comprised of data collected from 1981-2010. The historical averages were obtained from: https://www.ncdc.noaa.gov/cdo-web/datatools/normals

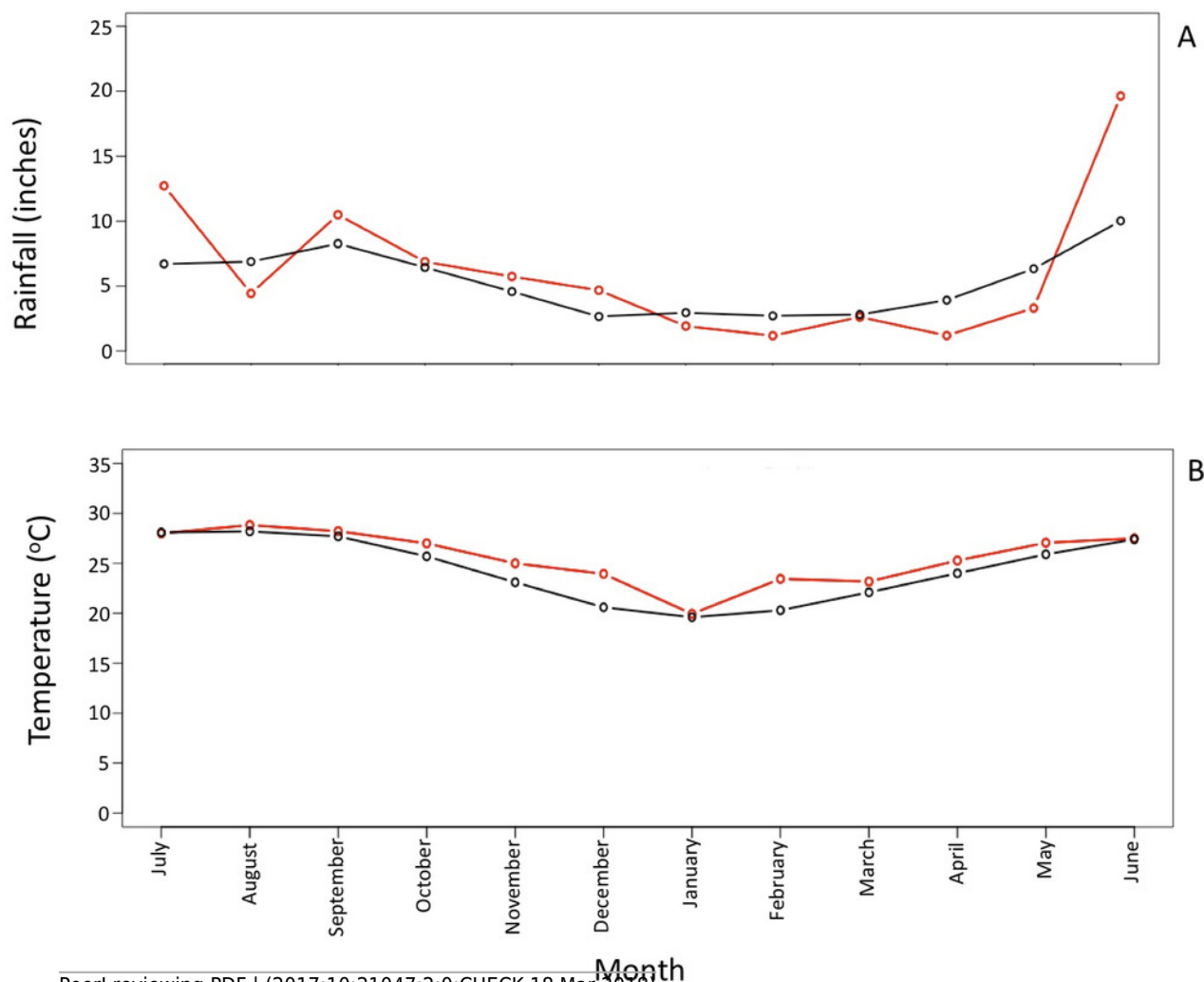
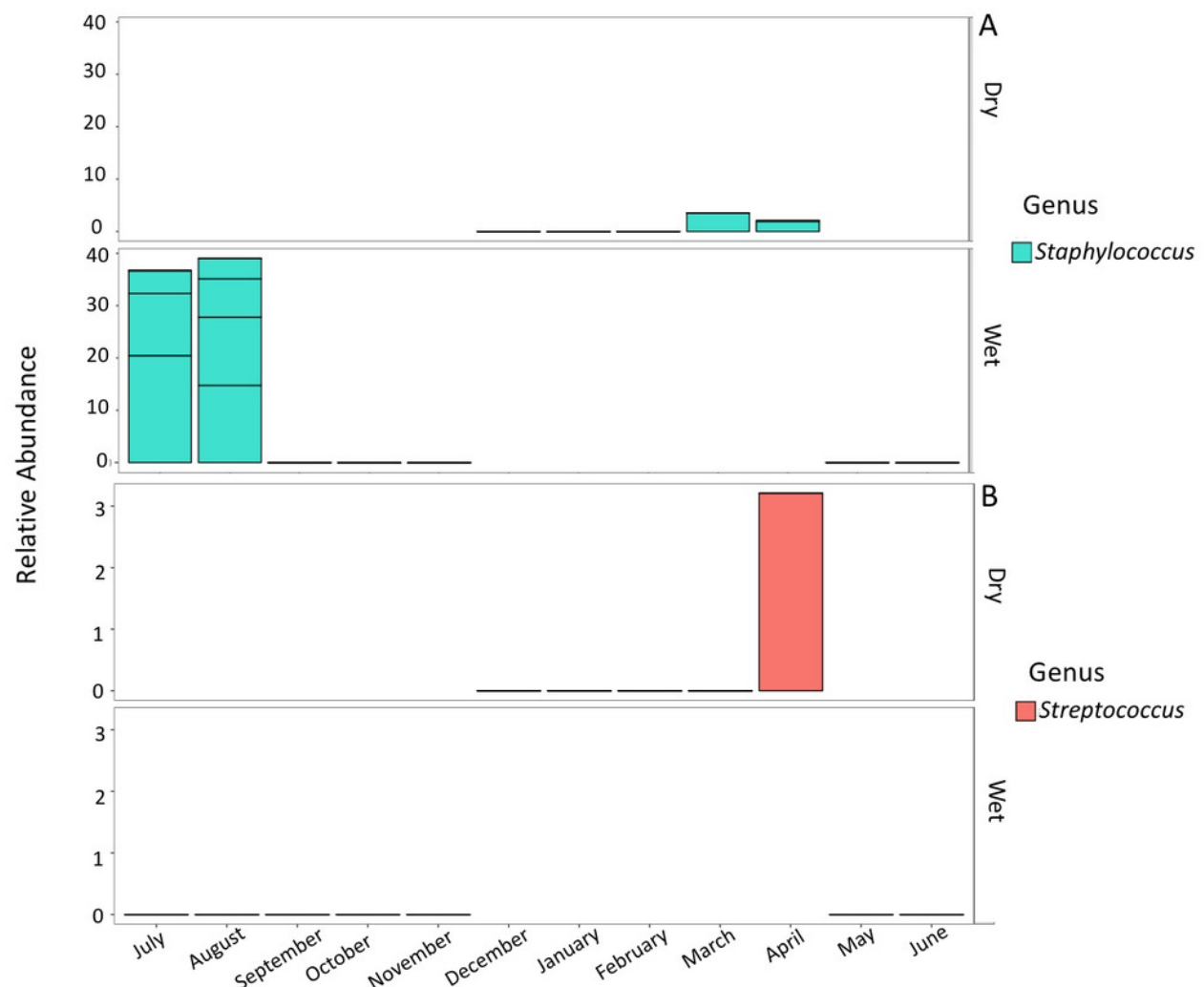



Figure 5

Comparison of relative abundance levels of potentially pathogenic genera in PEI.

(A) Stacked bar chart displaying the prevalence of *Staphylococcus spp*. between wet and dry season. Each box comprising the bars in the plots is a different OTU. (B) Stacked bar chart displaying the prevalence of *Streptococcus spp.*between wet and dry season. *Staphylococcus spp*. were present in much higher relative abundance levels than *Streptococcus spp.* as displayed by the different y-axis scales.

ANCOM test results for differential abundance of *Synechococcus spp.*

The ANCOM results for *Synechococcus spp.* that differed significantly in abundance levels between seasons. High W values indicate significant differences in abundance levels between seasons. The higher the W value the more significant the differences in abundance levels between seasons.

Percentile	0	25	50	75	100	0	25	50	75	100		
OTU	Dry	Dry	Dry	Dry	Dry	Wet	Wet	Wet	Wet	Wet	Reject null hypothesis	W
Unidentified *Synechococcus sp.* 1	1	1	42.5	60.5	159	31	86.25	120	159.75	313	TRUE**	14
Unidentified *Synechococcus sp.* 3	1	1	1	1	11	1	1	1	1	21	TRUE**	13
Unidentified *Synechococcus sp.* 5	1	1	1	8	57	1	23.75	41.5	79	132	TRUE**	14
Unidentified *Synechococcus sp.* 7	86	160.25	378	539.5	2172	231	822	1725	2411.25	4189	TRUE**	11
Unidentified *Synechococcus sp.* 8	1	1	1	1	34	1	1	1	1	57	TRUE**	13
Unidentified *Synechococcus sp.* 9	1	1	1	1	1	1	1	1	1	25	TRUE**	13
Unidentified *Synechococcus sp.* 11	1	1	1	1	1	1	1	1	1	27	TRUE**	13
Unidentified *Synechococcus sp.* 13	1	1	1	1	1	1	1	26.5	41.75	114	TRUE**	14
Unidentified *Synechococcus sp.* 15	1	1	1	1	1	1	1	51.5	123.25	237	TRUE**	18
Unidentified *Synechococcus sp.* 18	1	1	1	1	1	1	1	12.5	51	98	TRUE**	11
Unidentified *Synechococcus sp.* 20	1	1	1	1	1	1	1	32.5	64.25	213	TRUE**	15
Unidentified *Synechococcus sp.* 21	97	164.25	202.5	292.75	539	44	129.25	179	301.75	752	TRUE**	13
Unidentified *Synechococcus sp.* 24	1	1	1	1	1	1	1	30.5	47.75	105	TRUE**	16
Unidentified *Synechococcus sp.* 25	1	1	1	1	29	1	1	1	1	1	TRUE**	14
Unidentified *Synechococcus sp.* 29	1	1	1	1	1	1	1	1	1	19	TRUE**	13

Linear regression analysis of environmental metadata in relation to microbial taxa observed.

Multiple least squares regression analysis at class level taxonomy. Gammproteobacteria, Alphaproteobacteria, & Betaproteobacteria were abbreviated for space. There were statistically significant relationships with specific taxa and salinity, water temperature, rainfall, and chloride ion concentration. Alpha = 0.10.

	Gamma	Flavobacteriia	Acidomicrobiia	Alpha	Beta	Synechococcophycideae	Actinobacteria	Unclassified
R-Squared	0.21	0.21	0.21	0.13	0.14	0.2	0.21	0.07
Salinity	0.017**	0.101	0.0013**	0.0008**	0.0005**	NA	0.051**	NA
Water Temperature	0.060**	0.020**	NA	NA	NA	0.001**	0.0001**	NA
Chloride	NA	NA	NA	NA	NA	0.016**	NA	NA
Sulfate	NA	NA	NA	NA	NA	NA	NA	NA
Rainfall	NA	NA	NA	NA	NA	NA	NA	0.0184**