Bacterial Diversity by Time of Day in Marine Samples Near Puerto Rico

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INTRODUCTION

Oceanic biogeochemical processes that regulate levels of organic matter and nutrient cycling are heavily influenced by regional bacterial composition. Past studies have indicated that production and bacterial composition vary with specific regions in the oceans and that this can influence which biogeochemical processes are upregulated. This can influence the levels of organic matter and nutrient cycling (Schattenhofer et al. 2009; Yokokawa et al. 2013). Previous research has indicated that in the Atlantic Ocean there are specific patterns of bacterial diversity over vertical and horizontal distributions (Tada et al. 2016; Teira et al. 2006). This suggests that regional features of the oceanic regions contribute to the distribution pattern of bacterial diversity in the Atlantic Ocean. This study investigates the horizontal distribution of bacterial species in the epipelagic layers of the regions of the Atlantic Ocean and Caribbean Sea surrounding Puerto Rico, and considers how bacterial diversity differs during the day as compared to the night. This region is of specific interest because of the proximity of our sampling sites to the coast of Puerto Rico. Because the sites are close to Puerto Rico, nutrient composition of the waters is different than the nutrient composition of the open ocean. Specifically, extremely high levels of phosphate in this region may impact how the bacteria is distributed, because phosphate is an important component of proteins and DNA. This investigation of the regional distribution of specific bacterial diversity during the day and night will facilitate a better understanding of the linkage between microorganisms and biogeochemical processes in the waters around Puerto Rico.

GOALS

I. Compare bacterial diversity between marine samples collected during the day and night.

II. Compare how environmental factors such as phosphate influence community diversity and chlorophyll levels.

HYPOTHESES

I. Bacterial diversity will be distributed differently during the day and night.

II. Higher phosphate levels will influence community diversity and chlorophyll levels.

METHODS

A. Sample collection

Marine sample collection occurred during a cruise which began at San Juan harbor and circumnavigated Puerto Rico as part of a 2017 J-term Study Abroad course at Miami University called "SEA Miami". Day samples were collected at approximately 10 am, while night samples were collected at approximately 12 am. 200 mL of sea water was collected using Niskin bottles, filtered onto 0.2 µm PALL membrane filters and hand-carried to the US lab on ice. Samples were kept at -80 C until extraction. Phosphate concentration was determined from 200 mL of sea water filtrate using the ascorbic acid method. Chlorophyll a fluorescence was monitored using a diving fluorometer attached to the hydrocast. To measure Chlorophyll a, 20 mL of sea water were collected from Niskin bottles, filtered onto 25 mm GFF filters, and extracted in 95% Ethanol. Fluorescence of extracted samples was measured on a Turner fluorometer.

Fig. 1: Map of sampling locations around Puerto Rico. White boxes indicate day samples; gray boxes indicate night samples.

B. Sample processing

Fig. 2: Workflow of sample processing. After samples were collected, DNA was isolated, sequenced, and analyzed for bacterial species present. Diversity between the samples was then compared using VAMPS (Huse S, et al. 2014).

SAMPLING COMPLETENESS

A. Rarefaction curve

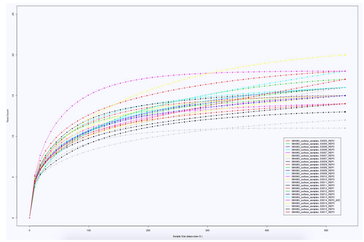
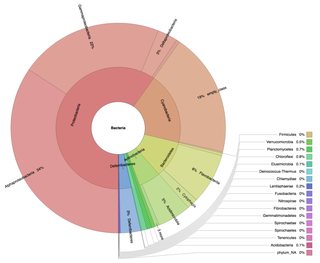


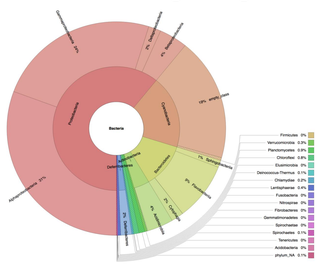
Fig. 3: Aggregated rarefaction curve generated for all surface station samples. The rarefaction curve to the right illustrates the species richness of the surface samples. The horizontal leveling off of the curves signifies that further intensive sampling would be unlikely to reveal any more common species and as such, only the rarest species are left unknown. The sampling in this experiment was adequate in terms of the species richness found in the surface samples, and no further sampling is necessary for complete results.

DISTRIBUTION OF MAJOR PHYLA

A. Collected during the day

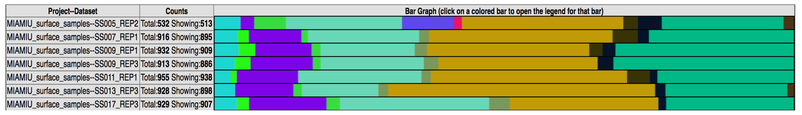


B. Collected during the night



C. Community comparison day samples

D. Community comparison night samples



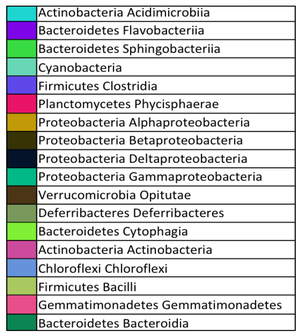


Fig. 4: Various alpha diversity measures of surface station samples. The first pie chart shows the alpha diversity of sample SS006, which was collected in the day (**A**). The second pie chart shows the alpha diversity of sample SS007, which was collected at night (**B**). The community bar graphs, made with use of VAMPS software, show the alpha diversity of SS005-SS017 (excluding SS0116) and are split up by day and night, as well (**C** and **D**). The most common classes seen in the surface samples were Flavobacteria, Cyanobacteria, Alphaproteobacteria, and Gammaproteobacteria. Samples 5, 12, and especially 14 show major differences in alpha diversity compared to the other samples, but for the most part, the composition of each sample remains relatively constant. All of the figures above are highly suggestive that there is not much change in terms of the diversity of microbial life in comparing day to night.

ACKNOWLEDGEMENTS

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RELATIONSHIPS BETWEEN THE SAMPLES

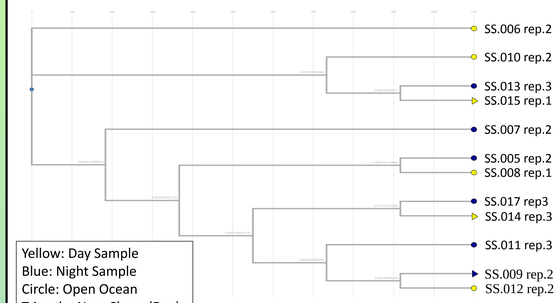
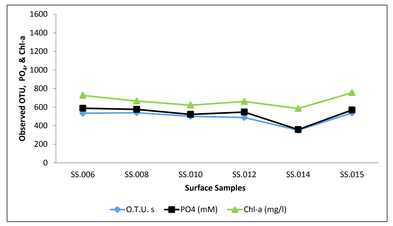


Fig. 5: Beta Diversity Dendrogram. Demonstrates the relatedness of our samples. Compares the surface samples in terms of distance to shore as well as the time of day each sample was taken. Sample repetitions were chosen at random. SS006 was very different from SS007, which was surprising because they were only 1 mile apart from each other. SS017 had greater similarity to close shore samples than open ocean samples, which is odd because SS017 was from the open ocean.

IMPACT OF PHOSPHOROUS

A. Bacterial abundance during the day



B. Bacterial abundance at night

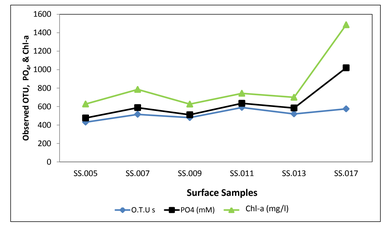


Fig. 6: Comparison of day (A) and night (B) surface samples in phosphate levels (PO_4), Chlorophyll-a (Chl-a), and observed transcriptional units (OTUs). Demonstrates how phosphate levels correlate with bacterial diversity. As phosphate levels increase, bacterial diversity concentration of chlorophyll-a increases in both day and night samples. Sample SS017 shows the positive relationship between phosphate and chlorophyll-a. SS017 is also unique because it was from the open ocean, but it still has very high levels of phosphate.

CONCLUSIONS

It can been seen that time of day of sampling does not influence the community structure as much as other factors examined. When comparing the alpha diversity of a day and night sample from a similar location, almost no difference is seen. From the community composition, the day samples had differences in community structure within themselves. In SS.012 rep.2, there was an increased presence of Gammaproteobacteria and Chloroflexi, and in SS.014 rep.2, Gemmatimonadetes and Deferribacteres presence was increased. The night samples were mainly homologous, the one major difference being the presence of both Clostridia and Phycisphaerae in sample SS.005 rep.2. Samples located close together were more related than samples far apart, and samples taken southeast of Puerto Rico were particularly similar. However, it is shown that samples taken in open ocean and close to shore do not always have similar community structures. Phosphate levels showed to have the greatest impact of our variables studied on bacterial diversity and community structure. As phosphate levels increase so do bacterial diversity and levels of chlorophyll-a. This suggests that phosphate is a limiting nutrient, which is comparable to existing research on the Southern Ocean (Falkowski, 1998).

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