Metabarcoding Aquatic Microbial Communities at Clove Lakes Park, Staten Island

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Abstract

We aim to measure the fluctuations in bacteria populations over time, specifically, as the weather grows warmer. We collected water samples from a small stream in Clove Lakes Park on Staten Island, NY. Utilizing centrifugation methods to pellet microorganisms from the water samples, we were able to extract DNA and sequence it to assess the estimated abundance of the microbial populations residing in the samples. By viewing the effects of increasing temperatures on the amounts of bacteria concentrated in water, we can draw conclusions as to how environmental factors influence the composition of aquatic microbial communities.

Introduction

- Clove Lake is a freshwater lake located on Staten Island
- We sought to assess the microbial makeup of Clove Lake through metabarcoding over two months in Spring
- We hypothesized that the warmer temperatures at the second collection date will impact microbial diversity *(Currie, et al, 2017)*
- Previous research (Tsuji et al., 2017) stated that bacterial abundance is higher at higher water temperatures
- Ambient air temperatures leading up to sample collection, as well as precipitation were recorded to see if there was a correlation between microbial diversity and weather
- We also hypothesized that human factors can also have an impact on microbial makeup. For example, salt is used during winter months to prevent ice. Runoff from paved areas to Clove Lake could impact bacterial makeup of the water

Materials & Methods

- Water samples from Clove Lake were collected on March 9th and April 6th, 2024.
- The samples were centrifuged multiple times to concentrate bacterial cells, and DNA extraction was performed using a Qiagen PowerSoil kit.
- Metabarcoding with 16s primers was conducted, followed by PCR and gel electrophoresis to check for DNA
- Illumina Amplicon sequencing was performed by GeneWiz
- Data analysis was done by using the ScienSano (Belgian Public Health Department) 16s pipeline on Galaxy
- The results included NCBI blast summaries and a Krona pie chart
- Additionally, weather data from Weather Underground was used for analysis on how environmental factors such as precipitation may impact the bacterial populations in the aquatic environment of Clove Lake.

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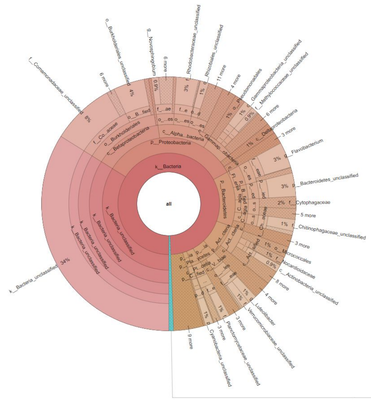
Table 1- Weather events for March 6-9 2024 (Source- Weather Underground Station KNYNEWYO1176)

Date	High temperature	Low temperature	Precipitation
March 6th	48.2°F	45.2°F	1.56 inches
March 7th	57.6°F	51.5° F	0.11 inches
March 8th	57°F	38.7°F	0.0 inches
March 9th* Sample collection date	46.6°F	39.7° F.	1.30 inches

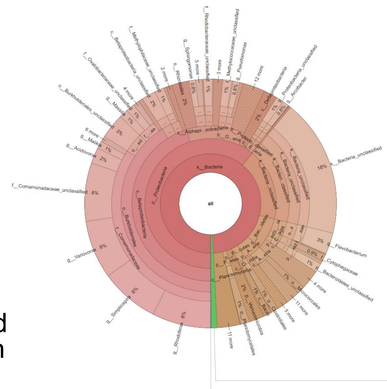
Table 2- Weather events for April 3-6 2024 (Source- Weather Underground Station KNYNEWYO1176)

Date	High temperature	Low temperature	Precipitation
April 3rd	43.5°F	40.3°F	1.42 inches
April 4th	51.4°F	35.6°F	0.33 inches
April 5th	52.7°F	36.7°F	0.00 inches
April 6th* Sample collection date	55.6°F	37.8°F	0.00 inches



Graph 1: Krona table of largest Genera bacteria compositions + Archaea

Composition	Genera
34%	Unclassified bacteria
8%	*Comamonadaceae*
4%	*Burkholderiales*
3%	*Rhodobacteraceae*
3%	*Flavobacterium*
3%	*Bacteroidetes*
2%	*Cytophagaceae*
0.6%	*Archaea*



Graph 2: Krona table of largest Genera bacteria compositions + Archaea

Composition	Genera
18%	Unclassified bacteria
8%	*Comamonadaceae*
8%	*Variovorax*
8%	*Simplicispira*
8%	*Rhodoferax*
3%	*Burkholderiales*
0.8%	*Archaea*
0.05%	Unknown

Results and Discussion

- Over time, unclassified bacteria percentages decreased while the proportion of Camamonadaceae increased. We also saw changes in the percentages of Archaea DNA
- genera Variovorax, Simplicispira, and Rhodoferax all individually increased to 8% (a sum of 24%) by 4/6/2024 from a total 8% of unclassified bacteria under the Comamondacaeae family on 3/9/2024. This points to the conclusion that these genera prefer warmer temperatures over colder ones.
- Archaea are a separate kingdom from bacteria, but are also single-celled prokaryotes. They thrive in warm environments (Chaban B et al., 2006). This supports their increase from 0.6% in March to 0.8% in April, since the weather was warmer in April
- Averaging temperatures from 3/9/24 and 4/6/24, we can see a 7°F increase
- Cytophagaceae and Burkholderiales genera decreased, from 2% and 4%, to 0.9% and 3%, respectively. Although Burkholderiales decreased by only one percent, which could be considered negligible, Cytophagaceae was nearly completely eliminated.
- This could indicate that these bacteria prefer colder climates
- These results show bias towards bacteria over Archaea species. There is also a bias towards the reference genomes available on NCBI. Other strains of common species could have been present but unable to be speciated on NCBI (van der Loos et al., 2020).

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