The COMMUNITY Foundation for Greater New Haven
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# QUINNIPIAC RIVER FUND FINAL REPORT- 2019

Please complete and submit completed form via e-mail to dcanning@cfgnh.org at The Community Foundation for Greater New Haven by March 31, 2020 (or as otherwise stated on the terms of grant).

Date: 24 February 2023

Group/Organization Name: University of New Haven

Address: 300 Boston Post Road

City, State, & Zip: West Haven, CT 06516

Telephone #: 203-479-4257

Project Name: Cyanobacterial biodiversity and nutrient usage in the Quinnipiac River

Grant Number: 20190125

Name & title of person completing this form: Dr. Amy Carlile (Associate Professor) and Dr. Nik Stasulli (Assistant Professor)

E-mail address: acarlile@newhaven.edu, nstasulli@newhaven.edu

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Please respond to the following statements:

1. List the specific objectives/outcomes of the project and tell how they were met during the grant period. Also, provide an update on any special conditions of the grant (if applicable).

In this project, we aimed to study the cyanobacterial communities of the Quinnipiac River by addressing the objectives below. We sampled four sites (Hamlin Pond, Southington Canoe Launch, Sindall Rd., and Hanover Pond) between April and August 2019. At each site, we collected three samples from the water column and three from the benthos (where possible) for a total of 107 samples.

1) Characterize cyanobacterial communities along the Quinnipiac River and monitor for bloom-forming species.

Several methodologies were used to characterize the cyanobacterial communities in the Quinnipiac River. Flow cytometry was used to measure the count of cells in samples that contained allophycocyanin, a pigment found in cyanobacteria. Cyanobacteria were found at all sites but were in the highest abundance in benthic samples at Hanover Pond and the stream at Sindall Road.

A portion of each sample was used for culturing using streak plating on enriched media. Single colony isolates were grown in media to obtain enough material for DNA extraction. We had difficulty in obtaining unialgal culture isolates, and using the 23S rDNA plastid marker (I.e. UPA, Sherwood and Presting 2007) only 13 isolated were successfully sequenced; six were cyanobacterial species. No bloom-forming or toxin-producing species were isolated.

Environmental DNA was extracted from all samples and used for metabarcode analysis. Published primers designed to amplify cyanobacteria and plastid 16S rRNA were used to generate sequences using the MiSeq Illumina platform (Nubel et al., 1997). The analysis picked up several lineages of cyanobacteria and other bacterial species. Most lineages were found in all samples, although some lineages were greater in abundance in either pond or stream sites. For example, *Microcystis* was found in greater abundance in Hanover Pond (Figure 1). A taxonomic annotation of the sample with the greatest *Microcystis* abundance (NS077) shows that this taxon makes up 15% of the sequences in the sample (Figure 2). Species within this genus can produce microcystins, harmful toxins that can negatively impact human health. While many samples had some trace of this genus, the highest abundance was found in Hanover Pond surface water in June and July.

2) Determine the extent to which biofilm communities in the benthos seed cyanobacterial populations in the water column. These pelagic communities have the potential to produce blooms.

We saw differences in benthic and surface water cyanobacterial communities, although these were more pronounced at pond sites. Of the unique sequences generated in our metabarcode analysis, approximately 41% were shared between benthic and surface water habitats and 50% were only in surface waters. The taxon of greatest interest due to its potential to produce harmful algal blooms, *Microcystis*, was found in both benthic and surface waters. It was present in all benthic samples from Hanover Pond, but at low relative abundance to the other sequences obtained from these samples. Its relative abundance fluctuated much more in surface water samples during the sampling period. This could indicate that there is a latent community of this taxon in the benthos that could seed greater numbers in the water column.

3) Identify correlations between the nutrient and pollutant inputs into the riverine system and the metabolic utilization of those types of nutrient sources by cyanobacterial species in situ.

The final objective of our project sought to identify correlations between the nutrient and pollutant inputs into the riverine system and the metabolic utilization of those types of nutrient sources by cyanobacterial species in situ. Specifically, we tested for phosphate, nitrate, nitrite, ammonium, dissolved oxygen, salinity, pH, and temperature during each of the visits to all four sites. This was the stage of our project that we had the most difficulty with and where our experimental sampling did not produce results that were easily analyzed to achieve this goal. The typical chemicals that indicate contamination of a water source did not have an identifiable trend across our sites. Individually, we could see some trends. During our May sampling, Sindall Rd. had particularly high levels of reduced phosphorous and nitrogen compounds. The Hanover Pond site had relatively high levels of all phosphorous and nitrogen compounds tested for during the final August sampling. The Southington site had relatively low levels of inorganic compounds except nitrate, which was quite variable throughout the sampling times. Hamlin Pond was the most variable site with little discernable trends over the sampled dates. The variability of the inorganic compound sampling did not allow us to successfully identify the correlation we proposed. Even though we weren't able to look specifically at cyanobacteria, the microbial metabolic data we did collect, indicating the metabolic capacity of the microbes present as a whole, was also variable over time based on the location in the water column where samples were collected from (e.g. carbon utilization potential in Hanover pond in June indicated that the surface water sampled had higher metabolic potential than benthos sampled. This pattern was completely the opposite from the results after our mid-July sampling). As part of our experiments, we did end up culturing cyanobacteria isolates that could be tested in the future for their individual metabolic potential, but as a community of microbes, it became difficult to correlate metabolic potential to nutrient pollution do to an abundance of environmental variability.

2. Please share your successes, challenges and any lessons learned through the implementation of your project. Were there any unintended consequences or lessons learned that may affect how you operate your program moving forward?

Some of our bigger challenges had to do with the inherent variability of natural environments. The frequency of our sampling did allow us to sample the microbiomes of the sites at various time points, but the variability in the inorganic compounds we tested made it difficult to make correlations between microbial populations and inorganic pollutants like phosphate and various oxidative states of nitrogen. Various environmental factors like most recent rainfall and water flow rate could shift these concentrations quickly and drastically, especially at the more riverine sites.

3. What are the opportunities and needs of your organization as it continues to move forward with its work to positively impact the Quinnipiac River?

Our results highlight the need for more sampling of cyanobacterial communities in non-river sites, especially Hanover Pond. A variety of recreational activities take place in this area that might contribute to cyanobacterial blooms and monitoring is needed to track growth of any

toxin-producing species. Our work found the presence of *Microcystis* sp. in both benthic and surface water samples. While this taxon is not producing harmful blooms at this time, its consistent presence over the course of our sampling warrants tracking. Along with *Microcystis* sp. tracking, establishing a more continuous nutrient sampling strategy would greatly improve the information that we're able to obtain about the status of the Quinnipiac River. Because of the listed above *Microcystis* sp. implications we would propose that this monitoring occurs at Hanover Pond where a high potential for blooms and human/animal interactions may occur. Developing a water and *Microcystis* sp. monitoring strategy at Hanover Pond could ensure an early alert of high pollutant or harmful algal bloom levels.

Also, please email a photo or image that can be uploaded along with your report to The Quinnipiac River Fund website to dcanning@cfgnh.org.

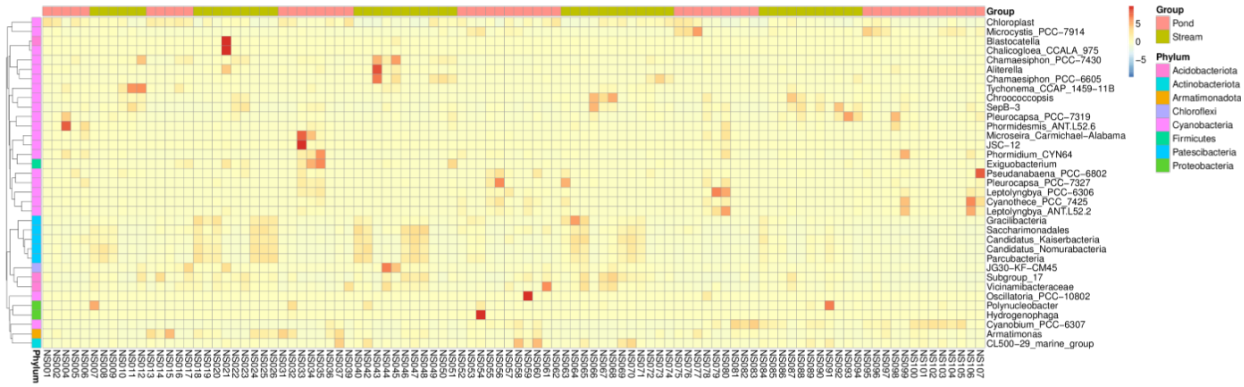


Figure 1. Taxonomic abundance cluster heatmap. Sample names are on the x-axis and genus names are on the y-axis. The main habitat type (pond vs. stream) is indicated at the top of the heatmap.

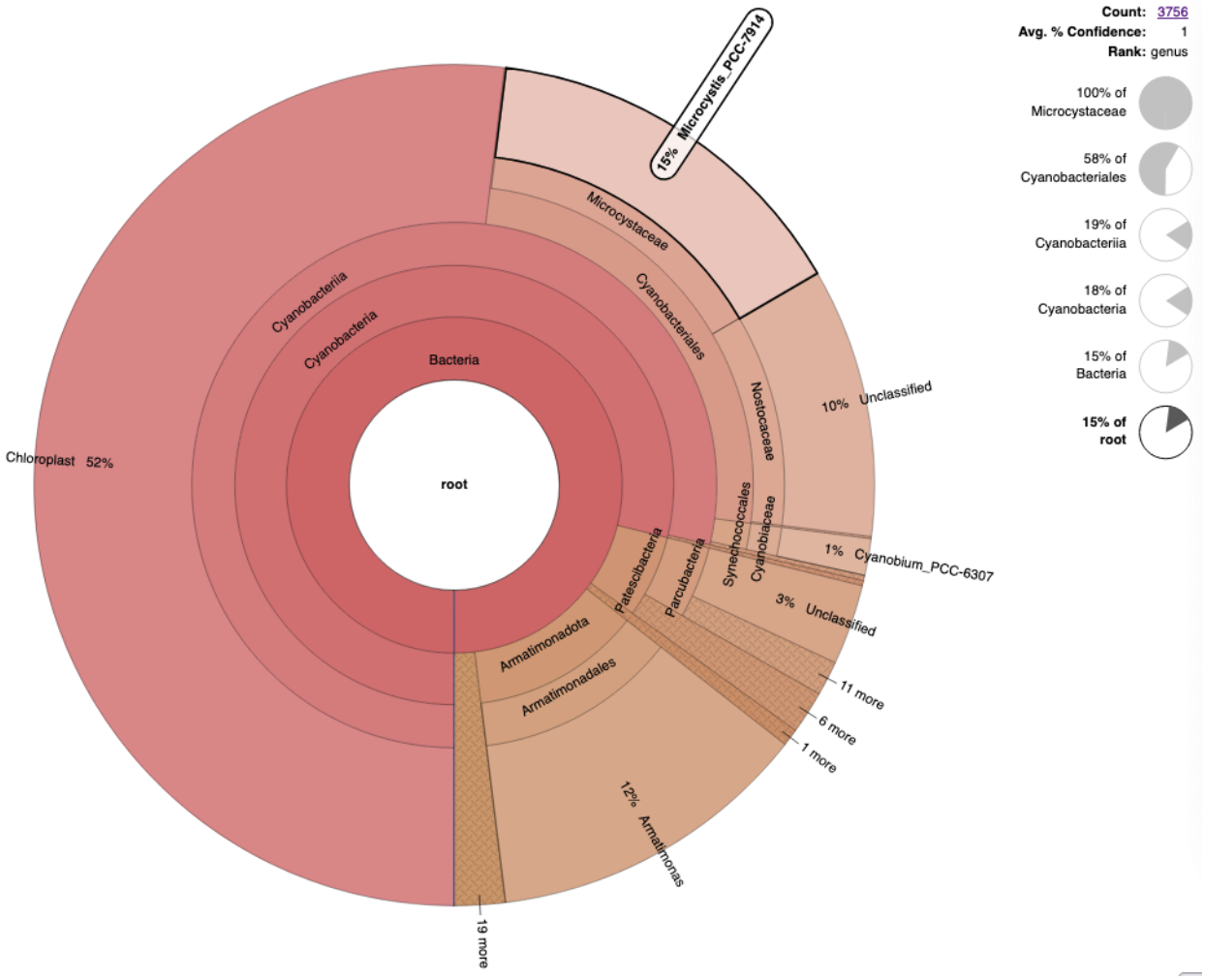


Figure 2. Taxonomic annotation of sequences from sample NS077, taken from the surface water of Hanover Pond in July 2019.