Metabarcoding of Bacterial Species in the Hudson and East Rivers

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Abstract

Many efforts by the government have been made to clean up the pollution accumulated in the Hudson and East Rivers. However, the presence of fecal indicator and antibiotic-resistant bacteria indicate the rivers are still fairly contaminated. The objectives of our research project were to determine the bacterial species present in the Hudson and East Rivers using metabarcoding, compare the populations of bacteria, and potentially identify any harmful bacteria that cause waterborne illnesses. Samples from the Hudson and East River were collected and filtered, and extracted for DNA using the Qiagen DNeasy PowerSoil Kit.

Introduction

Hudson and East Rivers

The Hudson and East Rivers in New York City span a total of 331 miles and are home to numerous species of fish, birds, and other aquatic animals (The Editors of Encyclopaedia Britannica, 2016). However, the rivers have been centers of pollution since the 19th century. Although many efforts to reduce the contamination have been made, the waters of the Hudson and East Rivers are still heavily polluted today.

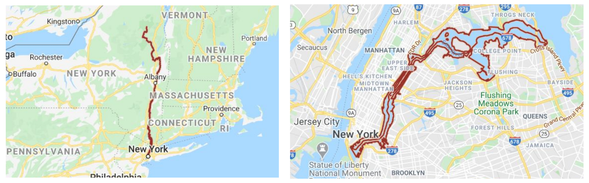


Figure 1: Map of the Hudson River (Right) and the East River (Left)
(Google Maps)

In the 30 years leading up to 1977, when the EPA banned the production of polychlorinated biphenyls (PCBs), approximately 1.3 million pounds of PCBs entered the Hudson River from two General Electric manufacturing sites in New York. In addition to being probable carcinogens, PCBs in the river sediment affected the fish and wildlife of the Hudson River (EPA, Hudson River Cleanup).

The East River was also a common place to dump waste and sewage, especially during American industrialization. As a result, the populations of fish and other organisms in the East River diminished. In addition, the pollution of the East River led to typhoid outbreaks due to the bacterial contamination (East Harlem Studio, 2013).

In 1972, Congress passed the Clean Water Act to make US waters safe by placing requirements on removing pollution from rivers. As a result, the Hudson and East River's water quality has improved since the 1970s. But the CWA is stated to have been unable to resolve stormwater runoff and combined sewer overflows (CSOs), which allow the rivers' pollution to persist.

DNA Metabarcoding

DNA metabarcoding is the use of subsets of genes from organism samples in an ecosystem to identify species, document species diversity, and find rare taxa. DNA metabarcoding uses the Next Generation Sequencing (NGS) technology, which consists of three different processes: PCR amplification, amplicon multiplexing and sequencing, and data analysis. Metabarcoding multiplexes and sequences amplicons use short homologous gene fragments from different samples all at once to increase efficiency and lower costs. Factors such as mutation rate, universal primers, and marker choice must be considered before a genomic region is amplified. The efficacy of primers, which depends on barcode coverage and barcode specification, can also affect the accuracy of metabarcoding (Pavan-Kumar, Gireesh-Babu, and Lakrak, 2015).

Objectives

- Determine the bacterial species present in the Hudson and East Rivers using metabarcoding
- Compare the populations of bacteria present in each rive
- Compare the current safety of the rivers today and identify which river has had a more effective cleanup effort.

Materials & Methods

Our plan for this project was as follows:

Collect water samples — We collected three samples each from the Hudson River at Pier 40 Dock and the East River at the Brooklyn Bridge Beach.

Extract DNA from water samples — Afterward, we filtered the water samples through vacuum filtration. We then isolated and extracted the DNA from the samples using the Qiagen DNeasy PowerSoil kit.

Conduct a PCR — We then perform a PCR in order to make copies of the target gene from the extracted DNA. We would test the success of this PCR using a gel electrophoresis.

Sequence DNA samples — After verifying our results, we would send our DNA samples to a lab in order to sequence them.

Analyze the results — We would then analyze our results using DNA subway, utilizing the Metabarcoding Analysis track.

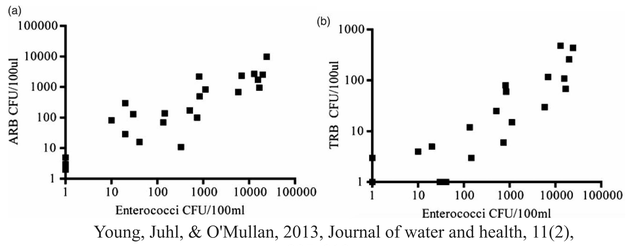
Sample	Location	Coordinates	pH	Temperature (C)
1	East River	40.696712, -73.998911	7.5	9
2	East River	40.696712, -73.998911	7.5	9
3	East River	40.696712, -73.998911	7.5	9
4	Hudson River	40.728261, -74.013769	N/A	7
5	Hudson River	40.728261, -74.013769	N/A	7
6	Hudson River	40.728261, -74.013769	N/A	7

Figure 2: Sample Data

Literature Review (in lieu of results)

Due to conditions caused by COVID-19, we were not able to get any results from our extracted DNA. Instead, we performed a literature review regarding previous work done on the two rivers:

A study was conducted in June 2013 examining antibiotic-resistant microbes and its distribution throughout the Hudson River, as well as testing the correlations of sewage and weather conditions to antibiotic-resistant microbe concentrations (Young, S., Juhl, A., and O'Mullan, G. D., 2013). Water samples were collected at 10 different sites throughout the lower Hudson River Estuary during both dry and wet weather conditions, and the samples were isolated and analyzed using 16S RRNA gene sequence analysis. Bacteria resistant to antibiotics tetracycline and ampicillin were detected in all 10 sites, with ampicillin-resistant bacteria having a 46% higher abundance than tetracycline-resistant bacteria. The results also revealed positive correlations between concentrations of fecal indicator bacteria, such as Enterococcus, and the concentrations of antibiotic-resistant bacteria. Thus, antibiotic resistant bacteria was more prevalent in sewage areas. In addition, there was a 70% higher concentration of antibiotic-resistant and fecal indicator bacteria in nearshore areas than mid-channel areas, as well as 70% higher sewage contamination and abundance of antibiotic-resistant bacteria in wet weather compared to dry weather.



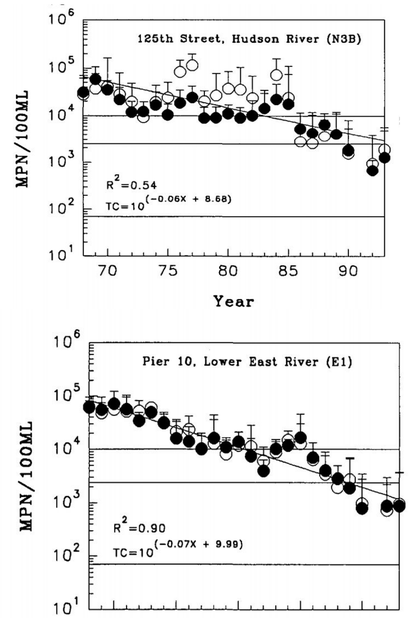
Young, Juhl, & O'Mullan, 2013, Journal of water and health, 11(2), 297–310.

Figure 3: Graph of colony forming units of ampicillin-resistant and tetracycline-resistant bacteria to colony forming units of Enterococci

Another research paper we analyzed was a study on "Fecal indicator bacteria, fecal source tracking markers, and pathogens detected in two Hudson River tributaries"(Brooks, Y. M., Spirito, C. M., Bae, J. S., Hong, A., Mosier, E. M., Sausele, D. J., Fernandez-Baca, C. P., Epstein, J. L., Shapley, D. J., Goodman, L. B., Anderson, R. R., Glaser, A. L., & Richardson, R. E., 2020). Volunteer monitoring of the Hudson River have found elevated concentrations of Enterococci since 2012 at the Wallkill River and Rondout Creek tributaries. As a result, the monthly volunteer monitoring had decided to identify culturable measurements of Enterococci and use nanoscale qPCR for quantification of enterococcus and e. Coli markers, microbial source tracking (MST), and quantification of 12 different gastrointestinal pathogens. The results of this procedure were that three MSTs–HumM2, HF183, and B. theta–present in the Wallkill River and the Rondout Creek tributaries were connected to human pollution. The presence of these MSTs, especially B. theta, had a positive correlation with the concentration of E. coli. The results also detected genes from the adenovirus 40 and 41 conserved region, rotavirus A NSP3, E. coli eae and stx1, and Giardia lamblia 18S rRNA in over 45% of the samples. The high concentrations of rotavirus A NSP3 genes was suspected to be correlated to the bovine marker gene, CowM3.

Literature Review (continued)

In "Sewage abatement and coliform bacteria trends in the lower Hudson-Raritan Estuary, since passage of the Clean Water Act" by Thomas M. Brosnan and Marie L. O'Shea, scientists studied the Hudson-Raritan system (which includes the Hudson River and East River) between the summers of 1968 to 1993. They monitored the amount of coliform bacteria present in water samples of the estuary across 40 stations (more stations were added in 1984 for a total of 52 stations), approximately once every other week. They collected water samples from the surface (1 m below the water surface) as well as from the river bottom (1 m above the sediment surface). Over 26 years, 16000 total coliform analyses were performed. It was reported that untreated wastewater discharge into the Hudson river dropped dramatically between 1930 to 1990, approaching 0 m3/s. The results show that in every collecting station, there has been a large downward trend in coliform bacteria present in the water samples (Brosnan & O'Shea, 1996). Another research paper, "Combined Sewer Overflow Abatement: The East River Project" agrees with these findings (Protopapas , 1999).



Brosnan & O'Shea, 1996, Water Environ. Res.. 68, 25

Figure 4: Total coliform trends at selected sites expressed as summer surface (0) and bottom water (●) geometric means.

Discussion

Through our literature review, we've been able to rethink parts of our research project. For example, originally, we wanted to see whether there was more bacteria in the Hudson and East rivers due to contamination and waste water discharge. However, the articles "Sewage Abatement and Coliform Bacteria Trends in the Lower Hudson-Raritan Estuary since Passage of the Clean Water Act" and "Combined Sewer Overflow Abatement: The East River Project" tell us that both of these had already significantly declined by 1993, so it is not likely that we would have found clear evidence of the impact of the pollution of the rivers on the bacterial biodiversity present. However, we could have possibly analyzed our results to determine the presence of coliform bacteria in our samples and compare them against the samples from 1968 to 1993 in order to determine if the contamination of the rivers has gotten worse recently. In addition to this, through analysis of"Antibiotic-resistant bacteria in the Hudson River Estuary linked to wet weather sewage contamination" and "Fecal indicator bacteria, fecal source tracking markers, and pathogens detected in two Hudson River tributaries" we were able to further evaluate certain procedures we could have executed better, such as gathering a larger sample size. We could have also tested other variables that may have contributed to the concentrations of bacteria present in these rivers. Furthermore, we could potentially determine how these factors affect the concentrations of bacteria in the rivers.

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