

Abstract

Estuaries are unique bodies of water that contain a mixture of both salt and freshwater, thus containing bacteria that will be common in both types of waters. Here, we present our findings resulting from the metagenomic data obtained from DNA purification followed by 16S RNA amplification and sequencing of deep-water samples from East River in NYC and relate the bacterial biodiversity to the physical and chemical characteristics determined over the course of the semester. We compare our results to those obtained by previous students in the Spring semester. We hypothesized a larger diversity in classes of bacteria in the fall water sample in relation to dissolved oxygen (DO) and carbon dioxide (CO₂) levels. Using DNA purification & amplification, titration, osmosis, salinity and inoculation, our results supported our hypothesis, showing an increase of biodiversity in the fall samples in comparison to spring samples.

Introduction

There are many chemical and physical factors that contribute to the flourishing of a marine ecosystem, including levels of dissolved oxygen (DO), dissolved carbon dioxide (CO₂), salinity, pH and temperature. In relation to marine ecosystems, the presence of certain bacteria can be indicative of the quality of the water. Pollution can alter the composition of the water and occurs in estuaries by a combination of sewage overflow and storm water runoff (Masseria & Hesse, 2013). Sewage overflow relates to the collection of domestic sewage, wastewater, and runoff (excess water from storms that aren't absorbed) and leads to an abundance of pathogens and bacteria sent to open waters (Masseria & Hesse, 2013). There are several classes of bacteria that are conducive to the recovery and degradation of a marine ecosystem. For example, planctomycetes contribute to remedy the effects of nitrogen-rich wastewater (Fuerst, Sagulenko, 2011), and cyanobacteria contribute to DO levels by photosynthesis (Heirdorn, et. Al, 2011). Other bacteria, including species of the gammaproteobacteria such as fecal coliforms (Donnenberg, 2015), and actinomycetes, which are pathogenic to animals and humans (Sowani et al, 2017), are indicators of water contamination by sewage overflow (Divya, Solomon, 2016) and can contribute to degradation of water quality. Here, we present the results of our research into the properties of East River NY bottom waters and compare the bacterial biodiversity found in water samples collected three months apart.

Materials and Methods

Water Analysis:

Water samples were tested for salinity, dissolved oxygen and dissolved carbon dioxide and alkalinity. Temperature and pH were tested at the time of collection. Salinity was measured by evaporation and a standard curve determined by dialysis bags containing known NaCl solutions. Dissolved oxygen and alkalinity were measured using LaMotte titration kit. Dissolved carbon dioxide was measured using Hach titration kit (Model CA-23). Lactose broth was inoculated with 100 µl of water collected during fall, to test for coliform bacteria.

DNA Purification & Analysis:

DNA was isolated using MoBio WaterPower Kit and amplified using Polymerase Chain Reaction (PCR). Genes were sequenced using Basic Local Alignment Search Tool (BLAST).

Results

Metagenomic Analysis Suggests Contaminants Account for Differences in Classes Of Bacteria Found in Spring & Fall Samples Collected

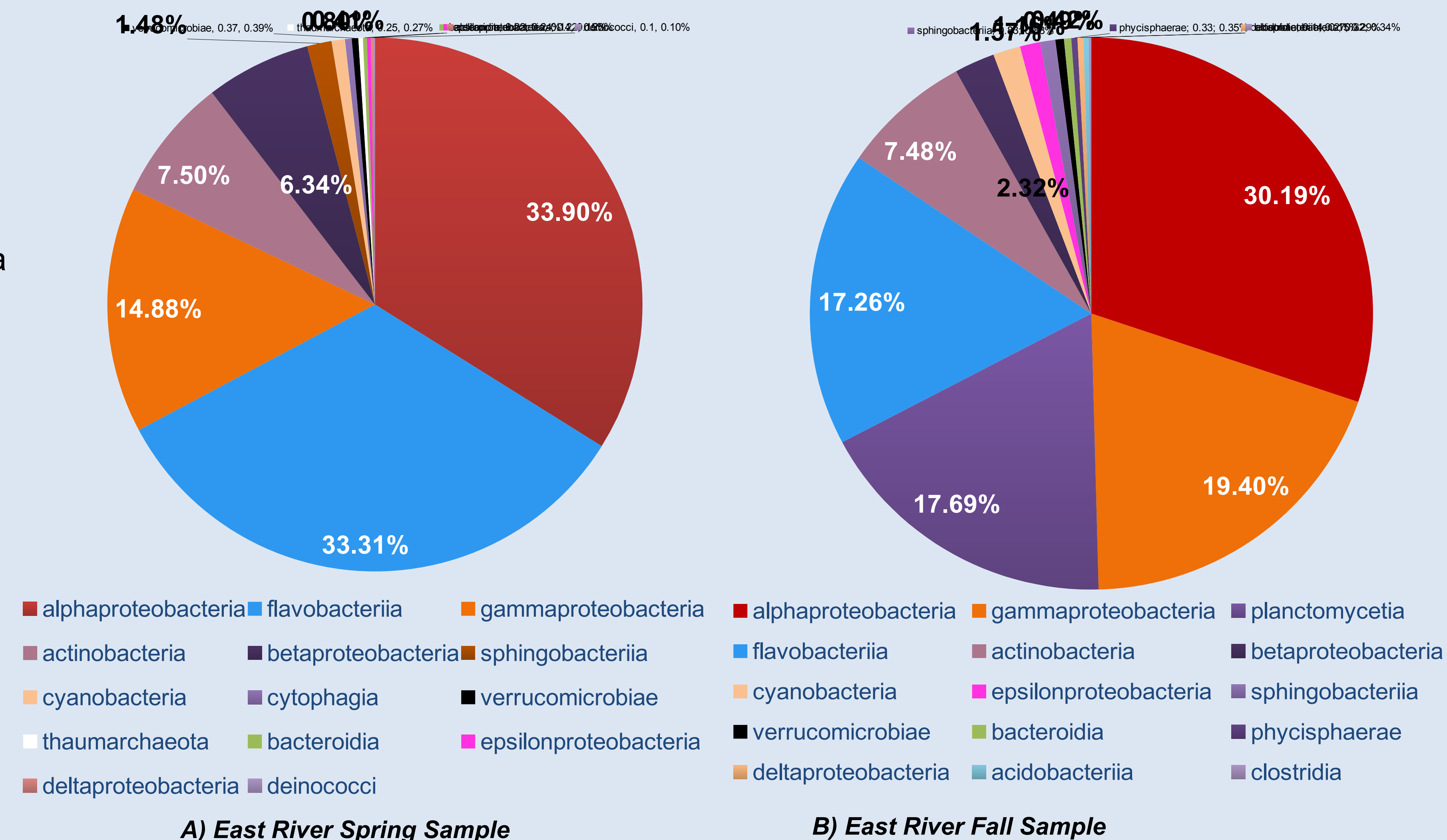


Figure 1. Bacterial class diversity was greater in the fall (B) than in the spring (A) sample. Fall samples also had a greater abundance of gammaproteobacteria while in the spring sample alphaproteobacteria and flavoproteobacteria were more abundant. Planctomycetes were only present in the fall sample.

Collection Time/Date	Temperature	pH	Salinity	Dissolved Oxygen	Dissolved CO ₂
Spring: 5/16/2018	14 °C	7.0	25,000 mg/L	6.1 ppm = 30.1 mg/L	16 mg/L
Fall: 9/26/2018	24 °C	7.8	22,000 mg/L	5.0 ppm = 25 mg/L	6.25 mg/L

Table 1. Water samples from East River, at 34th Street deep levels, presented higher temperatures and alkalinity in the fall compared to the spring sample. However, values of DO, CO₂ and salinity were higher in the spring sample.

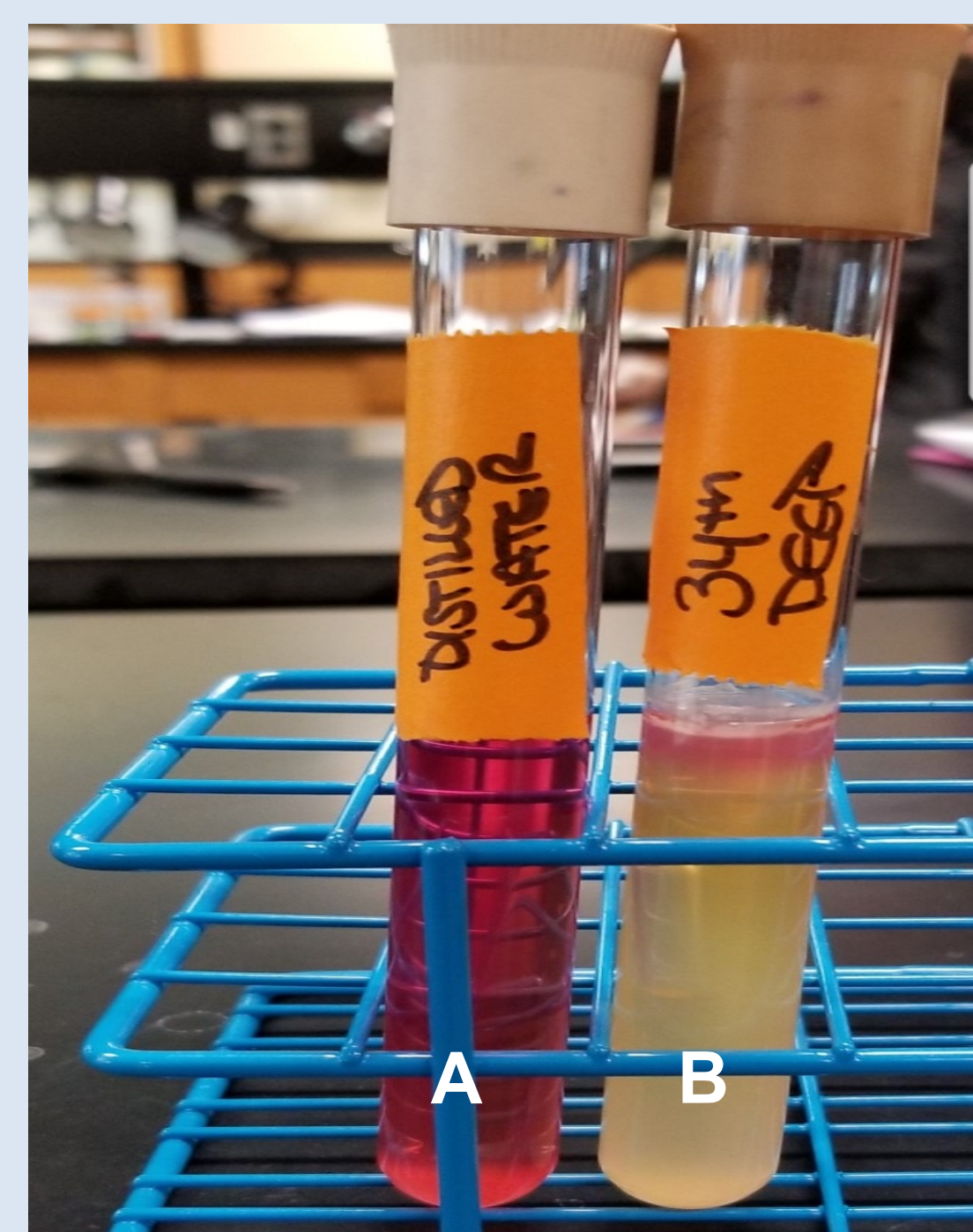


Figure 2. The presence of Coliform bacteria was tested by inoculation and ON incubation of lactose broth with the fall water sample; turbidity and color change in the inoculated (B) compared to the un-inoculated tube (A) confirmed the presence of such bacteria.

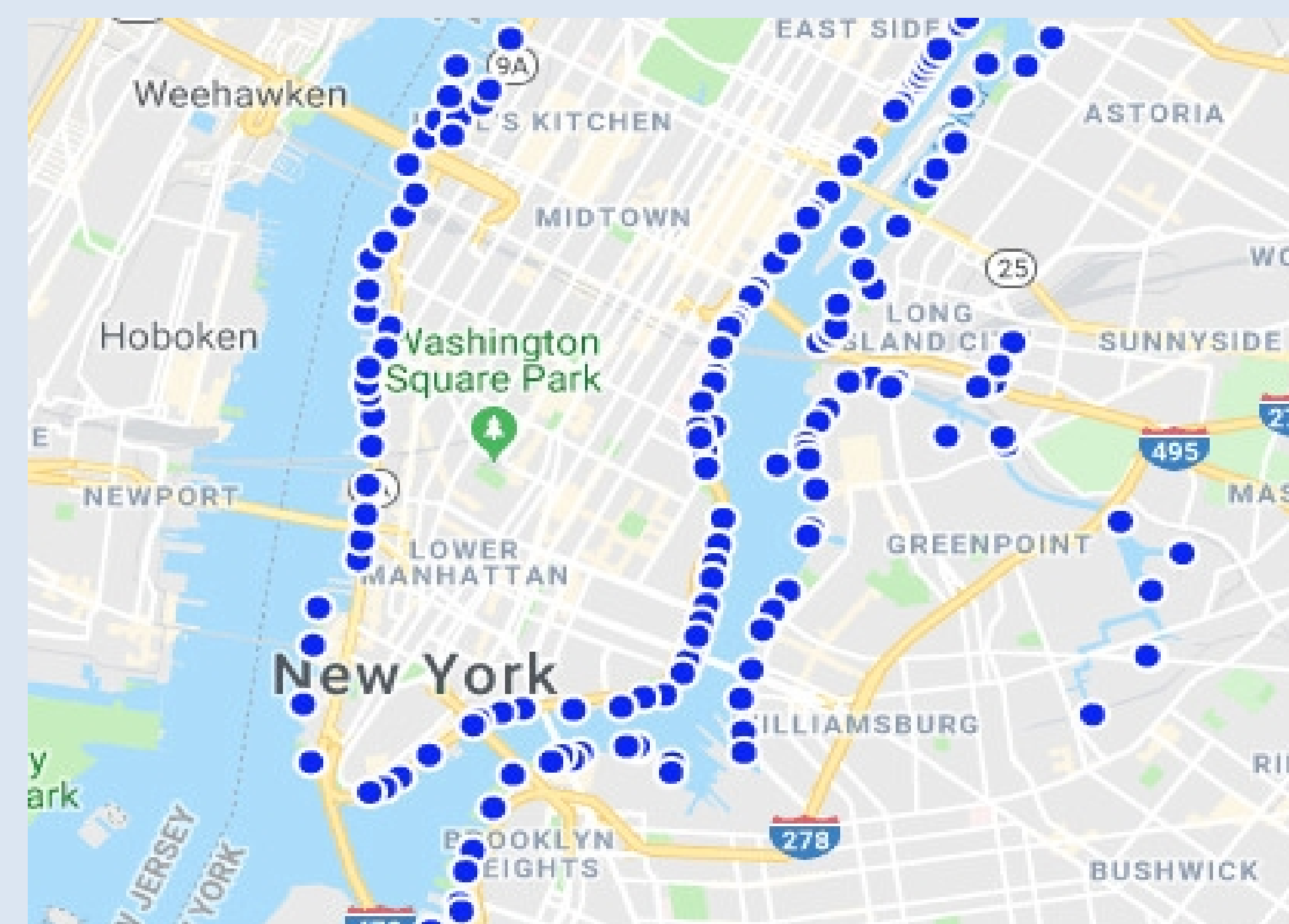


Figure 3. Areas in which combined sewer overflow is larger (https://ny.curbed.com/2018/3/30/17178662/new-york-waterways-combined-sewer-overflow-risks)

Discussion

- ❖ Greater abundance of proteobacteria, specifically alphaproteobacteria, in both water samples, is consistent with wastewater contamination.
- ❖ The presence of Actinobacteria can be explained by soil contamination in both deep-water samples.
- ❖ Green algae such as cyanobacteria were present in both samples consistent with the turbidity of the water and effluence wastewater.
- ❖ The presence of planctomycetes in the fall sample is consistent with the increase in water temperature which promotes blooming of phytoplankton,
- ❖ The presence of flavobacteria is consistent with the aerobic conditions indicated by greater levels of dissolved oxygen in the spring.
- ❖ Time elapsed between collection and measurements of CO₂ could account for low levels of carbon dioxide in fall samples.

Conclusion

- ❖ Our hypothesis was accurate; the increase in temperature and pH of the water in the fall resulted in greater diversity of bacteria compared to spring.
- ❖ The presence of coliform bacteria are indicative of sewage contamination and this is consistent with the bacterial profiles identified through metagenomic analysis.
- ❖ Temperature and pH differences appear to have an effect on bacterial diversity, with higher temperatures and more alkaline conditions in the Fall resulting in greater class diversity.

Future work

- Further sampling during fall, spring and summer in order to allow for a more complete comparison of seasonal variations in bacterial diversity.
- Research meteorological data for information regarding precipitation and ambient temperatures at time of collection.
- Obtain records of sites of sewage discharge/ overflow and dumping permits in order to find possible contamination sources as well as types of contaminants.

References

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