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Abstract

Protection of the water quality by humans has been a long learning process shaped by loss of life, scientific innovation, citizen organization, infrastructure, government oversight, politics and money. Clean waterways can provide, food, water, recreation, connection to nature, transportation and beauty while contaminated waterways result in deleterious effects on both aquatic organisms as well as the surrounding environments including human communities. Here we present the results of our study of chemical and biological characteristics of samples collected from The East River (NY) surface water over a three months period. We determined oxygen and carbon dioxide saturation by titration and also measured the pH and temperature at time of collection. Salinity was measured directly by evaporation and indirectly by titration. We also purified DNA from these samples and did a metagenomic analysis, using 16SrRNA probes, of the water microbiome. We found a large diversity of bacteria, including *Chlamydia* and *Candidatus saccharibacteria*, bacteria which are not characteristic of brackish waters and whose presence can be explained by sewage contamination. In future, we expect this data will also allow us to determine correlations between changes in salinity and oxygen and carbon dioxide saturation with bacterial class abundance, permitting the establishment of probable sources of contamination and changes in the aquatic microbiome. The East River is far from its once lush bio-diverse history and New York needs to continue to undertake improvements in wastewater treatment, dumping regulation and consistent monitoring.

Introduction

During the early industrial development of NYC there was little concern for approximately 600 million gallons of waste dumped, every day, directly to the city's East River. This pollution caused cholera epidemics and the disappearance of aquatic species, such as oysters and other shellfish. The city eventually developed wastewater treatment plants, and in 1909 the New York Metropolitan Sewerage Commission started collecting data on water quality. By 1987, with 14 wastewater treatment plants, the city was finally able to treat all of the dry-weather waste. This caused up to a 99% drop in coliform bacterial count and it also allowed for monitoring of illegal dumping that was previously concealed by the general poor quality of the waterways (New York City Department of Environmental Protection, 2009). In 2017 approximately 60% of the sewer system combined storm water and wastewater, so when plants reach twice the dry-weather input, the excess flow is released directly into harbors to prevent sewage backup (NYC Department of Environmental Protection, 2017).

In our study, we obtained data on levels of carbon dioxide (CO₂), dissolved oxygen (DO), salinity, pH, and temperature to determine the physical and chemical characteristics of surface water samples from the East River on the Manhattan side (at 34th street), and the Long Island City (LIC) side over a three-month period. In order to determine the presence of coliform bacteria, we inoculated lactose broth with water samples. We extracted DNA from water samples to do metagenomic analysis and determine bacterial diversity. Since the East River is a tidal estuary containing brackish water -salinity between 0.5 to 35ppm-, with pH ranging between 5.0-9.0 and dissolved oxygen average of 6.54 mg/ml (National Oceanic and Atmospheric Administration, 2017; NYC Dept. of Environmental Protection, 2017; United States Environmental Protection Agency, 2006), we expected to find bacteria adapted to such environments in both samples, and hypothesized that any differences in bacterial classes would be due to differential contamination from different sources particular to sewage or waste dumping on one shore, since both samples came from the East River and were collected at the same time. We posit that studies of the microbiome can provide valuable information as to the types of contaminants in the water as well as in future, become a tool to study the changes that occur seasonally and over longer periods of time, in water bodies.

Materials and Methods

DNA Analysis: DNA from LIC surface water sample was isolated using MoBio WaterPower Kit. The results of the sequences of DNA found in this sample were analyzed using the Basic Local Alignment Search Tool (BLAST).

Water Environmental Characteristics:

La Motte titration kit was used to measured the levels of CO₂, DO and alkalinity. Salinity was measured directly thought evaporation method (Fig. 3) and indirectly by observing the change in mass of dialysis bags of known concentration of sodium chloride (NaCl) (Fig. 2) to determine the Total Dissolved Salts (TDS) present.

Results

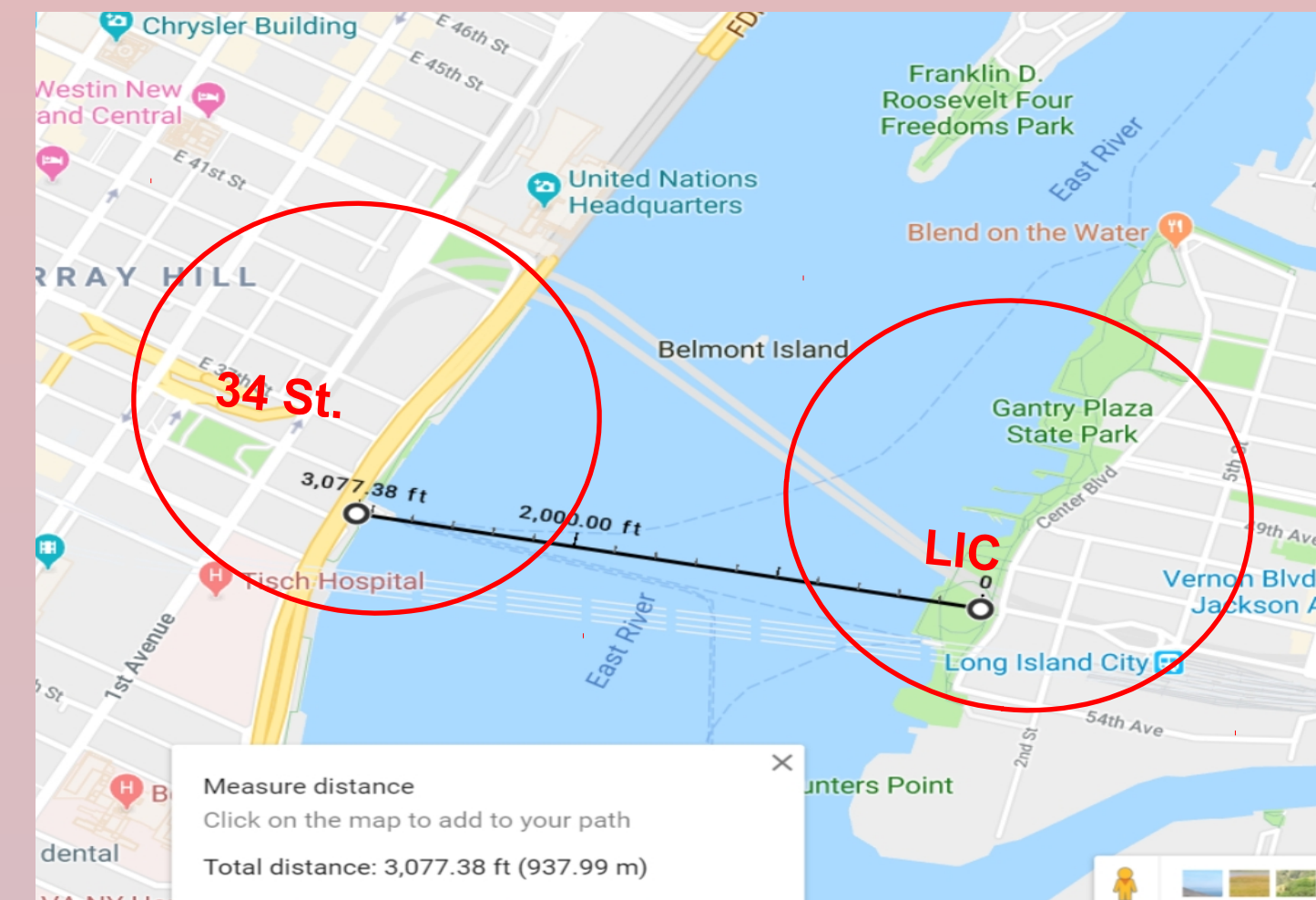


Fig 1. Map with the locations where surface water samples were taken during Fall 2018. The distance between this two points is 938 m. Photo taken from google.

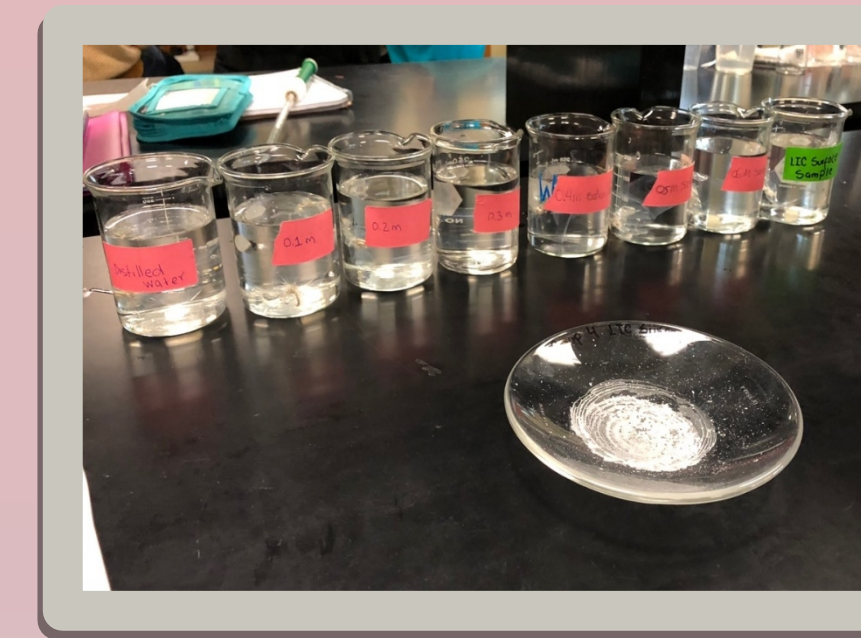


Fig 2: Photo of the dialysis bags filled with different NaCl concentrations and submerged in distilled water to establish a standard curve.

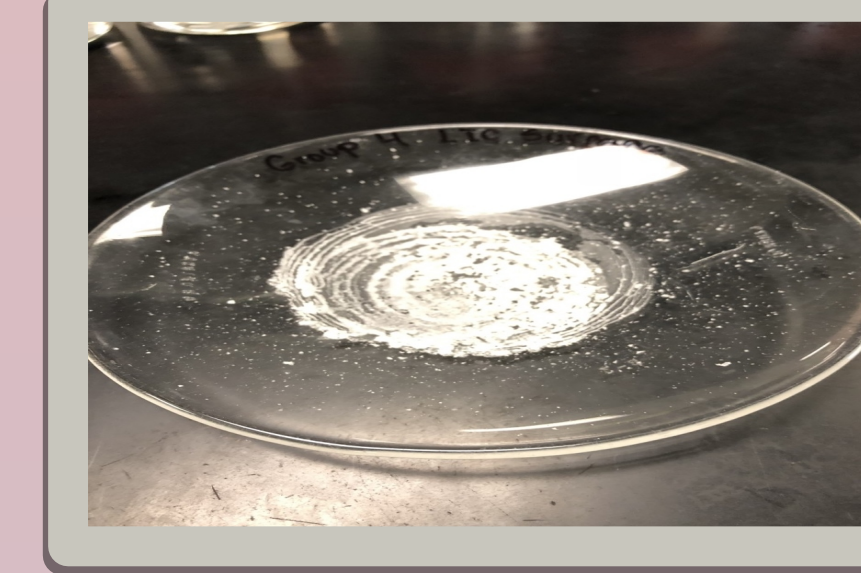


Fig 3 Photo of salt remaining in watch glass after evaporation. Total mass was calculated directly.

Metagenomic analysis of surface-water samples from opposite shores of the East River show differences in relative class abundance and types of Bacteria

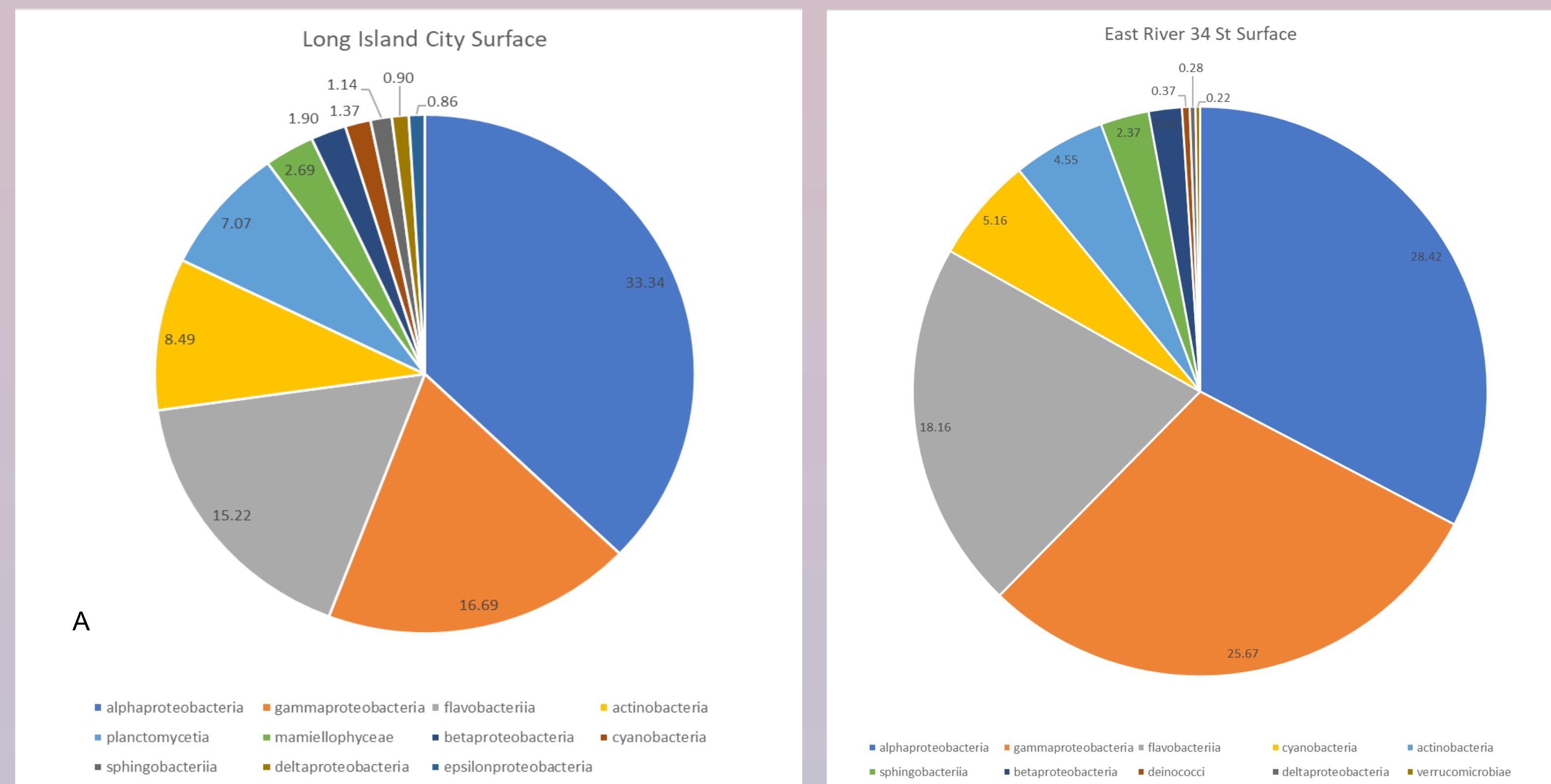


Fig 4 Pie charts with the relative amounts of Bacterial classes derived from metagenomic analysis of DNA from samples collected in Fall 2018 from A) Long Island surface water and B) East River 34th Surface water. Relative abundance of Alphaproteobacterial, Gammaproteobacterial, Flavobacteria, Actinobacteria, and Betaproteobacteria is similar for both sites. The LIC shore presents a higher diversity in bacterial classes than the East River Shore sample, with presence of bacteria from classes Mamiellophyceae, Planctomycetia, Cyanobacteria and Epsilonproteobacteria. On the other hand, bacteria classes Deinococci and Verrucomicrobiae were present in East River 34th water but not in the LIC shore sample.

Physicochemical characteristics of water samples from LIC and 34St

Place of Collection	Date of Collection	Temperature	pH	Salinity	Dissolved Oxygen	Dissolved Carbon dioxide	Alkalinity
Long Island City Surface	09/27/18	22°C	7.7	40,000 mg/L	6 ppm	18.75 mg/m	12 ppm
	10/14/17	21°C	7.67				
East River 34th St. Surface	09/27/18	22°C	7.6	29,740mg/l	5.4 ppm	15 mg/ml	NA
	10/14/18	21°C	8.0				

Table 1. Physical and chemical characteristics were determined for samples taken at different times between the months of September and December 2018. Temperature, pH and dissolved Carbon dioxide were determined for samples taken on Sept 27th, which were used for DNA purification a subsequent metagenomic analysis. Salinity for the Long Island City shore water exceeded the limit characteristic of brackish waters; the pH of the LIC sample was slightly more alkaline at the time salinity was measured. Temperature at the time of collection of samples used for DNA purification and metagenomic analysis was the same for both shores, while dissolved oxygen and carbon dioxide were higher in the samples taken from the Long Island City shore were both higher.

Chlamydia and Candidatus sp., not normally found in uncontaminated brackish water, were detected in water samples

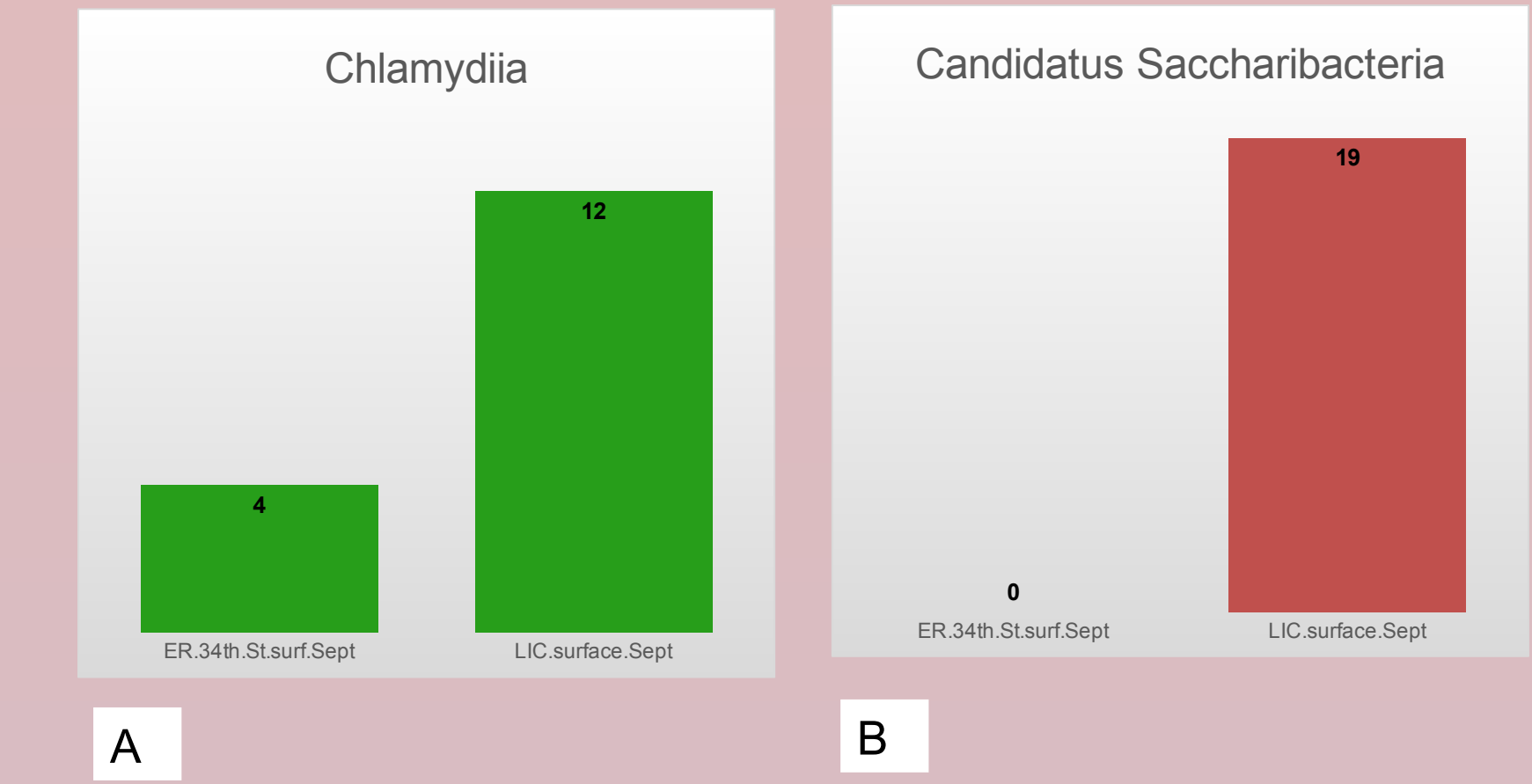


Fig 5. Graph of the bacteria found on LIC and 34St Surface A) Chlamydia B) Candidatus saccharibacteria

NYC Dep Wastewater Treatment Plants and CSOs

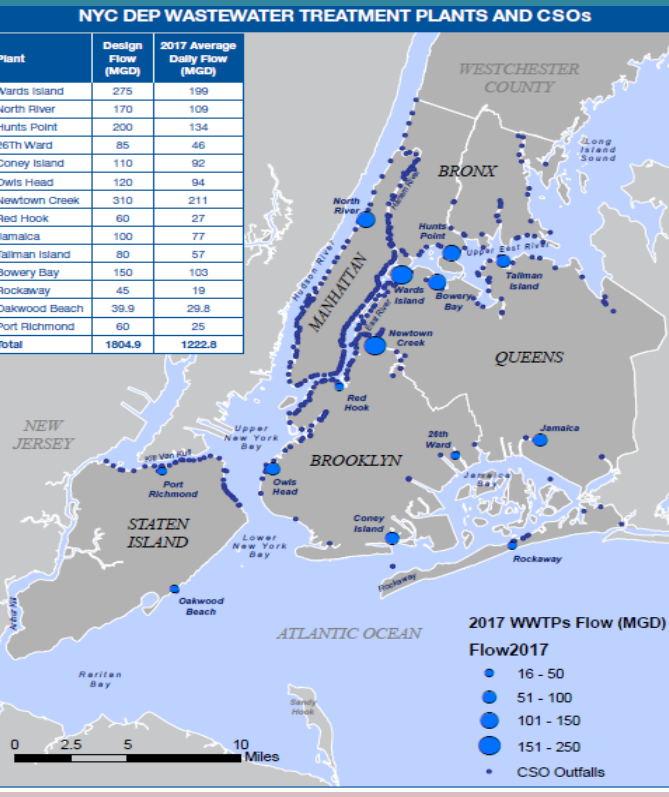


Fig 6. Capacity of the fourteen wastewater treatment plants in NYC

Discussion

- Although the relative abundance of the most abundant bacterial classes – Alphaproteobacterial, Flavobacteria and Gammaproteobacterial- is similar for both sites, the bacterial makeup of surface water from LIC presents a higher bacterial class diversity compared to the 34th street (Fig 4) although these two shores are only 940 meters away (Fig. 1).
- The differential presence of relatively abundant Planctomycetia in the LIC sample (Fig. 4A), could be related to the presence of macroalgae in the area, as has been reported elsewhere (Faria et al, 2018).
- Differences in the physicochemical characteristics between both sides of the river (Table 1), including the higher pH and TDS on the LIC shore, require further investigation to be explained. The range of total dissolved salt (TDS) for brackish water is between 5,000/35,000 mg/L putting the LIC side above brackish water thresholds (Table 1).
- Both coasts have the appropriate levels of dissolved oxygen for survival of aquatic species (between 5.5 ppm and 6.5 ppm) with the 34th street side having .6 ppm less than LIC (Table 1).
- Examples of bacteria not naturally found in brackish water and indicative of sewage contamination were abundant. *Candidatus saccharibacteria* found in the LIC side (Fig 5B), plays a role in the degradation of various organic compounds and is present in water treatment plant sludge (Soro, et al., 2014), which is consistent with the proximity of the Newtown water treatment plant. *Chlamydia*, found in both samples (Fig 5) is responsible for sexually-transmitted infection; once in the human body, it can be expelled through human fluids that are usually transported in the sewage system of the city (Avert, 2018).

Conclusions

- The East River contains a large bacterial population with many bacteria which normally are not found in non-contaminated brackish water.
- While the differences on each side of the river are multiple, the physicochemical characteristics cannot account for the presence of nonaquatic bacterial populations. For example, the presence of *Candidatus saccharibacteria* and *chlamydia* can be explained by waste products entering the waterway at different points of entry.
- Our preliminary data suggests that differences in bacterial populations on either shore can partially be accounted for by a) differences in incoming waterways, b) sources of and nature of discharge (dumping) in the area and c) presence/absence of macroscopic algae and plants which represent niches for the development of biofilms.

Future work

- Thorough analysis of the metagenomic data is needed in order to identify all brackish and non-brackish water bacteria and identify their natural habitat.
- Investigate the amount of contamination flowing into the East River from the known contaminated areas of Newton Creek and Gowanus Canal.
- Using the date and time of sampling, gather meteorological data including tides and precipitation, which can provide insight into the factors affecting the microbiome.
- Analyze data collected in previous years and look into patterns and trends that may help correlate the presence of particular bacteria with possible sources of contamination.

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