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Project Report:

Real-time analysis of Hudson River water quality using Nanopore DNA sequencing technology

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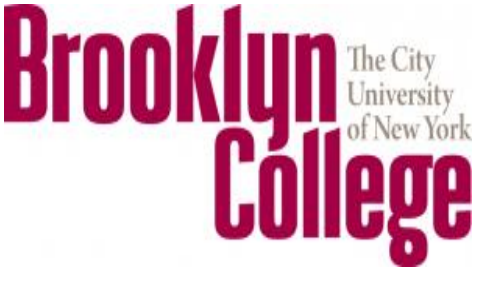

Abstract:

The funding provided by the Hudson River Park Trust CUNY Research Alliance Scholars Program was used to support the *Real-time analysis of Hudson River Water Quality Using Nanopore DNA Sequencing Technology* research proposal. This research proposal was designed to test novel approaches in assessing the water quality in Hudson River Park using Oxford Nanopore DNA sequencing technology. The project focused on two primary research objectives: 1) sequencing bacterial communities from the Hudson River that are indicators of water quality, and 2) assessing the connection between microplastics and the presence of antibiotic resistance genes (ARGs) within microbial communities that grow on these microplastics. Project methodology included the use of a Nanopore MinION sequencer, a small and inexpensive DNA sequencing platform that can be used in the field and is able to provide real-time data. Between May and December 2024, water samples were collected from several locations within the Park at various time points. Total DNA was extracted from these samples and a 16S amplicon DNA sequencing approach, alongside whole metagenome analyses, were used to explore the bacterial communities in the Hudson River. Although work on these samples and data analysis is ongoing, the preliminary findings suggest that the Nanopore MinION sequencer is effective in addressing both primary research objectives. Diverse bacterial communities were detected in the Hudson River water samples, including bacterial taxa that serve as indicators of water quality. Additionally, complex bacterial communities were identified on microplastics placed in the Hudson River, along with ARGs present in these bacterial communities. Although sample processing and data analysis are preliminary, initial findings suggest that Nanopore sequencing is a viable method for assessing Hudson River water quality and characterizing microplastic-associated microbiomes. The 80 samples analyzed thus far show complex bacterial community

composition, and further analysis is underway to determine whether location, depth, season, or tide cycle have an influence on the bacterial community composition. Fecal indicator bacteria have been detected suggesting that the Nanopore sequencing approach could be adapted for routine water quality measurements. Initial findings demonstrate that microbiomes colonizing various types of microplastics incubated in the Hudson River for three to six weeks can be collected and analyzed. The microplastic microbiomes from the Hudson River are complex and appear to be influenced by microplastic location within the river. This report outlines the initial findings and provides an overview of the additional analyses currently underway.

Introduction:

Hudson River Park (the Park) is a four-mile waterfront park on Manhattan's west side. The Park's 400-acre Estuarine Sanctuary offers vital habitat that supports a great diversity of wildlife and serves as a key site for river research. Situated in one of the largest cities in the world, the Park, since its origin, has been subject to a wide range of environmental and anthropogenic stressors, including sea level rise, warming temperatures, intense rain events, microplastic contamination, invasive fish and insects, and more (Wirgin et al., 2023; Lane et al., 2013; Gonzalez et al., 2021; O'Neil et al., 2020). Monitoring the population dynamics and health of organisms such as animals, fish, plants, insects within the Park, along with surveying overall biodiversity, represents a significant undertaking that requires effort, expertise, funding, and time.

Over the last 20 years, researchers have explored the potential of DNA sequencing as a tool to monitor the presence, abundance, and activity of plant, animal, insect, and microbial communities. Both environmental DNA (eDNA) and metagenomic strategies use DNA (or RNA) collected from environmental samples such as water, soil, or air, as a proxy to identify organisms within a given environment (Deiner et al., 2017; Ficetola et al., 2008; Thomsen et al., 2015). For example, eDNA has been used to monitor fish presence and diversity, and others in the region have used DNA-based approaches to monitor fecal coliform bacteria in water quality assessments (HRP ESMP-2022; HRP eDNA; Stoeckle et al., 2017; Stoeckle et al., 2018; O'Mullan et al., 2019; Brooks et al., 2020). While DNA-based approaches are powerful, they rely on expensive sequencing instruments that require skilled technicians to run, limiting their use to research laboratories and commercial sequencing facilities. The Nanopore MinION DNA sequencing instruments are small, inexpensive, and comparatively easy to use – they represent a significant step forward in making DNA sequencing accessible and field-deployable, akin to

tools like microscopes or pH meters (Nanopore website; Bass et al., 2023; Werner et al., 2022; Banerjee et al., 2021). This technology offers exciting opportunities for students and community members to engage directly with environmental research by deploying Nanopore technology to address environmental questions and collect crucial data as the Park and its diverse assembly of organisms adapt to environmental changes (Pomerantz et al., 2021; Prost et al., 2020; Wolf et al., 2023).

However, as a relatively new technology, Nanopore sequencing differs from the more widely used platforms like Illumina next-generation sequencing and requires validation to determine if it can produce the same depth and quality of DNA sequence data needed to address challenging research objectives. This study aims to address key questions about Hudson River water quality and the microbiomes associated with microplastics, while also serving as a proof of concept for the use of Nanopore technology in these applications. If successful, this work can serve as a foundation for exploring more intricate questions in the future. Furthermore, this technology could serve as a cornerstone for engaging students and the public in scientific research, reinforcing the Park's role as a hub of urban resiliency and sustainability.

Methods:

The experimental design of this study was divided into two distinct projects: water quality and microplastics. However, several methodologies, including Nanopore sequencing and data analysis, were shared across both projects. All samples were collected in Hudson River Park with the help of Park staff, and samples were prepared and analyzed at either the Muth lab at CUNY Brooklyn College, or the Park's Pier 40 Wetlab.

Water quality analysis: In 2024, a total of 220 water samples were collected over 20 sampling days from three locations (i.e., Pier 26, Pier 40, Gansevoort Peninsula) and at two depths – surface and five feet below surface. Microbial communities were concentrated using vacuum filtration: 0.22um filters were used to collect all microbes on all sampling days and locations, and on select days, 10um filters were additionally used to collect only the particle-associated microbes. DNA extraction was performed on approximately 80 samples using the Power Water DNeasy kit (Qiagen), followed by amplification and sequencing of full-length 16s rRNA genes using the Nanopore MinION. Further work on the remaining samples will include additional 16S metabarcoding and whole metagenome sequencing, informed by the preliminary findings from the analyses of the initial 80 samples.

Microplastics, microbiomes, and ARGs: Following discussions with Park staff, it was determined that collecting existing microplastics from the Hudson River was not feasible due to several challenges: 1) a sufficient quantity of microplastics could not be collected and separated using nets from the shore, 2) microplastics would not easily separate out from other, similar sized debris, and 3) the available equipment was not capable of identifying the specific polymer types of microplastics collected. As an alternative, we used commercial (ResinZone, Cincinnati, OH) microplastic pellets of ~2-4 mm in diameter. We used Acryrex, high-density polyethylene (HDPE), clear polypropylene (PP), and polyactic acid (PLA) microplastics in this study. Prior to experimentation, the microplastics were rinsed with deionized water in the lab, air-dried in a laminar flow hood, and exposed to UV light for ~20 minutes to reduce any existing bacterial contamination from the commercial supplier. Depending on the experimental setup, the four types of microplastics were either mixed at a 1:1:1:1 ratio and 16 grams (4 grams of each type) and placed in a sterilized aluminum screw-top tea infuser/strainer, or 16 grams of an individual microplastic type were added to a tea infuser (Figure 1).

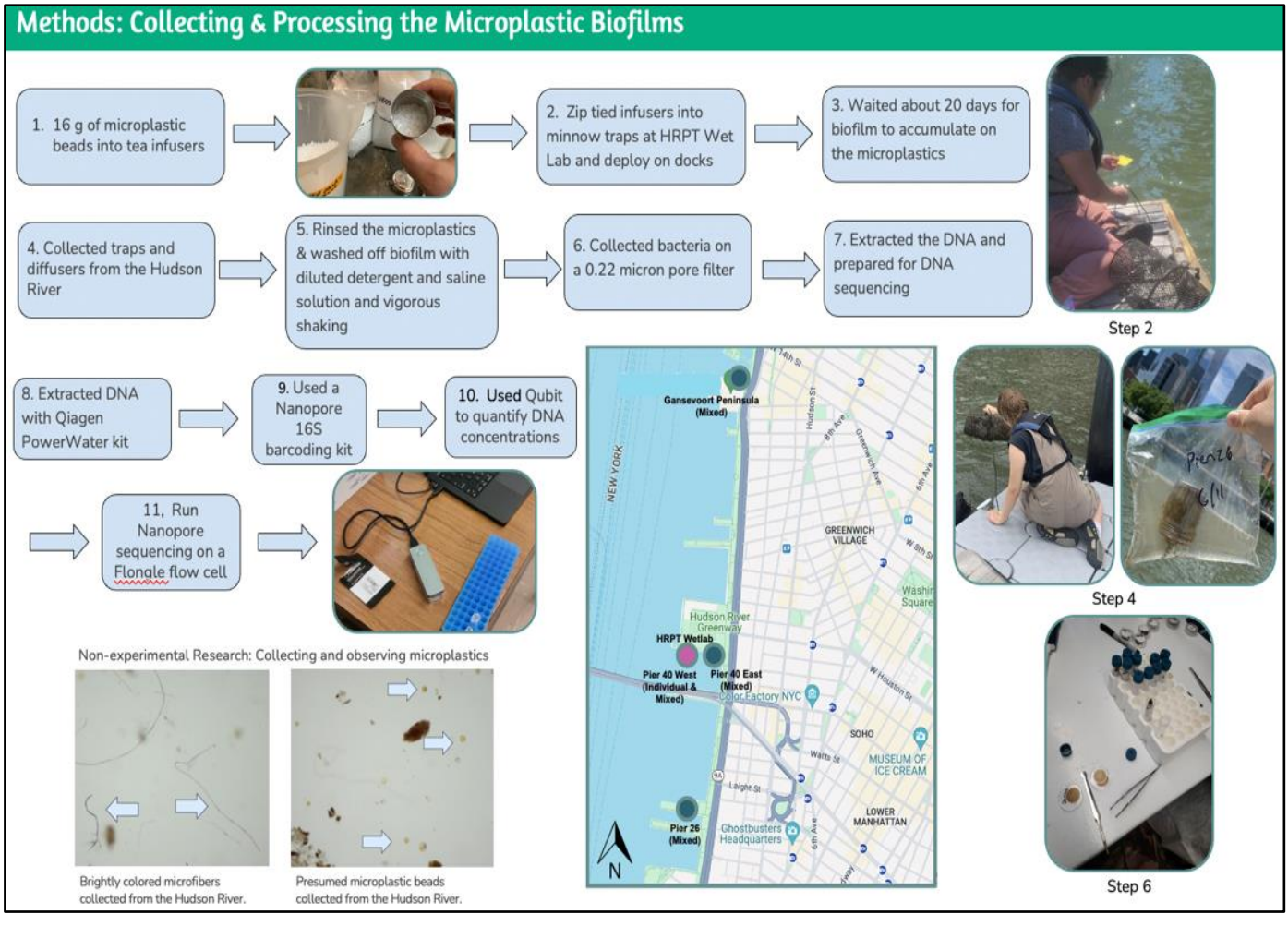

Figure 1: Microplastic microbiome sample collection protocol. The map shows the sampling sites in the Park and the images show steps in the collection at Pier 40 and in the Muth Lab. The two pictures in the lower left corner show probable existing microplastic fibers and beads that were collected in Hudson River samples.

In some treatments, microplastics were abraded and deformed using a Ninja-brand blender to create a rough, non-uniform surface, better simulating the weathered condition of microplastics collected directly from the Hudson River. On the day of deployment, the microplastics in tea infusers were brought to Pier 40 and placed in minnow traps using small zip-ties. Four to eight tea infusers were placed in a single minnow trap. The minnow traps were then closed with the trap clip, and zip-tied closed for additional security. To prevent fish and other organisms from becoming trapped, mesh was placed over the trap openings. The minnow traps were deployed at a depth of approximately 3-4 feet from the surface with a nylon line, either from a floating dock

(when available) or, in cases where no dock was accessible (e.g., at Gansevoort Peninsula), from a pier or riprap shoreline. Microplastics remained submerged for two to six weeks, depending on experimental design, with occasional checks by Park staff. In one instance, an entire minnow trap containing six tea infusers was lost due to fraying of the nylon line.

Microplastics were collected at regular intervals with the help of Park staff. At Pier 40, tea infusers were rinsed off with river water and sediment and fouling organisms (e.g., sea squirts) were removed. The tea infusers containing microplastics were then placed in clean sample bags or Ziploc bags with enough river water to keep them submerged during transport to the Muth Lab at Brooklyn College. In the lab, microplastic microbiome material was collected within 24 hours of sampling. Samples were stored at 4°C until processing. To extract microbiome material, the microplastics were gently rinsed in a 50 mL Falcon tube filled to the 45 mL line with sterile water and then drained. This step was repeated twice to remove river water from the sample and any loosely attached debris. Next, 20 mL of sterile wash buffer (0.1M NaCl, 0.1% Tween 20) was added to each tube, and the mixture was shaken on a platform shaker at 450 rpm for 30 minutes at room temperature to release microbiome bacteria from the microplastics. Microorganisms were collected from ~15 mL of the wash solution using a 20 mL syringe with 0.22 µM Millipore pre-wetted filter. A 1500 µl aliquot was used for plating onto marine agar and/or R2A media plates and for BioLog Ecoplate assays. Filters were stored at -20°C if DNA extraction was not performed immediately. DNA was extracted using the Qiagen DNeasy Power Water or Power Soil Pro kits with slight modifications: filters were placed in vials with collection surface facing inward, beads were added before the first vortex session, vortexing was extended to 20 minutes (sealed with Parafilm), and the extracted liquid volume ranged from 300-400 µl due to the larger vial size. Extracted microplastic microbiome DNA was stored at -20°C and its quantity and quality were assessed using a Qubit 2.0 fluorometer.

DNA Sequencing: Nanopore DNA sequencing library preparation was conducted using the 16S kit (SQK-16S114.24; R10 chemistry) for all 16S amplicon sequencing, and either the native barcoding kit (SQK-NBD114.24) or ligation sequencing kit (SQK-LSK114) for metagenomic sequencing. Manufacturer protocols were followed with minor modifications. For 16S library preparation, samples were barcoded and amplified using Takara Premix Taq™ DNA Polymerase Hot-Start Version (#R028A) with ~7 µl barcoded 16S primers from the Nanopore kit (instead of 15 µl). PCR products were assessed by gel electrophoreses on a 1% agarose gel for ~ 1500 bp bands before pooling at roughly equimolar concentrations. Barcoded and prepped DNA samples were run on Nanopore Flongle flowcells on a MinION Mk1B sequencer using the MinKNOW software. Initial sequence data were carried out using the Nanopore EPI2ME software, R and Excel.

Results:

Water quality analysis: This study focused on implementing Nanopore DNA sequencing as a method to assess water quality in Hudson River Park's Estuarine Sanctuary, specifically examining the influence of location, seasonality, and tide on both water quality and the river's microbiome. Initial analyses of 80 samples revealed a highly diverse Hudson River microbiome, dominated by taxa expected, such as Pelagibacteraceae, Alteromondadaceae, and Coleofasciculaceae, in a marine estuary environment (Figure 2). As is typical of marine water samples, the data shows a lack of evenness in the relative abundance of bacterial taxa, with ~70% of all identified sequence reads belongs to the 10 most abundant bacterial families, while the remaining ~30% of reads are distributed across more than 20 additional families at lower abundances (listed as "Other" in Figure 2).

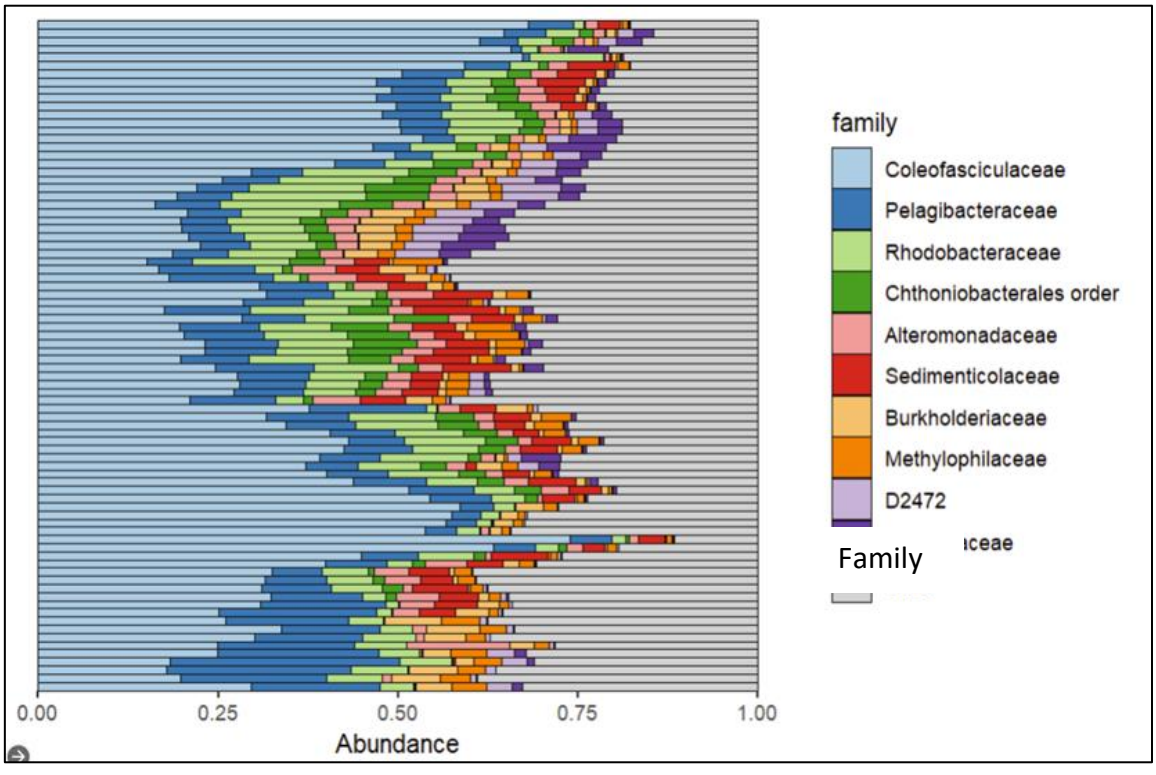

Figure 2: Relative abundance of the ten most abundant bacterial families detected in the Hudson River, 2024. Horizontal bars represent individual samples (n=80).

When examining these results at lower taxonomic ranks, the data suggests that there are several genera present including fecal indicator bacteria species which serve as indictors of water quality (Figure 3).

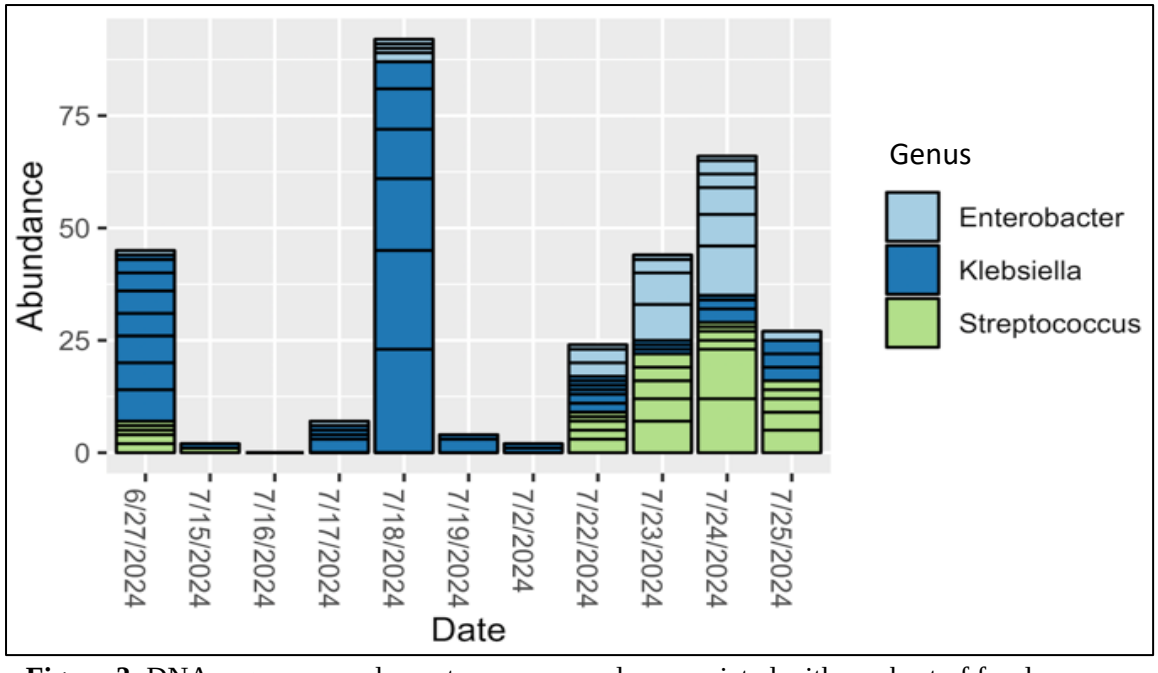

Figure 3: DNA sequence read counts across samples associated with a subset of fecal indicator bacteria.

These findings are currently being compared to conterminous water quality analyses using standard fecal indicator bacteria detection methods employed in the Park (IDEXX Enterolert). Alpha diversity analyses of the Hudson River water samples confirmed high levels of bacterial

Figure 4: Shannon Diversity data of four treatments rarefied to 2k reads. Each point represents one sample of the respective treatment.

diversity in bulk (total) water samples (Figure 4). When the water was separated into particle-associated and free-living fractions by filtration, the free-living component had greater alpha diversity than both the particle-associated component and microplastic-associated component.

The effects of location, seasonality, tide, and depth on the Hudson River microbiome within the Park were also investigated. Preliminary data (not shown) suggest that bacterial microbiome community composition varies over time and by location. However, tide, depth, and location do not appear to be the primary factors influencing microbiome composition. These findings indicate that bacterial community distribution may be highly heterogeneous or influenced by additional, unaccounted-for variables contributing to observed differences in community composition.

Microplastics and microbiomes: This study evaluated the ability of commercial microplastic pellets incubated in the Hudson River to be colonized by microorganisms present in the river water. Results indicate that for the four locations within the Park tested, the microplastic microbiomes isolated at all sites contained a diverse community of bacteria and, across the sites tested, shared many of the same abundant genera (Figure 5).

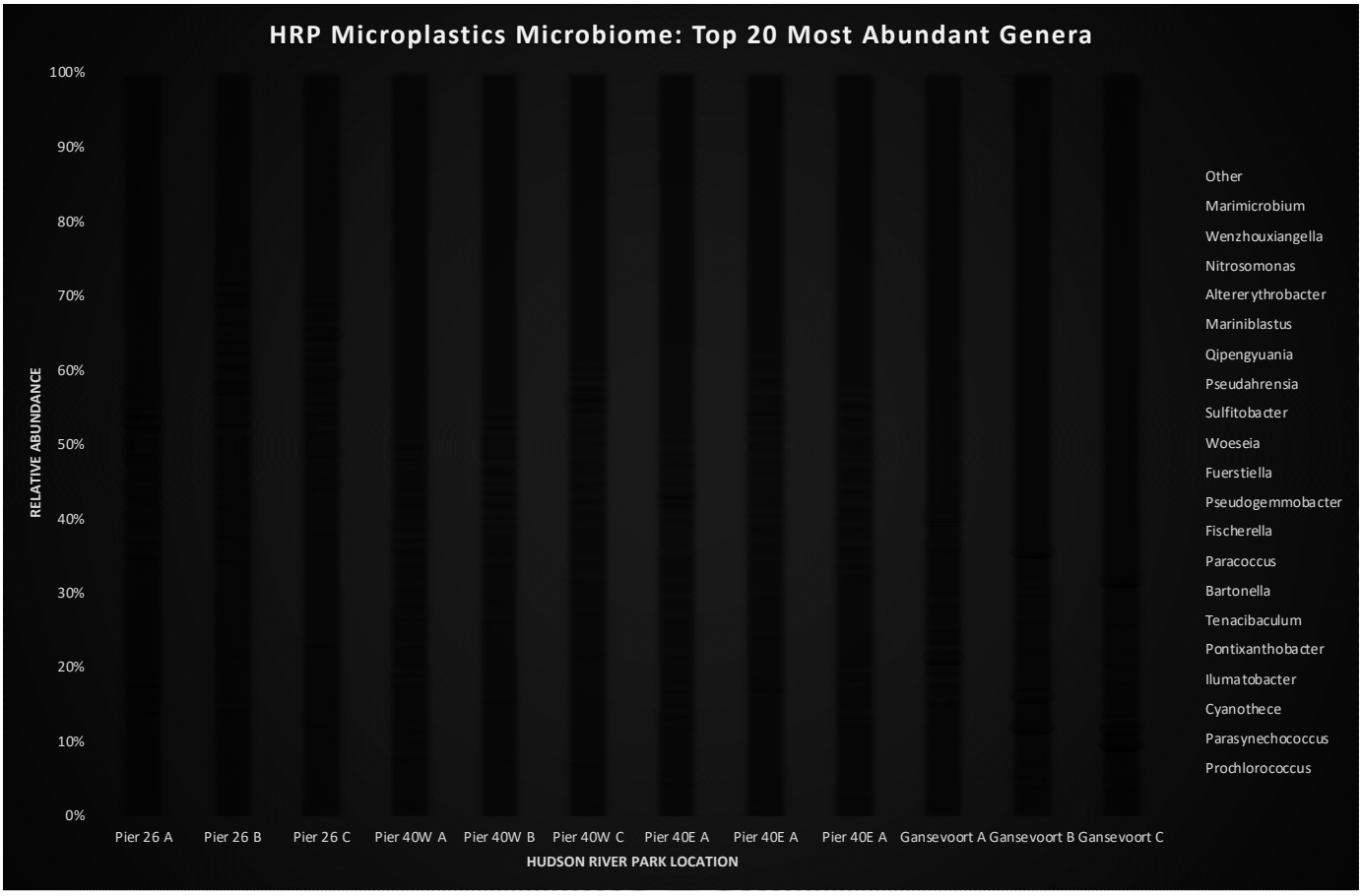

Figure 5: Relative abundance of the 20 most abundant genera of bacteria isolated from mixed microplastics at four locations within the Park (Pier 26, Pier 40 East, Pier 40 West, and Gansevoort Peninsula). The data are in triplicate (A, B, C). Bacterial genera below the cutoff threshold are not included.

The triplicate samples from each site exhibited similar microbial communities, except for those collected from Gansevoort Peninsula, which showed a significantly different composition of abundance genera. This variation may be explained by the fact that the microplastics at this location were not placed off a floating dock and were therefore subject to greater tidal influence and were exposed to air and sediment during the experiment duration. When incubated individually in the Hudson River, all four types of microplastics pellets accumulated complex and similar bacterial communities (see Figure 6).

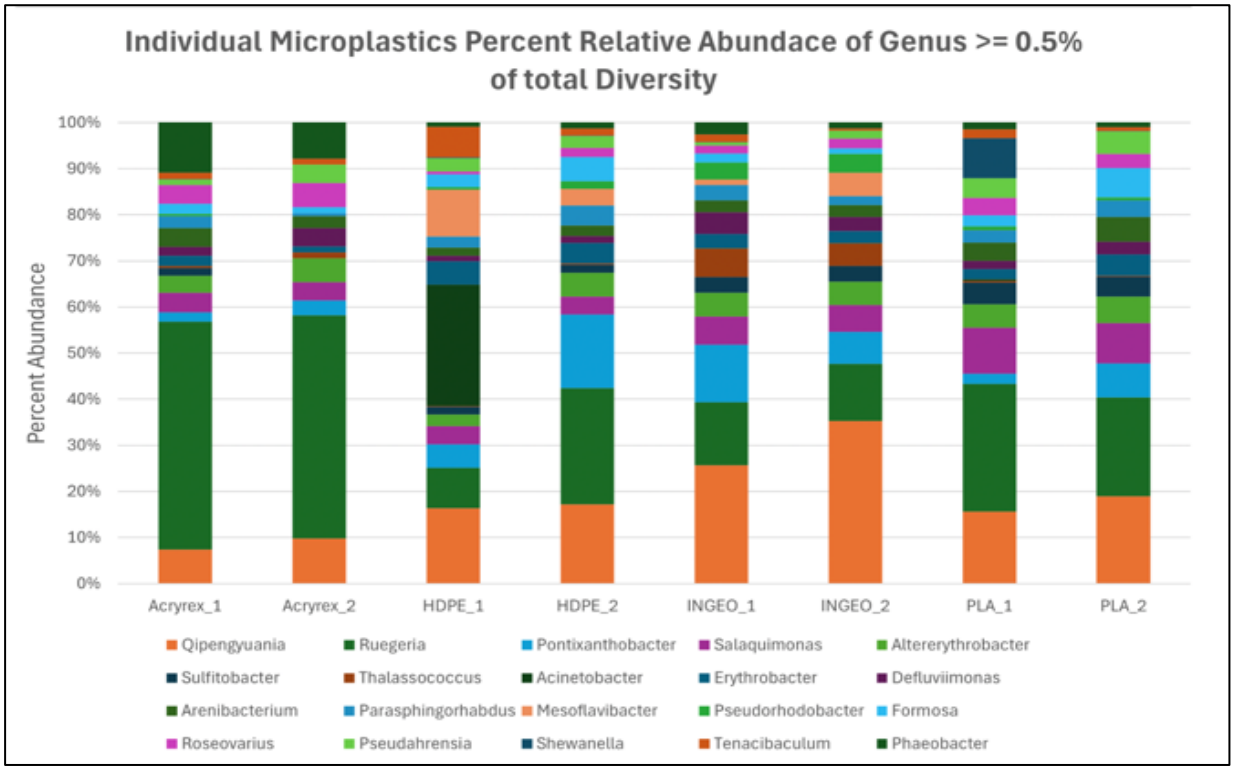

Figure 6: Most abundant genera of bacteria by microplastic type (Acryrex, HDPE, INGEO, PLA). Only genera >0.5% relative abundance of the total are shown. Microplastic type is indicated on the x-axis. Samples in duplicate (1,2).

There were significant overlaps in bacterial colonization across different microplastic types, with over 30% of identified species present on all microplastic surfaces (Figure 7). Notably, microplastics that were roughened in a blender accumulated a larger microbiome mass compared to smooth, untreated microplastic pellets. Ongoing analysis is focused on determining whether microbial communities on roughened microplastics differ from those on smooth microplastics.

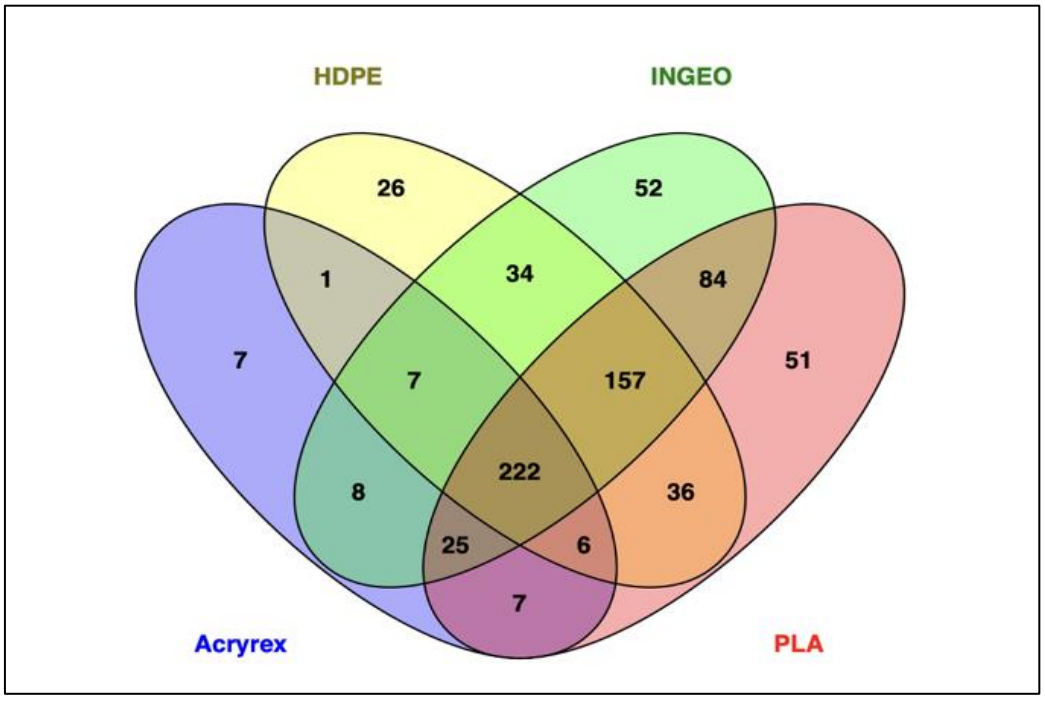

Figure 7: The distribution of bacterial species across four types of microplastics.

In addition to the 16S DNA sequencing used to analyze microplastic bacterial communities, whole metagenome sequencing was employed to detect the presence of ARGs in the sampled microbiomes. Preliminary analyses have identified several genes predicted to confer antibiotic resistance (data not shown).

Discussion:

This report outlines progress in developing a novel approach to assessing water quality in in the Lower Hudson River Estuary using Oxford Nanopore DNA sequencing technology. The research focused on two primary objectives: 1) sequencing bacterial communities from the Hudson River that serve as indicators of water quality and 2) assessing the relationship between microplastics and the presence of ARGs within microbial communities colonizing these microplastics.

Although sample processing and data analysis are preliminary, findings suggest that Nanopore sequencing is a viable method for assessing Hudson River water quality and characterizing

microplastic-associated microbiomes. For water quality testing, sufficient bacterial DNA has been successfully extracted from filtered water samples, and the 16S gene has been amplified and sequenced. Initial results indicate a bacterial community composition that is diverse and includes abundant genera, such as *Prochlorococcus*, *Cyanothece*, and *Tenacibaculum*, similar to other marine samples analyzed using other DNA-based approaches. The 80 samples analyzed thus far show variations in bacterial community composition, and further analysis is underway to determine whether these differences correlate with location, depth, season, or tide cycle. Additionally, fecal indicator bacteria have been detected as a measure of water quality, and future work will compare this detection method with other methods including membrane filtration and IDEXX Enterolert previously used in the Park.

Findings demonstrate that microbiomes colonizing various types of microplastics incubated in the Hudson River for three to six weeks can be successfully collected and analyzed. These microplastic microbiomes are complex, influenced by microplastic location within the river, and contain bacteria within these microbiomes that carry several different ARGs.

Moving forward, data analysis will continue for both research objectives. Future collaboration with Hudson River Park will provide an opportunity to share this data and findings with the greater Park community and could help inform future decisions and practices in the Park.

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