

18S rRNA amplicon sequence data (V1–V3) of the Bronx river estuary, New York

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Abstract

Characterising and monitoring biological diversity to foster sustainable ecosystems is highly recommended as urban centres rapidly expand. However, much of New York City's biodiversity remains undescribed, including in the historically degraded, but recovering Bronx River Estuary. In a pilot study to identify organisms and characterise biodiversity patterns there, 18S rRNA gene amplicons (V1–V3 region), obtained from river sediments and surface waters of Hunts Point Riverside and Soundview Parks, were sequenced. Across 48 environmental samples collected over three seasons in 2015 and 2016, following quality control and contaminant removal, 2,763 Amplicon Sequence Variants (ASVs) were identified from 1,918,463 sequences. Rarefaction analysis showed sufficient sampling depth, and community composition varied over time and by substrate at the study sites over the sampling period. Protists, plants, fungi and animals, including organisms of management concern, such as Eastern oysters (*Crassostrea virginica*), wildlife pathogens and groups related to Harmful Algal Blooms, were detected. The most common taxa identified in river sediments were annelid worms, nematodes and diatoms. In the water column, the most commonly observed organisms were diatoms, algae of the phylum Cryptophyceae, ciliates and dinoflagellates. The presented dataset demonstrates the reach of 18S rRNA metabarcoding for characterising biodiversity in an urban estuary.

Key Words

eDNA, metabarcoding, New York City, next-generation sequencing, QIIME2, urban ecology

Introduction

With the extensive modification of ecosystems by people and increasing urbanisation, the disrupted ecology of cities is garnering substantial research interest (Alberti 2008). Understanding how urban life forms are responding to habitat alteration, invasion by non-native species, pollution, human population growth, overexploitation, disease, climate change and/or interactions amongst these threats are key areas of urban ecology research. New York City is one of the world's great metropolises, yet much of its biodiversity remains to be identified and described, especially the microfauna and microflora of its rivers and estuaries.

The once highly polluted, 23 mile-long (ca. 37 km), Bronx River is currently considered “impaired”, with pollutants including faecal coliforms, garbage, refuse, polychlorinated biphenyls (PCBs) and other toxins, coming from a combination of urban and stormwater runoff, Combined Sewer Overflow (CSO) outfalls, contaminated sediments, and other sources (BRA 2021; NYSDEC 2020). These waters have been impacted by invasive species, such as green (*Carcinus maenas*) and Asian shore (*Hemigrapsus sanguineus*) crabs. They are also affected by harmful algal blooms (HABs), including *Gymnodinium* dinoflagellates (Fuss and O'Neill 2015). Eukaryotic pathogens include *Cryptosporidium parvum*

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(an intestinal parasite that can affect humans), and the oyster pathogens *Perkinsus marinus* and MSX (*Haplosporidium nelsoni*). However, the recovering area now hosts several clean-up and revitalisation programmes, such as targeted restoration of American eels (*Anguilla rostrata*), river herring (*Alosa pseudoharengus*, *A. aestivalis*) and eastern oysters (*Crassostrea virginica*). In the Bronx River Estuary (Fig. 1), Hunts Point Riverside Park used to be an illegal garbage disposal site, but is now an integral part of the Bronx River Greenway (Kimmelman 2012). Soundview Park waters contain a successfully-restored oyster reef identified as a key research site (Grizzle et al. 2012).

Although the first step in any ecological study is the correct identification of organisms in the focal system, there are numerous challenges in conducting biodiversity inventories (Bik et al. 2012; Bohmann et al. 2014; Taberlet et al. 2018; Deiner et al. 2021). Traditional surveys involving morphological classification and other techniques provide essential data, including abundance information. However, many taxa remain difficult to detect and describe due to a combination of their microscopic or cryptic natures, collection logistics, lack of taxonomic expertise and labour-intensive morphological assessments, leading to uncertain species identifications and biodiversity estimates. Rapid, effective and standardised approaches are needed to guide more detailed investments and cost-effectively complement morphological data for comprehensive management through improved biodiversity information (Bik et al. 2012; Bohmann et al. 2014; Rees et al. 2014; Taberlet et al. 2018; Deiner et al. 2021).

Recently developed, non-invasive and transformative environmental DNA (eDNA) metabarcoding technology provides volumes of data for biodiversity assessment via next-generation sequencing. DNA barcoding was the first worldwide effort to document life and identify species using genetic sequences from a standard DNA segment (Hebert et al. 2003). Approaches such as metabarcoding, a high-throughput extension of DNA barcoding, offer orders of magnitude more data than traditional morphological or DNA barcoding research. Multiple organisms can be identified simultaneously from genetic material extracted from environmental samples (e.g. water, air and sediment) by sequencing and analysing specific marker genes using primers that target their conserved flanking regions (Taberlet et al. 2018; Deiner et al. 2021).

Environmental DNA has numerous advantages, offers high-throughput presence, absence and relative abundance data, and can improve representation of microscopic or cryptic taxa (Bik et al. 2012; Bohmann et al. 2014; Taberlet et al. 2018; Deiner et al. 2021). Environmental DNA metabarcoding is low-impact, efficient, cost-effective, rapid and replicable. The method has been effectively used in estuaries (Chariton et al. 2010, 2015; Leray and Knowlton 2015; Taberlet et al. 2018; Afzali et al. 2021; Carraro et al. 2021; García-Machado et al. 2021) and has found previously undetected or poorly characterised organisms, in particular bacterial, protist and invertebrate

taxa (Bik et al. 2012; Bohmann et al. 2014; Goldberg et al. 2015; Taberlet et al. 2018; Deiner et al. 2021; Leese et al. 2021). Laboratory, computational and data storage limitations exist and reference data for taxonomic assignment of many groups are lacking, but the methods are continuously improving (Valentini et al. 2016; Taberlet et al. 2018; Deiner et al. 2021).

One advancement that facilitates biodiversity assessment and monitoring is state-of-the-art bioinformatics pipeline development to perform quality-control and large-volume data analysis (Taberlet et al. 2018), such as for the 18S rRNA amplicon datasets presented here. Some of the advantages offered by 18S rRNA metabarcoding are broad amplification across eukaryotic kingdoms, a rapidly growing reference database due to wide marker use, as well as conservation within and divergence amongst species genetic profiles (Leray and Knowlton 2016; Taberlet et al. 2018). This marker was selected here to provide a broad overview of estuarine eukaryotic biodiversity, including microorganisms, other algae and invertebrates, that would mirror our prior 16S rRNA metabarcoding work on prokaryotes (Naro-Maciel et al. 2020). Within the 18S rRNA gene, several markers for metabarcoding are being used. For protist taxa, the V4 and the V9 regions are utilised especially often (Stoeck et al. 2009; Dunthorn et al. 2012; de Vargas et al. 2015; Boenigk et al. 2018). Here, the V1–V3 region was targeted due to the high phylogenetic resolution available using hypervariable segments V2–V4, previously demonstrated in dinoflagellates (Ki 2012) and copepods (Wu et al. 2015). Initial checks against published databases and preliminary laboratory tests supported our choice of the V1–V3 region for common taxa or those of management concern, including Eastern oysters (*Crassostrea virginica*) and HAB-related taxa. Thus, the 18S rRNA dataset, presented here, was used to identify organisms, explore biodiversity patterns and establish a baseline for future work in the Bronx River Estuary.

Methods

Study sites and sampling

The Bronx River Estuary was sampled from August 2015 to September 2016, monthly from May to October during low tide (Fig. 1). Samples were collected from Reach 1 (NYCParks 2021) at Hunts Point (HP, 40.82°N, 73.88°W, $n_{\text{sediment}} = 9$; $n_{\text{water}} = 8$) and Soundview (SVP, 40.81°N, 73.87°W). At Soundview, samples were obtained along a restored oyster reef (SVP-BRO: $n_{\text{sediment}} = 8$; $n_{\text{water}} = 7$) and at another estuarine site about one tenth of a mile (ca. 0.16 km) away where wild oysters were observed (SVP-BRC: $n_{\text{sediment}} = 8$; $n_{\text{water}} = 8$). To investigate two key habitats of estuarine organisms and complement ongoing conventional surveys (NYCParks 2021; Fitzgerald 2013), surface waters and benthic sediments were sampled. The former were sampled by dipping a 1-litre autoclaved jar

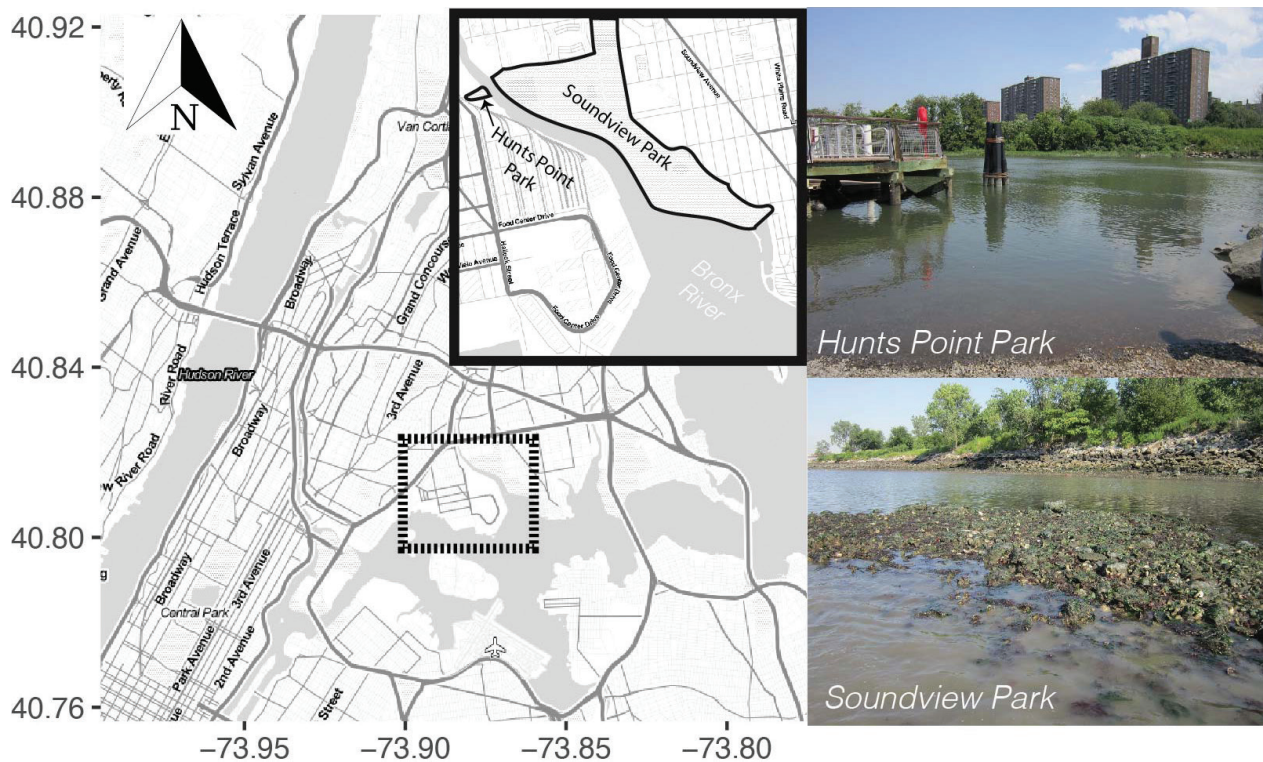


Figure 1. Sampling site map depicting greater New York City waterways. Inset: Detail map of sample area showing Soundview Park (SVP) and Hunts Point Riverside Park (HP) in bold outline. Right: site photos of the SVP and HP estuaries on the Bronx River. Map data 2019 (C) Google.

horizontally into the river before sediment core collection, in order to avoid contamination (Naro-Maciél et al. 2020). A polyvinyl chloride (PVC) pipe (6-inch length, 2-inch diameter) and a pallet shovel were used to sample river sediments (about 100 g) from the surface sediment layer following standard procedures, including use of disposable gloves and individual, sterilised material for each sample (Fitzgerald 2013). The samples were stored in a cooler and then moved to a laboratory refrigerator (Naro-Maciél et al. 2020).

Environmental DNA analysis

All materials were processed within 24 hours of sampling (Naro-Maciél et al. 2020). The water samples ($n = 23$) were divided equally between two funnels (500 ml) and filtered with 0.45 μm Whatman Cellulose Nitrate Sterile filters (Cytiva, USA) using a standard laboratory vacuum pump (Airtech, USA; Type L-250D-G1). Filters were placed into PowerWater DNA Isolation Kit bead tubes (Qiagen, USA) and DNA was extracted following the manufacturer's instructions. A randomly selected 0.25 g soil subsample of each sediment sample ($n = 25$) to be used for extraction was placed in 2 ml collection tubes and centrifuged. The sediment was then transferred into PowerSoil bead tubes and extracted as instructed (Qiagen, USA). Following DNA extraction and quantification using a NanoDrop 2000 Spectrophotometer or a Qubit Fluorometer (Thermo Scientific, USA), the samples were stored frozen at -20°C (Naro-Maciél et al. 2020). No ex-

traction blanks or positive controls were included in this pilot study and the turtle-focused molecular biodiversity research lab was not PCR-free. However, contaminant prevention, disinfection, decontamination and sterilisation procedures, standard for a university molecular lab, were assiduously used (e.g. bleach or alcohol disinfection and surface sterilisation, single-use molecular-grade disposable material utilisation, autoclaving, UV-irradiation of supplies etc.) and state-of-the-art *in silico* quality control including contaminant identification and removal was later carried out as discussed below.

All remaining lab work (amplification, purification and sequencing) was conducted in a commercial laboratory (MRDNA, Molecular Research LP, Shallowater, TX, USA) using previously described industry-standard procedures and controls (MRDNA 2021; Dowd et al. 2008; Naro-Maciél et al. 2020). Preliminary runs with small sample sizes were conducted first to confirm primer amplification efficiency, followed by the full sample sets. Through polymerase chain reaction (PCR), about 563 bp of the 18S rRNA gene V1–V3 region was amplified using primers Euk7F (Medlin et al. 1988) and Euk570R (Weekers et al. 1994) (Euk7F: AACCTGGTT-GATCCTGCCAGT + a unique 8 bp identifier barcode; Euk570R: GCTATTGGAGCTGGAATTAC). PCRs were run in 20 μl volumes using the Qiagen HotStarTaq Plus Master Mix Kit (Qiagen, USA) as follows: 94°C for 3 minutes, 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute and a final elongation step at 72°C for 5 minutes (MRDNA 2021, Dowd et al.

2008). The number of cycles was determined by initial testing to optimise product detection versus errors from over-amplification.

After 2% agarose gel checks, the uniquely barcoded PCR samples were pooled in equal proportions, based on a combination of electrophoresis-based size and density estimations and DNA concentrations. The pooled samples were then purified with calibrated Ampure XP beads (Agencourt Bioscience, USA); the ratio of beads to PCR products used for purification was $0.75\times$ as per Illumina manufacturer guidelines. Next, an Illumina DNA library was created from these purified and pooled PCR products ligated to Illumina adapters using the Illumina TruSeq DNA library preparation protocol. Finally, sequencing was performed on an Illumina MiSeq according to manufacturer's instructions, using paired-end 2×300 bp v.3 chemistry (MRDNA 2021, Dowd et al. 2008).

Amplicon sequence variants (ASVs)

The FASTQ Processor was used to extract barcodes and sort forward and reverse reads into distinct files (MRDNA 2021). Raw reads were then processed using the QIIME2 v. 2019.1 pipeline (Bolyen et al. 2018) (Suppl. material 1: Document S1). The demultiplexed reads were not merged due to insufficient overlap. As quality statistics were high for forward reads, but more variable for reverse, only the forward reads were analysed as single-end. The DADA2 plug-in was then used in QIIME2 to de-noise and quality-filter the forward sequences, assign amplicon sequence variants (ASVs or features), include only Eukaryotes and generate a feature table of ASVs and metadata (Callahan et al. 2016). Primers and low-quality base calls (5–10 bp) were trimmed at the ends of each single-end sequence during the DADA2 step and reads were truncated at 260 bp, based on examination of quality scores, to account for typically observed end-of-sequence decline in quality. All other parameters, including culling short and otherwise low-quality sequences, identifying and deleting chimeras etc. were run as default (QIIME2 2021). After DADA2 filtering, the average percentage of sequences retained was 79%, with a median of 39,758 sequences kept per sample (Table 1). To taxonomically classify the 18S reads, the q2-Naïve Bayesian classifier, as implemented in QIIME2, was employed, using the SILVA 138 reference database (Quast et al. 2013) trained on the entirety of the 18S rRNA gene (Bokulich et al. 2018; Karst et al. 2018). This reference database was selected because it is a comprehensive, frequently updated and quality-curated resource for identifying eukaryotic 18S rRNA gene sequences. ASV taxonomy was manually inspected to ensure adequate taxonomic resolution was achieved.

Once taxonomic annotation was complete, R Studio v. 1.2.1335 (R Core Team 2008) was used to perform statistical analysis (Suppl. material 1: Document S2). The 'DECONTAM' programme v. 1.8.0 was run on the feature table to remove potential contaminants (Davis et al. 2018). The programme's frequency method option works

Table 1. Summary of sample data, including sample ID and statistics on the recovery of reads per sample after filtering, de-noising and chimeric sequence removal.

Sample	Input	Filtered	% input passed filter	De-noised	Non-chimeric	% of input non-chimeric
S.B.BRC	61653	51307	83.22	49865	49355	80.05
S.B.BRO	57043	46675	81.82	45055	43716	76.64
S.B.HP	41172	34193	83.05	32911	31537	76.60
S.C.BRC	65637	54030	82.32	52074	50699	77.24
S.C.BRO	53923	43911	81.43	42387	41716	77.36
S.C.HP	48441	40413	83.43	38983	37581	77.58
S.D.BRC	37670	31600	83.89	29892	29647	78.70
S.D.BRO	39984	32324	80.84	30829	30329	75.85
S.D.HP	38926	32241	82.83	30923	29822	76.61
S.E.BRC16	81853	67172	82.06	65692	64195	78.43
S.E.BRO16	64498	52646	81.62	51301	49170	76.23
S.E.HP16	50365	41017	81.44	39681	38571	76.58
S.F.BRC16	74188	62748	84.58	61411	60144	81.07
S.F.BRO16	72355	59701	82.51	57927	57472	79.43
S.F.HP16	56688	47187	83.24	46094	44870	79.15
S.G.BRC16	60173	50378	83.72	48470	47934	79.66
S.G.BRO16	59639	50520	84.71	49155	45773	76.75
S.G.HP16	62125	49689	79.98	48429	45891	73.87
S.H.BRC16	84518	62017	73.38	60776	60186	71.21
S.H.BRO16	64609	53593	82.95	51590	50524	78.20
S.H.HP16	48136	40675	84.5	39217	37346	77.58
S.I.BRC16	63314	53030	83.76	51647	51166	80.81
S.I.BRO16	54232	44896	82.79	43223	42157	77.73
S.I.HP16	51530	40901	79.37	39682	37770	73.30
S.J.HP16	55575	44475	80.03	43004	41364	74.43
W.B.BRC	66549	57336	86.16	49282	46759	70.26
W.B.BRO	66937	57216	85.48	49679	47022	70.25
W.B.HP	50568	43407	85.84	37591	35988	71.17
W.D.BRC	17978	15045	83.69	14346	14173	78.84
W.D.BRO	33475	27576	82.38	26316	25681	76.72
W.D.HP	23573	19891	84.38	17931	17902	75.94
W.E.BRC16	39716	34005	85.62	32661	31003	78.06
W.E.BRO16	37689	32271	85.62	30832	29645	78.66
W.E.HP16	39206	33090	84.4	31243	30725	78.37
W.F.BRC16	37895	32172	84.9	30931	30462	80.39
W.F.BRO16	43923	36032	82.03	34993	34027	77.47
W.F.HP16	70651	57008	80.69	56284	52129	73.78
W.G.BRC16	50138	42646	85.06	41774	38491	76.77
W.G.BRO16	47758	40433	84.66	39051	36563	76.56
W.G.HP16	54025	45735	84.66	44776	40909	75.72
W.H.BRC16	41440	35408	85.44	34245	32717	78.95
W.H.BRO16	42214	34834	82.52	33777	32871	77.87
W.H.HP16	55457	46005	82.96	44750	41631	75.07
W.I.BRC16	47134	39762	84.36	37335	35006	74.27
W.I.BRO16	48683	40787	83.78	38592	35516	72.95
W.I.HP16	46537	38277	82.25	37216	35306	75.87
W.J.HP16	41706	34926	83.74	33496	32281	77.40
W.J.SVP16	57638	48829	84.72	47289	42721	74.12
Totals	2509137				1918463	

by inferring potential contaminants using a simple inverse linear correlation between initial sample DNA concentration and the frequency of each ASV. Contaminants should behave such that their relative proportion increases as sample concentration decreases (Davis et al. 2018). Using a threshold of $p < 0.10$, the programme filtered ASVs meeting this criterion from the dataset. In total 28 contaminants were removed from the feature table, representing just 1% of the dataset (Suppl. material 2: Table S1).

The PHYLOSEQ v. 1.28.0 package was then used for basic data manipulation and, visualisation and community-level statistical analyses were performed using tools available in the VEGAN v. 2.5.5 package (McMurdie and Holmes 2013; Oksanen 2019). Observed ASV richness and the Shannon Diversity Index (Shannon

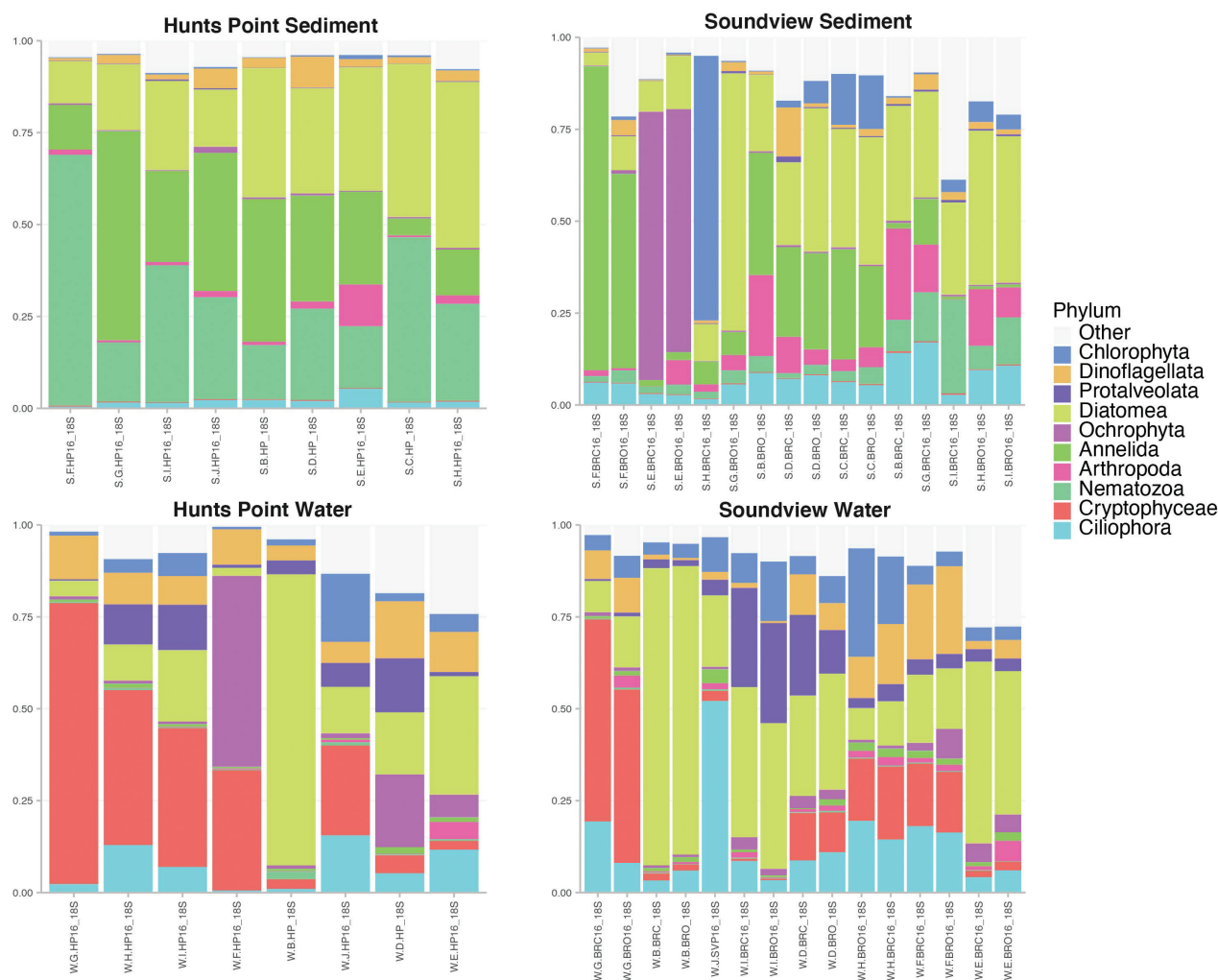


Figure 2. 18S rRNA community profiles of Amplicon Sequence Variants (ASVs) in sediment and water samples from Hunts Point Riverside and Soundview Parks shown at the level of phylum. Bar heights show relative abundance of sequences from each taxon.

1948) were computed to summarise alpha diversity of the eukaryotic communities. Differences in alpha diversity amongst sediments and water from both sites were assessed using a Kruskal-Wallis test with non-parametric pairwise comparisons. For beta diversity comparisons, the data were normalised using a Hellinger Transformation, which takes the square root of each ASV's relative abundance and bounds it between 0 and 1 (Legendre and Gallagher 2001; Lahti et al. 2017). Beta diversity, or turnover between sites, was summarised using the Bray-Curtis Index. To visualise differences in beta diversity, non-metric multidimensional scaling (NMDS) ordination (stress = 0.13), based on Bray-Curtis Dissimilarity was carried out using several random starts and stress assessment through the metaMDS command (Oksanen 2015) and ellipses were drawn using the stat_ellipse function.

Results and discussion

This pilot study identified key organisms, explored biodiversity patterns and established a baseline for future work in the area, but the data must be interpreted with

caution considering methodological issues. In total 48 environmental samples were successfully collected and sequenced for the 18S rRNA gene ($n_{\text{water}} = 23$; $n_{\text{sediment}} = 25$). Within these samples, protists, plants, fungi and animals encompassing 2,763 ASVs were recovered from a total of 1,918,463 post-quality-control sequences (Suppl. material 2: Table S2). Species accumulation curves of each sample reached an asymptote, indicating that the communities were surveyed with sufficient depth to detect robust differences in community structure and composition (Suppl. material 2: Fig. S1). At the study sites over the sampling period, community composition varied over time and by substrate (Fig. 2).

Several organisms of known occurrence, including taxa of management concern, were detected. Commonly observed species identified in this survey included soft-shell clams (*Mya arenaria*) and blue mussels (*Mytilus edulis*) (Suppl. material 2: Table S1). Oyster DNA (*Crassostrea virginica*) was detected in both Soundview waters and sediments, but not in Hunts Point waters or sediment. Similarly, oysters have been observed at Soundview, but not at Hunts Point; the oyster parasite genus *Perkinsus* was detected only in the water at Soundview Park. The

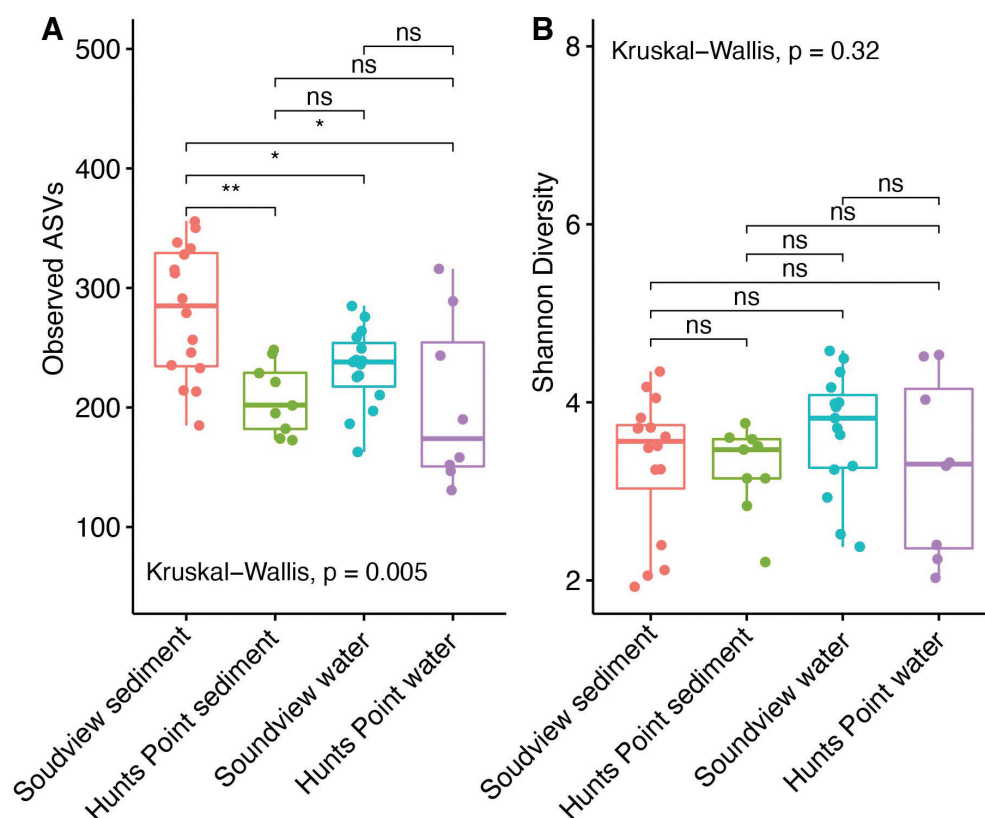


Figure 3. Alpha diversity comparison between sediment and water samples from Hunts Point Riverside and Soundview Parks computed using A) Observed ASVs and B) Shannon Diversity. Pairwise comparisons are indicated as follows: * = $p < 0.05$, ** = $p < 0.001$, ns = non-significant.

oyster pathogen MSX (*Haplosporidium nelsoni*) was not identified at any site, but the crustacean parasite *Haliphthoros* was found in Hunts Point sediments. Dinophyceae dinoflagellates (*Gymnodinium*, *Heterocapsa*, *Karlodinium*) and Raphidophyceae (*Heterosigma akashiwo*), which may cause Harmful Algal Blooms (Hara and Chihara 1987; Faust and Gullede 2002; Millette et al. 2015; Lin et al. 2018), were sequenced from the Bronx River Estuary. Macroinvertebrate taxa considered to be indicators of estuary pollution (Pelletier et al. 2010; Smith et al. 2015) were not commonly found, except for the various small aquatic worms (Nematoda), some of which are consistent with poor water quality (Fig. 2). American eels (*Anguilla rostrata*) and herring (*Alosa pseudoharengus* and *A. aestivalis*), key organisms being restored and monitored in the Bronx River, were not detected.

In terms of alpha diversity, Soundview Park sediments were significantly higher in the observed number of ASVs compared with all other sites (Kruskal-Wallis, $p = 0.05$, Fig. 3A). While Soundview water also trended towards higher biodiversity compared with Hunts Point water, the difference was non-significant. However, none of the sites differed significantly in Shannon Diversity ($p > 0.05$, Fig. 3B). Sediment communities between Hunts Point and Soundview were differentiated by the presence of several key taxa missing or less proportionally abundant at Hunts Point. For example, Soundview sediments had higher proportions of arthropod DNA detected than

those at Hunts Point (Figs 2 and 3). In agreement with our results on overall alpha diversity metrics, Soundview Park sediments were more taxonomically diverse when compared with Hunts Point. Water samples from both sites were not apparently different in taxonomic composition (Figs 2–4). However, there were clear differences between the taxonomic make-up of sediments and water column samples, driven mostly by the more frequent detection of annelid worms and nematodes in sediments and larger proportions of diatoms, dinoflagellates and Protalveolata in water samples (Fig. 2). The community turnover (i.e. beta diversity) of eDNA from water samples was significantly different from that of sediment ($r^2 = 0.169$, $P = 0.001$; Fig. 4). Water samples were homogeneous amongst sites ($r^2 = 0.069$, $P = 0.834$). In contrast, sediment samples from Soundview Park were distinct from those at Hunts Point ($r^2 = 0.245$, $P = 0.001$; Fig. 4).

Future metabarcoding work in the area would benefit from lessons learned during and resources developed since this pilot study. The high quality, comprehensive protocols now available to standardise and ensure eDNA metabarcoding excellence should be carefully followed (Taberlet et al. 2018; Minamoto et al. 2021). To better incorporate unicellular organisms and viruses, specialised methods, including use of filters with finer pore size, should be employed. While state-of-the art bioinformatics work conservatively identified and removed errors and contaminants, ongoing research would additionally

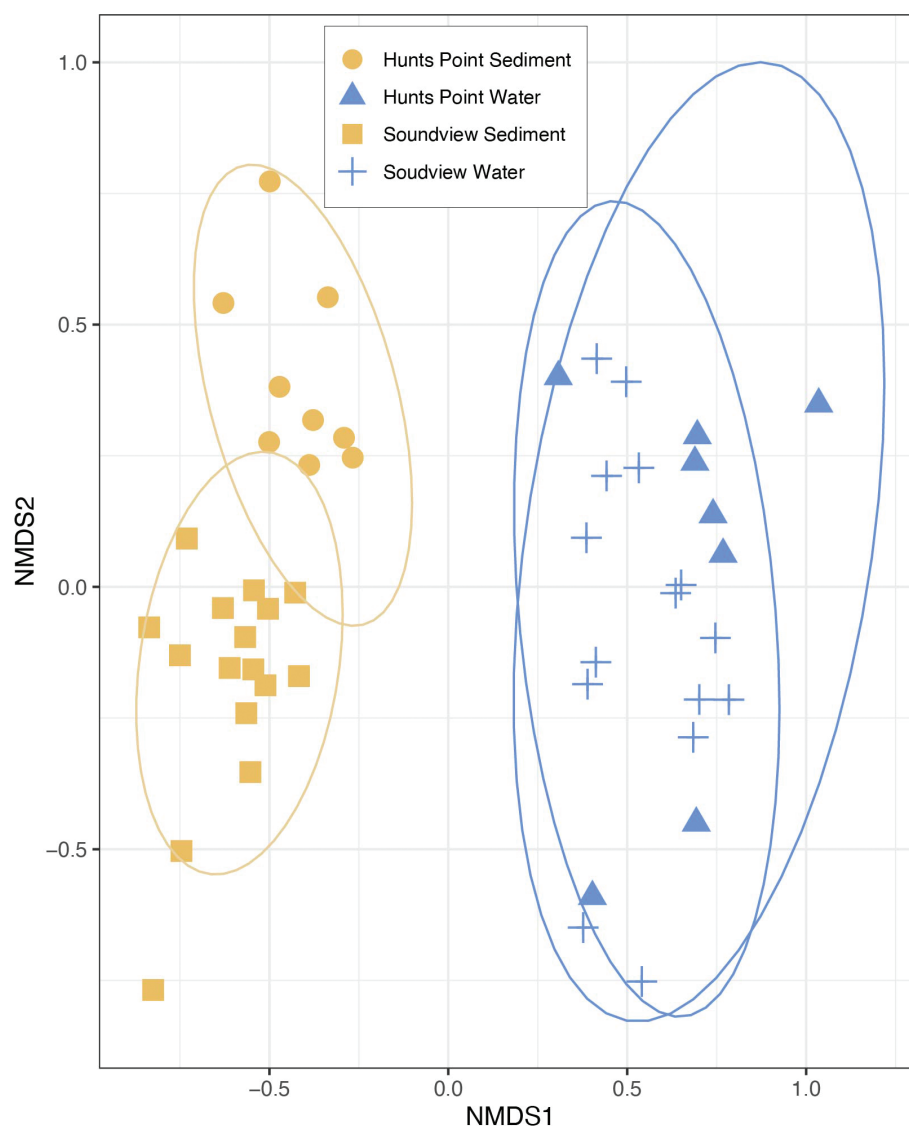


Figure 4. Community comparisons amongst substrates (sediment and water) and sites (Hunts Point Riverside and Soundview Parks), based on Amplicon Sequence Variants (ASVs) and using Non-metric multidimensional scaling analysis (NMDS).

benefit from stringent laboratory checks. These include the use of extraction blanks in a PCR-free lab, positive controls to assess amplification efficiency and negative controls to identify contaminants directly. Technical replicates address contamination, errors including rare taxa detection, and PCR amplification and sequencing variation. Further, although the V1 – V3 segment did capture known organisms and those of management interest, reference data bases for the increasingly used V4 or V9 regions may be more complete, thus resulting in more identifications. Finally, organismal abundance does not necessarily correlate with sequence abundance given amplification biases and errors such as primer-template fidelity and suboptimal annealing temperatures. Thus even inferences about relative abundance should be interpreted with caution (Fonseca 2018; Taberlet et al. 2018). Comparing metabarcoding results to conventional survey data will continue to be essential for ground-truthing and optimising both methods (Fediajevaite et al. 2021).

In conclusion, the 18S rRNA V1 – V3 dataset, presented here, complements our prior study, “16S rRNA Amplicon Sequencing of Urban Prokaryotic Communities in the South Bronx River Estuary”. Future work will comparatively analyse information from these two genetic regions and new data from Cytochrome Oxidase I, the standard locus for animal barcoding (Hebert et al. 2003). Despite its advantages, the 18S rRNA marker alone is insufficient to fully characterise biodiversity and a suite of markers would provide a more complete profile (Leray and Knowlton 2016; Taberlet et al. 2018) to further describe life in a complex urban estuary.

Data availability

All 18S rRNA amplicon gene sequences from this study are posted on the NCBI Sequence Read Archive (SRA) under BioProject PRJNA606795 accession numbers

SAMN19729835–SAMN19729882 (Table 1). All DNA extracts are stored at the American Museum of Natural History. Bioinformatics and statistical scripts are available as a supplement to this article (Suppl. material 1: Documents S1, S2).

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Conflicts of Interest

The authors declare no competing interests.

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Supplementary material 1

Scripts used for metabarcoding analysis

Author: Melissa Ingala

Data type: QIIME and R Scripts (in zip. archive)

Explanation note: **Document S1.** QIIME2 workflow. **Document S2.** R script.

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Link: <https://doi.org/10.3897/mbmg.5.69691.suppl1>

Supplementary material 2

Figure S1, Tables S1, S2

Author: Melissa Ingala

Data type: jpg. file, docx. file and xlsx. file (in zip. archive)

Explanation note: **Figure S1.** Sample-based species accumulation curves of total 18S rRNA diversity by substrate type (sediment, water) for each site (Hunts Point Riverside and Soundview Parks), calculated using the Vegan 2.4-3 package for Amplicon Sequence Variants (ASVs). **Table S1.** Taxonomies of contaminating ASVs removed by “decontam” analysis. **Table S2.** Taxonomic Assignment including ASV ID to Domain (d), Phylum (p), Class (c), Order (o), Family (f), Genus (g) and Species (s), as applicable.

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