HUDSON RIVER PARK
2024 METABARCODING REPORT

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Abstract

Environmental DNA (eDNA) metabarcoding offers a non-invasive approach to assess aquatic biodiversity, providing insights into species presence without the need for extractive sampling. This pilot study explores the potential of eDNA metabarcoding to reveal fish diversity detectable in water samples collected at Pier 40 in Hudson River Park, and at partner sites in Yonkers, Piermont, and Norrie Point. Sequencing and analysis identified 84 fish species, including native and non-native species to the Lower Hudson River watershed, across 77 samples. Sequencing reads of Atlantic menhaden (*Brevoortia tyrannus*) emerged as the most abundant of any species, indicative of its key role and prevalence in this ecosystem. Additionally, the detection of species such as Atlantic sturgeon (*Acipenser oxyrinchus*) and shortnose sturgeon (*Acipenser brevirostrum*) underscores the value of eDNA metabarcoding for monitoring protected species. Pond loach (*Misgurnus anguillicaudatus*), a species first detected in the Lower Hudson River watershed in 2009, was detected in Norrie Point, New York, indicating its ongoing presence in this system.

Introduction

In recent years, environmental DNA (eDNA) metabarcoding has emerged as a tool for studying aquatic biodiversity without the need for traditional sampling methods that may disrupt ecosystems (Hoffmann et al., 2016). eDNA analysis is a method for monitoring organismal

presence through the detection of taxon-specific DNA fragments in environmental samples, offering a non-extractive and minimally invasive means of assessing species presence and diversity. Traditional methods of fish biodiversity assessment often involve time-consuming and labor-intensive sampling techniques such as electrofishing, netting, trapping, or trawling. These methods can be invasive to aquatic habitats and fish populations. Gear biases may preclude detection of the full spectrum of species present, underrepresenting harder to capture species. In contrast, eDNA metabarcoding utilizes genetic markers to identify species based on detection and sequencing of DNA purified from environmental samples, providing a snapshot of biodiversity with minimal disturbance to habitat and biota. Using "universal" PCR primers (Miya et al., 2015; Riaz et al., 2011) designed to amplify DNA from many species, metabarcoding can produce detections across a taxonomic breadth that rivals or exceeds more established capture survey methods (Jiang et al., 2023; McColl-Gausden et al., 2021). Furthermore, eDNA samples collected for other analyses, such as water quality assessment or pollution monitoring, can be repurposed for metabarcoding analysis. This dual-use approach maximizes the value of collected samples, providing additional insights into fish diversity with reduced need for additional fieldwork.

This pilot project explores the potential value of eDNA metabarcoding for studying fish diversity in the Lower Hudson River. Samples collected at Hudson River Park (the Park) and partner locations for analysis by end-point PCR, a single-species eDNA assay, were further processed by 12S metabarcoding to assess the efficacy of this analysis. By leveraging this innovative technique, this project aims to add value to existing Park samples and surveys, enhance understanding of the Hudson River ecosystem, inform fish monitoring strategies, and contribute to the sustainable management of this vital aquatic resource.

Methods

1.0 L surface water samples were collected during the spring and summer from the Hudson River at Hudson River Park's Pier 40 in Manhattan, Sarah Lawrence College's Center for the Urban River at Beczak in Yonkers, Lamont Doherty Earth Observatory in Piermont, and the New York State Department of Environmental Conservation in Norrie Point from June 2021 to September 2024 (Table 1). Filtration occurred on site via 0.45 µm filters which were then shipped overnight, on ice, to Hudson River Park. Extractions were performed at the Park's Pier 40 facilities using Qiagen DNeasy Blood & Tissue kits with modifications outlined in Stoeckle et al., 2018. For samples 01 to 57, aliquots of extracted DNA were amplified with Mifish primers targeting a fragment of the 12S ribosomal region of the mitochondrial genome (Miya et al., 2015) as the first stage of a nested end-point PCR analysis (Stoeckle et al., 2018) testing for the presence of American eel (*Anguilla rostrata*), Atlantic sturgeon (*Acipenser oxyrinchus*), and striped bass (*Morone saxatalis*). Mifish PCR products were diluted 1:20 in molecular grade water and submitted to BAS Lab at the University of Maryland for 2x300 sequencing by Illumina Miseq. Samples 58 to 97 were sent to BAS Lab as DNA extracts, where Mifish PCR was performed before sequencing.

Processing of sequencing data was performed in DADA2 (Callahan et al., 2016) in R. Sequence reads were trimmed, filtered for quality and chimeric sequence removal, and dereplicated before being matched to a reference library to assign taxonomy by *assignTaxonomy* and *assignSpecies*. Initial assignments to species were made at 100% match to reference sequences. The reference library was prepared using CRABS (Jeunen et al., 2023) to perform *in silico* PCR with the Mifish primer sequences on the Mitofish database (v 3.99), a curated archive of fish mitogenomes representing 4,129 species. Secondary assignments of detected sequences that

passed quality filtration, but were not assigned to a single species were submitted as BLAST (Sayers et al., 2021) queries of NCBI and Mitofish databases. Matches to species known in the region with no more than one mismatched base were assigned to that species. Matches that could not be resolved to a single species by these steps were manually assigned to their genus (these nonspecific assignments can be distinguished by "*spp.*" following genera names in scientific names and "unk" in common names, indicating that species is unknown). Matches to database sequence records of hybridized fishes were assigned as unique taxa.

Results

A total of 8,023,344 reads were generated from 77 of the 97 samples submitted. 20 samples submitted for sequencing failed to form clusters during sequencing and generated no data. 3,649,149 sequencing reads passed all filtering and denoising steps and were assigned to fish taxa. The quantity of post-filtration reads produced varied widely between samples (Tables 1, 2), from 671 reads for the July 2023 Yonkers sample, to 245,323 reads for the July 2021 Norrie Point sample. The mean quantity of reads produced per sample was 47,391 (sd= 47,712). The median read quantity was 36,036 per sample.

After dereplication, 283 unique sequences were assigned to 95 fish taxa, including 84 unique fish taxa (species or genus level assignments) and 11 multispecies assignments inclusive of uniquely detected fishes (Figure 1, Table 3). Detected fishes include members of 26 orders, 47 families, and 74 genera. Most taxonomic assignments were made unambiguously to single species, but in cases where two or more species were matched to the same barcode sequence, multispecies assignments were used (e.g., "alosa spp." or "pumpkinseed or bluegill", Table 3). 11 of the 14 multispecies taxonomic assignments reported here were to genera or pairs of species also represented by unambiguously assigned reads. Multispecies assignments to yellow or brown

bullhead (*Ameiurus natalis or Ameiurus nebulosus*), blue or southern blue catfish (*Ictalurus furcatus or Ictalurus meridionalis*), and unknown flying fish (*Hirundichthys spp.*) did not include any species detected separately. Species included in multispecies assignments are not distinguishable by the MiFish barcode by any sequence variant detected. These ambiguous assignments are the result of high mitochondrial DNA similarity, which is a feature of recently diverged species.

Atlantic menhaden (*Brevoortia tyrannus*) was the most abundantly detected species (Table 3, Figures 1, 2, 3), with 893,780 reads assigned, accounting for 24.5% of all assigned reads. The top ten most abundant species detections accounted for 73.8% of all assigned reads and further include white perch (*Morone americana*), pumpkinseed or bluegill (nonspecific assignment due to identical barcode for *Lepomis gibbosus* or *Lepomis macrochirus*), common carp (*Cyprinus carpio*), American eel (*Anguilla rostrata*), bay anchovy (*Anchoa mitchilli*), mummichog (*Fundulus heteroclitus)*, striped bass (*Moron saxatalis*), white sucker (*Catostomus commersonii*), and banded killifish (*Fundulus diaphanus*).

The detected species (Table 3) span the range of salinity preferences with 21 freshwater and 20 marine species joining 52 species with intermediate or facultative salt tolerances. Native species comprised the majority (67 of 84, 79.8%) of detected fishes. 22 nonnative species were detected, excluding multispecies assignments that include species detected separately. The most abundantly sequenced nonnative species detected were common carp (*Cyprinus carpio*, 270,739 reads), followed by detections assigned to common carp or goldfish (matched to *Cyprinus carpio* x *Carassius auratus* hybrid, 83,872 reads), and channel catfish (*Ictalurus punctatus*, 39,233 reads. Pond loach (*Misgurnus anguillicaudatus*), a relatively recent introduction to the Hudson River system, was detected at Norrie Point.

Several fishes without known occurrence in the Hudson River were detected, including Atlantic salmon (*Salmo salar*), tilapia (*Oreochromis spp.*), grass carp (*Ctenopharyngodon Idella*), blue or southern blue catfish (*Ictalurus furcatus or Ictalurus meridionalis*), and European pilchard (*Sardina pilchardus*).

Reflecting the estuarine nature of the Lower Hudson River, at least 12 diadromous species were detected: Atlantic menhaden, white perch, American eel, striped bass, Atlantic salmon (*Salmo salar*), Atlantic tomcod (*Microgadus tomcod*), shads and herrings (*Alosa* spp.), rainbow trout or steelhead (*Oncorhynchus mykiss*), Atlantic sturgeon (*Acipenser oxyrinchus*), shortnose sturgeon (*Acipenser brevirostrum*), rainbow smelt (*Osmerus mordax*), and flathead mullet (*Mugil cephalus*).

Between sites, assigned fish richness ranged from 52 to 62 taxa detected (Table 2), with a mean of 57.5 assigned fishes per site (sd = 3.87). Samples from Yonkers (n=19, 62 assigned taxa, Table 5) and Piermont (n=20, 59 assigned taxa, Table 6) yielded the largest fish assemblages. Samples from the Hudson River Park site (n=20) produced detections of 56 fish taxa (Table 5). Norrie Point samples, the least numerous set analyzed (n=18), produced detections of 53 fish taxa (Table 7).

All sites produced unique species detections. Six species were detected exclusively at Hudson River Park: skilletfish (*Gobiesox strumosus*), American conger (*Conger* oceanicus), smallmouth flounder (*Etropus microstomus*), cunner (*Tautogolabrus adspersus*), Atlantic herring (*Clupea* harengus), and northern pipefish (*Syngnathus fuscus*).

The Yonkers site yielded unique detections of ten species: longnose dace (*Rhinichthys cataractae*), Acadian redfish (Sebastes fasciatus), red snapper (*Lutjanus campechanus*), tilefish

(*Lopholatilus chamaeleonticeps*), blue or southern blue catfish (*Ictalurus furcatus or Ictalurus meridionalis*), eastern blacknose dace (*Rhinichthys atratulus*), spotted goatfish (*Pseudupeneus maculatus*), red hake (*Urophycis chuss*), pompano (*Trachinotus spp.*), and king mackerel (*Scomberomorus cavalla*).

Samples from the Piermont site revealed unique detections of seven species: Atlantic croaker (*Micropogonias undulatus*), European pilchard (*Sardina pilchardus*), longfin rivoliana (*Seriola rivoliana*), rough silverside (*Membras martinica*), flathead grey mullet (*Mugil cephalus*), and western mosquitofish (*Gambusia affinis*), and rainbow trout (*Oncorhynchus mykiss*).

The Norrie Point site produced nine unique detections: rainbow smelt, (*Osmerus mordax*), johnny darter (*Etheostoma nigrum*), fourspine stickleback (*Apeltes quadracus*), grass carp, satinfin shiner (*Cyprinella analostana*), striped or common shiner (*Luxilus chrysocephalus* or *Luxilus cornutus*), round goby (*Neogobius melanostomus*), and pond loach (*Misgurnus anguillicaudatus*).

Discussion

The results from environmental DNA (eDNA) sampling and Mifish metabarcoding analysis of water from four sites in the Hudson River estuary provide insight into the composition of local fish communities. The fish taxa detected reflect the diverse ecological habitats of the Lower Hudson and underscore the importance of the river for both resident and migratory species. The species detected at Hudson River Park, Yonkers, Piermont, and Norrie Point vary widely in salinity preference, conservation status, ecological niche, and recreational value.

Two Endangered Species Act listed species were detected: shortnose sturgeon (*Acipenser brevirostrum*) and Atlantic sturgeon (*Acipenser oxyrinchus*). Non-extractive survey methods,

such as eDNA metabarcoding, enable the monitoring of protected species without risking harm to the fish or requiring permits for capture. The abundance of sturgeon reads was low compared to other species (891 for shortnose sturgeon in four samples, 2,725 for Atlantic sturgeon in five samples). This may reflect the true relative abundance of sturgeons in the Lower Hudson at the times sampled, but several factors may also have contributed. Sturgeons are evolutionarily distant from other teleost fishes, which might reduce the affinity of the MiFish primers for their DNA, especially in the presence of large abundances of other readily detected species such as common carp and menhaden. It is also possible that sturgeon DNA is more concentrated in the deeper channel waters they prefer rather than nearshore surface waters sampled. Collection of water samples at depth and in channel by using Kemmerer or Niskin bottles would allow investigation of the distribution of sturgeon DNA throughout the water column.

The relative abundance of metabarcoding reads has been shown to approximate the true relative abundances of fish species present, but this relationship tends to be stronger for more abundant species (Skelton et al., 2023; Stoeckle et al., 2021). The high variability of assigned reads produced by samples in this study reduces the comparability of the assemblages detected in each. Samples with low read totals likely underestimate fish diversity. For instance, only six species were detected in the 671 reads produced by one of the July 2023 samples from Yonkers, while 16 were detected in a duplicate, from 38,107 reads. To increase the ability of future metabarcoding by the Park to resolve spatiotemporal variation in assemblages, and to better detect rare species, efforts should be made to increase the minimum yield of reads per sample. Many factors contribute to final read yields, including the volume of water sampled, PCR setup, and sequencing depth. As many samples produced ample reads, sequencing depth does not appear to have been a limiting factor in this analysis. Increasing the volume of water sampled could be

achieved with almost no additional cost, but samples with high particulate content may clog filters. The use of filters with larger nominal pore sizes (≥1.2µm) can allow for processing of larger volumes without clogging, which can allow for collection of more eDNA per sample (Turner et al., 2014). The variation in read yields over several orders of magnitude, and the failure of 20 samples to produce sequencing data suggests that adjustments to PCR and post-PCR protocols may be needed. Pooling the products of multiple reactions per sample can compensate for stochastic variation of PCR itself, but multiplies cost and effort needed. Visual examination of PCR products by gel electrophoresis for bands corresponding to the total length of the MiFish barcode (160-190 bp) and primers (49bp for the MiFish-U pair, 110bp if Nextera adapters are included) can ensure that the desired amplicons have been produced and are more abundant than primer dimer (short fragments resulting from PCR primers amplifying themselves, which waste sequencing resources). The analysis of multiple samples per site appears to have compensated for the high level of variation in read abundances per sample, as large total quantities of reads were produced for all sites. No site produced less than 500,000 reads, and high species richness was detected at all sites (min=53 assigned taxa, max=62 assigned taxa).

Detections at Hudson River Park

The metabarcoding fish assemblage produced from samples collected at Pier 40 in Hudson River Park reveals a diverse fish community indicative of a dynamic estuarine ecosystem (Figures 4,5, Table 4). Reads of two forage fishes, Atlantic menhaden (*Brevoortia tyrannus*) (238,852 reads) and bay anchovy (*Anchoa mitchilli*) (104,667 reads) were the most abundant, together accounting for 42% of all fish reads from this site. These detections, along with diadromous fishes like American eel (*Anguilla rostrata*), striped bass (*Morone saxatilis*) and Atlantic sturgeon, demonstrate the River's importance as a migratory corridor, connecting freshwater and

brackish habitats to the marine environment. The six detected fish unique to this site were all marine species, further emphasizing this site's proximity to the Atlantic Ocean. Several species detected are of conservation interest, such as the Atlantic and shortnose sturgeons.

Predator-prey dynamics are suggested by detections of forage fish like Atlantic silversides (*Menidia menidia*) and bay anchovies, which are prey for larger predators such as bluefish (*Pomatomus saltatrix*) and summer flounder (*Paralichthys dentatus*). Species like the winter flounder (*Pseudopleuronectes americanus*) and tomcod (*Microgadus tomcod*) further indicate the system's ecological complexity and show that the sampling approach used is capable of detecting members of both benthic and pelagic food webs.

From a recreational perspective, detected fishes include popular sportfishing targets such as striped bass, bluefish, and tautog (*Tautoga onitis*). These species support local fisheries and recreational angling, contributing to the site's economic and cultural significance.

Detections at Yonkers

The eDNA detections at Yonkers reflect its role as habitat for resident estuarine and freshwater species and as a migratory stopover for diadromous and marine species (Figures 6,7, Table 5). This site produced an assemblage of 62 assigned fish taxa (species and multispecies assignments). Similar to Hudson River Park, reads of Atlantic menhaden were the most abundant (209,880 reads, 22.9% of site total). American eel reads were next most abundant (119,272 reads, 13% of site total). The site supports species of conservation interest, including the shortnose sturgeon and Atlantic sturgeon. The presence of diadromous species like American eel and striped bass underscores the role of Yonkers as a critical migratory corridor connecting marine and freshwater habitats.

Sportfishing species are also well represented, with detections of red snapper (*Lutjanus campechanus*), largemouth bass (*Micropterus salmoides*), black sea bass (*Centropristis striata*), bluefish, tautog, and summer flounder.

Salinity preferences among the detected species reflect the estuarine nature of the Yonkers site. Tolerant estuarine residents such as mummichog (*Fundulus heteroclitus*), Atlantic silverside, and bay anchovy thrive in variable salinity conditions, while freshwater species like creek chub (*Semotilus atromaculatus*), blacknose dace (*Rhinichthys atratulus*), and common carp (*Cyprinus carpio*) point to freshwater inflows from nearby tributaries.

Many exclusively marine species were detected, notably king mackerel (*Scomberomorus cavalla*) and Acadian redfish (*Sebastes fasciatus*), the only member of *Sebastes* in the Atlantic. Such detections could result from transient occurrences of marine fishes or the influence of tidal mixing on both fish and DNA presence. Some detections likely result from exogenous sources of DNA such as wastewater, runoff, or discards. The detection of Atlantic salmon (*Salmo salar*) may be attributable to this effect, as this species has not been known to occupy the Lower Hudson River system in recent history (Henderson et al., 2023).

Detections at Piermont

Fish detections from samples collected at the Piermont site reflect a mix of ecological and anthropogenic influences (Figures 8,9, Table 6). Atlantic menhaden was the most abundantly read species at this site, with 435,974 reads accounting for 50.8% of all reads from this site. White perch (*Morone americana*) was next most abundant with 123,929 reads, 14.6% of site total. Species such as the flathead grey mullet (*Mugil cephalus*) and rough silverside (*Membras martinica*), both tolerant of brackish conditions, indicate the transitional estuarine habitat at

Piermont. Rainbow trout (*Oncorhynchus mykiss*), a species once widespread in the Lower Hudson but diminished in recent years, was also detected. This detection may reflect local presence, but as this species is also produced and consumed at commercial scale, the possibility of exogenous DNA sources must be considered. Exogenous sources also likely account for the detection of Nile tilapia (*Oreochromis niloticus*), another common foodfish, at this site.

The site's community reflects a wide range of salinity preferences. Estuarine residents such as Atlantic silverside, mummichog, and bay anchovy thrive in the variable salinity conditions of this habitat. Meanwhile, freshwater species like creek chub, pumpkinseed (*Lepomis gibbosus*), and golden shiner (*Notemigonus crysoleucas*) point to freshwater inputs influencing the site. Marine visitors, including Atlantic needlefish (*Strongylura marina*) and Atlantic menhaden, illustrate tidal connectivity with coastal waters.

Several species with significance to sportfishing were also identified, including striped bass, bluefish, largemouth bass, and tautog.

Detections at Norrie Point

The fish detections at Norrie Point are indicative of a site that supports both freshwater and estuarine species (Figures 9,10, Table 7). In contrast with all other sites, reads for pumpkinseed or bluegill (*Lepomis macrochirus*) were the most abundant (257,245 reads, 24.3% of site total) rather than those of Atlantic menhaden (9,074 reads, 0.86% of site total). Reads assigned to common carp were next most abundant (165,371 reads, 15.6% of site total). Key species of conservation interest include both the Atlantic sturgeon and shortnose sturgeon, as well as the diadromous American eel. Several popular targets for anglers were also detected, including striped bass, largemouth bass, and smallmouth bass (*Micropterus dolomieu*), as well as yellow

perch (*Perca flavescens*) and walleye (*Sander vitreus*). These species support both local fisheries and recreational activities, contributing to the site's economic and cultural significance.

As with the three other sites, the mixed salinity preferences of the species detected at Norrie Point reflect the site's estuarine nature. Marine species such as spot and tautog were still detected, though a shift toward freshwater species is evident. Species such as mummichog and golden shiner are well adapted to brackish conditions, while freshwater species like creek chub and fathead minnow (*Pimephales notatus*) point to freshwater inflow from tributaries.

The detection of nonnative species like goldfish (*Carassius auratus*), European rudd (*Scardinius erythrophthalmus*), and pond loach (*Misgurnus* anguillicaudatus) reflect human influence on local biodiversity. Pond loach was first reported in the Lower Hudson system in 2009 in Dwaar Kill (Schmidt & Schmidt, 2014). Dwaar Kill is tributary to the Wallkill River, which empties into the Hudson River via Rondout Creek at Kingston Point, NY, less than 10 km upstream of Norrie Point. It is possible that the detection of pond loach at this site originated in the Wallkill system, as downstream transport of detectable quantities of eDNA over several kilometers has been documented (Deiner & Altermatt, 2014; Villacorta-Rath et al., 2021). Another species of interest/concern, the round goby (*Neogobius melanostomus*), was also detected in one 2023 sample. Detections of the loach and goby highlight the potential use of these methods to aid in the tracking introduced species, especially those small and cryptic, in the Lower Hudson.

Conclusions

All of the samples analyzed here were collected and extracted as part of the Park’s preexisting eDNA program aimed at detecting striped bass, American eel, and Atlantic sturgeon via Go Fish nested PCR methods. The analysis described in this report built upon the collection,

transportation, and laboratory work already coordinated by the Park and partner organizations. By applying metabarcoding to these samples, a broader view of the fish diversity of the Lower Hudson River was revealed.

A total of 84 fish species spanning a variety of habitat preferences and migration strategies were detected, showcasing the value of eDNA metabarcoding for providing a comprehensive snapshot of aquatic biodiversity. Highly abundant reads of Atlantic menhaden and bay anchovy reflect species known to occur in large abundances in the Hudson, but many lower-abundance reads were also noteworthy.

The detections of endangered sturgeons highlight the potential for eDNA as a nonextractive, minimally invasive method for monitoring protected and at-risk species, reducing the need for disruptive survey techniques. Additionally, the detection of pond loach and round goby, two species recently introduced to the Hudson, demonstrates the utility of metabarcoding for tracking the occurrence of rare, nonnative, and potentially invasive species. Unique detections at each site, including species with recreational or ecological importance, further emphasize the value of spatially distributed sampling to capture localized biodiversity.

This study also revealed areas for methodological refinement. Variability in read yields among samples, and the failure of 20 samples to yield sequencing data suggests a need for optimization of PCR protocols and sampling approaches to improve the consistency of data between samples. Increasing water sample volumes or modifying filtration methods may improve read abundance, enabling better detection of rare species and increasing resolution for spatiotemporal comparisons. Future studies incorporating sampling at different depths and proximity to the channel could enhance the detection of species like sturgeons that may prefer these environments.

In conclusion, the application of eDNA metabarcoding has demonstrated its potential to advance fish biodiversity monitoring in the Lower Hudson River, complementing and enhancing existing survey efforts in the system. By leveraging this technology, the Park can gain insights into species assemblages, monitor conservation priorities, and detect early signs of invasive species, ultimately supporting the sustainable management of this vital ecosystem.

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Figures and Tables

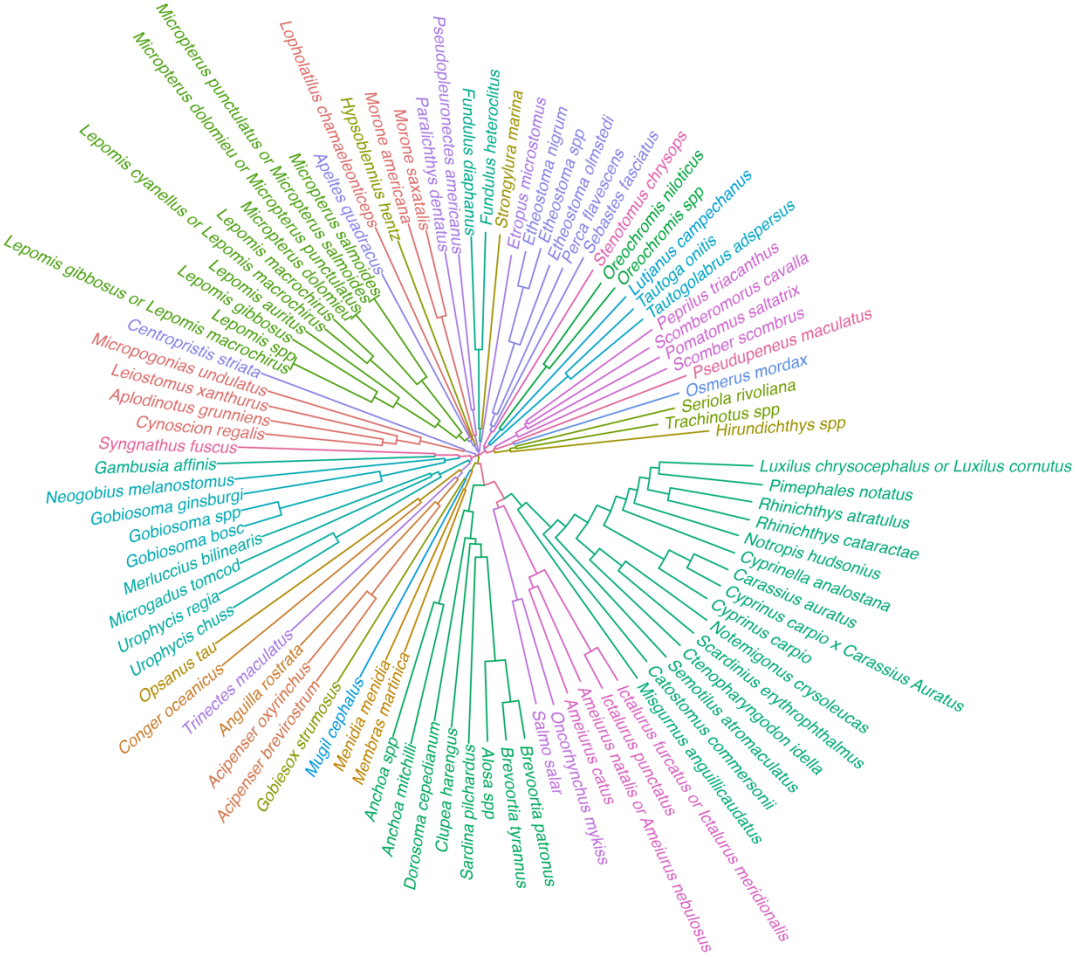

Figure 1 Neighbor-joining tree displaying fish taxa detected by MiFish eDNA metabarcoding of Hudson River water samples. Shorter branch lengths indicate more similar barcode sequences. Color-coded by taxonomic order.

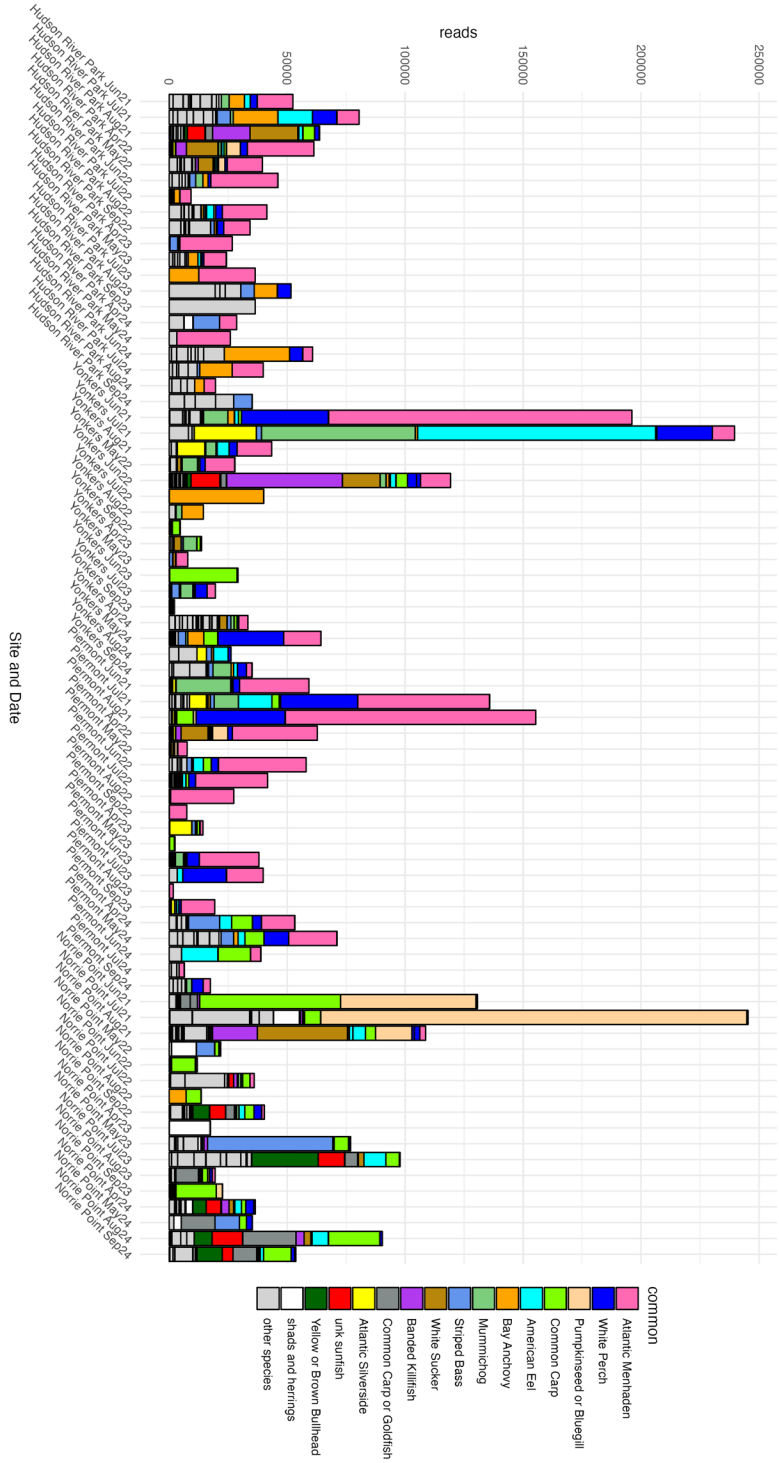

Figure 2 Sequencing read counts for all samples showing high variation in total yield of reads per sample. Only 15 most sequenced taxa are color-coded.

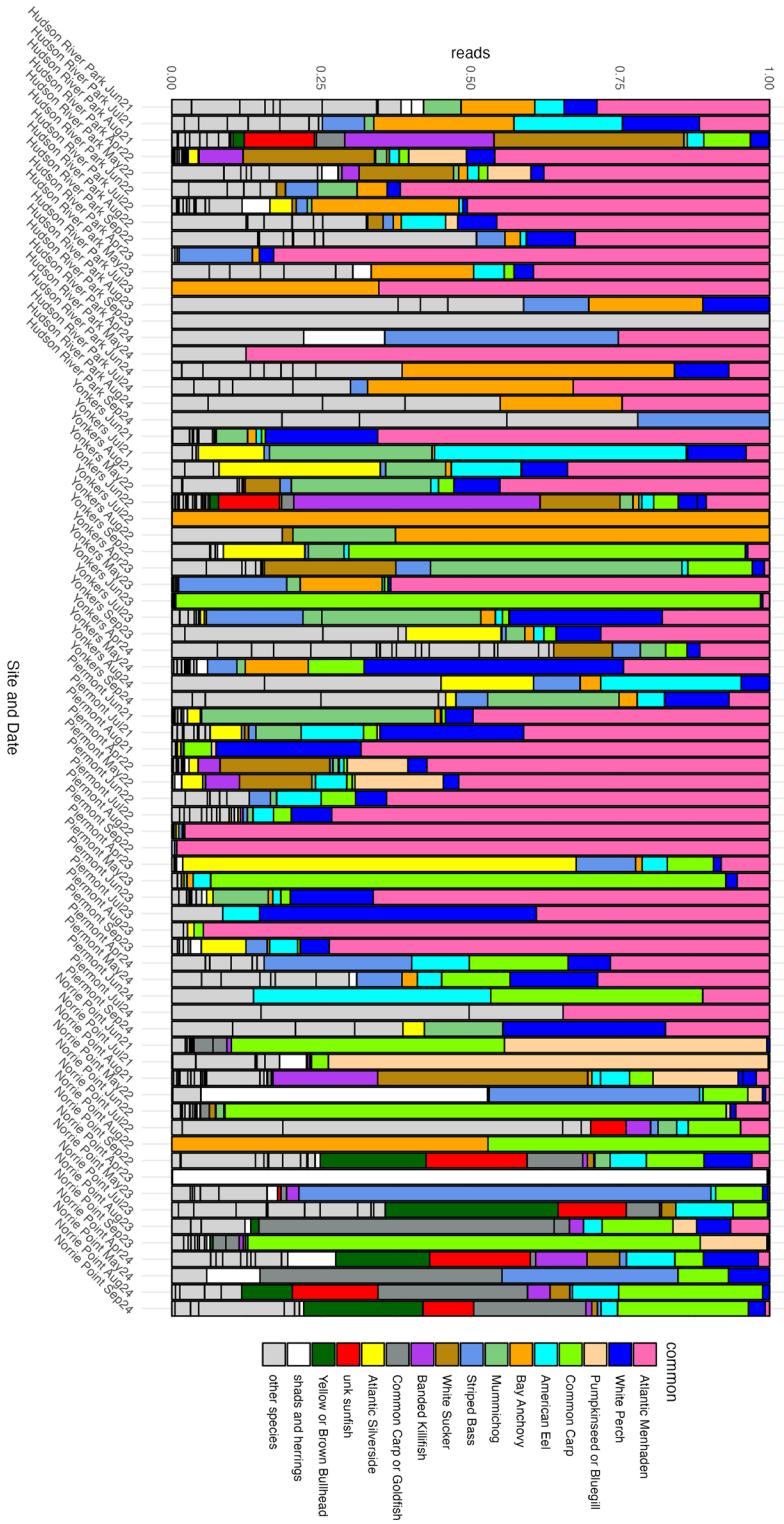

Figure 3 Relative read abundances for assigned species in all samples. Only 15 most sequenced taxa are color-coded.

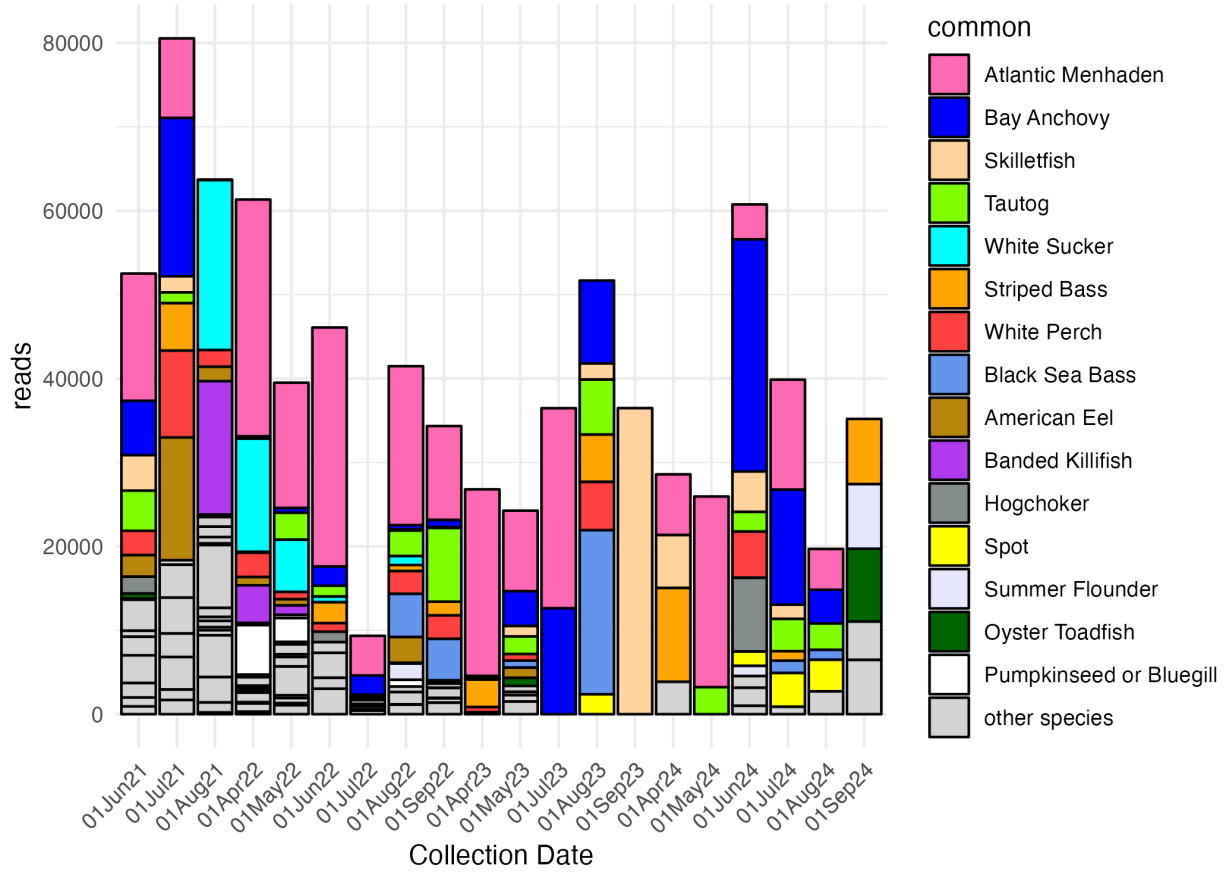

Figure 4 Sequencing read counts for samples collected at Pier 40 in Hudson River Park. 15 most sequenced taxa for this site are color-coded.

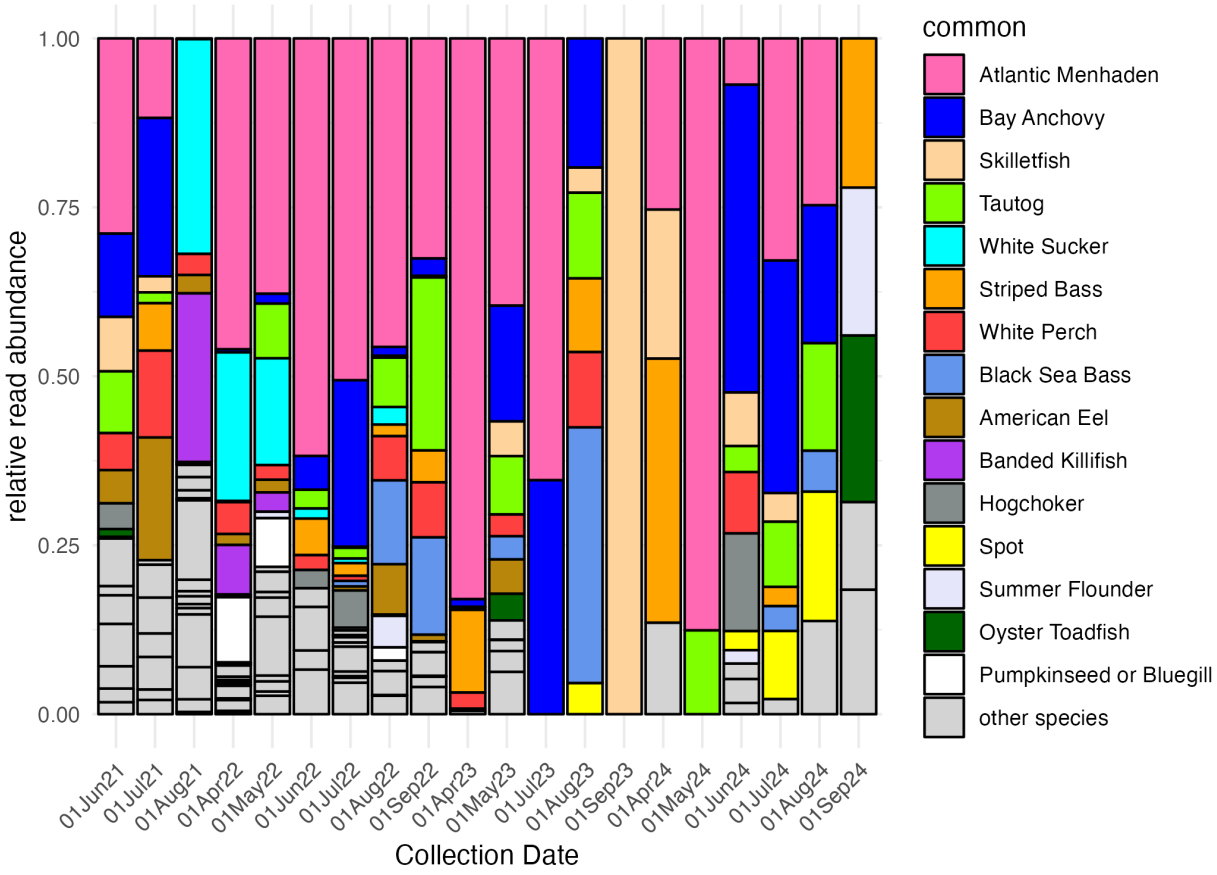

Figure 5 Relative read abundances for samples collected at Pier 40 in Hudson River Park. 15 most sequenced taxa for this site are color-coded.

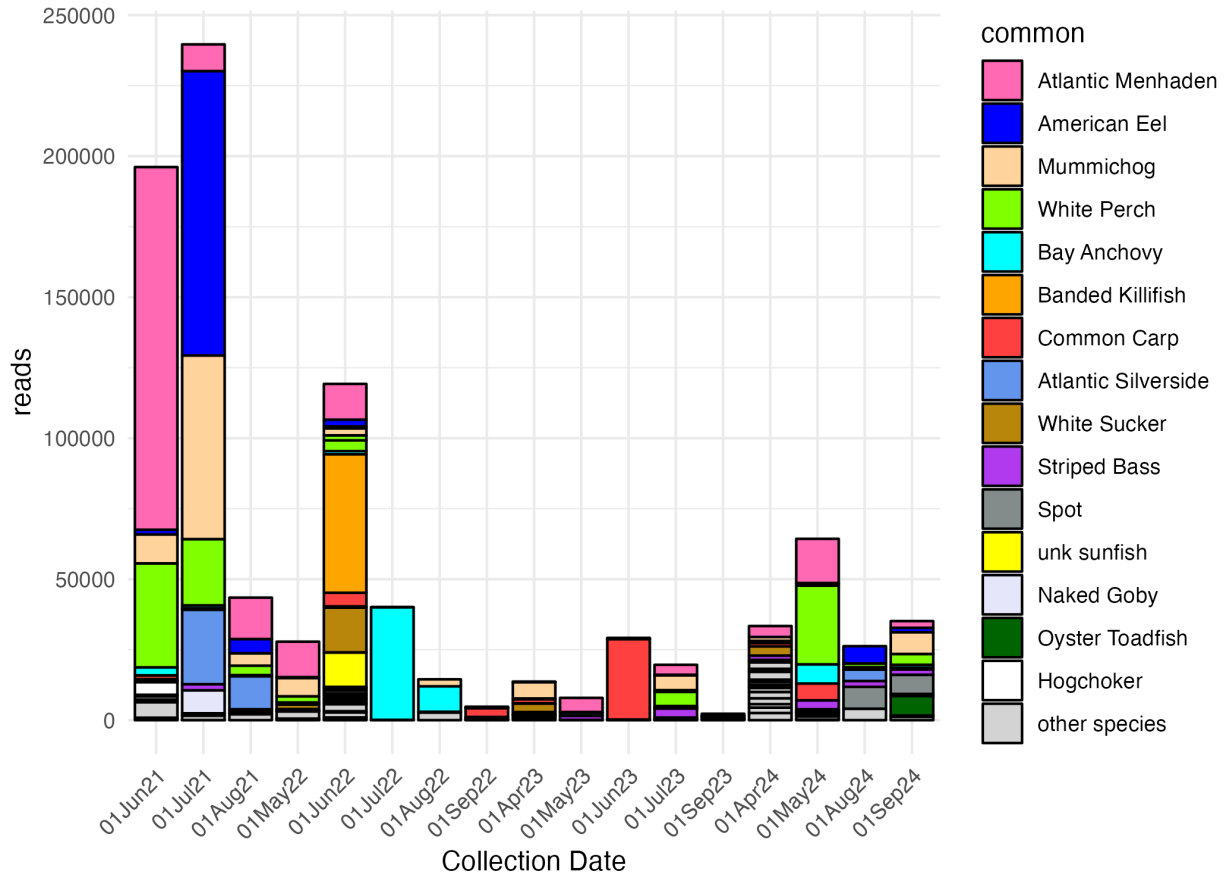

Figure 6 Sequencing read counts for samples collected at Yonkers site. 15 most sequenced taxa for this site are color-coded.

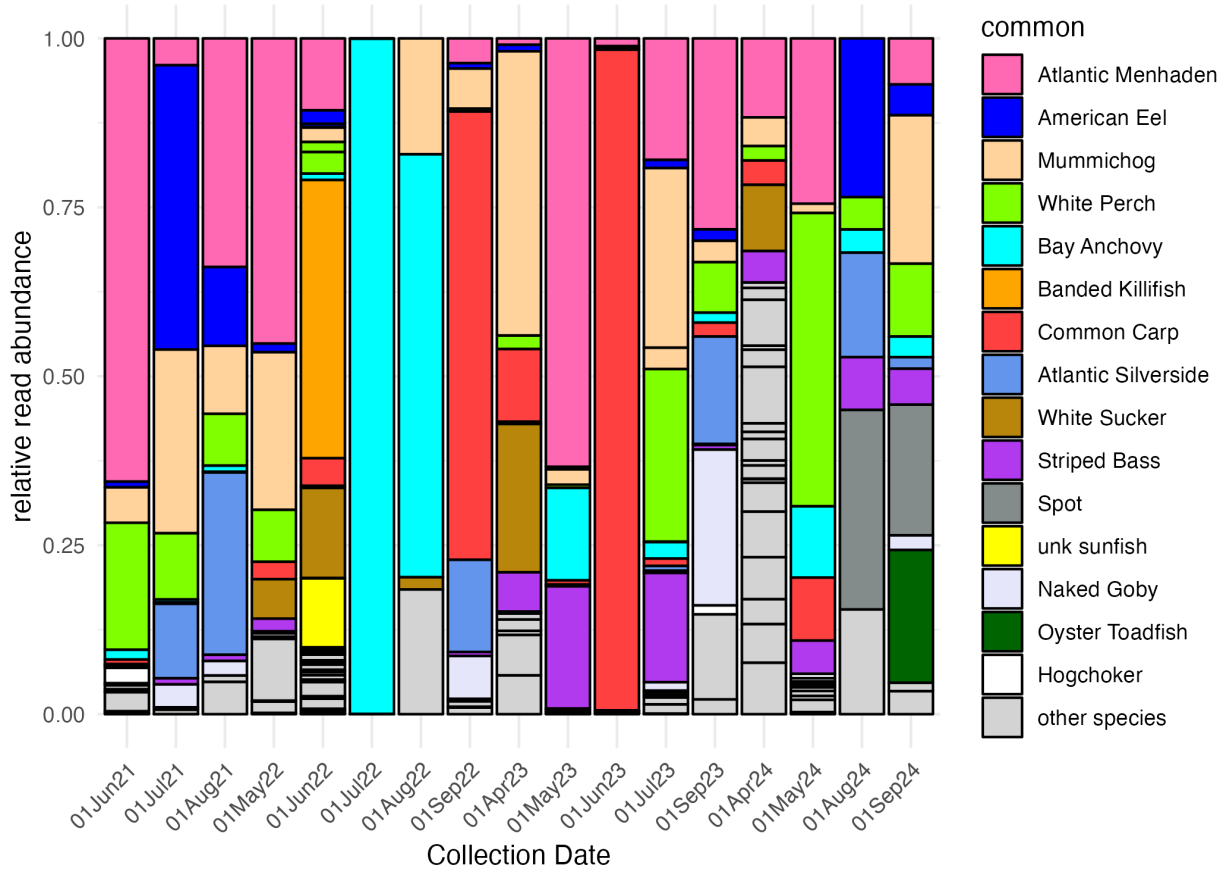

Figure 7 Relative read abundances for samples collected at Yonkers site. 15 most sequenced taxa for this site are color-coded.

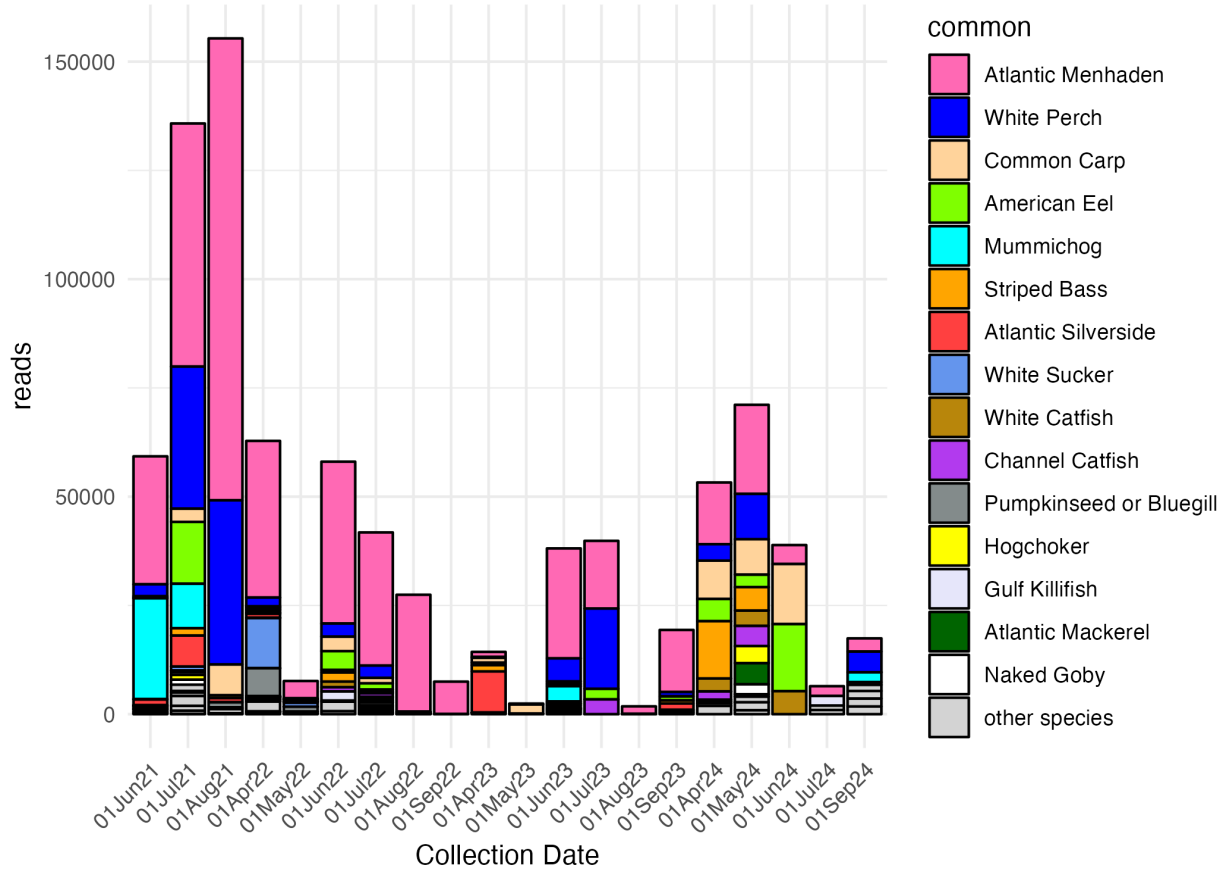

Figure 8 Sequencing read counts for samples collected at Piermont site. 15 most sequenced taxa for this site are color-coded.

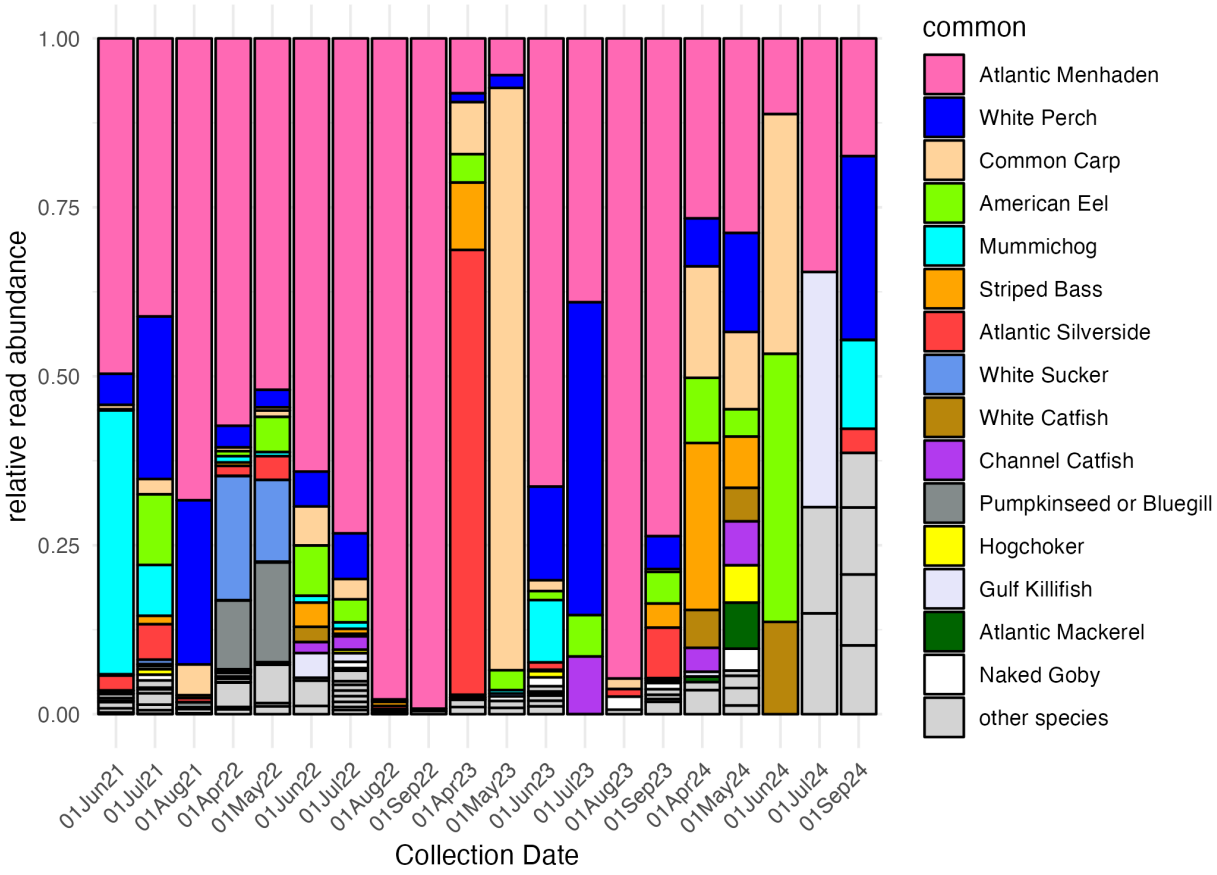

Figure 9 Relative read abundances for samples collected at Piermont site. 15 most sequenced taxa for this site are color-coded.

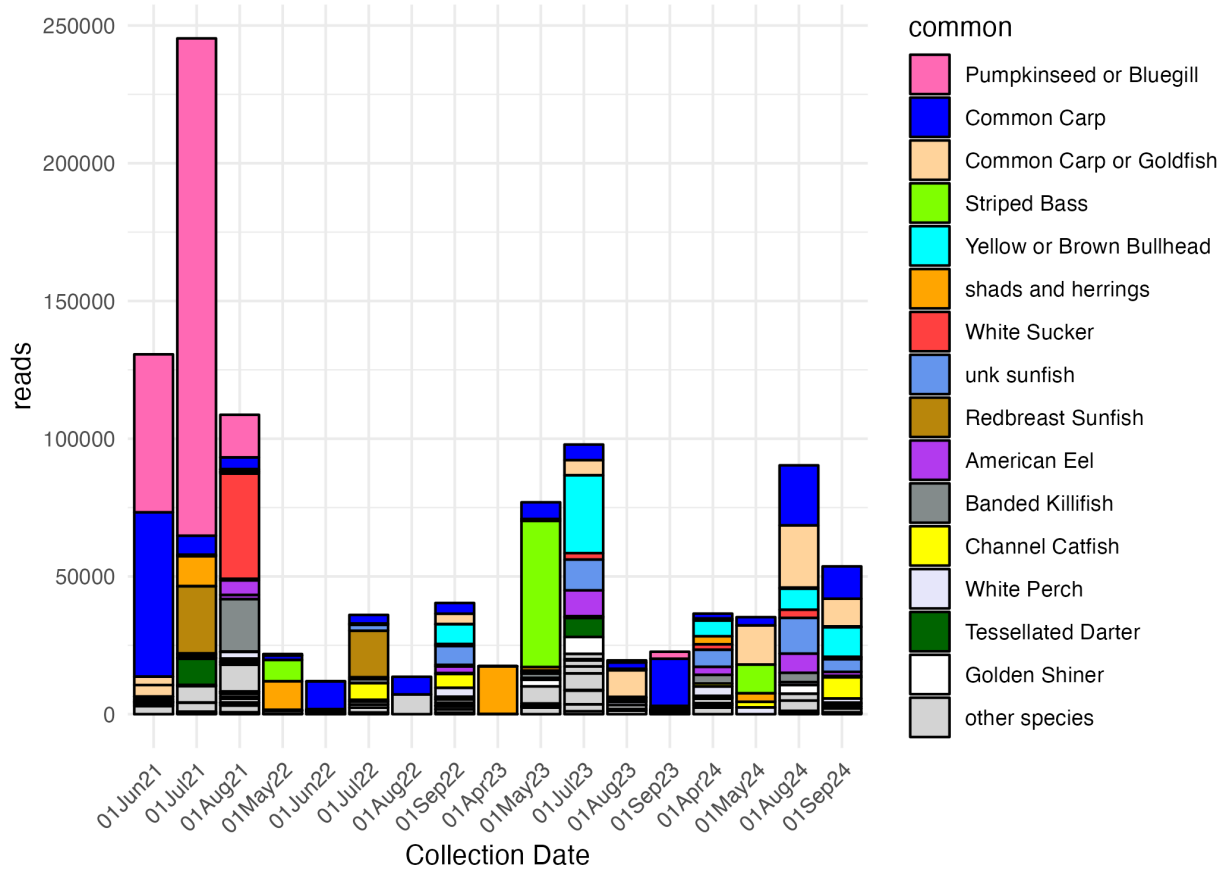

Figure 10 Sequencing read counts for samples collected at Norrie Point. 15 most sequenced taxa for this site are color-coded.

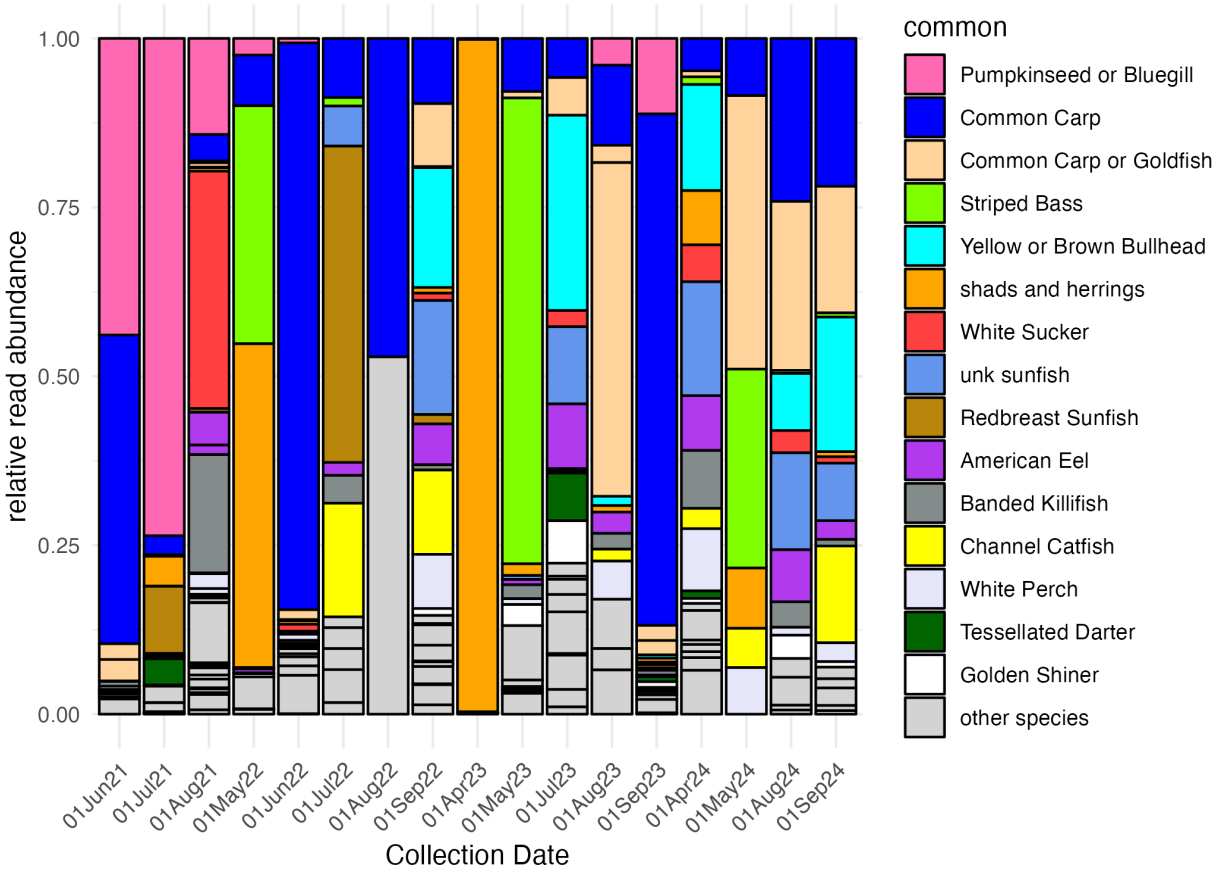

Figure 11 Relative read abundances for samples collected at Norrie Point. 15 most sequenced taxa for this site are color-coded.

Table 1 List of samples producing sequences, date and location of collection, number of sequence variants recovered, and fish taxa detected by Mifish metabarcoding.

Sample ID	Date	Site	Sequences	Reads	Fish Taxa Detected
HRP-001	July, 2021	Hudson River Park	16	80,530	14
HRP-002	April, 2022	Hudson River Park	42	61,326	32
HRP-003	May, 2022	Hudson River Park	41	39,503	18
HRP-004	June, 2022	Hudson River Park	22	46,076	11
HRP-005	July, 2022	Hudson River Park	25	9,338	21
HRP-006	August, 2022	Hudson River Park	25	41,470	17
HRP-007	September, 2022	Hudson River Park	23	34,334	18

Sample ID	Date	Site	Sequences	Reads	Fish Taxa Detected
HRP-008	April, 2023	Hudson River Park	13	26,791	8
HRP-010	July, 2023	Hudson River Park	5	36,463	2
HRP-013	June, 2021	Piermont	47	59,276	17
HRP-014	June, 2021	Yonkers	30	196,139	20
HRP-015	June, 2021	Norrie Point	15	130,632	14
HRP-016	July, 2021	Piermont	29	135,793	20
HRP-017	July, 2021	Yonkers	22	239,639	13
HRP-018	July, 2021	Norrie Point	40	245,323	27
HRP-019	August, 2021	Piermont	21	155,350	11
HRP-020	August, 2021	Yonkers	19	43,459	11
HRP-021	August, 2021	Norrie Point	30	85,344	23
HRP-022	April, 2022	Piermont	44	62,822	33
HRP-023	May, 2022	Norrie Point	19	21,842	13
HRP-024	May, 2022	Piermont	24	7,630	13
HRP-025	May, 2022	Yonkers	25	27,836	14
HRP-026	June, 2022	Norrie Point	26	11,966	23
HRP-027	June, 2022	Yonkers	24	24,125	16
HRP-031	July, 2022	Yonkers	4	40,072	4
HRP-033	August, 2022	Piermont	20	27,456	15
HRP-036	September, 2022	Piermont	4	7,487	3
HRP-037	September, 2022	Yonkers	12	4,767	12
HRP-038	April, 2023	Norrie Point	9	17,451	9
HRP-039	April, 2023	Piermont	15	14,320	12
HRP-040	April, 2023	Yonkers	19	13,692	15
HRP-042	May, 2023	Piermont	10	2,443	9
HRP-043	May, 2023	Yonkers	12	7,909	12
HRP-044	June, 2023	Yonkers	10	29,187	10
HRP-045	June, 2023	Piermont	21	38,107	16
HRP-048	July, 2023	Yonkers	6	671	6

Sample ID	Date	Site	Sequences	Reads	Fish Taxa Detected
HRP-049	July, 2023	Yonkers	19	18,963	16
HRP-050	August, 2023	Norrie Point	14	19,530	12
HRP-051	August, 2023	Piermont	5	1,803	5
HRP-054	September, 2023	Norrie Point	19	22,670	18
HRP-055	September, 2023	Piermont	17	19,366	14
HRP-056	September, 2023	Yonkers	14	2,280	13
HRP-058	May, 2023	Hudson River Park	13	24,255	13
HRP-059	August, 2023	Hudson River Park	7	51,670	7
HRP-060	September, 2023	Hudson River Park	1	36,471	1
HRP-061	June, 2021	Hudson River Park	16	52,506	15
HRP-062	August, 2021	Hudson River Park	22	63,711	22
HRP-063	August, 2021	Norrie Point	12	23,341	12
HRP-064	June, 2022	Yonkers	21	95,118	21
HRP-065	June, 2022	Piermont	12	58,045	12
HRP-066	July, 2022	Norrie Point	12	36,036	12
HRP-067	July, 2022	Piermont	20	41,782	20
HRP-068	August, 2022	Norrie Point	2	13,580	2
HRP-069	August, 2022	Yonkers	4	14,491	4
HRP-070	September, 2022	Norrie Point	23	40,370	23
HRP-071	May, 2023	Norrie Point	15	76,921	15
HRP-072	July, 2023	Norrie Point	19	97,888	19
HRP-073	July, 2023	Piermont	5	39,845	5
HRP-075	April, 2024	Norrie Point	20	36,510	20
HRP-076	April, 2024	Piermont	11	53,264	11
HRP-077	April, 2024	Yonkers	25	33,388	25
HRP-078	April, 2024	Hudson River Park	4	28,582	4
HRP-079	May, 2024	Norrie Point	6	35,225	6
HRP-080	May, 2024	Piermont	14	71,119	14
HRP-081	May, 2024	Yonkers	17	64,308	17

Sample ID	Date	Site	Sequences	Reads	Fish Taxa Detected
HRP-082	May, 2024	Hudson River Park	2	25,938	2
HRP-083	June, 2024	Piermont	4	38,878	4
HRP-085	June, 2024	Hudson River Park	11	60,745	11
HRP-087	July, 2024	Piermont	4	6,438	4
HRP-089	July, 2024	Hudson River Park	8	39,862	8
HRP-090	August, 2024	Norrie Point	14	90,338	14
HRP-092	August, 2024	Yonkers	7	26,247	7
HRP-093	August, 2024	Hudson River Park	6	19,687	6
HRP-094	September, 2024	Norrie Point	17	53,654	17
HRP-095	September, 2024	Piermont	9	17,429	9
HRP-096	September, 2024	Yonkers	12	35,142	12
HRP-097	September, 2024	Hudson River Park	5	35,184	5

Table 2 Detected fish species richness (including nonspecific assignments), total samples analyzed, number of unique sequences detected, and per-sample summary statistics of sequencing reads for all sites

Site	Richness	# samples	# sequences	Total reads	Min reads	Max reads	Mean reads	SD
Hudson River Park	56	20	307	814,442	9,338	80,530	40,722	17,160
Yonkers	62	19	302	917,433	671	239,639	48,286	64,278
Piermont	59	20	336	858,653	1,803	155,350	42,933	41,380
Norrie Point	53	18	312	1,058,621	11,966	245,323	58,812	57,834

Table 3 List of fish species detections by eDNA metabarcoding of Hudson River water samples, 2021-2023. Number of samples producing detections and total counts of reads assigned to each species or group displayed.

Order	Family	Species	Common	Nativity	# samples	Reads
Acanthuriformes	Malacanthidae	*Lopholatilus chamaeleonticeps*	Tilefish	Native	1	2,075
Acanthuriformes	Moronidae	*Morone americana*	White Perch	Native	62	292,079
Acanthuriformes	Moronidae	*Morone saxatalis*	Striped Bass	Native	46	155,831
Acanthuriformes	Sciaenidae	*Aplodinotus grunniens*	Freshwater drum	Native	5	4,812
Acanthuriformes	Sciaenidae	*Cynoscion regalis*	Weakfish	Native	3	5,088
Acanthuriformes	Sciaenidae	*Leiostomus xanthurus*	Spot	Native	12	29,974
Acanthuriformes	Sciaenidae	*Micropogonias undulatus*	Atlantic Croaker	Native	1	1,775
Acipenseriformes	Acipenseridae	*Acipenser brevirostrum*	Shortnose Sturgeon	Native	4	891
Acipenseriformes	Acipenseridae	*Acipenser oxyrinchus*	Atlantic Sturgeon	Native	5	2,725
Anguilliformes	Anguillidae	*Anguilla rostrata*	American Eel	Native	54	226,512
Anguilliformes	Congridae	*Conger oceanicus*	American Conger	Native	1	6,483
Atheriniformes	Atherinopsidae	*Membras martinica*	Rough Silverside	Native	3	1,316
Atheriniformes	Atherinopsidae	*Menidia menidia*	Atlantic Silverside	Native	26	68,710
Batrachoidiformes	Batrachoididae	*Opsanus tau*	Oyster Toadfish	Native	7	17,392
Beloniformes	Belonidae	*Strongylura marina*	Atlantic Needlefish	Native	2	207
Beloniformes	Exocoetidae	*Hirundichthys spp.*	unk flying fish	Undetermined	1	90
Blenniiformes	Blenniidae	*Hypsoblennius hentz*	Feather Blenny	Native	6	4,042
Blenniiformes	Gobiesocidae	*Gobiesox strumosus*	Skilletfish	Native	12	58,811
Carangiformes	Carangidae	*Seriola rivoliana*	Longfin rivoliana	Native	1	1,405
Carangiformes	Carangidae	*Trachinotus spp.*	Pompano	Native	1	264
Centrarchiformes	Centrarchidae	*Lepomis auritus*	Redbreast Sunfish	Native	20	47,908

Order	Family	Species	Common	Nativity	# samples	Reads
Centrarchi-formes	Centrarchi-dae	*Lepomis cyanellus or Lepomis macro-chirus*	Bluegill or Green Sunfish	Nonnative	14	12,391
Centrarchi-formes	Centrarchi-dae	*Lepomis gibbosus*	Pumpkinseed	Native	10	11,840
Centrarchi-formes	Centrarchi-dae	*Lepomis gibbosus or Lepomis macro-chirus*	Pumpkinseed or Bluegill	Undeter-mined	18	276,193
Centrarchi-formes	Centrarchi-dae	*Lepomis macro-chirus*	Bluegill	Nonnative	14	6,619
Centrarchi-formes	Centrarchi-dae	*Lepomis spp.*	unk sunfish	Undeter-mined	10	63,922
Centrarchi-formes	Centrarchi-dae	*Micropterus dolo-mieu*	Smallmouth Bass	Nonnative	7	646
Centrarchi-formes	Centrarchi-dae	*Micropterus dolo-mieu or Micropterus punctulatus*	Smallmouth or Spotted Bass	Nonnative	6	12,930
Centrarchi-formes	Centrarchi-dae	*Micropterus punctu-latus or Micropterus salmoides*	Spotted or Large-mouth Bass	Nonnative	8	9,899
Centrarchi-formes	Centrarchi-dae	*Micropterus salmoides*	Largemouth Bass	Nonnative	7	806
Cichliformes	Cichlidae	*Oreochromis nilot-icus*	Nile Tilapia	Nonnative	2	36
Cichliformes	Cichlidae	*Oreochromis spp.*	unk Tilapia	Nonnative	2	730
Clupeiformes	Clupeidae	*Alosa spp.*	shads and herrings	Native	33	62,010
Clupeiformes	Clupeidae	*Brevoortia patronus*	Gulf Killifish	Nonnative	6	6,044
Clupeiformes	Clupeidae	*Brevoortia tyrannus*	Atlantic Menha-den	Native	66	893,780
Clupeiformes	Clupeidae	*Clupea harengus*	Atlantic Herring	Native	1	1,011
Clupeiformes	Clupeidae	*Dorosoma cepedi-anum*	Gizzard Shad	Nonnative	5	4,733
Clupeiformes	Clupeidae	*Sardina pilchardus*	European Pilchard	Nonnative	1	1,728
Clupeiformes	Engraulidae	*Anchoa mitchilli*	Bay Anchovy	Native	38	180,792
Clupeiformes	Engraulidae	*Anchoa spp.*	unk anchovy	Native	1	50

Order	Family	Species	Common	Nativity	# samples	Reads
Cypriniformes	Catostomidae	*Catostomus commersonii*	White Sucker	Native	31	126,415
Cypriniformes	Cobitidae	*Misgurnus anguillicaudatus*	Pond Loach	Nonnative	1	34
Cypriniformes	Cyprinidae	*Carassius auratus*	Goldfish	Nonnative	7	4,703
Cypriniformes	Cyprinidae	*Cyprinus carpio*	Common Carp	Nonnative	50	270,739
Cypriniformes	Cyprinidae	*Cyprinus carpio x Carassius Auratus*	Common Carp or Goldfish	Nonnative	20	83,872
Cypriniformes	Leuciscidae	*Cyprinella analostana*	Satinfin Shiner	Native	2	1,392
Cypriniformes	Leuciscidae	*Luxilus chrysocephalus or Luxilus cornutus*	Striped or Common Shiner	Native	1	391
Cypriniformes	Leuciscidae	*Notemigonus crysoleucas*	Golden Shiner	Native	14	15,249
Cypriniformes	Leuciscidae	*Notropis hudsonius*	Spottail Shiner	Native	10	8,637
Cypriniformes	Leuciscidae	*Pimephales notatus*	Bluntnose Minnow	Nonnative	5	6,453
Cypriniformes	Leuciscidae	*Rhinichthys atratulus*	Eastern Blacknose Dace	Native	2	504
Cypriniformes	Leuciscidae	*Rhinichthys cataractae*	Longnose Dace	Native	6	3,266
Cypriniformes	Leuciscidae	*Scardinius erythrophthalmus*	Rudd	Nonnative	13	3,583
Cypriniformes	Leuciscidae	*Semotilus atromaculatus*	Creek Chub	Native	2	375
Cypriniformes	Xenocyprididae	*Ctenopharyngodon idella*	Grass Carp	Nonnative	4	2,146
Cyprinodontiformes	Fundulidae	*Fundulus diaphanus*	Banded Killifish	Native	21	105,937
Cyprinodontiformes	Fundulidae	*Fundulus heteroclitus*	Mummichog	Native	38	166,313
Cyprinodontiformes	Poeciliidae	*Gambusia affinis*	Western Mosquitofish	Nonnative	1	316
Gadiformes	Gadidae	*Microgadus tomcod*	Atlantic Tomcod	Native	13	4,850
Gadiformes	Phycidae	*Merluccius bilinearis*	Silver Hake	Native	2	2,141

Order	Family	Species	Common	Nativity	# samples	Reads
Gadiformes	Phycidae	*Urophycis chuss*	Red Hake	Native	1	302
Gadiformes	Phycidae	*Urophycis regia*	Spotted Hake	Native	3	802
Gobiiformes	Gobiidae	*Gobiosoma bosc*	Naked Goby	Native	23	20,761
Gobiiformes	Gobiidae	*Gobiosoma ginsburgi*	Seaboard Goby	Native	5	7,730
Gobiiformes	Gobiidae	*Gobiosoma spp.*	unk goby	Native	1	135
Gobiiformes	Gobiidae	*Neogobius melanostomus*	Round Goby	Nonnative	1	237
Labriformes	Labridae	*Tautoga onitis*	Tautog	Native	18	45,907
Labriformes	Labridae	*Tautogolabrus adspersus*	Cunner	Native	7	1,052
Lutjaniformes	Lutjanidae	*Lutjanus campechanus*	Red Snapper	Native	1	2,252
Mugiliformes	Mugilidae	*Mugil cephalus*	Flathead Grey Mullet	Native	2	476
Osmeriformes	Osmeridae	*Osmerus mordax*	Rainbow Smelt	Native	1	6,182
Perciformes	Gasterosteidae	*Apeltes quadracus*	Fourspine Stickleback	Native	1	2,374
Perciformes	Percidae	*Etheostoma nigrum*	Johnny Darter	Nonnative	2	2,563
Perciformes	Percidae	*Etheostoma olmstedi*	Tessellated Darter	Native	15	20,027
Perciformes	Percidae	*Etheostoma spp.*	unk darter	Native	1	16
Perciformes	Percidae	*Perca flavescens*	Yellow perch	Native	4	569
Perciformes	Sebastidae	*Sebastes fasciatus*	Acadian Redfish	Native	1	2,268
Perciformes	Serranidae	*Centropristis striata*	Black Sea Bass	Native	9	33,989
Pleuronectiformes	Achiridae	*Trinectes maculatus*	Hogchoker	Native	20	24,493
Pleuronectiformes	Cyclopsettidae	*Etropus microstomus*	Smallmouth Flounder	Native	1	1,696
Pleuronectiformes	Paralichthyidae	*Paralichthys dentatus*	Summer Flounder	Native	17	16,798
Pleuronectiformes	Pleuronectidae	*Pseudopleuronectes americanus*	Winter Flounder	Native	6	7,256
Salmoniformes	Salmonidae	*Oncorhynchus mykiss*	Rainbow Trout (Steelhead)	Nonnative	1	17

Order	Family	Species	Common	Nativity	# samples	Reads
Salmoniformes	Salmonidae	*Salmo salar*	Atlantic Salmon	Nonnative	13	20,871
Scombriformes	Pomatomi-dae	*Pomatomus saltatrix*	Bluefish	Native	18	11,671
Scombriformes	Scombridae	*Scomber scombrus*	Atlantic Mackerel	Native	3	5,483
Scombriformes	Scombridae	*Scomberomorus ca-valla*	King Mackerel	Native	1	121
Scombriformes	Stro-mateidae	*Peprilus triacanthus*	American Butter-fish	Native	3	2,869
Siluriformes	Ictaluridae	*Ameiurus catus*	White Catfish	Native	16	21,440
Siluriformes	Ictaluridae	*Ameiurus natalis or Ameiurus nebulosus*	Yellow or Brown Bullhead	Native	14	63,023
Siluriformes	Ictaluridae	*Ictalurus furcatus or Ictalurus merid-ionalis*	Blue or Southern Blue Catfish	Nonnative	1	1,909
Siluriformes	Ictaluridae	*Ictalurus punctatus*	Channel Catfish	Nonnative	26	39,233
Spariformes	Sparidae	*Stenotomus chrysops*	Scup	Native	6	2,398
Syngnathiformes	Mullidae	*Pseudupeneus macu-latus*	Spotted Goatfish	Native	1	349
Syngnathiformes	Syngnathi-dae	*Syngnathus fuscus*	Northern Pipefish	Native	1	14

Table 4a Metabarcoding read totals for assigned fish taxa detected at Pier 40 in Hudson River Park from June, 2021 to September 2022.

Species	Common	Jun21	Jul21	Aug21	Apr22	May22	Jun22	Jul22	Aug22	Sep22
Acipenser oxyrinchus	Atlantic Sturgeon				11					
Alosa spp.	shads and herrings	1,993		213	131	1,077		435		
Ameiurus catus	White Catfish				51					
Ameiurus natalis or Ameiurus nebulosus	Yellow or Brown Bullhead			1,194						
Anchoa mitchilli	Bay Anchovy	6,481	18,881		287	582	2,307	2,302	547	888
Anguilla rostrata	American Eel	2,584	14,630	1,738	1,003	746		57	3,093	344

Species	Common	Jun21	Jul21	Aug21	Apr22	May22	Jun22	Jul22		Aug22	Sep22
Brevoortia tyrannus	Atlantic Menhaden	15,160	9,470	75	28,207	14,921		28,467	4,723	18,932	11,175
Catostomus commersonii	White Sucker			20,244	13,488	6,244		694	65	1,079	
Centropristis striata	Black Sea Bass								76	5,147	4,945
Cynoscion regalis	Weakfish	1,735									
Cyprinus carpio	Common Carp			4,977	981	593					
Cyprinus carpio x Carassius Auratus	Common Carp or Goldfish			3,028	111	250					
Dorosoma cepedianum	Gizzard Shad				24						
Etheostoma olmstedi	Tessellated Darter			596	129						
Etropus microstomus	Smallmouth Flounder		1,696								
Fundulus diaphanus	Banded Killifish			15,897	4,495	1,128				80	
Fundulus heteroclitus	Mummichog	3,289	1,252	372	1,142	339		3,040	69		
Gobiesox strumosus	Skilletfish	4,229	1,915						14	111	84
Gobiosoma bosc	Naked Goby		3,875		65						
Gobiosoma ginsburgi	Seaboard Goby	2,224									
Hypsoblennius hentz	Feather Blenny				10				22	1,131	1,384
Ictalurus punctatus	Channel Catfish				40						
Leiostomus xanthurus	Spot			94							
Lepomis auritus	Redbreast Sunfish			744	220						
Lepomis cyanellus or Lepomis macrochirus	Bluegill or Green Sunfish			476	200						
Lepomis gibbosus	Pumpkinseed					3,442		1,305	64		

Species	Common	Jun21	Jul21	Aug21	Apr22	May22	Jun22	Jul22		Aug22	Sep22
Lepomis gibbosus or Lepomis macrochirus	Pumpkinseed or Bluegill				5,928	2,855				821	6
Lepomis macrochirus	Bluegill			1,078	293	1,116					
Lepomis spp.	unk sunfish			7,494							
Menidia menidia	Atlantic Silverside			190	1,012				344		
Merluccius bilinearis	Silver Hake	715									
Microgadus tomcod	Atlantic Tomcod							2,972	57	13	518
Micropterus dolomieu	Smallmouth Bass				92						3
Micropterus dolomieu or Micropterus punctulatus	Smallmouth or Spotted Bass			753							
Micropterus punctulatus or Micropterus salmoides	Spotted or Largemouth Bass			1,244							
Micropterus salmoides	Largemouth Bass				36						
Morone americana	White Perch	2,884	10,351	1,980	2,894	854		1,022	72	2,716	2,797
Morone saxatalis	Striped Bass		5,659		92			2,483	173	704	1,619
Notemigonus crysoleucas	Golden Shiner			1,147	35						
Notropis hudsonius	Spottail Shiner			39							
Opsanus tau	Oyster Toadfish	609									13
Paralichthys dentatus	Summer Flounder				153	368			40	1,926	
Peprilus triacanthus	American Butterfish		2,804								48
Pimephales notatus	Bluntnose Minnow					329					
Pomatomus saltatrix	Bluefish							1,276	74	34	1,204

Species	Common	Jun21	Jul21	Aug21	Apr22	May22	Jun22	Jul22		Aug22	Sep22
Pseudopleuronectes americanus	Winter Flounder		4,269			1,184			25	1,471	
Salmo salar	Atlantic Salmon	3,697	3,923		58				4		
Scardinius erythrophthalmus	Rudd			138	20						2
Stenotomus chrysops	Scup									636	500
Syngnathus fuscus	Northern Pipefish				14						
Tautoga onitis	Tautog	4,787	1,274			3,194		1,271	147	3,029	8,783
Tautogolabrus adspersus	Cunner	122	531		21	281			62		21
Trinectes maculatus	Hogchoker	1,997			83			1,239	513		

Table 5b Metabarcoding read totals for assigned fish taxa detected at Pier 40 in Hudson River Park from April 2023 to September 2024.

Species	Common	Apr23	May23	Jul23	Aug23	Sep23	Apr24	May24	Jun24	Jul24	Aug24	Sep24
Alosa spp.	shads and herrings		746				3,871					
Ameiurus catus	White Catfish		1,520									
Anchoa mitchilli	Bay Anchovy	314	4,159	12,629	9,878				27,673	13,719	4,020	
Anguilla rostrata	American Eel		1,237									
Brevoortia tyrannus	Atlantic Menhaden	22,227	9,588	23,834			7,240	22,722	4,154	13,098	4,859	
Centropristis striata	Black Sea Bass		830		19,560					1,474	1,196	
Clupea harengus	Atlantic								1,011			

Species	Common	Apr23	May23	Jul23	Aug23	Sep23	Apr24	May24	Jun24	Jul24	Aug24	Sep24
	Herring											
Conger oceanicus	American Conger											6,483
Cynoscion regalis	Weakfish								2,153			
Cyprinus carpio	Common Carp		399									
Gobiesox strumosus	Skilletfish	5	1,245		1,923	36,471	6,305		4,816	1,693		
Gobiosoma ginsburgi	Seaboard Goby									896		4,564
Hypsoblennius hentz	Feather Blenny								1,390			
Leiostomus xanthurus	Spot				2,373				1,722	4,010	3,768	
Lepomis auritus	Redbreast Sunfish	134										
Morone americana	White Perch	645	784		5,764				5,519			
Morone saxatalis	Striped Bass	3,279			5,630		11,166			1,131		7,769
Opsanus tau	Oyster Toadfish		956									8,672
Paralichthys dentatus	Summer Flounder								1,201			7,696
Pomatomus saltatrix	Bluefish										2,714	
Tautoga onitis	Tautog	104	2,091		6,542			3,216	2,332	3,841	3,130	

Species	Common	Apr23	May23	Jul23	Aug23	Sep23	Apr24	May24	Jun24	Jul24	Aug24	Sep24
Tautogolabrus adspersus	Cunner		14									
Trinectes maculatus	Hogchoker								8,774			
Urophycis regia	Spotted Hake	83	686									

Table 6a Metabarcoding read totals for assigned fish taxa detected at Yonkers from June, 2021 to September 2022.

Species	Common	Jun21	Jul21	Aug21	May22	Jun22	Jul22	Aug22	Sep22
Acipenser brevirostrum	Shortnose Sturgeon	268							
Acipenser oxyrinchus	Atlantic Sturgeon					126			
Alosa spp.	shads and herrings	604			54	812			47
Ameiurus catus	White Catfish	5,503	32			27			
Ameiurus natalis or Ameiurus nebulosus	Yellow or Brown Bullhead					1,763			
Anchoa mitchilli	Bay Anchovy	2,871	1,050	403		1,138	40,051	9,072	
Anguilla rostrata	American Eel	1,700	100,868	5,079	361	2,985			39
Aplodinotus grunniens	Freshwater drum					456			
Brevoortia tyrannus	Atlantic Menhaden	128,608	9,476	14,695	12,566	12,673	16		174
Catostomus commersonii	White Sucker	279			1,627	15,954		262	
Centropristis striata	Black Sea Bass	147							

Species	Common	Jun21	Jul21	Aug21	May22	Jun 22	Jul2 2	Aug22	Sep 22
Cyprinus carpio	Common Carp	1,294	541	45	718	4,8 78			3,1 63
Cyprinus carpio x Carassius Auratus	Common Carp or Goldfish					2,4 77			
Etheostoma olmstedi	Tessel- lated Darter	751				145			
Fundulus diaphanus	Banded Killifish					49, 081			
Fundulus hetero- clitus	Mummi- chog	10,27 5	65,141	4,367	6,492	2,6 27		2,483	283
Gobiosoma bosc	Naked Goby	482	8,157	945		422			303
Ictalurus punctatus	Channel Catfish					51			
Leiostomus xanthu- rus	Spot					3			
Lepomis auritus	Red- breast Sunfish	1,136			466	252			
Lepomis cyanellus or Lepomis macro- chirus	Bluegill or Green Sunfish				40	780			
Lepomis gibbosus	Pump- kinseed				2,543			2,674	
Lepomis gibbosus or Lepomis macro- chirus	Pump- kinseed or Blue- gill		5						
Lepomis macro- chirus	Bluegill					533			
Lepomis spp.	unk sun- fish				90	12, 150			
Menidia menidia	Atlantic Silver- side		26,394	11,722		384			650
Microgadus tomcod	Atlantic Tomcod	69			88	259			
Micropterus dolo- mieu	Small- mouth Bass					168			

Species	Common	Jun21	Jul21	Aug21	May22	Jun 22	Jul2 2	Aug22	Sep 22
Micropterus dolomieu or Micropterus punctulatus	Small-mouth or Spotted Bass					911			
Micropterus punctulatus or Micropterus salmoides	Spotted or Large-mouth Bass					407			
Morone americana	White Perch	36,827	23,438	3,328	2,136	5,561			19
Morone saxatalis	Striped Bass	361	2,134	391	519				28
Notemigonus crysoleucas	Golden Shiner					343			
Paralichthys dentatus	Summer Flounder			2,083					6
Pimephales notatus	Blunt-nose Minnow				136				
Pomatomus saltatrix	Bluefish			401		1,012			38
Pseudopleuronectes americanus	Winter Flounder					305	2		
Rhinichthys cataractae	Longnose Dace	30							
Salmo salar	Atlantic Salmon	465	1,563						
Scardinius erythrophthalmus	Rudd					352			
Semotilus atromaculatus	Creek Chub	34							
Tautoga onitis	Tautog								17
Trinectes maculatus	Hog-choker	4,435	840			208	3		

Table 7b Metabarcoding read totals for assigned fish taxa detected at Yonkers from April 2023 to September 2024.

Species	Common	Apr23	May23	Jun23	Jul23	Sep23	Apr24	May24	Aug24	Sep24
Acipenser oxyrinchus	Atlantic Sturgeon							200		

Species	Common	Apr23	May23	Jun23	Jul23	Sep23	Apr24	May24	Aug24	Sep24
Alosa spp.	shads and herrings							1,165		
Ameiurus catus	White Catfish							375		
Anchoa mitchilli	Bay Anchovy		1,084	14	478	34		6,796	905	1,079
Anchoa spp.	unk anchovy					50				
Anguilla rostrata	American Eel	135	31	28	236	39			6,164	1,607
Aplodinotus grunniens	Freshwater drum						2,546			
Brevoortia patronus	Gulf Killifish							471		
Brevoortia tyrannus	Atlantic Menhaden	127	5,013	332	3,532	644	3,903	15,730		2,391
Catostomus commersonii	White Sucker	3,008	23		68	4	3,280			
Cynoscion regalis	Weakfish									1,200
Cyprinus carpio	Common Carp	1,476	47	28,542	215	47	1,200	5,992		
Etheostoma olmstedi	Tessellated Darter	788	8	46						
Fundulus heteroclitus	Mummichog	5,759	179		5,839	72	1,417	887		7,717
Gobiosoma bosc	Naked Goby		13	38	250	526				769
Ictalurus furcatus or Ictalurus meridionalis	Blue or Southern Blue Catfish						1,909			
Ictalurus punctatus	Channel Catfish				4			341	4,068	
Leiostomus xanthurus	Spot								7,753	6,794
Lepomis auritus	Redbreast Sunfish	819	24	11			1,229			
Lopholatilus chamaeleonticeps	Tilefish						2,075			

Species	Common	Apr23	May23	Jun23	Jul23	Sep23	Apr24	May24	Aug24	Sep24
Lutjanus campechanus	Red Snapper						2,252			
Menidia menidia	Atlantic Silverside	42		7	135	362			4,058	598
Merluccius bilinearis	Silver Hake						1,426			
Microgadus tomcod	Atlantic Tomcod						175	138		
Micropterus salmoides	Largemouth Bass				283					
Morone americana	White Perch	271	36	113	5,028	170	708	27,898	1,254	3,784
Morone saxatalis	Striped Bass	796	1,429		3,172	15	1,555	3,166	2,045	1,866
Opsanus tau	Oyster Toadfish									6,905
Oreochromis niloticus	Nile Tilapia						27			
Oreochromis spp.	unk Tilapia				78		652			
Paralichthys dentatus	Summer Flounder						247	141		432
Pomatomus saltatrix	Bluefish				199	287	1,064			
Pseudupeneus maculatus	Spotted Goatfish						349			
Rhinichthys atratulus	Eastern Blacknose Dace	82					422			
Rhinichthys cataractae	Longnose Dace	230	22		67		2,791	126		
Salmo salar	Atlantic Salmon						846	169		
Scomber scombrus	Atlantic Mackerel						199			
Scomberomorus cavalla	King Mackerel	121								
Sebastes fasciatus	Acadian Redfish						2,268			
Stenotomus chrysops	Scup	5			50		584			

Species	Common	Apr23	May23	Jun23	Jul23	Sep23	Apr24	May24	Aug24	Sep24
Trachinotus spp.	Pompano						264			
Trinectes maculatus	Hogchoker	33		56		30		411		
Urophycis chuss	Red Hake							302		

Table 8a Metabarcoding read totals for assigned fish taxa detected at Piermont from June, 2021 to September 2022.

Species	Common	Jun21	Jul21	Aug21	Apr22	May22	Jun22	Jul22	Aug22	Sep22
Acipenser brevirostrum	Shortnose Sturgeon	154								
Alosa spp.	shads and herrings	362			455	89		255		
Ameiurus catus	White Catfish	13	34		46		1,318			
Anchoa mitchilli	Bay Anchovy	550		225	96					
Anguilla rostrata	American Eel	113	14,218		472	399	4,329	1,430	33	
Brevoortia patronus	Gulf Killifish						2,133	545		
Brevoortia tyrannus	Atlantic Menhaden	29,422	55,888	106,186	36,014	3,968	37,207	30,604	26,858	7,427
Carassius auratus	Goldfish		94							
Catostomus commersonii	White Sucker		911		11,544	925				
Cyprinus carpio	Common Carp	385	3,065	7,101	357	106	3,347	1,256		
Cyprinus carpio x Carassius Auratus	Common Carp or Goldfish		721		38	37				
Dorosoma cepedianum	Gizzard Shad		1,107	964						
Etheostoma olmstedi	Tessellated Darter			147	67					

Species	Common	Jun21	Jul21	Aug21	Apr22	May22	Jun22	Jul22	Aug22	Sep22
Etheostoma spp.	unk darter				16					
Fundulus diaphanus	Banded Killifish				2,285	432		192		
Fundulus heteroclitus	Mummichog	23,145	10,224		584	46	587	398	22	
Gambusia affinis	Western Mosquitofish							316		
Gobiosoma bosc	Naked Goby		1,167		20		248	388	39	
Gobiosoma ginsburgi	Seaboard Goby				6					
Hypsoblennius hentz	Feather Blenny	105								
Ictalurus punctatus	Channel Catfish	55	345	62		9	935	831		
Leiostomus xanthurus	Spot						709	356		
Lepomis auritus	Redbreast Sunfish				323				21	
Lepomis cyanellus or Lepomis macrochirus	Bluegill or Green Sunfish	246	2,281		167					
Lepomis gibbosus	Pumpkinseed				51					
Lepomis gibbosus or Lepomis macrochirus	Pumpkinseed or Bluegill		652	1,152	6,382	1,126			21	
Lepomis macrochirus	Bluegill		797		337					
Membras martinica	Rough Silverside								9	
Menidia menidia	Atlantic Silverside	1,297	7,127	931	939	267		158	123	
Microgadus tomcod	Atlantic Tomcod				18				5	
Micropterus punctulatus or Micropterus salmoides	Spotted or Largemouth Bass							344		

Species	Common	Jun21	Jul21	Aug21	Apr22	May22	Jun22	Jul22	Aug22	Sep22
Micropterus salmoides	Large-mouth Bass				32					
Morone americana	White Perch	2,717	32,641	37,716	1,997	198	2,994	2,819	47	
Morone saxatalis	Striped Bass	106	1,662	627	310		2,074	302	159	26
Mugil cephalus	Flathead Grey Mullet							354		
Notemigonus crysoleucas	Golden Shiner				69					
Opsanus tau	Oyster Toadfish							225		
Oreochromis niloticus	Nile Tilapia				9					
Paralichthys dentatus	Summer Flounder	348			14				10	
Peprilus triacanthus	American Butterfish								17	
Perca flavescens	Yellow perch				15					
Pimephales notatus	Blunt-nose Minnow				12					
Pomatomus saltatrix	Bluefish			239	38		2,164	648	88	34
Salmo salar	Atlantic Salmon	72			35			152		
Scardinius erythrophthalmus	Rudd				21					
Semotilus atromaculatus	Creek Chub		341							
Strongylura marina	Atlantic Needle-fish								4	
Tautoga onitis	Tautog		1,435			28				
Trinectes maculatus	Hog-choker	186	1,083		53			209		

Table 9b Metabarcoding read totals for assigned fish taxa detected at Piermont April 2023 to September 2024.

Species	Common	Apr23	May23	Jun23	Jul23	Aug23	Sep23	Apr24	May24	Jun24	Jul24	Sep24
Acipenser brevirostrum	Shortnose Sturgeon		23	446								
Alosa spp.	shads and herrings	151		315			355		920			
Ameiurus catus	White Catfish	54					5	2,991	3,540	5,308		
Ameiurus natalis or Ameiurus nebulosus	Yellow or Brown Bullhead				7							
Anchoa mitchilli	Bay Anchovy	154	25	293			80		1,834			
Anguilla rostrata	American Eel	604	72	511	2,449		907	5,138	2,890	15,429		
Aplodinotus grunniens	Freshwater drum										961	
Brevoortia patronus	Gulf Killifish							388			2,241	
Brevoortia tyrannus	Atlantic Menhaden	1,164	133	25,277	15,560	1,708	14,266	14,189	20,482	4,358	2,225	3,038
Catostomus commersonii	White Sucker		11									
Cyprinus carpio	Common Carp	1,103	2,105	609		28	83	8,793	8,128	13,783		11
Dorosoma cepedianum	Gizzard Shad							1,902				
Fundulus heteroclitus	Mummichog		12	3,516								2,287
Gobiosoma bosc	Naked Goby		16	505		35	182		2,316			
Gobiosoma spp.	unk goby			135								
Ictalurus punctatus	Channel Catfish	4		86	3,392		59	1,878	4,626			
Leiostomus xanthurus	Spot										1,011	
Lepomis auritus	Redbreast Sunfish			76								

Species	Common	Apr23	May23	Jun23	Jul23	Aug23	Sep23	Apr24	May24	Jun24	Jul24	Sep24
Membras martinica	Rough Silverside			15					1,292			
Menidia menidia	Atlantic Silverside	9,425		408		20	1,447					623
Microgadus tomcod	Atlantic Tomcod								533			
Micropogonias undulatus	Atlantic Croaker											1,775
Morone americana	White Perch	185	46	5,277	18,437		940	3,771	10,411			4,733
Morone saxatalis	Striped Bass	1,426					688	13,156	5,379			
Mugil cephalus	Flathead Grey Mullet						122					
Oncorhynchus mykiss	Rainbow Trout (Steelhead)	17										
Paralichthys dentatus	Summer Flounder			292		12						1,829
Pomatomus saltatrix	Bluefish						157					
Sardina pilchardus	European Pilchard											1,728
Scomber scombrus	Atlantic Mackerel							435	4,849			
Seriola rivoliana	Longfin rivoliana											1,405
Stenotomus chrysops	Scup							623				
Trinectes maculatus	Hogchoker			346			75		3,919			
Urophycis regia	Spotted Hake	33										

Table 10a Metabarcoding read totals for assigned fish taxa detected at Norrie Point from June, 2021 to September 2022.

Species	Common	Jun21	Jul21	Aug21	May22	Jun22	Jul22	Aug22	Sep22
Acipenser oxyrinchus	Atlantic Sturgeon					9			
Alosa spp.	shads and herrings		10,988	569	10,469	64			341
Ameiurus catus	White Catfish						623		
Ameiurus natalis or Ameiurus nebulosus	Yellow or Brown Bullhead	104	12	40		8			7,158
Anchoa mitchilli	Bay Anchovy			710				7,183	
Anguilla rostrata	American Eel		554	6,834	128	20	693		2,445
Aplodinotus grunniens	Freshwater drum								561
Brevoortia tyrannus	Atlantic Menhaden	9	138	2,451	154	681	1,755		1,205
Carassius auratus	Goldfish	2,942	131	303		167			
Catostomus commersonii	White Sucker		17	38,172	32	122			439
Centropristis striata	Black Sea Bass			614					
Ctenopharyngodon idella	Grass Carp		146						52
Cyprinus carpio	Common Carp	59,679	6,883	4,274	1,640	10,034	3,155	6,397	3,890
Cyprinus carpio x Carassius Auratus	Common Carp or Goldfish	7,178	485	996		178			3,770
Etheostoma nigrum	Johnny Darter		38						
Etheostoma olmstedi	Tessellated Darter		9,518	319					
Fundulus diaphanus	Banded Killifish	951	988	19,027	29		1,483		315
Fundulus heteroclitus	Mummichog		77	53		160	1,126		1,035
Gobiosoma ginsburgi	Seaboard Goby			40					
Hirundichthys spp.	unk flying fish			90					
Ictalurus punctatus	Channel Catfish		28	127		20	6,063		5,037

Species	Common	Jun21	Jul21	Aug21	May22	Jun22	Jul22	Aug22	Sep22
Leiostomus xanthurus	Spot			1,381					
Lepomis auritus	Redbreast Sunfish		24,310	574	10	15	16,873		563
Lepomis cyanellus or Lepomis macrochirus	Bluegill or Green Sunfish			666		54			273
Lepomis gibbosus	Pumpkinseed	369	188			90	1,114		
Lepomis gibbosus or Lepomis macrochirus	Pumpkinseed or Bluegill	57,336	180,528	15,447	538	80			
Lepomis macrochirus	Bluegill	555	91	1,145	12				56
Lepomis spp.	unk sunfish						2,135		6,806
Menidia menidia	Atlantic Silverside			47					
Microgadus tomcod	Atlantic Tomcod					5			
Micropterus dolomieu	Smallmouth Bass		62	303	11	7			
Micropterus dolomieu or Micropterus punctulatus	Smallmouth or Spotted Bass						569		941
Micropterus punctulatus or Micropterus salmoides	Spotted or Largemouth Bass								1,211
Micropterus salmoides	Largemouth Bass		25	355					
Misgurnus anguillicaudatus	Pond Loach		34						
Morone americana	White Perch	617	424	3,301	105	108			3,233
Morone saxatalis	Striped Bass			35	7,687	10	447		59
Notemigonus crysoleucas	Golden Shiner		356	291		51			416
Notropis hudsonius	Spottail Shiner	314	3,310	17	1,027	42			85
Opsanus tau	Oyster Toadfish			12					
Perca flavescens	Yellow perch		16	91					
Pimephales notatus	Bluntnose Minnow		5,959			17			

Species	Common	Jun21	Jul21	Aug21	May22	Jun22	Jul22	Aug22	Sep22
Salmo salar	Atlantic Salmon	234		9,653					
Scardinius erythrophthalmus	Rudd	141	17	62		24			479
Strongylura marina	Atlantic Needlefish	203							
Tautoga onitis	Tautog			686					

Table 11b Metabarcoding read totals for assigned fish taxa detected at Norrie Point April 2023 to September 2024.

Species	Common	Apr23	May23	Jul23	Aug23	Sep23	Apr24	May24	Aug24	Sep24
Acipenser oxyrinchus	Atlantic Sturgeon		2,379							
Alosa spp.	shads and herrings	17,366	1,345		193	135	2,937	3,148		405
Ameiurus natalis or Ameiurus nebulosus	Yellow or Brown Bullhead			28,299	265	112	5,730		7,645	10,686
Anguilla rostrata	American Eel	10	615	9,385	613	80	2,963		6,985	1,489
Apeltes quadracus	Fourspine Stickleback						2,374			
Aplodinotus grunniens	Freshwater drum									288
Brevoortia patronus	Gulf Killifish		266							
Brevoortia tyrannus	Atlantic Menhaden	21	233		1,283	47	686			411
Carassius auratus	Goldfish				617	449				
Catostomus commersonii	White Sucker	6		2,346		85	1,993		2,979	514

Species	Common	Apr23	May23	Jul23	Aug23	Sep23	Apr24	May24	Aug24	Sep24
Ctenopharyngodon idella	Grass Carp								560	1,388
Cyprinella analostana	Satinfin Shiner			1,071			321			
Cyprinus carpio	Common Carp	14	6,044	5,650	2,316	17,159	1,747	2,976	21,775	11,738
Cyprinus carpio x Carassius Auratus	Common Carp or Goldfish		740	5,472	10,144	987	330	14,263	22,609	10,058
Dorosoma cepedianum	Gizzard Shad									736
Etheostoma nigrum	Johnny Darter			2,525						
Etheostoma olmstedi	Tessellated Darter	7		6,924		167	415			
Fundulus diaphanus	Banded Killifish		1,587	290	457	160	3,130		3,404	526
Ictalurus punctatus	Channel Catfish				345		1,095	2,047		7,685
Lepomis auritus	Redbreast Sunfish					108				
Lepomis cyanellus or Lepomis macrochirus	Bluegill or Green Sunfish			4,972	1,424	154			658	
Lepomis gibbosus or Lepomis macrochirus	Pumpkinseed or Bluegill	11			773	2,532				
Lepomis macrochirus	Bluegill		279	232		95				
Lepomis spp.	unk sunfish		437	11,161			6,152		12,935	4,562
Luxilus chrysocephalus or Luxilus cornutus	Striped or Common Shiner						391			
Micropterus dolomieu or	Smallmouth			6,026					3,730	

Species	Common	Apr23	May23	Jul23	Aug23	Sep23	Apr24	May24	Aug24	Sep24
Micropterus punctulatus	or Spotted Bass									
Micropterus punctulatus or Micropterus salmoides	Spotted or Largemouth Bass		741	2,525					2,509	918
Micropterus salmoides	Largemouth Bass					75				
Morone americana	White Perch	8	666	344	1,100	55	3,356	2,428	1,055	1,497
Morone saxatalis	Striped Bass		53,008				410	10,363	392	323
Neogobius melanostomus	Round Goby						237			
Notemigonus crysoleucas	Golden Shiner		2,399	6,146		194	270		3,102	430
Notropis hudsonius	Spottail Shiner	8		2,196			1,599			
Osmerus mordax	Rainbow Smelt		6,182							
Perca flavescens	Yellow perch			447						
Scardinius erythrophthalmus	Rudd			1,877		76	374			