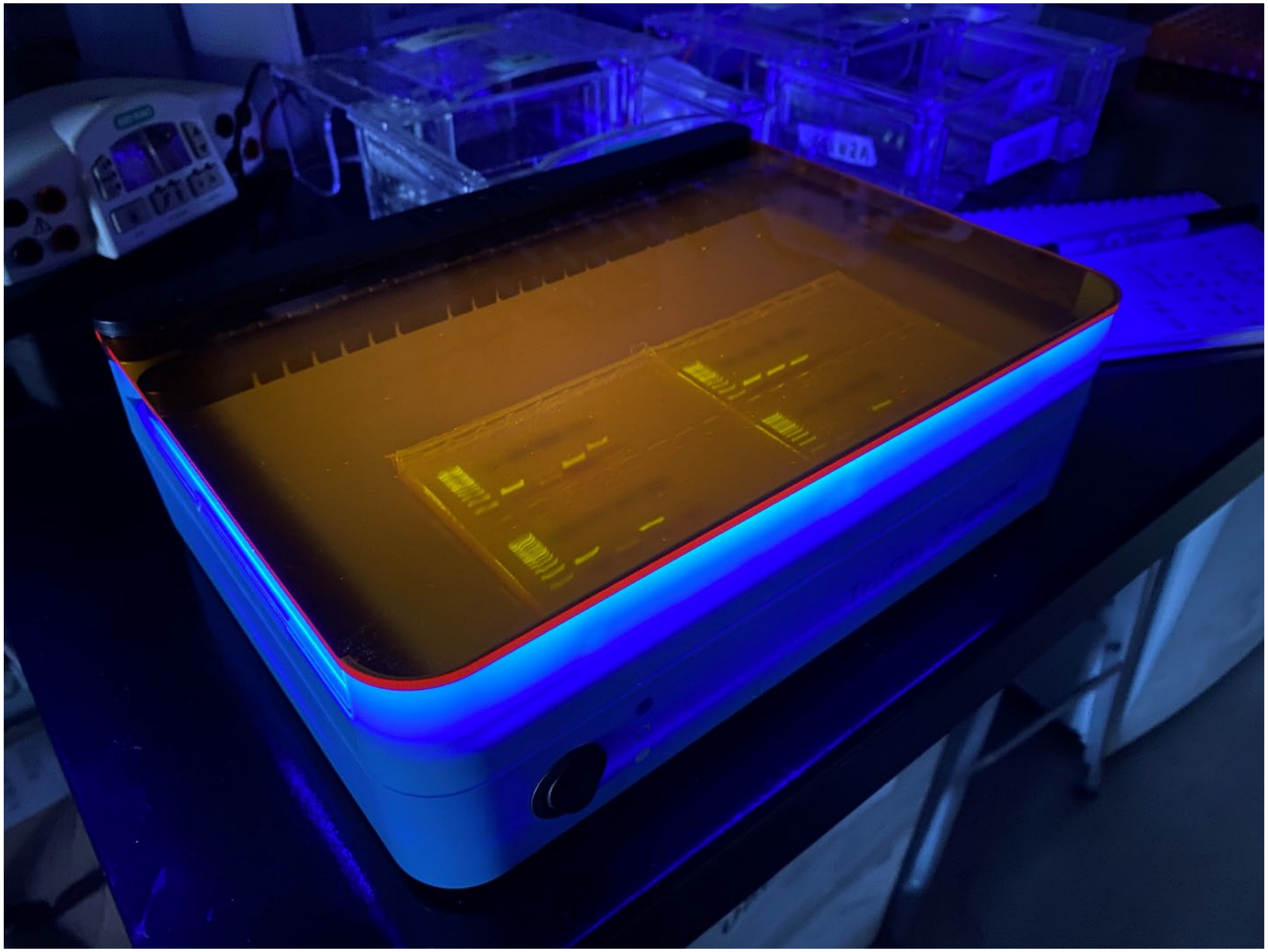
HUDSON RIVER PK
RIVER PROJECT

eDNA Report 2021-2024



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Purpose

The Hudson River Estuary serves as an important spawning ground and migratory corridor for diadromous, or migratory, fish such as the American eel (*Anguilla rostrata*), striped bass (*Morone saxatilis*), and Atlantic sturgeon (*Acipenser oxyrinchus*).

Since 2021, Hudson River Park has implemented environmental DNA (eDNA) sampling to identify species and track their migratory patterns with the help of upriver partners: the Department of Environmental Conservation (DEC) at Norrie Point, Lamont Doherty Earth Observatory (LDEO) in Piermont, and the Center for the Urban River at Beczak (CURB) at Yonkers. Sampling involves isolating DNA in water left behind by organisms in the form of scales, tissue, feces, or decaying material. This method allows for non-invasive detection and monitoring of fish populations and is complementary to the Park's long-running fish ecology trap survey.

With new funding secured to conduct next generation sequencing and metabarcoding analysis, the Park's eDNA survey is taking its next steps to become a more holistic diagnostic fish survey of the Lower Hudson. This report outlines initial findings from sequencing samples between 2021-2024.

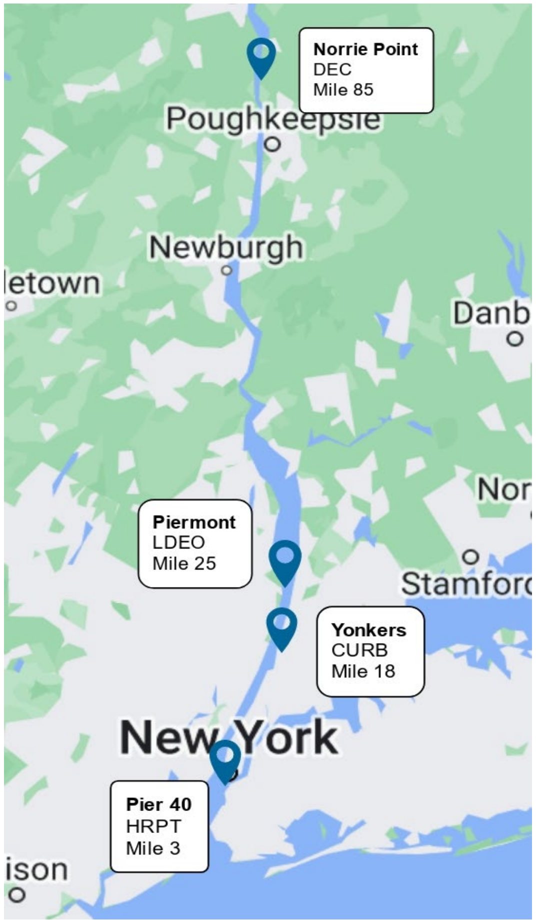


Fig. 1 | Map of the four sampling locations, 2021-2024.

Key Research Questions

- Can eDNA be used to track presence/absence of key diadromous fish species in the Lower Hudson Estuary?
- How can metabarcoding more comprehensively sample for fish diversity in the Lower Hudson Estuary?
- How does eDNA compliment traditional fish trapping surveys?

Methods

- **Collection:** From April to September, 1L surface water samples are taken monthly at four different sites along an 85-mile-long stretch of the Hudson River within a 48hr span **(Fig. 1)**.

- **Filtration:** Samples are filtered via vacuum pump through a 0.45*μ*m filter to separate solid debris from the macromolecules in the water samples.

- **Extraction:** DNA is extracted via DNeasy PowerSoil or PowerWater kit. This is a series of steps that separates DNA molecules from other macromolecules on the filter and results in an elution or "extract" that contains any DNA molecules present in the sample.

- **Amplification:** The elutions are subjected to two rounds of polymerase chain reaction (PCR) according to Stoeckle et al. Go Fish methodology (2018). This step amplifies DNA by making billions of copies of what is present in the elution to allow for subsequent detection and identification using 12S MiFish primers **(Appendix).**

- **Electrophoresis:** The PCR product is run through 2% agarose gels, 1X TBE buffer for 30 mins at 130V and read via UV transilluminator. The resulting bands (or lack thereof) on the gels indicate the presence/absence of each target species at each site **(Fig. 2).**

- **Sequencing & Metabarcoding:** Amplicons and/or extracts are additionally then shipped to a laboratory facility and sequenced via Illumina Miseq, a technique that identifies all DNA fragments present in a sample. The resulting sequences can then be matched to a reference library of known species sequences.

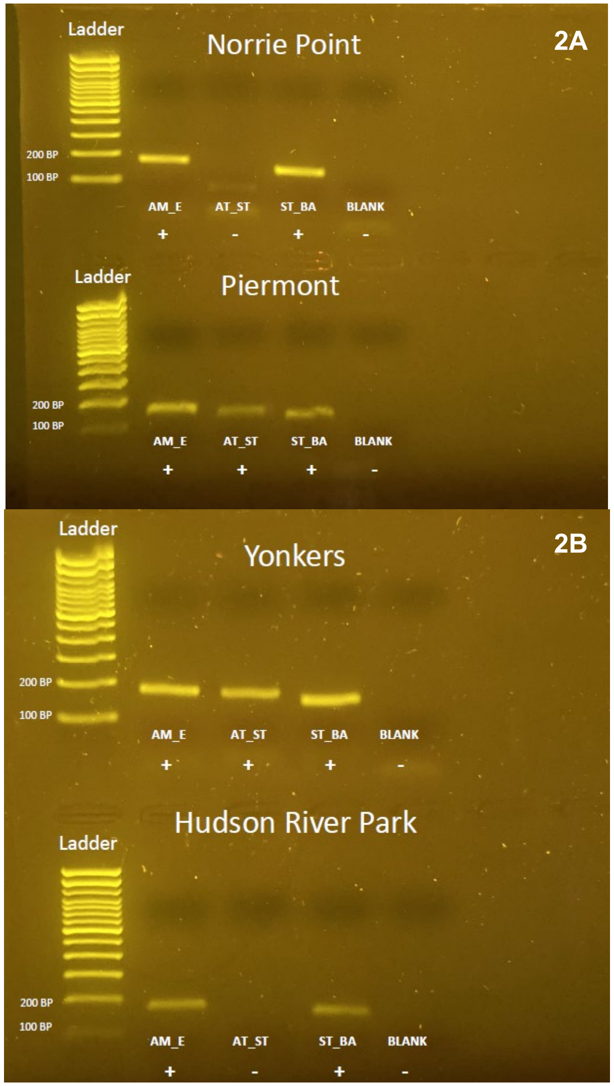


Fig. 2 | Annotated photo of gel results from Norrie Point (**A**) and Yonkers (**B**), May 2024. Species codes are as follows: AM_E = American eel; AT_ST = Atlantic sturgeon; ST_BA = Striped bass.

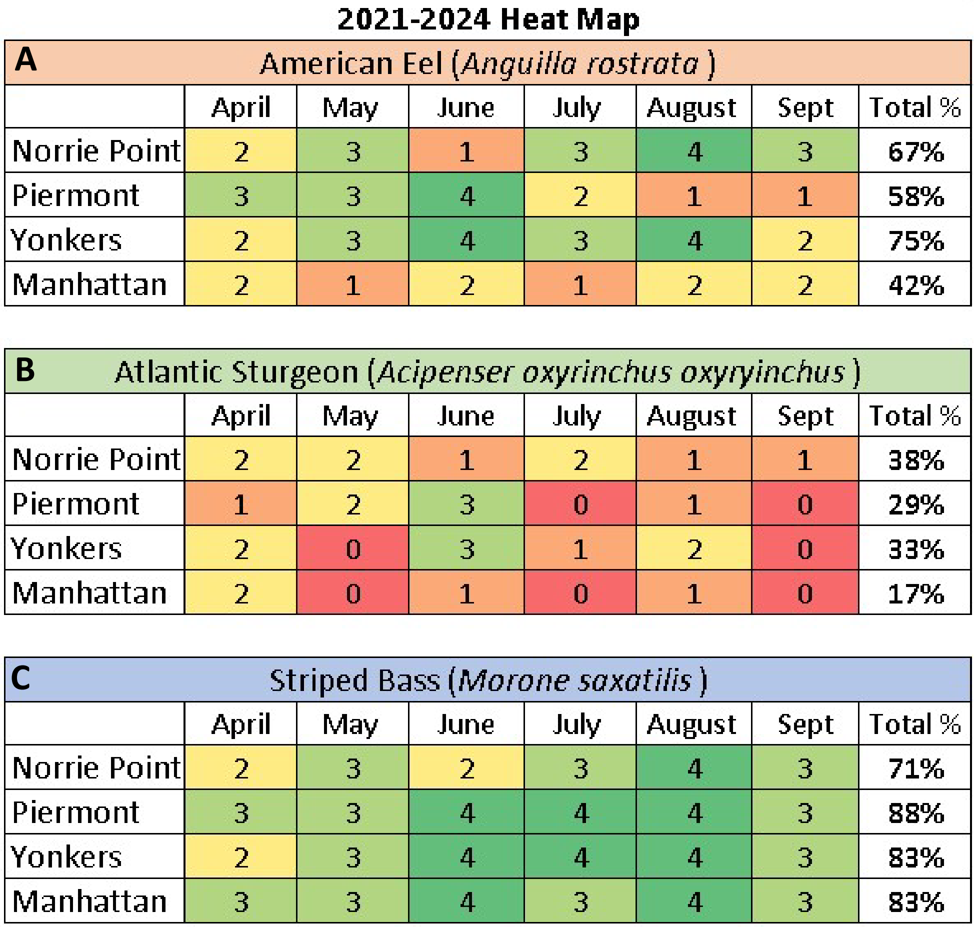
Major Findings – Nested PCR

Through monthly, conterminous sampling at four sites along 85 river miles, general trends of diadromous fish migration can be observed. Note variance in site distance, Norrie to Piermont = 60mi., Piermont to Yonkers = 7mi., Yonkers to Manhattan = 15mi.

Striped bass were ubiquitous, detected in nearly all samples (~80%) at nearly all sites, especially in the summer months (**Fig 3.C**). Unlike the other two species, they were slightly less common at the more northern sites, reflective of their preference for more coastal, estuarine waters even as juveniles.

American eels were detected in nearly half to three-quarters of samples (**Fig 3.A**), with less clear preference for one site over another. They flourish in the Lower Hudson and likely move between sampled areas regularly as they mature, perhaps explaining the lack of observed trends.

Sturgeon proved to be the most elusive of the species, only detected in a third of samples, if that (**Fig 3.B**). Their widest distribution across sites was observed in April, June, and August, perhaps indicating waves of migration from further offshore to fresher upstream waters, and vice versa, but difficult to determine from these data alone.



2021-2024 Heat Map

A American Eel (*Anguilla rostrata*)

	April	May	June	July	August	Sept	Total %
Norrie Point	2	3	1	3	4	3	**67%**
Piermont	3	3	4	2	1	1	**58%**
Yonkers	2	3	4	3	4	2	**75%**
Manhattan	2	1	2	1	2	2	**42%**

B Atlantic Sturgeon (*Acipenser oxyrinchus oxyryinchus*)

	April	May	June	July	August	Sept	Total %
Norrie Point	2	2	1	2	1	1	**38%**
Piermont	1	2	3	0	1	0	**29%**
Yonkers	2	0	3	1	2	0	**33%**
Manhattan	2	0	1	0	1	0	**17%**

C Striped Bass (*Morone saxatilis*)

	April	May	June	July	August	Sept	Total %
Norrie Point	2	3	2	3	4	3	**71%**
Piermont	3	3	4	4	4	3	**88%**
Yonkers	2	3	4	4	4	3	**83%**
Manhattan	3	3	4	3	4	3	**83%**

Fig. 3 | Monthly eDNA positive detections of American eel (**A**), Atlantic Sturgeon (**B**), and striped bass (**C**), April-September 2021-2024 (sampling only occurred June-Aug 2021). Gross latitudinal & seasonal distribution of target diadromous fishes through presence/absence sampling, numbers indicate how many samples across all years were positive for each species.

Major Findings – Sequencing

Presence/absence sampling via nested PCR and gel electrophoresis can provide quick results, but findings are limited, especially in low-frequency sampling schemes. With new funding for sequencing, the Park was finally able to send all four years of samples to the BioAnalytical Services Laboratory (BAS Lab) at the University of Maryland, and work with Dr. Sam Chew Chin to metabarcode & identify the full fish diversity captured in the water samples. From 77 samples that produced 3,649,149 reads, 95 unique fish taxa were identified across all 4 sites, with 53-61 taxa per site. Of these, 84 were unique species and 11 were indeterminate, multi-species assignments (same genus but unclear which of two species), inclusive of 21 freshwater, 20 marine, and 52 estuarine fishes.

Atlantic menhaden (*Brevoortia tyrannus*) were by far the most abundantly detected species, comprising the majority of reads of all but the northernmost site, where genus *Lepomis* sunfish (e.g., pumpkinseed & bluegill) were predominant, alongside various carp (**Fig 4**).

All sites exhibited detections of unique species. Those unique to Hudson River Park included – in descending abundance – skilletfish (*Gobiesox strumosus*), conger eel (*Conger oceanicus*), smallmouth flounder (*Etropus microstomus*), cunner (*Tautogolabrus adspersus*), Atlantic herring (*Clupea harengus*), and Northern pipefish (*Syngnathus fuscus*). These species are all squarely estuarine/marine, with low tolerance for fresh water. Many of them are observed in the Park's fish ecology survey, though conger eels and herring remain elusive, the former only typically seen as juveniles curled up in Park oyster enhancements, and the latter when a stray from a feeding school accidently swims into a trap.

Further north at river mile 85, Norrie point also had a number of unique species of interest: rainbow smelt (*Osmerus mordax*), pond loach (*Misgurnus anguillacaudatus*), and round goby (*Neogobius melanostomus*). Rainbow smelt populations have fallen dramatically in the Hudson since 1995, though they have been seeded into some NYC reservoirs. It is difficult to determine what the signal means without further detections. Pond loach and Round Goby are two introduced species of concern, currently being tracked through other eDNA sampling efforts by NYS DEC. Their detections highlight the potential use of his technique to monitor for regulated species.

By itself, eDNA can be broadly diagnostic, but an underlying familiarity of ground-truthed fish species presence is key to is use as a diversity sampling tool. eDNA signals can be detected sometimes from sources that may be a good distance away, such as freshwater fish signals detected in Manhattan samples that likely came from adjacent tributaries.

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Major Findings – Sequencing

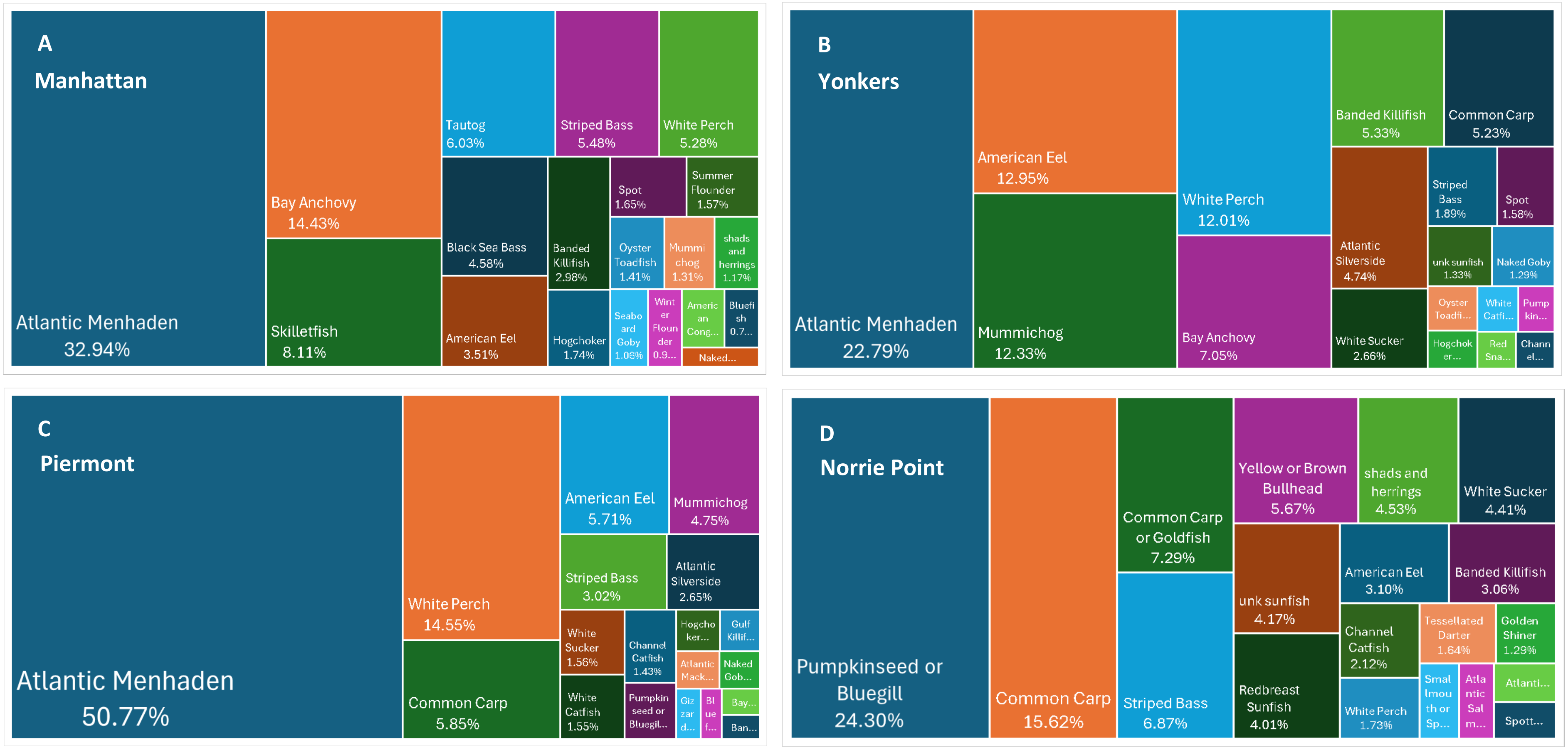
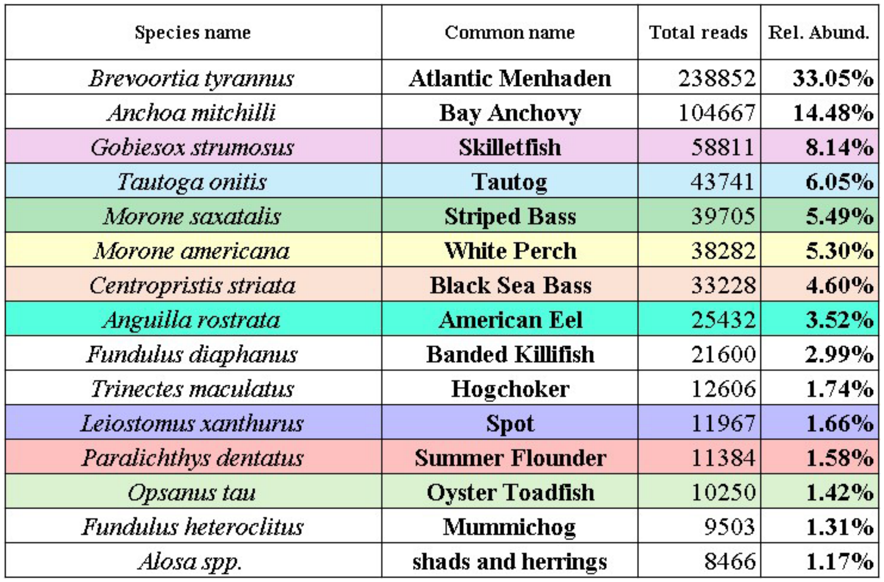


Fig. 4 | Total relative abundance of top 20 species by sequence reads at Pier 40, Manhattan (**A**), Yonkers (**B**), Piermont (**C**), and Norrie Point (**D**). Sites exhibit total DNA reads over the four-year period between 751,161 and 1,058,62, and 53-61 total species detected.

Major Findings – Sequencing

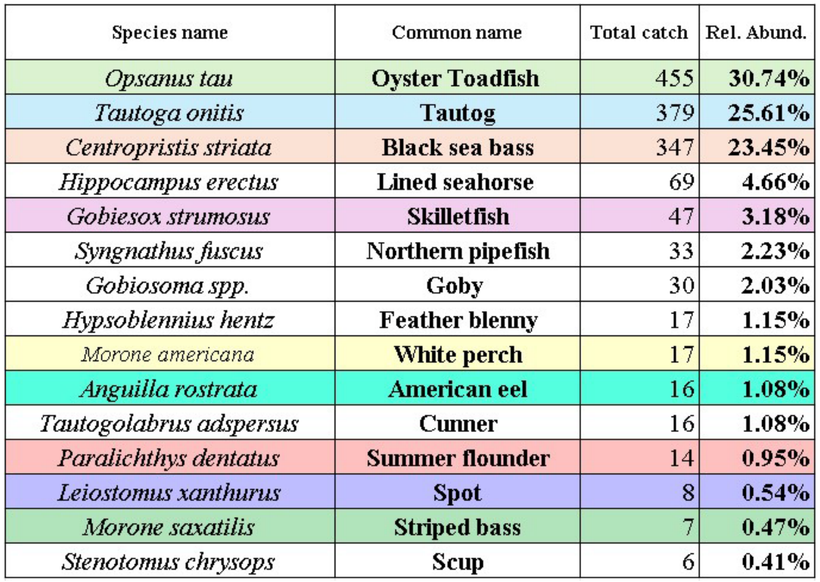
By comparing eDNA reads with fishes caught in the same location over the same time period, such as in the Park's fish ecology survey, a fuller picture of Lower Hudson fish diversity can be painted, each sampling technique complimenting the other. When looking at the 15 most abundant species in each survey, relative abundances are significantly different, species to species, with only a few exceptions (**Tables 1, 2**). The trap survey is biased toward benthic, demersal, and shallow water fishes, whereas the eDNA reads indicate a large presence of pelagic fishes, which is known from other studies and observations. Large numbers of schooling fish such as menhaden and anchovy are readily detected by eDNA due to their sheer biomass and proximity to the water's surface, whereas more sedentary fish like toadfish and seahorses stay hidden near the bottom and likely shed less DNA either due to behavior, physiology, or simply body size, though eDNA still picks up some small, cryptic species, such as skilletfish. More data is needed to understand how eDNA detections can best inform and supplement traditional surveys.

Table 1 | Relative abundance of fishes detected in the Park's eDNA survey, 2021-2024.



Species name	Common name	Total reads	Rel. Abund.
Brevoortia tyrannus	**Atlantic Menhaden**	238852	**33.05%**
Anchoa mitchilli	**Bay Anchovy**	104667	**14.48%**
Gobiesox strumosus	**Skilletfish**	58811	**8.14%**
Tautoga onitis	**Tautog**	43741	**6.05%**
Morone saxatalis	**Striped Bass**	39705	**5.49%**
Morone americana	**White Perch**	38282	**5.30%**
Centropristis striata	**Black Sea Bass**	33228	**4.60%**
Anguilla rostrata	**American Eel**	25432	**3.52%**
Fundulus diaphanus	**Banded Killifish**	21600	**2.99%**
Trinectes maculatus	**Hogchoker**	12606	**1.74%**
Leiostomus xanthurus	**Spot**	11967	**1.66%**
Paralichthys dentatus	**Summer Flounder**	11384	**1.58%**
Opsanus tau	**Oyster Toadfish**	10250	**1.42%**
Fundulus heteroclitus	**Mummichog**	9503	**1.31%**
Alosa spp.	**shads and herrings**	8466	**1.17%**

Table 2 | Relative abundance of fishes caught in the Park's fish ecology survey, 2021-2024.



Species name	Common name	Total catch	Rel. Abund.
Opsanus tau	**Oyster Toadfish**	455	**30.74%**
Tautoga onitis	**Tautog**	379	**25.61%**
Centropristis striata	**Black sea bass**	347	**23.45%**
Hippocampus erectus	**Lined seahorse**	69	**4.66%**
Gobiesox strumosus	**Skilletfish**	47	**3.18%**
Syngnathus fuscus	**Northern pipefish**	33	**2.23%**
Gobiosoma spp.	**Goby**	30	**2.03%**
Hypsoblennius hentz	**Feather blenny**	17	**1.15%**
Morone americana	**White perch**	17	**1.15%**
Anguilla rostrata	**American eel**	16	**1.08%**
Tautogolabrus adspersus	**Cunner**	16	**1.08%**
Paralichthys dentatus	**Summer flounder**	14	**0.95%**
Leiostomus xanthurus	**Spot**	8	**0.54%**
Morone saxatilis	**Striped bass**	7	**0.47%**
Stenotomus chrysops	**Scup**	6	**0.41%**

Takeaways

eDNA sampling is a broadly diagnostic and non-invasive technique that allows for relatively low-cost and low-effort detection of aquatic species, making it accessible to groups with varying levels of funding and bandwidth. Processing requires specific equipment, materials, and technical expertise; however, our partnership model delineates ways in which networks can work together to collect sampling through collaboration.

While DNA analyses can be difficult to use to estimate abundance, they are excellent at sampling for diversity and therefore relative abundance. Four years and the better part of 100 samples indicate a huge spread of biodiversity across the Lower Hudson River Estuary. More analysis is required to elucidate seasonal changes between months and years as well as the effects of tides and site conditions.

eDNA can be a powerful tool to supplement traditional fish sampling by detecting species not caught via traps due to behavior, low abundance, physiology or other factors. This highlights the usefulness of a synthesis of various sampling techniques to wholistically study species abundance and diversity. Steps of course must be taken to flag and discount erroneous signals, such as those that may come from human activity (e.g., fish markets, sewer effluent, etc.) and signals from further upstream or tributaries. As such, the “calibration” of this technique for use in coastal, estuarine systems is still underway.

Fig. 5 | River Project staff performing vacuum filtration of May 2025 Pier 40 & Governor’s Island water samples.

Future Directions

The Park's River Project is continuing to work with BAS Lab & Dr. Sam Chew Chin to sequence and barcode amplicons and extracts from the 2025 season.

This year also marks the newest addition to the Lower Hudson River Estuary sampling network: Governor's Island, sampled monthly in 2025 with partners from NYU's Gallatin House. This addition extends the survey an additional 2.5 miles south and fully into the New York Harbor proper.

In addition to this monthly survey, the Park has begun to incorporate eDNA sampling into habitat enhancement monitoring projects, namely the work being done at Gansevoort Peninsula. Partners from Monmouth University sampled in summer 2025 in the vicinity of the artificial reef on the north side of the peninsula made up of reef balls and gabions to supplement other fish survey work being performed via baited remote underwater video. In an effort to align with this work and eDNA surveys in the region more broadly, the Park is exploring changing primers from MiFish, which has been used to-date, to Riaz primers. Consistency in primer sets use in DNA amplification will ensure valid comparison between studies and collectively strengthen our understanding of fish and other marine organism assemblages in the Lower Hudson.

Fig. 6 | Striped bass (above) and black sea bass (below) observed via Go Pro camera during underwater video trials at the Gansevoort Habitat Enhancement Area.

Future Directions

With funding awarded by the Lounsbery Foundation, the Park's eDNA network will not only sequence water samples but create educational and professional resources for students, teachers, and the public to engage in real, place-based data, and environmental science learnings.

Special thanks to
Norrie Point DEC
Center for the Urban River at Beczak
Lamont Doherty Earth Observatory
NYU Gallatin school
BAS Lab
Dr. Sam Chew Chin
Dr. Mark Stoeckle
Dr. Jesse Ausubel
The Richard Lounsbery Foundation

Appendix

Species	Primer Name	Primer Sequence	Amplicon Size (bp)	Annealing Temp (C)
Vertebrate Fish	MiFish-U-F	GTCGGTAAAACTCGTGCCAGC	~220	60
	MiFish-U-R2	CATAGTGGGGTATCTAATCCCAGTTTGT		
American Eel	AM_E_F	TGTAAAACGACGGCCAGTGGGCTCAAATTGATATTACA	~175	60
	AM_E_R	CAGGAAACAGCTATGACCGTGAGTTCAAAGGTGT		
Atlantic Sturgeon	AT_ST_F	TGTAAAACGACGGCCAGTCGTAAAGCGTGATTAAAGGATATC	~162	60
	AT_ST_R	CAGGAAACAGCTATGACGTTCAAGGGGTTCTTGTTAGG		
Striped Bass	ST_BA_F	TGTAAAACGACGGCCAGTGGTTAAGGGCCCAACTTTTAT	~148	60-65
	ST_BA_R	AGGAAACAGCTATGACTTTCGTGGGGTCAGGTTTGAG		

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