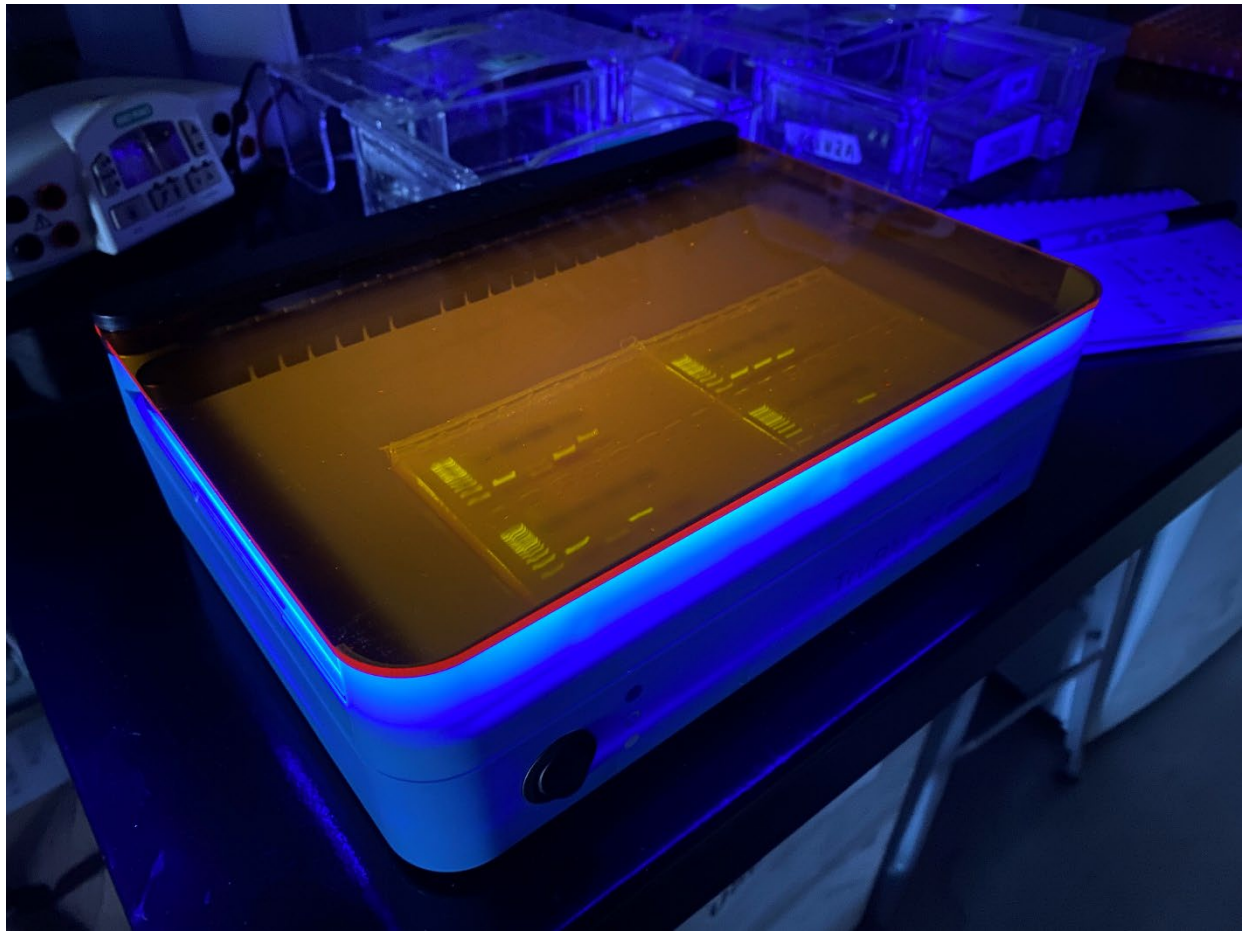
HUDSON RIVER PK
RIVER PROJECT

Environmental DNA Report 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*). Striped bass and Atlantic sturgeon are anadromous fish that spend their adult lives in the sea and migrate to freshwater to reproduce, whereas the American eel is a catadromous fish that spends its adult life in freshwater and migrates to the sea to spawn. These fish thus spend varying portions of their life cycles in the Hudson River. Sturgeon is particularly elusive as it only briefly passes through the lower Hudson during its migratory season.

Hudson River Park has implemented environmental DNA (eDNA) sampling to identify species and track their migratory patterns since 2021 with the help of upriver partners. This process 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.

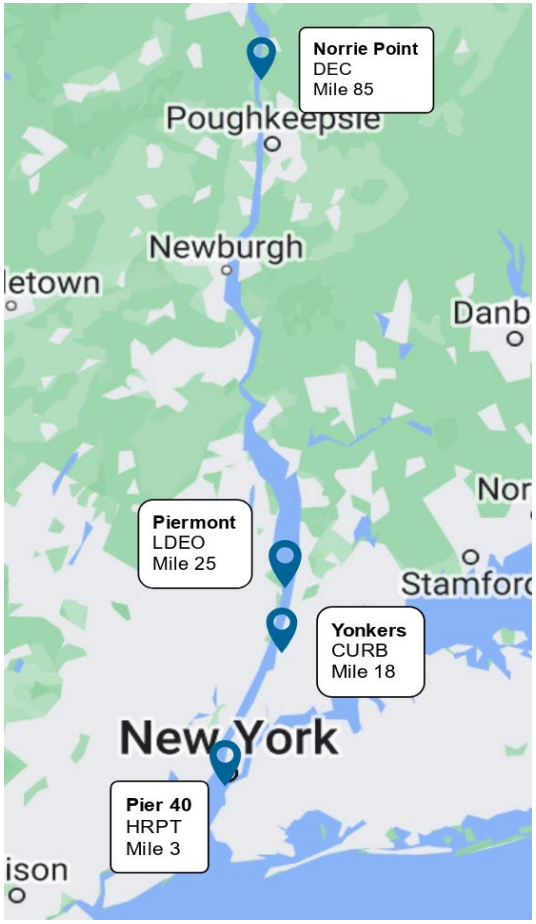

Fig. 1 | Map of the four sampling locations.

Key Research Questions

- Can eDNA be used to track presence/absence of key diadromous fish species in the Lower Hudson Estuary?
- Are migration patterns of three key species observable over a ~85mi. stretch of river?
- How can metabarcoding more comprehensively sample for fish diversity in the Lower Hudson Estuary?

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Methods

- **Collection:** From May 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 0.45μm filter to separate solid debris from the macromolecules in the water samples.

- **Extraction:** DNA is extracted via DNeasy PowerSoil Pro 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 that were extracted.

- **Amplification:** The elutions are subjected to two rounds of PCR (Polymerase Chain Reaction) 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.
    - The first round uses MiFish 12S vertebrate fish primers to amplify all fish DNA in the sample.
    - The second round uses species-specific MiFish primers for the target species: American Eel, Striped Bass, and Atlantic Sturgeon **(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).**

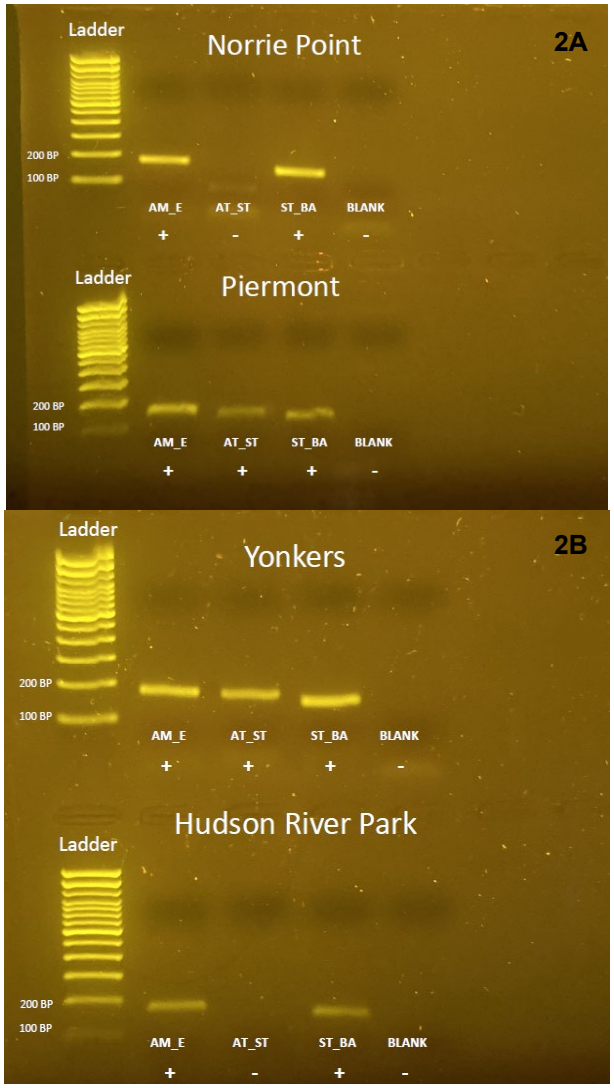

Fig. 2 | Annotated photo of gel results from Norrie Point (**A**) and Yonkers (**B**), May 2024. Species codes are as follows: AM_E = American eel; AT_ST = Atlantic sturgeon; ST_BA = Striped bass.

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Major Findings

All three target species were observed over the course of the 2024 sampling season, with Sturgeon proving the most elusive.

American Eel

American eels (*Anguilla rostrata*) were prevalent throughout the season, present in 14 of 23 samples (**Fig. 3**). This year appeared to show potentially fewer eels down south near Manhattan as the summer progressed, with detections at Pier 40 relegated to June and August, although three eels were caught via traps on site in April and one was caught in May. Eels were also seen during mobile species monitoring further offshore in partnership with Rutgers University throughout the summer. The absence of eel positives in the samples may be indicative of overall low critical mass of eels during these times compared to other species (such as menhaden), or other confounding factors such as variability in tide during sampling. More sampling will be needed to ascertain specific findings and account for interannual variation.

2024 eDNA Results

American Eel (*Anguilla rostrata*)	April	May	June	July	August	Sept.
Norrie Point	✓	✓		✓	✓	✓
Piermont	✓	✓	✓	×	×	×
Yonkers	✓	✓	✓	×	✓	×
Pier 40	×	×	✓	×	✓	×

Atlantic Sturgeon (*Acipenser oxyrinchus*)	April	May	June	July	August	Sept.
Norrie Point	✓	✓		✓	×	×
Piermont	×	✓	✓	×	×	×
Yonkers	✓	×	✓	×	×	×
Pier 40	×	×	×	×	×	×

Striped Bass (*Morone saxatilis*)	April	May	June	July	August	Sept.
Norrie Point	✓	✓		✓	✓	✓
Piermont	✓	✓	✓	✓	✓	✓
Yonkers	✓	✓	✓	✓	✓	✓
Pier 40	✓	✓	✓	✓	✓	✓

Fig. 3 | Monthly eDNA samples, April-September 2024. Norrie Point samples were not taken in June. Checks represent positive bands observed during gel electrophoresis, x's, no bands or bands at incorrect base pair fragment length.

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Major Findings

Atlantic Sturgeon

Atlantic sturgeon (*Acipenser oxyrinchus*) continues to be the least observed of the three species, with their DNA present in only 7 of 23 samples, and even when present, often only in trace amounts. Sturgeon were observed at Norrie Point and Yonkers at the start of the sampling season (April) and absent in every site by the end (September). Overall, positive samples were sporadically distributed throughout the middle of the season but tended to be more northerly (**Fig. 3**), especially in the warmer summer months. These data may indicate an adult migration upstream in the late spring, but more samples are required to understand fluctuations. Sturgeon can reside in brackish/fresh water for up to 6 years before they return to the sea, therefore, it is not surprising to see the presence of sturgeon further up the estuary.

Striped Bass

Striped Bass (*Morone saxatilis*) DNA was found in all 23 samples in 2024 (**Fig. 3**). Even though they are a diadromous species whose adults spend their time in deeper waters, there are populations of young stripers throughout the LHRE. Striped bass make use of the estuary as a nursery ground as far south as Manhattan (Grothues and Abel, 2010) and the Park's trap survey corroborates their presence into the winter months. Being a pelagic – free swimming – species, striped bass are often underrepresented in the Park's fish ecology survey, highlighting the usefulness of eDNA for overcoming study design & equipment-specific limitations by broadly sampling the environmental medium.

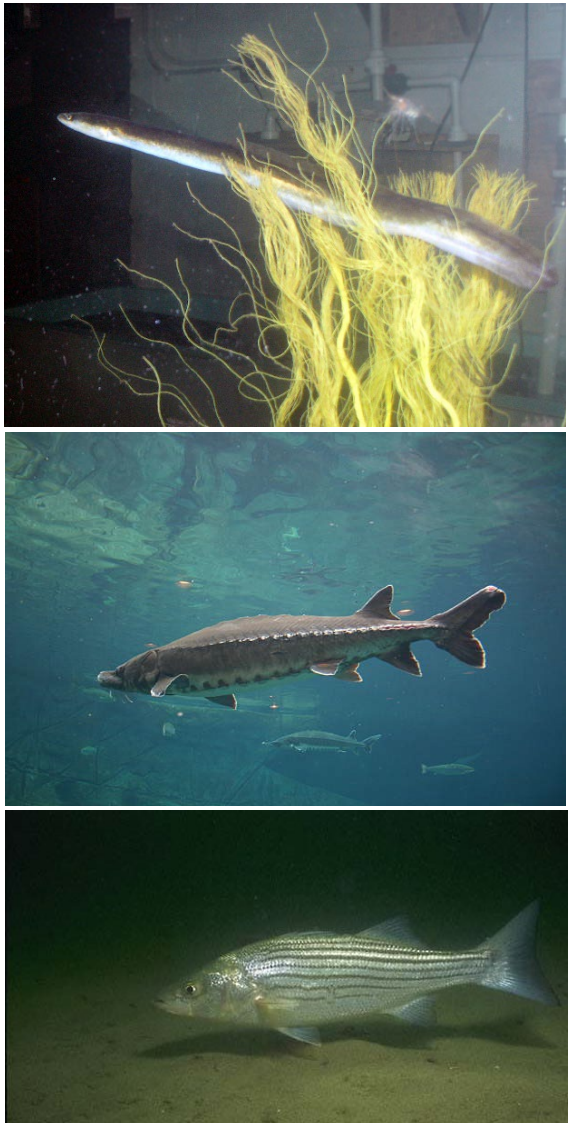

Fig. 4 | American eel (top), Atlantic sturgeon (middle), and striped bass (bottom).

Takeaways

These data show that American eels and striped bass appear to be dwelling in the Lower Hudson Estuary during the warmer months, while sturgeon travel through the more northern sample sites earlier in the season and do not reside long-term at the southerly sites.

As we are only collecting and processing samples monthly, it is possible that we are seeing data discrepancies due to gross sample timescale, variation in sampling times, and tidal effects between sites. For example, American eels were caught in small numbers in fish traps in April and May but were not detected by eDNA sampling. It is possible that, while present, their relative abundance is too low to produce the requisite amounts of material necessary for DNA detection, especially amongst the noise of more numerous and less cryptic species that occupy the surface of the water where samples are taken. It is also likely that due to differences in sampling times, tides are affecting the samples' contents depending on the ebbing or flooding, making samples potentially less consistent than if they were standardized to a specific point in the tide cycle across sites, which is difficult to coordinate.

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.

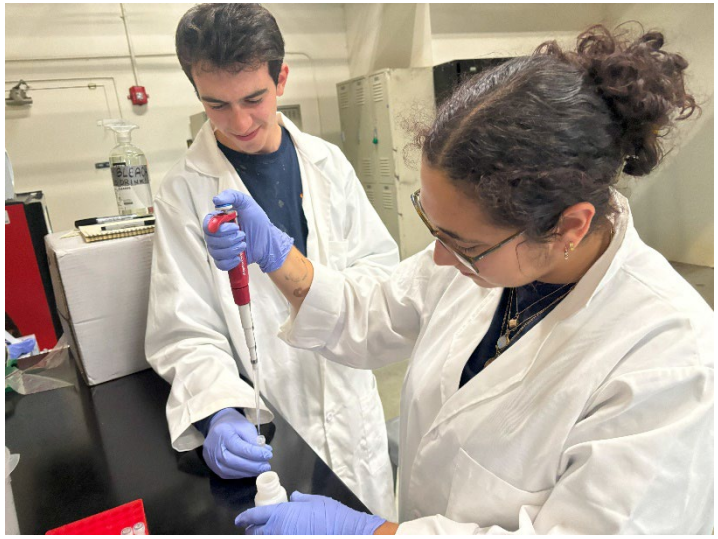

Fig. 5 | River Project staff extracting samples using DNEasy Power Soil protocols

Fig. 6 | River Project staff loading thermocycler with DNA extractions for PCR.

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Future Directions

The Park's River Project is currently working with Dr. Sam Chew Chin of NOAA to sequence and barcode amplicons and extracts taken within the Park to better understand the presence of fish in the Park not typically caught in standard collection gear.

Through metabarcoding of 42 eDNA samples from 2021 to 2023, preliminary results indicate 61 detected species across all sites, 47 within the Park, including many species not observed in the fish ecology trap survey. For example, in most samples, Atlantic menhaden (*Brevoortia tyrannus*) had the highest abundance of sample reads, often making up ~30% of all DNA detections, despite almost never observing the fish in the survey traps. Menhaden, like all river herring, are pelagic fish that school near the water's surface in vast numbers for safety instead of hiding amongst substrate, like many other fishes. Some benthic species observed in the trap survey are only minimally observed in eDNA sampling due to low relative biomass and cryptic behavior, such as oyster toadfish. This dichotomy highlights the usefulness of a synthesis of various sampling techniques to wholistically study species abundance and diversity.

The Park plans to continue to incorporate broad spectrum DNA analysis via metabarcoding to paint a more complete picture of the biodiversity present in the Estuarine Sanctuary, and study changes over time. A full metabarcoding report will be posted on the Park's website in the coming months.

The Park is also looking to investigate more species via presence/absence sampling in coming years, especially taxa of interest and/or concern to the State, such as the invasive round goby (*Neogobius melanostomus*).

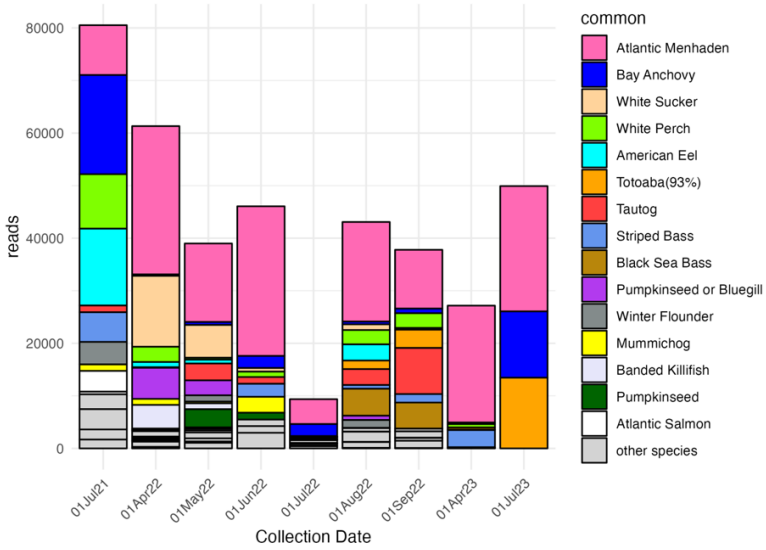

Fig. 7 | Sequencing reads for samples collected at Hudson River Park. Top 15 of the most abundant taxa are color-coded. Courtesy Sam Chew Chin, PhD.

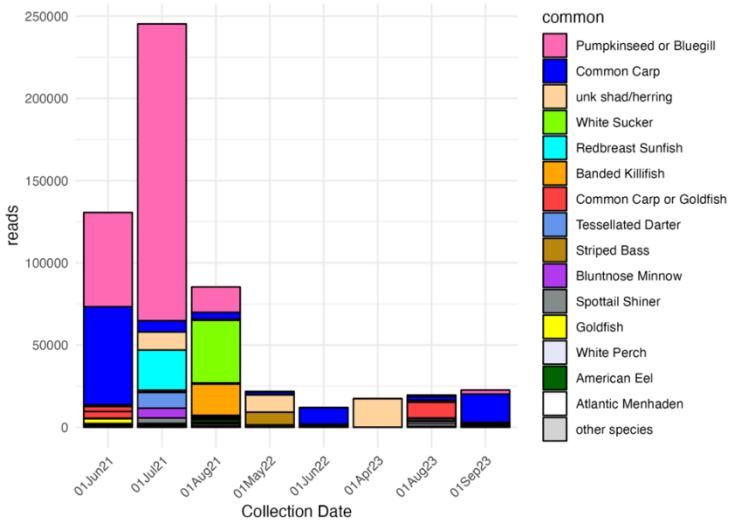

Fig. 8 | Sequencing reads for samples collected at Norrie Point. Top 15 of the most abundant taxa are color-coded. Courtesy Sam Chew Chin, PhD.

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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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