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RIVER PROJECT

Environmental DNA Report 2023

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Purpose

The Hudson River Estuary serves as an important spawning and migratory corridor for diadromous 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 reproduce. These fish spend varying portions of their life cycle in the Hudson River. Sturgeon in particular are elusive as they only briefly pass through the lower Hudson during their migratory season.

For these reasons, Hudson River Park has implemented environmental DNA (eDNA) sampling to identify species and track their migratory patterns since 2021 with the help of partners upriver. This process involves isolating DNA in water, soil, etc., left behind by organisms in the form of scales, tissue, faeces, or decaying material. This method allows for efficient, non-invasive monitoring of fish populations and is complementary to the Park's long-running fish ecology trap survey

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?

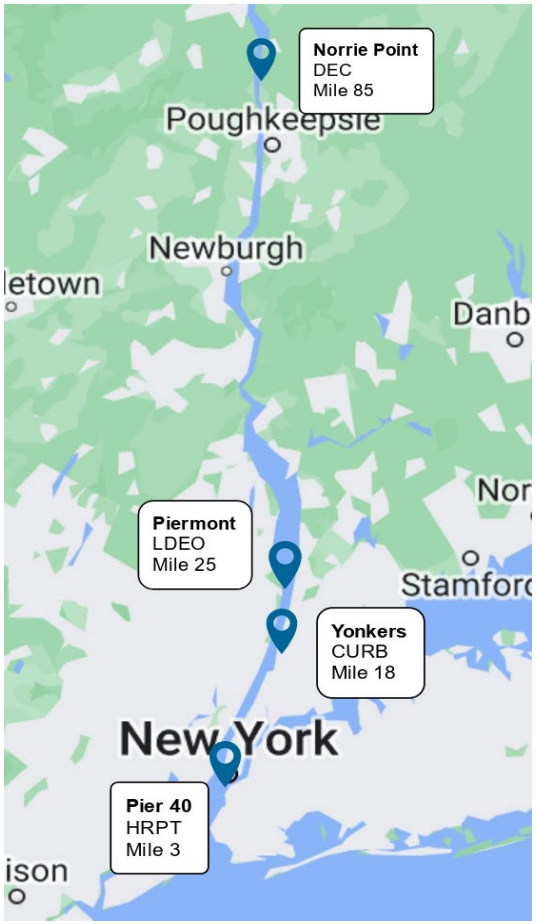

Fig. 1 | Map of the four sampling locations.

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Methods

- **Collection:** From May to September, 1L surface water samples are taken at four different sites along an 85-mile-long stretch of the Hudson River **(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 kits. This is a series of steps that separates DNA molecules from other macromolecules on the filter and results in an elution that contains any DNA molecules that were extracted.

- **Amplification:** The elutions are subjected to two rounds of nested PCR (Polymerase Chain Reaction) according to Stoeckle et al., (2018) Go Fish methodology. This step "amplifies" DNA by making billions of copies of the DNA in the elution for better detection and identification.
    - First round uses MiFish 12S vertebrate fish primers.
    - 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).**

April 2023 eDNA Results

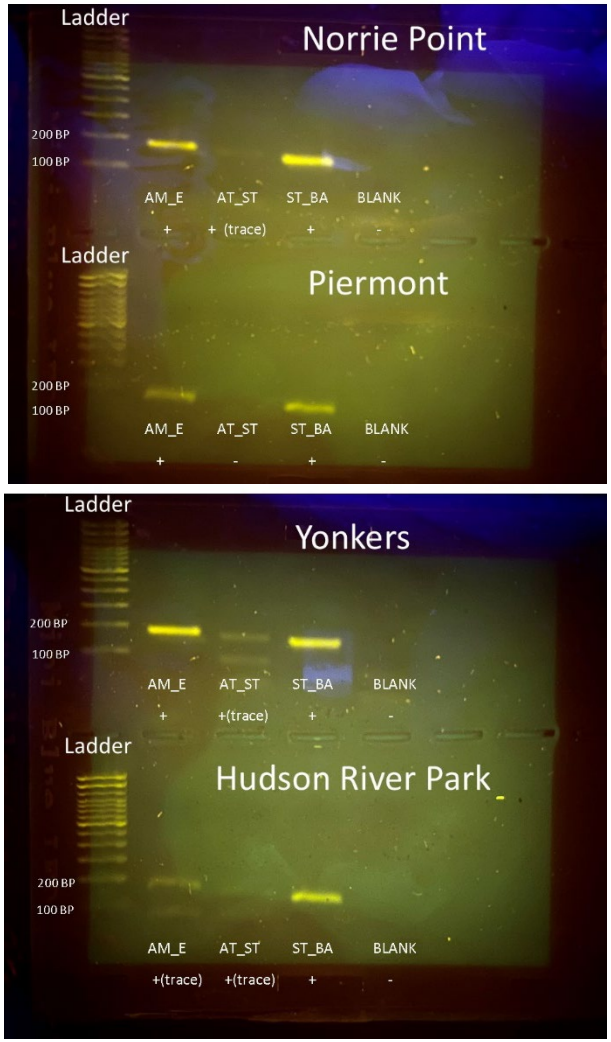

Fig. 2 | Annotated photo of gel results from April 2023. Sites arranged North to South.

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Major Findings

All three target species were observed over the course of the 2023 sampling season, with Sturgeon proving the most elusive.

American Eel

American eels were highly prevalent at most sites throughout the season (**Fig. 3**), as expected from previous years' findings. This year appeared to show potentially fewer eels down south near Manhattan in the summer, with detections at Pier 40 relegated to April and September, though a single large female was caught via traps on site in August. Eels have also been reported by contracted divers within the Park during these months, so absence of eDNA may be indicative of overall lower critical mass of eels during these times, or some other confounding factor. More sampling will be needed to ascertain specific findings and account for interannual variation.

2023 eDNA Results						
American Eel (*Anguilla rostrata*)						
	April	May	June	July	August	Sept.
Norrie Point	✓	✓		×	✓	✓
Piermont	✓	✓	✓	✓	×	×
Yonkers	✓	✓	✓	✓	✓	✓
Pier 40	✓	×	×	×	×	✓
Atlantic Sturgeon (*Acipenser oxyrinchus oxyryinchus*)						
	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 2023. Norrie Point samples were not taken in June. Checks represent positive bands observed during gel electrophoresis.

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Major Findings

Atlantic Sturgeon

Atlantic sturgeon continued to be least observed of the three species, with their DNA present in less than half of the 2023 samples and even when present, often seen in trace amounts. Sturgeon were observed in every site besides Piermont 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 summer. These data may indicate adult migration upstream in the late spring, but more samples are required to understand fluctuations. Sturgeon will reside in brackish/fresh water for up to 6 years before they return to the sea, therefore, it is not surprising to see sturgeon presence further up the estuary.

Striped Bass

Striped Bass DNA was found in nearly all samples with only a few absences, in keeping with findings to date (**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.

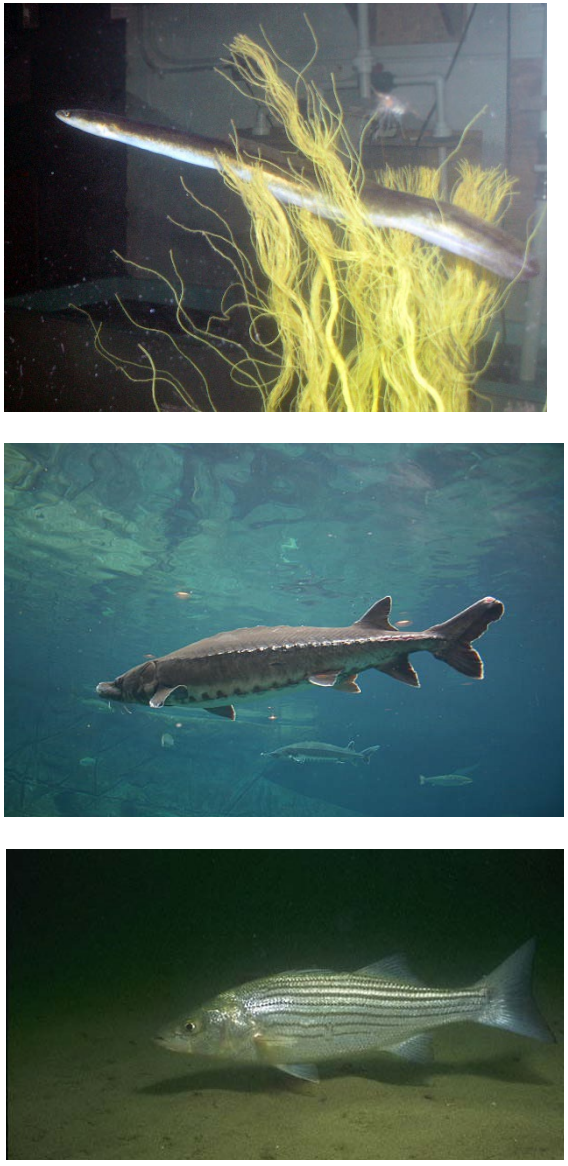

Fig. 4 | American eel (top), Atlantic Sturgeon (middle), and Striped bass (bottom).

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Takeaways

eDNA sampling is an effective, 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 more equipment, materials, and technical expertise; however, our partnership model delineates ways in which networks can work together to collect sampling through collaboration.

American eels and striped bass appear to be dwelling throughout the lower Hudson estuary, while sturgeon travel through the sample sites and do not reside long-term within the Lower Estuary during the sampling season.

Fig. 5 | River project staff collecting eDNA water samples, Pier 40.

Future Directions

The Park's River Project hopes to continue using eDNA analysis to supplement its fish ecology survey by providing additional presence/absence information on fish not typically caught in standard collection gear.

The Park is looking to investigate more species of interest in coming years, especially taxa of interest and/or concern to the State, such as the round goby (*Neogobius melanostomus*).
The Park is also seeking to conduct next-generation sequencing, a broad-spectrum diagnostic method of DNA analysis that can identify dozens or even hundreds of species through DNA present in a sample, to expound upon the findings and provide a wider picture of the fish species present in the Lower Estuary.

Fig. 6 | Preparing molds for gel electrophoresis.

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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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