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RIVER PROJECT

# Environmental DNA Report 2022


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# Purpose

Environmental DNA, otherwise known as eDNA, is an innovative method of using water samples to identify fish species through the extraction and amplification of DNA floating around naturally in water. This method allows for efficient, non-invasive monitoring of fish populations and is a useful complement to the biodiversity study the Park has had in place for over thirty years.

The purpose of this project is to examine the migration patterns of three different fish species in the Hudson River: the American eel, striped bass, and Atlantic sturgeon. We are partnered with three research facilities located along the Hudson River who send filter samples monthly during the field season. There are four locations in total where water samples are collected along an approximately 85-mile-long stretch of the Hudson River (**Fig. 1**) to test for the presence of these three fish species via the presence or absence of their DNA in the water.

# Key Research Questions

- How can eDNA be used to supplement traditional sampling methods?
- Can eDNA be used to track presence/absence of key diadromous fish species in the Lower Hudson Estuary?

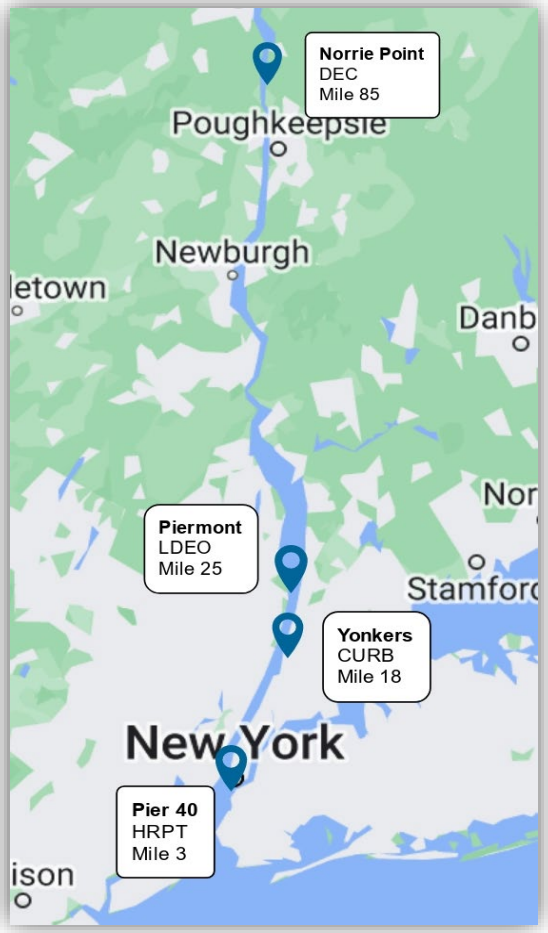


**Fig. 1** | Map of the four sampling locations. From North to South: Norrie Point Environmental Center (NYS Department of Environmental Conservation), Lamont-Doherty Earth Observatory at Piermont Pier, Sarah Lawrence College Center for the Urban River at Beczak in Yonkers, and Hudson River Park at Pier 40.

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# Methods

- From May to September, 1L surface water samples were taken at each of the four sites within a handful of days of each other; typically, on the same date.
  - Piermont and Pier 40 saw additional samples in April
- Samples were filtered same day via vacuum pump through a 0.45µm filter on site and mailed overnight on ice to Hudson River Park.
- Once received, DNA from both sample and blank filters from each site was extracted via QIAGEN PowerSoil Pro Kit to produce elutions ready for amplification
- Elutions were then subjected to two rounds of nested PCR according to Stoeckle et al., (2018) Go Fish methodology.
  - First round uses MiFish general vertebrate fish primers
  - Second round uses species-specific MiFish primers for the target species: American Eel, Striped Bass, and Atlantic Sturgeon. (**Appendix**)
- All PCR products were loaded into 2% agarose gels, ran in 1X TBE buffer for 30 mins at 130V (**Fig. 2**) and read via UV transilluminator.

July 2022 eDNA Results

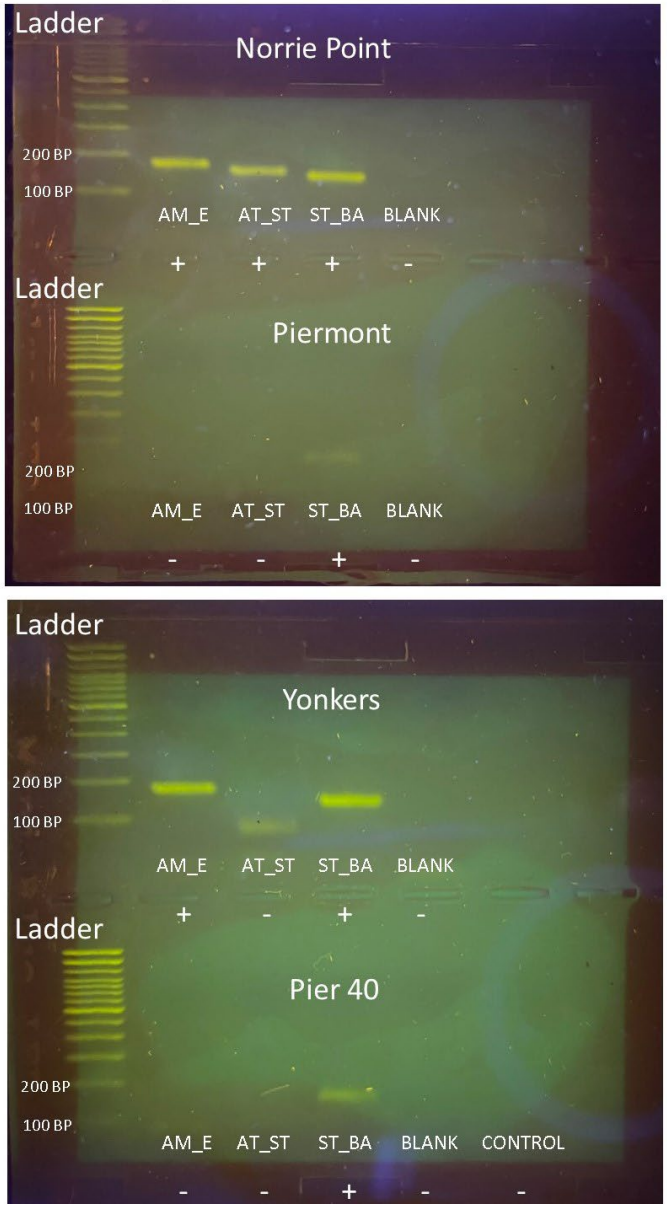


**Fig. 2** | Annotated photo of gel electrophoresis results from July 2022.

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# Preliminary Findings

All three target species were observed over the course of the 2022 sampling season.

## American Eel

American eel DNA was found in nearly all samples, with the only negatives in June & July at the southernmost site – Pier 40, as well as July at Piermont (**Fig. 3**). It is possible that the hits at Pier 40 in April/May and subsequently in August/September reflect an influx of elvers into coastal waters in spring, and migration of adults to the sea in fall, respectively. However, many more data will be needed to ascertain whether this is the case or simple inconsistency of sampling. Regardless, it is known through other survey methods that there are consistent eel populations at most sites throughout the sampling season, including Pier 40.

| 2022 eDNA Results | | | | | | |
|---|---|---|---|---|---|---|
| American Eel | | | | | | |
| | April | May | June | July | August | Sept. |
| Norrie Point | | ✓ | ✓ | ✓ | ✓ | ✓ |
| Piermont | ✓ | ✓ | ✓ | × | ✓ | ✓ |
| Yonkers | | ✓ | ✓ | ✓ | ✓ | ✓ |
| Pier 40 | ✓ | ✓ | × | × | ✓ | ✓ |
| Atlantic Sturgeon | | | | | | |
| | April | May | June | July | August | Sept. |
| Norrie Point | | × | ✓ | ✓ | × | ✓ |
| Piermont | ✓ | × | × | × | ✓ | × |
| Yonkers | | × | ✓ | × | ✓ | × |
| Pier 40 | ✓ | × | × | × | ✓ | × |
| Striped Bass | | | | | | |
| | April | May | June | July | August | Sept. |
| Norrie Point | | ✓ | ✓ | ✓ | ✓ | ✓ |
| Piermont | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Yonkers | | ✓ | ✓ | ✓ | ✓ | ✓ |
| Pier 40 | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |

**Fig. 3** | Chart of results from April-September 2022. Only Pier 40 & Piermont samples were taken in April.

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# Preliminary Findings

## Atlantic Sturgeon

Atlantic sturgeon proved to be the most elusive of the three species, with their DNA present in less than half of the 2022 samples, and even when present, often just barely detectible, indicating only trace amounts of DNA. The positive hits are sporadically distributed with sturgeon only observed in trace amounts at the southernmost site (Pier 40) in April and August, and by September only at the northernmost site (Norrie Point) (**Fig. 3**). This does, however, line up with the expected adult migration up and downstream in the spring and fall, with maturing populations remaining much further north up the Hudson. Sturgeon will reside in brackish/fresh water for up to 6 years before they return to the sea, so it is not surprising to see positive gels further up the estuary.

## Striped Bass

Striped bass were found to be the most prevalent with positive signals in all samples taken from all sites in both 2021 (June - August; see 2021 eDNA results for details) and 2022 (April – September) (**Fig. 3**). This suggests that, even though they are a diadromous species whose adults spend their time in deeper, coastal marine waters, there are populations of young stripers throughout the LHRE. It is known that 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.

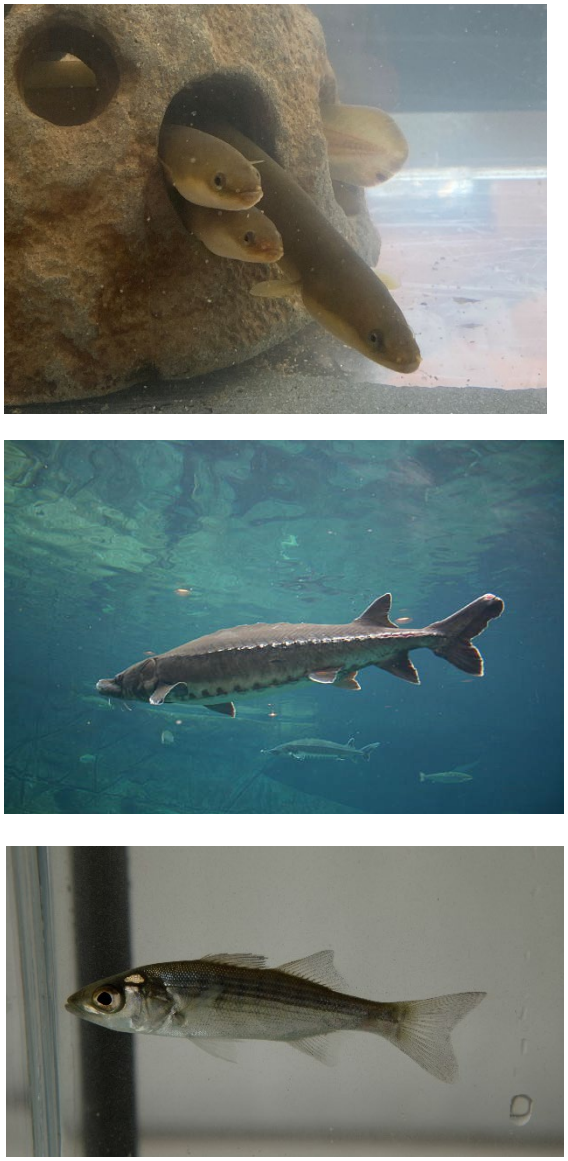

**Fig. 4** | American eel (top), Atlantic Sturgeon (middle), and Striped bass (bottom).

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# Takeaways

eDNA sampling is an effective, non-invasive sampling method that allows for relatively low-cost and effort observation of aquatic species, making it accessible to groups with varying levels of funding and bandwidth. Processing requires significantly more equipment, materials, and technical expertise, but our partnership model delineates ways in which networks can come together to collect and observe findings about their local species.

American eels and striped bass have been observed to be highly prevalent throughout the lower Hudson estuary, while sturgeon appear to be more difficult to track. This could be for a number of reasons: 1) their scutes – much larger and tougher than scales – don't shed or degrade as quickly and may release less DNA into the water, 2) they are only spending limited time in the lower Hudson as they pass through to the fresher waters of the north Hudson Valley and the Capital region, 3) when migrating, they likely keep to the center channel, which has deeper, faster moving water that may direct DNA particles away from the near-shore sampling areas, especially in wider sections of the River. Nevertheless, these practically living fossils were indeed detected at times that are reasonable to expect their presence.

The method is not without its challenges, however. Due to the relatively large time sink that is extraction and PCR amplification, this project began with a low frequency of water samples (monthly). More frequent sampling would provide much finer data on the incidence of species' presence and possibly inform more conclusive assessments of migration patterns, especially for Atlantic sturgeon.

**Fig. 5** | Forceps taking out a 0.45µm membrane filter

**Fig. 6** | A filter after a water sample was vacuum pumped through it

**Fig. 7** | Storing a run filter in a falcon tube for later extraction

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# Future Directions

The Park's River Project hopes to continue using eDNA analysis supplement its fish ecology survey by providing additional presence/absence information on fish not typically caught in standard collection gear. The sampling project has also brought together state agencies, academic institutions, and education centers to collaboratively investigate key fish species common to all parties; something the Park will strive to continue to facilitate in order to support regional peers and gather more data.

Contamination remains an occasional issue – especially for sites without full laboratory space, but 2022 saw dramatic reduction in contaminated blanks over the course of the season as we worked with sampling sites to enhance sterilization techniques.

The Park is looking to investigate more species of interest in coming years – such as the round goby which is being found with increasing frequency in the northern Hudson and will additionally consider sending samples to be analyzed via metabarcoding and next-generation sequencing to better utilize the samples and gain insight into the presence of many more species than would be feasible solely using gel electrophoresis. The Park will also consider additional sites and/or higher frequency of samples if funding and bandwidth allows.

**Fig. 8** | River project staff collecting water samples

**Fig. 9** | Setting molds in the gel electrophoresis chamber

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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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