Celebrities or "Shell"-outs? Incorporating Novel eDNA Analysis to Survey the Bronx River's Freshwater Turtle Species

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Introduction

The Bronx River flows south through Westchester County, the Bronx Zoo, and the New York Botanical Garden, before emptying into the East River. Impounding the Bronx River, however, lie nine dams that mitigate rapid water flow and prevent flooding, and highways that impede the welfare of its ecosystem by way of air, noise, and chemical pollution.[2,6] Despite these disturbances, the Bronx River serves as a habitat for riparian plant species, fish, macroinvertebrates, birds, and aquatic reptiles.

Img. 1 - *C. picta* (painted turtle), *exhibited at the Bronx Zoo. Credit: Aveena Khan*

While several species of freshwater turtles occupy the Bronx River, this study focuses on **three** of them: ***Trachemys scripta*** (slider turtle), ***Chelydra serpentina*** (common snapping turtle), and ***Chrysemys picta*** (painted turtle). While *C. serpentina* and *C. picta* are native to North America's northeastern waters, *T. scripta* was introduced from its native range in the southeastern and central United States and overlaps with the ecological niches of native freshwater turtles in the northeast.[4,5] One particular subspecies of *T. scripta,* named *T. s. elegans* or the red-eared slider, consistently outcompetes native species due to their higher resistance to pollutants, predation efficiency, and rate of generational growth, marking them as one of the region's most detrimental invasive species.[4,9]

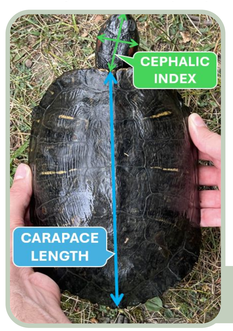
Although the exact population count of the turtle species is difficult to assess due to their elusive, aquatic nature, the presence of each species may now be confirmed with ***eDNA analysis***. eDNA, or *environmental DNA*, is deposited into an organism's surroundings as waste, reproductive substances, or fragments of dead epithelial cells, and can be amplified using standard PCR procedures.[3] This process has slowly been integrated into conservation endeavors, and so by collecting water samples from two Bronx River sites, they can then be filtered to isolate and amplify any DNA present to detect the three turtle species in question.[3]

Using traditional hoop-trapping techniques and piloting eDNA extraction for turtle-specific genomic sequences, this project aims to evaluate two primary predictions regarding the composition of turtle species within the Bronx River: **the invasive *T. s. elegans* will be more prevalent and therefore be captured at a higher rate than the native *C. serpentina* and *C. picta***, and that **positive detections of *T. s. elegans* eDNA will be highest out of all three studied species** for similar reasons.

Methodology

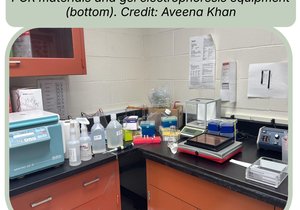
Site Selection - Two sites were selected along the Bronx River: the Twin Dams (Site TD) and the Mitsubishi Riverwalk (*Site MR-A* - eDNA collection; *Site MR-B* - hoop trap deployment). Site TD is closed to visitors and generally remains undisturbed, except for research activities. In contrast, Site MR is open to the public, which exposes its waters to human disturbance and pollution. Both sites have been previously used for hoop trap surveys in past years of Project TRUE.

Fig. 1 *Map of field research sites.*



Img. 2
*Female **T. scripta** biometrics at Site MR. Credit: Aveena Khan*

Turtle Biometrics - Turtles with a carapace length of **10 cm or more** were sexed by assessing physical dimorphic traits; a turtle whose measurements are *below* this threshold are more than likely juvenile, and thus not yet sexually matured.[1] Physical traits pertaining to the sex of each turtle vary per species, so the following presentations were examined: *claw length*, *tail length*, *carapace length*, and *approximate cephalic index*.[7] Carapace length was measured using a digital caliper. For safety purposes and the turtle's comfort, claw, tail, and cephalic measurements were estimated visually. The weight of each specimen was taken by using a lightweight bag to hold the turtle, then measured with a gravity scale. All specimen were released afterward.



Img. 3 & 4 *eDNA filter equipment (top); on-site PCR materials and gel electrophoresis equipment (bottom). Credit: Aveena Khan*

eDNA Analysis - By the end of this study period, 18 aquatic eDNA samples were collected; 9 from Site MR-A, 8 from Site TD, and 1 blank control. Each sample was approximately 1 liter in volume, and obtained *before* hoop traps were taken down to mitigate cross-contamination. Using manual vacuum filtration, the samples were passed through qualitative filter paper to collect concentrated sediment, which was then soaked in 2 mL of absolute, molecular bio grade ethanol immediately to preserve any eDNA caught in the filter. Samples were taken to the onsite Wildlife Health Center Molecular Lab to be refrigerated at -80°C to minimize DNA degradation until extraction and analysis.

Once eDNA was extracted, a *polymerase chain reaction* (PCR) was performed in order to amplify isolated DNA sequences for species detection. **Three unique primers** were designed and utilized to target *T. s. elegans, C. serpentina,* and *C. picta* multivariate CO1 genes within the samples, which were finally visualized via gel electrophoresis in **Fig. 4**.[3]

Abstract

The **Bronx River** is a habitat for a large index of native and non-native species. The river, being the only true freshwater river in New York City, contains a highly competitive ecosystem, including native populations of *Chelydra serpentina* and *Chrysemys picta* turtles and a detrimental rise of invasive *Trachemys scripta elegans* turtles. To survey these aquatic reptiles, this study used traditional hoop trapping methods, and piloted the implementation of eDNA analysis to confirm the presence or absence of the aforementioned species within two sites of the river. It was found that invasive turtles had a higher capture rate in comparison to native ones. This trend was *not* reflected after performing eDNA analysis, where there was an equal positive detection of native and invasive species. However, there were no traces of *C. picta* in the traps or eDNA samples, suggesting that the non-native *T. s. elegans* are outcompeting this sensitive native species. Additionally, the secluded, steadily-flowing Twin Dams region had a higher turtle capture rate than the publicly-accessible, stagnant Mitsubishi Riverwalk site; however, positive eDNA readings were exclusively found at the Mitsubishi Riverwalk. Thus, this data concludes that eDNA deposits are more potent in slower moving waters, and less so in faster currents.

Research Questions & Hypotheses

Question 1: **How does the capture rate of non-native turtle species (*T. s. elegans*) compare to the capture rate of native species within accessible sites along the Bronx River?**
Hypothesis 1: Because the invasive red eared slider *overlaps* the native turtles' *ecological niche* while possessing comparatively *faster generational growth and more efficient predation*, the rate of invasive species captured is expected to be *higher* than the rate of native species captured.

Question 2: **How do eDNA-positive detections compare between three different freshwater turtle species along accessible sites of the Bronx River?**
Hypothesis 2: The number of eDNA-positive *T. s. elegans* detections will be *higher* than those of the river's native turtle species (*C. serpentina and C. picta*).

Question 3: **How does the potency of aquatic eDNA vary between two accessible sites of the Bronx River?**
Hypothesis 3: Because the Mitsubishi Riverwalk section contains *more turbid, sediment-enriched* water than the Twin Dams site, there will be *more eDNA-positive samples* from Mitsubishi Riverwalk than from Twin Dams.

Results & Figures

Turtle Capture Statistics

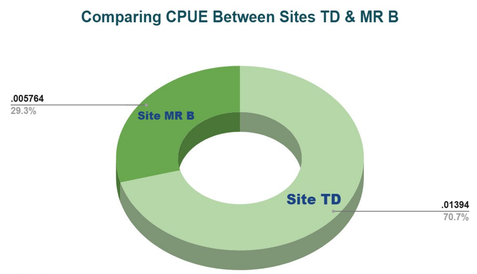


Fig. 2
*A formula adopted by Waye[11] was used to quantify the **CPUE**, or **catch per unit effort**, of Site TD and Site MR-B to assess their respective efficacy:*

$$\frac{(\#\ of\ turtles\ caught)}{(\#\ of\ traps) * (hours\ of\ deployment)}$$

As shown, the CPUE of Site TD is 242% higher compared to Site MR-B.

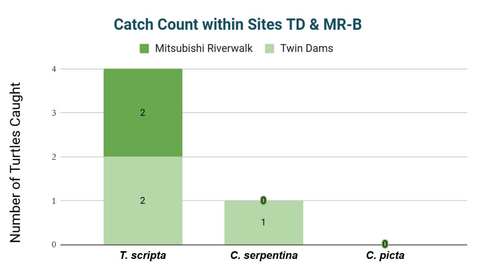


Fig. 3
Site TD had a greater capture count than Site MR-B while displaying greater species richness. The two catches at Site MR-B were identified as the same female T. scripta turtle.

Gel Electrophoresis

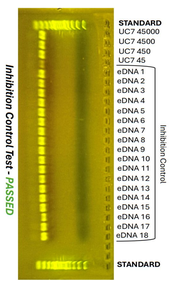


Fig. 4a
Each eDNA sample was tested for PCR inhibitors that may result in false negative results. No inhibitors were detected.

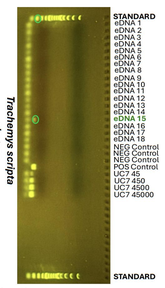


Fig. 4b
***One** positive **T. s. elegans** detection at 'eDNA 15'; this sample was collected at Site MR-A on July 24, 2025, immediately before capturing the same species.*

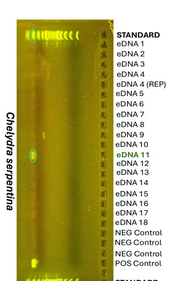


Fig. 4c
***One** positive **C. serpentina** detection at 'eDNA 11'; this sample was collected at Site MR-A on July 21, 2025. However, this species was not seen at this site for the duration of the study.*

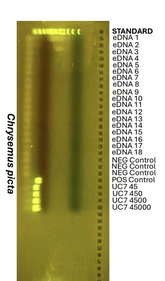


Fig. 4d
***No** positive **C. picta** detections.*

Discussion

Hoop Trap Interpretations - As displayed in **Fig. 3**, the capture count of native turtle species is significantly lower than those of non-native species, which is concurrent with the hypothesis. Our research results diverge from those of past turtle surveys in the region, where there was a higher capture count of native species at Site MR-B.[8] Further, this study processed three unique turtles at Site TD: two invasive ***T. s. elegans*** and one native ***C. serpentina***, as well as one ***T. s. elegans*** at Site MR-B that was captured twice. There were no captures of ***C. picta*** at either site.

It is important to note that out of **seven** overnight traps, the first three were baited with half a tin of sardines, and the other four a full tin. Those traps with less bait did not capture any turtles, while the traps with double the amount caught at least one turtle per deployment, which **may have skewed the calculated CPUE.** This oversight could have been responsible for the lack of attraction from the larger *C. serpentina*,[5] but, the volume of bait should not have affected the capture rate of the ***C. picta***, as it is similar in size to *T. scripta*.[10] Lastly, due to extended periods of torrential rain, only seven of our projected twelve traps were able to be deployed, thus reducing the sample size of our data.

eDNA Interpretations - Results following our eDNA analysis demonstrate that there was an even detection rate between native and non-native species, which refutes our initial hypothesis. Out of 18 samples, only **2 positive detections occurred**: one for *T. s. elegans* (**Fig. 4b**), the other *C. serpentina* (**Fig. 4c**). With no detection of *C. picta* (**Fig. 4d**), it is plausible that *T. s. elegans* is drastically outcompeting this species at both sites. The distribution of both detections occurred at **Site MR-A**; a lack of detection at Site TD is perhaps due to the faster waterflow sweeping away traces of eDNA. In contrast, Site MR-A's waterflow is much slower, with a visibly higher turbidity. The conditions at Site MR-A allowed for higher sediment buildup, which likely kept any deposited eDNA within the sample collection range.

On the other hand, some **human error** could have an effect on our PCR results as DNA is extremely sensitive. Sample 17, collected from Mitsubishi Riverwalk, may have been accidentally contaminated due to a faulty tip when pipetting. Our detergent (AL buffer) was excessively vortexed, resulting in overly-sudsy samples that may have lowered the amount of undamaged DNA we were able to extract from each conical tube, especially samples 1 - 5, 14, 15, and 17.

Img. 5
Team recovering a hoop trap at Site TD. Credit: Naima Hossain

Img. 6
***C. Serpentina** recovered from a hoop trap at Site TD. Credit: Aveena Khan*

Conclusions

This study supports further usage of eDNA within the Bronx River to detect its turtle species, but **only** at the Mitsubishi Riverwalk site. On the contrary, Twin Dams had a greater species richness and higher catch count than the Riverwalk. To maximize possible positive detections of any given aquatic species in these regions, the following best practices are suggested for future projects: *keeping water samples cooled while transporting* to minimize DNA degradation, *increasing the number and area of sampling sites* for eDNA analysis, and *ensuring hoop traps are sufficiently baited* to attract target species.

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