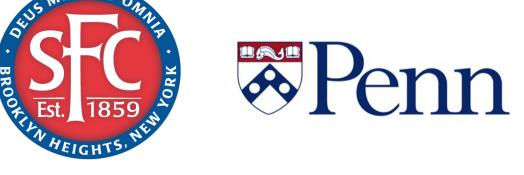
# Aquatic Biomonitoring: Analyzing Invasive Species in Urban Estuaries as Indicators of Ecosystem Recovery



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### Abstract

Aquatic ecosystems near urban centers are subject to countless negative environmental influences; this is especially true of Newtown Creek, New York. The creek is believed to support populations of pollution-tolerant invasive organisms while local biodiversity struggles to thrive. This investigation serves to identify invasive species via DNA barcoding and use their presence to assess the estuary's health. We hypothesized that numerous invasive bivalves/amphipods would be recognized due to their adaptations to pollution. DNA from the East River and Newtown Creek specimens was isolated through the DNALC protocol; Genewiz sequenced PCRs. Invasive bivalves from Newtown Creek indicate tolerance to relatively low dissolved oxygen levels as well as variable salinities. The amphipods/bivalves from the East River illustrate the estuary's healthier comparative oxygen levels and variable salinity. While the East River is home to numerous invasive species, its conditions are superior to Newtown Creek's where nonnative organisms dominate the nutrient deficient waters.

## Introduction

#### **Newtown Creek Background Information:**

- The heavy industrialization which transpired along the shores of Newtown Creek, an EPA Superfund site, resulted in polluted, stagnant waters. Newtown Creek is heavily contaminated by heavy metals, oil runoff, chemicals, and other toxicants from neighboring refineries and distilleries.
- 14,000 million gallons of rainwater runoff, raw domestic sewage, sewage overflow, and industrial wastewater are expelled into the creek annually. The pollutants, due to the lack of a current, are unable to exit making the concentration of harmful substances several times more dangerous (Figure 1).
- Despite recent progress in establishing small communities of wetland plants, blue crabs, fish, and waterfowl, it is clear that the creek has not achieved the level of recovery promised by the EPA.

#### **Biological Threats:**

- While it currently has limited ecological functionality, numerous non-endemic species are believed to be adapted to live in the unhealthy waters.
- Data collected by the NYC Department of Environmental Protection indicate less than or equal to 3.0 mg/L of dissolved oxygen. In addition, water collected at Plank Road indicates high levels of nitrates and phosphates which is problematic in terms of oxygen transport and availability
- These conditions, while detrimental for many species of fish, do not prevent the survival of many bivalves and amphipods. Due to their unique physiological structures, many amphipods and bivalves have been shown to survive in extremely variable or extreme waters.

#### **Specific Aims:**

While extensive water quality reports have been filed regarding Newtown Creek, information on the ecosystem's invasive species is scarce.

- This investigation, through the application of DNA barcoding, aims to identify and utilize invasive species as indicators of ecosystem recovery. By cross referencing identified species with nonnative species catalogues and toxicant tolerance literature, we present a preliminary assessment of Newtown Creek.
- The process of aquatic biomonitoring serves as a method to further understand the effects of pollution on urban estuaries. We hypothesized that numerous invasive species would be identified, especially amphipods and bivalves that are tolerant to variable toxicant levels.

## Materials and Methods

Samples were collected from Newtown Creek as well as the East River. These samples included bivalves and amphipods. We collected a variety of samples in the fall of 2015 (prior to the first frost). Samples were also collected from points on the Newtown Creek Nature Walk in the spring of 2016. We gathered a total of 22 samples from the East River in the fall (12 amphipods, 1 bivalve, 7 insects, and 2 plants) and two bivalves were collected at Newtown Creek in the fall. We collected more samples in the spring from Newtown Creek (6 plants and 5 bivalves). We collected samples while wearing nitrile gloves and placed each sample in a 1.5mL microcentrifuge tube which were subsequently labeled in ascending order.

The DNA extraction procedure outlined in the DNALC protocol was followed. PCRs were set up using: 10.5 microliters of water, 10 microliters of TAQ, 2.5 microliters of DNA template, and 2 microliters of primer (HCO2195 and LCO1490 - Invertebrate; a PCR was also set up using the mammalian primers from Genelink). A second set of PCR reactions was set up using the PCR beads provided in the Carolina DNA barcoding kits and the mammal primers. The unpurified PCRs were sequenced by Genelink and results were analyzed using DNA Subway.

# Results

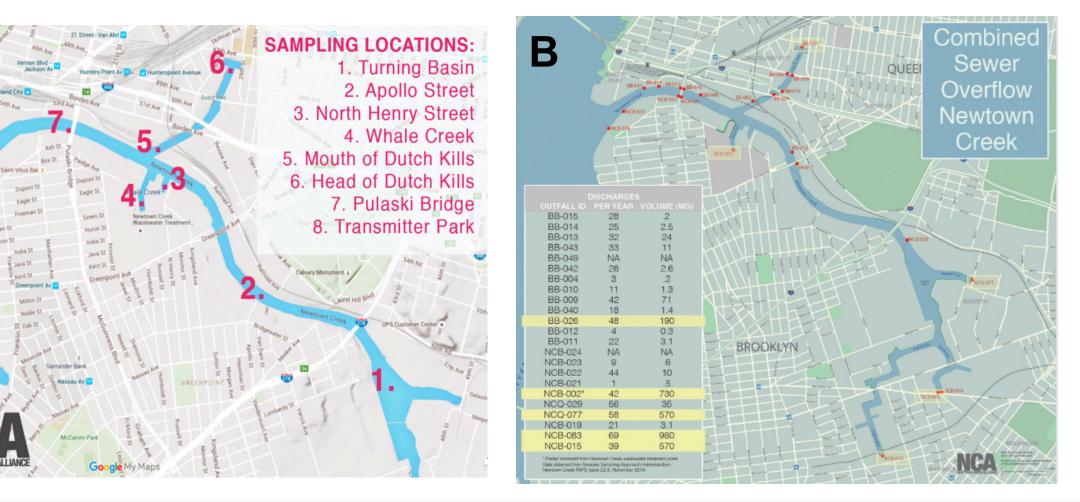


Figure 1. Specimen Collection: A. Sample locations along Newtown Creek. Samples were also collected from the East River at Transmitter Park and Brooklyn Bridge Park (Brooklyn side) and Near South Street Seaport (Manhattan side). B. Sewer outflow locations along the creek, one of many sources of pollution at this Superfund Site. Images modified from the Newtown Creek Alliance (NCA).

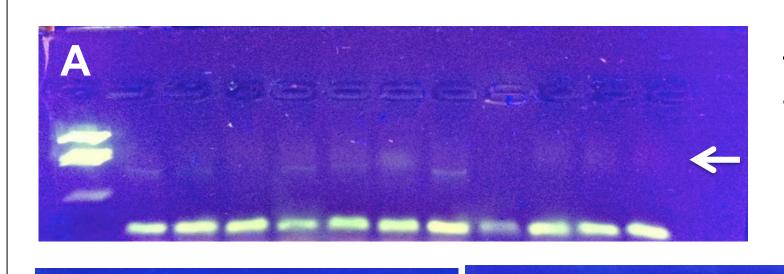
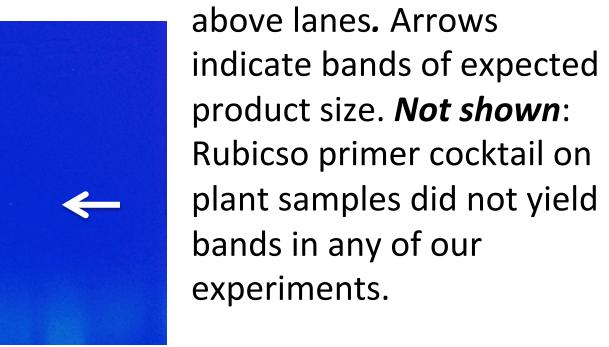
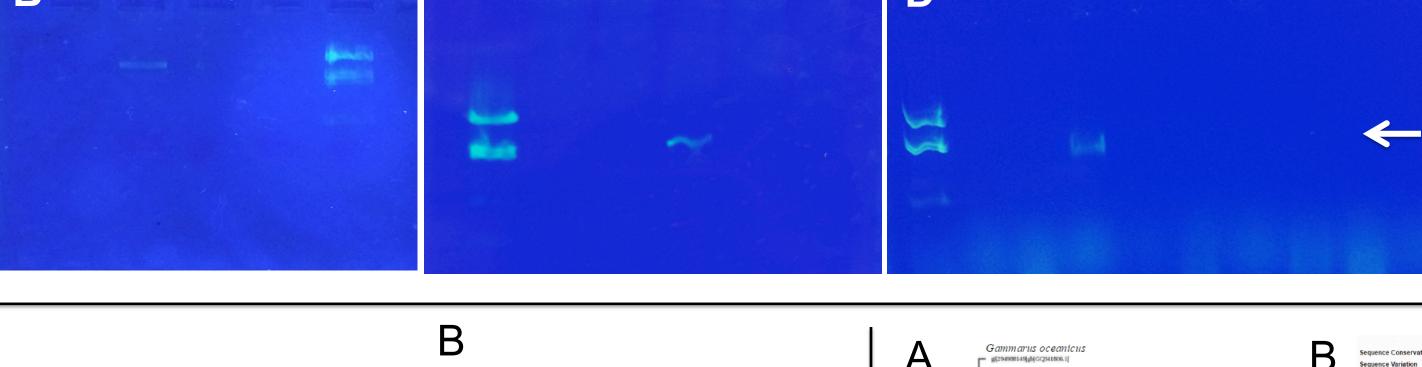


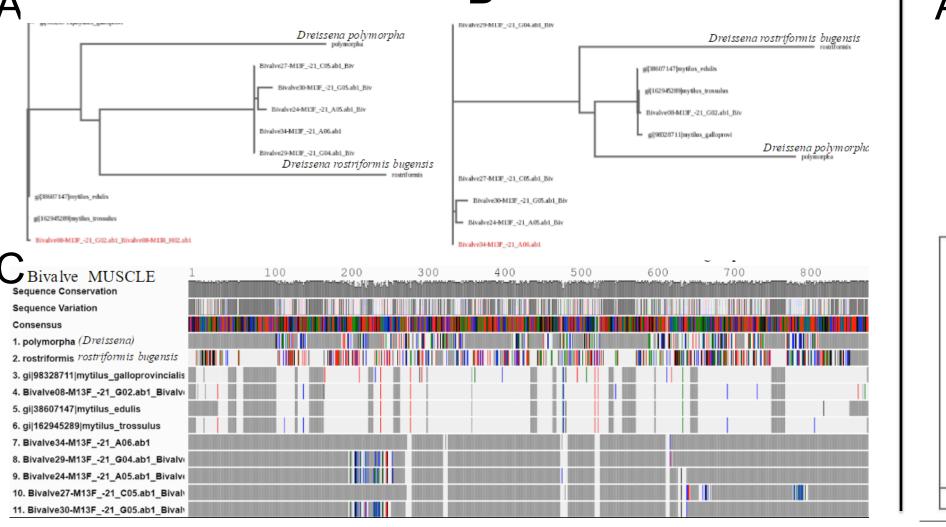
Figure 2. Representative PCR results from DNA isolated from collected **samples:** A. Carolina CO1 primer cocktail yielded the strongest results despite the presence of primer dimers. Additional reactions were performed using HCO2195 and LCO1490 primers for invertebrates (Genelink). B-D. Representative gels using invertebrate primers. Sample numbers are indicated

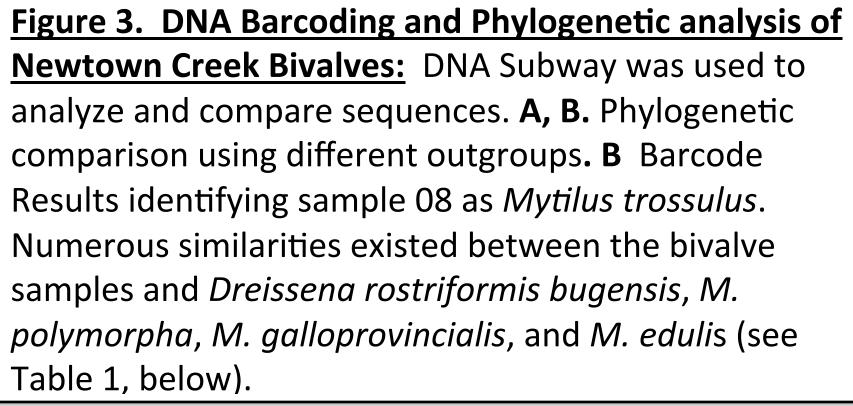


Amphipod13-M13F\_-21\_A03.ab1\_Am



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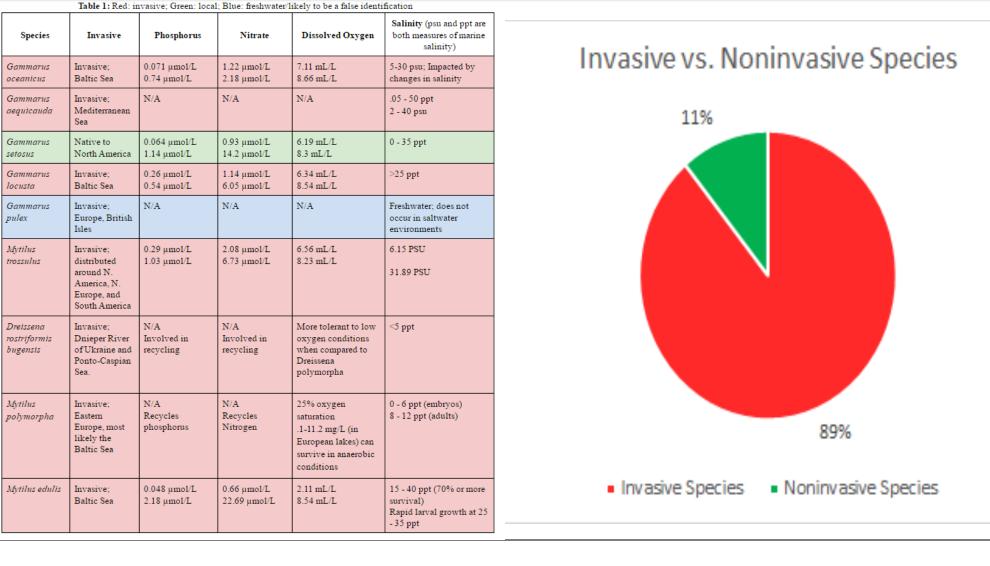


Figure 4. DNA Barcoding and Phylogenetic analysis of Newtown Creek Amphipods: DNA Subway was used to analyze and compare sequences. A, C. Phylogenetic comparison using different outgroups identified from literature. B. Barcode Results suggest samples: 16, 15, 07, 02, 14, 04, and 13 are closely related. This group can be subdivided into a more exclusive clade which includes amphipods: 07, 02, 14, and 04. The species we collected are novel to DNA Subway. To create a more accurate phylogenetic tree, we included accession numbers from the literature.

Table 1: Identification of invasive species. Selected amphipod species correlated with endemic habitats and tolerance to various environmental variables. Of the amphipods collected from the East River we identified three that were closely related to Gammarus aequicauda and G. oceanicus. Amphipod samples were shown to be closely related to the species G. setosus, G. pulex, and G. locusta Figure 5: Provisional/Tentative identification of Newtown Creek samples as "invasive" (non-endemic) or "noninvasive" (endemic) based on sequence relatedness shown in Figure 4 (above.)

#### Discussion

The DNA sequences of our amphipod samples are novel to DNA subway, and we did not identify a match using BLAST. Our analysis is based on the literature. We may have identified novel species, or were unable to discover a database match. Either is possible given the lack of genetic and morphological research conducted on Amphipoda. We base our provisional identification of invasive vs. endemic species on our literature search. Some of the bivalves we identified also were not found in the DNA subway analysis, though poor sequence quality may have complicated our analysis in this case. Coexisting populations of M. trossulus and M. edulis are known for hybridizing. Collection of hybrids could have further prevented specific species identification. Of the relative species, eight invasive species were identified.

Our results indicate a low recovery for Newtown Creek. In terms of the related species, there is a wide range of toxicant tolerance levels. Our data suggests that Newtown Creek has variable salinity levels, limited available dissolved oxygen, and unhealthy phosphorus and nitrate concentrations. The amphipods collected in the East River indicate that this urban estuary has a smaller saline range (due to the survival of *G. locusta*) and more than 6.19 mL/L of dissolved oxygen. The East River thus serves as a model of a healthier urban estuary when compared with Newtown Creek. The use of aquatic biomonitoring techniques put water quality data and EPA estimates into perspective. It is clear that the conditions of the creek are not only harmful due to their inability to allow populations of fish and other aquatic life, but they also foster beneficial environments for invasive species as was hypothesized. We discovered potentially new species of amphipods and hybrid populations of bivalves which would require further research. To bolster our results, we plan on investigating a larger amount of amphipods and bivalves. To ensure optimal results in the second stage of our experiment, we plan on extracting DNA sooner to prevent degradation.

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