Barcoding Plant Diversity Around Newtown Creek

Bonnie Chen[1] and Katya Naphtali[2], Lisa Bloodgood[3], Alison Dell[4,5]

1 Brooklyn Technical High School; 2 Institute for Collaborative Education; 3 Newtown Creek Alliance; 4 St. Francis College; 5 Department of Neuroscience; Perelman School of Medicine at the University of Pennsylvania

Abstract

Newtown Creek is a Superfund site polluted by centuries of continuing industrial use. Since ecosystems in areas of high industrial activity are destabilized by decreased biodiversity (Hautier, et al.) It was hypothesized that biodiversity along the creek would inversely correlate with ongoing human disturbance. To complement an existing citizen science plant survey; species diversity at two different sites were assessed during fall and spring. Our barcoding results validated and extended the observations of the citizen scientists and the Simpson Diversity Index (SDI) was used to quantify diversity along the creek. Similar SDI's were calculated across experimental and sampling conditions. UBRP sampling yielded an SDI of .09524 compared to the *iNaturalist* SDI calculated 0.98039 for Vernon Blvd. This initial finding using a small sample size suggests that both sites had similar levels of biodiversity.

Introduction

Newtown Creek is a tributary, branching off from the East River, that separates the boroughs of Brooklyn and Queens. Hautier described how human impact and pollution devastates biodiversity of plant and animal populations (Hautier, et al.). Hooper reported that a lack of biodiversity also leads into a lower habitat functionality and stability (Hooper, et al.). It was predicted that areas with lower human intervention will have higher biodiversity. It is also predicted that the locations analyzed in April would have a higher species diversity because of the time of year and would be a better representation of the area's species index. To analyze how biodiversity surrounding Newtown Creek, we used DNA barcoding to complement a citizen science biodiversity survey led by local organizations Newtown Creek Alliance (NCA) and Hudsonia. Participants in the survey used a plant identification app, *iNaturalist*, to document the species surrounding Newtown Creek. We used random quadrat sampling followed by barcoding to assess biodiversity at two different access points along Newtown Creek at two different times (November 2017 and April 2018). By analyzing the total diversity of these locations, we aimed to study ecosystem health and record prominent species of plants.

Materials & Methods

Sampling and Collection: We conducted random quadrat sampling at two Newtown Creek locations, Henry Street and Vernon Boulevard. We photographed, identified and geotagged plants using *iNaturalist*; following the protocol defined by NCA. Each sample was assigned a unique alphanumeric identifier. We preserved each plant for an herbarium, reserving a small portion of leaf tissue (about the size of a hole punch) for DNA barcoding. Herbarium samples were dried and pressed between paper and cardboard at room temperature. Barcoding samples were preserved in 1.5 ml centrifuge tubes at -20℃ until ready for DNA barcoding (approximately 7 days).

DNA isolation and amplification: We extracted genomic DNA from samples using the method described in Barcoding101 (DNALC 2014). We used this protocol for PCR amplification, using DNALC standard plant primers targeting the Rubisco gene (rbcLaF / rbcLa rev), together with PuReTaq Ready-To-Go™ PCR Beads (GE-Illustra) and 2.5ul template DNA in a 25ul rxn. We verified PCR results using agarose gel electrophoresis and GelGreen staining, visualized using a digital transilluminator (BioRad Gel Doc). GENEWIZ performed sequencing with universal M13F/R primers.

Sequence Analysis: Sequences were assessed, aligned, trimmed, and analyzed using the Blue Line of DNASubway. We used BLAST to identify each plant. These methods were repeated for both sampling sites in November and April to analyze pre- and post- winter biodiversity levels.

Biodiversity Assessment: We measured biodiversity using Simpson's Diversity Index (SDI) using an online calculator. The SDI allows for a comparative numerical analysis of both species diversity and richness resulting in a 0-1 ranking from least to most diversity.

Results

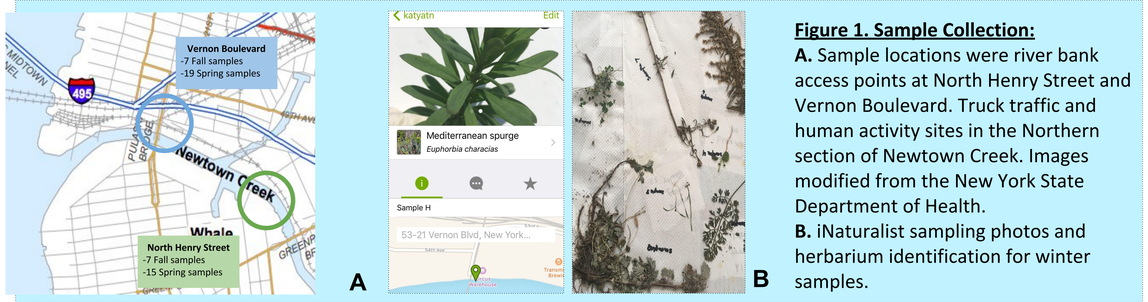


Figure 1. Sample Collection:
A. Sample locations were river bank access points at North Henry Street and Vernon Boulevard. Truck traffic and human activity sites in the Northern section of Newtown Creek. Images modified from the New York State Department of Health.
B. iNaturalist sampling photos and herbarium identification for winter samples.

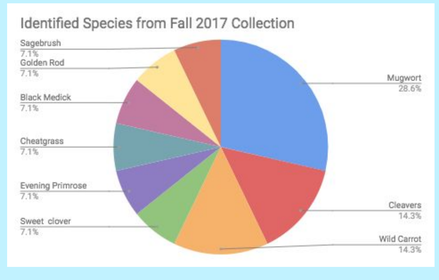
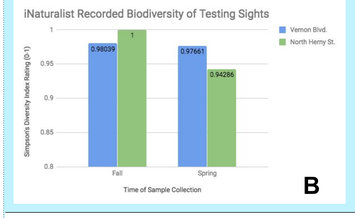


Figure 2. Species Identification: The above pie chart depicts the total amount of species identified and their proportions of diversity compared to other samples.

Sample #	iNaturalist App predicted:	DNA Blast /: Top result(s):
1-M13	Sweet clovers (*Melilotus*)	Sweet clovers (*Melilotus officinalis*)
2-M13	Common mugwort (*Artemisia vulgaris*)	Mugwort (*Artemisia vulgaris*) OR Sagebrush (*Artemisia tridentata*)
3-M13	Spotted Spurge (*Euphorbia maculata*)	Cleavers (*Galium aparine*)
4-M13	Pine (*Pinus*)	Cheatgrass (*Bromus tectorum*)
5-M13	Common Mugwort (*Artemisia vulgaris*)	Mugwort (*Artemisia vulgaris*) OR Sagebrush (*Artemisia tridentata*)
6-M13	Sassafras (*Sassafras albidum*)	Cleavers (*Galium aparine*)
7-M13	Butterfly Bush (*Buddleja davidii*)	Sagebrush (*Artemisia tridentata*)
A-M13	Wild carrot (*Daucus carota*)	Wild carrot (*Daucus carota*)
B-M13	Common Mugwort (*Artemisia vulgaris*)	Mugwort (*Artemisia vulgaris*) OR Sagebrush (*Artemisia tridentata*)
C-M13	Virginia Spring Beauty (*Claytonia virginica*)	Wild carrot (*Daucus carota*)
D-M13	Common Chickweed (*Stellaria media*)	Evening Primrose (*Oenothera bienalis*)
E-M13	Common Yellow Woodsorrel (*Oxalis stricta*)	Alfalfa (*Medicago sativa*) OR Medick (*Medicago truncatula*)
F-M13	Virginia Pepperweed (*Lepidium virginicum*)	Mugwort (*Artemisia vulgaris*) OR Sagebrush (*Artemisia tridentata*)
G-M13	Common Sow-thistle (*Sonchus oleraceus*)	Eaton's Aster (*Symphyotrichum eatonii*) Missouri Goldenrod (*Solidago missouriensis*)

Figure 3. Sample Identification: Table of the identification of winter collection samples with *iNaturalist* and Sequencing. Samples 1-7 were collected from North Henry. A-G were collected from Vernon Blvd. Bar color indicates DNA identified species corresponding with the pi chart (Figure 2).
Not shown: Samples collected in April 2018 are not included.

Testing Location	Fall 2017	Spring 2018 A
North Henry St.	Simpson's Index= 0.90476 (7 samples, 5 species)	Simpson's Index= _____ (15 samples, 11 species)
iNaturalist N Henry (excluding our data)	Simpson's Index= 1 (7 samples, 7 species)	Simpson's Index= 0.94286 (15 samples, 11 species)
Vernon Blvd.	Simpson's Index= 0.90476 (7 samples, 5 species)	Simpson's Index= _____ (19 samples, 16 species)
iNaturalist Vernon (excluding our data)	Simpson's Index= 0.98039 (18 samples, 15 species)	Simpson's Index= 0.97661 (19 samples, 16 species)



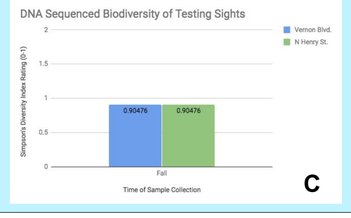


Figure 4: Simpson's Diversity Index:
A. Biodiversity calculation based on Barcoding and Citizen Scientist Observations. Using the Simpson's Diversity Index of 0-1, 0= least diverse 1= most diverse. Blue bars indicate barcoded observations at North Henry and Vernon while purple bars indicate local *iNaturalist* observations. Not shown: Samples collected in April 2018 are not included.
B. Diversity Index comparison of Citizen Scientist Observations Recorded using *iNaturalist*. Change seen in the North Henry St., Vernon Blvd. stayed relatively consistent.
C. Diversity Index comparison of sequenced identifications. Spring Samples not included.

It was hypothesized that ongoing ecosystem disturbance would decrease diversity so we chose sampling close to each other which differed in their accessibility (Figure 1). The sampling location at North Henry Street is subject to heavy local construction and truck traffic. On the other hand, the sampling location at Vernon Boulevard was chosen for its relative lack of truck traffic and human activity. Both locations were close enough in proximity that water pollution levels would be relatively similar. Using barcoding we were able to identify all sampled plants (n=14) at the genus or species level (Figure 2/Figure 3). For our fall collections, the species identified were Sweet Clover, Mugwort, Cleavers, Cheatgrass, Sagebrush, Wild Carrot, Evening Primrose, Medick, Eaton's Aster, and Goldenrod. The species with the highest abundance at these times were Mugwort and Cleavers. We calculated the biodiversity of each site using SDI. The biodiversity of the Vernon Boulevard and Henry Street testing sites were identical (SDI 0.90476). Seven samples were collected from each site with the total of five recorded species each (Figure 4, 6). To determine if our results differed from those of the Citizen Scientists participating in the NCA/Hudsonia survey we calculated SDI using the iNaturalist dataset. North Henry Street was rated a 1 on the Simpson's Diversity Index and Vernon Boulevard was rated as 0.98039 (Figure 4, 5) .

Discussion

We used DNA barcoding to complement a biodiversity survey in a disturbed ecosystem, the superfund site Newtown Creek. Two sites were assessed in both Fall 2017 and Spring 2018 using random quadrat sampling, recording observations using the app *iNaturalist*, preserving whole plants for an herbarium, and reserving a portion of leaf for barcoding. We predicted areas with less industrial activity would have higher biodiversity and that biodiversity would be greater in the spring. However, we did not observe a significant difference in biodiversity between these two sites. This may be due to several factors including small sample size at both locations; and late fall (many plants dead) and early spring (plants not yet sprouted) collection times. April's NCA observations suggest less diversity at North Henry St. (SDI 0.94286) than recorded at Vernon Blvd. (SDI 0.97661). However, in contrast to our samples, NCA sampling is not random. Survey participants may be less concerned with capturing overall biodiversity than with identifying novel plants. Citizen scientists may be familiar with common invasives such as *Artemesia vulgaris* (mugwort) and thus will not waste time and smartphone battery life by repeatedly photographing and uploading the entry to *iNaturalist*. Overall, barcoding was a valuable part of the NCA study - validating and extending results; and resolving identification disagreements. We will continue to work with the NCA study analyzing samples from more locations along the creek such as ExxonMobil, National Grid, or Maspeth. To deepen analysis of the ecosystem's biodiversity future barcoding studies might focus on identifying microorganisms within the testing-site's soil. Soil microorganisms support the health of local plant life because the plants are dependent on their secretion of nutrients (Maron et al.). Using barcoding of both plants and microorganisms within the soil, we can ask if the biodiversity of small organisms within the soil are correlated with the diversity of larger organisms. The diversity of both populations may also be influenced by local industrial activity.

References

1. Hautier, Y., et al. "Anthropogenic Environmental Changes Affect Ecosystem Stability via Biodiversity." Science, vol. 348, no. 6232, 2015, pp. 336–340., doi:10.1126/science.aaa1788.
2. Hooper, David U., et al. "A Global Synthesis Reveals Biodiversity Loss as a Major Driver of Ecosystem Change." Nature, Feb. 2012, doi:10.1038/nature11118.
3. Riverkeeper. "Greenpoint Oil Spill on Newtown Creek." Riverkeeper, <www.riverkeeper.org/campaigns/stop-polluters/newtown/>.
4. "Search Superfund Site Information." EPA, Environmental Protection Agency, 28 Dec. 2016, <http://cumulis.epa.gov/supercpad/SiteProfiles/index.cfm?fuseaction=second.Cleanup&id=0206282#bkground>.
5. "Water Quality Sampling." Newtown Creek Alliance, Newtown Creek Alliance, 2016, <www.newtowncreekalliance.org/water-quality-sampling/>.

Acknowledgements

We would like to thank Cold Spring Harbor Laboratory, the Urban Barcode Project, Science Sandbox and the Pinkerton Foundation for supporting this project. Special thanks to Dr. Christina Marizzi and Dr. Alison Dell for their consistent assistance and helpful guidance. Finally, we would like to thank the Newtown Creek Alliance for partnering with us.