

Cold Spring Harbor Laboratory DNA LEARNING CENTER One Burgtown Road, Cold Spring Harbor, NY 11724 Lichen Biodiversity of Long Island Parks in Response to Urbanization

Abstract

Lichen are an important bioindicator. When pollution in the ecosystem increases, lichen are adversely affected (Yang et al.) We hypothesized locations with higher population density (Eisenhower Park) will have a lower lichen biodiversity compared to others (Rocky Point Pine Barrens and Cold Spring Harbor Park) due to air quality. Lichen samples were extracted from every tree in a 144 meter quadrat at each location. Silica protocol was used to extract the DNA and PCR was used to amplify the ITS region. Most lichens we collected had high tolerance to pollution across all locations.



CYX-015 Trametes versicolor (Turkey Tail Fungus)

Introduction

Lichens are a complex symbiotic collection of organisms that are composed of fungus and algae. They are a food source to deer, birds, rodents and other species and are a pioneer species in primary succession. They use oxalic acid to chemically weather rock in order to create soil in newly developed ecosystems (National Park Service). Some lichens are sensitive to even small changes in nutrient nitrogen availability or pH changes (United States Forest Service). Lichens get nutrients from the air, and their sensitivity to air pollution allows them to be good air quality indicators (Askham). If lichen biodiversity decreases it could cause a domino effect and affect the abundance of other animals and ecosystems. We hypothesize parks in more densely populated areas such as Eisenhower Park will have a lower Simpson's Diversity Index due to pollution whereas parks in less populated areas such as Cold Spring Harbor State Park and Rocky Point Pine Barrens Preserve will have a higher diversity index, finding more tolerant lichens in urban areas.

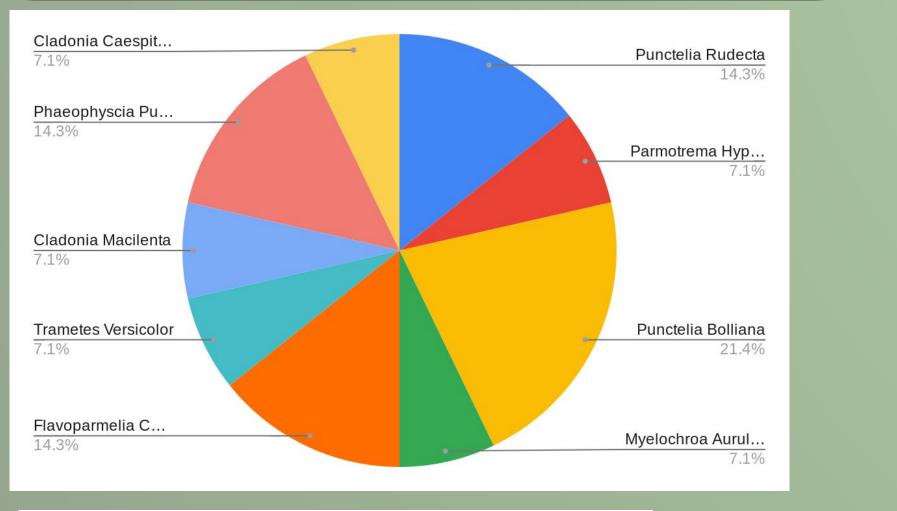


Figure 2: Lichen Diversity Across Sampled Parks



12 samples total were sequenced (Figure 4). 5 from Eisenhower Park, 2 from Rocky Point Pine Barrens, and 5 from Cold Spring Harbor Park. 9 distinct species were identified. CYX-15 which was collected from Rocky Point Pine Barrens came back as an unexpected species, Trametes versicolor, a fungi commonly known as turkey tail mushroom and not a lichen. Several gels produced double bands (Figure 3).

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Materials and Methods

Lichens were collected from Rocky Point State Pine Barrens Preserve, Cold Spring Harbor State Park, and Eisenhower Park. Ten lichens were collected from ten different trees in a 144 square meter quadrat at each location by being sprayed with water and scraped off the tree with a boxcutter. At first Chelex protocol was followed as per the Barcode Long Island protocol manual to extract DNA, however this left us with a faulty extraction and poorly visible bands. DNA was then extracted from tissue using the silica protocol. The ITS region was amplified using ITS primers to mark the target sequence in the lichen DNA. Sequencing data was analyzed using BLAST on the blue line of DNA Subway to identify species. To determine which lichens are tolerant or intolerant of various pollutants and environmental factors we referenced the U.S Forest Service website and Urban Lichens: A Field Guide for Northeastern North America (Allen 2021).

Double banding in lichen samples (CYX-023, CYX-024, CYX-026)



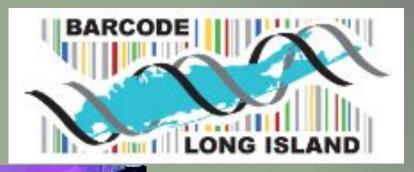
CYX-008 Punctelia boliana

| CYX-001 | Punctelia rudecta | (S) Tolerant (N) Tolerant | Eisenhower Park | CYX- 016 | Cladonia macilenta | (S)Tolerant (N)Tolerant | Rocky Poin Pine Barren |
|---------|---------------------------|---------------------------------|--------------------------------|-------------|-----------------------------|--------------------------------|----------------------------|
| CYX-005 | Parmotrema hypotropum | (S) Tolerant (N)Tolerant | Eisenhower Park | CYX- 023 | Phaeophyscia pusilloides | (S)Moderate (N) Moderate | Cold Spring Harbor Park |
| CYX-008 | Punctelia bolliana | (S)Sensitive (N) Moderate | Eisenhower Park | CYX- 024 | Phaeophyscia pusilloides | (S)Moderate (N)Moderate | Cold Spring Harbor Park |
| CYX-009 | Myelochroa aurulenta | (S)Tolerant (N)Tolerant | Eisenhower Park | CYX- 026 | Flavoparmeli a carpeta | (S)Moderate (N)Tolerant | Cold Spring Harbor Park |
| CYX-010 | Flavoparmeli a carpeta | (S)Tolerant (N)Tolerant | Eisenhower Park | CYX- 027 | Cladonia caespiticia | (S)Tolerant (N)Tolerant | Cold Spring Harbor Park |
| CYX-015 | Trametes versicolor | | Rocky Point Pine Barrens | CYX- 029 | Punctelia rudecta | (S)Tolerant (N)Tolerant | Cold Spring Harbor Park |

Figure 1: Sampled Lichens Tolerance Levels for Sulfur and Nitrogen From Each Park

References

- National Park Service. "Lichen the Little Things That Matter (U.S. National Park Service)." Nps.gov, 2018, www.nps.gov/articles/lichen-and-our-air.htm.
- Askham, Beth . "Nature and Pollution: What Lichens Tell Us about Toxic Air." *Www.nhm.ac.uk*, 4 June 2020, www.nhm.ac.uk/discover/nature-and-pollution-what-lichens-tell-us-about-toxic-air.html.
- Allen, Jessica L, et al. Urban Lichens : A Field Guide for Northeastern North America : Including New York City, Chicago, Toronto, Boston, New Haven, Philadelphia, Baltimore, Washington, D.C. New Haven, Yale University Press, 2021.
- Yang, Jin-Long, et al. "Lichen as Bioindicators: Assessing Their Response to Heavy Metal Pollution in Their Native Ecosystem." Mycobiology, vol. 51, no. 5, 3 Sept. 2023, pp. 343–353, https://doi.org/10.1080/12298093.2023.2265144.



Positive Control ITS band at ~550bp

Figure 3: Gel Electrophoresis of Lichen Samples

023 024 025 026 027 028 029

Discussion

Lichen tolerance to various pollutants is summarized in Figure 1. Lichen species found in Eisenhower were the ones that are more tolerant to various types of pollution.

An interesting finding from CYX-015 was sequenced as a fungus, Trametes versicolor. This could be the result of fungal spores landing on the lichen and because lichen are a symbiotic relationship between fungi and algae, the ITS primers would have also amplified the fungi. This data is interesting, but does not provide us with enough information to support our hypothesis.

From Cold Spring Harbor sample CYX-027 (Cladonia caespiticia) and CYX-029 (Punctelia rudecta) were tolerant to acid deposition and the rest were moderately tolerant. These results do not support our hypothesis because we believed that Cold Spring Harbor Park would have more lichen that are sensitive to pollution.

We had difficulty extracting DNA from lichen with Chelex, but had more success with silica. Occasionally we observed double bands, and a gel extraction was attempted by staff at DNALC to separate bands. We still had enough lichens to sequence, however, despite the double banded samples. Results of this project serve as a good foundation for future projects with a larger scope of park locations and sampling size. Air quality could also be measured longitudinally across all sampling locations as well to determine if air quality is correlated with population density or if lichen biodiversity fluctuates throughout the year.

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