Fungi as Indicators of Water Health in NYC Ponds

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Abstract

Urban aquatic ecosystems play a crucial role in maintaining water quality and biodiversity, and fungal diversity can serve as an important tool for assessing the ecological state of New York City ponds. However, this approach remains underexplored. This study aimed to evaluate fungal diversity in NYC ponds and establish a baseline profile to inform future monitoring efforts. Water samples were collected from Fairytale Pond and The Lake in Central Park, then concentrated using centrifugation before DNA extraction with a silica resin protocol. The fungal ITS region was amplified using ITS4 primers, and PCR products were analyzed via electrophoresis. Despite careful procedures and repeated trials, no fungal DNA amplification was observed, with only primer dimers detected. These results suggest potential limitations in sample processing, such as inadequate filtration or low fungal DNA concentration. This study highlights the methodological challenges of detecting fungal DNA in urban waters and the need for protocol optimization.

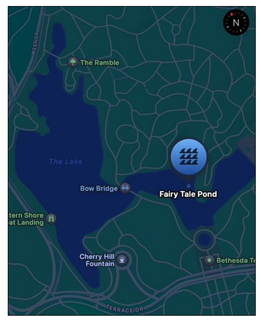
Introduction

Fungi play a significant role in the Kingdom of Fungi, a large and diverse group of organisms (Bridge et al.). Despite their importance in nutrient decomposition within aquatic ecosystems and their vital role in ecological processes, fungi often receive limited attention. New York City is teeming with life, much of which remains unseen in the city's thousands of puddles, lakes, and ponds (PondLife). Over 520 species of fungi have been documented in New York City ("Fungi of New York City | New York Botanical Garden"). Fungi are indispensable to life and serve many functional roles in various ecosystems.Obligate marine fungi are those that grow and sporulate exclusively in aquatic habitats, while facultative marine fungi are those originating from freshwater or terrestrial habitats that possess the ability to grow and possibly sporulate in marine environments (Kohlmeyer & Kohlmeyer, 1979).Molecular identification through DNA barcoding has revolutionized fungal ecology recently, providing new insights into fungal diversity and ecological functions (Bellemain et al.). This study aims to amplify potential fungal DNA specifically in the ITS region by using the ITS4 primer, a universal reverse primer designed to target the ITS2 region (a variable region of the DNA that helps identify fungal species), paired with ITS1, a forward primer for PCR. Underwater, trillions of fungi and other microbes form the foundation of our ecosystems. These fundamental organisms must not be overlooked, as they can serve as indicators of water health and quality. On the other hand, the presence of pathogenic or invasive fungi may pose ecological risks by degrading water quality or disrupting nutrient transport within ponds (Heller).

Materials & Methods

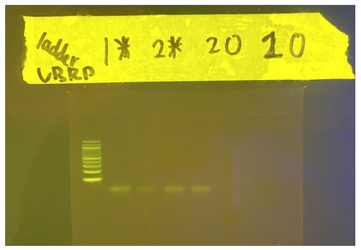
The materials required for the study include a centrifuge to concentrate fungal spores from water samples, sterile collection bottles for gathering water, and filter paper to remove large debris. The specific primer (ITS4) is used to target fungal DNA. A microscope is employed to analyze fungal spores during the study.

To begin, sterile centrifuge tubes were labeled with trackable tags, and 40 ml of two separate water samples from two distinct NYC ponds are collected to ensure a wide representation of fungi. The water samples are initially filtered using filter paper to remove larger debris. The filtered samples were then centrifuged at high speeds, typically between 3,000 to 5,000 x g for 10 to 15 minutes, to concentrate fungal spores at the bottom of the centrifuge tubes, effectively separating them from the water. DNA isolation was conducted with the silica DNA isolation protocol, which first involved adding 300 µL of lysis solution to our samples. After a 10 minute water bath incubation at 65 degrees Celsius, the tubes were placed into a centrifuge for one minute at maximum speed. Then 150 µL of the supernatant was added to a new tube and 3µL of silica resin was added, the new tube was then incubated for 5 minutes in a water bath at 57 degrees Celsius. The tube was then centrifuged for 30 seconds at maximum speed, before removing the supernatant and adding 500 µL of cold wash buffer. Together it was centrifuged at maximum speed, this process is called a wash and was done two times. After completing the washes, 100 µL of distilled water was added to the silica resin, followed by a 5 minutes incubation at 57 degrees Celsius, and centrifugation of 30 seconds at maximum speed. Then, only 2 µL of DNA was used for amplification by PCR using the ITS primer mix plus the Taq mix. After PCR completion the PCR product was analyzed by gel electrophoresis.



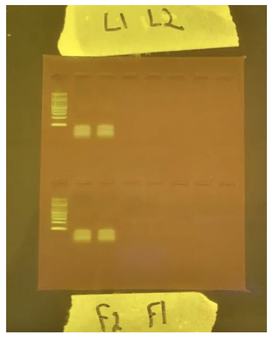
Results

The samples analyzed in this paper were collected from two lakes in Central Park: Fairytale Pond (Latitude: 40.775366 | Longitude: -73.969114) and The Lake (Latitude: 40.777358 | Longitude: -73.972733). The first group of samples was collected on March 15, 2025, in the morning, where 40 ml was sampled from these two lakes. It was brought back to the laboratory to be centrifuged, amplified, and subjected to DNA extraction and analysis. Following, DNA was extracted from beneath the filter of the collection bottles using the silica resin protocol, and was then amplified by the primer, ITS4, to target fungal species in the sample. PCR results were then analyzed, and throughout the entire process, each material had been properly sterilized to reduce external factors and contamination. However, despite the strict parameters and careful instructions followed, no notable data were found.



Across all samples tested, with two trials done for each lake, no notable band was shown. The last band on the bottom is negligible and is known as primer dimers, where primers bind to each other, and not their amplification.

Due to no significant analysis being concluded, a second trial was conducted, repeating the same steps in the experiment on April 5th, 2025. However, this time the sample was taken from above the filter of the collection tube after undergoing centrifugation rather than beneath it. It was suspected that the fungal components were not able to go through the filter, which led to no notable bands being observed. However, to our disappointment, no notable bands were found.



Discussion

The absence of notable DNA bands, even after a repeated trial, suggests a failure to detect fungal DNA—likely due to ineffective filtration or DNA extraction. The second trial extracted samples from above the filter to capture any fungal DNA missed earlier, but band visibility remained minimal. This suggests fungal concentrations in the water were too low for successful PCR amplification, possibly due to environmental factors like pollution.

Urban areas like New York City have long struggled with air and water pollution, which may reduce fungal biodiversity. Fungi, such as lichen—once rare in Central Park due to pollution—have reappeared as air quality improved, highlighting their sensitivity to environmental conditions. The lack of fungal DNA, including lichen typically found near freshwater, raises concerns about the ecosystem's health.

Pollutants, particularly pesticides, pose a major threat to fungal communities. Fungi play a critical role in decomposing organic matter and supporting nutrient cycling. Their absence can disrupt these processes, affecting the broader ecosystem.

A key limitation of this study was the lack of existing research on still water fungi, forcing reliance on studies of moving water systems and limiting result interpretation. Despite fungi's ecological importance, they remain understudied. This research aimed to assess fungal diversity in NYC ponds and provide a baseline for future monitoring. Findings suggest that urban environmental conditions may be silently reducing fungal biodiversity in freshwater ecosystems.

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