Using eDNA to determine how Pollution affects Biodiversity of Fish in Patchogue Bay Feeder Streams

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Abstract

We wished to determine the fish biodiversity of three streams emptying into Patchogue Bay: Great Patchogue Lake, Little Creek, and Swan Lake. Our two hypotheses were first to determine whether the eDNA technique would allow the detection of specific fish species, and second to examine whether water samples near more highly populated areas showed a difference in fish biodiversity. We collected water samples, prepared DNA, and used a fish-specific primer to amplify the fish DNA regions. The results of the BLAST analysis revealed the presence of common carp, killifish, and winter flounder species. BLAST also detected Homo sapiens and several species of bacteria and fungi. These results indicate that while the eDNA technique can detect species of fish in water samples, sample contamination is an issue. Lower fish biodiversity may be an early warning sign of pollution effects that might indicate the need for further testing of fish for pollutants that might be harmful to consume. Our most important finding was that eDNA allows for detection of individual fish species, suggesting that it might augment or replace more labor-intensive methods of determining fish biodiversity in natural waters.

Introduction

If an area becomes further industrialized, the pollution produced will increase (1). The chemicals released from machinery and physical debris can create a hostile environment for aquatic life and can cause marine animals to suffocate resulting from entanglement or ingestion (2). Long Island Sound has shown signs of severe long-term environmental damage since the late 1900's (3). The aim of our project was to use eDNA to measure the biodiversity of fish in three bodies of water (Great Patchogue Lake, Little Creek, and Swan Lake), each containing different levels of human activity, and use those values to determine each one's overall health. In addition, Patchogue Lake and Little Creek have experienced recent decreases in volume due to sedimentation and poor flow. A project was undertaken in 2016 to install water aeration equipment and pipes in both bodies of water to improve water flow and oxygenation and reduce pollutant levels (4). A 2016 DEC report lists Patchogue Lake and Swan Lake as Class B waterbodies, suitable for recreation purposes; however, Patchogue Lake was observed to be under threat by invasive species, while water quality was unassessed at Swan Lake (5). Our project was aimed at using eDNA techniques to detect the specific fish species present in our three lakes, comparing the relative fish biodiversity, and interpreting any biodiversity differences using available water quality data.

Materials & Methods

Water samples were collected from Great Patchogue Lake, Little Creek, and Swan Lake in early March 2019. We chose these lakes because they varied from low, medium, to high human population density. The samples were collected in triplicate at 25 feet distances from each lake and GPS data was recorded. Water samples were rated for low, medium, or high turbidity upon collection. Samples were filtered using eDNA filtration units supplied by the Barcoding LI laboratory, and controls of distilled water were run for each experimental sample. DNA was prepared from the filters at a Brookhaven National Laboratory Open Lab by the PowerSoil protocol, and specific regions were amplified using a fish-specific primer supplied by Barcoding LI. Gel electrophoresis was used to select the amplicons of interest and these PCR products were submitted for next-generation Illumina sequencing. The Purple Line routine of DNA Subway was used to analyze the data, and species were identified using the BLAST feature DNA Subway.

Results

We obtained a total of 25 sequences after carrying out the Demultiplex and Trimming steps in the Purple Line. Fourteen of these sequences were available in the Feature Table to run through the BLAST routine in order to identify the species present. The fish species detected included Cyprinus carpio (common carp), Fundulus heteroclitus (killifish), and Pseudopleuronectes americanus (winter flounder), with common carp present in two samples and killifish present in four samples (Fig. 1). We also detected Homo sapiens, yeast, and bacterial species. The species abundance heat maps of these species show differences in distribution between the three lakes (Fig. 2) and the estimated pollution (turbidity) level (Fig. 3). The species abundance in Greater Patchogue Lake appears lower than the abundance in Little Creek and Spring Lake (Fig.2). The species abundance is not easily correlated with turbidity, since the differential abundance in the least turbid water (Greater Patchogue Lake) appears to be lower than in the lakes with medium to high turbidity (Fig. 3).

BLAST Results, Longwood Lionfish eDNA Team

1	Cyprinus carpio (common carp)
2	Homo sapiens (human contamination)
3	Aureobasidium pullulans (yeast)
4	Fundulus heteroclitus (killifish)
5	Pseudopleuronectes americanus (winter flounder)
6	Homo sapiens (human contamination)
7	Fundulus heteroclitus (killifish)
8	Uncultured Finegoldia (human gram-positive bacterium, class Clostridia, causes infections such as skin ulcers)
9	Malassezia restricta (fungus, human pathogen, skin infections)
10	Fundulus heteroclitus (killifish)
11	Fundulus heteroclitus (killifish)
12	Uncultured bacterium clone LMP12_16S_OTU_1252
13	Cyprinus carpio (common carp)
14	Homo sapiens (human contamination)
15	Homo sapiens (human contamination)
16	Uncultured bacterium clone 02726

Figure 1

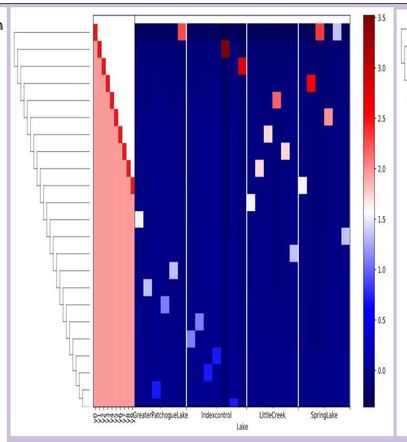

Figure 2

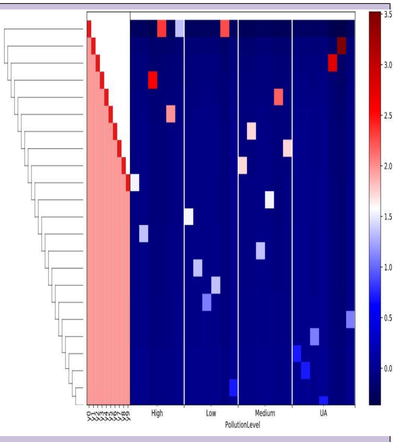

Figure 3

Discussion

Our results indicate that we were successful in detecting fish species using eDNA. We are attempting to interpret the differential abundance maps in order to reveal any differences in species distribution according to the lake source or the proximity to human activity or pollution levels. We were surprised by the small number of sequences yielded by the technique and do not currently have an explanation for this. Also, there is clearly an issue with contamination of the samples with the experimenters' DNA (or possibly from the water samples themselves) as shown by the detection of Homo sapiens in 4 samples (Fig. 1). In addition, we are not sure why the fish primer might generate sequences associated with bacteria and fungi. Interestingly, killifish were detected in four of the samples. Killifish (also known as mummichog) are well known to be highly resistant to pollution and low oxygen levels, which may explain their frequency of detection in the medium to high turbidity samples (6). In summary, we have shown that eDNA water sampling can successfully detect individual fish species, indicating that eDNA analysis may someday replace the current systems of monitoring bodies of water for fish biodiversity. Additionally, we have detected differences in the fish species distribution across the lakes and when grouped by estimated pollution levels. The eDNA technique may at some point be useful in monitoring total fish diversity in environmentally threatened bodies of water. Along with water quality data, fish diversity revealed by eDNA studies could assist in the evaluation of the overall health of individual bodies of water.

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