Brook Trout (*Salvelinus fontinalis*) Aquatic eDNA Degradation Analysis

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Abstract

Maine stands as the sole U.S. state harboring extensive, self-reproducing brook trout populations in its lakes and ponds, owing to its limited development (MDIFW). However, the state's growing population poses a threat to these habitats through increased development and potential impacts of climate change. This study dives into environmental DNA (eDNA) degradation and brook trout conservation in Maine, aiming to refine population assessment methods and identify areas requiring protection. The hypothesis asserts a decline in brook trout eDNA concentration with increasing distance downstream from the source population. The study design involved water sample collection from five sites at varying distances, followed by qPCR analysis. Although statistical analysis indicates no significant ties between distance and DNA amounts, there were certainly trends, hinting at a connection. To refine the study, diluted standard curves, a higher sample size, and rerun qPCR analysis should take place. These findings underscore the importance of comprehending eDNA dynamics for effective brook trout conservation in the face of impending environmental changes.

Introduction

Climate Impact on eDNA Surveys: Understanding the impact of climate changes on eDNA-based quantification surveys is crucial, given the potential threat to the brook trout population.

Emphasis on Conservation Strategies: The research aims to contribute to the comprehension of effective conservation strategies, specifically focusing on the dynamics of environmental DNA (eDNA) degradation.

Central Objective: The central objective of this study is to refine population assessment methods to help with swiftly identifying areas necessitating immediate protection.

Importance of eDNA Degradation: A critical aspect of the research effort involves comprehending the degradation of eDNA released by Brook Trout in their habitats.

Comprehensive Understanding Needed: Given the dynamic climate patterns, a comprehensive understanding is necessary to assess the potential impact on Brook Trout populations.

Study Focus: This study explores the complex realm of eDNA degradation, recognizing its role in supporting Brook Trout conservation efforts and its implications for the ongoing preservation of these ecologically vital populations in Maine.

Methodology

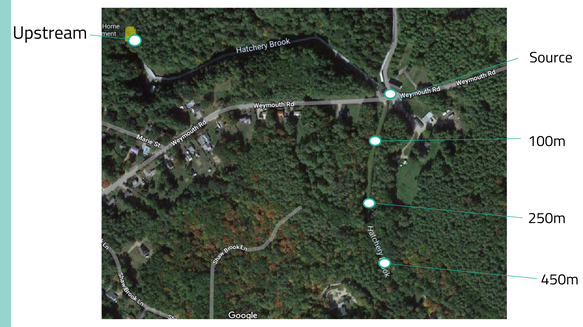

Figure 1. Sampling Sites at Dry Mills Hatchery in Gray, ME

1. Water Sample Collection: 500mL water bottles were employed to collect water samples from five distinct sites, including the source population, one site upstream (likely with minimal trout DNA), and three points downstream at increasing distances (100m, 250m, and 450m). (See Figure 1)

2. Filtration Process: A 0.45µm pore-sized filter, coupled with a suction pump, was utilized for the filtration process. Each water sample underwent filtration, and the filtered material was then added into separate tubes.

3. Manual Cutting and Lysis: The arranged tubes underwent manual cutting and lysis using a specialized lysis solution. This helps with the preservation and extraction of DNA.

4. qPCR Analysis: The extracted eDNA underwent qPCR analysis to determine its concentration; conditions below. Primers and conditions from (Baldigo et al. 2016). (See Figure 2)

5. Standard Curves: Five eDNA templates, serving as references, were created with known concentrations. These templates, along with the five site sample tubes, and a negative control tube containing water instead of DNA, constituted the 11 tubes used for the qPCR trial. Due to time constraints, only one tube from each site was arranged for further analysis.

Figure 2. qPCR Conditions

Results

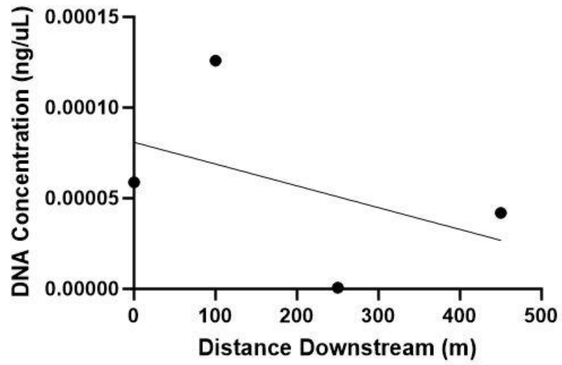

Figure 3. Relationship between DNA concentration and Distance. Spearman's r = -0.600. P (two tailed) = 0.4167

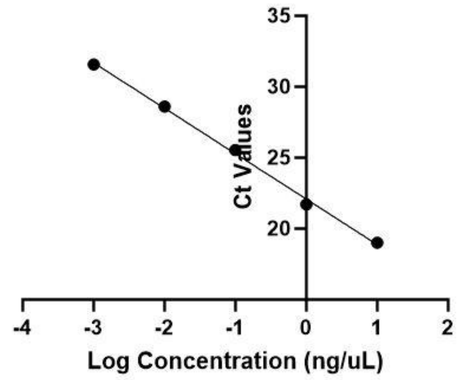

Figure 4. Linear Regression of Template DNA for Standard Curve Testing. r^2 = 0.9975. P = <0.0001

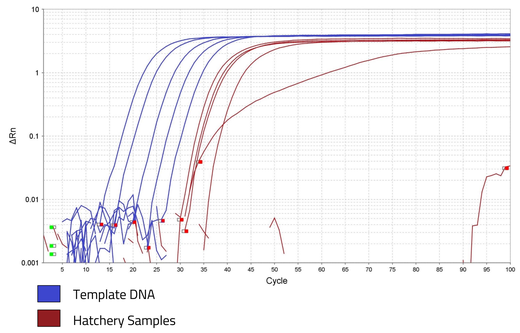

Figure 5. qPCR Amplification Curves

Conclusion

Spearman Correlation: Despite the moderate negative correlation, the non-significant p-value (0.4167) suggests a lack of strong evidence for a significant relationship between DNA concentration and distance downstream. The observed correlation may likely occur by random chance, and the sample size may lack sufficient power to detect a true relationship.

Refining the Study: Further dilution of standard curves with a follow up qPCR run is recommended for improved comparisons to the site-sampled amplification curves. A higher sample size would help to bolster the statistical efficacy of the study's results.

References

Baldigo, B. P., Sporn, L. A., George, S. D., & Ball, J. A. (2016). Efficacy of Environmental DNA to Detect and Quantify Brook Trout Populations in Headwater Streams of the Adirondack Mountains, New York. Transactions of the American Fisheries Society, 146(1), 99–111.

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