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**Genetic Assessment of Boardman River Fish Populations Before Dam Removal**

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**ABSTRACT:**

Fragmentation of river systems by dams can have a substantial genetic impact on fish populations. However, genetic structure can exist naturally at small scales through various processes such as isolation by adaptation (IBA) and spawning site fidelity, even in the absence of barriers. We sampled individuals from five native fish species with varying life histories above and below a dam in the lower Boardman River, Michigan, USA, and used RADseq to test whether genetic structure was influenced by the dam or other processes. Species assessed were white sucker *Catostomus commersonii*, yellow perch *Perca flavescens*, walleye *Sander vitreus*, smallmouth bass *Micropterus dolomieu*, and rock bass *Ambloplites rupestris*. We detected significant differentiation within each species, but this structure did not appear to be a result of fragmentation by the dam. Population groupings were not consistent with our original “above dam” and “below dam” sampled populations, but instead aligned with a Great Lakes (GL) group from Lake Michigan and a Boardman River (BR) group that appear to mix below the dam. We hypothesize that these groups formed prior to dam construction through IBA in these different habitats and further maintained divergence through spawning site fidelity. Additionally, GL fish for most species were significantly smaller in length than BR fish, suggesting a potential ontogenetic habitat shift of young GL fish into the lower river for feeding and/or refuge. Without our genetic assessment, the existence of these cryptic ecotypes likely would have continued undetected. Our study illuminates the importance of tributary habitats for GL fish and has major implications for the management of fish populations in the Great Lakes and beyond. Finally, our approach of combining genetic data, ecological data, and simulations to assess connectivity and identify cryptic diversity has far reaching applicability for understanding the potential genetic impacts of fragmentation in other systems.

Understanding biodiversity in aquatic systems is critical to ecological research and conservation efforts, but accurately measuring species richness using traditional methods can be challenging. Environmental DNA (eDNA) metabarcoding, which uses high-throughput sequencing and universal

primers to amplify DNA from multiple species present in an environmental sample, has shown great promise for augmenting results from traditional sampling to characterize fish communities in aquatic systems. Few studies, however, have compared exhaustive traditional sampling with eDNA metabarcoding of corresponding water samples at a small spatial scale. We intensively sampled Boardman Lake (137 ha) in Michigan, USA from May to June in 2019 using gill and fyke nets and paired each net set with lake water samples collected in triplicate. We analyzed water samples using eDNA metabarcoding with 12S and 16S fish-specific primers and compared estimates of fish diversity among methods. We captured a total of 12 fish species in our traditional gear and detected 40 taxa in the eDNA water samples, which included all the species observed in nets. The 12S and 16S assays detected a comparable number of taxa, but taxonomic resolution varied between the two genes. In our traditional gear, there was a clear difference in the species selectivity between the two net types, and there were several species commonly detected in the eDNA samples that were not captured in nets. Finally, we detected spatial heterogeneity in fish community composition across relatively small scales in Boardman Lake with eDNA metabarcoding, but not with traditional sampling. Our results demonstrated that eDNA metabarcoding was substantially more efficient than traditional gear for estimating community composition, highlighting the utility of eDNA metabarcoding for assessing species diversity and informing management and conservation.