The benefits and challenges of using environmental nucleotides for fish passage species detection and enumeration

Kristine N. Moody[1], Steven Gardner[1], Andrew Furtak[1], Xiu Marks[1], Ed Pearce[2], and Brenda Pracheil[3]*

[1]Environmental Sciences Division, Oak Ridge National Laboratory

[2]Michigan Department of Natural Resources

[3]Earth Systems Sciences Division, Pacific Northwest National Laboratory

**moodykn@ornl.gov*

ABSTRACT: Globally there is an urgent need to find solutions to balance energy-production and protection of vulnerable species and the maintenance of biodiversity. Although reliable and accurate ecological assessments are key to ensure protection of biodiversity, conventional biodiversity survey methods- electrofishing, seining, gill netting, snorkeling -rely on the physical capture or visual detection of fishes, which are costly, time-consuming, hazardous to personnel, cause habitat disturbance, and result in size, age, or species-biased estimates and misidentification. Newer, non-invasive methods such as environmental nucleic acids (eNA)- organismal DNA and RNA deposited into the environment from excretion, cellular discharge, and mortality- can be collected and analyzed from a water sample to identify species. Surveys using eNA thus have the potential to revolutionize species and biological community assessments, management planning, and decision-making procedures. In this study we used a combination of eDNA and eRNA sampling in the Grand and St. Joseph rivers in Michigan, USA, to determine if eNA can be a useful tool for species detection and enumeration in fish passages. We collected in total 678 samples across six dams and sampled upstream, downstream, and within fish passages across three months to characterize biodiversity before, during, and after spawning runs of potamodromous steelhead trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*), and largemouth bass (*Micropterus salmoides*). We analyzed both eDNA and eRNA using 12s MiFish primers and CO1 primers for metabarcoding, as well as species specific primers for qPCR for four genes (12s, CO1, 18s, and CytB). We compared our results to Michigan Department of Natural Resources fish ladder counts and electrofishing data. Concurrently, we conducted mesocosm tank experiments to determine differential decays rates between eDNA and eRNA to provide a calibration metric for field eDNA/eRNA results. The findings from this work will serve as foundational information to support further investigation into eDNA/eRNA dynamics and the utility of eNA in fish passages and hydropower. Further, this work will demonstrate the application of eNA for biomonitoring, hydropower planning, construction, operations, and (re)licensing that could streamline processes by elevating our understanding of potentially impacted biota and informing and evaluating mitigation of environmental impacts.