



## PROJECT ABSTRACT

**Grant Title:** 2011.1197 – *Estimating Asian Carp abundance using environmental DNA*

**Grantee:** David Lodge, University of Notre Dame, Center for Aquatic Conservation & Environmental Change Initiative

**Funding Amount:** \$249,999

### Project Summary

Experiments to test the hypothesis that the abundance of Asian carp eDNA in a water body reliably indicates the abundance of Asian carp. Development of a statistical calibration model relating eDNA abundance to Asian carp abundance. Application of the model to previous eDNA data for Asian carp to estimate 2009-2010 abundance in the upper reaches of the Chicago Area Waterway System.

### Project in Context

Both environmental DNA (eDNA) results (Jerde et al. 2013) and the capture of a bighead carp attest that Asian carp are on the Lake Michigan side of the electric barriers in the Chicago Area Waterway System (CAWS), and thus have access to Lake Michigan. However, the magnitude of the risk to the Great Lakes is unknown because it is uncertain if only a few or many Asian carp are responsible for the abundant positive DNA samples (Jerde et al. 2011). This uncertainty has led to indecisive management action, and to a broad consensus that experiments are required to calibrate the DNA signal against fish abundance (ACRCC 2010).

We conducted experiments and observations to address this major gap in management-relevant knowledge. First, we used experimental ponds at the University of Kansas Field Station (KUFS) in Lawrence, KS, to evaluate the spatial and temporal patterns of the eDNA signal across different stocked densities of bighead and silver carp. Because the rates at which fish release DNA into the environment and at which DNA subsequently degrades are largely unknown, experiments were necessary to quantify how the proportion of positive eDNA samples using a traditional PCR assay relates to fish abundance over space and time. We stocked artificial ponds with a gradient of bighead and silver carp densities and repeatedly sampled the ponds spatially and temporally over four months. Additionally, we developed and used quantitative PCR (qPCR) to measure the amount of DNA present in space and time.

Second, we returned to the CAWS, and compared detection rates from the PCR and qPCR assays in situ across a known gradient of Asian carp density. This information is essential to inform both

the ongoing Asian carp response in the CAWS, and to interpret ongoing GLRI-funded eDNA surveillance results in Great Lakes tributaries.

## **Results**

Results revealed seven key insights regarding Asian carp surveillance and detection:

1. A positive relationship exists between Asian carp abundance and the concentration of Asian carp DNA, but the strength of the correlation is coupled to exogenous factors that make estimating fish abundance difficult.
2. Many factors contribute to detection of environmental DNA and the quantity of eDNA recovered. These factors include, but are not limited to: water volume collected, life history and behavior of Asian carp, DNA degradation rates, and presence of PCR inhibitors in the water.
3. Relative to the PCR assay previously used (Jerde et al. 2011, Jerde et al. 2013), the qPCR assay for Asian carp detection developed in this study is more sensitive (i.e., more likely to detect Asian carp at low abundance).
4. Relative to the materials and methods we (and others) previously used, the use of polycarbonate track etched (PCTE) filters with CTAB extraction is cheaper, recovers more DNA, and reduces the risk of contamination by reducing the handling of samples.
5. In situ testing in the CAWS resulted in few detections using either the PCR or qPCR assay. However, qPCR positives below O'Brien, Dresden Island, Marseilles, and Starved Rock locks and dams confirm what PCR assays collected since 2009 have concluded: Asian carp DNA exists in the CAWS. Estimating Asian carp abundance using environmental DNA
6. The quantity of DNA recovered in the CAWS is indicative of a gradient of Asian carp abundance, with more DNA recovered per sample in areas of moderate Asian carp abundance and little DNA recovered per sample in areas with low Asian carp abundance.
7. While many have raised alarms about the issue of potential false positives (concluding Asian carp DNA is present in a sample when actually not), this study documents that the more likely bias is in favor of false negatives. For example, in the pond experiment, many samples were negative (using the PCR approach) in ponds known to contain Asian carp.

## **Significance**

This study provides a number of critical insights and advancements. First, because of the direct comparison of qPCR and PCR assays, it is now possible to deploy a more sensitive detection approach (the qPCR assay) to the surveillance of bighead and silver carp. Second, it is clear that the PCR assay used from 2009 to present in the CAWS and Great Lakes is more prone to false negatives than false positives. Third, while there is a positive relationship between Asian carp abundance and amount of DNA in the water, there is sufficient variability to make estimation of abundance from amount of DNA very difficult. Fourth, environmental conditions drive fluctuations in the amount of DNA present. Overall, considering previous PCR detections, the implications of false negatives, the screening of the CAWS using a new qPCR, Asian carp marker, and comparing eDNA concentrations we measured in the CAWS relative to those we measured in our experimental ponds, the most reasonable inference is that some Asian carp are present on the Lake Michigan side of the electric barrier in the CAWS.

## **Publications**

1. Barnes, MA, CR Turner, CL Jerde, MA Renshaw, WL Chadderton, and DM Lodge. 2014. Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science and Technology*. 48:1819-1827.
2. Turner, CR, MA Barnes, CCY Xu, SE Jones, CL Jerde, and DM Lodge. Particle size distribution and optimal capture of aqueous microbial DNA. In Revision
3. Mahon AR, CL Jerde, and L Waits. Investigating error and quality assurance in environmental DNA surveillance. Submitted
4. Renshaw, MA, BP Olds, CL Jerde, MM McVeigh, and DM Lodge. The room temperature preservation of filtered eDNA samples and assimilation into Phenol-Chloroform DNA extraction protocol. Submitted.
5. Turner, CR, DJ Miller, KJ Coyne, and J Corush. Improved methods for capture, extraction, and quantitative assay of environmental DNA from Asian bigheaded carp (*Hypophthalmichthys* spp.). Submitted.
6. Turner, CR, KL Uy, and RC Everhart. Fish environmental DNA is more concentrated in aquatic sediments than surface water. Submitted.
7. Turner, CR, et al. Using environmental DNA to assess fish abundance: integration of modeling and monitoring in semi-natural ponds. In preparation.
8. Jerde, CL, A Tucker, CR Turner, et al. Application of qPCR to detection of Asian carp in the CAWS and Great Lakes: The problems with zeros. In preparation.
9. Barnes, MA, CR Turner, CL Jerde, and DM Lodge. Environmental DNA particle size distributions: implications for collection and analysis. In preparation.

## **Presentations**

1. Jerde CL. 2013. Rensselaer Polytechnic Institute. The science of early detection of invasive species. November 18th. Darrin Freshwater Institute, Lake George, New York.
2. Jerde CL. 2013. University of Notre Dame. Asian carp in the Great Lakes. October 2nd. Teachers as Scholars Program. South Bend, Indiana.
3. Jerde CL. 2012. Central Michigan University. Finding something to carp about: Unraveling the controversies surround Asian carp invasion of the Great Lakes. December 6th. Mount Pleasant, Michigan.
4. Jerde CL. 2012. Loyal University. Conservation by the cup of water: The search for Asian carp DNA in the Chicago Area waterway system. October 31st. Chicago, Illinois.
5. Jerde CL. 2012. University of Montréal at Québec. Environmental DNA surveillance or rare and invasive species. October 16th. Montréal, Québec, Canada.
6. Barnes MA, CR Turner, CL Jerde, MA Renshaw, WL Chadderton, DM Lodge. 2013. Environmental conditions influence eDNA persistence in aquatic systems. American Fisheries Society 143<sup>rd</sup> Annual Meeting, Little Rock, AR.
7. Barnes MA. 2013. Prediction, detection, and management of aquatic invasive species. Indiana State University Department of Biology Departmental Seminar.

8. Lodge, DM. 2013. Congressional briefing on eDNA, US Capitol.
9. Lodge, DM. 2014. Biology, economics, and policy of invasive species. University of Michigan, Conservation Biology Series.