A Rapid and Accurate DNA Assay for Invasive and Native Fish Species from Water, Plankton, and Gut Content Samples

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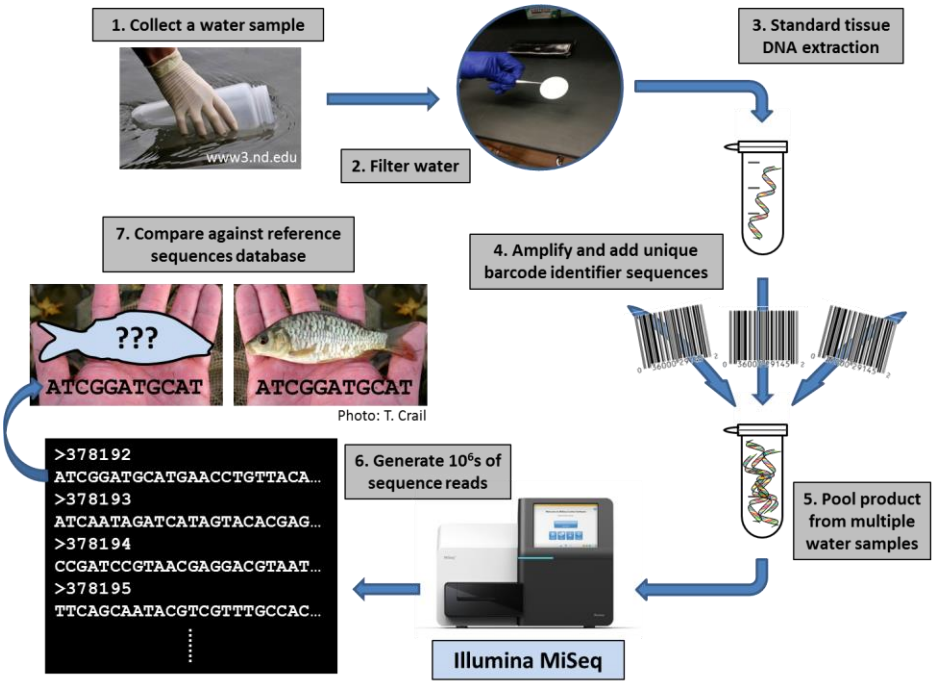
Overview and Objectives:

The goals of this project are to develop and evaluate an environmental (e) DNA and next-generation sequencing (NGS) assay to (1) detect the presence or absence, (2) identify to species, and (3) estimate the relative abundances of Great Lakes fish species (native and non-native) from water or plankton or gut content sample. The assay is based on a diagnostic region of the cytochrome b mitochondrial DNA gene (first evaluated on the cytochrome b gene and potentially will be expanded to the mtDNA COI gene, and possibly a nuclear gene to build redundancy. The targeted gene regions are selected to contain sufficient variability to correctly identify 200 potential Great Lakes fish species, including all existing invasive species, as well as high-risk potential invaders. The assay will be vetted on water samples from controlled lab aquarium experiments, as well as several field locations where conventional netting and electrofishing have occurred. We also plan to evaluate the assay's performance on gut contents of piscivorous species and on ichthyoplankton samples.

Specific end-point project outcomes are:

1) Design and evaluate an inexpensive, easy-to-use assay for managers and other sciences to assess water, gut content, and plankton samples for presence and abundance of high-risk invasive fish species using diagnostic DNA markers
2) Help stop the introduction of new aquatic invasive species (AIS) in the Great Lakes through enhanced surveillance (e.g., ballast water, harbor samples, plankton samples, gut contents of piscivores) to facilitate rapid response actions
3) Develop technology and sampling methodology to use eDNA testing to control and reduce the spread of AIS already in the ecosystem and expedite critical management information

During the fellowship, Carson Prichard worked to compile all known genetic information for two mitochondrial genes (barcode COI and cytochrome *b*) and the nuclear RAG1 gene for ~200 species of interest. The most informative small (<150bp) diagnostic sequence regions from these genes have been identified and ranked for mini-barcode marker development. Primers for these markers were developed to amplify across all species. These gene regions contain diagnostic single nucleotide polymorphisms (SNPs) that will accurately identify each species, and



Conceptual flow diagram of our proposed eDNA assay. Water is collected (1) and vacuum-filtered (2) to collect suspended DNA and cellular material. A standard tissue DNA extraction is conducted on a portion of the filter (3), and then the target marker is amplified. An additional PCR step is used to append unique oligonucleotide "barcode" identifier sequences (4) so that product corresponding to multiple water samples can be combined for a single Illumina sequencing run (5,6). A bioinformatics pipeline is then used to parse the resultant sequences and bin them according to their corresponding sample. Lastly, short-read alignment software is used to assign the target marker portion of each amplicon to its parent

additionally will yield diagnostic haplotypes to elucidate relative genetic variability within biological populations in the Great Lakes.

Thus far, for one 55 base marker within the cytochrome *b* gene we have conducted lab experiments to optimize PCR cycling conditions to ensure non-differential amplification between species. A next-generation sequencing assay performance trial was conducted via an Illumina Mi-seq run. Illumina libraries comprising seven "simulated fish communities" prepared from fish tissue DNA extractions from 10 species were mixed in known, varying concentrations to simulate the eDNA from a water sample. Observed relative abundances of aligned sequence outputs were highly correlated to expected relative abundances, confirming the assay's performance.

The assay's reliability has been tested using filtered water samples from aquaria housing single species (i.e., fathead minnow). Using Sanger sequencing, the output correctly identified one haplotype. Lab trials currently are underway to test the assay's performance on water samples collected from aquaria housing multiple fish species, as well as on filtered water samples collected from the Maumee River (by the Ohio EPA) during electroshocking fish sampling. We will be comparing the relative abundances of species-specific molecular markers from water samples against fish population abundance estimates from this traditional fish sampling method to pioneer the use of eDNA for estimating relative fish abundances.

Additionally, population genetics information for two fish species of high Great Lakes invasion risk – bighead and silver carps – is being collected. For five sampling locations spanning much of their invasive ranges throughout the Mississippi River basin, tissue samples for 250 silver carp and 197 bighead carp have been obtained. Ten nuclear microsatellite loci have been optimized and data have been collected for approximately half of all samples. COI and cytochrome *b* genetic sequence data have been generated for a limited number of individuals (*N*=10) across all sampling locations and previously unpublished haplotypes have already been identified. These vital population genetics data will (1) identify all COI and cytochrome *b* haplotypes for these species that may show up in a Great Lakes eDNA water sample, and (2) describe the structure and diversity of these two species to track their spreads over time, and influence control and eradication strategies in the future.

Future work will examine the eDNA assay's ability to identify and quantify relative fish species abundances from ichthyoplankton samples and fish gut contents – two sample types of immense fisheries research and management importance for which visual species identification is often difficult.

Accomplishments:

Carson Prichard was awarded the 2013 IAGLR Scholarship ($2,000), the 2014 Dr. Robert Brundage Scholarship ($500), and the 2014 Paul W. Rodgers Scholarship ($2,000) for his work on this project.

Publications: None to date

Presentations:

Prichard, C.G., Blomquist, T.M., Stepien, C.A. A new next-generation sequencing assay to identify and quantify all fish species from eDNA water samples. May 2014. Oral presentation at the Joint Aquatic Sciences Meeting, Portland, OR.

Prichard, C.G., Blomquist, T.M., Stepien, C.A. A new next-generation sequencing approach to simultaneously characterize entire fish communities from water samples. March 2014. Oral presentation at the Ohio Fish and Wildlife Management annual meeting. Columbus, OH.

Prichard, C.G., Blomquist, T.M., Stepien, C.A. A new environmental DNA approach to simultaneously characterize entire fish communities from water samples. March 2014. Oral presentation at the Midwest Graduate Research Symposium. Toledo, OH.

Stepien, C.A., Prichard, C.G., Blomquist, T. A new next-generation sequencing approach to simultaneously characterize entire invasive and native fish communities from water samples. February 2014. Marine Invasive Species: Management of Ballast Water and Other Vectors international conference. Muscat, Oman.

Prichard, C.G., Blomquist, T.M., Stepien, C.A. A new next-generation sequencing assay to characterize fish communities from water samples. January 2014. Oral presentation at the Lake Erie Inland Waters Annual Research Review. Columbus, OH.

Prichard, C.G., Blomquist, T.M., Stepien, C.A. Environmental DNA detection and quantification: A new test for Great Lakes native and high-risk invasive fish species. September 2013. Oral presentation at the American Fisheries Society annual conference. Little Rock, AR.

Prichard, C.G., Blomquist T., Willey, J.C., Sigler, V., Stepien, CA. *"Development of a Rapid eDNA test for Invasive Fish Species"*, poster presentations given at:

(1) Ohio Fish and Wildlife Management Association annual meeting, February 2013, Ohio State University, Columbus, OH.
(2) Joint Ohio-West Virginia Chapters American Fisheries Society annual meeting, March 2013, Marshall University, Huntington, WV.
(3) International Conference on Aquatic Invasive Species, April 2013, Niagara Falls, ON.
(4) International Association for Great Lakes Research annual conference, June 2013. West Lafayette, IN.

Stepien, Carol A. Invited research seminar presentations on "*Invasion Genetics: Tracing Pathways, eDNA, and Temporal Changes Across Aquatic Ecosystems*".

(highlighted example from this eDNA research project)

(1) Abu Dhabi Environmental Agency, January 22, 2013, Abu Dhabi, United Arab Emirates.
(2) Department of Biological Sciences, University of Warsaw, January 25, 2013, Warsaw, Poland.
(3) Department of Zoology, University of Tasmania, February 28, 2013, Tasmania, Australia.
(4) Department of Zoology, University of Melbourne, March 5, 2013, Melbourne, Australia.
(5) Victoria Museum, March 4, 2013, Melbourne, Australia.

Outreach Activities:

Research presentation by Carson Prichard to undergraduate students of chapter of Beta Beta Beta National Biology Honorary Society at Hillsdale College in Michigan. April 16, 2013.

Relevant Websites: http://www.utoledo.edu/nsm/lec/research/glgl/index.html