Environment and Natural Resources Trust Fund
2018 Request for Proposals (RFP)

Project Title: **ENRTF ID: 137-D**

Fish on a Chip: An AIS Detection Platform

Category: D. Aquatic and Terrestrial Invasive Species

Total Project Budget: $ 399,000

Proposed Project Time Period for the Funding Requested: 2 years, July 2018 to June 2020

Summary:

In this study we will develop and validate an new method for simultaneously determining the presence and relative quantity of 21 invasive fish species in any Minnesota waterway.

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Sponsoring Organization: U of MN

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Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Approximate Sampling Locations for Fish on a Chip Technology and Invasive Species to be Tested

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____%

PROJECT TITLE: Fish on a Chip: An AIS Detection Platform

I. PROJECT STATEMENT

A rapidly growing number of aquatic invasive species (AIS) threatens Minnesota's Upper Mississippi River and inland lakes. Once established, these species can cause significant ecological and economic harm, yet Minnesota's waterways are not routinely monitored for AIS. **In this project, we will use a new molecular detection tool to simultaneously track and quantify the growing number AIS in MN waterways**. Understanding the distribution and abundance of AIS (fishes, mussels and plants) **is** necessary to affect their control and our system improves upon earlier technology by allowing for the simultaneous detection and tracking of multiple AIS.

In the past decade, DNA released to the environment (eDNA) has gained favor for detecting AIS. This approach can easily detect organisms with great specificity and sensitivity and increased detection reliability. In fact, the U.S. Fish and Wildlife Service (USFWS) now employs eDNA technology to locate silver and bighead carp. However, this technique can only measure one to two species in a single water sample. This is a major limitation because collection and extraction of water samples for DNA is both time consuming and expensive, and multiple samples are required (costs for one species measurement is ~ $50-$100). This has meant that eDNA technology is not yet widely available – and routine monitoring is still not possible.

The proposed research will solve this problem by developing and using a rapid and quantitative DNA detection system, called MFQPCR, as a tool for simultaneously monitoring known AIS found in MN waterways. In MFQPCR technology, up to 96 detection reactions can run simultaneously in very small volume chambers embedded on a chip (*Figs. 1a* and *1b*). This "**Fish on a Chip" technology** can simultaneously measure eDNA from many species in each water sample. This novel chip system greatly reduces the time, labor, and costs when compared with the detection systems now is use. The substantial lower price, about $10 per species, brings routine monitoring within reach.

Our project will focus on developing and implementing this promising technique for all 21 invasive species potentially impacting Minnesota waterways. We will make the assay available to government biologists including the USFWS (a collaborator) and MNDNR, and train them and test samples they may collect in the first year. This will greatly aid state and federal agencies in strategies to control invasive species.

II. PROJECT ACTIVITIES AND OUTCOMES

The project will proceed in several distinct steps to quantitatively identify21 invasive species. We currently have markers for 12 invasive fish species, 2 mussels (zebra and quagga) and 1 plant (Eurasian milfoil). In this study we will expand our detection technology by identifying 6 new additional markers for other AIS fish species (Rainbow smelt, Rudd, Tench, White perch, Western mosquitofish, and Zander). We will then test all of these markers in the laboratory and in a field setting. Lastly, we will disseminate results and approaches to government biologists including the MN DNR and USFWS. We will establish the technique as a service for the state to use for a period of time. Key steps in our project are described below:

Activity 1: Marker and technology development. **Budget:** $146,000

While good markers already exist for 15 AIS species (fish, mussels, snails and plants) we will need to develop and validate 6 new ones.

Outcome	Completion
1. Modify and validate current markers for invasive species with MFQPCR, e.g., black carp, grass carp, sea lamprey and goldfish.	August 2017
2. Design and screen markers for invasive species that do not yet have genetic markers for e.g., rainbow smelt, rudd, tench, western mosquitofish, white perch, and zander.	December 2017
3. Test new markers for specificity and sensitivity with conventional qPCR and MQPCR.	June 2018
4. Optimize protocols to enhance detection sensitivity in several waterways.	September 2018

Activity 2: Assay validation **Budget:** $231,000

The MFQPCR assay will be fully evaluated and improved (if necessary) in several steps.

Outcome	Completion
5. Test Protocols using large fish tank waters containing known numbers of fish and species and correlate fish abundance to marker genes.	December 2018
6. Test the MFQPCR assay in the Mississippi River and 4 lakes in the state for several AIS including invasive carp, common carp, snakehead, round goby, and zebra and quagga mussels.	March, 2019

Activity 3: Dissemination of results **Budget:** $22,000

To be meaningful, the assay we develop needs to be used in the field by others.

Outcome	Completion
1. Publish results in peer-reviewed journals.	May, 2019
2. Sponsor a workshop to train DNR and USFWS biologists	June, 2019
3. Present results at scientific meetings and symposia.	June, 2019
4. Work with MAISRC and BTI outreach personnel to publicize our technology, including development of a web site resource.	June, 2019

III. PROJECT STRATEGY

A. Project Team/Partners

Our team consists of Michael Sadowsky, Peter Sorensen (FWCB), Satoshi Ishii (SCW), and Ping Wang (BTI). Dr. Sadowsky will coordinate all laboratory activities, Dr. Sorensen will coordinate fish studies, Drs. Ishii, and Wang will also work with a postdoctoral fellow to develop markers and implement MFQPCR assays using DNA sequence data. The research team assembled has all the necessary qualifications to carry-out the proposed studies. Their areas of expertise are complementary. Michael Sadowsky is a microbial ecologist with expertise in genomics and quantitative PCR. Peter Sorensen is an expert in invasive fish species, Satoshi Ishii is an expert in the use MFQPCR, and Dr. Wang has expertise in qPCR and the development of primers and probes to detect fish in aquatic systems.

B. Project Impact and Long-Term Strategy

Dr. Sorensen has already developed eDNA techniques to measure the presence of invasive common and bighead carp in inland lakes. This new work extends this technique using MFQPCR, to 21 AIS found in Minnesota lakes and in its rivers. We will conduct initial studies to show how it works in MN waterways. A next phase will be to work directly with DNR and USFWS biologists to implement this technology. We propose to train DNR and USFWS biologists in how to do these assays in workshops at the University of Minnesota. The USFWS has expressed interest. It is our goal to obtain a competitive national grant to continue this research.

C. Timeline Requirements: We are requesting two years for this project.

2018 Detailed Project Budget

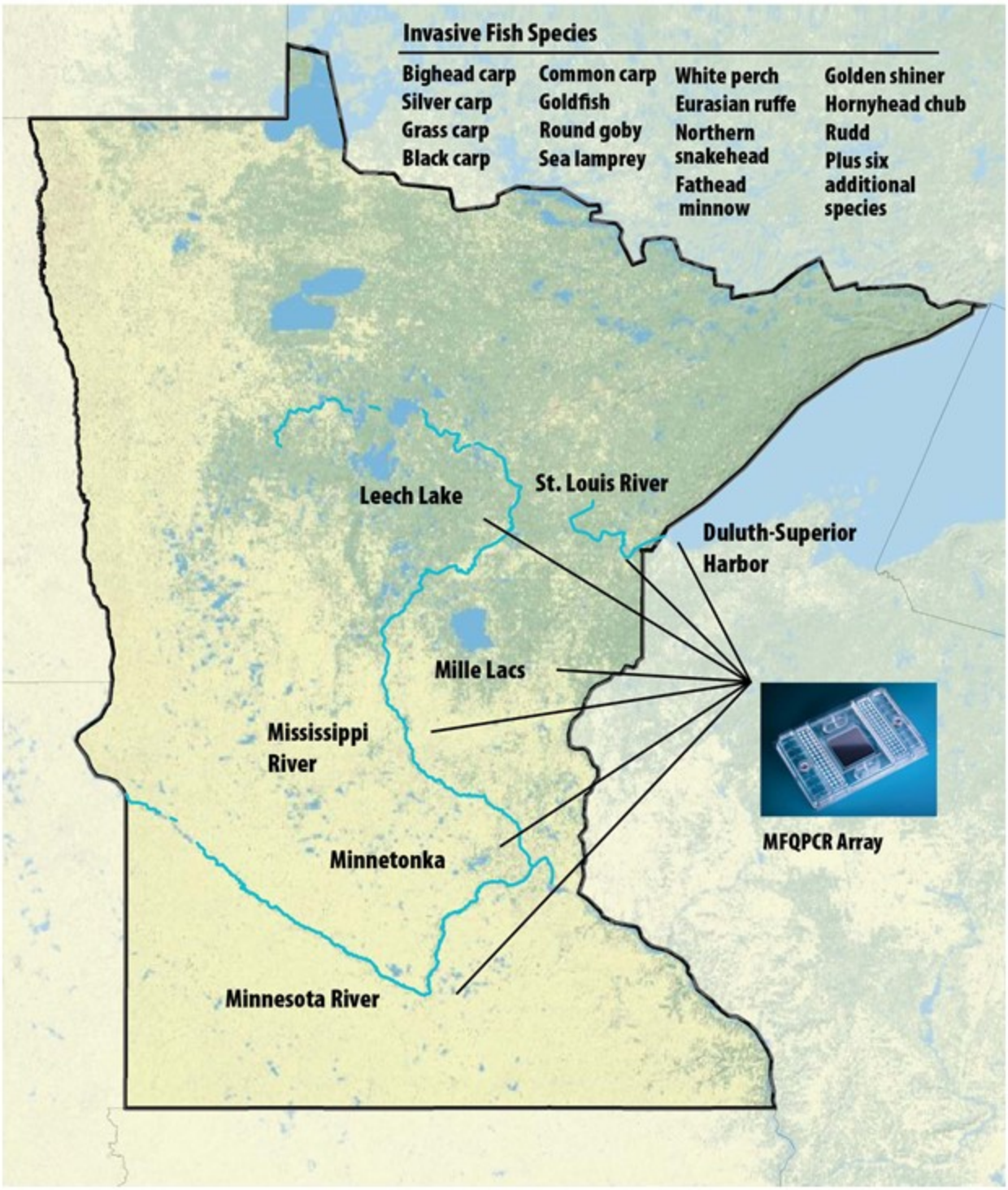
Project Title: Fish on a Chip: An AIS Detection Platform

IV. TOTAL ENRTF REQUEST BUDGET 2 years

BUDGET ITEM	AMOUNT
Satoshi Ishii, Assistant Professor (75% salary, 25% benefits); 8% FTE for two years; assay design, MFQPCR data analysis, project reporting.	$ 27,000
Peter Sorensen, Professor (75% salary, 25% benefits); 8% FTE for two years; supervision of research technician, data analysis, project reporting.	$ 38,000
Postdoctoral research associate (82% salary, 18% benefits); 100% FTE for two years; perform experiments, analyze data, write manuscripts	$ 120,000
Research technician (75% salary, 25% benefits); 100% FTE for two years; perform experiments, analyze data, engage in outreach activities.	$ 100,000
Research technician (75% salary, 25% benefits); 50% FTE for two years; maintain fish tanks, would get permits to squire fish, capture them and bring to lab, build holding facilities, maintain fish in lab and sample them and play a primary role in field sampling	$ 58,000
Professional/Technical/Service Contracts: University of Minnesota Genomics Center: MFQPCR ($980/run x 4 runs = $3,920), MiSeq ($3,000/run x 2 runs = $6,000), Sangar sequencing ($5/reads x 2 reads/sample x 200 samples = $2,000), Rental: MAISRC AIS holding facility ($3000)	$ 15,000
Equipment/Tools/Supplies: Lab and/ or Field: Field sampling ($3,000), water filtration ($20 x 200 samples = $4,000), DNA extraction ($5/sample x 200 samples = $1,000), PCR/qPCR primers and reagents ($6,000), qPCR standards ($95/assay x 21 assays = $2,000), fish feed ($5,000), chemicals ($2,000), disposable plasticware ($3,000), aquarium supplies ($4,000).	$ 30,000
Travel: MN: In-state sampling – Transportation: 5 field trips per year (~1000 miles) X $0.54/mile x 2 years = $1,080. Lodging and meals during field trips: $150/person/trip x 2 person/trip x 5 trips x 2 years = $3,000	$ 4,000
Shipping of AIS by FedEX	$ 2,000
Printing: Publication costs for dissemination. $500-$2000 per manuscript. Total: 3-4 manuscripts	$ 5,000
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 399,000

V. OTHER FUNDS

SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ To Be Applied To Project During Project Period:	N/A	
Other State $ To Be Applied To Project During Project Period:	N/A	
In-kind Services To Be Applied To Project During Project Period: The University of Minnesota does not charge the State of Minnesota its typical overhead rate of 54% of the total modified direct costs.	$ 195,000	Secured
Past and Current ENRTF Appropriation:	$303,217	ends June 30, 2017
Other Funding History:	N/A	



Approximate Sampling Locations for Fish on a Chip technology and Invasive Fish Species to be Tested

PROJECT TITLE: Fish on a Chip: An AIS Detection Platform

Project Manager Qualifications and Organization Descriptions

Michael Sadowsky
Michael Sadowsky is McKnight University Professor in the Dept. of soil, Water and Climate. He is also director of the BioTechnology Institute. He has been studying microbes in the environment for the last 38 years and has recently been working on microbiota associated with invasive species (fish, mussels, and plants). His laboratory has expertise in quantitative PCR and DNA sequencing technologies to track organisms in the environment. He has published over 305 peer-reviewed papers in scientific journals. The Sadowsky Lab is well is equipped with all the necessary items for the proposed research. In addition, his group has a full access to the microfluidics qPCR and related instruments in the University of Minnesota Genomics Center and access to the University of Minnesota supercomputing institute.

Peter Sorensen
Peter Sorensen is a professor in the Dept. Fisheries, Wildlife and Conservation Biology. He has been studying invasive fish for 28 years since he arrived at the University of Minnesota and has over 140 peer-reviewed articles in addition to nearly 50 book chapters and a patent on sea lamprey control. Dr. Sorensen founded MAISRC in 2012 and now focuses his efforts on developing controls for invasive carp but also has considerable experience measuring carp DNA, which was a prelude to this work. His laboratory is well equipped for this study and he also has access to two wet lab facilities to hold and handle invasive species. He also has all necessary permits for this work and government colleagues who could acquire them.

Satoshi Ishii
Satoshi Ishii is Assistant Professor in the BioTechnology Institute (BTI) and the Department of Soil, Water, and Climate (SWC) at the University of Minnesota. He has over 10 years of experiences on water quality microbiology. He has developed novel microfluidics tools to simultaneously quantify multiple pathogens and applied these tools to the risk assessment of water samples. The Ishii Lab (located in the St. Paul campus of the University of Minnesota) is equipped with all the necessary items for the proposed research. In addition, his group has a full access to the microfluidic qPCR and related instruments in the University of Minnesota Genomics Center.

Organization Descriptions
The University of Minnesota is the main research and graduate teaching institution in the state of Minnesota. The BioTechnology Institute provides advanced research, training, and university-industry interaction in biological process technology. In the Department of Soil, Water, and Climate, we seek to improve and protect the quality of soil, air, and water resources in natural and managed ecosystems, through research, reaching, and extension.

The mission of the Minnesota Department of Health is to protect, improve, and maintain the health of all Minnesotans.