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

Project Title: **ENRTF ID: 133-D**

Developing RNA Interference Genetic Controls for Zebra Mussels

Category: D. Aquatic and Terrestrial Invasive Species

Total Project Budget: $ 769,528

Proposed Project Time Period for the Funding Requested: 3 years, July 2018 to June 2021

Summary:

We will develop a microparticle using genetics (RNA interference) to specifically control zebra mussels.

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Sponsoring Organization: U.S. Geological Survey

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Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

The Central Dogma of Biology: (blueprints) DNA is the blueprint for the cell, arrow to bookshelf instructions schematic to represent RNAs are detailed instructions for individual parts, crossed out arrow to legos representing Proteins are the building blocks and machinery that carry out cell functions.

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

PROJECT TITLE: Developing RNA interference genetic controls for zebra mussels

I. PROJECT STATEMENT

We aim to develop a control tool for eliminating zebra mussels (ZM) that exploits natural gene regulation mechanisms (RNA-induced gene silencing; RNAi) to be specific to ZM without non-target effects. In addition to the nuisance they pose, Pimentel et al. estimated that ZM cost the U.S. economy over $1 billion annually. ZM are among the International Union for Conservation of Nature Global Invasive Species Database's 100 world's worst invasive alien species and are an emerging invasive species in Minnesota, found in over 200 lakes to date. Furthermore, ZM undergo a microscopic veliger stage where they are free-swimming throughout the water column for up to a month. This enables them to be easily spread into new waterways, making each currently infested lake a potential source for the next invasion. There are a few chemicals available for controlling ZM, but their use can affect the ecosystems when applied. As molecular tools are becoming more powerful and allowing broader-scale ecological studies, it is becoming apparent that changes to the microbiome can have significant and long-lasting impacts to an ecosystem's resilience to disease and future invasions. Such effects are not well studied as part of the chemical registration process. The use of a genetic control allows us to specifically target only ZM with no impact to non-target species.

- Zebra mussels have invaded over 200 lakes in Minnesota so far
- Zebra mussels are very easy to spread unknowingly by recreationists
- Control tools for zebra mussels are limited by cost and ecosystem impact
- Genetic control tools can target zebra mussels with greater specificity
- RNAi microparticles will be cheap to manufacture and safe to deploy
- RNAi microparticles will facilitate treatment in areas previously prohibited
- Unlike CRISPR gene drives, there is no risk of this control escaping back to native populations

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Identify and describe more zebra mussel genes. **Budget: $53,320**

Having accurate and well-understood gene sequence information is critical to enable this type of control. We will identify the essential and non-essential DNA sequences that make up suspected genes discovered as part of previous genome sequencing work. We will confirm that suspected genes are what they were predicted to be. This additional effort will give us flexibility for selecting gene targets for making the final product as effective as possible with as minimal dose as possible. The information gained by this activity can also be used for a wide range of future research applications.

Outcome	Completion Date
1. Contribution to 2019 genome symposium	*Spring 2019*
2. Publish sequence and descriptive information about genes discovered so they can be targeted for controls or other purposes.	*June 2021*

Activity 2: Develop RNA molecules that can turn off vital zebra mussel genes. **Budget: $599,338**

We will design silencing RNAs for critical ZM genes and clone them into microbial expression vectors as done by Timmons et al. for the worm *C. elegans*. We will feed the microbes expressing designed silencing RNAs to newly settled ZM juveniles and then analyze extracted RNA from the ZM by qPCR to experimentally determine if silencing RNAs can successfully knockdown target gene expression. Potential targets we may try have been successful against agricultural pests or have vital functions in digestive tract cells such as: ATPase, α-tubulin, aquaporin, cholecystokinin, leptin, and arginine kinase. We will determine lethality by feeding microbes expressing successful silencing RNAs to newly settled ZM juveniles and observing mortality.

Outcome	Completion Date

1. Design silencing RNAs and clone into microbial expression vectors based on available sequence information.	*April 2019*
2. Evaluate gene expression knockdown efficacy of silencing RNAs.	*September 2019*
3. Evaluate lethality of effective silencing RNAs.	*January 2020*
4. Design additional silencing RNAs and clone into microbial expression vectors based on gained sequence information.	*April 2020*
5. Evaluate gene expression knockdown efficacy of additional silencing RNAs.	*September 2020*
6. Evaluate lethality of effective additional silencing RNAs.	*January 2021*

Activity 3: Develop RNAi microparticles lethal to zebra mussels. **Budget: $116,868**

We will incorporate bacteria expressing effective and lethal silencing RNAs into microparticles of appropriate size for ZM to feed on. Microparticle lethality will be evaluated by feeding them to ZM in aquaria. This is the final product that will be used to control zebra mussel infestations.

Outcome	Completion Date
1. Incorporate effective silencing RNAs into microparticles.	*February 2021*
2. Evaluate ZM lethality of silencing RNA-laden microparticles.	*June 2021*

III. PROJECT STRATEGY

A. Project Team/Partners

This project will be conducted by scientists at the U.S. Geological Survey Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, Wisconsin; the Minnesota Aquatic Invasive Species Research Center (MAISRC) in St. Paul, Minnesota; and the U.S. Fish and Wildlife Service La Crosse Fish Health Center (LFHC) in Onalaska, Wisconsin. Dr. Jon Amberg (UMESC) will provide oversite of activities 2 and 3. Christopher Merkes and two technicians (UMESC) will develop the silencing RNAs and conduct knockdown and lethality trials. Diane Waller (UMESC), Eric Leis (LFHC), and two technicians will collect ZM and provide assistance in knockdown and lethality trials. Blake Sauey (UMESC) will incorporate silencing RNAs into microparticles. Mike McCartney (MAISRC) and researchers at the Minnesota Supercomputing Institute will conduct Activity 1.

B. Project Impact and Long-Term Strategy

This project will result in a cheap control tool that uses genetics to specifically target ZM. It will result in multiple peer-reviewed publications and conference presentations to share the information. This project will produce a method to manufacture microparticles that are lethal to ZM through RNA interference. We anticipate that additional funding will be needed to experimentally determine the efficacious dosing rates, and to demonstrate that the microparticles do not affect other species. This project represents the necessary initial investment to develop this technology for ZM. Once registration is completed, the RNAi microparticles have the potential to drastically improve ZM eradication efforts throughout Minnesota and across the globe.

C. Timeline Requirements

This project will be completed in three years. Genome annotation will be a continuous process throughout the length of the project, and we will publish results as we complete annotation for genes involved in particular biological pathways or some other sensible grouping. Newly settled juveniles will be available in May-September of each year. Gene silencing and lethality trials, as well as design and cloning of interfering RNAs will revolve around the ZM life cycle accordingly. Technical microparticle incorporation methods will be worked out as the lethality trials are being conducted, and particles for each lethal target will be made and tested as data comes in.

2018 Detailed Project Budget

Project Title: *Developing RNA interference genetic controls for zebra mussels*

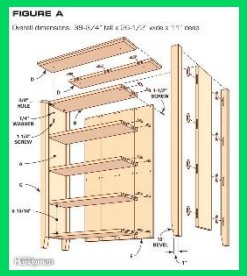
IV. TOTAL ENRTF REQUEST BUDGET [3] years

BUDGET ITEM	AMOUNT
Personnel:	$ -
Michael McCartney (MAISRC), Research Assistant Professor, (XX% salary, XX% benefits); 8% FTE	$ 15071
TBD, Research Informatics Solutions (RIS) Analyst, (XX% salary, XX% benefits); XX% FTE	$ 38250
Jon Amberg (USGS), Research Fish Biologist, (79% salary, 21% benefits); 17.5% FTE each year.	$ 94293
Christopher Merkes (USGS), Geneticist, (75% salary, 25% benefits); 46.8% FTE each year.	$ 180781
Matthew Hoogland (USGS), Geneticist, (81% salary, 19% benefits); 46.9% FTE year 1&2, 10.4% FTE year 3.	$ 81220
Tariq Tajjioui (USGS), Geneticist, (81% salary, 19% benefits); 46.9% FTE year 1&2, 10.4% FTE year 3.	$ 81220
Diane Waller (USGS), Fish Biologist, (71% salary, 29% benefits); 15.8% FTE each year.	$ 100316
Justine Nelson (USGS), Biologist, (81% salary, 19% benefits); 13.9% FTE each year.	$ 42304
Eric Lord (USGS), Biologist, (81% salary, 19% benefits), 13.9% FTE each year.	$ 41016
John Nelson (USGS), Biologist, (76% salary, 24% benefits), 2.9% FTE each year.	15411
Blake Sauey (USGS), Physiologist, (70% salary, 30% benefits), 12% FTE year 2&3	41477
Eric Leis (USFWS), Fish Biologist, (78% salary, 22% benefits), 12% FTE each year.	$ 38169
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 769,528

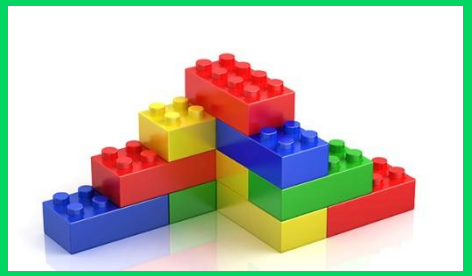
SOURCE OF FUNDS	AMOUNT	Status
Other Non-State $ To Be Applied To Project During Project Period:	N/A	
Other State $ To Be Applied To Project During Project Period:	N/A	
In-kind Services To Be Applied To Project During Project Period: USGS Overhead (55.5%): $ 427,088 Supplies: $ 100,000 Travel Expenses: $ 8,000 Previously purchased equipment: $ 243,000 (qPCR thermocyclers, incubators, biosafety laminar flow hood, large centrifuge, zebra mussel holding and culture facilities)	$ 778,088	*Secured*
Past and Current ENRTF Appropriation:	N/A	
Other Funding History:	N/A	

The Central Dogma of Biology

DNA is the blueprint for the cell



RNAs are the detailed instructions for individual parts

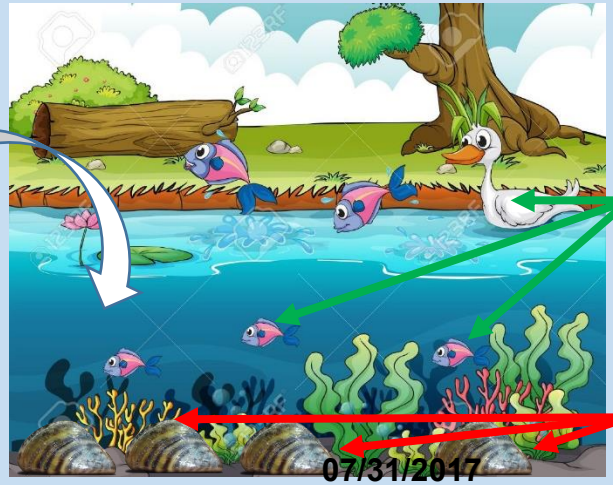


Proteins are the building blocks and machinery that carry out cell functions

RNAi blocks this process

Using RNAi microparticles to control zebra mussels

Spread microparticles over infested area



Harmless to fish and other non-target organisms

Lethal to zebra mussels

Developing RNA interference genetic controls for zebra mussels
Project Manager Qualifications and Organization Description

I earned my Bachelor of Science degree in Biology from the University of Wisconsin – Stevens Point in 2008. I earned my Master of Arts degree in Molecular, Cellular, and Developmental Biology from the University of Kansas in 2013. Since then, I have been working as a geneticist with the U.S. Geological Survey (USGS) in La Crosse, Wisconsin to develop molecular methods for detecting, assessing, and controlling invasive species. Part of my graduate research involved studying gene regulation (Merkes et al., 2015), and I completed coursework under Lisa Timmons who first developed the methods for using RNA interference (RNAi) to silence genes in *C. elegans* as part of her graduate work (Timmons et al., 2001). I have initiated studies to develop similar RNAi controls for Asian Carp at USGS. Developing this technology for zebra mussels as well would allow for some efficiencies to achieve cost savings on both projects. Including Mike McCartney from the Minnesota Aquatic Invasive Species Research Center on the team will also advance the starting point significantly for zebra mussels, because of the zebra mussel genome sequencing and annotation work he has been doing as that information is not available for Asian Carp.

The U.S. Geological Survey (USGS) is a federal research institution and our science center in La Crosse serves the ecosystems mission area. Ecosystems provides unbiased science, tools, and decision support to our Nation's natural resource managers, with particular focus on the science needs of the Department of Interior bureaus to conserve species, lands, and priority ecosystems; fulfill treaty obligations; provide water for irrigation and human consumption; and manage mineral and energy resources.