Environment and Natural Resources Trust Fund
2019 Request for Proposals (RFP)

Project Title: **ENRTF ID: 167-D**

Zebra Mussel Genetic Biocontrol: Methods and Public Engagement

Category: D. Aquatic and Terrestrial Invasive Species

Sub-Category:

Total Project Budget: $ 368,125

Proposed Project Time Period for the Funding Requested: June 30, 2022 (3 yrs)

Summary:

We develop techniques for precise genetic modification of zebra mussels, and at the same time engage the public in decisions on whether and how to apply these for biocontrol.

Name: Michael McCartney

Sponsoring Organization: U of MN

Title: Research Assistant Professor

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Location

Region: Statewide

County Name: Statewide

City / Township:

Alternate Text for Visual:

Genetic biocontrol of zebra mussels—current, proposed and future research

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ TOTAL	_____%
____ If under $200,000, waive presentation?			

PROJECT TITLE: Zebra mussel genetic biocontrol methods and public engagement

I. PROJECT STATEMENT

This project is the first phase of research required to modify zebra mussel (ZM) genes using modern tools for precision genetics, and to involve the public from the beginning of the research and development process. Prevention remains the most effective way to slow the spread of ZMs to new MN waters. However, for our several prized lakes in which ZMs are established, *essentially no options exist* to reduce populations and their ecologic and economic damages. Modifying crucial genes in ZMs and spreading the changes throughout large invasive populations is now an option within reach. Modern tools in genetic manipulation are providing new strategies to control pests with great flexibility and pinpoint accuracy for gene targets, while minimizing ecological risks. Yet none of these tools is near the stage of real application to ZMs. Inserting a fragment of DNA (which can be used to modify the gene of interest) into ZM eggs at frequencies high enough to counter the high mortality of larvae, as they develop into mussels, is a major hurdle. This project takes these first necessary steps—by developing DNA fragments and reagents to deliver to fertilized ZM eggs, by verifying stable incorporation, and by optimizing efficiency so that hundreds to thousands of larvae can be modified in a single experiment. Meanwhile, we bring select stakeholders to the table, and with state of the art communication methods, engage citizens of the state in the process of deciding if and how to go forward with ZM biocontrol.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Modifying DNA of ZM embryos and larvae, and testing for success **Budget:** $339,025

We will evaluate methods that have been successfully used to introduce fragments of DNA into other aquatic animals, e.g. transient electrical pulses that open cell membranes to DNA (Tsai et al. 1997; Collares et al. 2010) and a "gene gun" to bombard cells with DNA coated particles (Yamauchi et al. 2000). A particular focus will be given to methods (such as the ones listed above) that will allow us to modify hundreds of eggs at once to offset the high mortality experienced by ZM embryos and larvae. The tasks involved in this activity will combine bioinformatics and laboratory experiments and are: (i) searching the ZM genome (sequencing completed in our current ENRTF-funded project) for regions to target for proof-of-concept, (ii) design and synthesis of DNA constructs that make precise mutations or alter expression of gene products, (iii) fertilization of zebra mussel eggs in the laboratory, (iv) experiments on delivery of DNA to fertilized eggs, and (v) assessment of stable incorporation into embryos and larvae. This first activity is an essential step if we hope, one day, to go forward with ZM genetic biocontrol. None of the control strategies (shown as examples in the visual component), made possible by the modern genetic tools will become accessible if this first activity is not undertaken.

Outcome	Completion Date
1. Informatics; DNA-modification constructs designed, synthesized and purified	*June 2020*
2. Experiments on delivery of modified DNAs to ZMs, tests to verify incorporation completed	*June 2022*

Activity 2: Engage the public in decision-making for zebra mussel genetic biocontrol **Budget:** $ 29,100

As the potential has grown for genetic manipulation to address invasive species and other environmental problems, there have been clear calls for such technological advances to be accompanied by rigorous forms of engagement (NASEM 2016; Jasanoff & Hurlbut 2018). These calls recognize that the development and use of gene manipulation must not be seen as a narrow technical issue. Rather it is a values-laden societal endeavor requiring deliberation—throughout the technological research and development process—with a wide range of experts, stakeholders and decision makers (Stilgoe et al. 2013). Such inclusive engagement can help ensure that the highest risks of a control strategy are identified and assessed, and that any decision to apply it is informed by broad deliberation. The potential for the genetic biocontrol of ZMs is within reach, so now is the right time to begin the process needed to make this research technically *and* socially sound. The task of this activity will be to hold a 2-

day workshop (25-30 participants). The goals are: i) to bring together local and national NGO stakeholders, academics, and state and federal agency managers and decision makers to learn about and deliberate on biocontrol strategies; ii) to create a list of questions /research needs that participants feel need to be answered before any decision is made; and iii) to gain a better understanding of how participants differentiate and prioritize the potential biocontrol techniques. Small and large group deliberative exercises and a survey will be utilized to garner those insights. Conclusions from this workshop will be summarized in a workshop report/publication.

Outcome	Completion Date
1. Deliberative workshop convened	*September 2020*
2. Workshop report/publication completed	*August 2021*

III. PROJECT PARTNERS:

A. Partners receiving ENRTF funding (*M. Smanski is not receiving salary)

Name	Title	Affiliation	Role
Michael McCartney	Research Assistant Professor	MAISRC, UMN	PI, lab spawning and raising of larvae
Sophie Mallez	Postdoctoral Associate	MAISRC, UMN	DNA delivery to ZM and tests for success
Adam Kokotovich	Postdoctoral Associate	North Carolina State Univ., Genetic Engineering and Society Center	Deliberative workshop
Michael Smanski*	Assistant Professor	Biochemistry, Molecular Biology and Biophysics, UMN	Design and synthesis of precision genetic tools
Siba Das	Postdoctoral Associate	Smanski lab	Training on DNA delivery methods

IV. LONG-TERM- IMPLEMENTATION AND FUNDING:

This project is Phase I of a larger, longer-term and multi-phase project whose goal is to develop efficient, species-specific and environmentally-friendly ways to control zebra mussels. This phase has already received considerable state funding to sequence the ZM genome and identify target genes—paving the way for the project proposed here. This project will, for the first time, develop techniques for modifying the DNA of ZM embryos and larvae. The next phases will require rearing of larvae in a research-scale hatchery such that modified offspring can be raised routinely to the stage where they settle down and become juvenile mussels. This will require a facility for growing live algae and for rearing larvae. Genetic modification of target genes, and laboratory evaluation of phenotypes and transmission to offspring will then follow. We will seek funding for the hatchery from MAISRC and ENRTF, and from federal sources (NSF, Bureau of Reclamation) for laboratory trials on effects and transmission of engineered mutations. By this time, through stakeholder engagement and working with regulatory agencies, we will develop protocols for trial releases in isolated water bodies, such as infested mine pits in MN.

V. TIME LINE REQUIREMENTS:

Project duration is three years. DNA modification agents can only enter the germ line (embryonic cells giving rise to eggs and sperm) and be transmitted to offspring if they are introduced to early larvae, meaning that delivery must be done on eggs spawned and fertilized in the lab. Mussels can be induced to spawn June to late August each year in Minnesota. We intend to extend this window by inducing animals mailed from sites on the lower Mississippi River (which spawn in spring), and perhaps quagga mussels from the Colorado River (which spawn in winter). Bioinformatics and design of CRISPR-Cas9 and dCas9 elements will extend through year 1, with delivery experiments, followed by PCR and fluorescent microscopy to confirm successful incorporation taking the full three years to complete. The workshop will be planned during year one and held in September of 2020.

2019 Proposal Budget Spreadsheet

Project Title: Zebra mussel genetic biocontrol methods and public engagement

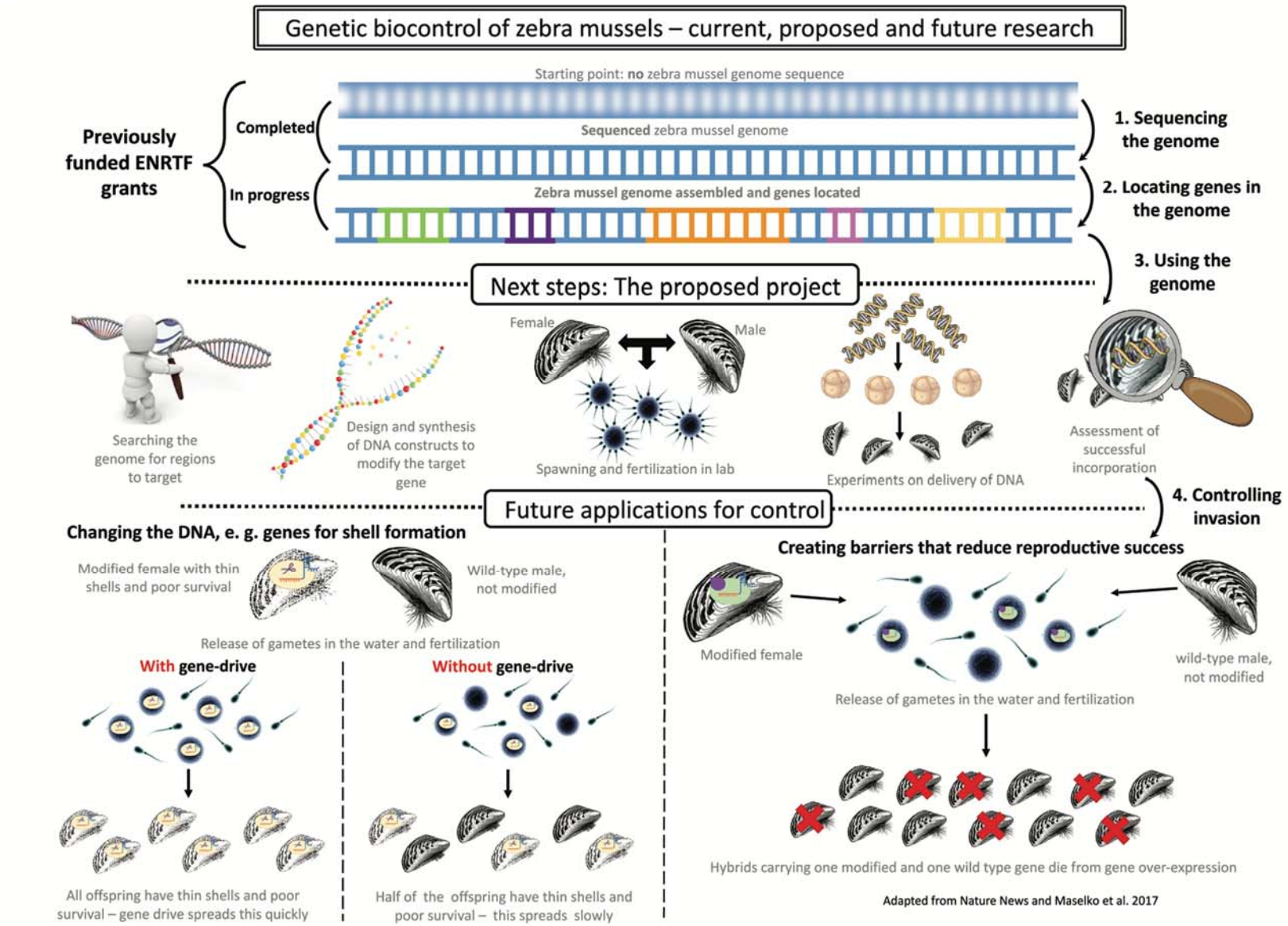
IV. TOTAL ENRTF REQUEST BUDGET 3 years

BUDGET ITEM (See "Guidance on Allowable Expenses")	**AMOUNT**
Personnel: 1. Michael McCartney, Research Assistant Professor, 25% FTE per year, 74.8% salary, 25.2% fringe benefits, 3 years. 2. Sophie Mallez, postdoctoral associate, 100% FTE per year, 81.7% salary, 18.3% fringe benefits, 3 years. 3. Siba Das, Postdoctoral associate in Smanski lab, 25% FTE, 81.7% salary, 18.3% fringe benefits, 1 year (2019 only). 4. Graduate student stipend support for note-taking at workshop = $750.	$297,019
Professional/Technical/Service Contracts: Subcontract to Adam Kokotovich, North Carolina State University to design and conduct workshop and publish results.	$9,500
Rental of invertebrate lab space in MAISRC Containment Lab to work with eggs, embryos and larvae (5 X 13-week rental periods @ $2,190 per 13 weeks)	$10,950
Use of growth chamber to grow larvae (150 days @ $3.00 per day)	$450
Honoraria for speakers	$1,000
Equipment/Tools/Supplies: 1. Glassware, supplies for growing algae and larvae 2. Supplies for purifying DNA modification agents 3. Other molecular biology supplies (CRISPR/Cas9 kits, transfection kits, genomic DNA extraction kits, PCR reagents) 4. Supplies for delivery of DNA to fertilized eggs	$25,354
Travel: MAISRC truck miles for collecting zebra mussels.	$5,002
Workshop travel: mileage, parking, lodging, airfare for workshop participants. $9000 of this is domestic travel for essential out of state participants from agencies and academia whose expertise is not represented in MN.	$13,900
Additional Budget Items: Shipping costs for live mussels	$1,500
Catering for workshop, room rental for workshop	$3,450
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$368,125

V. OTHER FUNDS *(This entire section must be filled out. Do not delete rows. Indicate "N/A" if row is not applicable.)*

SOURCE OF FUNDS	**AMOUNT**	**Status**
Other Non-State $ To Be Applied To Project During Project Period:	N/A	*N/A*
Other State $ To Be Applied To Project During Project Period:	N/A	*N/A*
In-kind Services To Be Applied To Project During Project Period:	N/A	*N/A*
Past and Current ENRTF Appropriation:	N/A	N/A
Other Funding History: MN Aquatic Invasive Species Research Center Sub-Project # 9.1: Zebra mussel investigations: pathways and mechanisms of spread, new molecular approaches for early detection, and methods for estimating population change in response to pesticide treatment. Funding for initial sequencing of zebra mussel genome (at low sequencing depth), SNP discovery and genotyping = $124,636.	$567,115	Completed
MAISRC Sub-Project #9.2: Population genomics of zebra mussel spread pathways, genome sequencing and analysis to select target genes and strategies for genetic biocontrol. Funding for seqeuenced and analyzed genome that will be used to build DNA modifying agents = $198,827.	$427,950	Secured

B. Visual Component. Zebra mussel genetic biocontrol methods and public engagement



D. Acquisition, Easements, and Restoration Requirements

This is not an acquisition or restoration proposal

F. Project Manager Qualifications and Organization Description

Michael McCartney is a Research Assistant Professor in the Minnesota Aquatic Invasive Species Research Center (MAISRC) and the Department of Fisheries, Wildlife and Conservation Biology at the University of Minnesota. He holds a Ph.D. in Ecology and Evolution from the State University of New York (1994), has 5 years of post-doctoral experience at the Smithsonian Tropical Research Institute, University of California and Florida State University, and spent 13 ½ years on the faculty at the University of North Carolina, Wilmington, studying marine and freshwater aquatic animals. His unique skills for this project include methods of fertilization and study of the development of bivalve molluscs, including laboratory culture of larvae, as well as molecular biology experience gained since 1985 on organisms ranging from bacteria through invertebrate animals and fishes.

McCartney leads the invasive mussel research program at MAISRC where he focuses on zebra mussel ecology, population genetics and genomics for the purpose of understanding and preventing spread, and he maintains active research directed at developing population controls. He will direct and assist with research on delivery of DNA modifying agents (CRISPR/Cas9 and dCas9 constructs) to zebra mussel fertilized eggs. He has led the zebra mussel genome project at UMN over the last 3 years, and is uniquely suited to coordinate this collaboration between zebra mussel biologists at MAISRC and synthetic biologists in the Smanski lab who are pioneering techniques for genetic modification of AIS. His outreach throughout the state and close relationships with LGUs in MN, MN DNR and federal agency personnel working on AIS will facilitate the public engagement work led by Dr. Kokotovich.

Minnesota Aquatic Invasive Species Research Center

In 2012, the Minnesota legislature appropriated funds to create an Aquatic Invasive Species Cooperative Research Center at the University of Minnesota, in collaboration with the Commissioner of Natural Resources (DNR). The Center's mission is to develop research-based solutions that can reduce the impacts of aquatic invasive species in Minnesota by preventing spread, controlling populations, and managing ecosystems; and to advance knowledge to inspire action by others. MAISRC's vision is to be a vibrant and durable research enterprise that advances the knowledge and builds the capacity that Minnesota needs in order to reduce the impacts of aquatic invasive species on our cherished lakes, rivers, and wetlands.