# Environment and Natural Resources Trust Fund (ENRTF)
# M.L. 2018 ENRTF Work Plan (Main Document)

**Today's Date:** February 16, 2018

**Date of Next Status Update Report:** January 31, 2019

**Date of Work Plan Approval:** 06/05/2018

**Project Completion Date:** June 30, 2021

**Does this submission include an amendment request?** No

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**PROJECT TITLE:** Developing RNA Interference to Control Zebra Mussels

**Project Manager:** Christopher M. Merkes

**Organization:** U.S. Geological Survey

**College/Department/Division:** Upper Midwest Environmental Sciences Center

**Mailing Address:** 2630 Fanta Reed Road

**City/State/Zip Code:** La Crosse / Wisconsin / 54603

**Telephone Number:** (608) 781-6316

**Email Address:** cmerkes@usgs.gov

**Web Address:** https://www.usgs.gov/staff-profiles/chris-m-merkes

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**Location:** Statewide

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**Total Project Budget:** $500,000

**Amount Spent:** $0

**Balance:** $500,000

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**Legal Citation:** M.L. 2018, Chp. 214, Art. 4, Sec. 02, Subd. 06d

**Appropriation Language:** $500,000 the second year is from the trust fund to the commissioner of natural resources for an agreement with the United States Geological Survey to develop a genetic control tool that exploits the natural process of RNA silencing to specifically target and effectively control zebra mussels without affecting other species or causing other nontarget effects. This appropriation is available until June 30, 2021, by which time the project must be completed and final products delivered.

**I. PROJECT STATEMENT:**

We aim to develop a control tool for eliminating zebra mussels that exploits natural gene regulation mechanisms (RNA-induced gene silencing; RNAi) to be specific to zebra mussels without non-target effects. In addition to the nuisance they pose, Pimentel *et al*. estimated that zebra mussels cost the U.S. economy over $1 billion annually. Zebra mussels 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 300 waters to date. Furthermore, zebra mussels 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 zebra mussels, but their use can affect the ecosystems when applied. One such chemical is EarthTecQZ. This is a copper-based compound, and it is also used to control algae as well as zebra mussels. At the recommended levels, it is not harmful to fish and other animals, and it has not been found to contaminate drinking water. It will kill other macroinvertebrates however, and when it is applied to areas with a lot of algae, that can result in dissolved oxygen crashes as the algae degrades which can kill many fish and other aquatic organisms not affected by the copper directly. Another chemical that is used for zebra mussel control is potash. Potash is not registered like EarthTecQZ is, but generally gets approved for use on waivers, because it is regarded as generally benign and commonly used for fertilizer. While potash does not seem to harm fish or other vertebrates, it is toxic to native mussels along with zebra mussels and it can persist in the system for a long time. Another available zebra mussel treatment is zequanox. Zequanox is an attenuated bacteria treatment, and is more selective for zebra mussels than either EarthTecQZ or potash. However, zequanox is mostly used for treating pipes rather than open water applications, because it is prohibitively expensive to treat a large area.

While these treatments have been tested well against fish and some other vertebrate species for lethality, sub-lethal effects and how they affect the microbiome are not as rigorously tested. 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. The use of a genetic control allows us to specifically target only zebra mussels with no impact to non-target species.

There are a couple options that can be considered for genetic control. One strategy is to use a gene drive to propagate a negative trait through a population, such as female sterility. Using a CRISPR mechanism or a selfish genetic element to carry defective alleles to all offspring with super-Mendelian inheritance, a few genetically modified individuals could be released to mate with wild type animals and change the genetic makeup of a population so that it is no longer viable. Another strategy is to use RNA interference (RNAi) to selectively turn off critical genes of the target organism. This strategy takes advantage of the natural gene regulation mechanism of RNA-induced gene silencing. We target unique genetic sequences of zebra mussels that do not occur in other species for critical genes provide the instructions (silencing RNA) to turn off those genes. Because the target sequences do not occur in other species, it makes the control tool very specific. Both strategies have advantages and disadvantages. Gene drives will require less application effort, because it involves releasing a few animals who do the work of spreading the control. Whereas, RNAi controls will require applying the control agent to all areas where the target organism occurs. RNAi control strategy gives managers better control over where and how it is applied though, whereas gene drives will spread wherever genetically modified individuals move to. This could result in accidental escape of a gene drive from a target population to another population of the same organism where locals may not wish for the same control to be applied (i.e. jumping back to the native range).

Methods for deliberate RNA-induced gene silencing were developed in the early 1990's, and this molecular tool has since been widely used to discover complex gene functions that could not be studied before. Over 3,000 peer-reviewed journal articles have been published using RNAi since that discovery, and the researchers responsible for it were awarded the Nobel Prize in 2006. Only recently, has its potential for controlling harmful pests and invasive species begun to be explored. There are a number of trans-genic crops and sprays in development and being tested that use RNAi to confer resistance to agricultural pests such as corn root worm and striped flea beetle. This project could lead to the first application of this technology by natural resource managers to extirpate an invasive species in an area.

- Zebra mussels have invaded over 300 waters 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. OVERALL PROJECT STATUS UPDATES:** See Activity-1 below

**III. PROJECT ACTIVITIES AND OUTCOMES:**

**ACTIVITY 1: Develop RNA molecules that can turn off vital zebra mussel genes.**
**Description:**

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.
**ENRTF BUDGET: $500,000**

| 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 a second round of silencing RNAs and clone into microbial expression vectors making refinements as needed of first-round RNAs and targeting additional genes. | April 2020 |
| 5. Evaluate gene expression knockdown efficacy of second round silencing RNAs. | September 2020 |
| 6. Evaluate lethality of effective additional silencing RNAs. | June 2021 |

**First Update January 31, 2019**
**Second Update June 30, 2019**
**Third Update January 31, 2020**
**Fourth Update June 30, 2020**
**Fifth Update January 31, 2021**
**Final Update June 30, 2021**

**IV. DISSEMINATION:**

**Description:**

This project will result in a peer-reviewed journal article describing our methods and results. Presentations will be given at a minimum of two scientific conferences as well as the Minnesota Aquatic Invaders Summit. Bacterial clones producing successful gene knockdown will be available upon request. USGS twitter and facebook posts will be used to promote the publication when it becomes available.

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**Final Update June 30, 2021**

**V. PROJECT BUDGET SUMMARY:**

**A. Preliminary ENRTF Budget Overview:** See Attached Budget Sheet

**Explanation of Capital Expenditures Greater Than $5,000:**
Not Applicable
**Explanation of Use of Classified Staff:**
Not Applicable
**Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation:**

| | |
|---|---|
| Enter Total Estimated Personnel Hours: 6,923 | Divide by 2,080 = TOTAL FTE: 3.33 |

**Total Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation:**

| | |
|---|---|
| Enter Total Estimated Personnel Hours: N/A | Divide by 2,080 = TOTAL FTE: N/A |

**B. Other Funds:**

| SOURCE OF AND USE OF OTHER FUNDS | Amount Proposed | Amount Spent | Status and Timeframe |
|---|---|---|---|
| **In-kind Services To Be Applied To Project During Project Period:** | | | |
| USGS Overhead (55.5%) | $277,500 | $0 | Secured |
| Supplies | $90,000 | $0 | Secured |
| Travel Expenses | $8,000 | $0 | Secured |
| Previously purchased equipment | $243,000 | $243,000 | Secured |

**VI. PROJECT PARTNERS:**

**A. Partners receiving ENRTF funding**

| Name | Title | Affiliation | Role |
|---|---|---|---|
| Jon Amberg | Research Fish Biologist | U.S. Geological Survey | Coordination with other projects to obtain additional sequencing information, development of delivery mechanism, and additional testing required for biopesticide registration. |
| Diane Waller | Research Fish Biologist | U.S. Geological Survey | Zebra mussel collection, care, and live zebra mussel testing. |

**B. Partners NOT receiving ENRTF funding**

| Name | Title | Affiliation | Role |
|---|---|---|---|
| | | | |

**VII. LONG-TERM- IMPLEMENTATION AND FUNDING:**

This project will result in a series of bacterial clones expressing silencing RNA molecules that are lethal to zebra mussels. The bacterial clones will be useable to develop a cheap control tool (i.e. microparticle or some other delivery mechanism) that uses genetics to specifically target zebra mussels. This project will result in multiple peer-reviewed publications and conference presentations to share the information. We anticipate that additional funding will be needed to optimize delivery, experimentally determine the efficacious dosing rates, and to demonstrate that the silencing RNAs do not affect other species. This project represents the necessary initial investment to develop this technology for zebra mussels. Once biopesticide registration is completed, the RNAi control tool will have the potential to drastically improve zebra mussel eradication efforts throughout Minnesota and anywhere they are invasive across the globe. Proposals to leverage this funding for additional funds will be sent to USGS ecosystems mission area, Great Lakes Restoration Initiative, Bureau of Reclamation, and to a new multi-agency Dreissenid mussel initiative developing in the Pacific Northwest who have all funded zebra mussel research in the past and would likely be interested in supporting this work.

**VIII. REPORTING REQUIREMENTS:**

- **The project is for 3 years, will begin on July/01/2018, and end on June/30/2021.**
- **Periodic project status update reports will be submitted January/31 and June/30 of each year.**
- **A final report and associated products will be submitted between June 30 and August 15, 2021.**

**IX. SEE ADDITIONAL WORK PLAN COMPONENTS:**

**A. Budget Spreadsheet**
**B. Visual Component**

**Attachment A:**
**Environment and Natural Resources Trust Fund**
**M.L. 2018 Budget Spreadsheet**

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**Project Manager: Christopher M. Merkes**
**Organization: U.S. Geological Survey**
**College/Department/Division: Upper Midwest Environmental Sciences Center**
**M.L. 2018 ENRTF Appropriation: $500,000**
**Project Length and Completion Date: 3 Years, June 30, 2021**
**Date of Report:** February 16, 2018

| ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET | Budget | Amount Spent | Balance |
|---|---|---|---|
| **BUDGET ITEM** | | | |
| **Personnel (Wages and Benefits) - Oveall** | $500,000 | $0 | $500,000 |
| *2 Research Fish Biologists, $129,740 (75% salary, 25% benefits), 7.7% FTE each per year for 3 years (Total amount estimated $129,740)* | | | |
| *3 Geneticists, $298,310 (78% salary, 22% benefits), 23.1% FTE each per year for 3 years Total amount estimated $298,310)* | | | |
| *3 Biologists, $71,950 (80% salary, 20% benefits), 8.8% FTE each per year for 3 years Total amount estimated $71,950)* | | | |
| **COLUMN TOTAL** | $500,000 | $0 | $500,000 |