

Date of Status Update Report: N/A Date of Next Status Update Report: December 31, 2013 Date of Work Plan Approval: June 11, 2013 Project Completion Date: June 30, 2016

Is this an amendment request? N

**PROJECT TITLE:** Zebra Mussel Control Research and Evaluation in Minnesota Waters

Project Manager: Mark Gaikowski

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Location:

Statewide

Total ENRTF Project Budget:	ENRTF Appropriation:	\$600,000
	Amount Spent:	\$0
	Balance:	\$600,000

Legal Citation: M.L. 2013, Chp. 52, Sec. 2, Subd. 06f

#### Appropriation Language:

\$600,000 the first year is from the trust fund to the commissioner of natural resources for an agreement with the United States Geological Survey, Upper Midwest Environmental Sciences Center, to assess the ecological impacts of a commercially available molluscicide formulation on the reproduction and development of native fish, as well as impacts on larval aquatic insect survival, and to evaluate the effectiveness of these treatment options for detection and control of zebra mussels. The United States Geologic Survey is not subject to the requirements in Minnesota Statutes, section 116P.10. This appropriation is available until June 30, 2016, by which time the project must be completed and final products delivered.

**II. PROJECT STATEMENT:** There is an immediate need for safe and effective control measures to reduce the impact of dreissenid mussels (zebra *Dreissena polymorpha* and quagga mussels *D. rostriformis bugensis*) whose attachment and feeding behavior disrupt aquatic food webs and foul spawning habitats, behaviors that threaten native aquatic species like mussels and fish. The range expansion of dreissenid mussels within Minnesota lakes and rivers continues (e.g. ~27 lakes were added to the list since 2009, bringing the total to about 90 waters with confirmed or interconnected dreissenid populations) while management agencies lack access to effective tools to control dreissenid mussel populations in open waters.

One potential tool for limited open-water control of dreissenid mussels is the commercially formulated product, Zequanox<sup>®</sup>, which contains the killed cells of a specific strain (*Pf*-CL145A) of the common soil bacterium *Pseudomonas fluorescens*. Zequanox<sup>®</sup> is produced by Marrone Bio Innovations (Davis, CA) and it is registered by the U.S. Environmental Protection Agency for control of dreissenid mussels in defined discharges (e.g. in cooling and service water systems for industrial facilities). Reference to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not constitute or imply its endorsement, recommendation, or favoring by the United States government. Throughout the remainder of this document the formulated *Pf*-CL145A product (Zequanox<sup>®</sup>) will be referred to as sprayed dried powder (SDP). A 3-year, multiagency (U.S. Geological Survey, U.S. Fish and Wildlife Service, and New York State Museum) research project is in progress to further assess the potential impacts of SDP on native fish and mussel species during open water applications (see <u>http://cida.usgs.gov/glri/projects/invasive\_species/zm\_control.html</u>).

The overall goal of the proposed project is to determine the safety and efficacy of SDP for control of dreissenid mussels in limited, high-value Minnesota waters. The existing non-target animal impacts database of SDP will be expanded by evaluating the impacts of SDP on the reproductive success of native fish populations and on the survival of native aquatic insects. Fathead minnows, a representative test species, and their eggs will be exposed to an environmentally relevant concentration and exposure duration of SDP and the impacts on spawning and development assessed. Separate but similar exposures will be completed to assess the potential acute effects of SDP to larval aquatic insects.

Adult fathead minnows (*Pimephales promelas*) will be exposed to SDP in outdoor mesocosms. Treatment groups will be exposed to a single dose of SDP at the expected environmental treatment concentration (e.g. 0, 50, and 100 mg/L active ingredient [A.I.]) for 8 hours. Fish in each treatment group will be observed for up to 30 days after exposure to assess reproduction. Fish in each treatment group will have access to spawning tiles and the number of eggs deposited on each tile will be determined. Resulting eggs will be placed into separate holding chambers and monitored to determine percent hatch. A subset of the resulting fry (F<sub>1</sub> generation) from each treatment concentration will be reared to adulthood to compare development and reproductive success to that of untreated controls.

Separately, eggs (at selected development stage[s]) from naïve fathead minnows will be exposed to a single static dose of SDP in an outdoor mesocosm setting at the expected environmental treatment concentration (e.g. 0, 50, and 100 mg/L A.I.) for 8 hours to assess the potential impact of SDP open water application on fish embryo development. A subset of the resulting fry from each treatment group will be reared to adulthood to compare development between exposed and control groups.

Laboratory and pond-scale studies will compare treatment application techniques (i.e., injection vs. whole water column treatment) to determine the minimum amount of SDP required during field applications. These studies will focus on determining techniques that maintain an effective concentration of SDP for the required exposure duration. Delivery techniques will be evaluated in the laboratory then evaluated under field conditions in 0.01-acre outdoor research ponds.

Detailed maps of two Minnesota lakes, or portions thereof, (1 high and 1 low level infestation, to be identified in 2013) will be prepared using a combination of bathymetric (using high-resolution sonar systems to characterize habitat), physical (to determine zebra mussel densities) and molecular surveys (using environmental DNA [eDNA]). The maps will be used to correlate zebra mussel populations within the lake to bathymetric and substrate data.

Habitats in Robinson's Bay, Lake Minnetonka (Deephaven, MN) will be selected to evaluate efficacy of SDP application techniques for controlling zebra mussels in limited, high-value, open water. Five replicated enclosures (~24 m<sup>2</sup>) will be prepared on 3 independent treatment days at selected locations and assigned 1 of 5 treatments (control, 50 mg/L A.I. SDP injection, 100 mg/L A.I. SDP injection, 50 mg/L A.I. SDP complete water column, or 100 mg/L A.I. SDP complete water column) according to a randomized study design. Treatment efficacy will be assessed from pre- and post-application surveys with a focus on impacts on zebra mussel survival and colonization rates. In addition to evaluating treatment efficacy, the project will evaluate the capacity of molecular monitoring (using eDNA) to identify locations where the application of a control, such as SDP, might have the greatest impact on zebra mussel populations.

#### **III. PROJECT STATUS UPDATES:**

Periodic work plan status update reports will be submitted not later than December 31, 2013, June 30, 2014, December 31, 2014, June 30, 2015, December 31, 2015, and June 30, 2016. A final report and associated products will be submitted by June 30, 2016 or as requested by the LCCMR.

Project Status as of December 31, 2013: Project Status as of June 30, 2014: Project Status as of December 31, 2014: Project Status as of June 30, 2015: Project Status as of December 31, 2015: Project Status as of June 30, 2016:

#### **IV. PROJECT ACTIVITIES AND OUTCOMES:**

#### **ACTIVITY 1:** Non-target animal impacts

**Description:** Three experimental trials will be conducted to evaluate the impacts of SDP exposure on non-target animals. The first trials will evaluate the potential acute toxicity of SDP to aquatic invertebrate larvae. Mayfly (*Order: Ephemerotera*) and amphipod (*Order: Amphipoda*) larvae will be collected in the upper Mississippi River basin and a known number of larvae placed into outdoor mesocosms (~1,000 L) containing sediment and water from a UMESC research pond. The larvae will be exposed to a single static application of SDP. Replicated exposures will be applied at expected environmental concentrations (e.g. 0, 50 and 100 mg/L A.I.) and at the expected environmental exposure to fathead minnows. Adult fathead minnows will be exposed to a single static application of SDP in outdoor mesocosms (~1,000 L) containing pond water from a UMESC research pond. The survival of larval aquatic insects. The second set of trials will evaluate the potential reproductive impacts of SDP exposure to fathead minnows. Adult fathead minnows will be exposed to a single static application of SDP in outdoor mesocosms (~1,000 L) containing pond water from a UMESC research pond. The replicated exposures will be conducted at expected environmental concentrations (e.g. 0, 50, and 100 mg/L A.I.) and at the expected environmental exposure duration (8h). Fish will be observed for reproductive activity for up to 30 days after exposure and the number of eggs deposited and the portion that hatches will be determined. A subset of the resulting fry (F<sub>1</sub> generation) from each treatment group will be

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reared to adulthood to compare development and reproductive success to that of untreated controls. The third set of trials will be conducted to evaluate the potential effects of SDP exposure on fathead minnow larval development. Fathead minnow eggs spawned from naive fish will be exposed to a single static application of SDP in outdoor mesocosms (~1,000 L) containing water from a UMESC research pond. The replicated exposures will be conducted at expected environmental concentrations (e.g. 0, 50 and 100 mg/L A.I.) and at the expected environmental exposure duration (8h) during selected embryo developmental periods to determine the impact on embryo development. A subset of the resulting fry from each treatment group will be reared to adulthood to compare development between exposed and control groups.

Summary Budget Information for Activity 1:

ENRTF Budget:	\$ 163,500
Amount Spent:	\$0
Balance:	\$163,500

#### **Activity Completion Date:**

Outcor	ne	Completion Date	Budget
1.	Determine the survival of aquatic invertebrates following	October 2014	\$35,270
	exposure to SDP in outdoor mesocosms		
2.	Determine reproductive success (egg deposition and % egg	October 2015	\$79,280
	hatch) of adult FHM ( $F_0$ generation) following exposure to SDP		
	pre-spawn in mesocosm		
3.	Determine reproductive success of fathead minnows embryo	October 2015	\$48,950
	survival following egg exposure to SDP in mesocosm tanks		
4.	Publish results	February 2016	\$ USGS

Activity Status as of December 31, 2013: Activity Status as of June 30, 2014: Activity Status as of December 31, 2014: Activity Status as of June 30, 2015: Activity Status as of December 31, 2015: Activity Status as of of June 30, 2016:

Final Report Summary: N/A

ACTIVITY 2: Bathymetric mapping, environmental DNA and physical surveys

**Description:** Detailed bathymetric maps of two Minnesota lakes or portions thereof, (1 high and 1 low level infestation, to be identified in 2013) will be prepared using high-resolution side-scanning sonar systems to characterize bottom substrate and vegetated habitat. Physical (e.g. divers) and eDNA sampling will be conducted over various habitat types determined from bathymetric survey. Survey (physical and eDNA) and bathymetry data will be compared to identify potential SDP application locations and to determine the potential for eDNA as a treatment prioritization and evaluation tool.

Summary Budget Information for Activity 2: E	NRTF Budget:	\$ 160,580
Α	mount Spent:	\$0
	Balance:	\$160,580

**Activity Completion Date:** 

Outcome		<b>Completion Date</b>	Budget
1.	Identify study lakes and sampling locations	July 2013	\$5,000
2.	Optmize zebra mussel eDNA primers and sampling protocol	May 2014	\$12,500
3.	Complete bathymetric surveys and data processing	January 2015	\$62,920
4.	Complete physical surveys and eDNA surveys and data	Eebruary 2015	\$80,160
	processing	Tebruary 2015	
5.	Publish results	June 2015	\$ USGS

Activity Status as of December 31, 2013: Activity Status as of June 30, 2014: Activity Status as of December 31, 2014: Activity Status as of June 30, 2015: Activity Status as of December 31, 2015: Activity Status as of of June 30, 2016:

Final Report Summary: N/A

**ACTIVITY 3:** SDP application technique development and validation and field efficacy

**Description:** Laboratory, pond-scale and field studies will be completed to develop and validate the use of injection versus whole water column treatment application techniques to achieve SDP treatment concentrations and to potentially reduce the quantity of SDP applied during field application. Laboratory studies will compare injection technology and techniques for treatment administration to reduce the quantity of SDP applied relative to whole water column treatments. Refined injection techniques will be further evaluated pond-scale (0.01 acre) to compare injection application methods with whole water column SDP application. The pond-scale studies will refine the selected injection techniques to confirm that effective concentrations of SDP are maintained for the required exposure duration. The efficacy of dreissenid mussel control through SDP application will be validated under field conditions through in-lake testing in Lake Minnetonka (Deephaven, MN). The developed/refined injection application technique and whole water column SDP application will be evaluated within replicated enclosures (~24m<sup>2</sup>). Applications will be conducted in September 2013 and treatment success will be evaluated through the completion of pre-and post-treatment assessments.

Summary Budget Information for Activity 3: ENRTF Budget:	\$ 275,920
Amount Spent:	<b>\$ 0</b>
Balance:	\$275,920

#### Activity Completion Date:

Outcome		Completion Date	Budget
1.	Identify enclosure areas and place colonization substrates	August 2013	\$10,500
2.	Complete laboratory and pond scale evaluations of SDP	atory and pond scale evaluations of SDP	
	injection application techniques	September 2013	
3.	Perform field treatments with SDP	September 2013	\$146,032
4.	Perform post-treatment assessments and compile data	February 2014	\$18,456
5.	Publish results	May 2015	\$USGS

Activity Status as of December 31, 2013:

Activity Status as of June 30, 2014: Activity Status as of December 31, 2014: Activity Status as of June 30, 2015: Activity Status as of December 31, 2015: Activity Status as of June 30, 2016:

#### **V. DISSEMINATION:**

**Description:** Results will be communicated to local groups, state agencies and national peer groups through presentations at regional and national meetings including state resource management meetings. Details of results will be available as a final project report to the LCCMR, fact sheet summaries and scientific journal articles.

Status as of December 31, 2013: Status as of June 30, 2014: Status as of December 31, 2014: Status as of June 30, 2015: Status as of December 31, 2015: Status as of June 30, 2016:

#### **Final Report Summary:**

#### VI. PROJECT BUDGET SUMMARY:

The project includes a combination of ENRTF funds in addition to USGS overhead (48%), USGS in-kind for project management and Marrone Bio Innovations in-kind for project facilitation, implementation, equipment, and test product. Additionally, see the attached Marrone Bio Innovations letter of commitment.

Α.	ΕN	RTF	<b>Budget:</b>
<b>~</b> .			Duuget.

Budget Category	\$ Amount	Explanation
Personnel:	\$387,800	6.7 FTE
Professional/Technical/Service Contracts:	\$102,500	Bathymetric mapping, SCUBA, technical support
Equipment/Tools/Supplies:	\$88,282	eDNA, enclosures, sampling, product, plumbing
Capital Expenditures over \$3,500:	\$0	Single item not anticipated >\$3,500
Professional Services for Acquisition:	\$ N/A	N/A
Printing:	\$ N/A	Covered by USGS
Travel Expenses in MN:	\$21,418	Invert collection, mapping, treatment,
		assessments, sampling, meals and lodging-MN
Other:	\$ N/A	N/A
TOTAL ENRTF BUDGET:	\$600,000	

Add or remove rows as needed

# Explanation of Use of Classified Staff: N/A

**Explanation of Capital Expenditures Greater Than \$3,500:** 

The purchase of a single item >\$3,500 is not anticipated

**Number of Full-time Equivalent (FTE) funded with this ENRTF appropriation:** 6.7 FTE

Number of Full-time Equivalent (FTE) estimated to be funded through contracts with this ENRTF appropriation:

0.5 FTE

#### **B. Other Funds:**

	\$ Amount	\$ Amount	
Source of Funds	Proposed	Spent	Use of Other Funds
Non-state			
Marrone Bio Innovations	\$39,500	\$	Project support, test product, equipment
USGS overhead expenses (48%)	\$288,635	\$	Project overhead costs
USGS in-kind	\$42,000	\$	Project management, computer
State			
	\$	\$	
TOTAL OTHER FUNDS:	\$	\$	

Add or remove rows as needed

#### **VII. PROJECT STRATEGY:**

**A. Project Partners:** This project is a continuing partnership between the United States Geological Survey (USGS), Western Technical College, New York State Museum, MN DNR and Marrone Bio Innovations. Team members from the USGS include Mark Gaikowski (USGS-UMESC project manager), Dr. Richard Kiesling (USGS-MN WSC, bathymetric mapping manager; \$60,000 ENRTF), Jim Luoma (USGS-UMESC project coordinator) and Dr. Jon Amberg (USGS-UMESC, eDNA manager). Dr. Diane Waller (Western Technical College/USGS-UMESC) will manage the fathead minnow life cycle and invertebrate toxicology. Dr. Denise Mayer (New York State Museum Field Research Laboratory \$20,000 ENRTF) will assist in project planning and execution of the application techniques and field exposures. Gary Montz (MN DNR- Ecological and Water Resources, Aquatic Invertebrate Biologist) and Nathan Olson (MN DNR- Ecological and Water Resources, Invasive Species Specialist) will assist in test lake selection, permitting and field applications. Carolyn Link and Megan Weber (Marrone Bio Innovations - Zequanox product development manager and open water development scientist, respectively; \$20,000 ENRTF) will provide test product, project support and field treatment equipment. All team members will participate in writing the final report and communicating results to state and national user groups.

# B. Project Impact and Long-term Strategy:

1) The project determines the potential magnitude of non-target animal responses to acute SDP exposure by assessing the potential effects of intermittant SDP exposure on the reproductive success of fathead minnows (*Pimephales promelas*) and survival of mayfly (*Order: Ephemerotera*) and amphipod (*Order: Amphipoda*), larvae common to Minnesota's aquatic ecosystems.

Much of the previous non-target animal impact data is limited to acute exposures in laboratory settings. The proposed work will expand the non-target animal database by assessing the potential effects of intermittant SDP exposure on reproduction and success of fathead minnows and on the survival of mayfly and amphipod larvae. Exposures will be completed in mesocosms at environmentally relevant concentration and duration.

# 2) The project directly provides treatment protocols and optimization techniques by assessing multiple treatment application techniques, development of high resolution baythmetric and environmental DNA maps, and field application to various substrates.

The application of SDP using injection techniques will be evaluated in laboratory and pond-scale trials then validated through in-lake field trials using multiple zebra mussel attachment substrates. The use of injection techniques has the potential to deliver effective SDP applications while significantly reducing the amount of applied product, lowering treatment costs and reducing potential impacts to non-target organisms. The use of eDNA to detect the presence of specific fish species is becoming widespread. The proposed work includes the use of eDNA to both determine the presence of zebra mussels and to potentially target treatment locations to optimize efficacy.

# C. Spending History:

Funding Source	M.L. 2007	M.L. 2008	M.L. 2009	M.L. 2010	M.L. 2011
	or	or	or	or	or
	FY08	FY09	FY10	FY11	FY12-13

(add or remove rows and columns as needed)

# VIII. ACQUISITION/RESTORATION LIST: N/A

# IX. MAP(S):

Maps of test lakes will be provided after creation for the test lakes upon completion of Activity 2 (Bathymetric mapping, environmental DNA and physical surveys).

# X. RESEARCH ADDENDUM:

# See Attachment B

# XI. REPORT REQUIREMENTS:

Periodic work plan status update reports will be submitted not later than December 31, 2013, June 30, 2014, December 31, 2014, June 30, 2015, December 31, 2015, and June 30, 2016. A final report and associated products will be submitted by June 30, 2016 or as requested by the LCCMR.

# Attachment A: Budget Detail for M.L. 2013 Environment and Natural Resources Trust Fund Projects

Project Title: Zebra Mussel Control Research and Evaluation in Minnesota Waters
Legal Citation: M.L. 2013, Chp. 52, Sec. 2, Subd. 06f
Project Manager: Mark Gaikowski
M.L. 2013 ENRTF Appropriation: \$600,000
Project Length and Completion Date: 3 yr, June 30, 2016
Date of Update: Initial

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Balance	Activity 2 Budget	Amount Spent	Balance	Activity 3 Budget	Amount Spent	Balance	TOTAL BUDGET	TOTAL BALANCE
	Non-larget Anni	nai inipacis		DNA and physi	cal surveys	onnentai	validation and	field efficacy	evelopment and		
Personnel (Wages and Benefits)	129.200		129.200	65.140		65.140	193.460		193.460	387.800	387.800
Amberg(UMESC)/Research Fisheries Biologist (PCR expert) \$13,000 (70% salary & 30% benefits) 15% FTE			-,								
Luoma(USGS)/Research Fisheries Biologist \$55,510 (80% salary & 20% benefits) 54% FTE											
Waller(WWTC)/Research Biologist \$98,570 (93% salary & 7% benefits) 100% FTE x 1.4											
Weber(USGS)/Biologist/project implimentation \$65,530 (75% salary & 25% benefits) 97% FTE											
UMESC GS-7 term research assistant/project implimentation \$100,565 (77% salary & 23% benefits) 100% FTE x 2											
UMESC GS-5 temp research assistant/project implimentation \$54,625 (93% salary & 7% benefits) 100% FTE x 1.6											
Professional/Technical/Service Contracts											
Minnesota Water Sciences Center (USGS) high resolution substrate mapping				<u> </u>		co 000				co 000	0000
SCUBA dive support				60,000		60,000	2 500		2 500	2 500	2 500
Mayer (NYSM) project design, implementation (salary and benefits 10% FTE)				5 000		5 000	15 000		15,000	2,300	2,300
Marrone Bio Innovations, permitting, project design and implimentation				5,000		3,000			20,000	20,000	20,000
Equipment/Tools/Supplies							20,000		20,000	20,000	20,000
eDNA kits, reagents, sampling equipment (600 samples x \$20 sample)				12.000		12.000				12.000	10.000
Laboratory tanks, mesocosm tanks, application equipment	10.000		10.000	12,000		12,000	10.000		10.000	20,000	20,000
Plumbing supplies and tubing	12 500		12 500				754		754	13 254	13 254
Pumps	2 300		2 300				704		704	2 300	2 300
Filter systems and hoses	5,000		5,000							5,000	5,000
Field invertebrate collection gear	3 500		3 500							3 500	3 500
Field enclosure supplies, samplers, sampling gear	0,000		0,000	2 000		2 000	12 478		12 478	14 478	14 478
Expendable supplies				1 850		1 850	4 650		4 650	6,500	6 500
SCUBA equipment				2 500		2 500	5 000		5,000	7,500	7 500
Survey/transect equipment, tools, nets				2,250		2,250	1,500		1,500	3,750	3 750
Travel expenses in Minnesota							.,		1,000	0,100	0,100
Invertebrate collection, mileage	1,000		1,000							1,000	1,000
Mapping \$123/day x 10 days x 4 people				4,920		4,920				4,920	4,920
Sampling, eDNA/conventional \$123/day x 20 days x 2 people				4.920		4.920				4.920	4.920
Pre-treatment work \$123/day x 5 days x 4 people				.,020		.,	2.460		2.460	2.460	2.460
Treatments \$123/day x 10 days x 5 people							6.150		6,150	6,150	6,150
Post- treatment surveys \$123/day x 4 days x 4 people							1,968		1,968	1,968	1,968
COLUMN TOTAL	\$163,500	\$0	\$163,500	\$160,580	\$0	\$160,580	\$275,920	\$0	\$275,920	600,000	\$600,000