Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2013 Work Plan

Date of Status Update Report: September 30, 2012

Date of Next Status Update Report: March 30, 2013

Date of Work Plan Approval: Pending

Project Completion Date: June 30, 2015 **Is this an amendment request? ____**

PROJECT TITLE: An evaluation of the efficacy and safety of a formulated *Pf*-CL145A product (Zequanox®) for control of zebra mussels in MN waters

Project Manager: Mark Gaikowski

Affiliation: USGS – UMESC

Mailing Address: 2630 Fanta Reed Rd

City/State/Zip Code: LaCrosse, WI

Telephone Number: (608) 781-6284

Email Address: Mgaikowski@usgs.gov

Web Address: http://www.umesc.usgs.gov

Location:

Statewide

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

Legal Citation: M.L. 2013, Chp. xx, Sec. xx, Subd. xx

Appropriation Language:

DRAFT

I. PROJECT TITLE: An evaluation of the efficacy and safety of a formulated *Pf*-CL145A product (Zequanox®) for control of zebra mussels in MN waters

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®, which contains the killed cells of a specific strain (*Pf*-CL145A) of the common soil bacterium *Pseudomonas fluorescens*. Zequanox® 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®) 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 http://cida.usgs.gov/glri/projects/invasive_species/zm_control.html).

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 will be assessed. Separate but similar exposures will be completed to assess the acute effects of SDP to larval aquatic insects.

Adult fathead minnows will be exposed to a single static dose of SDP in an outdoor mesocosm setting. Treatment groups will be exposed to a single dose of SDP at multiples of 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_1 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 stages) from naïve fathead minnows will be exposed to a single static dose of SDP in an outdoor mesocosm setting at multiples of 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 versus whole water column treatment) to determine the minimum amount of SDP required during field applications. These studies will determine which technique maintains an effective concentration of SDP for a sufficient

exposure duration. Different delivery techniques will be evaluated in the laboratory to determine the methods that will achieve the desired outcomes. The methods determined most successful in the laboratory will then be applied in 0.01 acre outdoor pond trials.

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 allow for the correlation of zebra mussel populations within the lake to bathymetric and substrate data.

We will select habitats (in the high level infestation lake) where the efficacy of SDP application techniques for controlling zebra mussels in limited, high-value, open water will be tested. Replicated enclosures (~24 m^2) will be prepared at selected locations to 1 of 3 treatments (control, SDP injection, or SDP complete water column) will be applied according to a randomized study design. Treatment effect 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 treatment like 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 12/31/13, 6/30/14, and 12/31/14. A final report and associated products will be submitted between June 30 and August 15, 2015 as requested by the LCCMR.

Project Status as of September 30, 2012: Pending funding

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Non-target Animal Impacts: Fathead Minnow Reproductive Success: Adult Reproductive Activity Description:

Adult fathead minnows will be exposed to a single static application of SDP in outdoor mesocosm tanks (~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 hatch will be determined. A subset of the resulting fry (F_1 generation) from each treatment group will be reared to adulthood to compare development and reproductive success to that of untreated controls.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 77,380
Amount Spent: $ 0
Balance: $77,280

Activity Completion Date:

Outcome	Completion Date	Budget

1. Determine reproductive success (egg deposition and % egg hatch) of adult FHM (F_0 generation) following exposure to SDP pre-spawn in mesocosm	October 2013	$30,902
2. Compare growth of fry from exposed and unexposed F0 adult FHM	July 2014	$15,476
3. Determine reproductive success (egg deposition and % egg hatch) of adult FHM (F_1 generation)	October 2014	$30,902
4. Publish results	December 2014	$ USGS

Activity Status as of September 30, 2012: Pending funding

Final Report Summary: N/A

ACTIVITY 2: Non-target Animal Impacts: Fathead Minnow Reproductive Success: Embryo Development
Description:
Fathead minnow eggs spawned from naive fish will be exposed to a single static application of SDP in outdoor mesocosm tanks (~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 2:

ENRTF Budget: $ 47,950
Amount Spent: $ 0
Balance: $47,950

Activity Completion Date:

Outcome	Completion Date	Budget
1. Determine reproductive success of fathead minnows embryo survival following egg exposure to SDP in mesocosm tanks	October 2013	$28,580
3. Determine development of embryo-adult fathead minnows following egg exposure to SDP in mesocosm tanks	October 2014	$19,370
4. Publish results	December 2014	$ USGS

Activity Status as of September 30, 2012: Pending funding

Final Report Summary: N/A

ACTIVITY 3: Non-target Animal Impacts: Invertebrate
Description:
Mayfly and caddisfly larvae will be collected in the upper Mississippi River basin. A known number of larvae will be placed into outdoor mesocosm tanks (1,000 L) containing sediment and water from a UMESC research pond. The larvae will be exposed to a single static application of formulated *Pf-CL145A*. The replicated exposure tanks 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) to assess the potential effect of open water application of SDP to control dreissenid mussels on larval aquatic insects survival.

Summary Budget Information for Activity 3:

ENRTF Budget: $ 33,270
Amount Spent: $ 0
Balance: $33,270

Activity Completion Date:

Outcome	Completion Date	Budget
1. Determine survival of mayflies exposed to SDP in mesocosm tanks	July 2014	$16,635
2. Determine survival of caddisflies exposed to formulated *Pf*-CL145Ain mesocosm tanks	July 2014	$16,635
3. Publication of results	Aug 2014	$ USGS

Activity Status as of September 30, 2012: Pending funding

Final Report Summary: N/A

ACTIVITY 4: Bathymetric mapping

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.

Summary Budget Information for Activity 4:

ENRTF Budget: $ 64,920
Amount Spent: $ 0
Balance: $64,920

Activity Completion Date:

Outcome	Completion Date	Budget
1. Identify study lakes and sampling locations	July 2013	$2,000
2. Complete bathymetric surveys	August 2013	$60,000
3. Complete bathymetric survey data processing	September 2013	$2,920
4. Publish results	June 2014	$ USGS

Activity Status as of September 30, 2012: Pending funding

Final Report Summary: N/A

ACTIVITY 5: Environmental DNA and physical surveys

Description:

Detailed eDNA maps of two Minnesota lakes, or portions thereof, (1 high and 1 low level infestation, to be identified in 2013) will be prepared. Physical (e.g. divers) and eDNA sampling will be conducted over various habitat types (determined from bathymetric survey). Physical and eDNA surveys will be compared to identify

SDP application locations and to determine the potential for eDNA as a treatment prioritization and evaluation tool.

Summary Budget Information for Activity 5:

ENRTF Budget: $ 97,120
Amount Spent: $ 0
Balance: $97,120

Activity Completion Date:

Outcome	Completion Date	Budget
1. Identify study lakes and sampling locations	July 2013	$3,000
2.Optmize zebra mussel eDNA primers	August 2013	$7,500
3.Optimize zebra mussel eDNA sampling protocol	August 2013	$5,000
4. Complete physical surveys	October 2013	$13,015
5. Complete eDNA surveys	October 2013	$59,605
6. Complete physical survey data processing	February 2014	$1,000
7. Complete eDNA survey data processing	February 2014	$8,000
8. Publish results	June 2014	$ USGS

Activity Status as of September 30, 2012: Pending funding

Final Report Summary: N/A

ACTIVITY 6: Application Technique Development - Laboratory

Description:

Laboratory studies will be conducted to evaluate 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. These studies will determine the techniques required to maintain effective concentrations for the required duration.

Summary Budget Information for Activity 7:

ENRTF Budget: $ 47,850
Amount Spent: $ 0
Balance: $47,850

Activity Completion Date:

Outcome	Completion Date	Budget
1. Complete laboratory scale evaluations of SDP injection application techniques	August 2013	$47,850
2. Publish results	February 2014	$ USGS

Activity Status as of September 30, 2012: Pending funding

Final Report Summary: N/A

ACTIVITY 7: Application Technique Development – Pond Scale

Description:

Outdoor pond scale (0.01 acre) studies will evaluate the use of injection versus whole water column treatment application techniques to achieve SDP treatment concentrations and to potentially reduce the amount of SDP applied during field application. These studies will determine the efficacy of injection techniques to maintain effective concentrations.

Summary Budget Information for Activity 7:

ENRTF Budget: $ 52,078
Amount Spent: $ 0
Balance: $52,078

Activity Completion Date:

Outcome	Completion Date	Budget
1. Complete outdoor pond (0.01 acre) evaluations of SDP application techniques	August 2013	$52,078
2. Publish results	February 2014	$ USGS

Activity Status as of September 30, 2012: Pending funding

Final Report Summary: N/A

ACTIVITY 8: Field Application and Monitoring

Description:

The efficacy of dreissenid mussel control using SDP treatments will be assessed in a selected Minnesota lake with a high zebra mussel population. Multiple treatment techniques will be evaluated within replicated enclosures (~24m^2). If possible, applications will be conducted in September 2013 and treatment success will be evaluated through the completion of pre-and post-treatment assessments. If September 2013 applications are not possible due to scheduling conflicts, a spring 2014 application will be conducted.

Summary Budget Information for Activity 8:

ENRTF Budget: $ 179,532
Amount Spent: $ 0
Balance: $179,532

Activity Completion Date:

Outcome	Completion Date	Budget
1. Identify location of enclosures in selected lake	August 2013	$5,000
2. Perform pre-treatment surveys of enclosures	September 2013	$7,500
3. Perform field treatments with formulated *Pf*-CL145A	September 2013	$152,032
4. Perform post-treatment physical and eDNA assessment	October 2013	$ 15,000

5. Publish results	May 2015	$ USGS

Activity Status as of September 30, 2012: Pending funding

Final Report Summary: N/A

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.

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.

A. ENRTF Budget:

Budget Category	$ Amount	Explanation
Personnel:	$385,300	2.4 FTE + 4.5 FTE @ 180 days
Professional/Technical/Service Contracts:	$95,000	Bathymetric mapping, SCUBA, technical support
Equipment/Tools/Supplies:	$97,178	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:	$22,522	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:
Single item >$3,500 not anticipated

Number of Full-time Equivalent (FTE) funded with this ENRTF appropriation:
2.4 FTE + 4.5 FTE @ 180 days

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

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
Marrone Bio Innovations	$36,350	$	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, Viterbo University, 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), Jim Luoma (USGS-UMESC project coordinator) and Dr. Jon Amberg (USGS-UMESC, eDNA manager). Dr. Kim Fredricks (Viterbo University) will manage the fathead minnow life cycle and invertebrate toxicology. Dr. Diane Waller (Western Technical College) and Dr. Denise Mayer (New York State Museum Field Research Laboratory) 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. Dr. Sarahann Rackl and Dave Roberts (Marrone Bio Innovations -Zequanox project manager and open-water manager, respectively) 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 caddisfly *(Order: Trichotera*), 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 caddisfly 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. The use of injection techniques has the potential to achieve efficacious treatments while significantly reducing the amount of applied product, thereby, loweringreducing treatment costs and the potential impacts to non-target organisms. The use of environmental DNA (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.

The project also compares treatment application techniques by conducting field treatments over multiple substrate types within defined and monitored enclosures.

C. Spending History:

Funding Source	M.L. 2007 or FY08	M.L. 2008 or FY09	M.L. 2009 or FY10	M.L. 2010 or FY11	M.L. 2011 or 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 selection in addition to bathymetric and eDNA maps that will be created during the conduct of the proposed work.

X. RESEARCH ADDENDUM:

2012 LCCMR Research Work Plan – An evaluation of the efficacy and safety of SDP for control of zebra mussels in MN waters

1. Overview of the study

1.1 Non-target Animal Impacts: Fathead Minnow Reproductive Success: Adult Reproductive Activity
Adult fathead minnows will be exposed to a single static application of SDP in outdoor mesocosm tanks (~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 hatch will be determined. A subset of the resulting fry (F_1 generation) from each treatment group will be reared to adulthood to compare development and reproductive success to that of untreated controls.

1.2 Non-target Animal Impacts: Fathead Minnow Reproductive Success: Embryo Development

Fathead minnow eggs spawned from naive fish will be exposed to a single static application of SDP in outdoor mesocosm tanks (~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.

1.3 Non-target Animal Impacts: Invertebrate

Mayfly and caddisfly larvae will be collected in the upper Mississippi River basin. A known number of larvae will be placed into outdoor mesocosm tanks (1,000 L) containing sediment and water from a UMESC research pond. The larvae will be exposed to a single static application of formulated *Pf*-CL145A. The replicated exposure tanks 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) to assess the potential effect of open water application of SDP to control dreissenid mussels on larval aquatic insect survival.

1.4 Bathymetric Mapping

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 sonar systems to characterize habitat).

1.5 Environmental DNA and physical surveys

Detailed eDNA maps of two Minnesota lakes, or portions thereof, (1 high and 1 low level infestation, to be identified in 2013) will be prepared. Physical (e.g. divers) and eDNA sampling will be conducted over various habitat types (determined from bathymetric survey). Physical and eDNA surveys will be compared to identify SDP application locations and to determine the potential for eDNA as a treatment prioritization and evaluation tool.

1.6 Application Technique Development: Laboratory

Laboratory studies will be conducted to evaluate 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. These studies will determine the techniques required to maintain effective concentrations for the required duration in order to achieve efficacious control.

1.7 Application Technique Development: Pond Scale

Outdoor pond scale (0.01 acre) studies will evaluate the use of injection versus whole water column treatment application techniques to achieve SDP treatment concentrations and to potentially reduce the amount of SDP applied during field application. These studies will determine the efficacy of injection techniques to maintain effective concentrations.

1.8 Field Application and Monitoring

The efficacy of dreissenid mussel control using SDP treatments will be assessed in a selected Minnesota lake with a high zebra mussel population. Multiple treatment techniques will be evaluated within replicated enclosures (~24m^2). If possible, applications will be conducted in September 2013 and treatment success will be evaluated through the completion of pre-and post-treatment assessments. If September 2013 applications are not possible due to scheduling conflicts, a spring 2014 application will be conducted.

2. Experimental Design

2.1 Non-target Animal Impacts: Fathead Minnow Reproductive Success: Adult Reproductive Activity

Eighteen replicate 1000-L mesocosm tanks will be placed in 0.01-acre outdoor concrete ponds containing water as a thermal buffer. Treatment will be randomly allocated to each tank according to a randomized block design. Test fish (reproductively active fathead minnows) will be distributed to the exposure chambers according to a completely randomized distribution scheme. A single SDP exposure (8-h duration) will be administered to each tank at nominal concentrations of 0, 50, and 100 mg/L A.I. Water inflow will be restored to each test replicate after the exposure period to simulate expected post-treatment environmental dissipation.

Exposures will be initiated 12-24 h after fish are placed in the exposure chambers. Exposures will be initiated by administering an aliquot of SDP stock solution to the appropriate test tank. The concentrations of SDP will be determined by spectrophotometry comparison to analytical standards. Exposures will be conducted at an environmentally relevant temperature and photoperiod.

After the exposure, PVC spawn tiles will be placed into each treatment replicate. Fish will be monitored for spawning activity for up to 30 days. Spawning tiles will be removed daily and the number of eggs will be enumerated and photographed prior to each tile be placed into a separate incubation chamber to assess hatch. A subset of fry (F_1 generation) from each treatment concentration will be reared to adulthood to compare fry development and reproductive success between exposed and unexposed treatment groups.

2.1.1 STUDY PROCEDURES

2.1.1.1 Test Animals

2.1.1.1.1 Description:

2.1.1.1.1.1 Species: Fathead minnow, (*Pimephales promela)*

2.1.1.1.1.2 Age – ~1 yr.

2.1.1.1.1.3 Sex – 1:2 ratio male and female

2.1.1.1.2 Number of animals: Approximately 1080 fish (20 males and 40 females per replicate x 6 replicates x 3 concentrations).

2.1.1.1.3 Source of animals: Fish will be obtained from the UMESC fish culture facility or other state or federal fish hatcheries and identified according to Eddy and Underhill (1978) by the UMESC fish culturist prior to entrance into the study. The fish source will be described in the study data management system.

2.1.1.1.4 Inclusion criterion: Fish will only be used only if their mortality is less than 0.2% per day for 3 consecutive days (UMESC SOP GEN 132).

2.1.1.1.5 Acclimation: Fish will be acclimated to test temperature at a rate not to exceed 3°C/day (SOP No. GEN 132).

2.1.1.1.6 Distribution to exposure chambers: After the acclimation period, a group of 10 fish will be placed into a bucket with water and transferred into a randomly chosen exposure chamber according to a pre-determined randomization scheme. The process will then be repeated until all exposure chambers designated for use receive a total of 6 distributions (2 male, 4 female) for a total of 60 fish per exposure chamber.

2.1.1.1.7 Feeding: Fish will be fed during the acclimation and post exposure periods by offering commercially-prepared dry feed, fresh or frozen natural food. The percentage body weight offered will vary between feed types and will be documented in the study records. The feed rate will be adjusted as necessary to account for fish growth. The exact procedures used will be documented in the study records. Feed offered will either be weighed or volumetrically measured for each holding chamber.

2.1.1.2 Water Chemistry

2.1.1.2.1 Dissolved oxygen: Dissolved oxygen will be measured and recorded weekly in acclimation tanks. Dissolved oxygen will be measured and recorded in each exposure chamber during the exposure period. Dissolved oxygen will be measured and recorded daily in one concentration replicate spawning chamber during the post exposure observation period (UMESC SOP AEH 394 or equivalent).

2.1.1.2.2 Temperature: Temperature will be measured and recorded weekly in acclimation tanks. Temperature will be measured and recorded in each exposure chamber during the exposure period. Temperature will be measured and recorded daily in one concentration replicate spawning chamber during the post exposure observation period.

2.1.1.2.3 pH: pH will be measured and recorded weekly in acclimation tanks. pH will be measured and recorded in each exposure chamber during the exposure period. The pH will be measured and recorded daily in one concentration replicate spawning chamber during the post exposure observation period (UMESC SOP AEH 335 or equivalent).

2.1.1.2.4 Hardness: Hardness will be measured and recorded prior to test initiation and during the exposure period from all replicate exposure concentrations. Hardness will be measured weekly throughout the observation period on one representative spawning chamber (UMESC SOP AEH 712).

2.1.1.2.5 Alkalinity: Alkalinity will be measured and recorded prior to test initiation and during the exposure period from replicate exposure concentrations. Alkalinity will be measured weekly throughout the observation period on one representative spawning chamber (UMESC SOP AEH 706).

2.1.1.2.6 Conductivity: Conductivity will be measured and recorded prior to test initiation and during the exposure period from all replicate exposure concentrations. Conductivity will be measured weekly throughout the observation period on one representative spawning chamber (UMESC SOP AEH 188 or equivalent).

2.1.1.3 Disposal: All live fish at the end of the post-exposure spawning observation period will be euthanized by MS-222 overdose (UMESC SOP GEN 132) and incinerated.

2.1.1.4 Study facilities:

2.1.1.4.1 Test Facility
U.S. Geological Survey, Upper Midwest Environmental Sciences Center
2630 Fanta Reed Rd
La Crosse, Wisconsin 54603

2.1.1.4.1.1 Exposure system: The test system is comprised of a series of 18 circular HDPE tanks (~ 175cm diameter x 64 cm high) containing ~1,000 -L of water. Each exposure chamber will receive a continuous supply of pond water. Each chamber will be uniquely identified (eg: A1) to allow for identification of treatment type and replicate number. Coding procedures will be documented in the study records.

2.1.1.4.1.2 Aeration: Supplemental aeration will not be supplied during the SDP exposures. Aeration may be supplied during the acclimation and post exposure observation periods.

2.1.1.4.1.3 Water supply: Pond water will be supplied continuously to each chamber at ~0.25 tank-volume exchanges/h (~ 4.2 L/min). Water flow will be interrupted during the 8-h exposure period.

2.1.1.4.1.4 Lighting: Natural photoperiod.

2..1.1.4.1.5 Exposure chamber dimensions: The exposure chambers are circular HDPE tanks (~ 175 cm diameter x 64 cm high) ~1,000- L of water.

2.1.1.4.1.6 Incubation chamber dimensions: The incubation chambers are glass aquaria (~51 x 25 x 33 cm) and each chamber will contain ~30 L of water.

2.1.1.4.1.7 Water discharge: All SDP treated water will be discharged into the UMESC carbon adsorption system. Untreated water will be recirculated to the source pond or discharged into the UMESC general effluent system.

2.1.1.4.1.8 Exposure and spawning chamber cleaning: During the pre-exposure and spawning observation period, each chamber or tank containing animals will be cleaned as needed to remove uneaten feed and feces. Cleaning will be conducted by removal

of the stand pipe and then brushing tank or by siphoning settled waste. No cleaning will be performed during the exposure period.

2.1.1.5 Observations:

2.1.1.5.1 Mortality: Fish without opercular movement or that do not respond to direct pressure to the caudal peduncle will be coded as a mortality and removed.

2.1.1.5.2 Behavioral: Behavioral responses (i.e.: guarding, courting, egg deposition, etc.) observed during the observation period will be documented in the study records.

2.1.1.6 Treatment administration:

2.1.1.6.1 Treatment: Fish will be exposed to six replicates of either 0 (control) and, approximately 50, and 100 mg/L A.I. SDP as an 8-h static exposure.

2.1.1.6.2 Route of administration: Exposures will be initiated by addition of a stock solution of SDP to the exposure chamber**.** The test concentrations will include 0 (control), and approximately 50 and 100 mg/L A.I. of formulated *Pf*-CL145A.

2.1.1.6.3 Concentration verification: Concentration will be determined by spectrophotometric comparison to a standard curve prepared from a known mass of formulated *Pf*-CL145A. The absorbance of exposure solutions will be compared to the standard curve to determine the exposure concentration. Absorbance will be determined using a Beckman DU 800 UV/VIS spectrophotometer (UMESC SOP AEH 303).

2.2 Non-target Animal Impacts: Fathead Minnow Reproductive Success: Embryo Development

Eighteen separate replicate 1000-L mesocosm tanks will be prepared to contain the exposures in an outdoor environment. The mesocosm tanks will be placed in 0.01 acre outdoor concrete ponds containing water as a thermal buffer. Treatment will be randomly allocated to each tank according to a randomized block design. Spawning tiles containing deposited fish eggs will be distributed to the exposure chambers according to a completely randomized distribution scheme. A single SDP exposure will be administered to each tank at nominal concentrations of 0, 50, and 100 mg/L A.I. The exposures will be static for 8h, then the tiles will be removed from the treatment and placed into incubation chambers to assess hatch.

Exposures will be initiated after the spawning tiles are placed in the exposure chambers. Each exposure will be a single 8-h static dose followed by a post exposure observation period. Exposures will be initiated by administering a single dose of a SDP stock solution. The actual concentrations of SDP will be determined by spectrophotometry comparison to analytical standards. The exposures will be conducted at an environmental relevant temperature and photoperiod.

Two separate sets of exposures will be performed; one set of exposures will be completed on newly deposited (≤24-h) eggs and one set of exposures will be completed on advanced (≥48-h) eggs.

For each set of exposures, eighteen separate replicate 45-L tanks will be prepared to contain the exposures. Treatment will be randomly allocated to each tank according to a randomized block design.

Spawning tiles containing fathead minnow eggs will be distributed to the exposure chambers according to a completely randomized distribution scheme. A single SDP dose will be administered to each tank to achieve nominal concentrations of 0, 50, and 100 mg/L A.I.

After exposure, the tiles will be transferred to clean temperature-adjusted well water in 4-L incubation chambers to access hatch. A subset of fry (F_1 generation) from each treatment concentration will be reared to adulthood to compare development between exposed and control groups.

2.2.1 STUDY PROCEDURES

2.2.1.1 Test Animals

2.2.1.1.1 Description:

2.2.1.1.1.1 Species: Fathead minnow, (*Pimephales promela)*

2.2.1.1.1.2 Age: Eggs (≤24-h & ≥48h)

2.2.1.1.2 Number of animals: Approximately 8100 eggs (~225 per replicate x 6 replicates x 3 concentrations x 2 age class) of each species.

2.2.1.1.3 Source of animals: Eggs resulting from brood fish obtained from the UMESC fish culture facility or other state or federal fish hatcheries and identified according to Eddy and Underhill (1978) by the UMESC fish culturist prior to entrance into the study. The fish source will be described in the study data management system.

2.2.1.1.4 Inclusion criterion: Spawning tiles will not be used if they contain insufficient numbers of eggs (<50) or if there are apparent health concerns (i.e.: fungal infection).

2.2.1.1.5 Acclimation: Any acclimation of brood fish or eggs will be at a rate not exceeding 3°C/day (SOP No. GEN 132).

2.2.1.1.6 Distribution to exposure chambers: A spawning tile with eggs will be placed into a bucket with water and transferred into a randomly chosen exposure chamber according to a predetermined randomization scheme. The process will then be repeated until all exposure chambers designated for use receive a total of 3 distributions for a total of 3 spawning tiles per exposure chamber.

2.2.1.1.7 Feeding: Subsets of the F_1 generation from each treatment group will be fed by offering commercially-prepared dry feed, fresh or frozen natural food. The feed type and rate will be adjusted as necessary to account for fish growth. The exact procedures used will be documented in the study records. Feed offered will either be weighed or volumetrically measured.

2.2.1.2 Water Chemistry

2.2.1.2.1 Dissolved oxygen: Dissolved oxygen will be measured and recorded in each exposure chamber during the exposure period. Dissolved oxygen will be measured and recorded daily in one concentration replicate

hatching chamber during the post exposure observation period (UMESC SOP AEH 394 or equivalent).

2.2.1.2.2 Temperature: Temperature will be measured and recorded in each exposure chamber during the exposure period. Temperature will be measured and recorded daily in one concentration replicate hatching chamber during the post exposure observation period.

2.2.1.2.3 pH: pH will be measured and recorded in each exposure chamber during the exposure period. The pH will be measured and recorded daily in one concentration replicate hatching chamber during the post exposure observation period (UMESC SOP AEH 335 or equivalent).

2.2.1.2.4 Hardness: Hardness will be measured and recorded during the exposure period from all replicate exposure concentrations. Hardness will be measured at least once during the observation period on one representative hatching chamber (UMESC SOP AEH 712).

2.2.1.2.5 Alkalinity: Alkalinity will be measured and recorded during the exposure period from all replicate exposure concentrations. Alkalinity will be measured at least once during the observation period on one representative hatching chamber (UMESC SOP AEH 706).

2.2.1.2.6 Conductivity: Conductivity will be measured and recorded during the exposure period from all replicate exposure concentrations. Conductivity will be measured at least once during the observation period on one representative hatching chamber (UMESC SOP AEH 188 or equivalent).

2.2.1.3 Disposal: All live fish and fish eggs not retained for grow-out at the end of the post-exposure observation period will be euthanized by MS-222 overdose (UMESC SOP GEN 132) and incinerated.

2.2.1.4 Study facilities:

2.2.1.4.1 Test Facility
U.S. Geological Survey, Upper Midwest Environmental Sciences Center
2630 Fanta Reed Rd
La Crosse, Wisconsin 54603

2.2.1.4.1.1 Exposure system: The test system will be a series of 18 circular HDPE tanks (~ 175cm diameter x 64 cm high) placed in a 0.01 acre pond containing a water bath to maintain temperature. Each exposure chamber will receive 1000 L of pond water. Each chamber will be uniquely identified (eg: A1) to allow for identification of treatment type and replicate number. Coding procedures will be documented in the study records.

2.2.1.4.1.2 Aeration: Supplemental aeration will not be supplied during the SDP exposures. Aeration will be supplied during the post exposure observation period.

2.2.1.4.1.3 Water supply: Pond water will be used during the exposure period and temperature-adjusted (~20-25 °C) well water will be used for the post exposure observation period.

2.2.1.4.1.4 Lighting: Exposure period; natural photoperiod. Observation period; overhead lighting (~18 h light: 6 h dark; 100-1000 lux) will be provided.

2.2.1.4.1.5 Exposure chamber dimensions: The exposure chambers are circular HDPE tanks (~ 175cm diameter x 64 cm high) and each chamber will contain ~1,000 L of water during the exposure period.

2.2.1.4.1.6 Hatching chamber dimensions: The hatching chambers are plastic buckets (~20.3 cm diameter x 16.5 cm high) and each chamber will contain 4 L of water during the observation period.

2.2.1.4.1.7 Water discharge: All SDP treated water will be discharged into the UMESC carbon adsorption system. Untreated water will be recirculated to the source pond or discharged into the UMESC general effluent system.

2.2.1.4.1.8 Exposure and hatching chamber cleaning: Throughout the observation period, each chamber or tank containing spawning tiles will be replaced daily until hatch commencement to reduce potential fungal infections. Chamber replacement will be conducted by removal of the spawning tile and then placing it into a clean chamber with 4 L of temperature-adjusted well water. No chamber replacement will be conducted after hatching is observed.

2.2.1.5 Observations:

2.2.1.5.1 Hatch Success: Each spawning tile will be uniquely coded to identified treatment and replicate number. The number of eggs deposited will be enumerated upon placement into the exposure chamber in addition to a photographic record of each egg mass. After hatch completion, any remaining eggs will be enumerated and photographed to determine the percentage of hatched fry. The resulting fry from each tile will be preserved for enumeration.

2.2.1.5.2 Mortality: Fish eggs that are observed to have fungal infections will be coded as a mortality, enumerated and removed.

2.2.1.6 Treatment administration:

2.2.1.6.1 Treatment: Fish eggs will be exposed to six replicates of either 0 (control) and approximately 50, and 100 mg/L A.I. SDP as an 8-h static exposure.

2.2.1.6.2 Route of administration: Exposures will be initiated by addition of a stock solution of SDP to the exposure chamber**.** The test concentrations will include 0 (control), and approximately 50 and 100 mg/L A.I. of formulated *Pf*-CL145A.

2.2.1.6.3 Concentration verification: Concentration will be determined by spectrophotometric comparison to a standard curve prepared from a known mass of formulated *Pf*-CL145A. The absorbance of exposure solutions will be compared to the standard curve to determine the exposure concentration. Absorbance will be determined using a Beckman DU 800 UV/VIS spectrophotometer (UMESC SOP AEH 303).

2.3 Non-target Animal Impacts: Invertebrate

Eighteen separate replicate 1000-L mesocosm tanks will be prepared to contain the exposures in an outdoor environment. The mesocosm tanks will be placed in 0.01 acre outdoor concrete ponds containing water as a thermal buffer. Treatment will be randomly allocated to each tank according to a randomized block design. A known number of mayfly and caddisfly larvae will be distributed to the exposure chamber according to a completely randomized distribution scheme. Exposures will be initiated by administering a single SDP dose to each exposure chamber to achieve nominal concentrations of 0, 50, and 100 mg/L A.I. The exposures will be static 8-h. After exposure, water inflow will resume to each exposure chamber to allow dilution to mimic expected post-treatment environmental dissipation.

The exposures will be performed to assess the acute toxicity of SDP exposure to mayfly (*Order: Ephemerotera*) and caddisfly *(Order: Trichotera*) larvae. Mayfly and caddisfly larvae will be collected in from the upper Mississippi River Basin. The exposures will be completed at multiples of the expected environmental concentration (e.g. 0, 50, and 100 mg/L A.I.) to assess the potential effect of open water application of SDP to control dreissenid mussels on larval aquatic insects. The exposures will be completed at an environmentally relevant temperature and duration. Treatment will be randomly assigned to each exposure chamber according to a randomized block design. Invertebrates will be distributed to the exposure chambers according to a completely randomized distribution scheme. A single SDP dose will be administered to each exposure chamber to achieve nominal concentrations of 0, 50, and 100 mg/L A.I.

Exposures will be initiated 12-24 h after the test animals are placed in the exposure chambers. Each exposure will be a single 8-h static exposure followed by 96 h post exposure observation period. Exposures will be initiated by administering a dose of SDP stock solution. The actual concentrations of SDP will be determined by spectrophotometry comparison to analytical standards. The exposures will be conducted at an environmentally relevant temperature and photoperiod.

After the post-exposure observation period, the test replicate will be drained and test animals assessed for survival.

2.3.1 STUDY PROCEDURES

2.3.1.1 Test Animals

2.3.1.1.1 Description:

2.3.1.1.1.1 Species: Mayfly (*Order: Ephemerotera*) and caddisfly (*Order: Trichotera*) larvae

2.3.1.1.1.2 Age: Larvae

2.3.1.1.2 Number of animals: Approximately 540 (~30 per replicate x 6 replicates x 3 concentrations) of each species.

2.3.1.1.3 Source of animals: The test animals will be collected from the Upper Mississippi River or other local streams. The test animal source will be described in the study data management system.

2.3.1.1.4 Inclusion criterion: The test will be acceptable provided the control mortality is < 20%.

2.3.1.1.5 Acclimation: Test animals will be acclimated to test temperature at a rate not to exceed 3°C/day (SOP No. GEN 132).

2.3.1.1.6 Distribution to exposure chambers: Invertebrates will be transferred from the holding tank by counting them into a bucket and immediately transferring into a randomly chosen exposure chamber according to a predetermined randomization scheme. The process will then be repeated until all exposure chambers designated for use receive a total of 3 distributions for a total of 30 test animals per exposure chamber for each species.

2.3.1.1.7 Feeding: Feed will not be offered.

2.3.1.2 Water Chemistry

2.3.1.2.1 Dissolved oxygen: Dissolved oxygen will be measured and recorded in each exposure chamber during the exposure period. Dissolved oxygen will be measured and recorded daily in one concentration replicate chamber during the post exposure observation period (UMESC SOP AEH 394 or equivalent).

2.3.1.2.2 Temperature: Temperature will be measured and recorded in each exposure chamber during the exposure period. Temperature will be measured and recorded daily in one concentration replicate chamber during the post exposure observation period.

2.3.1.2.3 pH: pH will be measured and recorded in each exposure chamber during the exposure period. The pH will be measured and recorded daily in one concentration replicate chamber during the post exposure observation period (UMESC SOP AEH 335 or equivalent).

2.3.1.2.4 Hardness: Hardness will be measured and recorded during the exposure period from a replicate exposure concentration. Hardness will be measured at least once during the post exposure observation period on one representative chamber (UMESC SOP AEH 712).

2.3.1.2.5 Alkalinity: Alkalinity will be measured and recorded during the exposure period from a replicate exposure concentration. Alkalinity will be

measured at least once during the post exposure observation period on one representative chamber (UMESC SOP AEH 706).

2.3.1.2.6 Conductivity: Conductivity will be measured and recorded during the exposure period from a replicate exposure concentration. Conductivity will be measured at least once during the post exposure observation period on one representative hatching chamber (UMESC SOP AEH 188 or equivalent).

2.3.1.3 Disposal: All live test animals will be euthanized by MS-222 overdose (UMESC SOP GEN 132) and incinerated.

2.3.1.4 Study facilities:

2.3.1.4.1 Test Facility
U.S. Geological Survey, Upper Midwest Environmental Sciences Center
2630 Fanta Reed Rd
La Crosse, Wisconsin 54603

2.3.1.4.1.1 Exposure system: The test system will be a series of 18 circular HDPE tanks (~ 175cm diameter x 64 cm high) placed in a water bath to maintain temperature. Each exposure chamber will receive 1000-L of pond water. Each chamber will be uniquely identified (eg: A1) to allow for identification of species, treatment type and replicate number. Coding procedures will be documented in the study records.

2.3.1.4.1.2 Aeration: Supplemental aeration will not be supplied during the SDP exposures. Aeration may be supplied during the post exposure hatching observation period.

2.3.1.4.1.3 Water supply: Pond water will be used for the exposure and the post exposure hatching observation periods. The exposure and post-exposure hatching periods will be static.

2.3.1.4.1.4 Lighting: The natural photoperiod will be utilized in the conduct of the exposures.

2.3.1.4.1.5 Exposure chamber dimensions: The exposure chambers are circular HDPE tanks (~ 175cm diameter x 64 cm high) and each chamber will contain ~ 1,000 L of water during the exposure period.

2.3.1.4.1.6 Water discharge: All SDP treated water will be discharged into the UMESC carbon adsorption system. Untreated water will be recirculated to the source pond or discharged into the UMESC general effluent system.

2.3.1.5 Observations:

2.3.1.5.1 Mortality: Invertebrates that do not respond to physical or mild electrical stimulus will be coded as mortality.

2.3.1.6 Treatment administration:

2.3.1.6.1 Treatment: Invertebrates will be exposed to six replicates of either 0 (control) and approximately 50, and 100 mg/L A.I. SDP as an 8 h static exposure.

2.3.1.6.2 Route of administration: Exposures will be initiated by addition of a stock solution of SDP to the exposure chamber**.** The test concentrations will include 0 (control), and approximately 50 and 100 mg/L A.I. of formulated *Pf*-CL145A.

2.3.1.6.3 Concentration verification: Concentration will be determined by spectrophotometric comparison to a standard curve prepared from a known mass of formulated *Pf*-CL145A. The absorbance of exposure solutions will be compared to the standard curve to determine the exposure concentration. Absorbance will be determined using a Beckman DU 800 UV/VIS spectrophotometer (UMESC SOP AEH 303).

2.4 Bathymetric Mapping

Zebra mussel habitat mapping surveys will be conducted using a high-resolution side-scanning sonar unit linked with real-time differential GPS data collection. Data will be collected in transects followed by compilation into a habitat map layer. A single survey will be conducted for each lake during mid-summer (July-August) to determine the substrate type, depth and vegetative cover during the veliger settling period. If the selected lake(s) are excessively large (ie: >2,000 acres) a portion of the lakes such as a bay may be mapped in lieu of the entire water body.

Data from surveys will be processed using a commercially available post-processing software tool that allows for near real-time uploading of transect data from the field. Data are processed on remote servers with same-day incorporation of recent transect data into a composite habitat map. The resulting habitat maps can then be assessed for desired levels of detail and resolution, often on the same day. This rapid feedback between data collection and preliminary map development will allow field crews to optimize survey efforts on each lake.

Once the habitat maps are available from the commercial software product, they will be evaluated for relationships with vegetation samples taken from ground-truth plots (GTP) and from diver surveys. If additional post-processing of the raw sonar data is required to improve the map, options will be explored using supervised classifications based on data from the GTP.

2.5 Environmental DNA and physical surveys

Currently, molecular survey tools (i.e. eDNA) are being used to monitor aquatic invasive species throughout the Great Lakes and Upper Mississippi regions. The development and use of an eDNA survey tool for zebra mussels may greatly improve identification of application sites and effectiveness of zebra mussel controls. Prior to implementation, zebra mussel specific-markers must be developed and the appropriate sampling depth determined. Once these are established, surveys will be completed for two infested Minnesota water bodies to determine the potential application of eDNA as an evaluation tool. Physical (e.g. divers) surveys will be completed to compare and identify SDP application locations and to determine the potential for eDNA as a treatment prioritization and evaluation tool. Transects selected from the bathymetric maps will be used to select sampling location for the eDNA and physical surveys.

After collection of the eDNA water samples for each location, a ground truthing physical survey will be conducted to determine the abundance of zebra mussels at that location.

2.5.1 STUDY PROCEDURES

2.5.1.1 Marker development:

More than 1100 sequences are currently uploaded into a public database (National Center of Biotechnology Information). These sequences will be evaluated relative to all other sequences in these public databases and only those unique to zebra mussels will be used as markers.

2.5.1.2 Appropriate sampling depth:

2.5.1.2.1 The test location will be determined in spring 2013 after consultation with the MN DNR and site visits.

2.5.1.2.2 Sampling: Environmental DNA sampling procedures outlined by the U.S. Army Corps of Engineers in the Quality Assurance Project Plan (QAPP) for monitoring Asian carp will be used, but with slight modifications. Ten water samples will be collected from each of 3 depths (surface, mid-level and bottom) above 3 to 4 well-established zebra mussel beds.

2.5.1.2.3 Sample processing: Methods described in the QAPP will be used to process all samples. Currently these include: filtration of water samples and the extraction of DNA using a commercially available kit like Mo-Bio PowerWater DNA Isolation Kit.

2.5.1.2.4 DNA Detection: Zebra mussel DNA will be detected using multiple species-specific markers (see Section 2.5.1.1).

2.5.1.2.5 Measurements: Detection of DNA will be determined as a positive or negative. Mean number of positives from each depth will be compared. A second method, quantification of DNA will be used if quantitative markers (Section 2.5.1.1) can be designed.

2.5.1.2.5 Analysis: Presence of zebra mussel DNA will be compared among depths. The depth with the most consistent and/or greatest number of positive detections will be used for mapping (Section 2.5.1.3).

2.5.1.3 Mapping:

2.5.1.3.1 Sampling location: Sampling locations will be determined after the completion of the bathymetric surveys in conjunction with determination of sampling quadrants for physical mapping of lakes. Transects will be established from the bathymetric data that represent a diversity of substrate type. Up to 300 locations will be selected, placed and assessed for zebra mussel populations on each lake.

2.5.1.3.2 Sampling: Environmental DNA sampling procedures outlined in the QAPP will be used with slight modifications. Water samples will be collected in triplicate at a depth determined in Section 2.5.1.2 at each sampling site followed by a physical survey of each location.

2.5.1.3.3 Sample processing: Methods described in the QAPP will be used to process all samples. Currently these include: filtration of water samples and the extraction

of DNA using a commercially available kit like Mo-Bio PowerWater DNA Isolation Kit.

2.5.1.3.4 DNA Detection: Zebra mussel DNA will be detected using multiple species-specific markers (see Section 2.5.1.1).

2.5.1.3.5 Measurements: Detection of DNA will be determined as a positive or negative. Mean number of positives from each depth will be compared.

2.5.1.3.5 Analysis: A probability map will be established based on the incidence of detection. This probability map will be compared with the physical abundance data.

2.6 Application Technique Development - Laboratory

Laboratory studies will be conducted to determine the best techniques (i.e.: injection depth, injection rate, and SDP stock solution concentration) for injection delivery of SDP which will achieve the desired treatment concentration and duration at the desired water level (i.e.: the bottom 50% of the water column).

A series of replicate exposure tanks (76.2 cm diameter x 91.4 cm depth fiberglass circular tanks) containing 350 L of 20 ± 2°C UMESC well water will be dosed to achieve either a 0 (control), a 50 and/or 100 mg/L whole tank treatment or a 50 and/or 100 mg/L bottom 50% injection tank treatment.

The whole tank treatments will be conducted by the addition of the appropriate amount of SDP stock solution to achieve the desire target concentration in the entire 350-L tank volume. After stock addition, the tank will be mixed to provide a uniform concentration.

The injection tank treatments will be conducted by delivering a selected concentration SDP stock solution using a peristaltic pump at the selected rate, delivery depth and method (i.e.: tubing size/terminal end configuration) to each replicate until the calculated dose is delivered to produce the desired concentration (50 or 100 mg/L A.I.) to the bottom 50% of the tank volume.

Each treatment (control, whole tank volume and injected) will be tested in triplicate for each treatment variable (i.e. delivery rate, stock concentration, etc). Identical SDP stock concentrations will be used in the whole tank and the injection treatments for each treatment trial.

Samples will be collected hourly from each replicate at every 10-cm of depth using a manifold system connected to a peristaltic pump. Collection lines will be flushed prior to sample collection. The absorbance of exposure samples will be compared to a standard curve prepared using a known mass of SDP determined using a Beckman DU 800 UV/VIS spectrophotometer. The best combination of injection depth, injection rate and SDP stock concentration required to achieve the desired treatment concentration will be determined and used in pond scale applications.

2.6.1 STUDY PROCEDURES

2.6.1.2 Water Chemistry

2.6.1.2.1 Dissolved oxygen: Dissolved oxygen will be measured and recorded on each exposure chamber prior to exposure initiation and upon exposure termination at the bottom of the exposure chamber (UMESC SOP AEH 394 or equivalent).

2.6.1.2.2 Temperature: Temperature will be measured and recorded each on exposure chamber prior to exposure initiation and upon exposure termination at the bottom of the exposure chamber.

2.6.1.2.3 pH: pH will be measured and recorded on each exposure chamber prior to exposure initiation and upon exposure termination at the bottom of the exposure chamber (UMESC SOP AEH 335 or equivalent).

2.6.1.2.4 Hardness: Hardness will be measured and recorded on each exposure chamber prior to exposure initiation and upon exposure termination at the bottom of the exposure chamber (UMESC SOP AEH 712).

2.6.1.2.5 Alkalinity: Alkalinity will be measured and recorded on each exposure chamber prior to exposure initiation and upon exposure termination at the bottom of the exposure chamber (UMESC SOP AEH 706).

2.6.1.2.6 Conductivity: Conductivity will be measured and recorded on each exposure chamber prior to exposure initiation and upon exposure termination at the bottom of the exposure chamber (UMESC SOP AEH 188 or equivalent).

2.6.1.3 Study facilities:

2.6.1.3.1 Test Facility
U.S. Geological Survey, Upper Midwest Environmental Sciences Center
2630 Fanta Reed Rd
La Crosse, Wisconsin 54603

2.6.1.3.1.1 Exposure system: The test system will be a series of circular fiberglass tanks. Each exposure chamber will contain 350-L of temperature-adjusted well water. Each chamber will be uniquely identified (eg: A1) to allow for identification of treatment type and replicate number. Coding procedures will be documented in the study records.

2.6.1.3.1.2 Water supply: Temperature-adjusted (20 ± 2 °C) well water.

2.6.1.3.1.3 Exposure chamber dimensions: The exposure chambers are circular fiberglass tanks (76.2 cm diameter x 91.4 cm depth) and each chamber will contain 350 L of water during the exposure period.

2.6.1.3.1.4 Water discharge: All SDP treated water will be discharged into the UMESC carbon adsorption system. Untreated water will be discharged into the UMESC general effluent system.

2.6.1.3.1.5 Exposure chamber cleaning: Each chamber will be thoroughly cleaned with a bristle scrub brush and rinsed after each use.

2.6.1.4 Observations:

2.6.1.4.1 Delivery: Observations on injection delivery (i.e.: dissipation, etc.) will be recorded for each trial.

2.6.1.4.2 Stock preparation: Observations on preparation (ie: clumping, ease of mixing etc) will be recorded for each trial.

2.6.1.5 Treatment administration:

2.6.1.5.1 Treatment: Each Exposure trial will consist of three replicates of 0 (control), 50 or 100 mg/L A.I. SDP whole tank treatment, and 50 or 100 mg/L A.I. SDP injection treatment as an 8-h static exposure.

2.6.1.5.2 Route of administration:

2.6.1.5.2.1 Whole tank volume treatment: Exposures will be initiated by addition of a stock solution of SDP to the exposure chamber followed by thorough mixing.

2.6.1.5.2.2 Injection tank treatment: Exposures will be initiated by addition of a stock solution of SDP to the exposure chamber through a delivery manifold attached to a peristaltic pump**.** The exact details (stock concentration, delivery rate, delivery depth, etc) of each trial will be recorded in the study records.

2.6.1.5.3 Concentration verification: Concentration will be determined by spectrophotometric comparison to a standard curve prepared using a known mass of formulated *Pf*-CL145A. Absorbance will be determined using a Beckman DU 800 UV/VIS spectrophotometer (UMESC SOP AEH 303).

2.7 Application Technique Development – Pond Scale

The application methods developed from the laboratory studies will be used compare injection and whole water volume treatment of replicated 0.01 acre (10.4m x 4.9m x 1.2m) concrete ponds.

A series of replicate 0.01 acre (10.4m x 4.9m x 1.2m) concrete ponds will contain approximately 1-m of well water and will be treated to achieve either a 0 (control), a 50 and/or 100 mg/LA.I. whole pond volume treatment or a 50 and/or 100 mg/L A.I. 50% injection treatment.

The whole pond volume treatments will be conducted by the addition of the appropriate amount of SDP stock solution to achieve the desire target concentration in the entire pond volume. After stock addition, the pond will be mixed to provide a uniform concentration.

The pond injection treatments will be conducted by delivering a selected concentration SDP stock solution using peristaltic pumps at the selected rate, delivery depth and method (i.e.: tubing size/terminal end configuration) to each replicate until the calculate dose is delivered sufficient to produce the desired concentration (50 or 100 mg/L A.I.) to the bottom 50% of the pond.

Each treatment (control, whole pond volume and pond injection) will be tested in triplicate. Identical SDP stock concentrations will be used in the whole tank and the injection treatment trials.

Samples will be collected hourly from each replicate every 10-cm of using a manifold system connected to a peristaltic pump. The collection lines will be flushed prior to sample collection. The absorbance of exposure samples will be compared to a standard curve prepared using a known mass of SDP using a Beckman DU 800 UV/VIS spectrophotometer.

2.7.1 STUDY PROCEDURES

2.7.1.2 Water Chemistry

2.7.1.2.1 Dissolved oxygen: Dissolved oxygen will be measured and recorded on each pond replicate prior to exposure initiation and upon exposure termination at the bottom of the pond (UMESC SOP AEH 394 or equivalent).

2.7.1.2.2 Temperature: Temperature will be measured and recorded on each exposure pond replicate prior to exposure initiation and upon exposure termination at the bottom of the pond.

2.7.1.2.3 pH: pH will be measured and recorded on each pond replicate prior to exposure initiation and upon exposure termination at the bottom of the pond (UMESC SOP AEH 335 or equivalent).

2.7.1.2.4 Hardness: Hardness will be measured and recorded on each pond replicate prior to exposure initiation and upon exposure termination from samples collected from the bottom of the pond (UMESC SOP AEH 712).

2.7.1.2.5 Alkalinity: Alkalinity will be measured and recorded on each pond replicate prior to exposure initiation and upon exposure termination from samples collected from bottom of the pond (UMESC SOP AEH 706).

2.7.1.2.6 Conductivity: Conductivity will be measured and recorded on each exposure chamber prior to exposure initiation and upon exposure termination from samples collected from the bottom of the pond (UMESC SOP AEH 188 or equivalent).

2.7.1.3 Study facilities:

2.7.1.3.1 Test Facility
U.S. Geological Survey, Upper Midwest Environmental Sciences Center
2630 Fanta Reed Rd
La Crosse, Wisconsin 54603

2.7.1.3.1.1 Exposure system: The test system will be a series of 0.01 acre (10.4m x 4.9m x 1.2m) outdoor concrete ponds. Each pond will be filled with well water 24-48h prior to exposure initiation.

Each pond is uniquely identified (e.g.: C1) to allow for identification of treatment type and replicate number. Coding procedures will be documented in the study records.

2.7.1.3.1.2 Water supply: Ambient well water will be used for the static exposures.

2.7.1.3.1.3 Pond replicate dimensions: Each pond is a 0.01 acre (10.4m x 4.9m x 1.2m) outdoor concrete pond.

2.7.1.3.1.4 Water discharge: All SDP treated water will be carbon filtered prior to discharge into the UMESC general effluent system. Untreated water will be discharged into the UMESC general effluent system.

2.7.1.3.1.5 Pond cleaning: Each pond will be thoroughly rinsed and/or power washed after each use to remove all SDP residues.

2.7.1.4 Observations:

2.7.1.4.1 Delivery: Observations on delivery (i.e.: dissipation, etc.) will be recorded for each trial.

2.7.1.4.2 Stock preparation: Observations on preparation (i.e.: clumping, ease of mixing etc.) will be recorded for each trial.

2.7.1.5 Treatment administration:

2.7.1.5.1 Treatment: Each Exposure trial will consist of three replicates of 0 (control), 50 and/or 100 mg/L A.I. SDP as an 8-h static exposure.

2.7.1.5.2 Route of administration:

2.7.1.5.2.1 Whole pond volume treatment: Exposures will be initiated by addition of a stock solution of SDP to the pond followed by thorough mixing.

2.7.1.5.2.2 Injection pond treatment: Exposures will be initiated by addition of a stock solution of SDP to the pond through a delivery manifold attached to a peristaltic pump**.** The test concentrations will include 0 (control), 50 and/or 100 mg/L A.I. of active formulated *Pf*-CL145A. The exact details (stock concentration, delivery rate, delivery depth, etc.) of each trial will be recorded in the study records.

2.7.1.5.3 Concentration verification: Concentration will be determined spectrophotometrically by comparison to a standard curve prepared using a known mass of formulated *Pf*-CL145A. Absorbance will be determined using a Beckman DU 800 UV/VIS spectrophotometer (UMESC SOP AEH 303).

2.8 Field Application and Monitoring

The efficacy of dreissenid mussel control using SDP treatments will be assessed in a selected Minnesota lake with a high zebra mussel population. Whole water column and injection treatment techniques will be applied within replicated enclosures and compared to untreated control enclosures. Necessary permitting will be the responsibility of the USGS with assistance from project partners.

The results from the ongoing efficacy studies and proposed treatment technique development will determine the exact SDP application methods used during the field treatments. The exact procedures used will be documented in the study management system.

A series of replicate ~ 24 m^2 (3m x 8m) enclosures will be placed into the designated lake after site selection is accomplished using bathymetric, physical and eDNA surveys. The enclosures will be treated to achieve either a 0 (control), a 50 and/or 100 mg/L A.I. whole water column treatment or a 50 and/or 100 mg/L A.I. bottom 50% injection treatment for a treatment duration of 8-h. After the exposure period the enclosure will be removed to allow for dissipation of the formulated *Pf*-CL145A. The enclosures will be situated to avoid cross contamination.

The whole water column treatments will be conducted by the addition of the appropriate amount of SDP stock solution to achieve the desire target concentration in the entire enclosure.

The injection treatments will be conducted by delivering a selected concentration SDP stock solution using peristaltic or other suitable delivery pump at the selected rate, delivery depth and method (i.e.: tubing size/terminating end configuration) to each replicate until the calculated dose is delivered sufficient to produce the desired concentration (50 or 100 mg/L A.I.) to the bottom 50% of the enclosure. After the delivery, the delivery apparatus will be removed.

Each treatment (control, whole water volume, and injection) will be tested in triplicate. Identical SDP stock concentrations will be used in the whole tank and the injection treatment trials. The treatments will be replicated over two types of substrate (i.e.: gravel, cobble, sand, etc.). If possible, the treatments will be conducted in September 2013 after the majority of the veliger settlement has been completed for the year. If fall treatments are not possible, spring 2014 treatments will be conducted prior to the majority of the veliger release.

Samples will be collected hourly from each replicate using a manifold system connected to a peristaltic pump or a similar system. The collection lines will be flushed prior to sample collection. The absorbance of exposure samples will be compared to a standard curve prepared using a known mass of formulated *Pf*-CL145A.

Pre-treatment surveys within each treatment and control replicate will be conducted to determine the pretreatment zebra mussel population. The replicate areas will be resurveyed ≥ 30-d after exposure to compare the treated and control areas.

2.8.1 STUDY PROCEDURES

2.8.1.1 Water Chemistry

2.8.1.1.1 Dissolved oxygen: Dissolved oxygen will be measured and recorded on each enclosure replicate prior to exposure initiation and upon exposure termination at the bottom of the enclosure (UMESC SOP AEH 394 or equivalent).

2.8.1.1.2 Temperature: Temperature will be measured and recorded on each exposure replicate prior to exposure initiation and upon exposure termination at the bottom of the enclosure.

2.8.1.1.3 pH: pH will be measured and recorded on each enclosure replicate prior to exposure initiation and upon exposure termination at the bottom of the enclosure (UMESC SOP AEH 335 or equivalent).

2.8.1.1.4 Hardness: Hardness will be measured and recorded on a representative enclosure replicate prior to exposure initiation and upon exposure termination on a representative enclosure replicate (UMESC SOP AEH 712).

2.8.1.1.5 Alkalinity: Alkalinity will be measured and recorded on a representative enclosure replicate prior to exposure initiation and upon exposure termination on a representative enclosure replicate (UMESC SOP AEH 706).

2.8.1.1.6 Conductivity: Conductivity will be measured and recorded on a representative enclosure replicate prior to exposure initiation and upon exposure termination on a representative enclosure replicate (UMESC SOP AEH 188 or equivalent).

2.8.1.2 Study facilities:

2.8.1.2.1 Test Facility
U.S. Geological Survey, Upper Midwest Environmental Sciences Center
2630 Fanta Reed Rd
La Crosse, Wisconsin 54603

2.8.1.2.1.1 Exposure system: The test system will be a series of ~24-m^2 (3m x 8m) enclosures within the selected lake. Each enclosure will contain lake water and zebra mussels. Each replicate will be uniquely identified (e.g.: W1) to allow for identification of treatment type, replicate number, substrate type, etc. A semi-permanent benthic marker (i.e.: staked chain) will be placed along the perimeter of each enclosure in addition to GPS coordinates at each corner. Coding procedures and GPS coordinates will be documented in the study records.

2.8.1.2.1.2 Water supply: Natural lake water will be used for the exposures and SDP stock preparation.

2.8.1.2.1.3 Enclosure replicate dimensions: Each enclosure is ~24-m^2 (3m x 8m) impermeable barrier.

2.8.1.2.1.4 Water discharge: All SDP treated water will be dissipated into the lake upon completion of the exposures.

2.8.1.3 Observations:

2.8.1.3.1 Delivery: Observations on delivery (i.e.: dissipation, etc.) will be recorded for each trial.

2.8.1.3.2 Stock preparation: Observations on preparation (i.e.: clumping, ease of mixing etc.) will be recorded for each trial.

2.8.1.4 Treatment administration:

2.8.1.4.1 Treatment: Each Exposure trial will consist of three replicates of 0 (control), 50 and/or 100 mg/L A.I. SDP as an 8-h static exposure.

2.8.1.4.2 Route of administration:

2.8.1.4.2.1 Whole water column treatment: Exposures will be initiated by addition of a stock solution of SDP to the enclosure.

2.8.1.4.2.2 Injection pond treatment: Exposures will be initiated by addition of a stock solution of SDP to the enclosure through a delivery manifold attached to a peristaltic or other suitable pump**.** The exact details (stock concentration, delivery rate, delivery depth, etc) of each trial will be recorded in the study records.

2.8.1.4.3 Concentration verification: Concentration will be determined spectrophotometrically. A standard curve will be prepared using a known mass of formulated *Pf*-CL145A. The absorbance of exposure solutions will be compared to the standard curve to determine the exposure concentration. Absorbance will be determined using a Beckman DU 800 UV/VIS spectrophotometer (UMESC SOP AEH 303).

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted not later than 12/31/13, 6/30/14, and 12/31/14. A final report and associated products will be submitted between June 30 and August 15, 2015 as requested by the LCCMR.

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

Project Title: An evaluation of the efficacy and safety of Pf-CL145A for control of zebra mussels in MN waters
Legal Citation: Pending
Project Manager: Mark Gaikowski
M.L. 2013 ENRTF Appropriation: $600,000 pending approval
Project Length and Completion Date: 2 yr, June 30, 2015
Date of Update: September 30, 2012

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	Activity 4 Budget	Amount Spent	Balance	Activity 5 Budget	Amount Spent	Balance
BUDGET ITEM	***Non-target Animal Impacts: Fathead Minnow Reproductive Success: Adult Reproductive Activity***			***Non-target Animal Impacts: Fathead Minnow Reproductive Success: Embryo Development***			**Non-target Animal Impacts: Invertebrate**			**Bathymetric mapping**			**Environmental DNA Physical Surveys**		
Personnel (Wages and Benefits)															
Amberg(UMESC)/Research Fisheries Biologist (PCR expert) (70% salary & 30% benefits) 15% FTE													10,500		
Fredricks(VU)/Research Biologist (93% salary & 7% Benefits) 100% FTE	39,130			15,050			6,020						4,300		
Luoma(USGS)/Research Fisheries Biologist (80% salary & 20% benefits) 40% FTE	6,500			2,250			1,250						5,940		
Waller(WWTC)/Research Biologist (93% salary & 7% benefits) 60% FTE													12,800		
UMESC Biologist/project implimentation (70% salary & 30% benefits) 25% FTE	6,500			5,500			2,000						8,000		
UMESC research assistant/project implimentation (93% salary & 7% benefits) 100% FTE of 4.5 180-d tempory appointments	15,000			15,000			15,000						20,600		
Professional/Technical/Service Contracts *(List out proposed contracts. Be clear about whom the contract is to be made with and what services will be provided. If a specific contractor is not yet determined, specify the type of contractor sought. List out by contract types/categories—one row per type/category. Add rows as needed)*															
Minnesota Water Sciences Center (USGS) high resolution substrate mapping										60,000					
SCUBA dive support													7,500		
Mayer (NYSM) project design, implementation (salary and benefits 10% FTE)													5,000		
Equipment/Tools/Supplies *(List out general descriptions of item(s) or item type(s) and their purpose—one row per item/item type. Add rows as needed. If a single piece of equipment will exceed $3,500, list it under "Capital Expenditures over $3,500" instead.)*															
Test material (Pf-CL145A)															
eDNA kits, reagents, sampling equipment (600 samples x $20 sample)													9,000		
Laboratory tanks, mesocosm tanks, application equipment	4,000			4,000			2,000								
Plumbing supplies and tubing	5,000			5,000			2,500								
Pumps	1,150			1,150											
Field invertebrate collection gear							3,500								
Field enclosure supplies, sampling gear													2,000		
Expendable supplies													1,850		
Survey/transect equipment, tools, nets													2,250		
Capital expenditures over $3,500 *(List specific items. Add rows as needed.)*															
Travel expenses in Minnesota (*Specify types of travel expenses, e.g., mileage, lodging, meals. Per diems are not allowed.)*															
Invertebrate collection, mileage							1,000								
Mapping $123/day x 10 days x 4 people										4,920					
Sampling, eDNA/conventional $123/day x 30 days x 2 people													7,380		
Pre-treatment surveys $123/day x 5 days x 5 people															
Treatment surveys $123/day x 5 days x 5 people															
Post- treatment surveys $123/day x 5 days x 5 people															
Other *(Describe the expense—one row per type/category. Add rows as needed. Be specific.)*															
COLUMN TOTAL	**$77,280**	**$0**	**$0**	**$47,950**	**$0**	**$0**	**$33,270**	**$0**	**$0**	**$64,920**	**$0**	**$0**	**$97,120**	**$0**	**$0**

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

Project Title: An evaluation of the efficacy and safety of Pf-CL145A for control of zebra mussels in MN waters
Legal Citation: Pending
Project Manager: Mark Gaikowski
M.L. 2013 ENRTF Appropriation: $600,000 pending approval
Project Length and Completion Date: 2 yr, June 30, 2015
Date of Update: September 30, 2012

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 6 Budget	Amount Spent	Balance	Activity 7 Budget	Amount Spent	Balance	Activity 8 Budget	Amount Spent	Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	**Application Technique Development - Laboratory**			**Application Technique Development – Pond Scale**			**Field Application and Monitoring**				
Personnel (Wages and Benefits)											
Amberg(UMESC)/Research Fisheries Biologist (PCR expert) (70% salary & 30% benefits) 15% FTE							7,000			17,500	
Fredricks(VU)/Research Biologist (93% salary & 7% Benefits) 100% FTE	2,150			2,150						68,800	
Luoma(USGS)/Research Fisheries Biologist (80% salary & 20% benefits) 40% FTE	4,950			4,950			23,760			49,600	
Waller(WWTC)/Research Biologist (93% salary & 7% benefits) 60% FTE	6,400			6,400			19,800			45,400	
UMESC Biologist/project implimentation (70% salary & 30% benefits) 25% FTE	7,000			7,000			20,000			56,000	
UMESC research assistant/project implimentation (93% salary & 7% benefits) 100% FTE of 4.5 180-d tempory appointments	18,025			18,025			46,350			148,000	
Professional/Technical/Service Contracts *(List out proposed contracts. Be clear about whom the contract is to be made with and what services will be provided. If a specific contractor is not yet determined, specify the type of contractor sought. List out by contract types/categories—one row per type/category. Add rows as needed)*										0	
Minnesota Water Sciences Center (USGS) high resolution substrate mapping										60,000	
SCUBA dive support							7,500			15,000	
Mayer (NYSM) project design, implementation (salary and benefits 10% FTE)	2,500			2,500			10,000			20,000	
Equipment/Tools/Supplies *(List out general descriptions of item(s) or item type(s) and their purpose—one row per item/item type. Add rows as needed. If a single piece of equipment will exceed $3,500, list it under "Capital Expenditures over $3,500" instead.)*										0	
Test material (Pf-CL145A)	1,000			4,000			15,000			20,000	
eDNA kits, reagents, sampling equipment (600 samples x $20 sample)							3,000			12,000	
Laboratory tanks, mesocosm tanks, application equipment	5,000			4,000			1,000			20,000	
Plumbing supplies and tubing										12,500	
Pumps										2,300	
Field invertebrate collection gear										3,500	
Field enclosure supplies, sampling gear				1,978			15,500			19,478	
Expendable supplies	825			825			1,400			4,900	
Survey/transect equipment, tools, nets				250						2,500	
Capital expenditures over $3,500 *(List specific items. Add rows as needed.)*										0	
Travel expenses in Minnesota (*Specify types of travel expenses, e.g., mileage, lodging, meals. Per diems are not allowed.)*										0	
Invertebrate collection, mileage										1,000	
Mapping $123/day x 10 days x 4 people										4,920	
Sampling, eDNA/conventional $123/day x 30 days x 2 people										7,380	
Pre-treatment surveys $123/day x 5 days x 5 people							3,074			3,074	
Treatment surveys $123/day x 5 days x 5 people							3,074			3,074	
Post- treatment surveys $123/day x 5 days x 5 people							3,074			3,074	
Other *(Describe the expense—one row per type/category. Add rows as needed. Be specific.)*										0	
COLUMN TOTAL	**$47,850**	**$0**	**$0**	**$52,078**	**$0**	**$0**	**$179,532**	**$0**	**$0**	**600,000**	**$0**