

**Main Request for Funding Form**

**Lessard-Sams Outdoor Heritage Council  
Fiscal Year 2013**

**Program or Project Title:** EVALUATION OF ZEQUANOX  ASA ZEBRA MUSSEL CONTROL AGENT  
**Funds Requested:** \$ *350000*

**Manager's Name:** Pam Marrone ,Dennis Bitter  
**Organization:** MARRONE BIO INNOVATIONS

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**Organization Web Site** [.marronebioinnovations.com](http://marronebioinnovations.com)

**County Location:** *DOUGLAS*

**Ecological Planning Regions:**

x Forest/Prairie Transition

**Activity Type:**

Protect - Other

x Restore x Enhance

**Priority Resources addressed by activity:**

x Wetlands

**Project Abstract**

The objective of this research proposal is to describe MBI's collection and research interests in Open Water Treatment and Application in Minnesota lakes in order to establish necessary cooperative research agreements with interested parties.

*Due its significant environmental and economic impacts, the zebra mussel is widely recognized as the poster child of aquatic invasive species in North America. Lakes in Douglas County have zebra mussel infestations, there is interest in having the effectiveness of Zequanox □ a new, promising, environmentally friendly biopesticide – tested as a control agent in small-scale demonstration projects in several of its lakes .*

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## Project Narrative

### Design and scope of work

Although Zequanox □ has proven its effectiveness in treatment of zebra mussel infested pipes within industrial facilities, its effectiveness in controlling zebra mussels in open water environments (e.g., lakes and rivers) has yet to be evaluated. This document presents an overall research plan that would be carried out in June through October of this year by the commercial developer of Zequanox □, Marrone Bio Innovations (MBI). Execution of the plan is dependent on: 1) funding to be awarded by DCLA and 2) regulatory approval of such a testing program by the Minnesota Department of Natural Resources. The testing program outlined herein would take place in three sequential phases. Each of the test phases is designed to confirm high rates of mussel kill in incrementally larger volumes of lake water and under increasingly more realistic “field-like” treatment conditions. These phases are: 1) initial laboratory (indoor) “jar tests” in June (purpose: mussel kill assessed in glass jars containing ~0.5 liters of water); 2) subsequent laboratory (indoor) “biobox tests” in July (purpose: mussel kill assessed in glass aquaria containing ~30 liters of water); and finally 3) a series of small-scale “mesocosm” field trials in August-October (purpose: mussel kill assessed in shallow, physically isolated sections of water column along the lake shore containing ~300 liters of water). The indoor laboratory tests are critically important steps toward designing treatment protocols to use in the field trials to ensure high mussel kill, e.g., learning which of several Zequanox □ formulations is best to use in the field trials, how many hours and at what concentration should the mussels be exposed, etc.). Thus, although small-scale, the proposed field trials this year would provide valuable information to the DCLA as to the effectiveness of Zequanox □ in the open water environment of their lakes.

## SUMMARY

Due its significant environmental and economic impacts, the zebra mussel is widely recognized as the poster child of aquatic invasive species in North America. Lakes in Douglas County have zebra mussel infestations, and the Douglas County Lake Association (DCLA) has expressed interest in having the effectiveness of Zequanox □ – a new, promising, environmentally friendly biopesticide – tested as a control agent in small-scale demonstration projects in several of its lakes (e.g., Carlos, Darling, and Le Homme Dieu). Although Zequanox □ has proven its effectiveness in treatment of zebra mussel infested pipes within industrial facilities, its effectiveness in controlling zebra mussels in open water environments (e.g., lakes and rivers) has yet to be evaluated. This document presents an overall research plan that would be carried out in June through October of this year by the commercial developer of Zequanox □,

Marrone Bio Innovations (MBI). Execution of the plan is dependent on: 1) funding to be awarded by DCLA and 2) regulatory approval of such a testing program by the Minnesota Department of Natural Resources. The testing program outlined herein would take place in three sequential phases. Each of the test phases is designed to confirm high rates of mussel kill in incrementally larger volumes of lake water and under increasingly more realistic “field-like” treatment conditions. These phases are: 1) initial laboratory (indoor) “jar tests” in June (purpose: mussel kill assessed in glass jars containing ~0.5 liters of water); 2) subsequent laboratory (indoor) “biobox tests” in July (purpose: mussel kill assessed in glass aquaria containing ~30 liters of water); and finally 3) a series of small-scale “mesocosm” field trials in August-October (purpose: mussel kill assessed in shallow, physically isolated sections of water column along the lake shore containing ~300 liters of water). The indoor laboratory tests are critically important steps toward designing treatment protocols to use in the field trials to ensure high mussel kill, e.g., learning which of several Zequanox<sup>™</sup> formulations is best to use in the field trials, how many hours and at what concentration should the mussels be exposed, etc.). Thus, although small-scale, the proposed field trials this year would provide valuable information to the DCLA as to the effectiveness of Zequanox<sup>™</sup> in the open water environment of Minnesota lakes.

### **Relationship to Other Constitutional Funds**

No other funds will be sought for field trials. Zequanox<sup>™</sup> product will be manufactured commercially. The purpose of having the trials in Minnesota is to become the leader in the cutting edge product and to have the possibility to bring a manufacturing arm here to our state and possibly create job. The DNR will need to monitor the ongoing aspects of the results of this trial for possible use State wide for a green control of zebra mussels. The AIS funding that was placed in the DNR budget as a line item can be subsequently be a source to infested lakes for the control of invasive zebra mussels.

### **Relationship to Current Organizational Budget**

*The request is a one time request for initial field trials. The entire project to complete the Open Water Management Program will need to be defined as it progresses. Douglas County Lakes Association is a non profit, volunteer based organization with yearly dues as the source of income. Our participation in the DNR AIS Prevention Grants for the last two years shows our dedication to combating the AIS issue facing Minnesota lakes..*

### **Sustainability and Maintenance**

At the conclusion of the trials, Marrone Bio Innovations will need to continue studying a delivery method specific to a lake application. Those studies are taking place currently as the progression of Zequanox evolves.

The entire Project will exceed the \$350K (with the desire to define deliverables for future \$dollars from Legacy funds to continue / complete a comprehensive Open Water Mussel Management Program). Many elements to a complete Open Water Mussel Management Programs will need to be defined, formulated and developed (or even discovered). The desired objective is to have working programs within 3 years with detailed or comprehensive programs within 5 years. In this estimate, it is a 3-5 year journey. These initial funds will start the process sooner, rather than later, and move the process forward towards successful Programs.

### **Outcomes**

The final outcome revolves around having an environmentally safe product to use to control the destruction caused by zebra mussels in our fish growth and personal property damage. Once zms are in the water body they cannot be eradicated, only controlled.

### **Activity Type Detail**

#### **Fee Acquisition Projects**

Will local government approval be sought prior to acquisition?

Yes

If no, please explain here:

Is the land you plan to acquire free of any other permanent protection?

Yes

If no, please explain here:

**Easement Acquisition Projects**

Will the eased land be open for public use?

Yes

If no, please explain here:

Will the conservation easement be permanent?

not applicable

If no, please explain here: Not a conservation easement program

**Restoration and Enhancement Projects**

Is the activity on permanently protected land and/or public waters?

Yes

**If no, please explain here:**

Does the activity take place on an Aquatic Management Area (AMA), Scientific and Natural Area (SNA), Wildlife Management Area (WMA), or State Forests?

No

**Past Outdoor Heritage Fund Appropriations Received for this program**

ML 2009	ML 2010	ML 2011
\$0	\$0	\$0

**Accomplishment Timeline**

Activity	Milestone	Date
<p><input type="checkbox"/> <i>Field site visit to Lakes Carlos, Darling, and Le Homme Dieu.</i></p> <p><input type="checkbox"/> <i>Choose a location and establish indoor laboratory for jar and biobox testing.</i></p> <p><input type="checkbox"/> <i>Initiate contacts to secure all necessary permits (if permits are needed) from the MN DNR for collection of zebra mussels from the lakes and the conduct of the laboratory trials starting in June and the field testing scheduled to begin in August.</i></p> <p><input type="checkbox"/> <i>Conduct laboratory jar tests whose purpose is to assess mussel kill in glass jars (~0.5 liters of water) under ideal treatment conditions; this type of testing is extremely valuable to quickly and economically evaluate ZQ formulations and treatment doses (length of exposure and product concentration)</i></p>	<p>Laboratory jar testing whose purpose is to assess mussel kill in glass jars (~0.5 liters of water) under ideal treatment conditions; this type of testing is extremely valuable to quickly and economically evaluate ZQ formulations and treatment doses (length of exposure and product concentration)</p>	<p>July 2012:</p>
<p><i>Continue jar testing as needed.</i></p> <p><input type="checkbox"/> <i>Conduct laboratory biobox tests whose purpose is to assess mussel kill in a larger (deeper) quantity of water (within aquaria containing ~30 liters of water); this type of testing is extremely valuable to confirm the jar test result, thereby increasing the probability of successful achieving high kill in the next phase of testing, i.e., actual field trials starting in Sept</i></p>	<p>To assess mussel kill in a larger deeper quantity of water confirming the probability of achieving high kill in the next phase.</p>	<p>August 2012</p>
<p><i>Continue jar and biobox testing as needed.</i></p> <p><input type="checkbox"/> <i>Conduct the series of small scale “mesocosm” field trials in shallow sections of water along the lake shore where ~300 liters of water can be isolated by a surrounding barrier and treated, e.g.,</i></p>	<p>To demonstrate the technical feasibility of ZQ to achieve high mussel kill under realistic lake conditions.</p>	<p>September-October 2012 November thru January 2013</p>

<p><i>cylinders) which are open at the top and bottom could be embedded in the lake bottom or curtain barriers installed; following the treatment exposure period (e.g., ~6 to 24 hours), the barrier would be removed; this type of field trial (albeit quite small-scale) is extremely valuable in demonstrating the technical feasibility of ZQ to achieve high mussel kill under realistic lake conditions. Completion of all data analysis and project report issued</i></p>		
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**Attachments:** *[Attach the spreadsheet to the web application form.]*

**A. Budget**

- B.**
- B. Proposed Output Tables 1-5**
- C. Parcel List**

**BUDGET:**

**Personal (\$150K):**

**Sr. Level Project Manager - MBI plans to hire a Sr. level Project Manager dedicated to the Open Water Platform. Pending resumes and experience, it is desired that this person is a Master or Ph.D. in the Sciences. This person will be responsible for the complete management of the deliverables for a Complete Zequanox Open Water Mussel Management Program.**

**Formulation / Application – This is a team of Scientists (including Ph.D’s). Currently, the team is approximately 12 (with an additional 4-8 interns at any point in time). The entire team is dedicated to formulations / applications of all varieties. With this program, the entire team can be utilized to help in the Open Water deliverables with a desired 1-2 dedicated to the development of an Open Water Mussel Management Program.**

**Analytical Team / Jay Assays – The Analytical department and testing is an entire group without one person dedicated to any one particular Project. Assignments will be made based on deliverables defined from the Open Water Platform Project Manager.**

**Field Team / Biobox Trials / Open water Trials – Currently, a three (3) person field Team exist dedicated to Zequanox. All on this Team will be utilized with one person dedicated to the Open Water Trials (on site person).**

**Operations Team: currently, a team is being developed for the future manufacturing and production of Zequanox. This team and operations will expand with “economies of scale” of the Zequanox production.**

**Equipment / Supplies / Misc. (\$75K):**

**Laboratory & Testing Equipment / Analytical lab tests & supplies / Formulation supplies & Equipment / Jay test equipment & Supplies / Biobox Trial Equipment & Supplies / Field Equipment & supplies - Mesocosm Supplies / Report writing, research and documentation / Application Equipment & supplies**

**Travel & Living / Field shipments / car rentals (\$25K):**

**Oversite: of project by Dr. Dan Molloy developer of the bacterium strain. Molloy & Associates (\$50,000)**

**Attachment A. Budget Spreadsheet**

Name of Proposal:

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Date:


[Link HERE to definitions of the budget items below.](#)

**Total Amount of Request**      \$      1,500,000 *From page 1 on the funding form.*

**Personnel**

Position breakdown here	FTE	Over # of years	LSOHC Request	Anticipated Cash Leverage	Cash Leverage Source	Total
	<i>Manager of Programs</i>	0.5		\$ 17,000		
<i>Admin Asst</i>	0.15		\$ 5,000			\$ 5,000
<i>position 3</i>						\$ -
<i>position 4</i>						\$ -
<i>position 5</i>						\$ -
<i>position 6</i>						\$ -
<i>position 7</i>						\$ -
<b>Total</b>	0.65		\$ 22,000	\$ -	\$ -	\$ 22,000

**Budget and Cash Leverage** *(All your LSOHC Request Funds must be direct to and necessary for program outcomes.)*

*Please describe how you intend to spend the requested funds.*

Budget Item	LSOHC Request	Anticipated Cash Leverage	Cash Leverage Source	Total
	<b>Personnel - auto entered from above</b>	\$ 22,000	\$ -	\$ -
<b>Contracts</b>	\$ 7,500			\$ 7,500
<b>Fee Acquisition w/ PILT (breakout in table 7)</b>	\$ 160,000			\$ 160,000
<b>Fee Acquisition w/o PILT (breakout in table 7)</b>				\$ -
<b>Easement Acquisition</b>	\$ 1,300,000			\$ 1,300,000
<b>Easement Stewardship</b>				\$ -
<b>Travel (in-state)</b>	\$ 1,000	\$ 1,500	<i>federal grant match</i>	\$ 2,500
<b>Professional Services</b>				\$ -
<b>Direct Support Services</b>				\$ -
<b>DNR Land Acquisition Costs (\$3,500 per acquisition)</b>	\$ 3,500			\$ 3,500
<b>Other</b>				\$ 31,500
Capital Equipment ( <i>auto entered from below</i> )	\$ 15,000	\$ 7,000	<i>private source</i>	\$ 22,000
Other Equipment/Tools	\$ 5,900			\$ 5,900
Supplies/Materials	\$ 1,100	\$ 2,500	<i>local fundraising</i>	\$ 3,600
	\$ 1,516,000	\$ 11,000	\$ -	\$ 1,527,000

**Capital Equipment** *(single items over \$10,000 - auto entered into table above )*

Item Name	LSOHC Request	Leverage
<i>Truck</i>	15,000	7,000
<i>Item 2 enter here</i>		
<i>Item 3 enter here</i>		
<i>Item 4 enter here</i>		
<i>Item 5 enter here</i>		
<i>Item 6 enter here</i>		
<i>Item 7 enter here</i>		
<i>Item 8 enter here</i>		
<b>Total</b>	15,000	7,000

## Attachment B. Output Tables

<b>Name of Proposal:</b>	Evaluation of Zequanox as a Control Agent for Zebra Mussels
<b>Date:</b>	13-Jul-11

*Table 1 and Table 3 column totals should be the same AND Table 2 and Table 4 column totals should be the same*

*If your project has lakes or shoreline miles instead of land acres, convert miles to acres for Tables 1 and 3 using the following conversion:*

*Lakeshore = 6 acres per lakeshore mile / Stream & River Shore = 12 acres per linear mile, if both sides*

**Table 1. Acres by Resource Type**

Describe the scope of the project in acres (use conversion above if needed)

	Wetlands	Prairies	Forest	Habitats	Total
Restore					0
Protect Fee					0
Protect Easement					0
Protect Other					0
Enhance					0
<b>Total</b>	0	0	0	0	0

Total Acres (sum of Total column)	0	These two cells should be the same figure.
Total Acres (sum of Total row)	0	

**Table 2. Total Requested Funding by Resource Type**

	Wetlands	Prairies	Forest	Habitats	Total
Restore					\$ -
Protect Fee					\$ -
Protect Easement					\$ -
Protect Other					\$ -
Enhance				\$ 350,000	\$ 350,000
<b>Total</b>	\$ -	\$ -	\$ -	\$ 350,000	\$ 350,000

Total Dollars (sum of Total column)	\$ 350,000	These two cells should be the same figure.
Total Dollars (sum of Total row)	\$ 350,000	

Check to make sure this amount is the same as the Funding Request Amount on page 1 of Main Funding Form.

**Table 3. Acres within each Ecological Section**

	Metro/Urban	Forest/Prairie	SE Forest	Prairie	Northern Forest	Total
Restore						0
Protect Fee						0
Protect Easement						0
Protect Other		13,000				13000
Enhance						0
<b>Total</b>	0	13000	0	0	0	0

Total Acres (sum of Total column)	13000	These three cells should be the same figure.
Total Acres (sum of Total row)	13000	
Total Acres from Table 1.	0	

**Attachment B. Output Tables**

**Table 4. Total Requested Funding within each Ecological Section**

	Metro/Urban	Forest/Prairie	SE Forest	Prairie	Northern Forest	Total
Restore						\$ -
Protect Fee						\$ -
Protect Easement						\$ -
Protect Other		\$ 350,000				\$ 350,000
Enhance						\$ -
<b>Total</b>	\$ -	\$ 350,000	\$ -	\$ -	\$ -	

Total Dollars (sum of Total column)

\$ 350,000

*These two cells should be the same figure.*

Total Dollars (sum of Total row)

\$ 350,000

Check to make sure these amounts are the same as the Funding Request Amount on page 1 of Main Funding Form.

**Table 5. Target Lake/Stream/River Miles**

# miles of Lakes / Streams / Rivers Shoreline

**Table 6. Acquisition by PILT Status (enter information in acres)**

	Wetlands	Prairies	Forests	Habitats	Total
Acquired in Fee with State PILT Liability					0
Acquired in Fee w/o State PILT Liability					0
Permanent Easement <i>PILT Liability</i> <b>NO State</b>					0
	0	0	0	0	

**Table 7. Estimated Value of Land Acquisition by PILT Status (enter information in dollars)**

	Wetlands	Prairies	Forests	Habitats	Total	
Acquired in Fee with State PILT Liability					\$ -	\$ 160,000
Acquired in Fee w/o State PILT Liability					\$ -	\$ -
Permanent Easement <i>PILT Liability</i> <b>NO State</b>					\$ -	\$ 1,300,000
	\$ -	\$ -	\$ -	\$ -		

*FR: should match total in budget table that is auto entered below*

### Attachment C. Parcel List

Name of Proposal: \_\_\_\_\_  
 Date: \_\_\_\_\_

County	Township (25-258)	Range (01-51)	Direction most parcels are 2 with the exception of some areas of Cook County which is 1	Section (01 thru 36)	TRDS	# of acres	Budgetary Estimate (includes administrative, restoration or other related costs and do not include matching money contributed or earned by the transaction)	Description	Activity PF=Protect Fee PE=Protect Easement PO=Protect Other R=Restore E=Enhance	If Easement, what is the easement cost as a % of the fee acquisition?	Any existing protection? (yes/no)	Open to hunting and fishing? (yes/no)
<b>Parcel Name</b>												
<i>Example Lambertton WMA Addition</i>	<i>Redwood</i>	<i>109</i>	<i>37</i>	<i>2</i>	<i>13</i>	<i>10937213</i>	<i>114</i>	<i>\$5,500,000</i>		<i>P</i>		

*Information provided will be used to map project locations. Incomplete or inaccurate information will result in that parcel or program not being mapped.*