

**DON'T
MOVE
A
MUSSEL!**



Zebra Mussel Collection & Zequanox™ Efficacy Evaluation

Douglas County Citizen's Committee

DNR

June 1, 2011 Research Proposal



Tom Landwehr, Commissioner
Minnesota Department of Natural Resources
500 Lafayette Road
St. Paul, MN 55155

Dear Tom,

Re: Research Proposal - Zebra Mussel Collection & Zequanox™ Efficacy Evaluation in Douglas County

Thanks for squeezing in another phone call. And, thank you for your continued interest and support of the control, containment, and eradication studies and recommendations of the Douglas County citizens' committee on zebra mussel (and other AIS).

As you are aware, our Board of Commissioners took action on April 12, 2011, to:

- support the passage of SF0847;
- request the State Legislature make available dedicated funding from the Clean Water, Land and Legacy Act, devoted to control, containment and eradication of AIS, particularly zebra mussels;
- urge the State Legislature to continue support of the DNR AIS initiative by providing ample and continued funding.

On April 26, 2011, the Douglas County Commissioners resolved to:

- commit to no further spread of zebra mussels beyond the currently infested lakes;
- fund the first watercraft decontamination station in the county;
- appoint a county zebra mussel "czar";
- expand the education strategy/ increased signage "Don't Move a Mussel!"
- work cooperatively with the DNR to implement strategies to address AIS
- and to continue to seek research technologies for eradication

While efforts at control and containment are noble, they pale in light of the size of the problem upon us. Eradication is the answer. But how? After a year's time of looking we believe there is a solution near ready for the marketplace – you and I have touched on this potential solution called Zequanox™. We are prepared to continue in a piloting role in Minnesota, within the traditional regulations and parameters of the DNR, and arrange for testing the efficacy of this all-important possible solution. Sampling (jar assays, biobox trials, and mesocosms) intended to take place this summer – beginning this month -- June.

Per your recommendation noted during our phone conversation this morning, we submit this research proposal prepared for Douglas County by Marrone Bio Innovations (Zebra Mussel Collection & Zequanox™ Efficacy Evaluation in Douglas County) for the timely review by your senior staff.

- What role would you have the DNR play?
- What permitting must the early sampling efforts (beginning in late June) require?

Let us work together and look for the day we can celebrate that together, we made a positive difference for Minnesota and our nation. With every good wish, from my fellow Douglas County citizens' committee members, Carl Towley and Bonnie Huettl, I am

Very truly yours,

Lois Sinn Lindquist, Chair, Douglas County Citizens' Committee
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Alexandria, MN 56308
651-283-5647

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RESEARCH PROPOSAL

Evaluation of Zequanox™ as a Zebra Mussel Control Agent

Submitted by:
MARRONE BIO INNOVATIONS

Financial sponsorship sought from:
DOUGLAS COUNTY LAKE ASSOCIATION

Permitting and other regulatory approvals sought from:
MINNESOTA DEPARTMENT OF NATURAL RESOURCES

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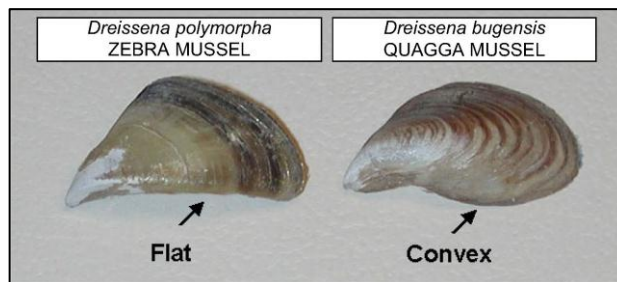
SUMMARY

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™ 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.

Introduction

Marrone Bio Innovations (MBI), a Davis California company specializing in development of natural biopesticides, is in the process of demonstrating efficacy and commercial viability of an invasive zebra and quagga mussel control product, Zequanox™. At the request of the Douglas County Lake Association (DCLA), MBI is interested in conducting zebra mussel collection and small-scale product efficacy evaluations in 3 lakes within Douglas County (Lakes Carlos, Darling, and Le Homme Dieu are currently under consideration). The objective of this research proposal is to describe MBI's collection and research interests in Open Water Treatment and Application in these three lakes in order to establish necessary cooperative research agreements with interested parties.

Zequanox™ is an effective, safe and environmentally friendly product for control of invasive zebra and quagga mussels (dreissenid species). The microbial active ingredient, *Pseudomonas fluorescens* in Zequanox™ (USEPA registration # 84059-U), can be found worldwide and is present in all North



American bodies of water and commonly found in food. Only the specific environmental isolate used in Zequanox™ (*Pseudomonas fluorescens* strain CL145A), however, is active against dreissenid mussels. In nature, *Pseudomonas fluorescens* is a harmless, plant growth promoting bacterial species that is known to live in association with plant roots, protecting them from disease. Zequanox™ will significantly reduce the use of polluting chemicals in industrial facilities and will be the first environmentally safe method considered for invasive mussel control in open waters.

MBI ON-SITE EVALUATIONS & STUDIES

Industrial Trials

MBI is currently conducting field studies with Zequanox™ under a Cooperative Research & Development Agreement at the Bureau of Reclamation's Davis Dam on the lower Colorado River, where cooling water systems and water supply lines are heavily infested with quagga mussels. Based on current studies, MBI will conduct a full scale demonstration and apply in the summer of 2011.

In a parallel path, MBI is conducting field studies with Zequanox™ at the Ontario Power Generation DeCew facility in St. Catherines, Canada. Based on current studies, MBI will conduct a full scale demonstration and apply in the summer of 2011 (after the Davis Dam Demonstration).

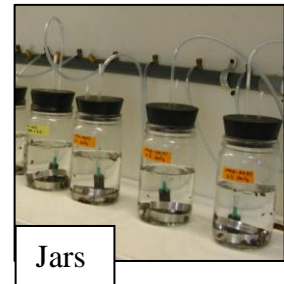
Open Water Trials

MBI has also begun research at the San Justo Reservoir in California. The zebra mussel infestation at San Justo Reservoir is an ideal location for developing a better understanding of the efficacy of Zequanox™ on planktonic larval and attached (juvenile and adult) life stages of zebra mussels. This open water type of research is the next level in understanding the mussel's life development and the affects of Zequanox™. Research in the State of Minnesota would also develop efficacy evaluations of Zequanox for open water applications. This research would give the state a leadership position and help all agencies and interested parties.

SCHEDULE OVERVIEW

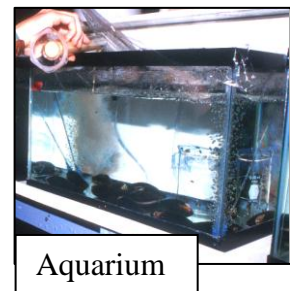
June 2011:

- Field site visit to Lakes Carlos, Darling, and Le Homme Dieu.
- Choose a location and establish indoor laboratory for jar and biobox testing.
- 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.
- 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) to develop a protocol that will likely maximize zebra mussels kill in the next phase of testing – the bioboxes.



July 2011:

- Continue jar testing as needed.
- 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 August.



August through October 2011:

- Continue jar and biobox testing as needed.
- 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., cylinders (see photo below) 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.



December 2011

- Completion of all data analysis and project report issued

January through May 2012

- Prepare application for Legacy funding to expand the project to larger size lake applications in 2012 and 2013.

INTELLECTUAL PROPERTY

MBI will retain sole ownership of all discoveries, improvements, inventions, patents, trademarks, copyrights, know-how, trade secrets, or other intellectual property rights associated in any way with the Products. The parties specifically agree that all improvements, inventions, discoveries and copyright in works of authorship, including those in formative stages, made by either party hereto (either alone or jointly with others) improving upon or related to the Products shall from the time of conception or, in the case of works of authorship, from the time of creation, be the property of MBI.

All devices, equipment, designs (including drawings, plans and specifications), estimates, prices, notes, electronic data and other documents or information prepared or disclosed by MBI in connection with the Products provided hereunder shall remain MBI's exclusive property. Participants shall not disclose any such material to third parties without MBI's prior written consent.

Cooperating parties to date:

New York State Museum, Cambridge Research Institute

Marrone Bio Innovations

Molloy and Associates

Board of Commissioners, Douglas County

Douglas County Lakes Association

Douglas County Citizens' Committee

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