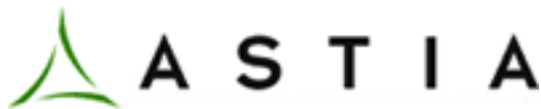


Evaluation of a Promising, Environmentally Safe, Invasive Mussel Control Solution for Freshwater Habitat Restoration



Marrone Bio Innovations

We discover, develop, and market effective and environmentally responsible natural products (biopesticides) that fill unmet needs for weed, pest and plant disease management



2008 Cleantech Innovator Award



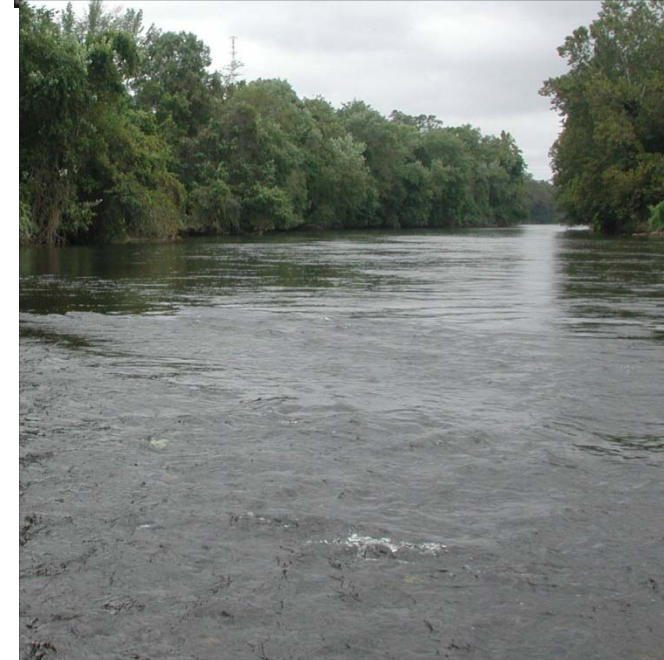
Natural Product Chemistry

- ❑ Safer alternatives to standard chemicals to protect public health, applicators and the environment
- ❑ Highly specific to target organisms
- ❑ Well established technology in the pharmaceutical and agriculture industry
 - Isolation and product development
- ❑ Biological control history
 - *Bacillus thuringiensis (israelensis)* used to control black fly larva in open waters throughout North America

ZEQUANOX™



- ❑ Product is comprised of *Pseudomonas fluorescens* CL 145A
 - Strain discovered and patented by Dan Molloy, New York State Museum
 - MBI is the license holder for the commercial product
- ❑ Product is a dead microorganism
- ❑ *Pseudomonas fluorescens* is ubiquitous in the environment
 - One of the most common bacterial species in water and soil, found throughout the world
 - **Plant growth promoting bacterium** in the rhizosphere - protect the roots of certain crops



Operator and Environmental Safety

- ❑ Completed required USEPA environmental toxicity and human safety testing for pesticide registration
- ❑ Low human safety risk for manufacturing and application
- ❑ Highly selective efficacy against zebra and quagga mussels only
 - Of those tested, it does not kill native North American bivalve (clams/mussels) species or the Asian clam



Ecotoxicology



Zooplankton
Daphnia magna



Freshwater shrimp
Hyalella azteca



Fathead minnow
Pimephales promelas



Sunfish
Lepomis macrochirus



Trout
Salmo trutta



Freshwater ciliate
Colpidium colpoda

7 Bivalve
species



Mytilus edulis, *Pyganodon grandis*,
Lasmigonacompressa, *Strophirus undalatus*,
Lampsilis radiata, *Pyganodon cataracta*, *Elliptio complanata*

Additional Non-Target Toxicology Work

- ❑ 30-day exposure
 - Assess long term impact of Zequanox on fish populations
 - Refreshed water and product every 48 hours at 1, 6, and 36 mg/L
 - Evaluating impact of both live and dead bacteria
 - Measuring growth and fish health
 - 1 fish died in 30 days exposed to the 36 mg/L of product
- ❑ 96 hr LC50 trials
 - Based on initial pilot data some fish may require doses >1000 mg/L to achieve mortality
 - Mortality may be a cause of water quality rather than product (static 8 L tanks)
- ❑ Will conduct additional trials with fish avoidance behavior

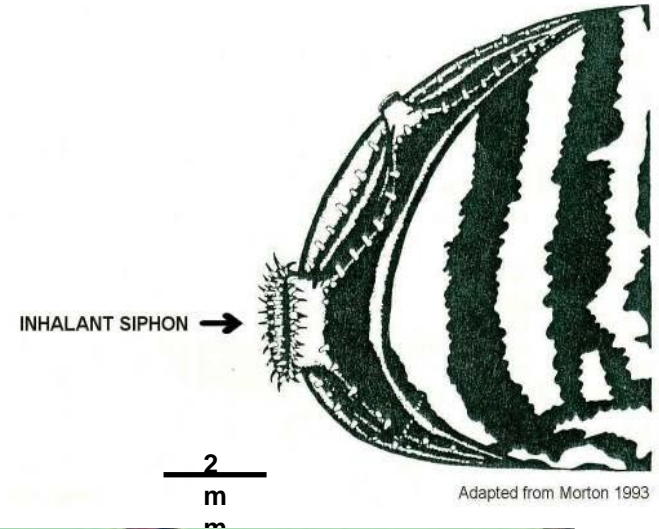
Demonstration Trials

- ❑ **Overall objective:** Test and demonstrate product prototypes under realistic treatment conditions
 - Currently conducting extensive trials in industrial (hydropower) facilities (U S Bureau of Reclamation and Ontario Power Generation, Canada)
 - Biobox and cooling water systems
 - **Next step: Open Water Demonstration trials**
- ❑ Effective for adult mussel control (> 90 % mortality) and settlement prevention
 - Adult treatments require 6 -8 hours of exposure at 50 – 100 ppm
 - Low pulsed doses (5 ppm 1 hr per week) effective for settlement prevention

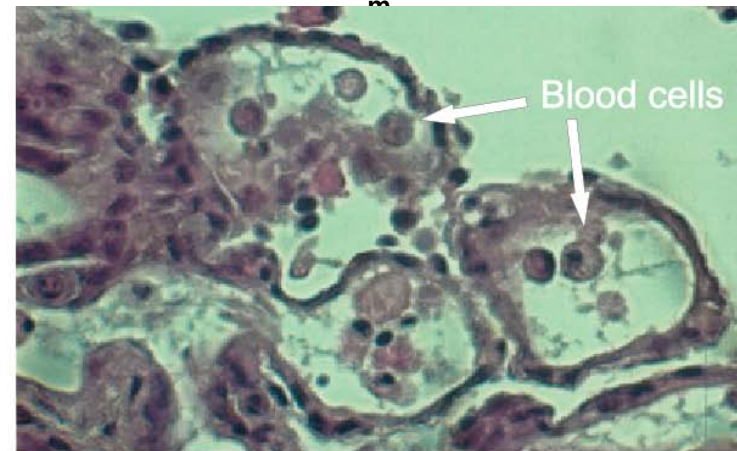


Mode of Action for Adult Mussels

- *Dreissena* spp. (zebra and quagga) mussels die following ingestion
- Mussels do not close shells in reaction to presence of Zequanox™
- Can visually see “bloating” upon death



In healthy mussels, epithelial cells (arrows) appear as a thick layer lining the tubules of the digestive gland.



Following treatment, epithelial cells are destroyed. Blood cells are abundant as the digestive gland hemorrhages.

Timeline and Regulatory Status

- ❑ Complete USEPA registration
 - PRIA date was May 2010, however EPA requested an extension
- ❑ Section 18 Emergency Use Permit
 - Initial one submitted by U.S. Bureau of Reclamation for areas along the Colorado Approval
 - Approval is currently pending
 - **MBI would be supportive of other states submitting Section 18 requests**
- ❑ Objective remains to conduct open water demonstration trials Summer and Fall 2010



SePRO-MBI Partnership for Open Water Development

- ❑ SePRO...leading US company for management of aquatic invasive plants
 - Hydrilla, Eurasian milfoil, giant salvinia, egeria, curly-leaf PW
 - Past aquatic biopesticide research experience (*Mt*)

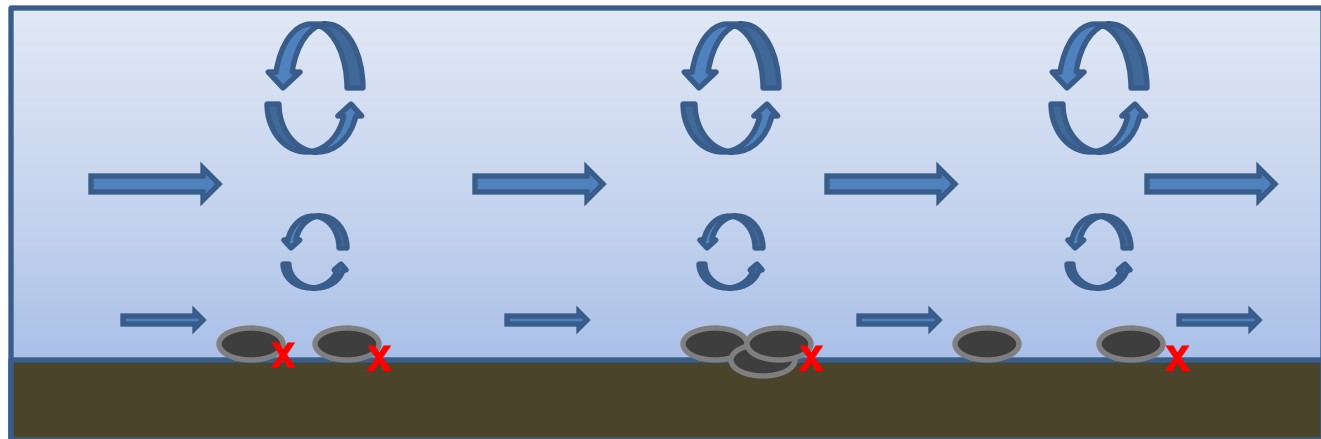


SePRO-MBI Partnership for Open Water Development

- ❑ Fall 2009 – Collaborative Development
 - Open water of lakes and reservoirs and other natural aquatic habitats.
 - Use Pattern(s) of Novel Treatment Solution
 - Formulation
 - Delivery System Concepts
 - Ecological Compatibility
 - Integration into Management Programs
 - Commercial Fit
- ❑ Tremendous interest from natural resource managers throughout the Great Lakes region

Considerations for Use of Zequanox for Aquatic Habitat Restoration

- ❑ **Flowing System (industrial use or natural habitat)**
 - Constant Re-Suspension (both)
 - Injection or repeated application needed for exposure (both)
 - Some settling in more quiescent, 'backwater' areas (natural)
 - Microzones of reduced flow (natural)

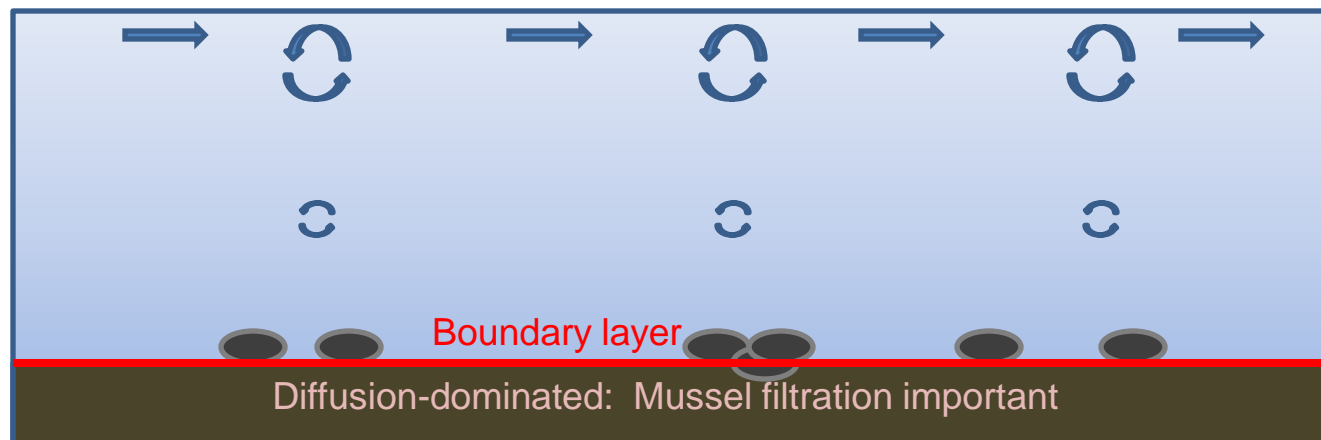


Flowing water conditions

Considerations for Use of Zequanox for Aquatic Habitat Restoration

❑ Static System (quiescent lake / reservoir)

- Potential for product settling to bottom
- Exposure scenarios for various mussel populations
 - Position in water column
- Will settling work for or against management effort?
 - No longer available or maximizes exposure?



Static or quiescent conditions

Primary Development Areas

1. Determine optimal methods for effective delivery of Zequanox to target mussels growing primarily on bottom substrate of lake, reservoir, stream habitat.
 - Treat bottom water versus entire water column
 - Dose
 - Exposure period requirements
2. Confirm treatment selectivity to native fauna with new use patterns of Zequanox.

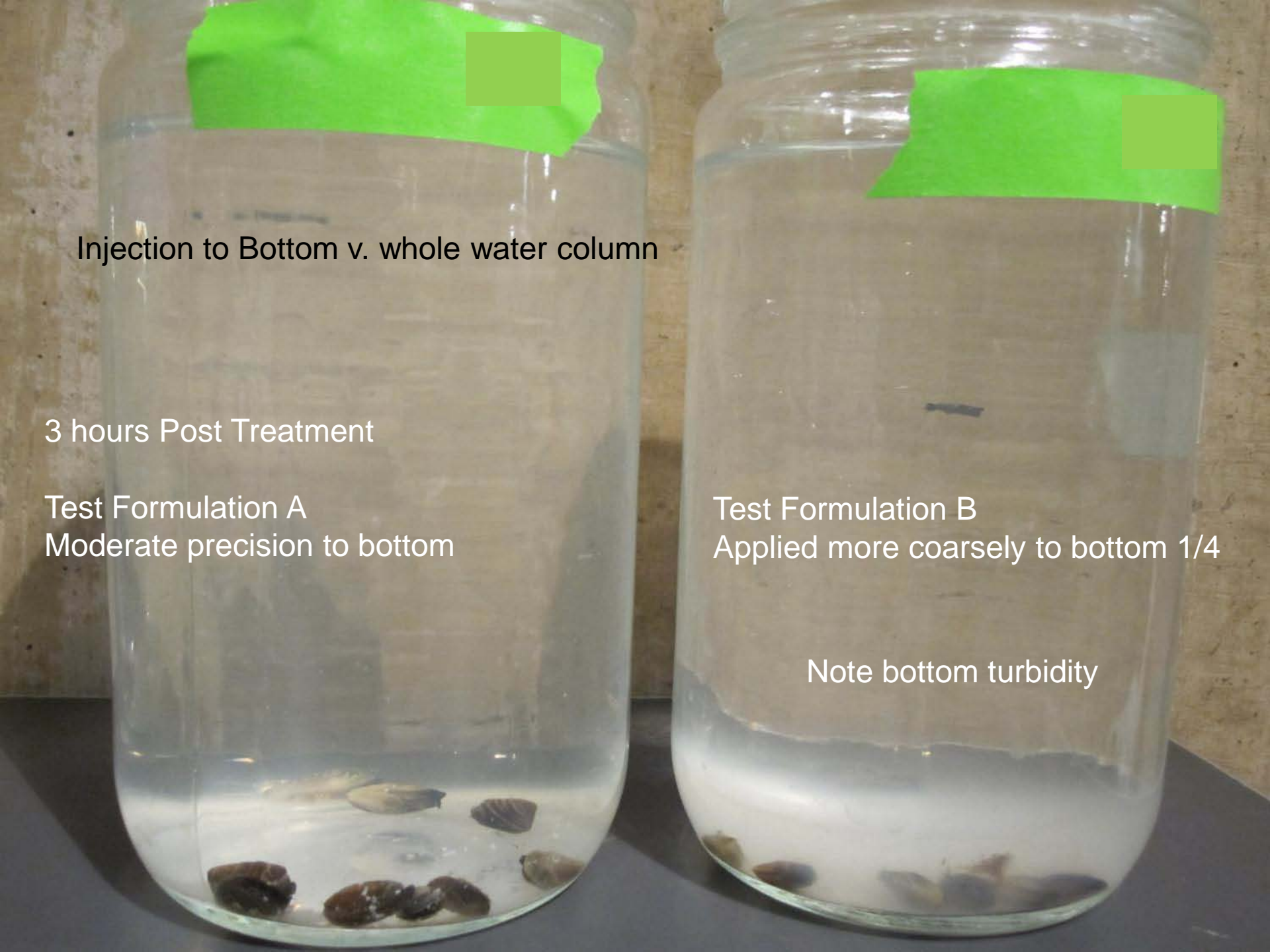


From USGS GLSC Fact Sheet 2000-6

Evaluation Plan

- ❑ Small-scale lab and mesocosm studies
 - Method development with MBI
 - SePRO - SRTC (NC)
 - USGS (WI) (*pending*)
 - Others
 - Basic CET simulations for invasive mussel control performance and native selectivity





Injection to Bottom v. whole water column

3 hours Post Treatment

Test Formulation A
Moderate precision to bottom

Test Formulation B
Applied more coarsely to bottom 1/4

Note bottom turbidity

- 
- A vertical blue decorative bar with a wavy, water-like texture is located on the left side of the slide.
- Video of mussel feeding on Zequanox
 - 1 minute and 30 seconds approximately

Evaluation Plan

□ Initial Field Development

- Scale dependent on regulatory status but no more than several acres total anticipated for 2010 season
 - Emergency use conditions?
- Confirm smaller scale use pattern
- Inform scale-up of field development in 2011



Potential Use Patterns

□ Control of Adults

- Higher use rates / longer exposures can control adult populations
- Goal: Selective Control of Invasive Dreissenids and allow Native Flora/Fauna Re-Establishment
- Characterize:
 - Speed and consistency of control
 - Fate of dead mussels (water quality)
 - Economics



Potential Use Patterns

❑ Veliger Settlement / Establishment Prevention

- Lower use rates / exposures appear to prevent settlement
- Goal: Keep valuable habitat free of mussels or greatly reduce rate of infestation
- Characterize:
 - Partial v. full prevention of establishment
 - Integration with control of adults
 - Economics and Performance for Longer-Term Maintenance Control



Potential Great Lakes Habitat Restoration

❑ Off-Shore Reef Habitats

- Important fish spawning areas impacted by zebra mussel infestation
 - Lake trout and yellow perch
 - Natural (USGS example) and artificial reef zones
- Other biotic/abiotic interactions on reefs

❑ Critical shoreline / harbor habitat

❑ Inlet streams

❑ Others?

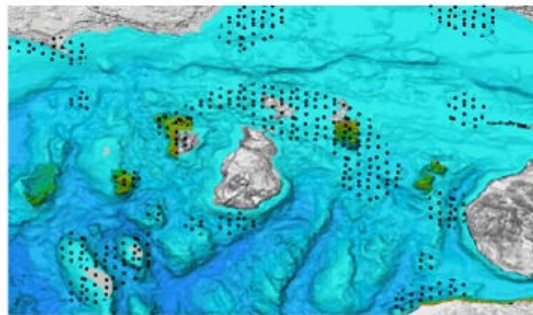
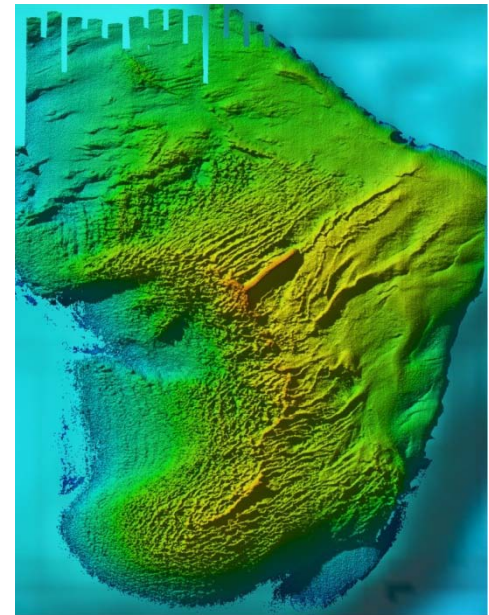


Figure 2. - Location map and spawning and nursery areas for lake trout and yellow perch (modified after Goodyear et al., 1982).



Shaded Relief Map
Gull Island Reef,
N. Lake Michigan

Barnes et al. 2003

USGS Open File Report 03-120

Developmental Collaboration

- ❑ Zequanox is a brand new technology for effective, selective mussel control and habitat restoration.
- ❑ Regulatory support needed for field evaluations.
- ❑ Great Lakes Restoration Initiative Grant Program
 - 2011 cycle on horizon
 - Will support university and government agencies in proposal development and implementation
- ❑ Please Contact Us!

Acknowledgements



NEW YORK State
Museum

- ▣ NYSM discovered *Pseudomonas fluorescens* CL145A (Dan Molloy).
Marrone Bio Innovations in



National Science Foundation
WHERE DISCOVERIES BEGIN



ONTARIO **POWER**
GENERATION

Questions?

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