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# Developments in Bio-based Management of Juvenile and Larval Stage Zebra and Quagga Mussels in Industry and Natural Resource Management


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# Forward Looking Statement

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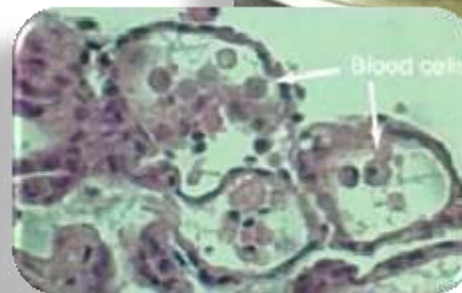
- About Zequanox
  - Juvenile Mussel Control
  - Veliger Testing
  - Other Updates & Next Steps
- 

# Zequanox: The Industry's Only Biopesticide for Invasive Mussel Control

**ZEQUANOX**<sup>®</sup>  
Invasive Mussel Control

- Environmentally friendly
  - Derived from soil microbe (*Pseudomonas fluorescens*) discovered by NYSM
  - Composed of 100% dead cells
  - Biodegradable
- Controls mussels in all life stages
  - Perceived as food source—destroys the mussel's digestive system
  - Occurs over time (days to weeks after treatment)
- Highly selective toward zebra/quagga mussels
- Effective in a broad range of water conditions
- Noncorrosive to infrastructure, equipment

**NYS**  
**M** NEW YORK State Museum



# Extensive Ecotox Studies Show No Impact to Other Aquatic Species



## FISH

- Bluegill sunfish (*Lepomis macrochirus*)
- Channel catfish (*Ictalurus punctatus*)
- Chinook Salmon (*Oncorhynchus tshawytscha*)
- Coaster brook trout (*Salvelinus fontinalis*)
- Common Carp (*Cyprinus carpio*)
- Fathead Minnow (*Pimephales promelas*) \*
- Klamath Suckers (*Catostomus sucker spp*)
- Lake sturgeon (*Acipenser fulvescens*)
- Largemouth bass (*Micropterus salmoides*)
- Rainbow Trout (*Oncorhynchus mykiss*) \*
- Sacramento Splittail (*Pogonichthys macrolepidotus*)
- Smallmouth bass (*Micropterus dolomieu*)
- Striped Bass (*Morone saxatilis*)
- Walleye (*Sander vitreus*)
- Yellow perch (*Perca flavescens*)



## OTHERS

- Mallard Duck \*
- Midge (*Chironomidae*)
- Mayfly (*Baetis*)
- Amphipod (*Hyalella azteca*) \*
- European Freshwater Crayfish (*Austropotatamobius pallipes*)
- Freshwater Crustacean (*Asellus aquaticus*)
- Freshwater Water Flea (*Daphnia magna*) \*



## MOLLUSCS

- Blue Mussel (*Mytilus edulis*) \*
- Freshwater Mussel - Duck Mussel (*Anadonta*)
- Freshwater Mussel - Black Sandshell (*Ligumia recta*)
- Freshwater Mussel - Fatmucket (*Lampsilis siliquoidea*)
- Freshwater Mussel - Pink mucket (*Lampsilis abrupta*)
- Freshwater Mussel - Hickorynut (*Obovaria olivaria*)
- Freshwater Mussel - Higgins Eye (*Lampsilis higginsii*)
- Freshwater Mussel - Mucket (*Actinonaias ligamentina*)
- Freshwater Mussel - Paper Pond Shell (*Utterbackia imbecillis*)
- Freshwater Mussel - Plain Pocketbook (*Lampsilis cardium*)
- Freshwater Mussel - Washboard (*Megaloniaias nervosa*)
- Freshwater Snail (*Lymnaea peregra*)



## PLANTS AND ALGAE

- Algae \*
- Bindweed (*Convolvulaceae*)
- Common Water Plantain (*Alisma subcordatum*)
- Curly Dock (*Rumex crispus*)
- Mallow (*Malvaceae*)
- Nightshade (*Solanaceae*)
- Smallflower Umbrella Sedge (*Cyperus difformis*)



\* EPA required



## Juvenile Mussel Control in Industrial Settings

# Why Juvenile Control?

- Less product annually, less overall cost
- Lower concentration and shorter treatment times needed
- Manages extent of debris release
  - Reduces maintenance and staffing after treatments
- Controls pipe restriction year-round
  - Improves flow reliability
  - Reduces unexpected shut-downs



# Developing a Juvenile Control Program for Zequanox (Lab Testing)



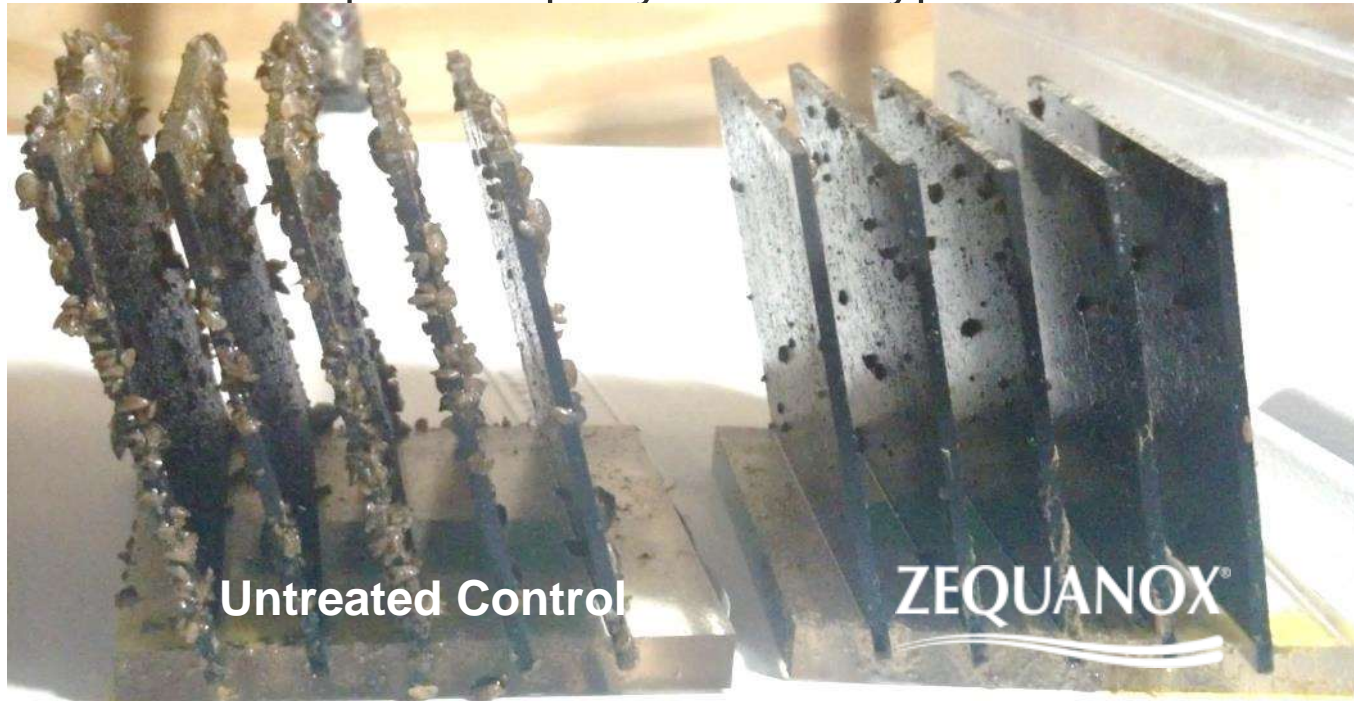
- Created test matrix of exposure time vs. concentrations vs. frequency to test
  - Examples:
    - monthly, 50 mg a.i./L, 4 hours
    - Bi-weekly 20 mg a.i./L, 2 hours
- Paired control/treated settlement plates
  - Shared space in same biobox and were controlled against each other
- Analyzed settlement via microscopy
  - Photographed subsections to allow for non-destructive analysis





# Results

- Best treatment regime (level of control vs. product required for 10 treatments) was 10 mg a.i./L for 2 hours, every other week
  - Only need to conduct treatments during mussel settlement season
  - Requires 60% less product per year than typical annual treatment

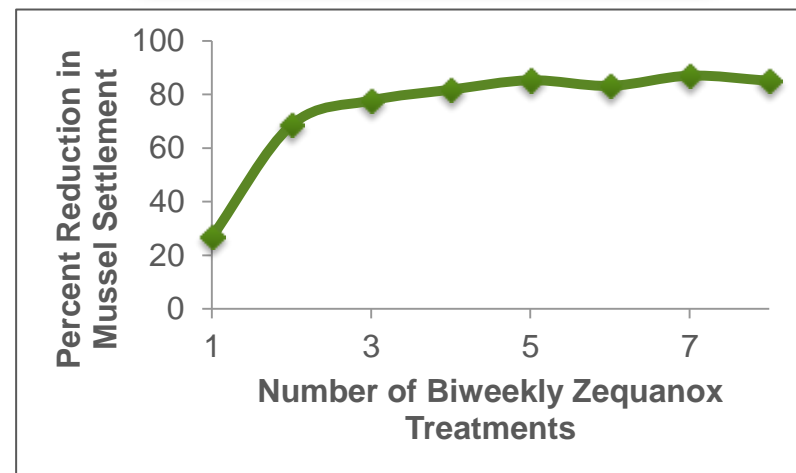


**87% Juvenile Settlement Control**

# Case Study: Settlement Prevention at Hoover Dam

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- **Hoover Dam**
  - Boulder City, NV (Colorado River)
  - 2,080 MW hydropower facility
  - 1,110 gpm cooling water system
- **Bi-weekly Treatment Program**
  - Minimal impact on operations
  - Minimal space
  - No system alterations
  - Minimal debris management
- **Results:**  
**85% Reduction in Settlement**



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## Veliger Control for Natural Resource Management

# Why Veliger Control?

- Veligers can be spread to non-infested waterways via fish stocking, native mussel relocation, baitfish harvest, aquaculture, etc
  - Commercial operations are often limited in infested waters because of the risk of transporting of mussel larvae
- Provides a method of managing risk of veliger transport without harming fish/mussels



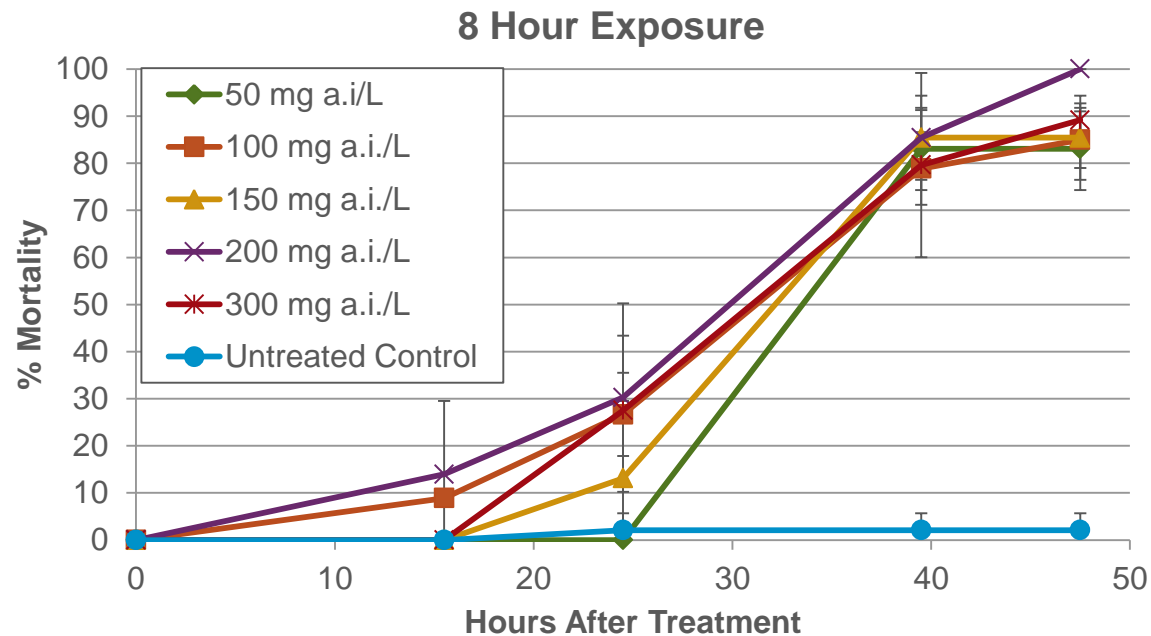
# Veliger Test Methods

- Veligers collected via plankton tow
  - San Justo Reservoir, CA
- Fine mesh filters to remove debris and isolate veligers
- Veligers put into 6-well plate
- Apply Zequanox solution
- Move veligers to clean water after assigned treatment
- Monitor mortality



# Early Results (2013)

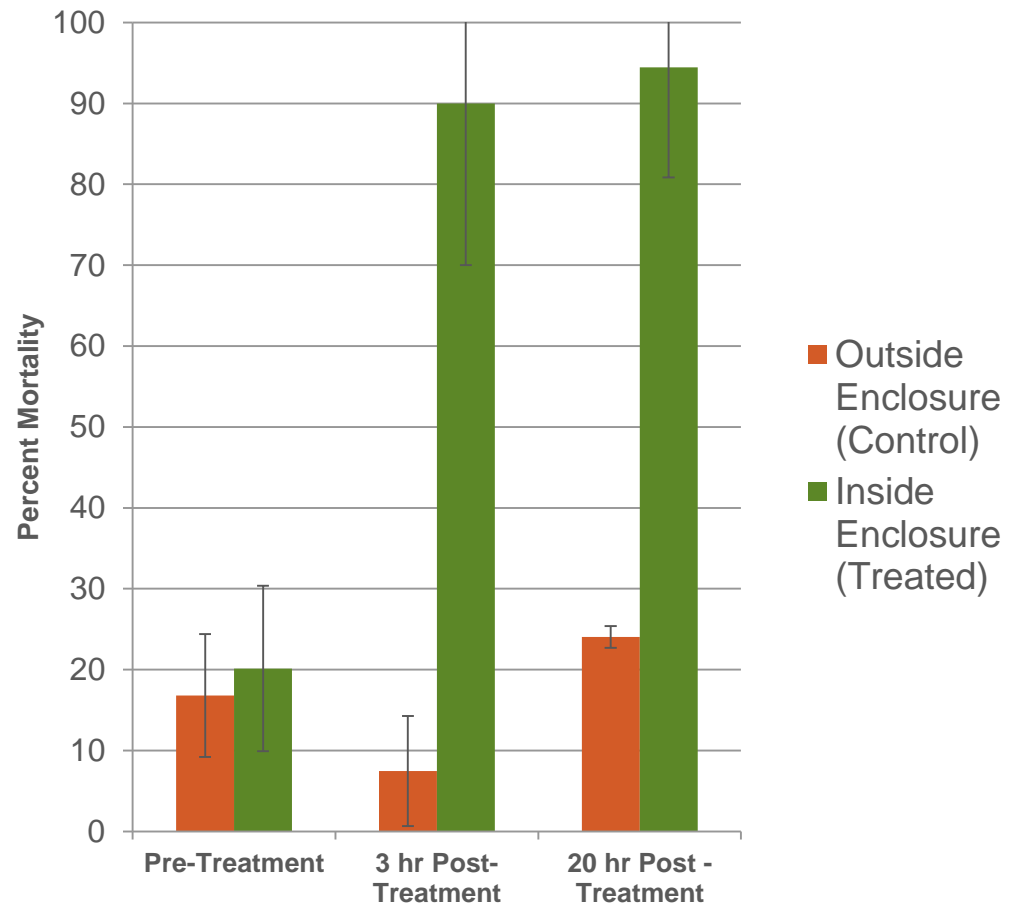
- Monitored mortality for 48 hours
  - Good results
  - Low control mortality
  - No repeatable 100% mortality treatment identified



# Deep Quarry Lake Field Trial - Veligers



- 2013 monitoring of in-lake treatment in Illinois
- Monitoring to 20-hours post-treatment
- High veliger mortality



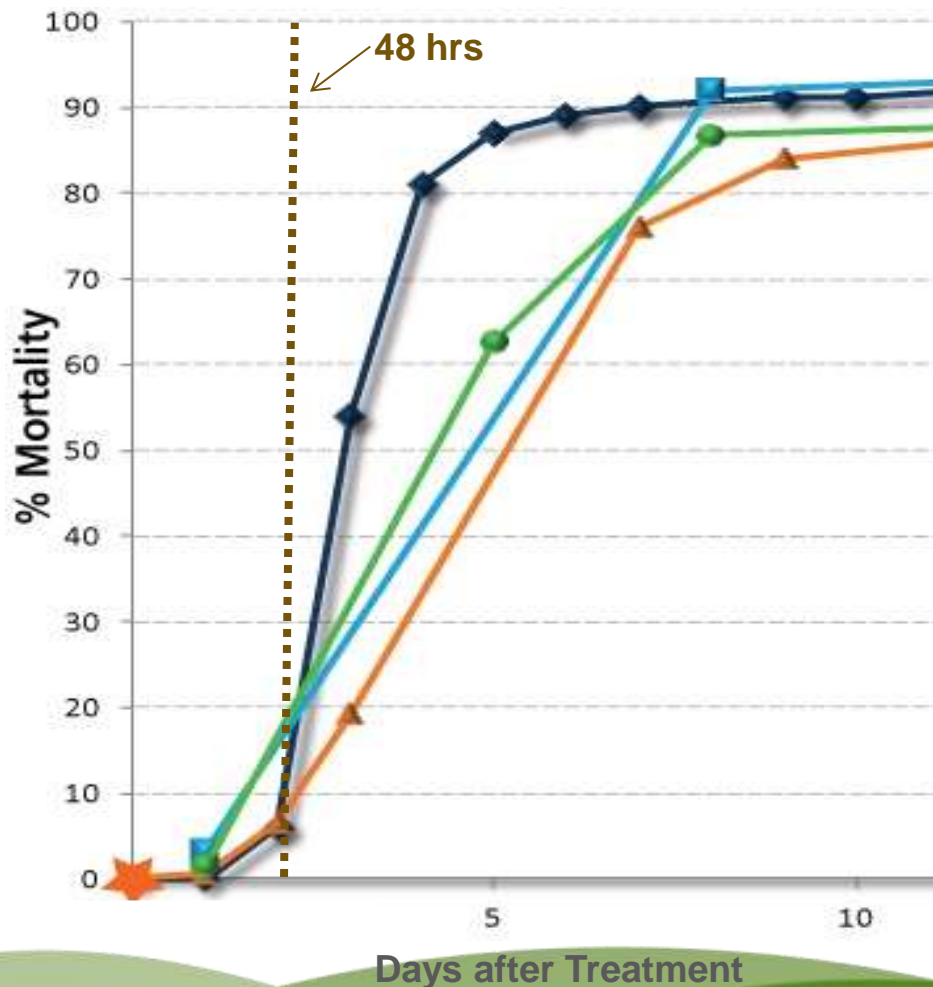
# Re-evaluating Mortality Monitoring Methods



- Adult mortality occurs over a period of up to 30 days
  - Perhaps at 48 hours all veligers aren't dead YET?

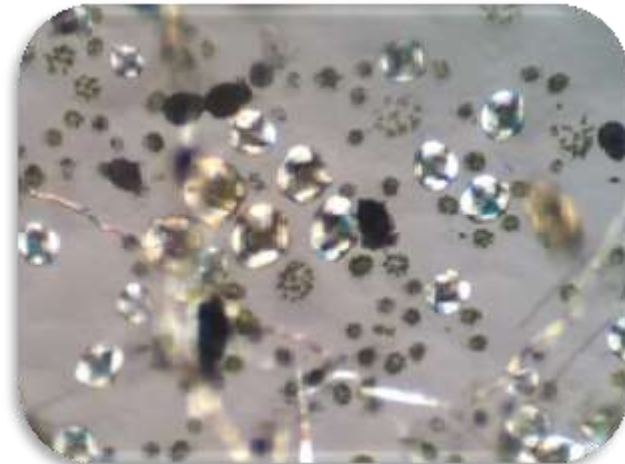
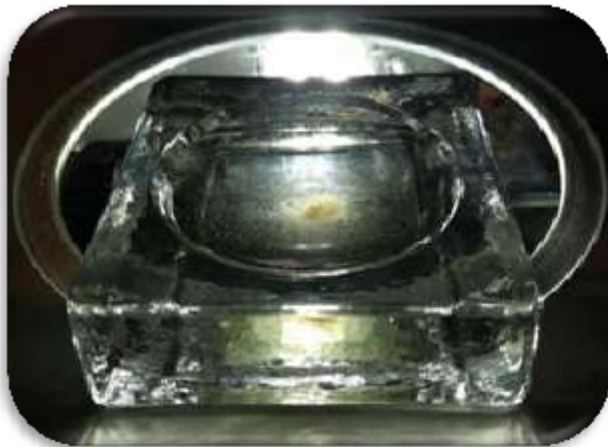


Example Adult Mussel Case Studies

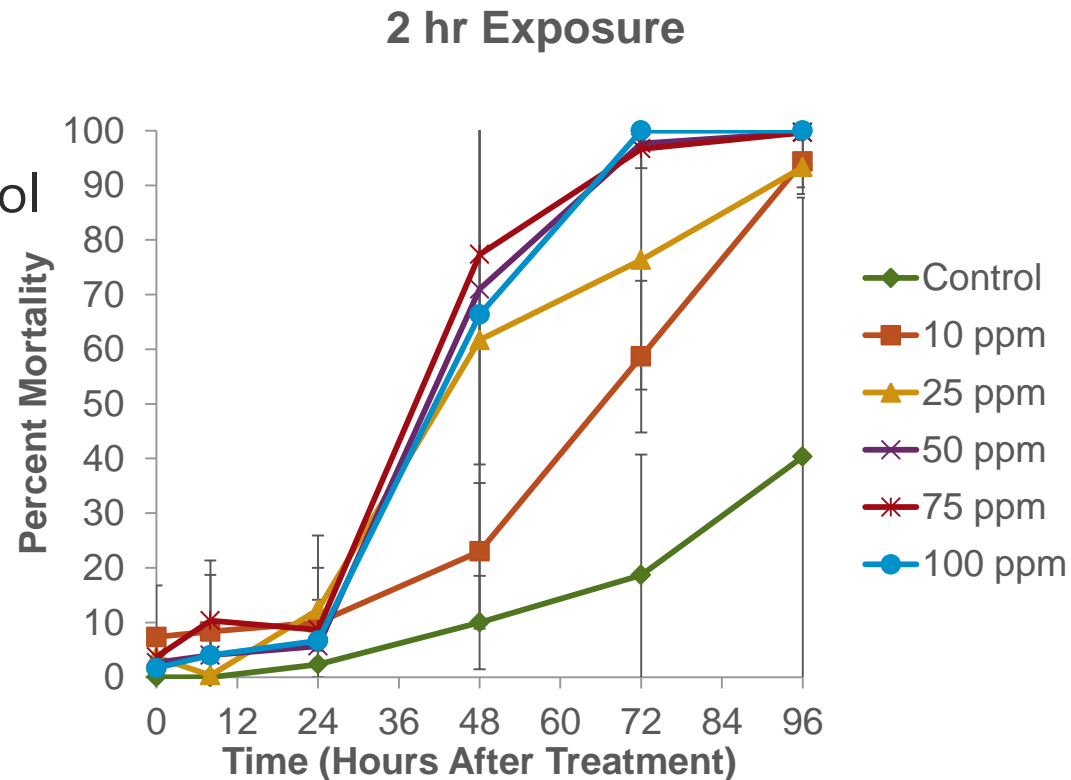




- Extended monitoring period to 96 hours
  - Will un-treated veligers survive in 6-well plates that long?
- Initial screen
  - 2 – 48 hour exposures
  - 10 – 100 mg a.i./L concentrations
- Added scale-up after initial screen of selected combinations



- Promising Results
  - Saw more consistent candidates for 100% control
- Untreated control mortality
  - Grows after 48 hours
  - Scaled-up most promising treatments to offer more stable environment



# Scale-Up of Select Treatments

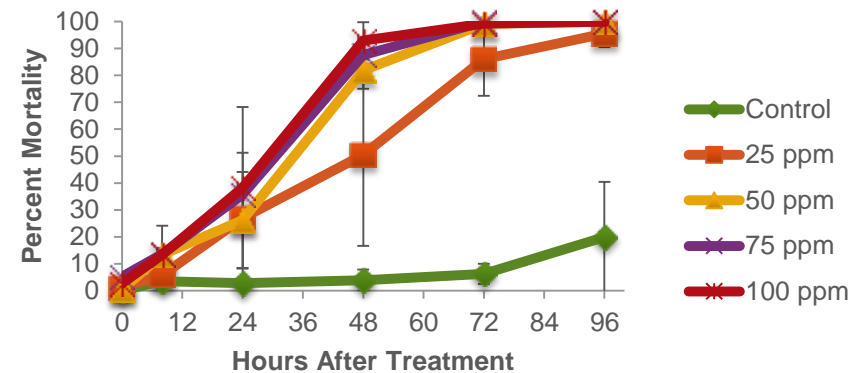
- Treated beakers
  - Larger water volumes (150 mL compared to 10 mL)
  - Better able to maintain water quality
  - More veligers in each replicate
- After treatment, passed through fine mesh to collect veligers and place in clean recovery water



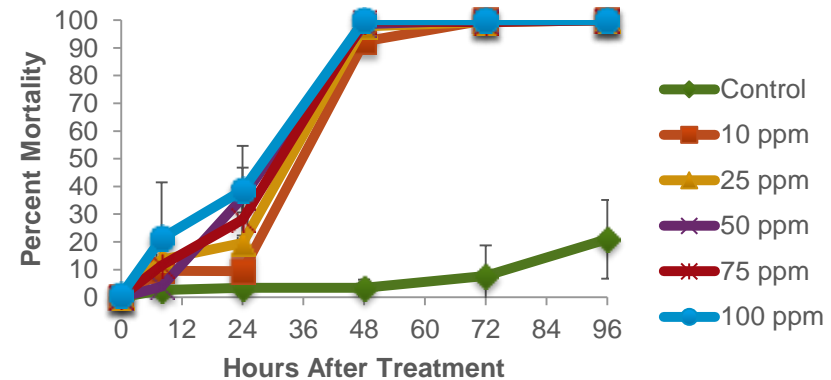
# Jar Test Results

- More consistent results
  - Smaller standard deviations
- Generally reduced control mortality during monitoring
  - Below 20 % at 72 hrs
- Candidate treatments that yield 100% veliger mortality
  - 2 hour treatment at 50+ mg a.i./L
  - 8 hour treatment at 10+ mg a.i./L

## 2 hr Exposure



## 8 hr



# Other Updates

- Continued facility treatments
  - Bi-weekly (juvenile) program currently an available option for treatments
- Continued cost improvement
  - 50% reduction in 2015 with improved manufacturing efficiency
  - Manufacturing in-house at Marrone Michigan Manufacturing
- Open water label granted in United States June 2014
  - Working with lakes to evaluate most ecologically and economically sound treatment regimes
- Canadian PMRA label expansion in progress



# Next Steps

- MBI open to partnerships for field studies for veliger uses field validation
- Distributor and applicator partnerships
- Further research
  - Control necessary to achieve natural resource management goals
  - Potential HABs management tool
  - Native mussel rehabilitation goals
  - Collaboration with regulatory agencies on acceptable veliger protocols





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