Effectiveness of an algaecide for killing invasive quagga mussels and preventing their colonization

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Outline

- Introduction
- EarthTec® on killing quagga mussels
- EarthTec® in preventing colonization
- Discussion
- Conclusion

Chemical Control

- **Oxidizing**
  - Chlorine, chlorine dioxide, chloramines, bromine, and potassium permanganate
- **Non-oxidizing**
  - Quaternary and polyquaternary ammonium compounds, potassium, and copper

Oxidizing Chemical Control

- **Chlorination** (Jenner and Janssen-Mommen, 1993)
  - Most popular and least expensive
  - Kills adult mussels and prevents colonization of veligers
  - Damages membranes by diffusing through the cell wall and disrupts enzyme activities
  - Mussels react by closing their valves, stop filter feeding, forced to survive off stored food reserves and anaerobic respiration
  - THMs: Linked to adverse health effects and may even be carcinogenic to animals (Cotruvo & Regelski, 1989)

Non-chlorine Oxidizing Chemicals

- **Bromine** (Fellers, Flock, & Conley, 1988)
  - Destroys vital tissues when water pH>8.0
  - Rapid effects in veligers versus adult mussels
  - Not very effective in preventing colonization of mussels
- **Potassium permanganate** (San Giacomo & Wymer, 1997)
  - Oxidizer used in municipal water treatment facilities for purification
  - Best used during the veliger settlement phase
  - Disadvantage: it can produce a pink or yellow color in treated water

Non-oxidizing Molluscicides

- Developed for bacterial disinfection and algae control
- Generally more potent, more easily and safely handled and more easily applied to raw water systems than chlorine (Claudi & Mackie, 1994)
- Higher cost compared to chlorination strategies
- Used in closed loop systems because of environmental concern

Polyquaternary Ammonium Molluscicide

- Polyquats induce mortality in mussels when given as a semi-continuous dose
- Binds to negatively charged surfaces on the mollusk membrane
- The mussel does not detect the polyquat; therefore, the organism does not close its valves and quickly dies (Sprecher & Getsinger, 2000)

Potassium

- Potassium ions interfere with membrane integrity and respiration
- Kills adult mussels by destroying the membrane integrity of the gill epithelium, making it impossible for the mussel to respire
- Non-toxic to larger organisms, but toxic to other bi-valves
  (Waller et al., 1993)

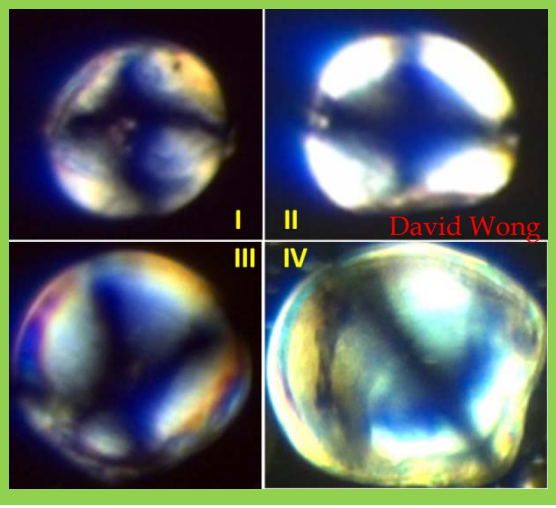
Copper

- Added to antifouling coatings for ships to prevent barnacle growth and as an antifouling agent on underwater pipes (Claudi & Mackie, 1994)
- Copper ions leach from the coatings and result in unacceptable copper concentrations in water systems
- Minimal effective copper concentration: 5-10 ppb for bluegreen microalgae (Horne and Goldman 1974; Demayo et al. 1982)
- US EPA's Drinking Water Regulation for copper is a maximum containment level of 1.3 ppm (US EPA, 1991)

EARTHTEC®

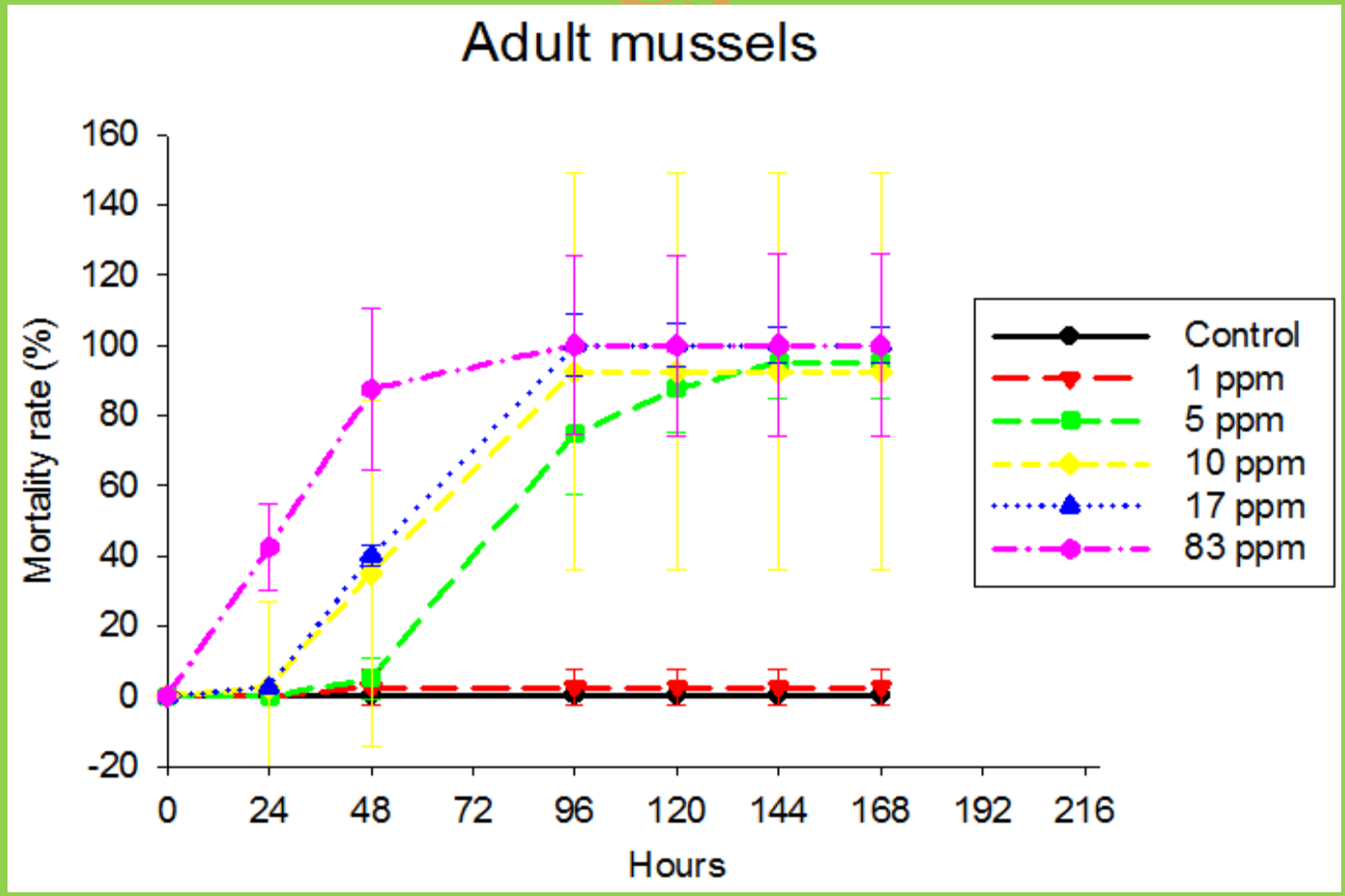
- EarthTec® is a low pH algicide/bactericide designed for use in lakes, ponds, reservoirs, and other water systems
- Active ingredient is a biologically active form of cupric ion (Cu^{2+})
- Cu^{2+} remains unattached to inorganic elements in waters, which allows copper to exert toxic effects to microorganisms (Earth Science Laboratories, 2010)
- Copper is no longer biologically active
  - Due to the proprietary copper "carrier" that holds Cu^{2+} in solution
  - Prevents the cupric ion from chelating with other organic molecules (Earth Science Laboratories, 2010)

EARTHTEC®



Lethal concentration to adults
Lethal concentration to juvenile
Lethal concentration to veligers
Concentration to prevent colonization

Cumulative mortality of adult *D. rostriformis bugensis* at different EarthTec® concentrations



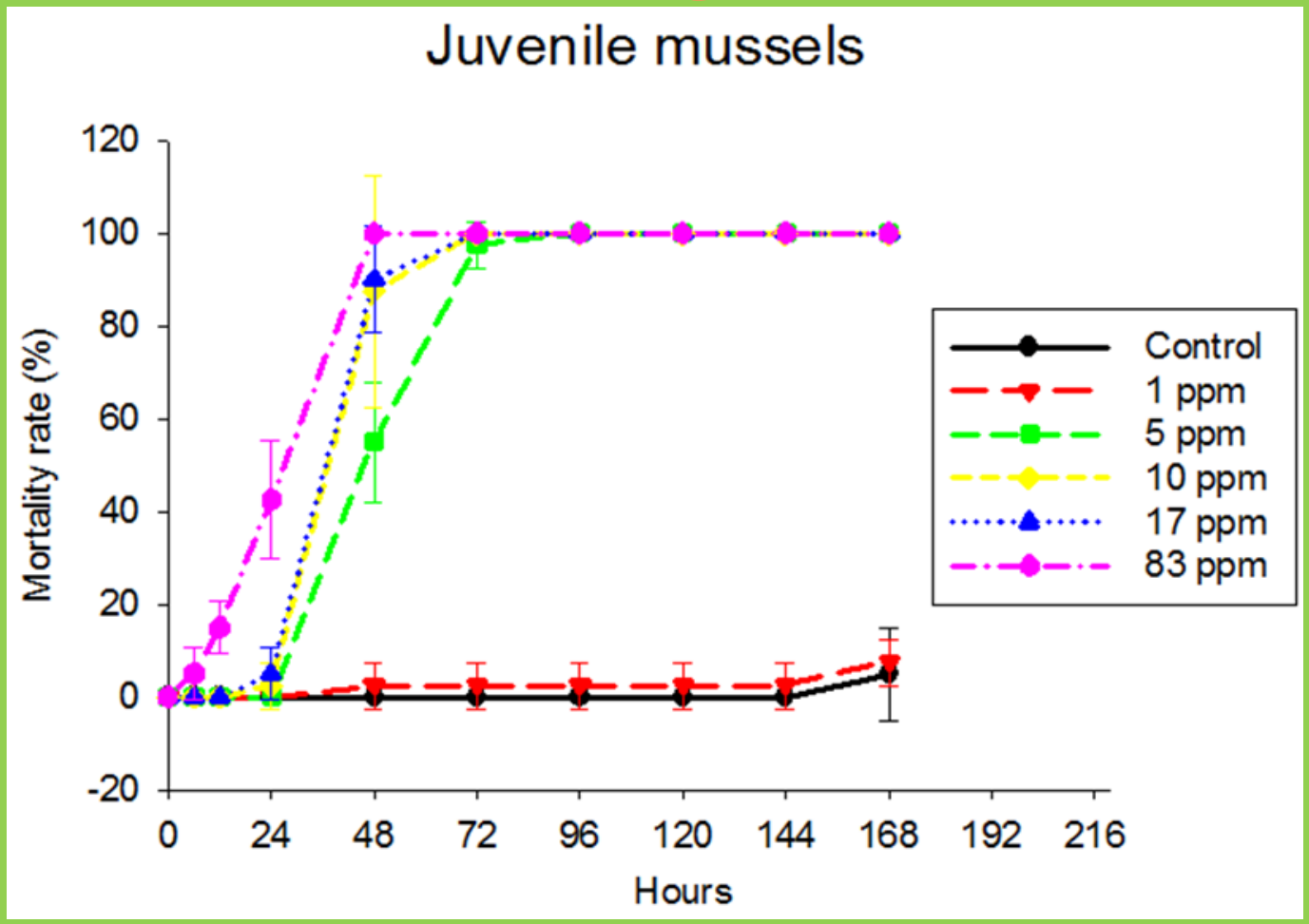
Adult Toxicity Test

- 30 h→ 50% of the mussels in the 83 ppm group of EarthTec® were dead
- 96 h→ >50% of the mussels in the 5 ppm and 10 ppm groups were dead
- 96h→ all mussels in the 17 and 83 ppm groups were dead
- 168 h→ 5% of the mussels in the 5 ppm group were alive and 7.5% in the 10 ppm group were alive

Results for Adults

- ANCOVA shows that the concentrations of EarthTec® affected survival of adult mussels with duration time as a significant covariant ($p < 0.0001$)
- Higher concentrations of EarthTec® resulted in lower numbers of mussel survival
  - the increased duration time led to higher numbers of mussel mortality
- Time to 100% mortality of quagga mussels decreased with increasing EarthTec® concentration for both adults and juveniles
- Higher concentrations of EarthTec® and the increased duration time both led to a higher mortality rate (ANCOVA, $p < 0.0001$).

Cumulative mortality of juvenile *D. rostriformis bugensis* at different EarthTec® concentrations



Juvenile Toxicity Test

- 6h→ 5% in the 83 ppm group were dead
- 12→ 5% more in the 83 ppm group were dead
- 24 h→ > 50% were remaining in the 83 ppm group
  - 5% were dead in the 17 ppm group
- 48 h→ all were dead in the 83 ppm group
  - 90% were dead in the 17 ppm group
  - 87% were dead in the 10 ppm group
  - almost 50% were dead in the 5 ppm group
- 72 h→ all mussels in the 17 and 10 ppm groups were dead, 98% in the 5 ppm group were dead, and 3% were dead in the 1 ppm group.
- 96 h→ all the mussels were dead except for the controls and the 1 ppm group.
- 168 h→ 5% of the control groups and 1 ppm groups were dead.

Veliger Toxicity Test

EarthTec®	Minutes (Mean	±	Standard deviation)	Replicates
0ppm	*	±	*	8
1ppm	*	±	*	6
3ppm	27.5	±	7.5	4
5ppm	20.3	±	8.1	3
10ppm	6.0	±	2.0	3
17ppm	6.0	±	1.0	3
83ppm	5.7	±	4.5	3

* Mortality rate was 0

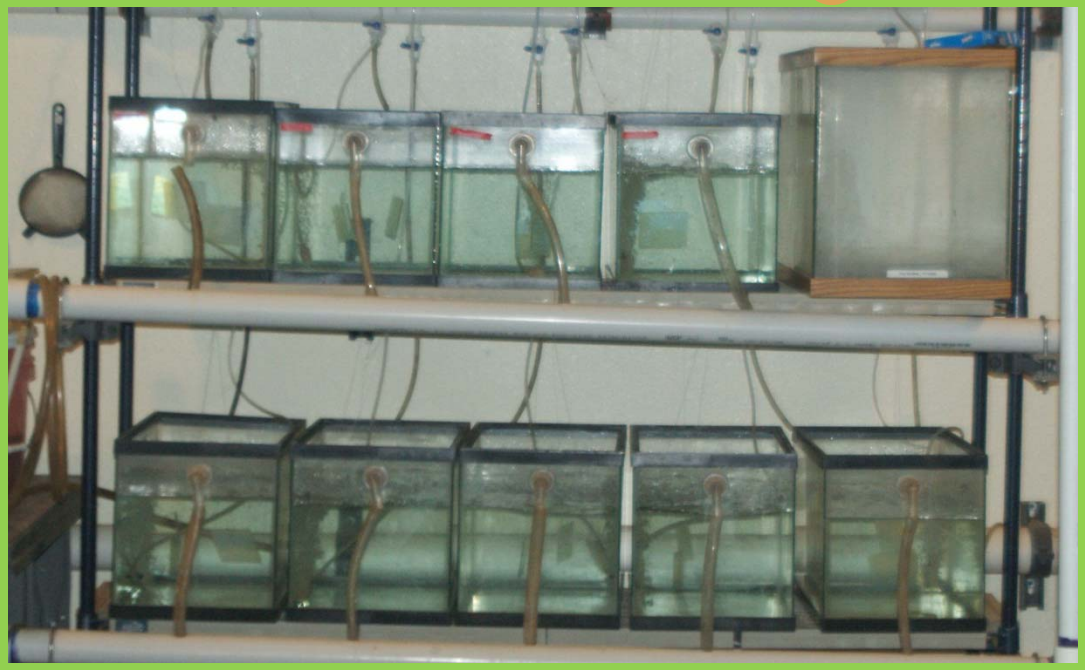
Veliger Toxicity Test

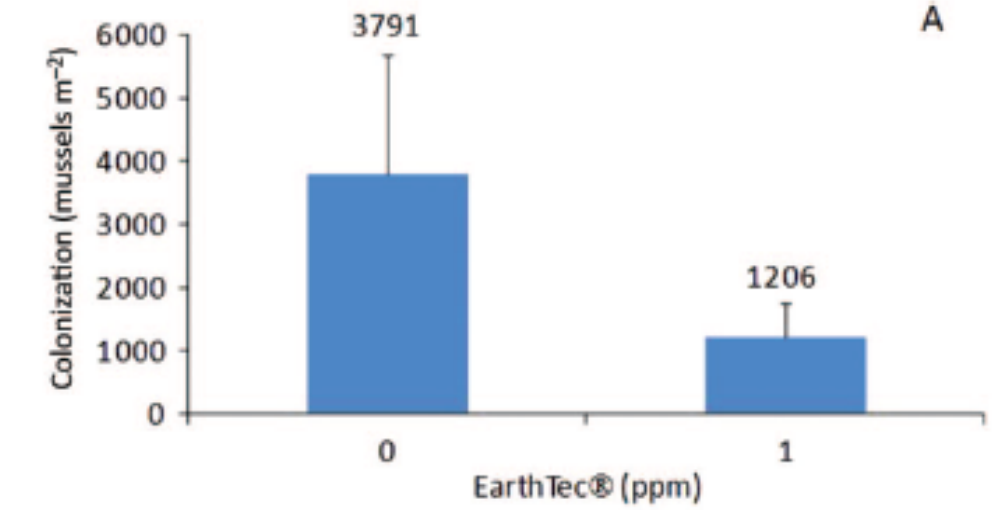
- > 10 min→ 83, 17, and 10 ppm groups died
- >20 min→ 5 ppm groups died
- >30 min→3 ppm groups to die
- The experiment was completed after 36 h, and all the controls and individuals in the groups with 1 ppm were still alive.
- To reach a 100% mortality rate, the minutes needed were significantly different (ANOVA, $p < 0.001$)

The use of EarthTec® in preventing veliger colonization

- **Hypotheses**
  - EarthTec® will be effective below 3 ppm (0.18 ppm of Cu^{2+}) in preventing veliger quagga mussel colonization on fiberglass substrates
  - The dose to prevent veliger colonization will be below the US EPA's Drinking Water Regulations for copper at 1.3 ppm in an experimental situation

Methods





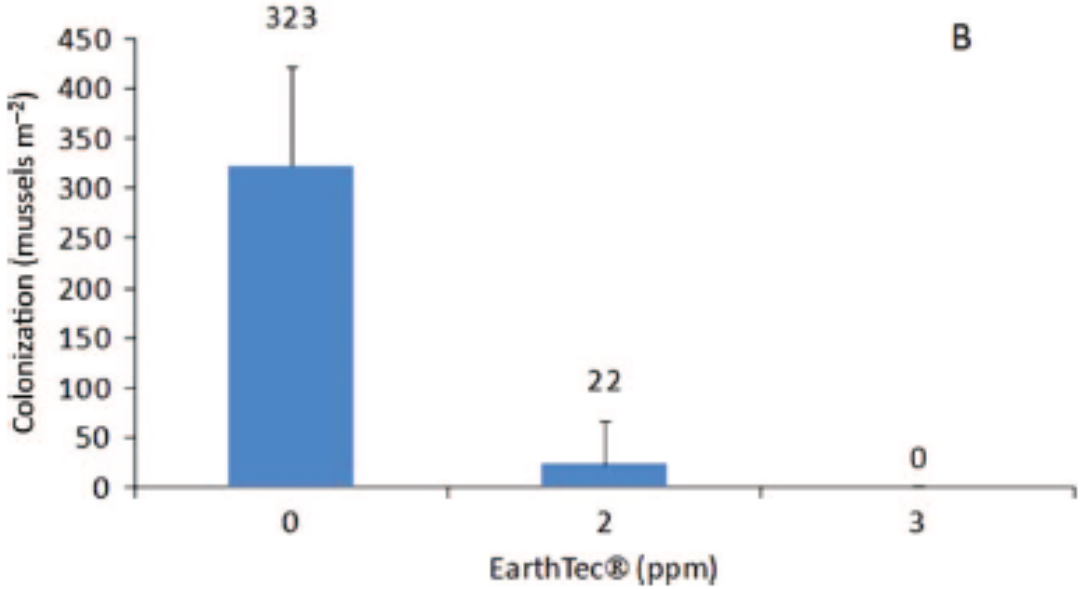


Figure 2. Colonization density of quagga mussels treated with 0 and 1 ppm (A) and 0, 2, and 3 ppm (B) of EarthTec®. Mean ± 1 SD; values shown on top of the bars are means.

Log (Colonization Rate % + 1) = -0.7 × Dose + 2.0 (R = 0.98, $p < 0.01$)
To get a 0 colonization rate, the estimated dose is **2.8 ppm of EarthTec®**

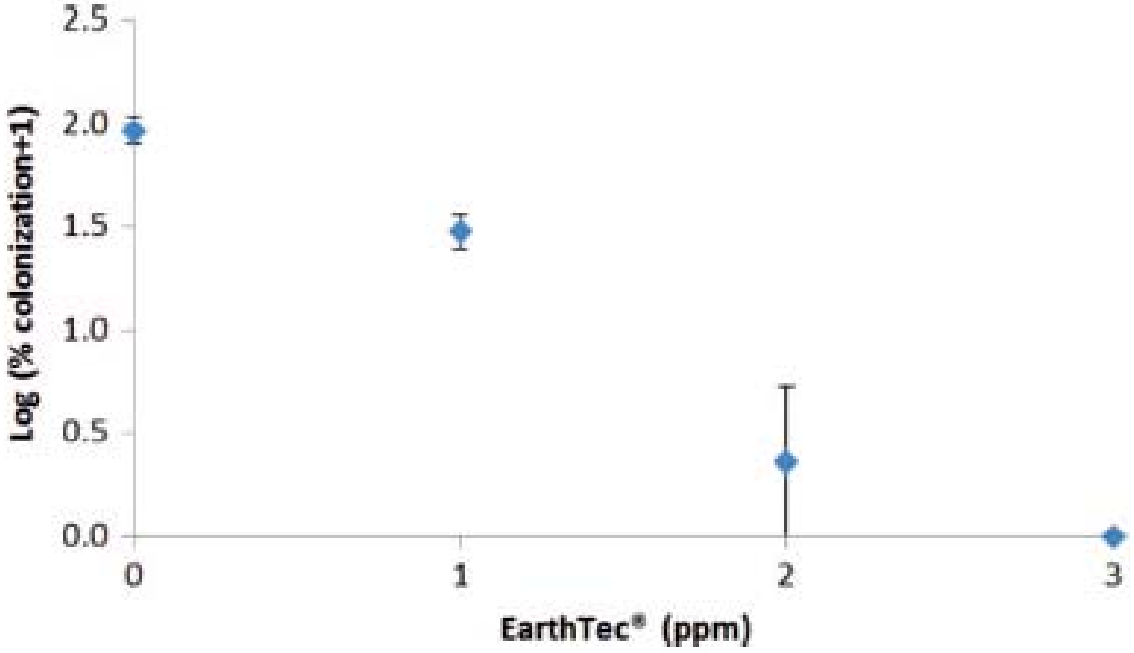


Figure 3. Relationship between the percentage colonization rates and EarthTec® dose in quagga mussel colonization. Mean ± 1 SD. Note that the data on y-axis are log (y + 1) transformed.

Summary

- To reach 100% mortality, adult quagga mussels would need to be continuously exposed to 5 ppm of EarthTec® (0.30 ppm Cu^{2+}) for 168 h; juveniles need 48 h exposure
- 100% mortality of veligers occurs when exposed to 3 ppm of EarthTec® (Cu^{2+} 0.18 ppm) > 30 min
- Veliger life stage is the most sensitive to EarthTec®
- Statistical analysis shows that 2.8 ppm can prevent veliger colonization under the experimental conditions
- Targeting veligers to reduce colonization in water treatment facilities is cost effective because the dose is low, and application duration would be minimized

Future Research

- Chemical management strategies targeting early larval stages of quagga mussels are more likely to be cost efficient and less prone to non-target environmental impact
- Determine the lowest dose of EarthTec® that can result in zero colonization in different seasons
- Copper toxicity increases with an increase in water temperature (Rao & Khan, 2000)
- Quagga mussel spawning

Thank you

EarthTec® and Microorganisms

- Cu^{2+} reacts with organic molecules that form living tissues
- Cu^{2+} affects the permeability of the cell membrane
- Results in a loss of potassium ions
  - Cu^{2+} accumulates in cell walls, membranes, and organelles which contain negatively charged molecules
- Cu^{2+} is chelated by negatively charged components
  - Cu^{2+} moves to the chloroplast of the cell
  - Cu^{2+} becomes chelated by the compounds in the chloroplast membranes, and photosynthesis is inhibited (Pasek, 1993)

EarthTec® and the Environment

- Copper remains chelated to the negatively charged components of the cell walls, which degrade over time, and the copper chelates will settle to the bottom of the water
- Copper is no longer biologically active
  - Due to the proprietary copper “carrier” that holds Cu^{2+} in solution
  - Prevents the cupric ion from chelating with other organic molecules (Earth Science Laboratories, 2010)

The effectiveness of EarthTec® on killing quagga mussels

- **Questions**
  - Can the US EPA-registered and National Sanitation Foundation (NSF)-certified algicide/bactericide, EarthTec®, be used as an effective molluscicide?
  - What is the lowest concentration of EarthTec® that is effective in killing adult, juvenile, and veliger quagga mussels?
  - What life stage of the quagga mussel is most sensitive to EarthTec®?

The effectiveness of EarthTec® on killing quagga mussels

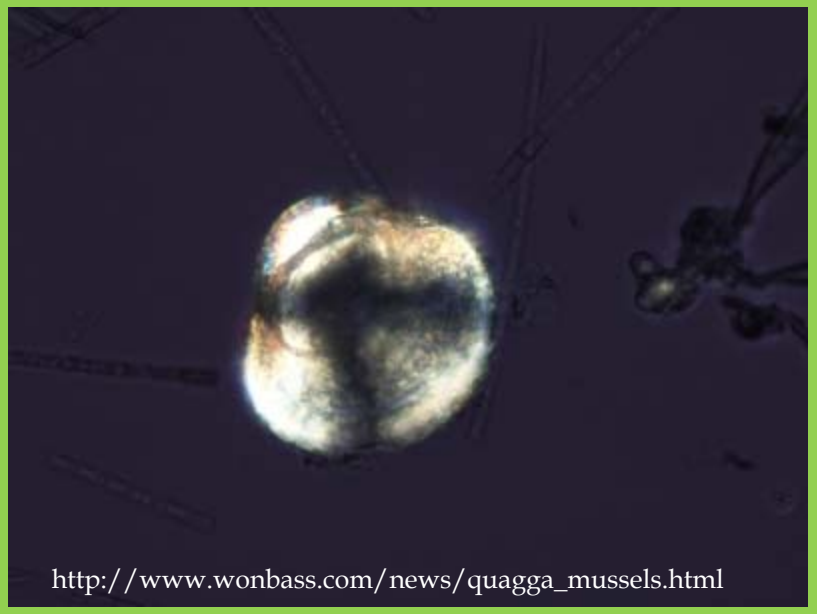
- **Hypotheses**
  - EarthTec® can kill adult, juvenile, and veliger quagga mussels at or below the US EPA's Safe Drinking Water Regulations at 1.3 ppm of copper
  - The effective dose of EarthTec® in killing quagga mussels will be dependent on the stage of the quagga mussel's lifecycle, where veligers will be more sensitive to EarthTec® than adults

Adult and Juvenile Mortality

- Assessed via gaping valves
- Adults: 24 hour
- Juveniles: 6, 12, 24 hour

Veliger Mortality

- Assessed via ciliary movement of body or observations of internal organs moving (Britton & Dingman, 2011)
- Monitored immediately and every 5 min, up to 36 h



http://www.wonbass.com/news/quagga_mussels.html

http://www.iisgcp.org/exoticsp/zebra_mussel.htm

Results

Adult, Juvenile, and Veliger Toxicity Tests

Analysis of Data

- Toxicity tests of EarthTec® were performed with different stages of quagga mussels
  - Adults (shell length > 11 mm; mean 17.99 mm ± 3.51, N = 240)
  - Juveniles (shell length < 11 mm; mean 7.48 mm ± 1.87, N = 240)
  - Veligers (mean size 178.30 µm ± ---56.59, N = 23).

The use of EarthTec® in preventing veliger colonization

- **Questions**
  - Is EarthTec® effective in preventing veliger quagga mussels from colonizing fiberglass substrates?
  - What concentration of EarthTec® will be effective in preventing veligers from colonizing fiberglass substrates?

The use of EarthTec® in preventing veliger colonization

- **Objective**
  - To determine the lowest concentration of EarthTec® that is effective in preventing veliger quagga mussels from colonizing fiberglass substrates

Methods

- **Phase I experimental design**
  - 1 month
  - 6 controls and 6 treatments of 1 ppm of EarthTec® (0.06 ppm of Cu^{2+})
- **Phase II experimental design**
  - 1 month
  - 4 controls, 4 treatment groups of 2 ppm of EarthTec® (0.12 ppm of Cu^{2+}) and 4 treatment groups of 3 ppm (0.18 ppm of Cu^{2+})

Methods

Experimental design

- 12 tanks with 25 L of raw Lake Mead water
- 3 fiberglass substrates (79 x 68 x 1.66 mm) (with biofilm)
- Each week, ½ the water in the tanks was exchanged and replaced with fresh, raw Lake Mead water
- To prevent the loss of the veligers, the water was filtered in the cone portion of the plankton net
- At least 25 veligers per L of water (25 x 25 L = 625) was added each week
- EarthTec® concentration was added to the treatment tanks
- pH was recorded

Methods

- **Substrate analysis**
  - Substrates in control tanks were assessed first
  - All 6 sides were analyzed
  - Photographed and recorded

Treatment 6B

Control 4C

Combining Phase I and II

- Assuming the control (0 ppm) treatment had 100% colonization rate in Phase I, an average 32% colonization rate was found for 1 ppm
- The same assumption was applied for the Phase II experiment where the colonization rates for 0 ppm, 2 ppm, and 3 ppm treatments were 100%, 7%, and 0%, respectively
- Therefore, EarthTec® with 1 ppm, 2 ppm, and 3 ppm had reduced colonization rates at 68%, 93%, and 100%, respectively

Health Implications of Copper

- MCL of copper in drinking water at 1.3 ppm (US EPA, 1991)
- As of 2009, the average level of copper detected in the Southern Nevada Water Authority (SNWA) distribution system was 0.8 ppm (SNWA, 2010)
- 1 ppm of EarthTec® (0.06 ppm Cu^{2+}) can deter veliger settlement
  - Copper output would still be below the US EPA's MCL
- Excess copper in the body can cause stomach and intestinal distress such as nausea, vomiting, and stomach cramps (Pizarro, Olivares, Uauy, Contreras, Rebelo, & Gidi, 1999)