

# Use of a Differential Simple Stain to Confirm Mortality of Dreissenid Mussels in Field Research Experiments



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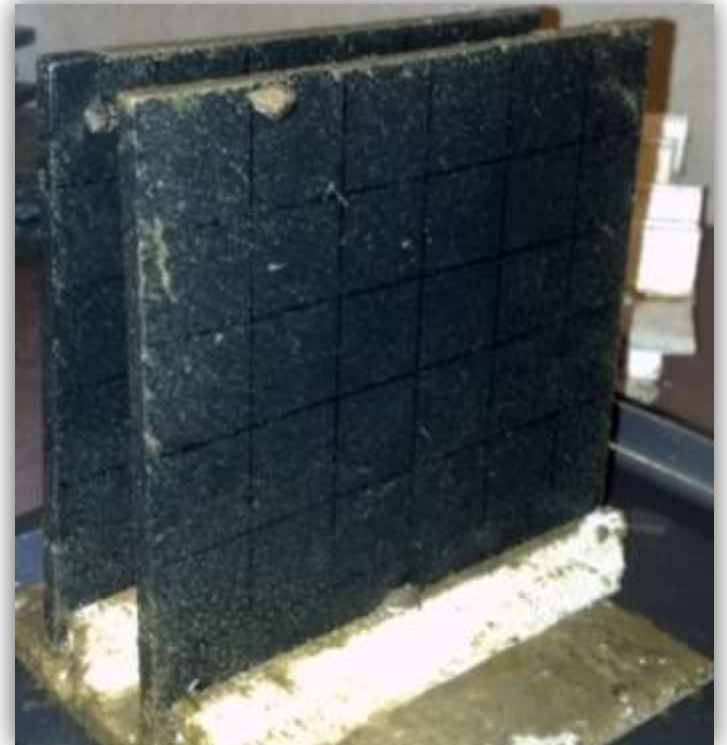
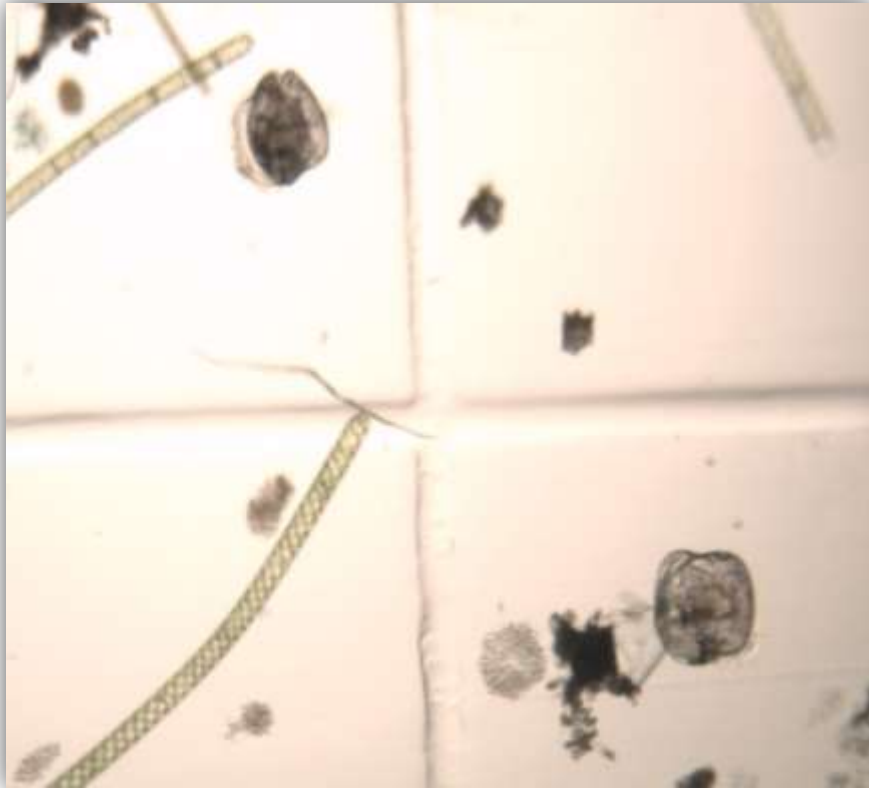
Renata Claudi



*19<sup>th</sup> International Conference on Aquatic Invasive  
Species 2016*

# Veliger Toxicity Studies

- Determination of live and dead veligers
  - Observe each individual veliger for cilia or organ movement up to 5 min
  - Hold for a period of time to determine if settlement will occur



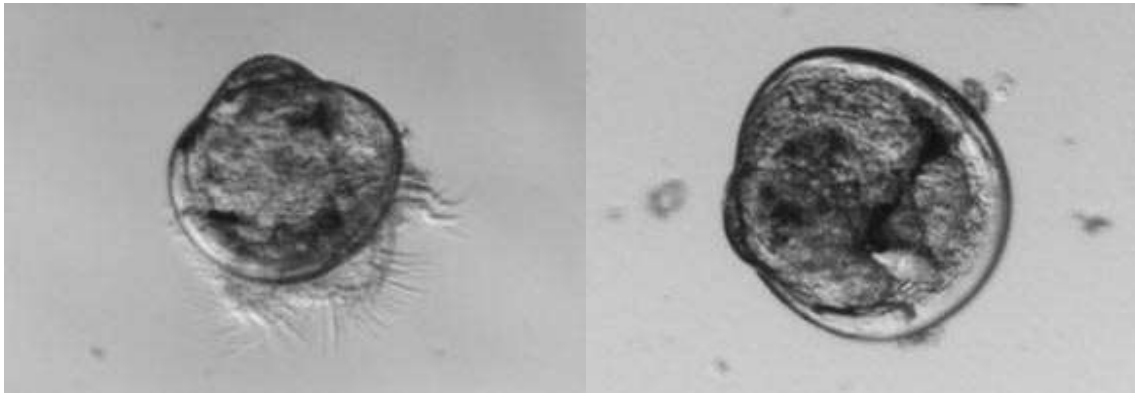
# Z/Q Veliger Toxicity Studies — Problem Definition

- Long hours staring into microscope
- Low sample sizes=low statistical certainty
- Low confidence of mortality due to movement by post-mortem decomposers



# Potassium Toxicity Studies

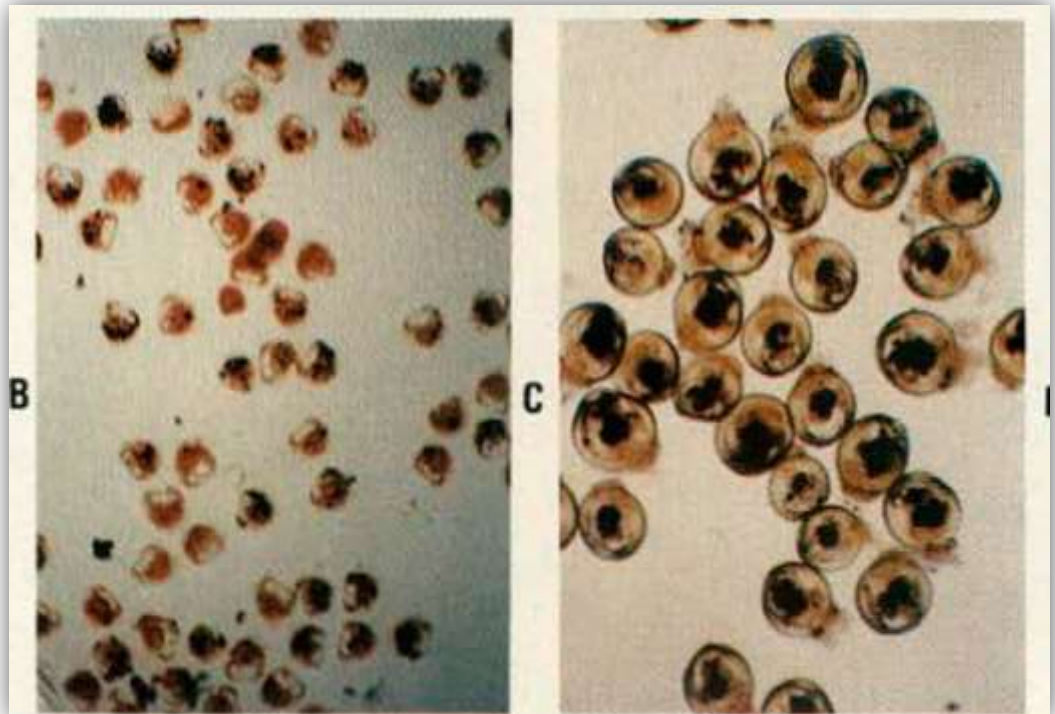
- Potassium found to relax mussels
  - Vellum completely out of shell
- Treated veligers had no movement = dead?
  - After 24+ hours in recovery they were living



Sykes et al. reported both veliger conditions as dead

# Biological Staining Techniques

- Neutral Red (stains living tissue)
  - Found hard to differentiate in samples stained for less than 3 hours
  - Easy to differentiate for up to 24 hours after stained for 3 hours
- Other stains?



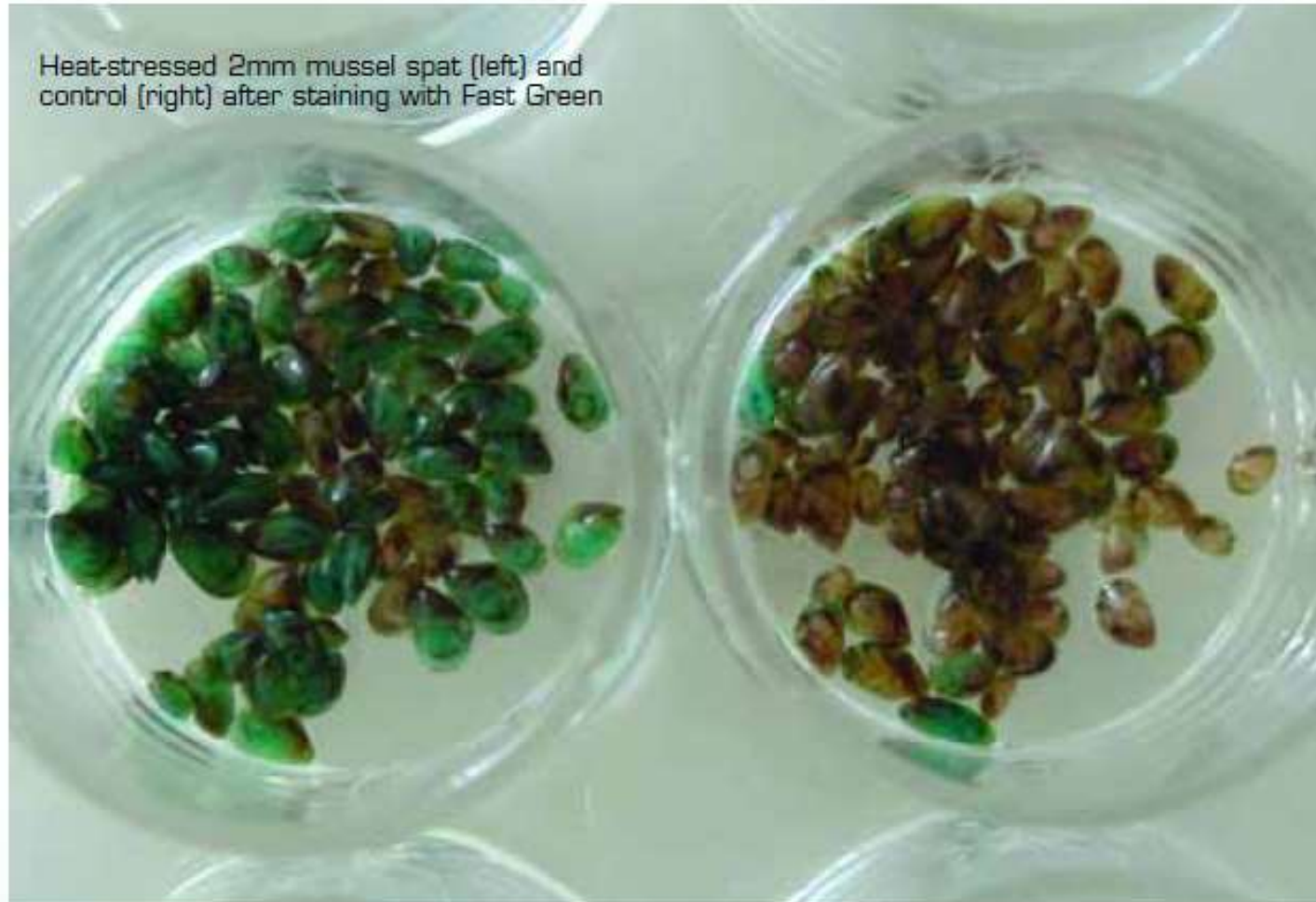
# Background on Fast Green

- Commercial food dye
- Biological viability stain to determine dead tumor cells
- Non-lethal marker for arthropods
- Detection of skin injury sites on Chinook salmon smolts



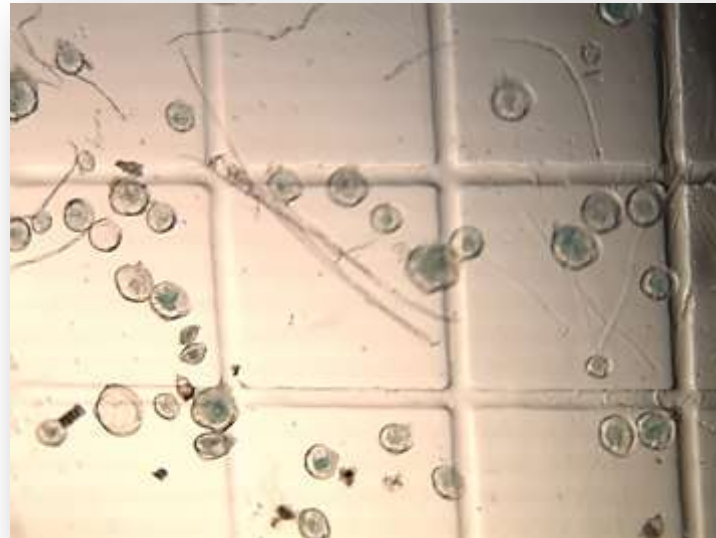
# Mollusks and Fast Green Dye

- Marine mussel spat assessment staining



# Dreissenids and Fast Green Dye

- Method development for z/q mussels veligers & juveniles (Link, Claudi, & Taraborelli ICAIS 2013)
  - 4% solution of Fast Green for 20-30 min
  - Rinse well and observe with 40x microscope or hand held
  - Confirmed “no impact” of fast green stain on live veligers





# Latest Application of Fast Green Staining Technique

Objective – Assess condition of veligers exposed to potassium chloride to build a toxicity curve and end point with high certainty

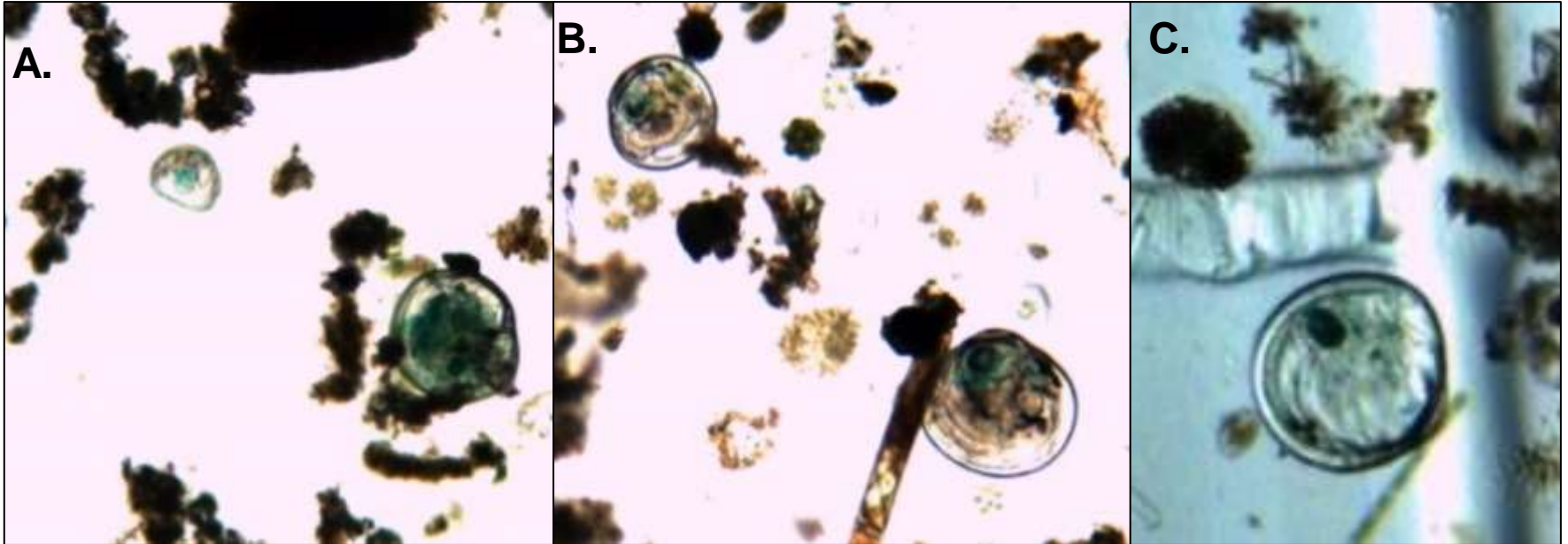


# Methods

- Collect veligers- sieving out large algae or zooplankton
- Divide out concentrated veligers into test beakers
- Exposure dose: 960 mg/L KCL solution
- After exposure time, rinse off test solution
- Place in 4% Fast Green solution for 20-30 min
- Rinse off dye solution well and put into beaker
- Analyze with 40x-100x microscope within 2 hours



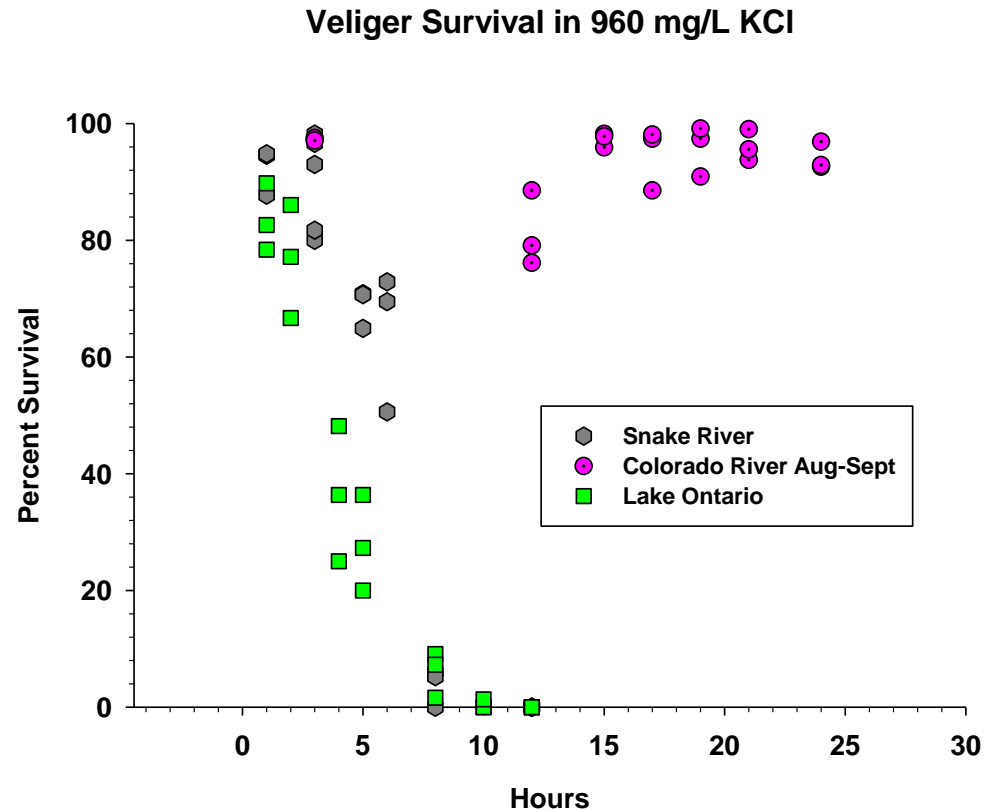
# Veliger Stain Analysis



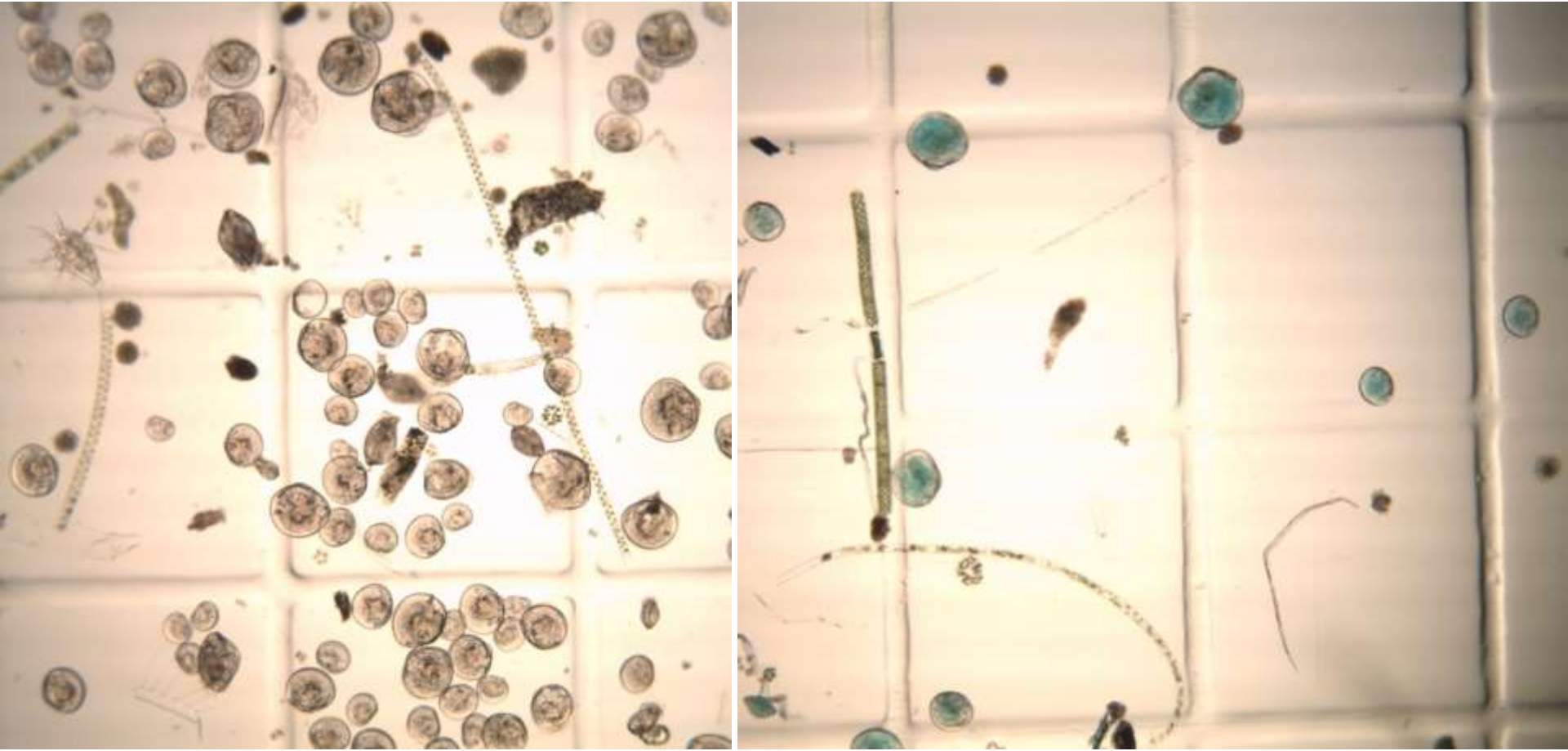
**A. Dead    B. Live    C. Empty**

# Results

- Used two different filtered water sources to dissolve dye
  - No difference in staining effect
- > 100 veligers analyzed in each of three replicates per exposure time period
- 5-15 min analysis time for each replicate

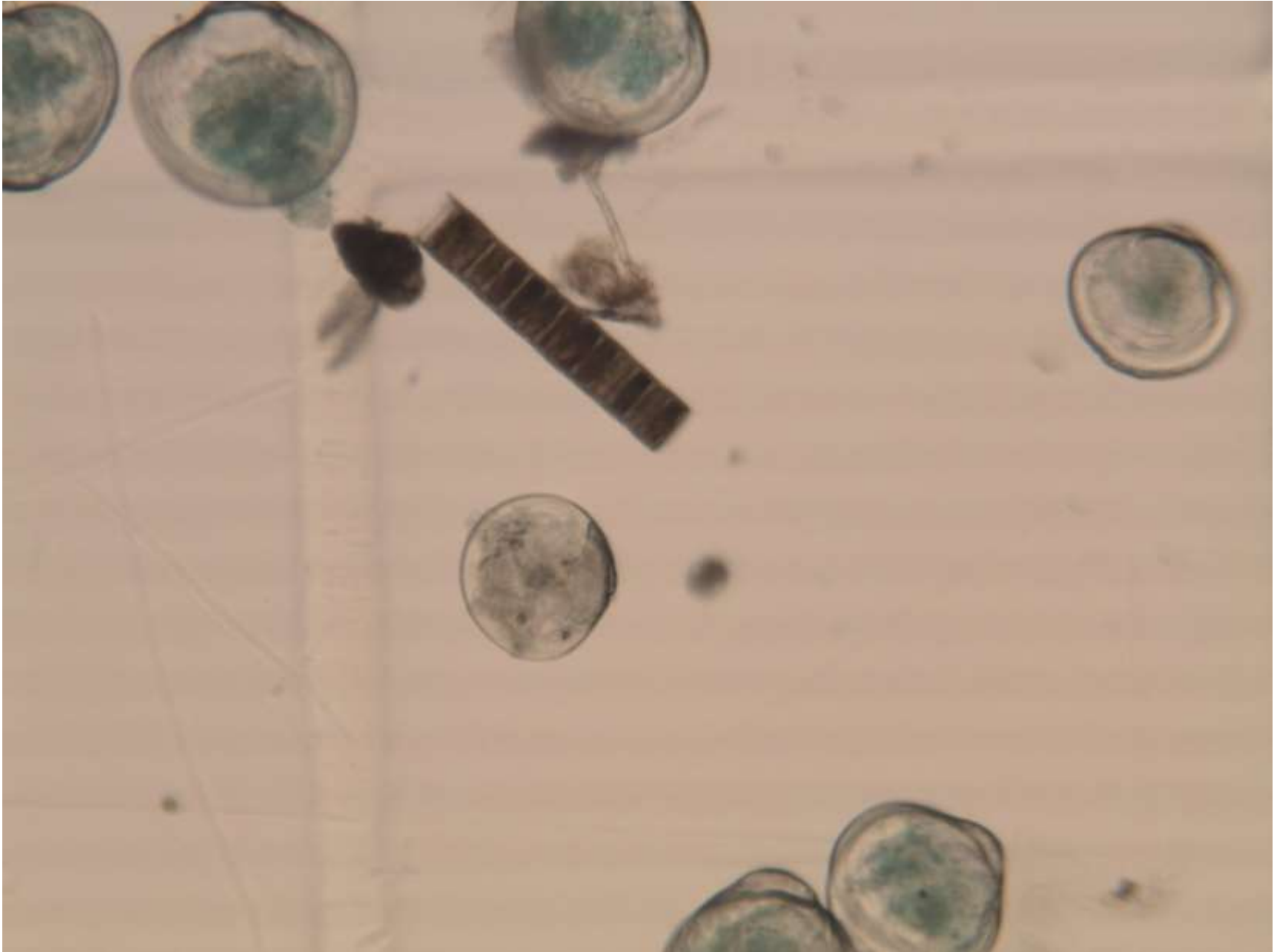


# Confidence in Analysis

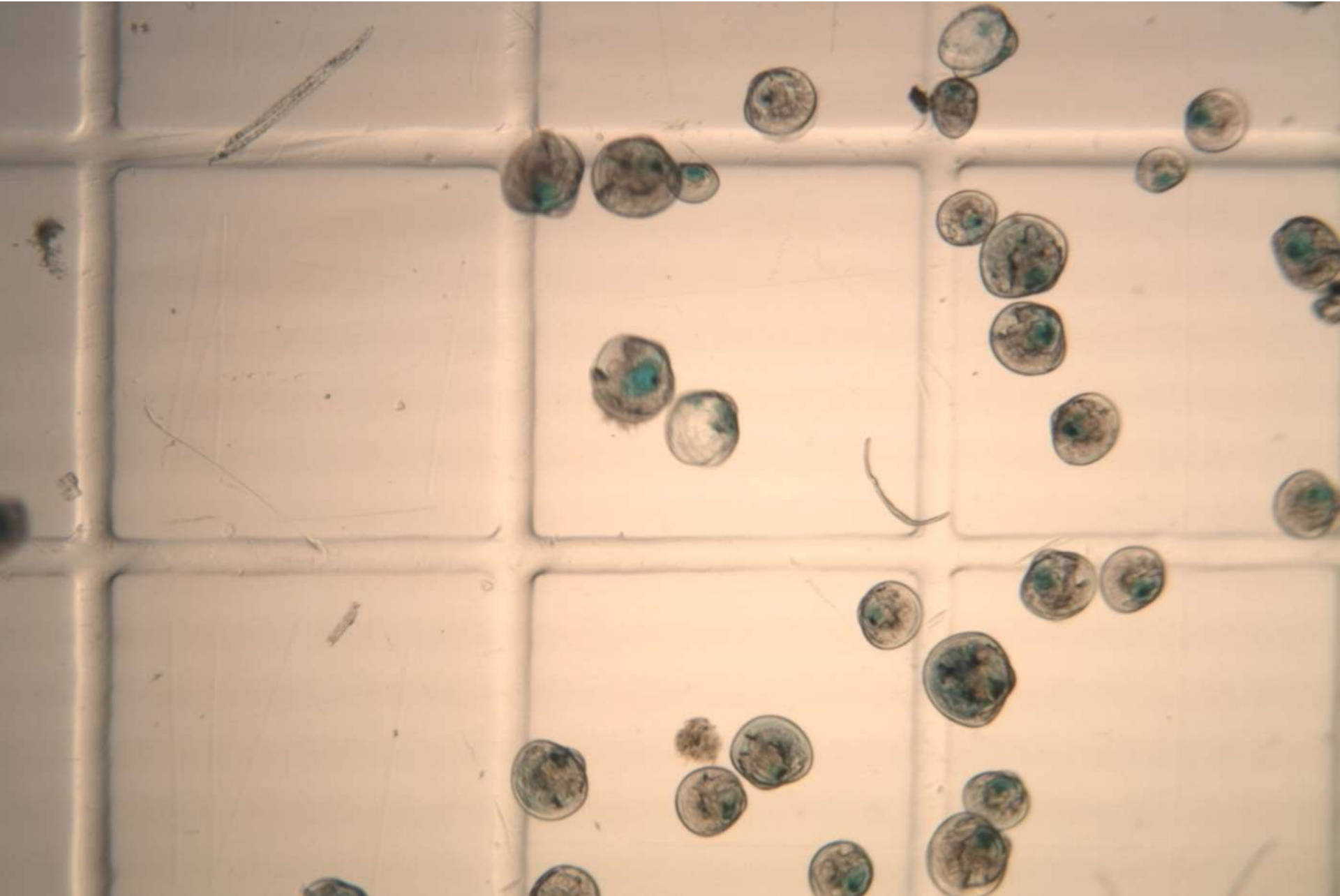


Quickly see which are dead out of a group of veligers

**Very Apparent at 100X**



# Live Veligers Stain Partially



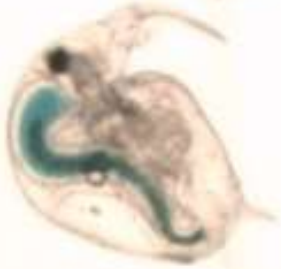
# Easy to Find in Plankton





# No Harm to Zooplankton

*Bosmina*



*NZMS neonate*



*Moina*



*Copepod*

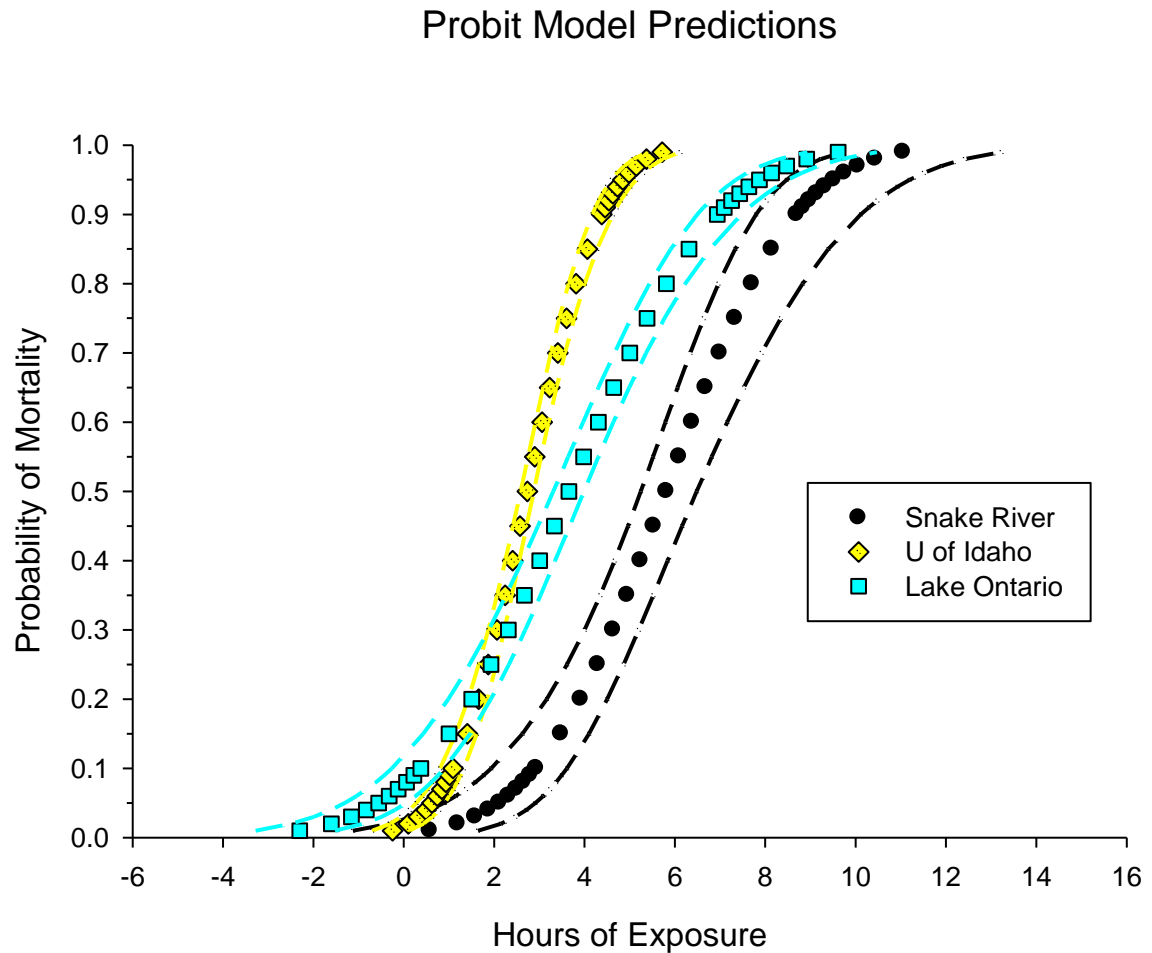


*Tardigrade*



# Statistical Analysis

- Probit model was constructed with small confidence intervals
  - High sample size
  - Low variation across replicates



# Conclusions

- Fast Green Stain can be used to determine mortality
  - Low processing time (20 to 30 min)
  - Quickly (5-15 min) to count over 100 veligers
  - Easily pick out from other debris or organisms
  - Highly confident in assessment of mortality
- Eliminates the need for recovery period or prolonged studies to determine the status of the veliger
- Toxicity studies using Fast Green Stain method should be regarded as accurate for use in rapid response of eradication efforts

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