Effects of water calcium concentrations on fertilization and early development of zebra mussels

Julianna Martinez, Dr. Mike Misamore

Department of Biology, Texas Christian University

INTRODUCTION

- The zebra mussel (*Dreissena polymorpha*) is an invasive species of freshwater bivalves that have recently spread into bodies of water across North America.
- Zebra mussels attach to substrates via strong byssal threads and grow in tightly packed colonies in very high densities. They are very effective filter feeders, filtering up to a liter of water per day, resulting in reduced phytoplankton levels, increased water clarity and a cascade of water quality problems. (Griffiths)
- Calcium is a critical component of their survival due to its function in numerous cellular processes and in shell production for both larval and adult stages. Current models of predicted spread are based primarily on minimum calcium levels.
- Zebra mussels are broadcast spawners that release their gametes directly into the water column.
- The viability of north Texas zebra mussel gametes and survivability of larvae were assessed when spawned in a calcium free medium compared to a control medium with calcium.
- The results of this experiment, along with future research, will help to determine which waterways may or may not be suitable for zebra mussel reproduction and subsequent infestation.

METHODS

- Zebra mussels were collected from Lake Bridgeport, TX on April 6th, 2018 then stored at 9°C.
- Animals were separated, rinsed, and placed in individual specimen cups in order to be brought up to room temperature.
- Mussels were placed in individual test tubes in a solution of 1mM serotonin and pond water in order to induce the release of gametes.
- Mussels will be spawned in water containing various levels of calcium ranging from 0 to 30 mg/L.

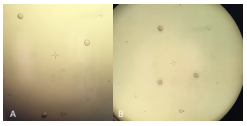

Figure 1. Zebra mussel gametes. A) Unfertilized eggs. B) Fertilized eggs. Sperm can be seen concentrated around eggs due to chemoattraction.

- Samples of both eggs and sperm will be analyzed for viability including motility (sperm), acrosome integrity (sperm), and membrane integrity (eggs). (Fig. 1)
- Samples of eggs and sperm will be combined in order to assess factors of fertilization including egg activation. (Fig. 1)

REFERENCES

Griffiths, R.W., D. W. Schloesser, J.H. Leach, and W.P. Kovalak. 1991. Distribution and dispersal of the zebra mussel (*Dreissena polymorpha*) in the Great Lakes region. *Canadian Journal of Fisheries and Aquatic Sciences* 48: 1381-1388.

Veliger photo source - https://www.wildlife.ca.gov/Conservation/Laboratories/Shellfish-Health/Detection-Program

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EXPECTED RESULTS

- Male zebra mussels released sperm after 20-30 minutes in the serotonin solution and females released eggs after 60-75 minutes in solution.
- The eggs and sperm were observed under a compound microscope to assess their viability.
- Eggs and sperm were combined to allow for fertilization and larvae were observed over multiple days.
- Role of Calcium in Gamete Integrity
  - Sperm
    - Sperm Motility – calcium is assumed to play a role in sperm guidance; normal motility is assumed
    - Acrosomal Integrity– calcium involved in acrosome reaction
  - Eggs
    - Membrane Integrity (examined using vital dyes)
    - Artificial activation (polar body formation) – calcium plays in a role in induction of spontaneous polar body formation
- Role of Calcium in Fertilization
  - Chemoattraction – calcium plays a known role in urchins; it is assumed to be similar in mussels and will be tested
  - Sperm Binding – major class of cell-cell adhesion molecules (caherins) require calcium; presence in mussels?
  - Egg Activation – calcium waves common in many animals; assumed also in mussels
  - Sperm entry into egg
- Role of Calcium in Cell Division
  - Cleavage furrow formation
- Role of Calcium in Larval Stage Formation and movement
  - Trochophore Larval Stage
  - Veliger Larval Stage

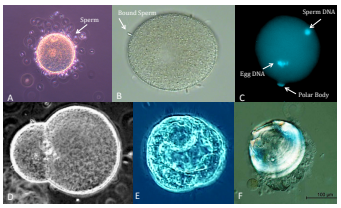

Figure 2. Stages of Mussel Fertilization and Development A) Chemoattraction of sperm concentrated around egg. B) Bound sperm. C) DNA labeled with Hoechst 33342 showing sperm inside egg. D) 2-cell stage. E)Trochophore larval stage. F) Veliger larval stage

FUTURE IMPLICATIONS

- Reproduction is essential for establishment of viable populations of mussels
- Calcium is known to play a prominent role in many reproductive events. Zebra mussels cannot survive in water containing less than 12mg/L
- If reproduction is inhibited by absence of calcium, then population establishment could become inhibited as a result.
- Further research could potentially strengthen current understanding of calcium being a limiting resource for zebra mussel spread.

FUTURE RESEARCH

In my future graduate research I will further test the effects of a wider spectrum of calcium concentrations on the mussels ability to spawn and larval survivability.

Additionally, research will also be conducted on Quagga mussels in order to compare across species.