Determination of gamete viability in the dreissenid zebra and quagga mussels.

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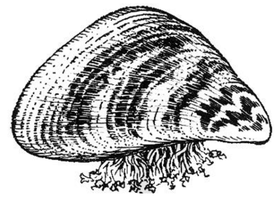


Abstract

Zebra mussels are an invasive species known to cause adverse ecological impacts by outcompeting native species, disrupting the food web, and destruction to aquatic habitats. Zebra mussels often aggregate on hard surfaces, clogging pipes, damaging boats and infrastructure, etc., leading to costly economic challenges. As broadcast spawners, they release eggs and sperm into the water column where fertilization and larval development occurs. During this process, the larvae may travel long distances enabling their spread into new locations, including Texas. These early stages of the lifecycle (gametes, larvae) zebra mussels will be most sensitive to external factors and conditions. They may also be the most sensitive to control mechanisms such as copper or bleach treatments. Little is known about exactly how long gametes remain in the water prior to fertilization and how long after spawning are they viable. The objective of this research project is to gain a deeper understanding of Zebra mussel reproduction with a focus on gamete viability. I will assess sperm viability using multiple assays including established procedures such as sperm motility using video analysis, gamete and sperm longevity. We have developed novel a fixed egg assay that allows analysis of sperm binding to eggs without the need for freshly spawned eggs. These assays will allow us to determine how long zebra mussel sperm and eggs are viable after release in order to address how environmental factors such as temperature, calcium, or copper affect gamete viability.

Introduction

Background

●Originated in Eastern Europe

● Introduced to U.S in 1991 and Texas in 2021 (*May & Marsden, 1992; McMahon, 2011)*

Impacts

● Consume phytoplankton, leading to increased water clarity and overgrowth of aquatic plants (*Fahnenstiel et al., 2010)*

● Competes with native species, decreasing biodiversity *(Mahdenjian et al., 2015)*

● Attaches to hard surfaces, which can clog pipes and damage boats

● Damage costs us hundreds of millions of dollars annually in the U.S

Spawning

● Broadcast spawners: release gametes into water column, external fertilization *(Ackerman et al., 1994)*

●Planktonic larvae: remain suspended in water column for long periods of time *(Misamore et al.)*

Means of spreading

●Ballast water and bait wells

●Attachment to hard surfaces, such as the bottom of boats

Gamete Structure

● Sperm
  - Made up of head, midpiece and flagellum *(Walker et al., 1996)*●

● Egg
  - Surrounded by jelly layer, which possibly serves as a chemoattractant for sperm *(McAnlis et al., 2010; Miller et al., 1994)*

Fertilization*(Misamore et. al, 1994)*

● Sperm attracted to egg surface by jelly layer surrounding egg

- Sperm binds perpendicular to egg surface *Sperm enter into egg cytoplasm*
- *Egg is activated, resuming meiosis and producing a polar body*
- *Zygotes cleave approximately 60 min after fertilization*

Objective

To determine the viability of gametes in freshwater at different time points after being released from adults.

Experimental Design

- Zebra and quagga mussels were isolated and warmed to room temperature
- Individual mussels were place in test tubes and induced to spawn by exposure to serotonin
- Males were allowed to spawn in test tubes while females were transfer to spawning dishes to disperse eggs.
- Fertilization were performed at various times point with recently and aged gametes at various timepoints.
- Several aspects of fertilization was analyzed including:
  - Sperm binding to the egg at 5 minutes postinsemination
  - Sperm enter into the egg cytoplasm at 30 min postinsemination
  - Zygote division producing a 2-cell embryo at 90 min

Male spawning in test tube

Female spawning in spawning dish

Zebra mussel
(*Dreissena polymorpha*)

Quagga mussel
(*Dreissena rostriformis bugensis*)

Motility of aged sperm

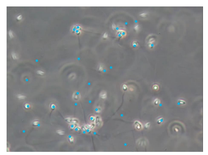
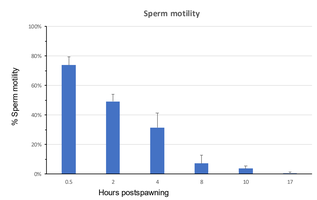


Image showing movement of sperm. Dot indicates location of sperm in image 5 seconds prior. Location where dot overlaps sperm indication no sperm motility.



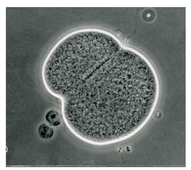
Sperm motility

Sperm motility was examined for up to 17 h postspawning. Three randomly selected fields of view were digitally recorded and total motility was determined based on percent of sperm moving in a given field of view. (n=3 replicates from 3 different males)

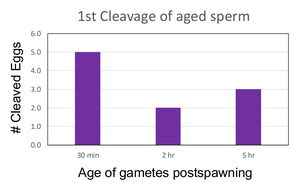
Cleavage of zygote from aged gametes @ 120 min PI

First cleavage by aged gametes

Eggs and sperm of various ages postspawning were fertilized. Inseminated eggs were examined after 2 hr postinsemination and the number of eggs that successfully completed first cleavage was determined.



Light micrographs of eggs dividing to form two-cell embryos.



Numbers of eggs exhibiting first cleavage at 2 hr PI. Three trials of 30 eggs involving fertilizations between unique males and females.

Conclusions

- Sperm remained motile for 8 hours postspawning
- Sperm binding decreased significantly after 5 hrs
- Gametes 5 hours postspawn were still able to divide.
- Sperm were still able to bind to fixed eggs. Aged sperm showed a similar pattern of decreased sperm binding with time after spawning independent of egg age.

Acknowledgements:
Maddie Comeaux
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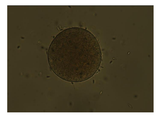
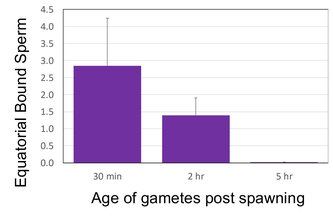
Sperm binding by aged gametes @ 5min PI



Sperm-egg binding by aged gametes

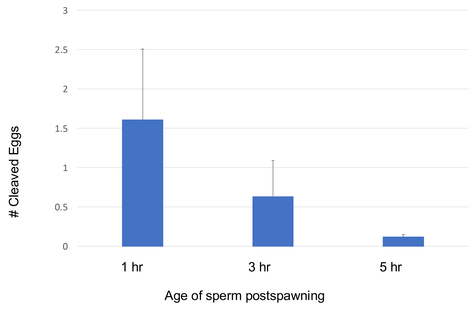
Eggs and sperm of various ages postspawning were fertilized. Inseminated eggs were examined 5 min postinsemination and the number of equatorial bound was determined.



Mean number of equatorial bound sperm from three trials involving fertilizations between unique males and females. Thirty eggs were evaluated for sperm binding for each trial. Bar – SE, n=3,

Aged sperm binding to fixed eggs

Freshly spawned eggs (<1 hr) were fixed and subsequently washed and stored for later use. Sperm were spawned and used to inseminate the fixed eggs. This showed the ability of aged sperm to bind to fixed eggs at a single, recently spawned timepoint.



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