DEPARTMENT OF BIOLOGY

Final Report – April 19, 2022

Growth, survival, and reproductive success of zebra mussels in Texas lakes

Principal Investigator – Michael Misamore, PhD. Department of Biology, Texas Christian University

TPWD Contract Number – 529527

OBJECTIVE

The objective of this study was to determine the viability of zebra mussels in various Texas waters with differing calcium concentrations. Fifteen lakes/reservoirs were utilized with calcium levels above or below the 12 mg Ca/L threshold level. These sites include water infested with mussels (positive control), positive for mussels above the threshold, above the threshold but uninfested, and water below the calcium threshold. Seven river basins were represented in this sample group. Water from these locations was evaluated for its ability to maintain adult/juvenile zebra mussels for extended periods of time in a laboratory setting. I also evaluated if fertilization and early development can occur in these waters of varying calcium concentrations. Lastly, I evaluated veliger survival and development in these various waters. The goal of the study was to develop a better understanding of the potential viability of mussels in Texas waters of varying calcium concentrations, which will allow for further confirmation and refinement of predictive models for zebra mussel spread in Texas.

INTRODUCTION

Since their appearance in North American in the mid 1980's, the impact of zebra mussels (*Dreissena polymorpha*) on North American waterways has been well documented. Their ecological impact has been profound due to their high filtration rates and biofouling effects. It is estimated they can filter upwards of 1.5L/hr. This high filtration leads to decreased available phytoplankton and zooplankton and increased water clarity - both negatively impacting aquatic ecosystems. The loss of available plankton impacts subsequent trophic levels while the increased water clarity can lead to elevated water temperatures, decreased hypolimnion, and increased aquatic plants leading to altered habitats (MacIsaac 1996). Furthermore, they

physically impact other aquatic life, primarily freshwater clams, by literally smothering them (Schloesser et al., 1996).

Economically, they have been just as costly. Estimates between 300 million to 1 billion dollars has been spent on zebra mussel control between 1998-2004 alone, with estimates of 60 million annually to deal with this problem (Pimentel et al., 2005; Connelly et al., 2007). These damages result primarily due to biofouling of pipelines, machinery, watercraft, and other hard substrates where the mussel like to attach. Numerous methods, and subsequent costs, to prevent attachment of mussels by industrial facilities have been employed including chemical treatment using oxidizing and non-oxidizing chemicals, antifouling paints, mechanical filters, UV radiation, electric currents, acoustics, and mechanical fields (Chakraborti et al. 2014). Additionally, zebra mussels can impact recreation and tourism by encrusting on rocks, piers, docks, and watercraft, negatively affecting recreational users.

Zebra mussels were first reported in North America in the Great Lakes in 1989 (Hebert and Muncaster 1989). They have since spread through much of the Great Lakes region and the length of the Mississippi River as far south as New Orleans. In 2009, zebra mussels were found in Texas in Lake Texoma and have fully infested 28 Texas lakes from six river basins. Five additional lakes having repeated detections and, in most cases, likely in the process of developing established populations (Texas Parks and Wildlife, 2022). In contrast to European distribution patterns, zebra mussels in North American have spread significantly further particularly, into warmer water climates (Strayer, 1991; McMahon, 1996; Mackie and Schloesser, 1996). Thus, predicting the ultimate distribution of these mussels can be challenging. Several environmental parameters have been shown to indicate potential spread of zebra mussels.

Temperature

Being a cold-water species, temperature is believed to a significant factor that might limit mussel distribution (Strayer 1991). In North America, the upper limit of mussel survivability is believed to be 28 to 32 °C (Spidle et al., 1995, McMahon, 1996) with temperatures above 25 °C having a negative effect on the mussel physiology including decreased growth rates and increased respiration (Elderkin and Klerks, 2005). McMahon (2015) used mean August water surface temperature (with >32°C as unsuitable) as one parameter for identifying invasion risk in Texas lakes.

Dissolved Oxygen

Zebra mussels require dissolved oxygen greater than 3 mg/L (Johnson and McMahon, 1998; Mackie and Claudi, 2010).

pH

Ramcharan et al. (1992) reported that the optimal pH of the water for zebra mussel is 8.4 and they are absent below 7.3. Sprung (1987) found no larval production occurred at pH above 9.4 or below 7.4, with an optimum of 8.4.

Eutrophication.
Dreissena are rare or absent in lakes with high eutrophication including high levels of both phorphorus and nitrogen (Ramcharan et al., 1992).

Suitable substrate
Zebra mussel survival in dependent on appropriate substrate for veliger settlement. Inorganic hard surfaces serve as the predominate substrate (Nalepa and Schloesser 1993), while certain organic hard substrates, such as aquatic plants, may also be sufficient for veliger settlement and adult development. Conversely, Berkman et al. (1998) found that zebra mussels in Lake Erie were able to colonize softer sediments by creating conglomerates with their byssal threads. Mellina and Rasmussen (1994) found most of the variability in zebra mussel abundance was attributed to substrate size in the St. Lawrence and Hudson Rivers and Lake Oneida.

Calcium
Lastly, water hardness and specifically calcium, is believed to be one of the most significant factors limiting zebra mussel distribution (Mellina and Rasmussen 1994; Whittier et al. 2008). Calcium plays a critical role in several aspects of the biology of zebra mussels. Physiologically, it is an essential ion in many cellular processes. Dietz et al. (1994) showed that mussels maintain a blood calcium level of 1 mM and are able utilize calcium from their shells to elevate blood calcium levels when exposed to low calcium levels. Calcium is also a critical component of bivalve shells in the form of calcium carbonate (Dietz et al., 1994). Vinogradov et al. (1993) found calcium levels below 10-12 mg/L was unsuitable for normal mussel calcium metabolism. Hincks and Mackie (1997) found that increased shell growth occurred with increasing calcium levels. This is particularly significant during early larval stages, where veligers first develop a calcium carbonate shell to survive. Sprung (1987) found that larvae failed to develop in the absence of calcium. Hincks and Mackie (1997) found in water with calcium levels below 10 mg/L, no veligers were produced, there was little to no growth in juveniles, and adults died within 35 days.

Numerous studies have indicated varying thresholds of calcium needed for mussel survival. European studies reported that zebra mussels required high calcium thresholds. Ramcharan et al. (1992) reported calcium threshold levels of at least 28.3 mg/L calcium. In a survey of 527 lakes in Belarus mussels were only found in lakes with at least 25.4 mg/L calcium (Karatayev 1995, cited in (Cohen and Weinstein 2001). In North America, reported minimal calcium thresholds are lower. Mellina and Rasmussen (1994) found limited mussel abundance in the St. Lawrence River when with calcium levels below 15 mg/L. (Whittier et al. 2008) classified ecoregions at risk as very low (<12mg/L), low (12-20 mg/L), moderate (20-28 mg/L), and high (>28mg/L). McMahon (2015) used 12 mg/L as the critical calcium threshold. Hincks and Mackie (1997) reported calcium threshold levels as low as 8.5 mg/L. Based on an extensive literature review, (Cohen and Weinstein 2001) concluded that >28 mg/L calcium allowed for abundant, reproducing populations. Calcium between 20-28 mg/L allowed for good adult survival and embryonic development and growth rates comparable to higher calcium levels. There was little experimental or field data regarding zebra mussel survival or reproductive capabilities between

15-20 mg/L. Below 15 mg/L, some experiments showed good adult survival but lower growth rates and reported loss of calcium. Minimal, to no, reproductive success either in larval development or veliger survival was found below 15 mg/L (Cohen and Weinstein 2001).

One potential explanation for the difference between European and North American thresholds is that higher European levels may represent concentrations needed for successful reproduction and larval development while the lower North American thresholds predict mussel survival but not necessarily reproductive populations (Cohen and Weinstein 2001). Alternatively, North American zebra mussels may be genetically distinct (e.g. founder effects) from European mussels and have a lower calcium threshold (Cohen and Weinstein 2001).

It is important to note the critical relationships with calcium concentrations and other viabilities. First, pH can have profound effects on calcium utilization with calcium thresholds declining with increasing pH levels (Ramcharan et al., 1992). Thus, maintenance of pH within the parameters conducive to zebra mussel survival is critical to their ability to become established and survive. Second, magnesium and calcium can have synergistic effects, with improved survival in lower calcium water with sufficient magnesium levels (Dietz et al., 1994). McMahon (2015) concluded that calcium concentration, along with pH and summer surface water temperature, were the most critical risk assessment factors.

METHODS

Selected Reservoirs

Fifteen lakes/reservoirs, termed lakes for this report, were selected for use in this study (Table 1). The lakes were selected based on three criteria: (1) Calcium levels above or below the 12 mg Ca/L threshold (2) Lakes that were currently infested or have had no reported sighting of zebra mussels, and (3) Lakes from diverse Texas river basins (Table 1). These sites include water infested with mussels (positive control), positive for mussels above the threshold, above the threshold but uninfested, and water below the calcium threshold. Seven river basins are represented in this sample group. The lakes were placed into three Risk Groups: High – Calcium levels reported to be above 20 mg/L or are infested with zebra mussels, Moderate – Calcium levels between 12 and 20 mg/L and have no reported zebra mussel infestations, and Low – calcium levels below 12 mg/L (Table 1). The calcium range of 12 - 20 mg/L encompasses the two reported lower limits (12 mg/L and 20 mg/L) for zebra mussels (Neary and Leach 1992; McMahon 1996). This grouping mirrors the US Army Corps of Engineers Risk Status (USACE 2013) assessment for reported lakes, except that Lakes Palestine and Fork were placed in a Moderate based on reported calcium levels.

Water Collection

Waters at all locations were collected in the following manner. Surface water (< 1m) was collected from piers extending into lakes to avoid shoreline sedimentation. Water was collected using surface casts of a 5-gallon bucket. Collected water was passed through a 55-um felt filter bag into 7-gallon Reliance Aqua-Trainer carboys. Between 28-35 gallons of water was collected per lake. Water temperature and dissolved oxygen was measured at each site using a YSI EcoSense ODO 200 Dissolved Oxygen/Temperature meter. The collections consisted of five,

1-2 day collection trips with each trip typically involving 3 locations. At the conclusion of each collection trip, water was returned to Texas Christian University and stored in 20-gallon Bure plastic containers with lids and in a cold room at 5°C with aeration. Water from the 15 lakes was collected twice. Collection I occurred between May 28 to June 7, 2021. Specific location and collection dates are found in Table 2. Collection II occurred between September 27 to October 8, 2021. Specific location and collection dates are found in Table 2. Water collection I was used for Experiments I and II while water collection II was used for Experiment III and reproductive studies.

Mussel Collection

Mussels were collected from Lake Travis, TX near the Mansfield Dam. They were transported to TCU and maintained at room temperature (~20°C) in dechlorinated tap-water for 1-2 weeks prior to use.

Table 1. Grouping of 15 evaluated lakes.

Lake	Risk Grouping	Basin	Calcium Level	USACE Risk Status[a]	TPWD Invasion Status[b]
Belton	High	Brazos	Above (>20mg/L)	Not evaluated	Infested
Possum Kingdom	High	Brazos	Above (>20mg/L)	Not evaluated	Inconclusive
Waco	High	Brazos	Above (>20mg/L)	Not evaluated	Undetected/Negative
Buchanan	High	Colorado	Above (>20mg/L)	Not evaluated	Infested
Eagle Mountain	High	Trinity	Above (>20mg/L)	High	Infested
Joe Pool	High	Trinity	Above (>20mg/L)[a]	High	Undetected/ Negative
Worth	High	Trinity	Above (>20mg/L)	Not evaluated	Infested
Tawakoni	High	Sabine	Above [a]	High	Inconclusive
Coffee Mill	Moderate	Red	Low range 12-20 mg/L	Not evaluated	Unknown/ Not Monitored
Bonham	Moderate	Red	Low range [a] 12-20 mg/L	Moderate[a]	Unknown/ Not Monitored
Fork	Moderate[b]	Sabine	Low range [a] 12-20 mg/L	Low	Inconclusive
Palestine	Moderate[b]	Neches	Low range [a] 12-20 mg/L	Low	Inconclusive
Cedar Creek	Moderate	Trinity	Low range [a] 12-20 mg/L	Moderate	Undetected/ Negative

Caddo	Low	Cypress	Below (<12 mg/L)	Not evaluated	Unknown/ Not Monitored
Sam Rayburn	Low	Neches	Below a	Low	Undetected/ Negative
High – Calcium levels > 20 mg/L or infested status Moderate – Calcium levels between 12 and 20 mg/L, no reported infestation Low - Calcium levels below 12 mg/L, no reported infestation					
a – USACE reported values b – Grouping differs from USACE risk status					

Table 2. Lake Water Collection II – Water was collected from the 15 lakes from Sept. 27 to Oct. 8, 2021.

Date	Reservoir	Temp. (°C)	DO (%)	Conductivity (µS/cm)	Notes
9/27/21	Joe Pool	25	79.7	NA	Lloyd Park Boat Ramp
9/28/21	Cedar Creek	95.5	25.9	172	Tom Finley Park
9/28/21	Palestine	27.6	108	209	Lake Palestine Motor Lodge
9/28/21	Sam Rayburn	253.9	94.3	126	Cassels-Boykin Boat Ramp
9/30/21	Waco	25.7	79.6	2967	Midway Park Pier
9/30/21	Belton	27.5	79	385	Temple State Park
9/30/21	Buchanan	27.8	86	483	Big Chief RV Resort
10/4/21	Bonham	26.8	102.9	134	Lake Bonham Rec Area
10/4/21	Coffee Mill	26.7	98	175	Coffee Mill Lake Rec Area
10/7/21	Tawakoni	25.7	107	189	Tawakoni City Park
10/7/21	Fork	26.5	105.9	162	FM 17 Public Boat Ram
10/7/21	Caddo	27.4	93.8	120	Johnson Ranch Marina
10/8/21	Possum Kingdom	26.6	162	162	BRA Area #3 – 1775 FM 2951
10/8/21	Eagle Mountain	25.7	73.3	381	Twin Points Park
10/8/21	Worth	26.5	97.3	386	Fort Worth Boat Dock

Mussel Measurements

Mussel weight was determined by drying individual mussels using Kimwipe tissues and weighing using a Mettler Toledo™ MS-TS Analytical Balance. Depending on experiment, either individual weights (Experiments I, III) or groups weights (Experiment II) were measured. Mussel height, width and length was measured by determining maximum linear diameter. Measurements were determined using either a Mitutoyo Digital Caliper with 0.001" accuracy or digital image analysis. For digital image analysis, Digital photographs were taken of each mussel with a Shinwa H-3412A metric engineering rule in the image for scale. Mussel width and length were determined using ImageJ Image Processing and Analysis in Java software

(Schneider, Rasband et al. 2012) by the determining maximum length utilizing the ruler image to set calibration (Figure 1).

Figure 1. Digital image of a zebra mussel with lines indicating length (yellow) or width (teal). Markings to the right is the scientific ruler used to determine scale.

Feeding and Monitoring

Mussels were monitored three times weekly which included feeding, removal of dead mussels, and changing/adjusting water levels as needed. Mussels were fed 18 µl of Shellfish Diet 1800 (Reed Mariculture) per container with a calculated rate of 0.06ml algae/gm wet weight of mussel tissue per feeding. Dead mussels were removed, and their date of death recorded. For the growth study (Experiment III), length and width was determined using the digital calipers as described above (digital imaging of dead mussels was not possible due to gaping). No new mussels were added. At the end of the trials, the remaining mussels were collected, and final survival rates determined.

Water Quality Analysis

Temperature and dissolved oxygen were determined using a YSI EcoSense ODO 200 Dissolved Oxygen/Temperature meter. Conductivity was determined using a YSI 30 Salinity/Conductivity/Temperature meter. pH was determined using a Mettler Toledo SevenCompact pH meter. Total dissolved calcium was determined using a YSI TruLab pHISE1320 with a calcium ion selective electrode probe using standard additional procedure. Due to complicating events described below (Appendix I) and the need to commence work, water quality analysis capabilities was limited and varied by experiment as described for each experiment.

Survival Experiment I

Experimental Design

This initial experiment measured survival of mussels over an 84-day period. To hold mussels during the survival trial, 750-ml rectangular (22 x 16 x 5 cm) polypropylene containers with lids were filled with water from each of the 15 lakes to a volume of approximately 500-ml. Each container was loosely covered to reduce evaporation and gently aerated with an air stone.

Containers were kept in an environmental chamber at 19 °C in a 14/10 hour light/dark cycle. Ten mussels ranging in size from 10 – 30 cm were added to each of the containers. Each mussel was placed in a 50-mm Beautyflier Mesh Net Plant aquaponic cup to keep them separated. A total of 4 replicates of each lake were established for a total of 40 mussels per reservoir and a total of 600 mussels. Each trial of 15 lakes was randomly distributed on a single shelf within the environmental chamber. A fifth replicate was started but terminated due to an unknown contamination effecting a portion of the containers that were nearest a recirculating fan for the chamber – the probable source. None of the other replicates showed any signs of contamination.

Water Quality Analysis

Due to unavailability of equipment described below (Appendix I) and the need to commence work, Survival Experiment I had to be conducted with limited water quality analysis capabilities. Ultimately, temperature, dissolved oxygen, and conductivity were measured weekly during the trial. As desired, there was no significant difference in temperature (mean = 19.5±0.7°C) (ANOVA, P=0.9188) or dissolved oxygen (mean = 84.7±1.0%) (ANOVA, P=0.6080) between containers. I was only able to provide a calcium and pH analysis at the completion of Experiment I of the water in the holding barrels (termed source water) used to supply water to the containers (Table I). This indicates the water condition of the water supplied to the containers for the trials (source water) but not any potential changes within the individual container during the trials. The source water calcium levels for each lake fell within the reported or expected range for their groups (High – Calcium levels > 20 mg/L; Moderate – Calcium levels between 12 and 20 mg/L; Low - Calcium levels < 12 mg/L) except for Moderate lakes (Palestine, Bonham, Fork) which were slightly below the 12 mg/L threshold.

Table 3. Water quality analysis of holding barrels at the end of Survival Experiment 1.

Lake	pH	Conductivity (µS/cm)	Calcium (mg/L)	Risk Group
Belton	8.11	380.2	44.8	High
Possum Kingdom	8.02	1037	58.11	High
Waco	8.13	351.4	41.6	High
Buchanan	8.22	503	34.18	High
Eagle Mountain	8.14	391.9	36.02	High
Joe Pool	8.05	431.6	43.53	High
Worth	8.12	401.3	40.9	High
Tawakoni	7.94	203.6	20.27	High
Coffee Mill	7.84	140.3	13.08	Moderate
Palestine	7.43	171.4	7.63	Moderate
Bonham	7.7	113.8	8.79	Moderate
Fork	7.58	166.8	8.53	Moderate
Cedar Creek	7.73	173.2	15.05	Moderate
Caddo	7.36	128.4	6.13	Low
Sam Rayburn	7.75	132.9	5.5	Low

Survival Experiment II

Experimental Design

This experiment measured survival of mussels over a 45-day period. There are several differences in design from Survival Experiment I. First, mussels were held in tall (14 cm tall x 10 cm in diameter) cylindrical polypropylene containers (Reditainer 32oz containers) with lids. The containers were filled with 500 ml of water from each of the 15 lakes. Each container was loosely covered to reduce evaporation and gently aerated with an air stone. Containers from each trial were randomly distributed on a shelf in the lab (not the environmental chamber used in Experiment I) with an ambient room temperature (18-19 °C) with natural light/dark cycle. Ten mussels ranging in size from 10-30 mm in length were added to each container. Mussel size range was evenly distributed into each container and the total weight of the mussels was determined before placement into the containers. Indicating equivalent biomass, no significant difference in group weight was found between containers in the three trials (Figure 2) (ANOVA, P=0.5083). A total of 3 replicates of each lake were established for a total of 30 mussels per reservoir and a total of 450 mussels.

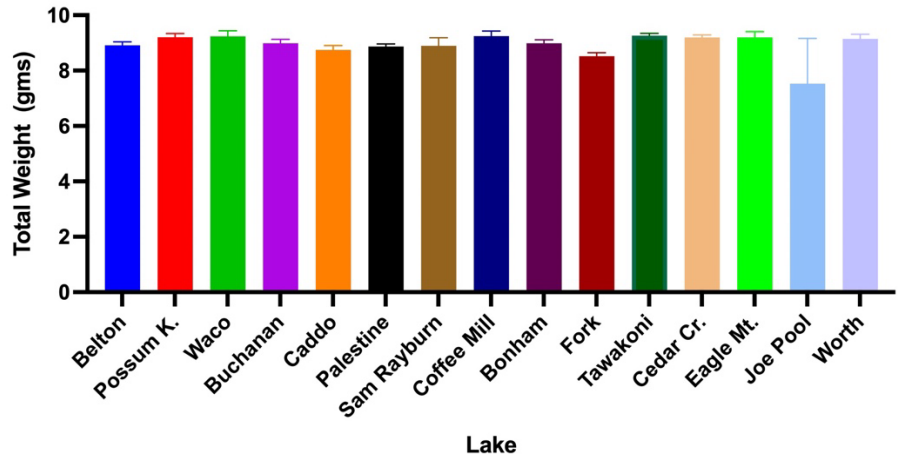

Figure 2. Mean weight of 10 mussels placed collectively into each 1-Qt container. There was no significant difference in total mussel weight between the containers. Bar- SEM

Survival and Growth - Experiment III

Experimental Design- This experiment looked at both survival and growth of mussels over a 63-day period. Mussels were individually weighed, and length and width determined using digital analysis described above (Figure 1). Individual mussels were placed into labeled 50-mm Beautyflier Mesh Net Plant aquaponic cups arranged in two tiers of four cups (Figure 3) to keep them separated. Semirigid polyethylene mesh (Pentair) covers were used to help restrict mussel movement.

Water analysis for Experiment III

Water analysis was performed on the source water (Water collection 2 (Table 4) at the start of the experiment. All High Risk lakes had calcium levels above the 20 mg/L threshold, all Moderate Risk lakes fell between 12-20 mg/L except for Lake Fork (slightly below 12 mg/L), and both Low Risk lakes were below 12 mg/L in the source water. Dissolved oxygen, temperature,

and conductivity were measured for all trials every 1-2 weeks. Calcium levels were measured for an individual trial each week with each trial being measured at least twice.

Figure 3. Two-tiered isolation compartments. Hydroponic cups were arranged in tiers of 4 cups with a central air stone (not visible at end of airline positioned in between lower 4 cups). A total of 3 replicates of each lake were established for a total of 24 mussels per lake and a total of 360 measured mussels.

Table 4. Water quality for Collection Trip 2. Water was used for both the Survival and Growth Experiment III and the Veliger and Reproduction experiments. No appreciable change in water quality (Ca, pH, Conductivity) occurred during storage in the cold room.

Lake	pH	Conductivity (µS/cm)	Calcium (mg/L)	Risk Group
Belton	7.82	381.2	38.8	High
Possum Kingdom	7.94	1491	78	High
Waco	7.61	326.2	34.1	High
Buchanan	8.2	465.5	29.04	High
Tawakoni	7.15	197.1	20.77	High
Eagle Mountain	7.53	361.7	27.76	High
Joe Pool	8	430.6	43	High
Worth	7.68	373.3	39.67	High
Coffee Mill	7.9	178.6	12.12	Moderate
Cedar Creek	7.28	203	16.07	Moderate
Palestine	6.95	174.9	10.29	Moderate
Bonham	6.95	138.7	12.14	Moderate
Fork	7.23	161.9	8.635	Moderate
Caddo	7.08	121.2	5.997	Low
Sam Rayburn	7.46	128.3	8.307	Low

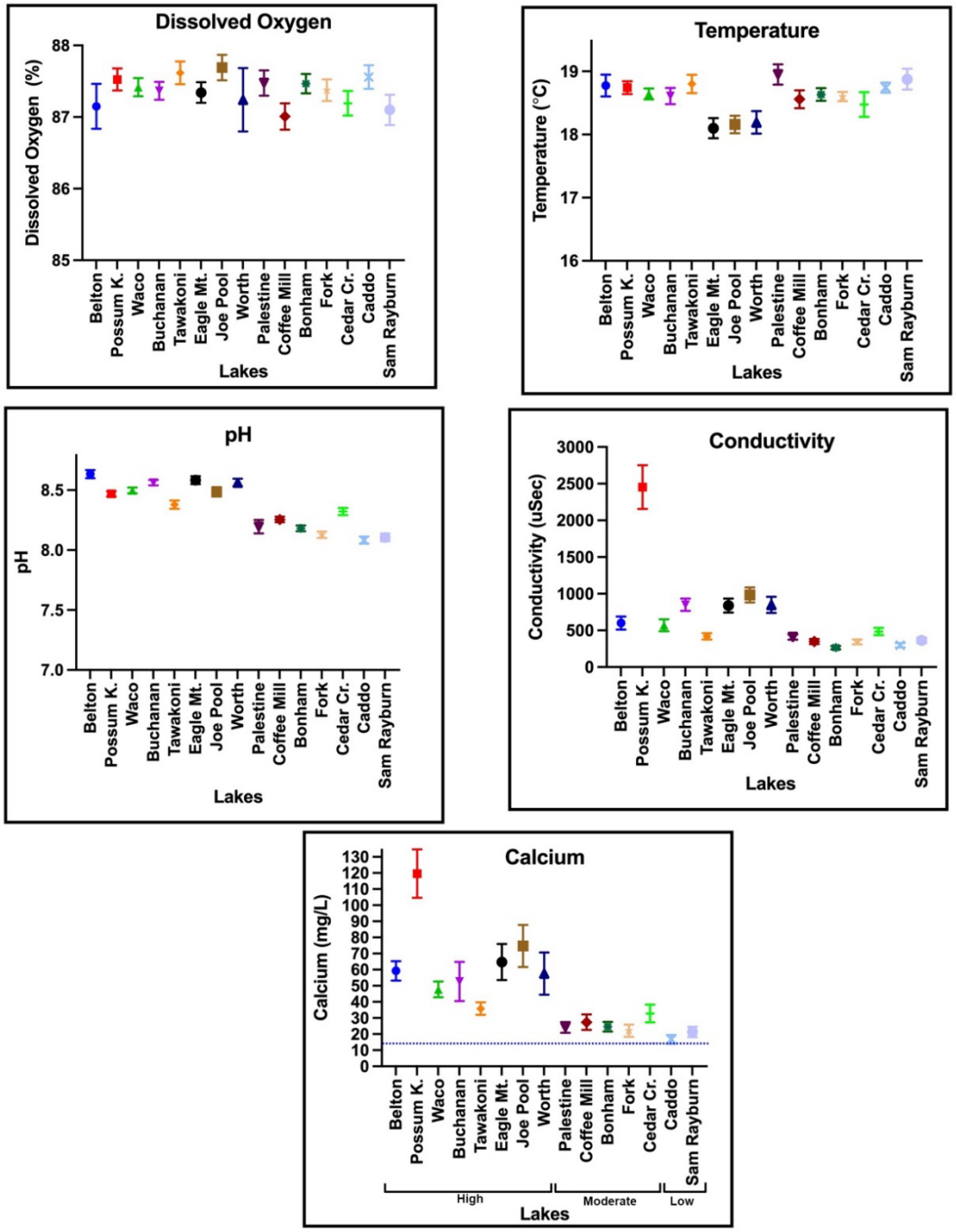

Figure 4. Water quality comparison between lakes during Experiment III. Samples were taken from each of the 45 containers during the experiment. Symbols indicate mean (± SEM) for each parameter for each lake. Replicates are as follow: Dissolved oxygen n=13, Temperature n=13, pH n=12, Conductivity n=12, Calcium n=6. Line on calcium graph indicates the 12 mg/L threshold. Risk grouping indicated on calcium graph.

Veliger Survival Trial

Veligers collection

Veligers were collected from Lake Travis near Mansfield Dam using a 64-μm plankton net on Dec.7, 2021. Collected veligers were transfer to Texas Christian University and maintained overnight in Lake Travis water with aeration.

Veliger identification and distribution

Prior to distributing veligers, 250-ml flasks containing 200 ml lake water for each of the target lakes were covered with a foam stopper and aerated with a Pasteur pipet attached to an airline for gradual aeration and water circulation. The day following collection, water containing the veligers was poured into a 60-mm glass petri dishes for selecting veligers. These aliquots were viewed using a Nikon stereomicroscope equipped with cross-polarizing filters. The cross-polarized light was utilized to help identify veligers due to the birefringent nature of their shells (Figure 5). They were confirmed as veligers and not birefringent ostracods due to the presence of a ciliated velum in veligers and the prominent eyespots and jointed appendages characteristic of ostracods. Live veligers were individually removed from the petri dish using a glass Pasteur pipet and transferred to the 250-ml flasks. Twenty-five live veligers were transferred into each flask. To verify that only live veligers and not dead or empty shells were transferred, each veliger identified by cross-polarized light was observed for one minute or until detectable movement was observed. A veliger that was motile or exhibited obvious internal movement (distinguished from bacterial or Brownian motion) was considered live and utilized. Any veliger/shell that did not move was discarded. All veligers transferred were early veliger stages (D-shaped or umbonal stages).

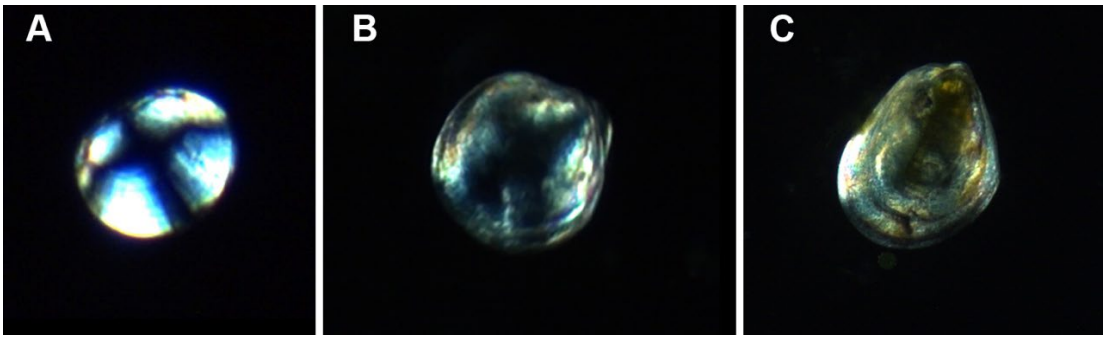

Figure 5. Representative example of a transferred veligers showing birefringent cross under cross- polarized light. (A) Empty veliger shell. (B) Representative photo of live, early veliger at transfer (day-2 veliger from Possum Kingdom trial) (C) Representative photo of a larger, later staged veliger (from Possum Kingdom trial).

Veliger maintenance and evaluation

The flasks containing veligers were transferred to an environmental chamber at 18°C and a 14:10 light/dark cycle. Veligers were fed Isochrysis microalgae (ISO 1800, Reed Mariculture) (approximately 40 million cells) every other day. Survival of veligers in the various lake waters

was examined over 19 days. Early (D-shaped or umbonal) veligers were observed for survival. Veliger cultures were examined at 2, 6 and 19 days for survival. A minimum of 10 veligers were randomly sampled and examined to determine live/dead status on days 2 and 6. To reduce handling stress on veligers, determination of veliger survival was conducted periodically by pouring a subsample of the flask water into a 60-mm petri dish and observing veligers under the Nikon Stereoscope. After counting, the veligers were returned to their flasks. Survival was determined as described above. The trial was terminated after 19 days due to typically low survival rates of cultured veligers (Stoeckel et al. 2004).

Fertilization Trials

Spawning - Spawning of mussels followed standard techniques used in our lab (Misamore, Silverman et al. 1996). Briefly, gravid zebra mussels were collected from Lake Travis in June, 2021 and stored at 10°C until use. The day prior to spawning, individual mussels are separated and placed into individual cups containing cold artificial pondwater and allowed to warm up to room temperature overnight. This helps stimulate spawning as well as ensure that no unexpected gametes are present prior to the experiment. The day of spawning, the zebra mussels were rinsed with deionized water and place into 24x95-mm, flat-bottom test tubes. The mussels were covered in a 1-mM solution of serotonin made with the various lake waters. The mussels remained in the serotonin/lake water solution for 20 minutes then were rinsed twice with deionized water and resubmerged in the appropriate lake water (minus serotonin). A total of 24 mussels were spawned per lake and used for fertilization trials. Males started spawning approximately 10 minutes after transfer back into the lake water (minus serotonin) and were allowed to spawn in the test tubes. Once females started spawning, typically after 45 min, they were removed from the test tubes and transferred individually into 70x50-mm crystalizing dishes containing the appropriate lake water. The larger spawning dishes are required to reduce egg clumping and damage during spawning (Misamore et al. 1996).

Fertilization

To perform fertilizations, eggs and sperm collected from isolated mussels in the same lake water were mixed. Eggs were collected from the crystalizing dishes using a 1-ml wide bore pipet. A total of 4 ml of lake water containing eggs was transferred to a 10-ml glass beaker. Sperm suspension was collected for the male spawning tubes and sperm concentration determined using a hemocytometer. The sperm concentration was adjusted to 2 x 106 sperm by adding additional lake water. For inseminations, 1 ml of sperm suspension was added to the 4 ml of eggs in the 10 ml beakers. A fertilization trial consisted of a single male and female. Replicates were performed using different males and females. After insemination, 0.5-ml aliquots were taken from the dishes and fixed at various time points postinsemination. Samples were fixed 1:1 with 3.2% paraformaldehyde in mussel buffer (Misamore and Lynn 2000). Samples were fixed at 5 min to determine sperm binding, 20 min to determine sperm entry, and 90 min to determine cleavage at the two-cell stage. Approximately 24 hr later, the number of developing embryos was evaluated in the fertilization beakers.

Sperm Binding

To evaluate sperm binding to eggs while in the various lake waters, the numbers of sperm bound within an equatorial focal plane were determined from the 5 min postinsemination time points. Using an equatorial focus allows for precise determination of sperm binding to the egg surface in contrast to sperm just closely associated with the egg surface but not bound (Fallis, Stein et al. 2010). A minimum of 30 eggs from three independent crosses were scored for numbers of bound sperm. The three replicate crosses were performed between unique individuals.

Sperm Entry

To evaluate sperm entry, samples fixed at 20 min postinsemination were stained with the DNA-specific fluorochrome Hoechst 33342 (1μg/ml) for 15 min followed by two washes with mussel buffer (Misamore and Lynn 2000). Eggs were viewed using a Zeiss Axiovert 200 epifluorescent microscope to determine if sperm were incorporated into the egg cytoplasm. Sperm that have entered the egg cytoplasm will begin nuclear decondensation inside the egg cytoplasm. This gives incorporated sperm DNA a very characteristic diffuse-circle appearance relative to the compact nucleus of bound sperm (Misamore, Stein et al. 2006). Thirty eggs were counted for each cross.

Zygotic Cleavage

Approximately 60 min after fertilization, zebra mussel zygotes (fertilized eggs) divide for the first time. Cell cleavage is an early indicator that fertilization was success and embryo development is commencing. To evaluate first cleavage, eggs were fixed at 90 min postinsemination and the number of two -cell embryos was determined. A minimum of 30 eggs per trial were examined.

Lakes Evaluated

Due to the delayed timetable described below (Appendix I), I was only able to evaluate five of the lakes before reproduction stopped. The following lakes were evaluated: Belton, Possum Kingdom, Waco, Buchanan, Caddo. After this time (late July), the gravid mussels collected in late spring and held in my spawning tanks failed to spawn. Repeated attempts at spawning in several of the remaining lake waters, as well as in artificial pondwater (as a control to confirm the issue was mussel reproductive status), yielded too weak of gametic output to conduct experiments. Multiple attempts to collect fresh mussels at varying locations from August – November did not yield gravid individuals. This was unfortunately not unexpected given the seasonality of reproduction particularly in late summer months. Thus, I was only able to get to the first 5 of the 15 lakes to be examined. As described below, I plan to complete the fertilization trials in the remaining lakes starting in spring 2022. As a side observation, many of the larger, surface mussels collected at <10 ft depth from Lake Travis were dead by mid-summer. These mussels previously were strong spawners. Only smaller, presumably < 1 year old, mussels appeared to survive the elevated surface summer temperatures.

RESULTS

Survival Experiment I

After 84 days, mean mussel survival was greater than 50% of mussels in water from all lakes. As indicated, the only specific calcium numbers I was able to obtain during the trial were the end values of the supply water used to fill the containers (Table 3). Using these values as an indicator of minimum calcium levels, a simple linear regression showed a significant relationship between calcium and survival. Linear regression analysis performed on arcsin transformation of percent survival showed a significant relationship between calcium concentration and survival ($p<0.001$). Figure 6 graphs the untransformed data. It must be re-emphasized that the calcium levels used are minimum calcium levels of the source water used to fill the containers and does not account for potential changes in calcium in the 60 individual containers.

To evaluate survival rates in the specific lakes, the date of death of mussels was recorded. The data from all four trials for each lake were pooled and the survival probability rates for each lake were generated by a Mantel-Cox test using Graph Pad Prism. A statistically significant difference was found between the lakes ($p<0.0001$)(Figure 7).

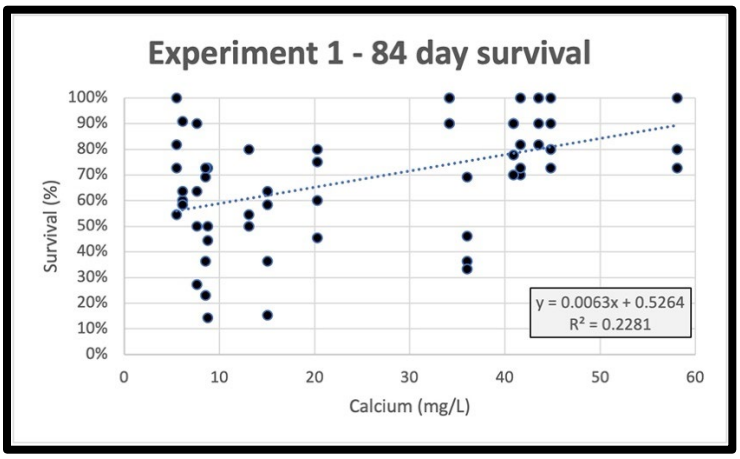

Figure 6. Simple linear regression of percent survival of mussels after 84 days relative to source water calcium levels (Table 2).

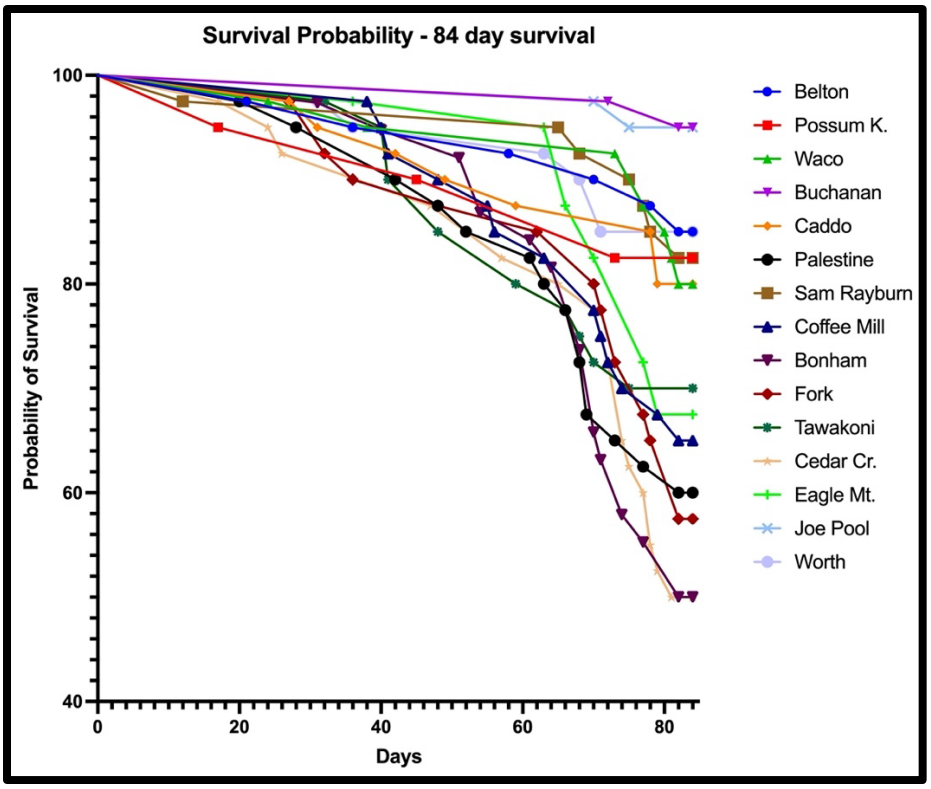

Figure 7. Probability of survival for individual lakes after 84 days based on a Mantel-Cox test. Data from all four trials was pooled so that each lake consisted of survival times of 40 mussels.

Individual Mantel-Cox comparisons between lakes found significant differences in survival probability. Table 5 shows the significant differences between each lake. Rankings of lakes based on their mean percent survival can be found in Appendix 2. When viewed by Risk Groups (Table 1), the mean survival rates were as follows (mean±SEM): High= 78.56±3.06 % (n=8 lakes), Moderate – 51.1±4.72% (n=5 lakes), Low – 72.75±5.89 % (n=2 lakes). There was a significant difference between groups with the Moderate Risk group significantly lower than the High and Low Risk groups (ANOVA, Tukey Multiple comparisons, $p<0.0001$). Focusing specifically on the two low calcium lakes grouped as Low Risk (Caddo, Sam Rayburn), they had mean survival after 84 days was 75 % and 82.5% respectively (Figure 8). Caddo had a significantly lower survival probability than two of the High Risk lakes (Buchanan, Joe Pool) but a significantly higher survival rate than three Moderate Risk lakes (Bonham, Fork, Cedar Creek) (Figure 7, Appendix 2). Lake Sam Rayburn had a significantly higher survival rate than three Moderate Risk lakes (Bonham, Fork, Cedar Creek) but was not significantly different than the Higher Risk lakes.

Table 5. Individual Mantel-Cox comparisons between lakes found numerous significant differences in survival probability. X - significant difference between lake ($p<0.05$). ns- no significant difference. Letter indicates Risk Grouping: L-low risk (<12 mg/L Ca), M – Moderate 12-20mg/L Ca, H- >20mg/L Ca.

	Possum K. H	Waco H	Buchanan H	Caddo L	Palestine M	Sam Rayburn L	Coffee Mill M	Bonham M	Fork M	Tawakoni H	Cedar Cr M	Eagle Mt. H	Joe Pool H	Worth H
Belton H	ns	ns	ns	ns	X	ns	X	X	X	ns	X	ns	ns	ns
Possum K. H		ns	ns	ns	X	ns	ns	X	X	ns	X	ns	X	ns
Waco H			X	ns	X	ns	ns	X	X	ns	X	ns	X	ns
Buchanan H				X	X	ns	X	X	X	X	X	X	ns	ns
Caddo L						ns	ns	X	X	ns	X	ns	X	ns
Palestine M						X	ns	ns	ns	ns	ns	ns	X	X
Sam Rayburn L							ns	X	X	X	ns	ns	ns	ns
Coffee Mill M								ns	ns	ns	ns	ns	X	X
Bonham M									ns	ns	ns	ns	X	X
Fork M										ns	ns	ns	X	X
Tawakoni H											ns	ns	X	X
Cedar Cr M												ns	X	X
Eagle Mt. H													X	ns

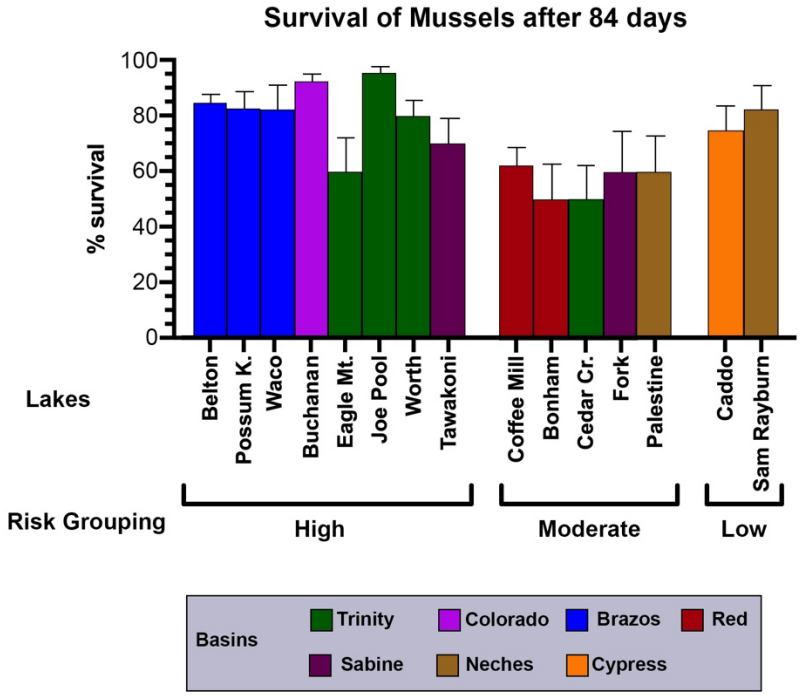

Figure 8. Mussel survival after 84 days in water from the various lakes. The mean value represents survival percentage of 10 mussels/container with 4 replicates per lake. Lakes are arranged by Risk Group described in Table 1. Bar-SEM.

Survival Experiment II

After 45 days, mean mussel survival ranged from a low of 70% (Cedar Creek) to a high of 96% (Joe Pool). As indicated, the only specific calcium numbers I was able to obtain during the trial was the end values of the source water used to fill the containers (Table 3). Using these values as an indicator of minimum calcium levels, a simple linear regression showed no significant relationship between calcium and survival. Linear regression analysis performed on arcsin transformation of percent survival showed no significant relationship between calcium concentration and survival (P=0.3643). Figure 9 graphs the untransformed data. It must be re-emphasized that the calcium levels used are minimum calcium levels of the source water used to fill the containers and does not account for potential changes in calcium in the 45 individual containers.

Unlike Experiment I which lasted 84 days, there was no significant difference in survival probability in Experiment II after 45 days between the lakes based on a Mantel-Cox test (P=0.3317) (Figure 10). All lakes showed relatively high survival in the 45-day time frame. Focusing specifically on the two low calcium/Low Risk lakes (Caddo, Sam Rayburn), their mean survival after 45 days was 90% and 80% respectively (Figure 11).

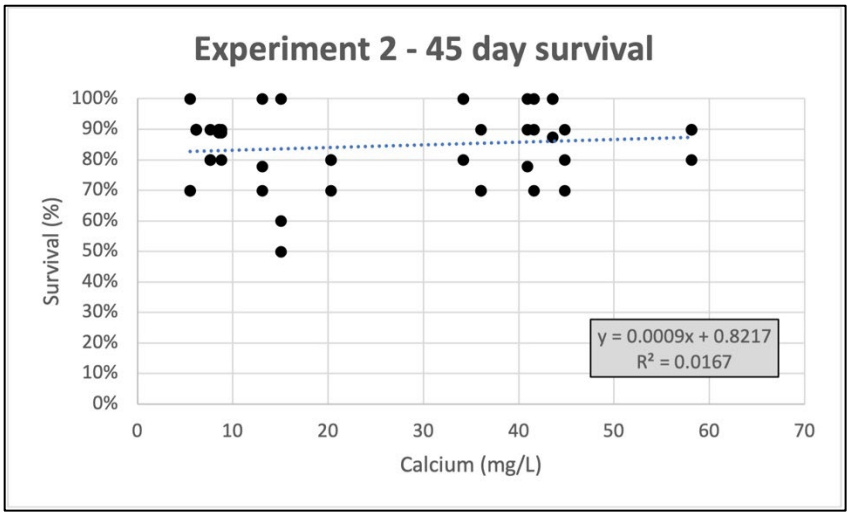

Figure 9. Simple linear regression of percent survival of mussels after 45 days relative to source water calcium levels (Table 4).

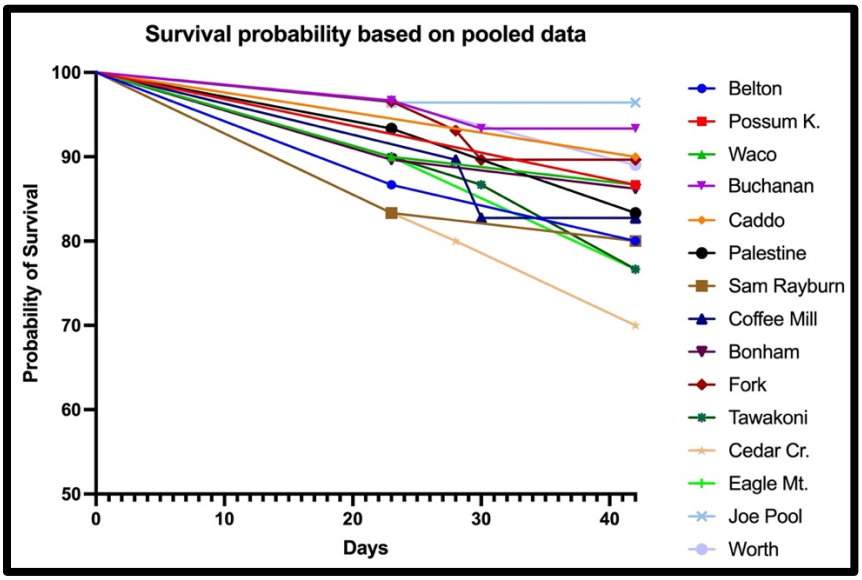

Figure 10. Probability of survival for individual lakes after 45 days based on a Mantel-Cox test. Data from all three trials were pooled so that each lake consisted of survival times of 30 mussels.

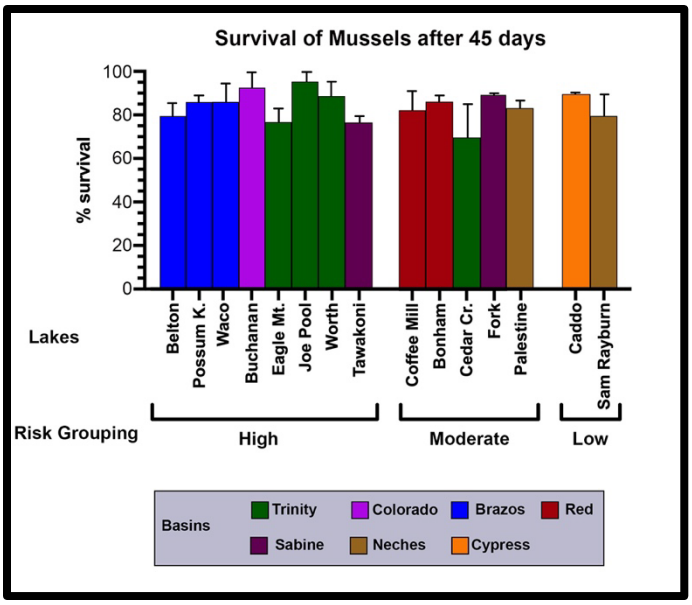

Figure 11. Mussel survival after 45 days in water from the various lakes. The mean value represents survival percentage of 10 mussels/container with 3 replicates per lake. Lakes are arranged by Risk Group described in Table 1. Bar-SEM.

Survival and Growth Experiment III

Survival

Survival over a 63-day period was examined. Linear regression analysis performed on arcsin transformation of percent survival showed no significant relationship between calcium concentration and survival (P=0.2494). Figure 12 graphs the untransformed data. The calcium levels were the average recorded calcium levels for each specific container.

To evaluate survival rates, the date of death of mussels was recorded. The data from all three trials was pooled and the survival probability rates for each lake was determined using a Mantel-Cox test. A significant difference was found between the lakes (Figure 13) ($p<0.0001$) with regards to mortality. Individual Mantel-Cox comparisons between lakes indicates significant differences particularly for Bonham, Buchanan, Sam Rayburn, Possum Kingdom, and Tawakoni.

Rankings of lakes based on their mean percent survival can be found in Appendix 3. When viewed by Risk Groups, the mean survival rates were as follows (mean±SEM): High= 55.84±5.0 % (n=8 lakes), Moderate – 47.37±5.17% (n=5 lakes), Low – 50.42±11.1 % (n=2 lakes). There was no significant deference in means between these 3 groups (ANOVA, P=0.5612). Focusing specifically on the two low calcium/Low Risk lakes (Caddo, Sam Rayburn), they had mean survival after 63 days was 44 % and 56% respectively (Figure 14). Caddo had a significantly lower survival probability than two of the High Risk lakes (Buchanan, Joe Pool) but a

significantly higher survival rate than three Moderate Risk lakes (Bonham, Fork, Cedar Creek) (Figure 7, Appendix 2). Lake Sam Rayburn had a significantly higher survival rate than three Moderate Risk lakes (Bonham, Fork, Cedar Creek) but was not significantly different than the Higher Risk lakes.

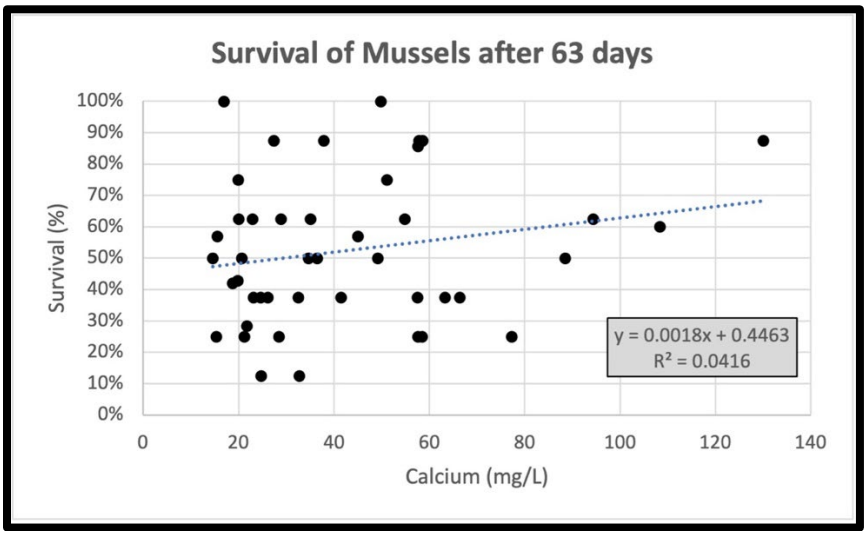

Figure 12. Simple linear regression of percent survival of mussels after 63 days. Individual points represent the percent survival of a container of 8 mussels. A total of 3 replicates of 15 lakes are included. Calcium levels are average recorded levels of calcium from the individual containers.

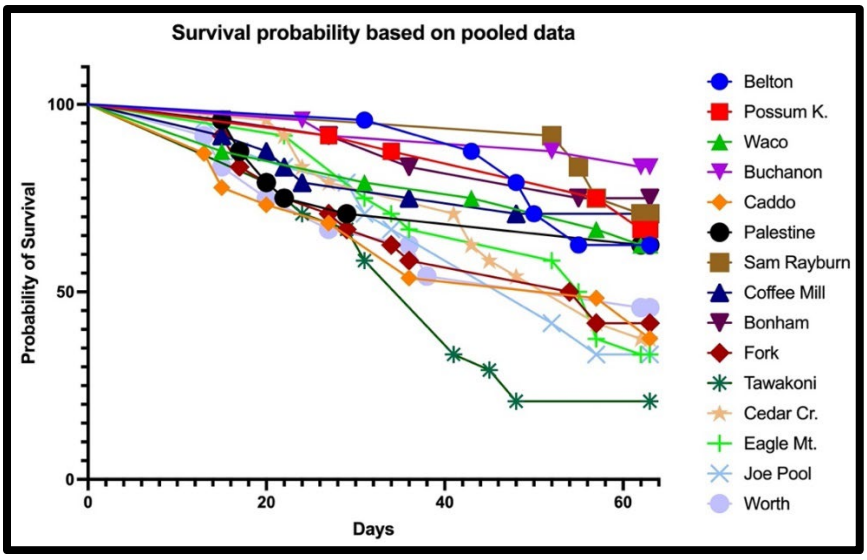

Figure 13. Probability of survival for individual lakes after 63 days based on a Mantel-Cox test. Data from all three trials were pooled so that each lake consisted of survival times of 30 mussels.

Table 6. Individual Mantel-Cox comparisons between lakes found numerous significant differences in survival probability. X - significant difference between lake ($p<0.05$). ns- no significant. Letter indicates Risk Grouping: L-low risk (<12 mg/L Ca), M – Moderate 12-20mg/L Ca, H- >20mg/L Ca.

	Possum K.[H]	Waco [H]	Buchanan [H]	Caddo[L]	Palestine [M]	Sam Rayburn [L]	Coffee Mill[M]	Bonham [M]	Fork [M]	Tawakoni [H]	Cedar Cr. [M]	Eagle Mt[H]	Joe Pool [H]	Worth [H]
Belton[H]	ns	ns	ns	X	ns	ns	ns	ns	X	X	ns	ns	X	ns
Possum K. [H]		ns	ns	X	ns	ns	ns	ns	X	X	X	X	X	ns
Waco[H]			ns	ns	ns	ns	ns	ns	ns	X	ns	ns	ns	ns
Buchanan[H]				X	ns	ns	ns	ns	X	X	X	X	X	X
Caddo[L]					ns	X	X	X	ns	ns	ns	ns	ns	ns
Palestine[M]						ns	ns	ns	ns	X	ns	ns	ns	ns
Sam Rayburn[L]							ns	ns	X	X	X	X	X	X
Coffee Mill[M]								ns	ns	X	ns	X	X	ns
Bonham[M]									ns	X	X	X	X	X
Fork[M]										ns	ns	ns	ns	ns
Tawakoni[H]											ns	ns	ns	ns
Cedar Cr. [M]												ns	ns	ns
Eagle Mt. [H]													ns	ns
Joe Pool[H]														ns

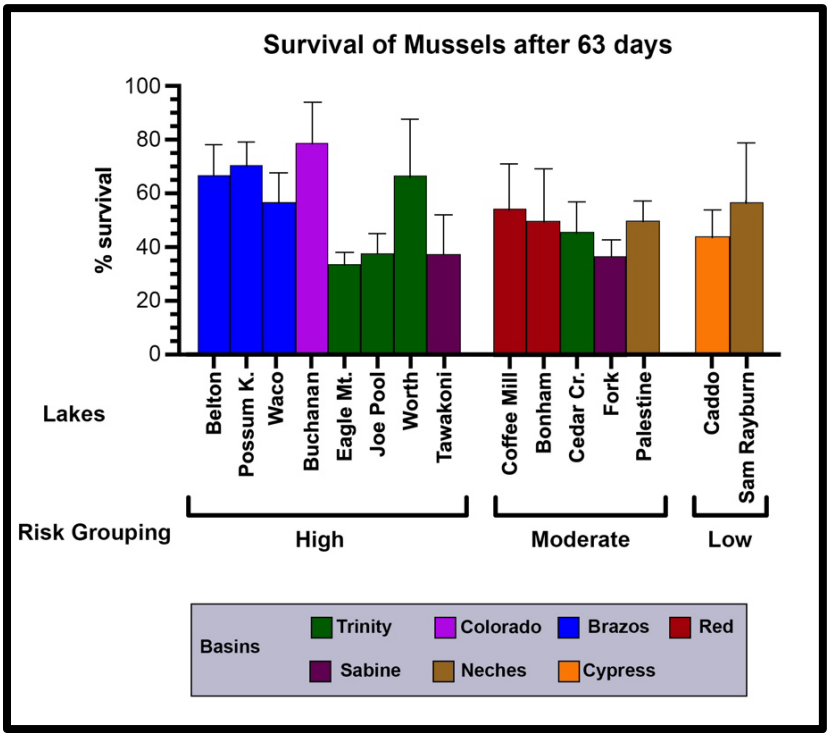

Figure 14. Mussel survival after 63 days in water from the various lakes. The mean value represents survival percentage of 8 mussels/container with 3 replicates per lake. Lakes are arranged by Risk Group described in Table 1. Bar-SEM.

Growth - Weight

To evaluate any change in growth, wet weights of mussels at the being and end of each trial were compared. Only mussels alive at the conclusion of the trial were weighed. Percent change in weight = (Initial Weight – final weight)/initial weight. There was no significant difference in weight change between lakes based on a Kruskal Wallis test (Figure 15) (P=0.2696).

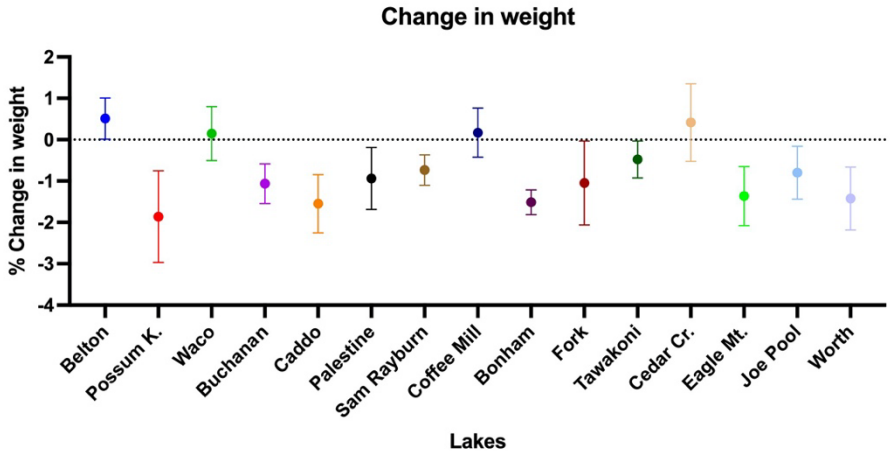

Figure 15. Change in weight during the 63-day trial. The change between initial and final weights of all mussels surviving the 63-day trial were calculated. Symbol indicates mean, bar - SEM.

Growth – Shell Length

To evaluate potential growth, we measured the change in shell length and shell width between the start and finish of the experiment (Figure 16). There was a significant difference in shell length between the lakes based on a Kruskal Wallis test (P=0.0103). Based on multiple comparisons, the one significant difference was found between the highest change (Coffee Mill) and lowest (Belton). The change in shell length was less than 2% for any lake.

We also measured the change in shell width between the start and finish of the experiment (Figure 17). There was no significant difference in shell width between the lakes based on a Kruskal Wallis test (P=0.3517).

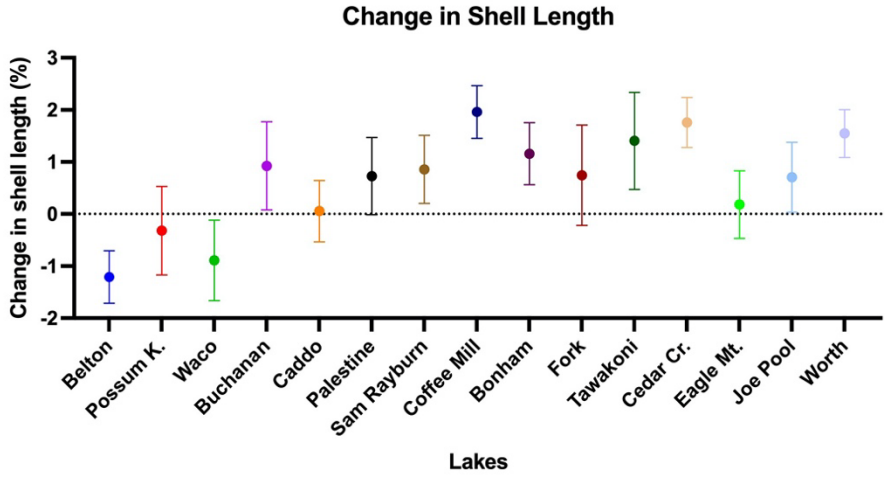

Figure 16. Change in shell length during the 63-day trial. The change between initial and final shell lengths of all mussels surviving the 63-day trial were calculated. Symbol indicates mean, bar - SEM.

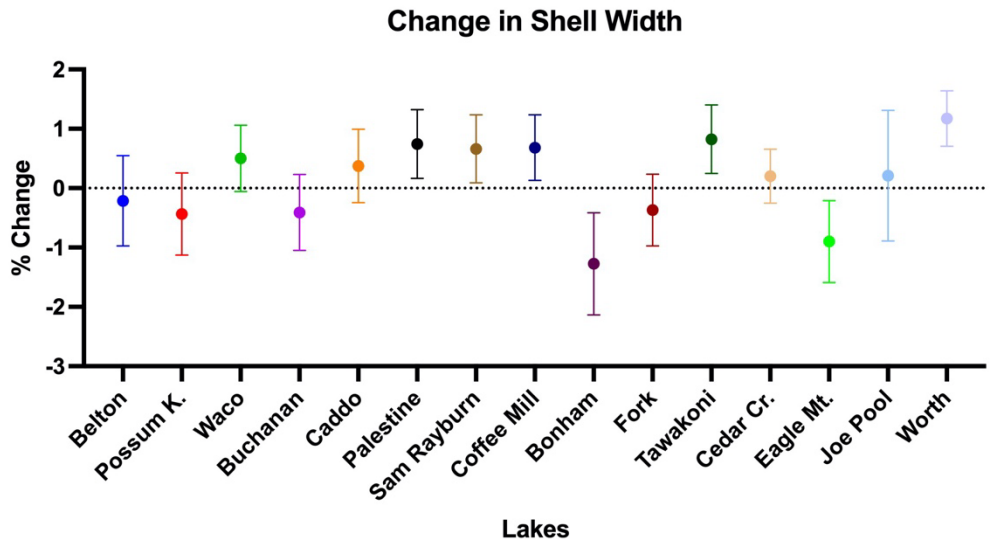

Figure 17. Change in shell width during the 63-day trial. The change between initial and final shell widths of all mussels surviving the 63-day trial were calculated. Symbol indicates mean, bar - SEM.

Veliger Survival Experiment

All lake showed relatively high survival after 2 days in the various lake waters (Figure 18). All veligers at this stage were small, D-shaped to Umbonal stage veligers (Figure 5). Based on a Mantel-Cox test there was a significant difference in survival between lakes (P=0.0005). At day 2, survival was high in all lake waters. By day 19, the highest survival was observed in Palestine and Sam Rayburn. Although only live/dead status was quantified, both lakes exhibited a high number of larger, later veliger stages (Figure 5). The lowest survival was observed in Lake Belton, Lake Caddo, and Lake Fork. All veligers in these groups were either empty shells (Belton) or dead early veligers. All others exhibited a mix of live veligers that included earlier stages as well as some with continued development. Several lakes had veligers that had transitioned (attached) to the bottom (Tawakoni, Eagle Mountain, Joe Pool). Most lakes showed a decline in survival at 19 days with survival less than 50%. When evaluating final percent survival after 19 days relative to calcium level, there was no significant relationship. Linear regression analysis performed on arcsin transformation of percent survival showed no significant relationship between calcium concentration and survival (P=0.9486). Figure 19 shows the untransformed data.

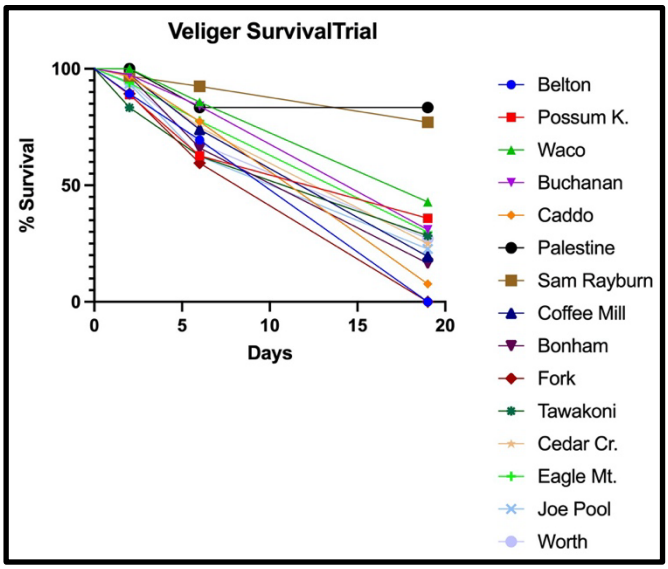

Figure 18. Survival of veligers in various lake waters. A single 19-day trial of veliger survival involving 25 live veligers per sample was conducted. Percent survival based on 10 randomly selected veligers on day 2 and 5. On day 19, all veligers were counted (minimum of 10 for any trial).

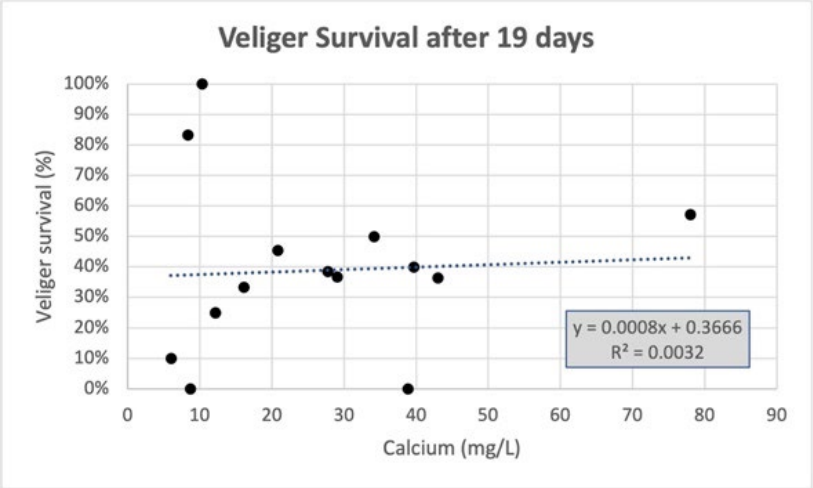

Figure 19. Veliger survival after 19 days relative to calcium concentration on the waters used for the experiment.

Spawning, Fertilization, and early development

Sperm binding

Both male and female zebra mussels successfully spawned in the water from the tested lakes (Figure 20). In all lake waters tested, sperm were able to swim and bind to eggs. The eggs showed no morphologically deformation suggesting no obvious acute effects of lake water quality on egg morphology, such as osmotic swelling or shrinking. There was no significant

difference between lakes when looking at mean number of equatorial bound sperm (Figure 21). This indicates that sperm were equally successful at reaching and binding to the egg surface in the tested lake waters. When looking a percentage of eggs had at least one bound sperm, there was a significant difference (Figure 21)(ANOVA, P=0.0043) between lakes. It must be noted that the quality of gametes observed in the Waco and Buchanan trials was poorer than the other lakes. This was evidenced by several eggs that prematurely activated (an event typically induced by sperm binding) prior to fertilization. This is often a sign that there were potential issues with the eggs being released at that time. Additional replicates for both Lakes Waco and Buchanan are needed before assumptions on their possible decrease in sperm binding. Nevertheless, sperm were able to successfully bind to the surface of the eggs in all lakes examined.

Sperm Entry

Sperm entry occurred in fertilizations in all lake waters. Sperm incorporated into the egg cytoplasm were visible as decondensing nuclei (Figure 22). There was a significant difference between lakes in percentage of eggs that contained sperm inside the cytoplasm (ANOVA, P=0.0008) (Figure 23). Possum Kingdom lake was significantly higher than the other lakes. Less than 12% of the eggs in both Caddo exhibited sperm entry. It is worth noting that Lake Caddo was significantly lower than Belton and Possum kingdom with regards to sperm entry, but was similar in the earlier sperm binding stage. Additionally, there were markedly more eggs (approximately 15-30%) with polar body formation but without sperm incorporation in the Lake Waco and Lake Buchanan samples relative to the other three lakes (less than 5%). This could indicate greater variability in gametes in the Lake Waco and Lake Buchanan trials relative to the other lakes.

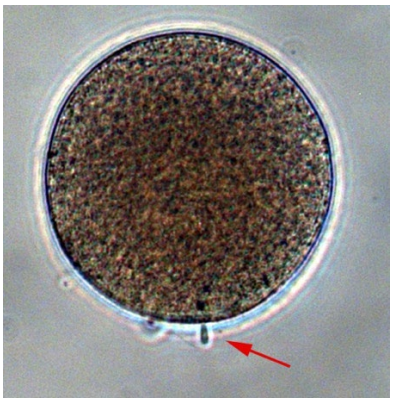

Figure 20. Micrograph of fertilized egg at an equatorial focus with a sperm bound to the egg surface (arrow).

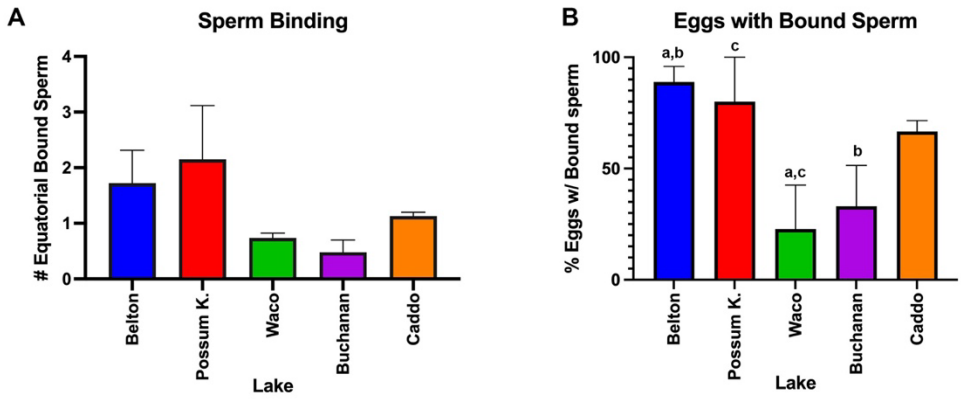

Figure 21. (A) Mean number of equatorial bound sperm per egg. There was no significant difference between lakes (P=0.3501). (B) Percentage of eggs that had at least one bound sperm. There was a significant difference between lakes (letters indicate significant difference. Bar – SEM, n=3 (except of Buchanan and Caddo, n=2).

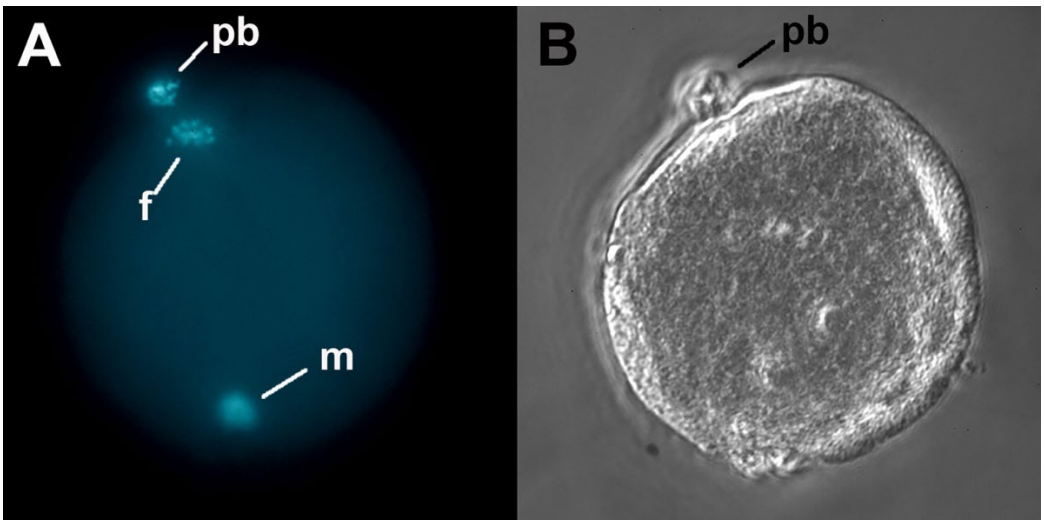

Figure 22. Fluorescent (A) and corresponding Phase Contrast (B) micrograph of a fertilized egg 20-min Postinsemination. (A) DNA specific staining shows the egg DNA (f) and extruded polar body (pb) indicated the egg has been activated. The sperm DNA (m) is evident inside the egg cytoplasm and has begun decondensing in preparation for uniting with the egg DNA. Pictured egg is from a Lake Belton fertilization.

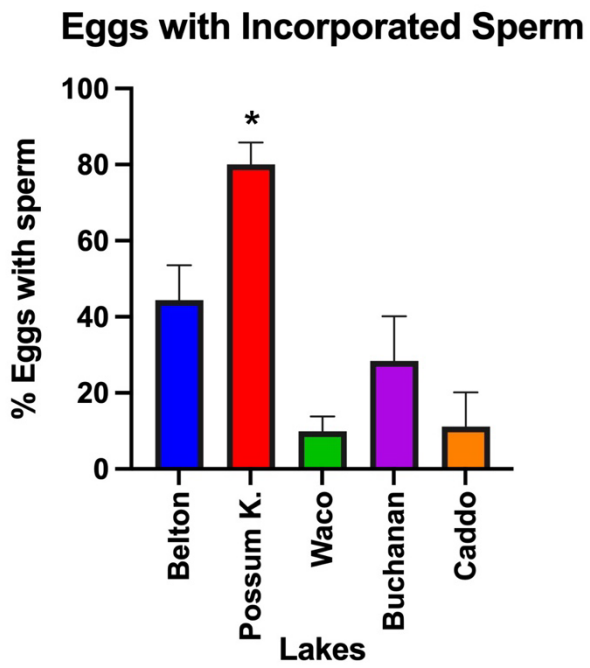

Figure 23. Sperm entry into the egg cytoplasm. Sperm were able to enter the egg cytoplasm in fertilizations in all four lakes tested. Possum kingdom was significantly different (*, $p<0.05$) than the other treatments. Mean numbers of 30 eggs with bound sperm, n=3 (except n=2 in Buchanan, Caddo).

Zygotic Cleavage

Approximately 60 min after fertilization, zebra mussel zygotes (fertilized eggs) divide for the first time (Figure 24). Cell cleavage is an early indicator that fertilization was successful and embryo development was commencing. Four of the five lakes were evaluated for cleavage. Due to the fewer eggs spawned during the Lake Buchanan trials, there were insufficient numbers of eggs remaining at this last sample point in the Lake Buchanan trials to adequately estimate zygote cleavage.

As seen with earlier stages of the fertilization process, Possum Kingdom Lake was significantly higher in cleaved egg relative to the other lakes (Figure 25) (ANOVA, P=0.0003). This would be expected given the higher rate of sperm entry seen in fertilization in Possum Kingdom water. Eggs were able to continue development following fertilization and cleave in Possum Kingdom Lake, Lake Belton, and Lake Waco. Lakes Belton and Waco were less than 20%. Interestingly, there were no observed 2-cell embryos in any of the Lake Caddo trials. Given the presences of sperm inside the egg cytoplasm (Figure 23), you would expect a subset of eggs to divide. Lake Caddo water was the only lake of the four lakes evaluated that had a calcium level below 10 mg/L (Table 3).

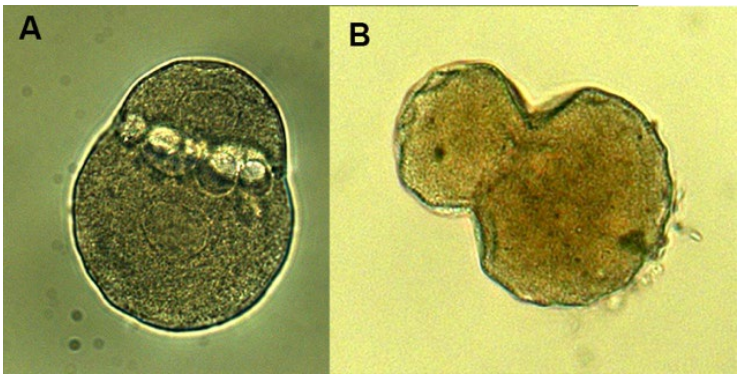

Figure 24. Light micrographs of eggs dividing to form two-cell embryos. (A) Early stage of cell cleavage with numerous circular vesicles at the site of cell division. (B) Cleavage completed forming a 2-cell embryo. A – Lake Belton specimen, B - Possum Kingdom specimen.

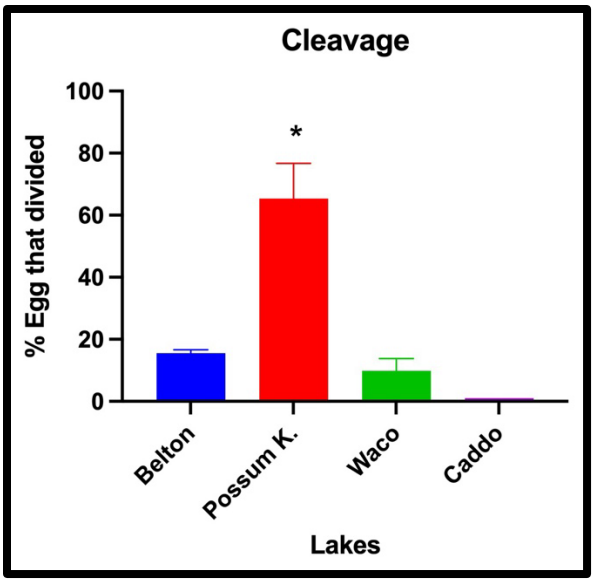

Figure 25. Fertilized eggs undergoing 1st cleavage. Percentage of eggs that divided to form 2-cell embryos. Mean numbers of thirty eggs that exhibited 1st cleavage, n=3 (except n=2 Caddo). Bar – SEM, *- significant difference ($p<0.05$)

DISCUSSION

The present study looked at the survival and reproduction of zebra mussel adults and larvae in varying water conditions found in 15 Texas lakes under laboratory conditions. The lakes represent 7 major river basins in north Texas. In this study, zebra mussel adults were able to survive for at least 45 days in waters from all 7 basins. A detailed analysis of each basin is provided in Appendix 4. Within the selected lakes were 8 lakes representing 4 basins that are viewed as High Risk for potential zebra mussel infestation, 5 lakes representing 4 basins with

Moderate Risk, and two lakes from 2 basins viewed as Low Risk (Table 1). These Risk factors are based primarily on calcium concentration, the main environmental factor indicating the potential risk for infestation. Numerous studies have proposed critical levels of calcium needed for zebra mussel survival and reproduction (Sprung 1987; Ramcharan et al. 1992; Hincks and Mackie 1997; Cohen and Weinstein 2001; McMahon 2015). Most studies indicate that zebra mussel adults will be able to survive, grow, and reproduce at calcium levels above 28 mg/L. Between 20-28 mg/L, zebra mussel adults appear to be able to grow and reproduction and embryonic develop occurs at rates comparable to higher calcium concentrations. Several studies suggest that below 12 mg/L calcium, zebra mussels are unable to reproduce (see Cohen and Weinstein 2001). Either veligers fail to develop, or fertilization and early development may be disrupted. This would effectively be the lower limit to establish independent, reproductively isolated populations. The absolute lower level of zebra mussel adult survival is more unclear with some studies indicating as low as 4 mg/L (reported in Cohen and Weinstein 2001) being the lower limit and minimal survival (67% mortality in 7 days) in deionized water (Ram and Walker 1993).

Three independent experiments were performed to address adult survival in waters with various calcium conditions. In Experiment I, an 84-day survival study, greater than 50% of survived regardless of calcium concentration. I was only able to determine specific calcium concentrations in the original source water. These calcium concentrations represent the minimum calcium concentrations during the experiments, but level may have increased during the 84 days. Using these minimum calcium values, there was a significant relationship between calcium and survival after 84 days (Figure 6). The High Risk lakes, with source calcium levels above the 20 mg/L threshold, had survival rates above 60% survival (Figure 7, Figure 8). The Moderate risk lakes (with source calcium levels below 20 mg/L) showed reduced survival relative to the High Risk Lakes, with the exception of Eagle Mountain (36 mg/L) and Coffee Mill (which had lower than expect calcium concentration). The two Low Risk lakes (Caddo and Sam Rayburn) had source water calcium concentrations below 12 mg/L, but had higher survival rates than several of the Moderate Risk lakes with higher calcium levels (Bonham, Fork, and Cedar Creek). There are several possible explanations for the higher survival probabilities in the two low calcium lakes (Caddo, Sam Rayburn). The most probable explanation is that the specific calcium levels in the containers rose above the 12 mg/L threshold allowing continued survival. Possible causes of this elevated calcium are discussed later. Alternately, the 84-trial period may not be sufficiently long enough to allow for potential increased mortality due to calcium deficiency. Dietz et al. (1994) showed that zebra mussels were able to mobilize calcium from their shells and utilize Mg to accommodate for depressed external calcium levels. However, Hincks and Mackie (1997) reported 100% mortality in mussels in calcium levels below 8 mg/L after 35 days. Furthermore, if calcium levels remained below the 12 mg/L threshold for the duration of the experimental period, the mussels may be able to survive as suggested by (Baldwin, per comm, as reported in Cohen, 2001), but they would be unable to establish a reproductive population.

Experiment II consisted of a 45-day survival trial with greater than 70% survival by mussels in all lake waters regardless of calcium concentration. As indicated, there was no significant relationship between calcium and survival after 45-days (Figure 9) or survival probability between the lakes. The most likely explanation for the lack of difference in survival

was the shortened time frame. The 45-day time period may be insufficient to discern any possible effect of calcium concentration and survival or mortality rate. This experiment ran concurrent with Experiment I (but started approximately 30 days later). It was terminated at 45 days due to logistical needs so that Experiment III could start with greater ability to monitor calcium levels.

Survival Experiment III looked at survival in lake waters after 63 days. There was no significant relationship between calcium and survival (Figure 12). Furthermore, there was no significant difference between the three risk groups (High, Moderate, Low) when comparing pooled groups of lakes. There were significant differences in survival probability between the lakes. Most of the High Risk lakes (with calcium levels greater than 30 mg/L) had higher survival probabilities relative to the other lakes (Figure 13, Appendix 3). As in Experiment I, the Moderate group lakes (with calcium levels above 12 mg/L but below 27 mg/L) showed the lowest survival probability. And the two Low Risk lakes (Caddo, Sam Rayburn) had higher survival probabilities than most of the Moderate Risk lakes. This pattern of higher survival probabilities in the High Risk and Low Risk lakes relative to the Moderate Risk lakes was similar, but not as pronounced, as in Experiment I.

The changes in growth, whether by wet weight or shell size, detected no appreciable change over time. There was no significant difference with most lakes showing a small (<1%) decrease in weight (Figure 15). This is not unexpected as minimal or negative growth in laboratory experiments has been previously reported (Dorgelo 1993; Hincks and Mackie 1997). Similarly shell length and width did not change significantly (<1%) with most mussels remain at or slightly larger in size. A few lakes had slight decreases in shell length or width (Figure 16, 17). This may be due to slight variations in measurements calculations or may represent a small reduction in shell size. Given that lakes with lower calcium levels (Caddo, Sam Rayburn) showed a slight increase in shell size while a few lakes with higher calcium levels (Eagle Mountain, Possum Kingdom) had slight decreases, the slight decreases due to loss of calcium, and shell mass, due to low external calcium seems doubtful.

The adult survival trials failed to detect a noticeable drop-off in mussel survival particularly in lakes with calcium levels below 12 mg/L (Low Risk Group) (Hincks and Mackie 1997). One question that needs further investigation is the precise calcium levels in the holding containers during the experiment. The calcium levels were lower in the source water but gradually increased in the individual containers in Experiment III (Figure 4). This very likely could have raised the calcium level high enough to not avoid the die-off reported by Hincks and Mackie (1997). While it does not show the pronounced die-off reported by Hincks and Mackie, it does illustrate the relatively strong survival in the 12-20 mg/L range. Mussel survival in the 15-20 mg/L range is less documented then the <12 mg/L and >20 mg/L Ca ranges (Cohen and Weinstein, 2001).

The potential increase in calcium could be from several sources. The mostly likely is concentration during evaporation. Efforts were taken to minimize evaporation (covered containers, reduced aeration) and partial water changes. Hincks and Mackie (1997) reported 100% mortality after 35 days in mussels housed in lakes with calcium less than 10 mg/L. However, they were able to utilize a flow-through system which was beyond the scope of this study. They required substantially more water with weekly collections of 50 L of water from each lake, compared to the single water collection used per experiment in this study. More

significantly, no mention is made regarding decontamination of effluent from the flow-through system prior to its disposal. Current permitting requires a minimum of 24-hour isolated bleach treatment before disposal of any water exposed to zebra mussels, making a flow-through system problematic. Calcium reading in the Hincks and Mackie 1997 study were only done on source water, not the individual tanks, so it is assumed that calcium levels did not change. As described below, I am currently performing experiments to hopefully address this situation. Additional sources of increased calcium may include calcium leaching directly from the mussels (Vinogradov et al 1987) or possible introduction of calcium during feeding of the Isochrysis. These seem less likely given the relatively small size of the zebra mussels and small volume of algae introduced relative to water volume of the containers.

Ideally, the best way to evaluate survival of zebra mussel in low calcium lake waters would be in situ cage experiments in low calcium lakes. This design is obviously not possible as it would require to placement of live mussels into uninfested waters. Alternately, on-site biobox experiments would allow for a more real-time assessment of dynamic water quality effects and would eliminate the need for placement of mussel directly into lakes. But like the laboratory flow-through experiments of Hincks and Mackie (1997) study, it would require on-site decontamination of effluent. In a recent biobox experiments utilizing zebra mussels I conducted near Lake Benbrook, permitting required effluent be processed through 3 separate 50-um filters followed by a chlorination treatment before the effluent could be added to sewage lines for further processing. This required on-site daily maintenance of filters and would be problematic at multiple lake locations. Hopefully the proposed experiment described below will allow for minimal change in calcium while also permitting acceptable processing of experimental water.

Reproductive Studies

I was only able to get preliminary data regarding reproductive ability in waters with varying calcium concentrations. In the single trial of veliger survival over 19 days, I found no significant relationship between calcium concentration and veliger survival (Figure 18). Lakes with the lowest veliger survival include two lakes with low levels of calcium Lake Caddo and Lake Fork (6 and 8 mg/L respectively) and third lake that had a high calcium concentration Lake Belton (39 mg/L). Conversely, the Low Risk, low calcium Lake Sam Rayburn (8 mg/L) had the second highest survival rate and had numerous veligers that had grown in size. Sprung (1987) found that normal veliger larvae developed at calcium levels above 40 mg/L. Below that level, veliger production decreased especially with the appearance of “crippled” veligers. Hincks and Mackie (1997) found that no veligers were produced by mussels housed in waters below 8.3 mg/L and the highest production was seen in lakes above 40 gm/L. All but Possum Kingdom, Joe Pool, and Worth had calcium concentrations below 40 mg/L. These findings are based on a single trial and subsequent interpretation must be limited. Some possible explanations need consideration for the upcoming trials described below. One is that the 19-day survival time is not sufficient to detect noticeable differences in survival with varying concentrations. The complicating challenge of rearing veliger for extended periods of time (Wright et al. 1996; Stoeckel et al. 2004) may need to be resolved. The possibility of increased calcium levels in the culture flasks as discussed for the adult survival trials is possible, although the shortened time period (19 days versus 84 days) and different culture containers (larger containers versus small,

tightly sealed flasks) makes this less likely. But additional steps will be taken to verify no significant change in calcium during the culture time. Another possibility is the veligers may be able to maintain their current size but not grow and transition into later stages in lower calcium concentrations. However, this was not the case in the lone trial involving the low calcium, Lake Sam Rayburn. Additional replicates are need as described below.

As with the veliger trials, I was only able to address spawning and fertilization in a few lakes. Two of the lake trials (Waco and Buchanan) had suspect gamete qualities due to end of spawning season as previously discussed. These will need to be replicated before conclusions can be drawn. The three remaining lakes consisted of two High Risk lakes, Lake Belton and Possum Kingdom Lake (38 and 78 mg/L calcium respectively) and a Low Risk Lake Caddo (8 mg/L calcium). Sperm binding occurred at comparable rates between all three lakes (Figure 21). This shows that sperm can swim, chemotactically locate the egg (Miller et al. 1994), and bind to the egg surface (Misamore et al. 1996). Hincks and Mackie (1997) found that male zebra mussels would not spawn in waters below 15 mg/L. Based on the Lake Caddo trial, we found that male zebra mussels could be chemically induced to spawn in waters with calcium levels below 10 mg/L and that sperm are able to locate and bind to eggs. Additionally, eggs in all three waters kept intact egg jelly layers and vitelline envelopes (Fallis et al. 2010) to allow sperm binding to occur, even in the low calcium waters of Lake Caddo. Once sperm bind to the egg, they must be incorporated into the egg cytoplasm where the sperm nucleus begins to decondense (Misamore et al. 2006). While this occurred in both the high calcium and low calcium lakes, it was significantly reduced in the low calcium Lake Caddo (Figure 23).
The first step in the developmental process is first cleavage. This event indicates the sperm has fertilized the egg, the arrested egg has resumed meiosis, and the developmental process has begun (Misamore et al. 1996) . Continuing the trend seen with sperm incorporation, both High Risk lakes showed cleaved zygotes with the high-calcium Possum Kingdom Lake (78mg/L) having the greatest cleavage success. Conversely, no cleavage whatsoever was observed in the low calcium Lake Caddo (Figure 25). This apparent failure to continue the developmental process beyond fertilization supports the theory that reproduction below 12 mg/L calcium cannot occur (Sprung 1987). These initial findings suggest it may be an issue of preventing early development as much as failure of veliger stages to progress. The continuation of these experiment as described below will address this question in greater detail.

Current and Future Experiments

Presented here are my finding regarding the growth, survival and reproductive success of zebra mussels in Texas lakes within the allotted time frame. As indicated, I am clearly not finished with this project. Here is a summary of current and future plans to continue this study.

Adult survival

I am currently conducting a fourth adult survival trial but finally with full water monitoring capabilities from start to finish. I am also specifically addressing the concern regarding potential rising calcium level in the individual containers. The current experiment varies slightly from the previous studies. I have 15 containers containing 15 mussels each filled with water from the various lakes. There are three replicate trials (45 total containers). Prior to filling the containers, the source water was measured for calcium, pH, and conductivity (15 total analyses)

prior to splitting into the three replicates. Aeration was reduced to further limit potential evaporative loss and dissolved oxygen will be monitor weekly in all 45 containers. After 7 days, water samples will be removed from each of the containers and analyzed for calcium concentration, pH, and conductivity (45 total samples). This will give me an accurate reading of the weekly change in these parameters. After sampling, I will do a 100% water change with the new source water eliminating any concentrating effects beyond one week. As at the start of the experiment, the source water will be analyzed prior to dividing among the three treatments. While this eliminates any potential long-term concentrating effects, it does raise the potential for effects from 100% water changes. But it is the best solution short of a flow-through system which is not feasible as described above. All other aspects (feeding, monitoring for dead, etc.) will be continued as with the other experiments.

Veliger Survival

I am continuing the veliger survival trials as described above. I will be collecting veligers and transferring verified live veligers into the culture flasks as previously described. I will monitor veliger survival at 7, 14, and 21 days by examining a subsample and determining percent survival. I am not conducting more frequent sampling to avoid additional handling stress. After the 21 days I will determine a total percent survival. I am adding an additional measure and will get sizes of all veligers (live and dead) to verify any differences in larval growth in addition to overall survival. I will also conduct a full water analysis at the end of 21 days to ensure no calcium concentrating is occurring.

Spawning and Fertilization

I will complete the spawning and fertilization trials as described above. I will redo both Lake Waco and Buchanan as well as Caddo Lake to confirm the absence of embryo development.

Calcium specific requirement on zebra mussel reproduction

I am planning to conduct experiments that will specifically target the effects of calcium on zebra mussel reproduction. It will mirror the experimental design of the present study but with a major difference. Instead of using differing lake waters with diverse calcium concentrations, I will be using artificial "lake water." Several other zebra mussel labs use an artificial pondwater formula originally develop by (Misamore et al. 1996; Byrne and Dietz 2006). I will generate artificial pondwater with calcium levels of 0, 5, 10, 15, 20, 25, and 30 mg/L. Initially, I will conduct the spawning and fertilization trials. Eventually, pending reliable supplies of veligers, I will conduct veliger survival trials. This will allow me to systematically determine the calcium levels that are essential for fertilization. This study will expand on initial observations by Sprung (1987) who found that low calcium resulted in defective veligers.

Once completed, I should be able to fill the gaps in the project to date and addressed questions that arose from this project. I plan to submit a supplemental report to TPWD with these finding.

IN SUMMARY

Some interesting trends, surprising inconsistencies in established lakes, holes that need to be filled, and questions that need to be addressed came from the project to this point. In general, Low Risk Group lakes to zebra mussels performed lower than their counterparts. However, there was no pronounced die off after rearing in low-risk waters. This may be due in part to unintended elevations in calcium levels during the experiment. While I was only able to conduct a few veliger and fertilization trials, the beginnings of some interesting trends are present. It is unclear what is the lowest level of calcium required for fertilization and veliger development. Some studies say 12 mg/L is sufficient where other studies say levels need to be 24 or even 40 mg/L (Sprung 1987, Nichols 1996, Cohen and Weinstein 2001). It is interesting to note the lack of fertilization and cell division in the Lake Caddo water that were below the 12 mg/L threshold. This, as much as failure for veligers to develop, may account for the poor reproduction reported in studies below 12 mg/L calcium.

APPENDIX 1.

Extenuating Circumstances and Effects of Outcomes

Multiple unexpected and unprecedented events effected the timing, experimental design, and progression of this project.

COVID-19

This project started in Fall 2019 with the initial collection of most water samples, mussel collection, initial holding facility design, and personnel recruitment and training. The arrival of the pandemic lead to the suspension of all research activities (both lab and field work) for several months that lead to the extension of the project deadline. All on-campus and off-campus research activities was suspended on Mar. 24, 2020 corresponding with the City of Ft. Worth "stay home, stay safe" order. Limited off-campus activities began in April, 2021 but unrestricted (use of student workers) did not resume until May 2021. When restriction gradually started lifting in Spring 2021, the previous water collections were no longer useful, and all previously collected mussels had been disposed during the shutdown. This resulted in an additional series of water collection trips not in the original design and budget. All lab personnel graduated, and a new set of student-workers needed to be recruited and trained. With distancing restrictions and limited accessibility, I was able to recruit and train students at the end of spring 2021 for work in summer of 2021.

Winter Storm, February 2021

The winter storm in early February 2021 had an even more profound impact on this project. The Winton-Scott Science Building on the TCU campus suffered significant flood damage due to ruptured pipes. The building was closed to all activity for several weeks, then reopened gradually as repairs progressed. My laboratory was hit significantly hard. I lost approximately 1/3 of my research equipment and supplies including most of the equipment essential for this project. This included all my analytical balances and water analysis equipment including calcium meter and probes, pH meter and probe, essential chemicals, and calibration standards. It took several months for damage assessment and insurance processing before lost equipment could start to be replaced. Various pieces of equipment were replaced starting May 2021, but I did not get minimal analysis ability until August, 2021.

Supply Chain Disruption due to COVID-19

The combination of lost equipment and COVID-19 induced disruption in supply chains made restoring my research lab unexpectedly more difficult. Numerous items were not available or took weeks-to-months to replace instead of the pre-pandemic days to 1-2 weeks. This was especially true for specialty items such as the calcium meter and probe and even chemicals like calibration standards. Some items were not fully available until September/October, 2021 and many are still in limited supply.

Mussel Collection Site Changes

When designing this project and budget, Lake Bridgeport was intended to serve as the source for zebra mussels and veligers. It had served as a reliable source for reproductively active

adults and veligers for several years prior. When the research project resumed in summer of 2021, the mussel population in Lake Bridgeport collapsed. Shoreline sites I frequently used to collect mussels were devoid of sufficient numbers of mussels and none we of reproductive status. The veliger numbers in the lake did not rise to previous levels, thereby not providing sufficient quantities of gametes. Similar phenomena have been occasionally observed in Texas and Oklahoma lakes in the past. This required me to identify and relocate my collection sites to Lake Travis (several hours away and where I no longer had multiple connections for collecting mussels, particularly veligers). This further hindered efforts particularly once the academic school year began making day-long trips more problematic. It added additional costs for travel and boat rental that were not originally budgeted.

Timeline Impact

The major impact of combined factors listed above was the drastic alteration of the research timeline as outline in previous reports. Most significantly, I was unable to get a fully functional lab and work force in place to utilize the spring 2021 spawning season. As detailed above, this greatly impacted several aspects of the project particularly the reproductive components. I was able to utilize the tail end of the spring spawning season to some extent, but not unexpectedly, attempts to prolong reproduction into the summer months had mixed results. I hoped to utilize the smaller fall spawning season and was able to in a limited capacity. But obtaining reproductively active adults for shoreline collections proved unsuccessful due to the high mortality of shallow-water mussels over the summer months. Furthermore, several aspects of the project originally intended to occur in summer months were forced into the Fall 2021, when academic commitments created additional complications.

APPENDIX 2.

Ranking of lakes based on their mean percent survival (% survival) after the 84-day trial. Lakes significantly different are listed based on the Mantel-Cox comparisons. Risk grouping as defined by Table 1.

Lake	% Survival	Risk Group	Significantly Different lakes. [1]Lakes with Lower Survival Probability [2]Lakes with Higher Survival Probability
Joe Pool	95	High	[1]Possum K., [1]Waco, [1]Caddo, [1]Palestine, [1]Coffee Mill, [1]Bonham, [1]Fork, [1]Tawakoni, [1]Cedar Cr., [1]Eagle Mt.
Buchanan	92.5	High	[1]Waco, [1]Caddo, [1]Palestine, [1]Coffee Mill, [1]Bonham, [1]Fork, [1]Tawakoni, [1]Cedar Cr, [1]Eagle Mt.
Belton	85	High	[1]Palestine, [1]Coffee Mill, [1]Bonham, [1]Fork, [1]Cedar Cr
Possum K.	82.5	High	[1]Palestine, [1]Bonham, [1]Fork, [1]Cedar Cr, [1]Joe Pool
Waco	82.5	High	[2]Buchanan, [1]Palestine, [1]Bonham, [1]Fork, [1]Cedar Cr, [1]Joe Pool
Sam Rayburn	82.5	Low	[1]Palestine, [1]Bonham, [1]Fork, [1]Tawakoni
Worth	80	High	[1]Palestine, [1]Coffee Mill, [1]Bonham, [1]Fork, [1]Tawakoni, [1]Cedar Cr
Caddo	75	Low	[2]Belton, [2]Possum K, [2]Buchanan, [2]Joe Pool, [1]Bonham, [1]Fork, [1]Cedar Cr
Tawakoni	70	High	[2]Buchanan, [2]Sam Rayburn, [2]Joe Pool, [2]Worth
Coffee Mill	62.5	Mod.	[2]Belton, [2]Buchanan, [2]Joe Pool, [2]Worth
Palestine	60	Mod.	[2]Belton, [2]Possum K., [2]Waco, [2]Buchanan, [2]Sam Rayburn, [2]Joe Pool, [2]Worth
Fork	60	Mod.	[2]Belton, [2]Possum K., [2]Waco, [2]Buchanan, [2]Caddo, [2]Joe Pool, [2]Worth
Eagle Mt.	60	High	[2]Buchanan, [2]Joe Pool
Bonham	50	Mod.	[2]Belton, [2]Possum K., [2]Waco, [2]Buchanan, [2]Caddo, [2]Sam Rayburn, [2]Joe Pool, [2]Worth
Cedar Cr.	50	Mod.	[2]Belton, [2]Possum K., [2]Waco, [2]Buchanan, [2]Caddo, 2Joe Pool, 2Worth

APPENDIX 3.

Ranking of lakes based on their mean percent survival (% survival) after the 63-day trial. Lakes significantly different are listed based on the Mantel-Cox comparisons. Lakes significantly different are listed. Risk grouping as defined by Table 1.

Lake	% Survival	Risk Group	Significantly Different lakes. [1]Lakes with Lower Survival Probability [2]Lakes with Higher Survival Probability
Buchanan	78.60%	High	[1]Waco, [1]Fork, [1]Tawakoni, [1]Cedar Cr., [1]Eagle Mt., [1]Joe Pool, [1]Worth
Possum K.	70%	High	[1]Fork, [1]Tawakoni, [1]Cedar Cr., [1]Eagle Mt., [1]Joe Pool
Worth	66.70%	High	[1]Buchanan, [1]Sam Rayburn, [1]Bonham
Belton	66.67%	High	[1]Fork, [1]Tawakoni, [1]Joe Pool
Palestine	62.50%	Mod.	[1]Tawakoni
Sam Rayburn	56.80%	Low	[1]Caddo, [1]Fork, [1]Tawakoni, [1]Cedar Cr., [1]Eagle Mt., [1]Joe Pool, [1]Worth
Waco	56%	High	[1]Buchanan, [1]Tawakoni
Coffee Mill	54%	Mod.	[1]Caddo, [1]Tawakoni, [1]Eagle Mt., [1]Joe Pool
Bonham	50%	Mod.	[1]Caddo, [1]Fork, [1]Tawakoni, [1]Cedar Cr., [1]Eagle Mt., [1]Joe Pool, [1]Worth
Cedar Cr.	45.80%	Mod.	[2]Possum K., [2]Buchanan, [2]Sam Rayburn, [2]Bonham
Caddo	43.48%	Low	[2]Sam Rayburn, [2]Coffee Mill, [2]Bonham
Joe Pool	37.50%	High	[2]Belton, [2]Possum K., [2]Buchanan, [2]Sam Rayburn, [2]Coffee Mill, [2]Bonham
Tawakoni	37.50%	High	[2]Belton, [2]Possum K., [2]Waco, [2]Buchanan, [2]Palestine, [2]Sam Rayburn, [2]Coffee Mill, [2]Bonham
Fork	36.80%	Mod.	[2]Belton, [2]Possum K, [2]Buchanan, [2]Sam Rayburn
Eagle Mt.	33.33%	High	[2]Possum K., [2]Buchanan, [2]Sam Rayburn, [2]Coffee Mill, [2]Bonham

APPENDIX 4. BASIN OVERVIEW

Brazos River Basin.
Three lakes were selected from the Brazos Basin: Lake Belton, Lake Waco, and Possum Kingdom. Lake Waco is located along the Bosque River Watershed.

Belton Lake - is located on the Leon River, a tributary of the Little River. It is considered fully infested with established reproductive populations. It showed mean survival of 85% after 84 days in Survival Experiment I (Figure 7, 8, Table 5) and 80% after 45 days in Survival Experiment II (Figure 10, 11) and 67% in the Growth Experiment after 63 days (Figure 13). Mussels had a minimal positive weight gain (Figure 15) but slightly lower shell length (Figure 16) and shell width (Figure 17). These differences are minimal and may be within the variability of measurements. In the veliger trial, 70% of the veligers were alive after two days which decreased to 60% by 6 days and dropped to 0% after 19 days (Figure 18). Of the live veligers observed, most were small, earlier-stage veligers. The reason for the sharp decline between 6-19 days is unclear and additional replicates need to be performed before any question of veliger viability in Lake Belton water can be interpreted. Given the established population, I expect additional veliger trials to support the assumption that veligers will successfully develop. As one of the lakes evaluated for fertilization and early development, sperm were able to function in Lake Belton water and reach eggs and most eggs exposed to sperm showed high levels of sperm binding (Figure 21). Sperm successfully entered into the eggs (Figure 23) and fertilized eggs were able to divide (Figure 25).

Lake Waco - is located along the Bosque River tributary of the Brazos Watershed. At one time was considered positive for zebra mussels but is now considered non-infested following a successful eradication effort. Adult mussel survival was high in Survival Experiment I with a mean of 82% after 84 days (Figure 7, 8, Table 5) and in Survival Experiment II at 80% after 45 days(Figure 10, Figure 11) and 56% after 63 days in the Growth Experiment (Figure 13). Mussels had a slightly positive weight gain (Figure 15) and shell width (Figure 17) but slight decrease shell length (Figure 16). These differences are minimal and may be within the variability of measurements. In the veliger trial, there was 50% survival after 19 days (Figure 18) and several later veliger stages (Figure 5) developed, but additional replicates need to be performed before any question of veliger success can be interpreted. Sperm were able to function in Lake Waco water and reach eggs. Significantly fewer eggs had bound sperm relative to the other lakes (Figure 21). As described above, this may be attributed to the quality of gametes and replicating these trials with health gravid females in the spring should clarify this discrepancy. While the levels were lower, sperm successful in entering into the eggs (Figure 23) and fertilized eggs were able to divide (Figure 24a and Figure 25).

Possum Kingdom - is located along the Brazos River upstream of the other Brazos sites. Zebra mussels nor or veliger have been detected at this time. It showed mean survival of 82.5% after 84 days in Survival Experiment I (Figure 7, Figure 8) and 86% after 45 day in Survival Experiment II (Figure 10, Figure 11) and 70% after 63 days in the Growth Experiment (Figure 13). Mussels had a slightly negative weight loss (Figure 15) but slightly lower shell length (Figure 16) and

shell width (Figure 17). These differences are minimal (<3%) and may be within the variability of measurements. In the lone veliger trial, 57% of veligers were alive after 19 days with a range from early to later veliger stages (Figure 18), but additional replicates need to be performed before any question of veliger success can be interpreted. Sperm were able to function in Possum Kingdom Lake water and reach eggs and most eggs exposed to sperm showed high levels of sperm binding (Figure 21). Sperm successfully entered the egg cytoplasm at a significantly higher rate than the other lakes (Figure 23). Fertilized eggs were able to divide (Figure 24b and Figure 25) with more than 60% of the eggs dividing. Calcium and pH levels, the two key indicators of zebra mussel survival, were within acceptable levels when measured in collected water (and in-trial measurements (Figure 4). Calcium (>50 mg/L), and especially conductivity (>1000 µS/cm), were particularly high relative to other lakes (Table 3, 4). This is expected given its slightly saline nature (Leifeste and Popkin 1968).

Basin Summary. As expected, Lake Belton, given its infested status, showed high survival and reproductive ability. Similarly, Lake Waco also showed high levels of survival. Fertilization and veliger survival was also successful. Interestingly, Possum Kingdom Lake show no significant difference in survival relative to Lake Belton and Lake Waco given its high conductivity (Table 5). Possum Kingdom Lake also showed a high degree of reproductive success in fertilization and veliger survival relative to the other lakes. Even with its higher conductivity, it initially appears the gametes will be able to function in the waters. While zebra mussels are sensitive to salinity effects (Fong et al. 1995), initial findings suggest fertilization should proceed unimpeded in Possum Kingdom waters. Two additional lakes (Georgetown, Stillhouse) within the Brazos Basin are also considered infested.

Colorado River Basin.

Lake Buchanan - was the only lake in the Colorado River basin studied. When this project was initially proposed in 2019, Lake Buchanan had no documented zebra mussels. It has since become fully infested with established reproductive populations. It showed mean survival of 92% after 84 days in Survival Experiment I (Figure 7,Figure 8, Table 5) and 93% in Survival Experiment II (Figure 10, Figure 11) and 78% in the Growth Experiment (Figure 13). Lake Buchanan was exhibited significantly higher survival rates than many of the other lakes (Table 5). As with the other lakes, change in weight (Figure 15), shell length (Figure 16) and shell width (Figure 17) were less than 2%. In the veliger trial, there was 38% survival after 19 days (Figure 18) and with a few later veliger stages (Figure 5). Additional replicates need to be performed before any question of veliger success can be interpreted. Given the established population, I expect additional veliger trials to show good veliger survival. As mentioned above regarding Lake Waco, the suboptimal gametes at the end of the breeding season may account for the reduced fertilization success. Nevertheless, sperm were able to bind to eggs (Figure 21) and enter the egg cytoplasm (Figure 23).

Basin Summary.

Numerous lakes along the Colorado River Basin in Texas contain well established populations of zebra mussels including Austin, Lady Bird, Travis, Brownwood, Inks, LBJ, Marble falls, O.H. Ivie,

and Pflugerville. Originally chosen as a lake within the basin without zebra mussels, in the last 2 years the situation along this region has changed dramatically.

Cypress River Basin

Lake Caddo - is the only lake studied within the Cypress Basin. It is considered of low risk due to low calcium levels (below 10 mg/L), and pH (below 7). Lake Caddo showed mean survival of 75% after 84 days in Survival Experiment I (Figure 7, Figure 8) and 90% after 45 day in Survival Experiment II (Figure 10, Figure 11). This value dropped to 44% after 63 days in the Growth Experiment III, but this followed the general trend of slightly lower overall survival rate among all lakes in Experiment III (Figure 13). The probability of survival was significantly lower in Caddo than several other lakes viewed as more favorable to zebra mussels including Sam Rayburn, Buchanan, Coffee Mill, Bonham (Appendix 3). There was no significant difference in change of weight, shell length, or shell height relative to other lakes and the overall changes was less than 3% (Figure 15, Figure 16, Figure 17).

In the veliger trial, they show good survival (>75%) up to day 6, but only 10% survival at day 19 (Figure 18). Additional replications are needed to verify this trend which will be of great interest. During fertilization trials, sperm were successful in sperm-egg binding and greater than 75% of eggs had bound sperm egg (Figure 21) suggesting gametes bind in Lake Caddo waters. However, even with a high number of eggs with bound sperm, less than 11% of eggs had sperm what was able to transition from binding to the egg to being able to fuse and enter the egg cytoplasm. This suggests that there may be some obstacles that could potentially reduce fertilization efficiency. This may be especially true of the egg as sperm can routinely bind to eggs (even if disrupted/broken) if the sperm receptors on the egg surface are intact. However, the mechanism for transporting sperm across the cell membrane and into the cytoplasm is much more dependent on the egg metabolic activity (Misamore, Silverman et al. 1996, Misamore and Lynn 2000). Further emphasizing this absence of fertilization is that no eggs in Caddo water cleaved (Figure 25). While not measured directly before the start of the experiment, the calcium levels in the holding barrels remained relatively constant and below the 12 mg/L threshold.

A possible explanation of the suggested success of zebra mussels in Lake Caddo waters during this experiment is water composition. pH from Lake Caddo waters ranged from 7.08 to 7.36 (Table 3, 5) for the collected waters to near 8.0 during the growth experiment (Figure 4). These values are all slightly higher than the 6.7 range reported for Lake Caddo. Similarly, calcium levels were 6.13 and 6.0 (Table 3, 5) from collected waters but reached a mean of 18 mg/L in the individual containers (Figure 4). While the collected waters were below the 12 mg/L threshold, the container levels were above. Adding to the complex situation is the questionable ability for fertilization and early development to occur.

Neches River Basin

Lake Palestine - is viewed as a low-risk lake due to lower calcium levels (reported 19.1mg/L and lower pH (7.3). Neither adult zebra mussels nor veliger have been detected at this time. It showed mean survival of 60% after 84 days in Survival Experiment I (Figure 7,Figure 8, Table 5)

and 83% after 45 day in Survival Experiment II (Figure 10) and 62.5% after 63 days in the Growth Experiment (Figure 13). Probability of survival after 84 days was significantly lower than several lakes (Belton, Possum K., Waco, Buchanan, Sam Rayburn Joe Pool, Worth) (Table 5). As with the other lakes, change in weight (Figure 15), shell length (Figure 16) and shell width (Figure 17) were less than 2%. In the veliger trial, 100% of veligers were alive after 19 days with numerous later veliger stages (Figure 18). Additional replicates are needed to verify larval survival, but in this initial trial, Lake Palestine had very high survival. This is somewhat surprising given the expected low calcium levels would expectantly negatively impact veliger survival and development especially formation and growth of the shell. Plans to replicate the veliger studies as well as conduct spawning and fertilization trials are planned for spring 2022. Calcium levels were low in the source water that supplied the water at 10.29 mg/L (Table 3, Table 4).

Lake Sam Rayburn - is views as a low-risk lake due to lower calcium levels (reported 6.9 mg/Land lower pH (7.3). Neither zebra mussels nor veliger have been detected at this time. It showed mean survival of 82.5% after 84 days in Survival Experiment I (Figure 7,Figure 8, Table 5) and 80% after 45 days in Survival Experiment II (Figure 10) and 56% after 63 days in the Growth Experiment (Figure 13). As with the other lakes, change in weight (Figure 15), shell length (Figure 16) and shell width (Figure 17) were less than 2%. In the veliger trial, 83% of veligers were alive after 19 days with numerous later veliger stages (Figure 18). Additional replicates are needed to verify larval survival, but in this initial trial, Lake Sam Rayburn had very high survival. This is somewhat surprising given the expected low calcium levels would expectantly negatively impact veliger survival and development especially formation and growth of the shell. Plans to replicate the veliger studies as well as conduct spawning and fertilization trials are planned for spring 2022. Calcium levels were low in the holding tanks at 8.307 mg/L (Table 3, Table 4) that supplied water for the veliger study.

Basin Summary

Both Lakes Sam Rayburn and Palestine exhibited greater than 60% survival in all three experimental trials. However, there survival curves were mid-level compared to the other lakes in the study. In all trials, Sam Rayburn had higher overall survival rates (and significantly higher in Experiment I. Veliger survival and development was strong in both lakes; however, more replicates are needed before confirming this observation. Planned reproductive studies will also be interesting given the potential inhibition of fertilization and development seen in Lake Caddo, which like Sam Rayburn and Palestine has low calcium levels. No lakes in the basin have reports of zebra mussels.

Red River Basin

Coffee Mill Lake – was formed from Coffee Mill Creek, a tributary of Bois d'Arc Creek which feeds to the Red River. No zebra mussels nor veliger have been reported but this lake is not actively monitored. Water collected from the lake had a pH of 7.8-8 and calcium levels around 12-13 mg/L putting it roughly at a moderate risk. It showed mean survival of 62% after 84 days in Survival Experiment I (Figure 7,Figure 8, Table 5) and 82% after 45 days in Survival Experiment II (Figure 10Figure 11) and 54% after 63 days in the Growth Experiment III (Figure

13). As with the other lakes, change in weight (Figure 15), shell length (Figure 16) and shell width (Figure 17) were less than 2%. In the veliger trial, 90% of veligers were alive after 2 days but only 30% after six days and less than 25% after 19 days (Figure 18). Most were small veligers (live or dead) but 1 larger veliger was present. The lower-level survival might be expected given the lower calcium levels.

Lake Bonham – was formed from Timber Creek, a tributary of Bois d'Arc Creek which feeds to the Red River. It is currently not infested by zebra mussels but rated as moderate risk due to lower calcium (15 mg/L) and pH (7.2). No zebra mussels nor veliger have been reported but this lake is not actively monitored. Zebra mussel reared in Lake Bonham water showed mean survival of 50% after 84 days in Survival Experiment I (Figure 7,Figure 8, Table 5) and 86% after 45 days in Survival Experiment II (Figure 10Figure 11) and 50% after 63 days in the Growth Experiment (Figure 13, 14). As with the other lakes, change in weight (Figure 15), shell length (Figure 16) and shell width (Figure 17) were less than 2%. In the veliger trial, 90% of veligers were alive after 2 days but only 30% after six days and less than 25% after 19 days (Figure 18). Most were small veligers (live or dead) but 1 larger veliger was present. The lower-level survival might be expected given the lower calcium levels.

Basin Summary

Both Coffee Mill Lake and Lake Bonham are low to moderate levels for potential infestation. Their survival rates place them mid-range relative to the other lakes in the study. The lower survival rates correspond to the expected effects of both lower calcium and pH. In the veliger trial, both lakes had relatively poor survival. Again, a factor that mirrors the lower calcium levels. Lake Texoma is the only reported lake in the Red River basin in Texas with zebra mussel infestation.

Sabine River Basin

Lake Fork – is located on the Lake Fork Creek and Caney Creek, a tributary of the Sabine River. It is currently not infested by zebra mussels but at one time was suspect due to the detection of a single veliger. It is considered a low risk due to low calcium levels (12 mg/L) and pH (7.3). Zebra mussels reared in Lake Fork water showed mean survival of 60% after 84 days in Survival Experiment I (Figure 7,Figure 8, Table 5) and 89.6% after 45 days in Survival Experiment II (Figure 10Figure 11) and 36% after 63 days in the Growth Experiment (Figure 13). Lake Fork had significantly lower survival rates relative to the infested or high-risk lakes such as Lakes Buchanan, Belton, Possum Kingdom, and Waco. As with the other lakes, change in weight (Figure 15), shell length (Figure 16) and shell width (Figure 17) were less than 2%. In the veliger trial, 70% of veligers were alive after 2 days but only 40% after six days and there were no live veligers after 19 days (Figure 18). All were small veligers (live or dead) with no larger, late-stage veligers present. The lower-level survival might be expected given the lower calcium levels.

Lake Tawakoni – is located north of Willis Point on the Sabine River. It is currently not infested by zebra mussels. It is considered a high risk due to calcium levels (28.5 mg/L) and pH (8). My collections were closer to 20 mg/L calcium. Zebra mussel reared in Lake Tawakoni showed mean survival of 70% after 84 days in Survival Experiment I (Figure 7,Figure 8, Table 5) and

76.6% after 45 days in Survival Experiment II (Figure 10Figure 11) and 37.5% after 63 days in the Growth Experiment (Figure 13, 14). Lake Tawakoni had significantly lower survival rates relative to the infested or high-risk lakes such as Lakes Buchanan, Belton, Possum Kingdom, and Waco. As with the other lakes, change in weight (Figure 15), shell length (Figure 16) and shell width (Figure 17) were less than 2%. In the veliger trial, live veliger dropped to 50% of veligers alive after 2 days but remained steady at 45% for 19 days (Figure 18). All were small veligers (live or dead) with no larger, late-stage veligers present. The lower-level survival might be expected given the lower calcium levels.

Basin Summary

Both lakes were in the lower half of lakes with regards to zebra mussel survival. Lake Fork was particularly low in both studies – not unexpected given their relative calcium levels. While Lake Tawakoni had higher survival rates relative to Lake Fork, the difference was not statically significant – surprising given the difference in risk level and calcium level. No Texas lakes in the basin are known to have zebra mussels.

Trinity River Basin

Cedar Creek Lake – is located on Cedar Creek, a tributary of the Trinity River. It is considered a moderate to high-risk lake due to its reported calcium levels (19 mg/L) and high pH (8.0). Neither zebra mussels nor veliger have been detected at this time. Cedar creek showed mean survival of 50% after 84 days in Survival Experiment I (Figure 7,Figure 8, Table 5) and 70% after 45 days in Survival Experiment II (Figure 10Figure 11) and 45% after 63 days in the Growth Experiment (Figure 13, 14). As with the other lakes, change in weight (Figure 15), shell length (Figure 16) and shell width (Figure 17) were less than 2%. In the veliger trial, veligers showed a steady decline from 90% of veligers were alive after 2 days, to 50% at 6 days to 33% live at 19 days (Figure 18). This is somewhat surprising given the expected acceptable calcium levels would support veliger development. The planned additional replicates of the veliger studies as well as conduct spawning and fertilization trials will provide better insight into potential reproductive success in Cedar Creek Lake.

Eagle Mountain Lake – is located on the West Fork Trinity River. It is categorized as an infested lake. Given the presence of zebra mussels in the lake, it exhibited surprisingly low survival rates. Its mean survival after 84 days was only 50% in Survival Experiment I (Figure 7,Figure 8, Table 5) and 76% after 45 days in Survival Experiment II (Figure 10Figure 11) and only 33% after 63 days in the Growth Experiment (Figure 13). As with the other lakes, change in weight (Figure 15), shell length (Figure 16) and shell width (Figure 17) were less than 2%. In the veliger trial, veligers showed a steady decline from 80% of veligers were alive after 2 days, to 60% at 6 days to 38% live at 19 days (Figure 18). This is somewhat surprising given the established populations in the lake. However, veliger abundance is lower in Eagle Mountain relative to other infested lakes such as Lake Bridgeport or Lake Travis. Plans to replicate the veliger studies as well as conduct spawning and fertilization trials are planned for spring 2022. Calcium levels were high in the holding tanks at 20 - 36 mg/L (Table 4,Table 4) that supplied water for the survival and veliger studies.

Joe Pool Lake – is located on the Walnut Creek and Mountain Creek, which are tributaries of the Trinity River. It is categorized as an infested lake with reported calcium levels above 50 mg/L and a pH of 8.0. Neither zebra mussels nor veliger have been detected at this time. Joe Pool Lake was highly contradictory. In Experiments I (84 days) and II (45 days) it had some of the highest survival rates (>90%), but had one of the lowest rates at 33% survival after 63 days (Figure 13, 14). As with the other lakes, change in weight (Figure 15), shell length (Figure 16) and shell width (Figure 17) were less than 2%. In the veliger trial, veligers showed a precipitous decline from 80% of veligers were alive after 2 days, to 30% at 6 days to 36% live at 19 days (Figure 18).

Lake Worth – is located on the West Fork Trinity River, the upper tributary of the Trinity River. It is classified as an infested lake with high calcium levels. Its mean survival after 84 days was 80% in Survival Experiment I (Figure 7,Figure 8, Table 5) and 89% after 45 days in Survival Experiment II (Figure 10Figure 11) and 66.7% after 63 days in the Growth Experiment (Figure 13, 14). As with the other lakes, change in weight (Figure 15), shell length (Figure 16) and shell width (Figure 17) were less than 2%. In the veliger trial, veligers showed a rapid decline from 90% of veligers were alive after 2 days, to 30% at 6 days to 40% live at 19 days (Figure 18). This is somewhat surprising given the established population of zebra mussels in the lake. Additional replicates will help clarify the initially perceived drop in veliger survival.

Basin Summary

As indicated by the number of infested lakes, the Trinity River basin is very susceptible to zebra mussels. In the present study, Cedar Creek showed the lowest survival rate relative to the other three Trinity lakes studied. Eagle Mountain performed somewhat surprisingly lower than expected given the infested nature of the lake. Additional veliger trials will be very interesting to see if the depressed trend has merit. Joe Pool, while inconsistent, did show 100% survival in 2 or 3 trials.

LITERATURE CITED

Berkman PA, Haltuch MA, Tichich E, Garton DW, Kennedy GW, Gannon JE, et al. 1998. Zebra mussels invade lake erie muds. Nature 393:27-28.

Byrne RA, Dietz TH. 2006. Ionic and acid-base consequences of exposure to increased salinity in the zebra mussel, dreissena polymorpha. Biol Bull 211:66-75.

Chakraborti RK, Madon S, Kaur J, Gabel D. 2014. Management and control of dreissenid mussels in water infrastructure facilities of the southwestern United States. In: Biology and management of invasive quagga and zebra mussels in the western united states, (Wong WH, Gerstenberger S, eds): CRC Pres, 349-374.

Cohen AN, Weinstein A. 2001. Zebra mussel's calcium threshold and implications for its potential distribution in north america. (A report for the California Sea Grant College Program, La Jolla CA, and the Department of Energy, National Energy Technology Center, Morgantown WV San Francisco Estuary Institute, Oakland, CA).

Connelly NA, O'Neill CR, Knuth BA, Brown TL. 2007. Economic impacts of zebra mussels on drinking water treatment and electric power generation facilities. Environ Management 40:05-112.

Dietz TH, Lessard D, Silverman H, Lynn JW. 1994. Osmoregulation in dreissena polymorpha : The importance of Na, Cl, K and particularly mg. Biol Bull 187:76-83.

Dorgelo J. 1993. Growth and population structure of the zebra mussel (Dreissena polymorpha) in dutch lakes differing in trophic state. In: Zebra mussel biology, impacts and control, (Nalepa T, Schloesser D, eds). Ann Arbor, MI:Lewis Publishers, 79-94.

Elderkin CL, Klerks PL. 2005. Variation in thermal tolerance among three Mississippi River populations of the zebra mussel, Dreissena polymorpha.J Shellfish Res 24:221-226.

Fallis LC, Stein KK, Lynn JW, Misamore MJ. 2010. Identification and role of carbohydrates on the surface of gametes in the zebra mussel, dreissena polymorpha. Biol Bull 218:61-74.

Fong PP, Kyozuka K, Duncan J, Rynkowski S, Mekasha D, Ram, JL. 1995. The effect of salinity and temperature on spawning and fertilization in the zebra mussel Dreissena polymorpha (Pallas) from North America. Biol Bull 189:320-329.

Hebert PDN, Muncaster BW. 1989. Ecological and genetic studies on Dreissena polymorpha (pallas): A new mollusc in the great lakes. Can J Fish Aquat Sci 46:1587-1989.

Hincks SS, Mackie GL. 1997. Effects of ph, calcium, alkalinity, hardness, and chlorophyll on the survival, growth, and reproductive success of zebra mussel (Dreissena polymorpha) in ontario lakes. Can J Fish Aquat Sci 54:2049-2057.

Johnson PD, McMahon RF. 1998. Effects of temperature and chronic hypoxia on survivorship of the zebra mussel (Dreissena polymorpha) and Asian clam (Corbicula fluminea). Can J Fish Aquat Sci 55:1564-1572.

Karatayev AY. 1995. Factors determining the distribution and abundance of Dreissena polymorpha in lakes, dam reservoirs and channels. IN: Proceedings of the Fifth International Zebra Mussel and Other Aquatic Nuisance Organisms Conference, February 1995, Toronto ON. Pages 227-243.

Karatayev AY, Burlakova LE, Padilla DK. 1998.
MacIsaac HJ. 1996. Potential abiotic and biotic impacts of zebra mussels on the inland waters of north america. Amer Zool 36:287-299.

Leifeste DK, Popkin, B. 1968. Quality of water and stratification of Possum Kingdom, Whitney, Hubbard Creek, Proctor, and Belton Reservoirs. U.S. Geological Survey and Texas Water Development Board and the Brazos River Authority. 116p.

MacIsaac HJ. 1996. Potential abiotic and biotic impacts of zebra mussels on the inland waters of North America. Amer Zool 36:287-299.

Mackie GL, Claudi R. 2010. Monitoring and Control of Macrofouling Mollusks in Fresh Water Systems, 2nd Edition. CRC Press. 508p.

Mackie GL, Schloesser, DW. 1996. Comparative biology of zebra mussels in Europe and North America: An overview. Amer Zool 36:244-258.

McMahon RF. 1996. The physiological ecology of the zebra mussel, dreissena polymorpha, in north america and europe. Amer Zool 36:339-363.

McMahon RF. 2015. Implementation of a cost-effective monitoring and early detection program for zebra mussel invasion of texas water bodies. In: Biology and management of invasive quagga and zebra mussels in the western united states, (Wong WH, Gerstenberger S, eds): CRC Pres, 349-374.

Mellina E, Rasmussen JB. 1994. Patterns in the distribution and abundance of zebra mussel (dreissena-polymorpha) in rivers and lakes in relation to substrate and other physicochemical factors. Can J Fish Aquat Sci 51:1024-1036.

Miller RL, Mojares JJ, Ram JL. 1994. Species-specific sperm attraction in the zebra mussel, Dreissena-polymorpha, and the quagga mussel, Dreissena-bugensis. Canadian Journal of Zoology-Revue Canadienne De Zoologie 72:1764-1770.

Misamore M, Silverman H, Lynn JW. 1996. Analysis of fertilization and polyspermy in serotonin-spawned eggs of the zebra mussel, Dreissena polymorpha. Molecular Reproduction and Development 43:205-216.

Misamore MJ, Stein KK, Lynn JW. 2006. Sperm incorporation and pronuclear development during fertilization in the freshwater bivalve Dreissena polymorpha. Mol Reprod Dev 73:1140-1148.

Nalepa TF, Schloesser DW. 1993. Zebra mussels. Biology, impacts, and control:CRC Press.

Neary BP, Leach JH. 1992. Mapping the potential spread of the zebra mussel (dreissena-polymorpha) in ontario. Can J Fish Aquat Sci 49:406-415.

Pimentel, D, Zuniga, R, Morrison, D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol Econ 52:273-288.

Ram JL, Walker JU. 1993. Effects of deionized water on viability of the zebra mussel, Dreissena-polymorpha. Comparative Biochemistry and Physiology C-Pharmacology Toxicology & Endocrinology 105:409-414.

Ramcharan CW, Padilla DK, Dodson SI. 1992. Models to predict potential occurrence and density of the zebra mussel, Dreissena-polymorpha. Can J Fish Aquat Sci 49:2611-2620.

Schloesser DW, Nalepa, TF, Mackie, GL. 1996. Zebra mussel infestation of unionid bivalves (Unionidae) in North America. Amer Zool 36:300-310.

Schneider CA, Rasband WS, Eliceiri, KW. 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods 9:671-675.

Spidle AP, Mills EL, May B. 1995. Limits to tolerance of temperature and salinity in the quagga mussel (Dreissena bugensis) and the zebra mussel (Dreissena polymorpha). Canadian Journal of Fisheries & Aquatic Sciences 52:2108-2119.

Sprung M. 1987. Ecological requirements of developing Dreissena polymorpha eggs. Arch Hydrobiol 79:69-86.

Stoeckel JA, Padilla DK, Schneider DW, Rehmann CR. 2004. Laboratory culture of dreissena polymorpha larvae: Spawning success, adult fecundity, and larval mortality patterns. Canadian Journal of Zoology - Revue Canadienne de Zoologie 82:1436-1443.

Strayer DL. 1991. Projected distribution of the zebra mussel, Dreissena polymorpha, in north america. Canadian Journal of Fisheries & Aquatic Sciences 48:13891395.

USACE. 2013. Zebra mussel resource document - trinity river basin, texas. W9126G-09-D-0067.US Army Corp of Engineers.

Texas Parks and Wildlife. 2022. https://tpwd.texas.gov/huntwild/wild/species/exotic/zebramusselmap.phtml Accessed Apr. 17, 2022.

Vinogradov GA, Smirnova NF, Sokolov VA, Bruznitsky AA. 1993. Influence of chemical composition of the water on the mollusk Dreissena polymorpha. In: Zebra mussels: Biology, inpacts and control. Boca Raton, FL:Lewis Publishers, 283-293.

Whittier TR, Ringold PL, Herlihy AT, Pierson SM. 2008. A calcium-based invasion risk assessment for zebra and quagga mussels (Driessena spp). Frontiers in Ecology and the Environment 6:180-184.

Wright DA, Setzler-Hamilton EM, Magee JA, Harvey HR. 1996. Laboratory culture of young zebra (Dreissena polymorpha) and quagga (D. Bugensis) mussels using estuarine algae. Journal of Great Lakes Research 22:46-54.