Impact of zebra mussels on unionid mussels, population dynamics and limiting factors for growth and survival

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Executive Summary

This report summarizes our work carried out during FY 2020 and 2021, but also includes data from previous years for comparison (especially Chapter 3).

The first chapter focuses on the impact of zebra mussels on native unionid mussels for which the impact of the presence of zebra mussels and their infestation on the physiological condition of unionid mussels was examined with samples from the field and lab experiments. Lab experiments showed that both direct and indirect interactions with zebra mussels can significantly reduce glycogen storage, but zebra mussel infestation on unionid shells had a significantly stronger effect on unionid glycogen stores than indirect competition for food under similar zebra mussel densities. Field data indicated that zebra mussels showed similar detrimental effects on native unionid mussels as in other states despite their lower densities. The impact of zebra mussels was likely exacerbated by the additional metabolic costs associated with higher water temperatures in water bodies in Texas near the southern extent of the invaded range. We also collected baseline data on native unionids for potential monitoring of future impacts of zebra mussels at various sites that could be used for long-term studies.

The second chapter discusses the potential impacts of summer mortality of zebra mussels on native unionid mussels and on nutrient cycling in Canyon Lake. Nutrient release in decaying mussels was examined in the laboratory and combined with field observations of zebra mussel density and mortality to estimate the amount of nutrients released during summer mortality events. Summer mortality associated with higher water temperature can cause considerable releases of nutrients while mussels die in large numbers and decay. This includes release of ammonia, which is highly toxic to juvenile unionid mussels.

The third chapter summarizes our ongoing long-term monitoring of population dynamics of zebra mussels in Canyon Lake, which started soon after the lake was invaded in 2017. Although higher summer temperatures seem to limit zebra mussels, their population has continued to increase and expand in Canyon Lake. Highest recruitment success and lowest mortality was detected in 2020, the year with the lowest number of days with high temperatures.

Chapter I

Impact of zebra mussels on physiological conditions of unionid mussels in Texas

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Abstract

Zebra mussels (*Dreissena polymorpha*) are an aquatic invasive species known to detrimentally affect native unionid mussels, a highly imperiled group of organisms. Yet, no study has compared the impact of infestation (i.e., direct attachment to unionid mussel shells) and presence of zebra mussels on glycogen storage under controlled conditions, nor examined the impacts of zebra mussels at the southern edge of their North American distribution. Hence, the objectives of this study were to 1) examine the impacts of infestation versus presence of zebra mussels with experiments in the laboratory and 2) in the field by collecting data on glycogen concentrations of unionid mussels at field sites with and without zebra mussels. In the laboratory experiment, unionid mussel tissue samples were collected after 30 days from treatment tanks where 1) Threeridge (*Amblema plicata*) were artificially infested with zebra mussels, 2) zebra mussels were present with similar biomass, but shells of *A. plicata* were not infested, and 3) control tanks where zebra mussels were absent. Results from the experiments showed zebra mussel presence and infestation reduced glycogen by 38% and 66% respectively. Results from the field supported these findings. Variation in glycogen concentrations of mussels collected in the field was best explained by chlorophyll-a concentrations (coarse measure of food resource) and total number of zebra mussels found in the sampling quadrats. Zebra mussels showed similar detrimental effects as in other states despite their lower densities, likely exacerbated by the additional metabolic costs associated with higher water temperatures in Texas water bodies near

the southern extent of the invaded range. Our study suggests that the combined impact of invasive species and rising temperatures due to global warming needs to be considered for conservation and management plans.

Introduction

Freshwater mussels (Family Unionidae) are filter feeders that provide crucial ecosystem services by filtering the water (biofiltration), recycling and storing nutrients, and creating and structuring habitat (Vaughn 2018). North America has the highest diversity of freshwater mussels in the world; however, they are also considered one of the most imperiled groups of organisms in North America (Haag 2012; Lopes-Lima et al. 2018). Texas has roughly 50 species of unionid mussels, 15 of those being state threatened, and 5 being candidates for federal listing (TPWD 2018). Invasive zebra mussels (*Dreissena polymorpha*) can pose a severe threat to unionid mussels (see below). Zebra mussels invaded Texas in 2009 and are currently reproducing in five different basins and 33 different Texas lakes and rivers downstream of infested lakes (TPWD 2019) despite original beliefs that they would be unable to survive high temperatures.

Zebra mussels are a successful freshwater invader that can reach high densities and reduce phytoplankton due to their filtering activity. They also redirect nutrients and energy from the water column to the bottom of the system, causing benthification, a decrease in pelagic production and an increase in benthic production (Strayer 2009; Higgins and Vander 2010; Karatayev et al. 2015). Maturation of zebra mussels is reached within one to two years and each adult female can produce over a million eggs per spawning event (Higgins and Vander 2010). After a brief planktonic veliger stage, juveniles adhere to hard surfaces and stay relatively sessile through adulthood (Higgins and Vander 2010; Karatayev et al. 2015). Infestation is achieved

through the production of byssal threads with which they attach to hard surfaces including epizoic colonization of unionid shells (i.e., infestation; Eckroat et al. 1993).

Zebra mussel invasion of the Great Lakes led to severe decline of unionid mussels and corresponded to observed infestation of zebra mussels on unionid shells (Schloesser and Nalepa 1994; Gillis and Mackie 1994). Because unionid mussels evolved in the absence of fouling organisms, they carry no line of defense when zebra mussels attach (Wahl 1989; Haag et al. 1993), although their burrowing behavior can protect them against zebra mussel infestation (Nichols and Wilcox 1997). Infestation of unionid mussels by zebra mussels can potentially lead to suffocation, death, altered locomotion and burrowing, shell deformities, and interference with normal functioning of the siphons and valve opening (Mackie 1991; Haag et al. 1993; Schloesser and Nalepa 1994).

Another effect of zebra mussel infestation is direct competition with native unionids for food resources. Through their filtering activity, zebra mussels can alter available food resources for native mussels; systems invaded by zebra mussels have experienced declines of up to 50-75% of phytoplankton biomass which resulted in more than a 50% population decline of filter feeding zooplankton and native mussels (Gillis and Mackie 1994; Karatayev et al. 1997; Higgins and Vander 2010). The competition for food can occur when zebra mussels are present in the same system but may be more severe when they are attached to the shells of unionid mussels (Strayer and Malcom 2018). Considerable attention has been given to the documentation of unionid survival after zebra mussel invasion in the northern regions of the United States and Canada, but little research has been conducted on the effects of zebra mussels at the southern edge of their North American distribution which was invaded more recently.

The warmer water temperatures, especially in summer, have metabolic consequences for invertebrates, which may exacerbate the physiological effects of competition for food with zebra mussels on unionid mussels. However, zebra mussels occur at lower densities in Central Texas (e.g., Locklin et al. 2020; chapter 3, this report) compared to the northern regions of North America (e.g., Griffiths et al. 1991, Hebert et al, 1991, Haag et al. 1993, Schloesser et al. 1996) which could potentially reduce their impact on unionid mussels but impacts of zebra mussels on native unionids has not previously been studied in Texas.

In addition to observed declines of unionid mussel populations, physiological measures have also shown that zebra mussels have a detrimental effect on unionid mussels. A common metric for assessing physiological stress in unionid mussels is through glycogen concentrations. Glycogen is the main storage of carbohydrates and studies have shown that glycogen concentrations in unionid mussels are sensitive to zebra mussel infestation (e.g., Haag et al. 1993; Hallac and Marsden 2000; McGoldrick et al. 2009; Sousa et al. 2011; Table 1.1). A study conducted in Lake Champlain, Vermont, USA found as the ratio of attached zebra mussel/unionid mussel mass increased, glycogen stores decreased for *Lampsilis radiata* (Hallac and Marsden 2000). Similar results were found in Lake Erie with individuals of *Amblema plicata* and *L. radiata* (Haag et al. 1993). Other studies have found the tribe Lampsilini to be less vulnerable to zebra mussels than *Elliptio complanata* (Hunter and Bailey 1992; Strayer and Smith 1996). A study conducted across six United Kingdom localities found that infested unionid mussels had lower glycogen stores than uninfested unionids and glycogen measures were independent of unionid size (Sousa et al. 2011). Previous studies have shown that both infestation of zebra mussels and the presence of zebra mussels in the same system can affect the body condition of unionid mussels (e.g., Haag et al. 1993; Hallac and Marsden 2000;

McGoldrick et al. 2009; Sousa et al. 2011; Table 1.1), but to the best of our knowledge no study has compared the impacts of these different scenarios on glycogen storage under controlled conditions.

The first objective of this study was to gather baseline data on glycogen concentrations of unionid mussel between lower and higher zebra mussel sites and control sites in Central Texas, the current southern edge of zebra mussel ranges. We predicted that unionid mussels at sites with higher densities of zebra mussel would have the lowest glycogen concentrations (lower energetic stores) while control sites would have the highest and sites with lower zebra mussels would have intermediate values (Table 1.2).

The second objective was to test the effects of zebra mussel presence and attachment on unionids experimentally with *Amblema plicata* in treatment tanks where (1) *A. plicata* was artificially infested with zebra mussels, (2) zebra mussels were present in similar biomass as in the first treatment, but no infestation occurred, and (3) control tanks where no zebra mussels were present. We predicted mussels on which zebra mussels were attached would have the lowest glycogen concentrations, control mussels the highest, and mussels in tanks where zebra mussels were present would have intermediate values (Table 1.2). In addition, it was examined whether the burrowing behavior would differ between treatment and control tanks in the experiment and different field sites.

Methods

The focus of this study was on *Amblema plicata,* because it is a common species that often occurs in higher abundances in Central Texas. *A. plicata* is also larger in size than other common species (*Lampisilis teres, Cyclonaias pustulosa*) and have ridges on their shells which allows more surface area for zebra mussels to attach. Additionally, *Amblema plicata* mussels

were the most used species across previous glycogen studies, thus this species was chosen to draw comparisons between studies.

Study Area and site selection

A total of ten sites were chosen along the Brazos, Colorado, and Guadalupe river basins in Central Texas, USA, where a sufficient number of *Amblema plicata* for comparative glycogen analysis were found (Figure 1.1, Table 1.3).

Zebra mussels were present at four of these sites (Table 1.3). Twelve additional sites were surveyed where zebra mussels and unionid mussels had been found by state agencies and other entities, but only few or no live unionid mussels and many dead unionid mussel shells were found at those sites.

Four control sites without zebra mussels were located in the Guadalupe River. Guadalupe 1 and 2 were about 260 and 195 kilometers respectively downstream of Canyon Lake (infested with zebra mussels since 2017) and Guadalupe 3 and 4 were directly downstream of Lake Wood (not infested by zebra mussels), where flow was minimal (Figure 1.1). The other two control sites were located in the San Antonio River and Yegua Creek (Brazos River watershed, Figure 1.1).

Two sites with lower zebra mussel densities in 2020, LBJ 1 and 2, were in Lake Lyndon B. Johnson, an impoundment of the Colorado River in the Highland Lakes chain that has a surface area of approximately 24 sq km and was invaded by zebra mussels in 2019. Although all lakes in this chain are now infested, zebra mussels were not present upstream of this lake until Lake Buchanan—two impoundments upstream—was invaded in 2020 and subsequently Inks Lake immediately upstream was invaded in 2021.

Lastly, two sites with higher zebra mussel densities in 2020, Belton 1 and 2, were in Lake Belton a reservoir on the Leon River in the Brazos River Basin (Figure 1.1). The reservoir is approximately 50 sq km and was invaded in 2013.

Environmental variables

Temperature (°C), specific conductivity (μS cm^{-1}), and dissolved oxygen (mg L^{-1}) were measured at each site using a YSI 143 556 MPS. Average velocity (m/s) was measured at each mussel sampling site at 60% depth in the middle of the stream using an electromagnetic flow meter (HACH, model number FH950). Substrate composition was examined visually to determine the dominant substrate size according to a Modified Wentworth scale.

Chlorophyll samples were taken at each site at the date of glycogen clips following the standard operating procedure for collecting water samples in the field (Oklahoma Water Research Board 2018). Water samples were kept in a cooler on ice and filtered as soon as they arrived back to the lab (2-3 hours after collection). Chlorophyll-a was measured using the in vivo method (Adamczyk and Shurin 2015). This was completed by using a Turner Trilogy® Laboratory Fluorometer and the following equation (determined from the regression relationship):

***Chlorophyll*-a (μg/L) = [0.0559 * RFU] – 4.3228.**

Where RFU is the measure of raw fluorescence.

Mussel Survey

At all sites, unionids and zebra mussels (if present) were surveyed within 30 sampling quadrats (0.5m x 0.5 m for unionid mussels, 0.25 x 0.25 m for zebra mussels) placed randomly along 6-10 transects, and the total number of mussels were determined. A substrate depth of at least 10 cm was searched for unionid mussels. Zebra mussel densities at sites in Lake Belton

were > 200 individuals per m^2 and these were designated higher density zebra mussel sites, whereas zebra mussel densities in Lake LBJ were < 200 individuals per m^2 and these were designated lower density zebra mussel sites.

Burrowing depths of all sampled mussels was estimated by measuring the posterior part that was exposed to the water and which was encrusted with algae and calcium and the total mussel shell length (to the nearest mm, Figure A1). Mussels that did not have calcification build up on their shells were not used, however only nine total mussels showed no calcification on their shells. No sampled mussels were completely burrowed at the time of sampling.

Mussel Processing and Tissue Clips

Due to seasonal variation in food resources, all field site glycogen clips were taken within one month between September 16th, 2020 and October 5th, 2020 when glycogen has been found to be highest (Hummel et al. 1988). After mussels were collected, they were kept in the water in buckets with holes in them to keep emersion to a minimum during measuring, tagging, and muscular foot clipping. Infested mussels were weighed before and after zebra mussels were removed to calculate the wet-weight zebra mussel biomass to the nearest tenth of a gram. Foot tissue of 20 randomly chosen individuals of *A. plicata* at each field site were sampled for glycogen analyses with sterilized dissecting scissors. Tissue samples were stored on dry ice and placed in the freezer (-20C) upon return to the laboratory. After processing, mussels were gently placed back into the substrate, siphons up, to ensure minimal disturbance.

Lab Experiment

The lab experiment was conducted from November 20th, 2020 to December 20th, 2020. A total of twelve 31 L tanks were used in the experiments, four for each treatment (treatment 1: with zebra mussels attached, treatment 2: zebra mussels present, and treatment 3: control, no

zebra mussels). Sixty adult *A. plicata* were collected from control site Guadalupe 3 on November 6th, 2020 and were transported in a cooler with substrate and aerated river water. Mussels were placed in tanks placed in a flow through system for 14 days before the start of the experiments. Mussels were fed a 2:1 diet of shellfish and *Nannochloropsis* on days 3, 6, 9, and 12. During the feeding days, the flow through system was stopped for 24 hours and the same amount of food was given to each tank. All experimental tanks were set up one day before the start of the experiments. They were filled with 10cm of substrate from the collection site, well water, and were aerated with air stones. Zebra mussels were collected from Canyon Lake, TX and brought back to the lab in aerated coolers. After returning to the lab, 20 randomly picked individuals of *A. plicata* were placed in an aerated 31 L tank at room temperature with zebra mussels (~4,500) for 12 hours to allow the zebra mussels to attach to the shells of the *A. plicata*. The infestation resulted in 8.8 ± 2.8 g wet zebra mussel biomass per unionid mussel, which was comparable to the average wet zebra mussel biomass attached on unionid mussel shells in the field (both higher and lower zebra mussel sites combined). All other unionids were handled the same way but no zebra mussels were added. Mussels did not receive food during this time. After this, all mussels were placed in the experimental tanks, 5 unionids per tank.

Treatment 1 tanks contained on average 44 g ± 14 g wet zebra mussel biomass. A similar zebra mussel biomass was created in all treatment 2 tanks by adding between 100 and 125 zebra mussels (i.e., on average 44 ± 14 g wet biomass). The mussels were fed a 2:1 diet of shellfish and *Nannochloropsis*. Food was dispensed every hour via a Bubble Magus BM-T11 Dosing Pump by Bubble Magus to ensure that feeding rates were consistent throughout the experiment and similar to natural chlorophyll-a concentrations at the collection site, Guadalupe 3. Chlorophyll-a was measured a total of eight times during the experiment to ensure the equipment

was functioning properly and dosages were correct. Temperature (^{0}C) and dissolved oxygen (mg L^{-1}) was measured daily with a YSI 143 556 MPS. Burrowing behavior was also documented daily by noting down the number of mussels which were burrowed at least 90% below the substrate. Room temperature well water was periodically added to tanks to balance loss via evaporation and one-third volume water changes were completed every seven days until the experiment was completed after 30 days.

Any live zebra mussels used in experiments and any water that was in contact with zebra mussels was treated with a 10% chlorine bleach solution after use before being disposed. Any items that had been in the water with the mussels, including the tanks, were treated as well. A water-proof tub was used to soak aquaria and water in the 10% bleach solution for a minimum of 30 minutes (Coon 1993). This solution ensured that any larvae, juvenile, or adult zebra mussels were killed.

Changes in glycogen concentrations have been seen in as little as seven days (Patterson et al 1997) or three months (Haag et. al 1993). For this laboratory study, we sampled foot tissue 30 days after the experiment was initiated from all unionid mussels in the experiment (see tissue sampling procedure above).

Glycogen Quantification

Glycogen analysis was completed using a procedure adapted from a method to quantify glucose in potatoes (Bethke and Busse 2008). First, glycogen was extracted from mussel foot tissue by homogenizing the sample in 10% ethanol and centrifuging the sample to obtain the supernatant. Second, the glycogen content was quantified by adding amyloglucosidase into one replicate of the sample wells to transform glycogen into glucose monomers. An enzyme mix of glucose oxidase, 10-acetyl-3,7-dihydroxyphenoxazine (ampliflu Red), and horseradish

peroxidase (HRP) was then added to cause a color change in the samples to a pinkish resorufin in the microplate wells. The resorufin has an absorbance of 560 nm and is proportional to glucose concentration. Spectrophotometer absorbance values of sample wells with amylglucosidase were subtracted from wells without amyloglucosidase to compute the amount of glycogen (mg/g) in the mussel tissue (Bethke and Busse 2008).

Data Analysis

Analysis of Variance (ANOVA) was used to examine differences in burrowing depth measured in the field (percentage of mussel shell burrowed below substrate), chlorophyll-a concentrations, and glycogen concentrations of lab samples. Data were tested for normality using a Shapiro-Wilks test and homogeneity of variances was confirmed through a Levene's test. A non-parametric Kruskal-Wallis test was used to examine differences in glycogen concentrations of field samples and between field and lab samples because homogeneity of variance was not met. To determine which differences were significant, a post-hoc Tukey's honest significance test (for ANOVA) and a Dunn test (for Kruskal-Wallis) were used. Burrowing depth was logit transformed to change percent data (bound from 0 to 100) into data that had no upper or lower limits and laboratory glycogen data were log transformed to increase normality and meet criteria of homogeneity of variance. Differences in the number of mussels burrowed per treatment were determined through a generalized linear mixed effect model (glmer) accounting for day as a factor (Table A1). An additional model was used to determine if there was an interaction between treatment and day. Estimated marginal means were then calculated to determine significant differences between treatments and control.

General linear models were also used to determine which variables were most strongly correlated with glycogen concentrations in the field. Variables tested in the model included:

chlorophyll-a concentrations, total number of zebra mussels present in the sampling quadrats (not attached), total number of unionid mussels, and zebra mussel infestation rate. An Akaike's information criterion (AIC) was performed to select the best models by comparing each of the candidate models simultaneously. We converted AIC to small-sample AICc and calculated Akaike weights (w_i). The model having the lowest AICc was selected because it identifies the main explanatory variables while providing the best compromise between predictive power and model complexity (Johnson and Omalnd 2004). In addition, the best performing models are those with the lowest AICc and the highest weight (w_i) and models with $\Delta_i < 2$ are generally considered to have substantial support (Burnham & Anderson 2002). The Δ_i is the difference between the AICc of the best fitting model and that of model *i*.

R 4.0.5 (https://cran.r-project.org/) and R studio (https://www. rstudio.com/) were used for all statistical analyses. Additionally, packages car (for Levene's test), FSA (for Dunn test), MuMIN (for model selection analysis), and lme4 and emmeans (for linear model analysis) were used.

Results

Environmental Variables

Environmental variables were similar between field sites except for chlorophyll-a concentrations. All field sites were similar in temperature (19.3-21.8 ºC, range), pH (7.2-7.6), DO concentrations (6.5-7.7 mg L^{-1}), and specific conductivity (680 and 832 µS cm^{-1}, Table 1.3). Average velocity ranged between 0 and 0.69 m s^{-1}. A total of five sites were dominated by sand, three sites were dominated by finer substrates such as silt and clay, and two sites were dominated by a mixture of gravel, cobble, and/or boulders (Table 1.3).

Chlorophyll-a significantly differed between sites ($F_{9, 40} = 217.9$, $p = <0.001$) (Figure 1.2). Chlorophyll-a concentrations ranged from (4.0 ± 0.4 µg/L, mean ± SD) to (22.1 ± 1.3 µg L-1) with the lowest concentrations found at Guadalupe 1, Guadalupe 2, and San Antonio and the highest concentrations found at LBJ 1, LBJ 2, and Yegua (Figure 1.2).

Mussel Surveys

Infestation rates at the lower density zebra mussel sites (LBJ) were about 6 zebra mussels per unionid mussel, whereas they were up to 7 times higher in Lake Belton (31-42 zebra mussels per unionid mussel, Table 1.3) at the high density zebra mussel sites. Zebra mussel densities ranged between 186/m^2 and 191/m^2 at LBJ sites and 885/m^2 and 968/m^2 at Belton sites. Unionid mussel densities ranged between 2.3/m^2 at Belton 2 and 25/m^2 at Guadalupe 3 (Table 1.3), and were <10 individuals/m^2 at zebra mussel sites, whereas densities were usually > 10 individuals/m^2 at control sites (except Guadalupe 2 and San Antonio, Table 1.3).

Burrowing

In the field, most unionid mussels had about 75% or more of their shell burrowed, but mussels tended to burrow less deeply at the higher zebra mussel sites (Belton 1 and Belton 2, Figure 1.3). Statistically significant differences were only detected between mussels at Belton 1 and mussels at all other sites except Belton 2, and Belton 2 and all other sites except for Belton 1, Guadalupe 3 and LBJ sites ($F_{9,599} = 30.3$, p-value <0.001, Figure 1.3). There was no obvious relationship with substrate type.

In the lab, differences in burrowing behavior were more pronounced compared to the field. The lowest percentage of burrowed mussels (mussels burrowed at least 90% below the substrate) were found in the control tanks. The highest percentage of burrowed mussels was found in the tanks where zebra mussels were directly attached to the shells, and an intermediate

percentage was found in the other treatment tanks where zebra mussels were present (Figure 1.4). All differences in laboratory burrowing behavior were statistically significant (z= -6.2 to 14.7, p-value = <0.001 in all cases, n= 360, Table A1). Mussels began to burrow within the first six days of the experiment and then burrowing behavior plateaued and varied only slightly within treatments for the remainder of the experiment. Day was found to be a significant factor in the linear model (Table A1). There was not a significant interaction between treatment and day (z= 0.4-0.9, p-value = >0.1 in all cases, n= 360).

Glycogen

In accordance with prediction 1, mean glycogen concentrations in the field samples were lowest at the two high density zebra mussel sites (Belton 1 and Belton 2) and highest at the control site Yegua. However, several control sites also had intermediate values similar to the sites with lower density of zebra mussels. The control sites with the lowest chlorophyll-a concentrations (Guadalupe 1, Guadalupe 2, and San Antonio) had lower mean glycogen concentrations compared to the control sites Guadalupe 3 and 4 and the lower density zebra mussel sites (LBJ 1 and LBJ 2, Figure 1.5A, Figure 1.2). Several differences between sites were statistically significant (indicated by different letters in Fig. 5). For example, differences were statistically significant between the higher density zebra mussel sites (Belton 1 and Belton 2) and all other sites except for the two control sites with the lowest chlorophyll-a concentrations (Guadalupe 2 and San Antonio, $X^2_9 = 177.54$, p-value = <0.001, Figure 1.5B, Figure 1.2).

In accordance with prediction 2, glycogen concentrations in the laboratory experiment samples were lowest in mussels on which zebra mussel were attached (4.8 ± 0.6 mg/g, n= 20, mean ± SD), highest in control mussels (14.0 ± 1.8 mg/g, n=20), and intermediate in tanks where zebra mussels were present but not attached to the shells of unionid mussels (8.8 ± 1.2 mg/g,

n=20). All differences between treatments were statistically significant ($F_{2,57}$ = 357.2, p-value = < 0.001, Figure 1.5A).

Glycogen concentrations measured in the laboratory were comparable with those in the field. Glycogen concentrations in mussels from laboratory control tanks (14.0 ± 1.8 mg/g, n=20) were similar (and not statistically different, P >0.05) to control mussels from the collection site, Guadalupe 3 (13.4 ± 1.8 mg/g, n=20), indicating that experimental conditions did not affect glycogen concentrations. In addition, glycogen concentrations in mussels with attached zebra mussels (4.8 ± 0.6 mg/g, n=20) were also similar to concentrations at high zebra mussel density field sites at which mussels were also infested with zebra mussels (4.3 ± 0.8 mg/g and 4.3 ± 0.7 mg/g for Belton 1 and 2 respectively, n=20, Figure 1.5). However, glycogen concentrations in laboratory mussels where zebra mussels were present but not attached (8.8 ± 1.2 mg/g, n=20) were comparable to glycogen concentrations at field sites with the lowest chlorophyll concentrations (Guadalupe 1 and 2, and San Antonio, range of averages: 8.4 to 9.9 mg/g, n=20), but lower compared to zebra mussel low density field sites.

Model selection

A large proportion of the variation in glycogen in mussels from the field was explained by chlorophyll-a concentrations and the total number of zebra mussels present. Based on the average Akaike weights (w_i) from AICc selection, the model combining chlorophyll-a concentrations and total number of zebra mussels present (Table 1.4) predicted glycogen concentrations better than any other model. Chlorophyll-a concentrations and total number of zebra mussels present explained 93% of the variation in glycogen concentrations (adjusted R^2= 0.93). The Δ_i of the next best model, chlorophyll-a and total unionid mussels, was 11.35 which

does not meet the suggested criteria for having substantial support to fit the data, even though it had a relatively high adjusted R squared value (0.73).

Discussion

This is the first study to compare the effects of infestation and presence of zebra mussels on the physiological condition of unionids in a controlled setting. We found that both direct and indirect interactions with zebra mussels can significantly reduce glycogen storage, but zebra mussel infestation of unionid shells had a significantly stronger detrimental effect on unionid glycogen stores than indirect competition for food under similar zebra mussel densities. Tissue samples of mussels collected at different field sites where zebra mussels were present and absent supported these findings.

Similar impacts of zebra mussel infestation on glycogen storage have been reported in other field studies of unionid mussels (Haag et al. 1993; Hallac and Marsden 2001; Sousa et al. 2011; Table 1.1). In our study, unionid mussels from a control site with no zebra mussels were infested with zebra mussels in the laboratory and had on average 66% lower glycogen concentrations when compared to control mussels, which is a bit higher compared to Lake Champlain, where infested mussels experienced a 50% and 46% reduction in glycogen (*A. plicata* and *L. radiata* respectively) when compared to uninfested control mussels from the Lamoille River delta, USA (Hallac and Marsden 2001). In contrast to our laboratory study, their control mussels were obtained from a different location and different environmental conditions, such as food availability (see below discussion about chlorophyll-a), may have affected the results. In Lake Erie, USA, infested unionid mussels experienced a 35% (*Amblema plicata*) and 62% (*Lampsilis radiata*) reduction in glycogen when compared after three months to control mussels from the same lake from which zebra mussels had been removed (Haag et al. 1993).

Zebra mussel infested unionid mussels (*Anodonta anatina* and *Unio pictorum*) in River Stour, Suffolk, UK had lower glycogen concentrations (~15 and ~35% respectively) when compared to uninfested mussels from the same sites (Sousa et al. 2011). As the control mussels in the latter two were from the same system, their results are more comparable to the 46% difference between means in the infested treatment versus zebra mussels present treatment in this study, which is somewhat comparable to the Lake Erie study (Haag et al. 1993), although it was carried out over a longer time period (3 months vs. 30 days in this study), but higher compared to the River Stour study (Sousa et al. 2011).

Interestingly, we found similar impacts of zebra mussels compared to other studies, although zebra mussels occurred at lower densities at our study sites compared to most other studies. The metabolic cost of higher water temperatures will deplete glycogen reserves more quickly, as has been shown in freshwater and marine mussels (Andrade et al. 2018, Clements et al. 2018). A study with unionid mussels (*Amblema plicata*, *Elliptio complanata*, *Fusconaia flava* and *Lampsilis cardium*) found an increase in oxygen consumption with increasing water temperatures, likely to maintain basal metabolic rates at the elevated temperatures (Ganser et al. 2015). Thus, the additional physiological stress of higher water temperatures in summer (see also discussion about mortality in Lake Belton below) may have contributed to the low glycogen concentrations of mussels sampled in infested lakes in early fall. Water temperatures are projected to rise due to global warming (e.g., Czernecki and Ptak 2018, Kedra 2020, Lall et al. 2018), which may render unionid mussels and other invertebrates more vulnerable to invasive species. Therefore, the combined impact of invasive species and changing climatic conditions need to be considered when developing conservation and management plans.

Zebra mussels can drastically reduce phytoplankton populations and are more efficient at differentiation between nutritious and less nutritious particles (Baker and Hornbach 2000; Baker and Levington 2003; Qualls et al. 2007; Higins and Vander 2010) which can increase food stress in unionids, reducing glycogen stores. This study measured the impact of such competition for food on the physiological condition of mussels in the lab and found a 38% average reduction in glycogen when zebra mussels were present compared to control mussels, which has not been measured before to the best of our knowledge. Other studies did not measure glycogen but observed symptoms of starvation and stress of infested unionid mussels (Baker and Hornbach 1997) and found a correlation between unionid body condition and zebra mussel filtration rates (Strayer and Malcom 2018). The impact of zebra mussel infestation may be most detrimental in low flow conditions due to limited replacement of food particles (Strayer and Malcom 2018) especially when thermal stratification impedes vertical mixing (Schwalb et al. 2013).

Variation in mussel glycogen concentrations from field sites were best explained by chlorophyll-a concentrations and total number of zebra mussel. Chlorophyll-a is a coarse measure of food resource (Vaughn et al. 2004; Roznere et al. 2014; Strayer and Malcom 2018) and differed significantly between several field sites, including between control sites. Glycogen concentrations were highest at a control site (Yegua) that was 2nd highest in chlorophyll-a concentrations suggesting that unionid mussels were experiencing low stress and sufficient food resources. Unionid mussels at field control sites with the lowest chlorophyll-a concentrations also had some of the lowest glycogen concentrations (Figure 1.5). This is in accordance with a study that moved unionid mussels from a river with chlorophyll-a levels of 51 ± 9 µg/L to a pond with chlorophyll-a levels of 11 ± 4 µg/L, where mussels experienced a 56% reduction in glycogen (Naimo and Monroe 1999). In the present study, lower zebra mussel field sites were

highest in chlorophyll-a which may have resulted in the higher glycogen concentrations in the tissue of unionid mussels sampled at field sites compared to the mussels in our lab experiment where food was limited and not replaced as consistently as food resources in a reservoir. This may have forced unionid mussels in the lab to use their glycogen reserves to maintain basal metabolic rates (Baker and Hornbach 1997).

Burrowing of mussels may help to control zebra mussel infestation (Nichols and Wilcox 1997; Schwalb and Pusch 2007) and to avoid unfavorable environmental conditions such as cold temperature (Amyot and Downing 1997; Watters et al. 2001). Thus, the significantly higher burrowing activity in the infestation and zebra mussel present treatments may have been caused by mussels trying to escape stress caused by starvation. In contrast, mussels in the field at higher zebra mussel sites (Belton 1 and 2) were burrowed less deeply than other field sites. These mussels were more heavily infested than mussels in our experiment and the substrate in the lab was finer (very fine sand) facilitating their burrowing. In addition, temperatures were high in Lake Belton the summer prior to the fall sampling which resulted in mass die-off of zebra mussels (Jason Locklin, pers. comm.). The high temperature likely also stressed unionid mussels (see also discussion about additional physiological stress of higher temperatures above) and may have caused changes in their burrowing behavior and increases in their mortality. This would explain why we observed that roughly 50% of unionid mussels in the sampling area at Belton 1 had recently died and tissue was still attached to the shells. Each dead unionid mussel had roughly 40-60 zebra mussels attached.

Glycogen is a vital physiological substance that drives multiple physiological processes and can enable unionid mussels to survive emersion and reduction in food availability. Thus, the observed decline in glycogen due to zebra mussel presence and infestation suggests that unionid

mussels affected by zebra mussels may not have enough energetic stores to survive long-term food shortages during winter months or prolonged temperature and low oxygen stress during the summer (Bayne 1976; Gabbott 1973; Hummel et al. 1988) which is likely to occur in Texas waters. Furthermore, depleted glycogen can reduce long-term fitness in unionids by reducing fecundity and growth rates of offspring (Helm et al. 1973; Bayne et al. 1975). Given that unionid mussels are already highly imperiled, any additional stressors such as zebra mussel attachment may lead to further population declines as observed in the Great Lakes and elsewhere (Strayer and Smith 1996; Schloesser and Nalepa 1994; Strayer and Malcom 2018) especially when unionid mussels are exposed to additional human stressors such as climate change and pollution. Adapting effective management practices such as periodic cleaning of unionid shells has been shown to be effective in reducing mortality (Schloesser 1996; Hallac and Marsden 2001) and could help to mitigate effects of zebra mussels on unionids but it is extremely labor and time intensive. Another approach could be to quarantine, clean, and relocate unionid mussels however quarantine periods reduce glycogen (Patterson et al. 1997; Hallac and Marsden 2000) and relocation may not be successful and lead to reduced survival (Dunn 1993; Cope and Waller 1995). Additionally, studies show that Zequanox, a specific strain (CL145A) of the common soil bacterium (*Pseudomonas fluorescens*) successfully and selectively kills zebra mussels but caution must be taken to ensure that dissolved oxygen levels do not fall below minimum requirements for aquatic life such as unionid mussels (Whiteledge et al. 2015, Luoma and Severson 2016).

Ultimately, prevention of zebra mussels infesting a new water body would be the best solution and strict guidelines and regulation, combined with education and outreach can help minimize the spread of zebra mussels (Balcom and Rohmer 1994; Strayer 2009).

Future studies should leverage controlled conditions in the laboratory to examine how the impact of zebra mussels on glycogen varies between species and how low vs. high flow conditions and food sources such as bacteria (not captured by chlorophyll-a concentrations) may interact with the impact of zebra mussels.

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Tables

Table 1.1: Overview of effects of zebra mussels on unionid mussels examined by different studies.

* This value was calculated based on medians from Figure 1.4.

Zebra Mussel Effects on Unionid Mussels	**Studies**	**Results**
Starvation and Stress	Hebert et al.1991	Lipid reserves of highly infested unionids less than half compared to control mussels.
	Baker and Hornbach 1997	Nutritive stress in infested unionid mussels indicated by shifts to lower metabolic rates, more protein-based metabolism (lower O:N ratios), and compensatory increases in grazing rates.
	Baker and Hornbach 2000	Infested specimens had higher ammonia excretion rates, lower carbohydrate and protein, lower respiration to nitrogen excretion ratios and lower clearance rates than non-infested specimens.
Depletion of Glycogen	Haag et. 1993	Infested unionid mussels experienced a 35% (*A. plicata*) and 62% (*L. radiata*) reduction in glycogen when compared to control mussels of which zebra mussels had been removed.
	Hallac and Marsden 2001	Infested mussels experienced a 50% and 46% reduction in glycogen (*Amblema plicata* and *Lampsilis radiate* respectively) when compared to uninfested control mussels
	Sousa et al. 2011	Unionid mussels (*Anodonta anatina* and *Unio pictorum*) experienced reductions in glycogen (~15 and ~35% respectively)* when compared to uninfested mussels from the same site.
Unionid Feeding	Baker and Levinton 2003	Native mussels must compete with zebra mussels for many of the same food types and are less efficient than zebra mussels at differentiating between nutritious and less nutritious particles.
	Strayer and Malcom 2018	Impact of zebra mussel infestation may be most detrimental in low flow conditions due to limited replacement of food particles.

Table 1.2: Predictions and results of glycogen concentrations for both study objectives. ZM – zebra mussel.

	Field Survey	**Lab experiments**
Prediction	Control > Lower ZM sites > Higher ZM sites	Control > Unattached > Attached
Results	Control ≥ Lower ZM Sites > Higher ZM sites	Control > Unattached > Attached

Table 1.3: Environmental parameters for all field sites on the day of tissue collection. All readings were taken within one month at 10am CST at each site. Sites were in the Guadalupe, Brazos, and Colorado watersheds. Belton 1 and 2 were sites with higher zebra mussel densities, LBJ 1 and 2 were sites with lower zebra mussel densities, all other sites were control sites. Infestation rate is the number of zebra mussels per unionid mussels. Unionid density is the number of individuals per m^2.

Date	**Site**	**Unionid Density [mean ± SE]**	**Infestation Rate [mean ± SD]**	**Temper-ature (°C)**	**pH**	**Avg. Velocity (m/s)**	**DO (mg/L)**	**Sp. Cond. (µS/cm)**	**Substrate**
5-Oct-20	San Antonio	5.6 ± 1.7	0	20.4	7.3	0.48	7.2	731	Gravel, cobble, and/or boulders
23-Sep-20	Guadalupe 1	11.7 ± 3.5	0	20.5	7.4	0.23	6.9	774	Silt/clay
23-Sep-20	Guadalupe 2	9.3 ± 2.3	0	20.1	7.2	0.69	7.7	722	Gravel, cobble, and/or boulders
5-Oct-20	Guadalupe 3	24.9 ± 2.6	0	20.8	7.2	0.31	7.4	794	Silt/clay
5-Oct-20	Guadalupe 4	22 ± 3.4	0	20.8	7.2	0.29	7.4	782	Silt/clay
30-Sep-20	Yegua	14.8 ± 2.6	0	19.6	7.6	0.4	6.9	755	Sand
16-Sep-20	LBJ 1	8.4 ± 2.2	6.3 ± 2.6	21.8	7.3	0	6.5	812	Sand
16-Sep-20	LBJ 2	6 ± 1.7	6.4 ± 3.3	21.8	7.3	0	6.5	832	Sand
19-Sep-20	Belton 1	3.1 ± 0.9	37.1 ± 12.9	19.3	7.5	0	7.4	694	Sand
19-Sep-20	Belton 2	2.3 ± 1.1	41.8 ± 12.3	19.4	7.5	0	7.4	680	Sand

Table 1.4: Summary of small-sample Akaike information criterion (AICc) selection of models predicting variation in glycogen concentration. ZM – zebra mussel.

Model	R^2	K	Δ_i	w_i
Chlorophyll+TotalZM	0.93	4	0	0.996
Chlorophyll+TotalUnionid	0.73	4	11.35	0.003
TotalZM	0.30	3	15.13	0.001
Chlorophyll*TotalZM	0.30	5	16.58	0
TotalUnionid	0.30	3	19.01	0
Chlorophyll	0.37	3	236.32	0

Figures

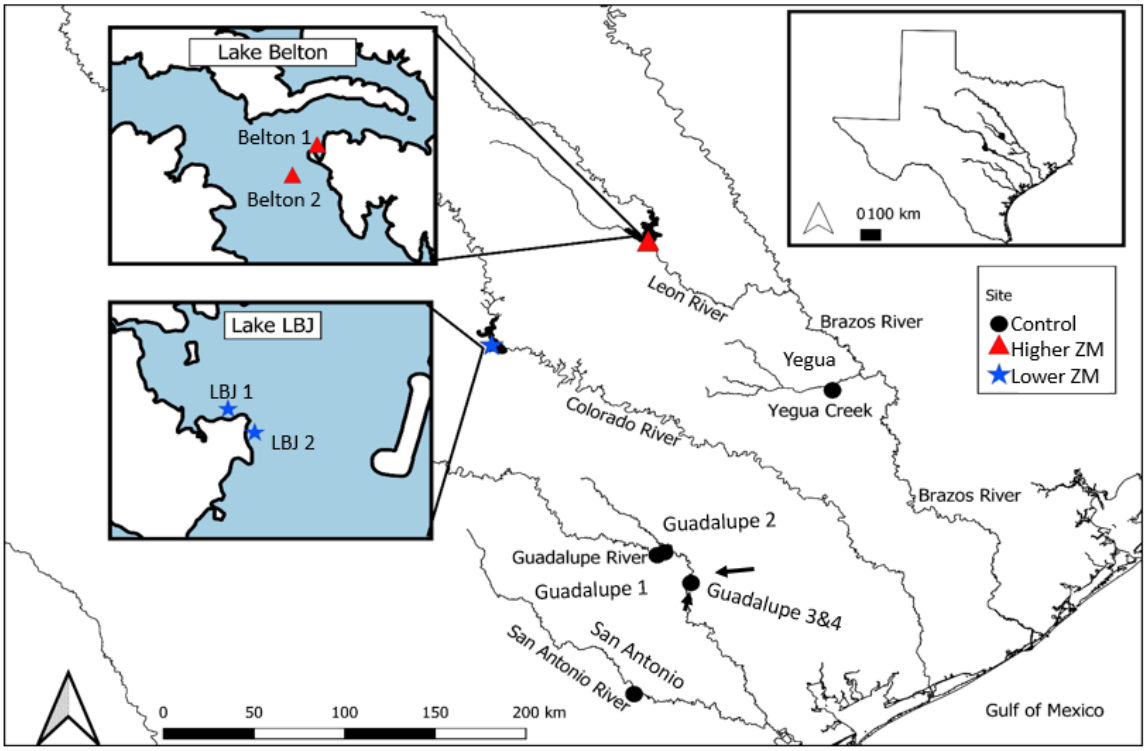

Figure 1.1: Study area in Central Texas showing control sites (black circle), sites with lower zebra mussel densities (blue star), and sites with higher zebra mussel densities (red triangle).

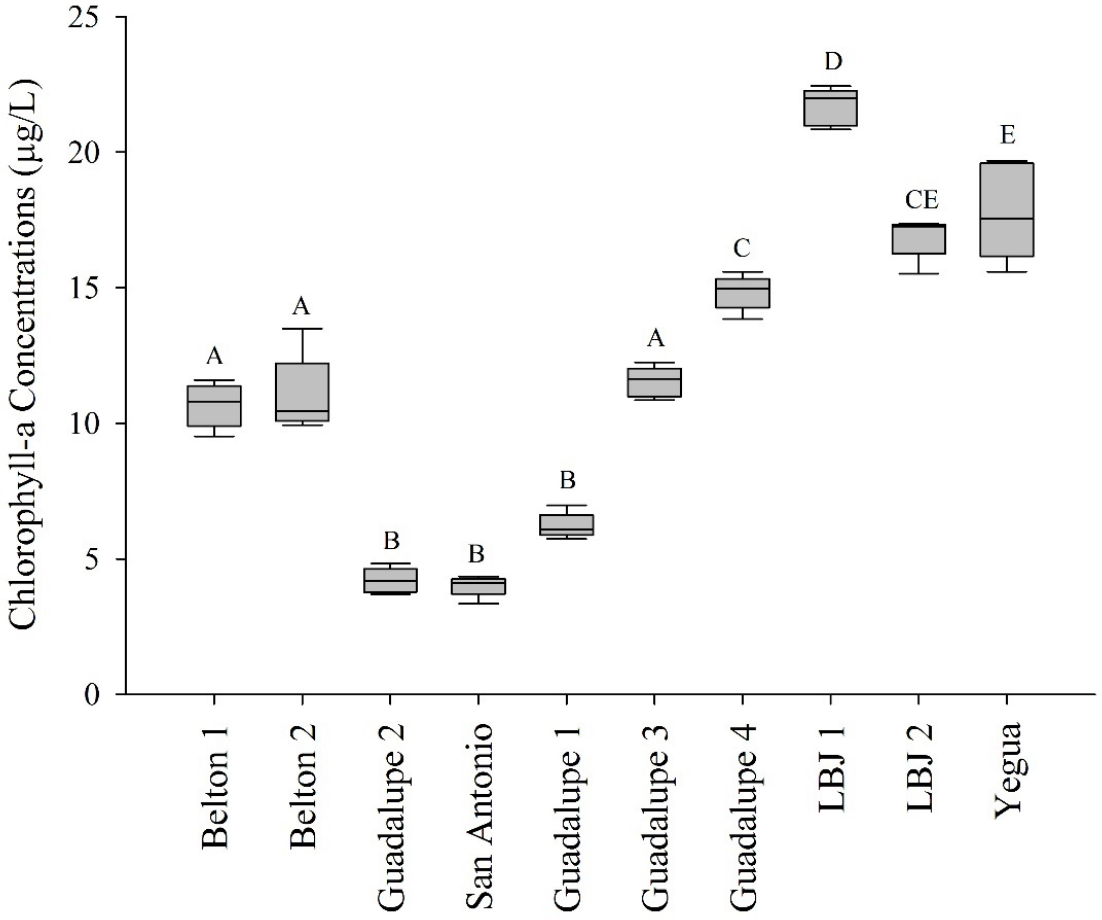

Figure 1.2: Chlorophyll-a concentrations at each field site. Boxplots indicate the 5th, 25th, 50th, 75th and 95th percentiles of the observations. The mean is indicated by the black line. Five measurements were taken at each site. All collections were taken within a one month. Different letters indicate statistically significant differences (Tukey Test, $P<0.05$). Figure is arranged from sites with lowest to highest glycogen concentrations.

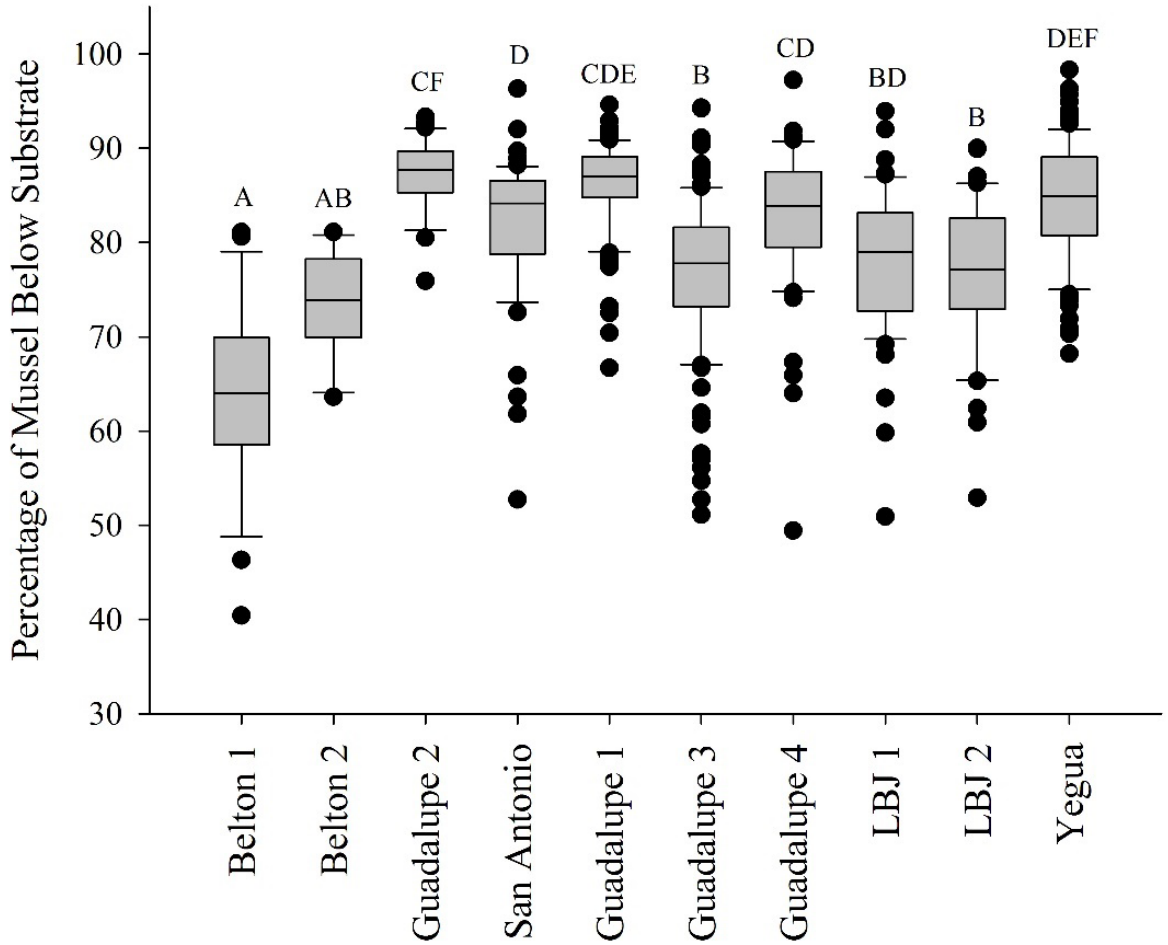

Figure 1.3: The percentage of mussel shell burrowed in the substrate at all field sites. Boxplots indicate the 5th, 25th, 50th, 75th and 95th percentiles of the observations. The mean is indicated by the black line and open circles represent any outliers. All burrowing depths were collected within one month. Different letters indicate statistically significant differences (Tukey Test, $P<0.05$). Figure is arranged from sites with lowest to highest glycogen concentrations.

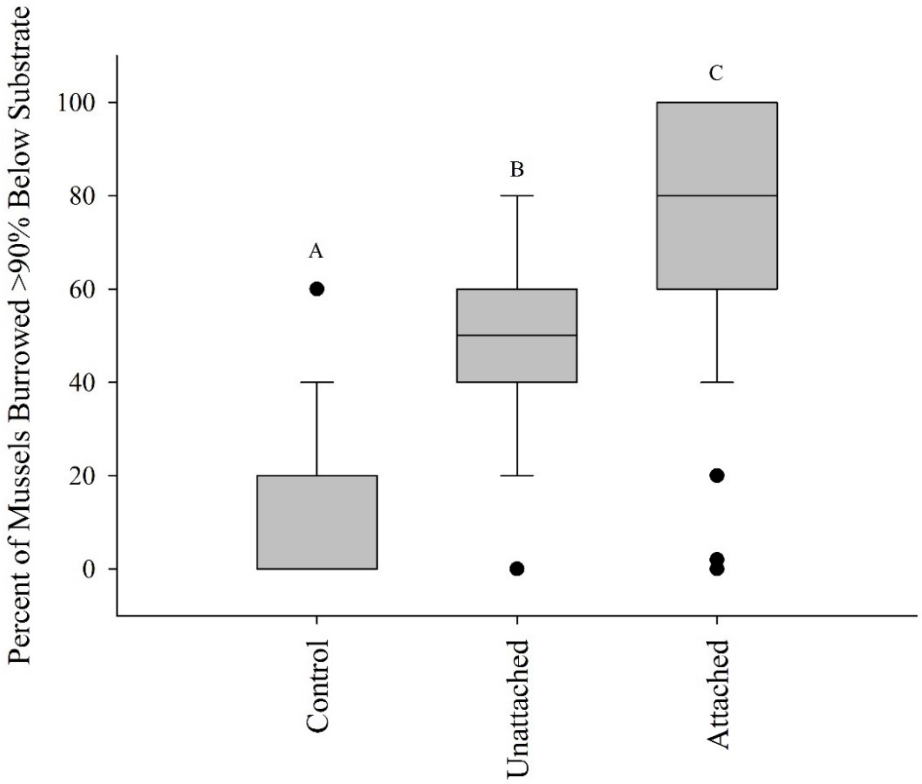

Figure 1.4: The percentage of mussels burrowed in the control and treatment tanks for the experiment. Boxplots indicate the 5th, 25th, 50th, 75th and 95th percentiles of the observations. The mean is indicated by the black line and open circles represent any outliers. Different letters indicate statistically significant differences (emmeans, $P<0.05$).

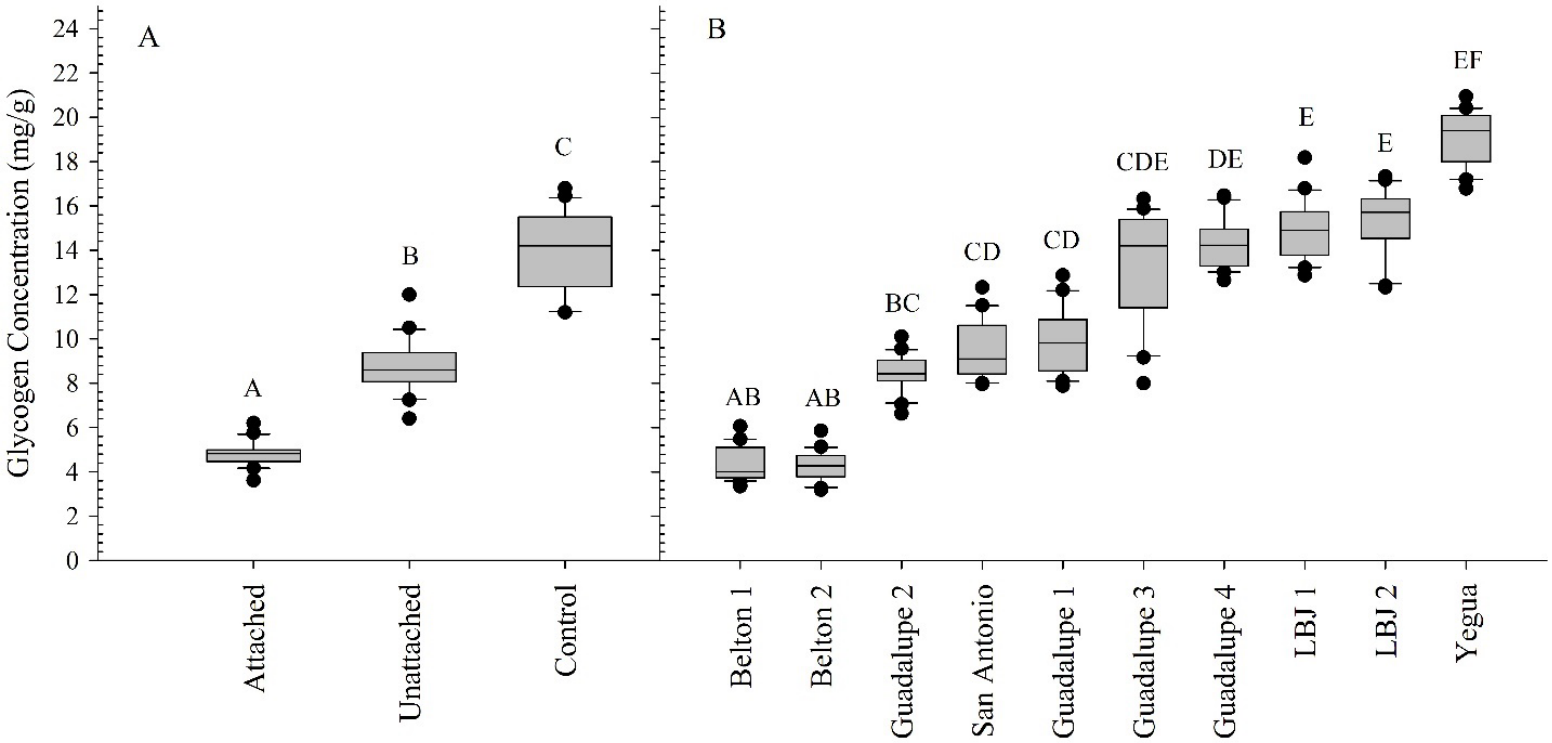

Figure 1.5: Glycogen concentrations (mg/g) of mussels from A) laboratory experiments and B) field sites. Boxplots indicate the 5th, 25th, 50th, 75th and 95th percentiles of the observations. The mean is indicated by the black line and open circles represent any outliers. Each lab treatment and field site had 20 tissue samples collected and analyzed. All field samples were collected within a one-month time period. Different letters indicate statistically significant differences in A) Tukey Test, $P<0.05$ and B) Dunn Test, $P<0.05$. Figure is arranged from sites with lowest to highest glycogen concentrations.

APPENDIX SECTION

Table A1: Summary of fixed effects output from the general linear mixed effects (glmer) model testing the difference of burrowing among treatments.

	estimate	S.E.	z	P
Intercept	-0.84939	0.137315	-6.186	< 0.001
Unattached	1.40028	0.132502	10.568	< 0.001
Attached	1.875047	0.127453	14.712	< 0.001
Day	0.019979	0.004066	4.914	< 0.001

Figure A1: Technique for measuring unionid mussel burrowing depth. The yellow line indicates total mussel shell length while the red line indicates total mussel burrowing depth.

Chapter II

The impact of summer mortality of invasive zebra mussels on native unionid mussels.

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Abstract

Large mortality events can cause nutrient pulses that affect nutrient cycling within a system and ecosystem functioning. Invasive zebra mussels (*Dreissena polymorpha*) in Canyon Lake, Texas occur at the southern edge of their North American distribution and high temperatures during summer can lead to high mortality. The goal of this study was to examine nutrient release in decaying mussels in the laboratory and to combine this with field observations of zebra mussel density and mortality. Zebra mussels were collected from Canyon Lake and ammonia release of decaying mussels was measured over four weeks. In another set of decaying experiments, mussels were decayed at 30°C in lab to determine mass loss and nutrient release rates. Dive surveys along several transects in July and October 2019 and 2020 were used to estimate population size of zebra mussels at different depths throughout the lake. Cages with smaller (<15mm) and larger (> 15mm) zebra mussels were placed at three marinas and monitored bimonthly to determine mortality rates. The decline of zebra mussels in summer 2019 was larger compared to 2020, which was associated with a longer period of high water temperatures (27 vs. 17 days over 30°C respectively). Mortality in the cages varied with mussel size, depth, and location. Temperature was likely the most important driver, but other factors such as total suspended solids and dissolved oxygen likely also played a role. Estimated nutrient releases cause by mortality events may exceed inputs from the Guadalupe River and we found

that zebra mussel decay in a laboratory setting resulted in total ammonia nitrogen concentrations that exceeded both the acute and chronic criterion maximum concentration. This suggests that zebra mussel mortality events may cause unionid mussels to decline

Introduction

Zebra mussels (*Dreissena polymorpha*) originate from Western Asia/Eastern Europe and are prolific freshwater bivalves that were first found in 1988 in the North American Great Lakes (Benson 2013). Within 3 years of establishment, they were found throughout the Great Lakes region. From this region they subsequently and quickly spread to the Mississippi, Arkansas, Cumberland, Illinois, Missouri, Ohio, and Tennessee river basins (Benson 2013). Beyond these connected waterways, invasion of zebra mussels from one unconnected water body to another has been facilitated by transfer of both planktonic larvae in ballast water and adult individuals attached to boats (Bossenbroek et al. 2001, Johnson et al. 2006, Bossenbroek et al. 2007, Strayer 2009, Kelly et al. 2013, Robertson et al. 2020).

Once introduced, zebra mussels cause a variety of ecological and economic problems. Zebra mussels are known to be a virulent bio-fouler and have caused damage to industry, recreation and drinking water infrastructure (Bobat et al. 2004, Connelly 2007 et al., Strayer 2009). Between 1993 and 1999, the economic impact of zebra mussels was estimated to have totaled more than $5 billion throughout the United States (De Leon 2008), and in 2013 annual economic costs across eastern North America were estimated at $100 million (Benson 2013). Furthermore, zebra mussels act as ecosystem engineers by altering the environments they invade (Karatayev et al. 2002, Sousa et al. 2009). Their efficient filter feeding can cause declines in phytoplankton (Raikow et al. 2004, Caraco et al. 2006) which results in increased water clarity (Caraco et al. 1997, Strayer 2009). This increase in water clarity increases benthic photosynthesis

and alters submerged macrophyte densities and composition (Chambers and Kalff. 1984, Vanderploeg et al. 2002, Zhu et al. 2006). In addition, zebra mussel beds often increase benthic macroinvertebrate densities (Stewart et al. 1998, Ricciardi et al. 1997, Vanderploeg et al. 2002, Mortl and Rothhaupt 2003). These alterations to invaded ecosystems can result in "benthification," a shift of energy production from the pelagic zone to the benthic region (Vanderploeg et al. 2002). Finally, studies have shown zebra mussels can affect nutrient cycling in aquatic systems, leading to large scale changes in ecosystem processes (Arnott and Vanni 1996, Li et al. 2021).

Nutrient cycling through animals can increase primary productivity, recycle nutrients within habitats, and translocate nutrients across habitats (Arnott and Vanni 1996, Vanni 2002, Vanni et al. 2006). Animals can also be a source of nutrient pulses, which can have significant impacts on nutrient cycling (Polis et al. 1997, Yang et al. 2008, Hsieh et al. 2012). For example, the massive mortality event following salmon spawning can introduce large quantities of nutrients directly into the surrounding environment (Gende et al. 2002), spread nutrients significant distances through connected water ways (Cak et al. 2008), and even influence a system for months or years later (Verspoor et al. 2011). While mass mortality of salmon after their spawning is a regular annual event, mass mortality of native unionid mussels only occurs during drought or periods of high water temperatures (Dubose et al. 2019, Mitchell et al. 2021)

In the short-term such mortality events increase ammonium and phosphorus (soluble reactive phosphorus (SRP)) and the shells of dead mussels could have significant impacts on long term nutrient release (Dubose et al. 2019). Mass mortality events of invasive mussels, e.g., *Corbicula fluminea*, can also increase nutrients in a system (McDowell et al. 2017), and represent a new source of pulsed nutrient input into an ecosystem, which has the potential to alter

nutrient cycling within a system. Even low levels of ammonia are known to be toxic to unionid mussels (Newton et al. 2003, Augspurger et al. 2003). Previous studies on Corbicula (another invasive clam) have suggested that the release of ammonia due to mass mortality of Corbicula may have negative impacts on native unionid mussels (Cherry et al. 2005; Cooper et al. 2005). Increase of nutrients during mass mortality events of zebra mussels could affect unionid mussels and lead to unwanted algae blooms.

Zebra mussels in Texas are at the southern edge of their distribution range and were first reported in Canyon Lake, TX in 2017. Zebra mussels can experience high mortality during summer in this southern range which has been linked to extended periods of high summer temperatures (White et al. 2015). A large mortality event was observed in Canyon Lake in late summer 2018, indicated by a considerable decline in zebra mussel densities observed by both dive surveys throughout the lake and mortality on artificial substrate installed at the JBSA Marina to monitor cumulative settlement rates (Robertson and Schwalb 2019). The goal of this study was to examine how much nutrient was release from summer mortality of zebra mussels in Canyon Lake. To accomplish this goal we (1) estimated zebra mussel densities and size distribution in Canyon Lake; (2) quantified summer mortality with *in situ* cage experiments; (3) examined with lab experiments how much ammonium, carbon, nitrogen, and phosphorous was released from zebra mussel mortality and whether nutrient release rates and ratios varied with mussel size; (4) estimated the amount of nutrients released in Canyon Lake from summer mortality of zebra mussel based on findings from objectives 1-3; and discussed potential impacts on unionid mussels.

Methods

Canyon Lake and Cage Locations

Canyon Lake is a reservoir in the Texas Hill country fed by the Guadalupe River with a surface area of ~33 km^2 and a volume of ~0.471 km^3. Canyon Lake is a monomictic lake, stratifying in the summer months and mixing between late fall and early spring. Cages for the *in situ* mortality experiments were placed at the JBSA, Canyon Lake, and Crane's Mill marinas (Figure 2.1). JBSA and Canyon Lake marinas are situated in relatively deep parts of the north shore (compared to Crane's Mill). JBSA is the smallest marina and is closest to the dam, Canyon Lake Marina is the largest marina and is the second closest to the dam, and Crane's Mill Marina is the farthest away. Crane's Mill Marina is situated on the south shore of Canyon Lake and is much closer than the other two marinas to the river/lake interface. All three marinas have boat slips for long-term storage of boats and are heavily trafficked during the summer.

Environmental Data

All environmental data was collected from the mortality cage locations (Figure 2.1). Onset pendant temperature loggers were attached to cages at 1 and 9 m depths, and recorded temperatures every two hours from June 16th to October 11th. Monthly (twice in September, with the second sample taken during additional field work related to surveys of dive site locations) water samples were collected in 1000 mL opaque sampling bottles and were kept on ice or in a fridge and filtered within 72 hours of collection. Samples were taken at cage locations (1 and 9 m) over the course of the summer and tested for both chlorophyll-*a* (chl-*a*) and total suspended solids (TSS). Crane's Mill and Canyon 9 m locations were not sampled in July due to a mulfunctioning pump used to retrieve water from 9 m depths. Chl-*a* was calculated by measuring the relative fluorescence units (RFU) using a Trilogy Fluorometer model 7200-000, then converting RFU into chl-*a* concentration using a predetermined regression relationship between the two methods (Robertson and Schwalb 2019).

To calculate TSS, water was filtered through pre-weighed glass microfiber filters until the filter noticeably changed color. The filter was then placed in an oven and heated to 101°C for one hour. Filters were allowed to cool to room temperature then were measured again for a final weight value. TSS was then calculated using initial weight of the filter, final weight after filtration and drying and total volume of water filtered (Standard Methods for the Examination of Water and Wastewater 1998).

Marina profiles were taken at all three marinas during each sampling event with a YSI model ProDDS, where temperature, dissolved oxygen (mg/L and % saturation), specific conductance and pH were measured every meter from 1 to 9 m.

Field Survey Methods

To estimate population densities and size frequency distributions (Objective 1), scuba surveys were conducted in July and October of 2019 and 2020 at 8 established transect sites that ranged from 0.5 to 14.7 rkm from the dam at Canyon Lake (Figure 2.1). The transects were located perpendicular to the Canyon Lake shoreline along a depth gradient, where three replicate quadrats (0.25 x 0.25 m) were placed every 3 m until a depth of 20 m was reached or no zebra mussels were found. Mussels were counted in each quadrat. A random subsample of 50 mussels were collected to determine the size distribution at each depth at all sites in July 2020, and at three sites (close to the dam, middle of lake and closer to the lake/river interface) in October 2020. Mussels collected for size frequency distribution estimates were measured from the anterior to the posterior end to the nearest tenth of a millimeter with vernier calipers. Mussels were grouped in two size classes, smaller (<15 mm) and larger (>15 mm) mussels and the proportion of each size class was computed for each sampling depth and location. To determine total nutrient release from summer mortality of zebra mussels, a total population value was

calculated by taking the highest and lowest average site densities from July 2020 and multiplying them by the total surface area of Canyon Lake to get high and low population values. The high and low population values were then averaged to get an average population value for Canyon Lake. This average was multiplied by the average shell distribution frequency of the entire lake (Small: 70.7%; Large: 29.3%) to determine the total number of small and large individuals. Zebra mussel population estimates were then used in scenario modeling below to estimate total nutrient release from summer mortality.

To determine mortality rates of zebra mussels in Canyon Lake (Objective 2), cages were suspended in the water column at two depths (1 and 9 m) and at each of 3 sites (JBSA, Canyon Lake and Crane's Mill marinas in Canyon Lake, TX). Preliminary cage experiments were run from September to October 2019 and April to May 2020. Summer mortality of zebra mussels was determined from cage experiments that ran from June to October 2020. Zebra mussels were removed by hand from marina substrates within arm's reach at the JBSA marina. Mussels were sorted into larger and smaller size classes and placed inside mesh bags (9 x 6.5 x 5 cm). Two bags containing smaller zebra mussels (5-15 mm) and two containing larger mussels (15-25 mm) were then placed inside conical mesh cages (40 cm long, 10 cm diameter, 1 cm wide holes). These cages were monitored every 2 weeks during the 2019 and June to October 2020 periods, however cages were only monitored once a month during the April to May 2020 period due to COVID-19 complications. At each sampling event, dead zebra mussels were counted and removed from cages.

Laboratory Methods

Two different kind of decay experiments (Objective 3) were carried out, one in which the focus was on ammonia release, whereas the other decay experiments followed methods similar to

those describe by Pray et al. (2009, see below). For the ammonia release experiments mussels in a size range of 10-20mm and water for the experiment were collected at the JBSA marina at Canyon Lake, TX on the same day as the experiment started. Half of the mussels were placed in tanks containing heated well water (60°C) for several minutes resulting in 100% mortality. Mussels were then placed in 2.5 L experimental tanks filled completely with Canyon Lake water. Two different treatment tanks with lower (350 individuals/m^2) and higher zebra mussel density (1,050 individuals/m^2) were used, for which 10 and 30 dead zebra mussels were added respectively. Similarly for control 1 and 2, 10 and 30 live mussels were added, respectively. Six tanks were used as replicated for each treatment and control resulting in a total of 24 tanks.

Tanks were sampled once a day for the first week of the experiment, then once a week for 4 weeks afterwards. Temperature and pH were measured directly before each sampling event. Tanks were stirred lightly before sampling, then 25 mL of water (the amount needed for the ammonium sampling procedure) was sampled directly from the tanks via micropipette. Samples were filtered through glass microfiber filters (47 mm diameter, 1-µm nominal pore size Pall A/E filters) to remove any particulate matter. After filtering, water samples were tested for total ammonia nitrogen (TAN; Wetzel and Likens 2000).

For the other decay experiments, live zebra mussels were collected from the JBSA marina in Canyon Lake, TX, and transported back to the lab and placed in the incubator on the same day as collection. Lake water filtered through 70 µm mesh was collected and transported on the same day as mussel collection to serve as the medium in which to conduct experiments. Mussels were separated into 2 size classes (5 to 15 mm and 15 to 25 mm shell lengths), and 5 replicates were used for each size class. Beakers consisted of five individuals (n = 25 for each size class) and 275 mL of filtered lake water. Beakers were sealed with parafilm and placed in an

incubator at 30° C with no light exposure. Treatments were pulled from the incubator at 1, 2, 4, and 8 days, and shells were removed after vigorous shaking to dislodge any particulate matter stuck to the shell. The beaker was then homogenized with a stir bar and plate, after which the water in the beakers was filtered through pre-weighed, ashed glass microfiber filters (47 mm diameter, 1-µm nominal pore size Pall A/E filters) to catch remaining mussel tissue. The captured tissue was dried at 60°C for 24 h to determine dry mass and then analyzed for C, N and P content. P content of captured mussel tissue was measured with a particulate phosphorus analysis involving an HCl digestion and an ascorbic acid/molybdenum blue spectrophotometric method. Spectrophotometry was performed in a Varian UV-Visible Spectrophotometer.C and N content of captured mussel tissue was analyzed using a FlashEA 1112 Series NC Soil Analyzer. Mass loss rates of zebra mussels during decay experiments were estimated from plots of the natural log-transformed % initial dry mass remaining as a function of time (days). Initial dry mass of each size class of zebra mussel was determined from additional sets of zebra mussels (n = 5 for each size class).

Total nutrient release from summer mortality (Objective 4) was estimated with the estimated total number of zebra mussels in the lake (see above, Objective 1), the nutrient release rates of N and P (Objective 3, for both smaller and larger mussels) and three different mortality scenarios (high, moderate, and low) across three different population sizes (high, average, and low). The highest mortality rate (88% over 16 days), was based on mortality observed in cages with larger mussels at 9 m depth at Crane's Mill, the low mortality rate (8% over 16 days) had been observed JBSA, and an intermediate value was assumed as 48% mortality (i.e., moderate mortality)

Data Analysis

Cage mortality data, mass loss data and nutrient ratio data were tested for normality and homogeneity of variances with a Shapiro-Wilks test and a Bartlett test respectively. If data was not normally distributed, it was log-transformed to improve normality. All tested data met the assumption of homogeneity of variances.

To determine what factors had significant impacts on mortality, a linear mixed effects model was run with survival as the dependent variable, time (days), size class (larger vs. smaller), depth (1 m vs. 9 m), and the number of degree days over 30°C as fixed factors and location (marina) as a random factor. Temperature data at the 9 m location at the Crane's Mill marina was lost as water infiltrated the logger and corroded the electronics. Temperature regimes between Canyon Lake and Crane's Mill marinas were similar, with an average difference of 0.29 ± 0.22°C so temperature for Crane's Mill 9 m was substituted with Canyon Lake 9 m temperature data. In addition, a repeated measures analysis of variance was run with temperature as the dependent variable and depth as the independent variable to determine if temperature regimes differed between depths.

To determine an accurate acute and chronic criterion maximum concentration (CMC) that is adjusted to the pH and temperature of our study we used the table provided in the 2013 EPA aquatic life ambient water criteria for ammonia – freshwater manual (USEPA 2013). Based on a pH of 8.6 and a temperature of 19 degrees Celsius (parameters from this study), acute and chronic CMCs were determined to be 1.3 mg/L total ammonia nitrogen (TAN) and 0.31 mg/L TAN respectively. Total ammonia nitrogen concentrations (mg/L) for all treatments and controls were averaged across the 35-day trial to determine if concentrations met or exceeded the acute and chronic ammonia CMCs.

Analysis of Variance (ANOVA) was used to examine differences in total ammonia nitrogen concentrations among treatments. Data was tested for normality using a Shapiro-Wilks test and homogeneity of variances was confirmed through a Levene's test. R 4.0.5 (https://cran.r-project.org/) and R studio (https://www. rstudio.com/) were used for all statistical analyses. Additionally, the R package car was used for Levene's test.

To assess potential differences in decomposition of the size classes in the laboratory experiments, percent initial dry mass remaining of both size classes was plotted as a function of time (d) and an exponential decay model was fitted to the data to produce a decay constant (k) (Prey et al. 2009) for both size classes. To determine whether mass loss rates differed between size classes, percent initial dry mass remaining was ln-transformed and compared with a repeated measures ANCOVA. Percent dry mass remaining was the dependent variable, size was the independent (categorical) variable and time was the covariate.

Changes of C:N ratios over time were analyzed with ordinary least squares regression, and an exponential decay model was fitted to C:P and N:P ratios. Rates of change in nutrient ratios of the 2 size classes (slopes of the regressions) were compared over the course of the experiment with ANCOVA. Ratio data was the dependent variable, size was the independent (categorical) variable, and time was the covariate).

Changes in mass and nutrient content of both size classes were used to calculate release rates (mg/d) of C, N, and P as:

$$RR\ (mg/d) = [(DM_0 * Nut_0) - (DM_f * Nut_f)]/d$$

where DM is the dry mass (mg) of items on the first (DM_0) and last (DM_f) days of the experiment and Nut is the proportional nutrient content (C, N, or P μg/mg dry mass) on the same

days. Initial dry mass concentrations for nutrient release calculations were derived from previously derived shell-mass relationships (Robertson and Schwalb, 2019).

Total Nutrient Release from Summer Mortality

Different mortality scenarios were used: low, medium, and high. The highest mortality in this study was found at 9 m at Crane's Mill, where 87.5% of large mussels died within 16 days (between sampling events). Lowest mortality was found 1m JBSA, where 7.5% of large mussels died over the same time period (16 days). Percent mortality was multiplied by the estimated population size in the lake (for both smaller and larger mussels, see above) and then multiplied by the nutrient release per individual mussel from laboratory decay experiments to determine the amount of nutrient (metric tons) released by mortality of each size class. Nutrient release from zebra mussel mortality was then compared to nutrient loading from the Guadalupe River. Average monthly values of nitrogen and phosphorus from river inputs were calculated using flow data from USGS gage 08167500 and water quality data from a 2018 TCEQ report on Canyon Lake. Average daily flow of the Guadalupe was taken from USGS gage 08167500 and multiplied by total P values reported by TCEQ to calculate average daily P inputs from the Guadalupe River, while the same flow data was multiplied by the combined values for ammonia and nitrate nitrogen from the same TCEQ report to calculate average daily nitrogen inputs. These daily values of P and N were then multiplied by 16 to arrive at the values used in the nutrient release scenarios (objective 4).

Results

Environmental Data

Across the entire experiment, temperature at 1 and 9 m differed on average by 0.6 to 1.0 degrees (range 0.1 to 5.6°C). These differences were statistically significant (ANOVA: $F_{1,700}$ =

33.85, $p < 0.001$). There were also difference in the number of degree days over 30 ranging between 17 (JBSA) and 40 (Canyon) at 1 m, and 0 (JBSA) to 4 (Canyon) at 9 m. In addition, the number of degree days over 30°C were nearly double in 2019 (27 days) compared to 2020 (17 days), but the number of degree days over 25°C (95 vs. 94 in 2019 and 2020 respectively) and 28°C (75 vs. 79 in 2019 and 2020 respectively) were similar in both years.

Crane's Mill 9 m had higher TSS values compared to all other sites, with an average of 9.1 ± 4.5 (mean ± SD) mg/L, whereas all other sites ranged between 2.0 ± 1.7 mg/L and 4.0 ± 2.0 mg TSS/L (Table A1). Chl-*a* concentrations from the same water samples taken showed little variation across all sites, including Cranes Mill 9 m, ranging on average from 0.9 ± 0.3 to 1.7 ± 1.3 µg/L (Table A2).

In 2020, average DO values at all cage sites were similar except at 9 m depth at Crane's Mill (Table A3), where average DO was considerably lower (5.8 ± 1.4 mg/L, minimum: 3.5 mg/L) compared to the other locations (range throughout summer: 7.3 ± 0.4 mg/L and 7.9 ± 0.3 mg/L, minimum 6.1-6.8 mg/L). In the Sep-Oct 2019 cage experiment, average DO values at all cage sites were similar except at 9m depth at Crane's Mill, where average DO was slightly lower (6.4 ± 0.8 mg/L, minimum: 5.80 mg/L) compared to other locations (range: 7.89 ± 0.69 and 7.53 ± 0.37 mg/L, minimum: 6.97 mg/L).

Objective 1 - Zebra mussel population estimates and size distributions

In July 2019, higher mussel densities were found closer to the dam and in deeper water, whereas higher densities were also found further away from the dam in both July and October 2020 (Fig 2.2). In July 2019, average mussel densities across all depths ranged from 47 ± 22.47 (mean ± SE) to 1208 ± 279 ind/m^2, with average densities > 1,000 ind/m^2 occurring at 9, 15 and 18 m depths. Mussel densities in July 2020 were generally higher compared to July 2019; for

example, densities of > 1,000 ind/m^2 were found at twice as many sampling points (at 6 m depth and deeper) when compared to July 2019. The spatial distribution of zebra mussels also changed between years: mussel densities were lower closer to the dam and in deeper water in 2020 (when compared to July 2019) and densities were greater in shallower depths and farther upstream from the dam (Figure 2.2).

Summer mortality occurred in 2019 and 2020 but was more intensive and widespread in 2019 (Figure 2.3) and the spatial distribution of mortality patterns in the lake differed between years. Between July and October 2019 mussel densities overall declined considerably, declining on average by -47 to -967 ind/m^2 (range: -35% to -100%) at depths $\geq$ 9 m (Figure 2.3), whereas densities increased on average at 3 and 6 m depths (+ 82 and 199 ind/m^2 (+ 27 and 74 %) respectively) with most increases observed at sites farther upstream (> 5.1rkm from the dam). In contrast, mussel densities did not decline at most depths between July and October 2020. Declines occurred at 3, 6 and 12 m depths (-360, -299, -533 ind/m^2; -38, -24, -29% respectively), however densities increased at 9, 15, 18 and 20 m (84, 254, 774, 280 ind/m^2; + 5, 27, 105, 3% respectively). The larger decline in summer 2019 compared to 2020 was associated with a longer period of extremely high temperatures (27 days > 30°C in 2019; 17 in 2020).

Size frequency distributions taken from both 2020 sampling events showed the Canyon Lake population was composed of predominately smaller (<15 mm) sized individuals (Figures 2.3 and 2.4). Overall, smaller zebra mussels comprised a larger proportion of the population. Across both sampling events, the average proportion of individuals <15 mm ranged from 65 ± 23% (mean ± SD) to 80 ± 19%. In July 2020, there was a higher proportion of larger individuals at some locations (BR 1: 3, 6, 9 m; Jacob's Creek: 3 m; BR 7: 3 m), and only at a few sites in October 2020 (BR 1: 3 m, Jacob's Creek: 3 m).

Objective 2 – Mortality

Similar to the dive surveys, higher zebra mussel mortality was detected during preliminary cage experiments in September to October 2019 (at 9 m depth) compared to the same time interval (42 days) in 2020 at two of the three marinas (declines in survival at JBSA: -92.5 ± 7.5 % in 2019 vs. -36.5 ± 6 % in 2020, and Canyon Lake: -50.0 ± 8 % in 2019 vs. -31.2 ± 17 % in 2020). At Crane's Mill survival declined quickly in both years (-92.5 ± 3 % decline 2019, 67.2 ± 25 % decline 2020).

In contrast, survival in spring (April to May 2020, 49 days) did not decline as quickly (-10% or less) in 20 out of 24 cages across all marinas (5 and 9 m depths). Highest declines were detected in three cages at JBSA 9m, where survival declined by -17.5 ± 10 to -25 ± 7% over 49 days.

For data collected in summer 2020, all tested variables (time, number of degree days over 30°C, size, and depth) had a significant effect on mortality (Table 2.1). At 1 m depth, survival decreased by -3.8% (Canyon) to -11.9% (Canyon and Crane) in the first 2 weeks, with similar decreases recorded at 9 m depth (-5% (Canyon) to -12.5% (JBSA)). The decline in survival accelerated at 1m depth after water temperature reached 30°C (after 26 days (Canyon) to 27 days (Crane's Mill and JBSA). Although water temperature did not reach 30°C until later in the summer (75 days (Canyon)) at 9 m depth, survival declined most rapidly at 9 m at Crane's Mill. Large mussels declined -88% within the first sampling period (16 days) and smaller mussels declined -20 ± 15% per week. All larger mussels were dead by day 74 after deployment of cages and smaller mussels by day 54. The average decline at 9 m depth at Crane's Mill was (-4.6 ± 0.07 %/week vs -5.3 ± 0 %/week, at 1m depth). In contrast, at two of the three marinas, mussel survival declined faster at 1 m compared to 9 m depth, which was most pronounced between day

32 and 89, when survival declined by an average of -16.3 ± 3 (JBSA) to -21.3 ± 8% (Canyon) at 1m and -11.7 ± 3 (JBSA) to -20.0 ± 4% (Canyon) at 9m.

The average survival at the end of the experiment (October 11th, 2020) was low, but slightly higher for smaller mussels (11 ± 10 (mean ± SD) to 21 ± 18%) compared to larger mussels (3.0 ± 3.0 to 6.0 ± 5.0%). Higher survival of smaller mussels compared to larger mussels occurred at 4 out of 6 sampling points (JBSA: 1, 9m; Canyon: 9m; Crane's Mill: 1m) (Fig. 2.5).

Objective 3 – Decay and ammonia release

Both treatments 1 (350 dead mussels/m^2) and 2 (1,050 dead mussels/m^2) exceeded the chronic CMC threshold beginning on day 2 after death and remained above the chronic threshold for the remainder of the experiment. Only treatment 2 exceeded the acute CMC threshold (days 2-28); however treatment 1 reached a maximum of 1.24 mg/L TAN on day 6 after death. There were significant differences between control and treatment tanks ($F_{5,24} = 51.8$, p-value: < 0.001). Average total ammonia nitrogen levels for both treatments were more than a magnitude greater than their respective control (Fig. 2.6). There were also significant differences between treatment 1 (0.5 mg/L ± 0.44) and treatment 2 (1.73 mg/L ± 1.79, $F_{5,24} = 51.8$, p-value: < 0.001).

The total ammonia nitrogen concentrations in both treatments were highest on day 6 and day 7 after death (Fig 2.7, treatment 1: 1.24 mg/L TAN and 1.05 mg/L TAN respectively and treatment 2: 5.33 mg/L TAN and 4.44 mg/L TAN respectively). Total ammonia nitrogen concentrations in both controls with alive mussels (control 1: 350 individuals/m^2 and control 2: 1050 individuals/m^2) remained low throughout the experiment. There were no significant differences between control 1 and control 2 ($F_{5,24} = 51.8$, p-value: 0.999).

Objective 3 – Decay and Nutrient Release

Both size classes lost the majority (~65%) of soft tissue mass within the first 48 hours of the experiment. Multiple functions were fit to the relationships between time and % initial mass remaining (e.g., linear, quadratic, exponential) to see which function best described the data (exponential) (Figure 2.8). Mass loss rates (based on % decline) did not differ significantly between size classes (small: k = -0.32, large: k = -0.28; ANCOVA: F = 0.047, p = 0.8345). The majority (~65%) of mass was lost within the first 48 hours for both larger and smaller mussels. Over the 8 days of decay, smaller mussels lost on average 4.4 ± 1.3mg of soft tissue per individual and large mussels lost 12.1 ± 5.9mg of soft tissue per individual. Mass loss across multiple preliminary experiments was consistent with results presented here and decay constants ranged between -0.23 and -0.32.

Molar ratios relating to phosphorus (C:P, N:P) decreased over the course of the experiment, while molar C:N ratios first decreased slightly day 0 to day 1 and increased afterwards (Figure 2.9). Thus, both N and C were lost at a faster rate from decomposing mussel soft tissue when compared to P. There were no significant differences detected in the rate of change of any nutrient ratio between the two size classes of mussel. (ANCOVA: $F_{1,7}$ = 0.131, p = 0.73 (C:N), $F_{1,7}$ = 3.335, p > 0.11 (C:P), $F_{1,7}$ = 1.804, p > 0.22 (N:P)). In addition, the average P release rate calculated for both size classes showed similar rates of P release (Large: 2.53 ± 3.51 µgP/mussel/day, Small: 2.10 ± 0.90 µgP/mussel/day). Calculated P release rates of large mussels varied by orders of magnitude (39.55 to 0.21 µgP/mussel/day), with one of the five calculated rates suggesting P was sequestered by mussel tissue (net uptake of (-4.75 µgP/mussel/day). This large variation in large mussel P release rates (and net uptake value) likely resulted in the average release rate for large mussels being lower, and thus similar to the release rates of small mussels. Unlike P, N and C were found to be released faster from large

zebra mussels when compared to small individuals (Large: 0.16 ± 0.11 mgN/mussel/day, 0.57 ± 0.46 mgC/mussl/day; Small: 0.07 ± 0.03 mgN/mussel/day, 0.26 ± 13 mgC/mussel/day).

Objective 4 - Summer Nutrient Release

Carbon release from summer mortality of zebra mussels (over 16 days) ranged from 5.4 to 240 t (metric tons) across all mortality scenarios (Table 2.2). Nitrogen release ranged from 1.2 to 47 t (52 to 2,000% increase compared to river inputs) and was higher than river inputs in eight out of nine mortality scenarios (Low Population Low Mortality Scenario: 1.2 t; River Loading: 2.3 t) (Table 2.2). Phosphorus release ranged from 0.04 to 14 t (6 to 240% increase compared to river inputs) but was higher than river inputs in only three scenarios (High Population High Mortality: 14 t; Average Population High Mortality: 0.92 t; High Population Intermediate Mortality: 0.78 t; River Inputs: 0.58 t). Eight of nine mortality scenarios predicted that nitrogen released by decaying zebra over 16 days would be higher compared to nitrogen loading from the Guadalupe River into Canyon Lake over the same time span, with even low mortality in the average population value resulting in an increase of 120% compared to river inputs (Table 2.2).

Discussion

This study supported previous findings that zebra mussel summer mortality is correlated with higher water temperatures (White et al. 2015); however, the thermal limit was higher in Canyon Lake when compared to reports from more temperate lakes (Griebeler and Seitz 2007, Feng et al. 2020), but similar to reports from other Texas lakes (Morse 2009, Locklin et al. 2020), where mussels occur at the southern edge of their distribution. Such mortality can cause considerable releases of nutrients while mussels die in large numbers and decay.

Although water temperature was linked to summer mortality of zebra mussel populations, the thermal threshold for populations may also depend on local adaptation. A study of zebra

mussel mortality in Gull Lake, Michigan showed a relationship between zebra mussel mortality and the number of degree hours above 25°C (White et al. 2015). In contrast I found that the higher mortality in 2019 (detected in both the dive surveys and cage experiments) compared to 2020 was associated with a roughly double the number of days over 30°C in 2019. In 2020, when days over 30°C occurred less often compared to 2019 (17 vs 27 respectively), zebra mussel mortality was more restricted to shallower locations and sites near the river/lake interface, where water temperatures tended to be slightly higher than in the deeper parts of the lake. Similarly, the absence of days over 30°C at 9 m depth was associated with lower mortality at that depth especially between days 25 and 80) at two of the three marinas, when average daily temperatures often exceeded 30°C. Although actual temperature differences between 1 and 9 m depth were small (0.6 ± 0.5 to 1.1 ± 1°C), the differences in degree days over 30°C likely contributed to the higher mortality, as even small differences at high temperatures may be relevant for zebra mussel mortality.

Apart from temperature, there are other factors that may potentially increase mortality. For example, mortality was higher at 9 m depth at Crane's Mill, which was associated with high values of total suspended solids (TSS) and lower DO, whereas chlorophyll-*a* showed little variation between sites. Studies have shown low levels of dissolved oxygen (DO) can result in mortality of zebra mussels (Karatayev et al. 1998, Garton et al. 2014, Robertson and Schwalb 2019) and larger values of total suspended solids (TSS) can have negative effects on zebra mussels, provided those higher TSS values do not represent higher concentrations of food particles (Madon et al. 1998, Allen et al. 1999, Chakraborti et al. 2002).

Summer mortality of zebra mussels may result in a nutrient pulse that exceed river inputs (see below). If this nitrogen is released in the form of ammonia, summer mortality of zebra

mussels could be especially relevant for juvenile unionid mussels, as even low levels of ammonia are known to be toxic (Newton et al. 2003: 93–165 µg, Cherry et al. 2005: 0.11-0.62 mg/L, Wang et al. 2007: 7.8-10.0 mg/L). Our study found that zebra mussel decay in a laboratory setting resulted in total ammonia nitrogen concentrations of up to 5.33 mg/L TAN which exceeded both the acute (1.3 mg/L TAN) and chronic CMC for aquatic life (0.31 mg/L TAN). The mixing of water in a lake, however, may dilute ammonia, although mixing is usually reduced in the hypolimnion. The magnitude of dilution is difficult to estimate and would require a detailed hydrodynamic model of the lake. Zebra mussels can occur in higher densities in Canyon Lake and other lakes than densities tested in the experiment. Zebra mussels reach densities of up to 6,000 mussels/m^2 in Canyon Lake (Lorkovic and Schwalb, unpublished data) and this experiment's high density treatment represented a density of 1,050 mussels/m^2. Thus, ammonia releases due to high mortality in areas with high zebra mussel densities may be especially lethal for nearby unionid mussels and impacts may be more severe in water bodies with higher pH and temperature.

Our results are comparable with another study examining ammonia release of *Corbicula fluminea,* where *Corbicula* densities of 10,000 mussels/m^2 resulted in peak NH3-N values of up to 5.0 mg/L after 100% mortality (Cherry et al. 2005). This is about 10-fold higher than our peak NH3-N value of 0.68 mg/L, but densities were also 10-fold higher in the other study (1050 vs. 10,000 mussels /m^2). Another study found that unionid mussel decay at a density of 11.9 mussels/m^2 resulted in peak NH4-N concentrations of ~0.35 mg/L (determined from Figure 3, DuBose et al. 2019), which is lower than in our study

Nitrogen release from summer mortality exceeded river inputs in eight out of nine scenarios, and even released half as much as river inputs in the lowest possible population and

mortality estimates. Phosphorus release from summer mortality was predicted to exceed river inputs in only three scenarios: High Population High Mortality, High Population Intermediate Mortality, and Average Population High Mortality). While these scenarios estimated P release that exceeded river inputs, these values are unrealistic as high population and mortality values were not seen throughout Canyon Lake. Average and low population values with intermediate mortality, however, were predicted to release phosphorus equal to 38 and 86% of river inputs respectively, which may or may not affect primary productivity in the lake, as Lake productivity and algal growth is usually phosphorus limited (Holdren et al. 2001, Kalff 2002, Wagner 2010).

It should be noted that all estimates from this study have a wide range of possible values due to the uncertainty connected with the estimated population size. As the population size was simply based on the total lake surface area, the estimated population size may be an underestimate of the actual population size, however zebra mussels were not found in parts deeper then 24 (July 2019 survey only, not used in any calculations presented here) meters and need hard substrate to settle, which could also lead to an overestimate of the populations size.

The estimated increase in phosphorus due to intermediate summer mortality in average and low population sizes in this study (38 and 86%) is similar to those found in other studies. For example, a mortality event of ~100 million Corbicula in Broad River in Georgia was estimated to increase phosphorus by 50% (McDowell et al. 2017) and mesocosm experiments with induced unionid mortality found a 38% increase in phosphorus (Dubose et al. 2019). Extensive modeling in the Laurentian Great Lakes predicted zebra mussel soft tissue released roughly 0.5-1.0 µgP/L (Li et al. 2021), and intermediate mortality in average and low populations from this study predicted similar increases in water column P concentrations (0.5-1.0 µgP/L). Phosphorus release from the same scenarios as above fell within the range of P fluxes from zebra mussel

excretion plus degradation of egesta found in the Great Lakes (Li et al. 2021: 0.05 to 9 $mg/m^2/day$; This study: 0.4 to 0.9 $mg/m^2/day$) but fell short of other rates found in the Mississippi River (James et al. 2000: 3 $mg/m^2/day$) and laboratory experiments (James et al. 2001: 0.5-2 mg/L).

Despite the similarities to some studies, summer mortality of zebra mussels in Canyon Lake is less substantial when compared to mass mortality events of larger organisms, such as salmon spawning and mortality of bison and caribou during migrations. Studies performed on salmon spawning have reported increases of dissolved ammonium by 30-350% and SRP by 14-130% (Cak et al. 2008), and inputs of 180 t of N and 24 t of P into systems (Gende et al. 2002). This study found increases in nitrogen and phosphorus similar to the lower range of increases from salmon spawning. Estimates of historic mass drownings during bison and caribou migrations have been calculated to total up to 50% of a river's annual P load, and introduce thousands of tons of C, N and P into aquatic systems across the ranges of these animals (Wenger et al. 2019). While zebra mussel mortality in Canyon Lake released fewer overall amounts of nutrients compared to larger native organisms, nutrient release from zebra mussel mortality represents a "new" source of inputs for systems such as Canyon Lake. In addition, the timing of these mortality events (both time of year (summer) and rate of release (can be quick, 16 days in scenarios) can potentially influence large scale ecosystem processes.

Provided P limitation exerts the strongest influence on system dynamics in Canyon Lake, the relatively small amount of P released by zebra mussel mortality may not have immediately noticeable impacts. However, when considered alongside other impacts of zebra mussels, mortality events may help accelerate the changes zebra mussels cause. For example, the small but relatively short-term P release by zebra mussel summer mortality may influence primary

productivity, as higher levels of nutrient input have been shown to increase primary productivity (Polis et al. 1997, Lurling et al 2018, Ferriera et al. 2020). This potential increase in primary productivity combined with the zebra mussels' selective consumption of phytoplankton (Caraco et al 2006, Fishman et al. 2010) could result in summer phytoplankton blooms dominated by grazing resistant and potentially toxic species.

In addition to the changes brought on by the zebra mussel invasion, climate change will modify the ecosystem as well, potentially altering the dynamics of zebra mussel mortality observed in this study. While mortality naturally occurs in any system, the "new" mortality from the invasive zebra mussel population will likely worsen as climate change progresses. Warming global temperatures will cause water temperatures to rise (Poff et al. 2002), resulting in more days during the summer where water temperatures reach or exceed 30°C. If the number of degree days > 30°C is an important factor for zebra mussel mortality in this region, as suggested by this study, an increase in the frequency of degree days > 30°C will likely increase both the frequency and magnitude of the mortality events, resulting in larger nutrient releases occurring more often and potentially a higher risk of toxic algal blooms.

Future studies will need to examine to what degree the warming climate will affect frequency and magnitude of mortality events to determine a trajectory for future nutrient release from zebra mussel mortality. Additional studies should also examine shell decomposition, as scenarios in this study estimate billions of dead individuals in one summer and research has shown nutrient release from shell decomposition can be a significant source of long-term (5-30 years) phosphorus release (Dubose et al 2019, Wenger et al. 2019). Future studies should also determine how long elevated total ammonia nitrogen levels from zebra mussel decay persist,

which could be used to determine the duration of time following mass zebra mussel mortality events during which there could be acute and chronic affects on unionid mussels.

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Tables

Table 2.1: Results of the linear mixed effects model for zebra mussel mortality over the course of the summer 2020 showing *t* and *p* values of various factors and interactions.

Factor/Interaction	***t* Value**	***p* Value**
Intercept	13.56	<0.001
Size	4.44	<0.001
Depth	-2.78	< 0.05
Deg. Days >30	-4.15	<0.001
Time	-7.76	<0.001
Time:Deg. Days >30	3.73	<0.001
Time:Depth	1.60	0.11
Depth:Deg. Days >30	-2.42	< 0.05

Table 2.2: Estimated amounts of C, N and P in tons released by decaying zebra mussels in Canyon Lake and how it would increase the loading from the Guadalupe River (%).

Estimated Population size		**Estimated release (t) due to mussel decay**			**Percentage of Guadalupe River loading**	
Total number of zebra mussels	Mortality	C	N	P	N	P
High	High (**88%**)	207	47	14	2,000	240
Average		133	30	0.92	1,300	160
Low		60	13	0.41	570	70
High	Intermediate **(48%)**	113	25	0.78	1,100	130
Average		73	17	0.50	700	86
Low		33	7.3	0.23	310	38
High		19	4.2	0.13	180	22
Average	Low **(8%)**	12	2.7	0.08	120	14
Low		5.4	1.2	0.04	52	6

Figures

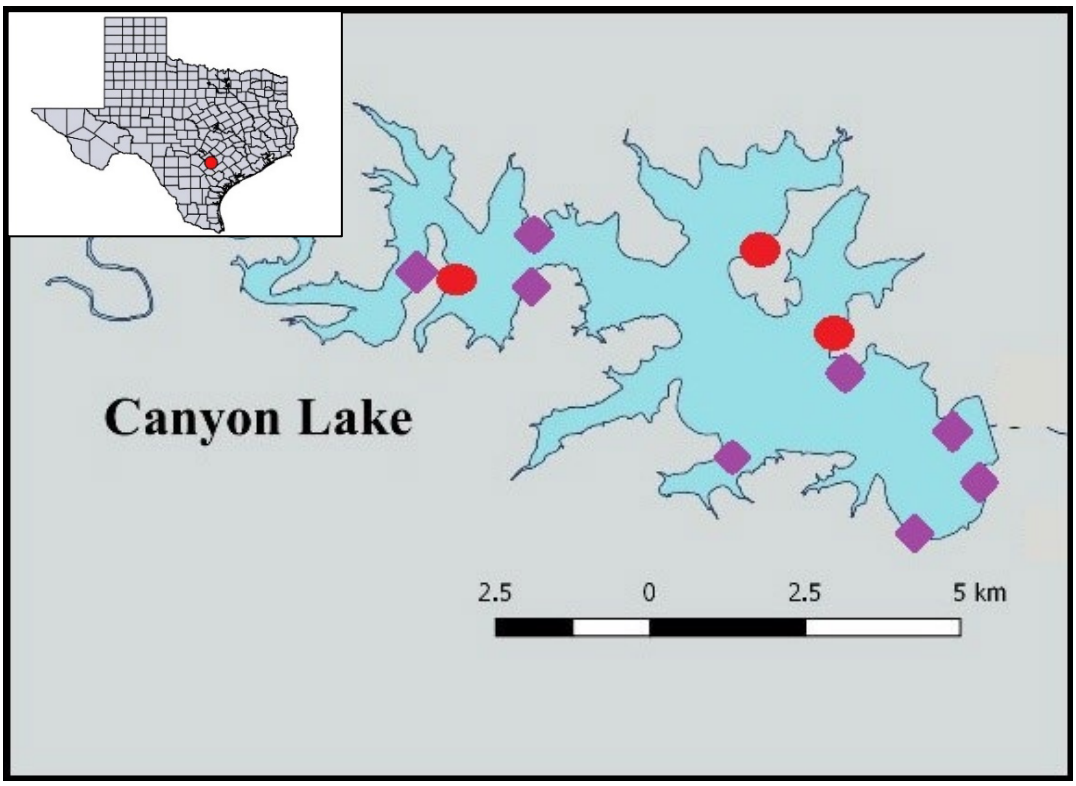

Figure 2.1: Map of settlement monitoring sites in Canyon Lake (red circles) and diving transects (purple diamonds).

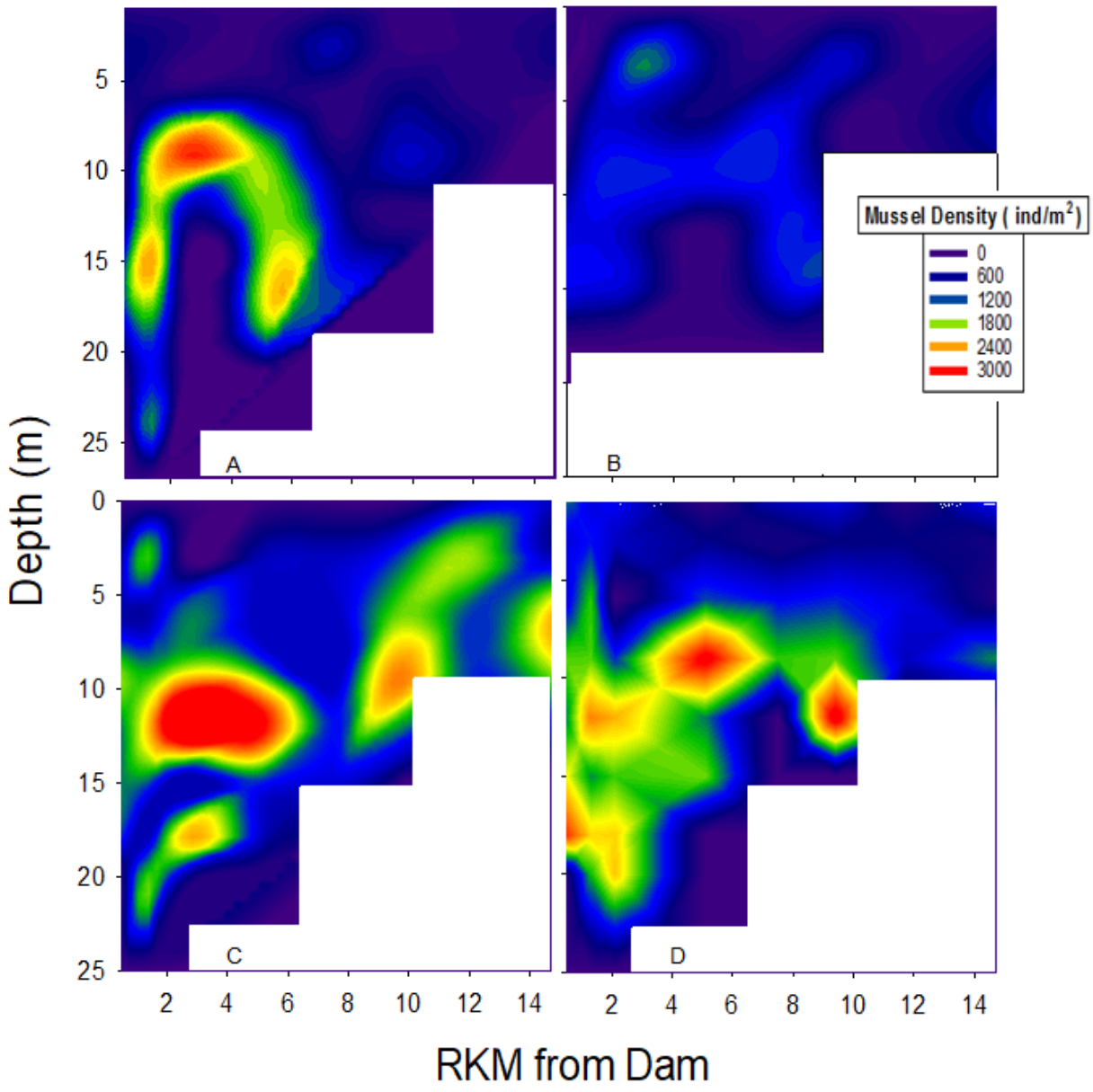

Figure 2.2: Density (ind. per m^2) distribution of zebra mussels surveyed via dive transects in A) July 2019, B) October 2019, C) July 2020, and D) October 2020. The July 2019 survey was conducted by Josi Robertson (Robertson and Schwalb 2019), the remaining dive surveys were collected specifically for use in this study.

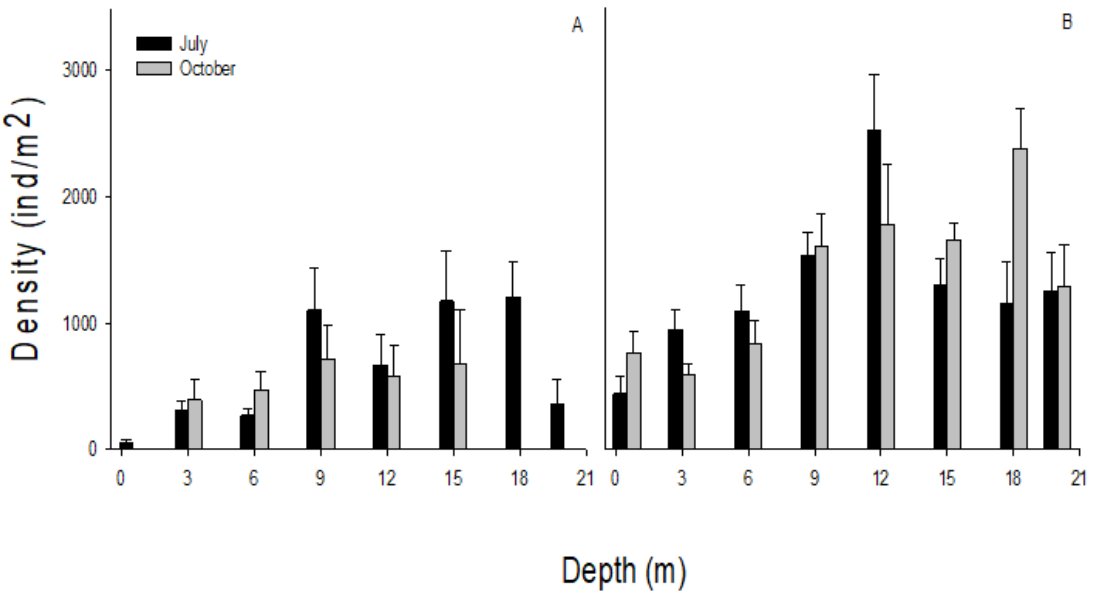

Figure 2.3: Densities (mean ± SE) at all sampled depths in A) July and October 2019 and B) July and October 2020.

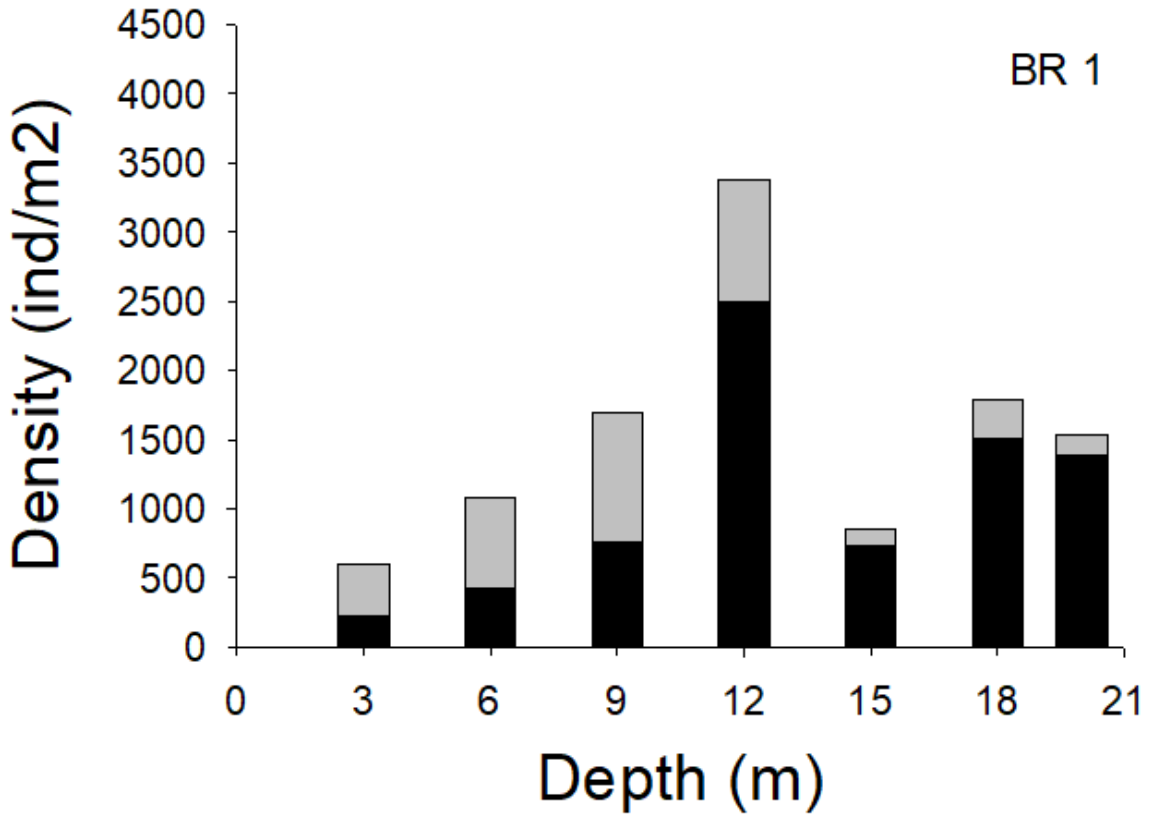

Figure 2.4: Size frequency distribution at Boat Ramp 1, 2.1rkm from the dam, presented as an average profile for near dam sites. Black bars represent the number of small individuals, grey bars represent the number of large individuals.

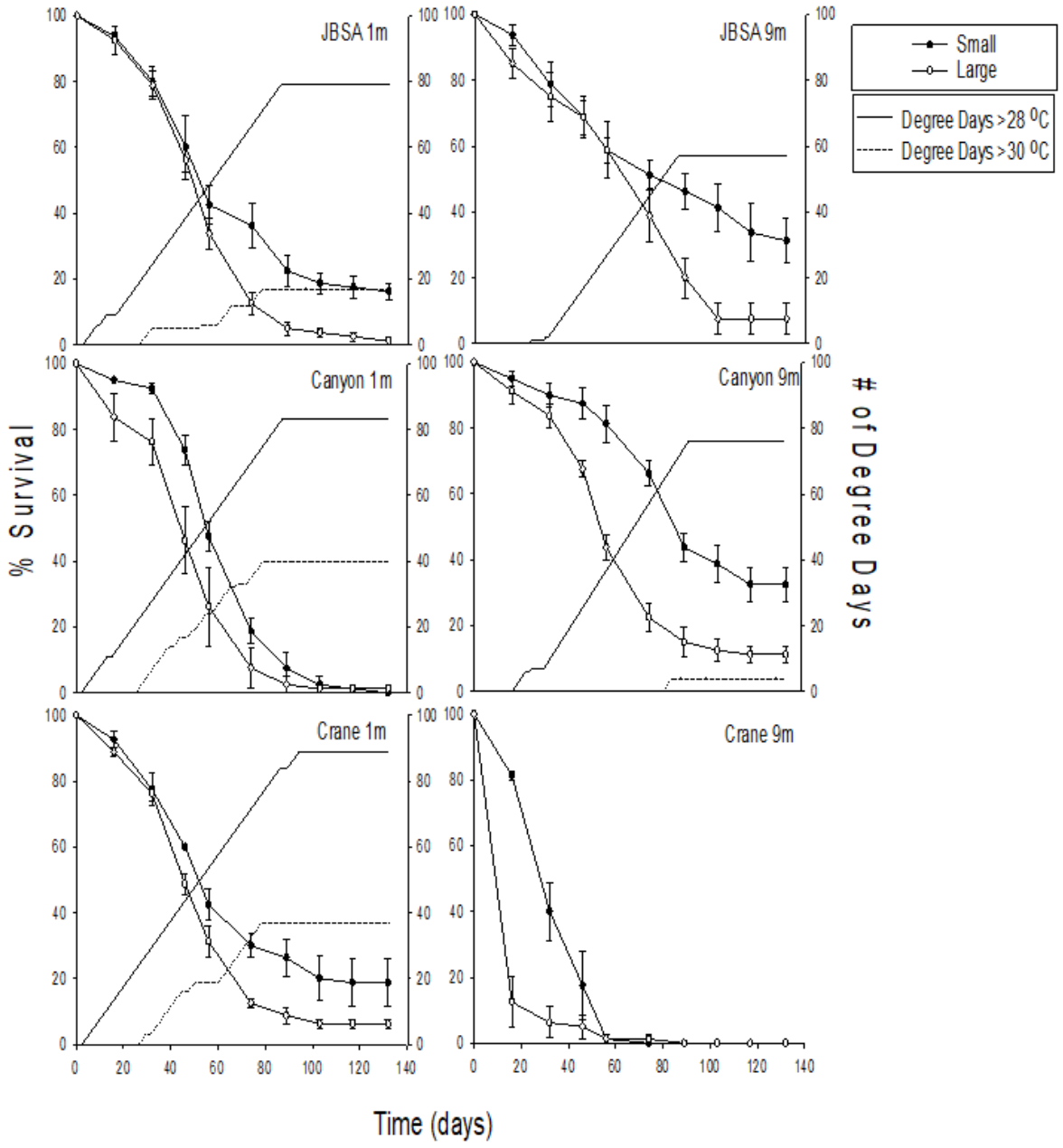

Figure 2.5 Percent survival (mean ± SE) of small (black dots) and large (white dots) mussels at each marina and each depth. Solid lines represent cumulative degree days over 28°C, dashed lines represent cumulative degree days over 30°C. No temperature data is available for Crane's Mill 9m as the logger was lost to corrosion near the end of the experiment.

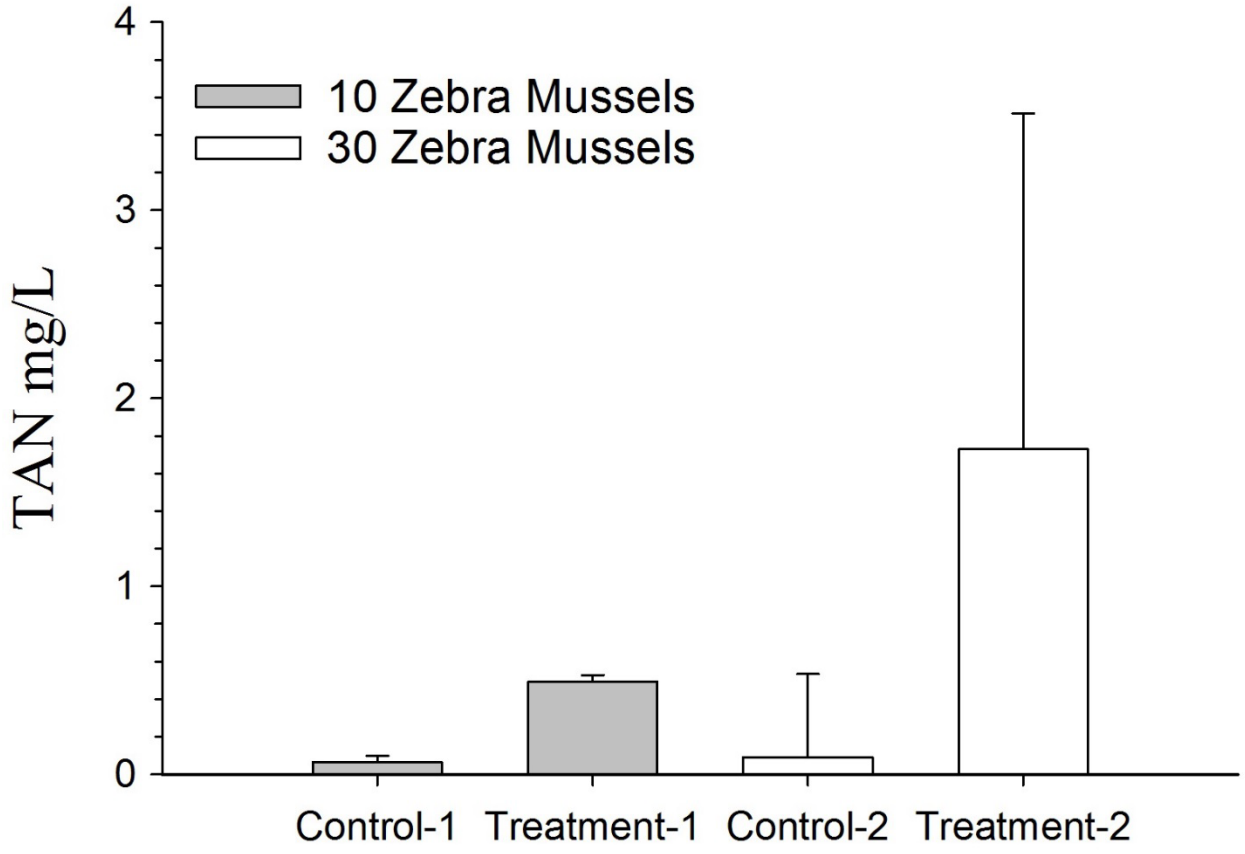

Figure 2.6: Total Ammonia Nitrogen concentrations (mean ± SD) for all treatments and controls averaged across the 35-day laboratory trial.

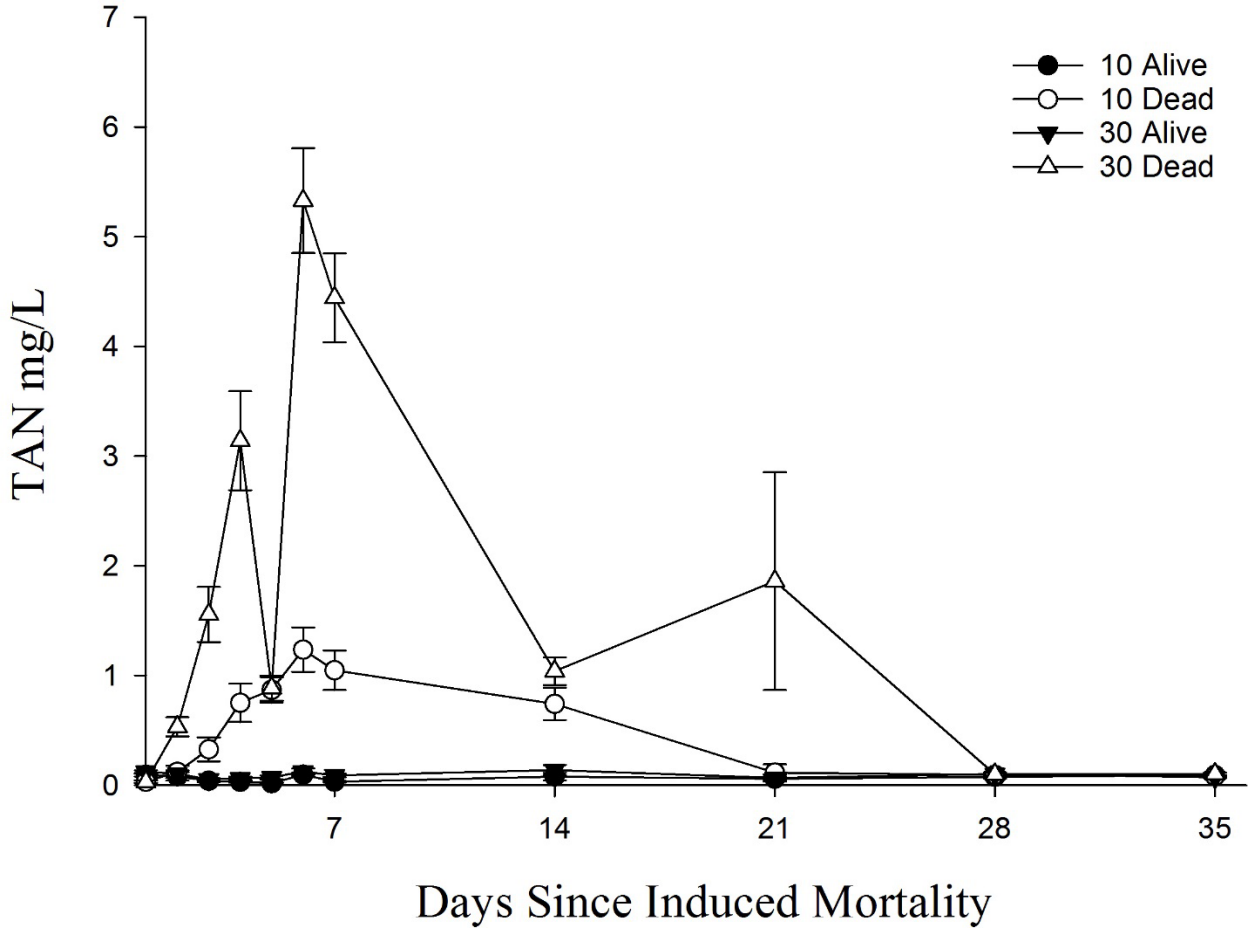

Figure 2.7: Total Ammonia Nitrogen (mean ± SD) per treatment per day across the 35-day laboratory trial.

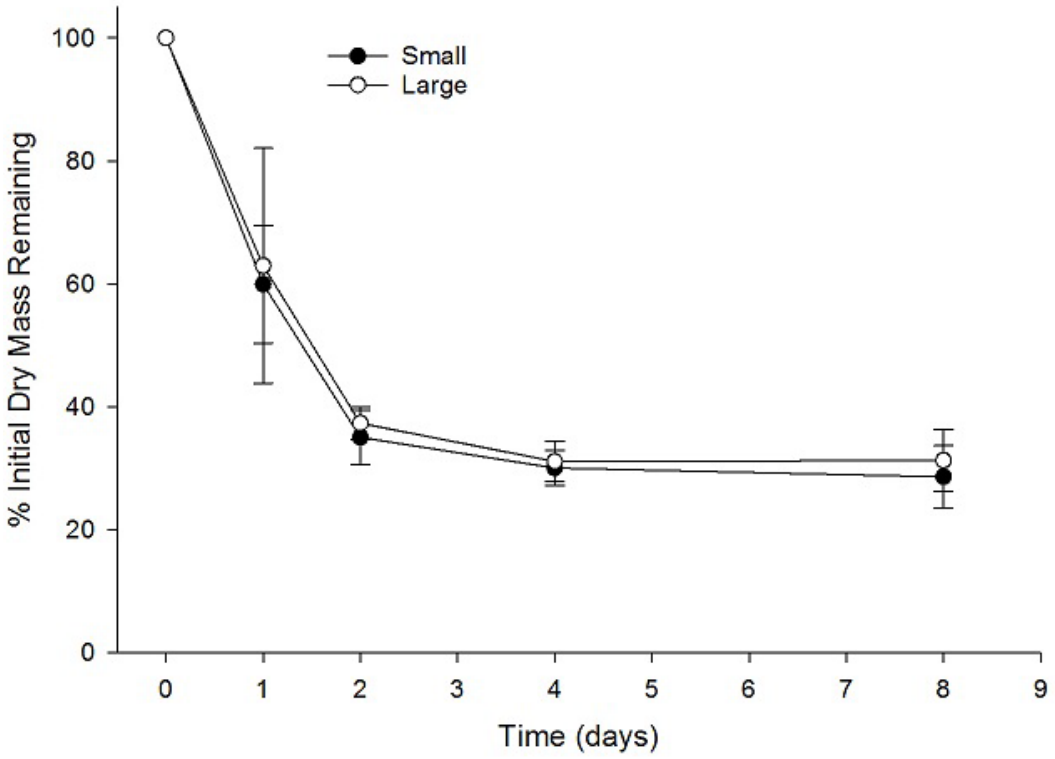

Figure 2.8: Percent initial dry mass remaining (mean ± SD) of large and small size classes of zebra mussels over 8 days of decomposition.

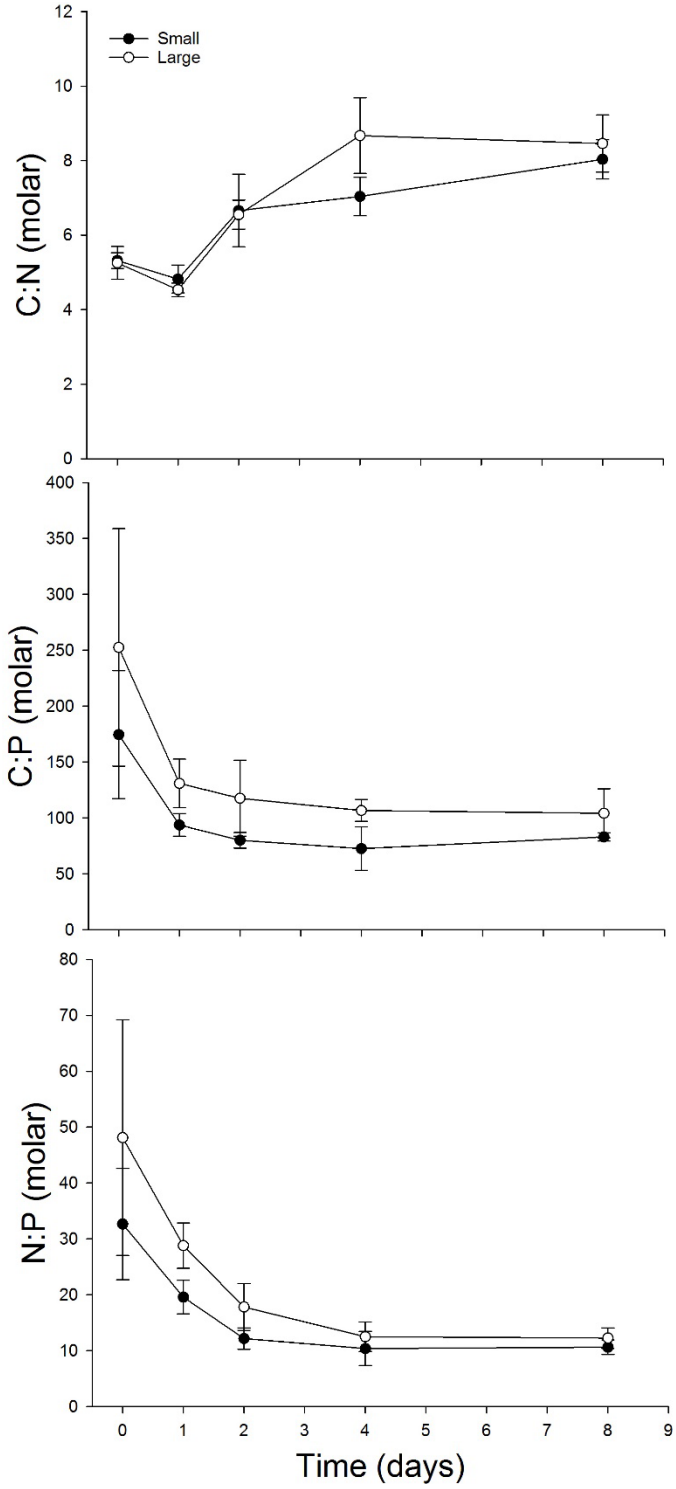

Figure 2.9: Ratios of C:N, C:P, and N:P (mean ± SD) in small and large mussel soft tissue over 8 days of decomposition.

APPENDIX SECTION

Table A1: Total suspended solid values (mg/L) from water samples taken across all marinas.

	TSS (mg/L)					
Date	JBSA 1m	JBSA 9m	Canyon 1m	Canyon 9m	Crane 1m	Crane 9m
6/17/2020	1.6	5.6	3.2	3.2	0.4	14
7/3/2020	3.6	7.6	4.8		3.6	
8/13/2020	2.4	3.2	2.4	3.2	4.8	7.2
9/13/2020	4.0	3.6	1.6	2.0	2.0	5.2
9/27/2020	0.8	3.6	1.2	4.4	1.0	14.4
10/11/2020	2.8	2.8	0.4	2.0	2.8	10.4
11/14/2020	1.0	1.6	0.8	0.8	1.6	3.6

Table A2: Chlorophyl-a values (µg/L) from water samples taken across all marinas.

	Chl-a (µg/L)					
Date	JBSA 1m	JBSA 9m	Canyon 1m	Canyon 9m	Crane 1m	Crane 9m
6/17/2020	0.16	0.02	1.05	0.84	0.0	14
7/3/2020	1.26	0.99	0.18		1.49	
8/13/2020	2.33	2.74	2.33	2.74	4.68	7.2
9/13/2020	1.79	2.0	0.99	0.55	1.47	5.2
9/27/2020	1.31	0.86	1.20	1.60	0.94	14.4
10/11/2020	0.77	0.65	1.14	2.18	0.86	10.4
11/14/2020	0.61	0.96	1.02	1.41	0.24	3.6

Table A3: Dissolved oxygen values (mg/L) from marina profiles.

	DO (mg/L)					
Date	JBSA 1m	JBSA 9m	Canyon 1m	Canyon 9m	Crane 1m	Crane 9m
6/1/2020	8.15	6.84	8.13	7.07	8.22	6.65
6/17/2020	8.15	6.84	7.93	7.58	7.66	3.5
7/3/2020	7.87	7.85	7.75	7.85	7.63	7.09
7/17/2020	7.67	7.07	7.61	6.02	7.80	7.51
7/27/2020	7.91	7.36	7.69	7.62	7.33	6.84
8/13/2020	7.69	7.28	7.52	7.54	7.42	5.05
8/29/2020	7.78	7.09	7.76	7.67	7.60	3.91
9/13/2020	7.60	7.42	7.37	7.23	7.24	6.59
9/27/2020	8.10	7.81	7.98	7.72	7.61	6.11
10/11/2020	8.50	8.13	8.96	7.79	8.32	5.05

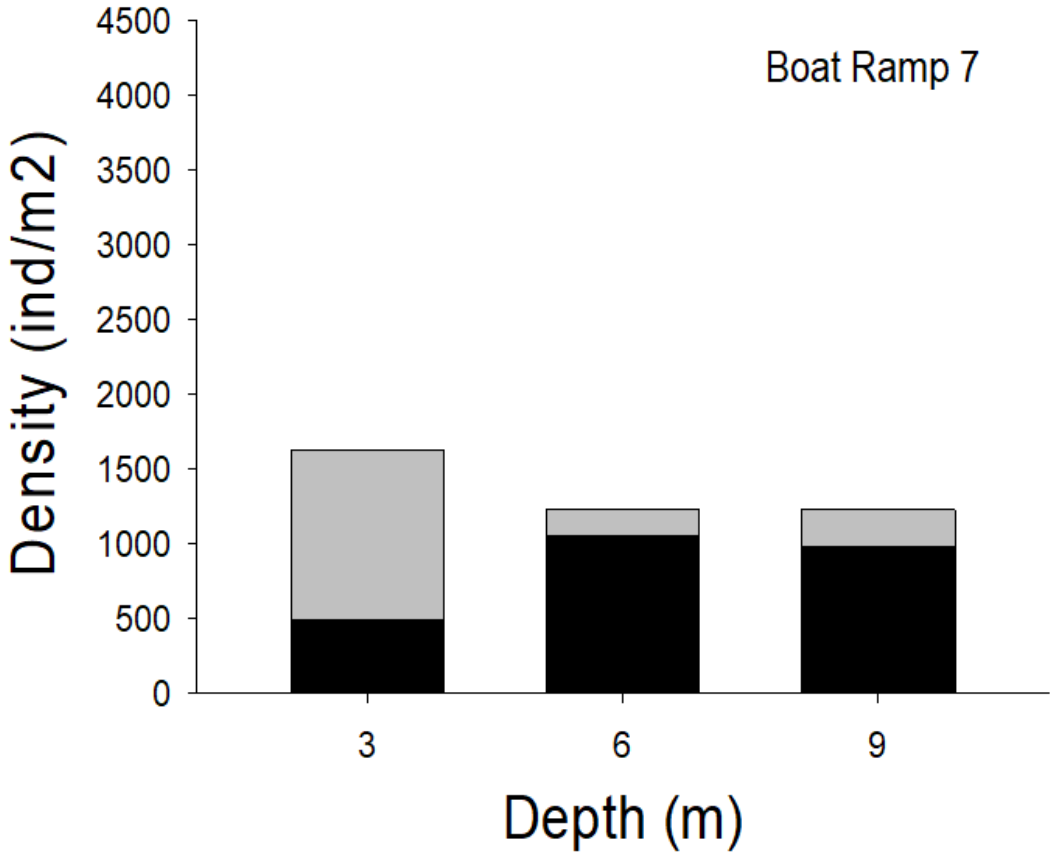

Figure A1: Size frequency distribution at Boat Ramp 7, 11.7rkm from the dam, presented as an average profile for sites closer to the river/lake interface. Black bars represent the number of small individuals, grey bars represent the number of large individuals.

Chapter III

Population dynamics of zebra mussels in Canyon Lake

Astrid N. Schwalb and David Swearingen

Introduction

Temperature is known to be an important driver for the growth and reproduction of zebra mussels (Sprung 1987). As temperatures in the southern United States, including Texas, routinely exceeded the recorded upper tolerance limit it was originally thought that zebra mussel populations could not be supported in these waters (Strayer 1991; Drake and Bossenbroek 2004; McMahon 2015). However, the occurrence and persistence of zebra mussel populations at lower latitudes than previously predicted suggest that these populations have adapted to warmer temperatures (Allen et al. 1999; Elderkin and Klerks 2005; Morse 2009; Churchill 2013; Churchill et al. 2017). Indeed, several studies indicate that southern populations do possess increased upper thermal tolerances compared to their northern latitude counter parts (Matthews and McMahon 1999; Morse 2009; Churchill 2013; Churchill et al. 2017).

Zebra mussel populations can experience vast temporal fluctuations (Strayer et al. 2019; Strayer & Malcom 2006), but oscillation in population numbers may occur at an even higher rate in southern populations (McMahon per comm.), most likely as result of higher temperatures increasing growth rates (Churchill et al. 2017). Additionally, mass-mortality events have been observed in both northern and southern populations and have primarily been attributed to environmental conditions such as increased water temperatures or low dissolved oxygen levels

(White et al. 2015; Boeckman 2011; Mihuc et al. 1999). Such events have also been observed in Texas reservoirs (e.g., Lake Texoma in 2011 (Churchill et al. 2017), Lake Belton in 2015 (Olsen et al. 2018; McMahon per. comm.), and Canyon Lake and Stillhouse Hollow Reservoir in 2018 (personal observation)).

Several lakes in Central Texas currently contain some of most southwestern reproducing zebra mussel populations in North America (TPWD 2021), for which temperature is likely an important driving factor in the population dynamics of this prominent invasive species. A better understanding of the population dynamics of zebra mussels in southern lakes and their driving and limiting factors will help predict their spread and inform management decisions.

The objectives of this study were to examine (1) seasonal variation in juvenile settlement and (2) changes in distribution and densities of zebra mussels within 4 years after invasion in Canyon Lake in relation to temperature, especially high temperatures during summer.

Methods

Environmental variables

Vertical profile readings of dissolved oxygen and temperature values were taken using a multisonde (YSI ProDSS 2030) at the deepest portion of Canyon Lake once a month from May-November 2018, March-September 2019, and May-November 2020. Temperature loggers were deployed at three marinas (Fig. 3.1, monitoring sites inside Canyon Lake) to continuously record hourly surface water temperature (1 and 9m) from September 2017-August 2021. Chlorophyll-a samples were also taken alongside lake profiles at 1, 15m in 2018 and additionally at 30m in 2019. In 2020, chlorophyll levels were measured at 1 and 9m at all three marinas where mortality cages were present.

Juvenile settlement

Sampling of juvenile settlement in Canyon Lake was conducted monthly in fall 2017 (Sep-Dec), spring to fall in 2018 (Mar-Nov), 2019 and 2020 (Mar, May-Nov), and spring to summer in 2021 (Mar-Aug) at the JBSA marina and at two additional marinas (Canyon Lake and Crane's Mill since September 2019; Figure 3.1). Juvenile settlement monitoring was conducted by suspending four brick blocks approximately 3-5 m deep. At each sampling event, all zebra mussels were removed to determine recently settled juvenile (i.e. <6 mm shell length) settlement rates. Additionally, four other bricks were deployed and counted each time, without removing individuals, to keep track of accumulative density of attached mussels. Juvenile settlement rates were also monitored seasonally downstream of Canyon Lake at established monitoring sites in the Guadalupe River (Figure 3.1) with methods described above.

Distribution and density of zebra mussels

Scuba surveys were conducted initially in October 2017 along 8 transects, approximately 6 months after the establishment of zebra mussels was detected in the lake. Additional surveys were carried out in October 2018, 2019, and 2020, and July 2019, 2020, and 2021 (Figure 1). At each site, transects were conducted perpendicular to the lake shoreline along a depth gradient. Quadrats (0.25 x 0.25 m) were placed approximately at every 3 m depth (i.e., 3, 6, 9, 12, etc.) until either the deepest depth of that site was reached or until no mussels were found. At each depth, three (since October 2019) to four (Oct 2018, Jul 2019) quadrats were randomly placed against the substrate and all mussels within the quadrat were counted, and the average density at each depth and each sampling location was computed. One site (Potters Creek) was not accessible in October 2018 due to damage caused by flooding. Excessive aquatic macrophyte

growth hindered surveys at 1-3 m depth at two sites (Boat Ramp 1 in October 2020 and Crane's Mill in July 2021).

Results

Temperature and oxygen conditions in Canyon Lake

Stratification of the lake usually occurred between May and October, while the depth of the epilimnion with higher water temperatures varied between years (Figure 3.2a). The hottest summer occurred in 2019 when temperatures over 30°C were measured up to 18m depth, and 20-25°C in up to 25 m depth (Figure. 3.2a). Temperature in the surface waters (i.e., approximately 3-5 m depth) of Canyon Lake reached a daily average temperature of at least 28°C throughout the summer months in 2018 and 2019, but 28°C was reached later in 2020 and ended sooner in early September compared to late September in previous years (Table 3.1). The largest number of days ≥ 30°C occurred in 2019 (56 days), followed by 2018 (48 days) then 2018 (16 days), with a similar pattern for days ≥ 31°C (2019: 13 days; 2018: 10 days; 2020: 0 days). Temperatures above 32°C, the temperature shown to be the upper thermal limit of zebra mussels (McMahon and Ussery 1995), were not recorded during any year.

Dissolved oxygen (DO) levels were generally high (6-10 mg/L) at all depths in the lake during the early months of the year (Figure 3.2b). Decline in DO levels to < 4mg/L in deeper waters of the hypolimnion (>26m) was recorded as early as late May (2019) or started during summer in June (2018) or July (2020). DO levels of ≤ 2mg/L usually occurred 1-2 months later but were recorded as early as late May (2019). Towards the end of the summer (late August) low DO levels (< 4mg/L) were found in depths of ≥ 13 m in 2018 and 2020 and ≥ 15m in 2019 and DO levels of ≤ 2mg/L were found in similar or deeper depths 1-2 months later (mid-September in depths of ≥ 15m in 2019 and October in (October) in 2018 (≥15m) and 2020 (≥18m).

Chlorophyll a was usually extremely low (< 2 µg/L) at all sampling dates and locations, except for August 2019 and 2020 when values between 2.3 and 2.8 µg/L were measured, and up to 4.7 µg/L at Crane's Mill Marina in August 2020 (1 m depth).

Juvenile settlement in Canyon Lake

Between September 2017 and August 2021 increased juvenile settlement typically coincided with increasing early summer temperatures and decreased as summer temperatures reached about 30°C, followed by an additional, usually smaller, peak in fall, except for 2020 (when overall cooler temperature occurred) when high settlement rates were detected in fall (Figure 3.3).

Juvenile settlement rates were comparable in both early summer 2018 and 2019 (200-330 ind. m^{-2} $week^{-1}$ June-July 2018 and 275-370 ind. m^{-2} $week^{-1}$ June-July 2019, Figure 2d). In 2020 and 2021, a smaller peak compared to summer was already detected in March (88 to 561 ind. m^{-2} $week^{-1}$) and a higher peak in July (265 to 832 ind. m^{-2} $week^{-1}$) in 2020, the year with the lowest number of days with high temperatures (see above). At Crane's Mill the peak was highest in March with 561 ind. m^{-2} $week^{-1}$ (Figure 3.3). Fall peaks were lower in 2018 and 2019, the years with hotter summers (< 200 ind. m^{-2} $week^{-1}$), and higher after the cooler summer of 2020 (> 600 ind. m^{-2} $week^{-1}$in November 2020 at two marinas) and intermediate in November 2017 (>400 ind. m^{-2} $week^{-1}$).

Juvenile settlement rates were also monitored downstream of the lake and were highest at the dam outlet, where higher densities on rocks were observed as well. However, no settlement was detected at sites beyond 2.5 rkm downstream (Figure 3.4), although zebra mussels were found at 7rkm on natural substrate, not our settlement monitors. We will discontinue the monitoring of settlement downstream in the future (fall 2021).

Change in distribution and density of zebra mussels within Canyon Lake

Densities of zebra mussels have generally increased in Canyon Lake since zebra mussels were first detected in the lake in 2017, although summer declines occurred between surveys in July and October at some depths and some sites (see below) across all years and between October 2017 and October 2018 especially at < 9m depths (Figure 3.5). When comparing sites which were sampled in both 2017 and 2018 near the dam (0.5, 1.3, and 5.1 rkm upstream of the dam) average densities at moderate depths (3 to 6 m depth) declined from 595 individuals m^{-2} in October 2017 to 148 in October 2018, whereas densities remained similar at deeper depths (12-15m; 857 and 833 individuals m^{-2} respectively). The lower number of mussels found at moderate depths in October 2018 also almost exclusively consisted of smaller individuals (i.e., < 15mm), suggesting a mass mortality event of adult mussels had occurred.

The highest increase over all sampling events occurred between October 2019 and July 2020, when densities were about 2 times higher at most depths (up to 3.6 times at 12 m depth) and consistently increased at all sites. Densities of > 1,000 ind/m^2 were found at twice as many sampling points (at 6 m depth and deeper) in July 2020. The largest decline was found between October 2020 and July 2021, when densities declined by 20 to 80% at 4 out of 8 sites (Figure 3.5). Nevertheless, overall densities increased between July 2019 and July 2021 by 3-4 times at 6 out of 8 sites (exception 2.1 and 7.5 rkm sites, Figure 3.5). A similar increase was also observed between October 2018 and October 2020 at sites up to 5.1 rkm from the dam resulting in a 3-4 fold increase at all sites closer to the dam, up to 6 fold at Crane's Mill, 14.7 rkm, and < 2 fold at 7.5 and 11.7 rkm upstream.

Mussels declined at some sites and some depths between July and October in both 2019 and 2020, but increased at others, so that the overall average density did not change much. In 2019, summer declines occurred mostly at sites near the dam (0.5, 1.3 and 2.1 rkm from the dam). Declines were most pronounced at the overlook site (1.3 rkm downstream at depths ≥ 9m), and most increases occurred at ≤ 6 m depth. In 2020, such declines also occurred farther upstream from the dam (9.4-14.7 rkm).

The spatial distribution changed throughout the years. Surveys in 2017-2019 showed that the greatest mussel densities (i.e., ≥1000 ind. m^{-2}) were found at sites within the first 7.5 rkm upstream of the dam (average density range: 0-2,693 ind. m^{-2}), while densities at sites further from the dam (i.e., 9.4-14.7 rkm) were lower (average density range 0-760 ind. m^{-2}, Figure 3.5). This pattern was different in July and October 2020, when higher average densities of zebra mussels than in previous years were found across all sites ranging from 0-3,381 ind. m^2. In addition, densities in 2020 were higher in shallower depths and farther away from the dam compared to July 2019 (Figure 3.5).

Lower DO concentrations at deeper depths in Canyon Lake did not seem to limit mussels as they were often found at deeper depths (>15 m) despite low DO conditions ≤ 4.0 mg/L, the reported lower tolerance for DO (Sprung 1987; Figure 3.2).

Changes in survival of zebra mussels

The dive surveys found some indications of declines during summer, at least at some sites and some depths in October 2018, 2019, and 2020 (see above). Monthly sampling of cumulative settlement monitors within Canyon Lake captured some of these declines. Larger declines (i.e., zebra mussels declining by more than half) occurred during hot summer months in August of 2018 (-58%), 2019 (-54%), and 2021 (-85%, only at Crane's Mill Marina) (Figure

3.6). Note that only 1 marina (JBSA) was monitored between October 2017 and September 2019. A larger decline was also observed in March 2021 (-55%) at one of the marinas (Crane's Mill) indicating a winter die-off. Such larger declines, however, were not observed during the cooler summer of 2020. More moderate declines (-20 to -35%) were observed in March and July 2019, and in October 2018 (at JBSA) and 2020 (at Crane's Mill and Canyon Lake marinas). At the JBSA marina where cumulative settlement was monitored since October 2017, declines of more than -10% were detected most frequently (5 months: March and monthly between July and October) during 2019, the year with the highest summer temperatures. In 2018 this occurred 3 times (June, August, and October), and in 2021 twice (March and August). However, in 2020, the year with the coolest summer, such a decline was only detected once at JBSA (October).

Summary

Between September 2017 and August 2021 increased juvenile settlement generally coincided with increasing early summer temperatures, but it was sometimes and at some marinas detected as early as March (2019, 2020 and 2021; Fig 3.3). Declines in settlement occurred as summer temperatures reached about 30°C, followed by an additional, usually smaller peak in fall (but higher peak in 2020). Highest juvenile settlement rates in summer and fall were detected in 2020, the year with the lowest number of days with high temperatures during summer compared to 2018 and 2019.

Downstream dispersal of zebra mussels was limited over the course of the sampling period for this report (Fig. 3.4). Juvenile settlement was only reliably seen at 0.3 rkm and 2.5 rkm downstream of dam outlet, however detection at the 2.5 rkm was often extremely low (< 1 ind/m^2/week) and settlement rates overall declined from 2019 to 2020 and from 2020 to 2021 (Fig. 3.4).

Dive surveys showed that the densities of zebra mussels have generally increased in Canyon Lake since zebra mussels were first detected in the lake in 2017, and the spatial distribution changed throughout the years, generally spreading from the dam farther upstream. While densities of zebra mussels at shallower upstream sites gradually increased at first (Fig. 3.5, panel A vs B and C vs D), the most significant increase occurred between fall 2019 and summer 2020 (panel D vs E). This larger increase compared to the trend of previous years was recorded during the relatively cooler summer of 2020, where a lower number of high degree days were recorded (and started later than) the previous years.

The dive surveys also found some indications of declines during summer, at least at some sites and some depths. Larger (by >50%) declines on the cumulative settlement monitors occurred during hot summer months in August of 2018, 2019, and 2021 (at 1 out of the 3 marinas), but not during the cooler summer of 2020. More moderate declines (-20 to -35%) were also observed in March and July 2019, and in October 2018. Declines may not only be caused by hot temperature, but also by ducks and catfish consuming zebra mussels.

In conclusion, although higher summer temperatures seem to limit zebra mussels, their population has continued to increase and expand in Canyon Lake. Highest recruitment success and lowest mortality was detected in the year (2020) with the lowest number of days with high temperatures.

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Tables

Table 3.1: Table summarizing the number of days the daily average temperature in surface waters (i.e. approximately 3-5 m depth) was ≥ 28°C, 30°C, and 31°C in Canyon Lake in the summer of 2018-2021.

	Days over threshold temperature			
Daily average temperature >=	**2018**	**2019**	**2020**	**2021**
28°C	119 (May 27-Sep 22)	116 (Jun 3- Sep 30)	91 (Jun 6, Jun 8-23,Jun 25-27, July-Sep 9)	70 (July 3-Sep 10)
30°C	48 (Jul 11 – Aug11)	56 (Jul 8, 10, 13-24, July 30-Sep 9, Sep 13, 15-19)	16 (July 12-16, August 14-19, August 28-Sep 1)	19 (July 3, July 29-Aug 2, Aug 7, 14, 24-26, Aug 31- Sep 7
31°C	10 (July 20-24, 26, 28-30)	13 (Jul 20-22, Aug 13-20, Sep 1-3)	0	0

Figures

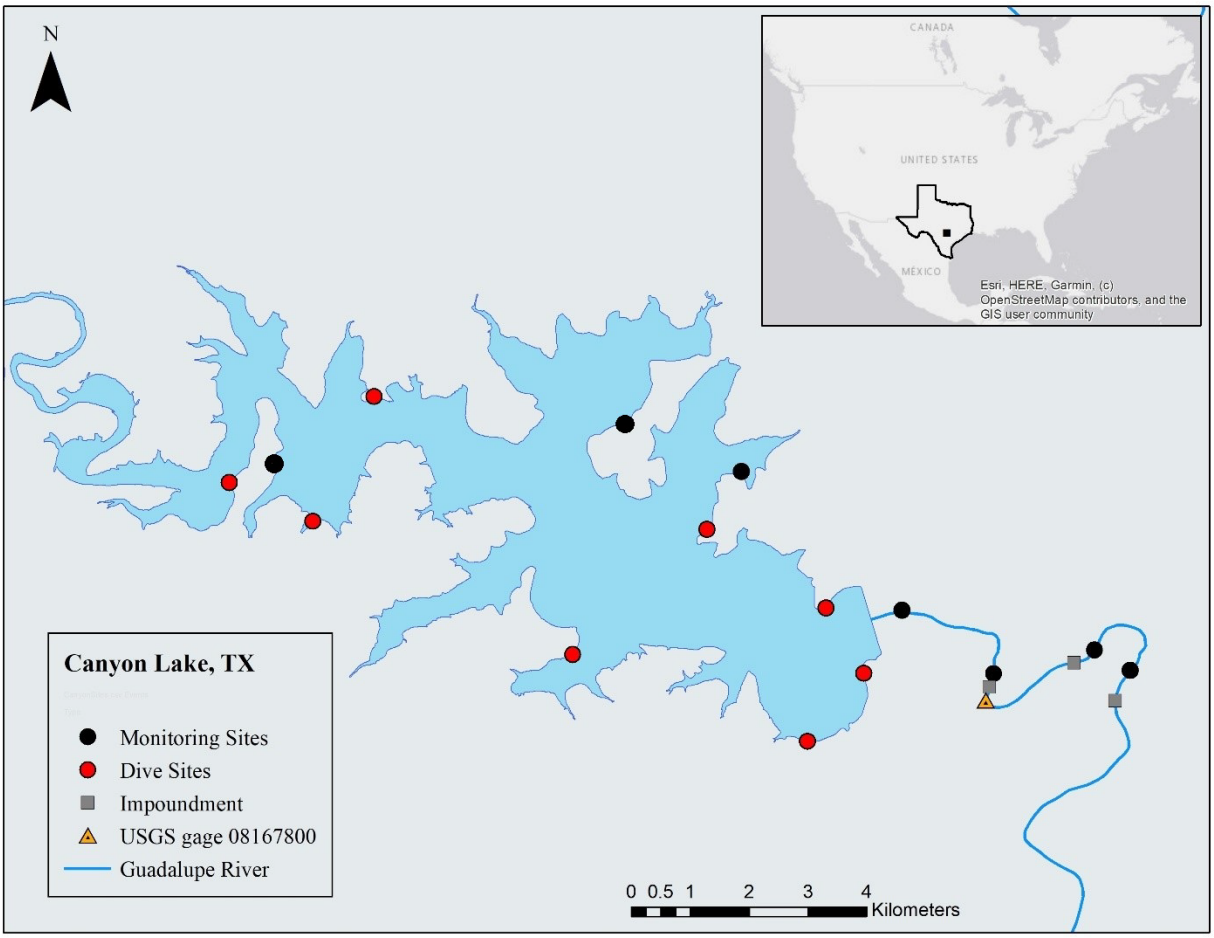

Figure 3.1: Map of settlement monitoring sites in Canyon Lake and the Guadalupe River (black circles) downstream of Canyon Lake. River impoundments (grey squares) occur adjacent to sites at 2.5, 4.5, and 7.0rkm downstream. USGS gage (08167800) is represented by the orange triangle at 2.5rkm. Sites within Canyon Lake represent sites of juvenile settlement monitoring (black circle) as well as monitoring of adult densities using diving transects (red circles). Additionally, live adult individuals used in laboratory experiments will be collected from the juvenile settlement monitoring site within the lake (black circle).

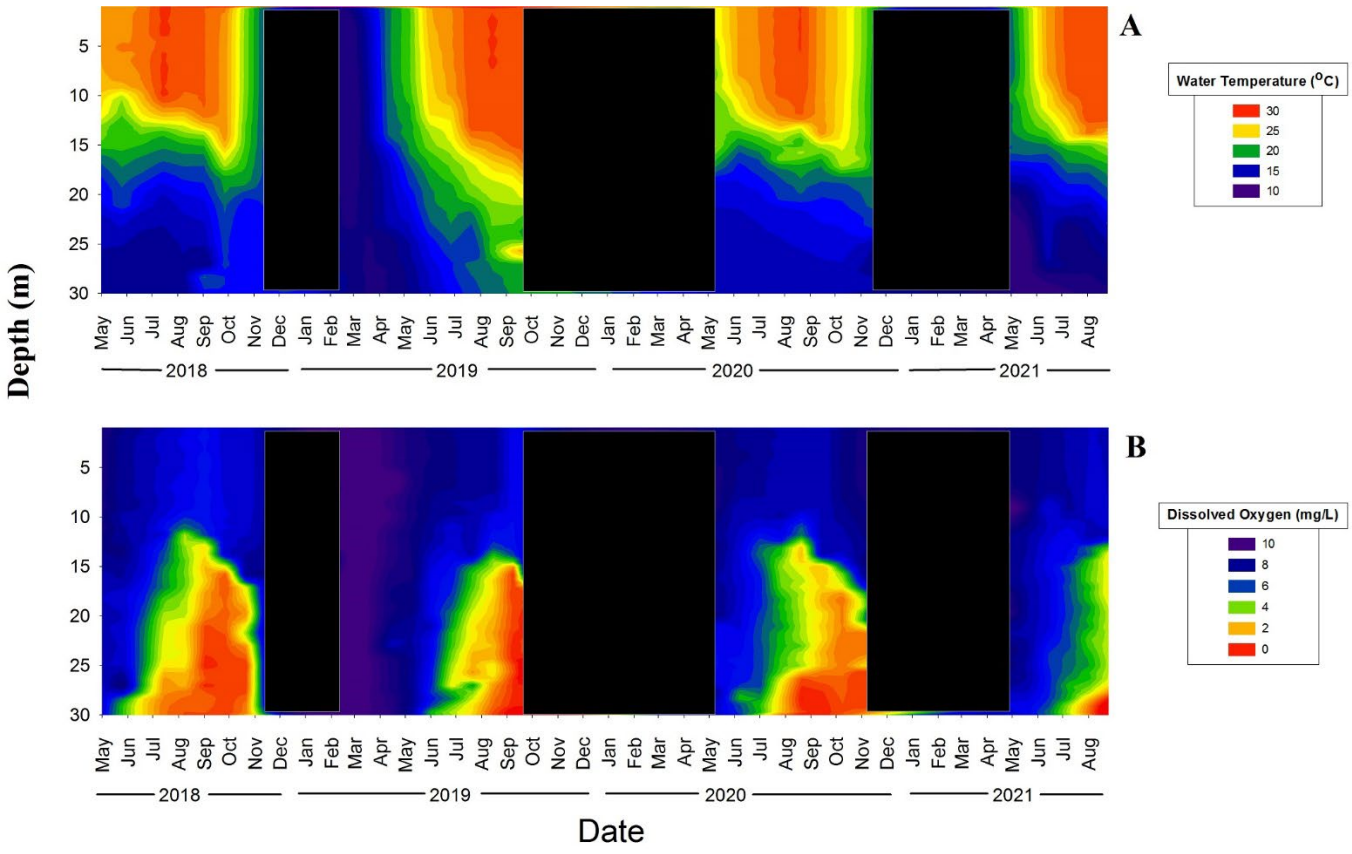

Figure 3.2: A) Variation of temperature (°C) and **B)** dissolved oxygen (mg/L) with depth in Canyon Lake from May 2018-September 2021. Black box represents period of time when monthly vertical measurements were not taken.

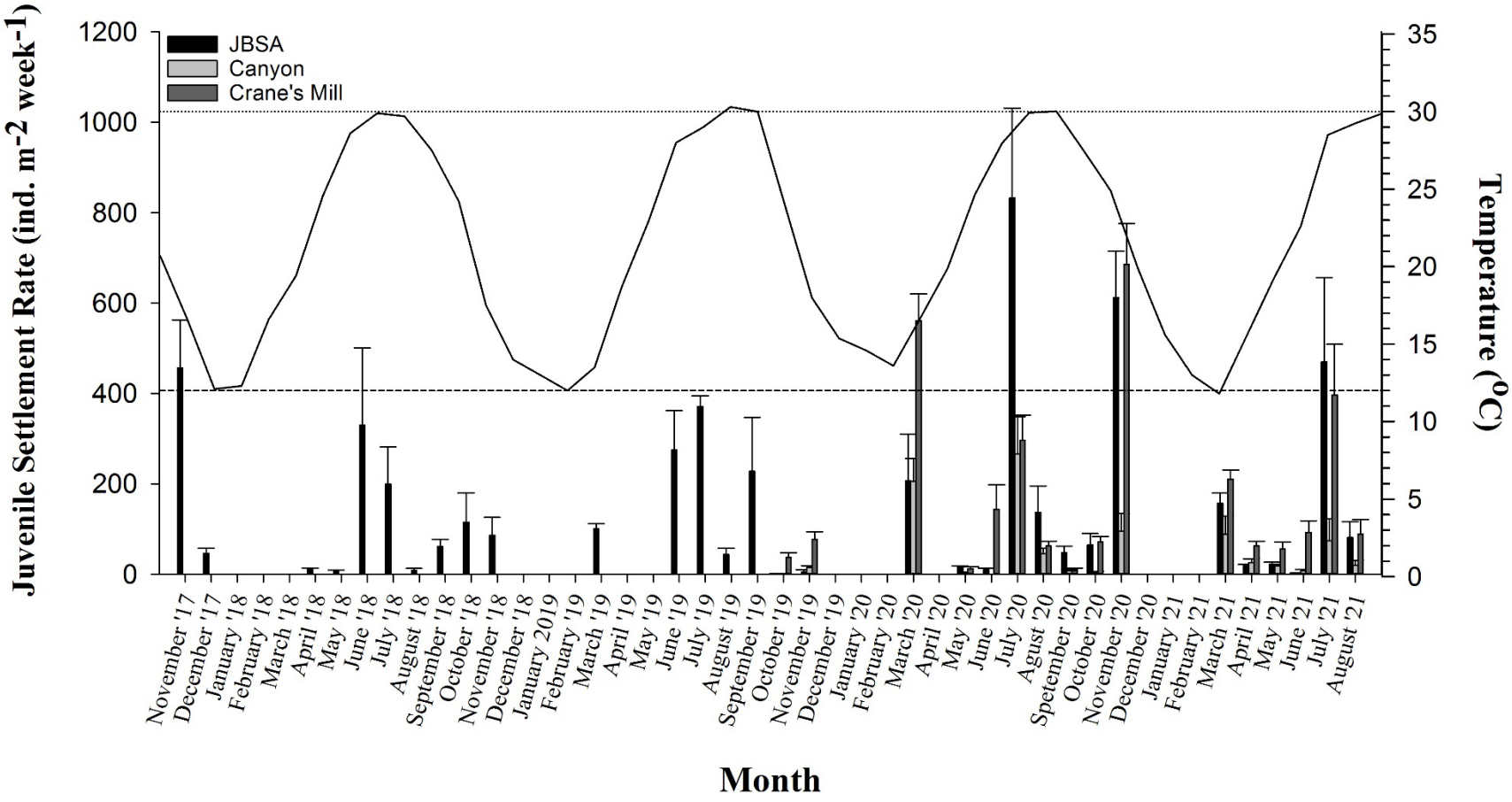

Figure 3.3: Juvenile settlement rate (ind. m^{-2} $week^{-1}$, mean ± 1 SE) in Canyon Lake, Texas from November 2017 - August 2021. Months that were not sampled are indicated with NA. Dashed lines represent upper and lower thermal reproduction limits from literature while solid line represents average monthly surface lake temperature.

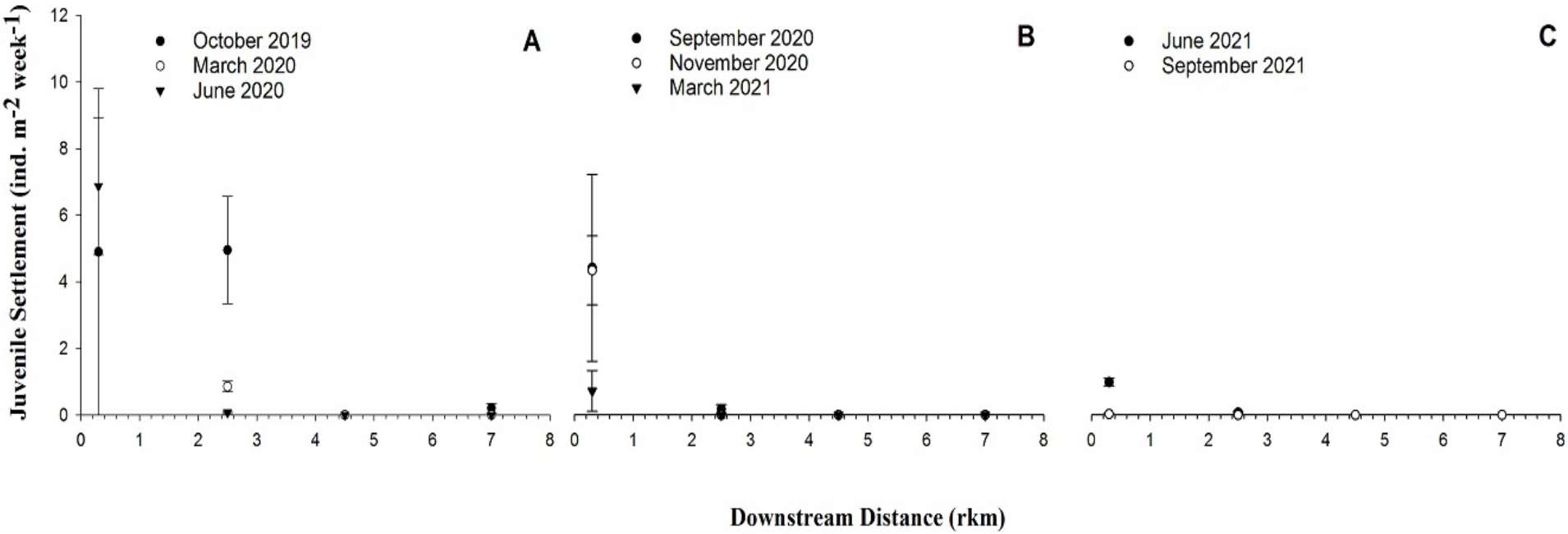

Figure 3.4: Juvenile settlement rates (individuals per m^{-2} $week^{-1}$ ± SE) at monitoring sites 0.3 to 7.0 rkm downstream of Canyon Lake in the Guadalupe River. A: October 2019, March 2020, and June 2020. B: September 2020, November 2020, and March 2021. **C**: June 2021, and September 2021.

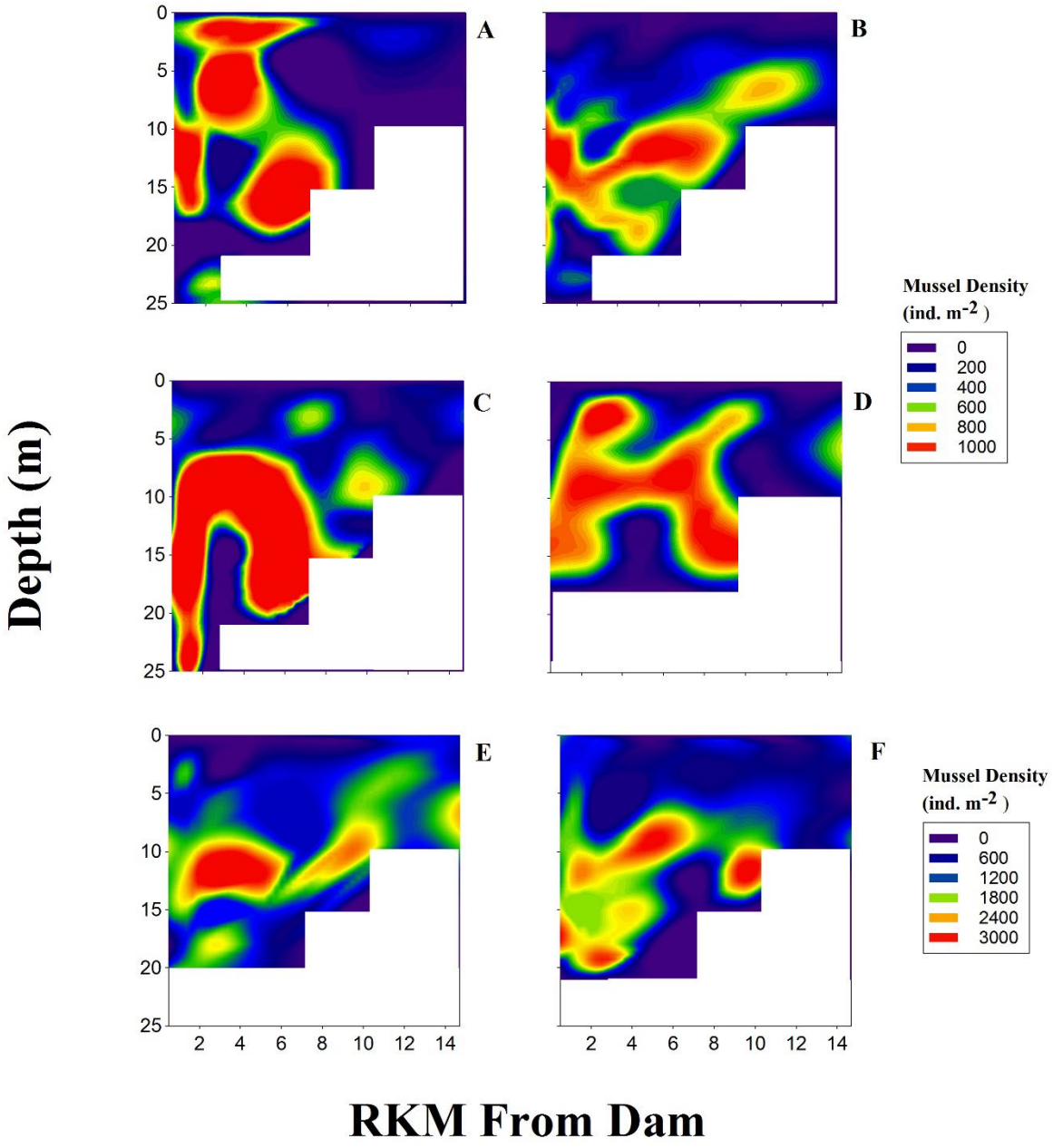

Figure 3.5: Density (ind. per m^{-2}) distribution of settled zebra mussels surveyed via dive transects in A) October 2017, B) October 2018, C) July 2019, D) October 2019, E) July 2020, and F) October 2020. Note the different scales between years prior to 2020 and after.

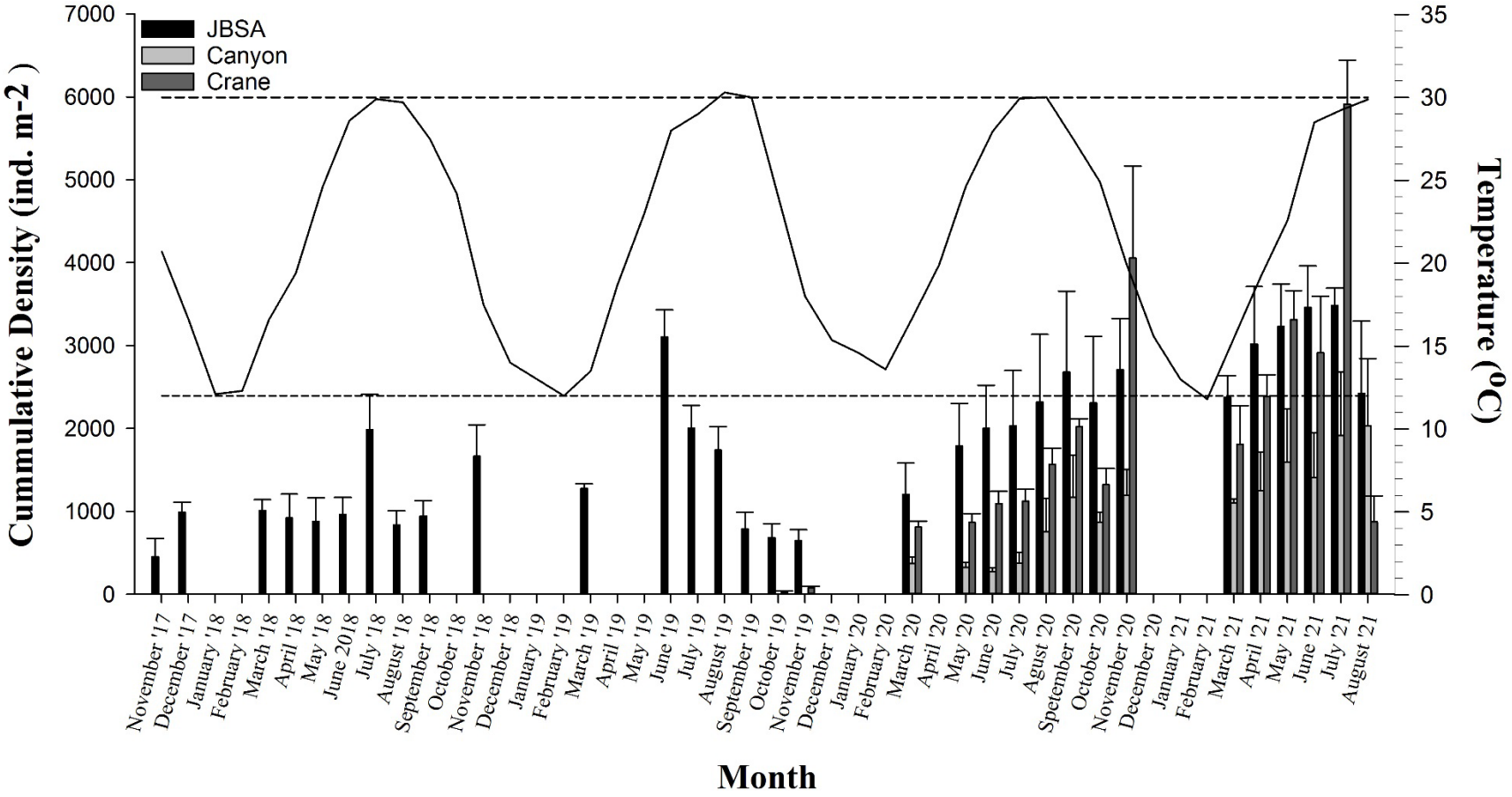

Figure 3.6: Average density (ind. m^{-2} ± 1SE) of settled adult individuals at monitoring sites in Canyon Lake from November 2017 – August 2021. Dashed lines represent upper and lower thermal reproduction limits from literature while solid line represents average monthly surface lake temperature.