IMPACTS OF ZEBRA MUSSELS ON TEXAS UNIONID MUSSELS

by

Ericah Beason, B.S.

A thesis submitted to the Graduate Council of
Texas State University in partial fulfillment
of the requirements for the degree of
Master of Science
with a Major in Aquatic Resources
August 2021

Committee Members:

Astrid N. Schwalb, Chair

Benjamin Hutchins

Clint Robertson

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ACKNOWLEDGEMENTS

I would like to thank my advisor Dr. Astrid Schwalb for her advice these past two years and for reading through countless drafts of this. In addition, I would like to thank my committee members Dr. Benjamin Hutchins and Clint Robertson for their input on this thesis and expertise in the field.

A big thank you to my lab mates: Leah Woolam, Zachary Mitchell, Kayla Hayes, Montana Stevens, and David Swearingen without whom this would not have been possible.

I would also like to thank my mom Brandy, my dad Chris, my sister Hannah, my brother Luke, Thakk, MaKayla, and the rest of my family and friends that reminded me every day that I was capable of completing this work.

Finally, the biggest thank you to my number one supporter, friend, and companion, Hudson LeRoy. We did it bubba.

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ABSTRACT

Zebra mussels (*Dreissena polymorpha*) are an invasive species known to detrimentally affect native unionid mussels, a highly imperiled group of organisms. Zebra mussels infest (directly attach to) the shells of unionid mussels and compete with them for food as both unionid and zebra mussels are filter feeders. Previous studies, mostly from the northeastern US, have shown that both competition for food (indirect interaction) and zebra mussel infestation (direct interaction) can affect the body condition of unionid mussels. However, no study has examined this in Central Texas, the southern edge of zebra mussel distribution nor compared the impact of zebra mussel presence (in the same system as unionid mussels) and infestation (direct attachment to unionid mussel shells) on glycogen storage under controlled conditions. Hence, the objectives of this study were to 1) examine the impact of infestation versus presence of zebra mussels with experiments in the laboratory and 2) collect data on glycogen concentrations of unionid mussels at different field sites with and without zebra mussels. In the experiment, 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 their biomass being comparable with the first treatment (44 ± 14 g wet biomass), but shells of *A. plicata* were not infested, and 3) control tanks where zebra mussels were absent. Tissue samples from *A. plicata* were collected from 20 individuals at each of the 10 field sites. Results from the experiments showed zebra mussel presence

and infestation reduced glycogen 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 zebra mussels. A larger percentage of infested mussels were burrowed in the lab experiment compared to control mussels, potentially to avoid unfavorable conditions created by both the presence and infestation of zebra mussels. Reduction of glycogen stores by zebra mussels can reduce short- and long-term fitness of unionid mussels which needs to be considered when creating and implementing management practices.

1: INTRODUCTION

Freshwater mussels (Family Unionoidae) are inconspicuous filter feeders that play vital roles in aquatic food webs as consumers of organic particles such as bacteria, algae, and diatoms, for nutrient cycling, and as food resources for opportunistic feeders such as raccoons (Watters 1999, Vaughn et al. 2008, Atkinson and Vaughn 2015). Depending on mussel densities and environmental factors, mussels can improve physical structure of freshwater habitats by stabilizing and bioturbating sediments (Vaughn and Hakenkamp 2001, Vaughn et al. 2008). Freshwater mussels also influence food availability both directly via filtration of organic particles and indirectly through expelling fecal material (Watters 1999, Covich et al. 1999, Hoellein et al. 2017, Vaughn and Hakenkamp 2001, Vaughn et al. 2008, Haag 2012). There are roughly 300 unionid species within North America and about fifty of those species are found within Texas (USFWS, TPWD 2018). Fifteen are listed as state threatened in Texas and one species as federally endangered (*Popenaias popeii*). Unfortunately, freshwater unionid populations have been in decline since the early 1900s and within the past fifty years, mussel populations have plummeted due to degradation of habitats, overharvesting, pollution, siltation, and invasive species such as zebra mussels (Bogan 1993, Vaughn and Hakenkamp 2001, Burlakova et al. 2011). Texas waters were invaded with zebra mussels in 2009 and populations have rapidly spread within the last ten years with current reproducing populations in five basins. (USFWS, TPWD 2019).

Zebra mussels (*Dreissena polymorpha)* are a successful freshwater invader proficient in filter feeding, infestation, and reproduction (Nalepa and Schloesser 1992, Strayer 2009, Higgins and Vander 2010, Karatayev et al. 2015). Capable of reaching

upwards of 100,000 individuals per m^2, zebra mussels can efficiently filter large bodies of water depleting local populations of phytoplankton, redirecting nutrients from the water column to the bottom, 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 1993).

Zebra mussel invasion to 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, Morton 1993, Schloesser and Nalepa 1994). Although some of the above effects have been documented, the more apparent effect of zebra mussel infestation is the direct competition for food resources.

Through their filtering activity, zebra mussels can alter available food resources for native bivalves; 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 bivalves (Gillis and Mackie 1994, Nalepa 1994, MacIsaac 1996, Karatayev et al. 1997, Higgins and Zanden 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 region 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.

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 is 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). A study conducted in Lake Champlain, Vermont, USA found as 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). Although 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), but to the best of our knowledge no study has compared these different impacts on glycogen storage under controlled conditions.

The first objective of this study was to gather baseline 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 numbers of zebra mussels 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 2). The second objective was to test the effects of zebra mussel presence and attachment to 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, 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 2). In addition, it was examined whether the burrowing behavior would differ between treatment and control tanks in the experiment and different field sites.

2. MATERIALS AND METHODS

Study Area and site selection

A total of ten sites were chosen along the Brazos, Colorado, and Guadalupe river basins in Central Texas, zebra mussels were present at four of them (Figure 1, Table 3). Twelve additional sites were surveyed where zebra mussels had been noticed and where unionids mussels had been found by state agencies and other entities, but only few or no alive unionid mussels and a large number of dead unionid mussel shells were found at those sites.

The Guadalupe River flows from Kerr County, Texas south to the Gulf of Mexico totaling roughly 400km (TPWD 1974). This river has a major reservoir which is infested with zebra mussels (Canyon) and several run of the river impoundments, some of which are also known to have zebra mussels (TPWD 1974). Guadalupe site 1 is located in Hoccheim, TX off of bridge crossing 183 and roughly 10 m downstream. Guadalupe site 2 is located in Gonzales, TX about 50 m upstream of bridge crossing 183. Guadalupe sites 3 and 4 are located in Gonzales, TX roughly 50 m (Guadalupe 3) and 150 m (Guadalupe 4) downstream of the reservoir Wood Lake where flow is very minimal and there is little riparian vegetation. The Yegua control site is located in Yegua Creek, Somerville, TX about 50 m upstream of bridge crossing 462. Yegua Creek that begins in Lee County, Texas about three kilometers west of Somerville Lake and flows east for approximately fifty kilometers until its junction with the Brazos River in Washington County, Texas (Chin et. al. 2002, Martinez 2010). This section experiences intermittent flow and flows through flat terrain and farmland with shallow depressions scattered throughout (Chin et al. 2002, Martinez 2010). The San Antonio control site is located in

the San Antonio River roughly 1750 m downstream of bridge crossing 239 near Angel City, TX. The San Antonio River that begins in Bexar County, Texas flowing approximately 386km until converging with the Guadalupe River (SARA 2021). The study site is located along a stretch of river used for agriculture and has steep banks surrounded dense riparian coverage. The two lower density sites, LBJ 1 and 2, are located near McNair Park within Lake LBJ, a reservoir along the Colorado River that has a surface area approximately 24 sq km. Lake LBJ begins near Horseshoe Bay in Llano and Burnet counties, Texas formed by the Wirtz dam and reaches to Kingsland, Texas also in Llano County, Texas (De Jesus and Farooqi 2012). The study sites are located near a recreational park with little bank vegetation. Lastly, the two higher zebra mussel sites, Belton 1 and 2, are located within Lake Belton a reservoir on the Leon River in the Brazos River Basin in Bell County, Texas (Tibbs and Baird 2018). The reservoir is approximately 50 sq km and consists mainly of bluffs and is dominated by rocky and sandy shores (Tibbs and Baird 2018). These study sites are also located near a recreational park with little bank vegetation.

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. Substrate composition was examined visually to determine the dominant substrate size according to a Modified Wentworth scale (Hauer and Lamberti 2011). 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 2).

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 (OWRB 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, unionid 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 along 6-10 transects, and the total number of mussels were determined. A depth of at least 10 cm was searched for unionid mussels (Hornbach and Deneka 1996). Zebra mussel densities at sites in Lake Belton were > 200 individuals per m^2 and were considered higher zebra mussel sites, whereas zebra mussel densities in Lake LBJ were < 200 individuals per m^2 and considered lower zebra mussel sites. A sufficient number of unionid mussels for comparative glycogen analyses were not found in other locations where zebra mussels occurred. Twelve sites were surveyed where zebra mussels had been noticed and where unionid mussels had been found by state agencies and other entities,

but only few or no alive unionid mussels were found at those sites and a large number of dead unionid mussel shells were found.

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

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 (Mackie et al. 2008). 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 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, in order 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 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 (Mackie et al. 2008). 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* (Mair 2015) 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 for 12 hours in order 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 mussels 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* (Mair 2015). 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 (TPWD 2013, 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

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). 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 amylogucosidase 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 amylgucosidase 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 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 general 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, total number 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.

3. 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 ^{o}C, 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 3). Average velocity ranged between 0 and 0.69 m s^{-1}. Most sites were characterized by finer substrate (silt and sand) except for San Antonio and Guadalupe 2 which were dominated by coarser substrate type (Table 3).

Chlorophyll-a significantly differed between sites ($F_{9, 40} = 217.9$, $p = <0.001$) (Figure 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 2).

Mussel Surveys

Infestation rates at the lower zebra mussel sites (LBJ) were about 6 zebra mussels per unionid mussel, whereas it was up to 7 times higher in Lake Belton (31-42 zebra mussels per unionid mussel, Table 3). Zebra mussel densities ranged between 186/m^2 and 191/m^2 at LBJ sites and 783/m^2 and 800/m^2 at Belton sites. Unionid mussel densities ranged between 3/m^2 at Belton 2 and 25/m^2 at Guadalupe 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 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 expect for Belton 1, Guadalupe 3 and LBJ sites ($F_{9,599}$ = 30.3, p-value <0.001, Figure 3).

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 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, median glycogen concentrations in the field samples were lowest at the two high 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 zebra mussels. The control sites with the lowest chlorophyll-a concentrations (Guadalupe 1, Guadalupe 2, and San Antonio) had lower median glycogen concentrations compared to the control sites Guadalupe 3 and 4 and the lower zebra mussel sites (LBJ 1 and LBJ 2, Figure 5A, Figure 2). Several differences

between sites were statistically significant (indicated by different letters in Fig. 5). For example, differences were statistically significant between the higher zebra mussel sites 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 5B, Figure 2).

In accordance with prediction 2, glycogen concentrations 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 mussel were present but not attached to the shells of unionid mussels (8.8 ± 1.2 mg/g, n=20). All differences were statistically significant ($F_{2,57} = 357.2$, p-value = < 0.001, Figure 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). In addition, glycogen concentrations in mussels with attached zebra mussels (4.8 ± 0.6 mg/g, n=20) were also similar to concentrations at field sites at which mussels were also infested with zebra mussels at higher densities (4.3 ± 0.8 mg/g and 4.3 ± 0.7 mg/g for Belton 1 and 2 respectively, n=20, Figure 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).

Model selection

A large proportion of the variation in glycogen in mussels from the field was explained by chlorophyll-a concentrations and total number of zebra mussels. Based on the average Akaike weights (w_i) from AICc selection, the model combining chlorophyll-a concentrations and total zebra mussels (Table 4) predicted glycogen concentrations better than any other model. Chlorophyll-a concentrations and total zebra mussels 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).

4. 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 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 has been reported in other studies of unionid mussels (Haag et al. 1993, Hallac and Marsden 2001, Sousa et al. 2011). In our study, unionid mussels infested with zebra mussels in the laboratory 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 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 to control mussels of 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, table 1) when compared to uninfested

mussels from the same sites (Sousa et al. 2011). As the control mussels in the latter two were still in the same system, their results are more comparable to the 46% difference between treatment 1 and 2 means 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).

Zebra mussels can drastically reduce phytoplankton populations and are more efficient at differentiation between nutritious and less nutritious particles (Mellina et al. 1995, Baker and Hornbach 2000, Baker and Levington 2003, Qualls et al. 2007, Higins and Zanden 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 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 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 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 and may have caused changes in their burrowing behavior and increases in their mortality. This would explain why we observed that roughly 50% of mussels in the sampling area at Belton 1 had recently died and tissue was still attached to the shells. All dead unionid mussels 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 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 1983, Bayne et al. 1985, 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 (Bayne 1972, Helm et al. 1973, Bayne et al. 1975). Given that unionid mussels are already highly imperiled, any additional stressors (such as zebra mussel attachment) will lead to further population declines as observed in the Great Lakes and elsewhere (Strayer 1996, Schloesser and Nalepa 1994, Strayer and Malcom 2018) especially when 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). 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.

Table 1: Overview of effects of zebra mussels on unionid mussels examined by different studies.

* This value was calculated based on medians from figure 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 comtrol 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 noninfested 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 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 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 within the Guadalupe, Brazos, and Colorado watersheds. Zebra mussel presence was none (control), lower (200 individuals or less), or higher (201 individuals or more). ZM – zebra mussel.

Date	Site	Water Body/Watershed	ZM Presence	Infestation Rate (no. of ZM/unionid mussel; mean ± SD)	Temperature (ºC)	pH	Avg. Velocity (m/s)	DO (mg/L)	Sp. Cond. (µS/cm)	Substrate
5-Oct-20	San Antonio	San Antonio River/Guadalupe	Control	0	20.4	7.3	0.48	7.2	731	Gravel, cobble, and/or boulders
23-Sep-20	Guadalupe 1	Guadalupe River/Guadalupe	Control	0	20.5	7.4	0.23	6.9	774	Silt/clay
23-Sep-20	Guadalupe 2	Guadalupe River/Guadalupe	Control	0	20.1	7.2	0.69	7.7	722	Gravel, cobble, and/or boulders
5-Oct-20	Guadalupe 3	Guadalupe River/Guadalupe	Control	0	20.8	7.2	0.31	7.4	794	Silt/clay
5-Oct-20	Guadalupe 4	Guadalupe River/Guadalupe	Control	0	20.8	7.2	0.29	7.4	782	Silt/clay
30-Sep-20	Yegua	Yegua Creek/Brazos	Control	0	19.6	7.6	0.4	6.9	755	Sand
16-Sep-20	LBJ 1	Lake LBJ/Colorado	Lower	6.3 ± 2.6	21.8	7.3	0	6.5	812	Sand
16-Sep-20	LBJ 2	Lake LBJ/Colorado	Lower	6.4 ± 3.3	21.8	7.3	0	6.5	832	Sand
19-Sep-20	Belton 1	Lake Belton/Brazos	Higher	37.1 ± 12.9	19.3	7.5	0	7.4	694	Sand
19-Sep-20	Belton 2	Lake Belton/Brazos	Higher	41.8 ± 12.3	19.4	7.5	0	7.4	680	Sand

Table 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	0.3	3	15.13	0.001
Chlorophyll*TotalZM	0.3	5	16.58	0
TotalUnionid	0.3	3	19.01	0
Chlorophyll	0.37	3	236.32	0

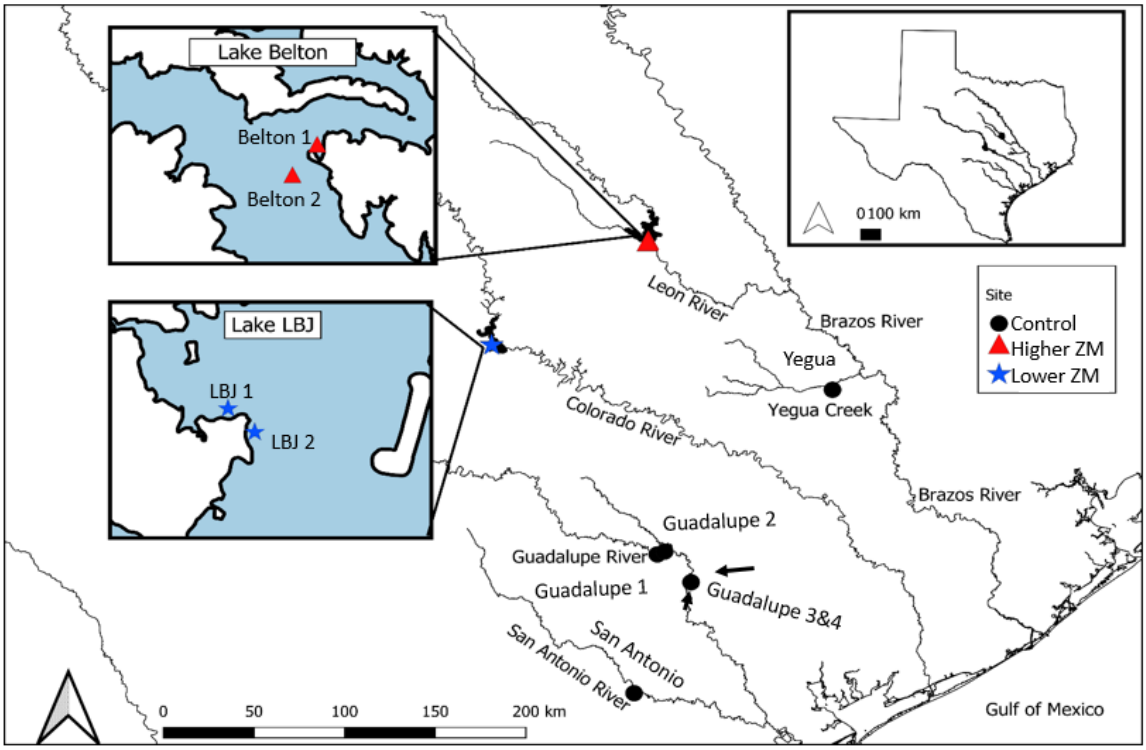

Figure 1: Study area in Central Texas showing control sites (black circle), sites with lower zebra mussels (blue star), and sites with higher zebra mussels (red triangle).

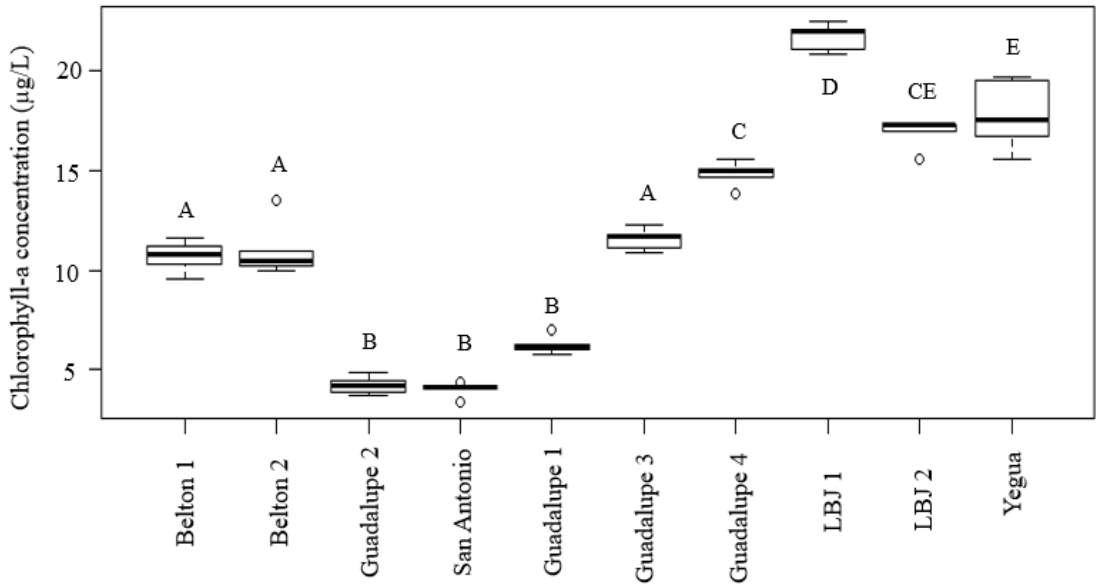

Figure 2: Chlorophyll-a concentrations at each field site. Boxplots represent the 25th and 75th percentile, the black line is the median, the error bars represent 1.5 times the interquartile range, and open circles represent any outliers. Five measurements were taken at each site. All collections were taken within a one month. Different letters indicate statistically significant differences (Tukey Test, $P<0.05$). Figure is arrange from sites with lowest to highest glycogen concentrations.

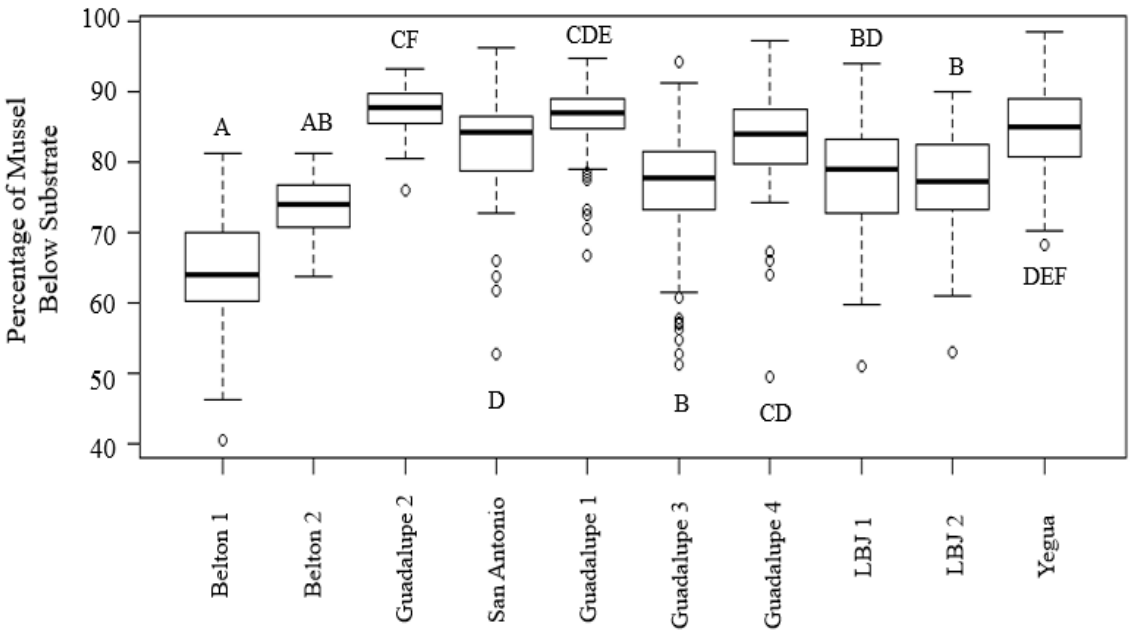

Figure 3: The percentage of mussel shell burrowed in the substrate at all field sites. Boxplots represent the 25th and 75th percentile, the black line is the median, the error bars represent 1.5 times the interquartile range, and open circles represent any outliers. All burrowing depths were collected within one month. Different letters indicate statistically significant differences (Tukey Test, $P<0.05$). Figure is arrange from sites with lowest to highest glycogen concentrations.

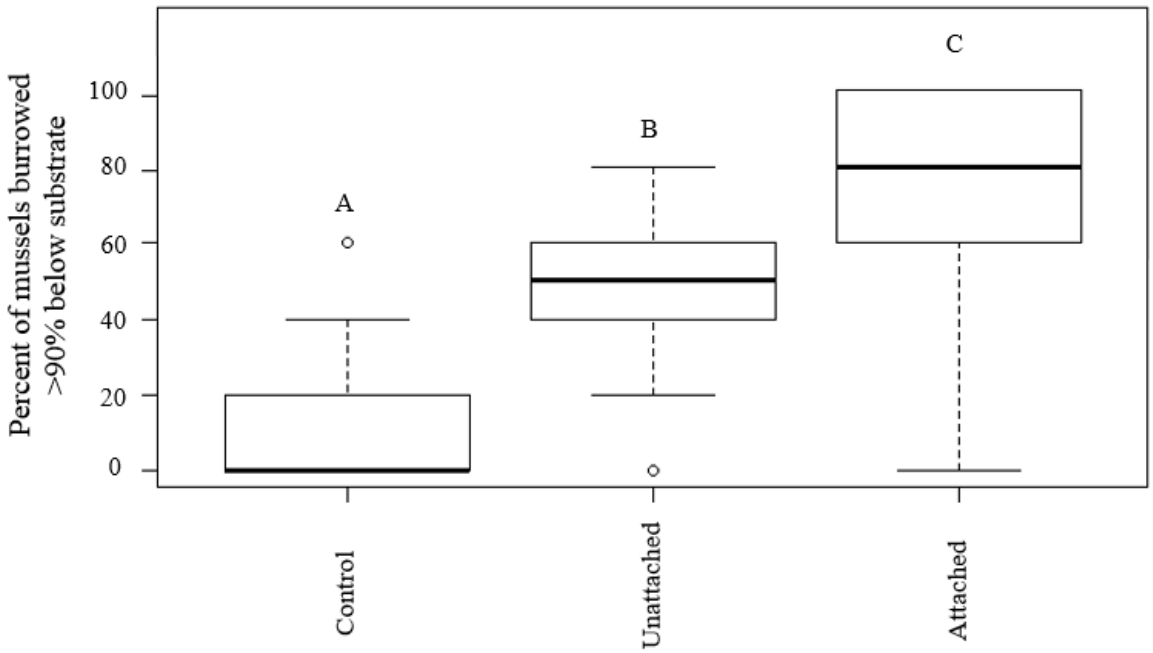

Figure 4: The percentage of mussels burrowed in the control and treatment tanks for the experiment. Boxplots represent the 25th and 75th percentile, the black line is the median, the error bars represent 1.5 times the interquartile range, and open circles represent any outliers. Different letters indicate statistically significant differences (emmeans, $P<0.05$).

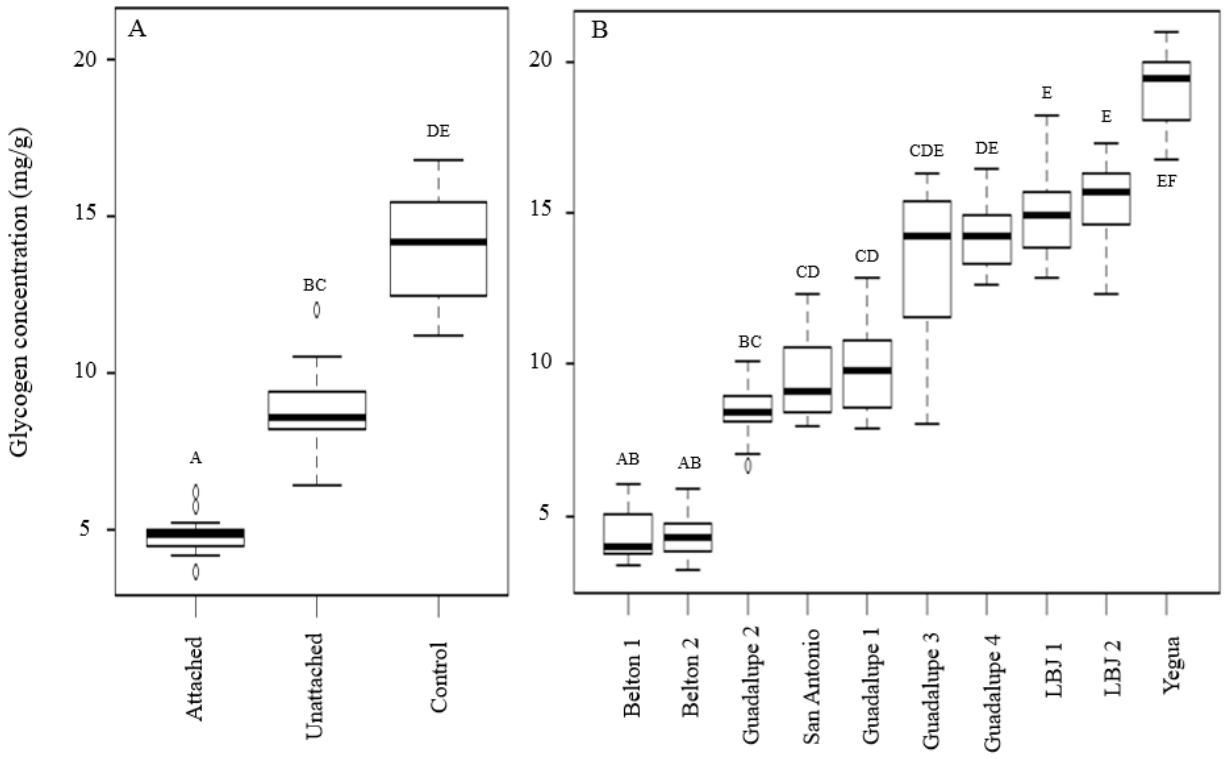

Figure 5: Glycogen concentrations (mg/g) of mussels from A) laboratory experiments and B) field sites. Boxplots represent the 25th and 75th percentile, the black line is the median, the error bars represent 1.5 times the interquartile range, and open circles represent any outliers. Each lab treatment 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 arrange 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.

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