EFFECTS OF ELEVATED TEMPERATURE ON INVASIVE FRESHWATER DREISSENIDS

by

Courtney Koplyay

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FRESHWATER DREISSENIDS

Project Approved:

Supervising Professor: Michael Misamore Ph.D.

Department of Biology

Mark Demarest Ph.D.

Department of Biology

Daniel Williams Ph.D.

Department of the John V. Roach Honors College

ABSTRACT

Dreissena polymorpha (zebra mussels) and *Dreissena bugenis* (quagga mussels) are invasive freshwater species native to Eastern Europe. In the 1980s, commercial shipping vessels introduced these dreissenids to the Great Lakes region, and they have since made their way throughout the United States. Originally thought to be a cold-water species, these mussels have invaded warmer waters of the southern US. The survival of *Dreissena polymorpha* and *Dreissena bugenis* were examined at various temperatures to determine the upper limit of their temperature tolerance. In experiments aimed at specifying their survival range, the mussels exhibited decreased survival at temperatures greater than 32°C and no survival at temperatures over 35°C. Long-term maintenance at 30°C and 32°C reduced survival rates compared to mussels maintained at low and mid-range temperatures. Repeated trials showed that quagga mussels and zebra mussels have similar survival rates at comparable temperatures. Smaller mussels also exhibited greater survival than larger mussels. The results indicate that the upper-temperature limit of Texas-obtained dreissenids is 34°C. Lakes in the southern regions of the United States can reach up to 30°C in the summer, which suggests that warming waters in the summer months may have a limited effect on the survival rate of dreissenids. However, elevated temperatures in shallow waters or isolated conditions may affect the further spread of these invasive species.

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INTRODUCTION

Dreissena polymorpha (zebra mussel) and *Dreissena bugenis* (quagga mussel) are small freshwater shellfish native to the freshwater lakes of Ukraine and Russia. These dreissenids were first introduced to the United States in the late 1980s via commercial shipping vessels traveling from Europe. Ships crossing the Atlantic would release their ballast water into the Great Lakes, which is considered the most likely means of the introduction of the species to the United States (May and Marsden, 1992). This may have introduced adult mussels and/or microscopic veligers into the American water system, from which they quickly began spreading throughout the United States (Hebert and Muncaster, 1989).

As invasive species, *D. polymorpha* and *D. bugenis* are disruptive to the ecology of the lakes they inhabit. Dreissenids utilize byssal threads, which are strong fibers that allow bivalves to stabilize and attach themselves to almost any object (Brazee and Carington, 2006). They attach themselves to rocks, boats, pipes, and other native mussels. Zebra and quagga mussels can incapacitate other mussel species and render underwater infrastructure unusable (Ram and Palazzolo, 2008). Dreissenids are filter feeders and can harm native fish and other species by filtering out algae that they rely on for sustenance (Ludyanksiy, et al. 1993).

Due to early European studies that indicated a maximum temperature limit of 28°C, *D. polymorpha* and *D. bugenis* were originally thought to be strictly cold-water species, (Jenner and Hanssem-Mommen, 1992). It was thus assumed that they lacked the thermal adaptation capabilities to spread from the Great Lakes to the warmer regions of the United States. However, zebra and quagga mussels have since made their way throughout the Midwest, the southern states of Louisiana and Texas, and even further west into Colorado, Utah, and California. As the species began inhabiting warmer water lakes throughout the southern and western United States, it became clear that the thermal capabilities of dreissenids had been greatly underestimated.

Prior studies have extensively examined the thermal tolerance of *D. polymorpha* and *D. bugenis*, but the results are widely inconsistent. Numerous studies have examined the lower-temperature limit of dreissenids, yet there are limited studies that examine the upper-temperature limit of North American mussels (Mackie and Claudi, 2009; Hernandez et al., 1995). Dreissenids were not originally thought to have the capability to survive outside of their native temperature range, so their spread throughout the United States has been unpredictable. It is vital to understand the extent of their extreme thermal capabilities on both ends of the spectrum to limit and prevent the further spread of the invasive species throughout the United States.

In this study, the thermal tolerance and survival of Texas-obtained zebra mussels and Nevada-obtained quagga mussels were examined. The goal of the study was to determine the upper-temperature lethal limit of *D. polymorpha* and *D. bugenis.* The survival rates were examined over time to determine the short-term and long-term effects of elevated temperatures on zebra and quagga mussels.

<u>METHODS AND MATERIALS</u>

Collection and Maintenance of Mussels

The zebra mussels used in this study were originally collected from Lake Bridgeport, TX, and the quagga mussels were originally collected from Lake Mead, NV, and have since been maintained in collecting tanks held at 15°C. Approximately 300 mussels were removed from the maintenance tanks and transferred into a smaller cooler containing pond water according to the protocol of Dietz, et al. (1994). The mussels were supplied with air through an air stone and allowed to gradually warm up to room temperature (20°C). Any dead mussels were removed from the cooler, and the remaining mussels were separated by size. Ten mussels of various sizes were placed into 1-L beakers. 25 beakers were utilized, resulting in 250 total mussels per experiment. The remaining mussels were then returned to their original maintenance tanks.

For each experiment, five beakers each containing ten mussels were separated into five different coolers, which would be set to different experimental temperatures. Each cooler contained approximately 30 L of distilled water and a line was drawn inside each cooler to ensure that the water level remained consistent throughout the different experiments. The beakers were placed on a tray made of PVC piping so that the opening of each beaker remained above the water level of the cooler. A Hygger Titanium Digital aquarium heater was placed at the bottom of each cooler and held in place via a suction cup. A Sun Microsystems 800GPH submersible circulating powerhead was similarly fastened to the side of each cooler via suction to circulate water and ensure that a consistent temperature was maintained throughout the water bath. An Inkbird Temperature Datalogger was also placed inside each cooler to measure the rate at which the heaters brought the water in each bath to the desired experimental temperature. An air stone connected to an air supply via rubber tubing was placed into each beaker to provide sufficient oxygen to the mussels.

Following the termination of each experiment, the contents of each beaker were placed into a bucket containing a bleach-water mixture to ensure the termination of any surviving mussels.

Zebra Mussel Temperature Survival Range

To determine the crude temperature survival range of the zebra mussels, the aquaria heaters in each of the coolers described above were set to different experimental temperatures: 20°C, 25°C, 30°C, 35°C, and 40°C. The beakers containing the mussels were then placed into the coolers and examined after 24 hours, 48 hours, one week, and two weeks to determine the number of dead mussels in each beaker. Mussels were labeled as dead based on a gapping response assay. Normally, healthy mussels will gape open to feed and respire, and snap closed as soon as they are disturbed. Mussels without this response were determined dead. During each check-up, dead mussels were removed and stored in the freezer for later analysis. After the two-week experiment, the mussels were disposed of properly.

To refine the temperature range of zebra mussels found in the initial broad-range temperature trials, a second experiment was conducted utilizing a narrower range of temperatures. The initial setup for this experiment followed the protocol from the first, with the exception that the mussels were initially placed in the beakers in each cooler at room temperature and then slowly brought up to their respective experimental temperature. The four aquaria heaters (not including room temperature control) were slowly brought up to 24°C. After acclimating for 24 hours at 24°C, the experimental heaters were each ramped up to their effective temperature at a rate of 2°C every 24 hours. This resulted in five different coolers set to five different temperatures (20°C, 28°C, 30°C, 32°C, or 34°C). Once a cooler had reached its set temperature, mussel survival in the given cooler was examined after 24 hours. The dead mussels were placed in a 20°C water bath to allow any thermally stressed mussels to recover and ensure that the dead mussels were correctly identified. The survival results were recorded, and the mussels were disposed of properly.

Quagga Mussel Survival and Recovery

The thermal capabilities of quagga mussels were determined using the same procedure explained in the narrow-range temperature experiment. The quagga mussels were removed from 15°C water and allowed to acclimate to room temperature (20°C) for 24 hours. The beakers were then placed into each cooler and slowly brought to their effective temperatures during a week-long ramp-up. Mussel survival was examined 24 hours after the final temperature increase. The survival results were recorded. The dead mussels were then placed in a 20°C water bath to allow any thermally stressed mussels to recover and ensure that the dead mussels were correctly identified. The mussels were maintained at 20°C for an additional 24 hours and then their survival was examined. The results were recorded, and the mussels were disposed of properly.

RESULTS

The first experiment utilized a broad range of temperatures to determine the thermal tolerance and survival of zebra mussels at elevated temperatures (Fig. 1). After 24 and 48 hours, the mussels held at 20°C, 25°C, and 30°C had a 100% survival rate and the mussels held at 35°C and 40°C had a 0% survival rate. After one week, the mussels held at 20°C and 25°C maintained a 100% survival rate, and the survival rate of the mussels held at 30°C decreased to 72%. After two weeks, the mussels held at 20°C and 25°C maintained a 100% survival rate, and the survival rate of the mussels held at 30°C decreased to 30%.

The second experiment expanded upon the first experiment and tested a narrower range of temperatures to determine a specific upper-temperature limit of survival of zebra mussels (see Figure 2). The mussels were slowly acclimated to their testing temperatures for two weeks and showed 100% survival through 30°C. After 24 hours, the mussels at 20°C, 28°C, 30°C, 32°C, and 34°C had survival rates of 98%, 96°%, 88%, 92%, and 50% respectively. None of the dead mussels returned to the 20°C water bath recovered, suggesting that all of the mussels were correctly presumed dead.

The third experiment utilized quagga mussels with the same narrow temperature range to compare the survival of zebra mussels and quagga mussels in similar conditions (see figure 3).

The mussels were slowly acclimated to their testing temperature and showed close to 100% survival through 30°C. Only one mussel out of the fifty had died during the ramp-up. After 24 hours, the mussels at 20°C, 28°C, 30°C, 32°C, and 34°C had survival rates of 100%, 94°%, 53%, 44%, and 0% respectively. Following the temperature decrease to 20°C, mussels in the 28°C, 30°C, and 32°C temperatures exhibited signs of recovery. After 24 hours at the recovery temperature, the remaining mussels from the initial temperatures of 20°C, 28°C, 30°C, 32°C, and 34°C had survival rates of 100%, 93°%, 83%, 88%, and 0% respectively. Approximately 60% of the presumed dead mussels in the 30°C and 32°C temperature groups recovered and were responsive to touch after 24 hours.

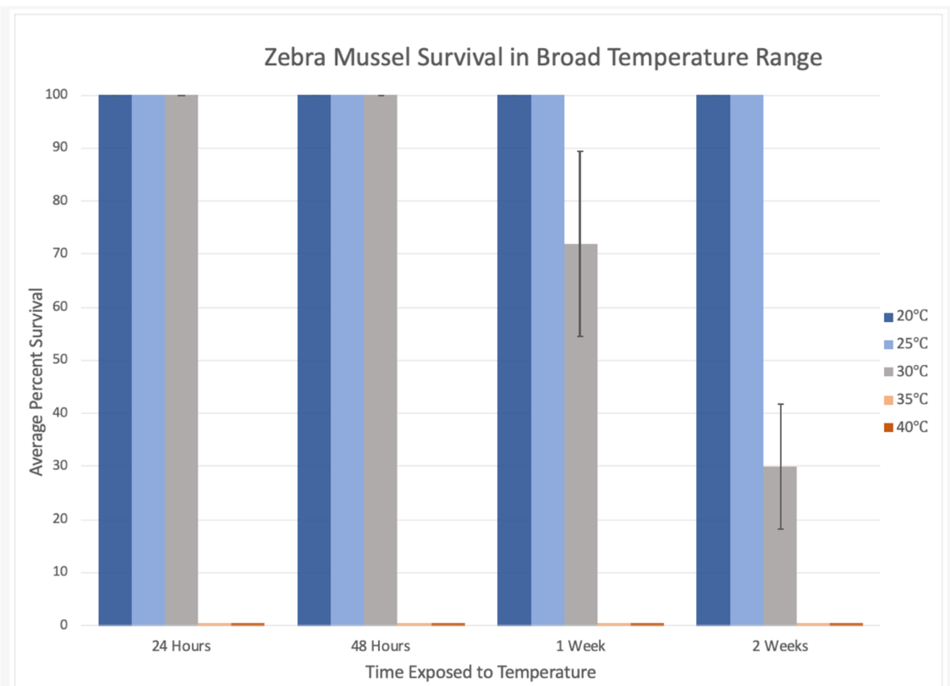

Figure 1: Average zebra mussel survival in a broad temperature range from 20-40°C. Error bars indicate standard error between each trial for a given temperature; n= 5 trials per bar.

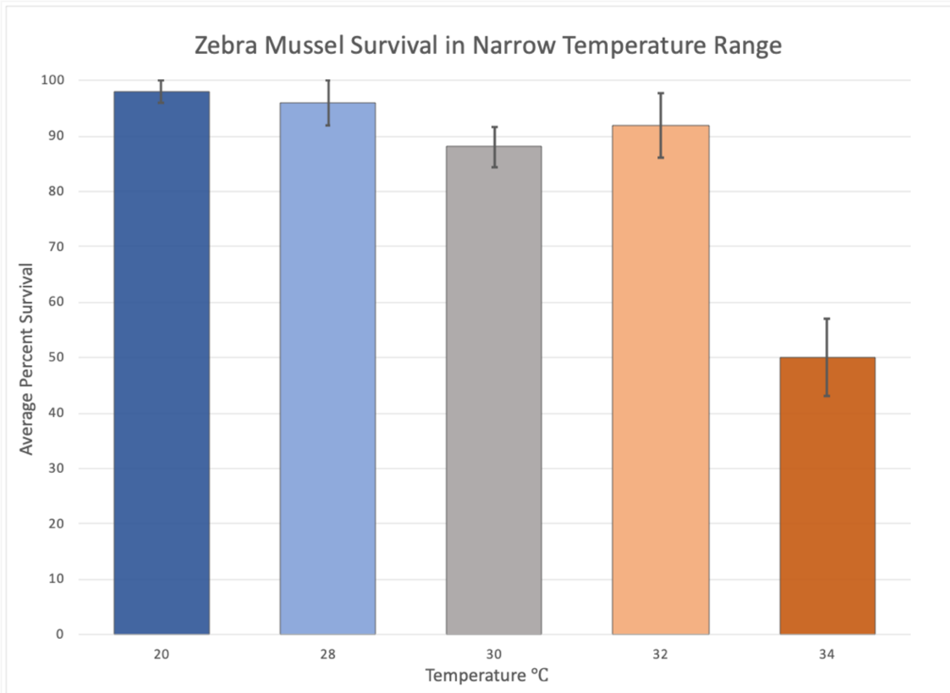

Figure 2: Average zebra mussel survival in a narrow temperature range from 28-34°C as compared to a 20°C control. Error bars indicate standard error between each trial for a given temperature; n= 5 trials per bar.

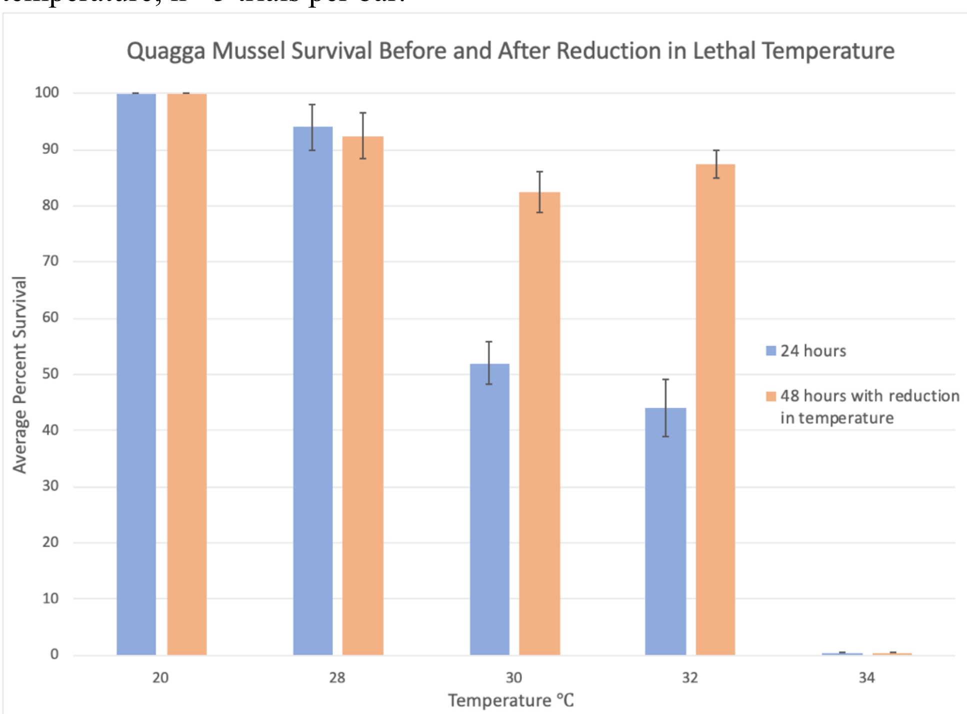

Figure 3: Quagga mussel survival in a narrow temperature range from 28-34°C compared to a 20°C control. Error bars indicate standard error between each trial for a given temperature and recovery condition; n= 5 trials per bar.

DISCUSSION

The results of this study show that zebra mussels were able to survive at 30°C, yet unable to survive at 35°C, suggesting that the lethal temperature limit lies below 35°C. During the broad range temperature experiment (Fig. 1), the mussels were brought up to their testing temperature immediately from room temperature, which took approximately four hours. This most likely shocked the mussels, which could have exacerbated the death rates of the mussels at warmer temperatures. Although the mussels at warmer temperatures of 25°C and 30°C had a high survival rate initially, their long-term survival was decreased (Fig. 1). The mussels held in warmer waters were most likely more stressed than mussels held at the control, decreasing their long-term survival. After the two-week trial, the water in each beaker was murkier and the surviving mussels were slower to respond when disturbed with a metal probe. Normally, healthy mussels use active adductor muscular contraction to hold their shells closed and use a passive ligamental force to open for respiring or feeding. Mussels will close their shells for protection when disturbed upon contact. To open, mussels relax their adductor muscles and the opposing hinge ligament opens the shell. When dead, the functioning hinge ligament causes the shells to open, but the mussels cannot close due to an absence of muscular contraction (Mackie, 1991).

To determine a more specific upper limit of survival, a narrower temperature range was used. A slow ramp-up was utilized to prevent premature mortality from thermally related shock and help acclimate the mussels to their experimental temperature. The slow acclimation may allow the experimental survival rates to be more representative of mussel death due to temperature, rather than heat shock. The slow ramp-up greatly increased the survival of zebra mussels at warmer temperatures. The data from the narrow-range experiments showed that zebra mussels have an upper-temperature limit of 34°C.

Quagga mussels appear to be less thermally capable than zebra mussels. Although they maintained a high-temperature survival rate throughout the temperature ramp-up, their survival rates plummeted after being set to the high experimental temperatures. The quagga mussels had been stored at 15°C longer than the zebra mussels, which could have contributed to their lack of ability to adapt to warmer environments. The data suggest that the lethal temperature limit of quagga mussels is 32°C. The water temperature was reduced back down to 20°C to determine if the mussels could recover. The quagga mussels did appear to recover after substantial time in the cooler water, suggesting that they were not dead following the first 24 hours. The mussels were most likely extremely stressed and not responsive, making them appear as if they were dead.

The thermal capabilities of *D. polymorpha* and *D. bugenis* from this study are comparable to results found in prior studies, although such studies have fairly inconsistent findings. Iwanyzski and McCauley (1993) suggested that the lethal temperature threshold of *D. polymorpha is* around 30°C. This value was widely accepted when dreissenids were considered to be a cold-water species. However, McMahon and Ussery (1995) determined the upper lethal temperature limit of zebra mussels to be between 37-40°C, which is much higher than previously accepted temperatures. We also found that a slow rate of temperature acclimation increased the thermal tolerance of both *D. polymorpha* and *D. bugenis*, which is consistent with the findings of previous studies. Garton and Haag (1993) and Spindel, et al. (1995) suggest a similar thermal tolerance when *D. polymorpha* are slowly adapted to high temperatures. McMahon and Ussery (1995) also found that slower acclimation increased the thermal survival range of the species, which increased the temperature required to achieve 100% mortality.

Zebra and quagga mussels reared in warmer southern waters may have a higher thermal tolerance, but mussels from other regions may not have the same thermal capabilities. White and Hamilton (2015) conducted a study of zebra mussels in the Great Lakes and found that mass mortality occurred at temperatures between 25-30°C. This is much lower than the lethal temperatures found in Texas-derived zebra mussels and Nevada-derived quagga mussels from this study, which may suggest mussels from southern regions have adapted to survive at warmer temperatures. Contrarily, Hernandez et al. (1995) reported no significant difference in thermal tolerance between mussels collected in New York and Baton Rouge. This study seemed to suggest that thermal tolerance was seasonal, rather than based on location.

It would be interesting to see how mortality rates of mussels reared in colder waters would compare to mussels reared in warmer southern waters. A potential experiment could create a direct comparison between the survival of zebra and quagga mussels from Texas and mussels from the Great Lakes, given that Texas lakes can reach up to around 31°C while the Great Lakes can only reach up to 24°C in the heat of the summer (West Fork Trinity; US Department of Commerce). While it is unlikely that water temperatures in either region will reach the 34°C temperature survival limit found in this study, smaller contained quantities of water, such as bait containers, boat ballasts, or standing water in various watercraft could potentially reach lethal temperatures. The elevated temperatures of these contained water sources in the warmer summer months, when boat traffic is particularly high, might aid in preventing the further spread of invasive species throughout the southern United States.

Further analysis of the reproductive capabilities of dreissenids at elevated temperatures could provide insight into their capability to survive as a species. More specifically, looking at gamete fertilization and early embryogenesis at elevated temperatures would be an excellent follow-up for further examination of the thermal tolerance of *D. polymorpha* and *D. bugenis*.

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