The effects of thermal acclimation on feeding rates and thermal tolerance in the invasive zebra mussel (Dreissena polymorpha) in Lake Champlain, VT, USA

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The effects of thermal acclimation on feeding rates and thermal tolerance in the invasive zebra mussel (*Dreissena polymorpha*) in Lake Champlain, VT, USA

Marissa Ng

Advisor Dr. Brent Lockwood
Abstract

Zebra mussels (*Dreissena polymorpha*) are an invasive and widespread species in North America, where they have large ecological and economic impacts. In the context of global climate change, it is uncertain how higher temperatures will affect the invasive success of zebra mussels. I tested the effects of acclimation temperature on thermal tolerance and filtration (i.e. feeding) rates of zebra mussels in Lake Champlain to shed light on the future success of this species in the lake and offer potential insight on how lake ecology may be affected with continued climate warming. Mussels were acclimated to four temperatures (10, 15, 22, 30°C) for a period of 1.5 weeks. Following this acclimation treatment, filtration rate was measured via absorbance spectrophotometry. Acute heat tolerance was scored by assaying mussel mortality during a heat ramp, in which temperature was increased at a rate of 0.15°C/min for 2.5 hours. I found no significant effect of acclimation temperature on filtration rate, although there was a trend in which filtration rate increased with temperature until 22°C after which filtration rate declined. Acclimation temperature did significantly affect survival after an acute heat shock event. The acute lethal temperature at which 50% of the mussels died (LT$_{50}$) was approximately 37°C, and acclimation to 30°C led to an increase in LT$_{50}$ of 0.65°C. My results indicate that zebra mussels have a heat tolerance that far exceeds the warmest temperatures likely to be experienced in Lake Champlain and therefore will successfully survive warming waters induced by climate change. However, increased temperatures do cause a decline in filtration rate, which may affect zebra mussel growth and reproductive capabilities and change the way they impact phytoplankton community composition and nutrient availabilities potentially exacerbating harmful cyanobacteria blooms, which are projected to occur with increasing frequency. These results offer insight on the response of Lake Champlain zebra mussels to thermal stress and the potential consequences to lake ecology due to climate change.
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**Introduction**

Climate change is the pressing issue of our time. It has produced conditions around the world that have facilitated the introduction and continued success of invasive species (Dukes and Mooney, 1999). In aquatic ecosystems climate change results in warmer water temperatures, reduced ice coverage, and increased storm events. These changes are expected to affect the probability of establishment of invasive species and alter the impacts of already established invasives, thus furthering the need for effective control methods (Rahel and Olden, 2008).

Zebra mussels (*Dreissena polymorpha*) are an invasive species to North America (McMahon, 1996) and have a significant impact on the ecosystems to which they are introduced (MacIsaac, 1996). Warmer water temperature facilitates their continued success by allowing them to begin growth earlier and maintain it later into the fall. A shorter period of ice coverage also potentially eliminates winter hypoxia allowing the expansion of invasive populations that would otherwise die in the winter (Rahel and Olden, 2008).

The success of zebra mussels is of concern because of their huge ecological and economic impacts. Zebra mussels pose problems for marine structures such as boats, dock and canal walls, and water intake structures for industrial, municipal, and hydroelectric plants (MacIsaac, 1996). The water intake structures are especially impacted because the mussels form dense mats blocking pipes (Ram and McMahon, 1996) and even lead to water outages (LePage, 1993). The financial impact of zebra mussel fouling is estimated at one billion dollars annually (Pimentel et al., 2005).

The ecological impacts are just as drastic. Zebra mussels negatively affect native unionid bivalve species by settling on native species’ shells and outcompeting them for food, which may result in native species population decline and possible extirpation (Ram and McMahon, 1996). In addition, zebra mussels accumulate environmental contaminants, which results in the increase of tissue contaminant loads in their predators, negatively impacting these species as well (de Kock and Bowmer, 1993; Ram and McMahon, 1996). Zebra mussels also drastically reduce phytoplankton biomass, which increases water clarity (MacIsaac, 1996). This results in changes to aquatic ecosystem
communities, such as benthic fauna, as well as having an oligotrophic effect in some lakes (Griffiths, 1993).

The ecology of Lake Champlain is expected to change with climate change increasing water temperatures (Stager and Thill, 2010) and invasive species impacting ecosystem processes. Zebra mussels in Lake Champlain have caused a decline in phytoplankton biomass as well as altering nutrient cycling and threatening the survival of native bivalve species (Miller and Watzin, 2007). The objective of this study was to shed light on the future success of zebra mussels in Lake Champlain and offer potential insight on how lake ecology may be affected with continued climate warming.

I sought to determine if zebra mussels acclimated to different temperatures had different filtration, or feeding, rates. I used absorbance spectrophotometry to quantify filtration rate based on the methods of Pestana et al. (2009) and created a thermal performance curve. Q_{10} coefficients (i.e. the measure of the rate of change in a biological system as an effect of increasing the temperature 10°C) were also used to gauge thermal performance. Previous studies have found a connection between temperature and filtration ability in zebra mussels, wherein filtration rates were shown to be optimal within a certain temperature range and declined at high and low temperatures (Aldridge et al., 1995; Lei et al., 1996; MacIsaac, 1996). Miller and Watzin (2007) showed how zebra mussels affect the planktonic food web of Lake Champlain but no studies have looked at how temperature affects filtration rate in Lake Champlain specifically.

I also sought to measure the thermal tolerance of zebra mussels acclimated to different temperatures. I used an acute heat shock event to test thermal tolerance based on the methods of McMahon and Ussery (1995). I used LT_{50}, or the lethal temperature at which 50% of the population dies, as an indicator of the point at which survival declined to determine the effect of acclimation temperature on survival. Previous studies have tested the thermal tolerance of zebra mussels found in various locations around the US and Europe (Elderkin and Klerks, 2005; Griebeler and Seitz, 2007; McMahon, 1996; Spidle et al., 1995). However, the thermal tolerance of zebra mussels in Lake Champlain has not been studied. Past studies have examined both chronic and acute upper lethal temperatures. Acclimation temperature and rate of temperature increase are important factors influencing lethal temperatures (Iwanyzki and McCauley, 1993; McMahon and...
North American zebra mussels tend to be more thermally tolerant than their European counterparts as the mussels introduced to North America came from the warmest part of the European mussels’ range and seasonal acclimatization may play an important role in thermal tolerance (Griebeler and Seitz, 2007; McMahon, 1996).

The filtration rate of zebra mussels was expected to increase with acclimation temperature. It was also expected that mussels acclimated to higher temperatures would have increased acute thermal tolerance. Because research examining the effect of temperature on multiple responses has not been done for zebra mussels in Lake Champlain, this study provides information on how zebra mussels respond to thermal stress and how that response may affect the lake ecosystem. The results give insight on how changes in zebra mussel filtration rates and survival may affect the planktonic food web, overall lake ecology and human systems, such as industrial plants and recreational activities like swimming and boating. In broad terms, this research allows predictions to be made about how global climate change will affect the Lake Champlain ecosystem.

Methods

Study Organism and Collection Site

Zebra mussels are native to the Black Sea region but are an invasive species in many parts of the world. They were introduced to North America in the 1980s through ballast water and are now found in many waterways throughout the United States (Ram and McMahon, 1996). On average zebra mussels are 2.5-3.8 cm long (USGS, 2015) and are filter feeders, primarily eating algae and can filter at rates of one (Reeders et al., 1989) to over five liters per day per mussel (Horgan and Mills, 1997). Their filtration ability impacts native species of mussels (Strayer and Malcom, 2007) as well as lake ecology in general by increasing water clarity (Maclsaac, 1996). They also exist across a wide range of thermal environments (Elderkin and Klerks, 2005).

Zebra mussels for this experiment were collected at Red Rocks Park in South Burlington, VT (GPS coordinates N 44.44247, W 073.22601) and transported to the Lockwood lab in Marsh Life Science on the University of Vermont campus. Three hundred mussels were collected on October 20, 2015. At the time of collection, the in situ lake temperature was 11.67°C (NWS, 2015).
Experimental Setup

Mussels were divided equally into twelve, one-liter beakers. Each beaker contained 800 ml of tap water treated with water conditioner (Seachem) with an air stone connected to an air pump (Tetra) to keep the water oxygenated. Three beakers were placed in each of four acclimation temperatures (10, 15, 22, and 30°C) to produce three replications for each temperature group for the experiments. Water temperatures were maintained using water baths or incubators. The 10°C and 15°C acclimation groups were kept in water baths in the cold room using immersion circulators to heat the water from the surrounding temperature of 4°C to their respective experimental temperatures. The 22°C acclimation group was kept in an incubator set at 22°C and the 30°C acclimation group was kept in a water bath in the lab that warmed the water from room temperature (23°C) to the experimental temperature of 30°C.

Mussels were brought to their respective acclimation temperatures over a period of three days. After collection, three beakers of mussels were placed in the 10°C water bath while the remaining nine were kept in 15°C baths for one day. A water bath in the lab had ramp capabilities so the beakers could be brought to acclimation temperatures over a set amount of time. On average, beakers were warmed 5°C over a period of twelve hours and remained at that temperature for another twelve hours. Once three beakers reached each acclimation temperature, they were maintained while the remaining beakers were warmed. Mussels were kept at acclimation temperatures for one week before experiments began.

Approximately two liters of treated water were kept at each acclimation temperature so water could be changed every four or five days. Water was added to beakers when needed due to evaporation. Mussels were fed freshwater *Nannochloropsis* grown in Alga-Gro® freshwater medium from Carolina Biological Supply Company (Burlington, NC), as per the manufacturer’s recommendations. Mussels were given the equivalent amount of algae food to 5% of total water volume every two days. Beakers were checked for mortalities every two days and removed if found.
**Filtration Rate**

Filtration rate was measured using a SPECTRONIC™ 200 Spectrophotometer from Thermo Scientific (Waltham, MA). 1:2 serial dilutions were made to create a standard curve. The series of concentrations (30, 15, 10, 7.5, 5, 3.75, 2.5, 1.875, 1.25, 0.9375, 0.625, 0.3125%) were measured on the spectrophotometer at 640 nm (Pestana et al., 2009) and a standard curve was created from the resulting absorbances.

Beakers were brought to room temperature over a period of two hours. The beakers were then emptied and gently rinsed if necessary to remove any waste and any dead mussels were removed. The total number of mussels was noted and 300 ml of room temperature (23°C) water were added along with 30 ml of algae food for a final concentration of 10% algae. 300 ml of water was used in the filtration assays to decrease error in the spectrophotometric readings. Any mussels above the water level were not counted, as they would have no impact on filtration. 4 ml samples were taken every thirty minutes for two hours (Horgan and Mills, 1997) for a total of five samples for each acclimation temperature. Absorbance of these samples was then measured on the spectrophotometer and the concentrations extrapolated using the standard curve. Filtration rates and $Q_{10}$ values were calculated to assess thermal performance. Here, $Q_{10}$ is defined by the equation, $Q_{10} = \left(\frac{R_2}{R_1}\right)^{10/(T_2-T_1)}$, where $R_2$ and $R_1$ are the filtration rates measured after acclimation to higher and lower temperatures, respectively, and $T_2$ and $T_1$ are the corresponding higher and lower acclimation temperatures, respectively, for a given acclimation temperature range. Thus, $Q_{10}$ is an index of the relative rate of change in filtration rate between each acclimation temperature. Following these filtration assays, beakers were filled to 800 ml and placed back into their acclimation temperatures overnight prior to the heat shock experiment.

**Thermal Tolerance**

To measure thermal tolerance, an acute heat shock experiment was performed, in which the water temperature was increased at a rate of 0.15°C per minute and survival was assayed during the heat ramp. Beakers were brought to room temperature over a period of two hours and then placed in a room temperature (23°C) water bath. The original ramp rate was based on the methods of McMahon and Ussery (1995), which
increased temperatures by 0.2°C per minute for one hour. The first replication served as a trial run to determine the most suitable ramp rate and make any necessary adjustments. Data was collected for the other two replications in which temperature increased by 0.15°C per minute for a duration of 2.5 hours. Mussel viability was observed every three degrees once the water bath reached 30°C and every one degree after 40°C. A mussel was deemed viable if tactile stimulation elicited a valve closure response or if the shell remained closed during and/or after the heat shock. If vigorous tactile stimulation did not elicit valve closure the mussel was determined to be dead (McMahon and Ussery, 1995). Tactile stimulation was done with a scoopula.

Statistical Analysis

Data analysis was performed in GraphPad Prism. An analysis of covariance (ANCOVA) was used to calculate filtration rates and assess the effect of acclimation temperature on filtration rate. A nonlinear regression analysis with a logistic model was used to analyze the results from the heat shock experiment and determine the LT$_{50}$s (i.e. the temperature along the heat ramp that elicited 50% lethality, extrapolated from the logistic regression curve fits). Extra sum of squares F test and Akaike’s Informative Criteria (AICc) test were used to determine statistical significance among LT$_{50}$s.

Results

Filtration Rate

Overall, acclimation temperature did not have a significant effect on filtration rate (Fig. 1; ANCOVA, $F(3,52) = 0.733781$, $p = 0.5366$). However, there was a trend among the acclimation groups, such that filtration rate increased with acclimation temperature up to 22°C (Fig. 2). Mussels acclimated to 30°C, on average, showed a decrease in filtration rate compared to mussels acclimated to 15°C and 22°C (Fig. 2). This trend was seen again when Q$_{10}$ temperature coefficients were calculated (Table 1). The Q$_{10}$ coefficient between 10°C and 15°C was 19.962, 1.349 between 15°C and 22°C and 0.478 between 22°C and 30°C. The decrease in Q$_{10}$ between 15°C and 22°C to a value between 1 and 2 indicates an approach to optimal filtration rate. The further decrease in Q$_{10}$ between 22°C and 30°C to a value less than one indicates an overall decrease in filtration rate across the highest temperature range of acclimation.
Thermal Tolerance

Acclimation temperature had a significant effect on acute thermal tolerance (i.e. survival after an acute heat shock treatment) (Fig. 3). Acclimation at 10°C, 15°C, and 22°C resulted in similar survival in response to heat shock, with LT$_{50}$s of 37.07°C, 36.95°C, and 37.26°C, respectively (Figs. 3 and 4). However, acclimation at 30°C led to a significant increase in LT$_{50}$ of 0.65°C (LT$_{50}$ = 37.74°C after 30°C acclimation), compared to the other three acclimation groups (Figs. 3 and 4; Nonlinear regression: F-test, $F(3,52) = 4.526$, p = 0.0068, AICc: 94.58% probability that LT$_{50}$ was different for the 30°C acclimation group). Maintaining the mussels in their respective acclimation temperatures for the duration of the experiment also impacted survival; acclimation to higher temperatures caused more mussel deaths during the acclimation period. Acclimation to 30°C resulted in the death of 22 mussels or 35% of the total number of mussels at 30°C while acclimation to 10°C, 15°C, and 22°C resulted in 2 (3%), 3 (5%), and 4 (7%) mussel deaths respectively over the three weeks mussels were kept in the lab.

Discussion

The response of zebra mussels to thermal stress will determine their success under climate change conditions. Here I studied the effect of acclimation temperature on filtration rate and thermal tolerance of zebra mussels in Lake Champlain. My results indicate that zebra mussels will be able to survive warming waters. However, declines in filtering ability due to thermal stress will potentially alter lake ecology in new ways.

Filtration Rate

Overall, I found that acclimation temperature had subtle effects on mussel feeding, as measured by filtration rate; however, this effect was not statistically significant (Figs. 1 and 2). This trend is illustrated in the thermal performance curve of Figure 2, which shows that acclimation to increasing temperatures led to increased filtration rates, up until 22°C. Acclimation to the higher temperature of 30°C caused an average decrease in filtration rate. These data suggest that the optimal temperature for zebra mussel feeding is 22°C. This conclusion is also supported by the Q$_{10}$ coefficients (Table 1). A Q$_{10}$ coefficient of approximately 1.0 indicates temperatures that support optimal physiological performance. The Q$_{10}$ coefficient found between 15°C and 22°C
was 1.349 (Table 1). $Q_{10}$ values greater than 2.0 indicate suboptimal performance at the lower temperature, as seen in the positive slope between 10°C and 15°C in the thermal performance curve (Fig. 2). The $Q_{10}$ coefficient for this temperature range was 19.962 (Table 1). A $Q_{10}$ coefficient of less than 1.0 indicates that the higher temperature in the range is detrimental and leads to declines in physiological performance. The $Q_{10}$ coefficient between 22°C and 30°C was 0.478 (Table 1).

Similar results have been found in other studies. Aldridge et al. (1995) tested zebra mussels from Lake Erie near Buffalo, New York and determined that the filtration rate did not significantly differ for mussels acclimated to 20°C and 24°C. Filtration rate did decline, however, in mussels acclimated to 28°C and 32°C. Lei et al. (1996) also looked at filtration rates in terms of $Q_{10}$ coefficients and found that optimal filtration in zebra mussels from the Niagara River in New York occurred between 14°C and 26°C. Therefore, it seems that the optimal temperature for zebra mussel feeding is similar among Lake Champlain zebra mussels (22°C), as I measured in this study, to that of zebra mussels in other locations.

Filtration experiments have also been done in *Limnoperna fortunei*, a mussel that has very similar life histories and ecological impacts as *D. polymorpha. L. fortunei*, or golden mussels, is a species of mussel from mainland China that has been introduced to Taiwan, Japan, and several South American countries. These mussels are potentially of even more concern because they have higher upper thermal and salinity limits and are more tolerant of lower pH and more resistant to polluted waters than zebra mussels (Karatayev et al., 2007). Temperature was expected to have a similar effect on filtration rate in golden mussels as it did in zebra mussels (i.e. filtration rate would increase until a certain point after which high temperatures would cause a decline). However, two studies that measured this found that filtration rate did not decline at temperatures where zebra mussel filtration rates typically declined. Sylvester et al. (2005) used 25°C as the highest acclimation temperature for golden mussels collected from the Paraná River in Argentina and saw no decline in filtration rate up to that point. Filtration rate did not decline at 30°C either for golden mussels collected from the Itaipu reservoir in Brazil (Pestana et al., 2009). Both studies may not have tested golden mussels at their upper thermal limits and so did not see a decline in filtration rate. For example, golden mussels can experience
water temperatures between 30°C and 32°C on a regular basis during the summer in Brazil. Golden mussels also clear more algae than zebra mussels with average filtration rates of five liters per day (Sylvester et al., 2005). As waters warm with climate change, the range of golden mussels will likely expand and may even overlap with the southern range of zebra mussels in the future. The high filtration rates and thermal tolerance of golden mussels may pose significant problems for regions previously inaccessible to this invasive species (Karatayev et al., 2007).

The decline in filtration rate in zebra mussels as a response to warming waters also has potential serious implications for aquatic ecosystems. Because zebra mussels have high clearance rates, they generally cause a decrease in phytoplankton biomass and increase in water clarity. This results in changes to phytoplankton community structure, food sources for other species, and habitat structure as a result of deeper light penetration (MacIsaac, 1996). Zebra mussels are used in some cases for water quality management because of their ability to increase water clarity. They tend to create oligotrophic environments (Griffiths, 1993) and so have been introduced into eutrophic bodies of water to control phytoplankton growth (Reeders et al., 1993). This use of zebra mussels may not be applicable with climate warming as filtration rate declines with increasing temperatures.

Zebra mussels also filter other single-celled organisms such as protozoa and rotifers as well as various kinds of phytoplankton, including cyanobacteria (Miller and Watzin, 2007). Cyanobacteria are of special interest as they are a type of phytoplankton that have harmful forms whose proliferation may be promoted by climate change (O’Neil et al., 2012). Toxic cyanobacteria negatively impact public health, tourism, and ecosystems (Anderson et al., 2012). The interaction between nutrient concentrations, especially phosphorous, in water bodies and increasing water temperatures promotes more toxic cyanobacteria blooms (Davis et al., 2009).

In Lake Champlain, zebra mussels have been found to alter nutrient cycling. They disrupt the microbial loop, which changes protozoan composition resulting in changes to the remineralization process, thus changing the availability of soluble nutrients. Increases in soluble reactive phosphorous and a decrease in the nitrogen to phosphorous ratio can favor cyanobacteria dominance (Miller and Watzin, 2007). The temperature of Lake
Champlain has also been increasing by 0.035-0.085°C per year for the past fifty years or so (Smeltzer et al., 2012) and may warm even more with climate change (Stager and Thill, 2010). Warming waters, resulting in decreased zebra mussel filtration ability, and altered nutrient availabilities may produce conditions that are advantageous to potentially harmful cyanobacteria populations.

However, there is conflicting evidence about whether zebra mussels suppress or promote cyanobacteria growth that must be considered before predictions can be made regarding how the impact of warming waters on zebra mussel filtration will change this aspect of lake ecology (Bastviken et al., 1998; Miller and Watzin, 2007; Reed-Andersen et al., 2000; Vanderploeg et al., 2001). One study found that zebra mussels from the Hudson River in New York caused a shift in phytoplankton composition from cyanobacteria to diatoms (Bastviken et al., 1998). Another study found that zebra mussels in Lake Mendota, Wisconsin could be expected to remove large amounts of cyanobacteria and may even preferentially remove them (Reed-Andersen et al., 2000). However, filtering of cyanobacteria was dependent on conditions, which Miller and Watzin (2007) found as well. Cyanobacteria removal depended on size of phytoplankton and mixing of the water column, as well as nutrient availabilities and ratios. Vanderploeg et al. (2001) found that in Lake Huron and Lake Erie zebra mussels rejected toxic strains of cyanobacteria, which resulted in more toxic blooms and phytoplankton concentrations in general.

Despite the uncertainty surrounding zebra mussels’ impact on cyanobacteria, the results of this study suggest that declining filtration rates due to higher temperatures will decrease zebra mussels’ ability to control phytoplankton abundance and may exacerbate harmful algal blooms, which are expected to worsen as climate change progresses. Future studies should look at the effect of temperature on zebra mussels’ influence on nutrient availabilities to determine if it will compound the effects of decreased filtration rates.

**Thermal Tolerance**

Acclimation temperature had a significant effect on zebra mussel survival after an acute heat shock treatment. Acclimation to higher temperatures increased the probability of survival, specifically for mussels acclimated to 30°C. The LT50s, extrapolated from the regression curves, were determined to be approximately 37°C. However, the survival
curve for the 30°C acclimation group is shifted further to the right in Figure 3 indicating an increased LT\textsubscript{50} and enhanced thermal tolerance. Accordingly, the LT\textsubscript{50} for mussels acclimated to 30°C is 0.65°C greater than the average of the other three acclimation groups.

Other studies found similar thermal tolerance results. McMahon and Ussery (1995) found that the acute upper lethal temperature for zebra mussels from the Niagara River in New York was dependent on acclimation temperature and the rate of temperature increase. In their experiment, temperature was raised at a rate of 0.2°C/min, compared to this experiment’s rate of 0.15°C/min. They determined the LT\textsubscript{50}s to be between 36°C and 40°C for acclimation temperatures between 10°C and 30°C, respectively. On average, they showed that LT\textsubscript{50} increased by 0.13°C for every 1°C increase in acclimation temperature. Spidle et al. (1995) also found that increased acclimation temperature increased acute thermal tolerance. They tested zebra mussels from both Lake Ontario and Lake Erie, New York and found slightly higher LT\textsubscript{50}s; mussels acclimated to 15°C had an LT\textsubscript{50} of 37.2°C. Interestingly, they did not observe any mortality for mussels acclimated to 20°C when tested between 30°C and 39°C with a heat ramp rate of 0.2°C/min, suggesting a high thermal tolerance. However, this result may have been due to the interaction between this higher heat ramp rate and temperature, and must be considered accordingly. In general, slower heat ramp rates have been shown to produce lower LT\textsubscript{50}s in laboratory acute thermal tolerance experiments, presumably because the organisms spend a longer time at each temperature along the ramp (Terblanche et al., 2011). On average, Spidle et al. (1995) observed LT\textsubscript{50}s between 35.003°C and 37.203°C using the same heating rate (0.2°C/min) with different acclimation temperatures and the same acclimation temperature (20°C) with different rates.

Two other studies used time to death rather than LT\textsubscript{50} as a measurement of thermal tolerance, but produced results that suggest similar conclusions as the previous studies. Elderkin and Klerks (2005) tested zebra mussels found in the Mississippi River but from different geographic locations i.e. Minnesota, Illinois and Louisiana. They tested thermal tolerance at 32°C and found that mussels from Louisiana had a significantly longer time to death compared to the mussels taken from more northern
locations. Their study suggests that perhaps zebra mussel populations have some degree of local adaptation across broad latitudinal ranges that experience different thermal environments. Beyer et al. (2011) tested mussels collected from the Fox River in Wisconsin at temperatures ranging from 32°C to 54°C. They found that zebra mussel mortality increased significantly after 38°C, especially with lengthened exposure time. They also observed 100% mortality at 43°C after only five minutes of exposure. These results are similar to the LT50 results from other studies.

Acute upper lethal temperature experiments have also been done in L. fortunei. Acclimation temperature and heat ramp rate were expected to affect thermal tolerance in similar ways to zebra mussels. Perepelizin and Boltovskoy (2011) acclimated golden mussels from the Río de la Plata estuary in Argentina to 12°C, 23°C and 28°C from in situ temperatures ranging from 12°C to 28°C representing winter and summer temperatures respectively. They increased temperature at the same rate of 0.2°C/min. LT50s were much higher than the LT50s of zebra mussels and were determined to be around 46°C. However, surprisingly, acclimation temperature had no effect on LT50. These results, and evidence that golden mussels can survive at low temperatures as well, suggest that this species will be able to greatly expand their range with continued climate warming.

There are many possibilities that serve as the basis for zebra mussel thermal tolerance that may help them adapt to increased water temperatures. Griebeler and Seitz (2007) suggest that zebra mussels are either able to tolerate a broad range of temperatures or have the ability to rapidly evolve locally adapted genotypes. They state that the latter ability is more likely as zebra mussels from different geographic locations have different thermal performances. They find that zebra mussel survival under climate change conditions will vary for each population and depend on the trade-offs or existing genetic variability that might limit adaptation. McMahon (1996) describes similar traits. He suggests that North American zebra mussels may be genetically more thermally tolerant because they originated from the warmest part of the European zebra mussel range as well as having rapid temperature adaptation abilities. However, he also describes the influence of seasonal acclimatization, which may impact experimental results. He found that even after laboratory acclimation thermal tolerance remained positively correlated
with water temperature at time of collection. Seasonal acclimatization may have impacted the results of this study, as ambient temperatures of Lake Champlain in October were low (approx. 12°C), suggesting LT\textsubscript{50} may be even higher than 37°C during the summer months. In any case, the data presented herein, as well as those of previous studies (as discussed above), suggest that there is some degree of physiological plasticity of acute thermal tolerance in zebra mussels, such that increasing acclimation temperature leads to increases in acute thermal tolerance. Thus, there is likely to be some combination of acclimatization and local adaptation in zebra mussel populations in response to climate warming.

Even if seasonal acclimatization impacted the LT\textsubscript{50}s found in this study, the temperatures that Lake Champlain currently reaches are well below the zebra mussels’ thermal limits. However, these results must be viewed in the context of short-term thermal stress on the order of minutes and hours. Although extreme temperature shifts are predicted to become more frequent as a result of climate change (Stager and Thill, 2010), chronic thermal stress experiments may be more applicable in simulating the effects of longer term warming from climate change on heat tolerance. Iwanyzki and McCauley (1993) found that zebra mussels from Lakes Erie and St. Clair had an upper incipient lethal temperature of 30°C. McMahon et al. (1995) found that zebra mussels from the Niagara River near Buffalo, New York could not survive for long periods of time at temperatures over 34°C and that acute upper lethal temperatures were higher than chronic upper lethal temperatures. McMahon and Ussery (1995) found that slower rates of temperature increase greatly decreased survival, which also may be more representative of temperature regime changes expected under climate warming.

Chronic thermal stress, besides lowering LT\textsubscript{50}, also has negative consequences on general organismal health, even if the temperatures experienced are not lethal (Jost et al., 2015). The thermal performance curve and Q\textsubscript{10} coefficients give some insight on this decline. Along with filtration rate, both adult and larval zebra mussel growth rates and successful spawning and fertilization are dependent on temperature (McMahon, 1996). As the thermal performance curve produced from the results of this study indicate, increasing temperatures improve performance only up to a point. For example, Griebeler and Seitz (2007) found that in the River Rhine zebra mussel spawning began earlier in
the year with warmer water temperatures allowing greater population growth. However, reproductive output only increased until 28°C. Ram et al. (1996) found similar results in the Great Lakes in which temperatures around 30°C inhibited gametogenesis and possibly contributed to decreased veliger densities. A similar effect of temperature is seen on zebra mussel growth. Allen et al. (1999) found that in the Lower Mississippi River, which experiences consistently high temperatures around 30°C, both shell growth stopped and tissue condition declined, though this tended to be a seasonal effect. The similar responses of multiple physiological variables to increasing temperature and the effects of their likely interactions (i.e. a decline in filtration rate due to increased temperatures will likely compound the decline in growth and reproductive abilities at high temperatures as well), suggest the thermal performance curve can be used to predict how zebra mussels will respond to climate warming.

Chronic and acute thermal stress cause declines in general zebra mussel performance. For thermal tolerance specifically, the LT₅₀s found in this study may be higher than what zebra mussels may actually experience with climate change. However, both these acute lethal temperatures and the chronic lethal temperatures found in other studies are well above what zebra mussels experience currently. Over the past 45 years or so, Lake Champlain’s warmest water temperatures have been around 22°C (NWS, 2016). With current increases in temperature of 0.035-0.085°C/year (Smeltzer et al., 2012) zebra mussels will be successful through the turn of the century, even if lethal temperatures are closer to 30°C rather than 37°C. This means that zebra mussels will continue to impact ecological and human systems, and preventing their spread and controlling established populations will remain a priority.

Conclusion

The results of this study indicate that zebra mussels in Lake Champlain will be successful in the face of climate change though responses to thermal stress may alter lake ecology in different ways. The thermal tolerance of zebra mussels, determined by LT₅₀s of approximately 37°C, was well above temperatures they experience now. To expand on this result, future experiments should explore the effect of seasonal acclimatization and collect mussels throughout the year to test the effect of ambient water temperatures on thermal tolerance and filtration rate despite laboratory acclimation. Although survival to
warming waters will not be a problem for zebra mussels, increased temperatures do cause a decline in zebra mussel filtration rate. Decreased feeding rates may affect zebra mussel growth and reproductive abilities and change the way they impact phytoplankton community composition and nutrient availabilities. It may also exacerbate harmful cyanobacteria blooms, which are projected to occur with increasing frequency with climate warming. These results offer insight on the response of Lake Champlain zebra mussels to thermal stress and the potential consequences to lake ecology due to climate change.

Bibliography


Table 1 $Q_{10}$ coefficients calculated for the temperature change between acclimation groups. Filtration rate is optimized between 15°C and 22°C.

<table>
<thead>
<tr>
<th>Acclimation temperature range (°C)</th>
<th>$Q_{10}$</th>
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<tbody>
<tr>
<td>10 - 15</td>
<td>19.962</td>
</tr>
<tr>
<td>15 - 22</td>
<td>1.349</td>
</tr>
<tr>
<td>22 - 30</td>
<td>0.478</td>
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</table>
Figure 1 Filtration rate measured as the change in concentration of algae in water media over time, following acclimation to 10°C, 15°C, 22°C, or 30°C (ANCOVA, $p = 0.5366$). Bands represent 95% confidence intervals.
Figure 2 The effect of acclimation temperature on filtration rate. Overall, the filtration rate increased with acclimation temperature until 22°C after which filtration rate decreased. Error bars represent 95% confidence intervals.
Figure 3 Mussel survival by acclimation group as heat shock temperature increased. Acclimation temperature affected survival to an acute heat shock event. Bands represent 95% confidence intervals.
Figure 4 LT₅₀ for each acclimation group with error bars representing 95% confidence intervals. LT₅₀ of the 30°C acclimation group was significantly higher than all of the other groups (Nonlinear regression F-test, p = 0.0068).