The Impact of Global Warming on Freshwater Bivalves Utterbackia imbecillis and Corbicula fluminea

Abstract

Air and water temperatures are rising at an alarming rate. For many aquatic organisms, global warming can have disastrous effects on physiological processes. Many individuals have heat thresholds and are unable to tolerate temperature changes without becoming heat stressed. For suspension feeding bivalves, heat stress can lead to greater oxygen consumption rates and inhibit nutrient excretion and egestion. We hypothesized that if native Utterbackia imbecillis and exotic Corbicula fluminea were exposed to rising temperatures then their oxygen consumption would increase and nutrient clearance would decrease. We also hypothesized that C. fluminea would respond better to higher temperatures because they are exotic and have adapted to a wide range of climates. These hypotheses were tested by assigning 9 jars of U. imbecillis and C. fluminea each to 3 temperature treatments (Control: 21°C, Medium: 22°C, and High: 23°C). Over the course of a month we took 3 separate measurements of water temperature (°C), dissolved oxygen (mg L⁻¹), nitrites/nitrates (NO₂⁻ and NO₃⁻ mg L⁻¹) and phosphates (PO₄³⁻ mg L⁻¹) then divided the measurements by each jar's total mussel dry weight. Temperature did not have an effect on oxygen consumption or excretion and egestion. However, dissolved oxygen and phosphate concentrations differed significantly between species. The average results suggest that U. imbecillis consumed more oxygen and sequestered more phosphorous compared to C. fluminea. Therefore, U. imbecillis and C. fluminea respond to the same conditions in different ways. In order to better understand the effects of climate change and heat stress on U. imbecillis and C. fluminea, they should be exposed to a wider range of temperatures for a longer period of time.

Introduction

The impacts of climate change can be seen in many ways. One main issue stemming from climate change is global warming. It's predicted that by 2050, air temperatures in the continental United States will increase up to 1.6°C. Consequently, water temperatures are also likely to increase in a similar fashion since air and stream temperatures are often related (Malish and Woolnough, 2019). The potential ramifications of global warming on freshwater ecosystems could be detrimental to many aquatic organisms. Heat stress due to rising water temperatures can negatively impact physiological and metabolic processes because most individuals have heat thresholds. Therefore, it is often hard for many organisms to acclimate to changes in water temperature because they have tolerances that limit how they interact with their environment and survive (Claësson et al., 2016).

It is often difficult to study the impacts of climate change on aquatic organisms because many factors work together that may place stress on an individual at any particular time. In the wild, aquatic organisms aren't just subjected to thermal stress as a result of climate change. Aquatic organisms also experience stress due to limited food availability, changes in weather patterns, and much more. However, we can still make strong inferences in case-by-case examples, especially if multiple factors can be controlled in a laboratory setting. While it is difficult to account for every variable that may cause an individual stress, many researchers have been making novel discoveries about the impacts of climate change by testing organisms that are already well known (Zippay and Helmuth, 2012).

Suspension-feeding bivalves are great candidates for experiments on the effects of climate change because there are many existing studies that have measured the impact of temperature on metabolic and physiological processes (Kittner and Riisgård, 2005). Furthermore,

in the context of limnology, freshwater mussels are very important because they act as water quality biomonitors and provide vital information about the state of the systems around them (Duxbury et al., 2004; Loayza-Muro and Elias-Letts, 2007). Mussels are also vital to many freshwater ecosystems because they perform tasks such as facilitating nutrient cycling, biodeposition, filtering bacteria and harmful pollutants from the water column, and much more (Malish and Woolnough, 2019). Despite being very important to freshwater systems, mussel populations are drastically declining due to stressors from climate change, pollutants, and other abiotic and biotic factors. Of all the freshwater bivalves from North America, about 70% are either threatened, endangered, or extinct (Burket et al., 2019; Malish and Woolnough, 2019). With the threat of climate change increasing every year, it is especially important now that we work towards understanding and protecting freshwater mussels.

Utterbackia imbecillis is a species of freshwater mussel that is commonly known as the paper pondshell due to its uniquely thin shell (Weinstein, 2009). *Utterbackia imbecillis* belong to the family Unionidae and can be commonly found in many watersheds, in abundance, throughout North America where they are native (Krebs et al., 2003). *Corbicula fluminea* are another species of freshwater bivalves that are widely distributed throughout North America. However, unlike *U. imbecillis, C. fluminea* is an invasive bivalve from Asia that has spread across multiple continents (Karatayev et al., 2007). *Utterbackia imbecillis* and *Corbicula fluminea fluminea* are commonly found together in many freshwater bodies throughout Florida which make them easy to collect for the purpose of our study. However, despite their abundance throughout North America, there is limited research available on how rising temperatures impact the oxygen consumption and nutrient clearance of *U. imbecillis and C. fluminea*.

Based on the literature cited above, we hypothesized that if *U. imbecillis* and *C. fluminea* were exposed to rising temperatures over the span of a month then the efficiency of their physiological processes would decline. The dissolved oxygen concentration in the water would decline because heat stress causes individuals to consume more oxygen. Furthermore, the mussel's excretion and egestion would decrease due to heat stress in an effort to conserve energy. We also predicted that *C. fluminea* would respond better than *U. imbecillis* to temperature treatments because they are able to thrive in many different climates around the world.

Methods

Study species and mussel collection

All freshwater mussels used in this manipulative study were hand-collected from Blue Lake in Deland, Florida on February 19th, 2020. We chose to collect two different species to see if higher temperatures would impact native and exotic species differently. In total, we collected 30 native *Utterbackia imbecillis* and 30 exotic *Corbicula fluminea* and transported them back to Stetson University's lab in a 5-gallon bucket along with enough lake water to fill eight 1-gallon jars. We collected more mussels than we intended to use in the event that mussels died during the acclimation period. We also collected extra lake water to replace water in the test tanks as it evaporated.

Tank set up and experimental design

Mussels that were used throughout the study were kept in tanks in between measurements. Each tank was a 1-gallon glass jar and was filled with water from Blue Lake and 1000 mL of playground sand to allow the mussels to burrow. Prior to the experiment, the

playground sand was sifted with 2mm mesh and rinsed thoroughly to remove any foreign particulates. We used 9 tanks per species and each tank held 2 mussels for a total of 18 tanks and 36 mussels. Extra mussels that weren't used were kept together in a 5-gallon bucket with the same playground sand. Each of the tanks had its own bubbler aerator to oxygenate the water.

All mussels were randomly divided into three temperature treatment groups. The control group was kept at the lake temperature which was 21°C. The medium temperature group was kept at 22°C and the high temperature group was kept at 23°C. We controlled the temperature of each tank using individual heat mats with thermostats set for each of the temperature treatments. The mussels were then given a week to acclimate to their surroundings.

Feeding regimen

Mussels were fed on a daily basis, typically in the afternoon or the evening. The mussels were all fed a mixture of 0.275 mL of Shellfish Diet 1800 and 0.15 mL of Nanno 3600 algae purchased from Reed Mariculture. The mixture was then added to 250 mL of lake water to form a solution. The mixture was evenly distributed to each of the tanks. All of the tanks received 10 mL of the food solution and any excess was given to the extra mussels in the 5-gallon bucket.

Data collection

After the week-long acclimation period, the water in the mussel containers was tested for dissolved oxygen (DO) and nutrient concentrations produced by excretion and egestion. In order to test this, we measured each tank's temperature (°C), DO (mg L⁻¹), nitrites/nitrates (NO₂⁻ and NO₃⁻ represented as NO_x mg L⁻¹) and phosphate (PO₄³⁻ mg L⁻¹) concentrations. After waiting for 30 minutes, measurements were taken using a YSI Multiparameter probe to measure the temperature and dissolved oxygen (mg O₂/L). In order to measure excretion and egestion, we

measured the concentrations of nitrites/nitrates and phosphates from the water with a Seal AQ 300 autoanalyzer after all the algae had been consumed.

Each step was repeated every 9-10 days over the course of a month for a total of 3 measurement days. When all DO (mg), NO_x (mg), and PO₄³⁻ (mg) measurements had been collected, we measured the volume of the remaining water in each tank to account for evaporation. In addition, each mussel was patted dry and then weighed. The measurements were then multiplied by the water volume and divided by each of the replicate's total weight in grams to get DO (mg g⁻¹), NO_x (mg g⁻¹), and PO₄³⁻ (mg g⁻¹). We also calculated the N:P ratio for each day's nitrogen and phosphate measurements. Once the data collection portion of the experiment concluded, all mussels were released back to Blue Lake in the same area they were collected from.

Data analysis

We ran two-way ANOVA statistical analyses for each of the dependent variables to determine if there was a significant effect of temperature groups and mussel types (native versus exotic species) on each of the variables measured. The temperature treatments and mussel species acted as the independent variables and the temperature (°C), DO (mg g⁻¹), NO_x (mg g⁻¹), PO4³⁻ (mg g⁻¹), and N:P ratio measurements acted as the dependent variables. We also ran linear regressions to determine the relationship between temperature (°C) and DO (mg g⁻¹), NO_x (mg g⁻¹), PO4³⁻ (mg g⁻¹), and N:P ratio, respectively. These values were used to give us insight on how changes in temperature are related to physiological and metabolic processes such as oxygen consumption, excretion, and egestion.

Results

The heating pads for each tank were set for 21°C, 22°C, or 23°C according to the treatment it was in. However, the measurements we took with the YSI probes revealed that the actual temperature of the water in the tanks varied from 20.6°C - 25°C. Despite the variations between each treatment's replicates, the result of the two-way ANOVA for water temperature showed that the average water temperature aligned with the temperatures intended (21°C, 22°C, 23°C) for the treatment groups throughout the study (p = 9.36 x 10⁻⁵, F = 11.32). Furthermore, the average water temperatures did not vary greatly between the *Utterbackia imbecillis* and *Corbicula fluminea* treatments (p = 0.45, F = 0.57; Fig 1).

Although the effect of the temperature treatments was significant on the water temperatures, the other two-way ANOVA statistical analyses showed that the effect of the temperature treatments (control, medium and high) was not significant on DO (mg g⁻¹), NO_x (mg g⁻¹), PO₄³⁻ (mg g⁻¹), or N:P ratio (Table 1). These results were further confirmed through linear regressions of temperature and DO (mg g⁻¹) (R² = 0.01, p = 0.99), NO_x (mg g⁻¹) (R² = 0.01, p = 0.25), PO₄³⁻ (mg g⁻¹) (R² = 0.02, p = 0.57), and N:P ratio (R² = 0.014, p = 0.23). These linear regressions indicate that the water temperature did not contribute to all the variables that were measured.

However, the two-way ANOVA statistical analyses on dissolved oxygen, nitrates, phosphates, and N:P ratio showed that the different mussel species had a significant effect on the DO concentration (mg g⁻¹) (p = 5.14×10^{-10} , F = 60.17). On average, the native freshwater mussel *Utterbackia imbecillis* had much lower DO concentrations, regardless of temperature treatment (Fig 2). Similarly, the effect of the different mussel species was also significant on the PO₄³⁻ (mg g⁻¹) measured (p = 0.02, F = 5.26; Fig 3). The findings showed that mean phosphate concentrations were higher in *C. fluminea* treatments than *U. imbecillis* treatments. All other variables and interactions yielded no statistically significant results (Table 1).

Table 1. The results of the two-way ANOVA statistical analyses with mussel species and temperature groups (control [21°C], medium [22°C], and high [23°C]) acting as the independent variables. Underlined values indicate statistically significant relationships at the $\alpha = 0.05$ level.

Dependent	F value:	P-value:	F value:	P-value:	F value:	P-value:
Variable	Mussel	Mussel	Temperature	Temperature	Interaction	Interaction
	Species	Species	Treatments	Treatments		
Water	0.57	0.45	11.32	9.36 x 10 ⁻⁵	1.67	0.20
Temperature						
(°C)						
DO (mg g ⁻¹)	<u>60.17</u>	<u>5.14 x 10⁻¹⁰</u>	1.29	0.28	0.79	0.46
$NO_x (mg g^{-1})$	0.00077	0.98	0.57	0.57	1.08	0.35
$PO_4^{3-}(mg g^{-1})$	<u>5.26</u>	0.02	0.49	0.61	1.36	0.27
N:P Ratio	0.85	0.36	0.41	0.67	0.61	0.55



Figure 1. The average water temperature (°C) of native (*Utterbackia imbecillis*) and exotic (*Corbicula fluminea*) freshwater mussels and temperature treatments (control, medium, and high) which were set using heating pads. All averages were taken from 3 testing days over the course of a month. Water temperatures ranged from 20.6°C - 25°C. Error bars indicate standard error.



Figure 2. The average dissolved oxygen concentration, given the weight of each replicate (mg g⁻¹) of native (*Utterbackia imbecillis*) and exotic (*Corbicula fluminea*) freshwater mussels and temperature treatments (control, medium, and high). All averages were taken from 3 testing days over the course of a month. DO concentrations ranged from 0.102 - 1.103 mg g⁻¹. Error bars indicate standard error.



Figure 3. The average concentration of phosphates from test tank jar water measured by a Seal AQ 300 Autoanalyzer and then divided by each replicates weight. Blue bars represent the North-American native *Utterbackia imbecillis* treatment averages and orange bars represent the exotic *Corbicula fluminea* treatments averages given 3 different temperature treatments (control, medium, and high). Error bars indicate standard error.



Figure 4. The average results of dissolved oxygen (mg g^{-1}), nitrates (mg g^{-1}), phosphates (mg g^{-1}), and N:P ratio over time from native *Utterbackia imbecillis* and exotic *Corbicula fluminea* in three different temperature treatments: Control (21°C), medium (22°C), and high (23°C).

Discussion

Overall, the initial hypotheses were partially supported. The results of the linear regressions between temperature and dissolved oxygen, nitrates, phosphates, and N:P ratio showed that temperature did not contribute to the outcome of any of the measured variables. These findings were further supported by the results from the two-way ANOVA statistical analyses that showed that the temperature treatments did not have a significant effect on any of dependent variables. However, our hypothesis that the exotic *Corbicula fluminea* would respond better than the native *Utterbackia imbecillis* was partially supported.

When interpreting the average results from the dissolved oxygen concentrations, the treatments containing *U. imbecillis* had much lower DO (mg g-1) than those containing *C. fluminea*. This finding shows that the native freshwater mussels consumed much more oxygen than the exotic mussels did. This result could be due to the *U. imbecillis* having a much thinner shell than the *C. fluminea*. Since *U. imbecillis* is commonly known as the Paper Pondshell due to its uniquely thin shell, it could have possibly felt the impacts of the warmer temperatures more than the exotic species did. Mitton (1977) found that phenotypic variations in the mussel *Mytilus edulis* contributed to greater heat stress or mortality for individuals with certain colors or patterns in their shells. He also theorized that different characteristics, such as shell thickness, could result in different mussels tolerating temperatures in different ways. Based on these findings, I theorize that the *U. imbecillis* experienced greater heat stress at higher temperatures as expected but the average difference was not great enough to be statistically significant.

Similarly, the native and exotic freshwater mussels also differed significantly in the amount of phosphates they egested throughout the study. On average, the *Corbicula fluminea* treatments had greater concentrations of phosphates. Since the feeding regimen was kept

constant between each species and all the food was consumed, one would assume the native and exotic species would have similar phosphate concentrations in their tanks. However, this was not the case for this study which leads me to the conclusion that *U. imbecillis* sequesters more phosphorous than *C. fluminea*.

Freshwater mussels contribute greatly to how nutrients cycle through their surroundings. After filtering different materials, freshwater mussels are able to produce nitrates and phosphates which are then available for other organisms to use (Nalepa et al., 1991). However, not all species produce similar amounts of phosphorous and the amount that one individual produces can vary seasonally (Nalepa et al., 1991; Lauritsen and Mozley, 1989). Phosphorous is important for many different processes necessary for life, including respiration. While a large percentage of phosphorous is egested by mussels, some phosphorous is also stored in the mussel's tissues (Kuenzler, 1961). Based on this information, it's possible that our findings suggest *U. imbecillis* stores more phosphorous in its tissues than *C. fluminea* but further studies should be done to support this theory.

Despite our best efforts to reduce confounding factors, quite a few unforeseeable events impacted this study which may have skewed the data. Namely, in between March 11^{th} and 20^{th} we found that many of the jars were leaking and the water levels were much lower than they had been originally. We immediately moved each replicate that was leaking into plastic containers of similar size, where they were kept for the remainder of the experiment. Switching the mussels into new containers could have led to the data being skewed because there was an uneven ratio of species in glass jars compared to species in plastic containers. In total, 5 of the 9 replicates of *U. imbecillis* were moved to plastic bins and 7 out of 9 replicates of *C. fluminea* were moved. In

addition, moving the mussels could have led to the treatments responding differently due to the added stress of moving into different conditions.

Similarly, another unaccounted variable was discovered on March 13th. After feeding the mussels we noticed that there were a significant number of worms in one of the replicates. Upon further inspection, we identified that the worms were likely *Chaetogaster limnae* and they were in all of the replicates in varying and unquantified densities. *Chaetogaster limnae* are a common worm found in populations of freshwater snails and mussels (Ibrahim, 2007). Although it's impossible to know the degree to which the presence of the *Chaetogaster limnaei* impacted the data, we can infer that it did in some way since there were more organisms in each replicate than we intended.

In the future, I think it would be advantageous to conduct further trials of this experiment but with some modifications. For example, I believe that it would be interesting to see how the oxygen consumption and filtration rates of *U. imbecillis* and *C. fluminea* vary in response to a wider range of temperatures with greater difference between temperature treatments. In addition, I would be interested in making the study period longer with more measurement days to get a better view of how DO, nitrates, phosphates, and N:P ratio vary over time.

Ultimately, I believe that it's very important that we understand how freshwater mussels respond to global warming now before it's too late. As previously stated, 70% of freshwater mussels in North America are either threatened, endangered, or extinct (Burket et al., 2019; Malish and Woolnough, 2019). Mussels are extremely important to our freshwater systems through the services they perform but even now the biodiversity of these systems are rapidly declining at an alarming rate. Furthermore, North American freshwater mussels are being outcompeted by exotic species which even further impacts the functionality of our freshwater systems (Vaughn and Hakenkamp, 2008). Through understanding mussel responses to climate change and how they compare to exotic competitors, such as *Corbicula fluminea*, we can be more equipped to protect the remaining bivalves before it's too late.

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