5-2020

Investigating the Behavioral Response of Lampsilis ovata to Various Salinity Conditions

Victoria Good

Investigating the Behavioral Response of *Lampsilis ovata* to Various Salinity Conditions

Victoria G. Good, Department of Biological Sciences, College of Arts and Sciences, East Tennessee State University, Johnson City, TN

Abstract

The Pocket-book mussel, *Lampsilis ovata*, is a native freshwater bivalve species that is endemic to North America. The salinity tolerance of this species is of interest because anthropogenic salinization events and climate change factors threaten their natural freshwater habitats. Furthermore, the invasive freshwater bivalve species *Corbicula fluminea* has been shown to display significant salinity tolerance, which may lead to negative competitive interactions with native freshwater bivalve species if the salinization of freshwater habitats exceeds thresholds beyond which native species can effectively cope. It was hypothesized that *L. ovata* would be sensitive to salinity conditions above 1 g/L and respond by closing their valves. To investigate this, juvenile pocket-book mussels were subjected to three experiments which measured tissue-water content, hemolymph osmolality, and oxygen consumption after salinity exposure to 0, 2.5, 5, and 10 g/L. The 96-hour exposure study showed that the 2.5 g/L and 5 g/L treatment groups had significantly lower average percent tissue-water content than the control group. The average percent tissue-water content for mussels exposed to 2.5 g/L and 5 g/L dropped 2.4% and 2.2%, respectively. In the 24-hour time-course study, it was observed that changes in the average percent tissue-water content for all treatment groups primarily occurred after four hours of exposure. In the same study, the osmolality of the control group maintained an average of 31.2 mOsm/kg over the 24-hour period, despite the osmolality of the treatment water being 2 mOsm/kg. The hemolymph osmolality concentration of mussels exposed to the 2.5 g/L and 5 g/L treatments increased to osmotically conform to their treatment waters. After 24 hours, the hemolymph osmolality of the 2.5 g/L and 5 g/L treatment groups was 79 mOsm/kg and 163 mOsm/kg, respectively. Contrastingly, the osmolality of mussels exposed to the 10 g/L treatment maintained an average hemolymph osmolality of approximately 132 mOsm/kg, while the osmolality of the treatment water was 320 mOsm/kg. Lastly, the oxygen-consumption study showed that mussels exposed to the 5 g/L treatment consumed a significantly lower amount of dissolved oxygen than that of the control and the 2.5 g/L treatment by an average of 1.6 mg O_2/mg/h. The control group consumed an average of 4.66 mg O_2/mg/h, while the 2.5 g/L treatment group consumed the highest amount of dissolved oxygen with an average of 5.05 mg O_2/mg/h. The data collected from these studies suggest that juvenile *L. ovata* might not be able to tolerate salinities greater than 2.5 g/L for an extended amount of time. Mussels exposed to the 5 g/L treatment and the 10 g/L treatment demonstrated varying degrees of behavioral avoidance and much higher morbidity rates. In contrast, the 2.5 g/L treatment group showed minimal behavioral avoidance and an elevated oxygen consumption rate. When compared to similar studies performed on *C. fluminea,* these results support the hypothesis that *L. ovata* is more sensitive to saline conditions than the invasive species and could be replaced by the invasive species if habitat conditions exceeded 2.5 g/L salinity.

Introduction

Freshwater ecosystems are home to a variety of plants and animals that are adapted to living in low salt concentration conditions—on average, below 1% salinity. Freshwater bivalve mollusks are one such group and are found in freshwater lakes, rivers, and streams. These organisms play vital ecological roles within their communities, such as acting as a purification system by filtering pollution and detritus, releasing nutrients into the system via bioturbation, and serving as a food source for many animals, both aquatic and terrestrial (Vaughn et al., 2008). Freshwater mollusks are considered key indicators of water quality and overall ecosystem health because of these important functions (Williams et al., 1993).

North America has the greatest diversity of freshwater bivalve mollusks in the family Unionidae, with the Appalachian region exhibiting particularly high diversity (Williams et al., 1993). Around 71% of these Unionidae species, however, are reported as extinct or imperiled (Williams et al., 1993). Native freshwater mollusk species have been facing pervasive population decline and extinction for many years (Bogan, 1993). Some of the primary causes of their decline include pollution, sedimentation, habitat loss and degradation, invasive species, and climate change (Bogan, 1993; Williams et al., 1993; Woodward et al., 2010). Because of the vital ecological roles of freshwater mollusks within the water column, it is of concern that negative impacts to their freshwater ecosystems may result from such stress.

As filterers, mollusks are especially vulnerable to water contamination. One anthropogenic pollution factor that threatens to undermine their natural habitat is increased salinization from road de-icing (Kaushal et al., 2005). Road de-icing is prevalent in regions where winters with snowfall and freezing temperatures are common. Roadway runoff containing de-icing products can rapidly disperse and pollute both groundwater and freshwater systems

(Kaushal et al., 2005). The most widespread de-icing product in the United States is sodium chloride, which is estimated to be used at an annual rate of 21 million tons and rising (Kelly et al., 2019). If the current trend in road de-icing continues, sodium chloride may accumulate to harmful levels within freshwater systems and negatively affect their biodiversity.

In combination with existing anthropogenic stressors like road de-icing, climate change further threatens the function of natural freshwater ecosystems (Woodward et al., 2010). Climate change is characterized by increased temperature, elevated atmospheric CO_2, and more extreme and frequent hydrological events (Woodward et al., 2010). The effects of climate change may exacerbate the impacts of increased salinization via highway runoff by increasing pollution concentrations within freshwater systems. The coalescence of climate change and anthropogenic pollution may lead to significant increases in the salinization of freshwater systems, which has the potential to be catastrophic for organisms that have not evolved adaptations for dealing with salinity concentrations greater than current freshwater standards.

Aquatic organisms reconcile with changing osmotic pressure through the process of osmoregulation. This mechanism provides aquatic organisms with the means necessary to balance water and maintain proper cell volumes. Freshwater organisms must overcome the issue of being hyperosmotic to their environment, meaning that they contain a higher internal solute concentration than their surroundings. Water and solutes tend to move down their concentration gradients. So, without proper mechanisms to control water flow, water will move into hyperosmotic cells and cause them to swell and burst. Freshwater mollusks must therefore prevent excess water from flowing into their tissues and necessary ions from moving out. Relative to saline populations, freshwater mussels have lowered hemolymph osmolality, which functions to lessen the osmotic gradient between their internal and external environment. This

response most likely evolved to reduce the energetic cost of being hyperosmotic (Lee et al., 2012). As salinity conditions increase, however, the internal osmotic pressure of freshwater mollusks eventually match that of their external environment and when this occurs, there is no further osmotic movement. With additional increases in salinity concentration, however, mollusks become hypoosmotic to their environment, meaning that their internal osmotic pressure is lower than that of the ambient medium. Water must then be prevented from leaving their cells and excess ions from moving in. Typically, freshwater mollusks will attempt to mitigate this osmotic concentration gradient by increasing the concentration of their cytoplasmic osmolytes (Deaton, 2009).

Freshwater bivalves are primarily sessile, which limits their ability to escape stressful environmental conditions—like increased salinity—so possessing mechanisms for tolerating such events are critically important. Research regarding the effects of increased salinity on freshwater mussels (Bivalvia: Unionidae) has been performed on less than 5% of the 300 species native to the United States and less than 1% of the species known world-wide (Johnson et al., 2018). It has been found that exposure to salinized conditions, both acute and chronic, influences all of their life stages, impacting their growth, reproductive success, and overall survival (Johnson et al., 2018). Freshwater mussels have a unique and complex life cycle. Eggs develop first within the mother and are carried inside her shell (Bogan, 1993). Soon, the eggs develop into glochidia, or parasitic larvae, that leave the mother to find a host fish. Once a host is found, glochidia attach to the gills or fins to complete their metamorphosis into independent juveniles (Bogan, 1993). Most Unionids are dependent upon a narrow range of fish species for glochidia attachment, so this specialized reproduction method increases their vulnerability to

extinction and imperilment when changes occur within their ecosystem, such as impoundments, siltation, and pollution.

Salinity tolerance has been shown to vary by species, owing largely to differences in life history (Johnson et al., 2018). In a study evaluating the salinity tolerance of three native freshwater mussel species (*Utterbackia imbecillis*, *Elliptio jayensis*, and *Glebula rotundata*) with different distributions and shell morphologies, it was found that salinity tolerance was highly variable among the species (Johnson et al., 2018). The test salinities used were 0, 6, 12, 18, and 24 parts per thousand [ppt] (Johnson et al., 2018). *U. imbecillis*, which typically inhabits strictly freshwater systems throughout eastern North America, had the lowest salinity tolerance of the three species, surviving for only a few days in salinities equal to 6 ppt and greater (Johnson et al., 2018). *E. Jayensis*, which is found in freshwater systems regionalized to peninsular Florida, survived salinities comparable to that of *U. imbecillis*, except for about 4 days longer (Johnson et al., 2018). *G. rotundata*, on the other hand, which inhabits freshwater systems closer to the coast in Gulf of Mexico drainages, had the greatest tolerance, surviving in the 6 and 12 ppt salinities as well as it did in the control (0 ppt) (Johnson et al., 2018). *G. rotundata* also tolerated 18 and 24 ppt for approximately 7-8 days before succumbing to osmotic stress, which is remarkable for a Unionid (Johnson et al., 2018). While the shell morphologies of *E. Jayensis* and *G. rotundata* enable them to fully close their shells to escape environmental stressors, *U. imbecillis* is unable to completely close its valve, therefore severely limiting its ability to protect its tissues (Johnson et al., 2018).

The Asian clam, *Corbicula fluminea*, has shown significant salinity tolerance to conditions ranging from an optimum salinity concentration of 1.5 ppt to a maximum salinity concentration of 15 ppt (Bertrand et al., 2017). In a study conducted by Bertrand et al. (2017), *C.*

fluminea were capable of surviving exposure to 15 ppt salinity conditions for the full duration of a 7-day salinity exposure experiment. The results of the study showed that the clams primarily responded to the high salinity concentration by valve closure and anaerobic respiration (Bertrand et al., 2017). *C. fluminea*'s capacity to survive at higher salinity conditions may be due to its recent evolutionary history dealing with estuarine environments. *C. fluminea* is a freshwater bivalve species that is native to freshwater environments in East Asia and Africa (Foster et al., 2019). After being introduced to the United States in the early twentieth century, *C. fluminea* is now present in 46 states (Foster et al., 2019). The ability of invasive freshwater bivalve species like *C. fluminea* to quickly proliferate and compete for resources in freshwater habitats in the United States has severely altered native populations. The competitive edge that the euryhaline *C. fluminea* holds over native bivalve species in terms of salinity tolerance could lead to severe consequences for native populations.

The freshwater bivalve species observed in this study, *Lampsilis ovata*, is in the family Unionidae and is native to the Appalachian region. As juveniles, this species has a thin, fragile shell that becomes solid and heavy in adulthood (Parmalee & Bogan, 1998). These mussels can be found in depths of water ranging from 15-20 feet in reservoirs to less than two feet in streams (Parmalee & Bogan, 1998). Although *L. ovata* prefers moderate to strong currents, the species can survive for a short period of time in lentic conditions. In vivo, the *L. ovata* population is stable, although continued decline of mature individuals has been observed (Woolnough & Seddon, 2017). *L. ovata* was chosen for this study because of its salinity tolerance not being well understood and the availability of individuals for experimentation. The goal of this study was to determine the physiological response of *L. ovata* to various salinity conditions by measuring percent tissue-water content, hemolymph osmolality, and oxygen consumption. The hypothesis

was that *L. ovata* will be sensitive to salinity conditions greater than 1 g/L and will demonstrate valve closure response. If this native freshwater bivalve species is unable to cope with increased salinization of its freshwater habitat, then further population declines or even extinctions may be observed when critical thresholds are reached. Likewise, if the sensitivity of *L. ovata* to saline conditions is much greater than that of *Corbicula fluminea*, the salinization of freshwater habitats could lead to native populations of freshwater bivalve species becoming replaced by invasive freshwater bivalve species. This study may also call for greater regulations or conservation procedures regarding freshwater systems.

Materials and Methods

Collection and Holding

Juvenile *Lampsilis ovata* mussels were obtained from the Tennessee Wildlife Resource Agency Cumberland River Aquatic Center (C-RAC) in Gallatin, Tennessee, and promptly transported back to the laboratory at East Tennessee State University (ETSU) in Johnson City, Tennessee. Before experimentation began, the mussels were held for an acclimation period of three days and were kept in a 26.5-liter aquarium filled with moderately hard freshwater, a biological filter, and limestone substrate at room temperature—approximately 20°C. *Lampsilis sp.* are known to adapt well to laboratory conditions when provided with an adequate food supply in quality and quantity (Farris and Hassel 2007), so the mussels were fed a 1:1 mixture of Shellfish Diet 1800 and Nanno 3600 (Reed Mariculture, Incorporated, Campbell, CA) once every 24-hours.

Preparation of Test Solutions

All test salinities (0 g/L, 2.5 g/L, 5 g/L, and 10 g/L) were prepared in separate 18.9-liter carboys using moderately hard water. The amount of dehydrated Instant Ocean (Spectrum Brands, Blacksburg, VA) necessary to achieve each salinity was calculated by multiplying the number of liters of water within the carboy by the test salinity itself. A P-603D Precision Balance (Denver Instruments, Bohemia, NY), which measured to the nearest 0.01 g, was used to obtain the proper mass of Instant Ocean. The appropriate amount of Instant Ocean was then added to the corresponding labeled carboy and hand-shaken to ensure mixing of the Instant Ocean with the water. Lastly, the conditions of the mixtures were checked using a YSI Pro DSS handheld water quality meter (YSI Incorporated, Yellow Springs, OH) and adjustments were made as necessary to reach the desired test salinity.

96-Hour Salinity Exposure Study

To achieve a base understanding of how *Lampsilis ovata* responds to different salinity conditions, tissue water samples were taken from individuals after 96 hours of exposure to 0 g/L, 2.5 g/L, and 5 g/L salinities and the percent change in their wet mass (g) to dry mass (g) was calculated. Each test salinity (0 g/L, 2.5 g/L, and 5 g/L) was labeled on four 2-liter glass aquariums, and four random *L. ovata* individuals were placed into each aquarium. Over the course of the exposure period, temperature, dissolved oxygen content, salinity, and pH were measured and recorded using the YSI Pro DSS handheld water quality meter (YSI Incorporated, Yellow Springs, OH). Measurements were taken in the first replicate of each test salinity every 24 hours for 96 hours, beginning at time zero. Other observations, such as mortalities, were also recorded. At the end of 96 hours, all mussels were pulled from their test salinities and a scalpel was used to scrape their soft-tissue from their shells. After the wet soft-tissue was dabbed with a paper towel, each sample was placed on an individually labeled, pre-weighed slip of aluminum

foil, and its wet tissue mass (g) was recorded. A P-603D Precision Balance (Denver Instrument Company, Bohemia, NY) was used to record all mass measurements, which reported in grams to the nearest hundredth. All test subjects were then placed in a Thermo Electron Corporation Precision drying oven (Thermo Fisher Scientific, Waltham, MA) set at 60°C and dehydrated for at least 48 hours. After dehydration, the dry tissue mass (g) of the samples was recorded, and the percent change was calculated to represent the amount of water contained in the tissue at the time of removal from salinity treatment.

Oxygen-consumption Study

To investigate the effect of chosen salinities on the oxygen-consumption (mg O_2/mg/h) of *Lampsilis ovata* over a 24-hour period, four replicate BOD bottles were set-up for the salinities of 0 g/L, 2.5 g/L, 5 g/L, and 10 g/L. Two blanks, BOD bottles not containing any samples, were included for each test salinity as controls. A YSI 5010 BOD Probe (YSI Incorporated, Yellow Springs, OH) was used to measure and record the initial dissolved oxygen content and temperature of each replicate per salinity. Four individuals were added to each BOD bottle, which were then promptly secured with a glass stopper, covered with a black sheet, and left at room temperature. After 24 hours, the dissolved oxygen content and temperature of each BOD bottle was re-measured and recorded. Tissue-water content samples were collected from every individual in the same manner as previously described in the 96-hour exposure study. Lastly, the mg of oxygen consumed per mg of tissue per hour (mg O_2/mg/h) of the mussels in each replicate was determined by dividing the adjusted change in dissolved oxygen—which was found by subtracting the change in dissolved oxygen (mg/L) of each replicate by the change in dissolved oxygen (mg/L) of its corresponding blank—by the total dry tissue mass (mg) it contained. The

amount of oxygen consumed per mg per hour (mg O_2/mg/h) was then found by dividing the previously found value by the number of hours the experiment ran.

Time-Course Exposure Study

To investigate the response of *Lampsilis ovata* to the salinities of 0 g/L, 2.5 g/L, 5 g/L, and 10 g/L more closely, tissue-water content and hemolymph osmolality samples were taken every 4 hours over the course of 24 hours. Each test salinity included five 2-liter glass aquaria replicates with 15 randomly chosen *L. ovata* individuals in each. Initial water conditions of temperature, dissolved oxygen content, salinity, and pH were measured and recorded, again using the YSI Pro DSS water quality meter (YSI Incorporated, Yellow Springs, OH). Three individuals—one for tissue-water content and two for hemolymph osmolality—were pulled from each replicate at times 0, 4, 8, 12, and 24 hours. Tissue water samples were collected and calculated as previously described in the sections above. To collect hemolymph, the mantle cavity was gently wedged open and dabbed onto a paper towel to remove excess water. The adductor muscles were then slit and a micro-hematocrit capillary tube was used to extract hemolymph. After being transferred to a centrifuge tube, the samples were placed in a Spectrafuge 24D Digital Lab Microcentrifuge (Labnet International Incorporated, Edison, NJ) and spun for 10 minutes at 9200 x g to separate debris from the hemolymph sample. The supernatant was then extracted, placed into a fresh centrifuge tube, and spun again for 10 minutes at 9200 x g. A Fiske 210 Micro-Osmometer (Fiske Associates, Norwood, MA) was used to analyze the samples and obtain hemolymph osmolality (mOsm/kg).

Statistical Analysis

For tissue-water content data, proportions were transformed prior to statistical analysis by arcsine square root transformation. All data was statistically analyzed for parametric assumptions using SigmaPlot 11.0. A one-way analysis of variance (ANOVA) was used for the 96-hour salinity exposure and oxygen-consumption experiments. A two-way ANOVA was used for the time-course exposure experiment, with time and salinity as the main factors. The Holm-Sidak method was performed for all pairwise multiple comparison procedures with an overall significance level of $\alpha = 0.05$. All data analyzed passed both normality and equal variance tests.

Results

The 96-hour exposure study showed that the average percent tissue-water content for mussels exposed to 2.5 g/L and 5 g/L was significantly lower ($p<0.002$) than that of the control treatment (0 g/L). The average percent tissue-water content for the control was 85.2%, while the 2.5 g/L and 5 g/L treatments yielded average percent tissue-water contents of 82.8% and 83%, respectively. It was noted, however, that at 72-hours, one mortality occurred in the 2.5 g/L treatment salinity and nine mortalities occurred in the 5 g/L treatment salinity. Thus, 16 samples were analyzed for the control treatment, 15 samples were analyzed for the 2.5 g/L treatment, and 8 samples were analyzed for the 5 g/L solution.

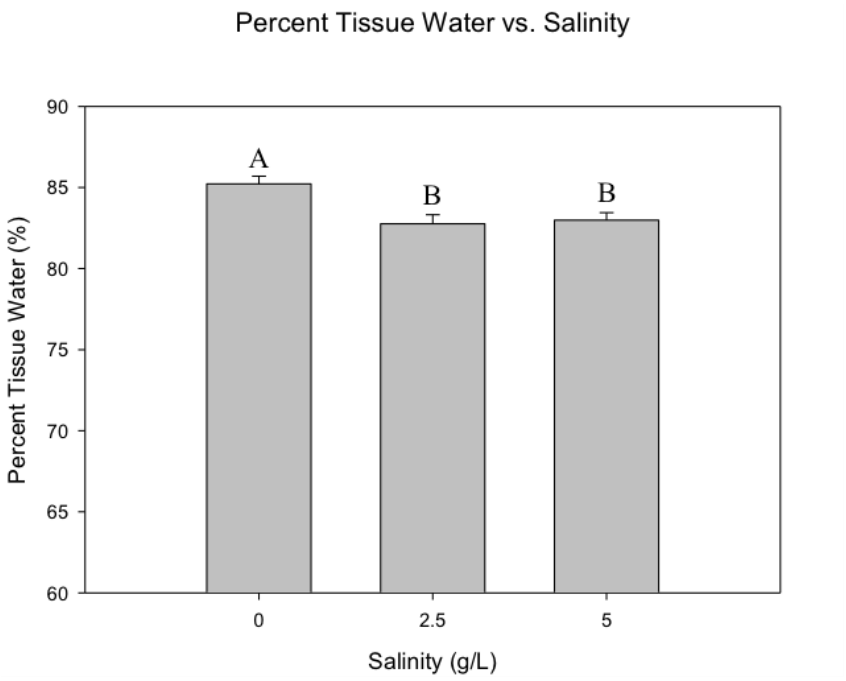

Figure 1: Mean percent tissue-water content for mussels after 96-hours of exposure to three different salinity levels. Error bars are included to show one standard error. Values not sharing a common letter are significantly different ($\alpha = 0.05$).

In the oxygen-consumption exposure study, it was observed that the average oxygen-consumption per mg of tissue for mussels exposed to the 5 g/L salinity treatment was significantly lower than that of the control treatment ($p<0.002$) and 2.5 g/L treatment ($p<0.001$). The average amount of oxygen consumed per hour for the control was 4.66 mg O_2/mg/h. The average oxygen consumption was 5.05 mg O_2/mg/h and 3.26 mg O_2/mg/h for the 2.5 g/L and 5 g/L treatments, respectively. It was also noted that two mortalities occurred in the 5 g/L treatment salinity over the course of the 24-hour experiment. Accordingly, 16 samples were analyzed for the control treatment, 16 samples were analyzed for the 2.5 g/L treatment, and 14 samples were analyzed for the 5 g/L treatment.

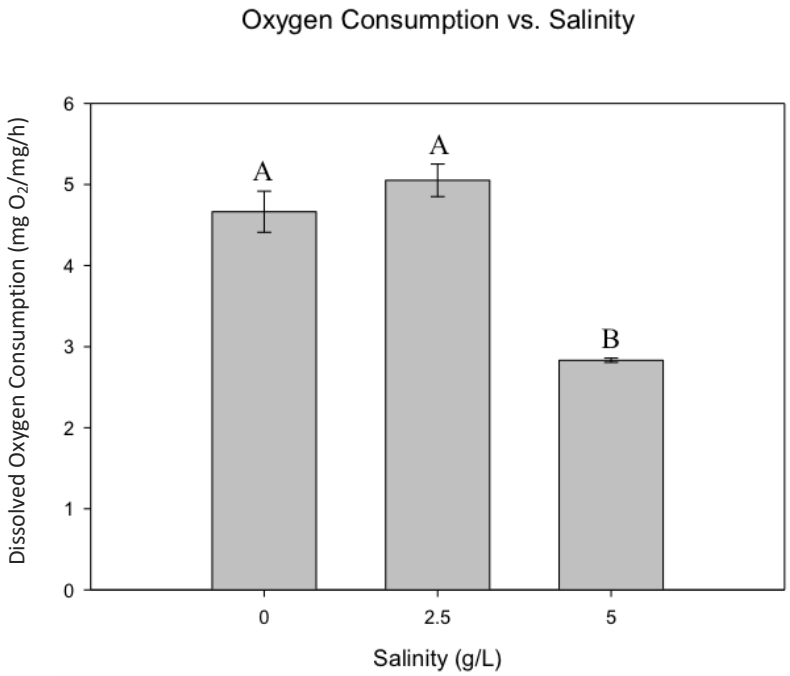

Figure 2: Mean dissolved oxygen consumption for mussels exposed to three different salinity levels over a 24-hour period. Error bars are included to represent one standard error. Values not sharing a common superscript are significantly different ($\alpha = 0.05$).

The time-course exposure study showed that significant change in the average percent tissue-water content of *Lampsilis ovata* for all salinity treatments primarily occurred within the first four hours of exposure and then became relatively more stable after 8 hours of exposure. The average percent tissue-water content for the control group decreased from 84.6% to 81.7% after four hours of exposure, increased slightly after eight hours of exposure, and then leveled off, ending the 24-hour experiment with an average percent tissue-water content of 81.8%. The 2.5 g/L treatment group showed a decrease in average percent tissue-water content from 84.7% to 80.7% after 4 hours of exposure and then mainly leveled off after eight hours of exposure. After 24 hours, the average percent tissue-water content for the 2.5 g/L treatment group was found to be 81.5%. Mussels exposed to the 5 g/L salinity treatment showed a steady decline in average percent tissue-water content from time zero to eight hours of exposure, decreasing from

83.8% to 77.8%. After 24 hours of exposure, the average percent tissue-water for the 5 g/L treatment was 78.5%. Lastly, the 10 g/L salinity treatment group showed a steady decline in average percent tissue-water content from time zero to 12 hours of exposure, decreasing from 81.4% to 73.9%. After 24 hours of exposure, the average percent tissue-water content for 5 g/L was found to be 75.4%.

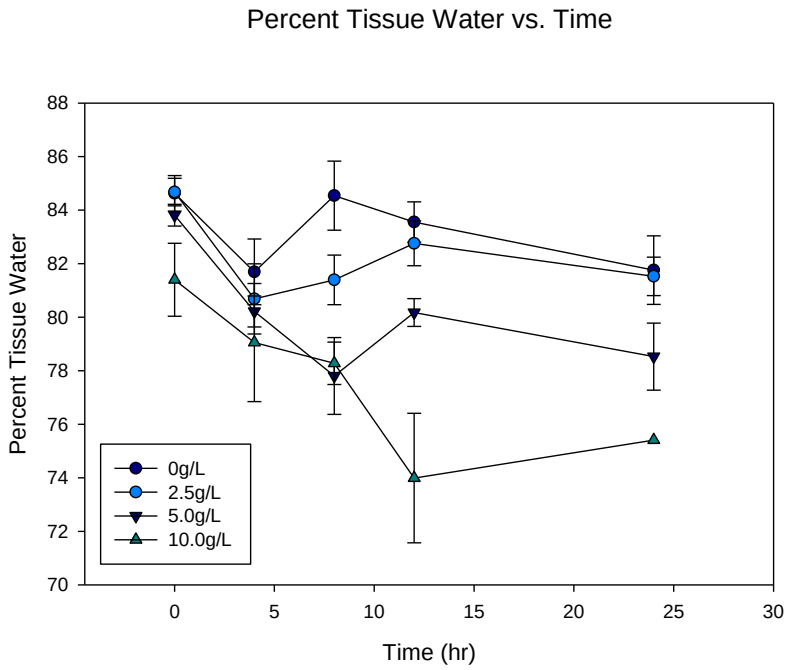

Figure 3: Average percent tissue-water content for four different levels of salinity over a 24-hour period. Error bars are included to represent one standard error.

The time-course experiment also showed that the average hemolymph osmolality for *Lampsilis ovata* in the control group remained steady around 31.2 mOsm/kg over the course of the 24-hour experiment, which was significantly higher than the average osmolality of the treatment water at 2 mOsm/kg. However, the average hemolymph osmolality of mussels exposed to 2.5 g/L and 5 g/L proceeded to increase within the first four hours of exposure and then mostly held steady at levels close to that of their respective treatment water after eight hours of exposure. The average hemolymph osmolality of mussels exposed to 2.5 g/L increased from 37.6

mOsm/kg to 69.5 mOsm/kg within the first four hours, while that of 5 g/L increased from 26.7 mOsm/kg to 117 mOsm/kg. After 24 hours, the average hemolymph osmolality of mussels in the 2.5 g/L treatment was 79 mOsm/kg, while the average osmolality of the treatment water was 74 mOsm/kg. Likewise, the average hemolymph osmolality of mussels exposed to 5 g/L after 24 hours was 163 mOsm/kg, while the average osmolality of the treatment water was 148 mOsm/kg. The average hemolymph osmolality of mussels exposed to 10 g/L increased from 30 mOsm/kg to 80 mOsm/kg within the first four hours. After 24 hours, the average hemolymph osmolality was found to be 132 mOsm/kg, while the average osmolality of the treatment water was 320 mOsm/kg. It is noted, however, that only one hemolymph sample could be recorded for the 10 g/L treatment group after 24 hours.

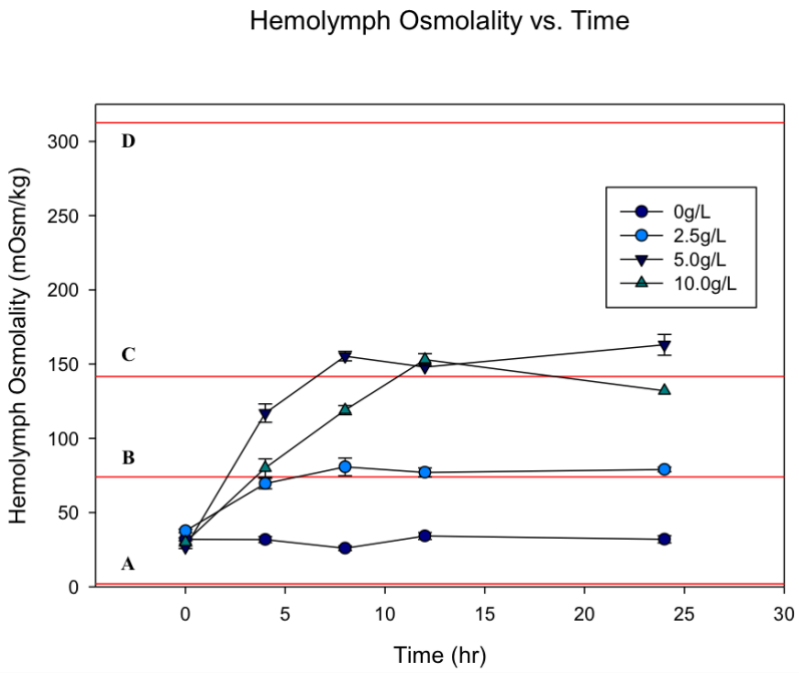

Figure 4: Mean hemolymph osmolality for mussels exposed to four different levels of salinity over a 24-hour period. Red lines labeled A, B, C, and D correspond to the osmolality of the treatment water for 0 g/L, 2.5 g/L, 5 g/L, and 10 g/L, respectively. Error bars are included to represent one standard error.

Discussion

The goal of this study was to better understand the salinity tolerance of *Lampsilis ovata* by evaluating its physiological response to various salinity conditions since research has suggested that freshwater mussel species with a relatively longer freshwater evolutionary history, like *L. ovata*, have lost mechanisms to effectively tolerate salinity stress (McMahon & Bogan, 2001). As a result of evolving a lower hemolymph osmolality concentration, these freshwater mussels have a limited free amino acid pool, thereby reducing their ability to effectively regulate cell volumes in salinized conditions (McMahon & Bogan, 2001). The original hypothesis of this study was that *L. ovata* would be sensitive to salinity conditions greater than 1 g/L and respond by demonstrating valve closure. The results of the experiments measuring *L. ovata*'s percent tissue-water content, oxygen consumption, and hemolymph osmolality in response to variable salinity concentrations suggest that *L. ovata* tolerates salinity stress up to 2.5 g/L with minimal behavioral avoidance. In contrast, mussels exposed to 5 g/L and 10 g/L salinity treatments demonstrate more significant avoidance behavior and far greater morbidity rates over the course of the three studies. Consequently, a salinity concentration of 2.5 g/L may be close to the maximum salinity concentration juvenile *L. ovata* can successfully tolerate for a prolonged amount of time.

In the time-course study, the average percent tissue-water content tended to decrease as salinity increased, indicating that the mechanisms *L. ovata* employed to prevent water loss from its cells were not completely effective. Measurements taken after four hours show an average 3.2% decrease in percent tissue-water content for all treatment groups. Notably, tissue-water recovery time is delayed as salinity increases. Figure 3 shows that mussels in the control group and in the 2.5 g/L treatment group began to recover tissue water after four hours of exposure,

with the 2.5 g/L samples showing a slightly longer delay. Likewise, mussels in the 5 g/L treatment group began to recover after 12 hours of exposure while mussels in the 10 g/L treatment group were largely ineffective at retaining water, especially after 12 hours of exposure. The delays observed here may be due to the time it takes for *L. ovata* to alter its intracellular concentration and restore the osmotic equilibrium. Likewise, 10 g/L salinity may be above the threshold that *L. ovata* can effectively adapt to. Existing experimental evidence regarding the salinity tolerance of adult unionid mussels suggests that the upper limit for long-term exposure is less than or equal to 6 ppt (Johnson et al., 2018).

In the 96-hour exposure study, statistical analysis showed no significant difference between the average percent tissue-water content between the 5 g/L treatment group and the 2.5 g/L treatment group. Therefore, since the 5 g/L treatment group was under increased osmotic stress, it is likely that this group employed some form of physiological response to prevent water loss. The most likely response that produced this outcome is behavioral avoidance by valve closure. The oxygen consumption study showed that the 5 g/L treatment group consumed the lowest amount of oxygen of the three groups over the 24-hour period. In contrast, the 2.5 g/L treatment group consumed the highest amount of oxygen. This data implies that mussels exposed to the 2.5 g/L salinity treatment demonstrated limited valve closure and perhaps experienced an elevated metabolic rate, while the 5 g/L treatment group demonstrated valve closure by respiring anaerobically. The higher oxygen-consumption rate of the 2.5 g/L treatment group, in correlation with its adjustment to water loss after four hours, may suggest that the mussels began utilizing active physiological mechanisms for ion and osmotic regulation.

Figure 4 shows that mussels in the control group maintained an average hemolymph concentration of 31.2 mOsm/kg over the course of the 24-hour experiment. Meanwhile, the 2.5

g/L and 5 g/L treatment groups osmotically conformed to their treatment water. The 10 g/L treatment group, on the other hand, delayed changes in its percent tissue-water content and hemolymph concentration, suggesting that the 10 g/L treatment group exhibited avoidance behavior. It took approximately four hours longer for mussels exposed to the 5 g/L treatment to osmotically conform than the samples exposed to the 2.5 g/L treatment. These results may be due to mussels in the 5 g/L treatment drastically altering their intracellular composition to reach ionic and osmotic equilibrium. Mussels exposed to the 10 g/L treatment reached an average hemolymph osmolality of approximately 132 mOsm/kg despite being placed in water with an average osmolality of 312 mOsm/kg. The average hemolymph osmolality of these mussels was similar to the average osmolality of the 5 g/L treatment water (148 mOsm/kg), despite the osmolality of the 10 g/L treatment water being twice as high. This could indicate that a critical threshold exists for juvenile *L. ovata* around 5 g/L.

Overall, a significantly greater amount of mortality occurred in the 5 g/L and 10 g/L treatment groups than occurred in the control or 2.5 g/L treatment groups over the duration of the three experiments, indicating that *L. ovata* is more sensitive to salinity stress at concentrations greater than 2.5 g/L.

To compare the salinity tolerance of *Lampsilis ovata* to that of the invasive freshwater bivalve species *Corbicula fluminea*, a study by Roden (2017) showed that *C. fluminea* tolerated salinity up to 5 g/L without significant behavioral avoidance. *C. fluminea* in the control group of Roden's study maintained an average hemolymph osmolality concentration of 50 mOsm/kg over the course of the 24-hour experiment despite the treatment water having an osmolality of 0.5 mOsm/kg, which is significantly higher than the average hemolymph osmolality maintained by *L. ovata* in the control group of this study. In both the 2.5 g/L and 5 g/L treatment groups, *C.*

fluminea and *L. ovata* were similar in that they osmotically conformed to their treatment conditions (Roden, 2017). Contrastingly, *C. fluminea* in the 10 g/L salinity treatment maintained an average hemolymph concentration value close to that of the starting value until 8 hours of exposure (Roden, 2017). Even after 24 hours, the average hemolymph osmolality concentration only rose to 87.5 mOsm/kg (Roden, 2017), which is far lower than the average hemolymph osmolality concentration of *L. ovata* in the 10 g/L salinity treatment, which rose to 132 mOsm/kg. These results suggest that *C. fluminea* is much more effective at behavioral avoidance by valve closure than *L. ovata*. As for oxygen-consumption, *C. fluminea* showed an elevation in oxygen consumption in the 5 g/L salinity treatment (Roden, 2017), while *L. ovata* showed a marked decline in oxygen consumption, indicating that *C. fluminea* might be better able to tolerate 5 g/L salinity concentrations than *L. ovata*. Therefore, these results also suggest that *C. fluminea* has a greater capacity to compensate for increased salinity than does *L. ovata*.

As for future studies, investigating the salinity tolerance of adult *Lampsilis ovata* may be worthwhile since salinity is known to affect all life stages. As previously mentioned, juvenile *L. ovata* have thin, fragile shells while adult *L. ovata* have solid and heavy shells (Parmalee & Bogan, 1998). There are several differences between juveniles and adults such as shell morphology, size, and progression of development that make further investigation of interest. Adults may also provide larger and more easily obtained samples sizes. Collecting samples from juvenile *L. ovata* proved to be a delicate task as they ranged in size from 10.9 mm to 16.7 mm. Perhaps adult *L. ovata* have a lower ionic and osmotic permeability, greater enzyme concentrations, and more developed physiological mechanisms that allow for a greater salinity tolerance than that of juvenile *L. ovata*.

References

Bogan, A. (1993). Freshwater Bivalve Extinctions (Mollusca: Unionoida): A Search for Causes. *American Zoologist, 33*(6), 599-609. https://www.jstor.org/stable/3883723

Bertrand, C., Devin, S., Mouneyrac, C., Giambérini, L., (2017). Eco-physiological responses to salinity changes across the freshwater-marine continuum on two euryhaline bivalves: *Corbicula fluminea* and *Scrobicularia plana*, *Ecological Indicators*, 74, 334-342. https://doi.org/10.1016/j.ecolind.2016.11.029.

Deaton, L. (2009). Osmotic and Ionic Regulation in Molluscs. In: *Osmotic and Ionic Regulation: Cells and Animals*. Boca Raton, FL: CRC Press, pp. 111.

Bishop, C. D., Hudson, R., & Farris, J. L. (2007). *Freshwater bivalve ecotoxicology*. Boca Raton: CRC Press. pp. 79

Foster, A.M., Fuller, P., Benson, A., Constant, S., Raikow, D., Larson, J., and Fusaro, A., 2020, *Corbicula fluminea* (O. F. Müller, 1774): U.S. Geological Survey, Nonindigenous Aquatic Species Database, Gainesville, FL, and NOAA Great Lakes Aquatic Nonindigenous Species Information System, Ann Arbor, MI, Available at: https://nas.er.usgs.gov/queries/GreatLakes/FactSheet.aspx?SpeciesID=92, Revision Date: 9/13/2019, [Accessed 21 Apr. 2020]

Johnson, N., Schofield, P., Williams, J., & Austin, J. (2018). Salinity tolerance among three freshwater mussels (Bivalvia: Unionidae) from Gulf Coastal Plain drainages. *Florida Scientist,81*(2-3), 61-69. doi:10.2307/26584956

Kaushal, S., Groffman, P., Likens, G., Belt, K., Stack, W., Kelly, V., . . . Fisher, G. (2005). Increased Salinization of Fresh Water in the Northeastern United States. *Proceedings of the National Academy of Sciences of the United States of America, 102*(38), 13517-13520. https://www.jstor.org/stable/3376665

Kelly, V. R., Findlay, S. E. G., Weathers, K. C. (2019). Road Salt: The Problem, The Solution, and How To Get There. *Cary Institute of Ecosystem Studies*. Retrieved April 14, 2020, from https://www.caryinstitute.org/sites/default/files/downloads/report_road_salt.pdf

Lee, Carol & Posavi, Marijan & Guy, Charmantier. (2012). Rapid evolution of body fluid regulation following independent invasions into freshwater habitats. *Journal of evolutionary biology. 25.* 625-33. 10.1111/j.1420-9101.2012.02459.x.

Mcmahon, R.F, & Bogan, A.E. (2001). "11-MOLLUSCA:BIVALVIA." In *Ecology and Classification of North American Freshwater Invertebrates*. (2nd edition). Retrieved from http://ebookcentral.proquest.com/lib/pace/detail.action?docID=300657.

Parmalee, P. W., & Bogan, A. E. (1998) *The Freshwater Mussels of Tennessee.* Knoxville: The University of Tennessee Press. pp. 131-133

Roden, J. W. (2017). *Determining the Physiological and Behavioral Aspects of Salinity Tolerance in the Asian Clam, Corbicula fluminea* (Undergraduate thesis). East Tennessee State University, Johnson City, TN.

Vaughn, C., Nichols, S., & Spooner, D. (2008). Community and foodweb ecology of freshwater mussels. *Journal of the North American Benthological Society, 27*(2), 409-423. doi:10.1899/07-058.1

Williams, J.D., Warren, M.L., Jr., Cummings, K.S., Harris, J.L. and Neves, R.J. (1993), Conservation Status of Freshwater Mussels of the United States and Canada. Fisheries, 18: 6-22. doi:10.1577/1548-8446(1993)018<0006:CSOFMO>2.0.CO;2

Woodward, G., Perkins, D., & Brown, L. (2010). Climate change and freshwater ecosystems: Impacts across multiple levels of organization. *Philosophical Transactions: Biological Sciences,365*(1549), 2093-2106. https://www.jstor.org/stable/25699227

Woolnough, D. & Seddon, M.B. 2017. *Lampsilis ovata*. *The IUCN Red List of Threatened Species* 2017: e.T11258A69490442. Retrieved from https://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T11258A69490442.en.