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## Abstract

The Asian clam, *Corbicula fluminea*, is an invasive bivalve species that now occurs through most of the lower 48 United States. While a significant degree of salinity tolerance has been observed in *C. fluminea*, owing to its estuarine lineage, the physiological and behavioral responses to changes in salinity by these organisms are not completely understood. It was hypothesized that *Corbicula* would initially avoid elevated salinity levels (>1 g/L) behaviorally through valve closure, but would eventually have to open to dispel anaerobic waste products and deal with the salinity. To explore this, *Corbicula* were collected and put through a series of experiments at salinity exposures of 0, 2.5, and 5.0 g/L, with tissue water content and hemolymph osmolality being measured. After an initial 96-hour exposure, it was observed that the percent tissue water content of clams in 2.5 g/L and 5.0 g/L water dropped 3.29% and 4.18%, respectively, below that of the control groups in 0 g/L. After a 24-hour time-course experiment, this change in tissue water was found to largely occur within the first eight hours of exposure for the 2.5 g/L and 5.0 g/L groups. It was also noted that the hemolymph osmolality of both the 2.5 g/L and 5.0 g/L groups rose to approximately 78 mOsm/kg and 148 mOsm/kg, respectively, matching the osmolality of their exposure water in roughly the same time span and indicating that little behavioral avoidance of the elevated salinity was occurring. The osmolality of the control group did not match the osmolality of the 0 g/L water at 0.5 mOsm/kg, but was held at a constant level around 50 mOsm/kg. In a later experiment measuring the same variables for clams in 10.0 g/L, it was found that the tissue water and osmolality did not begin to change significantly until after 12 hours, indicating behavioral avoidance at this salinity level. A context study was also conducted comparing oxygen consumption and percent tissue water between various salinities in a light and dark exposure to determine if ambient light influenced siphoning of the clams and exposure to the salt. In this experiment, it was observed that clams held in salinities of 5.0 g/L for 24 hours consumed roughly 1.90 mg $O_2$/L/g/h, whereas clams held in the control only consumed roughly 0.73 mg $O_2$/L/g/h. These findings suggest that *Corbicula* osmoregulate in freshwater but osmoconform at salinities of 2.5 g/L and 5.0 g/L. The data from the context study also suggests that this conformation comes at a significant metabolic cost. Furthermore, and in contrast to the results of some previous studies, a significant level of behavioral avoidance of elevated salinity does not appear to commence until the clams are at a salinity above 5 g/L.

**Introduction**

Freshwater bivalve mollusks play an important role in aquatic ecosystems around the globe. Mollusks not only act as a dietary staple for many fish and mammal species, but also serve to consume and recycle detritus and other particles from the waterway (Ward, 1996). In this manner, bivalve mollusks serve to filter the water, removing pollutants and improving water clarity (Sousa et al., 2009). Due to their filtering capacities, such mollusks are considered to be very reliable ecological bioindicators as to the wellbeing of their ecosystems (Oehlmann and Schulte-Oehlmann, 2003). While bivalve mollusks are an essential part of many aquatic ecosystems, invasion of these systems by non-native mollusk species can lead to a host of problems. Such problems include excessive competition with native species for food, invasion of water treatment and power plants, and disruption of natural ecosystem function (Sousa et al., 2008).

Invasive mollusk species are especially problematic due to their capacity for quick proliferation and competition with native species. In North America, invasive mollusk species have spread at an alarming rate over the past century. One such species is the Asian clam, *Corbicula fluminea*. The Asian clam is member of the family Corbiculidae. Adult Asian clams are hermaphrodites, capable of self-fertilization and mass reproduction, with an estimated production of over 68,000 offspring per year (Caffrey et al., 2011). Like many bivalve mollusks, the Asian clam lifecycle includes several stages of development. After internal fertilization, offspring are incubated inside the shell for a short period, in which they transform from trocophore, to veliger, to pediveliger larvae. After 4-5 days of incubation, they are released into the environment. The released larvae are very small, but fully formed and are capable of rapid dispersion before settling, usually in soft silty, sandy, or muddy substrates (Sousa et al., 2008). In

ideal conditions, populations can reach densities greater than 2,000 per square meter (Menninger, 2017). The Asian clam natively occurs in freshwater environments in East Asia and Africa. *C. fluminea* is thought to have been brought to North America through the United States in the early twentieth century by Asian immigrants that used the clam as a source of food (Sousa et al., 2008). Since its introduction, the Asian clam has spread throughout the US, and has now been observed in 46 states (Foster et al., 2016).

Asian clams are capable of significant primary dispersal into the water column in their early stages due to their small size and various dispersal mechanisms and also benefit greatly from human mediated secondary dispersal through the transportation of ballast water and water craft between various ecosystems (Sousa et al., 2008). Because of this, Asian clams have a very high invasive potential. As an invasive species, *C. fluminea* competes with native filtering species, alters sediment and water column composition, and alters the balance of native ecosystems. The invasion of Asian clams also causes significant economic impact, as the species frequently interrupts commercial activity in fisheries, aquaculture, and in water treatment and power facilities by water abstraction (Pimentel et al., 2005; Sousa et al., 2008). Due to the environmental and economic costs associated with their invasion, Asian clams are frequently targeted by environmental management projects, which are often costly and only serve as a short-term solution (Sousa et al., 2008).

While *C. fluminea* possesses a great capacity for rapid dispersal and range expansion, there are several limitations to the species' reproductive and dispersing success. Environmental factors such as dissolved oxygen levels, pollutant levels, temperature, and salinity all factor into the vitality of *C. fluminea* populations (McMahon, 2002). In this study, the effects of salinity will be examined in hopes of understanding the physiological and behavioral basis of salinity

tolerance in this invasive bivalve species. *C. fluminea* can occur in brackish environments such as estuaries and has been observed in natural salinities of up to 17 g/L (Foster et al., 2016; Verbrugge et al., 2011). The ability of *Corbicula* to survive in such conditions, combined with high population densities and fecundity, contributes greatly to the species' invasive potential. Tolerance to fluctuating and elevated salinity allows these clams to spread and establish populations further down river systems and into estuaries and marshes. *Corbicula* spreading into these environments not only poses a threat to potentially delicate ecosystems, but also amplifies risk of anthropogenic dispersal by moving into high-traffic areas such as ports and shipping fairways. Additionally, in freshwater environments that are at risk of salinization due to road runoff or rising sea levels, salinity tolerance gives *Corbicula* a competitive edge over native mollusk populations (Rahel and Olden, 2008).

*C. fluminea* has several methods to deal with environmental stressors such as salinity. Initially, clams may exhibit behavioral avoidance through valve closure as the primary defense mechanism when exposed to chemical stressors such as elevated salinity. Prolonged valve closure has associated negative tradeoffs in *Corbicula's* normal aerobic metabolic functions and in its capacity to feed. Because of this, behavioral avoidance is unfavorable and cannot be sustained indefinitely (Ferreira-Rodríguez and Pardo, 2016). However, studies have shown that *Corbicula* possess a remarkable capacity for prolonged valve closure. In a study conducted by Doherty et al. (1986), it was found that when exposed to lethal chlorine dosages, 95% mortality was observed after 32 days in *C. fluminea* samples at 20°C, suggesting that the sample populations could maintain valve closure for periods up to 32 days. Furthermore, *Corbicula*'s capacity for behavioral avoidance is more than adequate as a temporary means to cope with fluctuating salinity in estuarine environments (Ferreira-Rodríguez and Pardo, 2016).

At some point, the clams must open and siphon in order to expel internal metabolic waste products and feed, and, in so doing, cope with the environmental stressors through alternative methods. In a study conducted by Gainey and Greenberg (1977), it was found that *C. fluminea* behaves as an osmoregulator at salinities below 3 g/L, but osmoconforms at salinities above that. For organisms inhabiting freshwater, osmoregulation is a critical function in maintaining constant cell volume and necessary solute concentrations. Organisms living in freshwater are hyperosmotic to their surroundings, which means that they must keep water from flowing in along concentration gradients. Unchecked influx of water would result in cells swelling and bursting. Therefore, it is necessary for many freshwater species to regulate and hold their osmotic composition at a level above that of the surrounding freshwater. However, when exposed to increasing salinity, these organisms will reach a point where internal osmotic pressure matches that of the environment, and then fall below that of the environment as salinity rises. At this point, the organism becomes hypoosmotic to the environment. In this scenario, it is important for organisms to limit water loss in order to maintain cell volume (Deaton, 2009).

In *Corbicula*, the shift from osmoregulation to osmoconformity is curious because conforming would imply loss of water and a reduction in cell volume. Yet, it is known that *Corbicula* is capable of tolerating salinity up to certain levels, which raises questions about physiological repercussions not only at the organismal level, but also at the cellular level. In a series of studies conducted by Gainey (1978a;1978b), it was demonstrated that during osmotic conformation, *C. fluminea* begins to increase production of intracellular osmolytes, mainly composed of free amino acids. In doing this, *C. fluminea* is able to more closely match the osmotic pressure of its surroundings. This could partially explain how these clams are able to tolerate elevated salinities with minimal deleterious effect.

In a study conducted by Ortmann and Grieshaber (2003), it was found that fluctuations in metabolic rate and filtration were strongly correlated with valve movements. After observing differences in rate of osmotic conformation between clams held in the light and those held in the dark during initial time-course experiments, an exposure context study was developed. This study was designed to investigate the effects of light and dark exposure on the valve movements of *Corbicula*. As benthic organisms, *Corbicula* spend their lives buried in sediment and do not encounter high intensity light. While there has been no evidence to suggest the presence of photoreceptors in these organisms, the data from the previous experiments warranted further investigation. Due to the tight correlation between increased valve movement and increased metabolic rate, it was proposed that clams held in the dark would exhibit a higher metabolic rate than those in the light.

The purpose of this study was to examine the physiological and behavioral methods of salinity tolerance in Asian clams in order to gain a more complete understanding of how these bivalves handle increasing salinity stress. Additionally, this work could yield information on the tradeoffs the organisms may experience when remaining closed to avoid salinity stress versus coping with the physiological impacts of exposure to elevated salinity. It was hypothesized that clams would initially avoid any kind of salinity exposure until valve closure could no longer be maintained, and that after facing salinity stress, a decrease in tissue water content and elevation in hemolymph osmolality would be observed. It was also hypothesized that since *Corbicula* are freshwater organisms that do not osmoconform under normal conditions, they would display a significantly elevated metabolic rate as the clams faced the increased physiological strains associated with exposure to elevated salinity. Reaching a deeper understanding of the physiological and behavioral response of *C. fluminea* to various levels and contexts of salinity

exposure holds great potential for developing more practical and economical methods to manage and mediate the further dispersal of invasive aquatic mollusk species. It also can provide a better basic understanding of the physiological function of euryhaline organisms.

## Materials and Methods

### Collection and Holding

*Corbicula* were collected from the Clinch River at Clinchport, Virginia, and the Watauga River at Sycamore Shoals in Elizabethton, Tennessee, and were transported back to the laboratory for acclimation and experimentation. Specimens were pulled from areas of flowing freshwater, no more than a meter deep and were collected by using a shovel to excavate sediment from the bottom of the channel. Sediment was then sifted through wire mesh to remove dirt, and small rocks and clams were picked from the remaining material and placed in a bucket filled with stream water for transport. Depending on the temperature of the water at the time of collection, the clams would be acclimated in one of two ways. For those collected in the winter months when the water was significantly cooler than the room temperature of the laboratory, the clams would be placed in a Thermo Scientific 3990FL incubator (Thermo Fisher Scientific Incorporated, Waltham, MA) at 15°C and the temperature would be raised by 2-3°C per day until room temperature was reached. For specimens collected from water at or near the room temperature of the laboratory, the clams were held in the transport bucket and water until the water was at room temperature of approximately 22°C and then introduced into the holding tanks at the same temperature. Specimens were held in aquaria filled with dechlorinated tap water at room temperature.

## 96-Hour Exposure Study

To gain an initial understanding of the behavioral response of the clams to salinity stress, a comparison between pegged and unpegged individuals was set up at salinities of 2.5 g/L and 5.0 g/L and in a control treatment less than 1 g/L. Pegging was done to eliminate the clam's capacity for behavioral avoidance through valve closure (Gainey, 1978a). Wedging the valves of some clams open by pegging allowed for a comparison of the behavioral response between clams operating naturally and those being forced to deal with salinity stress under elevated exposure. Pegged individuals' valves were held open by inserting a short segment of toothpick between the valves and using a small drop of water-resistant glue to hold the peg to the shell. Five pegged individuals and five unpegged individuals were held in each of four, 2-liter glass aquaria at each salinity for 96 hours. During this time, the temperature, dissolved oxygen, salinity, and pH of each tank were recorded every 24 hours using a YSI Pro DSS handheld water quality meter (YSI Incorporated, Yellow Springs, OH). Any mortality within the same time period was recorded as well. After 96 hours, the clams were sacrificed and all soft tissue was removed from the shell and dabbed dry with a paper towel. Wet tissue was placed on a pre-weighed square of aluminum foil, and a wet tissue mass was then taken for each specimen. Tissue samples were then dehydrated in an oven at 60°C for a period of at least 48 hours, then removed and a dry mass was taken and a tissue water percentage was determined.

## Time-Course Study

A time-course experiment was also performed to monitor changes in percent tissue water content and hemolymph osmolality over a 24-hour time period between a control treatment of less than 1 g/L and salinities of 2.5 g/L, 5.0 g/L, and 10 g/L. Ten individuals were placed in each

of six, 2-liter glass aquaria for each salinity. Two individuals were removed from each tank in four-hour increments, starting at zero. One of the two individuals was used for hemolymph osmolality analysis, and the other for tissue water analysis. Hemolymph was collected from each specimen by wedging the clams open and pouring the water from the mantle cavity, then slitting the adductor muscles and collecting the hemolymph with a glass microtubule. Hemolymph samples were then centrifuged for five minutes at 6500 x G to separate cellular debris from the hemolymph. Hemolymph osmolality was obtained using a Fiske 210 Micro-Osmometer (Fiske Associates, Norwood, MA). Tissue water was obtained in the same manner as previously described.

Exposure Context Study

In initial runs of the time-course experiment, it was observed that clams held in open light conformed to elevated salinity at a significantly slower rate than those held in a covered, dark environment. Based on this, an experiment was conducted in which two groups of clams were held for 24 hours in either a light or dark environment, and within each context, clams were held in either a control treatment of less than 1 g/L or in 5.0 g/L salinity. Clam metabolic rates were also determined in this experiment to evaluate any energetic cost associated with the salinity exposure. The experiment was carried out in either complete darkness or with a 12h:12h light:dark cycle in separate Thermo Scientific 3990FL incubators (Thermo Fisher Scientific Incorporated, Waltham, MA) set at 20°C. Clam oxygen consumption was used to estimate metabolic rate and was determined in 300-mL BOD bottles. Bottles were first filled with either control or salt treatment water and initial oxygen levels determined using a YSI 5010 BOD Probe (YSI Incorporated, Yellow Springs, OH). Four individual clams were then placed in each of four replicate BOD bottles per salinity treatment and the bottles were sealed with glass

stoppers. Final dissolved oxygen levels were measured in each BOD bottle after 24 hours. The change in oxygen level over 24 hours was also determined in two BOD bottles in either light or dark with only treatment water to account for any factors influencing dissolved oxygen levels besides the respiration of the clams. After final oxygen measurements were made, clams were sacrificed for determination of tissue water content as described above. Tissue mass measured during tissue water analysis was also used in conjunction with dissolved oxygen consumption measurements to determine metabolic rate per tissue mass.

### Statistical Analysis

Statistical analysis for all data was done using SigmaPlot 11.0. All data were analyzed for parametric assumptions and passed both the normality and heterogeneity of variance tests. A one-way analysis of variance (ANOVA) was used for the second 96-hour exposure experiment without pegging. A Two-way ANOVA was used for the 96-hour exposure with pegging, the time-course experiments, and the context experiments. For the pegging experiment, pegging and salinity were the main effects, time and salinity were main effects for the time-course, and lighting regime and salinity were the main effects for the context experiment. All pairwise comparison tests were conducted using the Holm-Sidak method with an alpha error rate of 0.05.

## Results

In the initial 96-hour exposure experiment, it was observed that the percent tissue water content of both pegged and unpegged clams in 2.5 g/L and 5.0 g/L salinities was significantly lower ($p<0.001$) than that of their counterparts in the control treatment. For unpegged clams, the average percent tissue water content was 82.9% in the control, 81.6% in 2.5 g/L, and 80.7% in 5.0 g/L. For pegged clams, the percent tissue water content was 83.8% in the control, 81.9% in

2.5 g/L, and 81.3% in 5.0 g/L. It was also noted that no significant difference (p=0.088) in percent tissue water was observed between pegged and unpegged individuals in each treatment (Figure 1).

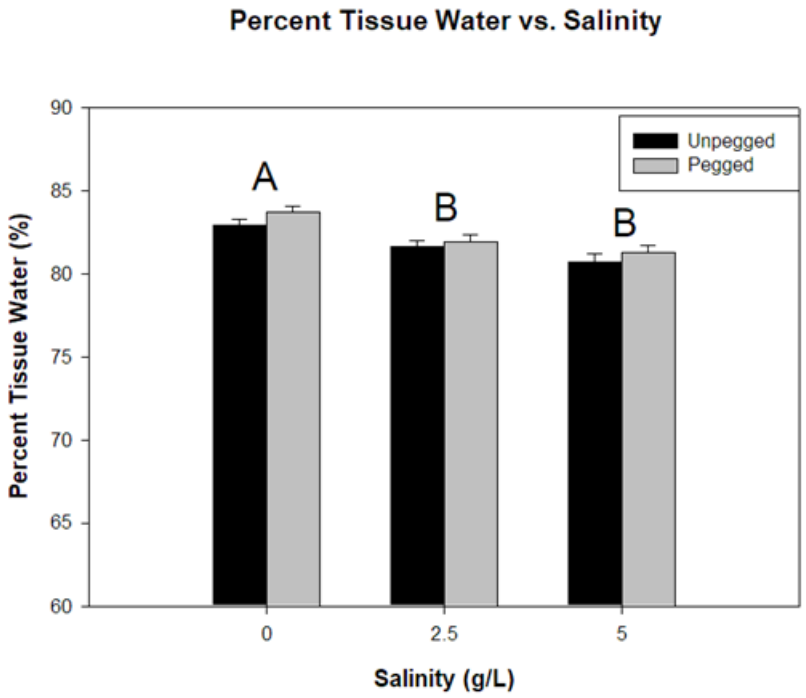


Figure 1: Mean percent tissue water content for pegged and unpegged clams exposed to three different salinity levels for 96 hours. Error bars represent one standard error. Non-matching letters denote a significant difference at $\alpha$=0.05.

Of the 120 individuals submitted to the experiment, 60 were unpegged and 60 were pegged. It was noted that 6 pegged individuals died in the course of the 96 hours. Since pegging seemed to have little influence on tissue water content percentage, the exposure experiment was run again with 48 new unpegged individuals with results similar to the first run. The average percent tissue water content for individuals in the control was 84.2%, 80.9% for 2.5 g/L, and 80.0% for 5.0 g/L, which were both significantly lower than the mean tissue water percentage of

the control group (Figure 2). It was also noted that 4 individuals died during the second trial of the 96-hour exposure.

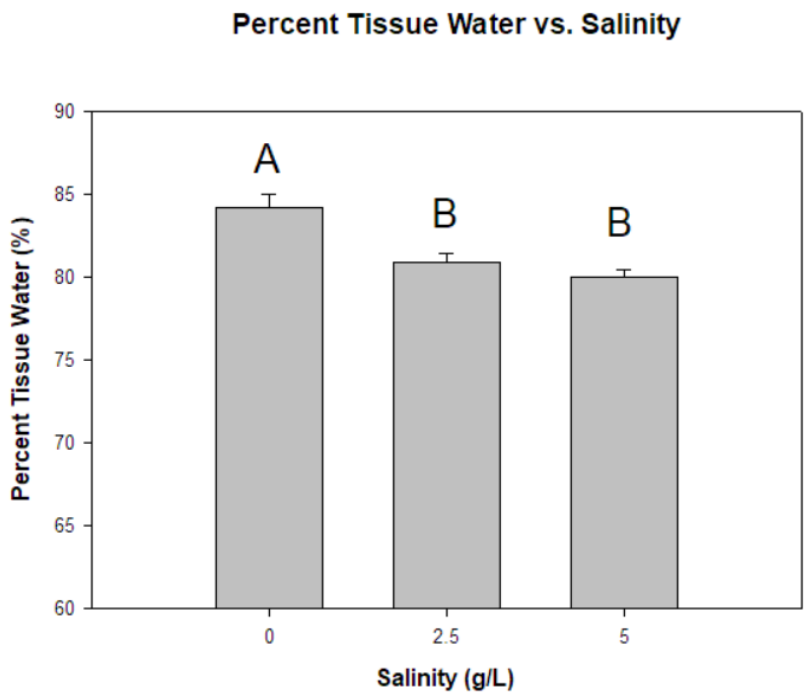


Figure 2: Mean percent tissue water content for clams exposed to three salinity levels for 96 hours with no pegging treatment. Error bars denote one standard error. Non-matching letters denote significance at $\alpha$=0.05.

In the time-course experiment, it was observed that changes in tissue water content occurred primarily within the first four hours of exposure for *Corbicula* exposed to salinities of 2.5 g/L and 5.0 g/L. However, for clams exposed to 10.0 g/L, it took significantly longer for such changes to occur. For the control group, it was observed that the average percent tissue water content held relatively steady around 82% throughout the 24-hour experiment. For the groups exposed to 5.0 g/L, percent tissue water content noticeably dropped within four hours of exposure and had largely leveled off within eight hours of exposure. In the first four hours, the average percent tissue water content of clams exposed to 5.0 g/L had dropped from 82.3% to 78.5%. Clams exposed to 2.5 g/L and 10 g/L showed a gradual decline in percent tissue water

content throughout the 24 hours of exposure. After 24 hours, the average percent tissue water content of clams exposed to 2.5 g/L was found to be 79.2%, that of clams exposed to 5.0 g/L was 78.6%, and that of clams exposed to 10 g/L was 79.8% (Figure 3).

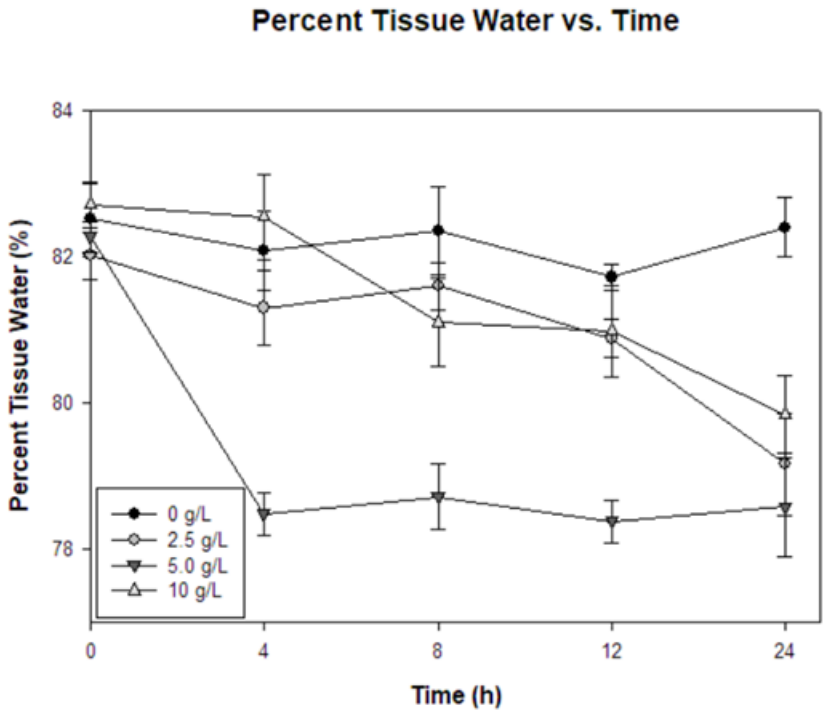


Figure 3: Mean percent tissue water of clams exposed to four different salinity levels over the course of 24 hours. Error bars represent one standard error.

The average hemolymph osmolality of the control group in the time-course experiment held steady around 50 mOsm/kg throughout the 24-hour time period; a level significantly higher than the average osmolality of the treatment water at 0.5 mOsm/kg. However, hemolymph osmolality of clams held in 2.5 g/L and 5.0 g/L salinities increased within the first four hours and had then leveled off after eight hours at a level nearly equivalent to the osmolality of their respective treatments. After four hours of exposure, the average hemolymph osmolality of clams exposed to 2.5 g/L had risen from 49.0 mOsm/kg to 69.8 mOsm/kg, and that of clams held in 5.0 g/L had increased from 52.0 mOsm/kg to 128.7 mOsm/kg. After 24 hours, the average

hemolymph osmolality of clams exposed to 2.5 g/L was found to be 81.3 mOsm/kg, with the average osmolality of the treatment water being 70 mOsm/kg. The average hemolymph osmolality of clams exposed to 5.0 g/L after 24 hours was 147.3 mOsm/kg, with the average osmolality of the treatment water being 141.7 mOsm/kg. In the 10 g/L exposure, the average hemolymph osmolality increased gradually over all the sample periods, but no significant difference from starting osmolality was observed until 8 hours of exposure. After 24 hours, it was found that the average hemolymph osmolality of clams held in 10 g/L had risen from 52.5 mOsm/kg to 87.5 mOsm/kg, whereas the average osmolality of the treatment water was 312.5 mOsm/kg (Figure 4).

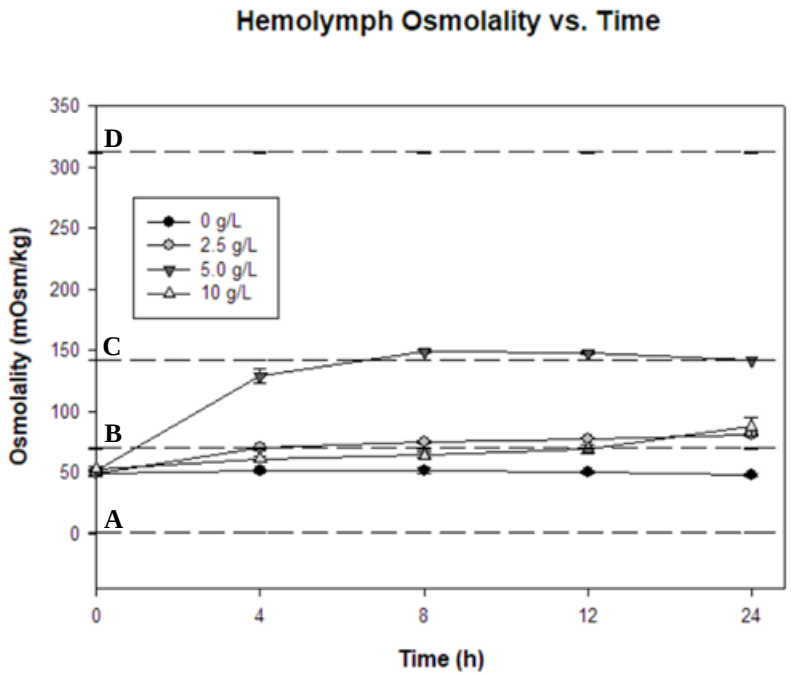


Figure 4: Mean hemolymph osmolality of clams exposed to four different salinity levels over the course of 24 hours. Error bars represent one standard error. Dashed lines labelled A, B, C, and D represent average treatment water osmolality of control, 2.5 g/L, 5.0 g/L, and 10 g/L, respectively.

In the context study, there was no significant difference in the response of clam tissue water to the salinity treatment between clams held in the dark or the light. After 24 hours of dark

exposure, the average percent tissue water of clams held in the control was 83.1% and that of clams held in 5.0 g/L was 80.3%. After 24 hours of light exposure, the average percent tissue water of the control group was 83.2%, and that of the 5.0 g/L group was 80.7% (Figure 5). No significant difference was observed between the light and dark exposures in terms of metabolic rate, as well, but there was a significant difference ($p<0.001$) in metabolic rate between clams held in control and those held in 5.0 g/L. In the control treatment, the average dissolved oxygen consumed per gram of tissue of clams held in the dark was 0.751 mg $O_2$/L/g/h and that of clams held in the light was 0.708 mg $O_2$/L/g/h. In the 5.0 g/L salinity treatment, the average dissolved oxygen consumed per gram of tissue was 1.85 mg $O_2$/L/g/h for clams held in the dark and 1.95 mg $O_2$/L/g/h for those in the light (Figure 6).

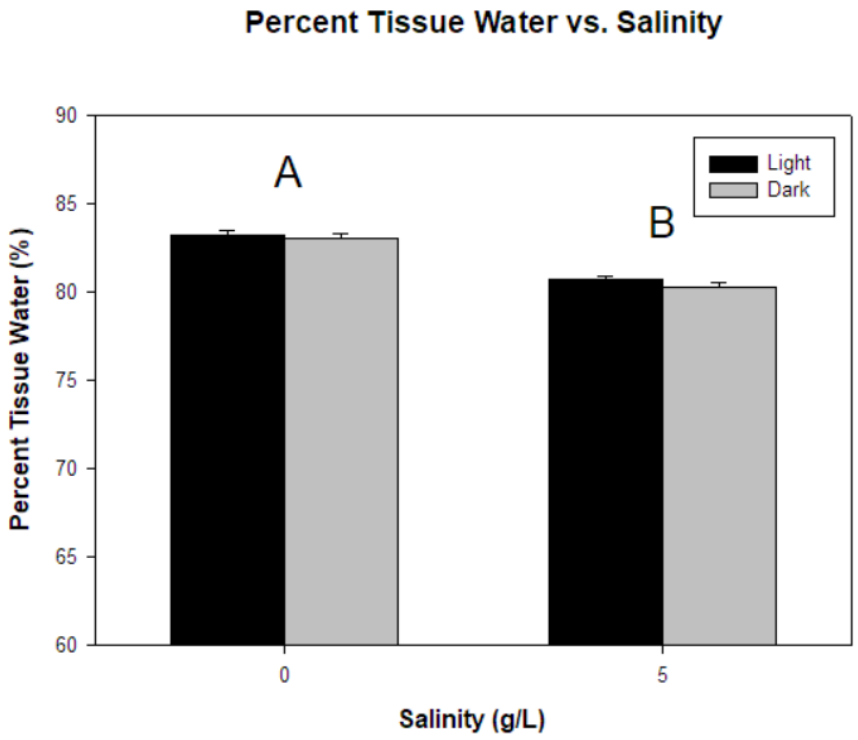


Figure 5: Mean percent tissue water of clams exposed to two different salinity levels in light and dark contexts for 24 hours. Error bars denote one standard error from the mean. Non-matching letters denote significance at $\alpha=0.05$.

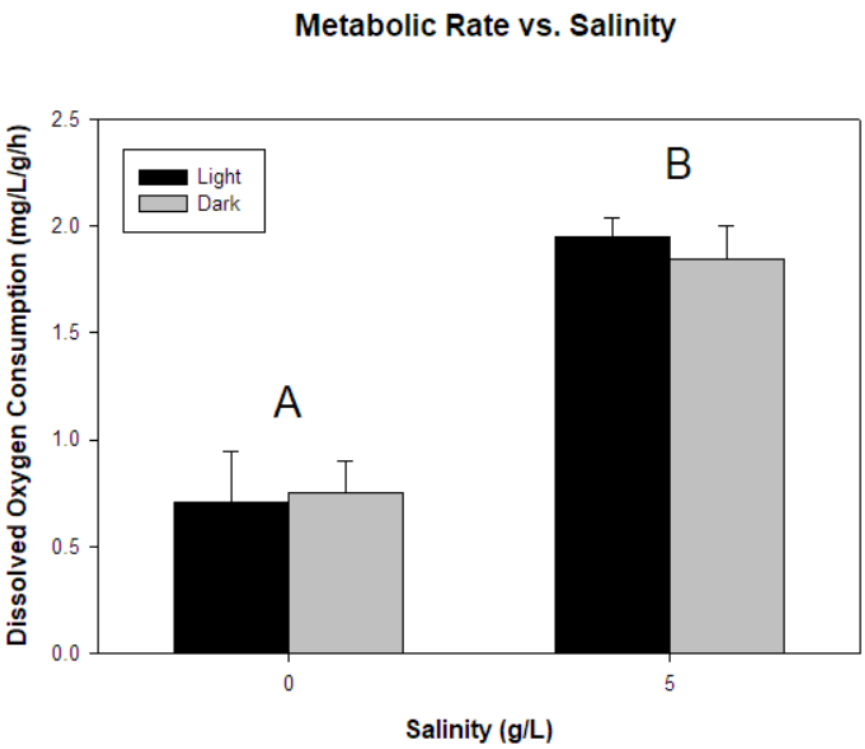


Figure 6: Mean dissolved oxygen consumed per gram of tissue per hour for clams exposed to two different salinity levels in light and dark contexts over 24 hours. Error bars denote one standard error. Non-matching letters denote significance at $\alpha$=0.05.

## Discussion

This study was carried out with the intent of gaining insight into how *Corbicula* use physiological and behavioral mechanisms to deal with salinity stress. *Corbicula* has been shown to tolerate fluctuating salinity levels in estuarine habitats through intermittent valve closure. However, prolonged valve closure results in depletion of substrates, buildup of anaerobic waste products, and eventually death (Ferreira-Rodríguez and Pardo, 2016; Ortmann and Grieshaber, 2003). So, it was initially thought that the clams would behaviorally avoid salinity stress through valve closure until the buildup of anaerobic waste products and lack of feeding necessitated opening and exposure to salinity stress. In a study conducted by Gainey (1978a), clams were pegged open to eliminate any capacity for behavioral avoidance through valve closure.

Following similar methods, in this study half of the clams in the first 96-hour exposure experiment were treated in the same way to examine the role of valve closure as an initial response to elevated salinities. However, it was observed that both pegged and unpegged *Corbicula* exposed to salinities of 2.5 g/L and 5.0 g/L had significantly lower tissue water content than control clams with no significant difference between the pegged and unpegged treatments after 96 hours. This suggests that while *Corbicula* are capable of behaviorally avoiding salinity stress for a time, salinity stress up to 5.0 g/L did not pose significant stress to cause behavioral avoidance. This also suggests that eliminating the capacity for behavioral avoidance played no role in how *Corbicula* dealt with salinity stress up to 5.0 g/L.

In the subsequent time-course experiment, it was found that *Corbicula* would begin to osmotically conform to salinities of 2.5 g/L and 5.0 g/L within four hours of exposure, suggesting little to no time spent avoiding the salinity stress. However, in the 10 g/L exposure, a delay in the change of tissue water content and plasma osmolality was observed that suggested behavioral avoidance. While the exact mechanism of salinity detection and response by *Corbicula* is not completely understood, it is known that hyperosmotic surroundings alter the ionic and osmotic gradients between extracellular fluid and cytoplasm, upsetting the osmotic equilibrium (Deaton, 2009). In Asian clams, it has been determined that alterations in intracellular amino acid concentrations are carried out to match osmotic pressure and minimize changes in cell volume, and also that these alterations are achieved through protein catabolism and anabolism (Gainey, 1978a). Mollusks are also known to be able to extract free amino acids from surrounding water, which can then be used in restoring osmotic equilibrium (Deaton, 2009). *Corbicula* held in 2.5 g/L and 5.0 g/L salinity readily conformed to the treatment water osmolality, but clams held in 10 g/L were much slower to adapt. This could suggest that

salinities above a certain threshold—somewhere between 5.0 g/L and 10 g/L—overwhelm *Corbicula*'s ability to alter free amino acid pools to adapt. When exposed to 10.0 g/L, it could be that changes in cell volume due to the hyperosmotic environment could outpace the reaction rate of proteins involved in nonessential protein catabolism, making it impossible for the clam to increase intracellular osmolyte concentrations quickly enough to prevent catastrophic cell volume loss. In this case, it would make sense that clams would avoid salinity exposure and limit water intake so that the organism could adapt at a rate that is manageable for the proteins involved in altering intracellular free amino acid pools. The increased activity of proteins associated with altering free amino acid pools would also explain the elevated metabolic rate of clams facing salinity stress observed in the context study, as increased activity comes with increased energetic demands. The increased metabolic rate could also be correlated with the activation of ATPases to drive transmembrane $Na^+/K^+$ ion exchanges to help initially balance osmotic gradients under increased salinity stress (Deaton, 2009).

In both the exposure experiments and the time-course experiments, percent tissue water content was observed to decrease in response to salinity exposure, implying that while *Corbicula* may attempt to match osmotic stress, it is not completely effective in stopping water loss. The tissue water content and hemolymph osmolality data seem to indicate that *Corbicula* will readily face salinity stress up to 5.0 g/L, resulting in tissue water loss, increased hemolymph osmolality, and increased metabolic rate associated with free amino acid pool alterations. It is unclear why the clams would opt for this option as opposed to behavioral avoidance. In periods of voluntary valve closure, it is known that *Corbicula* is able to maintain aerobic respiration for some time in normoxic conditions. However, when facing periods of prolonged valve closure, *Corbicula* deplete oxygen stores and must resort to anaerobic respiration, which is at most 20% as efficient

as aerobic respiration. Also, in order to conserve substrates, metabolic rate must be significantly lowered. Furthermore, it has been demonstrated that, in periods of valve closure under normoxic conditions, *Corbicula* will not carry out full anaerobic respiration to the end product of propionate, but rather accumulate the precursor succinate in their mantle cavity to be reabsorbed when siphoning activity is recommenced (Ortmann and Grieshaber, 2003). However, this incomplete anaerobic respiration is even less efficient than complete anaerobic respiration, making it difficult and unfavorable to maintain for extended lengths of time. This being the case, it seems that it could be that under the right conditions of food supply, dissolved oxygen, temperature, and other environmental factors, clams may have enough resources to adapt to the higher metabolic rate associated with osmoconformity, at least for a time and at salinity levels below a certain threshold, as opposed to resorting to severely inefficient anaerobic respiration. At least under the conditions in which the clams were held in the laboratory, it seems that dealing with the negative consequences of hyperosmotic stress is less costly than coping with potentially prolonged periods of behavioral avoidance.

Another interesting question brought up by this study relates to exposure context. Early in the study, a difference was observed in the rate of physiological changes related to salinity exposure between specimens kept in the dark as opposed to in the light. While there is not any evidence suggesting the presence of photoreceptors in *Corbicula*, it appears that exposure context played a role in the magnitude of changes related to salinity conformation. In the first run of the time-course experiment, clams were held in the open lab, with lights on during the day, but in later runs, the clams were kept under a black sheet. In both conditions, clams kept in 2.5 g/L and 5.0 g/L displayed the same changes in percent tissue water and hemolymph osmolality, but clams kept in the dark began displaying significant changes between four and eight hours of

exposure, whereas clams kept in the light did not begin to display significant changes until around twelve hours of exposure. This suggests that clams kept in the dark may have increased siphoning or valve movement activity relative to clams held in the light. While the metabolic and tissue water data from the context study did not show any significant difference between clams held in the light as opposed to in the dark, the initial time-course data still warrants further investigation into the role of exposure context in valve movements and siphoning activity. This possible sensitivity to exposure context holds serious implications to how studies on *Corbicula* and other bivalves are conducted in order to reach reliable conclusions.

Based on questions raised by this study, future studies might investigate the valve movements of clams exposed to salinities which they behavioral avoid, as was observed with the clams exposed to 10 g/L. In such a study, it would be interesting to monitor how often and how wide clams opened their valves and if siphoning activity occurred. Such information could provide insights on whether or not the clams are slowly acclimating to the salinity at a rate which they can tolerate, or if they are completely avoiding it. Another study might investigate the metabolic rate of clams that are behaviorally avoiding similarly high salinities to estimate *Corbicula*'s energetic demands while avoiding salinity. It would also be interesting to examine the physiological effects of salinities at 10 g/L and above on clams with their capacity for avoidance removed through pegging, as was done for clams in the first 96-hour exposure experiment. Such a study could yield relevant information regarding the survivorship of clams completely exposed to salinities that they would otherwise avoid. Additionally, further metabolic studies could be done to determine if the metabolic rates observed in *Corbicula* exposed to salinity are sustained at elevated rates, or if metabolic rates level off and begin to decline as clams adapt to the salinity.

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