12-2020

Determining the Physiological and Behavioral Responses to Elevated Salinity in the Freshwater Bivalves, Corbicula fluminea and Lampsilis ovata

John W. Roden
East Tennessee State University

Determining the Physiological and Behavioral Responses to Elevated Salinity in the Freshwater Bivalves, *Corbicula fluminea* and *Lampsilis ovata*

A thesis

presented to

the faculty of the Department of Biological Sciences

East Tennessee State University

In partial fulfillment

of the requirements for the degree

Master of Science in Biology

---

by

John Warren Roden III

December 2020

Dr. Joseph Bidwell, Chair

Dr. Thomas Laughlin

Dr. Thomas Jones

Keywords: freshwater, bivalve, salinity, response, physiology, behavior

ABSTRACT

Determining the Physiological and Behavioral Responses to Elevated Salinity in the Freshwater Bivalves, *Corbicula fluminea* and *Lampsilis ovata*

by

John Warren Roden III

Salinization has been identified as an increasing threat to freshwater mussel diversity in recent years. Native mussels have been observed to display reduced salinity tolerance in comparison to some invasive bivalve species, but methods by which organismal tolerance is achieved are not well understood. This study was designed to compare the behavioral and physiological responses of the native *Lampsilis ovata* to that of the invasive *Corbicula fluminea*. *Lampsilis* were found to exhibit strong behavioral avoidance to salinity exposure, whereas *Corbicula* displayed very weak avoidance to comparable salinity concentrations followed by indications of osmotic conformation through physiological mechanisms. Prolonged valve closure in *Lampsilis* could translate to adverse consequences related to feeding, waste removal, and energetics. Alternatively, while physiological osmotic conformation in *Corbicula* is associated with increased energetic costs, it allows continued respiration and feeding. These differences could convey a competitive advantage with the increasing prevalence and severity of freshwater salinization events.

DEDICATION

To Papaw, who gifted me with a passion for the woods and rivers of East Tennessee and showed me how to lead a life worth living.

Thank you.

"Eventually, all things merge into one, and a river runs through it. The river was cut by the world's great flood and runs over rocks from the basement of time. On some of the rocks are timeless raindrops. Under the rocks are the words, and some of the words are theirs.

I am haunted by waters."

– *Norman Maclean*

ACKNOWLEDGEMENTS

I would first like to acknowledge and thank my wife, Jasmine Roden, for her endless patience and support throughout the course of my education at East Tennessee State University. Through countless hours of planning, data collection, interpretation, and writing, she has been a constant source of reassurance and encouragement. Jasmine has always enthusiastically lent an ear for me to voice my speculations, concerns, and frustrations. At this point, she has probably learned as much about salinity responses of freshwater bivalves as I have through my ramblings. I cannot begin to adequately express how much her support and encouragement has meant to me through my academic and professional pursuits.

I would also like to acknowledge and thank my friends and fellow members of the Bidwell lab: Brian Dempsey, Trevor Chapman, Celeste Gallardo, Anna Grace Grizzard, Victoria Warren (Good), and Tyler Wicks. These excellent folks have always offered help, advice, encouragement, and commiseration, and are second to none in providing welcome distractions through shenanigans and general tomfoolery. I am incredibly grateful to have been afforded the opportunity to work with this fine group of miscreants, and I look forward to seeing where their ambitions take them in life.

I would also like to thank my committee members, Dr. Thomas Laughlin and Dr. Thomas Jones for their hand in the development and growth of my various research endeavors. Both Dr. Laughlin and Dr. Jones have helped to enrich my education and research by offering alternative perspectives and considerations relating to their respective areas of expertise. I am extremely grateful for their advice and the time they have invested in my project, and I believe that I have become a more well-rounded biologist and researcher having learned from their insights.

Finally, I would like to thank my committee chair and advisor, Dr. Joe Bidwell. Over the past four years, Dr. Bidwell has helped to guide and enrich my academic development and understanding of freshwater bivalve physiology, behavior, and ecology. He has also supported my professional development by working to provide me with opportunities to participate in research and work with professionals within the field of freshwater conservation. Through my time working with Dr. Bidwell, it has always been abundantly clear to me that he was invested not only in my education as a student, but ultimately in my success as a professional. It has been a privilege to call Dr. Bidwell not only an advisor and mentor, but also a friend.

TABLE OF CONTENTS

LIST OF FIGURES

PREFACE

Habitat quality of freshwater ecosystems across the US have been significantly degraded by human activity. Mining, agriculture, construction, and transportation are significant sources of heavy metal, inorganic ion, sediment, and nutrient pollution in many watersheds and alter freshwater systems, often at the expense of freshwater mussel diversity (Diamond et al. 2002). Agriculture land-use practices commonly result in riparian zone elimination, significant erosion of stream banks, severe sedimentation of river channels, and the introduction of pollutants from pesticides, fertilizer, and wastewater runoff (Diamond and Serveiss 2001; Williams et al. 1993). Mining activity similarly introduces sedimentation from runoff into waterways (Diamond and Serveiss 2001). Habitat degradation has also been reported as a product of increased runoff, wastewater treatment, and litter associated with urbanization (Gillies et al. 2003; Trombulak and Frissell 2001). Pollutants stemming from such commercial activity have been shown to have detrimental effects on freshwater aquatic habitat and drastically alter aquatic faunal assemblages in favor of more tolerant species (Clements et al. 1992; Diamond et al. 2002). Additionally, hydroelectric dams, weir dams, and other waterway obstructions such as bridge and dock pylons significantly alter the hydrologic makeup of ecosystems downstream, often harming aquatic fauna habitat and diversity (Magilligan and Nislow 2005; Williams et al. 1993).

North America contains remarkable freshwater mussel diversity. The continent holds over 300 species of freshwater mussels, with most of this diversity occurring in the southeastern United States (Neves et al. 1997; Williams et al. 1993). However, rapid declines in freshwater bivalve mollusk biodiversity and abundance have been observed over the past century (Diamond and Serveiss 2001). Of the 129 mussel species identified in Tennessee, 42 species are federally listed as endangered, with an additional two species listed as threatened. These declines in

mussel biodiversity are thought to be largely due to pollution and habitat degradation stemming from anthropogenic industries and commercial activities, such as those previously mentioned (Diamond and Serveiss 2001). This decline is of considerable significance due to the rate at which species are being lost and due to the loss of critical functions (water filtration, bioturbation, nutrient cycling, and others) that mussels provide in freshwater ecosystems (Vaughn 2018).

The majority of freshwater mussel diversity in North America is found in the family Unionidae. Unionid mussels primarily inhabit lotic freshwater ecosystems in the benthic substrate of riverbeds and feed by filtering phytoplankton, particulate matter, and nutrients from the water column (McMahon and Bogan 2001). Unionids prefer to occupy slightly rocky substrates, where they will orient to expose the posteriorly located incurrent and excurrent siphons to the water column. As filter feeding organisms, freshwater mussels play an important ecological role in nutrient cycling by removing and compartmentalizing particulate matter and nutrients from the water column which can then be consumed by fish and mammal species that predate bivalves (Ward 1996). This method of feeding also results in mussels removing ions and heavy metal pollutants carried in the water column as well as particulate matter that contributes to turbidity. While mussels serve to remove pollutants and improve clarity (Vaughn 2018), this activity also has detrimental effects on mussel health and viability as many unionid species are very sensitive to poor water quality (Watters 1999). This sensitivity makes unionids particularly useful as bioindicators (Van Hassel and Farris 2007).

Most unionid species are dioecious, although some unionids are hermaphroditic. Reproduction occurs when males release sperm into the current, which are then filtered out of the water column by females, at which point eggs are fertilized. Fertilized eggs are then incubated in

a modified gill pouch, called the marsupium, where they undergo a brief period of development into a larval stage, called glochidia (Graf and Foighil 2000). Glochidia are then expelled and attach to the gills or exterior of fish hosts as ectoparasites where they will develop into juvenile mussels before dropping off. Juveniles will then settle into the interstitial spaces of benthic substrate and develop into adults (Haag and Warren 1997; Wächtler et al. 2001). Unionid development is unique among other families of bivalves in that it requires an obligate fish host. In many instances, this host is species specific, and unionids hold a wide array of morphological adaptations to attract fish hosts for glochidial infestation. Many unionids possess intricate appendages that closely mimic potential prey for certain fish species and will open their valves and expose such appendages to attract hosts (Zanatta and Murphy 2006). While this parasitic life stage offers some advantages, such as dispersal, to the otherwise restricted organism, obligation to another species is not without problems. By having considerable host specificity, the fate of many unionid mussels is closely intertwined with that of their host, compounding potential threats stemming from habitat degradation and pollution.

Additionally, native mussels are threatened by the encroachment of invasive bivalve species introduced through human activity (Ricciardi et al. 1998; Sousa et al. 2008). The Asian clam, *Corbicula fluminea*, and the zebra mussel, *Dreissena polymorpha*, are invasive freshwater bivalves that have spread throughout much of the US (Benson et al. 2020; Foster et al. 2019). These species directly compete with native mussel populations for resources and habitat (Ricciardi et al. 1998; Sousa et al. 2008). *Corbicula* natively occur in East Asia and Africa but were deliberately introduced along the pacific coast of North America in the early twentieth century as a food source of Asian immigrants (Sousa et al. 2008). *Dreissena* natively occur in the Ponto-Caspian region of the Eurasian steppe and are thought to have been introduced to North

America in the Great Lakes in the 1980s through the transportation of ballast water (McMahon 1996; Son 2007). Since their respective introductions, both *Corbicula* and *Dreissena* have rapidly spread across the continent and can now be found in freshwater drainages throughout the eastern United States (Benson et al. 2020; Foster et al. 2019).

Corbicula and *Dreissena* share dietary requirements and habitat preferences similar to native unionids. Like unionids, *Corbicula* are infaunal; however, *Dreissena* are epifaunal and attach to substrate, aquatic vegetation and structures, and even other bivalves using byssal threads (Benson et al. 2020; Foster et al. 2019). Both species significantly differ from unionid mussels in that they do not develop through obligatory parasitism of a host species. The zebra mussel is dioecious, with eggs being expelled by females and fertilized externally, developing into free-swimming veliger larvae before attaching to suitable substrate (Benson et al. 2020). The Asian clam is hermaphroditic and capable of self-fertilization, featuring internal fertilization with the trocophore larvae being brooded on the gill before transformation and release as juveniles (Menninger 2019). Both reproductive strategies obviate the need of a host, thus precluding threats associated with loss of host species. Populations densities of *Corbicula* can reach 269,105 ind./m^2 (Cherry et al. 1986), while those of *Dreissena* have been observed in excess of 700,000 ind./m^2 in power plant water intake pipelines (Kovalak et al. 1993). Both species have been documented to greatly reduce concentrations of phytoplankton and seston and excessive competition from both species has been implicated in the declining abundance of unionid mussels (Cohen et al. 1984; Leff et al. 1990; Strayer and Smith 1996). Both species are also prone to mass die-offs which can results in pulse-release of nutrients and drastically alter water chemistry (Sousa et al. 2012; Sousa et al. 2014). Studies have indicated that increased ammonia stemming from *Corbicula* die-off events can hold adverse consequences for unionid condition

and viability (Cherry et al. 2005; Cooper and Bidwell 2005), and it has been suggested that *Dreissena* die-offs pose similar threats (Sousa et al. 2014).

Understanding the interactions between imperiled native mussels and their invasive counterparts is necessary to predict how bivalve assemblages may respond to impending climatic and habitat changes. Rising sea levels and increased droughts expected to occur with climate change are predicted to result in salinization of freshwater systems (Nielsen and Brock 2009). Freshwater salinization is expected to only be compounded by anthropogenic efforts to mediate the ill effects of climate change, such as using roadway deicing agents to maintain transportation routes with the onset of severe weather fluctuations predicted (Kaushal et al. 2005). Additionally, human activity in agriculture, resource extraction, and development industries has been shown to leach salts into freshwater bodies (Cañedo-Argüelles et al. 2016), and these industries will likely expand to meet the needs of a growing human populace. Salinity tolerance will likely play a vital role as freshwater ecosystems adapt to fluctuating and elevated salinities. *Corbicula* has been observed in estuaries at salinity concentrations exceeding 15 g/L (Ferreira-Rodríguez and Pardo 2016), and *Dreissena* has been observed in areas of the Caspian Sea approaching 10 g/L salinity concentrations (Ludyanskiy et al. 1993). Unionids, however, are considered to be largely intolerant of brackish water (Cvancara 1970; Verbrugge et al. 2011). With the increasing prevalence and severity of freshwater salinization, even marginal tolerance to salinity could convey a fitness advantage for invasive populations, allowing species such as *Corbicula* and *Dreissena* to outcompete already impaired native unionid populations for limited food and space. In time this could lead to a decrease in bivalve fauna diversity in affected ecosystems, resulting in less resilient ecosystems and impaired ecosystem services (Dudgeon et al. 2005; Jin 2007; Levin 1999).

The purpose of this project is to compare the salinity tolerance of the invasive clam, *Corbicula fluminea* to the native unionid, *Lampsilis ovata*. To better understand the response of each of these species to elevated salinity exposure, this study will investigate both behavioral and physiological responses, how these responses change with exposure duration, and how these responses may interact to provide a practical response. As more severe impacts of climate change are predicted to occur, and human population needs are projected to expand in the coming decades, salinization of freshwater systems is becoming of increasing concern. Freshwater salinization is predicted to drastically alter ecosystem dynamics and could cause the impairment or loss of the invaluable resources and services these systems provide (Kaushal et al. 2005; Kefford et al. 2016). Understanding how the constituent organisms of freshwater systems respond to elevated salinity exposure is integral to understanding how freshwater ecosystems will respond to increasing frequency and severity of salinization events. By comparing the salinity responses of the native *L. ovata* and the invasive *C. fluminea*, it is hoped that this study will contribute to the understanding of how freshwater bivalve assemblages will respond to salinization in the coming decades.

CHAPTER 1. BEHAVIORAL AND PHYSIOLOGICAL RESPONSES TO SALINITY

Introduction

Salinity pollution in the form of intermittent salinization through non-point runoff has been recognized as an increasing threat to freshwater ecosystems (Cañedo-Argüelles et al. 2013; Kaushal et al. 2005). Intermittent salinization events stem from runoff of road deicing agents, exposed minerals from mining operations, and construction material from areas of development. Such events can drastically alter the chemical and ionic composition of freshwater ecosystems and place significant stress on the resident biota, many of which lack the capacity to withstand extended periods of elevated salinity exposure (Kefford et al. 2016). In urbanized areas with extensive use of roadway deicing agents and highway runoff, intermittent salinization events have been documented to reach brackish chloride concentrations as high as 5 g/L (~8.24 g NaCl/L) during winter months Chloride concentrations remained as much as 100 times greater than those of unimpacted reference streams year-round (Kaushal et al. 2005). Even in less urbanized areas, chloride concentrations downstream from roadways have been documented to reach concentrations up to 31 times greater than chloride concentrations upstream during periods of deicing agent application, and elevated chloride levels have been observed to persist for up to 6 months following the last application of deicing agents (Demers and Sage 1990). With respect to freshwater bivalve fauna, salinization poses considerable threats relating to volume loss and ionic gradients, resulting in disruption of normal physiological processes (Ruiz and Souza 1991; Wehner et al. 2003). Differences in tolerance to elevated salinity exposure could give more tolerant species a competitive advantage in systems impacted by salinization events and may, in turn, alter community dynamics (Kefford et al. 2016).

Freshwater mussel diversity has experienced rapid declines over the past century due to pollution and habitat degradation (Diamond and Serveiss 2001). Most freshwater mussel diversity in North America is found in the family Unionidae. Unionid mussels are infaunal filter-feeding bivalves that primarily feed on algae and particulate matter in the water column (McMahon and Bogan 2001). Unionids provide valuable ecosystem services relating to nutrient cycling, water filtration and clarification, sediment stabilization, and bioturbation (Vaughn 2018). Many unionid species are useful bioindicators because they are very sensitive to poor and variable water quality conditions (Watters 1999) and are largely intolerant of brackish water (Cvancara 1970; Verbrugge 2011).

Corbicula fluminea is an invasive bivalve that natively occurs in freshwater environments in East Asia and Africa. *C. fluminea* were deliberately introduced to North America through the Pacific west coast of 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 their introduction, *Corbicula* have spread throughout the US, and now occur in 46 states (Foster et al. 2019). *Corbicula* share similar habitat and dietary preferences and directly compete with unionid mussels for resources in overlapping populations (Ricciardi et al. 1998; Sousa et al. 2008). Competition from *Corbicula* has been shown to have direct negative impacts on food availability and viability of juvenile and larval unionids in overlapping assemblages (Leff et al. 1990; Yeager et al. 1999). *Corbicula* are also prone to mass die-offs, which have been shown to release ammonia at concentrations detrimental to unionid condition and viability (Cherry et al. 2005; Cooper and Bidwell 2005). Compared to unionids, *Corbicula* are considerably more tolerant to salinity exposure and even occur naturally in estuaries with salinity concentrations exceeding 15 g/L (Ferreira-Rodríguez and Pardo 2016; Verbrugge 2011)

Previous studies have found that *Corbicula* has several methods of coping with salinity exposure. A study conducted by Gainey (1978) suggested that the initial response of clams exposed to salinity was to behaviorally avoid exposure through valve closure. However, while valve closure might effectively isolate soft tissue from salinity exposure, behavioral avoidance cannot be maintained indefinitely since it incurs costs related to metabolic function and feeding (Ferreira-Rodríguez and Pardo 2016). Specifically, behavioral avoidance necessitates that the bivalve terminate siphoning activity and oxygen uptake. There is some disagreement on how long bivalves can maintain aerobic respiration following valve closure. The partial pressure of oxygen in the mantle cavity of the marine bivalves *Mytilus edulis* and *Arctica islandica* has been found to decreases rapidly following valve closure (Davenport and Woolmington 1982; Taylor 1976). However, Ortmann and Grieshaber (2003) found that *C. fluminea* can maintain aerobic respiration from oxygen in mantle fluids for 5-10 hours at a reduced metabolic rate following valve closure. However, once mantle fluid oxygen is depleted, the bivalves must either initiate anaerobic respiration or open their valves to replenish oxygen, which could be problematic in the presence of environmental stress. Ortmann and Grieshaber (2003) noted reductions in metabolic rate in order to sustain aerobic respiration and suggested that similar reductions would be necessary to sustain anaerobic respiration as well. Despite metabolic reductions required to sustain avoidance, *Corbicula* have been shown to possess a remarkable capacity for prolonged valve closure. In response to emersion, Byrne et al. (1990) found that clams could maintain complete valve closure for up to 27.6 hours at 25°C and remain closed for an additional 93.4 hours by slightly protruding mantle tissue between the valves for oxygen uptake.

Corbicula also possess means to physiologically tolerate salinity exposure at certain concentrations. Gainey and Greenberg (1977) found that *Corbicula* osmoconform to salinities

above 3.0 g/L and osmoregulate below this threshold. Subsequent studies demonstrated osmotic conformation was accomplished by altering intracellular free amino acid (FAA) concentrations to balance osmotic gradients. Increases in FAA concentrations occurred by multicomponent systems thought to involve catabolic breakdown of proteins, de novo amino acid synthesis, and amino acid extraction from the external environment (Gainey 1978a). This physiological response allows *Corbicula* to effectively balance the internal osmotic gradient against hyperosmotic environments to minimize tissue volume loss (Ruiz and Souza 1991). However, upregulation of the physiological mechanisms needed to maintain volume has metabolic costs. While alterations of intracellular free amino acid pools help balance the osmotic gradient at cellular exchange surfaces, protein catabolism, amino acid synthesis, and extraction from the environment are energetically costly processes. Furthermore, changes in free amino acid concentrations do not efficiently control flux along ionic gradients, necessitating upregulation of ion exchange mechanisms (Deaton 2008; Pourmozaffar et al. 2020).

In contrast to what is known about *Corbicula*, the behavioral and physiological responses to salinity exposure in unionid mussels are less understood. Unionids have been found to have a significantly reduced capacity for amino acid accumulation in comparison to Corbiculids (Matsushima et al. 1987). Blakeslee et al. (2013) found that the unionid, *Elliptio complanata*, displayed significant metabolic depression in response to 28-day salinity exposures to 1.0 g/L and 2.0 g/L. Another study conducted by Hartmann et al. (2016) found that the European unionid, *Anodonta anatina*, displayed strong behavioral avoidance during periods of valve closure when exposed to salinities as low as 1.0 g/L, but that they also displayed periods of increased filtration activity as compared to control organisms during intermittent salinity exposures. The results of this study suggest that *Anodonta* strongly avoids even low-level

salinity, but this behavior is interrupted by frequent periods of filtration activity. Hartmann et al. suggested that these periods of increased filtration may be attempts by the mussel to expel excess salt rather than reflecting increased metabolic demands (Blakeslee et al. 2013).

The family Unionidae, a clade within the monophyletic order Unionida, has a long history of freshwater radiation tracing back to the early Jurassic period, roughly 177 million years ago (Bolotov et al. 2017; Combosch et al. 2017). Conversely, the family Cyrenidae, of which *Corbicula* is a member, is placed within the largely marine order Venerida, and represents a relatively recent freshwater colonization (Combosch et al. 2017). The family Cyrenidae is thought to contain multiple freshwater invasions because several of the freshwater members have marine sister taxa (Graf 2013; Taylor et al. 2009). *Corbicula* naturally occurs in salinity concentrations exceeding 15 g/L (Ferreira-Rodríguez and Pardo 2016; Verbrugge et al. 2011). Based on this observation, it is thought that *Corbicula* may still possess plesiomorphic mechanisms for physiological osmotic conformation. Alternatively, such mechanisms have likely been lost or reduced in unionids through their long freshwater history.

The invasion of non-native bivalve species, such as *Corbicula*, introduces novel competition on native bivalve assemblages. With the increasing prevalence of intermittent salinization events, *Corbicula's* greater capacity for salinity tolerance could provide a significant competitive advantage over native mussel populations. *Corbicula's* greater capacity for salinity tolerance could enable them to weather salinization events with lower mortality and in better condition than native species. Over time, this competitive edge could translate to the reduction of bivalve faunal diversity in freshwater ecosystems impacted by salinity. While the invasive *Corbicula* serves a very similar ecological role for native freshwater mussels, the loss of bivalve diversity could prove catastrophic for freshwater ecosystems because biodiversity has been

shown to be integral to more resilient ecosystems (Dudgeon et al. 2005; Jin 2007; Levin 1999). Furthermore, tolerance of salinity fluctuations also enables *Corbicula* to disperse into and establish in estuarine reaches and marshes, posing threats to potentially delicate ecosystems and amplifying the risk of human-mediated dispersal through shipping and transportation vectors associated with ports, shipping fairways, and other high-traffic avenues.

The goal of this study is to investigate and compare the behavioral and physiological responses of the native unionid pocketbook mussel, *Lampsilis ovata*, and the invasive Asian clam, *Corbicula fluminea*, to ecologically relevant salinity exposures. Assessing these differences is extremely pertinent to understanding how co-occurring native and invasive bivalve populations will respond to intermittent salinity exposure and the effects that such exposure might have on native mussel assemblages.

The specific goals of this project are to determine changes in volume regulation, hemolymph osmolality, oxygen consumption, and valve movement behavior in response to elevated salinity exposure in both *Corbicula* and *Lampsilis*. Since *Corbicula* have invaded freshwater much more recently than unionids (Bolotov 2017; Combosch et al. 2017), it is hypothesized that *Corbicula* will have much greater physiological capacity to tolerate salinity exposure owing to evolutionary holdover mechanisms. Alternatively, unionids would lack, or have significantly reduced, physiological capability to handle salinity exposure. This has been demonstrated in relation to the respective capacity for free amino acid accumulation of some unionids and Corbiculids (Matsushima et al. 1987). In the absence or reduction of physiological osmotic conformation mechanisms in unionids, it is thought that avoidance or modulation of salinity exposure through behavior will be the primary response of *Lampsilis*. Behavioral adjustments to modify exposure to stressors has been observed to effectively mediate biomarker

responses in bivalves (Cooper and Bidwell 2006; Doherty et al. 1987). Based on these assumptions, it is hypothesized that *Corbicula* will exhibit shorter durations of behavioral avoidance than *Lampsilis* at comparable salinities. Due to this it is expected that *Corbicula* hemolymph osmolality will match the treatment osmolality at a quicker rate than *Lampsilis*. It is also hypothesized that *Corbicula* will exhibit significantly increased oxygen consumption to meet the metabolic demands of upregulated physiological mechanisms. Alternatively, it is hypothesized that *Lampsilis* will display little to no increase in oxygen consumption if physiological mechanisms to tolerate salinity are reduced or absent. To accommodate prolonged avoidance behavior, it may be that *Lampsilis* even reduces metabolic rate in response to salinity, resulting in reduced oxygen consumption, which has been observed in *Elliptio complanate* (Blakeslee et al. 2013). If *Corbicula* exhibits little avoidance as hypothesized, it is predicted that the clams may initially experience some degree of volume loss due to the exposure of soft tissue as physiological mechanisms are initiated. However, once a new osmotic equilibrium is established, *Corbicula* will stabilize volume loss and potentially recover volume. Alternatively, *Lampsilis* is predicted to exhibit less initial volume loss if behavioral avoidance is effective. However, once avoidance can no longer be maintained it is predicted that *Lampsilis* will exhibit considerable volume loss owing to reduced capacity to establish osmotic equilibrium through physiological methods.

Methods

Collection and Housing of Organisms

Adult *Corbicula* ranging from 10-25 mm were collected from the Clinch River at Clinchport, VA, by excavating substrate using a shovel and then sifting the sediment through a wire screen to collect the organisms. Specimens were then transported back to the laboratory in buckets containing water from the site of collection. In the laboratory, the clams were allowed to acclimate to room temperature (~22 °C) before being placed in 38 L aquaria filled with moderately hard water (USEPA 2002). Juvenile *Lampsilis* ranging from 10-17 mm were supplied by the Tennessee Wildlife Resources Agency, Cumberland River Aquatic Center in Gallatin, TN. Specimens were transported back to the laboratory in 38 L coolers filled with water from the culturing facility under constant aeration. In the laboratory, the mussels were stored in 38 L aquaria in moderately hard water (USEPA 2002) at room temperature. All bivalves were fed daily with 5 mL of a 1:1 mixture of Shellfish Diet 1800 and Nanno 3600 (Reed Mariculture, Incorporated, Campbell, CA).

Treatment Salinities

Both *Corbicula* and *Lampsilis* were subjected to salinity concentrations of 2.5 g/L, 5.0 g/L, and 10.0 g/L. All test salinities were prepared as needed in 18 L increments using 19 L carboys. Moderately hard water was used as the control and diluent and Instant Ocean (Instant Ocean, Blacksburg, VA) was used as the salt. All salinity exposures were validated by measuring the actual salinity with a YSI Pro DSS handheld water quality meter (YSI Incorporated, Yellow Springs, OH). All test solutions were aerated for 24 hours to promote oxygen saturation and to dissolve the salt.

96-hour Exposure

Both *Corbicula* and *Lampsilis* were exposed to salinity concentrations of control, 2.5 g/L, and 5.0 g/L for a period of 96 hours, after which their tissue water content was determined. Salinity exposures were carried out in 2 L glass aquaria containing 4 individuals. Four replicates at each of the three exposure concentrations were conducted for a total of 16 individuals per exposure, 48 individuals total, per species. Temperature (°C), dissolved oxygen (%), specific conductance (uS/cm), salinity (g/L), and pH were monitored daily using a YSI Pro DSS handheld water quality meter. Mortality was assessed based on lack of response to gentle valve contact and dead individuals were recorded and removed from the treatment. Tissue water analysis was carried out by removing all soft tissue from the shells of each individual, then dabbing the tissue with a paper towel to remove excess water before placing on a pre-weighed aluminum square. The wet mass was taken to the nearest 0.001 g using a P-603D Precision Balance (Denver Instrument Company, Bohemia, NY). The tissue samples were then dried at 60 °C for at least 48 hours in a Thermo Electron Corporation Precision drying oven (Thermo Fisher Scientific, Waltham, MA) before being removed to determine dry weight. The wet and dry weights were then used to calculate tissue water proportion and percentage using the equation: $[(Mass_{wet} - Mass_{dry})/Mass_{wet}]$.

24-hour Oxygen Consumption

Both *Corbicula* and *Lampsilis* were exposed to the test salinity concentrations and a freshwater control for 24 hours, over which dissolved oxygen consumption was measured in order to investigate changes in metabolic rate associated with elevated salinity exposures. These exposures were carried out in sealed 300 mL Biological Oxygen Demand (BOD) bottles, with

four individuals in each bottle. Four replicates were conducted for each of the four salinity concentrations with a total of 16 individuals per exposure concentration and 64 individuals per species. Additionally, each test was conducted with two BOD bottles with no organisms to serve as blanks to monitor changes in dissolved oxygen independent of that consumed by the organism. Each bottle was filled to overflowing with the appropriate salinity solution, then initial dissolved oxygen and temperature readings were taken using a YSI 5010 BOD Probe (YSI Incorporated, Yellow Springs, OH). Following measurement, each bottle was replenished with the appropriate solution and sealed to prevent diffusion of oxygen. After 24 hours, final dissolved oxygen and temperature readings were taken in the same manner. Organisms were sacrificed for tissue water analysis as previously described. Dissolved oxygen consumption was normalized per unit mass based on the total dry weight of all organisms in each bottle and divided by the duration of each trial to develop rates of oxygen consumption in mg/L of dissolved oxygen consumed per gram of soft tissue per hour for each replicate.

24-hour Time-course Exposure

Both *Corbicula* and *Lampsilis* were also exposed to a series of salinities for a period of 24 hours, with individuals removed every four hours to test hemolymph osmolality and tissue water content. Organisms were exposed to all test salinity concentrations and freshwater control in 2 L glass aquaria with ten individuals in each aquarium. Six replicates were conducted for each of the four exposure concentrations for *Corbicula*, while five replicates were conducted for *Lampsilis* due to limited availability of specimens. Organisms were removed from their respective exposures at 0, 4, 8, 12, and 24 hours after the start of the exposure. Two organisms from each replicate were removed at each time interval; one being used to determine hemolymph osmolality, and the other to assess tissue water content. Hemolymph osmolality was determined

by draining the mantle cavity of residual water after slitting the adductor muscles and then collecting hemolymph in nonheparinized microhematocrit tubes (Thermo Fisher Scientific, Waltham, MA). Hemolymph samples were then transferred to centrifuge tubes and centrifuged at 6500 x G for five minutes using a Spectrafuge 24D Digital Lab Microcentrifuge (Labnet International Incorporated, Edison, NJ) to separate cellular debris from the hemolymph. Due to the small size of the *Lampsilis* specimens, hemolymph samples were contaminated with considerable cellular debris during collection. To remove debris, *Lampsilis* samples were centrifuged at 9200 x G for ten minutes, after which the supernatant was removed, transferred to a new centrifuge tube, and centrifuged again at 9200 x G for another 10 minutes. Following centrifugation, all hemolymph samples were stored on ice. The osmolality of hemolymph samples was then determined using a Fiske 210 Micro-Osmometer freezing point depression osmometer (Fiske Associates, Norwood, MA). Additionally, water samples were taken and combined from each replicate within respective salinity exposures. These combined water samples were centrifuged for five minutes at 6500 x G, and the osmolality was determined and compared to the osmolality of hemolymph samples. Tissue water content was determined following the methods previously described.

1-week Behavior Exposure

Corbicula and *Lampsilis* specimens were also subjected to a week-long salinity exposure trial in which valve activity was monitored using a Keithley 2400 Sourcemeter (Keithley Instruments, Cleveland, OH) to scan changes in voltages induced across a Hall Effect sensor (Allegro Microsystems, Manchester, NH) (Robson et al. 2006). All test salinity concentration and freshwater control exposures were conducted in 38 L aquaria with 10 individuals per salinity concentration and paired with a freshwater control conducted simultaneously in a separate

aquarium with an additional 10 individuals. To attach the Hall Effect sensor, bivalves were dried and a 5 mm x 2 mm cylindrical neodymium magnet (Eclipse Magnetics, Sheffield, UK) was attached to each individual approximately 1 mm from the right posterior valve margin using cyanoacrylate glue. A Hall Effect sensor was then glued to the left posterior valve, opposite the magnet. Before placement, each Hall Effect sensor was sealed to prevent water exposure using heat shrink tubing and hot melt polymer-based adhesive. Following attachment of magnets and sensors, organisms were placed in 100 mL plastic beakers filled with approximately 50 mL of aquaria substrate evenly spaced in the bottom of each aquaria. Each aquarium was then filled with 2.5 L of the appropriate salinity or freshwater. A surgical grade TDK-Lambda KMS15-5 five-volt power supply (TDK-Lambda Corporation, Tokyo, Japan) was used to supply a steady electric current to each of the Hall Effect sensors throughout each trial. Using a Raspberry Pi 3 Model B+ (Raspberry Pi Foundation, Cambridge, UK), the Sourcemeter was programmed to measure the voltage signal from each sensor every 60 seconds through the duration of each trial.

At the end of each exposure, the voltage readings were converted into gape measurements using linear calibration equations developed for each individual. In preliminary testing and calibration trials, three general size classes of *Corbicula* were observed among specimens: small (~13 mm), medium (~17 mm), and large (~22 mm). Multiple individuals representative of these classes were sacrificed and fitted with Hall Effect sensors and magnets to compare voltage measurements with physical gape measurements assessed with plastic feeler gages. It was observed that the voltage measurement corresponding with completely closed valve position varied widely within size classes and was largely a product of sensor and magnet placement in relation to distance from the valve margin, not the size of the individual. It was also noted that the voltage value corresponding to completely closed was strongly correlated with the

slope of the relationship between measured gape and observed voltage. Based on this relationship, repeated trials were conducted to validate this relationship (Figure 1). Linear calibration equations were developed for each individual in all trials by using the highest voltage value (corresponding to the lowest gape value) observed in the trial to obtain a slope value based on the equation of the linear relationship between voltage at closed and slope validated in the calibration trials. While Hall Effect sensors do not exhibit a linear decay in voltage as magnetic flux diminishes, the relationship was suitably linear for the realized gape distances observed during these trials to allow conversion between voltage and gape using a linear equation.

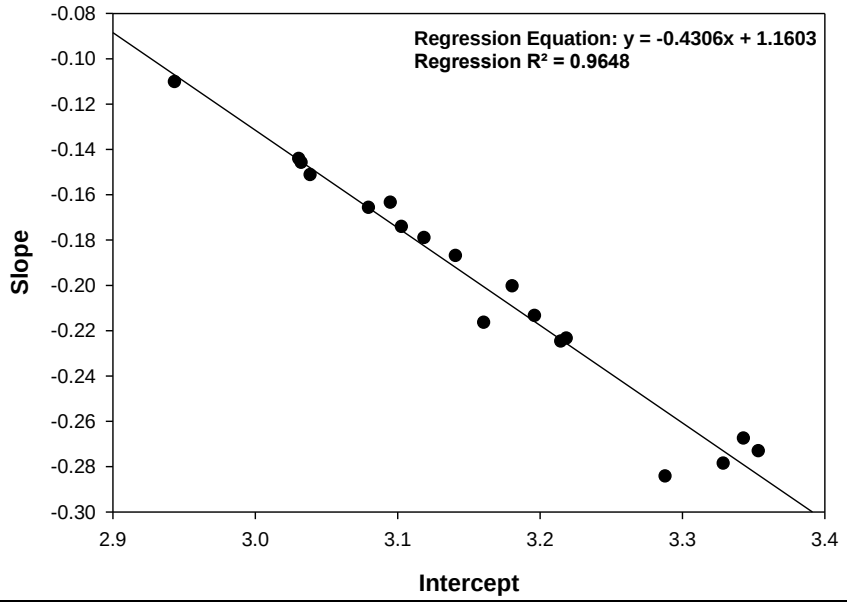

Figure 1: Linear regression of the relationship between the intercept and slope of the voltage-to-gape calibration curve generated for 18 *Corbicula*. The intercept corresponds to the voltage measurement when the valves are pressed shut (gape = 0 mm).

Unique thresholds were developed for each individual based on the average gape through the week-long trial. These thresholds were then used to assess the open or closed status of the bivalves at each measurement. Based on these thresholds, three behavioral metrics were

determined for each individual: time spent open, the number of transitions between open and closed, and latency to open. The time spent open and the number of transitions between open and closed states were assessed and developed into proportional time spent open and time spent transitioning between open and closed for five time periods corresponding to the time course of the experiments: 0 to 4 hours, 4 to 8 hours, 8 to 12 hours, 12 to 24 hours, and 24 hours through the end of the trial (168 hours).

Statistical Analysis

All statistical analyses were conducted using SigmaPlot 11.0 and significance in all tests was determined based on an alpha threshold of 0.05. All data were tested for parametric assumptions of normality and homogeneity of variance. Tissue water content collected in the 96-hour exposure was analyzed using separate one-way analysis of variance (ANOVA) tests for each species with exposure concentration as the fixed effect. For the 24-hour oxygen consumption experiment, separate one-way ANOVAs were used to compare the oxygen consumption and tissue water content across treatment groups of each species independently. This was done because all replicates of the 10.0 g/L salinity exposure of *Lampsilis* experienced at least one mortality – yielding unusable data with unbalanced factor levels – thus precluding the use of a two-way ANOVA. The hemolymph and tissue water data collected in the 24-hour time course were analyzed separately for each species using two-way ANOVAs with fixed effects of time and exposure concentration. All pairwise comparisons for two-way ANOVAs were conducted using Holm-Sidak post-hoc tests. In the behavior trials, strong significant interactions between treatment salinity and duration of exposure were expected, and much of the behavior data failed to meet parametric assumptions of normality and equal variance. Because of this non-normality, the analysis of each behavioral metric was blocked for time, using one-way

ANOVAs to compare behavior responses between salinities at each time period. The behavior data of each control group was first analyzed for each species separately to identify any differences between control groups that were conducted with each treatment salinity. If no significant difference was found between a specific behavior metric of the control groups, the control data were combined and compared against behavior metrics for all the treatment salinities. Kruskal-Wallis rank tests were conducted for non-parametric analyses followed by Dunn's post hoc tests for pairwise comparisons for data that did not meet parametric assumptions.

Results

96-hour Exposure

In the 96-hour exposure, treatment groups exposed to 2.5 g/L and 5.0 g/L salinity exhibited a significantly lower tissue water content than the freshwater control group for both *Corbicula* and *Lampsilis* ($p \leq 0.014$). In all tissue water analyses, the average tissue water content of *Lampsilis* was consistently greater than that of *Corbicula* in freshwater exposures, with differences ranging from approximately 1.0 – 3.0%. After 96 hours of exposure, the average tissue water content of *Corbicula* in the 2.5 g/L treatment (80.5±0.3%, n=14) and the 5.0 g/L treatment (80.0±0.4%, n=15) were both significantly lower ($p<0.001$) than that of the freshwater control (84.2±0.7%, n=15). However, there was no significant difference between the tissue water content of the 2.5 g/L and 5.0 g/L salinity treatments (Figure 2). Among the *Corbicula* specimens, 1 mortality was observed in the freshwater control, 2 in the 2.5 g/L salinity treatment group, and 1 in the 5.0 g/L treatment group, all occurring between 72 and 96 hours of exposure.

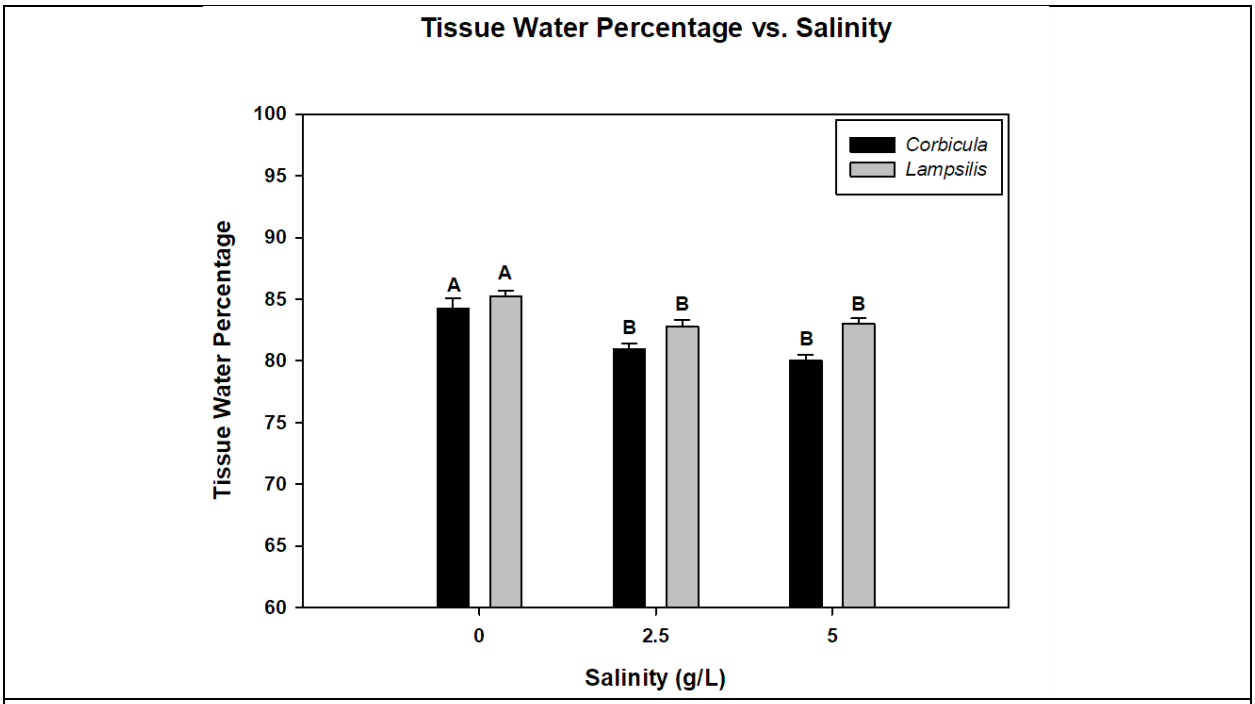

Figure 2: Average tissue water content of *C. fluminea* and *L. ovata* exposed to salinity concentrations of freshwater, 2.5 g/L, and 5.0 g/L for a period of 96 hours. Error bars denote standard error of the mean. Differing letters denote significance between salinity concentrations for each species based on an alpha threshold of 0.05.

Lampsilis exhibited a similar trend with average tissue water content of the 2.5 g/L treatment (82.8±0.6%, n=15) and of the 5.0 g/L treatment (83.0±0.5%, n=7) both being significantly lower (p=0.001 and p=0.014, respectively) than that of the control group (85.2±0.5%, n=16) with no significant difference between the two salinity exposures (Figure 2). Among *Lampsilis*, 1 mortality was observed in the 2.5 g/L treatment group, and 9 mortalities were observed in the 5.0 g/L treatment, all occurring between 48 and 72 hours of exposure.

24-hour Oxygen Consumption

In the oxygen consumption trials, all *Corbicula* salinity groups showed increased average oxygen consumption over the freshwater control (1.86±0.06 mg/L/g/h, n=4), but only the 5.0 g/L treatment group had a significantly greater oxygen consumption (2.41±0.12 mg/L/g/h, n=4) over the control group (p=0.008). In contrast, the average oxygen consumption of the 2.5 g/L group

(2.01±0.09 mg/L/g/h, n=4) and 10.0 g/L group (2.06±0.18 mg/L/g/h, n=4) was not significantly different from the control or 5.0 g/L treatment (Figure 3). Tissue water content of each treatment group decreased significantly with increasing salinity. The average tissue water content of the 2.5 g/L treatment (80.6±0.2%, n=16), 5.0 g/L (78.8±0.3%, n=16), and 10.0 g/L (76.1±0.3%, n=16) were all significantly lower than that of the control group (81.7±0.3%, n=16) and each increasing salinity treatment had significantly lower tissue water content than the preceding treatment ($p \leq 0.007$)(Figure 4).

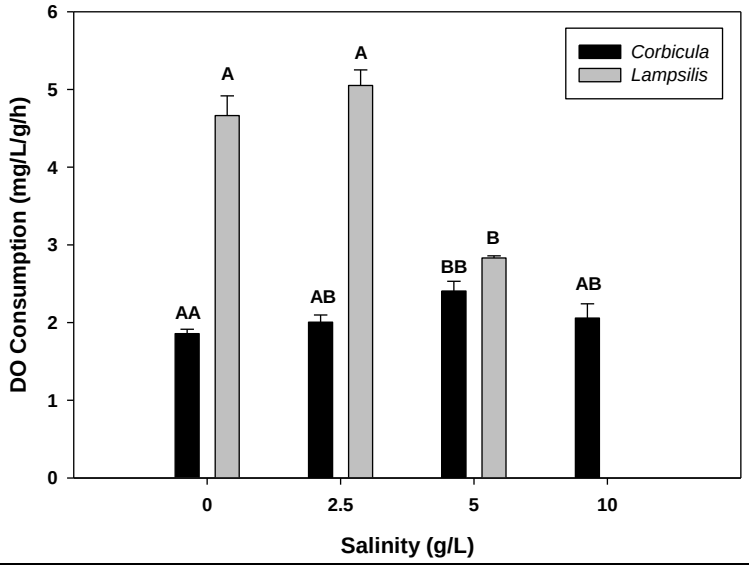

Figure 3: Average oxygen consumption per unit mass of *C. fluminea* and *L. ovata* exposed to salinity concentrations of freshwater, 2.5 g/L, 5.0 g/L, and 10.0 g/L over a period of 24 hours. Oxygen consumption in the *Lampsilis* 10.0 g/L treatment removed due to mortality. Error bars denote standard error of the mean. Differing letters indicate significance between salinity treatments for each species based on an alpha threshold of 0.05. Letter combinations indicate significance between the freshwater and 5.0 g/L treatments in *Corbicula*, but no significance between the 2.5 g/L and 10.0 g/L treatments and either of the other two significantly different treatments.

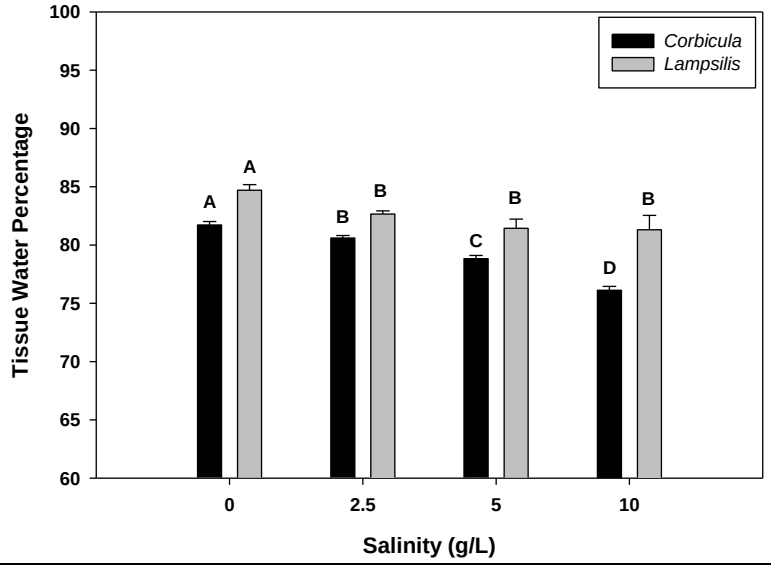

Figure 4: Average tissue water content of *C. fluminea* and *L. ovata* exposed to salinity concentrations of freshwater, 2.5 g/L, 5.0 g/L, and 10.0 g/L after 24 hours of exposure. Error bars denote standard error of the mean. Differing letters indicate significance between salinity treatments for each species based on an alpha threshold of 0.05.

In the *Lampsilis* oxygen consumption trial, considerable mortality was observed in the 10.0 g/L treatment group. While not all *Lampsilis* exposed to 10.0 g/L salinity concentration died over the course of the trial, each replicate did have at least one mortality and two replicates in the 5.0 g/L treatment group also experienced mortalities. These mortalities prevented accurate determination of oxygen consumption within affected replicates because the dead individuals consumed oxygen for only a portion of the trial, thus misleadingly decreasing oxygen consumption. Based on the usable data in the control, 2.5 g/L, and 5.0 g/L salinity treatments, the average oxygen consumption in the 2.5 g/L group (5.05±0.20 mg/L/g/h, n=4) was elevated above that of the control group (4.66±0.25 mg/L/g/h, n=4), but the difference was not significant, whereas oxygen consumption in the 5.0 g/L treatment (2.83±0.03 mg/L/g/h, n=2) was significantly lower than both the 2.5 g/L treatment ($p<0.001$) and the freshwater control ($p=0.002$) (Figure 3). The average tissue water content of *Lampsilis* in the 2.5 g/L treatment

(82.7±0.3%, n=16), 5.0 g/L treatment (81.4±0.8%, n=14), and 10.0 g/L treatment (81.3±1.2%, n=3) were all significantly lower ($p \leq 0.013$) than the freshwater control group (84.7±0.5%, n=16), but no significant difference in tissue water content was observed between the three salinity treatments (Figure 4).

24-hour Time-course Exposure

In the time-course experiment, 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 (Figure 5A). However, for the 10.0 g/L treatment group, it took significantly longer for such changes to occur. For the control group, no significant changes in tissue water content were observed between measurement intervals throughout the 24-hour experiment. In the 2.5 g/L treatment group, average tissue water content after 24 hours of exposure (79.2±0.7%, n=6) was significantly lower than at 0 (82.0±0.3%, n=6), 4 (81.3±0.5%, n=6), and 8 (81.6±0.3%, n=5) hours of exposure ($p \leq 0.002$). For the groups exposed to 5.0 g/L, percent tissue water content was significantly lower than the initial measurement (82.3±0.2%, n=6) after 4 hours of exposure (78.5±0.3%, n=6) and remained significantly lower at all subsequent measurements ($p < 0.001$) with no significant difference between 4, 8, 12, and 24 hours. Clams exposed to the 10.0 g/L salinity treatment showed a gradual decline in percent tissue water content throughout the 24 hours of exposure and did not show any significant decrease in tissue water content until after 24 hours of exposure. The average tissue water content of *Corbicula* in the 10 g/L treatment group after 24 hours (79.8±0.5%, n=6) was significantly lower than that at 0 (82.7±0.3%, n=6) and 4 (82.5±0.6%) hours of exposure ($p < 0.001$).

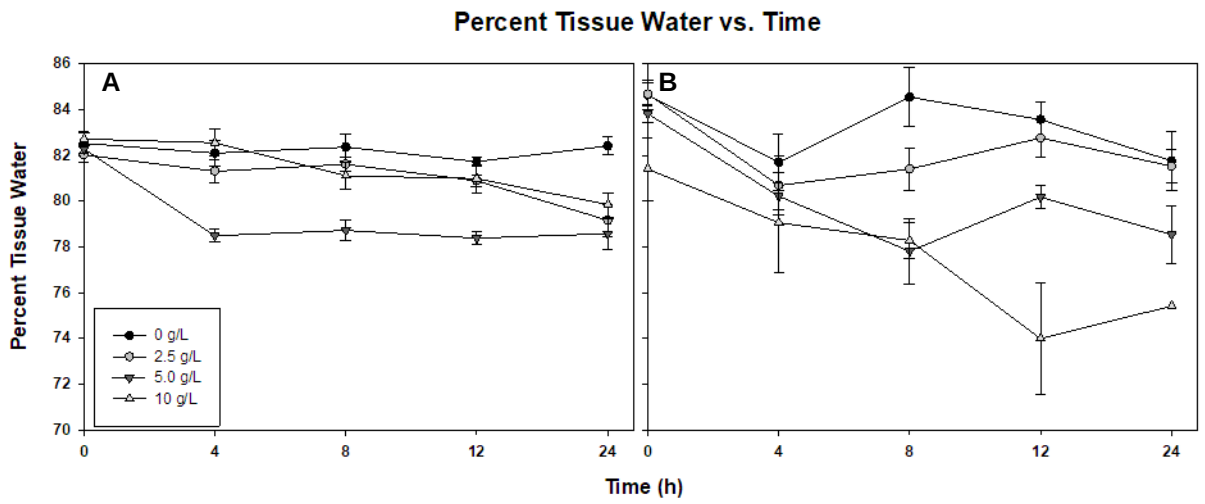

Figure 5: Average tissue water content of *C. fluminea* (A) and *L. ovata* (B) exposed to salinity concentrations of freshwater, 2.5 g/L, 5.0 g/L, and 10.0 g/L at time periods of 0, 4, 8, 12, and 24 hours. Error bars denote standard error of the mean. Absence of error bars denote only one surviving specimen available for measurement.

For *Corbicula*, hemolymph osmolality of the 2.5 g/L and 5.0 g/L treatment groups had also largely conformed to the osmolality of the respective salinity exposures within 4 hours, while the hemolymph osmolality of the 10.0 g/L treatment group only increased marginally throughout the 24 hours of exposure and never reached the osmolality of the exposure salinity (Figure 6A). In the 2.5 g/L treatment group, hemolymph osmolality after four hours of exposure (69.8±1.8 mOsm/kg, n=6) was significantly greater than the initial measurement (49.0±1.6 mOsm/kg, n=6) ($p<0.001$), as were the hemolymph osmolality measurements at each subsequent time interval ($p<0.001$), and had largely equilibrated with the osmolality of the salinity treatment (70.0±0.4 mOsm/kg, n=6). In the 5.0 g/L treatment group, a similar trend was observed, however, the average hemolymph osmolality rose through 8 hours of exposure, at which point it had largely equilibrated with the salinity exposure. In the 5.0 g/L treatment, the hemolymph osmolality at all measurement intervals after the start of the experiment were significantly greater ($p<0.001$) than the initial measurement (52.0±1.0 mOsm/kg, n=6), and the average

hemolymph osmolality at 8,12, and 24 hours was significantly greater ($p<0.001$) than that at 4 hours of exposure (128.7±5.9 mOsm/kg, n=6). After the initial increase in hemolymph osmolality in the first 8 hours of exposure at 5.0 g/L, no significant change was observed between 8, 12, and 24 hours of exposure, and hemolymph osmolality at each of these intervals had reached the osmolality of the treatment salinity (141.7±0.3 mOsm/kg, n=6). In the 10.0 g/L treatment group, hemolymph osmolality after 8 hours of exposure (64.0±2.4 mOsm/kg, n=6) was significantly greater ($p=0.003$) than at the initial measurement (52.5±2.3 mOsm/kg, n=6), as were all subsequent measurements ($p<0.001$). After 24 hours of exposure, the average hemolymph osmolality (87.5±7.7 mOsm/kg, n=6) was significantly greater ($p<0.001$) than that at all preceding measurement intervals but was still considerably lower than the osmolality of the treatment salinity (312.5±0.6 mOsm/kg, n=6).

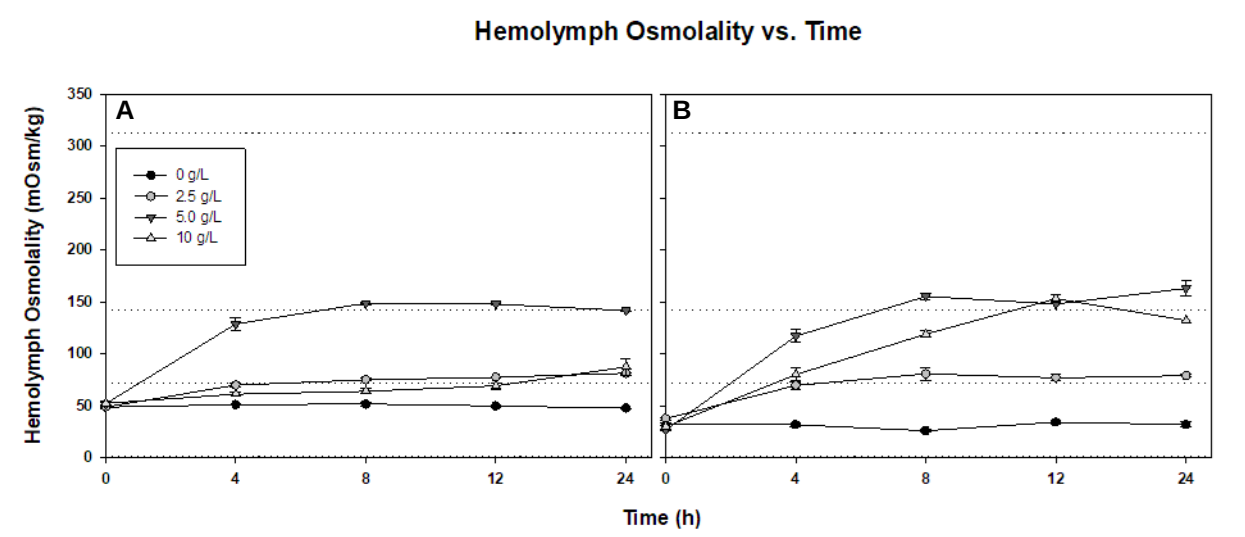

Figure 6: Average hemolymph osmolality of *C. fluminea* (A) and *L. ovata* (B) exposed to salinity concentrations of freshwater, 2.5 g/L, 5.0 g/L, and 10.0 g/L at time periods of 0, 4, 8, 12, and 24 hours. Error bars denote standard error of the mean. Absence of error bars denote only one surviving specimen available for measurement. Reference lines represent the average osmolality of freshwater, 2.5 g/L, 5.0 g/L, and 10.0 g/L treatments.

The tissue water content of *Lampsilis* illustrated a similar trend to that of *Corbicula* through the 24-hour time-course trial in that sharp declines in tissue water content were observed

after 4 hours of exposure across all treatment salinities. While the tissue water content of the 2.5 g/L and 5.0 g/L treatment groups seemingly leveled off after this initial decline, the tissue water content of the 10.0 g/L treatment group continued to decline through 12 hours of salinity exposure. Despite the initial decline in average tissue water content in the 2.5 g/L treatment group between the initial measurement (84.7±0.5%, n=5) and the measurement at 4 hours of exposure (80.7±1.3%, n=5), subsequent tissue water measurements increased slightly above that at 4 hours for all subsequent measurement intervals and no significant difference was observed between any of the time intervals. In the 5.0 g/L treatment the average tissue water content declined below the initial average (83.8±0.4%, n=5) for all subsequent measurement intervals, but the average tissue water content was only significantly lower ($p \leq 0.001$) than the initial measurement after 8 (77.8±1.4%, n=5) and 24 (78.5±1.3%, n=5) hours of exposure. Across all measurement intervals in the 10.0 g/L exposure, only the tissue water content after 12 hours of exposure (74.0±2.4%, n=4) was found to be significantly lower ($p<0.001$) than the initial measurement (81.4±1.4%, n=4). The average tissue water content after 12 hours of exposure was also significantly lower ($p=0.004$) than at 4 hours of exposure (79.1±2.2%, n=5) (Figure 5B).

The hemolymph osmolality of *Lampsilis* followed a trend consistent with that of *Corbicula* in that the 2.5 g/L and 5.0 g/L treatment groups had largely acclimated to the osmolality of their respective treatment salinities within 4 to 8 hours of exposure. In the 2.5 g/L treatment group, the average hemolymph osmolality after 4 hours of exposure (69.5±3.5 mOsm/kg, n=2) and at all subsequent measurements had significantly increased ($p<0.001$) over that of the initial measurement (37.7±1.2 mOsm/kg, n=3). In the 5.0 g/L treatment, the average hemolymph osmolality had significantly increased ($p<0.001$) over the initial measurement (26.7±0.9 mOsm/kg, n=3) after four hours of exposure (117.0±6.2 mOsm/kg, n=4). At 8 hours of

exposure, the average hemolymph osmolality in the 5.0 g/L treatment (155.3±3.2 mOsm/kg, n=3) was significantly greater ($p<0.001$) than that at 4 hours of exposure, as were all subsequent measurements ($p<0.001$). In the 10.0 g/L treatment, the average hemolymph osmolality after four hours of exposure (80.0±6.1 mOsm/kg, n=3) had significantly increased ($p<0.001$) over that of the initial measurement (30 mOsm/kg, n=1). Each subsequent measurement showed a significant increase ($p<0.001$) in hemolymph osmolality over the preceding measurement, except after 24 hours, at which point the single viable sample produced a hemolymph osmolality (132 mOsm/kg, n=1) significantly lower than the average at 12 hours of exposure (153.0±4.0 mOsm/kg, n=2) (Figure 6B).

1-week Behavior Exposure

Valve Opening Behavior. All *Corbicula* exposed to elevated salinity treatments of 2.5 g/L, 5.0 g/L, and 10.0 g/L survived through the week-long course of the exposure, as did those in the control groups. In general, it was observed that *Corbicula* exposed to salinity treatments of 2.5 g/L and 5.0 g/L concentrations displayed little behavioral avoidance and were open a greater proportion of the time open than the control group throughout the trial. While the 10.0 g/L treatment group displayed more prolonged avoidance initially, the proportion of time open was greater than that of the control group through most of trial.

Within the first four hours of exposure, clams exposed to 5.0 g/L (0.0900±0.0419, n=10) and 10.0 g/L (0.000±0.000, n=10) had significantly less ($p<0.05$) time open than the freshwater control (0.431±0.054, n=30) and the 2.5 g/L (0.455±0.081, n=10) salinity treatment groups. However, between 4 and 8 hours of exposure, the proportion of time open in the 5.0 g/L treatment group (0.299±0.089, n=10) increased, and by 12 hours of exposure, only the 10.0 g/L

treatment group (0.000±0.000, n=10) exhibited significantly less ($p<0.05$) proportional time open compared to all other salinity treatments. Between 12 and 24 hours of exposure, the proportion of time open in the 10.0 g/L treatment (0.196±0.081, n=10) began to increase, but was still significantly lower ($p<0.05$) than that of the 2.5 g/L (0.914±0.044, n=10) and 5.0 g/L (0.835±0.083, n=10) treatment groups. However, in the 12-24 hour interval, the 2.5 g/L and 5.0 g/L treatments also exhibited significantly greater ($p=0.002$ and $p=0.013$, respectively) proportional time open over the freshwater control, and that the 10.0 g/L treatment group had significantly less ($p=0.010$) proportional time open than the control group based on a parametric analysis. Through the remainder of the trial duration, the proportion of time open in the 10.0 g/L treatment group (0.680±0.019, n=10) rose to surpass all the other treatment groups, and was significantly greater ($p<0.05$) than the freshwater control (0.238±0.025, n=30) and 2.5 g/L (0.252±0.033, n=10) treatments. Parametric analysis also indicated that proportion of time open in the 10.0 g/L treatment was significantly greater ($p<0.001$) than that of the 5.0 g/L treatment (0.336±0.026, n=10) in this period (Figure 7A).

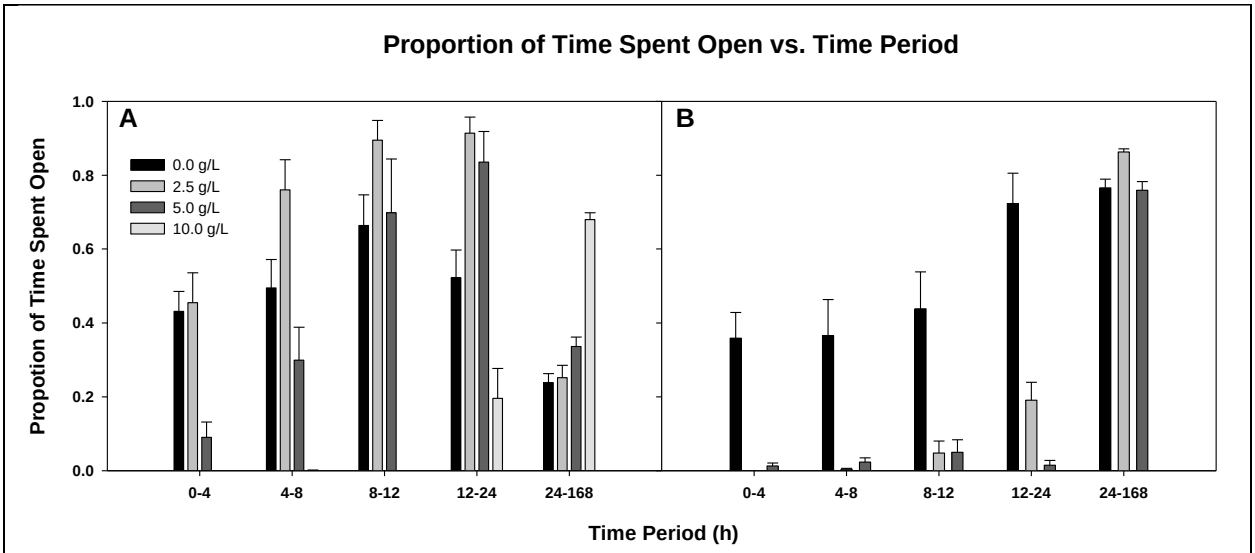

Figure 7: Average proportion of time spent open during the time periods of 0-4, 4-8, 8-12, 12-24, and 24-168 hours of exposure to salinity concentrations of freshwater, 2.5 g/L, 5.0 g/L, and 10.0 g/L in *C. fluminea* (A) and *L. ovata* (B). The proportion of time spent open could not be calculated for the *Lampsilis* 10.0 g/L exposure due to complete mortality. Error bars denote standard error of the mean.

Unlike *Corbicula*, considerable mortality was documented with *Lampsilis*, primarily in the 10.0 g/L treatment group in which no individuals survived the duration of the week-long trial. A single mortality was also observed in the 5.0 g/L salinity exposure, but no mortalities were observed in the 2.5 g/L treatment or in the freshwater control groups. Due to the complete mortality observed in the 10.0 g/L treatment, the average threshold to assess open and closed states for the individuals in the group could not be determined. Based on this, the 10.0 g/L treatment was removed from the *Lampsilis* behavioral comparisons.

In 0 to 4 hours of exposure, both the 2.5 g/L (0.000±0.000, n=10) and 5.0 g/L (0.0130±0.0078, n=9) salinity treatment groups had significantly less ($p<0.05$) proportional time open as compared to the freshwater control (0.359±0.069, n=20), and no significant difference was observed between the two salinity treatments. Between 4 and 8 hours of exposure, only the 2.5 g/L treatment group (0.00375±0.00252, n=10) had significantly less ($p<0.05$) proportional time open than the freshwater control (0.366±0.097, n=20). From 8 to 12 hours of exposure, no

significant difference in proportional time open was observed between any of the experimental groups. In 12 and 24 hours of exposure, only the 5.0 g/L treatment group (0.0150±0.0128, n=9) exhibited significantly less ($p<0.05$) proportional time open than the freshwater control (0.723±0.082, n=20). From 24 to 168 hours, the proportion of time open in the 2.5 g/L treatment group (0.863±0.008, n=10) increased to be significantly greater than both the freshwater control (0.766±0.024, n=20) and the 5.0 g/L treatment (0.759±0.024, n=9)($p<0.05$), with no significant difference being observed between the control and the 5.0 g/L treatment (Figure 7B). However, while no significant difference was observed between some salinity treatments and the freshwater control groups between 4 and 24 hours, the behavior data indicates that a strong difference does exist. The lack of statistical significance is likely due to high variability within the freshwater control based on floor and ceiling effects observed across multiple time periods within the group. These floor and ceiling effects are likely a product of individual variation in the timing of behavior cycles. In consideration of this, the average proportional time open in the 2.5 g/L and 5.0 g/L salinity groups was substantially lower than that of the freshwater control.

Transition Behavior. All transition behavior data collected for *Corbicula* failed parametric assumptions of normality and equal variance, so all analyses were conducted using Kruskal-Wallis one-way ANOVAs conducted on ranks. In the first four hours of the behavior trials, *Corbicula* exposed to the 2.5 g/L treatment (0.118±0.024, n=10) exhibited a significantly greater ($p<0.05$) proportion of time transitioning between open and closed states than the freshwater control (0.0283±0.0052, n=30), 5.0 g/L (0.0250±0.0084, n=10), and 10.0 g/L (0.000±0.000, n=10) treatments, with the control and 5.0 g/L treatment also showing significantly greater transitions than the 10.0 g/L treatment. Between 4 and 8 hours of exposure, the proportion of time transitioning between open and closed in the 2.5 g/L treatment group

(0.0104±0.0037, n=10) was no longer significantly different from the freshwater control group (0.00278±0.00064, n=30) but was still significantly greater than the 10.0 g/L treatment group (0.000833±0.000833, n=10). Also, the proportion of time transitioning in the 5.0 g/L treatment group (0.0462±0.0173, n=10) had increased to be significantly greater ($p<0.05$) than that of the control and the 10.0 g/L treatment. In the periods of 8-12 hours and 12-24 hours, no significant difference in the proportion of time spent transitioning was observed between any of the treatment groups. In the 24-168 hour period, the 10.0 g/L treatment group (0.00827±0.00133, n=10) showed a significantly greater ($p<0.05$) proportion of time spent transitioning between open and closed over the 2.5 g/L treatment group (0.00164±0.00022, n=10) and the freshwater control (0.00125±0.00016, n=30), and the 5.0 g/L (0.00249±0.00030, n=10) treatment group also exhibited significantly greater ($p<0.05$) transition activity over the control (Figure 8A).

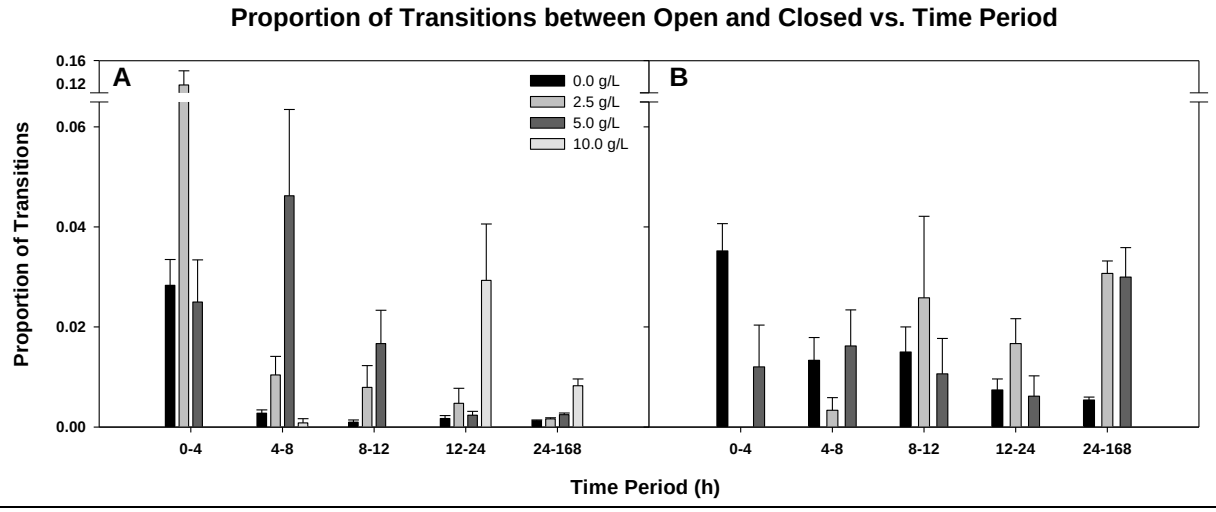

Figure 8: Average proportion of transitions between open and closed during the time periods of 0-4, 4-8, 8-12, 12-24, and 24-168 hours of exposure to salinity concentrations of freshwater, 2.5 g/L, 5.0 g/L, and 10.0 g/L in *C. fluminea* (A) and *L. ovata* (B). The proportion of transitions could not be calculated for the *Lampsilis* 10.0 g/L exposure due to complete mortality. Error bars denote standard error of the mean.

The transition behavior data for *Lampsilis* did not meet parametric assumptions of normality, equal variance, or both, in all time periods, so all analyses were conducted using

Kruskal-Wallis one-way ANOVAs conducted on ranks. In the first 4 hours of exposure, it was found that the 2.5 g/L treatment (0.000±0.000, n=10) exhibited a significantly lower ($p<0.05$) proportion of time spent transitioning than freshwater control group (0.0352±0.0055, n=20). From 4 to 8 hours, 8 to 12 hours, and 12 to 24 hours, no significant difference in the proportion of time spent transitioning was observed between any of the treatment groups. However, from 24 hours through the end of the trial, both the 2.5 g/L (0.0307±0.0025, n=10) and the 5.0 g/L (0.0300±0.0059, n=9) treatments exhibited significantly greater ($p<0.05$) transition activity over the freshwater control (0.00541±0.00059, n=20) (Figure 8B).

Latency to Open. Data collected on latency to open for *Corbicula* failed to meet assumptions of normality and equal variance. Based on non-parametric analysis, *Corbicula* exposed to the 10.0 g/L treatment (1275±184 min, n=10) took significantly longer ($p<0.05$) to open than those exposed to the freshwater control (35±7 min, n=30) and the 2.5 g/L salinity treatment (49±11 min, n=10), but not significantly longer than those in the 5.0 g/L treatment (190±51 min, n=10). *Corbicula* in the 5.0 g/L treatment also took significantly longer ($p<0.05$) to open than the freshwater control, but not significantly longer than the 2.5 g/L treatment group. No significant difference was observed between the latency to open of the 2.5 g/L treatment group and the freshwater control (Figure 9A). Latency data in the *Lampsilis* trials also did not meet assumptions of normality and failed assumptions of equal variance, so a non-parametric analysis was conducted. In *Lampsilis*, both the 2.5 g/L (879±131 min, n=10) and the 5.0 g/L (825±287 min, n=9) treatment groups took significantly longer ($p<0.05$) to open than the freshwater control (232±76 min, n=20), and no significant difference was observed in the latency to open between the two salinity treatments (Figure 9B).

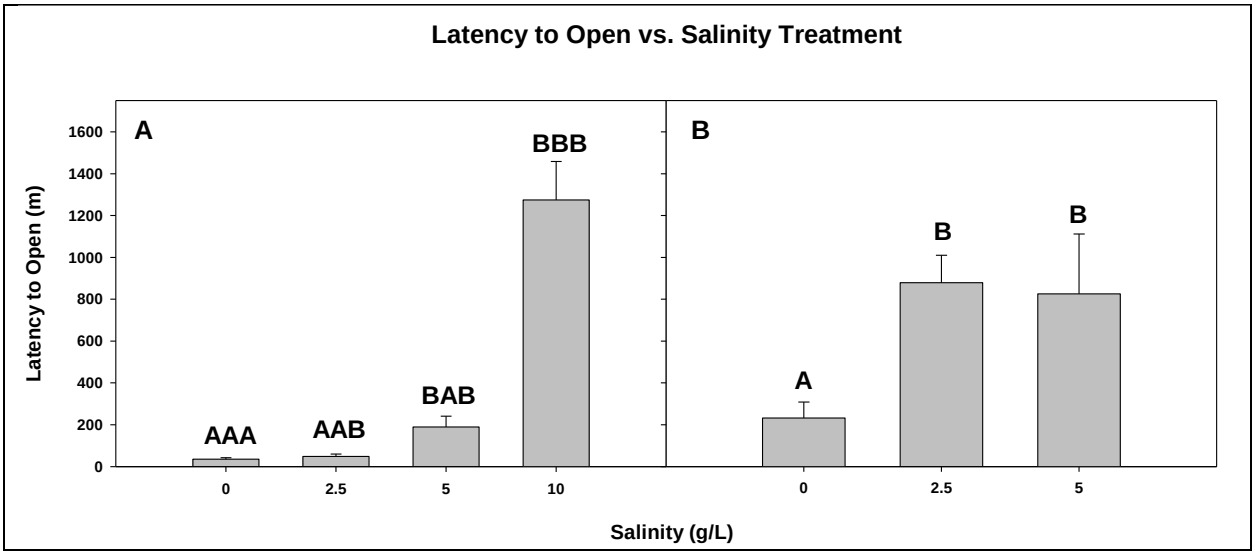

Figure 9: Average latency to open of *C. fluminea* exposed to salinity concentrations of freshwater, 2.5 g/L, and 5.0 g/L (A) and *L. ovata* exposed to salinity concentrations of freshwater, 2.5 g/L, and 5.0 g/L (B). Latency to open could not be determined for *Lampsilis* exposed to the 10.0 g/L treatment due to complete mortality. Error bars denote standard error of the mean. Differing letters indicate significance between salinity treatments for each species based on an alpha threshold of 0.05. For letter combinations, the first letter indicates significant differences in comparison to the freshwater control. The second letter indicates significance in relation to the 2.5 g/L treatment. The third letter indicates significance in relation to the 5.0 g/L treatment.

Discussion

Comparison of Salinity Responses

The objective of this study was to compare the behavioral and physiological responses to elevated salinity exposure of the unionid mussel, *Lampsilis ovata*, with that of *Corbicula fluminea*. Based on the relatively recent freshwater invasion of *Corbicula* (Combosch et al. 2017), it was hypothesized that the invasive clam may possess plesiomorphic physiological mechanisms allowing increased capacity for volume and ion regulation in hyperosmotic scenarios. Alternatively, the long freshwater lineages of unionids (Bolotov 2017; Combosch et al. 2017) may mean that *Lampsilis* would have lost such mechanisms. Unionids have been noted to exhibit reduced capacity for free amino acid accumulation—a primary mechanism to restore osmotic balance in bivalves (Deaton 2008)—in comparison to Corbiculids (Matsushima et al.

1987). To compensate for deficits in physiological capacity for salinity tolerance, unionids could avoid or modulate exposure through behavior, a known compensatory response in bivalves (Cooper and Bidwell 2006; Doherty et al. 1987). Based on these assumptions and observations, it was hypothesized that *C. fluminea* would display responses to elevated salinity exposure that would involve the incorporation of physiological mechanisms coordinated with behavioral adjustments whereas the *L. ovata* would rely almost exclusively on behavioral avoidance to limit salinity exposure because of the weakened or absent physiological capacity to cope with hyperosmotic stress. The findings of this study largely support this hypothesis.

Corbicula exhibited very weak avoidance behavior to salinity concentrations of 2.5 g/L and 5.0 g/L and began to open and exhibit proportionally more time open than the freshwater control group within 8 hours of exposure. In this same timeframe, physiological indications of conformity in hemolymph osmolality and regulatory volume decreases occurred and had largely equilibrated to the hyperosmotic conditions. Behavioral avoidance through valve closure and reduced siphoning activity have been observed in both veneroid (Kurihara 2017) and unionid (Hartmann et al. 2016) bivalves exposed to unfavorable osmotic conditions. The relatively short duration of valve closure in *Corbicula* following the onset of salinity exposure at 2.5 g/L and 5.0 g/L suggests that these concentrations do not impose sufficiently significant stress to trigger a strong avoidance response. In addition to complete valve closure, Hartmann et al. (2016) also identified increased valve closure frequencies as a form of avoidance behavior in the unionid *Anodonta anatina*. *Corbicula* exposed to 2.5 g/L and 5.0 g/L salinity concentrations initially displayed stark increases in proportional time transitioning between open and closed valve positions during the periods in which they first began to open and terminate complete valve closure. However, following this period and through the remainder of the trial, only marginal

increases in the proportional time spent transitioning were observed in comparison to the freshwater control. These findings further indicate that salinity concentrations of 2.5 g/L and 5.0 g/L do not illicit a strong behavioral avoidance response in *Corbicula*. The rapid abandonment of avoidance behavior in *Corbicula* exposed to 2.5 g/L and 5.0 g/L salinities indicates that physiological mechanisms used to cope with salinity exposure at these concentrations is a more favorable alternative. In response to hyperosmotic exposure, *Corbicula* are known to increase the concentration of intracellular free amino acid (FAA) pools through amino acid synthesis to match extracellular osmotic pressure and minimize volume loss (Gainey 1978a). These free amino acid pool increases were accomplished by increases primarily in glutamate and alanine concentrations. Gainey (1978a) speculated that these increases were the result of energy-dependent glutamate dehydrogenase (GDH) activity and pyruvate-consuming alanine aminotransferase (ALT) activity. This would equate to increased energetic demands and decreased energy production potential for clams using physiological approaches to cope with elevated salinity exposure.

While such physiological mechanisms of salinity tolerance are energetically costly, meeting these increased demands through aerobic respiration seems preferable to the anaerobic respiration that is necessitated by prolonged valve closure. Anaerobic respiration is at most 20% as efficient as aerobic respiration and prolonged anaerobic respiration requires reduced metabolic demands to adequately meet energy requirements (Grieshaber et al. 1994). Additionally, Ortmann and Grieshaber (2003) found that during periods of valve closure under normoxic conditions, *Corbicula* does not carry out full anaerobic respiration to the end product proprionate, but rather accumulates the precursor succinate in the mantle cavity to be reabsorbed once siphoning is resumed. This results in a net loss of 1 ATP per glucose molecule and also

does not fully utilize protons from metabolic intermediates for ATP production, making this incomplete form of anaerobic respiration at least 20% less effective than if propionate was formed (Müller et al. 2012). In the same study, it was found that even after the onset of valve closure, *C. fluminea* could maintain aerobic respiration at a reduced metabolic rate for several hours utilizing oxygen in the mantle fluid at the time of closure, and only resorting to anaerobic respiration after 5 to 10 hours of valve closure. In consideration of this, *Corbicula's* response to salinity concentrations up to 5.0 g/L seems to offer energetic benefits in both short- and long-term salinity exposure scenarios. In salinization events lasting up to 10 hours, *Corbicula* could simply avoid exposure while still maintaining aerobic energy production with reduced metabolism. However, in longer exposure durations, *Corbicula* would still be able to maintain aerobic respiration by terminating avoidance behavior and conforming, given adequate food, oxygen, and temperature conditions are met. This strategy may effectively enable *Corbicula* to avoid the inefficiencies of anaerobic respiration and the associated accumulation of harmful waste products by modulating behavior and metabolic demands.

However, salinity concentrations of 10.0 g/L triggered a substantially stronger avoidance response in *Corbicula*. The difference in duration of avoidance behavior observed between clams exposed to 2.5 g/L and 5.0 g/L and with those exposed to 10.0 g/L salinity concentrations suggests that at some salinity concentration between 5.0 g/L and 10.0 g/L, *Corbicula's* physiological capacity for osmotic conformation and ion regulation is overwhelmed, and triggers prolonged avoidance behavior necessitating anaerobic respiration. *C. fluminea* have been shown to modify exposure to pesticides through valve adjustments, effectively modulating biomarker responses (Cooper and Bidwell 2006). The slower rate of conformational changes and prolonged valve closure behavior in clams exposed to 10.0 g/L suggest that *Corbicula* similarly modify

valve behavior in response to salinity exposure. Since intracellular osmolyte increase is a product of enzymatic amino acid synthesis in *Corbicula*, it could be that the sharp osmotic gradient posed by 10.0 g/L salinity concentrations result in cell volume loss that outpaces the rate of amino acid accumulation. Alterations to intracellular FAA pools occur via multi-component systems with GDH playing a strong role in such alterations in many bivalves (Gainey 1978a; Pourmozaffar et al. 2020). However, increasing ionic concentrations, such as those posed by hypersaline exposure, have been shown to inhibit GDH activity in the marine oyster, *Crassostrea virginica* (Ballantyne and Berges 1991). Gainey (1978a) implicated GDH as the primary mechanism of initial increases in FAA concentrations in response to hypersaline exposure in *Corbicula*, followed by slower increases in ALT activity. If GDH activity is similarly inhibited by increased ionic concentrations in *Corbicula*, modulating behavior to modify the salinity exposure profile could be critical to allow osmotic conformation at a rate manageable by the decreased GDH activity and slower ALT activity.

Based on the long freshwater history of Unionidae, it was thought that *Lampsilis ovata* would lack or have reduced physiological capabilities to sustain prolonged osmotic conformation. It was hypothesized that *Lampsilis* would instead rely on behavioral avoidance to prevent exposure to elevated salinity. In this study, *L. ovata* displayed strong behavioral avoidance to all salinity treatments and no significant increase in oxygen consumption to suggest upregulation of any physiological mechanisms within 24 hours of salinity exposure. However, the slight increase in oxygen consumption of the 2.5 g/L treatment group suggests that once behavioral avoidance is terminated, *Lampsilis* begins to show increased metabolic demand. Unionids are known to alter FAA pools in response to hyperosmotic stress, but their capacity for FAA accumulation is markedly reduced in comparison to oligohaline and marine bivalves

(Deaton 2008). Matsushima et al. (1987) found that the unionid, *Anodonta woodiana,* displayed significantly reduced capacity for amino acid accumulation in isolated tissues compared to oligohaline and freshwater Corbiculid species, which suggests that unionids lack adequate physiological capacity to quickly balance osmotic gradients in hypersaline exposures. However, as in *Corbicula* exposed to 10.0 g/L salinity concentrations, *Lampsilis* could be modulating valve behavior in an attempt to conform at a more manageable rate. In *Lampsilis*, however, this process is much extended, likely owing to the relatively diminished capacity of unionids to accumulate FAAs. This would explain the much longer duration of avoidance behavior observed in *Lampsilis* compared to *Corbicula*.

The duration of avoidance behavior observed in *Lampsilis* raises questions regarding the exact nature of energy supply and demand in these organisms during prolonged valve closure. It is possible that *Lampsilis* could maintain aerobic respiration at a reduced metabolic rate for several hours using oxygen in the mantle fluid during valve closure, as noted in *Corbicula* (Ortmann and Grieshaber 2003). However, based on the small mantle cavity volume and high basal metabolic rate observed in the control groups of the oxygen consumption trials, the duration for which aerobic respiration could be maintained following valve closure is likely much shorter in the juvenile *Lampsilis* specimens used in this trial as compared to *Corbicula*. To continue aerobic respiration, avoidance behavior would have to be punctuated with brief periods of siphoning to release waste and replenish mantle oxygen before resuming avoidance. Hartmann et al. (2016) noted similar behavior patterns in adult *A. anatina* avoiding salinity exposure but suggested that these brief openings during avoidance are caused by adductor muscle fatigue and recovery. Regardless of the purpose, relaxation and even slight valve opening could replenish oxygen in the mantle cavity and allow continued aerobic respiration. However, this action also

requires tradeoffs, as it would expose soft tissues to elevated salinity. This could explain the rapid hemolymph conformation and volume loss observed even while *Lampsilis* were avoiding salinity exposure. Alternatively, *Lampsilis* could resort to anaerobic respiration during this long avoidance period. It has been suggested that some sphaeriid bivalves commence anaerobic respiration almost immediately following valve closure, even without stressors present (Holopainen and Penttinen 1993). The transitions between open and closed states during avoidance behavior in *Lampsilis* could support the thought that anaerobic respiration is utilized during avoidance, as these transitions could function to release anaerobic waste products. However, prolonged anaerobic respiration could be difficult to maintain based on the high basal metabolic rate of the juvenile *Lampsilis* observed in control group oxygen consumption. Further investigation is required to fully understand the metabolic and energetic condition of juvenile *Lampsilis* during avoidance to salinity exposure.

The findings of this study also raise questions regarding the efficacy of salinity response in mediating volume loss in juvenile mussels compared to adults. Previous studies demonstrated that juvenile mussels are more sensitive to exposure to cadmium, copper, and some pharmaceuticals than adults (Gilroy et al. 2016; Jacobson et al. 1993; Lasee 1991). Previous studies addressing salinity exposure in freshwater mussels have mainly focused on glochidia viability and attachment (Beggel and Geist 2015; Blakeslee et al. 2013; Gillis 2011), and adult behavior and physiological condition (Blakeslee et al. 2013; Hart et al. 2019; Hartmann et al. 2016). Patnode et al. (2015) found reduced survival of juvenile *Epioblasma torulosa rangiana* and reduced mussel diversity in a brine treatment facility mixing zone within a freshwater stream but did not investigate behavioral or physiological responses. The *L. ovata* specimens used in this study were juveniles (~1 year of age) ranging from 10 to 17mm in length, while mature

specimens grow up to 10 times this size (Parmalee and Bogan 1998). In this study, the juvenile *Lampsilis* displayed strong behavioral avoidance to all salinity exposure concentrations accompanied with substantially decreased oxygen consumption at salinity concentrations of 5.0 g/L. However, this response did not prevent changes in hemolymph osmolality or losses in tissue water content. The inefficiency of the salinity response observed in the juvenile *L. ovata* used in this study could be related to increased oxygen demands of juvenile in comparison to adult specimens. Oxygen consumption rate has been found to decrease with increasing size in several bivalve species (Peteiro et al. 2018; Xiao et al. 2014), and this elevated oxygen consumption in juveniles could translate to brief breaks in otherwise prolonged avoidance behavior to replenish oxygen for aerobic energy production as previously described. The inefficiency of this response has implications on both individual and population levels. For juvenile *L. ovata* exposed to hyperosmotic conditions, this inadequate response carries all the negative costs associated with avoidance with no substantial benefit, and means that juveniles would incur energetic costs associated with anaerobic respiration, metabolic depression, fasting, and waste accumulation while still experiencing volume loss and disruption of ionic gradients. The cost of this response to modulate salinity exposure and mediate volume loss could explain the increased sensitivity of juveniles exposed to various contaminants, such as that noted by previous studies. For *L. ovata* populations exposed to salinization events, this could result in significantly impaired recruitment.

Implications

The findings of this study suggest that the invasive *Corbicula fluminea* possess a markedly greater capacity for salinity tolerance through physiological mechanisms than the native unionid, *Lampsilis ovata*. However, both bivalves seemingly utilize behavior to modulate salinity exposure profile. This is likely done to allow osmotic conformation at a rate that is

manageable by their respective physiological capacities. *Corbicula* possess greater capacity to tolerate salinity exposure through physiological mechanisms. Such capacity is diminished in *Lampsilis*, but which can largely rely on behavior to avoid salinity exposure. This response is less sustainable due to energetic constraints, especially for juvenile mussels. This difference in response could be a competitive advantage for *Corbicula* in bivalve assemblages that include the clam and native mussels facing salinization events. With relatively less need for behavioral avoidance, *Corbicula* would likely be able to maintain aerobic respiration through brief periods of behavioral avoidance or modulation before osmotic conformation is accomplished and maintained through upregulated physiological mechanisms supported by aerobic respiration. Given adequate food, oxygen, and temperature conditions, this difference could allow *Corbicula* to effectively regulate volume while preventing energetic deficits. Alternatively, prolonged behavioral avoidance in *Lampsilis* exposed to comparable salinities would necessitate metabolic rate reductions to accommodate either anaerobic inefficiencies or to sustain prolonged aerobic respiration. Prolonged avoidance also restricts feeding and waste excretion and could result in depletion of energy stores and buildup of waste products. Furthermore, because of the inefficiency of avoidance behavior in mediating exposure, juvenile *Lampsilis* have particularly limited capacity to tolerate elevated salinity exposure. This places mussels emerging from elevated salinity exposures at a disadvantage. With increasing prevalence of intermittent salinization events, the difference in energetic and physiological states between native mussels and invasive competitors could convey a significant fitness advantage following such events to *Corbicula* over native mussel populations.

REFERENCES

Ballantyne JS, Berges JA. 1991. Enzyme activities of gill, hepatopancreas, mantle, and adductor muscle of the oyster (Crassostrea virginica) after changes in diet and salinity. *Canadian Journal of Fisheries and Aquatic Sciences, 48*(6), pp.1117-1123.

Beggel S, Geist J. 2015. Acute effects of salinity exposure on glochidia viability and host infection of the freshwater mussel Anodonta anatina (Linnaeus, 1758). *Science of the Total Environment*, *502*, pp.659-665.

Benson A, Raikow D, Larson J, Fusaro A, Bogdanof, A. 2020. *Dreissena polymorpha*. [online] USGS Nonindigenous Aquatic Species Database. Gainesville, FL. Available at: https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=5

Blakeslee CJ, Galbraith HS, Robertson LS, St. John White B. 2013. The effects of salinity exposure on multiple life stages of a common freshwater mussel, Elliptio complanata. *Environmental Toxicology and Chemistry*, *32*(12), pp.2849-2854.

Bolotov IN, Kondakov AV, Vikhrev IV, Aksenova OV, Bespalaya YV, Gofarov MY, Kolosova YS, Konopleva ES, Spitsyn VM, Tanmuangpak K, et al. 2017. Ancient river inference explains exceptional Oriental freshwater mussel radiations. *Scientific Reports*, *7*(1), pp.1-14.

Byrne RA, Gnaiger E, McMahon RF, Dietz TH. 1990. Behavioral and metabolic responses to emersion and subsequent reimmersion in the freshwater bivalve, Corbicula fluminea. *The Biological Bulletin*, *178*(3), pp.251-259.

Cañedo-Argüelles M, Kefford BJ, Piscart C, Prat N, Schäfer RB, Schulz CJ. 2013. Salinisation of rivers: an urgent ecological issue. *Environmental pollution*, *173*, pp.157-167.

Cañedo-Argüelles M, Hawkins CP, Kefford BJ, Schäfer RB, Dyack BJ, Brucet S, Buchwalter D, Dunlop J, Frör O, Lazorchak J, et al. 2016. Saving freshwater from salts. *Science*, *351*(6276), pp.914-916.

Cherry DS, Roy RL, Lechleitner RA, Dunhardt PA, Peters GT, Cairns Jr J. 1986. Corbicula fouling and control measures at the Celco Plant, Virginia. *American Malacological Bulletin. 1986.*

Cherry DS, Scheller JL, Cooper NL, Bidwell JR. 2005. Potential effects of Asian clam (Corbicula fluminea) die-offs on native freshwater mussels (Unionidae) I: water-column ammonia levels and ammonia toxicity. *Journal of the North American Benthological Society*, *24*(2), pp.369-380.

Clements WH, Cherry DS, Van Hassel JH. 1992. Assessment of the impact of heavy metals on benthic communities at the Clinch River (Virginia): evaluation of an index of community sensitivity. *Canadian Journal of Fisheries and Aquatic Sciences*, *49*(8), pp.1686-1694.

Cohen RR, Dresler PV, Phillips EJ, Cory RL. 1984. The effect of the Asiatic clam, Corbicula fluminea, on phytoplankton of the Potomac River, Maryland. *Limnology and Oceanography*, *29*(1), pp.170-180.

Combosch DJ, Collins TM, Glover EA, Graf DL, Harper EM, Healy JM, Kawauchi GY, Lemer S, McIntyre E, Strong EE, Taylor JD. 2017. A family-level tree of life for bivalves based

on a Sanger-sequencing approach. *Molecular Phylogenetics and Evolution*, *107*, pp.191-208.

Cooper NL, Bidwell JR. 2006. Cholinesterase inhibition and impacts on behavior of the Asian clam, Corbicula fluminea, after exposure to an organophosphate insecticide. *Aquatic toxicology*, *76*(3-4), pp.258-267.

Cvancara, AM. 1970. Mussels (UNIONIDAE) of the Red River Valley in North Dakota and Minnesota, USA.

Deaton, L. 2008. Osmotic and ionic regulation in molluscs. *Osmotic and Ionic Regulation: Cells and Animals*, pp.107-133.

Demers CL, Sage RW. 1990. Effects of road deicing salt on chloride levels in four Adirondack streams. *Water, Air, and Soil Pollution*, *49*(3-4), pp.369-373.

Diamond JM, Serveiss VB. 2001. Identifying Sources of Stress to Native Aquatic Fauna Using a Watershed Ecological Risk Assessment Framework. *Environmental Science & Technology*, 35(24), pp.4711–4718.

Diamond JM, Bressler DW, Serveiss VB. 2002. Assessing relationships between human land uses and the decline of native mussels, fish, and macroinvertebrates in the Clinch and Powell River watershed, USA. *Environmental Toxicology and Chemistry: An International Journal*, *21*(6), pp.1147-1155.

Dietz, TH. 1985. Ionic regulation in freshwater mussels: A brief review. *American Malacological Bulletin*, *3*(2), pp.233-242.

Doherty FG, Cherry DS, Cairns J. 1987. Valve closure responses of the Asiatic clam Corbicula fluminea exposed to cadmium and zinc. *Hydrobiologia*, *153*(2), pp.159-167.

Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard AH, Soto D, Stiassny ML, et al. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological reviews*, *81*(2), pp.163-182.

Ferreira-Rodríguez N, Pardo I. 2016. An experimental approach to assess Corbicula fluminea (Müller, 1774) resistance to osmotic stress in estuarine habitats. *Estuarine, Coastal and Shelf Science*, *176*, pp.110-116.

Foster A, Fuller P, Benson A, Constant S, Raikow D, Larson J, Fusaro A. 2019. *Corbicula fluminea*. [online] USGS Nonindigenous Aquatic Species Database. Gainesville, FL. Available at: https://nas.er.usgs.gov/queries/factsheet.aspx?speciesid=92

Gainey LF, Greenberg MJ. 1977. Physiological basis of the species abundance-salinity relationship in molluscs: a speculation. *Marine Biology*, *40*(1), pp.41-49.

Gainey Jr, LF. 1978a. The response of the Corbiculidae (Mollusca: Bivalvia) to osmotic stress: the cellular response. *Physiological Zoology*, *51*(1), pp.79-91.

Gainey Jr, LF. 1978b. The response of the Corbiculidae (Mollusca: Bivalvia) to osmotic stress: the organismal response. *Physiological Zoology*, *51*(1), pp.68-78.

Gillies RR, Box JB, Symanzik J, Rodemaker EJ. 2003. Effects of urbanization on the aquatic fauna of the Line Creek watershed, Atlanta—a satellite perspective. *Remote sensing of environment*, *86*(3), pp.411-422.

Gillis, PL. 2011. Assessing the toxicity of sodium chloride to the glochidia of freshwater mussels: Implications for salinization of surface waters. *Environmental Pollution*, *159*(6), pp.1702-1708.

Gilroy ÈA, Gillis PL, King LE, Bendo NA, Salerno J, Giacomin M, de Solla SR. 2017. The effects of pharmaceuticals on a unionid mussel (Lampsilis siliquoidea): An examination of acute and chronic endpoints of toxicity across life stages. *Environmental toxicology and chemistry*, *36*(6), pp.1572-1583.

Graf DL, Foighil DÓ. 2000. The evolution of brooding characters among the freshwater pearly mussels (Bivalvia: Unionoidea) of North America. *Journal of Molluscan Studies*, *66*(2), pp.157-170.

Graf, DL. 2013. Patterns of freshwater bivalve global diversity and the state of phylogenetic studies on the Unionoida, Sphaeriidae, and Cyrenidae. *American Malacological Bulletin*, *31*(1), pp.135-153.

Haag WR, Warren Jr ML. 1997. Host fishes and reproductive biology of 6 freshwater mussel species from the Mobile Basin, USA. *Journal of the North American Benthological Society*, *16*(3), pp.576-585.

Hart MA, Miller TD, Randklev CR. 2019. Salinity tolerance of a rare and endangered unionid mussel, Popenaias popeii (Texas Hornshell) and its implications for conservation and water management. *Ecotoxicology and environmental safety*, *170*, pp.1-8.

Hartmann JT, Beggel S, Auerswald K, Stoeckle BC, Geist J. 2016. Establishing mussel behavior as a biomarker in ecotoxicology. *Aquatic Toxicology*, *170*, pp.279-288.

Holopainen IJ, Penttinen OP. 1993. Normoxic and anoxic heat output of the freshwater bivalves Pisidium and Sphaerium. *Oecologia*, *93*(2), pp.215-223.

Jacobson PJ, Cherry DS, Farris JL, Neves RJ. 1993. Juvenile freshwater mussel (Bivalvia: Unionidae) responses to acute toxicity testing with copper. *Environmental Toxicology and Chemistry: An International Journal*, *12*(5), pp.879-883.

Jin, C. 2008. Biodiversity dynamics of freshwater wetland ecosystems affected by secondary salinisation and seasonal hydrology variation: a model-based study. *Hydrobiologia*, *598*(1), pp.257-270.

Kaushal SS, Groffman PM, Likens GE, Belt KT, Stack WP, Kelly VR, Band LE, Fisher GT. 2005. Increased salinization of fresh water in the northeastern United States. *Proceedings of the National Academy of Sciences*, *102*(38), pp.13517-13520.

Kefford BJ, Buchwalter D, Canedo-Argüelles M, Davis J, Duncan RP, Hoffmann A, Thompson R. 2016. Salinized rivers: degraded systems or new habitats for salt-tolerant faunas?. *Biology Letters*, *12*(3), p.20151072.

Kovalak WP, Longton GD, Smithee RD. 1993. Infestation of Power Plant Water Systems by the Zebra Mussel(Dreissena polymorpha Pallas). *IN: Zebra Mussels: Biology, Impacts, and Control. Lewis Publishers, Boca Raton, FL. 1993. p 359-380, 15 fig, 3 tab, 15 ref.*

Kurihara, T. 2017. Tolerance of the bivalve Trapezium liratum (Reeve, 1843) to decrease in salinity. *Plankton and Benthos Research*, *12*(1), pp.44-52.

Lasee, BA. 1991. Histological and ultrastructural studies of larval and juvenile Lampsilis (Bivalvia) from the upper Mississippi River.

Leff LG, Burch JL, McArthur JV. 1990. Spatial distribution, seston removal, and potential competitive interactions of the bivalves Corbicula fluminea and Elliptio complanata, in a coastal plain stream. *Freshwater Biology*, *24*(2), p.409.

Levin, SA. 1999. Fragile dominion: complexity and the commons. Perseus. *Reading*, *MA*.

Ludyanskiy ML, McDonald D, MacNeill D. 1993. Impact of the zebra mussel, a bivalve invader: Dreissena polymorpha is rapidly colonizing hard surfaces throughout waterways of the United States and Canada. *Bioscience*, *43*(8), pp.533-544.

Magilligan FJ, Nislow KH. 2005. Changes in hydrologic regime by dams. *Geomorphology*, *71*(1-2), pp.61-78.

Matsushima O, Katayama H, Yamada K. 1987. The capacity for intracellular osmoregulation mediated by free amino acids in three bivalve molluscs. *Journal of experimental marine biology and ecology*, *109*(1), pp.93-99.

McMahon RF, Williams CJ. 1984. A unique respiratory adaptation to emersion in the introduced Asian freshwater clam Corbicula fluminea (Müller)(Lamellibranchia: Corbiculacea). *Physiological Zoology*, *57*(2), pp.274-279.

McMahon, RF. 1996. The physiological ecology of the zebra mussel, Dreissena polymorpha, in North America and Europe. *American Zoologist*, *36*(3), pp.339-363.

McMahon R, Bogan A. 2001. Ecology and classification of North American freshwater invertebrates. *Choice Reviews Online*, 39(01), pp.39-0312-39-0312.

Menninger, H. 2019. *Asian clam (Corbicula fluminea)*. [online] New York Invasive Species Information. Available at: http://www.nyis.info/index.php?action=invasive_detail&id=52

Müller M, Mentel M, van Hellemond JJ, Henze K, Woehle C, Gould SB, Yu RY, van der Giezen M, Tielens AG, Martin WF. 2012. Biochemistry and evolution of anaerobic energy metabolism in eukaryotes. *Microbiology and Molecular Biology Reviews, 76*(2), pp.444-495.

Neves RJ, Bogan AE, Williams JD, Ahlstedt SA, Hartfield PD. 1997. Status of aquatic mollusks in the southeastern United States: a downward spiral of diversity. In *Aquatic Fauna in Peril: The Southeastern Perspective*. Chattanooga, TN: Southeast Aquatic Research Institute, pp. 43–86.

Nielsen DL, Brock MA. 2009. Modified water regime and salinity as a consequence of climate change: prospects for wetlands of Southern Australia. *Climatic Change*, *95*(3-4), pp.523-533.

Ortmann C, Grieshaber MK. 2003. Energy metabolism and valve closure behaviour in the Asian clam Corbicula fluminea. *Journal of Experimental Biology, 206*(22), pp.4167-4178.

Patnode KA, Hittle E, Anderson RM, Zimmerman L, Fulton JW. 2015. Effects of high salinity wastewater discharges on unionid mussels in the Allegheny River, Pennsylvania. *Journal of Fish and Wildlife Management, 6*(1), pp.55-70.

Parmalee PW, Bogan AE. 1998. *Freshwater mussels of Tennessee*. University of Tennessee Press.

Peteiro LG, Woodin SA, Wethey DS, Costas-Costas D, Martínez-Casal A, Olabarria C, Vázquez E. 2018. Responses to salinity stress in bivalves: evidence of ontogenetic changes in energetic physiology on Cerastoderma edule. *Scientific reports, 8*(1), pp.1-9.

Pourmozaffar S, Tamadoni Jahromi S, Rameshi H, Sadeghi A, Bagheri T, Behzadi S, Gozari M, Zahedi MR, Abrari Lazarjani S. 2020. The role of salinity in physiological responses of bivalves. *Reviews in Aquaculture*, *12*(3), pp.1548-1566.

Ricciardi A, Neves RJ, Rasmussen JB. 1998. Impending extinctions of North American freshwater mussels (Unionoida) following the zebra mussel (Dreissena polymorpha) invasion. *Journal of animal ecology*, *67*(4), pp.613-619.

Robson A, Wilson R, de Leaniz CG. 2007. Mussels flexing their muscles: a new method for quantifying bivalve behaviour. *Marine Biology*, *151*(3), pp.1195-1204.

Ruiz JL, Souza MMD. 2008. Osmotic stress and muscle tissue volume response of a freshwater bivalve. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *151*(3), pp.399-406.

Sousa R, Antunes C, Guilhermino L. 2008. Ecology of the invasive Asian clam Corbicula fluminea (Müller, 1774) in aquatic ecosystems: an overview. In *Annales de Limnologie-International Journal of Limnology* (Vol. 44, No. 2, pp. 85-94). EDP Sciences.

Sousa R, Gutiérrez JL, Aldridge DC. 2009. Non-indigenous invasive bivalves as ecosystem engineers. *Biological Invasions*, *11*(10), pp.2367-2385.

Sousa R, Varandas S, Cortes R, Teixeira A, Lopes-Lima M, Machado J, Guilhermino L. 2012. Massive die-offs of freshwater bivalves as resource pulses. In *Annales de Limnologie-International Journal of Limnology* (Vol. 48, No. 1, pp. 105-112). EDP Sciences.

Sousa R, Novais A, Costa R, Strayer DL. 2014. Invasive bivalves in fresh waters: impacts from individuals to ecosystems and possible control strategies. *Hydrobiologia*, *735*(1), pp.233-251.

Strayer DL, Smith LC. 1996. Relationships between zebra mussels (Dreissena polymorpha) and unionid clams during the early stages of the zebra mussel invasion of the Hudson River. *Freshwater Biology*, *36*(3), pp.771-780.

Taylor JD, Glover EA, Williams ST. 2009. Phylogenetic position of the bivalve family Cyrenoididae--Removal from (and further dismantling of) the superfamily Lucinoidea. *Nautilus*, *123*(1), p.9.

Trombulak SC, Frissell CA. 2000. Review of ecological effects of roads on terrestrial and aquatic communities. *Conservation biology*, *14*(1), pp.18-30.

United States. Environmental Protection Agency, 2002. *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms* 5th ed., Washington, D.C.

Van Hassel JH, Farris JL. 2007. A review of the use of unionid mussels as biological indicators of ecosystem health. *Freshwater bivalve ecotoxicology*, pp.19-49.

Vaughn, CC. 2018. Ecosystem services provided by freshwater mussels. *Hydrobiologia*, *810*(1), pp.15-27.

Verbrugge LN, Schipper AM, Huijbregts MA, Van der Velde G, Leuven RS. 2012. Sensitivity of native and non-native mollusc species to changing river water temperature and salinity. *Biological Invasions*, *14*(6), pp.1187-1199.

Wächtler K, Dreher-Mansur MC, Richter T. 2001. Larval types and early postlarval biology in naiads (Unionoida). In *Ecology and evolution of the freshwater mussels Unionoida* (pp. 93-125). Springer, Berlin, Heidelberg.

Ward, JE. 1996. Biodynamics of suspension-feeding in adult bivalve molluscs: particle capture, processing, and fate. *Invertebrate Biology*, pp.218-231.

Watters, GT. 1999, March. Freshwater mussels and water quality: A review of the effects of hydrologic and instream habitat alterations. In *Proceedings of the First Freshwater Mollusk Conservation Society Symposium* (Vol. 1999, pp. 261-274). Columbus: Ohio Biological Survey.

Wehner F, Olsen H, Tinel H, Kinne-Saffran E, Kinne RK. 2003. Cell volume regulation: osmolytes, osmolyte transport, and signal transduction. In *Reviews of physiology, biochemistry and pharmacology* (pp. 1-80). Springer, Berlin, Heidelberg.

Williams JD, Warren Jr ML, Cummings KS, Harris JL, Neves RJ. 1993. Conservation status of freshwater mussels of the United States and Canada. *Fisheries*, *18*(9), pp.6-22.

Xiao BC, Li EC, Du ZY, Jiang RL, Chen LQ, Yu N. 2014. Effects of temperature and salinity on metabolic rate of the Asiatic clam Corbicula fluminea (Müller, 1774). *SpringerPlus*, *3*(1), p.455.

Yeager MM, Neves RJ, Cherry DS. 1999, March. Competitive interactions between early life stages of Villosa iris (Bivalvia: Unionidae) and adult Asian clams (Corbicula fluminea). In *Freshwater Mollusk Symposium Proceedings—Part II: Proceedings of the First Freshwater Mollusk Conservation Society Symposium* (pp. 253-259).

Zanatta DT, Murphy RW. 2006. Evolution of active host-attraction strategies in the freshwater mussel tribe Lampsilini (Bivalvia: Unionidae). *Molecular Phylogenetics and Evolution, 41*(1), pp.195-208.

VITA

JOHN WARREN RODEN III

Education:	M.S. Biology, East Tennessee State University, Johnson City, Tennessee, 2020
	B.S. Biology, East Tennessee State University, Johnson City, Tennessee, 2018
	Chattanooga Christian School, Chattanooga, Tennessee
Professional Experience:	Graduate Assistant, East Tennessee State University, College of Arts and Sciences, 2018-2020
	Pest Survey Specialist, United States Department of Agriculture, Animal and Plant Health Inspection Service, 2019-2020
	Benthic Invertebrate Survey Specialist, Wetland and Stream Restoration Services, LLC, 2019
Honors and Awards:	Denise Pav Scholarship Award, East Tennessee State University, Department of Biological Sciences, 2020
	William Harvey Fraley and Nina M. Fraley Memorial Research Award, East Tennessee State University, Department of Biological Sciences, 2019
	University Honors Scholar, East Tennessee State University, 2014