FINAL PROJECT REPORT FOR THE SOUTHEAST CLIMATE ADAPTATION SCIENCE CENTER

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Project title: Impacts of sea level rise and associated salinity changes on at-risk native freshwater mussels and their habitats in Atlantic coastal rivers

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2. PUBLIC SUMMARY: Sea levels across the planet are rising, and particularly, along the eastern coast of the United States. Climate-induced sea level rise can result in the inundation and intrusion of seawater into freshwater drainages. This would alter salinity regimes and lead to the salinization of coastal freshwater ecosystems. Increased salinity levels in freshwater can negatively affect freshwater dependent species, including native mussels belonging to the order Unionida, which are highly sensitive to changes in water quality. Sea salt is largely made up of sodium (Na^{+}) and chloride (Cl^{-}) ions, forming sodium chloride (NaCl), a known toxicant to freshwater mussels. However, sea salt is a mixture that also contains other major ions, including potassium (K^{+}), sulfate (SO_4^{--}), calcium (Ca^{++}), strontium (Sr^{++}), and magnesium (Mg^{++}) among others. Freshwater mussels exposed to sea salt would be exposed to each of the sea salt ions at the same time, resulting in a mixture toxicity effect. The mixture toxicity of these ions on early life stages of freshwater mussels is largely unknown because most research to date has evaluated individual salt ions in relative isolation. Therefore, we conducted acute toxicity tests on early life stages (glochidia (larvae) and juveniles) of three freshwater mussel species that inhabit Atlantic Slope drainages (non-salinity adapted *Atlanticoncha ochracea*, salinity adapted *Atlanticoncha ochracea*, *Sagittunio nasutus*, and *Utterbackiana implicata*). Glochidia and juveniles of each species were exposed to a control and 6 concentrations of Instant Ocean® Sea Salt (IOSS), a synthetic sea salt that closely resembles the ionic composition of natural sea salt. Exposure concentrations were 1 part(s) per thousand (ppt), 2 ppt, 8.5 ppt, 12.5 ppt, 17 ppt, and 34 ppt. We calculated the median effective concentration (EC50) for each of the 8 acute toxicity tests and found that glochidia were more sensitive than juveniles to IOSS. EC50s at hour 24 for the glochidia ranged from 0.38-3.6 ppt, with the most sensitive freshwater mussel being the non-salinity adapted *Atlanticoncha ochracea*, exhibiting an EC50 of 0.38 ppt (95% C.I. = 0.33-0.44). Juvenile freshwater mussels exhibited EC50s at hour 96 ranging from 5.0-10.4 ppt, with the least sensitive freshwater mussel being the non-salinity adapted *Atlanticoncha ochracea*, exhibiting an EC50 of 10.4 ppt (95% C.I. = 9.1-12.0). Our results show that acute exposure to sea salt adversely affects freshwater mussel viability, particularly glochidia. This information can be used by natural resource managers and to enhance freshwater mussel conservation strategies in regions that are, or will be impacted by climate-induced sea level rise and associated freshwater salinization.

3. TECHNICAL SUMMARY: The aim of this research project was to investigate the adaptation and vulnerability potential of a native freshwater mussel living in coastal riverine drainages to climate-induced, sea level rise, specifically fluctuating salinity, temperature, and flow regimes. We accomplished this aim and more by conducting eight separate acute toxicity tests with sea salt and three species (from four populations) of Atlantic Slope mussels. In addition, we also completed two 28-day chronic toxicity tests on sub-adult mussels of two of the three Atlantic Slope species evaluating sublethal effects of sea salt when exposed in reconstituted water or in their natural waters. We discovered that that the glochidia (larvae) were the most sensitive life stage to sea salt and that they represent a critical life history and reproductive bottleneck, meaning that if the larvae cannot survive at these concentrations, then there will be no additional contribution of juveniles or adults to existing populations. Our results greatly advanced the understanding of the mixture toxicity of sea salt ions to native freshwater mussels. Moreover, they provide a set of benchmark values from which to judge future adverse effects on mussel populations in coastal drainages and to understand the critical threshold ion concentrations for their future protection and conservation. Our findings have regional, national, and international applicability because saltwater intrusions into mussel habitat is occurring worldwide.

4. PURPOSE AND OBJECTIVES: The objectives of our study were to: (1) assess the vulnerability of the Tidewater Mucket (*Atlanticoncha ochracea*), an imperiled freshwater mussel species that resides in lower Atlantic Slope coastal drainages, to salinity by conducting standard sensitivity tests with early life stages (e.g., larvae, juveniles) of the mussel under controlled laboratory conditions; (2) determine the potential effects of natural riverine salinity gradients on adult mussels by conducting a reciprocal transplant experiment with salinity adapted and non-salinity adapted mussels; and (3) develop a risk-based scenario of mussel salinity tolerances in existing occupied habitats incorporating predictions in sea level rise and projected salinity ranges. The project served the needs of the research community, as well as the collaborating state and federal natural resource management partners, the North Carolina Wildlife Resources Commission, the Virginia Department of Wildlife Resources, and the U.S. Fish and Wildlife Service. In our study, we were able to expand the number of Atlantic Slope mussel species tested from the one species originally proposed to three species, with the help of our hatchery partners in Virginia at the Harrison Lake National Fish Hatchery. The addition of these two other coastal species enabled us to provide more robust and value-added information about the effects of sea salt on mussels.

5. ORGANIZATION AND APPROACH:

Test organisms

Of the mussel species used in this study, the Tidewater Mucket that we tested came from two populations which were operationally defined as salinity adapted and non-salinity adapted. The *Atlanticoncha ochracea* collected from the Chowan River and Nottoway River, as well as their progeny, were referred to as salinity adapted, because a salt wedge could potentially reach the locations from where they were collected, and because salinity concentrations have been measured at a maximum of 4.7 ppt and 0.3 ppt, respectively, at or near their collection locations. The *Atlanticoncha ochracea* collected from Lake Gaston, as well as their progeny, were referred to as non-salinity adapted, since Lake Gaston was dammed in 1963, which eliminated the possibility of a salt wedge to move upstream, enter the lake, and affect mussel populations. In addition, the dam impounding Lake Gaston prevented

the upstream movement of host fish infected with larval *A. ochracea* from the lower portion of the river to potentially deposit salinity adapted juveniles in Lake Gaston. Lastly, salinity concentrations in Lake Gaston have not been measured above 0 ppt at or near the collection location of these mussels. For each species, gravid female mussels were collected in the wild via snorkel survey. Mussels were then transported to Harrison Lake National Fish Hatchery (HLNFH) for propagation, following handling and transport methods similar to those recommended by Cope et al. (2003). Once at the HLNFH, gravid females were kept in oxygenated tanks filled with water from a HLNFH pond. Non-salinity adapted *Atlanticoncha ochracea* were collected from Lake Gaston, NC, and salinity adapted *Atlanticoncha ochracea* were collected from the Chowan River, NC and the Nottoway River, VA. Salinity adapted *Sagittunio nasutus* were collected from the Nottoway River, VA, and salinity adapted *Utterbackiana implicata* were collected from the Rappahannock River, VA. Mussels collected from the Chowan, Nottoway, and Rappahannock were expected to be more tolerant to sea salt than mussels collected from Lake Gaston; therefore, mussels collected from the Chowan, Nottoway, and Rappahannock Rivers were operationally defined as salinity adapted. However, only *A. ochracea* collected from the Chowan River, and their progeny were referred to as salinity adapted within this manuscript.

Glochidia propagation and acclimation

Glochidia from each mussel species were extracted from the gravid females by flushing their marsupia with a syringe and water according to their population (salinity adapted *A. ochracea*, non-salinity adapted *A. ochracea*, *S. nasutus*, and *U. implicata*). Glochidia from at least three females of the same species were then pooled together for propagation and testing according to procedures at the HLNFH. *A. ochracea* from the Nottoway River, VA were not used in glochidia tests because we had a sufficient number of glochidia from the Chowan River, NC to conduct testing. In preparation for testing, glochidia were obtained from HLNFH on the same day as the female glochidia extraction and transported (~3 hour travel duration) to the Aquatic Toxicology Laboratory at NC State University (Raleigh, NC) in 50 mL centrifuge tubes filled with HLNFH pond water. Centrifuge tubes were placed within a cooler to maintain the water temperature within the centrifuge tubes. Approximately 2,000-5,000 glochidia were in each centrifuge tube. After initial water temperature was measured at HLNFH, glochidia mussels were acclimated to the test temperature of 25°C at a rate of no more than 3°C per hour. Mussels were also acclimated to the test water at a rate of 25% volume exchange per hour. The test water used was soft water formulated to ASTM International standards (ASTM, 2013). These acclimation processes were typically initiated upon mussel pick up at HLNFH to maximize time efficiency. Although all mussels were acclimated to ASTM test water for the testing duration, mussels retained the operational definition of salinity adapted or non-salinity adapted because of the mussels' expected tolerance or intolerance to sea salt due to each population's long-term genetic lineage. Acclimation to test water and temperature took no more than 8 hours for each species. After 100% ASTM test water concentration and test water temperature were reached, a subset of 250 mussels from each female (or pool) underwent an initial viability assessment by counting the number of closed glochidia after adding a saturated salt solution subtracted by the number of closed glochidia before adding the saturated salt solution divided by the number of open and closed glochidia after adding the saturated salt solution (ASTM, 2013). This was done at the NC State University Aquatic Toxicology Laboratory. If glochidia viability was >80%, mussels were distributed to test chambers.

Juvenile propagation and acclimation

Juveniles from each mussel species were transformed according to their population (salinity adapted *A. ochracea*, non-salinity adapted *A. ochracea*, *S. nasutus*, and *U. implicata*) by in-vivo propagation methods following HLNFH protocol, using glochidia obtained from our gravid females. Juvenile mussels were grown out in the HLNFH pond(s). Host fish used for *A. ochracea, S. nasutus,* and *U. implicata* transformations were *Morone americana* (White Perch), *Micropterus salmoides* (Largemouth Bass), and *Alosa aestivalis* (Blueback Herring), respectively. All juveniles for testing were obtained from HLNFH 3 months after their excystment from host fish, then transported to the Aquatic Toxicology Laboratory at NC State University (Raleigh, NC) in a 500-mL polyethylene Nalgene® bottle filled with HLNFH pond water. Approximately 250 juveniles were in each bottle. Juvenile mussels were acclimated by the same methods used during glochidia mussel acclimation and retained their operational definition of salinity adapted or non-salinity adapted based on the justification given for glochidia. After 100% ASTM test water concentration and test water temperature were reached (which took no more than 8 hours), mussels were held for 24 hours. After the holding time was complete, mussels were visually assessed for viability by looking for foot movement or heartbeat during a 5-minute observation period, and active mussels were placed in their test chambers (ASTM, 2013). In juvenile acute toxicity tests, 90% of control mussels are expected to survive until the end of test (ASTM, 2013). This can help gauge the initial condition of the mussels because there is no standard initial viability assessment aside from visual inspection. Viability was assessed at hour 48 and hour 96. Juvenile mussels were not feed during the acclimation period.

Photographs were taken for a subset of mussels at the time of arrival at the laboratory and the individual mussels were measured to the nearest millimeter using a Leica® EZ4 D stereo microscope with integral digital camera and Leica Application Suite EZ digital photographic software (Leica Microsystems, Ltd., Switzerland). Average shell lengths were 0.74 mm and 0.85 mm for non-salinity adapted and salinity adapted *A. ochracea*, respectively; 1.58 mm for *S. nasutus*; and 11.36 mm for *U. implicata.*

Glochidia toxicity testing

To determine the sensitivity of glochidia to sea salt, we exposed mussels from each of our four populations to six concentrations of IOSS (Spectrum Brands, Blacksburg, VA), totaling four separate tests. Each test consisted of a control with no sea salt and six treatment concentrations: 1 ppt, 2 ppt, 8.5 ppt, 12.5 ppt, 17 ppt, and 34 ppt. Mussels were tested at a temperature of 25 °C with a 12:12 hour light: dark photoperiod. Each treatment concentration and the control consisted of three replicate exposures with approximately 250 glochidia per replicate in 250-mL evaporating dishes. Tests were 48-hour non-aerated static tests, where viability was assessed at hour 24 and hour 48 using a subset of 50 impartially selected mussels per replicate. Test were conducted for 48 hours to simulate effects on glochidia that may have remained in the water column more than 24 hours after release from the female. Water quality conditions were measured at the beginning of each test, at hour 24 before the viability assessment, and at hour 48. Initial viability was calculated by counting the number of closed glochidia after adding a saturated salt solution subtracted by the number of closed glochidia before adding the saturated salt solution divided by the number of open and closed glochidia after adding the saturated salt solution (ASTM, 2013). The median effective concentration (EC50) of IOSS was calculated for each of the four tests. IOSS was selected as the toxicant because it closely resembles the ionic makeup of natural seawater. The six treatment concentrations were chosen based on salinity regimes that occur in lower coastal drainages of the Atlantic Slope, capturing a range of possible exposures.

Juvenile toxicity testing

To determine the sensitivity of juvenile mussels to sea salt, we exposed mussels from each of our four populations to six concentrations of IOSS, totaling four separate tests. Each test consisted of a control with no sea salt and six treatment concentrations: 1 ppt, 2 ppt, 8.5 ppt, 12.5 ppt, 17 ppt, and 34 ppt. Mussels were tested at a temperature of 25 °C with a 12:12 hour light: dark photoperiod. Each treatment concentration and the control consisted of three replicate exposures with 10 active mussels per replicate in 250-mL evaporating dishes. Tests were 96-hour non-aerated static-renewal tests, where a 90% water and toxicant renewal was conducted at hour 48. Water quality conditions were measured at the beginning of each test, at hour 48 before the water renewal, and at hour 96. Mussel mortality was assessed at hour 48 and hour 96. Mussel mortality was determined by observing foot movement, active syphoning, or heartbeat for 5 minutes, and examining the integrity of tissues within the shell (ASTM, 2013). The EC50 for IOSS was calculated for each of the four tests.

Quality assurance

All tests were conducted according to the ASTM International Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels (2013). All glochidia tests had >90% control viability at the end of hour 24 and all juvenile tests had >90% control viability at test termination (hour 96). Water quality conditions of temperature, conductivity, salinity, and dissolved oxygen were measured with a calibrated meter (YSI model 566 MPS multi-probe, Yellow Springs Instrument Co., Yellow 12 Springs, Ohio) from a composite water sample at each treatment concentration. pH was also measured from a composite water sample at each treatment concentration with a calibrated meter (Beckman Coulter model PHI410, Beckman Coulter, Inc., Brea, California). Alkalinity and hardness were measured using standard titrimetric procedures. Average water quality (and ranges) among all tests were: temperature 21.3 ºC (20.1-23.2); conductivity 16856 µS/cm (136-53697); dissolved oxygen 7.4 mg/L (3.8-13.6); pH 7.8 (6.7-8.7); alkalinity 42 mg/L $CaCO_3$ (18-138); hardness 51 mg/L $CaCO_3$ (16-92). The water test temperature was maintained at 25 ºC within each test incubator; however, water temperature of some samples collected for water quality measurements likely cooled to near ambient room temperature (~ 21 ºC) during the viability assessments and before measurement; hence the difference between incubator set temperature and average measured temperature. For juvenile tests, toxicant exposure concentrations were verified by RTI International (Research Triangle Park, NC) with a Thermo iCAP7600 ICP-OES equipped with an autosampler enclosed in a HEPA filtered exhaust box for Na^+, sulfur (S^{2-}), Ca^{++}, Mg^{++}, Sr^{++}, and K^+. Cl^- concentrations were verified with a Thermo Gallery Plus discrete analyzer. For glochidia tests, toxicant exposure concentrations were verified by NC State University Environmental and Agricultural Testing Service (Raleigh, NC) with a Perkin Elmer ICP-OES Optima 8000 for Na^+, S^{2-}, Ca^{++}, Mg^{++}, and Sr^{++} for glochidia tests. K^+ concentrations were not quantified for glochidia tests due to constraints associated with the analytical laboratory. Cl^- concentrations were verified with a Haake-Buchler model 442-5000 Digital Chloridometer. S^{2-} concentrations were multiplied by three for conversion to SO_4^{--} concentrations because the molar mass of SO_4^{--} is three times greater than that of S^{2-} due to the mass of the one S^{2-} atom and four adjoining oxygen atoms. After receiving the water chemistry results, each sea salt ion within each treatment concentration (1 ppt, 2 ppt, 8.5 ppt, 12.5 ppt, 17 ppt, and 34 ppt) was summed to calculate the total salt concentration of IOSS for that treatment concentration. This was done for each IOSS treatment for each mussel test. Briefly, the average percent recovery (and range) of each sea salt treatment as calculated by the sum of ions in the IOSS among all tests were as follows (all were in acceptable limits): 1 ppt 108% (76-142); 2 ppt 120% (90-176); 8.5 ppt

120% (105-135); 12.5 ppt 121% (106-135); 17 ppt 128% (106-151); 34 ppt 125% (102-148). All EC50 data are presented based on measured salt concentrations rather than target concentrations even though there was agreement between the two in analytical recoveries. The corresponding osmolarity for each nominal treatment concentration (1 ppt, 2 ppt, 8.5 ppt, 12.5 ppt, 17 ppt, and 34 ppt) were as follows: 33.2, 66.4, 282.0, 414.7, 564.0, and 1128.0 osmol/L, respectively.

Statistical analysis

The median effective concentration, or EC50, was the concentration of the toxicant producing an adverse effect to 50% of the test population in the specified time. EC50s were estimated with the Comprehensive Environmental Toxicity Information Software package (CETIS) (v1.9.7.10, Tidepool Scientific, LLC, McKinleyville, California). Confidence intervals of 95% were calculated for each EC50, and EC50s with non-overlapping confidence intervals were considered statistically different.

6. PROJECT RESULTS:

Glochidia acute toxicity tests

Salinity toxicity tests with IOSS and glochidia of four mussel populations (salinity adapted *A. ochracea*, non-salinity adapted *A. ochracea*, *S. nasutus*, and *U. implicata*) showed that EC50s at hour 24 ranged from 0.38-3.6 ppt. The most sensitive species was the non-salinity adapted *A. ochracea*, whereas the least sensitive species was the *U. implicata*. EC50s for all glochidia tests were statistically different.

Juvenile acute toxicity tests

The juvenile mussel IOSS toxicity tests revealed that EC50s at hour 96 ranged from 5.0-10.4 ppt. The most sensitive species was the *U. implicata*, whereas the least sensitive species was the non-salinity adapted *A. ochracea*. The EC50 for the non-salinity adapted *A. ochracea* was statistically different from each of the other three species. The EC50s for the salinity adapted *A. ochracea, S. nasutus, and U. implicata* were not statistically different from one another.

Proportion of individual ions in glochidia and juvenile IOSS treatments

Because we measured the concentrations of the individual major ions in the mixture of IOSS treatments to calculate the total salt concentration of IOSS for each treatment, we could also calculate the proportion of ions present in each treatment. Mean concentrations of each individual major ion was present in IOSS treatments proportionally according to the product formula. Major ion concentrations for IOSS treatments in glochidia tests ranged from 128.7-20913.4 mg/L for Cl^-, 136.5-13077.7 mg/L for Na^+, 11.9-486.6 mg/L for Ca^{++}, 20.8-1652.8 mg/L for Mg^{++}, 0.01-10.7 mg/L for Sr^{++}, and 74.8-3728.4 mg/L for SO_4^{--}. Major ion concentrations for IOSS treatments in juvenile tests ranged from 479.1-33056.4 mg/L for Cl^-, 320.6-18803.2 mg/L for Na^+, 24.4-614.0 mg/L for Ca^{++}, 44.6-2467.9 mg/L for Mg^{++}, 9.2-668.8 mg/L for K^+, 0.3-16.7 mg/L for Sr^{++}, and 119.6-4079.7 mg/L for SO_4^{--}. The ratios of Na^+ to K^+ in our treatment concentrations ranged from 28:1 to 35:1. Knowing the proportions of ions present in each IOSS treatment was useful when examining the calculated IOSS EC50s because we could observe the concentration of each measured ion at or near an EC50. These ion concentration data could also be used to compare with the published literature from ion-specific mussel toxicity studies, as a resource for expected ion concentrations at or near a particular salt (IOSS) EC50, depending on the toxicant.

7. ANALYSIS AND FINDINGS:

Our findings showed that IOSS is toxic to both glochidia and juvenile life stages of freshwater mussels, but that glochidia were the most sensitive life stage, representing a critical life stage and reproductive bottleneck. This is consistent with previous research that has shown glochidia to be the most sensitive life stage, and that glochidia are more sensitive to NaCl than juveniles. The greatest recorded salinity value nearest to each of our mussel collection sites were: 0 ppt, 4.8 ppt, 0.3 ppt, and 0.1 ppt for Lake Gaston, the Chowan River, the Nottoway River, and the Rappahannock River, respectively. It is unknown if glochidia and juvenile salinity tolerances would be different, if exposed to IOSS while in their source natural waters. This idea is worth exploring in the future because it could result in more environmentally relevant results. Limited previous research has found that glochidia exposed to salt in natural waters were 4x less sensitive to Cl^- than glochidia exposed to salt in reconstituted waters. Overall, we are unsure if exposure to IOSS enhances or reduces the toxicity of the individual IOSS component ions, but our findings suggest there may be substantial differences between individual salt ion toxicity depending on the other salt ions present at the time of exposure. It would be beneficial to conduct acute toxicity tests with each major salt ion and IOSS side-by-side under identical conditions. We suggest future exploration of individual salt ion toxicity compared to the mixed salt toxicity of IOSS.

In a natural riverine environment, glochidia EC50 results are most applicable to glochidia that have been released from a female and are not yet encysted onto a host fish, as occurred in our acute toxicity tests. Glochidia that are still brooding within the female marsupial gills or have encysted on a host fish are thought to receive some measure of protection from waterborne toxicants, such as sea salt, although very little is understood regarding these two stages of development in relation to toxicant exposures. Juvenile mussels have no “outside organismal” protection from waterborne toxicants, although they are thought to remain burrowed in the sediment for the first few years of their life. This may provide some protection from surface water exposures but would result in other exposures through sediment porewater. If surface water and porewater are converted from freshwater to saltwater via saltwater intrusion and inundation, juvenile mussels may be adversely affected even though they seem to tolerate higher salinity levels than glochidia. It would be beneficial to investigate the toxicity of sea salt to juvenile mussels burrowed in sediment, as well as the brooded and encysted glochidia. However, our research indicates that glochidia are the most vulnerable life stage regarding changes in water salinity.

8. CONCLUSIONS AND RECOMMENDATIONS:

The potential effects of prior salinity exposure and possible adaptation by mussels was addressed in our study by collecting *A. ochracea* broodstock from both an isolated freshwater ecosystem (Lake Gaston, with no salinity exposure for nearly 60 years) and salinity influenced lower coastal rivers. The mussel population that was most sensitive to IOSS was the non-salinity adapted *A. ochracea* glochidia, which were the progeny of gravid females collected from Lake Gaston. These glochidia had an EC50 value of 0.38 ppt (95% C.I. = 0.33-0.44) at hour 24. We expected and hypothesized that the non-salinity adapted *A. ochracea* would be the most sensitive due to its lack of previous exposure to sea salt. Mussels in Lake Gaston are prevented from sea salt exposure due to Lake Gaston’s geographical location in the upper to central portion of the Roanoke River watershed. Lake Gaston is the second reservoir in a series of three dammed impoundments on the Roanoke River. The construction of Lake Gaston dam in 1963 eliminated the possibility of a salt wedge to move upstream, enter the lake, and affect mussel populations. In addition, the dam impounding Lake Gaston prevented the upstream movement of host fish infected with *A. ochracea* from the lower portion of the river to potentially deposit salinity adapted juveniles in Lake Gaston. In contrast to our hypothesis, the non-salinity adapted *A. ochracea* was the least sensitive

juvenile mussel population, with an EC50 of 10.4 ppt (95% C.I. = 9.1-12.0) at hour 96. For comparison, the juvenile salinity adapted *A. ochracea* was twice as sensitive to IOSS with an EC50 of 5.5 ppt (95% C.I. = 4.9-6.2) at hour 96. Juvenile salinity adapted *A. ochracea* were the progeny of gravid females collected from the Chowan River, NC and the Nottoway River, VA. Water salinity concentrations near these mussel collection sites have reached documented concentrations of 4.8 ppt in the Chowan River and 0.30 ppt in the Nottoway River. Results from our *A. ochracea* glochidia toxicity tests with IOSS supported the salinity non-adaptation hypothesis, but the results from the corresponding juvenile toxicity tests did not. It is unknown why the non-salinity adapted *A. ochracea* was the most sensitive to sea salt as glochidia, but the least sensitive as juveniles. Because juvenile non-salinity adapted *A. ochracea* can tolerate salinity concentrations around 10 ppt, it is possible that individuals from their source population in Lake Gaston could be used to propagate juvenile mussels to restore or augment riverine *A. ochracea* populations in the event of extirpation due to river salinization. Replicating the glochidia and juvenile toxicity tests for non-salinity adapted and salinity adapted *A. ochracea* would be beneficial for further validation of our results. Little is known about the differences in toxicant sensitivities regarding riverine and lacustrine mussels of the same species. Exploring these possible differences with *A. ochracea* and/or different mussel species could increase knowledge and aid freshwater mussel conservation efforts.

9. MANAGEMENT APPLICATIONS AND PRODUCTS: Overall, our research demonstrated that sea salt is toxic to freshwater mussels at environmentally relevant salinities and that glochidia are more sensitive than juveniles, representing a critical life stage and reproductive bottleneck. Our findings provide natural resource managers with previously unknown information regarding the vulnerability of glochidia and juvenile freshwater mussels to sea salt. Results from this study can also guide conservation programs and allow decision makers to better plan and prepare for saltwater intrusion and inundation events because climate-induced sea level rise not only poses a risk to the survival and persistence of freshwater mussels in coastal drainages across the globe, but other salt sensitive species as well.

Below is a quote attributable to a stakeholder/partner in the project that describes the way(s) in which results of the project have or will be used.

"This research on the toxicity of sea salt to the early life stages of freshwater mussels provides valuable insight on the growing threat of sea level rise and other sources of salinity to imperiled mussel fauna. These findings contribute to the best available science to inform multiple aspects of work in the US Fish and Wildlife Service, such as threat analysis in Species Status Assessments or listing decisions for mussels, and selecting appropriate sites for activities that support species recovery, such as translocation or reintroduction to sites where animals will be protected from potentially toxic levels of salinity."

Jennifer M. Archambault, Deputy Field Supervisor, US Fish and Wildlife Service

10. OUTREACH AND COMMUNICATION:

PRESENTATIONS AT SCIENTIFIC MEETINGS

Explanation of Authorship: Presenting Author is Underlined

McIver, J. K., W. G. Cope, N. J. Hostetter, R. Boyles, T. J. Kwak, T. Ben-Horin, F. Weber, J. Nelson, A. Maynard, A. Glen, B. Watson, and M. Fisk. 2023. Acute and chronic effects of sea salt to freshwater mussels: implications for climate-induced sea level rise in coastal rivers. 13th Biennial Symposium of the Freshwater Mollusk Conservation Society, Portland, OR, April 10-14, 2023.

McIver, J. K., W. G. Cope, N. J. Hostetter, R. Boyles, T. J. Kwak, T. Ben-Horin, F. Weber, J. Nelson, and B. Watson. 2023. Chronic effects of sea salt on organ tissues of sub-adult freshwater mussels in reconstituted and natural waters. 34th Annual Meeting of the North Carolina Chapter of the American Fisheries Society, Durham, NC. February 21-23, 2023.

Cope, W. G., J. K. McIver, and R. Boyles. 2022. The salinization of coastal rivers from sea level rise: implications for freshwater mussels. 43rd Annual Meeting of the Society of Environmental Toxicology and Chemistry, Pittsburgh, PA. November 12-16, 2022.

McIver, J. K., W. G. Cope, T. J. Kwak, R. Boyles, A. Maynard, A. Glen, B. Watson, and M. Fisk. 2022. Assessing the toxicity of sea salt to freshwater mussels: implications for sea level rise in coastal rivers. American Fisheries Society North Carolina Chapter Annual Meeting, Morganton, NC. May 31-June 2, 2022. **Winner of the Richard L. Noble Best Student Platform Presentation Award**.

McIver, J. K., W. G. Cope, T. J. Kwak, R. Boyles, A. Maynard, A. Glen, B. Watson, and M. Fisk. 2022. Assessing the cumulative toxicity of major sea salt ions to freshwater mussels. Society of Environmental Toxicology and Chemistry Carolinas Chapter Annual Meeting, Research Triangle Park, NC. April 6-8, 2022.

RESEARCH PUBLICATIONS

McIver, J. K., W. G. Cope, R. B. Bringolf, T. J. Kwak, B. Watson, A. Maynard, and R. Mair. 2023. Assessing the toxicity of sea salt to early life stages of freshwater mussels: implications for sea level rise in coastal rivers. *Environmental Toxicology and Chemistry*. 42: in press.

McIver, J. K. 2022. Freshwater mussels and river salinization: potential impacts from climate-Induced sea level rise. M.S. Thesis, NC State University, Raleigh, NC, 135 p.

OUTREACH PRODUCTS

Torres-Molinari, Á., J. K. McIver, W. G. Cope, T. J. Kwak, R. Boyles, N. J. Hostetter, A. Maynard, A. Glen, B. Watson, and M. Fisk. 2023. Sea salt is lethal to early life stages of freshwater mussels in North Carolina. An infographic for state and federal natural resource management partners. United States Geological Survey (USGS) and USGS Southeast Climate Adaptation Science Center, Raleigh, NC, 1p.

McIver, J. K. 2023. Impacts of sea level rise and associated salinity changes on at-risk native freshwater mussels and their habitats in Atlantic coastal rivers. Invited seminar for the USGS Southeast Climate Adaptation Science Center, April 20, 2023.