Influence of Interstitial Sediments on an Endangered Freshwater Mussel Population

A Thesis
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Abstract

INFLUENCE OF INTERSTITIAL SEDIMENTS ON AN ENDANGERED FRESHWATER MUSSEL POPULATION

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Anthropogenic activities alter natural systems, resulting in both direct and indirect impacts to biota. Erosion and transport of sediment and associated pollutants to rivers and other aquatic systems are among the most commonly-reported yet poorly-understood water quality stressors. Freshwater mussels are endofaunal benthic invertebrates that spend much of their lives buried within sand and gravel substrates but appear to be sensitive to changes in concentrations of fine sediments associated with agriculture, urbanization and infrastructure development. I examined the role of sedimentation associated with a highway expansion project on an Appalachian elktoe (*Alasmidonta raveneliana)* mussel population in the South Toe River in western North Carolina. I compiled yearly mussel abundance data, used freeze cores to extract and quantify interstitial substrate and conducted paired field and lab experiments using juvenile mussels in order to better understand the degree to which sediment composition affects mussel population size as well as juvenile growth and survival. Population data reveal that only 1 of 6 long-term mussel monitoring sites appeared to have a stable Appalachian elktoe population. Populations at all other sites are very small and several appear to be currently experiencing declines. Freeze cores revealed that although fine sediment concentrations were higher downstream of the highway project, consistent differences were only observed at one monitoring site located immediately downstream of a heavily-impacted tributary that flows through the roadcut. Sentinel juvenile mussels at the three sites downstream of the highway crossing displayed reduced growth and survival compared to mussels at upstream sites. However, no differences in growth or survival were detected among sediment treatments in hatchery tank trials. Higher mortality rate, however, was observed in mussels grown in impacted site sediments relative to mussels grown in sediments from the upstream sites. Taken together, these data suggest that road construction may be contributing to Appalachian elktoe declines in the South Toe River but the mechanism does not appear to be direct impacts of fine sediments. Instead, the impacts of fine sediments are likely sub-lethal and may involve alteration of streambed microhabitats or exclusion of mussels from the hydraulic refugia that facilitate persistence in this high-gradient mountain stream.

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Dedication

I dedicate this thesis to Alton Harris, who instilled in me my love for the outdoors along with the importance of taking initiative, working hard and loving life.

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Foreword

The research detailed in this thesis will be submitted to the peer-reviewed journal *Freshwater Science*. This thesis has been formatted according to the style requirements for publication in this journal.

Introduction

Freshwater mussels are an often overlooked, yet ecologically-significant element of aquatic ecosystems. At least 840 taxa are currently recognized globally and the greatest concentration of freshwater mussel species (200+ taxa) occurs in southeastern North America (Graf and Cummings 2007). Freshwater mussel biomass may dominate benthic communities and mussels provide a link between the water column and both epi- and endo-benthic habitats (Negus 1966, Vaughn and Hakenkamp 2001, Haag 2012). Freshwater mussels also provide important ecosystem services including bio-filtration, nutrient sequestration, and habitat stabilization and serve as important links between filter-feeder and higher trophic levels in stream food webs (Vaughn 2018). Unfortunately, freshwater mussels are among the most imperiled groups of aquatic organisms. Approximately 10% of United States freshwater mussel species are extinct and ~28% are protected by the US Endangered Species Act of 1973 (United States 1973), with recent assessments suggesting that >65% of U.S. freshwater mussels may be imperiled (Haag and Williams 2014).

There are many anthropogenic stressors causing freshwater mussel population declines. Anthropogenic land use change is among the most widely-cited stressors in freshwater ecosystems (Kunz 1898, Ellis 1936, Strayer et al. 1999). Urban development, agriculture, and deforestation transform upland environments and the loss of forest cover and increase in impervious surface and sediment inputs can have profound implications for freshwater ecosystems and sensitive biota. Spatiotemporally localized disturbances including road construction are more widespread and affect many otherwise pristine river systems (Beschta 1978, Wheeler et al. 2005, Merz et al. 2006, Cocchiglia et al. 2012). As of 2014, there were ~6.5 million km of roads within the United States and $164.5 billion US state, local and federal dollars were spent on road construction, improvement, and repair in the United States during that one year alone (ASCE 2017). In North Carolina alone, ~$2.5 billion was spent on road construction during the 2018-19 Fiscal Year (NCDOT 2018).

Sedimentation is the most commonly-discussed environmental issues associated with road construction runoff (Henley et al. 2000, Wheeler et al. 2005, Hedrick et al. 2010). Sedimentation can impact sensitive biota in aquatic communities and has been a proposed cause of mussel population declines since the late 1800s (Kunz 1898, Henley et al. 2000). Although habitat use and substrate size preferences are variable among freshwater mussel species (reviewed in Ellis 1936 and Harman 1972), numerous studies have found that elevated concentrations of benthic and suspended fine particles appear detrimental to mussels. Silts and clays can occlude gill surfaces, interfere with filter-feeding and stress brooded larvae (Ellis 1936, Aldridge et al. 1987). Brim-Box and Mossa (1999) reported that sedimentation may indirectly affect mussel feeding by reducing the amount of photosynthetic food available. The increase of fine sediments may also reduce mussel recruitment (Kreutzweiser and Capell 2001, Österling et al. 2010). Juvenile mussels live buried beneath the surface of the river substrate (Cocchiglia et al. 2012), so deposition of fine sediments can impact their growth and survival by the formation of hard-pan (Gordon et al. 1992). Fine sediments deposition may thereby reduce the exchange of food and oxygen between interstitial substrates and the water column (Greig et al. 2005, Cocchiglia et al. 2012).

In addition to sedimentation, runoff of asphalt particles and associated pollutants is a potential stressor associated with road construction. Asphalt contains a range of inorganic and organic solids including polycyclic aromatic hydrocarbons (PAHs) and phenols compounds that have been shown to be harmful to freshwater biota (Beasley and Kneale

2002). Some pollutants including PAHs can be absorbed to and transported with fine sediments and exacerbate issues associated with construction-mediated siltation leading to impaired water quality, bioaccumulation and amplification within riverine food chains and ultimately impacts on sensitive biota including freshwater mussels (Beasley and Kneale 2002). Even pollutants indirectly related to road construction, such as heavy metals and road salts, which are deposited in soils near roadways due to vehicular travel, can become an issue as construction increases erosion of roadside soils. These heavy metals can decrease species abundance in streams, decrease macroinvertebrate biodiversity, alter food-webs, and decrease aquatic ecosystem services (Maltby et al. 1995, Clements et al. 2000, Hirst et al. 2002, Carlisle and Clements 2005). Schuler and Relyea (2018) suggest freshwater mussels could be especially vulnerable to these pollutants, as they are sensitive to salts and are filter feeders, which could cause them to accumulate pollutants.

Past studies have used controlled experiments to examine how sedimentation affects freshwater mussels, with in-situ field cages and ex-situ aquarium tank designs being two of the most commonly used techniques. Aldridge et al. (1987) used a controlled laboratory experiment to examine the effects of suspended solids and turbidity on mussels and found that intermittent exposure to high levels of suspended sediments altered mussel filtration rates and oxygen uptake eventually leading to decreased health. More recently, Gangloff et al. (2009) and Hoch (2012) used in-situ cage trials to examine effects of urbanized tributaries and beaver and mill dams on mussel survival and growth rates, respectively. Gangloff et al. (2009) found that sentinel adult mussels placed within and downstream of a wastewater discharge and sediment-impacted tributary exhibited lower survival than mussels in upstream cages and Hoch (2012) found that juvenile mussels at sites downstream from mill dams exhibited higher growth and survival compared with mussels grown at sites downstream from beaver dams.

Here I examine and discuss the potential impacts of a road construction project on a population of a federally endangered freshwater mussel in a western North Carolina river. I predict that sites downstream of the highway corridor will display elevated levels of fine sediments relative to upstream sites, and that mussels grown at these sites or exposed to sediments in the hatchery will experience slower growth rates and lower survival relative to mussels at sites upstream of the road construction. This study is one of the first to use a combination of long-term habitat and population monitoring combined with field and lab experiments to assess the impact of a localized habitat disturbance on a freshwater mussel population.

Methods

Study Site

The South Toe River is an oligotrophic tributary to the Toe and Nolichucky rivers that begins on the eastern slope of Mount Mitchell (elevation 2,037 m), the highest point in the eastern United States. The South Toe is considered one of the most pristine rivers in the southeastern United States and is classified as an outstanding resource water (ORW) by the North Carolina Department of Environmental Quality (NCDEQ) upstream of US Highway 19E. Much of the South Toe watershed is forested and protected by public lands including portions of the Pisgah National Forest, Mount Mitchell State Park and the Blue Ridge Parkway. The South Toe River supports numerous sensitive species including the Appalachian elktoe (*Alasmidonta raveneliana*), the Blotchside logperch (*Percina burtoni*) and the Eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*, NCWRC 2015). The freshwater mussel assemblage in the South Toe River is species-poor relative to other streams in the Tennessee Drainage. Only two mussel taxa, the Wavy-rayed lampmussel (*Lampsilis fasciola*) and the Appalachian elktoe, a federally endangered species, occur in the South Toe River (NCWRC 2015, Pandolfi 2016, Rondel 2019).

My research was conducted at seven sites in the South Toe River (Figure 1). Biologists with the United States Fish and Wildlife Service (USFW), North Carolina Wildlife Resources Commission (NCWRC), and Appalachian State University (ASU) have been regularly monitoring Appalachian elktoe populations at six of these sites since 2003 (Figure 1, Pandolfi 2016, Rondel 2019, J. Mays USFWS unpublished data), and I have been collecting sediment data at those 6 sites since May 2017. Each site is 150 m long and all sites are >1000 m apart. Three sites (Sites 0, 1 and 2) are located upstream of the US Highway 19E bridge, while Sites 3-6 are located downstream of the Highway 19E bridge.

Site 0 is located near the upstream limit of Appalachian elktoe within the South Toe River, just downstream of Celo, NC (NCWRC unpublished records). Site 0 is not one of the six historical monitoring sites, rather, it is used in the sentinel mussel trial to examine the potential for upstream dispersal of the Appalachian elktoe. Site 1 is located downstream of Site 0 and is directly upstream of the Blue Rock Road bridge crossing. Site 2 is located downstream of Site 1 and is ~500 m upstream of the US Highway 19E bridge crossing (Fig. 1). Prior to road construction, Sites 1 and 2 were chosen as relocation sites for mussels located within the bridge construction impact zone. Mussel relocations began in 2008 and continued through 2014 (NCDOT 2014). In 2012, NCDOT began expanding the footprint of US Highway 19E between Spruce Pine and Burnsville, North Carolina from a two-lane to a four-lane highway. A second bridge span was constructed across the South Toe River in 2018.

Three sites (Sites 3-6) are located downstream of the US 19E corridor (Figure 1). Site 3 is ~500 m downstream of the US Highway 19E bridge crossing and Site 4 is located ~50 m downstream of the confluence of Little Crabtree Creek (LCC), a third order tributary that flows parallel to the highway cut for several hundred meters before joining the South Toe River. Thompson (2018) found Site 4 is experiencing the greatest amount of siltation along with the highest rates of Appalachian elktoe population declines during the past decade. Site 5 is located ~500 m downstream of the new Yancey County Wastewater Treatment Facility and Site 6 is located ~2 km downstream of Site 5.

Focal species

Appalachian elktoe are endemic to the headwaters of the Tennessee River Drainage in western North Carolina and eastern Tennessee. This mussel was listed as endangered under the U.S. Endangered Species Act in November 1994 (Clarke 1981, USFWS 1994). Currently, seven highly-isolated Appalachian elktoe populations are extant and the Nolichucky Drainage contains one of the two largest known populations as well as one of the only two dendritic populations (USFWS 2017). The South Toe River is in the Nolichucky Drainage and supports one of the three largest extant populations (Pandolfi 2016). Appalachian elktoe populations in the South Toe River were not detected until 1998 (Fridell personal observation 1998), but by 2009 this population was considered to be one of only a few to show both widespread evidence of recruitment and recent growth (USFWS 2009). According to the most recent USFWS five-year review (2017) as well as more recent data in

Thompson (2018) and Rondel (2019), downstream populations have been in decline since 2015, likely due to road construction impacts.

The US Highway 19E corridor crosses the South Toe River and several tributaries including Crabtree and Little Crabtree creeks in Yancey County, North Carolina. The highway currently passes through what is believed to be the center of the Appalachian elktoe's distribution in the South Toe River and several large populations have been documented by recent surveys both up and downstream of the highway corridor (Pandolfi 2016, Rondel 2019). Sedimentation, geomorphic disturbance and other impacts (e.g., releases of contaminants from construction machinery) associated with this project have the potential to impact Appalachian elktoe populations in the South Toe River. A recent, more broadly-focused survey of mussel populations across the Nolichucky River Drainage found that occurrence was negatively correlated with the proportion of surface fines (i.e., sands and silts) within a site (Pandolfi 2016). It is likely that sediments and sediment-linked runoff originating from Highway 19E expansion may have significant implications for Appalachian elktoe populations in the South Toe River given that recent surveys suggest on-going population declines downstream of the highway corridor (USFWS 2017, Thompson 2018, Rondel 2019).

Freeze Core Samples

I used the freeze coring method described in Thompson (2018) to sample interstitial substrates seasonally from May 2017 to March 2020. Briefly, iron rods were hammered into gravel substrates and filled with crushed dry ice and allowed to sit for 20 minutes. Sediments adhering to the rod provide a vertical profile of interstitial substrate composition and can be used to quantify the concentrations of fine sediments (Marchant and Lillywhite 1989, Adkins and Winterbourne 1999). In the lab, sediment samples were dried and processed using a series of sieves and a shaker table. Sediment size categories were weighed using an Ohaus 6000 x 1g scale. The total weight of the sample was calculated and used to determine the percentage of each sediment size category within the sample. I predicted that the trend of increased concentrations of interstitial fine downstream of the US19E corridor found in Thompson (2018) would continue to be evident and that Site 4 should have higher concentrations of interstitial fines compared with up- and downstream sites.

Population Data

I used Appalachian elktoe survey data collected by USFWS, NCWRC, and ASU personnel to compile historical abundance data for each site dating back to 2003. Standardized collection methods were not used prior to 2015. Surveys conducted after 2015 all followed a similar protocol wherein searchers conducted snorkel surveys within a 150 m reach separated into 15 evenly-searched 10-m transects. Search time was recorded for each transect to calculate catch per unit effort, but I herein use raw mussel count data to account the inclusion of non-standardized, pre-2015 data.

Sentinel Mussel Trials

I followed the sentinel mussel protocols established by Gangloff et al. (2009) and Hoch (2012) to examine the effects of in-situ substrate composition on Appalachian elktoe growth and survival. Hatchery-cultured juvenile Appalachian elktoe (n = 5 per cage) were placed in each of six 18(W) x 18(L) x 10(H) cm mesh cages at seven sites in the South Toe River to examine how site position in the watershed and habitat conditions impacted mussel growth and survival (Figure 1). Juvenile Appalachian elktoe were propagated at the North Carolina Wildlife Resources Commission's Conservation Aquaculture Center (CAC) located at the Marion State Hatchery in Marion, NC using brood stock from the South Toe River. In April 2019, cages were placed at the six historical monitoring and freeze core sampling sites, along with one site located near the upstream limit of Appalachian elktoe in the South Toe River. Cages were filled with local river substrate and anchored into the substrate. Mussel length, width, and height were measured, and mortalities were removed during cage checks in June 2019, August 2019, November 2019 and March 2020. I predicted that mussels in cages upstream of Highway 19E would have higher growth and survival rates compared with those in cages downstream of the highway.

Hatchery Tank Trials

To control for potential effects of unmeasured parameters in situ experiments, I also held propagated juvenile mussels in 1.9 l (8.7 (W) x 25.9(L) x 15.6(H) cm) aquariums at the CAC to examine sediment impacts under more controlled conditions. Each tank was placed in a recirculating system and supplied filtered and ultraviolet light treated surface water (<45 µm) and commercially purchased algae (Nanno 3600 and Shellfish Diet® 1800, Reed Mariculture Pasadena, CA) at a concentration of 3×10^6 $\mu m^3/L$ (Mair et al. 2018). Temperature was held between 18 and 21°C. I used the oven-dried sediments obtained during freeze-core sampling for the hatchery experiment and grouped these into four sediment treatments: 1) upstream control group (sediments from South Toe sites 1 and 2); 2) a downstream control (South Toe sites 5 and 6); 3) a highway-impacted group (South Toe Site 4) and 4) a control group using standard hatchery sediments. Treatment sediments were limited to <500µm to follow. Each treatment had four replicate tanks and sediments in all treatments consisted of only <500 µm particles to follow typical NCWRC CAC protocols for my juvenile age class. Tanks contained ~65 mL of sediment and sediment were changed monthly. A twelve-month growing period required obtaining ~3,120 ml of sediment for each treatment. Additional sediment was obtained from subsequent rounds of freeze core sampling as needed. Ten mussels were placed in each tank for a total of 160 mussels. Mussel length, width, and height, and weight were measured at the beginning of the trial and monthly, and mortalities were removed monthly. I predicted that mussels grown in highway-impacted (Site 4) sediments would exhibit reduced growth and survival compared with mussels grown in control sediments.

Statistical Analyses

I analyzed the freeze core data using 1-way ANOVA and Bonferroni's Post hoc comparison to examine differences in the concentration of fine sediments across sites. Additionally, I used a univariate general linear model to examine the impact of site and season on the variation of concentration of fine sediments. All growth data were standardized to display relative change in length using the equation: (X – Y) / Y, where X = current growth measurement and Y = original measurement. Additionally, cages with 100% mortality were removed from the survival analyses as outliers due to the confounding factors that could have caused the complete mortality, such as burial in debris. Because of this, Site 5 was removed from the survival analyses due to only one mussel surviving.

In order to assess the effect of site or sediment origin on juvenile growth and survival, I used a 1-way ANOVA and a Bonferroni LSD Post hoc comparison to examine whether the final growth and survival data differed among and between sites. I used a linear mixed model to examine differences in growth rates while accounting for the nested study design. I coded time, site or treatment, and the interaction of time with site/treatment as fixed effects and cage or tank as a random effect. I used a Cox's proportional hazards mixed-effects model, which is a repeated measures analysis on individual survival, to examine differences in mortality rates among and between treatments. Fixed effects for the Cox's proportional hazards mixed effects model included site or treatment, and the random effect was cage or tank. For the Cox's proportional hazards mixed effects model on the cage trials, Site 0 was set as reference site, and in the tank trials, the impact treatment was set as the reference. All statistical analyses were conducted in SPSS (version 26; IBM 2019), except for the Cox's proportional hazards mixed effects analyses, which were conducted using the R package *coxme* (version 2.2-16; Therneau 2020).

Results

Freeze Core Samples

The univariate general linear model revealed that the proportion of fine sediments in interstitial substrate samples collected at each site varied significantly among seasons ($p = 0.002$, Table 1) and that there was an interaction between site and season ($p = <0.001$, Table 1). Similarly, the 1-way ANOVA with the Bonferroni Post hoc comparison revealed there was a significant difference among sites, but this was largely driven by one site. The proportion of interstitial fine sediments was only significantly different at the site downstream from the sediment-impacted tributary (Site 4) and the signal of elevated fine sediments at this site remained consistent across seasons ($p = <0.001$, Figure 2).

Population Data

The abundance of Appalachian elktoe in the South Toe River has oscillated at most sites during the last 15 years (Figure 3). However, sites downstream of the Highway 19E corridor display an overall decreasing trend of mussel abundance. Two of the most substantial declines were observed at Site 4 and Site 6. I found no mussels during May 2019 surveys at Site 4. During 2012 surveys, this site supported one of the largest Appalachian elktoe populations in the South Toe River (30+ mussels). Similarly, I found only two mussels in June 2019 at Site 6, which historically had the largest population in the South Toe River (60+ mussels) as recently as 2008. Site 2, on the other hand, has seen an increase in mussel detections from <5 individuals in 2008 to 260 mussels in June 2019.

Sentinel Mussel Trials

Examination of mussel growth revealed that sentinel Appalachian elktoe from Site 2 had significantly higher growth rates compared to mussels at all other sites ($p = <0.001$). Mussels at the three most downstream sites (Sites 4-6) all had significantly lower growth when compared to mussels at Sites 0-3 ($p = < 0.003$, Figure 4). The linear mixed model analysis revealed that site had a significant effect on growth rate ($p = < 0.001$, Table 2, Figure 5).

The final survival analysis revealed that sentinel mussels at Sites 4 and 6 both exhibited lower survival when compared to mussels at upstream sites 0 and 2 ($p = <0.03$,

Figure 6), but the Cox's proportional hazards mixed effects model revealed no significant differences in mortality rate among sites (Table 3, Figure 7). Additionally, although mortality was not significantly higher at Site 6 compared to other sites (p = 0.06) decreased survival at this site may be ecologically relevant and may help explain the dramatic changes in mussel abundance observed at this site during the last decade.

Hatchery Tank Trials

Analysis of Appalachian elktoe growth in hatchery trials found no differences among treatments (Figure 8). Similarly, the linear mixed-model revealed that only time had a significant effect on mussel growth ($p = <0.001$, Table 4), indicating mussels grew at the same rate across treatments (Figure 9). Survivorship analyses revealed no significant effect of sediment origin on mussel survival (Figure 10), but the Cox's proportional hazards mixed effects model revealed that mussels grown in sediments from Site 4 had a higher mortality rate than did mussels grown in sediments from Sites 1 and 2, upstream of the highway corridor (p = 0.018, Table 5, Figure 11).

Discussion

Mussel survey data revealed that Appalachian elktoe populations at 3 of the 4 sites downstream of the US 19E corridor in the South Toe River appear to have experienced dramatic declines over the past decade, whereas the population at the one densely-populated site located upstream of the highway crossing appears to be stable or increasing. Additionally, sentinel mussels placed at 2 downstream sites exhibited decreased growth and survival relative to upstream mussels. It was surprising then, that of the four sites downstream of the highway construction zone, only Site 4 exhibited elevated concentrations of interstitial fine sediments compared to other long-term monitoring sites. Hatchery trials revealed that although mussels grown in sediments from impacted sites had a higher mortality rate compared to mussels grown in upstream treatments there were no differences in growth rate. Taken together, these data suggest that fine sediment impacts on mussels in this system may be driven by sub-lethal effects of changes to sediment composition including changes to microhabitat conditions at and within the streambed.

Freeze Core Samples

Freeze core samples revealed that, despite substantial levels of seasonal variation, only Site 4 displayed elevated concentrations of interstitial fine sediments which is similar to my earlier observations (Thompson 2018). Siltation at Site 4 is likely due to its proximity to Little Crabtree Creek and the fact that it is a semi-depositional reach located just upstream of a sharp (~90 degree) bend in the river channel. Freeze core samples collected at Site 4 contained a higher percentage of fine sediments and also differed visually (Figure 12) suggesting compositional differences in the sediments that may be attributable to the influence of Little Crabtree Creek. Although fine sediments from Little Crabtree Creek may be contributing to Appalachian elktoe population declines at Site 4, hatchery tank trials using sediments from Site 4 do not support the hypothesis that sediment composition is directly responsible for observed changes. Rather, sediment composition may have sub-lethal impacts on mussel populations that are not captured by my experimental design.

One possibility is that fine sediments may be changing sediment composition of streambed microhabitats and flow refugia. Fine sediments may fill interstitial spaces and restrict vertical movements of juvenile mussels within the streambed (Gordon et al. 1992). Additionally, recently-deposited and unstable pockets of fine sediment are highly succeptable to being displaced and fine sediments increase the erosive capacity of streams which may exacerbate streambed scour during high-flow events (Jackson and Beschta 1984). Additionally, elevated levels of fine sediments can further impact mussel habitats by altering hyporheic transfer of oxygen and metabolic waste. Greig et al. (2005) found that fine sediments decrease interstitial flow rates and oxygen exchange, and Claret et al. (1997) found that sediment-impacted gravel bars acted as dissolved organic carbon and nitrate sinks, but that sediment occlusion induced hypoxic conditions favorable for denitrification. Denitrifying conditions in streambed sediments can lead to elevated concentrations of interstitial ammonia. Sediment-bound ammonia is toxic to a broad range of benthic organisms including freshwater mussels. Strayer and Malcomb (2012) examined sediment ammonia concentrations and found that in agricultural basins, toxic concentrations of ammonia are common and may limit mussel recruitment.

Mussel Populations

Appalachian elktoe populations in long-term monitoring sites upstream of the Highway 19E (Sites 1 and 2) crossing have remained stable over the last seven years. These sites historically supported low numbers (<6 mussels per site) of Appalachian elktoe, but were chosen as sites for relocation of mussels because local habitat conditions appeared suitable (i.e., both sites are characterized by an abundance of stable, coarse substrate and moderate flows, NCDOT 2014). The Appalachian elktoe population at Site 2 is now one of the largest in the South Toe and populations appear to be increasing. In contrast, populations at Site 1, the most upstream long-term monitoring site, have remained small (typically <10 mussels per 150-m reach) and exhibit dramatic year-to-year and even seasonal fluctuations in detectability. The year to year population fluctuations seen at these sites could be due to the variable detection rate of the survey methods (Rondel 2019). Other studies have shown that seasonal fluctuations in mussel detectability are most likely due to changes in surface activity related to the timing of reproduction or glochidia release (Watters et al. 2001, Schwalb and Pusch 2007, Meador et al. 2011, Annie et al. 2013).

At Site 3, immediately downstream of Highway 19E, Appalachian elktoe populations remained relatively small (<12 per site) prior to 2015, but numbers increased between 2015 and 2018, likely due to the use of standardized 150-m surveys. Mussel populations decreased sharply at Site 3 in 2019, and it is currently unclear if this is an outlier or a part of a larger trend. The sites downstream of 19E (Sites 4-6) historically supported the largest mussel populations in the South Toe but are experiencing ongoing population declines and in 2019, I did not find any Appalachian elktoe at Site 4. Rondel (2019) found nine new populations of Appalachian elktoe in the South Toe River. Eight of these were upstream of the Highway 19E whereas only one new population was detected downstream of the highway corridor. Taken together these data suggest that although Appalachian elktoe populations in some reaches of the South Toe are stable and seem likely to persist upstream of Highway 19E, downstream populations seem unlikely to persist if current trends continue. Although it is tempting to attribute these declines to highway construction activities, it is important to also note that Appalachian elktoe were absent from the South Toe River prior to 2000 and appear to have recently colonized this stream. It is also possible that populations are moving upstream, potentially in response to stream warming in lower reaches (Babaluk et al. 2000, Isaak and Rieman 2012). Pandolfi (2016) found that Appalachian elktoe populations in streams that have become warmer in recent decades have fared more poorly than those in streams, including the South Toe River, that have remained relatively cool over this time period.

Sentinel Mussel Trials

The sentinel mussel study found that juvenile mussels at sites experiencing the most dramatic long-term population declines (Sites 4-6) also had the lowest growth and survival rates, suggesting that fine sediments are indeed capable of affecting mussels in study reaches but do not provide many insights into the mechanisms driving these declines. It is possible that fine sediments may be reducing interstitial flow rates or dissolved oxygen concentrations and increasing ammonia concentrations (Claret et al. 1997, Greig et al. 2005), and this effect may be exacerbated by anchoring cages into the streambed.

There are some limitations to the sentinel mussel study that must be considered when interpreting these data. First, cage placement is frequently limited to flow and habitat refuges that are located close to the bank occasionally in habitats that may not be representative of habitats occupied by mussels. Additionally, cages may themselves create refugia from or exacerbate habitat impacts associated with sedimentation such as hard-pan and washout. Cages also prevent mussels from migrating vertically or horizontally in the substrate and this may reduce survival of caged individuals when cages are exposed to the atmosphere or buried by sediments or detritus accumulations. Despite these limitations, results of my sentinel mussel study appear to mirror responses of wild populations at these sites.

One interesting finding that was somewhat beyond the scope of my initial objectives is that sentinel juvenile Appalachian elktoe at Site 0 experienced growth rates that were similar to those observed in sentinel mussels at historical sites 1 and 3 and survival rates that were similar to those at Site 2. These results suggest that Appalachian elktoe may be able to survive in cooler reaches upstream from their current range in the South Toe River and it is possible that populations may continue to move upstream in the future.

Hatchery Tank Trials

In contrast to field trials, I did not observe any differences in growth or final survival that were attributed to treatment differences among hatchery tanks. The fact that I observed no difference in growth and final survival among treatments suggest that the composition of the sediments downstream of the US 19E crossing may not be driving mussel population declines, supporting Haag's (2012) hypothesis that potential sediment impacts to freshwater mussels in riverine ecosystems may be somewhat over-stated.

However, I believe it would be naive to dismiss sedimentation as a possible factor in Appalachian elktoe population declines in the South Toe River. I observed higher mortality rates in mussels grown in sediments from impacted sites compared to mussels grown in sediments from sites upstream of the highway corridor. Increased mortality rates of mussels in impacted site sediments occurred during the draining and refilling of the hatchery's water source and tanks went uncleaned during this time. However, all treatments experienced these factors equally so it is interesting that only mussels grown in impacted site sediments responded in this way. This seems to suggest that the health of the impact-sediment mussels was compromised, and that perhaps the extra stresses associated with water source changes was sufficient to induce mortality. Other studies have suggested that direct impacts of sediments are subtle and tend to be more chronic in nature with sub-lethal effects that may compromise individual health but which may not lead to immediate mortality (Ellis 1936, Aldridge et al. 1987, Naimo 1995, Brim-Box and Mossa 1999, Kreutzweiser and Capell 2001, Humphries 2006, Thorsen et al. 2007, Österling et al. 2010, Cocchiglia et al. 2012, Jorge et al. 2013).

Limitations of tank trials should also be considered when interpreting my results. First, interstitial sediments were collected with freeze cores and the amount of sediment that could be collected for use in tank trials as well as the number of times tanks could be cleaned was limited by time and financial resources. The limited depth of sediment in hatchery tanks may have also prevented mussels from moving vertically in the substrate or the effectiveness of pedal feeding. However all tanks were impacted equally by these constraints. Finally, propagation selection may have influenced these results, as all juveniles used in the study were produced from a small number of adults and were initially reared in fine sediments (<500 μm), potentially reducing treatment effects (Lynch and O'Hely 2009).

Taken together, the findings of my study suggest that road construction and resulting sediments have likely impacted Appalachian elktoe populations in the South Toe River downstream of the Highway 19E corridor. Downstream populations have experienced dramatic recent declines and sentinel mussels grew more slowly and survived at lower rates compared to individuals at sites upstream of the highway. However, my hatchery trials suggest that biotic impacts associated with sediments are likely not the direct cause of downstream population declines but are rather an indirect cause due to sub-lethal effects. Habitat factors associated with sedimentation, along with other anthropogenic impacts including perhaps stream warming due to climate change (Mohseni et al. 2003, Durance and Ormerod 2007, Isaak and Rieman 2013) appear more likely to be influencing variability in Appalachian elktoe populations in the lower South Toe River. If the current population trends continue, populations in the lower river may become extirpated unless habitat impacts can be mitigated. Efforts to augment existing populations using artificially-propagated individuals may help forestall extirpation but seem unlikely to recover populations if sediment impacts continue to affect reaches downstream of 19E. Fortunately, populations upstream of the 19E corridor appear stable and may contribute recruits to replenish downstream populations once habitat conditions have stabilized. Future studies should examine the mineralogical and chemical composition of sediments associated with road construction in order to exclude the role of ecotoxicological effects in Appalachian elktoe population declines. Additionally, the role of fine sediments on microhabitat conditions including interstitial temperatures as well as oxygen and nitrogenous waste diffusion should be investigated in this system.

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Tables

Table 1: Between-subject effects statistics for the univariate general linear model analysis examining the effects of site and season on percent fines (<125µm sediements) collected with freeze cores.

Source		*Type III Sum of Squares*	*df*	*Mean Square*	*F*	*Sig.*
Intercept	Hypothesis	3767.069	1	3767.069	955.560	.000
	Error	19.711	5	3.942[a]		
Season	Hypothesis	197.758	10	19.776	3.293	.002
	Error	300.298	50	6.006[b]		
Site	Hypothesis	1599.774	5	319.955	29.037	.000
	Error	275.474	25	11.019[c]		
Sample	Hypothesis	19.711	5	3.942	.330	.890
	Error	310.707	25.977	11.961[d]		
Season * Site	Hypothesis	773.918	46	16.824	3.396	.000
	Error	1139.390	230	4.954[e]		
Season * Sample	Hypothesis	300.298	50	6.006	1.212	.175
	Error	1139.390	230	4.954[e]		
Site * Sample	Hypothesis	275.474	25	11.019	2.224	.001
	Error	1139.390	230	4.954[e]		
Season * Site * Sample	Hypothesis	1139.390	230	4.954	.	.
	Error	.000	0	.[f]		

Table 2: Fixed effects statistics of the linear mixed model for juvenile growth rate (length, mm) in the sentinel mussel trials.

Source	*Numerator df*	*Denominator df*	*F*	*Sig.*
Intercept	1	35.266	1680.691	.000
Site	6	35.168	59.780	.000
Time	4	894.166	598.985	.000
Site * Time	24	893.358	31.052	.000

Table 3: Fixed Effects statistics of the Cox's proportional Hazards Mixed-Effects model for juvenile mortality rate in the sentinel mussel trials. Site 0 was set as the reference.

Predictors	*Estimates*	*CI*	*P*
Site [1]	4.77	0.45 – 50.41	0.194
Site [2]	0.27	0.02 – 4.90	0.379
Site [3]	3.11	0.32 – 30.28	0.327
Site [4]	4.13	0.47 – 36.62	0.202
Site [6]	8.20	0.91 – 73.79	0.060
Observations	147		

Table 4: Fixed effects statistics of the linear mixed model for juvenile growth rate (length, mm) in the hatchery tank trials.

Source	*Numerator df*	*Denominator df*	*F*	*Sig.*
Intercept	1	12.041	2175.507	.000
Treatment	3	12.040	1.262	.331
Time	12	1844.112	393.569	.000
Treatment * Time	36	1844.107	.816	.774

Table 5: Fixed Effects statistics of the Cox’s proportional Hazards Mixed-Effects model for juvenile mortality rate in the hatchery tank trials. Impact treatment was set as the reference.

Predictors	*Estimates*	*CI*	*P*
Downstream	0.55	0.24 – 1.28	0.165
Hatchery	0.75	0.35 – 1.62	0.465
Upstream	0.29	0.10 – 0.81	0.018
Observations	160		

Figures

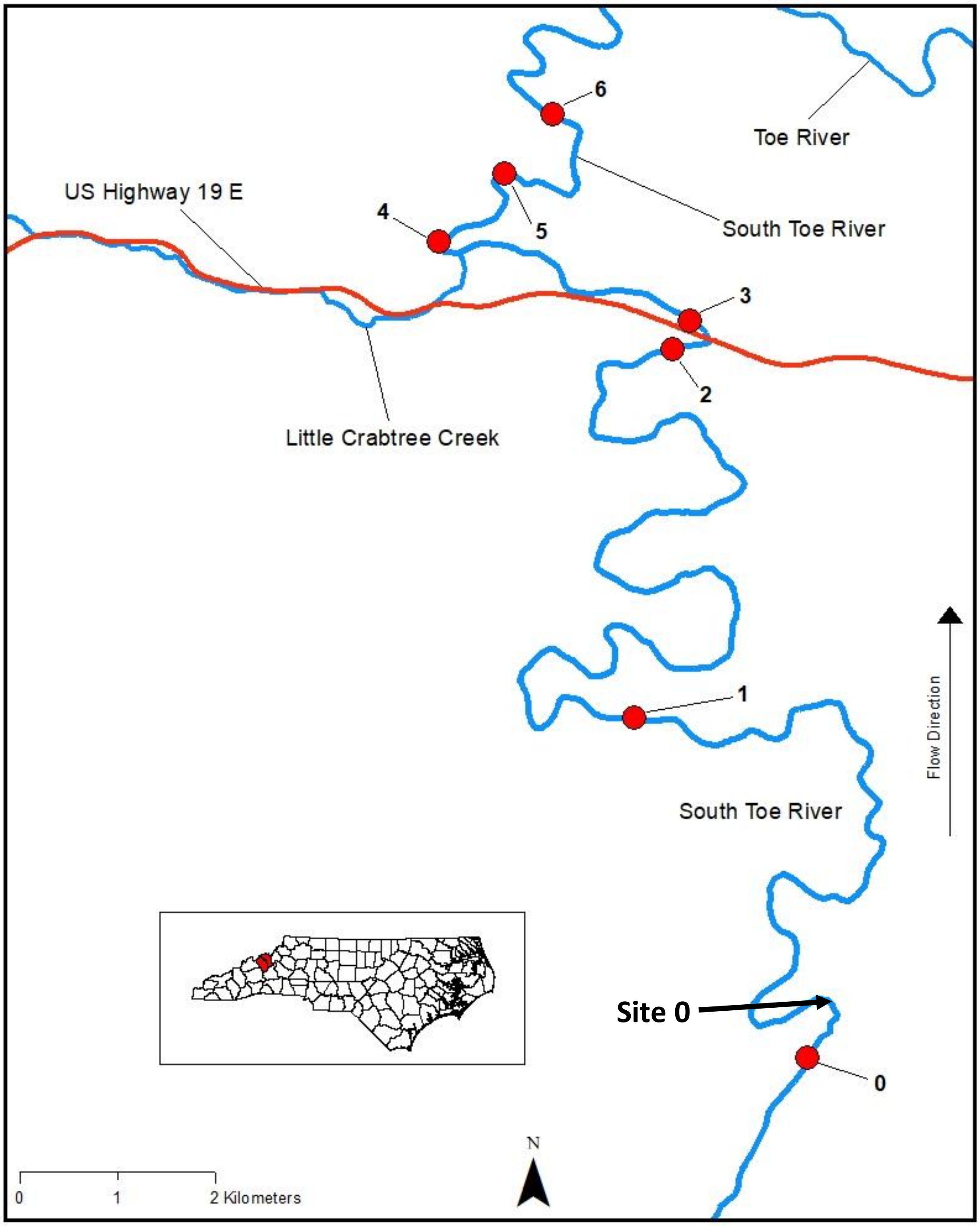

Figure 1: Map of study sites for freeze cores, population surveys, and sentinel mussel trials in the South Toe River, Yancey Country, NC. All sites were used for the sentinel mussel trials and Sites 1-6 were used freeze core and population surveys.

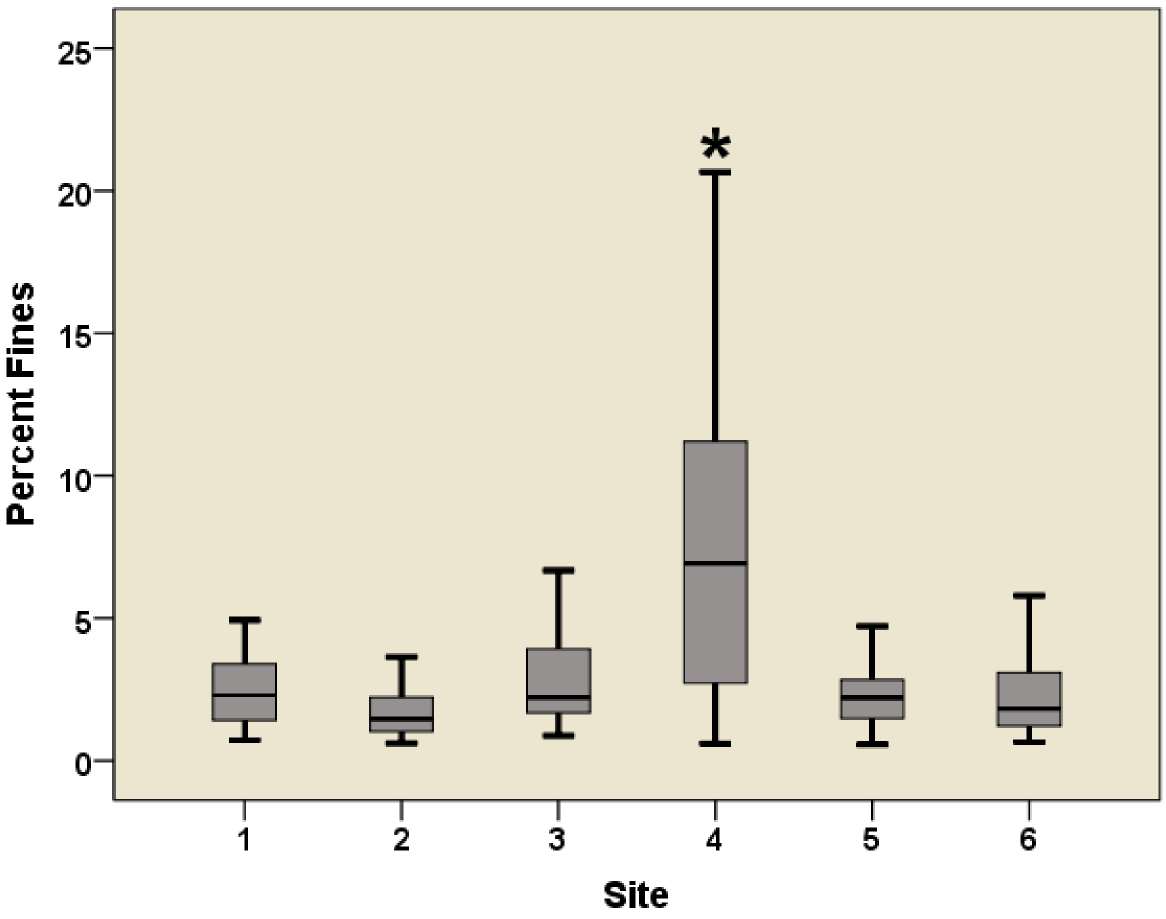

Figure 2: Percentage of fine sediment particles (<125 um) in freeze core samples collected seasonally at six sites in the South Toe River during 2017-2019. The asterisk represents significant difference in percentage of fine sediments on a site-level (1-way ANOVA, Bonferroni post hoc test, p = <0.001).

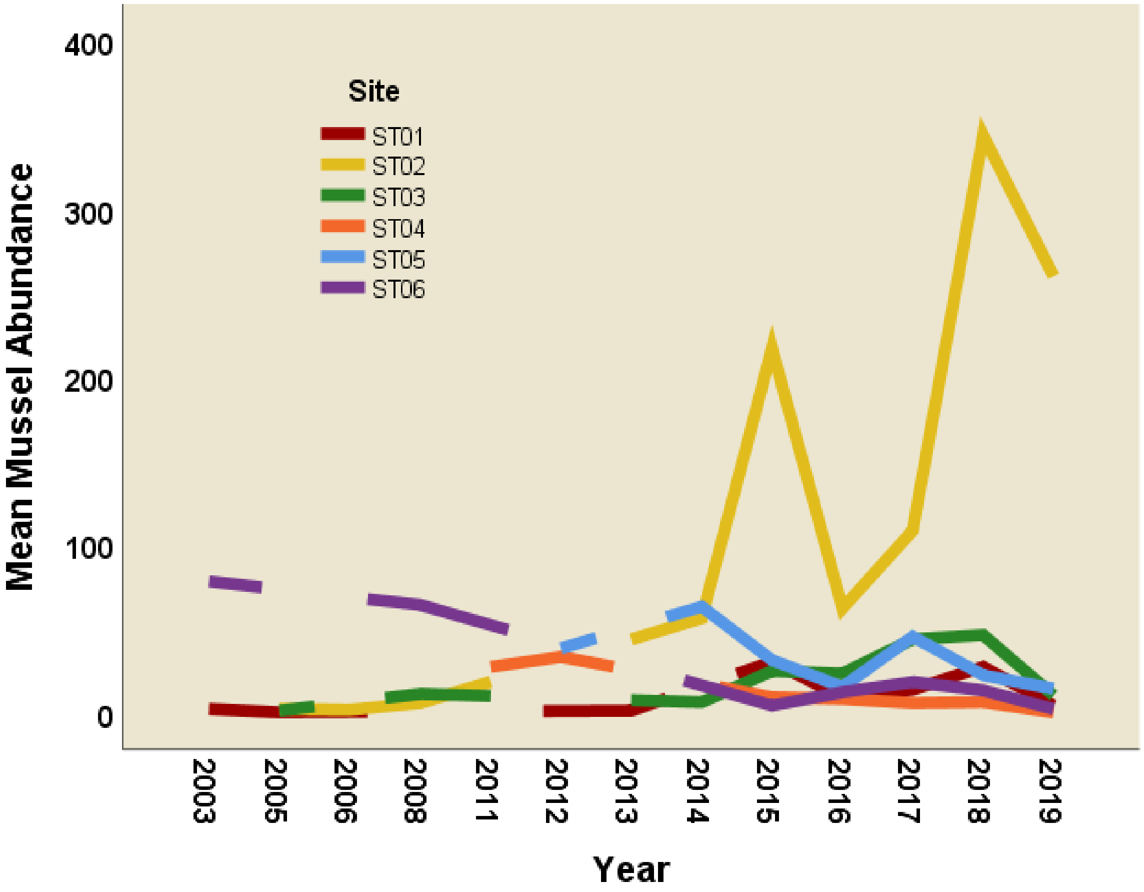

Figure 3: Yearly mean abundance of *A. raveneliana* observed at six sites in the South Toe River. Surveys were conducted between 2003 and 2019. Pre-2015 surveys were not standardized, but post-2015 surveys were standardized using 150m-timed surveys.

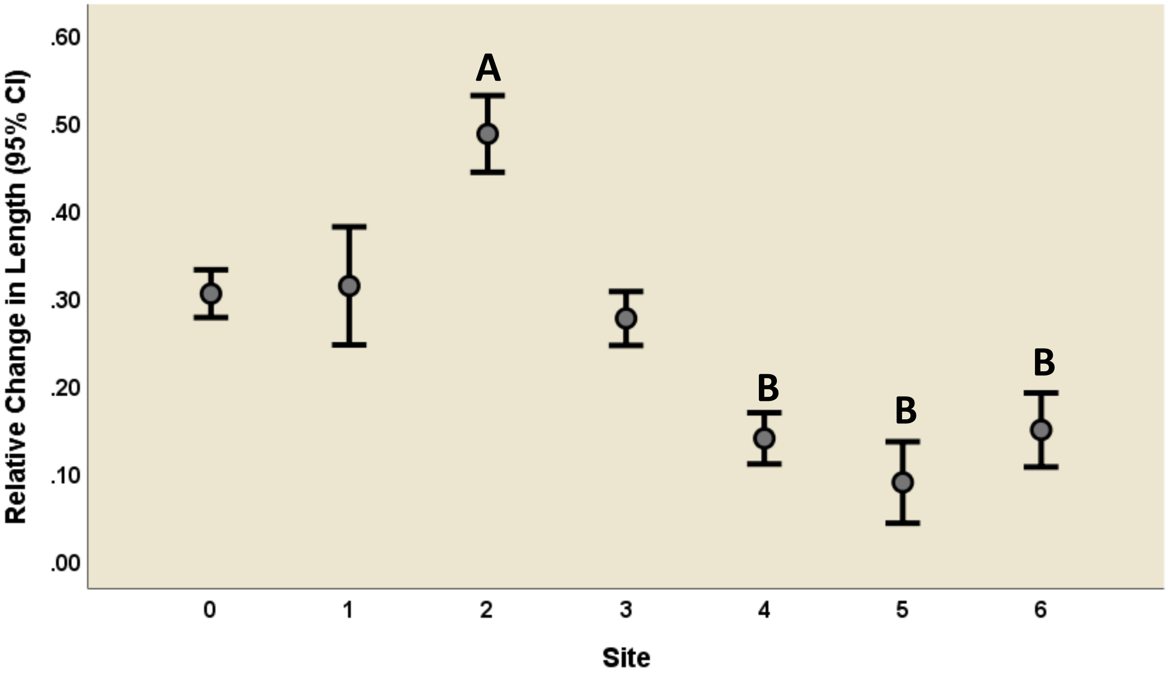

Figure 4: Final average (95% CI) relative change in length (mm) of juvenile *A. raveneliana* grown in cages at seven sites in the South Toe River during sentinel mussel trials from April 2019 to March 2020. Letters indicate significant differences in final relative change in length (1-way ANOVA, Bonferroni post hoc test, p = <0.003).

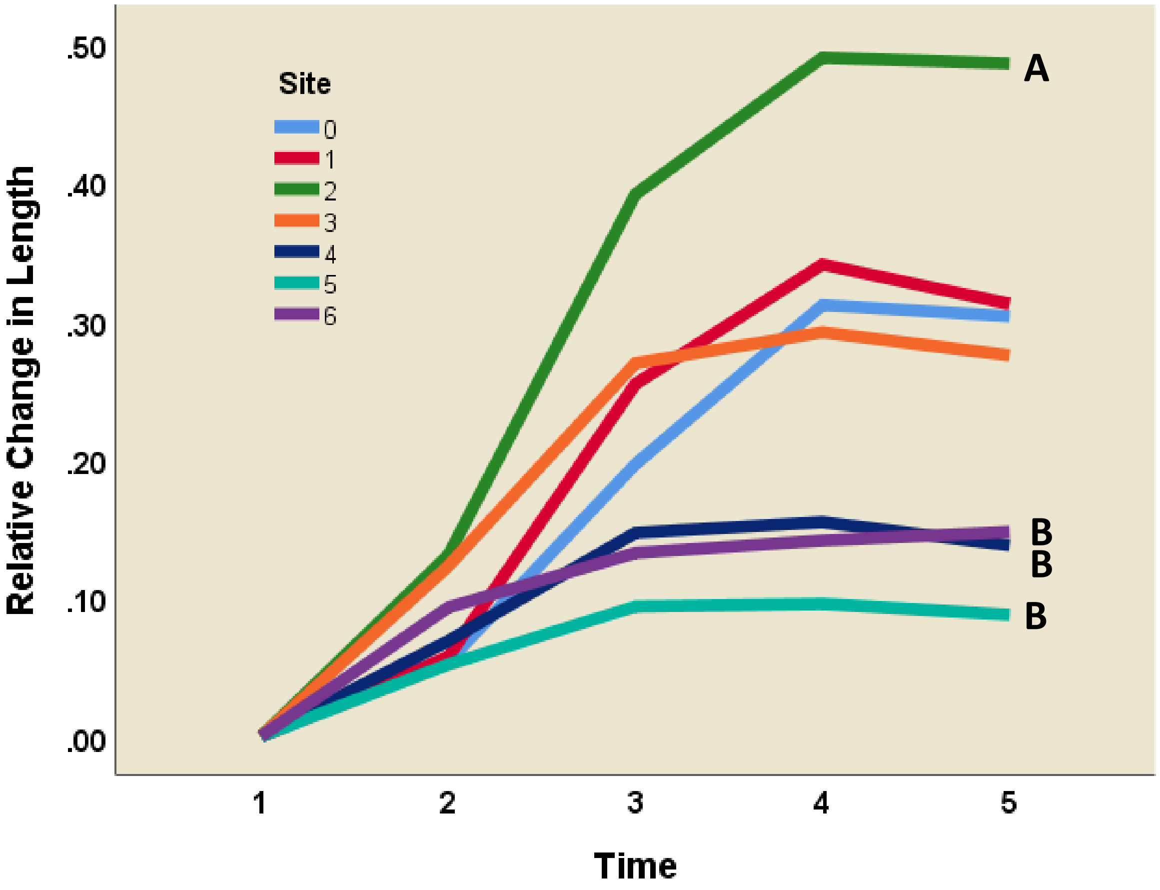

Figure 5: Relative growth rate (length, mm) of juvenile *A. raveneliana* grown in cages at seven sites in the South Toe River during sentinel mussel trials from April 2019 to March 2020. Letters indicate significant differences in growth rate (Linear Mixed Model Analysis, p = <0.001). Time values are not equal intervals.

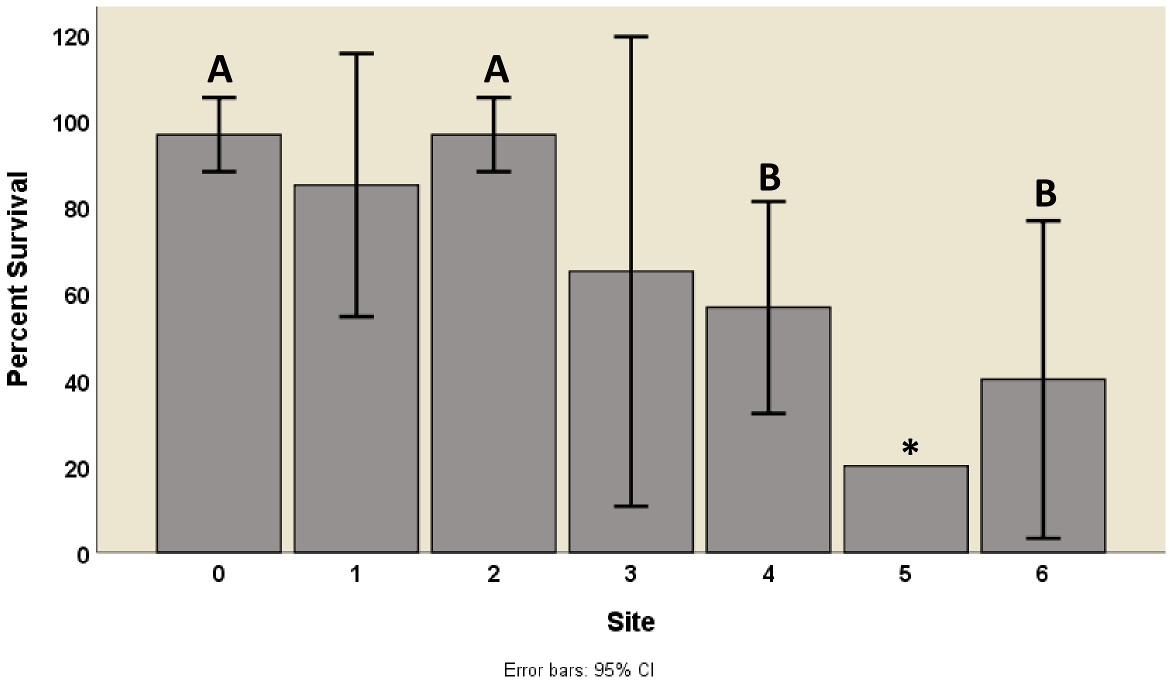

Figure 6: Final average (95% CI) percent survival of juvenile *A. raveneliana* grown in cages at seven sites in the South Toe River during sentinel mussel trials from April 2019 to March 2020. Letters indicate significant differences in final average percent survival (1-way ANOVA, Bonferroni post hoc test, p = <0.03). Asterisk indicates site excluded from analysis due to small sample size at the end of the trials (n = 1).

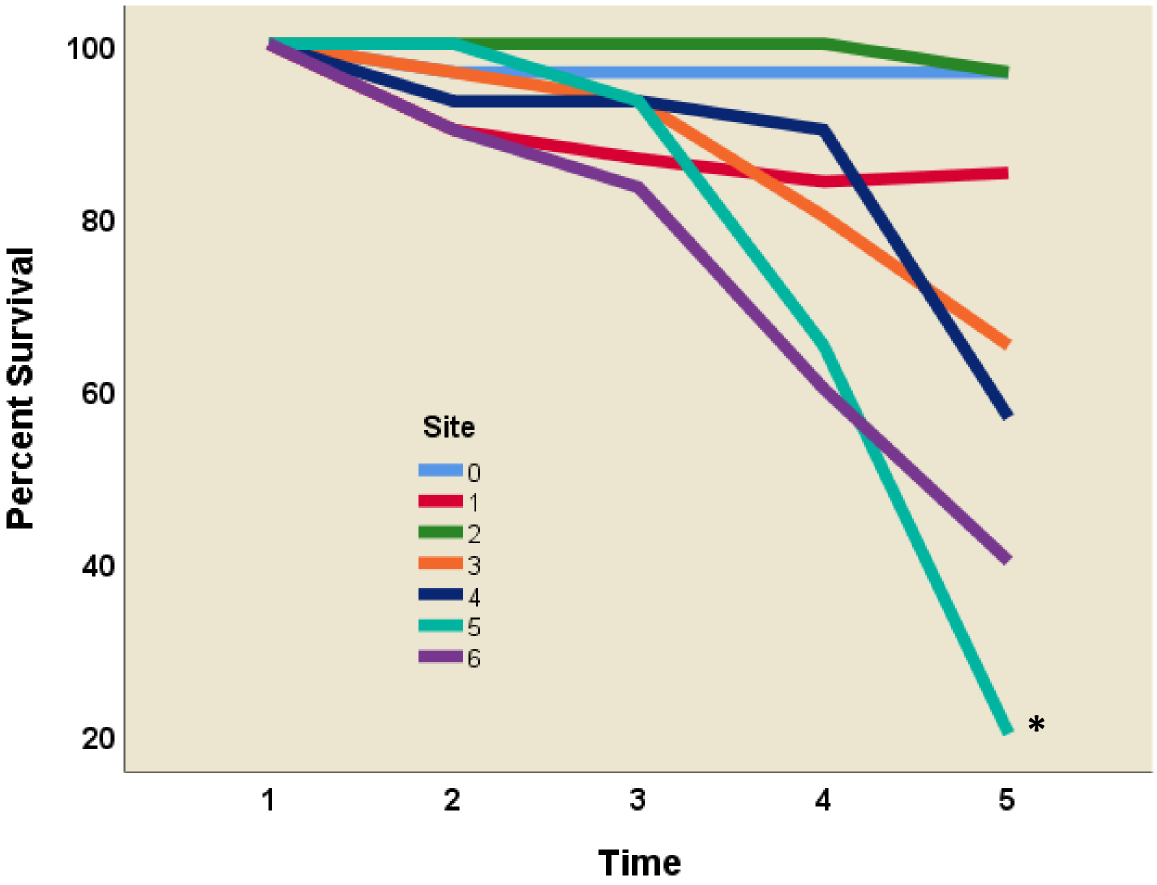

Figure 7: Mortality rate of juvenile *A. raveneliana* grown in cages at seven sites in the South Toe River during sentinel mussel trials from April 2019 to March 2020. There are no significant differences in mortality rate, however, Site 6 may be ecologically different (Cox's proportional hazards mixed effects analysis, $p = 0.06$). Time values are not equal intervals. Asterisk indicates site excluded from analysis due to small sample size at the end of trials ($n = 1$).

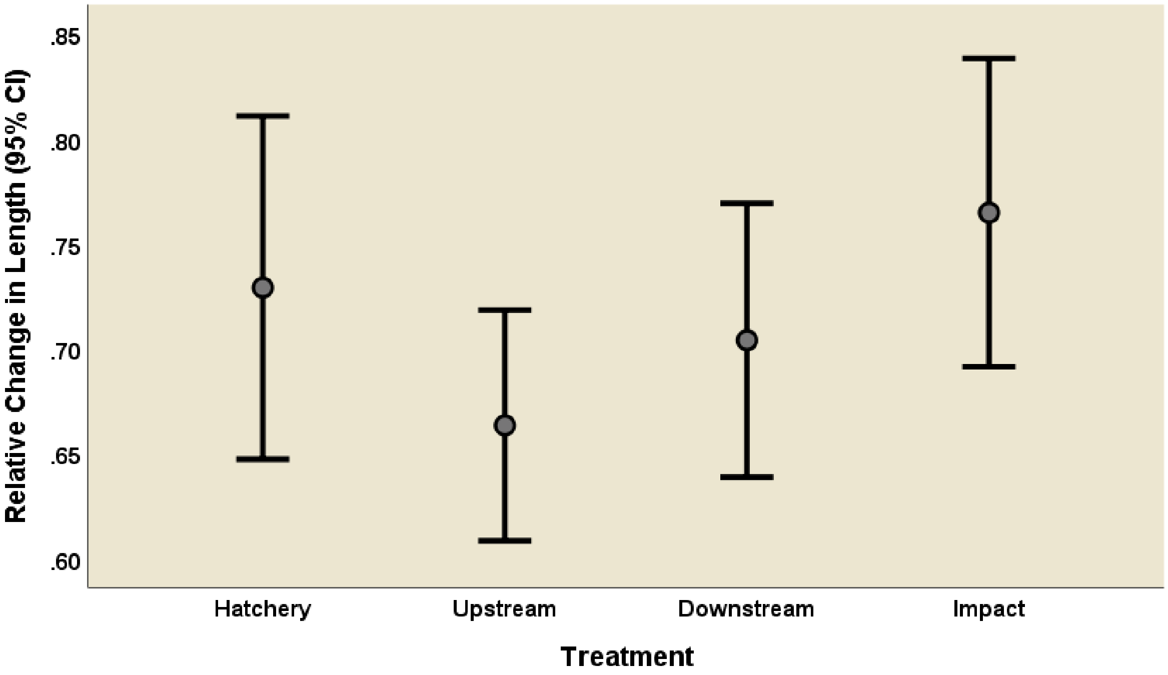

Figure 8: Final average (95% CI) relative change in length (mm) of juvenile *A. raveneliana* grown in four different sediment treatments during hatchery tank trials from April 2019 to March 2020. There are no significant differences in final relative change in length (1-way ANOVA, Bonferroni post hoc test).

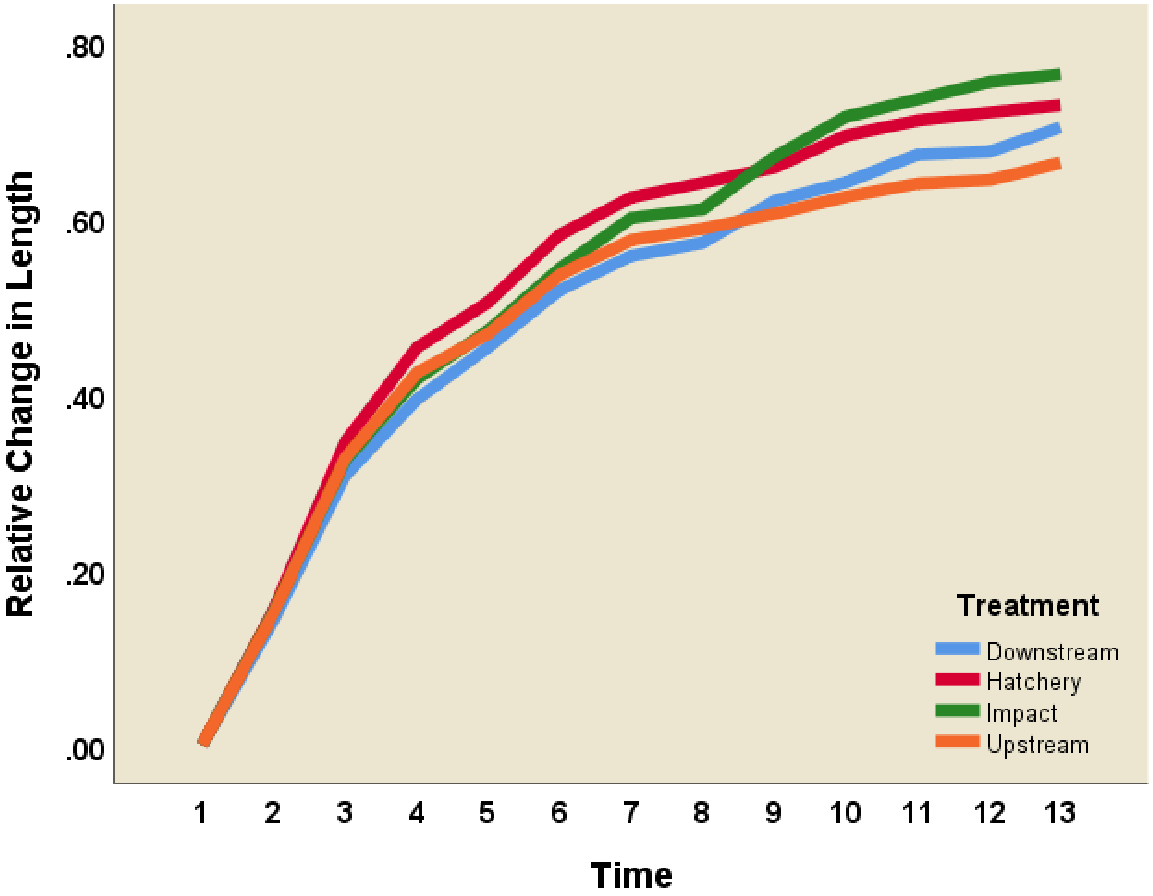

Figure 9: Relative growth rate (length, mm) of juvenile *A. raveneliana* grown in four different sediment treatments during hatchery tank trials from April 2019 to March 2020. There are no significant differences in relative growth rate (Linear Mixed Model Analysis).

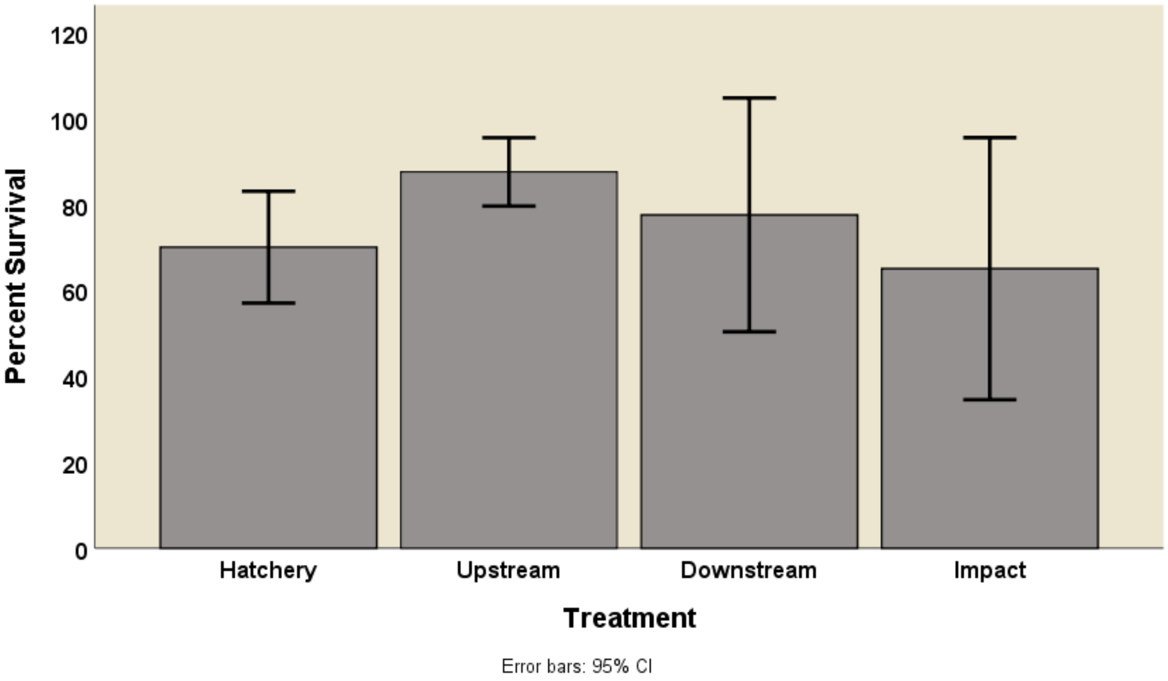

Figure 10: Final average (95% CI) percent survival of juvenile *A. raveneliana* grown in four different sediment treatments during hatchery tank trials from April 2019 to March 2020. There are no significant differences in final average percent survival (1-way ANOVA, Bonferroni post hoc test).

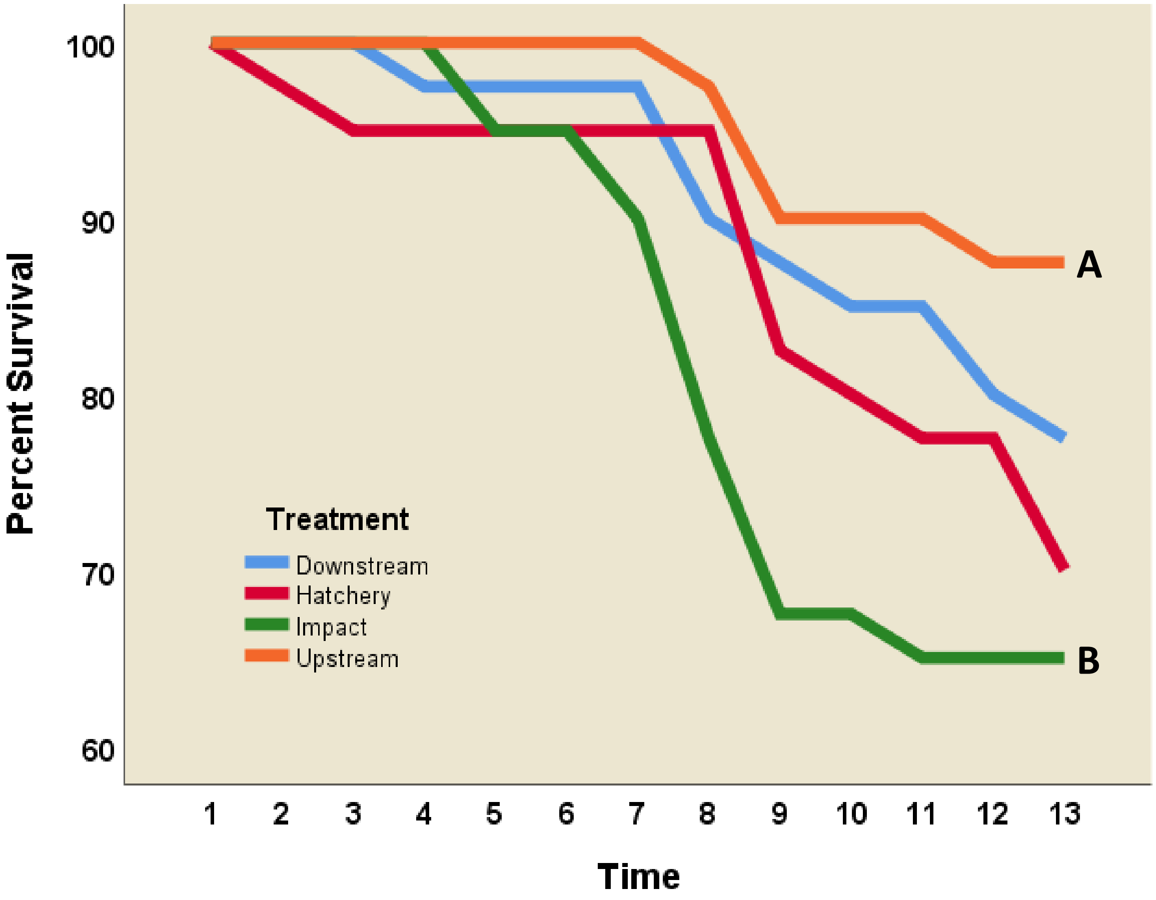

Figure 11: Mortality rate of juvenile *A. raveneliana* grown in four different sediment treatments during hatchery tank trials from April 2019 to March 2020. Letters indicate significant differences in mortality rate (Cox's Proportional Hazards Mixed Effects Analysis, p = 0.018).

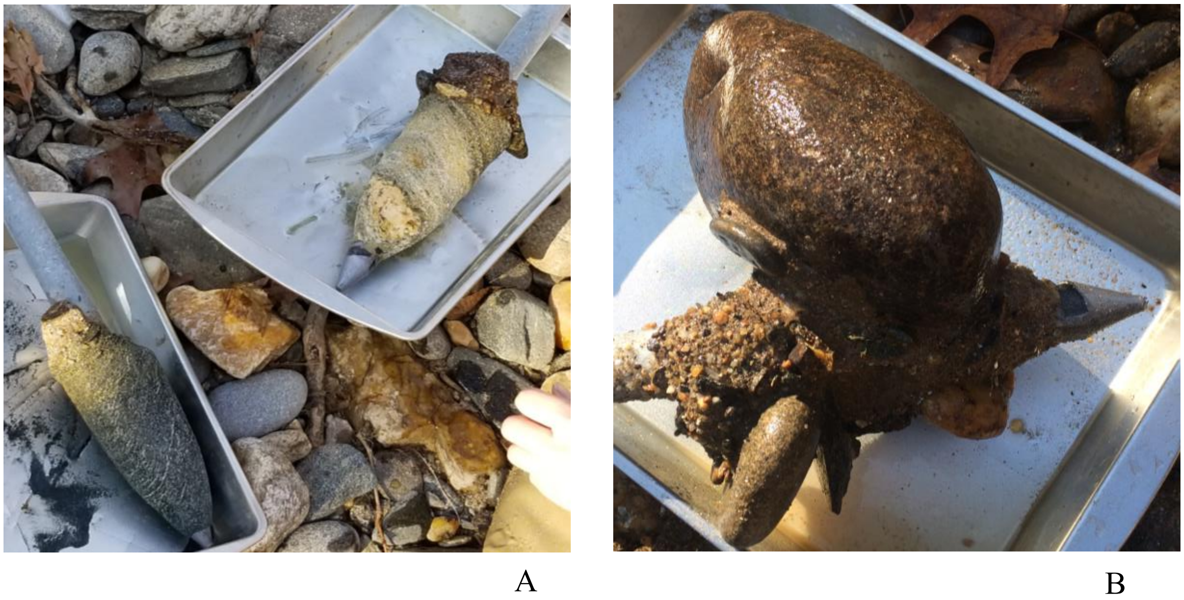

Figure 12: Images of cores collected in the South Toe River. A) Cores resembling concrete collected at Site 4 in the South Toe River (A) compared to a core collected at site 5 in the South Toe River exhibiting traits consistent with cores collecting in this river (B).

Appendix A

Table 1A: All 7 site localities throughout South Toe. Non-historical site is indicated with an asterisk next to its name. Sites are oriented from upstream to downstream (0-6).

Site Locality	Latitude	Longitude	Elevation (m)
Site 0*- Halls Chapel Road	35.839968	-82.179295	807.681
Site 1- U/S Blue Rock Road crossing	35.871221	-82.195219	782.383
Site 2- U/S US Highway 19 East crossing	35.905182	-82.191674	747.333
Site 3- Martin's Chapel	35.907769	-82.190096	746.419
Site 4- Wyatt Town Road	35.915051	-82.213237	739.104
Site 5- D/S waste water treatment plant on Wyatt Town Road	35.921330	-82.207110	736.970
Site 6- Baccus Siding Road	35.926794	-82.202692	732.399

Vita

Michael James Thompson was born in New Bern, North Carolina to Michael and Sherry Thompson. He graduated from New Bern High School in May 2014. The following fall, because of his love for hunting, fishing, and being outdoors, he entered Appalachian State University to study Evolutionary, Ecological, and Environmental Biology. Michael began working as undergraduate research assistant in Dr. Michael Gangloff's Aquatic Conservation Research Lab his Sophomore year. During his Junior year, Michael began working on an undergraduate honors thesis examining the effects of riverine sedimentation using a freeze coring method. In May 2018 he was awarded a Bachelor of Science degree and in the fall of 2018, he accepted a graduate research assistantship in Biology at Appalachian State University under Dr. Gangloff and began studying toward a Master of Science degree. He was awarded a Master of Science degree in May 2020 and now resides in eastern North Carolina where he can be found in the gym, on a ball field, in a deer stand or on the water.