HOST FISHES OF TWO IMPERILLED FRESHWATER MUSSELS: THE APPALACHIAN ELKTOE (*ALASMIDONTA RAVENELIANA*) AND THE LONGSOLID (*FUSCONAIA SUBROTUNDA*)

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Abstract

Freshwater mussels have complicated life histories and their larvae are briefly parasitic on fishes. This presumably enhances dispersal and apparently aids the development of juveniles. Despite many advances in mussel propagation, many mussel-host fish relationships remain poorly understood and the hosts of many at-risk species remain unknown. I assessed the ability of a suite of co-occurring fishes to serve as hosts for two imperiled freshwater mussels, the Appalachian Elktoe and the Longsolid. The Appalachian Elktoe (*Alasmidonta raveneliana*) is a freshwater mussel endemic to western North Carolina and eastern Tennessee. It is listed as endangered under the Endangered Species Act. The Longsolid (*Fusconaia subrotunda*) is a freshwater mussel found in the Ohio, Cumberland, and Tennessee river systems. It is currently proposed for threatened status under the Endangered Species Act. The only known host fishes for Appalachian Elktoe are Banded Sculpin (*Cottus carolinae*) and Mottled Sculpin (*C. bairdi*). Host fishes for the Longsolid were previously unknown. In March of 2022, host trials for the Appalachian Elktoe were conducted using 8 potential host fishes from 7 families at the North Carolina Wildlife Resources Commission's Conservation Aquaculture Center (CAC) in Marion NC. I determined that 6 of 8 species tested served as suitable hosts for Appalachian Elktoe. Glochidia transformed into juveniles on Northern Hogsucker (*Hypentelium nigricans*), Sicklefin Redhorse (*Moxostoma sp*.), Mottled Sculpin, Central Stoneroller (*Campostoma anomalum*), Smallmouth Bass (*Micropterus dolomieu*), and Blacknose Dace (*Rhinichthys atratulus*). However, there was no significant difference among host-specific survival rates because juveniles survived for only three weeks before they were consumed by flatworms. Longsolid host trials using 8 potential host fishes from 5 families were conducted in May 2022. I identified 5 potential hosts for the Longsolid: Central Stoneroller, Whitetail Shiner (*Cyprinella galactura*), Striped Shiner (*Luxilus chrysocephalus*), River Chub (*Nocomis micropogon*), and Warpaint Shiner (*Luxilus coccogenis*). These results make sense from a biogeographical perspective and will inform both captive propagation of and habitat management/enhancement for these imperiled freshwater mussels.

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Dedication

I would like to dedicate this thesis to my grandfather, Billy Randell Ewing (October 27th 1942 – September 7th 2021). Thank you so much for inspiring me, and always encouraging me to follow in your footsteps as a biology major.

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Foreword

This thesis will be submitted to *Freshwater Mollusk Biology and Conservation*, a peer review journal owned and published by The Freshwater Mollusk Conservation Society. It has been formatted according to the style guide for that journal.

CHAPTER 1

Host fishes of two freshwater mussels: the Appalachian Elktoe (*Alasmidonta raveneliana*) and the Longsolid (*Fusconaia subrotunda*)

Key Words: Appalachian Elktoe, Longsolid, glochidia, freshwater mussel, host fish

Introduction

Freshwater mussels in North America are highly imperiled, with 65% of mussel species being listed as threatened, endangered, or vulnerable (Haag and Williams 2014). Freshwater mussels have a unique lifecycle where they rely on a host fish to metamorphize from larvae (glochidia) to juveniles. Some of these mussels are host specialists, and can only transform on a suite of closely-related fishes, while others are generalists, and can transform on a variety of host fishes (Vaughn 2012). Approximately one-third of North American freshwater mussel species have known host fishes (Haag 2012). Diagnosing mussel-host fish relationships is important for the conservation of imperiled freshwater mussel species, and can be used to help bolster populations through captive propagation as well as by guiding management actions targeting host fishes.

The Appalachian Elktoe (*Alasmidonta raveneliana*) is a freshwater mussel endemic to the Tennessee River system in western North Carolina and eastern Tennessee (Parmalee and Bogan 1998). It was listed as an endangered species under the US Endangered Species Act in 1994 (USFWS 1994). Declines in Appalachian Elktoe populations have been documented from several former strongholds and while this phenomenon is poorly understood, it is likely the result of a variety of factors, including siltation, pollution, and anthropogenic habitat modifications (USFWS 1996; Pandolfi 2016; Pandolfi et al. 2022). Beginning in 2014, the North Carolina Wildlife Resources Commission (NCWRC) began captive propagation of

Appalachian Elktoe with the goal of augmenting existing populations and establishing new populations (Rachael Hoch, personal communication).

There are currently two documented host fishes for the Appalachian Elktoe. The Banded Sculpin (*Cottus carolinae*) was the first Appalachian Elktoe host identified (Gordon and Moorman 1998). Mottled Sculpin (*C. bairidi*) are also capable hosts for Appalachian Elktoe and are currently used in Appalachian Elktoe propagation at the NCWRC's Conservation Aquaculture Center in Marion, North Carolina (CAC, Rachael Hoch, NCWRC, personal communication). However, it remains unclear whether Appalachian Elktoe are host generalists or specialists. Like other members of the Tribe *Anodontini*, Appalachian Elktoe reproduce by releasing glochidia bound within a mucous net into the water column, a behavior typical of many host generalists (Haag 2012). Other mussels within genus *Alasmidonta* are also generalists, and use a variety of fish species, including darters (Percidae), daces (Cyprinidae), and redhorses (Catostomidae) as hosts (Michaelson and Neves 1995; Schulz and Marbain 1998; Bloodsworth et al. 2013).

Numerous studies have examined the impact of glochidia on host fishes, and are summarized by Rock et al. (2022). However, few studies have focused on the mussel side of this parasitic relationship beyond host trials. A study of the Freshwater Pearl Mussel (*Margaritifera margaritifera*) looked at the impact that length of excystment time on a host fish had on transformed juvenile size and growth (Marwaha et al. 2017). Lower water temperatures may increase the length of the transformation period and increase the percentage of glochidia that successfully transform into juveniles (Roberts and Barnhart 1999). Marwaha et al. (2017) found excystment time and both mean size and growth rate were positively correlated. Past host trials have shown different excystment periods for different host fishes. Host trials for the Triangular Kidneyshell (*Ptychobranchus greeni*) had transformation times ranging from 30-34 days on the Warrior Darter (*Etheostoma bellator*) and 28-54 days for the Tuskaloosa Darter (*Nothonotus douglasi*, Haag and Warren 1997). However few other studies have examined the impact of different host fishes on juvenile survival.

The Longsolid (*Fusconaia subrotunda*) is a freshwater mussel endemic to the Ohio, Cumberland-Tennessee and Great Lakes drainages (Simpson 1914; USFWS 2018). There are conflicting accounts about the preferred habitat for Longsolid. It is reported to both prefer strong currents (Gordon and Layzer 1989) and slower-flowing, deeper habitats (Ostby 2005). Longsolid occupancy has declined substantially range-wide during the past 100 years and it is currently proposed for listing as threatened under the Endangered Species Act (USFWS 2020).

The Longsolid is a tachytictic (short-term) brooder and is gravid from May through August (Heard and Guckert 1970; Gordon and Layzer 1989). Like other *Fusconaia*, the Longsolid reproduces by releasing pelagic conglutinates into the water column presumably targeting drift-feeding Cyprinidae and Leuscicidae (Haag 2012). Parasitism of *Cyprinella spp.* (Cyprinidae) is common among *Fusconaia* and its sister genus *Pleurobema* and viable hosts include taxa within the genera *Notropis*, *Luxilus*, and *Campostoma* (Kitchel 1985; Bruenderman and Neves 1993; Haag 2012; Bertram et al. 2017; Dudding et al. 2019).

The range and abundance of Longsolid appear to have declined in the last several decades (USFWS 2018). Suitable host fishes for Longsolid are unknown. Knowing these host fishes will be critical to future efforts to captively propagate and potentially augment existing Longsolid populations or to re-establish populations in historically-occupied watersheds.

There were three primary goals in my study. The first was to confirm the Mottled Sculpin as a host for the Appalachian Elktoe and to find additional host fishes for the Appalachian Elktoe. The second was to determine which fish were the most effective hosts for the Appalachian Elktoe by comparing the survival of juvenile mussels transformed on different host fishes. Finally, I wanted to identify suitable host fishes for the Longsolid.

Methods

Appalachian Elktoe Host Trial

I examined host suitability of 8 native fishes that co-occur with Appalachian Elktoe (Table 1). Northern Hogsucker (*Hypentelium nigricans*), Central Stoneroller (*Campostoma anomalum*), Mottled Sculpin, Smallmouth Bass (*Micropterus dolomieu*), and Gilt Darter (*Percina evides*) were collected from tributaries of the French Broad River (in the Ohio River Basin) in western North Carolina through a combination of seine netting and electrofishing. Blacknose Dace (*Rhinichthys atratulus*) were collected from tributaries of the Catawba River (an Atlantic Slope Drainage) in western North Carolina. Captive-reared Sicklefin Redhorse (*Moxostoma sp.*) and Lake Sturgeon (*Acipenser fulvescens*) were obtained from the USFWS Warm Springs National Fish Hatchery in Warm Springs, Georgia. Fishes were quarantined and treated with 170 ppm formalin solutions according modified from Zimmerman et al. (2003) to kill external invertebrate parasites and reduce the likelihood of disease transmission in the hatchery before the start of host trials.

Nine individuals from each species were used in host trials and housed in 30.28 L glass aquaria (3 fish per tank, 3 replicates). Northern Hogsuckers were housed individually because they have been observed removing glochidia from conspecifics in past trials and Smallmouth Bass were placed individually due to interspecific aggression (Rachael Hoch, personal communication). Sicklefin Redhorse were also placed two per tank due to low initial survival from parasite infections. Fishes were randomly assigned a tank using the random number generator from Microsoft Excel®.

On March 28th, 2022 seven gravid Appalachian Elktoe were collected by NC Wildlife Resources Commission biologists from the Little River, a tributary to the French Broad River (Tennessee-Ohio Drainage) in western North Carolina, and were held in the CAC until infestation trials began. Host fishes were infested in three batches, with each batch containing one replicate of each species. Host fish trials were conducted following guidelines in Eads et al. (2015).

Appalachian Elktoe glochidia were extracted from 7 gravid mussels using a water-filled syringe. Glochidia were removed from one gill of each female and tested for viability using a saturated salt solution (Zale and Neves 1982; Dudding et al. 2019). The glochidia from all 7 mussels were viable, and were combined and mixed before being divided into three batches. The three batches were subsampled to ensure that they were all approximately at the target concentration of 8000 glochidia/L. Host fishes were placed into a McDonald Jar, also known as a hatching jar, containing an 1.3 L of water for 25 minutes with an airstone to provide gentle aeration (Table 2).

Immediately after the infestation ended, fishes were transferred into clean water to remove excess glochidia (Johnson et al. 2012). The unattached glochidia were collected from

McDonald jars, nets, and buckets and then counted to estimate the total amount of unattached glochidia left after the infestation. Host fishes were then transferred to holding tanks. Each tank had a 125 µm screened collection cup placed at end of the outflow. Tanks were kept at 16 °C. Cups were rinsed daily Monday through Friday, and on either Saturday or Sunday while transformed juveniles were dropping off. Cups were checked three days per week before transformation began.

I used 1-way Analysis of Variance (ANOVA) to compare glochidia attachment from each of the three batches. The percentage of glochidia that transformed for each fish was also compare using 1-way ANOVA. Tukey's posthoc test was used to determine whether fish hosts had statistically different transformation rates. Percent metamorphosis was calculated using the following formula (Dudding et al. 2019):

$$\%\ \text{metamorphosis} = \frac{\#\ of\ juviniles}{(\#\ of\ juviniles) + (\#\ of\ sloughed\ glochidia)} * 100.$$

All percentages were arcsine square root transformed prior to analysis (Zar 1999). All statistical analyses were done using R Studio (Version 1.4.1717). A p-value of $\alpha = 0.05$ was used to determine statistical significance for all tests.

Juvenile Survival

Transformed juveniles were collected after transformation and placed in 400 mL beakers corresponding to their infestation batch and host fish. Beakers were filled with water and 14.8 mL of sediment. Sediment was sieved through a 125 µm screen and then frozen to remove any potential predators. Beakers were siphoned and filled twice to remove sediment particulates and increase water clarity. Each beaker was placed into a trough containing water that was used to maintain temperature. The trough was part of a recirculating system, equipped with a variable frequency drive pump, ultraviolet light, and drop-in chiller. Water from the Marion State Fish Hatchery's cold water was filtered to 30 µm and radiated with ultraviolet light in the pretreatment filtration building and then supplied to the common sump for the recirculating system. The water was distributed to a trough using a water manifold. The manifold supplied water to each beaker use nozzles fitted with airlines. The airlines supplied a constant drip of water to each beaker. The sump was fed a mix of commercially available algae daily (Reed Mariculture Shellfish 1800 and Nanochloroposis) with the target concentration of 2.5×10^6 $\mu m^3/mL$. Water temperatures were set at 16 °C and slowly raised to 19 °C in week 11 to maximize growth.

Sediment changes were conducted once per week. The water and sediment from the beakers were rinsed through a 125 µm sieve, which allows most of the sediment to pass through while catching the juveniles. Beakers were refilled with sediment using the same methods as the original set-up. Juvenile mussels had their survival recorded each week, and the dead juveniles were counted and removed. Infestation occurred on March 31st, which will be referred to as week 1. By week 7, most of the juvenile mussels had transformed to pediveligers. Juvenile mussel density per beaker was reduced to a target of 50 juveniles per beaker (Table 3). Mussels from hosts with low initial transformation were combined into one beaker. Mottled Sculpin, Northern Hogsucker, and Blacknose Dace had three beakers, whereas Smallmouth Bass, Sicklefin Redhorse, and Central Stoneroller were combined into one beaker. Survival was measured for 5 weeks following this consolidation. However, a flatworm infestation occurred between weeks 10 and 11 and resulted in high mortality. The Smallmouth Bass tank and two Blacknose Dace tanks had chironomids present in week 9, which may have contributed to juvenile mortality. Thus, survival was only analyzed from weeks 7 through 10. Percent survival was calculated at week 10 and survival rates among hosts examined using a 1-way ANOVA in R Studio (Version 1.4.1717). Percent survival data were arcsine square root transformed prior to analysis (Zar 1999). A p-value of $p < 0.05$ was used to determine statistical significance for all tests.

Longsolid Host Trials

Longsolid host trials were conducted in a similar manner to the methods described previously for the Appalachian Elktoe. I examined the suitability of eight fish species that historically co-occurred with the Longsolid (Table 4). Mottled Sculpin, Striped Shiners (*Luxilus chrysocephalus*), Warpaint Shiners (*Luxilus coccogenis*), Central Stonerollers, Whitetail Shiners (*Cyprinella galactura*), River Chubs (*Nocomis micropogon*), and Northern Hogsuckers were collected in tributaries to the French Broad River in western North Carolina using a combination of seine netting and electrofishing. Lake Sturgeon (*Acipenser fulvescens*) were obtained from Warm Springs National Fish Hatchery, in Warm Springs, Georgia. Fishes were quarantined and treated with 170 ppm formalin solutions according to methods from Zimmerman et al. (2003) to kill external invertebrate parasites and reduce the likelihood of disease transmission in the hatchery before the start of host trials.

Nine individuals from each fish taxon were used in host trials. Fishes were housed in 30.28 L glass aquaria (3 fish per tank, 3 replicates). Northern Hogsucker were housed individually because they have been observed removing glochidia from conspecifics in past host trials (Rachael Hoch, personal communication). Warpaint Shiner and River Chub were also housed at a density of two individuals per tank due to low initial survival while in quarantine. However, due to a misidentification prior to infestation, batch 1 contained three

River Chubs and two Central Stonerollers. Fishes were randomly assigned a tank using the random number generator in Microsoft Excel®.

Longsolids were collected by the North Carolina Wildlife Resources Commission from the Little River in the French Broad River Basin in April 2022. Female and male Longsolid were housed at the NCWRC's Conservation Aquaculture Center in Marion, North Carolina (USA). Mussels were held until the females became gravid and released their conglutinates. Infestations were conducted using methods outlined in Eads et al. (2015). Host fishes were infested in three batches, each containing a replicate of each species.

On May 3rd a female mussel released conglutinates at the CAC. The conglutinates were collected with a pipette and then sorted into two groups: well-developed conglutinates and poorly developed conglutinates. Well-developed conglutinates maintained a high glochidia to egg ratio, while poorly developed conglutinates maintained a low glochidia to egg ratio. Three well-developed conglutinates and three poorly developed conglutinates were rinsed through a 500 µm sieve to break them up and were counted to estimate the number of glochidia per conglutinate. Host fishes were placed into McDonald jars with 1.3 liters of water with gentle aeration. Fourteen well-developed conglutinates and 11 poorly-developed conglutinates were used during each infestation trial to create a target concentration of 2090 glochidia/L (Table 5). Conglutinates were rinsed through a 500 µm sieve to break up the external matrix of conglutinate and release free glochidia. Free glochidia were then added to McDonald jars. Infestations ran for 65 minutes, and 2 mL water withdrawals were made every 5 minutes to ensure glochidia remained open.

After 65 minutes, host fishes were immediately placed into clean water to remove excess glochidia (Johnson et al. 2012). After 65 minutes, host fishes were immediately placed into clean water to remove excess glochidia (Johnson et al. 2012). Unattached glochidia were rinsed from the nets, McDonald jars, and buckets and collected in order to enumerate the unattached glochidia post infestation. Fishes were then transferred to holding tanks in a recirculating system, which were kept at 21° C and supplied degassed well water. A 105 µm sieve was placed on the outflow of each tank and used to capture any glochidia or transformed juveniles.

I used a 1-way Analysis of Variance (ANOVA) to compare glochidia attachment rates from among the three batches. The percent of glochidia transformed for each fish taxon was compared using a one-way ANOVA. Tukey's post-hoc test was used to compare transformation rates across fish hosts. All percentage were arcsine square root transformed prior to analysis (Zar 1999). All statistical analyses were done using R Studio (Version 1.4.1717). An alpha value of $p < 0.05$ was used to determine statistical significance for all tests.

Results

Appalachian Elktoe Host Trials

There was no difference in glochidial attachment rates among the three batches (one-way ANOVA; df=1, 22; F= 0.072, p = 0.792). The excystment period for the Appalachian Elktoe lasted from 21 to 48 days (Table 6). The highest numbers of sloughs were present in the Lake Sturgeon (Fig. 1). The Mottled Sculpin and Northern Hogsucker had the highest numbers of transformed juveniles (Fig. 2). The Mottled Sculpin had the longest excystment period (27 days), whereas the Sicklefin Redhorse had the shortest excystment period, 12 days. Mottled sculpin had the highest average transformation percentage across the three batches (62.5%), whereas the Central Stoneroller had the lowest average transformation percentage (3.5%, Table 7). Based on the suite of host fishes examined, the primary hosts for the Appalachian Elktoe appear to be Mottled Sculpin and Northern Hogsucker. Both fishes exhibited statistically higher transformation rates relative to other hosts (Table 8).

Juvenile Survival

Survival at week 10 was highest with mussels transformed on Sicklefin Redhorses at 85.7%, and lowest for mussels transformed on Smallmouth Bass (10.6%, Table 9). However, the juveniles transformed from these host fishes only had one replicate, and were excluded from analysis. A one-way ANOVA was used to compare the survival of juveniles transformed on Mottled Sculpin, Northern Hogsuckers, and Blacknose Dace. There was no difference in juvenile survival rates among host fishes (Table 10).

Longsolid Host Trials

There was no difference in glochidial attachment rates among the three batches (one-way ANOVA; df=1, 22; F= 0.591, p = 0.45). Five of the eight fishes tested served as suitable hosts for the Longsolid. These are the Central Stoneroller, River Chub, Striped Shiner, Warpaint Shiner, and Whitetail Shiner (Fig. 3). The transformation period for the Longsolid lasted between 7 and 28 days (Table 11). The Striped Shiner had the longest excystment period (20 days), whereas the Warpaint Shiner had the shortest drop-off period (11 days). The River Chubs in batch 1 had the highest number of sloughs (Fig. 4). River Chubs in batch 3 had the highest number of transformed juveniles (Fig. 5). The Warpaint and Striped Shiner both had average transformation percentages over 50% (Table 12). Based on the suite of host fishes examined, the primary hosts for the Longsolid appear to be Warpaint and Striped shiners (Table 13).

Discussion

The Appalachian Elktoe appears to be a host generalist, similar to other *Alasmidonta* (Michaelson and Neves 1995; Schulz and Marbain 1998; Bloodsworth et al. 2013). Vaughn describes host generalists as mussels that use hosts from multiple fish families (2012). The Appalachian Elktoe uses host fishes from at least four families; Cottidae, Catostomidae, Leuciscidae, and Centrarchidae. Host generalist reproductive strategies are common among freshwater mussels that do not create mantle lures or conglutinate packages (Watters 1994). Additionally, generalist strategies are common among mussels that are capable of colonizing headwater streams. Appalachian Elktoe are headwater specialists and their life history in the wild likely involves utilization of a range of host fishes. Presumably, this strategy maximizes both colonization potential as well as the stability of established populations in highly variable headwater environments (Haag and Warren 1998).

Previous host trials found the Banded Sculpin was the only suitable host for Appalachian Elktoe from among 18 fish taxa tested (Gordon and Moorman 1998). However, while this study also examined whether Northern Hogsucker could serve as hosts, they did not observe any juvenile transformations. There are three possibilities why my results differed from Gordon and Moorman (1998). First, Gordon and Moorman (1998) conducted host trials during October 1992, whereas my trial took place in March 2022. Appalachian Elktoe are long-term brooders and are gravid from late summer through early spring. However glochidia are not fully mature until February or March in most streams. It is possible that the glochidia used by Gordon and Moorman (1998) may have not been fully mature in October and this may have limited their ability to attach to potential host fishes. Additionally, Gordon and Moorman did not report the number of fishes housed in each tank during their trial. If multiple Northern Hogsuckers were housed together, it is possible that individuals removed the glochidia from one other prior to excystment. Finally, the trial conducted by Gordon and Moorman was ran at 18° C, while my host trial was conducted at 16° C. Freshwater mussels transform at higher rates when temperatures are lower, which may be the result of immunosuppression of the host fish at lower temperatures (Roberts and Barnhart 1999).

Some of the host fishes in this study were collected from the Cane River, in the French Broad River Basin. The Cane River currently supports a small population of Appalachian Elktoe, and mussels are present at low densities at the location of one of my host fish collection sites. Fishes from this river may have past exposure to Appalachian Elktoe glochidia, which has been shown to create an immune response (Reuling 1919; O'Connel and Neves 1999). This could explain some of the large variations in glochidial transformation percentages between the different batches.

Central Stonerollers had glochidia present in their excrement, suggesting that they may, like Northern Hogsuckers, remove glochidia from each other in captivity and/or feed on the pediveligers in the tank. Future studies should house Central Stonerollers separately to see if Appalachian Elktoe transformation rates are higher on fish held in isolation and consider tanks with false bottoms. It is not clear whether this behavior occurs among wild fishes or is just an artifact of housing fishes in artificially close quarters. Allopreening behaviors among sympatric fishes and inter-species cleaning symbioses have been reported from marine systems, including most famously, coral reefs (Limbaugh 1961; Baliga and Law 2016). These behaviors have also been reported in some freshwater fishes. Centrarchids have been observed demonstrating these behaviors in the Florida Everglades during the dry season, and the behaviors were confirmed in laboratory experiments (Sulak 1975; French 1980). More research into glochidia removal, allopreening, and other grooming behaviors in freshwater fishes is needed.

Results of my host trials revealed that Mottled Sculpin appears to be the best host fish to use in the propagation of Appalachian Elktoe. Although there was no significant difference between transformation rates between Mottled Sculpin and Northern Hogsucker, Northern Hogsuckers are larger and must be housed individually to prevent them from removing glochidia from each other. Mottled Sculpin do not engage in grooming behaviors and multiple individuals can be placed in one tank, making it easier to maximize the production of juvenile Appalachian Elktoe during captive propagation. Additionally, there did not appear to be any difference in juvenile survival associated with the host fish. However, this part of the study was extremely limited due to the early mortality caused by flatworms and chironomids, which prevented any results from being conclusive. Future studies should see if differences in transformation periods corresponding to host fishes have a similar impact to juvenile survival as the differences caused by temperature seen by Marwaha et al. (2017).

Prior to this study there were no known hosts for the Longsolid. This mussel uses hosts from Families Cyprinidae and Leuciscidae, which are both in the Order Cypriniformes (Page et al. 2013). Vaughn (2012) describes host specialists as mussels that only use on fish species, or closely related fish species as hosts. The Longsolid appears to be a host-specialist, since both of its hosts are from the same order. Its hosts appear to be similar to other mussels within Genus *Fusconaia*. The Pearly Pigtoe (*Fusconaia cor*) has been observed naturally encysted on Warpaint and Whitetail Shiners (Kitchel 1985).

The Longsolid occupies a large range. Historically, this species occupied four river basins across 12 states, spanning from New York to Indiana to Alabama (USFWS 2018). This is similar to the range of the Striped Shiner: Striped Shiners are present through much of the Longsolid's current range, suggesting that it may be a primary host throughout most of the species range (NatureServe 2013a). However, not all of the hosts for this species cover the entire range. The Warpaint Shiner has a much smaller range than the Longsolid, and is limited to western North Carolina, western Virginia, northern Georgia, northern Alabama, and eastern Tennessee. (NatureServe 2013b). Future studies should be conducted in other states to see if any other cyprinids that occur within this region can serve as hosts for the Longsolid in these areas.

It is important to know the host fishes of the Appalachian Elktoe and the Longsolid for conservation of these species. Knowing that the Appalachian Elktoe is a generalist, and the Longsolid is a specialist, can help to make informed decisions about reintroductions and translocations for these species. Furthermore, knowing these hosts allows for captive propagation of these species.

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References

Baliga, V.B. and C. J. Law. 2016. Cleaners among wrasses: Phylogenetics and evolutionary patterns of cleaning behavior within Labridae. Molecular Phylogenetics and Evolution. 94: 424-435.

Bertram E. P., J. S. Placyk Jr., M. G. Williams, and L. R. Williams. 2017. Verification of two cyprinid host fishes for the Texas pigtoe, *Fusconaia askewi*. Freshwater Mollusk Biology and Conservation. 20: 65-70.

Bloodsworth, K.H., B.R. Bosman, B.E. Sietman, and M.C. Hove. 2013. Host fishes and conservation status of *Alasmidonta marginata* (Bivalvia: Unionidae) in Minnesota. Northeastern Naturalist. 20:49–68.

Bruenderman, S.A., and R.J. Neves. 1993. Life history of the endangered fine-rayed pigtoe, *Fusconaia cuneolus* (Bivalvia: Unionidae) in the Clinch River, Virginia. American Malacological Bulletin. 10:83-91.

Dudding, J., M. Hart, J. Khan, C. R. Robertson, R. Lopez, and C. R. Randklev. 2019. Host fish associations for two highly imperiled mussel species from the southwestern United States: *Cyclonaias necki* (Guadalupe Orb) and *Fusconaia mitchelli* (False Spike). Freshwater Mollusk Biology and Conservation. 22:12-19.

Eads, C. B., J. E. Price, and J. F. Levine. 2015. Fish hosts of four freshwater mussel species in the Broad River, South Carolina. Southeastern Naturalist. 14: 85-97.

French, D.P. 1980. Cleaning behavior in sunfish hybrids under laboratory conditions. Copeia. 1980: 869-870.

Gordon, M. E., and J. B. Layzer. 1989. Mussels (*Bivalvia: Unionoidea*) of the Cumberland River: review of life histories and ecological relationships. U.S. Fish and Wildlife Service Biological Report. 89: 99 pp.

Gordon, M. E., and J. R. Moorman. 1998. A glochidial host of *Alasmidonta raveneliana* (Bivalva: Unionidae). Malacological Review. 31: 31-33.

Haag, W. R. 2012. Host use and host infection strategies. Pages 140-179 *in* W. H. Haag. North American freshwater mussels: natural history, ecology, and conservation. Cambridge University Press, Cambridge, England.

Haag, W.R. and M.L. Warren. 1997. Host fishes and reproductive biology of 6 freshwater mussel species from the Mobile Basin, USA. Journal of the North American Benthological Society. 16: 576-585.

Haag, W.R. and M.L. Warren. 1998. Role of ecological factors and reproductive strategies in structuring freshwater mussel communities. Canadian Journal of Fisheries and Aquatic Sciences. 55: 297-306.

Haag, W. R., and J. D. Williams. 2014. Biodiversity on the brink: an assessment of conservation strategies for North American freshwater mussels. Hydrobiologia. 735: 45-60.

Heard, W.H. and R.H. Guckert. 1970. A re-evaluation of the recent Unionacea (Pelecypoda) of North America. Malacologia. 10:333-355.

Johnson, J. A., J. M. Wisniewski, A. K. Fritts, and R. B. Bringolf. 2012. Host identification and glochidia morphology of freshwater mussels from the Altamaha River Basin. Southeastern Naturalist. 11: 733-746.

Kitchel, H.E. 1985. Life history of the endangered shiny pigtoe pearly mussel, *Fusconaia edgariana*, in the North Fork Holston River, Virginia. Master's Thesis. Virginia Polytechnic Institute and State University, Blacksburg.

Limbaugh, C. 1961. Cleaning symbiosis. Scientific American. 205: 42-49.

Marwaha, J., K.N. Jensen, P.J. Jakobsen, and J. Geist. 2017. Duration of the parasitic phase determines subsequent performance in juvenile freshwater pearl mussels (*Margaritifera margaritifera*). Ecology and Evolution. 7: 1375–1383.

Michaelson, D.L., and R.J. Neves. 1995. Life history and habitat of the endangered dwarf wedgemussel *Alasmidonta heterodon* (Bivalvia: Unionidae). Journal of the North American Benthological Society. 14: 324-340.

NatureServe 2013a. Luxilus chrysocephalus. The IUCN Red List of Threatened Species. Version 2022-1. Available at :https://www.iucnredlist.org. (accessed on 20 October 2022.)

NatureServe 2013b. *Luxilus coccogenis*. The IUCN Red List of Threatened Species. Version 2022-1. Available at: https://www.iucnredlist.org. (accessed on 20 October 2022).

North Carolina Wildlife Resources Commission (NCWRC). 2021. Protected Wildlife Species of North Carolina. Available at:

https://www.ncwildlife.org/Portals/0/Conserving/documents/Protected-Wildlife-Species-of-NC.pdf (accessed October 19, 2022).

O'Connell, M.T. and R.J. Neves. 1999. Evidence of immunological responses by a host fish (*Ambloplites rupestris*) and two non-host fishes (*Cyprinus carpio* and *Carassius auratus*) to glochidia of a freshwater mussel (*Villosa iris*). Journal of Freshwater Ecology. 14: 71-78.

Ostby, B.J.K. 2005. Characterization of suitable habitats for freshwater mussels in the Clinch River, Virginia and Tennessee. Master's Thesis. Virginia Polytechnic Institute and State University, Blacksburg.

Page, L.M., H. Espinosa-Pérez, L.T. Findley, C.R. Gilbert, R.N. Lea, N.E. Mandrak, R.L. Mayden, and J.S. Nelson. 2013. Common and scientific names of fishes from the United States, Canada, and Mexico. American Fisheries Society, Special Publication 34, Bethesda, Maryland.

Pandolfi, G.S. 2016. Effects of climate, land use and in-stream habitat on Appalachian Elktoe (*Alasmidonta raveneliana*) in the Nolichucky River drainage, North Carolina. Master's Thesis. Appalachian State University, Boone, North Carolina.

Pandolfi, G. S., J. W. Mays, and M. M. Gangloff. 2022. Riparian land-use and in-stream habitat predict the distribution of a critically endangered freshwater mussel. Hydrobiologia. 849: 1763-1776.

Parmalee, P.W. and A.E. Bogan. 1998. The freshwater mussels of Tennessee. University of Tennessee Press, Knoxville.

Reuling, F.H. 1919. Acquired immunity to an animal parasite. The Journal of Infectious Diseases. 24: 337-346.

Roberts, A.D. and M.C. Barnhart. 1999. Effects of temperature, pH, and CO2 on transformation of the glochidia of *Anodonta suborbiculata* on fish hosts and in vitro. Journal of the North American Benthological Society. 18: 477-487.

Rock, S.L., J. Watz, P.A. Nilsson, and M. Osterling. 2022. Effects of parasitic freshwater mussels on their hosts fishes: a review. Parasitology. 1-18.

RStudio Team (2020). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA Available at: (http://www.rstudio.com/).

Schulz, C., and K. Marbain. 1998. Hosts species for rare freshwater mussels in Virginia. Triannual Unionid report 15: 32-38.

Simpson, C. T. 1914. A descriptive catalogue of the naiades, or pearly fresh-water mussels. Parts I-III. Bryant Walker, Detroit, Michigan.

Sulak, K.J. 1975. Cleaning behavior in the Centrarchid fishes, *Lepomis macrochirus* and *Micropterus salmoides*. Animal Behavior. 23: 331-332.

US Fish and Wildlife Service (USFWS). 1994. Endangered and threatened wildlife and plants; Appalachian Elktoe determined to be an endangered species. Federal Register 59:60324-60334.

US Fish and Wildlife Service (USFWS). 1996. Appalachian Elktoe recovery plan. Atlanta, Georgia.

US Fish and Wildlife Service (USFWS). 2018. Draft Species Status Assessment Report for the Longsolid Mussel (*Fusconaia subrotunda*), Version 1.X3. Asheville Ecological Services Field Office, Asheville, North Carolina.

US Fish and Wildlife Service (USFWS). 2020. Endangered and threatened wildlife and plants 12-month finding for Purple Lilliput; threatened species status with section

4(d) rule for Longsolid and Round Hickorynut and designation of critical habitat. Federal Register 85:61384-61458.

Vaughn, C.C. 2012. Life history traits and abundance can predict local colonization and extinction rates of freshwater mussels. Freshwater Biology. 57: 982-992.

Watters, G.T., 1994. An annotated bibliography of the reproduction and propagation of the Unionoidea:(primarily of North America) (No. 1). Ohio Biological Survey, Columbus, Ohio.

Zale, A. V., and R. J. Neves. 1982. Reproductive biology of four freshwater mussel species (Mollusca: Unionidae) in Virginia. Freshwater Invertebrate Biology. 1: 17-28.

Zar, J.H. 1999. Biostatistical Analysis. 4th Edition. Prentice Hall, Upper Saddle River, New Jersey.

Zimmerman, L. L., R. J. Neves, and D. G. Smith. 2003. Control of predacious flatworms *Macrostomum sp*. In culturing juvenile freshwater mussels. North American Journal of Aquaculture. 65: 28-32.

Tables

Table 1: Fishes tested as mussel hosts in Appalachian Elktoe host trials. Three replicates of the host trial were infested, with each replicate containing 19 fishes.

Host Fish	Number of Fish per replicate
Blacknose Dace (*Rhinichthys atratulus*)	3
Central Stoneroller (*Campostoma anomalum*)	3
Gilt Darter (*Percina evides*)	3
Lake Sturgeon (*Acipenser fulvescens*)	3
Mottled Sculpin (*Cottus bairdi*)	3
Northern Hogsucker (*Hypentelium nigricans*)	1
Sicklefin Redhorse (*Moxostoma sp.*)	2
Smallmouth Bass (*Micropterus dolomieu*)	1

Table 2: Estimated glochidia amounts for Appalachian Elktoe host trials. This includes the initial glochidia concentration for each batch (replicate), amount of water used, estimated glochidia/L concentration, number of fish used, and the amount of unattached glochidia at the end of the infestation period.

Batch	Initial Glochidia	Water (L)	Glochidia/L	# of Fish	Unattached Glochidia
1	12200	1.3	9384.6	19	3250
2	10260	1.3	7892.3	19	1867
3	13600	1.3	10461.5	19	3553

Table 3. Initial numbers of juvenile mussels placed into each beaker for survival experiments from each host fish. Each beaker had a target number of 50 juveniles, but fewer than 50 were placed in beakers with low juvenile numbers. (BND = Blacknose Dace; NHS = Northern Hogsucker; MOSC = Mottled Sculpin; CSR = Central Stoneroller; SFRH = Sicklefin Redhorse; SMB = Smallmouth Bass)

BND	NHS	MOSC	CSR	SFRH	SMB
50	50	50	43	14	47
50	50	50			
18	24	50			

Table 4: Table 1: Fishes tested as mussel hosts in Longsolid host trials. Three replicates of the host trial were infested, with each replicate containing 20 fishes.

Host Fish	Batch 1	Batch 2	Batch 3
Central Stoneroller (*Campostoma anomalum*)	2	3	3
Lake Sturgeon (*Acipenser fulvescens*)	3	3	3
Mottled Sculpin (*Cottus bairdii*)	3	3	3
Northern Hogsucker (*Hypentelium nigricans*)	1	1	1
River Chub (*Nocomis micropogon*)	3	2	2
Striped Shiner (*Luxilus chrysocephalus*)	3	3	3
Warpaint Shiner (*Luxilus coccogenis*)	2	2	2
Whitetail Shiner (*Cyprinella galactura*)	3	3	3

Table 5: Estimated glochidia for Longsolid host trials. This includes the initial glochidia concentration for each batch (replicate), amount of water used, estimated glochidia/L concentration, and the number of fish used.

Batch	Initial Glochidia	Water (L)	Glochidia/L	# of Fish
1	2718	1.7	1598.82	20
2	2718	1.75	1553.14	20
3	2718	1.8	1510.00	20

Table 6: Length of glochidia transformation times for Appalachian Elktoe juveniles. The day of infestation is day 1.

Host	Excystment Started	Excystment Ended	# of Days
Blacknose Dace	21	37	16
Central Stoneroller	21	33	12
Gilt Darter	N/A	N/A	N/A
Lake Sturgeon	N/A	N/A	N/A
Mottled Sculpin	21	48	27
Northern Hogsucker	21	38	17
Sicklefin Redhorse	24	36	12
Smallmouth Bass	21	41	20

Table 7: Transformation percentage of Appalachian Elktoe glochidia by host fish and batch, number attached, and number transformed.

	1	2	3	Average
Blacknose Dace	20.0%	3.7%	12.8%	12.2%
Central Stoneroller	3.5%	1.4%	5.6%	3.5%
Gilt Darter	0.0%	0.0%	0.0%	0.0%
Lake Sturgeon	0.0%	0.0%	0.0%	0.0%
Mottled Sculpin	63.6%	68.8%	55.2%	62.5%
Northern Hogsucker	74.9%	25.8%	47.3%	49.3%
Sicklefin Redhorse	9.5%	38.9%	34.0%	27.5%
Smallmouth Bass	11.9%	3.6%	20.9%	12.1%

Table 8: One-way ANOVA and Tukey Post Test comparing transformation percentages of glochidia for the Appalachian Elktoe. The homogeneous groups with the same letter are not statistically significant from each other (p= 0.05). The raw means are presented here.

Source	DF	SS	MS	F	P
Fish	7	2.39595	0.34228	18.05	0.0000
Error	16	0.30347	0.1897		
Total	23	2.69942			

Fish	Mean	Homogeneous Group
Mottled Sculpin	12.2%	A
Northern Hogsucker	3.5%	A
Sicklefin Redhorse	0.0%	AB
Blacknose Dace	0.0%	BC
Smallmouth Bass	62.5%	BC
Central Stoneroller	49.3%	BC
Gilt Darter	27.5%	C
Lake Sturgeon	12.1%	C

Table 9: Survival of Appalachian Elktoe juveniles for weeks 8 though 12. Percent survival is averaged for the Blacknose Dace, Northern Hogsucker, and Mottled Sculpin. Flatworms infested the trial between weeks 10 and 11, causing a spike in mortality. Two of the three Blacknose Dace replicates and the Smallmouth Bass replicate were infested with chironomids in week 9, which may have impacted their mortality.

Host	Week 8	Week 9	Week 10
BND	0.7253	0.4585	0.1156
NHS	0.9194	0.6833	0.3878
MOSC	0.9733	0.8400	0.5333
CSR	0.7674	0.3953	0.1163
SFRH	1.0000	1.0000	0.8571
SMB	0.7447	0.2553	0.1063

Table 10. One-way ANOVA comparing the survival of juvenile mussels transformed from Mottled Sculpin, Northern Hogsuckers, and Blacknose Dace at Week 10, where survival started at week 7.

Source	DF	SS	MS	F	P
Survival	2	0.3626	0.18129	4.656	0.0602
Error	6	0.2336	0.03894		
Total	8	0.5962			

Table 11: Length of glochidia transformation times for Longsolid juveniles. The day of infestation is day 1.

Host	Excystment Started	Excystment Ended	# of Days
Central Stoneroller	7	21	14
Lake Sturgeon	N/A	N/A	N/A
Mottled Sculpin	N/A	N/A	N/A
Northern Hogsucker	N/A	N/A	N/A
River Chub	12	27	15
Striped Shiner	8	28	20
Warpaint Shiner	15	26	11
Whitetail Shiner	7	19	12

Table 12: Transformation percentage of Longsolid glochidia by host fish and batch.

	1	2	3	Average
Central Stoneroller	4.4%	7.4%	1.3%	4.4%
Lake Sturgeon	0.0%	0.0%	0.0%	0.0%
Mottled Sculpin	0.0%	0.0%	0.0%	0.0%
Northern Hogsucker	0.0%	0.0%	0.0%	0.0%
River Chub	3.0%	5.1%	68.6%	25.6%
Striped Shiner	78.6%	28.1%	60.8%	55.9%
Warpaint Shiner	48.2%	80.0%	41.7%	56.5%
Whitetail Shiner	0.0%	16.9%	0.0%	5.6%

Table 13: Table 8: One-way ANOVA and Tukey Post Test comparing transformation percentages of glochidia for the Longsolid. The homogeneous groups with the same letter are not statistically significant from each other (p= 0.05). The raw means are presented here.

Source	DF	SS	MS	F	P
Fish	7	2.82396	0.40342	8.34	0.0002
Error	16	0.77393	0.04837		
Total	23	3.59789			

Fish	Mean	Homogeneous Group
Warpaint Shiner	4.4%	A
Striped Shiner	0.0%	A
River Chub	0.0%	AB
Central Stoneroller	0.0%	B
Whitetail Shiner	25.6%	B
Mottled Sculpin	55.9%	B
Northern Hogsucker	56.5%	B
Lake Sturgeon	5.6%	B

Fig. 1. Total numbers of Appalachian Elktoe Sloughs by host fish and infestation batch for each tank.

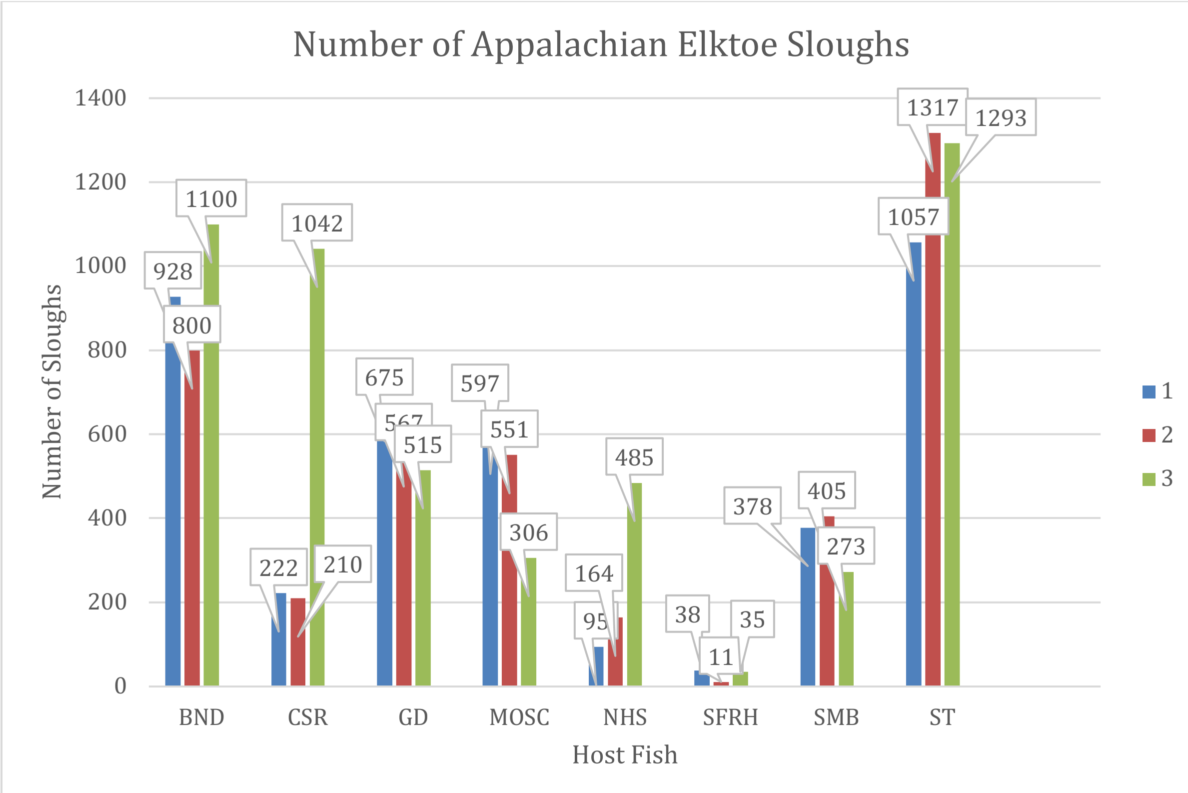


Fig. 2. Total numbers of transformed Appalachian Elktoe juveniles by host fish and batch for each tank.

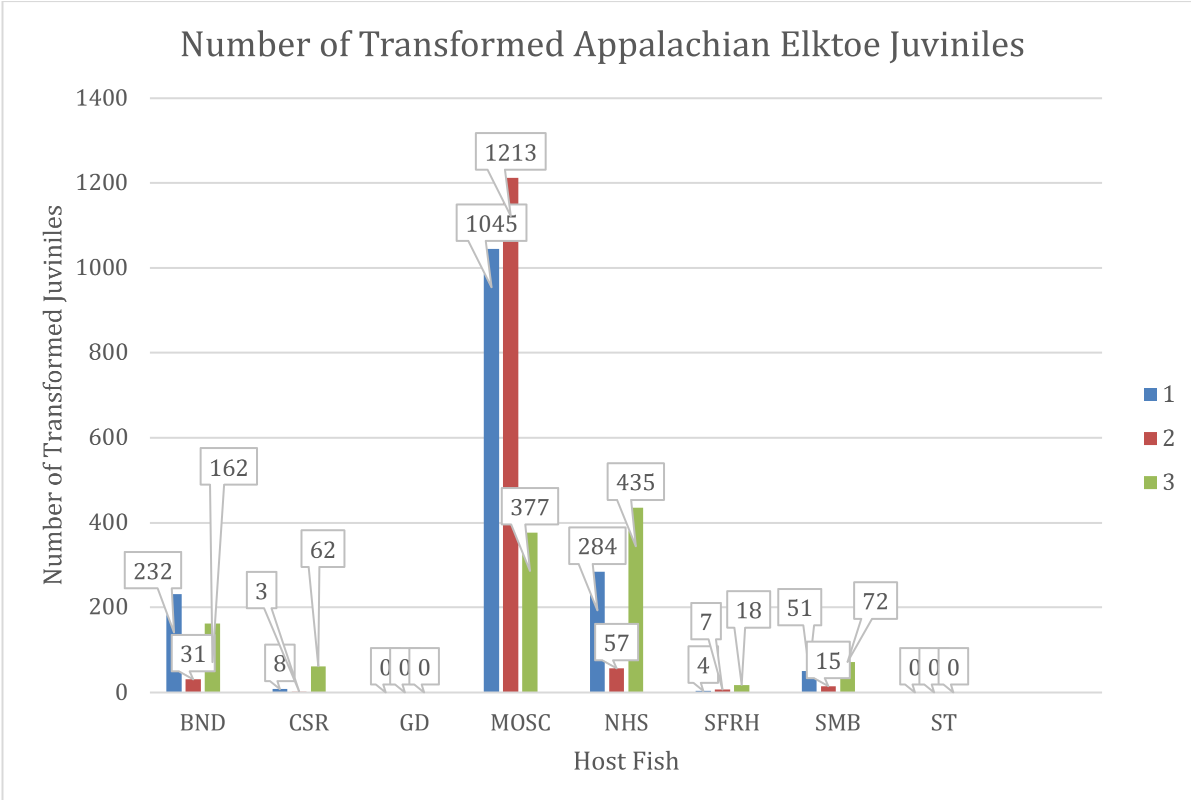


Fig. 3. Longsolid juvenile transformed on a Striped Shiner.

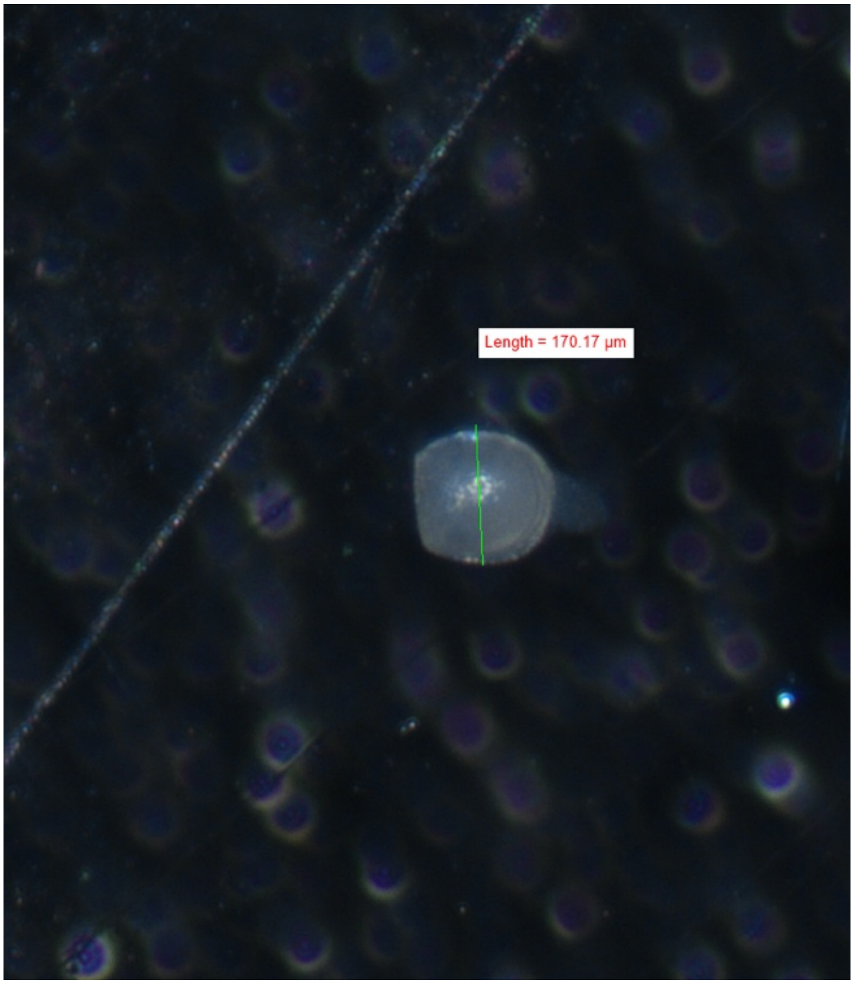


Fig. 4. Total numbers of Longsolid sloughs by host fish and infestation batch for each tank.

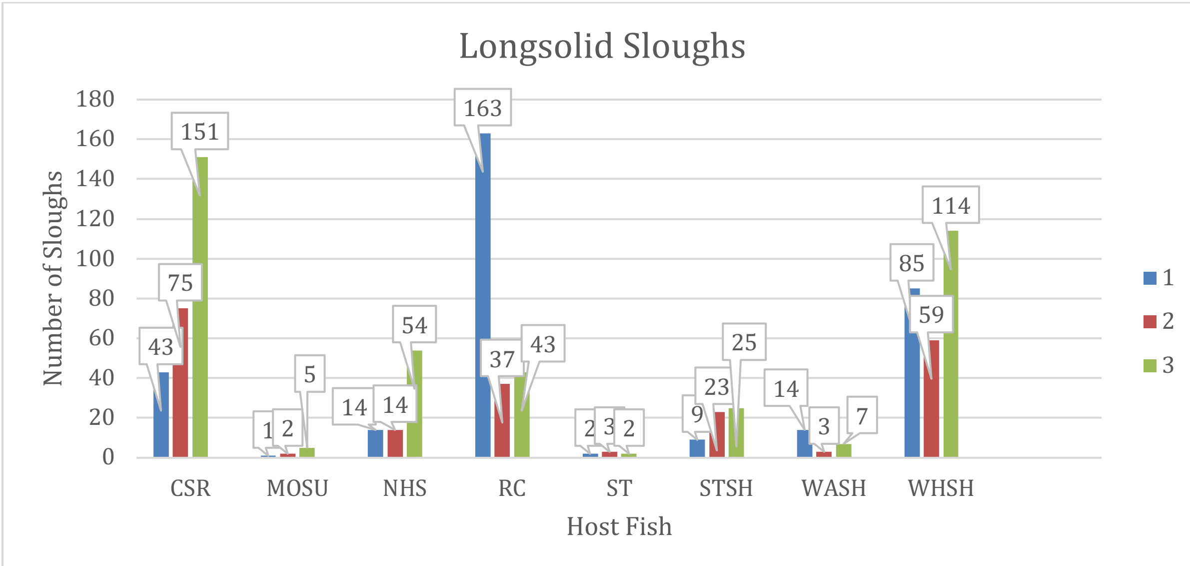
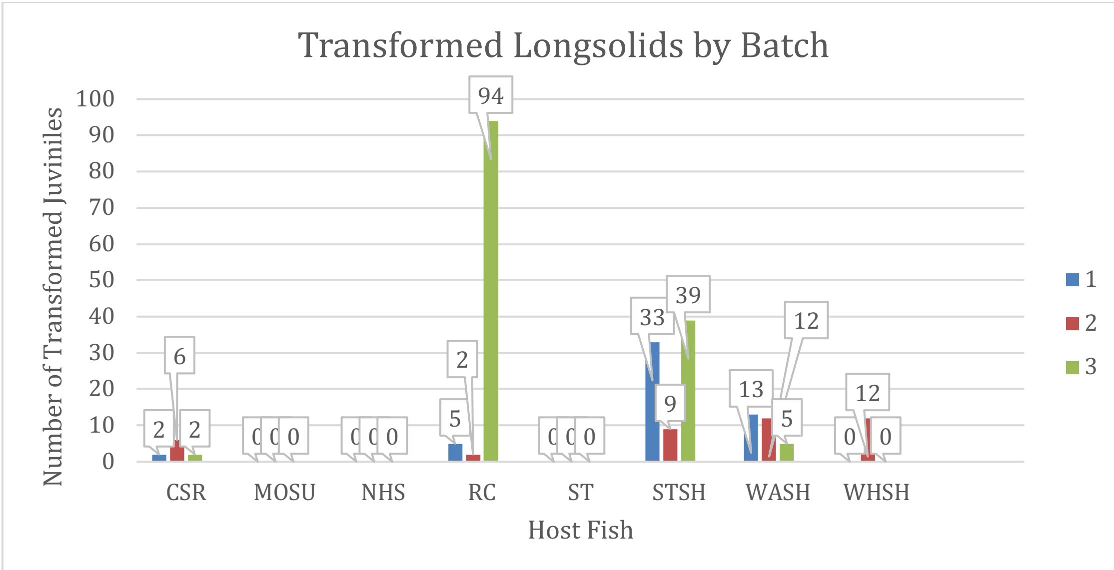


Fig. 5. Total numbers of transformed Longsolid juveniles by host fish and batch for each tank.



Vita

Rebekah Lynn Ewing was born in Morganton, North Carolina to Todd and Michelle Ewing. She grew up exploring the creeks of western North Carolina, and developed a love for aquatics. She graduated from Jimmy C. Draughn High School in June 2017. The following fall she enrolled at North Carolina State University to study Fish, Wildlife, and Conservation Biology, and was awarded a Bachelor of Science in December 2020. She began working on a Master of Science in Biology at Appalachian State in spring of 2021.

Rebekah is a member of the American Fisheries Society. In her free time she can be found cross stitching, exploring the parkway, baking, or hanging out with her cat.