Evaluation of Growth and Survival of Three Freshwater Mussel Species at Sites Targeted for Population Restoration in a North Carolina River

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Abstract: In North Carolina, wavyrayed lampmussels (*Lampsilis fasciola*) and spike (*Eurynia dilatata*) currently are state species of special concern, and rainbow mussels (*Villosa iris*) are state threatened. As a result of extensive conservation and management efforts, recovery of suitable habitat and improvements in water quality have made mussel restoration a possibility in the Oconaluftee River within lands owned by the Eastern Band of Cherokee Indians. As part of restoration efforts, we introduced propagated or translocated individuals of these three species into the Oconaluftee River. Individuals were marked and stocked at four sites as either free-living specimens or within silo enclosures, and monitoring took place over one growing season (April to October 2019) to record survival and growth. In addition, we included data from mussels in silos remaining on three of our four sites from a previous feasibility study. Survivorship of stocked mussels during the study period was 98.3% for wavyrayed lampmussels, 96.6% for spike, and 96.6% for rainbow mussels. Additionally, we detected growth for all species, though it differed among sites with individuals growing least at the most upstream site for both the wavyrayed lampmussel and rainbow mussel. Free-living individuals grew faster and suffered less valve damage than those held in silos. This information is useful to guide study design for further mussel restoration efforts and feasibility studies both in the Oconaluftee River drainage and in other high-gradient systems.

Key words: Unionids, conservation, aquatic diversity

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Freshwater mussels of the Order Unionida are represented by 302 recognized extant species in the United States and Canada (Graf and Cummings 2021). Over two-thirds of these species are thought to be critically imperiled, imperiled, or vulnerable (Bouska et al. 2018, Lopes-Lima et al. 2018), and at least 29 North American species have become extinct in the last 100 years (Haag and Williams 2014). Global declines have occurred in many in freshwater mollusk populations, even those that were previously robust in parts of their range (Lydeard et al. 2004, Spooner and Vaughn 2006, Lopes-Lima et al. 2018). River impoundment, increased point and nonpoint source pollution, and the introduction of invasive mussel species such as zebra mussels (*Dreissena polymorpha*) and Asian clams (*Corbicula fluminea*) are among the potential causes of decline, though the exact causes are not fully understood (Vaughn and Taylor 1999, Downing et al. 2010, Haag and Williams 2014).

Freshwater mussels fill highly important roles in river ecosystems. For example, their bioturbation of sediments oxygenates streambeds, encouraging growth and survival of diverse benthic communities of microbes and invertebrates (Vaughn and Hakenkamp 2001). These activities serve to make lotic ecosystems more resilient and speed recovery following disturbance but only if mussels are present in dense populations with significant biomass (Howard and Cuffey 2006). Established mussel beds provide stability for the substrate, preventing bedload shift, lessening the effect of high flow events, and allowing for easier re-establishment of benthic organisms after disturbance (Cowie et al. 2017). This could be of particular importance in mountainous streams where elevation changes and steep tributary topography can contribute to rapid fluctuations in water flow. By providing hard substrate, the valves of living mussels are regularly colonized by other organisms such as tardigrades, insect larvae, and bacteria (Spooner and Vaughn 2006).

Three species facing decline in western North Carolina are wavyrayed lampmussel (*Lampsilis fasciola*) and spike (*Eurynia dilatata*), which currently are listed as State Species of Special Concern, and rainbow mussel (*Villosa iris)* which currently is listed as State Threatened in North Carolina (North Carolina Wildlife

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Resources Commission [NCWRC] 2020, Ratcliffe 2021). These species are sympatric through a significant portion of their ranges and share similar habitat preferences where they co-occur. They are generally found in the lower Great Lakes and upper Mississippi River basins, including the Ohio and Tennessee river systems, where they inhabit small- to medium-sized rivers with stable gravel streambeds (Bogan 2017). They are currently found in the French Broad, Hiwassee, and Little Tennessee River systems of western North Carolina (Bogan 2017, NCWRC 2020). Wavyrayed lampmussels are most common in shallow rivers (depths less than 1 m) but unlike most lotic unionid species, they can also inhabit slow moving water and fine sediments (Alderman et al. 2001, Bogan 2017). Known fish hosts for wavyrayed lampmussels include smallmouth bass (*Micropterus dolomieu*) and largemouth bass (*M. salmoides*; Bogan 2017). Rainbow mussels are found in the Hiwassee, Little Tennessee, and French Broad drainages of North Carolina, and are most numerous in shallow, clearwater riffles with gravel substrate and strong flows (Alderman et al. 2001, Bogan 2017). Known fish hosts for rainbow mussels include smallmouth bass, largemouth bass, mottled sculpin (*Cottus bairdii*), and western mosquitofish (*Gambusia affinis*; Bogan 2017). Spike are found throughout the Mississippi River drainage, and in North Carolina can be found in the Hiwassee, Little Tennessee, French Broad, and New river drainages (Bogan 2017). These mussels are more generalized in habitat preference, living at a variety of depths and substrate types, but are thought to grow best in firm gravel substrates and moderate water flows (Elderkin et al. 2008, Bogan 2017). Known fish hosts for spike include gizzard shad (*Dorosoma cepedianum*), white crappie (*Pomoxis annularis*), and black crappie (*P. nigromaculatus*), as well as several sculpin (*Cottus* spp.) and darter species (*Etheostoma* spp.; Bogan 2017).

Most larger streams of the Upper Tennessee River Basin in western North Carolina have at least some populations of native mussels. However, mussels have likely been extirpated from the Oconaluftee River, the major tributary to the Tuckaseegee River (Fraley 2002, M. Cantrell, U.S. Fish and Wildlife Service [USFWS], personal communication). The exact causes of mussel extirpation from the Oconaluftee River are not fully known, but this drainage experienced changes in the early twentieth century including rapid riparian development with road and railroad construction, logging, and impoundment. Establishment of the Great Smoky Mountains National Park in 1934 provided some protection from anthropogenic disturbances for sub-watersheds within the Oconaluftee River drainage. However, establishment of the Park and construction of the nearby Blue Ridge Parkway resulted in the adjacent lands owned by the Eastern Band of Cherokee Indians (i.e., the Qualla Boundary) becoming the most visited Native American lands in the United States, leading to rapid development targeting tourists (Beard-Moose and Paredes 2009). Little data exists on historical distribution of mussels in the Oconaluftee River, as the earliest mussel surveys were conducted in the late 1990s by USFWS personnel (M. Cantrell, U.S. Fish and Wildlife Service, personal communication). However, several freshwater mussel species have names in Cherokee tribal language or are mentioned in tribal histories, and riverine health is very important to local traditions, so there is a desire for restoration of animals that were likely to have been historically present on the tribal landscape (C. Hickmann, Eastern Band of Cherokee Indians Natural Resources Department, personal communication).

Finigan (2019) explored the feasibility of restoring wavyrayed lampmussels and rainbow mussels in the Oconaluftee River by placing individuals in silos (described below in Methods) at three sites in the river and found that they survived well and grew at all three locations. The next step for restoration activities was to determine if this watershed is a viable candidate for further restoration efforts and if any of the selected sites is a better candidate for restoration efforts than the others based on mussel growth and survival rates. Thus, the objectives of this study were to 1) determine growth and survival of the three mussel species stocked at four sites on the Oconaluftee River, and 2) compare growth and physical condition of mussels held in silos with that of free-living mussels to determine potential limitations and trade-offs of the two stocking methods.

Study Area

Of our four study sites, three sites were previously used by Finigan (2019) and were chosen due to availability of adequate substrate for mussel bed establishment and because the sites collectively provided slightly different habitat conditions associated with their location in the watershed and proximity to development (Figure 1). Site 1 was located upstream of a reservoir created by a dam constructed in 1924–1925 on the Oconaluftee River near its confluence with the Tuckaseegee River and downstream of a wastewater treatment plant. Site 2 was located immediately upstream of the wastewater treatment plant but downstream of the confluence of Soco Creek, a tributary with significant development in its riparian zone. Site 3 was located upstream of Soco Creek but below the town of Cherokee, North Carolina, which is heavily used by tourists including extensive river fishing and recreation. We chose an additional site (site 4) that was located upstream near the border between the Qualla Boundary and Great Smoky Mountains National Park which had experienced few anthropogenic impacts.

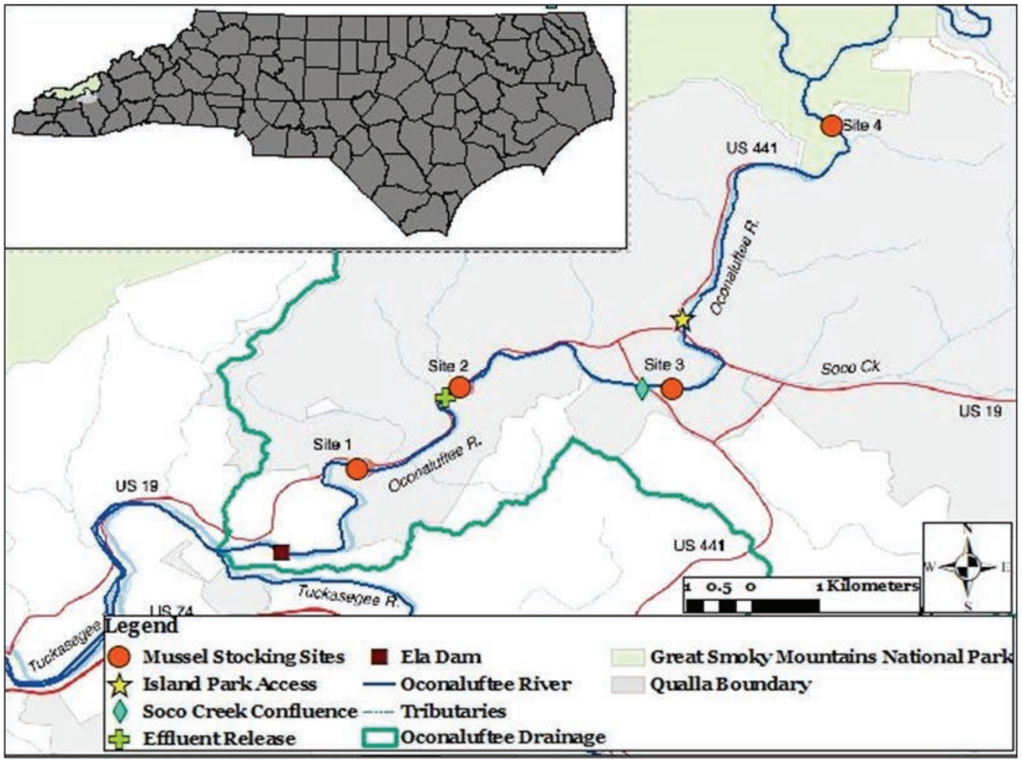

Figure 1. Study area for reintroduction of rainbow mussels, wavyrayed lampmussels, and spike in the Oconaluftee River, North Carolina. Also shown are major tributaries, Ela Dam, and the town of Cherokee, including the location of the effluent from its wastewater treatment plant.

Methods

Propagation and Field Protocols

The NCWRC Conservation Aquaculture Center in Marion, North Carolina, provided wavyrayed lampmussels and rainbow mussels that had been reared from gravid females collected from the Tuckaseegee River, with 2016 and 2017 cohorts used. One hundred and twenty mussels of each species were marked using Hallprint Shellfish tags (Hallprint Inc., Hindmarsh Valley, South Australia) fixed to their shells using waterproof super glue (Loctite Ultra Gel, Henkel Corporation, Rocky Hill, Connecticut) as described by Lemarié et al. (2000), allowing easy identification of individuals. Along with these shell tags, 60 juveniles ≥18 mm in length of each species also were tagged using Passive Integrated Transponder (PIT) tags (8-mm FDX-B, Oregon RFID, Portland, Oregon), which facilitated location of individuals. We fixed PIT tags to the right valve with superglue, and then covered them with JB Waterweld (J-B Weld, Sulphur Springs, Texas) molded to the shell.

Rainbow mussel and wavyrayed lampmussel juveniles were placed into study sites in April 2019. For the free-living group, we stocked 20 PIT-tagged individuals of each species into the substrate at each of sites 1–3, randomly allocating individuals to sites. The remaining wavyrayed lampmussels and rainbow mussels were placed in mussel silos, with three silos containing five individuals of each species placed at each of the four sites. Because Finigan (2019) found slower growth at upstream sites with their cooler temperatures and lower nutrient availability, at the most upstream site (site 4) we populated mussels only into silos because we wanted to be able to relocate individuals downstream at the conclusion of the study if it appeared that water conditions were not suitable upstream. Silos (modified from Barnhart et al. 2007) were dome-shaped concrete enclosures with a hollow column in the center; the hollow center contained a PVC inner chamber enclosed with screen on both sides so that mussels had access to the water column to feed. When installed, water would flow over the dome, creating negative pressure, then flow through the center column allowing the mussels to have continuous access to oxygen and food particles. In addition to the silos that we installed and stocked, we included 11 silos from the Finigan (2019) study that we had recovered through snorkel surveys at the three sites prior to beginning our data collection. This resulted in the addition of five silos (containing 24 mussels total when found) at site 1, three (containing 18 mussels total) at site 2, and three at site 3 (containing 16 mussels total).

For wavyrayed lampmussels, all individuals stocked in the free-living and silo groups were from a single 2016 cohort. However, the 2016 cohort of rainbow mussels did not contain enough individuals to meet desired sample sizes for each group, necessitating use of a 2017 cohort as well. Therefore, for rainbow mussels the 2016 cohort was used for the free-living group while those used in the silos were from the 2017 cohort.

In June 2019, adult spike were translocated from the Little Tennessee River to the Oconaluftee River as this species is not currently cultured at the Conservation Aquaculture Center. Spike were collected, tagged in the field with both PIT and Hallprint Shellfish tags similarly to the other two species, measured (length; 0.01 mm), and then immediately transported in a cooler and placed at sites 1–3. Translocation was completed in one day to reduce stress on the animals. We placed 32 spike at each site in already-established mussel beds; none were included in the silos because adults would not fit in the inner chamber of the silo.

Sampling Protocols

Surveys of free-living mussels.—We conducted monthly surveys of free-living mussels at each site beginning during initial stocking in April 2019 and ending in October 2019, resulting in seven total sampling events. The surveyed portion of each site was contained in a reach area approximately 10 by 10 m marked by flagging tape and bordered on two sides by the bank and a designated boulder. For each sampling event on each site, a 1-h snorkel survey was conducted beginning at the downstream end of the site reach and working in a zig-zag pattern upstream until the entire reach had been covered. The surveyor used a water-resistant PIT-tag reader wand (HPR Plus, Biomark Identification Solutions, Boise, Idaho) to locate free-living mussels, which were collected by hand and placed in a submerged mesh bag for measurement. Sampling was conducted during times when air and water temperatures were most similar and emersion times were minimized to reduce handling stress and therefore potential impacts to growth and survival (Bartsch et al. 2000, Ohlman and Pegg 2019).

Each animal was taken from its submerged bag, its species was identified, and then it was measured using digital calipers (0.01 mm). Qualitative observations were also recorded, including whether organisms were occupying mussel valves and the animal's overall body condition. Extent of valve damage was scored based on photos of the left and right valves of each mussel taken during the final sample in October 2019 (Table 1). Mild surface-level wear on and around the umbo of a mussel was not considered to be damage, as this area is naturally worn down by the regular activity of the animals (R. Hoch, NCWRC, personal communication). Any observed mortality (i.e., recovered shell) was recorded at each sampling event as well. After processing, the animals were returned by hand to the substrate with their siphons facing up, beginning at the upstream end of the reach and spreading them randomly across the entire marked area to the downstream end. The snorkel surveys and all mussel measurements were conducted by the same researcher throughout the study to reduce variation.

Table 1. Grading criteria for valve damage scores assigned to each individual mussel of all three species collected from the Oconaluftee River in the final sampling event in October 2019.

Score	Group	Criteria
1	No damage	no obvious scrapes, chips, or gouges into the valves
2	Mild damage	visible scraping on valves, no chips out of valve edges or deep gouges
3	Damaged	visible scrape marks and deep gouging into the valves or edges chipped
4	Severely damaged	valve is cracked, broken, or crushed in; some part of the animal inside may be visible

Surveys of mussel silos.—Following the snorkel survey, the mussel silos in each reach were collected and brought to the riverbank where they were kept submerged as much as possible. For each silo, the entire inner chamber was removed to allow access to the mussels within. The mussels were removed from the silos and processed exactly as described for free-living mussels. If any of the chambers had visible wear on the end mesh screens or if they appeared clogged with debris, they were replaced with a new one. Reused chambers were rinsed thoroughly in the river to clear out any accumulation of sediment or pseudofeces. After measurements had been taken, mussels were placed back into a random inner chamber which was then returned to a random silo dome and tied back into place. The silos were then returned to just below the downstream end of the snorkel reach, placed in a streamlined pattern with the upstream edge slightly buried to allow water to flow freely over the dome but prevent it from lifting the dome off of the substrate. Mortality was recorded if any valves were found empty or with partially decayed material in them. Final measurements of dead individuals were included in analysis if the valves were not obviously damaged.

Data Analysis

Survival was calculated and reported as the total percentage of mussels alive at the end of the study out of the total number stocked. In addition to observed mortalities, for free-living mussels we also categorized an individual's fate as a mortality if its tag number was not recorded in any surveys following initial stocking. Mussels from silos which washed away and were lost during our study were not included in analyses. These mussels were not considered to be mortalities given we found live mussels in previously lost silos from Finigan (2019).

Differences in growth among sites, sampling events, and stocking type (silo vs. non-enclosed substrate) were evaluated for each species using linear mixed-effects model analysis (i.e., repeated measures ANOVA) of lengths of individual mussels. In the mixed-model analysis, a random effect of individual mussel was included, with stocking type, site, and sample month as fixed effects. For spike, which were only placed on substrate, the stocking-type effect was omitted. In these analyses we used the individual mussel as the experimental unit and ignored potential correlation in growth among individuals in the same silo because there was no similar nesting structure for the free-living stocking type. Further, silos were not individually marked and mussels in the silos were occasionally moved among silos. For rainbow mussels caution is needed for interpreting effects of stocking type (silo vs. non-enclosed substrate), which were confounded by potential age class effects because we used younger, smaller mussels of this species in silos. Growth rate changes dramatically with age in freshwater mussels, especially between juveniles and sub-adults such as were used in this study (Kesler et al. 2007, Valdovinos and Pedreros 2007, Anthony et al. 2008).

Analysis of length data was conducted in R ver3.6.1 (R Core Team 2019) using the lme4 package (Bates et al. 2015), with lmerTest package (Kuznetsova et al. 2017) used to calculate *P*-values based on Type II SS and to estimate df with the Kenward-Rogers approximation. Relationships between valve damage scores and location (stocking type and site) were visualized with boxplots and tested using G-tests of independence from the DescTools package (Signorell et al. 2020), again using individual mussels as the experimental units. All tests were considered significant at $P < 0.05$.

Results

At the conclusion of the study, survivorship across both free-living and silo treatments was 96.6% for rainbow mussels, 98.3% for wavyrayed lampmussels, and 96.6% for spike. There was little evidence of mortality in mussels stocked either into the substrate or in silos: there was no shell material found in snorkel surveys and animals were consistently recaptured throughout the study (only four tag numbers were never recovered in the snorkel surveys). Four rainbow mussels, two wavyrayed lampmussels, and four spike died during our study in sites 1 and 3; there was no observed mortality at sites 2 and 4. We note, however, that the recapture of free-living PIT-tagged mussels was lower in the June sampling event because the PIT-tag reader stopped working properly, but the unit was repaired before the next sample. Six silos containing 38 of 394 mussels washed away during the study and were not recovered, including all Site 4 silos in July 2019.

Growth was detected in all three mussel species (Figure 2, 3, 4). Rainbow mussels and wavyrayed lampmussels stocked into the substrate achieved a larger size by the end of the study and changed in size earlier compared to those in silos (Figure 2, 3). We found

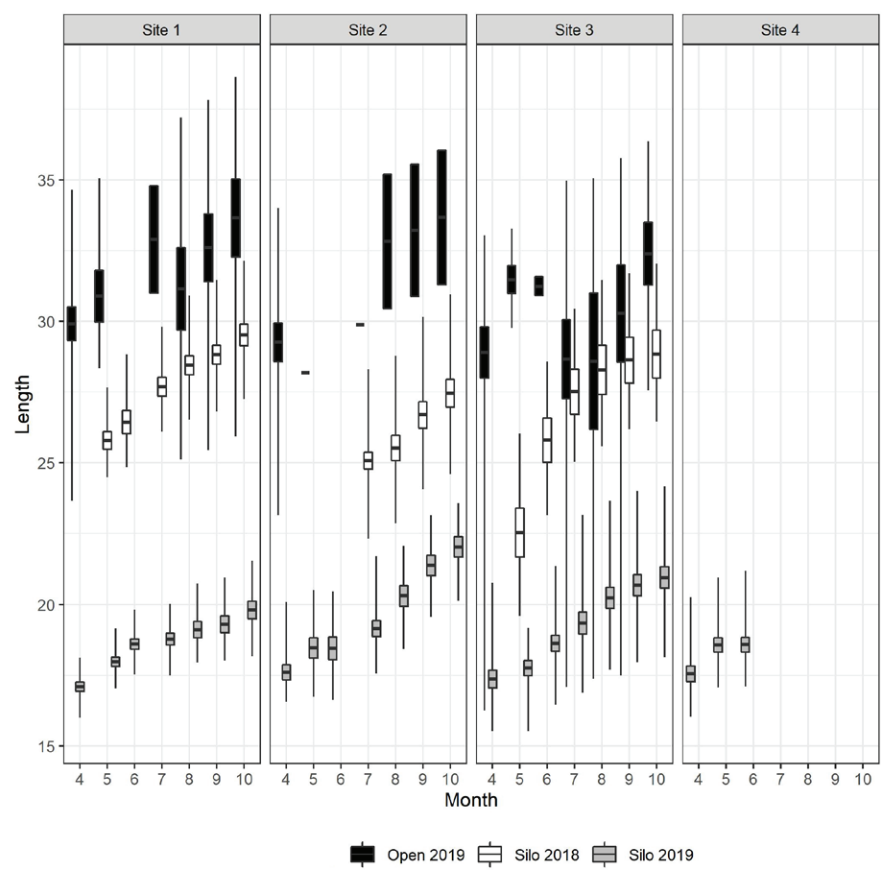

Figure 2. Length measurements for rainbow mussels by site and month of survey (April–October 2019) in the Oconaluftee River, North Carolina. "Open 2019" refers to free-living mussels placed at each site in April 2019; this group has missing values for the month of June due to PIT tag reader malfunction. The "Silo 2019" group were mussels placed in silos at the start of our study; "Silo 2018" refers to mussels in silos found from Finigan (2019). Silos at site 4 were washed away by high flows in July. Box center line, upper and lower box ends, and whiskers represent mean, one SE, and minimum and maximum measurements, respectively.

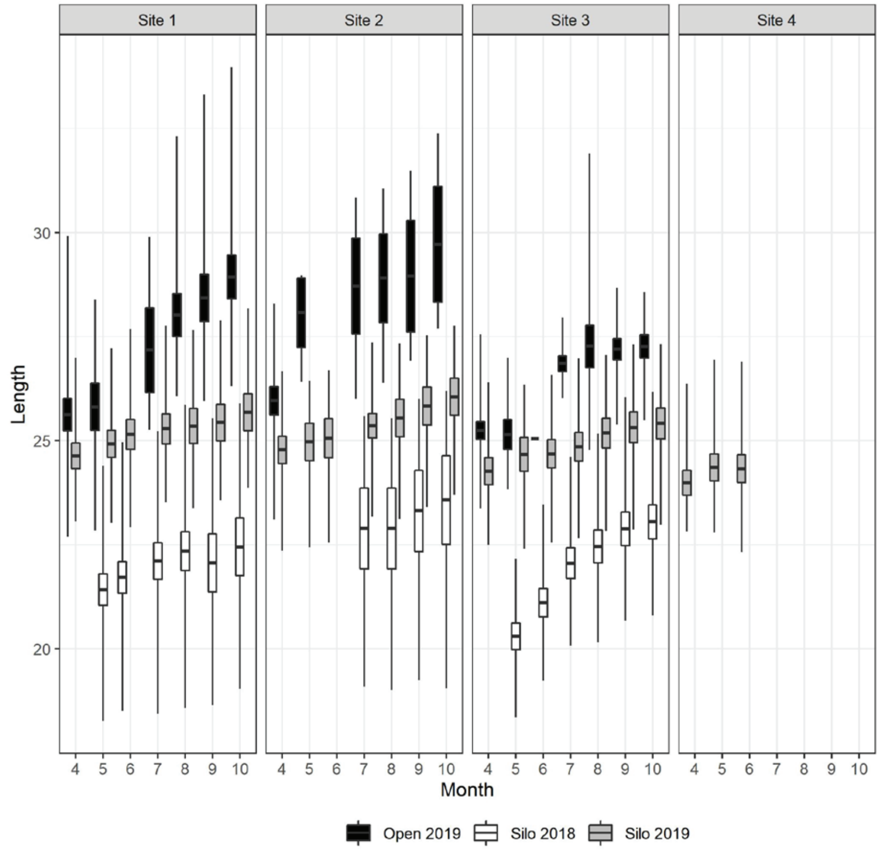

Figure 3. Length measurements for wavyrayed lampmussels by site and month of survey (April–October 2019) in the Oconaluftee River, North Carolina. "Open 2019" refers to free-living mussels placed at each site in April 2019; this group has missing values for the month of June due to PIT tag reader malfunction. The "Silo 2019" group were mussels placed in silos at the start of the study; "Silo 2018" refers to mussels in silos found from Finigan (2019). Silos at site 4 were washed away by high flows in July. Box center line, upper and lower box ends, and whiskers represent mean, one SE, and minimum and maximum measurements, respectively.

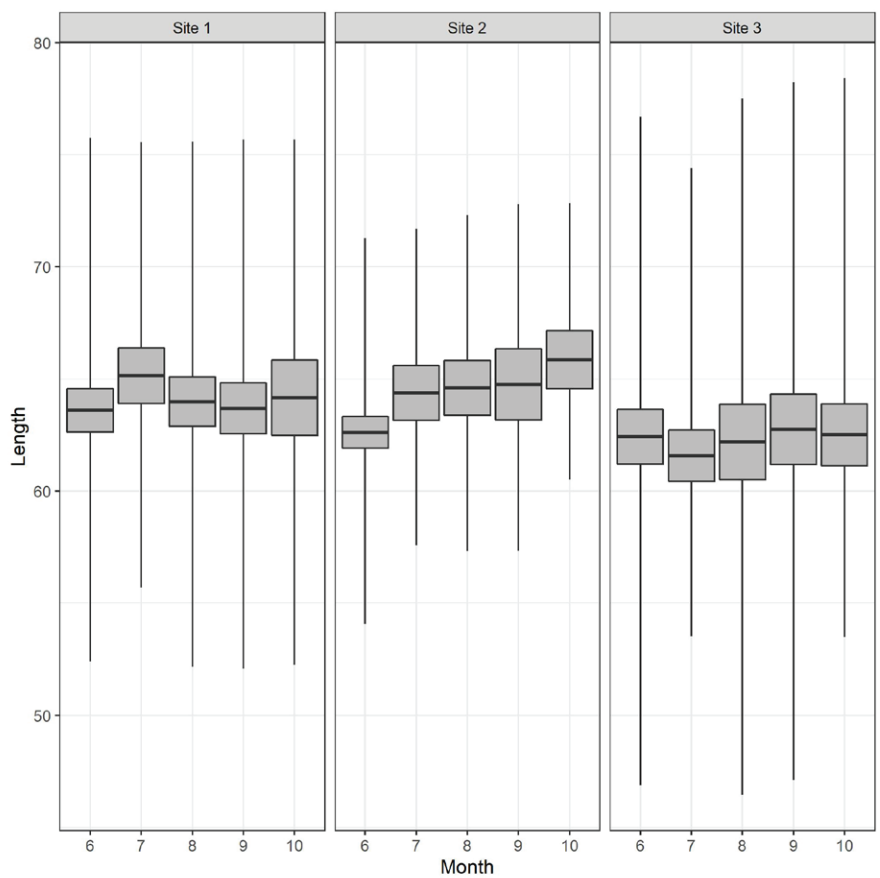

Figure 4. Length measurements of free-living spike placed into the substrate by site and month of survey (April–October 2019) in the Oconaluftee River, North Carolina. Box center line, upper and lower box ends, and whiskers represent mean, one SE, and minimum and maximum measurements, respectively.

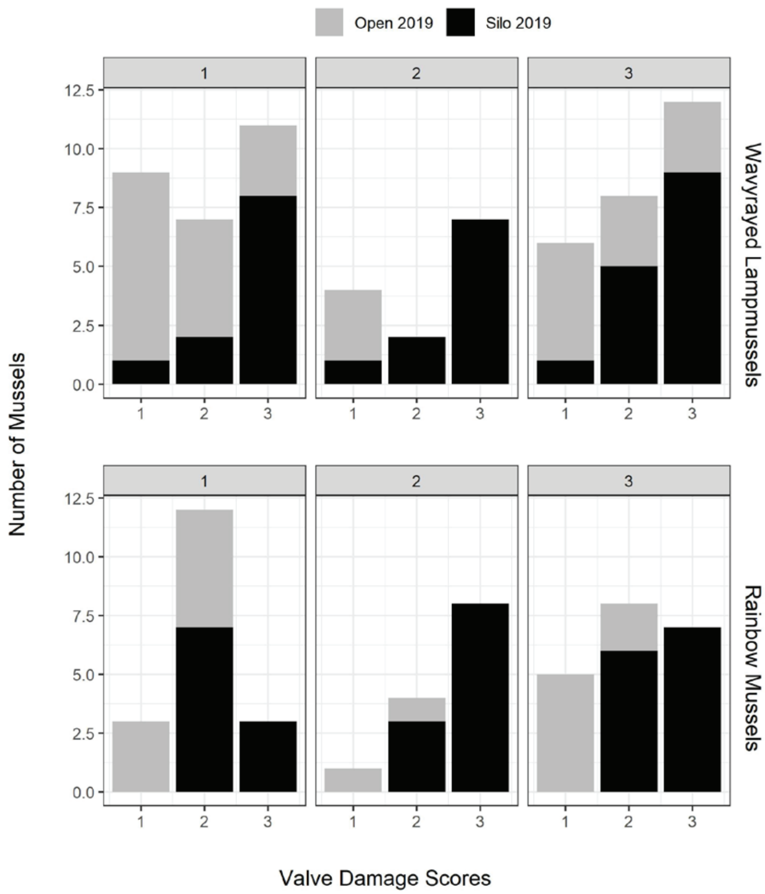

Figure 5. Valve damage scores (Table 1) by site (1–3) for rainbow mussels and wavyrayed lampmussels measured at the end of the study in October 2019. Light gray bars represent mussels that were free-living ("Open 2019"), and black bars represent those that were held in silos ("Silo 2019"). For rainbow mussels, individuals in the "Silo 2019" group were one year younger than the others.

significant interactions among sample month, site, and stocking type (silo or substrate) in growth of wavyrayed lampmussels ($F = 5.85$, df = 10, 337.2; $P < 0.001$) and rainbow mussels ($F = 8.30$, df = 18, 445.9; $P < 0.001$), and between month and site in growth of spike ($F = 2.00$, df = 8, 198.4; $P = 0.048$). Length differences between mussels stocked into the substrate and those stocked into silos increased over the course of the study, but this difference was smaller in more upstream sites (2 and 3) after early summer (Figure 2, 3). Thus, although mussels grew faster in the open substrate than in the silos, the disparity in length between treatments was also affected by site location along the river. Additionally, mussels at more downstream sites grew to larger sizes over the course of the study; however, for spike, the effect may have been driven by growth for site 2 only (Figure 4).

Valve damage scores varied from 1 to 3 for both wavyrayed lampmussels and rainbow mussels and were significantly lower in the free-living individuals than those in silos at all three sites ($G = 133.52$, df = 35; $P < 0.0001$ and $G = 128.47$, df = 35; $P < 0.0001$ respectively; Figure 5). The mean score for free-living wavyrayed lampmussels was 1.67 and in silos was 2.58. For rainbow mussels, the mean score of free-living mussels was 1.47 and of mussels in silos was 2.53. None of the mussels sustained enough damage to receive a score of 4.

Discussion

All three species survived and grew at all study sites, which suggests that the Oconaluftee River is a suitable location for restoration of these three species. Further, the mussels appeared to be performing ecological functions in the river as both free-living and enclosed mussels provided habitat for other macroinvertebrates. Mussels displayed typical growth patterns over the course of the growing season, with higher growth rates during early summer than later in the growing season (Augspurger et al. 2003, Beaty and Neves 2004, Finigan 2019). Slightly higher water temperature and potentially higher availability of suspended algae and microbes due to a more downstream position are likely the cause of more rapid growth at sites 1 and 2 in rainbow mussels and wavyrayed lampmussels. Further investigation into the potential for excess nutrient introduction or other similar impacts could inform whether the wastewater effluent release above site 1 could be impacting mussel growth rate there as well.

Each of our study sites had other unique traits relevant to our study. For example, site 2 consistently had noticeably higher velocity flows than at the other sites, likely due to a narrower channel. As a result, this site also had the greatest incidence of silo displacement, and the number of recovered mussels in the free-living treatment group was much lower. Mussels at this site were typically found 0.2–0.3 m below the substrate, whereas those at other sites were rarely deeper than 0.15 m. This may be due to greater penetration of oxygenated water into the substrate from the higher flows or upwelling of groundwater due to unique geology (Risse-Buhl et al. 2013). The habitat at site 3 changed significantly over the course of the study. Initially, this site was a fast-moving, shallow, and wide section in the river, but in May a large tree washed downstream and lodged in the river just downstream of the mussel site, resulting in slower velocity and deeper water. After this event, the mussels at this site were often found sitting on top of the substrate rather than buried or partially buried. In addition, the only known instance of predation occurred at this site, a single spike that was likely eaten by a river otter (*Lontra canadensis*). Further research into specific water chemistry and nutrient content differences among sites is needed to determine what parameters may have resulted in the varying behaviors of mussels observed in each site. Predation pressure and dissolved oxygen content may influence mussel behavior and linking these with stream characteristics may provide insight for biologists into potential causes of mussel behavior that could lead to lower detection rates, higher predation rates, or other obstacles to research and management (Uryu et al. 1996, Perles et al. 2003, Wilson et al. 2012). Temporal changes in study sites illustrate the plasticity of riverine habitats and emphasizes the need for selecting multiple restoration sites to account for potential impacts of stochastic events. Site selection for mussel placement should also consider the dynamic nature of the lotic system benthos.

The greatest challenge throughout the study was the repeated loss of silos due to high flow events. One enclosure traveled over 1 km downstream from its original placement site. The movement of silos and other enclosure types is not uncommon during in situ mussel studies and has been reported by many researchers (e.g., Huehner 1987, Bartsch et al. 2000, Elderkin et al. 2008, Haag and Commens-Carson 2008). In addition, we observed higher incidence of shell damage and lower growth rates for mussels in silos than those found in the stream substrate. The design of silos could cause them to become inhospitable during higher flows, as the water moves rapidly through the column and the mussels within have no means to mitigate impacts from sediment or other mussels inside the enclosure. Other researchers have found that simple handling, such as measurement with calipers, can cause scars and stunted growth in the annual rings of mussel valves in unionids (Haag and Commens-Carson 2008, Ohlman and Pegg 2019). Our results further suggest that the increase in damage to the valves of the mussels inside the silos may limit growth rate, rather than simply the limitation of feeding to the water column alone as some researchers have speculated (Barnhart et al. 2007, Bouska et al. 2018).

Exposure to high levels of water movement inside enclosed columns of silos during rainfall events could cause internal injuries as well, whereas free-living mussels may be able to escape much of this disturbance by burrowing deeper into the substrate. Mussel beds established in the substrate at the first three sites remained in their original locations throughout the study, even when some silos at these same sites were washed hundreds of meters downstream. The fact that the mussel beds remained in consistent locations despite bedload shifting may indicate that in particularly unstable river systems, keeping mussels in silos may place them at a greater risk for suffocation as they have no means of escape should the silos be buried during adverse weather events. Our findings suggest that use of silos in high-gradient systems could cause damage to the animals held within, and alternative methods (such as substrate placement of PIT tagged individuals) should be considered when designing future restoration feasibility studies in similar river systems.

The free-living mussels we placed in the Oconaluftee River appeared to naturally form multi-species beds, often found in clusters together as is consistent with what other researchers have found (Uryu et al. 1996, Alderman et al. 2001, Bogan and Roe 2008). Behaviors and conditions leading to aggregate formation are an area of active research (e.g., van de Koppel et al. 2008, Liu et al. 2014). Mussel aggregates may form due to some ecological benefit of clustering or may simply be the result of the animals independently seeking out the most suitable micro-habitats within the reach. Several researchers have suggested that multi-species mussel beds allow for greater resilience and positive ecological impact, as each species performs a unique ecological function (Liu et al. 2014, Cowie et al. 2017, Mitchell et al. 2018). Our three study species are known to aggregate in multi-species assemblages, and their tendency to form diverse benthic communities may indicate that each species uses resources in a sufficiently different manner to allow coexistence or that resources are not limiting (Vaughn and Hakenkamp 2001). Preserving biodiversity of these bivalves helps to ensure not only their persistence, but continuance of any ecosystem services provided. These findings suggest that future mussel restoration efforts may increase success by reintroducing multiple species in the same location.

The overall success of our stocking Oconaluftee River provides evidence that both translocating adult individuals and introducing hatchery-raised mussels can be used to increase mussel diversity and density in this watershed. The Oconaluftee River has protected headwaters and has been the focus of conservation efforts which have restored adequate mussel habitat and water quality to much of the drainage, further indicating that it should be an excellent candidate for establishment of populations of wavyrayed lampmussels, rainbow mussels, spike, and potentially other mussels. Further efforts with our three study species should focus on establishing populations of reproductive individuals, as the only introduced individuals of reproductive maturity in this study were spike. Known fish hosts for all three mussel species are reported to be present in the stream based on surveys conducted by Eastern Band of Cherokee Indians Natural Resources personnel. Documenting wild recruitment from the introduced mussels is an essential next step in confirming success in restoration of all three species. Given the dramatic declines of unionid mussels seen throughout their ranges, restoration and establishment of new populations in protected river systems such as the Oconaluftee River remain the best options for protecting this group of organisms in the future (Neves 1999, Lyons et al. 2007, Bogan and Roe 2008, Cowie et al. 2017, Brian et al. 2021).

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