IN-SITU FEASIBILITY STUDY OF FRESHWATER MUSSEL REINTRODUCTION: SURVIVAL AND GROWTH OF THE WAVY-RAYED LAMPMUSSEL (*LAMPSILIS FASCIOLA)* IN THE PIGEON RIVER, NC

A thesis presented to the faculty of the Graduate School of Western Carolina University
in partial fulfillment of the requirements for the degree of Master of Science.

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Western Carolina University (May 2010)

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The Pigeon River, North Carolina has a long history of habitat degradation due to water diversion and high levels of toxic effluents from a paper mill. Over the last 20 years the paper mill has modernized its processes and reduced water use and waste production greatly. Historically, the wavy-rayed lampmussel, *Lampsilis fasciola*, was believed to have been present throughout the river from Canton to its mouth in Tennessee, but it currently persists only upstream of Canton, NC. In this preliminary study of the feasibility of restoring the mussels to the downstream reach, I compared the survival and growth of *L. fasciola* placed in the Pigeon River downstream from Canton with those placed upstream. Captively propagated mussels were individually marked and placed in enclosures in the river at two upstream sites and three downstream sites in December 2008. They were monitored for survival and growth monthly from December 2008-November 2009. Mortality rates among sites were not significantly different; however, growth rates of mussels held in the downstream sites were significantly greater than for those held at upstream sites. Highest growth rates were observed at a site located approximately 18 km downstream from Canton. Several influences may have impacted these growth rates, such as elevated temperature due to heated effluent and agricultural runoff with elevated nutrients. Assessment of survival at other life stages is needed before

the full extent of potential for reintroduction of mussels to the studied reach of the Pigeon River is known.

INTRODUCTION

Freshwater mollusks are considered to be one of the most diverse and endangered assemblages of species in North America (Lydeard et al. 2004). The unionid mussels are a group of filter feeding species which are declining worldwide both in the number of species per area, and in abundance (Vaughn et al. 2004). Forty-four per cent of all known mollusk are threatened (IUCN 2008), thus protecting mollusk diversity represents a great challenge for the management of aquatic diversity. The main threat to unionid populations is habitat destruction by humans (Lyons et al. 2007). One of the underlying reasons for habitat destruction is urbanization, which has been shown to directly affect the freshwater fauna (Lyons et al. 2007).

Habitat destruction can occur in various ways including the construction of dams and canals, which alter the natural flow regime, stream depth, and sediment composition (Bogan 2008). Moreover, changes in stream power and concomitant adjustments to stream geomorphology associated with dams can result in direct loss of mussel colonies (Gangloff and Feminella 2007). Strayer and Fetterman (1999) found that mussels were limited by substrate type and stability. Weber (2005) found that even fairly small dissimilarities in habitat conditions can have a large effect on the species assemblage in Appalachian streams. Nutrient enrichment and organic matter contamination which remains trapped within the sediments has also been found to contribute to unionid decline (Weber, 2005).

Mussels have limited mobility, making them highly susceptible to toxic contaminants in the water column (Ellis, 1931, as cited in Goudreau et al. 1993). Their only defense against toxic effluents is closure of their valves. However, few species are able to maintain closure for very long, due to the need to obtain oxygen, food, and excrete waste (Horne & McIntosh 1979, as cited in Goudreau et al. 1993).

In North Carolina, the wavy-rayed lampmussel, *Lampsilis fasciola*, (Rafinesque, 1820) is restricted to Tennessee River tributaries in the Blue Ridge physiographic province. The species is currently found only in a few river reaches in the French Broad, Little Tennessee, and Hiwassee river basins in Cherokee, Swain, Macon, Jackson, Haywood, Henderson, Transylvania, Yancey, and Mitchell counties (Bogan 2002). Due to its limited range in western North Carolina, the wavy-rayed lampmussel is listed as a species of special concern in the state (LeGrand et al. 2006).

The wavy-rayed lampmussel was believed to have historically occurred throughout the lower Pigeon River in North Carolina and Tennessee (NCWRC 2009). However, only a small population exists in the river upstream of Canton, likely due to habitat and water quality degradation associated with the effluent from Champion Fibre Co. (later Champion International Corp. and Blue Ridge Paper, Inc., currently Evergreen Packaging) and urbanization of the greater Canton-Waynesville, NC area.

The Pigeon River in Haywood County, NC has a long history of high levels of toxic effluents and water quality and habitat degradation from a century of diversion for industrial use and waste disposal (Bartlett 1995). The river has also experienced hydrological and habitat damage from damming, channelization, and poor agricultural and forestry practices. In 1908, Champion paper mill began discharging waste into the Pigeon River, and fish kills occurred immediately. In the early 1920's, another major fish kill wiped out fish from Canton all the way downstream to Newport, TN. From 1908-1960 there was no treatment of effluent prior to its release into the Pigeon River. All native mussels and a significant proportion of fish species were extirpated downstream from Canton to the mouth of the river in Tennessee, a 101km section of stream (Bartlett 1995). In 1960 primary treatment of discharge was implemented, and in 1970 secondary treatment began. At low flow periods 100% of the Pigeon River was drawn in for the paper mill's use, and 100% of the river downstream was effluent. The paper mill's wastewater treatment plant releases heated effluent, and contains treated wastewater from the town of Canton, NC.

In 1980 Tennessee sued North Carolina, forcing Champion Paper Mill to clean up its practices (Bartlett 1995). Following the lawsuit, studies by North Carolina, Tennessee, and EPA investigated the state of the Pigeon River. In 1988, a fish consumption advisory based on high levels of dioxin contamination was posted. The Champion Paper Mill began a $300 million modernization project in 1990, discontinuing the use of elemental chlorine, and implementing the recycling of bleach filtrate. Blue Ridge Paper Products, Inc. took ownership of the paper mill in 1999 and completed the modernization project. The Rank Group took ownership of the paper mill in 2007, and now operates as a subsidiary of Evergreen Packaging Group.

Historically, paper mills used polychlorinated dibenzo-dioxins and furans to bleach wood pulp in the production of paper (Kalff 2002). These toxins are highly persistent and they tend to bioaccumulate in organisms and remain in the sediments (Kalff 2002). As habitat and water quality conditions have improved, aquatic communities have responded positively. Downstream from Canton, fish species richness and abundance have increased due to re-colonization and reintroduction efforts (Steve Fraley, NCWRC, pers. comm.). Recovery efforts enabled some fish species to return, but 24 species were still missing (Joyce Coombs, pers. comm.); however, the potential for survival of wavy-rayed lampmussel in the downstream area is still unknown. Successful mussel reintroductions have been made in the nearby French Broad River in Tennessee, where at least one translocated species has successfully reproduced (Layzer and Scott 2006). With augmentation of their host fishes, colonization and recruitment of additional mussel species are believed to have occurred (Layzer and Scott 2006). However, on the Pigeon River in 1927 Walters Dam was built creating a de-watered section, preventing movement of host fish upstream and downstream (Bishar et al. 1999). Therefore, natural re-colonization from downstream is prevented by the Walters Dam.

Of the 57 species of mussels known to be native to North Carolina, 43 (75%) are considered endangered, threatened, or of significant conservation concern and eight are extirpated from the state (Bogan 2002; LeGrand et al. 2006). Therefore, restoration of extirpated populations may play an important role in the conservation and management of North Carolina’s freshwater mussels.

Expanding the range of the wavy-rayed lampmussel downstream of the paper mill will help fulfill a goal of the NC Wildlife Action Plan to “keep common animals common” (NCWRC 2005). Reestablished native mussels would help further restore ecological functions (Vaughn et al. 2004) in the damaged reach of the Pigeon River and aid its continuing recovery. Also, knowledge gained in this study of wavy-rayed lampmussels could aid efforts to reintroduce the Appalachian elktoe, *Alasmidonta raveneliana*, a federal endangered species, to the same reach of the Pigeon River from which it is believed to have been extirpated.

In this study, I placed juvenile mussels cultured by North Carolina State University and North Carolina Wildlife Resource Commission (NCWRC) from gravid females collected from the upper Pigeon River in protected enclosures upstream and downstream of Canton, NC. My study objective was to determine if there was a difference in the survival and growth of the mussels at downstream sites versus those located upstream from Canton. Survival and growth was monitored on a monthly basis through a single growing season. I hypothesized that if mussel mortality is significantly higher, and instantaneous growth rates are significantly lower downstream, then urban and industrial effluents are negatively affecting the mussels and reintroduction would not be advisable at this time. If downstream individuals survived and grew comparable to experimental individuals placed upstream, then I could conclude that water quality had improved sufficiently to sustain late juvenile wavy-rayed lampmussels and may support their reintroduction to the reach downstream from Canton.

METHODS

Study Area

The Pigeon River is a large tributary of the French Broad River. The Pigeon River begins in southern Haywood County, NC and flows northward, converging with the French Broad just north of Newport, TN. My study sites were located in the upper Pigeon River near Canton, Clyde, and Crabtree, NC (Haywood County) (Figure 1). The Pigeon River's peak flows are in spring months, and lowest flows are in summer and fall. The drainage area upstream from my upstream study sites near Canton is approximately 135 km^2 with a mean annual discharge of 3.3 m^3/sec (USGS 2009). The drainage area and discharge is substantially greater in reach where my downstream study sites were located. Near Hepco, NC, seven river miles downstream from my lowest study site, the drainage area is 504 km^2, with a mean annual discharge of 14.3 m^3/sec (USGS 2009).

Preparation and Deployment

In April, 2008, a total of 20 mussel enclosures (silos) were fabricated following the design of Dr. M. Chris Barnhart, Missouri State University, with modifications by Virginia Game and Inland Fisheries (VGIF) (T.R. Russ, NCWRC, pers. comm.). The mussel silos were composed of a 10 kg concrete dome and a PVC inner chamber with standard 1x1.19 mm mesh size wire screen ends to house the mussels. The silos were designed so that as water flows over the silo it creates a Bernoulli effect which draws water up through the mussel enclosure, providing a continuous supply of fresh water and nourishment, while keeping mussels contained and easily retrieved for data collection. The mussel silos allow for containment of the mussels in the river, while avoiding cage design features which may collect debris.

Five study sites were chosen in the Pigeon River: two control sites upstream and three experimental sites downstream of Canton, NC (Table 1). The locations of the two control sites, (Site 1), and (Site 2), are approximately 4.0km, and 1.5km upstream from the paper mill in Canton, NC, respectively. The first downstream site, (Site 3) was located adjacent to downtown Clyde, incorporating the mill outflow mixed within the river. The other two downstream sites were chosen downstream from Richland's creek, (Site 4) and another downstream from Crabtree Creek, (Site 5) incorporating nutrient inputs from both agricultural areas and urban and suburban impacts.

Table 1. Location of mussel silo placement sites on the Pigeon River, NC.

Site	GPS Coordinates	Location: river kilometers (river mile)
1	N 35° 30.947: W 82° 51.248	105.9 (65.8)
2	N 35° 31.289: W 82° 50.908	104.1 (64.7)
3	N 35° 32.097: W 82° 54.664	94.0 (58.4)
4	N 35° 33.695: W 82° 57.236	83.8 (52.1)
5	N 35° 36.844: W 82° 57.937	79.2 (49.2)

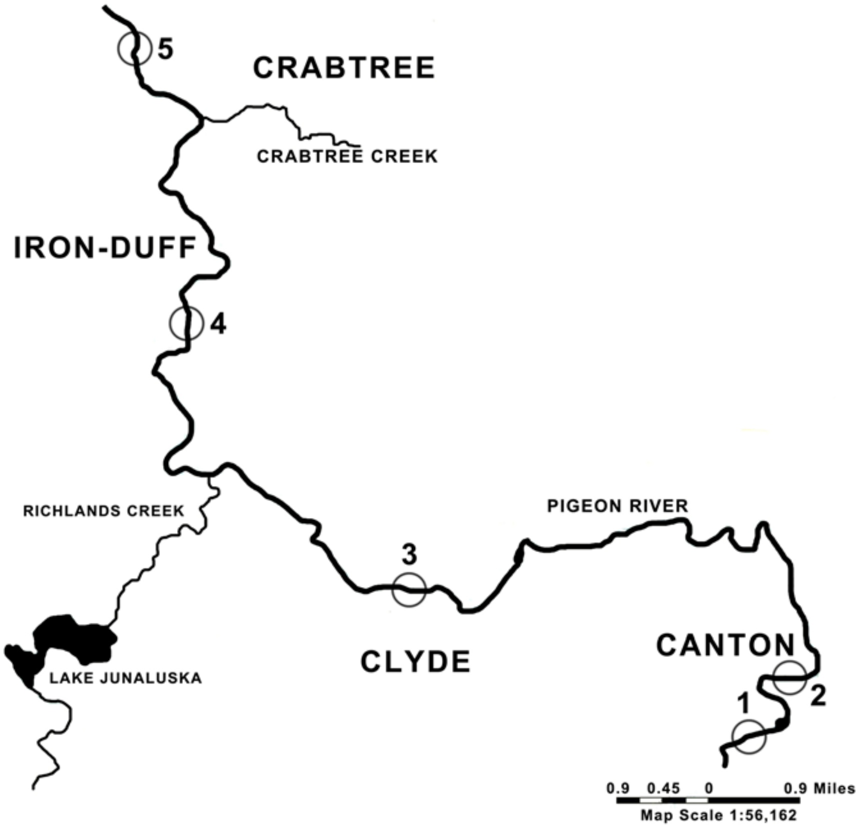

Figure 1. Map of mussel silo placement sites on the Pigeon River from Canton to Crabtree, NC

The juvenile mussels used in this experiment were propagated in captivity by Chris Eads, researcher at the Aquatic Epidemiology and Conservation Laboratory, North Carolina State University (NCSU), College of Veterinary Medicine. The mussels were propagated from 3 gravid adult female wavy-rayed lampmussels collected from the upper Pigeon River on 25 April 2007 (Chris Eads and William Russ, North Carolina Wildlife Resource Commission, pers. comm.). The glochidia were infested on 31 largemouth bass (*Micropterus salmoides*) on 26 April 2007, which were held at 19-21 °C. The juveniles dropped off the fish from 18 May to 13 June 2007. The juvenile mussels were reared at the NCSU facility for approximately one year. In the spring of 2008 the cohort of juvenile mussels was split; approximately one half of the juveniles (677) were transferred to the Table Rock State Fish Hatchery, Morganton, NC (this cohort split will hereafter be referred to as culture 1). The remaining juveniles (744) were transferred to the Marion State Fish Hatchery, Marion, NC (this cohort split will hereafter be referred to as culture 2). Culture 1 individuals experienced high mortality during summer 2008 due to warm temperatures and lowered dissolved oxygen levels. In August 2008 the surviving culture 1 mussels (310) were relocated to the newly constructed Marion Conservation Aquaculture Center, Marion, NC (CAC). Culture 2 individuals were transferred to the CAC in October of 2008. Due to the different (warmer) culture conditions under which they had grown, culture 1 individuals were approximately 10 mm longer than culture 2 individuals by December 2008.

In December 2008, 60 individuals from each culture (120 total) were individually marked with Hallprint’s type FPN glue-on shellfish tags (Hallprint Pty Ltd, 15 Crozier Rd, Victor Harbor, South Australia 5211) attached using cyanoacrylate-based glue. The mussels were held at the CAC until the start of the experiment. All mussels were measured (length, width, and height) prior to deployment.

In early December 2008 4 silos were placed at each of the 5 chosen sites. Six juvenile wavy-rayed lampmussels were placed in each silo, 3 from Culture 1 (average 25 mm in length), and 3 from Culture 2 (average 15 mm in length). At each study site, the mussel silos were placed at appropriate depths, approximately 0.5-0.75 m and velocities judged to provide adequate flows over the period between sampling.

Monitoring and Analysis

Once a month for the length of the study, the length, height, and width of each mussel was measured to the nearest tenth of a millimeter, using digital calipers and each mussel was observed for signs of gravidity. In addition to the measurements, any excess sediment in the PVC chambers was removed to ensure good flow conditions. Water quality measurements taken each month included temperature, dissolved oxygen concentration, conductivity, and pH at each of the sites. Temperature and conductivity was measured at each site using a Yellow Springs Instrument Model 85 meter and flow was recorded from the local USGS gauging station. A water sample was collected in early December 2009 upstream of Canton and one at each of the downstream sites, to be tested for total chlorides and total nitrogen. Water analysis was performed by Pace Analytical Services, Inc. in Asheville, NC. Lastly, each site was visually assessed for its quality of habitat based on the protocol for scoring level of urbanization developed by Lyons et al. (2007). Scores were visually determined based on surrounding land use: buildings, land use: roads, riparian zone condition, and river bank modification, presence of dam/spillway, erosion, and human trash. Scores ranged from 0-21, with higher scores being more urbanized.

I calculated the instantaneous growth rate (IGR) for each of the mussel's dimensions (D) over one growth year (t), where $IGR = (\ln D_2 - \ln D_1)/(t_2 - t_1)$. IGR was calculated for each individual mussel, and mean IGR of each culture within each site were used in the data analysis. I used a split plot analysis of variance and planned contrasts to examine differences among upstream and downstream IGR and mortality as well as to sort among the sites located at increasing distances downstream of Canton. The five experimental sites along the river formed the whole plots, while source culture formed the splits within sites. To determine if sites influenced the IGR of each culture I examined the interaction between culture source and site. The planned contrasts were between the upstream and downstream sites, to find if any variation existed between the two sections of the river. Orthogonal polynomial contrasts were used to test for linear and quadratic responses among the three downstream sites.

Dead individuals were found during our monthly sampling, so actual date of death wasn't determined. When dead individuals were found I compared the growth rate of that individual from the start of the experiment up to the previous sample period with the mean of the surviving individuals from that same site and culture using a t-test.

RESULTS

Out of 120 mussels used in the study, there were five mortalities, all among the downstream sites. Two deaths occurred in the first two months the mussels were in the river, at Site 4, another two between April and May, at Site 3 and Site 5, respectively and the last one was discovered in November, at Site 5. While all mortalities observed occurred in downstream sites, mortality rates were not significantly different between upstream and downstream sites (Table 2). The five deaths were all mussels from Culture 2; however, because there were so few deaths, the two cultures did not differ significantly in their mortality rates (Table 2).

Table 2. Summary from split plot analysis of variance (ANOVA) of mussel mortality rates to location (Site), in reference to the paper mill (upstream vs. downstream), comparison of controls, Cultures, and the 2-way interaction of (Culture X Site).

Source	df	SS	MS	F	P
Sites	4	0.5000	0.1250	0.7895	0.5498
upstream vs downstream	1	0.4167	0.4167	2.6316	0.1256
Control 1 vs. Control 2	1	0.0000	0.0000	0.0000	1.0000
downstream linear response	1	0.0625	0.0625	0.3947	0.5393
downstream quadratic response	1	0.0208	0.0208	0.1316	0.7219
Error (whole plot)	15	2.3750	0.1583		
Culture	1	0.6250	0.6250	3.9474	0.0655
Culture X Sites	4	0.5000	0.1250	0.7895	0.5498
Error (sub plots)	15	2.3750	0.1583		
Total	39	6.3750			

Only one mussel's IGR was significantly different from its population at the corresponding site prior to death (Table 3). Four of the five mussels trended towards lower growth (Figure 2).

Table 3. Summary of t-test results between IGR of deceased mussels and their corresponding site's population.

Site	Mussel	Mort IGR	Pop. IGR	Df	t score	p-value
3	A	0.041	0.052	10	-0.264	0.797
4	B	0.039	0.042	10	-0.049	0.962
4	C	0.000	0.017	10	-0.468	0.650
5	D	0.068	0.199	9	-2.797	0.021
5	E	0.639	0.491	9	1.462	0.178

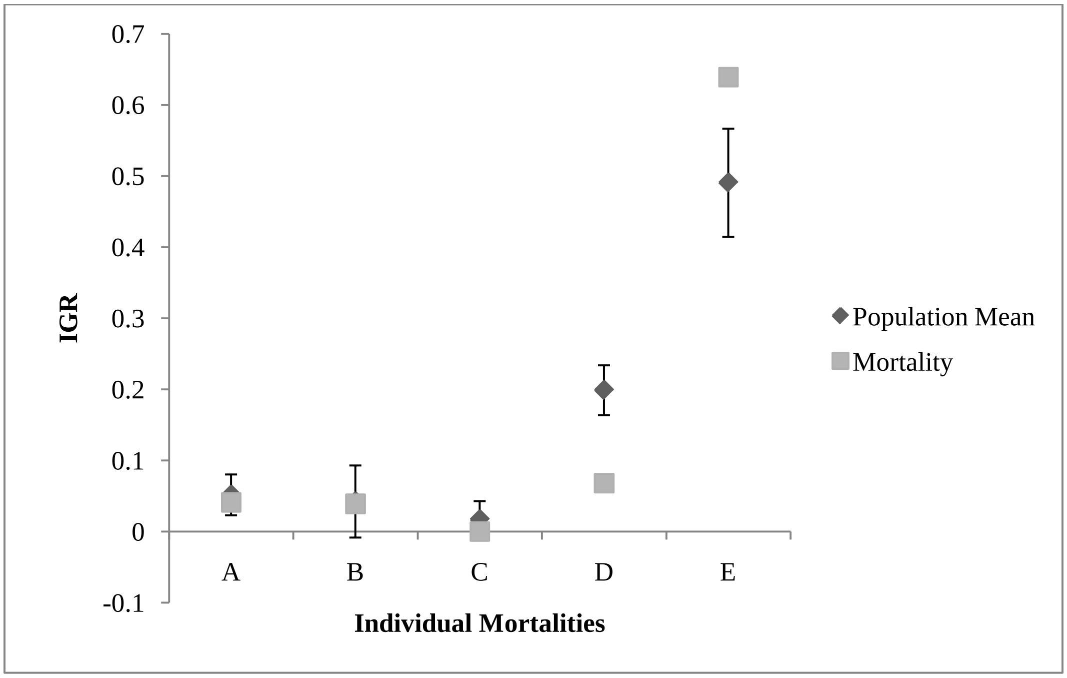

Figure 2. Comparison of deceased mussel's instantaneous growth rates (IGR) prior to their death to their corresponding populations with 95% confidence interval.

Overall, the IGR of the mussels as measured in three shell dimensions (length, width, and height), varied across all the sites on the Pigeon River ($P < 0.0001$). The mussel's IGR differed between downstream and upstream sites (Tables 4, 5, 6). Downstream sites trended toward faster growth for all three shell dimensions, suggesting there may be additional factors influencing the mussel's growth (Figure 3). There were no significant differences in IGR in any shell dimension between the two control sites (Tables 4, 5, 6). All downstream measurements of IGR exhibited a substantial linear relationship, or trending toward faster growth, suggesting relational differences among each site and IGR. A quadratic relationship also exists in all the downstream IGR measurements, implying a bend in the linear relationship. Site 4 mussels grew significantly more than those from Site 3 and 5 (Figure 3).

Culture 1 and 2 differed in their IGR for length, width and height ($P < 0.0001$) (Tables 4, 5, 6). Overall, Culture 2 trended toward faster growth than Culture 1 for all growth measurements (Figure 4). Length IGR of the mussels displayed no interaction between the two cultures and sites (Table 4). However, both width and height IGR varied with culture and site (Tables 5 and 6).

Table 4. Summary from split plot analysis of variance (ANOVA) of mussel length IGR to location (Site), in reference to the paper mill (upstream vs. downstream), comparison of controls, Cultures, and the 2-way interaction of (Culture X Site).

Source	df	SS	MS	F	P
Sites	4	0.2780	0.0695	23.9540	<0.0001
upstream vs downstream	1	0.0714	0.0714	24.6095	0.0002
Control 1 vs. Control 2	1	0.0041	0.0041	1.4243	0.2512
downstream linear response	1	0.0499	0.0499	17.2073	0.0009
downstream quadratic response	1	0.1526	0.1526	52.5749	<0.0001
Error (whole plot)	15	0.0435	0.0029		
Culture	1	0.3396	0.3396	171.4860	<0.0001
Culture X Sites	4	0.0221	0.0055	2.7913	0.0647
Error (sub plots)	15	0.0297	0.0020		
Total	39	0.7130			

Table 5. Summary from split plot analysis of variance (ANOVA) of mussel width IGR to location (Site), in reference to the paper mill (upstream vs. downstream), comparison of controls, Cultures, and the 2-way interaction of (Culture X Site).

Source	df	SS	MS	F	P
Sites	4	0.4286	0.1071	45.9039	<0.0001
upstream vs downstream	1	0.1369	0.1369	58.6494	<0.0001
Control 1 vs. Control 2	1	0.0030	0.0030	1.2982	0.2724
downstream linear response	1	0.0795	0.0795	34.0631	<0.0001
downstream quadratic response	1	0.2092	0.2092	89.6049	<0.0001
Error (whole plot)	15	0.0350	0.0023		
Culture	1	0.7093	0.7093	350.1524	<0.0001
Culture X Sites	4	0.0525	0.0131	6.4805	0.0031
Error (sub plots)	15	0.0304	0.0020		
Total	39	1.2558			

Table 6. Summary from split plot analysis of variance (ANOVA) of mussel height IGR to location (Site), in reference to the paper mill (upstream vs. downstream), comparison of controls, Cultures, and the 2-way interaction of (Culture X Site).

Source	df	SS	MS	F	P
Sites	4	0.2814	0.0703	28.2851	<0.0001
upstream vs downstream	1	0.1059	0.1059	42.5990	<0.0001
Control 1 vs. Control 2	1	0.0065	0.0065	2.6127	0.1268
downstream linear response	1	0.0317	0.0317	12.7611	0.0028
downstream quadratic response	1	0.1372	0.1372	55.1679	<0.0001
Error (whole plot)	15	0.0373	0.0025		
Culture	1	0.3515	0.3515	153.8512	<0.0001
Culture X Sites	4	0.0323	0.0081	3.5354	0.0319
Error (sub plots)	15	0.0343	0.0023		
Total	39	0.7367			

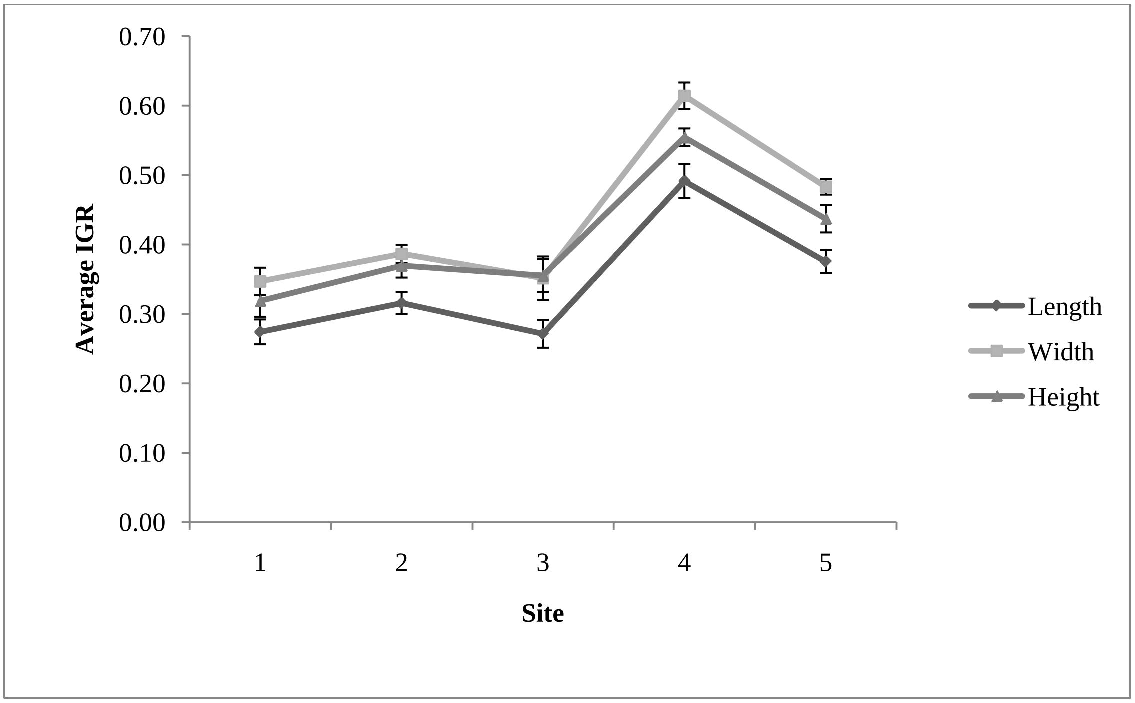

Figure 3. Differences in mussel instantaneous growth rate (IGR) measurements at each of the five sites along the Pigeon River, NC.

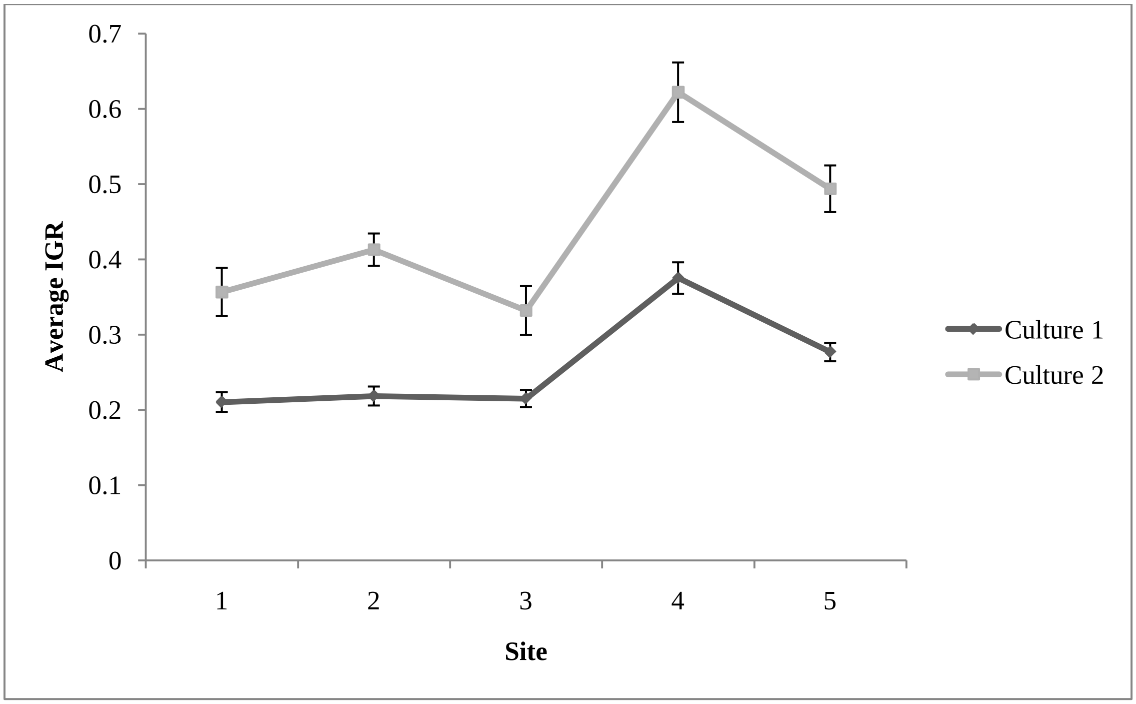

Figure 4. Average instantaneous growth rate (IGR) of length for mussels from culture 1 and 2 from December 2008 to November 2009.

Culture 1's growth remained stagnant during the winter months with little to no growth (Figure 5). Mussel growth started in April at the downstream sites, while upstream lagged behind by a month. IGR of downstream mussels were much higher than upstream in their main growth season (May-August). However, both areas began to plateau in the month of August. Culture 2's growth also remained idle during winter months in the river (Figure 6). The downstream mussel growth trend also began a month earlier than upstream from the paper mill in culture 2. However, differences in upstream vs. downstream IGR at the peak growth months were much greater in culture 2. Downstream mussels exhibited growth rates with much a steeper slope, while upstream rates modeled a logistic growth curve. Growth of mussels at both sites halted beginning in August.

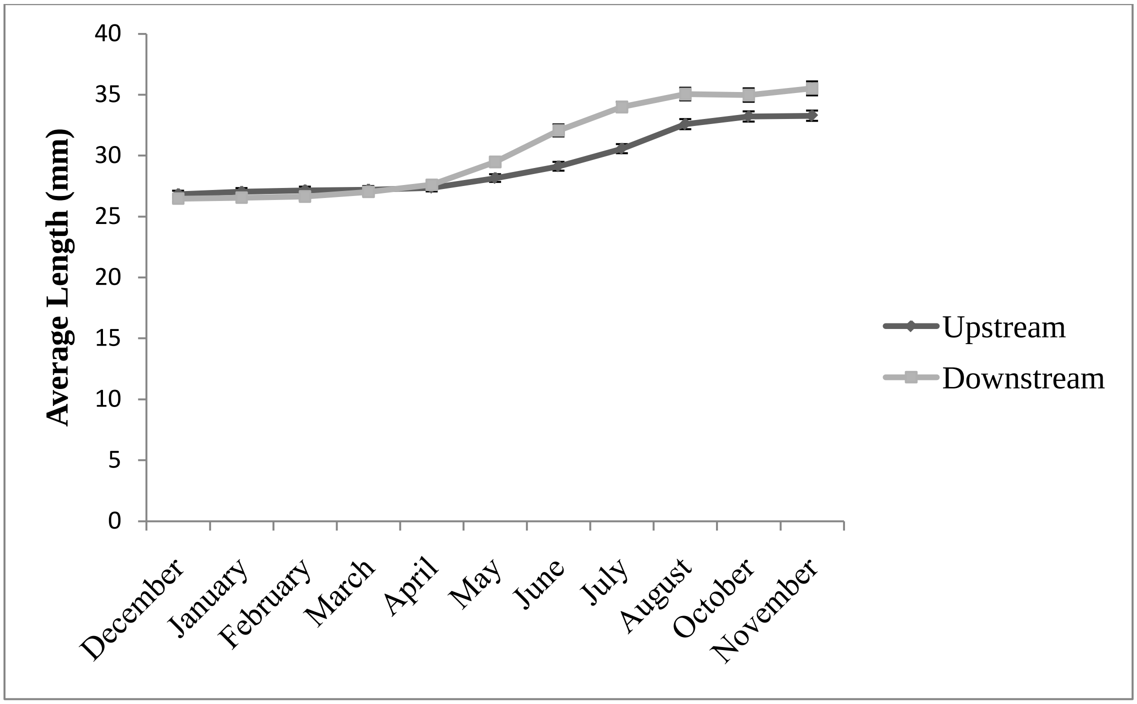

Figure 5. Average length (mm) of mussels from culture 1 from December 2008 to November 2009.

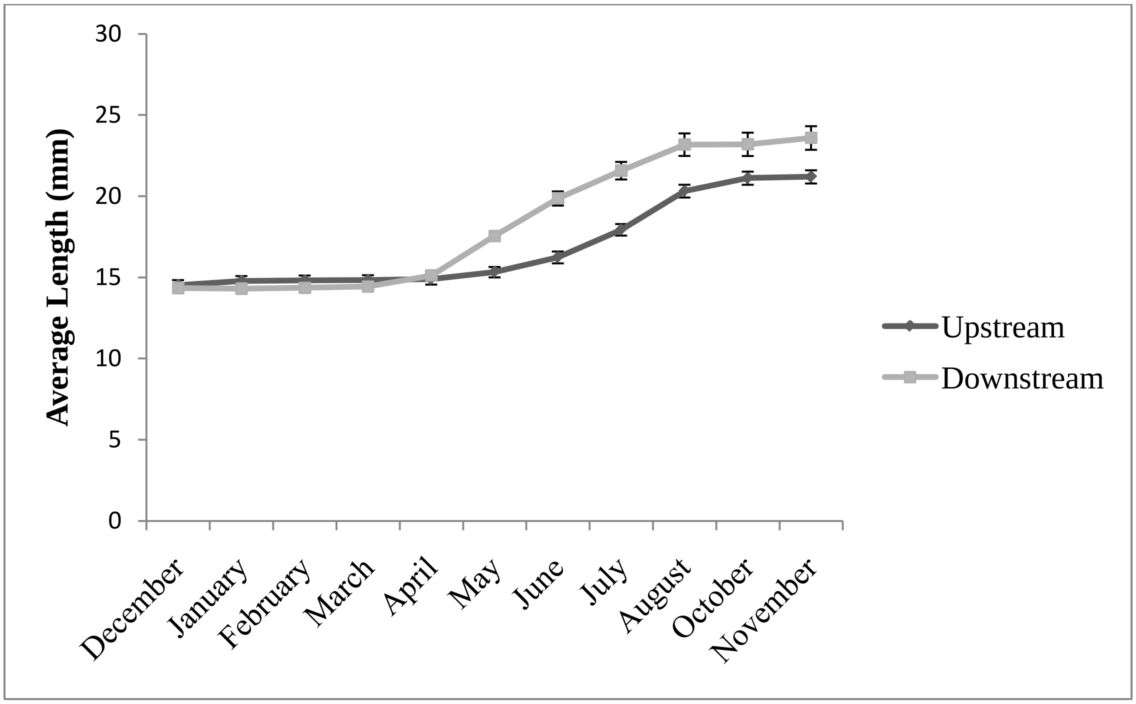

Figure 6. Average length (mm) for mussels from culture 2 from December 2008 to November 2009.

Over the course of the main growing season, temperature varied between 2-5 °C from the control to downstream sites (Figure 7). Dissolved oxygen however, displayed less variation but control sites tended to have higher concentrations than sites downstream (Figure 8).

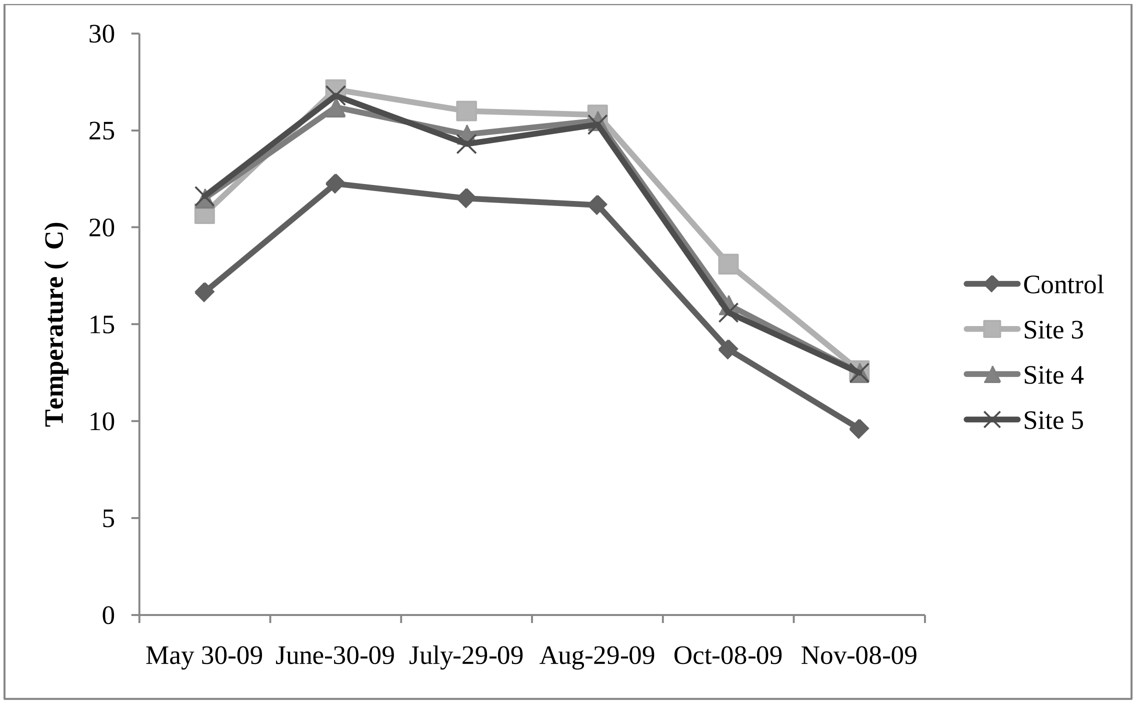

Figure 7. Temperature (°C) measured during the main mussel growth season at upstream control sites and each of the three downstream sites on the Pigeon River, NC.

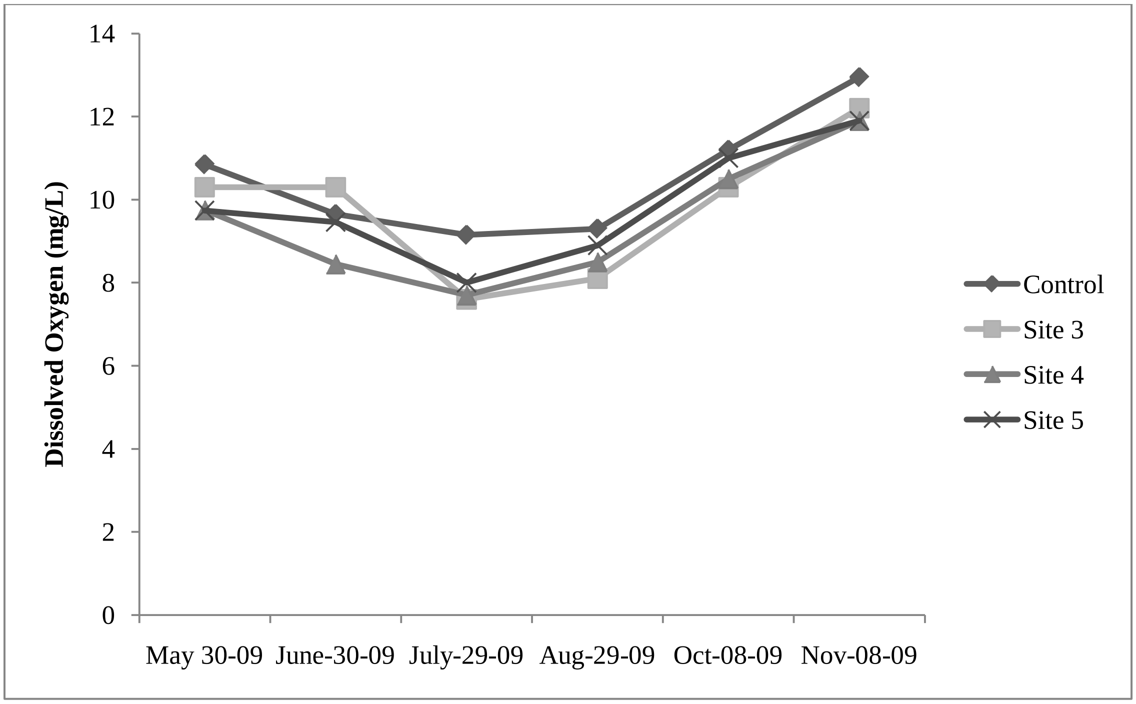

Figure 8. Dissolved oxygen (mg/L) measured during the main mussel growth season at upstream control sites and each of the three downstream sites on the Pigeon River, NC.

The parameter which exhibited the most notable variations was conductivity. Conductivity not only displayed differences between the control and downstream sites, but also amongst the downstream sites (Figure 9). Site 3, the first downstream from the paper mill, had the highest overall conductivity, peaking at just over 1700 µs/m during the month of August. There is a stark contrast between the control sites which had very little conductivity, to the downstream which displayed greater levels.

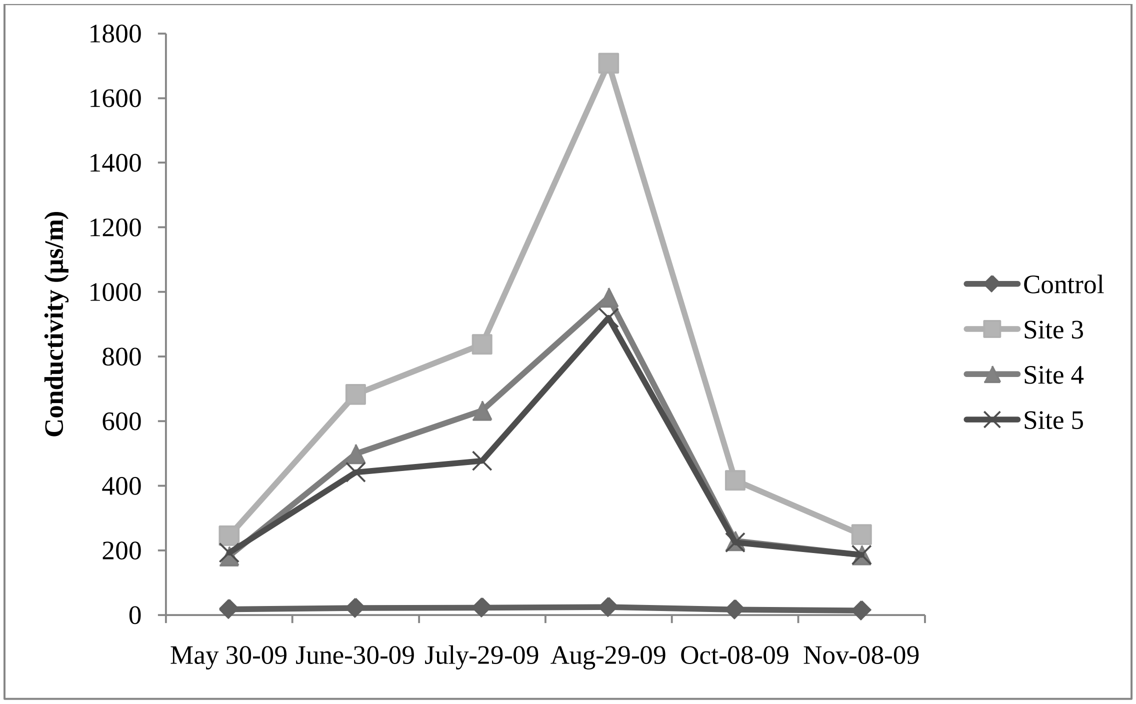

Figure 9. Conductivity (µs/m) measured during the main mussel growth season at upstream control sites and each of the three downstream sites on the Pigeon River, NC.

Salinity had very low levels across all the study sites in the river; however it demonstrated the same pattern as conductivity between controls vs. downstream and amid downstream sites (Figure 10). pH had little variation from upstream to downstream and between sites, maintaining values ranging from slightly below 7 (neutral) to slightly above 8 (Figure 11).

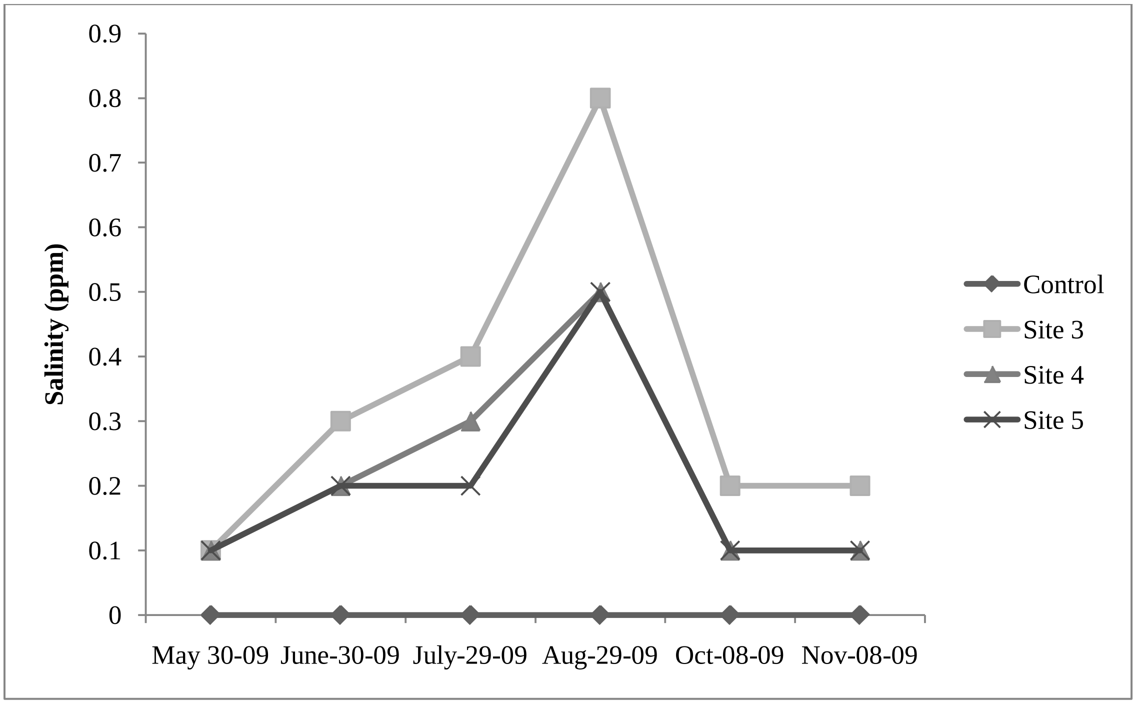

Figure 10. Salinity (ppm) measured during the main mussel growth season at upstream control sites and each of the three downstream sites on the Pigeon River, NC.

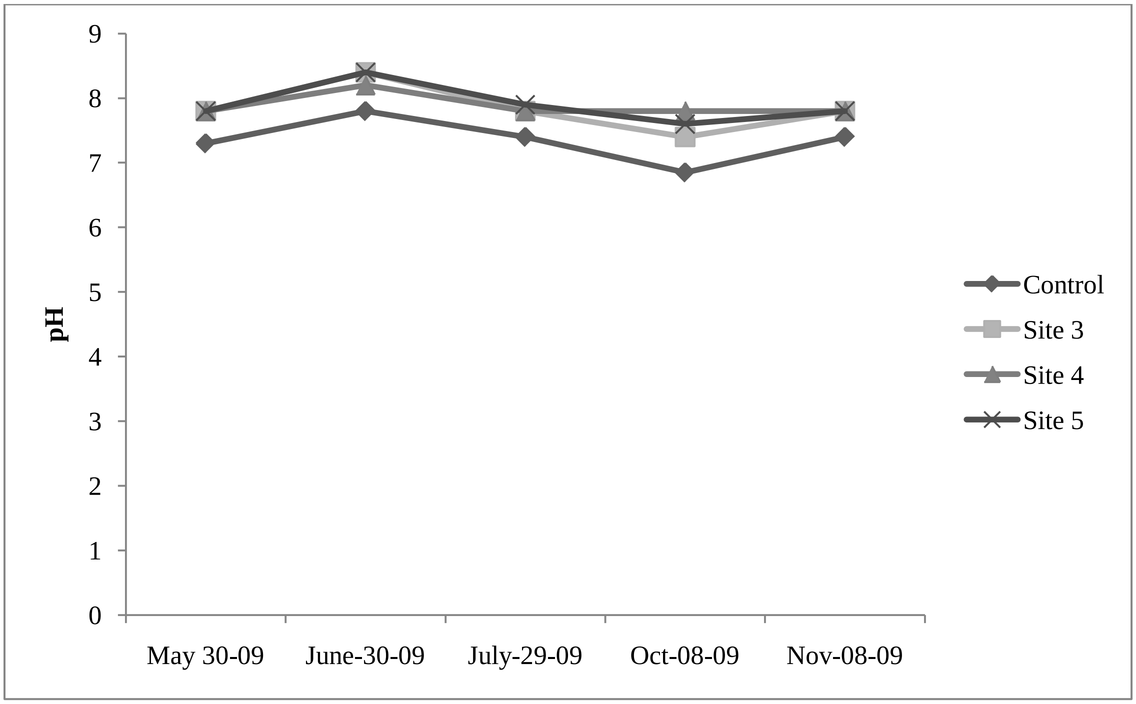

Figure 11. pH measured during the main mussel growth season at upstream control sites and each of the three downstream sites on the Pigeon River, NC.

There was little difference between the control and Site 3 for total nitrogen (Figure 12). Slightly higher levels were found at more downstream sites. Inputs from agricultural areas surrounding Sites 4 and 5 may be responsible for higher levels.

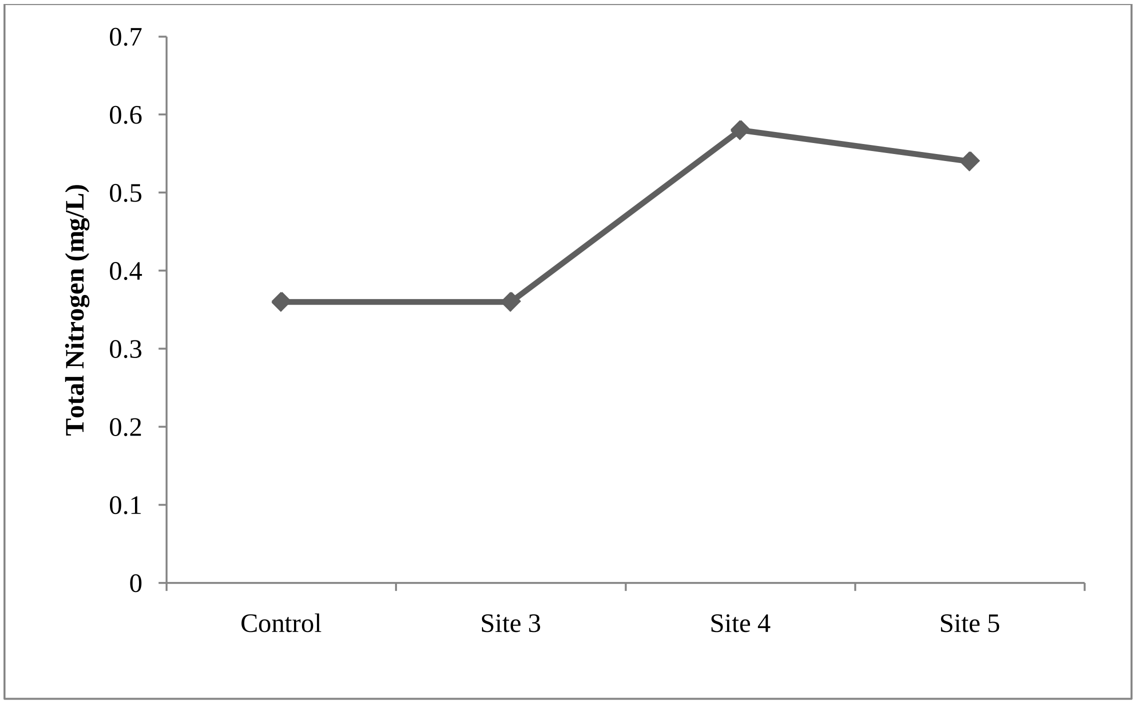

Figure 12. Total nitrogen from water samples taken in December 2009 at one of the controls and each of the downstream study sites in the Pigeon River, NC.

Analysis of water samples for total chlorides resulted in non-detectable or less than 0.5 (mg/L) at the control site (Figure 13). Highest levels of total chlorides were found at Site 3 and slightly lower levels at Sites 4 and 5 (Figure 13).

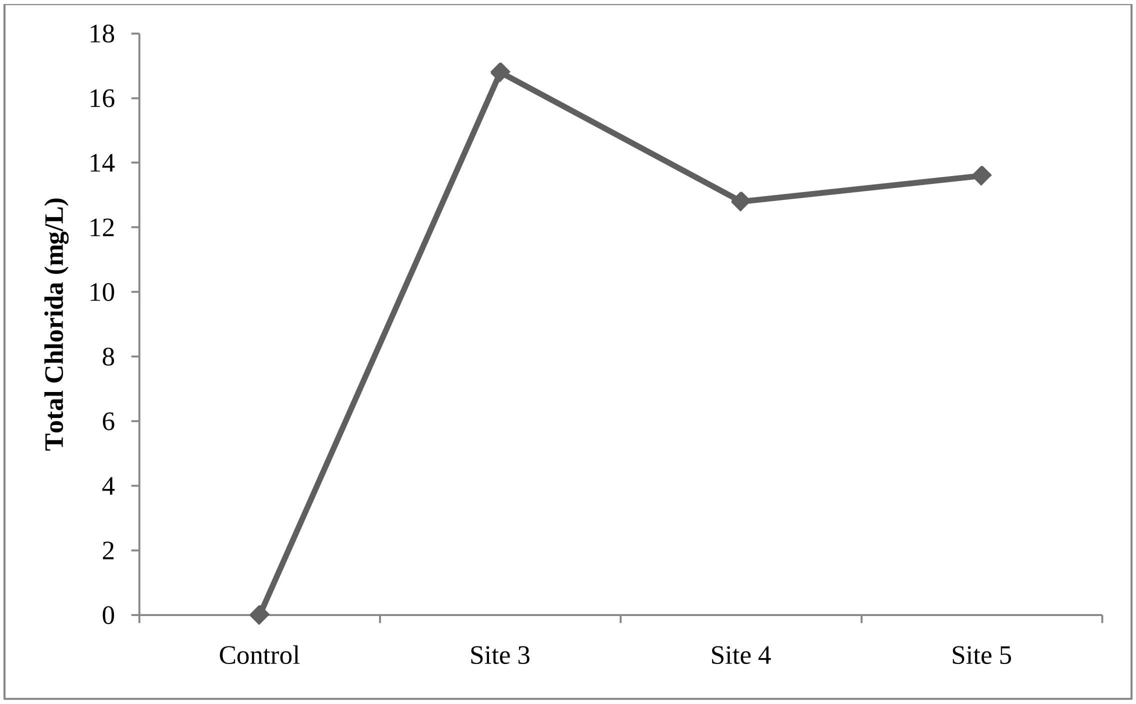

Figure 13. Total chlorides from water samples taken in December 2009 at one of the controls and each of the downstream study sites in the Pigeon River, NC.

Urbanization scores were determined at each of the five study sites, higher scores indicate greater urbanization. Sites 4 and 5 had the best scores, followed by the two control sites (Figure 14). Site 3's score was highest, due to its proximity to numerous buildings and roads. Also, there was a large amount of trash and debris on the banks and in the river. Sites 4 and 5 urbanization scores were lower than the controls, attributable to the larger amount of vegetation and only slight erosion along the banks.

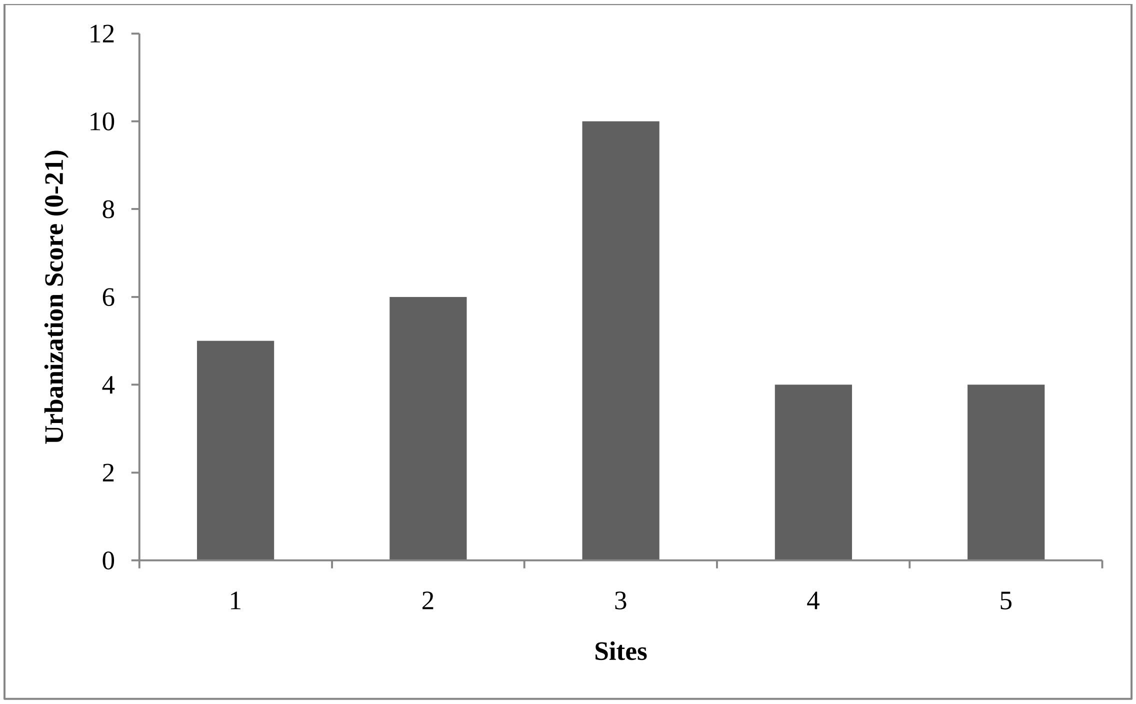

Figure 14. Urbanization rating for each of the five study sites on the Pigeon River, NC. A total score ranging from 0-21 was given based on seven visible factors typically associated with human presence and was assessed on a rating scale from 0 to 3, where a score of zero indicated little to no human effects and a score of three denoted extreme modification of the river: (a) the presence of buildings, (b) presence of roads, (c) depth and condition of the riparian zone, (c) condition of the stream bank, (d) presence of dams or spillways, (e) occurrence of erosion and (f) the quantity of trash in the river (Lyons et al. 2007).

DISCUSSION

Contrary to my expectation *L. fasciola* did not show a significant difference in mortality upstream and downstream from the paper mill. However, the few deaths that occurred were all from downstream sites. All mortalities were limited to mussels from Culture 2. Their slower growth under cooler, culture temperatures, may have led to less resilience to varying environmental stressors. However, Culture 1 experienced near lethal temperatures and weaker individuals may have died off resulting in a more heat tolerant population. Culture 1 may have had more robust individuals in my study and they may have been more resistant to environmental stressors in comparison to Culture 2. Therefore, varying culture conditions may affect the survivability and growth of the mussels once placed in their prospective habitats. The timing of the mortalities did not have any particular pattern. Some died in the early months, while others expired toward the end of the study. Thus, mortalities cannot be solely attributed to transportation and relocation of the mussels, or adjustment to the new environment. Only one mussel had significantly lower IGR than the other individuals at their site.

There was a significant relationship of IGR upstream and downstream, but in the opposite direction than what might be expected. The downstream mussels overall had higher IGR than upstream mussels. Increased growth rates at Site 4 and 5 may be attributed to warmer temperatures from the paper mill’s wastewater treatment plant heated effluent or urban impacts, such as precipitation run-off over concrete surfaces.

Warmer temperatures could increase the mussel's consumption rate (Rajagopal 2005), which may lead to higher growth rates. Another factor which could lead to higher growth rates is additional nutrients from surrounding agricultural areas, and from Lake Junaluska, which flows into Richland Creek. Additional nutrients supply the mussel's food source, e.g. algae, energy for increased growth and reproduction, in turn providing more algae for consumption by the mussels. Agricultural areas may use large volumes of fertilizer and manure, creating an excess of nitrogen which leaches into the aquatic environments (Carpenter et al. 1998). Analysis of water samples indicate there were higher levels of nitrogen in the downstream sites ranging from (0.36-0.58 mg/L), with the control containing only 0.36 mg/L. Runoff from agricultural sources feeds in from Richland Creek, just above Site 4 and Crabtree Creek above Site 5. NCDENR (2008) rated both creeks were rated ranging from poor-good, depending on sampling location, for overall condition in 2008, but lower ratings were given for fish community assessment. Overall scores were given out of 100 for water quality of both creeks, which flow into the Pigeon River. Richland's creek received a score of 83 while Crabtree creek scored only a 68 (NCDENR 2008).

Additional nutrients from agricultural runoff may be promoting the growth of algae or other organisms that mussels use for food. Possibly, resulting in the higher mussel growth rates I observed. However, higher concentrations of agricultural runoff such as nitrogen may not correspond to larger mussel populations. Nicklin & Balas (2007) found no correlation between nitrates and mussel density. Studies of negative agricultural impacts on mussel populations are focused primarily on land use changes. Changes of land use, from forested to agricultural resulted in extirpation of 47% of freshwater mussel species, in Iowa River reaches (Poole and Downing 2004). GIS analysis found decline was associated with sparse streamside woodlands, high siltation, and intensive agricultural land use (Poole and Downing 2004). A decrease in woodlands led to reduced species richness and high siltation (Poole and Downing 2004). GIS analysis comparing agricultural land use with mussel richness and diversity also showed a decline of mussels neighboring farmland (Arbuckle and Downing 2002). Agriculturally dominated watersheds with high slopes negatively impacted mussels through high siltation and deterioration of the stream substrate (Arbuckle and Downing 2002). Physical characteristics of the river tend to be the leading factors determining the success of mussel populations.

Nicklin & Balas (2007) found a positive correlation between mussel density and physical characteristics such as instream cover, embeddedness, velocity, depth, and sediment deposits. Unsurprisingly, areas with better habitat quality also had higher mussel densities. Despite higher mussel growth rates in my study, areas potentially affected by agriculture, mussels remained in chambers interacting with the water column, and some fine sediment in the water, but not directly with other external factors. If silos were filled with sediments, they were removed to maintain the proper flow rates. Frequency of sediments in the silos was variable, but was consistently found in Sites 4 and 5. I was unable to determine what proportion of the month the sediments were in the enclosures. Therefore, while I saw better growth of mussels at these sites, high levels of sedimentation may negatively impact the mussel’s ability to survive.

Higher IGRs at Site 4 and 5 may also be attributed to better urbanization scores. Decline of freshwater mussels has been directly related to habitat destruction, mainly through increasing urbanization (Lyons et al. 2007). A study investigating this hypothesis by Lyons et al. (2007), on the Black River, OH, tested the abundance of mussels in relation to their urbanization scores. Sites with urbanization scores less than 5 had abundant mussel populations, at urbanization scores of between 5-10 mussel abundance was dependent on the presence of riparian vegetation and at urbanization scores of 10 and above resulted in no mussels being found. This relationship corresponds to the IGRs at each of our study sites. Where the control sites had urbanization scores of 5 and 6, with lower IGRs, Site 3 had a score of 10 with the lowest IGR, and Site 4 and 5 had a score of 4, with the highest IGRs (Figure 13).

Site 3 had many factors which could have negatively affected the mussels and resulted in lower IGRs, than the other two downstream sites. First of all, there were lower levels of nitrogen than the other sites with levels were comparable to the control. Therefore, additional nutrients may have not been available to promote higher algae levels, then mussel growth rates. Site 3 had the highest levels of conductivity, and temperature, which may have influenced their difference in growth rates. Higher temperatures may increase their consumption rate, but with lower nutrients available to increase their food source, their growth rates may have suffered. Highest chloride concentration was also found at Site 3, which may originate from residual paper mill effluent (dioxins and furans), wastewater treatment effluent or urban runoff. However, what contaminants were present, their source, and how they impacted the development of our mussels was not determined from my study.

Exposure to dioxins and furans is believed to cause accumulation of these toxins in lipids of marine mussels (Abad et al. 2003). During the mussel's reproductive period lipids are converted to nutrients for offspring (Abad et al. 2003), which could adversely affect their survivability and successful development. A study by Abad et al. (2003), identified the isotopes of dioxins and furans and found their dioxin/furan profiles of sediments from the same coastal areas differed with those within the mussels, hypothesizing that the mussels may be obtaining them by metabolic processes, possibly through interacting with the water column. These findings suggest that if these contaminants still exist in the water column, mussels located at Site 3 may not be able to successfully reproduce, due to their exposure. However, I did not test for the presence of dioxins and furans; therefore, further research is needed to determine if contaminants exist and if their levels are a threat to mussel survival and reproduction.

Conversely, if the main source of contaminants results from metabolism food in the water column and not sediments, the mussels may have a better chance of survival at this site than predicted from the study above. These toxins have not been released at detectable levels since 1989 (NCDENR 2009); therefore any remaining dioxins and furans would most likely be trapped within the sediments. Toxins trapped within the sediments pose less of a threat than those within the water column used by the filter feeders. However, if flooding or high flows occur, these toxins may be re-suspended in the water column. Re-suspension of toxins may then result in mussel mortalities or adverse affects on their reproductive success. However, tests are needed to determine how long it takes for these contaminants to be reduced below levels that may affect the mussels, under average flow, and high flow periods. Sites 4 and 5 may be less impacted by remaining paper mill effluents, possible dilution of any residual paper mill contaminants by the additional inflows to the river downstream may lead to higher success of mussels at these sites.

Another possible threat to mussel survival and growth is the Waynesville Wastewater Treatment Plant (WWTP), which releases its waste into Richland's Creek, which then flows into the Pigeon River. WWTP's wastewater collection and treatment system report for July 2008-June 2009 explained there was only one negative incident. They reported at the end of August 2008, there was a discharge of 10,000 gallons of primary treated sewage into Richland's Creek (WWTP 2008-09). A wastewater treatment plant's (WTP) main toxicants are monochloramine (MCA) and unionized ammonia (Goudreau et al. 1993), however exact chlorine and nitrogen compounds used by WWTP were not determined. Most chlorine released from sewage treatment plants has undergone a de-chlorinization step; however trace amounts of chlorine are still released. Faulty equipment or exceeding maximum flow levels can result in the release beyond the limit of total residual chlorine. Most chlorine from WTPs is in the MCA form, which is less toxic but persists in the environment (Goudreau et al. 1993).

Goudreau et al. (1993), found sites on Clinch River,VA up to 3.7 km downstream of a WTP were devoid of freshwater mussels. They also found that glochidia were the most sensitive growth/development stage to these toxins. Chlorination was found to have a minimum of six effects on marine mussels (1) mortality, (2) pathology, (3) lower filtration rate, (4) decrease in growth, (5) decrease in settlement, and (6) extrication of settled larvae (Khalanski and Bordet 1980). Therefore, in the presence of chlorine concentrations near ~100 mg/L, mussels are not likely to thrive (Gillis et al. 2009, unpublished). The mussel’s complex life cycle (free swimming to sessile) exposes them to a wide range of areas within a freshwater ecosystem, which could make them more likely to be exposed to contaminants in their habitat (Cope et al. 2008). Goudreau et al. (1993) reported on bioasssays that showed glochidia were closed during 24h of exposure to MCA or unionized ammonia, and they did not reopen until having been in clean water for an additional 24h, 24h EC_{50} for MCA was 0.042 mg/L. This study also suggested that if glochidia were exposed to, and a particular level of those contaminants, even if not lethal, it could prevent them from infesting fish. However, our study tested for total chlorides, which ranged from 12-16 mg/L, which is much lower than EC_{50}s for total chlorides of 105 mg/L for glochidia found in another study by Gillis et al. 2009, unpublished. Therefore, based on their study, levels of total chlorides within the water column do not seem to pose a threat to our mussels.

In a related study by Cope et al. (2008), they tested the sensitivity of bivalves at their various life stages to environmental contaminants, each stage had universal and distinctive characteristics which led to their observed differences in the bivalves’ exposure and sensitivity. In short, the majority of unionid critical life stages are; sperm release into the water column, sperm siphoned into females, fertilization of ova, release of glochidia, encysted glochidia, and independent juvenile mussels (Cope et al. 2008).

At the adult stage, a freshwater mussel species, *Lampsilis siliquoidea*, exhibited a toxicant avoidance response, in which they close their valves to prevent exposure (Cope et al. 2008). However, this response could only be maintained for the first 24h, until they were seemingly forced to open their valves to meet their metabolic demands (Cope et al. 2008). Therefore, long-term exposure to contaminants at the adult stage could have significant impacts on their survivability and population success. Even at this most resilient stage, mussels can only close their valve for a limited period before metabolic demands outweigh exposure to toxins. However, they found that if exposed to low levels of contaminants (0.2 µg/L of Cd) over several days, mussels would open and close their valves, similar to pre-treatment conditions. The mussels were assessed to be risking slightly negative effects of contaminants in order to have adequate oxygen.

Juvenile mussels have relatively the same types of exposure as adult mussels (Table 6); however they differ in that adults exhibit vertical movement patterns in substrate, while juveniles tend to remain burrowed in sediments (Balfour & Smock 1995). Early stages of development are limited to pedal feeding, a type of deposit-feeding where cilia generates currents on the foot (Yeager et al. 1994). At this stage, mussels are more susceptible to contaminants trapped within the sediments. Therefore, residual toxins from industrial and urban effluent could produce greatest damage at this stage.

In my study I found successful survivability and growth of *L. fasciola* however, many other aspects need to be addressed to determine their likelihood of successful reintroduction. Besides study of the ability of mussels to withstand contaminants, research of successful reintroductions into areas they have been previously extirpated from is needed. Layzer & Scott (2006) successfully reintroduced mussels in the French Broad River and observed high survivability of translocated species. They also had successful reproduction of one of the translocated individuals. Furthermore, four of the mussel species naturally colonized in the river. The limiting factor for successful colonization and recruitment is increasing the presence of their host fish species (Layzer and Scott 2006).

Research is needed on the genetic and environmental implications of reintroduction. Hoftyzer et al. (2008) explains several genetic and environmental hurdles reintroductions face, leading to possible extinctions. Small populations of reintroduced mussels may have low genetic variability, resulting in low heterozygosity, increasing the susceptibility to extinction. Captive breeding may lead to reintroduced mussels being incapable of reproducing independently, and therefore may be unable to sustain their population. Despite genetic problems, reintroductions may cause unforeseen spread of diseases, parasites and exotic species from relocated individuals.

Successful mussel populations usually exist in multispecies assemblages, with species containing a wide range of traits (Vaughn et al. 2008). Therefore, restoration efforts should focus on community level approaches, instead of a single species to promote processes better and re-established ecological functions (Vaughn et al. 2008).

Mussels are also limited by the presence of suitable habitat, due to limited mobility. Therefore they are sensitive to habitat fragmentation because of their limited mobility, dispersal, longevity, and low juvenile survival (Newton et al 2008). Landscape ecology approaches to reintroduction should focus on maintaining suitable habitats and requiring connectivity of mussel populations. River channels act as corridors connecting patches (through host fish and nutrient movement), which augments restoration of species within a patch after extirpation and increases gene flow (Newton et al. 2008). Therefore, connectivity is important in the success of the population. Small populations in patches may result in local extinctions from demographic stochasticity, and will only be restored by colonization from other patches (Newton et al 2008). Moreover, the ultimate success of mussel populations in a connectivity scheme is solely dependent on the distribution of its host fish (Newton et al. 2008).

CONCLUSIONS

Mussels had high survivability and growth in The Pigeon River downstream of Canton, NC. This study provides evidence that if mussels survive until the juvenile stage, they have an excellent chance of surviving to reproductive age. But, the enclosures used in this study isolated mussels from the river substrate, so any effects of residual pollutants in river sediment or mussesls was no examined. Additional studies need to examine survivability of *L. faciola* at other sensitive life stages. Factors influencing successful colonization and growth of mussel populations and their limitations should be studied before reintroduction can be successfully implemented. Timing of the release of *L. fasciola* into the river should also be addressed. Release of *L. fasciola* in June in VA, resulted in the greatest growth and survival rates (Hanlon & Neves 2006).

Successful reintroduction of *L. fasciola* would improve habitat and possibly water quality in the Pigeon River. Mussels and their exhausted shells supply or augment habitat for other organisms by offering physical structure, stabilizing and bioturbating sediments, and impacting food accessibility through deposits of their organic matter and release of nutrients (Vaughn et al 2008). Reintroduction, including successful reproduction, could provide many ecosystem services to the Pigeon River that may further improve the river's overall quality.

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