Eads et al 2007

Carolina Heelsplitter (*Lasmigona decorata*) Conservation Measures

Final Report

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Introduction

The Carolina Heelsplitter (*Lasmigona decorata*) is a federally endangered freshwater mussel species that is known from only a few populations in North and South Carolina. Prior to this year, only two populations were known from North Carolina – one in Goose and Duck Creeks (Yadkin-Pee Dee) and one in Waxhaw Creek (Catawba) in Union County. In February 2007, three individuals were found by the Catena Group, a private consulting firm, in Sixmile Creek along the Union/Mecklenburg County Line in North Carolina. The Goose Creek population was determined by the North Carolina Freshwater Mollusk Scientific Council to be in imminent danger of extirpation due to the expansion of the Charlotte, NC metropolitan area. Degradation of both habitat and water quality have been observed over the past 13 years since it has been listed (John Fridell, US Fish and Wildlife Service, pers. comm.). Additionally, drought conditions in 2002 and 2005 may have severely impacted this population. Acting on recommendations from the Scientific Council, the NC Wildlife Resources Commission funded this project to initiate measures to help conserve the populations of the state's remaining *L. decorata*.

The objectives of this study were to:

1) Collect individuals from the Goose and Duck Creek population to bring individuals into captivity
2) Maintain animals in captivity long-term
3) Determine the required host fish species for the Carolina Heelsplitter through laboratory infections,
4) Attempt to grow out juvenile mussels produced in host trials, and
5) Identify potential relocation sites to establish a new population in a protected watershed in North Carolina.

The larvae (called glochidia) of freshwater mussels (Unionidae) require attachment to a host fish species to complete their life cycle. Often, this relationship is quite species-specific with a mussel being able to infect only one species of fish or a small group of closely related species. Prior to this study, the host fish for the Carolina Heelsplitter was unknown (Bogan 2002). A list of likely candidate hosts was generated by conducting fish surveys where *L. decorata* was known to occur (Starnes and Hogue 2005), but laboratory studies were necessary to definitively determine the needed host.

COLLECTION AND HOLDING OF ADULT *LASMIGONA DECORATA*

In August 2006, five *L. decorata* were collected from Duck Creek in Union County, NC and immediately transported to the Table Rock Fish Hatchery near Morganton, NC where we maintain a mussel culture trough. At the time of collection, one of the individuals was found stranded out of the water on a gravel bar. Later examination of USGS online stream gage data revealed that water had likely not covered that gravel bar for 11 days. This individual appeared to be alive at the time of collection; however, gaping, a clinical sign of impaired health, was observed following immersion in the stream. This individual was found dead and partially decomposed after four days at

the hatchery. The specimen was preserved in alcohol and deposited at the North Carolina Museum of Natural Sciences (NCSM). The four remaining individuals remained alive, and two were found to be gravid in January 2007.

We transported the gravid mussels to the mussel propagation laboratory at NC State University to conduct fish host trials. Adults were returned to the hatchery upon extraction of the glochidia. The first individual used in host trials was found dead and partially decomposed at the hatchery two weeks after it was returned from being used in propagation. We believe that trauma to the gill during extraction of the glochidia, rather than culture conditions, led to the mortality. The specimen was preserved in alcohol and deposited at the NCSM. The three remaining individuals were all alive at the time of this report, but no measurable growth was detected since their collection.

In February 2007, we acquired three adult *L. decorata* found in Sixmile Creek on the Union/Mecklenburg County Line in North Carolina. One of those individuals was gravid and was held in the lab for additional propagation work, and the other two were immediately taken to the mussel culture trough at Table Rock. The individual used for propagation was taken to the hatchery two weeks later. On 29 June 2007, one of these three individuals was found dead and partially decomposed. We believe that water supply problems and related temperature swings in the culture trough in the days preceding the discovery likely contributed to the mortality. The specimen was preserved in alcohol and deposited at the NCSM. The other two individuals were both alive at the time of this report, but no measurable growth was detected since their collection.

HOST FISH TRIALS

Methods

Trial 1 – On 18 January, 2007, host fish representing 20 species were collected by seine and backpack electrofishing from Big Bear and Island Creeks in Stanly County, NC and Irish Buffalo Creek in Cabarrus County, NC (all in the Yadkin-Pee Dee River Basin). Fish were transported live back to the propagation laboratory and maintained at 13 ± 1°C. A single gravid *Lasmigona decorata* from the Duck Creek stock (Tag # M323) was also transported from the Table Rock Hatchery to the propagation laboratory. On 23 January 2007, we extracted the glochidia by flushing the marsupium with a water filled syringe finding that mature glochidia adhered to unfertilized eggs forming conglutinates (Figs. 1-3). The size of these conglutinates made extraction somewhat difficult, and the repeated flushing necessary to complete the task caused some trauma to the gill tissue. Once tissue damage became apparent, we ceased extraction. We freed glochidia from the conglutinates by sucking the packets in and out of a plastic, 1-ml pipette. All fish were then placed together with the glochidia in approximately 12 liters of water for 30 minutes, and strong aeration was used to keep glochidia in suspension. After infestation, fish were divided into different aquaria by species and maintained at 13 ± 1°C. We used up to 3 aquarium replicates for certain species when enough fish of that species were available. We siphoned the aquaria routinely through a 150-μm-mesh sieve and counted

dead glochidia as well as transformed juvenile mussels to determine transformation rate. Individuals were considered to be transformed if two adductor muscles were visible or if there was foot movement.

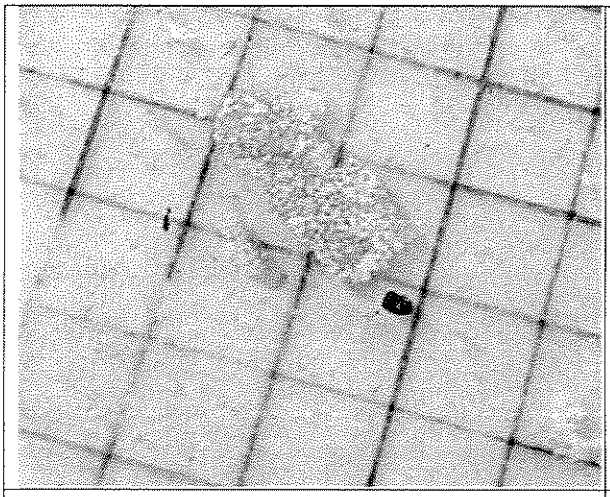

Figure 1. Conglutinate of *Lasmigona decorata* in a petri dish. Each brown spot represents one glochidium.

Figure 2. Conglutinate of *Lasmigona decorata*. Approx. 10x magnification

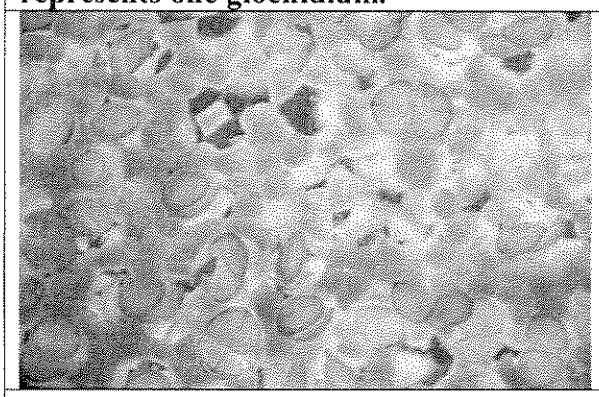

Figure 3. Glochidia of *Lasmigona decorata* packaged with unfertilized eggs.

Trial 2 – Based on early transformation results from Trial 1, we chose to collect bluegill (*Lepomis macrochirus*) to use in propagation of another *L. decorata* individual (Tag #V002) from the Duck Creek stock found gravid at the Table Rock Hatchery. On 20 February 2007, we collected bluegill by backpack electrofishing from Little Mountain Creek in Stanly County, NC (Yadkin-Pee Dee River Basin). Warmouth (*L. gulosus*) and rosyside dace (*Clinostomus funduloides*) were also collected from this location because they had not been tested in Trial 1. On 27 February 2007, we collected bluehead chub (*Nocomis leptocephalus*), white shiner (*Luxilus albeolus*), and rosyside dace from Morgan Creek (Cape Fear River Basin) in Orange County, NC. We also purchased Golden shiners (*Notemigonus chrysoleucas*) from a bait shop near Raleigh, NC. Because of the tissue damage done in extracting glochidia from the first *L. decorata* used in propagation, we chose to allow this individual to release glochidia without our

intervention. In the laboratory, we held the gravid mussel in an aquarium at 13 ± 1°C and siphoned its tank routinely to check for released glochidia. When glochidia were found, they were checked for viability by exposing a small subsample to a saturated salt solution. Glochidia were considered viable if their valves closed in response to the salt. The remaining collected glochidia were then immediately used to infest fish either by hand or by using a batch infestation method. Batch infestation involved putting host fish in a small volume of water with the glochidia and aerating vigorously to keep glochidia in suspension. Fish were monitored for attachment and removed from the treatment after approximately 30 minutes. Hand infestations were done with larger fish by anesthetizing them with MS-222 and pipetting the glochidia directly on to the fish's gills. Fish were infested with glochidia from this brood on 5 separate occasions from 20 February to 9 March 2007. Fish were divided by species and monitored as described in Trial 1.

Trial 3 – Three *L. decorata* were collected by a private consulting firm (The Catena Group) in Sixmile Creek (Catawba River Basin) on the Mecklenburg/Union County Line in North Carolina. These mussels were delivered to our lab, and one was found to be gravid. We subsequently collected host fish representing 17 species on 1 March 2007 by seine and backpack electrofishing in Waxhaw Creek and the Twelvemile Creek system (Catawba River Basin) in Union County, NC. Because the gravid mussel in Trial 2 only released a small number of glochidia at a single time, we needed to extract glochidia to conduct further host trials using this individual from the Catawba River Basin. To accomplish this, we immersed the mussel in 500 mg/L serotonin for 3 hours. The mussel was then removed from the serotonin and placed back in its aquarium overnight. By the following morning (6 March 2007), the entire brood had been released into the aquarium. We tested a subsample for viability using the salt test described in Trial 2 and used the harvested glochidia to batch infest our host fishes. Fish were divided by species and their aquaria were siphoned routinely to check for transformed juvenile mussels.

Results

Trial 1 - We found 6 species of fish served as successful hosts, 7 acted as poor hosts (only producing 1 or 2 juveniles), and 7 did not serve as hosts (Table 1).

Table 1. Results of Host Trial 1 for *Lasmigona decorata* (Yadkin-Pee Dee River Basin)

Species Common Name	Species Scientific Name	Total # of Juveniles Produced	Number of Aquaria Replicates	Total # of Fish	Mean # of Juveniles per Fish	Mean % Transformation
Successful Hosts						
Bluegill	*Lepomis macrochirus*	185	3	15	12.3	23.3
Golden shiner	*Notemigonus chrysoleucas*	64	1	1	64.0	64.0
Satinfin shiner	*Cyprinella analostana*	6	1	1	6.0	54.5
Bluehead chub	*Nocomis leptocephalus*	67	2	4	16.8	73.7
Spottail shiner	*Notropis hudsonius*	66	3	6	11.0	32.2
Highfin shiner	*Notropis altipinnis*	38	3	15	2.5	44.0
Poor Hosts						
Largemouth bass	*Micropterus salmoides*	1	3	3	0.3	4.8
Redlip shiner	*Notropis chiliticus*	1	2	7	0.1	3.1
Fantail darter	*Etheostoma flabellare*	1	4	12	0.1	0.1
Whitemouth shiner	*Notropis alborus*	2	3	9	0.2	16.7
Yellow bullhead	*Ameiurus natalis*	2	1	1	2.0	10.5
Tesselated darter	*Etheostoma olmstedi*	1	2	12	0.1	0.4
Pirate perch	*Aphredoderus sayanus*	1	3	5	0.2	0.9
Non-Hosts						
Green sunfish	*Lepomis cyanellus*	0	3	3	0	0
Redear sunfish	*Lepomis microlophus*	0	1	1	0	0
Redbreast sunfish	*Lepomis auritus*	0	3	3	0	0
Creek chubsucker	*Erimyzon oblongus*	0	2	5	0	0
Margined madtom	*Noturus insignis*	0	2	6	0	0
Piedmont darter	*Percina crassa*	0	2	5	0	0
Carolina darter	*Etheostoma collis*	0	1	1	0	0

Trial 2 – Overall production was decreased compared to Trial 1 (Table 2). Bluegill, and golden shiners produced transformed juveniles, but both species produced fewer than in Trial 1. Bluehead chubs from the Catawba system acted as poor hosts in this trial. A single Rosyside dace from the Cape Fear Basin produced more juveniles than a single Rosyside dace from the Yadkin-Pee Dee basin. The single warmouth tested produced no juveniles.

Table 2. Results for Host Trial 2 for *Lasmigona decorata* (Yadkin-Pee Dee River Basin).

Species Common Name	Species Scientific Name	Source	Total Number of Juveniles Produced	Total Number of Fish Used	# Juveniles Produced per fish
Bluegill	*Lepomis macrochirus*	Yadkin-Pee Dee	73	24	3.04
Bluehead chub	*Nocomis leptocephalus*	Catawba	6	16	0.38
Golden Shiner	*Notemigonus chrysoleucas*	Cape Fear	11	2	5.50
Golden Shiner	*Notemigonus chrysoleucas*	Purchased	73	14	5.21
Rosyside dace	*Clinostomus funduloides*	Cape Fear	13	1	13.00
Rosyside dace	*Clinostomus funduloides*	Yadkin-Pee Dee	1	1	1.00
White shiner	*Luxilus albeolus*	Cape Fear	1	3	0.33
Warmouth	*Lepomis gulosus*	Yadkin-Pee Dee	0	1	0.00

Trial 3 – A total of 1,140 juveniles were produced in Trial 3. There were seven species that served as successful hosts, five that served as poor hosts, and six that were non-hosts (Table 3).

Table 3. Results of Host Trial 3 for *Lasmigona decorata* (Catawba River Basin)

Species Common Name	Species Scientific Name	Total # of Juveniles Produced	Number of Aquaria Replicates	Mean # of Juveniles per Fish
Successful Hosts				
Golden Shiner	*Notemigonus chrysoleucas*	541	5	20.7
Bluehead chub	*Nocomis leptocephalus*	160	5	30.6
Sandbar shiner	*Notropis scepticus*	280	5	23.5
Whitefin shiner	*Cyprinella nivea*	29	1	14.5
Rosyside dace	*Clinostomus funduloides*	37	1	12.3
Creek chub	*Semotilus atromaculatus*	41	1	41
Warmouth	*Lepomis gulosus*	19	1	19
Poor Hosts				
Green Sunfish	*Lepomis cyanellus*	7	1	7
Flat bullhead	*Ameiurus platycephalus*	1	3	0.3
Tessellated darter	*Etheostoma olmstedi*	1	1	0.3
Redbreast sunfish	*Lepomis auritus*	1	1	0.1
Misc. Sunfish	*Lepomis spp.*	23	1	3.8
Non-Hosts				
White Sucker	*Catostomus commersonii*	0	2	0
Jumprock sp.	*Scartomyzon sp.*	0	1	0
Creek Chubsucker	*Erimyzon oblongus*	0	1	0
Bluegill	*Lepomis macrochirus*	0	2	0
Redear sunfish	*Lepomis microlophus*	0	1	0
Margined madtom	*Noturus insignis*	0	1	0

Discussion

Overall, 9 minnow species (Cyprinidae) served as successful hosts between the Yadkin-Pee Dee and Catawba River Basin individuals. Two sunfish species, bluegill and warmouth, produced some juveniles but with inconsistent results. We recommend golden shiners for use in future propagation efforts since they are a relatively large minnow and can be easily purchased in large numbers at a relatively low price. Bluehead chubs are also abundant and relatively easy to capture by electrofishing and can serve as good hosts. The use of serotonin was most effective in allowing the extraction of all glochidia in a brood without harm to the adult mussel. The adult used in Trial 1 was found dead at the hatchery soon after use in propagation. We believe this was likely due to gill tissue trauma experienced during the flushing of the glochidia. Allowing the gravid mussel to release glochidia on its own produced far fewer juveniles. Since the adult mussel's tank could not be checked every day, a portion of the glochidia may have sat in the aquaria for up to a few days between checks. Although all glochidia responded

to the salt viability test, they may have been less than completely healthy when attached to the fish.

CULTURE OF JUVENILE *LASMIGONA DECORATA*

Methods

Juveniles produced in the three host trials were cultured in 7.6-liter containers in either dechlorinated municipal water or creek water (collected from New Hope Creek in Orange County, NC). Each container held either fine sediment (< 200 μm), a commercial substrate (Caribsea Mineral Mud™, Aquatic Ecosystems), or coarse natural substrate (2-4 mm). Containers were held in a chilled water bath and began initially at 13 ± 1°C. By 4-months post-metamorphosis, temperature had been raised to 16 ± 1°C. Juveniles were fed daily a mixture of cultured green algae (*Scenedesmus* and *Franceia sp.*) and commercial algae (*Nannochloropsis, Isochrysis, Pavlova, Tetraselmis*, and *Thalassiosira weissflogii* from Reed Mariculture Inc., Campbell, CA) at approximately 50,000-100,000 cells/mL. A ¾ water change was performed weekly. Survival and growth assessments were performed minimally to avoid disturbance of the mussels. Additionally, those cultured in coarse sediment or Mineral Mud™ could not be assessed until they had grown to a visible size. On 29 May 2007, we placed 40 juveniles from Trial 3 (Sixmile Creek) at the Table Rock Hatchery. Those juveniles ranged from 1-1.5 mm when they were placed at the hatchery. All treatments that began in lab water were changed to creek water in May 2007 based on observed success at that time.

Results

At the time of this report (6-7 months post-metamorphosis), there were still surviving mussels from all 3 broods propagated in the laboratory. Survival and growth was comparable to other species propagated in the lab (Eads et al. 2007), and in some treatments, survival was much better (Table 4). The 40 juveniles taken to the hatchery at 1-1.5 mm had experienced complete mortality when checked after 1 month.

Table 4. Survival and growth of *Lasmigona decorata* juveniles cultured in the laboratory from 3-6 months post-metamorphosis. Survival is presented as the number alive divided by the number originally placed in the treatment. Length is presented as a range from smallest to largest individual.

Brood	Treatment	3-month Survival and Length	4-month Survival and Length	5-month Survival and Length	6-month Survival and Length
Duck Creek (M323) – Trial 1	Creek water	50/77 (64.9%)	42/77 (54.5%)		
	Fine sediment	800-950 µm	1.3-1.9 mm		
	Lab water	3/67 (4.5%)			
	Fine sediment	700-720 µm			
	Creek water				20/50 (40.0%)
	Mineral Mud™				1.8-3.7 mm
	Lab water				4/49 (8.2%)
	Mineral Mud™				2.4-3.4 mm
	Creek water				0/48 (0%)
	Coarse sediment				
	Lab water				0/47 (0%)
	Coarse sediment				
Duck Creek (V002) – Trial 2	Creek water	1/44 (2.3%)			
	Fine sediment	1.1 mm			
	Lab water	10/49 (20.4%)			
	Fine sediment	650-850 µm			
	Creek water			0/61 (0%)	
	Mineral Mud™				
	Lab water			18/45 (40%)	
	Mineral Mud™			1.9-3.0 mm	
Sixmile Creek - Trial 3	Creek water	203/248 (81.9%)			
	Fine sediment	0.75-1.4 mm			
	Creek water		222/365 (60.8%)		
	Fine sediment		1.4-2.6 mm		
	Creek water			64/280 (22.9%)	
	Mineral Mud™			1.9-3.9 mm	
	Creek water			69/249 (27.7%)	
	Mineral Mud™			1.9-3.0 mm	

Discussion

Water collected from New Hope Creek was more effective than dechlorinated municipal water at growing juvenile *Lasmigona decorata* in Trial 1 but less effective in Trial 2. The reason for this difference between trials is unknown and could be due to chance or to a bad batch of conditioned municipal water early in Trial 1. Because creek water was more effective in the culture of *Alasmidonta viridis* in another trial, we have continued to use that for all juvenile mussels. We have maintained relatively low temperatures over the culture period to try to maximize survival before mussels are transported to culture at the hatchery. Our experience has shown that mussels experience good survival at the Table Rock Fish Hatchery if they are taken there after reaching 2-3 mm in length. Smaller mussels, on the other hand, have always experienced complete

mortality at the hatchery. This could be due to silt loads, predation by some organism, or higher temperatures, but the true cause remains unknown. The 40 juveniles taken to the hatchery at 1-1.5 mm also experienced complete mortality after 1 month. Because of the time of year when propagation of these juveniles occurred, we anticipate that they will just be reaching the 3-mm threshold when the growing season at the hatchery is coming to a close (early October). Because mussels less than 5 mm seem to do poorly over the hatchery winter (Eads et al. 2007), we plan to wait until April 2008 to take these mussels to the hatchery.

DETERMINING POTENTIAL RELOCATION SITES

We evaluated several sites (Table 5) in the Yadkin-Pee Dee basin to examine the possibility of eventually stocking a new experimental population of *Lasmigona decorata*. Based on recommendations by the North Carolina Freshwater Mollusk Scientific Council, we primarily targeted Barnes Creek (Fig. 4) in Montgomery County, NC. Barnes Creek has an Outstanding Resource Waters (ORW) designation and has a substantial amount of land within the watershed (almost 30%) owned by the US Forest Service (Fig. 5). Additionally, virtually none of the watershed is urban or in intensive agriculture. Relative to most of the North Carolina Piedmont, the Barnes Creek watershed is well protected. As expected Barnes Creek had the best habitat and the most abundant and diverse mussel fauna of the streams surveyed. The reach between Tower Road and the confluence with Poison Fork offered the best habitat and mussel fauna. Based on available habitat and the diversity and abundance of resident mussel fauna, we recommend a reach approximately 1 km downstream from the confluence with Poison Fork be used as the primary site (Fig. 5). This reach contains 2-3 large pools that will hold water during extreme drought and contain an abundant and diverse mussel fauna. This reach lies on private property, but it remains well forested and is in good condition. Though Barnes Creek is relatively rocky and with a higher gradient and fewer mussels downstream of Tower Road, there is some potential habitat immediately upstream of the US Forest Service land. This could serve as a secondary stocking location to spread mussels further throughout the creek.

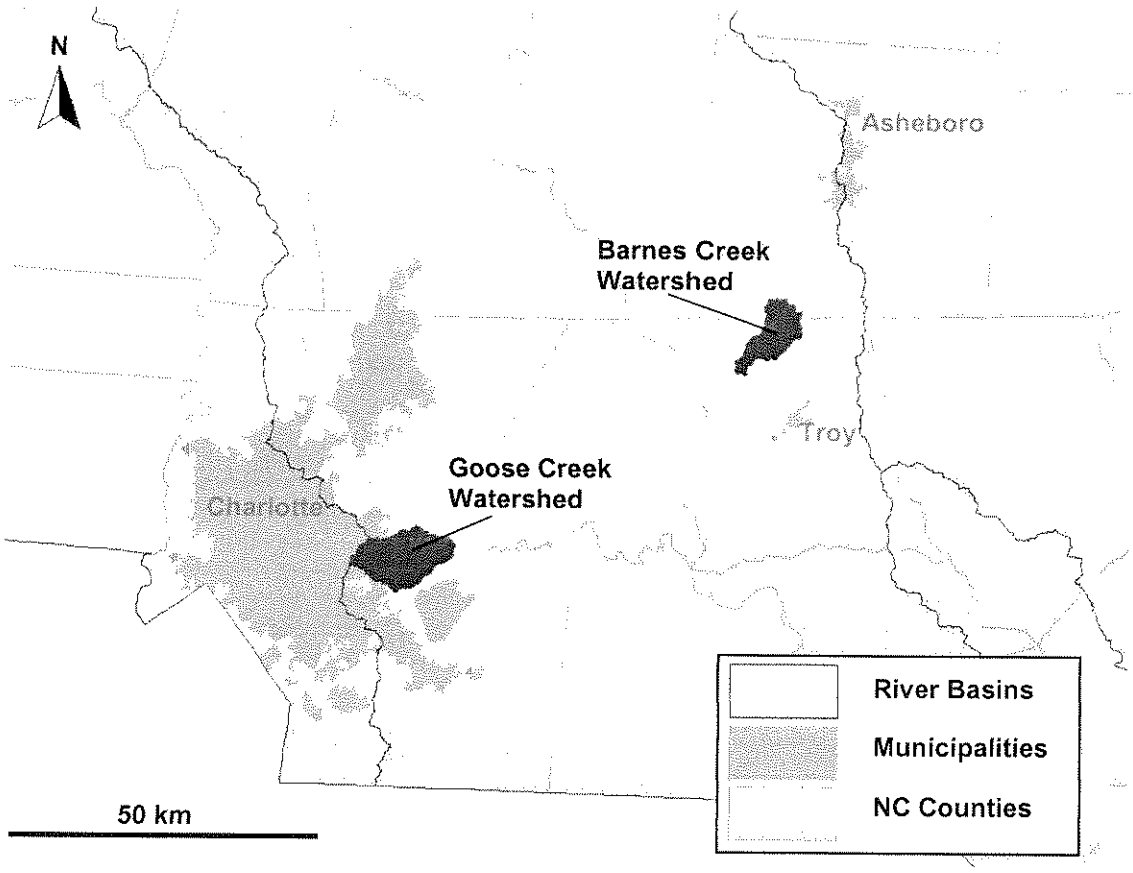

Figure 4. Map of Goose and Barnes Creek watersheds.

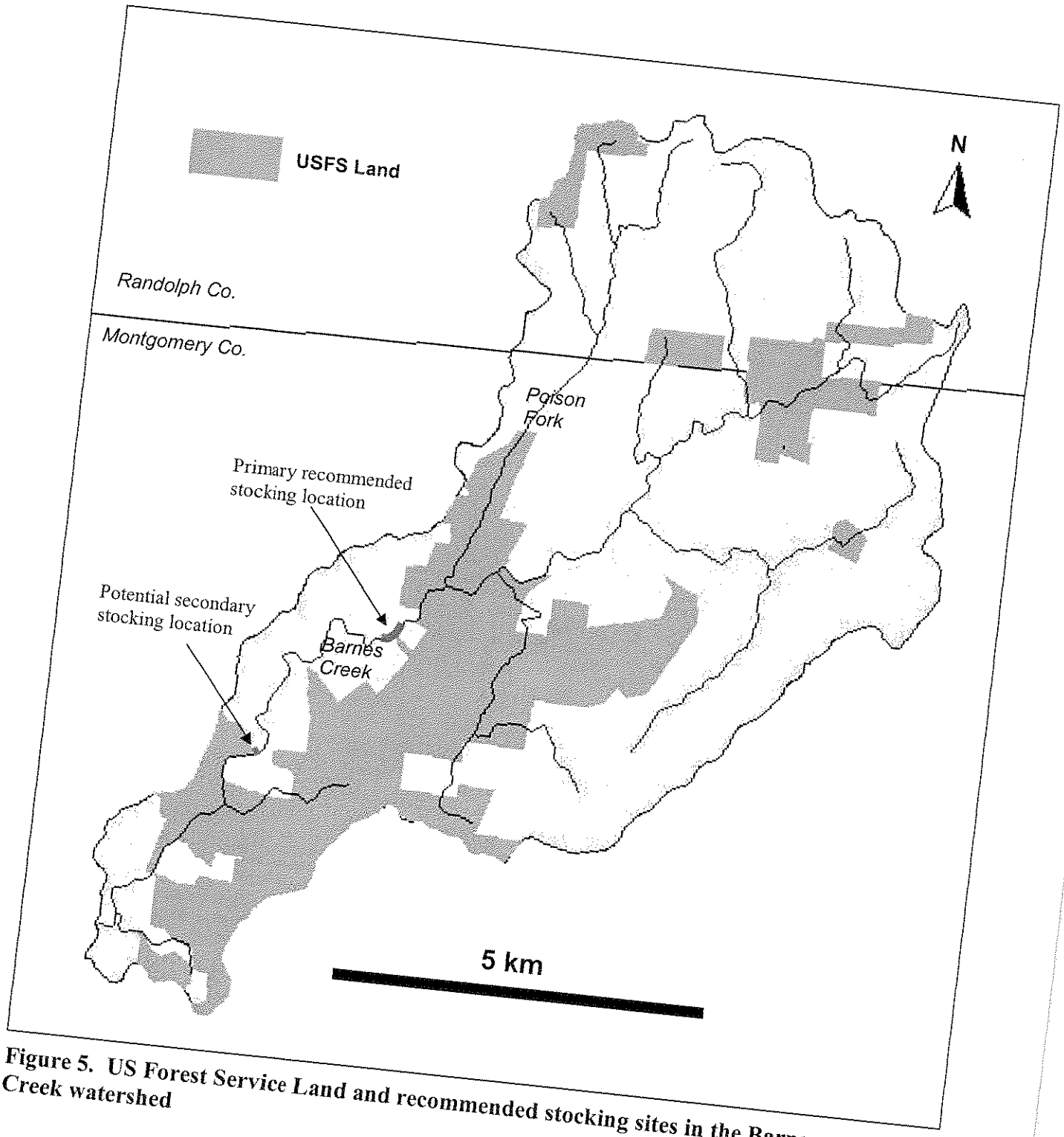

Figure 5. US Forest Service Land and recommended stocking sites in the Barnes Creek watershed

Table 5. Sites evaluated for potential mussel relocation sites

Stream	Location	Comments
Barnes Creek	Ophir Rd (Montgomery County)	Landowner may be uncooperative. Riparian zone very forested. Lots of slate and bedrock instream. Stream has fairly high gradient with 2-3 large pools that could possibly hold *L. decorata* but habitat not ideal. Bottom of pools filled with fine sediment. Mussels not abundant. Species found: *E. complanata, L. subviridis, V. vaughaniana,* and *P. cataracta*
Barnes Creek	Tower Rd (Montgomery County)	Lower stream gradient and better mussel habitat. Mussels more abundant than at Ophir Rd. There are no deeper pools to protect from drought. Best mussel habitat is shallower with mussels in gravel between large cobbles. Some potential for relocation based on the number of mussels found. Species found: *E. complanata, A. varicosa, V. constricta, V. delumbis,* and *V. vaughaniana*
Barnes Creek	Between Tower and Ophir Rd	Entered from Dolan Corbin property. Surveyed approximately 1 mile of stream walking downstream toward USFS property. Stream is relatively steep and dominated by bedrock and boulder. Most of habitat is inhospitable to mussels. Mussels diverse but not abundant with many occurring in depositional areas near banks. Stream banks are in good condition and riparian zone is well forested with no apparent threats to habitat. One pool specifically (N35.45601, W79.98243) has some potential for relocation.
Barnes Creek	Below confluence with Poison Fork.	Riparian zone forested. Good mussel habitat in long stretches. Stream banks in good condition. Mussels abundant and diverse. There are 2-3 pools that should be able to serve as good habitat for *L. decorata*. There is a variety of habitat available from sand-gravel to rocky pools. Good population of *A. varicosa*. Species found: *E. complanata, Elliptio* (lance), *V. constricta, V. delumbis, V. vaughaniana, A. varicosa,* and *Uniomerus carolinianus*
Clark's Creek	Hydro Rd	Lots of excess sediment. Stream banks in poor condition. Lots of development nearby. Not a good candidate for relocation
Mountain Creek	NC 73 and Green Rd	Stream banks in fair condition, but few mussels were present. Found a few *E. complanata* and 1 *V. delumbis*
Betty McGees Creek	Lassiter Mill Rd	Habitat is marginal, and stream banks are in fair to poor condition. A few *E. complanata* found. Likely too small to hold water during drought and not a good candidate for relocation.
Betty McGees Creek	Birkhead Wilderness	Upstream of Lassiter Mill Rd. Habitat is marginal at best. A few *E. complanata* found. Too small.
Spencer Creek	Ophir Rd	Too small. Too rocky with no fine sediment. Not good habitat. No mussels found. Not a good candidate for relocation.
Uwharrie River	Confluence w/ McClean's Creek on FR 555	Large patches of fair-good mussel habitat with many E. complanata found. No other species found. Not highly recommended as relocations site based on lack of other species.
Dutchman's Creek	River Road	Too small. No mussels found. Not a good candidate for relocation

Conclusion

We are optimistic that we can continue to culture this species in the laboratory and in a hatchery setting. If no unexpected problems arise with the current cultures, we can estimate, based on results with a species with similar growth (*Strophitus undulatus*), that these individuals will be approximately 30 mm in length by the fall of 2008. This size is large enough to tag and stock, but they could be vulnerable to predation by muskrats or raccoons at that size. They would likely reach approximately 50 mm by the fall of 2009. We plan on propagating this species again in 2008 using the Duck Creek stock at the Table Rock Hatchery.

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